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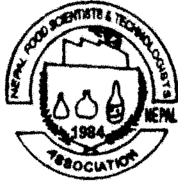

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Regd. No. 224/047/048

The Association is a professional and educational organization of Food Scientists and Technologists in Nepal with its central office in Kathmandu.

There are Two chapters- Purbanchal (Eastern Development Region) Chapter and Narayani (Mid Development Region) Chapter of the Association, which are located at Dharan and Hetauda respectively.

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- To facilitate the development and propagation of knowledge of Food Science and Technology.
- To provide a forum for discussion and exchange of the outcomes of research work in the field of Food Science and Technology.
- To create the supportive environment in order to encourage innovative works in Food Science and Technology.

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- Publication of Journal of Food Science and Technology Nepal (JFSTN), Food Nepal and News letters.
- Arranging lectures and seminars on different aspects of Food Science and Technology for the benefit of members and the Public

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- Membership is open to graduates in Food Science and Technology as well as to those engaged in these professional activities. Students studying in the related graduate course can be illegible to the student membership.
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Editorial

Development of Processing Technology has made tremendous divertification in food products. Newer and newer innovations in the field of Flavour Technology has been instrumental to present food items according to the tastes, pallets and choices of the consumers at every corner of the globe. Because of the rapid pace of innovation or new products and formulations, the business propogating dimension has been prosporous and brightened every hour. In the center of every activity of food Processing and marketing, there is the consumer. Behind the effort of serving taste, flavour, satiety and comfort to the consumer, there has to be a strong justification in the effort that consumer are addressed properly for their health and nutrition. However there are consumers representing significantly higher magnitude of world population at a choiseless circumstances to whom food means the ultimate bulk of staple available in front of them. Therefore while thinking, planning, acting and propogating for food, we have to focus both the chucks of world population those in the mainstream of world economy and those which are left behind from the mainstream. Nutrition and food safety should be thought as a central issue while working for food in terms of Research, Development, Innovation, Production and iversification.

Ganesh Dawadi
Editor-in-Chief

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Some Indigenous Limbu Foods of Dhankuta (district), Terhathum (district) and Dharan (municipality, Sunsari district)

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Some indigenous Limbu (an ethnic group of Nepal) foods of Dhankuta (district), Terhathum (district) and Dharan (municipality, Sunsari district) were studied. The annual production- and sales-volume of jand (cereal beer) and raksi (congeneric distillate from jand) were found to be the highest (totaling about 800MT of cereal). These products contribute significantly to their socio-economic status (annual income of about US $ 870,000). Other foods studied in detail were kinema (fermented soybean product), murcha (an amylolytic fermentation starter cake), yangben (edible lichen), lunghakcha (boiled product made from green maize paste), sargyangma (a type of sausage), womyuk (bitter dish made from chicken down-feather and offal), and papanda (finger millet bread). The nutritional values (in terms of proximate constituents) of these products were found to be quite impressive. Of them, kinema, yangben, jand, raksi and murcha have scope for commercialization if some improvement in production method can be carried out. Lunghakcha, sargyangma and womyuk also have scope for niche market because of their unique sensory properties.

Keywords: Ethnic group, Indigenous knowledge, Kinema, Limbu, Lichen, Murcha

Introduction

Today, indigenous knowledge (IK) is recognized as a critical factor for sustainable development (Gorjestani, 2004) because recognition of farmer's knowledge has been steadily gaining momentum in sustainable development and there is growing awareness among the development professionals of the value of enhancing scientific and professional understanding with the detailed knowledge held by the farmers. Local knowledge is not only the knowledge and beliefs transmitted from generation to generation, obtained as a result of experience but also a dynamic resource modified by contemporary experience and experimentation.

Indigenous technologies, practices and knowledge systems have been studied extensively by sector specialists and even more so by social anthropologists. However, most studies are descriptive: they concentrate primarily on the social or ethnological aspects rather than on the technical ones. Available literatures contain limited information on the systematic transfer of local knowledge across communities and cultures (ARWB, 1998).

Nepal is an excellent repository of cultural, ethnobotanical and indigenous foods heritage. There are 59 ethnic groups in Nepal. They speak over 75 different dialects and use over 800 plant species for various purposes (Manandhar, 1997). Limbus are one of the major ethnic groups of Nepal with a very rich cultural heritage (Bista, 1967; Bairagi Kainla, 1996; Subba, 1999; Sherma, 1999). They produce wide varieties of foods with an astounding range of tastes and flavors. These foods are consumed as staples, adjuncts to staples, condiments and as fermented beverages. A few fermented products are also used as starter for the main fermentation. The main categories of agricultural materials used for preparing indigenous foods include meat and meat by-products, soybean, lichens, cereals and other starchy materials, and many other wild and cultivated plant materials.

Due to rapid urbanization, many of the indigenous practices are on the verge of extinction. A greater awareness on the importance of IK is likely to help preserve the knowledge base for the use and benefit of the local community.

A comprehensive documentation of indigenous food practices can only be possible through a very rigorous study. Despite the plethora of traditional foods available in Nepal, unfortunately, no comprehensive data exist *vis a vis* their production profile, consumption pattern, safety and nutritional aspects, and contribution towards the rural economy. It is pertinent at this point that an exhaustive study be carried out to determine, qualitatively and quantitatively, the magnitude of impact these indigenous foods have on our lives.

Thus, the aim of this study was to collect information on some selected indigenous Limbu foods of Dhankuta (district), Terhathum (district) and Dharan (a municipality of Sunsari district) and peruse related literatures in order to shed light on scientific aspects of these foods.

Materials and Methods

The study was carried out by a multidisciplinary team consisting of Botanists (2), Sociologist/Anthropologist (1) and Food Technologist (1), all of them familiar with the Limbu dialect. Because of the time constraint, the present study was limited to three sites of Koshi zone, viz., Dhankuta (district), Terhathum (district) and Dharan (municipality, Sunsari district) only.

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Information on methods of preparation (and use of local resources), use and popularity and trade of each food were recorded from the key informants (local authorities, senior citizens, housewives, *phedangma* (Limbu priest), and *yeba/ yema* (shaman)) by open-ended conversations as well as semi-structured interviews. The key informants (10 from each site) were selected by free-listing technique (Martin, 1995; Rastogi, 1998). Selected key informants were also requested to demonstrate the actual methods of preparation and serving. Housewives were encouraged to participate in the discussion process. The nine popular food preparations (including alcoholic beverages and amylolytic fermentation starters) selected for the study in the present case were *kinema*, *jand*, *raksi*, *murcha*, *womyuk*, *lunghakcha*, *papanda*, *yangben* and *sargyangma*. List of ingredients and amounts used in the demonstration was prepared to facilitate estimation of nutritional value (crude protein, crude fat, crude fiber, ash, moisture and carbohydrate).

The scientific aspects of these foods were obtained from different published works. Data on nutritional composition were extracted from food composition tables (Rao and Polacchi, 1972; Mudambi and Rajagopal, 1990; HMG-N, 1994) to estimate the nutritional value.

Results and discussion

Details regarding the indigenous Limbu foods popular in Dhankuta, Terhathum and Dharan are presented and discussed in the following paragraphs:

Kinema

Kinema is a fermented soybean (*Glycine max*) product indigenous to Limbus. The food is popular in the eastern hills of Nepal and is valued by Limbu, Rai, Yakkha, Sunuwar and Dewan ethnic groups. It is commonly used as curry and *achar* (side dish used as appetizer). Black variety soybean (also called *Nepali Bhatmas*) is preferred to white variety, as exemplified by the term *chembikhik* (*chembi* = black variety soybean) for *kinema* in Limbu dialect.

Because of the peculiar smell, *kinema* can be revolting for a new user but when accustomed, it becomes highly appetizing (KC *et al.*, 2004).

Kinema is rich in protein, fat, minerals and carbohydrate (Karki, 1985). It is superior to the raw soybeans. *Kinema* from black soybean (with hull) contains 47.6% crude protein, 22.7% crude fat, 15.2% moisture and 6% ash (Shrestha, 1997). Few basic researches are available (Karki, 1985; Shrestha, 1989; GC, 1993; Sarkar *et al.*, 1996; Shrestha, 1997; Tamang, 1998; Moktan, 2001) on the biochemistry, chemistry, ingredient optimization, product diversification and microbiology of *kinema*. These studies show that fermentation of soybean helps remove toxins (trypsin inhibitors present in the raw beans) and improves nutritional value (increases water-soluble vitamins and free amino acids) and digestibility. Tamang (1998) has reported that the dominant microorganisms of *kinema* are strains of *Bacillus subtilis* and lactic acid bacteria.

Kinema is a value-added product. Therefore there is ample scope for *kinema* trade, both in the form of wage-employment and self-employment.

Official data on production- and sales-volume of *kinema* in the study sites could not be found. However, at a conservative, about 1000 kg of soybean is used per month for the production of *kinema* alone. Approximately 30% of the product is sold locally, about 30% sent abroad (for Nepalese residents and workers in Hong Kong and Brunei), 30% consumed at home, whilst the rest is shared with the neighbors. Dry form of *kinema* has has already been introduced in the market and is available in a number of groceries and stores.

A generic process of *kinema* production (observed in the study sites) is as follows:

Soybean → cleaning → steeping in water (overnight) → draining → boiling to cook → draining → crushing in *okhli* (large, wooden mortar-pestle, Figure 4) → placing in *dalo* (woven bamboo-strip basket, Figure 4) previously lined with banana (*Musa paradisaca*), *bharla* (*Bauhinia vahlii*) or *dhusure*(*Colebrookea oppositifolia*) leaves → sprinkling of a small amount (about 0.5%) of wood ash → wrapping the beans with banana leaves → blanketing with cloth pieces → incubation in a warm place (usually near the firewood oven) for 2-3 days → fresh *kinema* → eaten or dried to make dried *kinema*.

The role of wood ash in *kinema* preparation is still unclear. Since *kinema* is an alkaline fermented food, ash probably helps maintain favorable pH (~ 9) needed for initiating the fermentation. Additionally, ash may play a role in the development of unique taste of *kinema* and supply of mineral elements to the essential organisms.

Traditional *kinema* fermentation depends on natural flora of leaves used for lining *dalo* and the air-borne flora of the immediate surrounding. It is thus a spontaneous fermentation of mixed culture, giving rise to a product with properties somewhere between Japanese *natto* (bacterium-fermented soyfood) and Indonesian *tempeh* (mold-fermented soyfood). It is observed that the quality of *kinema* does not remain consistent, often leading to totally unacceptable (putrid) product. This variation is basically due to the variable microbial profile of the leaves used for wrapping the beans (KC *et al.*, 2004). Apart from this, sanitation, quality of raw material and time-temperature combination of fermentation also might affect the quality. With such inconsistent quality of *kinema*, commercial production would always be risky. Tamang (1998) and Dhungel (2000) showed that *kinema* starter cultures can be more reliable than the microflora of

the banana leaves. KC *et al.* (2004) suggested using a good quality *kinema* as a starter. A good quality fresh *kinema* is characterized by ammoniacal flavor and formation of stringy threads when touched with fingers (Tamang and Sarkar, 1988).

Although the quality of *kinema* is improved, one is still left with the problem of producing *kinema* in quantities large enough to take a commercial form. This is because *kinema* making is a solid-state fermentation where cooling and aeration problem is inevitable. In small lots, cooling is not necessary. In fact, a mild warming up may be needed. However, in bulk fermentation, the mass gets heated up due to bacterial activity and this can spoil the product. Some sort of improvement is necessary at this point.

Murcha, Jand, Nigar and *Raksi*

Murcha (also spelt *marcha*, *marchar* and *marchaa*) is an amylolytic fermentation starter cake (Tsuyoshi *et al.*, 2005) made by mixing selected wild plants (called *murcha* plants hereafter) in a starchy substrate. KC *et al.* (2001) have listed use of some 38 *murcha* plants in the Far East zones (Mechi and Koshi) of Nepal. *Murcha* serves as a source of microorganisms (a mixture of saccharifying molds, fermentative yeasts and acidifying lactic acid bacteria derived from *murcha* plants) for the fermentation (Rai and Subba, 2003) and has been reported to have a shelf life of about 1 year (Karki, 1986).

Some researches on *murcha* and similar starters are available (Gautam, 1987; Tamang and Sarkar, 1988; Verma, 1991; Tamang and Sarkar, 1995; Shrestha *et al.*, 2002; Shrestha and Rati., (2003); Dung *et al.*, (2005); Tsuyoshi *et al.*, 2005) but these studies are limited to either isolation and identification of *murcha* flora or assessment of brewing value. Study on the preparation of *murcha* using isolated cultures is relatively scarce. To this end, a simple protocol for the preparation of improved *murcha* described by KC *et al.*, (2004) might be useful.

A generalized method of *murcha* preparation observed in the study area is as follows:

Steeping (few hours to overnight) cereals (rice or maize grits) in water → draining → pounding the soaked cereals along with sun-dried *murcha* plants and a small amount of *murcha* seed from the previous batch → moist powder obtained → addition of water → mixing to form a stiff dough of about 50% moisture content → dividing the dough into small balls (~ 1.5 cm dia) or flat cakes (0.5-1 cm thick, 3-10 cm dia) → placing the cakes (in a single layer) on a mat previously lined with *pire unyu* (fern plant, *Christella appendiculata*) leaves or paddy straw (Figure 1) → blanketing with *pire unyu*, paddy straw or cloth → incubation for 2-3 days until the cakes swell and become distinctly moldy → sun-drying (to about 13% moisture content) → *murcha*.

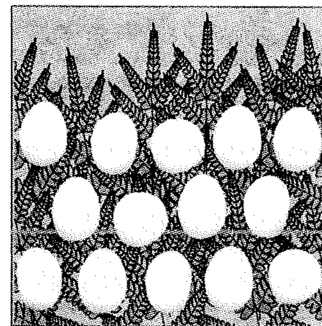

Figure 1: *Murcha* cakes spread on fern leaves for incubation

Data on *murcha* production profile is scanty. However, it can be safely assumed (based on interview) that the annual production would not less than 100MT. This trade is one of the important economic activities among not only Limbus but also *Matuwali* (collective term: those with the tradition of dr.nking alcohol). According to Karki (1984), 10% of *murcha* production has been industrialized.

The role of *murcha* in *jand* production is shown in Figure 2.

Jand (also spelt *jandh*, *jnar*, *jaanr*, *jnard*) is a generic term that refers to sweet-sour cereal beer made from grains like finger millet (*Eleusine coracana*), rice (*Oryza sativa*), wheat (*Triticum* spp) and maize (*Zea mays*) by using *murcha*. A generic process of *jand* production using finger millet as the raw material is as follows:

Finger millet → dehusking in *okhli* (Figure 4)→ winnowing in *nanglo* (a woven bamboo tray, Figure 4) → washing → cooking until gelatinized → spreading on bamboo mat to cool → addition of *murcha* powder (about 0.5%) and turning → biomass build up (aerobic fermentation for 1-2 days in a *dalo* lined with banana leaves) → filling in *ghyampo* (narrow necked earthen jar, Figure 4) → plugging → anaerobic fermentation for over a week (depending on temperature) → *jand* → serving as aqueous extract or as *tongba* (a variation of serving *jand*).

Jand finds a very prominent place in Limbu and Rai culture in particular and among other ethnic groups in general. The tradition of offering *jand* to guests is a unique way of showing hospitality. *Jand* is also used in several festive occasions, ritual rites, settling disputes and appeasing deities (Rai, 1991).

Nigar is the clear liquid that spontaneously accumulates upon prolonged fermentation (over several months) of *jand*. The product likens *sake* and is highly prized by the drinkers. *Nigar* can be classified as cereal wine, rather than beer.

Jand is served in different forms. Strained *jand* is prepared by leaching out the readily extractable contents from the mash with water (usually lukewarm). A strainer made of thin

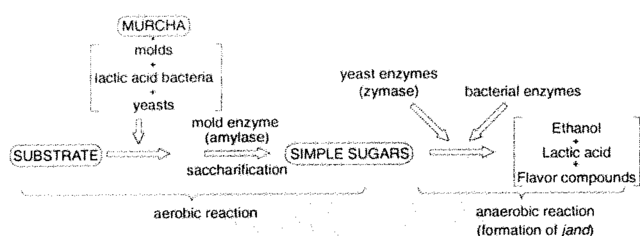

Figure 2: Fermentation process during *jand* production

bamboo strips or perforated aluminum strainer is normally used for straining the liquor. This form of beverage is drunk in *lumbha* (small brass bowl) or deep aluminum mugs until satiated (Rai, 1991). The beverage is cloudy in appearance and has a very short shelf-life, of about few hours. The self life of strained *jand* can be extended by pasteurization (Mongar and Rai, 2005).

Tongba is another variation of serving millet *jand*. About 500g of mash is transferred to a cylindrical bamboo or wooden barrel (Figure 4) previously filled (about one-fourth) with hot water. The juice gradually and spontaneously gets extracted, and after about 15 min, the extract is sucked in through a bamboo or metal pipe called *peepa*. The mash can be repeatedly steeped and sucked in to exhaust the extract.

Raksi (also spelt *rakshi, rukshi*) is an unaged congeneric spirit obtained by pot distillation of the slurry of *jand*. The product likens whiskey and has highly varying alcohol contents (KC *et al.*, 2004), generally between of 15 and 40% (depending on the degree of exhaustion). The traditional distillation assembly is shown in Figure 3. Several basic researches have been done on *raksi* production from different cereals using *murcha* as well as pure culture isolated thereof (Rai, 1984; Subba, 1985; Shrestha, 1985; Yadav, 1993; Bhandari, 1997) but there seems to be general lack of attention towards process development such as preparation of good starter culture, increasing efficiency of traditional distillation apparatus, and separation of fients and foreshots for improving quality of *raksi*.

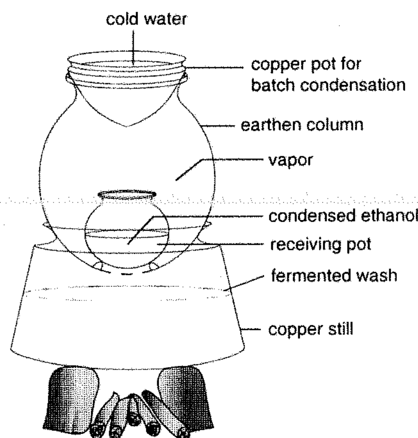

Figure 3: *Raksi* making equipment

With the advent of alcoholic beverages based on 'new' technology, *jand* has earned itself social stigma among the well-offs, simply because it happens to be a poor man's beer.

Although hated by local administration, production and sale of *jand* and *raksi* have remained one of the most lucrative activities among many ethnic groups. *Jand* trade is the sole means of earning livelihood of majority of these people.

It is estimated that 250, 225 and 325MT of cereal grains are used annually for *jand* and *raksi* making in Dharan, Dhankuta, and Terhathum respectively. Considering that half this amount is sold in the market places, the total annual income comes to about US $ 870,000 (as of 2006) which is indeed a huge sum.

Grains that are normally considered inferior for food use, e.g., finger millet, broken rice, low quality maize, can be used for *jand*. In this sense, it is a good option for by-product utilization. The spent grains (brewery residues) can be fed to cattle and pigs. Thus, *jand* trade can become a viable option for the rural mass if adequate attention can be given on its promotion, development and standardization.

Unlike other beverages such as wine and beer, *jand* contains large amounts of starch, which contributes to energy. It also contains other unrefined carbohydrates (e.g., resistant starch) that can have therapeutic value. It is generally believed (and sometimes manifested) that *jand* drinking in moderation can indeed have a beneficial effect on health.

Typically, *jand* contains 5-9% alcohol; 0.8-1.1% acidity (as lactic acid); 1.6-2.5% reducing sugar (as glucose); 1.6-2.8% total sugar (as sucrose); 12-14% starch; 76-80% water; and traces of methanol, esters, aldehydes and other flavor components (Upadhyaya, 2005).

The technology of production is rather straight-forward and requires minimum capital inputs. The basic problem, besides issues of legality in its indiscriminate production and sale, is the variability in quality. Quite often, the hard earned money goes waste because of unexpected spoilage. They attribute this to bad *murcha* or other superstitious factors.

Some researches are now available suggesting that quality of *murcha* can be kept consistent using simple scientific inputs such as sanitation and appropriate culture preservation techniques. KC *et al.* (2004) reported that *jand* of very consistent can be prepared by using pure cultures. The maintenance and preservation of culture can be modified to suit household-level know-how. Interest on study of *jand* in the recent years in different academic and research institutions probably stems from the success stories of African beer and Japanese *sake*. African beer is produced from sorghum whilst Japanese *sake*, a wine, from rice. The technologies of both of them are similar to *jand* making but today the production of *sake* and African beer has already reached a commercial scale.

Yangben

Yangben is a Limbu term that refers to certain species of lichens consumed as food by Limbus of eastern hills of Nepal. Of the estimated 20,000 species of lichens found in the world, 2000 (465 of them identified) of them are found in Nepal (Sharma, 1995). Most of the *yangben* are found in Dhankuta, Taplejung and Terhathum districts and belong to *Parmelia*, *Ramalina*, *Cladonia* and *Usnea* species.

Lichens have a wide range of uses, ranging from fabric dyeing, cosmetics, medicinal, hop substitute, alcoholic beverages to food use (Sastri, 1998; Brodo *et al.*, 2001). Some of the lichens used for food purpose in various parts of the world are species of *Parmelia, Endocarpon, Cetraria, Dermatocarpon, Ramalina Umbilicaria, Cladonia* and *Usnea* (Sastri, 1998). *Cladonia* and *Usnea* spp are common in Dhankuta district whilst *Ramalina* is found in Dhankuta, Taplejung and Terhathum districts in the eastern hills of Nepal.

Nepal exports about 250MT of lichens to India annually, 70% of which consists of *Parmelia* species, the rest being *Usnea* species (Subba Yashok, 2003).

The cell wall of lichen contains complex polysaccharides like lichenin, glucan, and isolichenin. Lichens also contain polyols, depsides and depsidones. Depsidone derivatives are responsible for the characteristic bitterness of lichens (Sastri, 1998).

Although lichens are flat-tasting or have an acidic taste, they have plentiful starch-carbohydrate which makes lichen useful for human food (Sastri, 1998). The proximate composition of raw (unprocessed) *yangben* reported by Subba Yashok (2003) is shown in Table 1.

Table 1: Proximate composition (g/100g dry basis) of unprocessed two varieties of *yangben*

Parameter	*Ramalina farinacea*	*Ramalina conduplicans*
Moisture	13.7	11.3
Carbohydrate	70.4	71
Protein	9.04	9.4
Fat	5.8	5.1
Crude fiber	10.0	11.6
Ash	3.95	2.5

As can be seen in Table 1, the protein and carbohydrate contents are quantitatively similar to those of conventional cereal grains, viz., rice, maize and wheat. *Yangben* has very high crude fiber content. Crude fiber has no nutritional implications but is an indispensable factor for maintaining healthy bowel function.

Yangben is probably never eaten alone. It is normally served as mixed curry with offal of animals including blood as the complement. *Yangben* curry goes well with alcoholic beverages like *tongba* and *raksi*. It can also be taken with the main dish.

Raw *yangben* is bitter in taste. Traditionally, the bitter principles are removed by boiling *yangben* in 10% wood ash solution in a closed vessel for 20-30 min. The dark gruel that gets extracted is drained away. The alkaline nature of ash probably helps neutralize the bitter principles present in raw *yangben*. Boiling under alkaline condition also helps lighten the color. Later on it is washed, sun-dried, packed and stored. It can be rehydrated before making curry mixture.

Due to the use of liberal amounts of wood ash and consequent inadequate washing, the ash content of *yangben* becomes appreciably higher (Subba Yashok, 2003). In relation to the amount of *yangben* consumed normally, the increased ash levels may not be harmful. However, the sensory and nutritional quality may be affected because of the variation in mineral composition and alkalinity of ash.

Although *yangben* is customarily taken as a delicacy it can also be a good source of income. A small amount of processed yangben is occasionally found in marketplaces for sale. The commodity is highly prized by Limbus. Collection and home processing of *yangben* does not entail full-time involvement. People going to forests for the collection of fodder and firewood, as also cowherds roaming the forest area (or in the vicinity) with their cattle, can readily collect a sizeable amount of *yangben*. *Yangben* collection can therefore be an important side job.

A few studies on *yangben* are available. Those of Dhungana (1985), Subba (1997) and Subba Yashok, (2003) have relevance to the present study as they relate to food use of *yangben*.

Subba Yashok (2003) carried out a comparative study of the efficacy of ash, sodium bicarbonate ($NaHCO_3$) and calcium carbonate ($CaCO_3$) for removing the bitter principles. His finding indicated that sodium bicarbonate can also be used for processing *yangben*. Since the quality of *yangben* can be materially affected by ash (because of variable mineral composition and alkalinity), supplanting it with cheap and readily available sodium bicarbonate can be an attractive proposition.

Lunghakcha

Lunghakcha is a Limbu name for a solid food made by boiling green maize paste. The food is eaten as breakfast or in the main course but can also be dried for future use. Normally, it has the shape of a rolling pin.

The traditional preparation of *lunghakcha* entails following steps:

Green maize → removal of the cob husk → shelling of grain → grinding in *janto* (hand operated stone attrition mill, Figure 4) or *okhli* (Figure 4) → dividing into lumps (~ 50g) → Packing (wrapping in green cob husk) → tying the packet → transferring to boiling water → boiling until cooked (10-15 min)→ straining → *lunghakcha* → serving with *achar*.

Lunghakcha preparation probably evolved in the period of food crisis. People might have resorted to this alternative to feed themselves until optimum harvest maturity of maize. Under normal condition, people generally do not prepare *lunghakcha*. The food has a unique taste and is palatable. The nutrient content of *lunghakcha* is similar to that of green maize. Green maize grain contains 67% moisture, 4.7% crude protein, 0.9% crude fat, 1.9% crude fiber, 0.8% ash and 24.7% carbohydrate. If some improvements can be done it may find a place in restaurant and hotels. For instance, grinding in *janto* is both tedious and unhygienic. Use of *okhli* can be a better option. The best option would be to use any of the commercial grinding machines, such as hand-operated attrition device, readily available in the market.

Sargyangma

This is a type of unsmoked sausage prepared by Limbus in freshly prepared pork intestine casing. It is normal practice to prepare *sargyangma* in the household whenever a pig is slaughtered. *Sargyangma* is a good example of by-product utilization. People of all age relish it.

The nutritional value of *sargyangma* depends on the amount of each ingredient used. The main ingredients influencing the proximate composition are quantities of *yangben* (60-70%), blood (10-20%), heart, liver, fat and meat used in the recipe. On an average, a typical *sargyangma* contains 12% crude protein, 41% moisture, 16% crude fat, 30% carbohydrate (from *yangben*) and 1% minerals. The dish is rich in iron because of the inclusion of blood.

Sausages prepared by Limbus are very unique and traditional. But it has yet to take a commercial form. Adopting basic technological inputs such as selection of raw materials, hygiene, 'adequate' heat treatment and suitable packaging can significantly improve the product quality.

Sargyangma is prepared as follows:

Large intestine of pig → cleaning → stuffing with seasonings (salt, chilli, ginger, garlic) to taste, chopped meat, offal (heart, liver, blood), fat, *yangben* → tying with thread → boiling until cooked (15-20 min) → *sargyangma* → chopping → serving.

The fundamental difference between a regular sausage and *sargyangma* is the use of *yangben* as the principle ingredient in the latter. Fresh *sargyangma* has a very short shelf life (1-2 days). Occasionally, people preserve the surplus *sargyangma* by immersing it in dry-rendered lard (from pig). This product remains stable for at least 6 months at temperatures below 25°C.

Womyuk

Womyuk is a special Limbu dish prepared from charred down-feathers, wings and offal (legs, intestines, gizzard, heart, head and liver) of local chicken. *Womyuk* is bitter in taste, goes well with alcoholic beverages, and also works as an appetizer. In the household, it is prepared every time a local bred chicken is dressed. The nutritional value depends on the amounts of individual ingredients used in the recipe. The dish is rich in minerals, particularly calcium and phosphorus due to the inclusion of fine bones. A typical *womyuk* recipe contains 50% moisture, 15% crude fat, 28% crude protein, traces of carbohydrates and 7% ash. Some key informants associate bitterness of *womyuk* to medicinal property.

Womyuk is prepared as follows:

Local chicken → killing → flaming and plucking away of pennae → flaming of down- feathers and filoplumes until charred → collection of charred residues → collection, cleaning, and chopping of wings and offal (heart, liver, head, gizzard, legs and intestines) → mixing with seasonings like garlic, ginger, onion, chilli and salt (to taste) → frying in oil → *womyuk* → serving.

Womyuk is not produced in large amounts, nor is it sold in the market. However, it can find a place in restaurants and hotels as a special dish.

Pyrolysis of organic matter (during charring) have been shown to form carcinogenic polycyclic hydrocarbons such as 3,4-benzpyrene (Bender, 1992), which implies that excessive consumption of *womyuk* may not be good for health.

Papanda

Papanda is one of the many types of breads made from finger millet. It is generally eaten as such or with some *achar*. It is generally sweet in taste and has a crumbly texture. *Papanda* is comparatively tougher to bite and is unsuitable for old people with weak teeth.

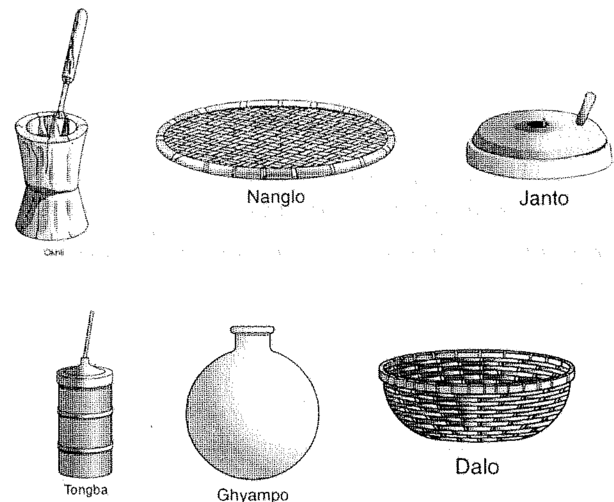

Figure 4: Some traditional materials described in the text

Finger millet is considered a poor man's cereal (BOSTID, 1996). Finger millets of both red and white varieties are available but the red millet is preferred for making *papanda*. All food items derived from red millet become black upon cooking. This is considered to be unappealing to many people. Finger millet contains 13% moisture, 7.3% crude protein, 1.5% crude fat, 3.5% crude fiber, 72% carbohydrate and 2.7%

minerals. Finger millet is known to be rich in iron. Thus, the black color notwithstanding, *papanda* is very nutritious.

Papanda is prepared as follows:

Finger millet flour → preparation of dough → dividing and patting it into shape → wrapping the cakes in banana leaf → burying inside hot cinders (in the active firewood oven) → checking for completion of cooking → *papanda* → serving with *achar*.

Papanda is limited to household level and is not available in the market. Improvement in its appearance and baking techniques are needed if it is to take a commercial shape.

The foregoing results show that indigenous Limbu foods consist of both fermented (*kinema*, *murcha*, *jand* and *raksi*) and non-fermented (*lunghakcha*, *papanda*, *sargyangma yangben* and *womyuk*) types. *Kinema* is beginning to find a place in the shelves of the stores and this is an encouraging sign. One of the challenges of *kinema* preparation (in commercial quantities) is the solid-state nature of fermentation that limits its batch size to less than 10-15 kg. Some researches, with emphasis on appropriate technology, are needed to address this problem. The variation in the quality of *kinema* can be eliminated and/or reduced by using starter cultures instead of depending on spontaneous fermentation.

The quality of *jand*, *nigar*, and *raksi* basically depends on the quality of *murcha*, whose traditional preparation in itself is prone to variation. Development of simple methods for the preparation or propagation of *murcha* and improvement of *raksi* distillation system is needed to address these problems. *Jand* and *raksi* trade are considered illegal by local governments and consequently lack good image among the urbanites. Overcoming this hurdle can tremendously increase the scope of these products.

Of the non-fermented indigenous Limbu foods, *yangben* is considered a delicacy. It is occasionally sold in local market. *Lunghakcha*, *womyuk* and *sargyangma* are other non-fermented Limbu foods that have scope for niche market because of their unique sensory quality.

Conclusions

Limbus have a very rich ethnobotanical and traditional food heritage. Some of their well known traditional foods (including alcoholic beverages and amylolytic starters) that have received renewed interest in the scientific community are *jand*, *raksi*, *murcha*, and *kinema*. These products contribute significantly to the community economy. The lesser known products, viz., *womyuk*, *sargyangma* also deserve attention because they entail by-product utilization. Likewise, *yangben* and *papanda* preparations are examples of how neglected food resources can be exploited. Since these indigenous foods are intimately associated with our identity - and even survival - any effort expended on any aspects of indigenous foods should be more than justified.

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References

ARWB. (1998). Indigenous Knowledge for Development: A Framework for Action, Africa Region World Bank

Bairagi Kainla. (1996). *Tonsin Mundhum* of the Limbu Ethnic Group in the Kirant *Mundhum*. *J. Nepalese Studies*

Bender, A. (1992). Meat and Meat Products in Human Nutrition in Developing Countries. Food and Nutrition Paper 53, FAO, Rome. (e-book)

Bhandari, S. (1997). Comparative Study on *Raksi* Production from Different Raw Materials Using *Murcha* and Pure Culture. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 28-31

Bista, D.B. (1967). People of Nepal. Ratna Pustak Bhandar, Nepal.

BOSTID. (1996). Board on Science and Technology for International Development, National Research Council. Lost Crops of Africa, Vol. 1., National Academy Press, Washington, D. C. (e-book)

Brodo, I. M., Sharnoff, S. D. and Sharnoff, S. (2001). Lichens of North America (e-book). http://www.lichen.com/index.html

Dhungana, K. P. (1985). Study of the Food Values of Lichens (*Jhyau*) of the Species *Cladonia* and *Usnea* found in Dhankuta District of Dadabazar Village Panchayat. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 12-35

Dhungel, Y. (2000). Preparation and Quality Evaluation of Starter Culture for *Kinema* Production. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, p 44

Dung, N. T. P., Rombouts, F. M. and Nout, M. J. R (2005). Development of Defined Mixed-culture Fungal Fermentation Starter Granulate for Controlled Production of Rice Wine. *Innov. Food Sci. Em. Technol.* 6: 429-41

Gautam, G. (1987). Efficiency of Ethanol Production by *Murcha* Yeast. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 33-40

GC, K. (1993). Study on Microbiology and Chemistry of *Kinema*: An Indigenous Fermented Food of Eastern Nepal. MSc Thesis, Tribhuvan Univ., Nepal, pp 25-36

Gorjestani, N. (2004). Indigenous Knowledge for Development: Challenges and Opportunities. The World Bank

HMG-N. (1994). Food Composition Table of Nepalese Foods. Ministry of Agriculture, Agriculture Development Department, Nutrition Program Section, Babarmahal, Kathmandu

KC. J. B, Rai, B. K. and Subba, D. K. (2001). Plants Used in *Murcha* Preparation in Eastern Nepal. *J Hill Research, Sikkim Science Soc.* 14(2): 107-09

KC. J. B., Rai, B. K., Ghimire, G. and Subba, D. K. (2004). Practicals in Basic Biochemistry and Industrial Microbiology, pp 70-75, 97-99, 111-113

Karki, T. B. (1984). Some Nepalese Fermented Foods and Beverages of Nepal. In: *Proceedings, First National*

Seminar on Food Industries and Food Technology. Central Campus of Technology, Dharan, Nepal
Karki, T. B. (1985). Microbiology of *Kinema*. In: *Proceedings, Asian Symposium on Non-salted Soybean Fermentation*. Tsuba, Japan.
Karki, T. B. (1986). *Murcha*. In: A Concise Handbook of Indigenous Fermented Foods in *the ASCA Countries*. Saono S., Hull R., Dhamcharee, D. (eds). The Govt. of Australia, Canberra, p 136
Manandhar, N. P. (1997). Ethnobotany in Nepal. In: *Proceedings, National Training Workshop in Nepal*, ESON, Nepal.
Martin, G. J. (1995). Ethnobotany: A Methods Manual. Chapman and Hall, London, pp 213-15, 96-112
Moktan, D. (2001). Preparation of Soy-maize *Kinema* using Pure Culture and its Quality Evaluation. B. Tech. (Food) Dissertation, Tribhuvan Univ., Nepal, pp 17-29
Mongar, G. and Rai, B. K. (2005). Preservation of Strained *Jand* by Pasteurization. *J. Food Sc. & Technol. Nepal,* 1: 58-61
Mudambi, S. R. and Rajagopal M. V. (1982). Fundamentals of Food and Nutrition. 3rd edn. Wiley Eastern Limited, New Delhi, pp 277-90
Rai, B. K. (1991). Preparation and Quality Evaluation of *Jand* from Malted and Non-malted Millet (*kodo*) by Using *A. oryzae* and *S. sake*. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, p 11
Rai, B. K. and Subba, D. K. (2003). Screening of Fermentative Yeasts from *Murcha* Plants and Assessment of their Brewing Value. *J. Food Sci. Technol.* 40(4): 382-85
Rai, R. K. (1984). To Study *Raksi* (Distilled Liquor) Making Process in Eastern Nepal. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 24-55
Rao, M. N. and Polacchi, W. (1972). Food Composition Table for Use in East Asia. FAO Food Policy and Nutrition Division, Rome (e-book)
Rastogi, A. (1998) Basic Survey and Assessment Methodology for Applied Ethanobotanical Research. In: *Proceedings, Ethnobotany for Conservation and Community Development.* Shrestha, K. K., Jha, P. K., Shengii, P., Rastogi, A., Rajbhandari, S. and Joshi, M. (eds). Ethnobotanical Society of Nepal (ESON)
Sarkar, P. K., Jones, J. L., Gore, W., Carven, S. G., and Somerset, M. S. (1996). Changes in Soybean Lipid Profile during *Kinema* Production. *J. Sci. Food Agri.* 71: 321-28
Sastri, B. N. (1998). The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products, Vol VI: L-M. National Institute of Sciene Communication, Council of Scientific and Industrial Research, New Delhi, India, p 83
Sharma, L. R. (1995). Innumeration of Lichens of Nepal. Biodiversity Profile Project Publication No. 3., Dept. of National Parks and Wildlife Conservation, Kathmandu, Nepal
Sherma, N. (1999). Marriage System and Death Rituals of the Limbu Tribe. In: *Nirman*. Nirman Publication, Sikkim
Shrestha, A. K. (1989). A Comparative Study on Preparation and Quality Evaluation of Natto and *Kinema*. B.Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 38-57
Shrestha, A. K. (1997). Preparation, Evaluation, and Utilization of *Kinema* (Solid State Fermented Soyfood from Nepal) Flour. MSc thesis, Asian Institute of Technology, Bangkok, Thailand, p 7
Shrestha, B. (1985). Studies on *Rukhsi* [sic] Production from Rice by Traditional Method. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 42-54
Shrestha, H., Nand, K. and Rati, E. R. (2002). Microbial Profile of *Murcha* Starter and Physicochemical Characteristics of *Poko* – A Rice-based Traditional Fermented Food Product of Nepal. *Food Biotechnol.* 16(1): 1-15
Shrestha, H. and Rati, E. R. (2003). Defined Microbial Starter Formulation for the Production of *Poko* – a Traditional Fermented Food Product of Nepal. *Food Biotechnol.* 17(1): 15-25
Subba Yashok, G. (2003). Evaluation of Nutritive Value and Processing Effect on Nutrients of *Yangben* (Edible Lichen). B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 22-30
Subba, B. (1999). The Importance of Folk Song in Limbu Culture. In: *Nirman*. Nirman Publication, Sikkim
Subba, C. (1985). *Raksi* Production from Finger millet (kodo) by Traditional Method. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 26-34
Subba, R. (1997). Evaluation of Nutritive Value of *Yangben* (Edible Lichen) and Effects of Processing on its Nutrients. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 22-26
Tamang, J. P. and Sarkar, P. K. (1995). Microflora of *Murcha*: An Amylolytic Fermentation Starter. *Microbios.* 81(327): 115-22
Tamang, J. P. (1998). Development of Pulverized Starter for *Kinema* Production. *J. Food Sci. Technol.* 36: 475-478
Tamang, J. P., Sarkar, P. K. (1988). Traditional Fermented Foods and Beverages of Darjeeling and Sikkim – A Review. *J. Sci. Food Agric.* 44: 375-85
Tsuyoshi, N., Fudou, R., Yamanaka, S., Kozaki, M., Tamang, N., Thapa, S. and Tamang, J. P. (2005). Identification of Yeast Strains Isolated from *Marcha* in Sikkim, a Microbial Starter for Amylolytic Fermentation. *Int. J. Food Micro.* 99(2): 135-46
Upadhyaya, A. (2005). Effect of Raw Materials on the Quality of *Jand*. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, p 28
Verma, S. K. (1991). Study on the Preparation and Efficacy of Starter Cake Prepared from *Aspergillus oryzae* and *Saccharomyces sake* Using Rice as Binder. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 28-43
Yadav, B. K. (1993). Study on some Physicochemical Indices of Locally Produced *Rakshi* Quality. B. Tech. (Food) Dissertation. Tribhuvan Univ., Nepal, pp 25-36

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An Overview of Malnutrition in Nepal

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This article presents a brief overview on the situation of malnutrition (protein energy malnutrition and micronutrient deficiencies) prevalent in Nepal. Available data from different surveys show that protein energy malnutrition and micronutrient deficiencies mainly Vitamin A deficiency, Iodine deficiency disorder and Iron deficiency anemia are the serious problems in the country and the most vulnerable groups are children of below 5 years of age, pregnant and nursing mothers.

Keywords: Malnutrition, Protein energy malnutrition, Vitamin A deficiency (VAD), Iodine deficiency disorder (IDD), Iron deficiency anemia (IDA).

Introduction

Malnutrition or undesirable physical condition or disease condition results when the cells do not receive an adequate supply of the essential nutrients because of poor utilization of food. Sometimes, it occurs because people do not or cannot eat enough food that provide the essential nutrients to satisfy body needs. Other times, people may eat full, well balanced diets, but suffer from disease that prevents normal usage of the nutrients. Persons most prone to malnutrition are infants, preschool children, adolescents, the elderly and pregnant women,(Carolynn,1994).

According to FAO, 815,000,000 people are suffering from hunger and malnutrition in the world. Among them 777,000,000 people are in developing countries. Recent findings show that 1,500,000,000 people are suffering from iron deficiency anemia (IDA), 742,800,000 children are suffering from blindness due to Vitamin A deficiency (VAD) and 200,000,000 people are suffering from VAD in the world. Hunger and nutritional problems are mainly found in developing countries of which densely populated South Asia is no exception, (HMG/MoAC, 2002). Major nutritional problems in Nepal are Protein energy malnutrition (PEM) and Micronutrient deficiencies namely Vitamin A deficiency (VAD), iodine deficiency disorder (IDD) and iron deficiency anemia (IDA). This article presents current situation of malnutrition in Nepal.

According to Nepal Demographic Health Survey (NDHS, 2001), overall 50% of the children under 5 are stunted and 21% are severely stunted. Stunting increases sharply from 10% among children under 6 months of age to 57% among children age 24-35 months. Female children are slightly more likely to be stunted (52%) or severely stunted (24%) than male children (49% and 19%, respectively).

Children in rural areas are more likely to be stunted than in children in urban areas (37%), and children in the mountain zones are more likely to be stunted (61%) than children in the hills (53%) and Terai zones (47%). Eastern Development Region has the lowest level of stunting (45%). Ten percent of children under 5 years age are wasted and 1% are severely wasted. Forty eight percent of Nepalese children are under weight and 13% are severely under weight (NDHS, 2001).

Under nutrition among mother is also widely prevalent in Nepal. Nationally, the prevalence of thinness among women (BMI <18.5) is 25% which is higher than WHO cut off point of 20% and is highest in the Terai and in rural areas. In the Eastern Development Region, it is lowest in the mountains (11.5%) followed by the hills (13%) and is highest in the Terai (42%), (Dhungana, 2003). The main BMI for women in Nepal is 23.3. One in four women (27%) in Nepal falls below cut off, indicating that the level of chronic energy deficiency is relatively high. Obesity among Nepalese women varies with age and as women get older, they are more likely to be obese (NDSH, 2001). Comparative chart of situation of malnutrition on different years is shown in Table1.

Table 1: Trends of nutritional status of children in Nepal

Survey	Year	Malnutrition/ Under weight (%)		
		Stunting	Wasting	Underweight
National Nutrition Survey (NNS)	1975	69.4	13.0	69.1
Joint Nutrition Support Project Survey (JNSP), 5 district	1986	37.4	4.9	-
Nepal Multiple Indicator Surveillance Survey (NMIS) cycle 1	1995	63.5	6.0	48.5
Nepal Family Health Survey (NFHS)	1996	54.8	12.7	54.2
Nepal Multiple Indicator Surveillance Survey (NMIS) cycle 4	1997	53.3	16.5	-
Nepal Micronutrient status survey (NMSS)	1998	54.1	7.0	47
Nepal Demographic and Health Survey (NDHS)	2001	50.0	10.0	48

Source: NDHS, 2001

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Iodine deficiency disorder (IDD)

Iodine is naturally present in top soil which is absorbed and utilized by plants. By eating food grains, vegetables and fruits, we get iodine. But due to heavy rain falls and land slides, top soil becomes deficient in iodine content and which ultimately lead to IDD.

Though iodine is needed in small quantity, it is essential for normal growth, physical and mental development, (NMSS, 1998).IDD can cause brain damage in fetus and nervous system disorders. Prolonged IDD is likely to be irreversible. IDD can also cause still births in fetus, miscarriages, cretinism, neonatal mortality and psychomotor defects,(HMG, MoAC, 2002).

IDD is the most common malnutrition problem in Nepal. Nepal Micronutrient Status Survey (NMSS), 1998 shows that IDD can be divided into two forms namely (i) excretion of iodine in urine and (ii) goiter. Almost 90% of the iodine is excreted in urine and hence it provides an estimate of the current iodine intake. The prevalence of urinary iodine excretion (UIE) value is 43.6% among women and 35% among school aged children. UIE level was low in rural areas of all region and terai region in both women and school aged children. UIE level recommended by WHO/UNICEF is shown in Table 2.

Table 2. UIE level recommended by WHO/UNICEF

UAE Level	Remarks
>300μg/l	More than adequate
100-299μg/l	Adequate
51-99μg/l	Mild deficiency
21-50μg/l	Moderate deficiency
<20μg/l	Severe deficiency

Source: (NMSS, 1998)

The proportion of household using adequate iodized salt overall is highest in the hills and mountains and lowest in Terai. About 83% of the population is using iodized salt. Goiter is enlargement of thyroid gland. Thyroid size can be measured clinically by inspection and palpation. When iodine intake is inadequate, automatically thyroid increases in its size to fulfill thyroxin hormone production. So if the amount of iodine intake decreases, thyroid size increases. Hence these two are inversely proportional to each other. It is noted that the entire goiter is due to iodine deficiencies but all the iodine deficiencies are not a goiter. Prevalence of goiter was 50% in women (however prevalence of visible goiter was only 1.3%) and 40% in school aged children. Goiter was found to be lowest in central and Midwest Terai. By ecological zone, prevalence of goiter among women was highest in the mountains (60.6%) in comparison to Terai(492%) and theHills(48.9%). This problem was found to be increased with the advancement of age in women and school aged children, (NMSS, 1998).

Prevention and control:

Iodization of salt : Government of Nepal has promulgated Iodized Salt Production and Distribution Act 1999. This Act prohibits selling of non-iodized salt. But non-iodized salt is still available in the market because of socio-cultural behavior attached to it. The Salt Trading Corporation is the main agency for distribution of iodized salt in the country. Iodization of salt at 50ppm at the point of production to retain 30ppm at retail level and 15ppm at household level. A government has also monitoring iodized salt regularly at points of impacts, distribution and consumption. Encouraging National and district level, mass media to promote use of iodized and creation of demand for crushed and other varieties of packed salt and awareness creation among health workers and general public, (NMSS, 1998). Again, in the mountain region too, women of reproductive age and children <15 years of age. Government of Nepal has provided iodine injection in every 5 years. Later on, it was replaced by iodized oil capsules. The capsules are distributed through health posts and health institutions,(Mathema, 2002).

Vitamin A deficiency (VAD)

Vitamin A is essential micronutrient involved in various biological functions including vision function, immune function, and maintenance of epithelial cellular integrity, growth, development and reproduction. Immune system of the with VAD is damaged and has a more infections, (HMG,MoAC, 2002). Most important cause of VAD is lack of dietary vitamin A(i.e. carotenoides from plant source and retinol from animal source such as egg, meat and milk) and persistent high infections load.

VAD is found into two forms:

i) Clinically visible forms: It is related to Night blindness and Bitot's spot, Xeropthalmia, Corneal score, corneal xerosis and or ulcerations.

ii)Sub-clinical VAD: It is related to deficiency of serum retinol in blood. Serum retinol value less than 0.7 micro mol per liter indicates the presence of sub-clinical VAD, (NMSS, 1998).

According to NMSS, ocurrence of night blindness is 4.7% with pregnant women having a high rate(6.1%)than non-pregnant(4.5%),0.27% in preschool children and 1.2% in school aged children. Bitot's spot is found in 0.33% preschool children and 1.95% among school aged children. Both of these values are below WHO cut-off points to designate a significant public health problem (1% for night blindness and 0.5% for Bito's spot).Occurrence of Bitot's spots among school-aged-children was higher than for preschool children. It was also observed from the survey that xeropthalmia, night blindness and Bitot's spots increases with age not only through the preschool period, but also into early adolescence. Prevalence of night blindness is highest in Terai showing alarmingly high rate of 13.4%.

Prevalence of sub-clinical VAD among women was 16.6%in with the prevalence among women living in the Terai and Mountains being twice that of women in the Hill zone. Highest prevalence of subclinical VAD was among women below 20years (19.5%) and the lowest, among women aged,30-40 years(13.3%).The overall prevalence of sub-clinical VAD among preschool children was 32.3%, one out of three children. The prevalence of sub-clinical VAD among age groups 6-11 and 12-23 months was alarmingly high. This is supposed to be due to decreasing breastfeeding frequency, insufficient vitamin A from complementary food and high rates of infections. Children in the rural areas had a higher prevalence of sub-clinical VAD than urban areas. Which is due to difference in dietary patterns and high rate of higher rate of infections than children in urban settings, (NMSS,1998). According to NDHS 2001, 20% of women reported night blindness during pregnancy and 8% of the women reported night blindness during last pregnancy.

Prevention and control of VAD

- Fortification of Vitamin A in food
- Twice a year high dose (100,000 IU) Vitamin A capsule supplementation program in children of 6-12 months of age and 200,000 IU for children of 12-60 months of age.
- Creation of taking Vitamin A rich food habits
- Promotion of breast feeding
- Improved methods for production, preparation and preservation of vitamin A rich food.
- Supplementation of vitamin A (200,000 IU) to postpartum women within 6 weeks of pregnancy.

Vitamin A distribution programme was started in 1993 to overcome vitamin A deficiency in children of age 6 month to 5 years specially to control night blindness and Bitot's spots. About 90% of the children of 6 months to 5 years of age are receiving vitamin A capsules. It has been estimated that this distribution has saved the life of 25,000 children every year. The programme of the project has been implemented by Nepal Government through a national NGO, named National Technical Association Groups (NTAG). In Year 2002, the project was implemented in 73 districts,(Mathema,2002). But according to NDHS 2001, 8% of children age 6-59 months received vitamin A supplementation. There is little difference in vitamin A supplementation by sex of the child. The urban rural children somewhat more likely to receive vitamin A capsules than urban children. Four out of five children in rural areas received vitamin A capsules, compared with three in four children in urban areas. Vitamin supplementation for child increases slightly with education of mothers. Overall, 10% of recent mothers received vitamin A supplement within 2 months of postpartum. Educated mothers are most likely to receive vitamin A than women with no education, (NDHS, 2001).

Iron deficiency anemia (IDA)

Body needs iron to make hemoglobin, a protein in red blood cells that carries oxygen to the brain, muscular system, immune system, and other parts of the body, (HMG, MoAC, 2002).Iron is a critical nutrient essential for the production of hemoglobin, which functions in the delivery of oxygen from lungs to body tissues. Iron works for cellular transport and in the synthesis of iron enzymes that are required for oxygen utilization and energy metabolism. IDA decreased physical activity and work output, impaired growth and development, increased risk of maternal, prenatal and prenatal mortality. The first stage in the development of iron deficiency is a decrease of stored iron. If intake of iron absorption cannot replenish the lost iron, then serum hemoglobin levels begins to decline following the complete depletion of stored iron. More severe stage of iron deficiency is iron deficiency anemia. In another words, reduction of red blood cells is called anemia which is most severe degree of iron deficiency.

The most important cause of IDA is an inadequate dietary intake of iron. Presence of parasitic infections such as malaria and intestinal helminthes, low bioavailability of iron in vegetable sources and consumption of food that inhibits the absorption of iron are also related causes of IDA. Pregnant women and children are greatest risk of iron deficiency because iron needed for rapid growth and building of the new cells,(NMSS,1988).

The prevalence of anemia is assessed through the measurement of concentration of hemoglobin. Using WHO cut off points for hemoglobin as shown in Table 3. The hemoglobin data presented as prevalence for both preschool children and mothers.

Table 3. Hemoglobin (Hb) cut-off points used to classify anemia

Physiological group	Hb cut-off points(g/dl)	
	Moderate	Severe Anemia
Pregnant women	7.0-10.9	<7.0
Non-pregnant women	7.0-11.9	<7.0
Preschool children(6-59 months)	7.0-10.9	<7.0

Source: (NMSS, 1998)

Survey conducted on different years revealed that IDA is the most common and serious malnutrition problem in Nepal. According to survey conducted by United Nutrition Assistance Program, 1980, iodine deficiency in women of child bearing age (6- 36 months old) was 71.95%. NMNS shows that overall national prevalence of anemia among women is 67.7% which is about two in every three women. Prevalence of severe anemia was 2.2%. Further, the prevalence of IDA is highest among pregnant women (74.6%) than men (66.7%). This problem is highest in Terai than in hills. Among children of age group 6-11 months, it is up to 90% and in pre-school children it is 78%. IDA has decreased with the increase in the age and is 59.3% in children of 48-59 months age group, (Mathema, 2002).

Prevention and control of IDA:

WHO has declared that anemia is considered as a significant public health problem in those population where the prevalence is greater than 20%, rate found in Nepal is four times higher. So, to prevent the high rates of anemia, the following policies and approaches are needed:

- Iron fortification in food
- Iron tablet supplementation: daily provision of 60 mg of iron to all pregnant women from second trimester to 45 days post partum.
- Modified plant breeding for self fortification: improvement of environmental sanitation and de-worming of children at local levels.

NDHS data 2001 show that more than three in four women who gave birth in the five years preceding the survey did not take iron/folic acid tablets during their pregnancy. 14% reported taking iron/ folic acid tablets for less than 60 days. Young women, women living in the urban areas and educated women are more likely to take iron/ folic acid tablets than other women.

Efforts in solving the problem of malnutrition in Nepal

Government's plans, policies and programs

Tenth five years plan has incorporated nutritional objectives into development plans and policies for solving nutritional problems, which is shown in Table 4.

Table 4. Proposed Target of the Tenth Plan

Year	2002	2006
Reduction severe and moderate malnutrition among under 5 years children by 50%	50	25
Reduction of IDA in women by 50%	25	12
Virtual elimination of VAD and its consequences.	1	0
Virtual elimination of IDD	78	48

(Source: Tenth plan, 2002)

Nutritional Policies of the Tenth Plan

Food storage capacity at local level will be increased and distribution system will be improved with a view to enhance nutritional status by increasing production of cereals of high nutrition value.

People's awareness regarding the importance of nutrition and availability will be increased through extensive communication.

Preventive vaccination will be given to maintain the nutritional status among the children. Arrangements will be made to maintain the nutritional status of pregnant and lactating mothers, and arranging medicinal facilities.

Nutrition education will be provided to the children from the primary school level, public awareness will be increased through training and seminars at local levels, and breakfast programme will be launched at the school levels with a view to enhancing nutritional status of the children.

Programmes of micro-nutrients (protein, vitamin A, iodine, iron, etc) for supplying in the acute problematic areas will be formulated according to the international commitments made for micro-nutrients. Similarly, legal arrangements will be made for sale and distribution of iodinated salt.

Quality of edible cereals will be improved to control the adverse impact on nutritional status, importance of balanced diet will be highlighted through public media, and use of low quality cereals will be discouraged.

National Nutrition Coordination Committee will be strengthened to implement nutrition activities operated by various agencies in an integrated way, and carrying out coordinated efforts for disseminating nutrition information, monitoring, and evaluation through developing inter-regional institutional arrangement.

Private and non-governmental sector will be encouraged to participate in the nutrition programmes.

Targeted programmes will be implemented for enhancing the nutritional status of the communities suffering from vulnerable situation.

- ***Millennium Development Goals(MDGs):***

At the Millennium Summit of September 2000, the member States of the United Nations adopted the Millennium Declaration, which aims to bring peace, security and development to all the people. The MDGs drawn from the Millennium declaration are ground breaking international development agenda for the 21st century to which all nations are committed. The MDGs outline major development priorities to be achieved by 2015. Numerical targets are set for each goal and are to be monitored through 48 indicators. The MDGs give people the power to claim their fundamental human rights, such as the right to food, education, health and shelter, and allow ordinary citizens to become active advocates for development.The millennium development goals are to:

Eradicate extreme poverty and hunger
Achieve universal primary education
Reduce child mortality
Improve maternal health

Steps adopted for the prevention of overall malnutrition

- Promoting appropriate diets and healthy life styles.
- Improving household food security.
- Focusing socio-economically deprived and nutritionally vulnerable groups.
- Awareness for preventing specific micronutrients deficiencies.
- Development of plans and policies in such a way to incorporate nutritional objectives.
- Prevention and control of infectious disease.
- Promoting breast feeding.
- Promoting consumer awareness about improved food quality and safety.
- Monitoring, analyzing and assessing situation.

Conclusions and Recommendations

Widely occurring problem of malnutrition has caused devastating effect among the Nepalese people. This review has attempted to address and enlist the ways and approaches taken by the responsible organizations to tackle to problem of malnutrition without further delay from the multi-sectoral approaches. About 50% of children of age below 5 years old are suffering from PEM. Vitamin A deficiencies (VAD), Iodine deficiency disorder (IDD), Iron deficiency Anemia (IDA) are most common micronutrient deficiencies. Developmental policies, plans and programmes of the government and other responsible organizations will help to improve the nutritional status of the population at risk. Integrated programmes aiming to increase food security in terms of quality will ensure the health security of the entire population.

- Revision of Nepal Plan of Action on Nutrition.
- Activation of National and District level communities.
- Establishment of National Institute of Nutrition.
- Incorporating nutrition as a part of functional literacy.
- Developing and functioning of a good coordination mechanism among Ministry of Health , Ministry of Agriculture and Cooperatives, Ministry of Education and Sports Vs directing nutrition related programes of UN agencies, NGOs, INGOs, CBOs etc as per the national need.

References

Carolynn, E.T. (1994), Nutrition and Diet Therapy, Sixth Edition, Delmar Publishers Inc.™, pp3-9.

Dhungana Pashupati, 2003, May 22, Solving Nutrition Problems through Private Sectors. *In Proceedings of Nutrition Advocacy Workshop, Regional Training Program on Food and Nutrition Planning.:* Biratnagar, Morang, NepalHMG/ MOAC, DFTQC, National Nutrition Program, Babarmahal, Kathmandu, Nepal UPLB College, Laguna, Philippines.

HMG/ MoAC, DFTQC, 2002; National Nutrition Program, Babarmahal, Kathmandu, Nepal, Food and Nutrition Planning Managenent.

HMG/ MOAC, DFTQC, 2059; Babarmahal, Kathmandu, Nepal, Samudaik Kahdhya thata Poshan Talim Pustika(in Nepali).

Mathema Padma, 2002, October, 2-4, Plans, Ploicies, Strategy and Programs on Nutrition in Nepal. *In Proceedings of Nutrition Advocacy Workshop, Regional Training Program on Food and Nutrition Planning.;*Kathmandu, Nepal. HMG/ MOAC, DFTQC, National Nutrition Program, Babarmahal, Kathmandu, Nepal UPLB College, Laguna, Philippines.

NDHS, 2001, Nepal Demographic Health Survey.

Nepal Micronutrient Status Survey (NMSS), 1998.

NMDs, 2002 (Nepal Millennium Development Goals), Progress Report.

Swami Nathan, M. (1985). Essentials of Food and Nutrition; Vol. 1. , Second Edition, The Bangalore Printing and Publishing Co. Ltd.

Tenth Plan 2002, HMG of Nepal, National Planning Commission.

UNICEF (1998): The state of the world's children 1998, Oxford. Oxford University Press.

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Noodle Technology in Nepal- A Brief Review

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Noodle is a well recognized food item consumed as a snack or full meal by all age groups especially popular in children and young in Nepal. Most of the food is being replaced by instant noodle even from rural areas to big cities which helps people to save time. Noodle business these days extending its roots from bigger cities to the rural areas and creating it market wider and wider everyday. This article briefly reviews the noodle processing technology as well as its quality status and marketing situation in the Nepalese context.

Keywords : Noodle, Processing Technology, Quality status, Marketing Situation, Nepal

Introduction

Noodle took birth in Chinese Community around 5000 Years back; it traveled out of China to various countries. The popularity of instant noodle has grown since packet instant noodle were first developed in 1958 followed by cup noodles in 1971 in Japan. (Thapa and Katuwal 2001) It has been gaining popularity everywhere. Many producers of different countries have been trying to modify the taste of the noodle in the way their ultimate consumers like. (Kun & Basiron, 1999)

Although noodle has not any traditional or cultural value in majority of Nepalese society, but everybody is cultured to its taste now. Till two decades back most of the people were not used to it and disliked the taste and appearance. The per capita consumption of noodle in Nepal is 9 packets. Noodles begun to gain popularity when modern technology is applied in its production and are presented in varieties of packets.

Noodle is not a'strange item in the consumer's perception now. It is now a well marketted food item, liked and consumed as snack or breakfast by all age groups especially popular in children and young. The instant noodles can be replaced by any food but the result is other way round. These days by instant noodle is come tiny with many of the indigenous snakes of Nepal. In cities, it has been helping people to save time and in the rural area it has become fashion and status symbol to consume such food. Therefore it has been extending its roots from city to the rural area and widening market. This article wishes to explore the existing Nepalese Noodle processing technology and market situation of Noodle.

Overview of Noodle industry in Nepal

The history of noodle industry indicated that noodle industry had begun with the Nepali Currency of five thousands (~US$ 70/-) only, has now crossed the boarder of twelve million thousand (> US$ 1, 70, 00, 000/-) which clearly shows the development of noodle industries in Nepal. Another considerable fact is that the price of the one packet noodle was NRS 10/- at the beginning time (1 US $=NRS 45) and the price of that packet is still the same NRS 10/- while the 1US $ equals NRS 75 now.

Out of total processed food products of Nepal, noodles, carries only 0.43% by weigh (HMG 1998). The following are the noodle industries in Nepal with their product brand names, years of entry into the industry, and origination of the machine they have been using in the factory (Table 1).

The beginning of the instant noodle industry in Nepal had taken place in 1983. For two years, Gandaki Noodles enjoyed the market alone (Table-1). Since the company had to start all the marketing activities from the scratch and introduced instant noodle in general mass. It contributed significantly for the familirization of the noodles in Nepalese markets .

Table 1: Noodle Industries in Nepal

Estd.Yr	Location	Name of company	Brand	Supplier of plant
1983	Pokhara	Gandaki Noodles	Rara, Ezee	Japan
1985	Kathmandu	Nepal Thai Food (Later renamed as CG Foods)	Waiwai, Golmol, Mama, Soalti	Taiwan
1990	Narayangh	General food industry *(Closed)*	Yumyum	Japan
1992	Pokhara	Pokhara Noodles	Ramba	Local Fabrication
1994	Pokhara	Everest Noodles	A-one, Aaha	Japan
1995	Kathmandu	Multi foods	Minmin, Khaja	China and Taiwan
1995	Nawalparasi	Fast Foods	Waiwai, Golmol, Mama, Soalti	Japan
1997	Pokhara	Anupam Foods *(Closed)*	Femee	Japan
1999	Sunsari	Asian Thai Foods	Rumpum, 2PM, Phuchhe, Phataphat	China
1999	Banepa	Himalayan Noodles	Mayose, Hurre, Ruchi	Japan
2000	Sunsari	Kanchanjungha Foods *(Closed)*	U Key	China

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The first company gave the product in its original form whereas the second company which started after two years, introduced differentiation. Nepal Thai Food product started the noodle factory with Taiwan machines and Thai taste with its soup in every packet. These two factories enjoyed the total market for five years until General Food Industry came up with new production differentiation in taste with Japanese machine and Thai Technology .

After 24 years of noodle production in Nepal there are six indigenous players with eight production units in the market with 24 fold incremunt of production volume production. Now the industry trying to provide product differentiation to the market. In this short period, the industry grew in such way that the play zone is more than sufficient inviting other to join in. Every year newly established noodle industries have added brands in the noodle array and still some industries are on the way to increase this number further. So far ten noodle industries are already in field (Table 2).

Table 2: Present Production capacity and Market Volume of Noodle in Nepal

Industries	Production Capacity		Market Volume	
	Daily Capacity	Annual Capacity	Daily Avg production	Tent Annual Production
	Tonne	Tonne	Tonne	Tonne
CG Foods Kathmandu	10	3000	7	2100
Fast Foods P Ltd,Nawalparasi	60	18000	40	12000
Himshree Foods Pokhara	30	9000	10	3000
Pokhara Noodles,Pokhara	30	9000	20	6000
Asian Thai Foods ,Biratnagar	45	13500	30	9000
Himalayan Noodles,Banepa	50	15000	40	12000
MultiFood Industries,Kathmandu	10	3000	4	1200
General Food,Chitwan	15	4500	0	0
Anupum Foods ,Pokhara	7	2100	0	0
Kanchanjungha Foods,Sunsari	20	6000	0	0
Total	**277**	**83100**	**151**	**45300**

All varieties of instant noodle manufactured by Nepalese industries belong to the class of alkaline noodle (pH 9-11.5) due to use of alkaline salts in their formulation. The noodles are available in two tastes- Vegetable and Chicken taste. The vegetable taste lacks the incorporation of fish and chicken products in their formulation and is meant for vegetarian. The usual consumer packet noodle is found in 75 g and 50g weight. The 75 g packet is found to contain sachets of separate tastemaker, oil and chilli powder in it.
Yum Yum, Hits, Wai Wai, Min Min, Aaha, Rum Pum, Mayose, Famee are the examples of alkaline brown colored instant noodles in 75 g packet. *Rodi, RARA, Rambha* are the examples of alkaline plain noodle. *Yum Yum* Snack, *MiMi, Phuche, Aaha* 50 are examples of alkaline brown colored noodles in 50 g packet (snack type).

Export Import Scenario

In the primary days of noodles in Nepal, Maggi two minute noodle from Nestle India has played the corner stone role to establish noodle consumption habit to Nepali consumer. Later, Nepali noodles established its strong market inside the country and swept out the Maggi from Nepalese market. However, Maggi two minute and other varieties of imported noodles from Thailand and Indonesia are seen in the rack of the super markets of the major cities of the country.

The export scenario of Nepalese noodle has been growing fastly since last few years. Initially in 1994 to 1997, General Food Industry exported to large volume of Maggi two minute of noodle to India as franschise of Nestle India.Late other industries has starrted to enter Indian market with Nepalease brown noodles. The major market of the Nepalese noodles are north Indian states. However Nepalese noodles are trying to grow with their market to northern India and metropolitian cities of India. Every year, these seems to grow up. Besides India, Nepalese noodles has been started to export to Bhutan Bangaladesh, Malaysia and UAE. More than 20% of total volume of production of noodle is exported to India and other countries. To fulfill the consumer demand of Nepalese noodles, CG Foods India already started production of Waiwai in Sikkim from this year 2006 in technical collaboration of CG Foods, Nepal.

Production Technology of Noodle in Nepal

Basically two types of noodles made from wheat flour are seen in Nepalese market. They are cooked and uncooked types. The instant noodle belongs to the former type. The example of later is SINKE CHAU CHAU. It needs to be cooked before consumption. Its manufacturing is solely confined to cottage level industry only. In the instant noodle class, two varieties-plain instant noodle and souped instant noodle (brown color) are found in Nepalese market. The plain instant noodle differs from that of brown type in its manufacturing stages in which souping step is lacking. The color of the plain noodle is white. So it is also known as white noodle. The average sales volume of white and brown types of noodle is about 10% and 90% respectively.

Noodle Ingredients

The commonly used ingredients in the instant noodle making are

- ***Noodle Cake Ingredients***: Wheat flour, Palm oil or palmolein (RBD or HVO), water, alkaline salts (sodium carbonaté, potassium carbonate, sodium hydroxide, sodium tri or polyphosphates), common salt, fresh chicken, dried shrimps, fresh spices (garlic, onion, ginger etc.), black pepper, soy sauce, monosodium glutamate (Azinomoto), wheat gluten, gums (CMC, guar gum) eggs, antioxidants etc.
- ***Tastemaker ingredients***: Dehydrated powder of various spices (garlic, onion, ginger), black pepper, chicken powder, hydrolyzed vegetable proteins, salt,, MSG, Red chilli powder, flavored oil (onion, garlic or leek) citric acid, maleic acid, sugar, anti-caking agents etc.

All industries use almost the same ingredients and their unit operations are more or less identical. The difference lies in their formulation. A typical production flow diagram of noodle is given in Fig 1.

The sifted and preweighed quantity of wheat flour is fed to the mixture. Required quantity of dough mixing solution (prepared in separate tank) is pumped to the mixture for a period of 15-20 minutes to form dough of desired properties. Well-formed dough is easily pressed and cut, doesn't break and cling to the roller and results noodle with desired texture. The moisture content is maintained around 30%. Required amount of wheat gluten is also mixed together which increases the protein content of the noodle. Gums, eggs, stabilizers can be added separately or by dissolving into water.

The quality of wheat flour is most important to manufacture noodle with desired texture. The aesthetic contribution is associated with both the texture and appearance of the noodle and both factors are strong drivers of consumer preference. The texture should be elastic and relatively firm. Appearance should be bright with clean colors. The major polymeric components of the flour (starch and protein) have been shown to be important in determining the texture. The less abundant component (lipids and pentosans) should again have an influence on texture but their effects may be modified by the increased pH.

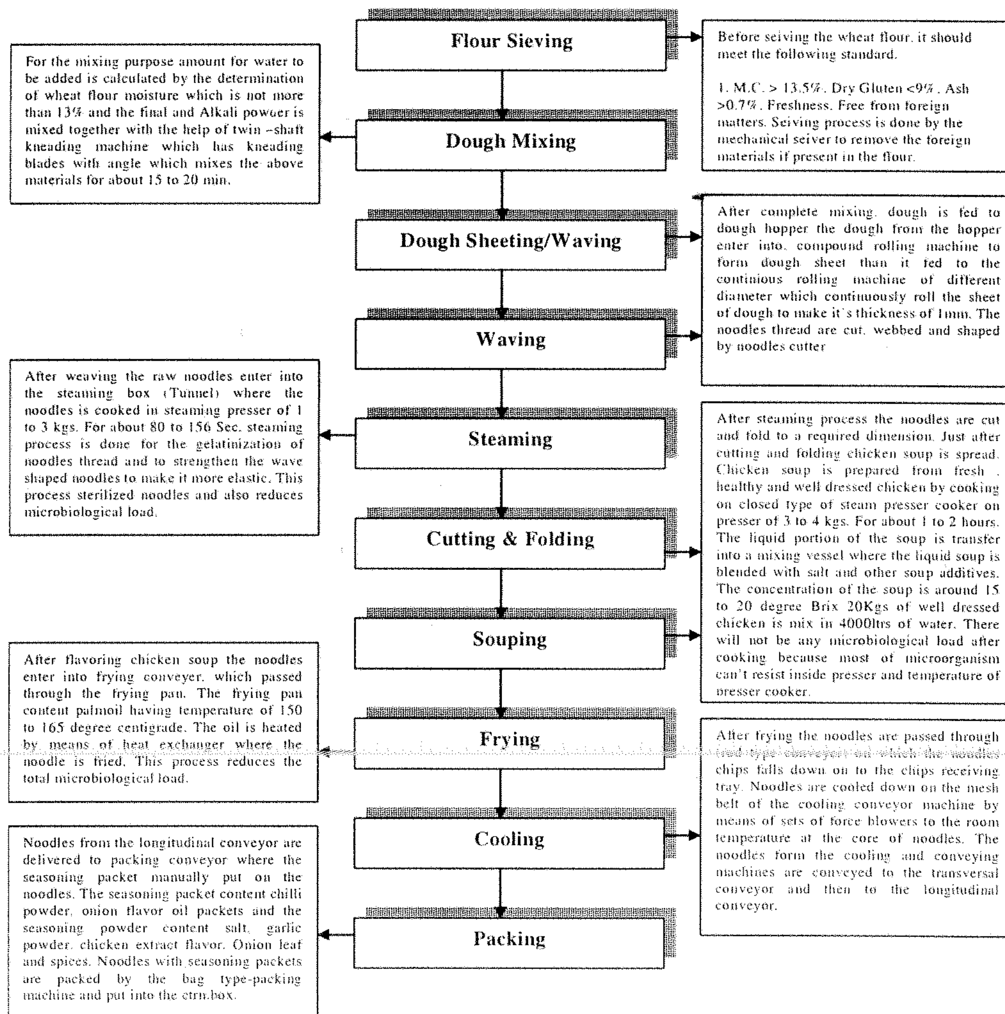

Fig 1: A Typical Production Flow Chart of Instant Noodle in Nepal

At the pH values and ionic strength commonly associated with alkaline noodle manufacture, starch paste viscosity and swelling volume both increase but the onset of gelatinizaton is delayed with carbonates and accelerated with sodium hydroxide. Increased flour protein content and higher protein quality' have also been associated with firmer and more elastic alkaline noodle texture.

The dough is sheet mould about 10 mm thick. It then goes through another successive rollers (5-6 pairs of rollers) each will make the sheet thinner to emerge as a 0.8-1.3 mm thick sheet mould. The sheet is cut into noodle strands b the slitter. The strands are shaped into noodle wave by the waving unit, which also divides the waved noodle into separate lanes (2-6). The wave conveyer conveys them and transfers to the steaming conveyor.

The noodle is steamed cooked for a period of about 2 minutes at a temperature of 100°C. The heat treatment homogenizes the flour and starch to obtain a correct noodle texture.

The half cooked noodles wave (emerging as continuous lanes) are cooled, cut and folded as per the final weight required. These cakes are wetted by the stream of chicken or vegetable soup (by variety), when they move forward. Excess soup is removed from the cake by blowing air across them crosswise before delivering to the retainer of the frying conveyor. In plain noodle manufacturing, this souping step is lacking.

The soup for noodle is prepared separately from the soluble extract of fresh chicken, shrimps and fresh spices (for chicken flavor). For vegetarian noodle, chicken and fish is excluded. The soluble extract is mixed with salt, MSG, soy sauce etc.. The color, flavor and the specific gravity of the soup is adjusted to the desired level.

The souped noodles are deep fat fried continuously at a temperature of 130-180°C for a period of 1-2 minutes depending on the variety, fryer's size, plant speed and the temperature maintained. Among the unit processes, frying is the most critical because it greatly affects the quality and the shelf life of the final product. Frying reduces the moisture in the noodles to a level where growth of moulds is prevented. It imparts flavor and texture and improves the nutritional content by incorporating fat into the noodles.

The choice of the oil use in frying determines the quality of the finished product. The taste and flavor contributed by the noodles are very promionent for the acceptance. Hence the stability of the oil or fat used in frying and as a consequence the final product stability will determine the quality of oil-fried instant noodle. All noodle industries use RBD palm oil or palmolein as a frying medium. Studies have shown that palm oil and palmolein have better frying stability and behaviour at a high temperature because of their fatty acid composition and the presence of natural anti-oxidants.

The bland flavor of palm oil and palmolein is ideally suitable to incorporate the desired flavor through souping. Compared with the liquid vegetable oils (which have to be hydrogenated to an appreciable degree) palm and palmolein oil don't require such processing. This makes them not only cost effective but also free of trans fatty acids, which as new cardiovascular heart disease than saturated fatty acids.

The fried noodle cakes emerged from the fryer are very hot, which are cooled down to room temperature by the air in cooling chamber. The cooled noodles cakes are wrapped airtight. Before wrapping, each packet is provided with a sachet of tastemaker (seasoning powder, flavored oil and chilly). The wrapper composition, thickness and sealing quality are important to preserve the product ups to the period of entire shelf life of 9 months.

The taste and flavor of different brands of noodle differ from one another. However, all brands of noodle should comply with mandatory standards in addition to their factory norms.

About 70-80 % of the total weight of instant noodle is derived from wheat flour, remaining 20-30% is made up of by fat, salt and other permitted food additives. The average proximate composition of instant deep fat fried noodle is 18% fat, 10% protein, 58% total carbohydrate, 0.8% dietary fiber, 4.5% ash, 2.0 % moisture and a net calorific value of 466 calories per 100g.

Quality Aspects of Noodle in Nepal

Wheat flour and hydrogenated vegetable oil (especially RBD palm oil) are the major raw ingredients of noodle. The existing quality standards of these ingredients in Nepal are presented in Table 3. Whole wheat flour (also known as *ATTA*) is the raw ingredient of stick noodle (also know as *SINKE CHAU CHAU*- the Nepali local word).

Table 3: Mandatory Quality Standards of Major Raw Ingredients of Noodles in Nepal

Parameters	Whole Wheat flour (ATTA)	Wheat flour (MAIDA)	Hydrogenated Vegetable Oil
Moisture % Max	14.0	14.0	0.25
Ash % Max, db	2.0	0.70	---
Acid Insoluble Ash %, db	0.15	0.10	---
Gluten % Min, db	6.0	8.0	---
Alcoholic acidity % as H_2SO_4	0.18	0.12	---
Melting Point (°C)	---	---	31-41
Refractive index at 40°C, Max	---	---	1.4580
Unsaponifiable matter % Max	---	---	1.25
Acid Value (mg KOH/g), Max	---	---	0.50
Peroxide Value (meq O_2/Kg), Max	---	---	10.0
Nickel Content (mg/Kg), Max	---	---	1.5

[Source: Nepal Gazette, 2000]

Nepal Government has explained the defination of instant noodle(Nepal Gazette, 2002). The defination is that it should be maid from wheat flour prepared as curled thread shape which may content different seasonings, egg, mushroom, Vegetables, Chicken, or its extract, shrimb and other ingredients and it should not be infected by fungus or should not be damaged by any insects. Similarly standard declared that there should not be monosodium glutamate added if it is prepared for infant below 12 months where as Indian Gazette mentioned that monosodium glutamate should not be added as additives in noodle but it may be added in the noodle seasoning only (Gazette of India, 2004)

The final products of Noodle should have the quality characteristics as given in Table 4. The quality standards of *SINKE CHAU CHAU* is also presented (Table 4). Few of the instant noodles manufacturing units have taken Nepal standard (NS marks) where as few other are certified with ISO 9001.

Table 4: Quality Standard of Noodle in Nepal

Parameters	Noodles (SINKE CHAU CHAU)	Instant Noodles GOV/Nepal	Instant Noodles NS
Moisture % Max	12.5	5.0	5.0
Ash % Max, db	1.0	4.0	4.0
Acid Insoluble Ash %, db	0.1	0.1	0.1
Total Protein % Min, db	8.0	10.0	10.0
Acid Value of Extracted Fat, Max	---	1.0	1.0
Peroxide Value(meq O_2/Kg), Max	---	10.0	10.0
Monosodium Glutamate* %, Max	---	1.0	
Packet oil	----	As per the GOV standard of the concerned oil used	
Gruel Content %, Max	---	---	9.0

Note: The packet of instant noodle must be labeled as vegetarian or non-vegetarian.*-If intended for children below 12 months of age, it must be absent.

[Source: Nepal Gazette, 2000, Thapa and Katuwal-2001]

In all of the larger units, there are experienced Food Technologists to look afer the process and quality with quality control laboratories acompanial with modern food analytical equipments.

Market Situation of Noodle in Nepal

In 1983, Gandaki Noodles of Pokhara tried to capitalize the market initiated by imports of instant noodles as 'tourist food' from India and abroad. Along with this, it worked much to create now market. With the beginning of production in Nepalese themselves. Sensing the rapid growth of market and wide possibilities in product diversification, different other firms set-up the factory and started production.

The growth of market of the product is experienced very rapid since 1993. At the present situation only about 5-6 % people in Nepal consume noodles. Increment of demand of the product was 4% in the year 1992. The growth trend was not the same every year, but it is ever increasing. In thé year 1998 the market expanded by 50% which is a massive and tall increment for the whole industry to fulfill the demand.

Some facts can be traced as the causes of high demand of noodles.

- It saves time of busy people.
- Distribution system of the industry is good and has good access even to the remote areas.
- The cost is affordable to every income groups.
- It has unique blends of seasoning
- It has realtively longer keeping quality

Table 5: Market Expansion of Noodle in Nepal

Years	Increment in Demand (%)
1992	4.0
1993	19.5
1994	19.5
1995	21.0
1996	12.0
1997	25.0
1998	50.0

Source: Department of Industry, HMG

Besides the increasing domestic demand, the Thai and Indonesian type taste noodles are exported outside of the country. The private source says that about 25% of the total domestic products of instant noodles of Nepal is exported to the neighboring countries including Tibet. Supply of the product in different forms and tastes by the noodles industry of Nepal is 4500 MT. Manufacturere trying to up their sales volume with cheaper and low value noodles. Besides this, the concept of health noodles also seem in the Nepalese market from this year. 2 PM Atta noodles with aata, real chicken cube and dried vegetables of Asian Thai Foods and Wai Wai noodles enriched with extra protein and calcium from CG Foods are the examples of this kinds of noodles.

References

BS (2000) Budget speech for the fiscal year 2056/57, HMG/N, Ministry of Finance P 6

HMG (1998) Department of Industry, HMG

Economic Survey, Fiscal Year 1998/99, HMG/N, Ministry of Finance pp 27,36

Gazette of India(June 25, 2004). Ministry of Health and Family Welfare, Government of India p28-29.

Nepal Gazette, 2000

Noodle Plant : An explanation(1990). Fuji Manufacturing Co. Ltd. Japan

SPB (1998) Statistical Pocket Book Nepal -1998

Teah Yau Kun and Yusof Basiron (1999). Palmolein Capture the market for instant noodle Frying. Frying Oil with reference to Palm Oil. PORIM, Malaysia p24-30.

Thapa H and Katuwal M B (2000). Additional wheat Gluten Required for Quality Noodle Production in the context of Nepal. Food Nepal, Vol 4: 1-4

Thapa H and Katuwal M B (2001). Instant Noodle Technology: In context of Nepal. Food Nepal, Vol 5: 33 35

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Probiotics: Selection and Health Benefits

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Fermented dairy products by probiotic lactic acid bacteria and Bifidobacteria have long been used for their proposed health promoting effects and in the treatment of some diseases. Based on the corresponding health effects, each strain may have specific properties. Besides those properties all organisms selected for probiotics should meet at least some criterion such as viability, acid/bile tolerance, and adherence to colon (colonization) to impart health benefits. The relief from lactose intolerance symptom, shortening of rotavirus diarrhoea and thus functioning in gut health is widely accepted probiotic functions of selected probiotics by defined mechanism. The mechanism involved in anti-cancer property, immune modulation, cholesterol reduction in hypercholesterolemic cases and in the treatment against allergy, candidiasis, and in hypertension needs further in vivo tests and human trials. The selection criteria and possible mechanism of health benefits by probiotic lactic acid bacteria, Bifidobacteria and some other genera have been summarized in present review.

Keywords: Probiotics; Lactic acid bacteria; Health effects, Selection.

Introduction

Probiotic can be defined as the fermented food containing specific live microorganism or live microbial food or feed supplement which has beneficial effects to human by improving its intestinal balance (Fuller, 1989). It was probably Vergio in 1954, who first introduced the term 'probiotic' in his report 'Anti- und Probiotika'. He compared the detrimental effects of antibiotics and other antimicrobial substances on the gut microbial population, with factors (Probiotika) favorable to the gut micro flora (Holzapfel & Schillinger, 2002). Lilly and Stillwell (1965) referred the term 'probiotic' in reference to substances produced by protozoa, which stimulated the growth of other organisms (Kaur, et al., 2002). The concept of probiotic, however, was proposed at the beginning of 20th century when the Nobel Prize-winning Russian scientist Elie Metchnikoff (1845-1916) described the importance of the intestinal microflora on the general health status of the human body (Cruce and Goulet, 2001).

The widely accepted Fuller's definition is restricted to only the feed supplements, so the term probiotic can not be used for live microorganisms administered in any form other than food system. A modified definition for probiotic means a mono or mixed culture of live microorganisms which applied to animals or man has beneficial effects by improving the properties of the indigenous microflora (Havenaar and Veld, 1992).

Lactic acid bacteria and species of Bifidobacteria isolated from human intestine have long been used as probiotics. The Yogurt starter bacteria and some other lactic acid bacteria (LAB) present in milk and fermented milk products have potential of being probiotics (Salminen et al., 1998). Although many of the works on probiotics and their health benefits have been reviewed, the key factors for selection of probiotic strains have not been summarized yet. This article summarizes the selection criteria with their justification and possible mechanism of health benefits attributed by consumption of probiotic food.

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Probiotic microorganisms

Fermented dairy products such as Bulgarian milk, now known as yogurt maintain a good equilibrium of the intestinal microflora and minimize putrefactive fermentations lids to the discoveries of probiotic effect of microorganisms (Metchnikoff, 1908).

The first probiotic organism considered was probably the *Lactobacillus* sps from Bulgarian milk. Although microbes from many different genera such as t *Escherichia coli* Nisse 1917 (Malchow, 1997; Kruis et al., 1997), *Saccharomyces boulardii* (McFarland et al., 1994; Guslandi et al., 2000) are being used as probiotics, the most commonly used strains are members of heterogeneous group of lactic acid bacteria: lactobacilli (Aiba et al., 1998; Anderson & Gilliland, 1999; Hallen et al., 1992), enterococci (Sanders & in't Veld, 1999), Lactococci (Salminen & von Wright, 1998), propionibacterium (Grant & Salminen, 1998; Adachi, S., 1992) and Bifidobacteria (Prasad et al., 1998).

Commonly used probiotic microorganisms are listed in Table 1. Not all, out of 56 species of lactobacilli and 29 species of Bifidobacteria are the probiotic. Yogurt starter bacterial species, *Streptococcus thermophilus* (Collins et al., 1998; Naidu et al., 1999; Sreekumar & Hosono, 2000) and *Lactobacillus delbrueckii* subspecies. *bulgaricus* (Naidu et al., 1999; Sreekumar & Hosono, 2000), are also considered probiotics since they are able to release, among other compounds, enzyme (^{2}-galactosidase) that improve the digestion of lactose and are biological barrier resistant (Vinderola & Reinheimer, 2003).

Probiotic characteristics

The probiotic bacteria not only needed to remain viable during processing, handling and storage of the food it contains also till it reaches the intestinal tract and colonizes the surface of human intestine (Cruce & Goulet, 2001). A viable cell population of 10^7-10^9 colony forming unit (CFU) per day is necessary for beneficial effects to human (Salminen et al., 1998). In strict sense, probiotic viability would be a reasonable measure of probiotic activity, but there are some situations in which cell viability is not required for health benefits such as improved digestion of lactose, anti-hypertensive effects, some immune system modulation activities and resistance to infections. In these cases, health beneficial effects have been linked to non-viable cells or to cell components, enzyme activities or fermentation products (Vinderola & Reinheimer, 2003). However, Lankaputra and Shah (1998) reported that live bacterial cells showed higher antimutagenic activity than killed cells.

Selection criteria of probiotic bacteria

The choice of microbes to be used as a probiotic is determined by in vitro tests (Mishra and Prasad, 2005). Once the targeted groups are able to survive their possible health effect are evaluated. The selection of probiotics based on survival and functionality are summarized as follows:

Normal habitat of human intestinal tract: The probiotic works actively in the intestine of human and prevents from disease. Probiotic bacteria isolated form human intestine could have better viability. Human isolates such as *L. johnsonii (acidophilus)* LA1, *L. crispatus* BG2FO4, *L. acidophilus* NCM#2, *L. paracasei* subsp. *paracasei* DSM 20312, *L. rhamnosus* 271, *L. plantarum* 299, and *L. rhamnosus* LGG are active probiotic cultures. However, species other than intestinal isolates such as *L. plantarum* 299v from sourdough, *L. reuteri* R2LC from rat colon, *L. reuteri* DSM12246 from pig feces are considered probiotics (Jacobsen et al., 1999).

Viability: Viable Probiotic bacteria are claimed to have significant health benefits. At least 10^8-10^9 live bacteria should reach the small intestine daily to be functional. In vitro model showed 10-40% of probiotic bacteria survive. This difference in number of consumed viable cells and their viability range signifies the proliferation and growth of probiotic in gastrointestinal tract (Sanders and in't Veld, 1999). In contrast, effective hypocholesterolemic effect has been found by consuming 10^4 cells/d of L. reuteri 1098 in mice. This dose is the lowest reported found to be effective in decreasing serum lipids without producing side effects like bacterial translocation (Taranto et al., 2000). Different metabolites of non-viable bacteria have also shown some activities in case of lactose intolerance by epithelial lactase-deficient subjects. The fresh yogurt containing viable probiotics is more efficient in facilitating the case (Marteau et al., 1990; Shermak et al., 1995). The trials in gnotobiotics (germ free animal) have clearly demonstrated the value of live bacteria (Fuller, 1992). Probiotic viability, stability and activity in food systems can be retained or improved by microencapsulation technology (Cruce & Goulet, 2001).

Survive the upper gastrointestinal tract (Acid and bile tolerance): A good probiotic organism should tolerate the low pH of approximately 2, solution of pepsin of 0.3% w/v and solution of NaCl 0.5% w/v–which are the normal conditions of normal human stomach (Vinderola & Reinheimer, 2003). Bile salt tolerance is directly related to deconjugation of bile salt by the organism that possess bile salt hydrolase (BSH) a factor for desirable health effect in reducing serum cholesterol in blood (Corzo & Gilliland, 1999 and duToit, et al., 1998; Fukushima & Nakano, 1996). Similarly, acid tolerance is contributed by Arginine deiminase (ADI) pathway to catabolise arginine into ammonia, ornithine, ATP, carbamyl phosphate and citrulline (Liu et al., 1994; Rollen et al., 2003; Tonon & Lonvaud-funel, 2002).

Easy colonization and/or proliferation: The ability of bacteria to adhere to mucus in high level is of ecological importance when colonizing the gut mucosa (Mikelsaar et al., 1998). Colonization in the intestine is the important property of probiotic bacteria to establish the persistent health benefit. The ability of probiotic bacteria to adhere to the intestinal wall improves their chances of winning the competition against other bacteria. Species specificity is regarded for colonization rather than host specificity (Salminen et al., 1998; Nikoskelainen et al., 2001). The production of lactic acid, antibiotic related compounds and bacteriocins help the probiotic bacteria to create the environment (Kaur, et al., 2002; Tuomola et al., 1999). Surface components of the bacterial cell; polysaccharides and proteins play an essential role in this adhesion phenomenon which is important for colonization of host surfaces by both pathogens and resident microorganisms. Exopolysaccharides (EPS) produced by *Lactobacillus* sps isolated from human intestine may also assist the colonization (LipiDski et al., 2003 Whitfield and Valvan, 1993).

Potential to perform some other health beneficial effects: Well recognized probiotic bacteria are found to improve intestinal gut function and stimulate immune system. *Lactobacillus rhamnosus* GG (*L.* GG), and L. reuteri are effective against rotaviral diarrhea in children, also the prevention of gastrointestinal infection in mice has been recorded while milk fermented by *L. casei* and *L. acidophilus* were fed (Pochapin, 2000; Perdigon et al., 1990). The increased antibody response was found against cholera by yogurt cultures in viable or non-viable form and non viable cells of *L. gasseri* were found to induce IFN± in murine macrophage cultures (Portier et al., 1993; Kitazawa et al., 1994). Cancer controlling action *of L. paracasei, L. acidophilus* and bifidobacteria has gained attention to use probiotic in cancer therapy by oral administration of probiotics (Hirayama & Rafter,1999; Fernandes et al., 1992, Rafter, 2002). Alteration of physicochemical condition in the colon,

Table 1: Commonly used probiotic cultures around the world.

Species	Strains	Source Company
Lactobacillus acidophilus	Johnsonii (La1, also known as La5, Lj1)	Chr. Hansen, Inc.(Milwaukee, wis.)
	La2	
	NCFM	Rhodia, Inc. (Madison, Wis.)
	DDS-1	Nebraska cultures, Inc. (Lincoln, Neb.)
	SBT-2062	Snow Brand Milk Products Co. Ltd. (Tokyo, Japan)
L. bulgaricus	Lb12	
Lactobacillus delbrueckii subsp. *bulgaricus*	2038	Meiji Milk Products, Tokyo, Japan
L. lactis	La1	Essum AB (Umea, Sweden)
L. plantarum	229v	Probi AB (Lund, Sweden)
	Lp01	
L. rhamnosus	GG	Valio Darty (Helsinki, Finland)
	GR-1	Urex Biotech (London, Ontario, Canada)
	271	Probi AB (Lund, Sweden)
	LB21	Essum AB (Umea, Sweden)
L. reuteri	SD2112 (also known as MM2)	Biogaia (Raleigh, N.C.)
L. casei	shirota	Yakult (Tokyo, Japan)
	Immunitass	Danone (Paris, France)
	744	
	01	
L. paracasei	CRL431	Chr. Hansen, Inc. (Milwaukee, Wis.)
L. fermentum	RC-14	Urex Biotech (London, Ontario, Canada)
L. helveticus	B02	
L. salivarius	UCC118	University College (Cork, Ireland)
Streptococcus thermophilus	1131	Meiji Milk Products ((Tokyo, Japan)
Bifidobacterium adolescentis	-	
B. longum	BB536	Morinaga Milk Industry Co., Ltd. (Zama-City, Japan)
	SBT-2928	Snow Brand Milk Products Co. Ltd. (Tokyo, Japan)
B. breve	Yakult	Yalult (Tokyo, Japan)
B. bifidus	Bb-11	
B. essensis	Danone (Bio Activia)	
B. lactis	Bb-02	
	Bb-12	Chr. Hansen, Inc.(Milwaukee, wis.)
B. infantis	Shirota	
	Immunitass	
	744	
	01	
B. laterosporus	CRL 431	
B. subtilis	-	
Saccharomyces cerevisiae	boulardii[a]	Biocodex, Inc., Seattle, Washington, USA

a against Candida infection not used in food systems yet.

Source: Krishnakumar and Gordon (2001); Young et al., (1999);Sanders & in't Veld, (1999)

enzyme deactivation, carcinogen binding, immune activation has been proposed as cancer controlling mechanism (Hirayama & Rafter, 1999). Kato et al. (1994) have studied viable *L. casei* YIT 9018 potentiated systemic immune responses that modified T-cell functions in tumor bearing mice.

Antimutagenic activity of probiotic is related to prevention of cancer. Oral administration of *L. rhamnosus* GG in yogurt, *L. gasseri* ADH, suppress the enzymes (nitroreductase, ²-glucuronidase, azoreductase, 7-±-dehyrozylase, glycocholic acid hydrolase) which are present in feces and are involved in conversion of procarcinogens into carcinogens (Ling et al., 1994; Pedrosa et al., 1995). Oral administration of *L.*

acidophilus was effective on chemically induced DNA damage in colon cells in vivo (Pool-zobel et al., 1996). Some probiotics also perform cholesterol lowering action by cholesterol assimilation or by deconjugation of bile acids (Fukushima & Nakano, 1996). The increased excretion of bile acids might lead to a faster rate of systemic cholesterol catabolism of cholesterol to bile acids. Oral administration of viable and non-viable cells of *L. casei* BDII in small dose (10^4 cells/d) were effective to reduce total bile acid (TBA) and the relation between effect and dose was in inverse manner (Yong-Shi & Ben-Heng, 2003). *L. casei* has been demonstrated to suppress IgE responses and systemic anaphylaxis in a murine model of food allergy (Isolauri, 2003). Some probiotics strains of *Enterococcus faecium, L. acidophilus* and *Saccharomyces boulardii* appear to have anti-Candida activity (Shalev, et al., 1996; Satonaka et al., 1996). The role of probiotics on synthesis of vitamins: folic acid, niacin, vitamin K, vitamin B6, increased calcium absorption rate determined by elevated level of calcium after consumption of both yogurt and heat treated yogurt when compared to a control group refraining from yogurt consumption, preserving nutrients are the attributes for probiotic selection for commercial use (Ouwehand and Salminen, 1998).

Others: The selected probiotic organisms should be non infectious, non invasive, non toxic, non carcinogenic and not absorbed in digestive tract (Kalantzopoulos, G, 1997). Stability in wide variation of temperature and pH, genetically stable, lack of mutation, ease of production at large scale, utilization of nondigestible fiber and oxygen tolerance are other properties to be considered in probiotic selection.

Probiotic foods

Probiotic microorganisms consist mostly of the strains of *Lactobacillus*, *Bifidobacterium* and *Streptococcus*, the bacterial types which have been used in the production of fermented dairy products such as yogurt, fermented milk for centuries back knowingly or unknowingly. However, probiotic strains have been isolated from various other sources except dairy products. Thus, any kind of food that contains higher number of live probiotic microbial supplement like bacterial lyophilizates, capsules, infant formula, unfermented milk, juices and candy have been considered probiotic foods (Heyman & Ménard, 2002). There are more than 70 bifidus- and acidophilus-containing products produced world wide, including sour cream, buttermilk, yogurt, powdered milk and frozen desserts. More than 53 different types of milk products that contain probiotic organisms are already marketed in Japan alone. Probiotics are very popular in Europe but their use is largely restricted to fermented milk and yogurt (Hilliam, 2000). The new non milk based probiotic functional food launched in Sweden in 1994 for the first time is a *L. plantarum* 299v fermented oatmeal gruel that is mixed with fruit drink (Molin, G., 2001). Besides the fermented milk products, some of the lactic acid fermented foods of plant origin such as brined olives, salted gherkins, and sauerkraut and *togwa*- a Tanzanian beverage made from sorghum or maize containing high number of viable cells are consumed after any further processing (Molin, G., 2001). The application of probiotic organisms in such foods and in cereal based fermented rice beverage of traditional Nepalese diet, *poko*, has also been suggested (Shrestha & Rati, 2003).

Effects on human health

The beneficial effects of probiotics are contributed by a direct antagonism against specific groups of microorganisms (enteropathogenes) by an effect on the metabolism in the gut or by a stimulation of systemic or mucosal immunity and other different physiological enhancement. The health benefits from in case of urogenital infection caused by *Helicobacter pylori*, gastrointestinal dysfunction and hepatic encephalopathy are attributed to direct competitive exclusion or inhibition of corresponding bacteria (Sanders & in't Veld, 1999). The beneficial effects in human health and their possible postulated mechanism by consuming probiotics are given in Table 2. However, concrete mechanism of health benefits need further research studies in vivo and in human trials.

Heart disease: The coronary heart disease (CHD) and major risk of heart attacks in hypercholesterolemic individuals can be significantly reduced by lowering their serum cholesterol by feeding probiotic fermented food (Sindhu and Kheltarpaul, 2003). Bile salt hydrolase (BSH) activity of lactic acid bacteria has been directly related to the cholesterol reduction (28.8-47.2%) and bile tolerance in vitro with no or very little reduction by BSH negative individuals (Saavedra et al., 2003). The in vivo tests in human feeding fermented milk products showed significant lowering of cholesterol ranged from 2.4-23.2% for total cholesterol and 9-9.8% for low-density lipoprotein cholesterol (Sanders, 1999). Bile salt hydrolase activity was more pronounced in species of *Bifidobacterium* than in species of *Lactobacillus*. The 98% of tested species isolated from feces of human, animals and birds were BSH positive while only 83% of others isolated from milk products or starter culture were BSH positive suggesting that BSH activity is highly correlated with the habitat of genus, species or strains (Tanaka et al., 1999). *L. reuteri* CRL 1098 and *L plantarum* 229v have been reported to modulate the lipid effectively and beneficial on heart disease (Toranto et al., 2000; McNaught & McFie, 2001).

Intestinal health: The intestinal health benefits by consuming large number of probiotic in the range of 10^9-10^{11} cells/day have been directly associated with the proteolytic and ^{2}-galactosidase activity of *L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus*, *B. longum* and other *Lactobacillus* sps in case of lactose intolerance (Martini et al., 1991; Jiang et al., 1996). Antagonism against putrefactive or harmful bacteria in intestine by the bactericidal agents or bacteriocins: lactacin B and Acidolin of L. acidophilus (Zamfir et al., 1999) and

production of rotavirus specific IgA by *L. rhamnosus* GG, *L. reuteri*, *L. casei* shirota & GG, *B. lactis* Bb12 have been speculated to effect on acute gastroenteritis, antibiotic associated diarrhoea and inflammatory bowel diseases. Other probiotics associated with intestinal health such as *B. breve*, *B. bifidum*, *L. johsonii* La1, *L. gasseri*, *Saccharomyces cerevisiae* (boulardii), *Enterococcus faecium* SF68, *L. salivarius* UCC118 & WB1004, *L. plantarum* 229v, *E. coli* Nissle 1917 have already been reviewed by Heyman & Ménard, 2002 and Ouwehand et al.,2002.

Cancer prevention: There are some evidences that consumption of fermented milk containing probiotic bacteria has antitumor and antimutagenic effect. The in vitro and animal tests has shown that the probiotic bacteria reduce risk of cancer possibly by counteracting mutagenic and genotoxic effects in the colon and other organ sites. Hirayama & Rafter (1999) has summarized the possible mechanisms of probiotic lactic acid bacteria and *Bifidobacterium* in reducing colon. cancer risks. In vivo study in rat showed dietary supplementation with *Lactobacillus acidophilus* suppressed total number of colon cancer cells in dose dependent manner. *Lactobacillus* GG has been shown to inhibit incidence and number of tumors of colon and *B. longum* to inhibit the incidence of tumors in colon, small intestine, liver and mammary in rats (Pool-Zobel et al., 1996; Reddy & Rivenson, 1993). A few clinical studies on mechanistic action of probiotics reveal *L. casei* strain postponed the recurrence of bladder tumors and *L. acidophilus* and *L. rhamnosus* GG reduce the colon cancer risk by mutagen binding and degradation (De Roos & Katan, 2000). However, extensive clinical trials should be done to reach to confirmation.

Immune system modulation: Various properties of probiotic bacteria have been proposed as an explanation for their multiple beneficial effects. Reinforcement of the intestinal barrier capacity to exogenous antigens and an increase in humoral immunity and mucosal secretory IgA response are the properties of most of the immune active probiotics to counteract imbalance in gut flora. In addition to strengthening the specific immunity, probiotic lactic acid bacteria also seem to reinforce the non-specific mechanism of defense such as phagocytosis (engulfment) and cytokine production. Fermented milk supplemented with *L. acidophilus* LA1 and bifidobacteria produced a specific serum IgA response against attenuated *Salmonella typhi* strain ingested to mimic an enteropathogenic infection in human volunteers (Link et al., 1994). Also the immune system modulation by translocation of a small number of ingested bacteria via M cells to the Payer's patches of the gut-associated lymphoid tissue in the small intestine has been proposed. Yogurt containing *S. thermophilus*, *L. bulgaricus*, *B. infantis*, *L. acidophilus* NCFM tested for its effect on mucosal and systemic IgA and IgG response of mice orally immunized with cholera toxin showed higher intestinal and serum IgA specific for cholera toxin than in mice received only *S. thermophilus*, *L. bulgaricus*. The result suggested that the combined use of probiotics increase immune response to oral antigens (Tejada-Simon et al., 1999).

Antihypertensive effect: The control of hypertension by dietary recommendations lead to test the food products derived from probiotic cultures for possible contribution to blood pressure control. The antihypertensive effect has been already documented in hypertensive rats and clinical test in human (Hata et al.,1996). *Saccharomyces cerevisiae* and *Lactobacillus helveticus* fermented milk which contained two tripeptides, valine-proline-proline and isoleucine-proline-proline, have been identified as angiotensin-I-converting enzyme inhibitors. In this regard, the antihypertensive effect is mediated by fermentation end product rather than direct viable cells functioning. Similarly, non viable cell associated effect has been proposed by Sawada et al. (1990) in placebo-controlled human trials with Lactobacillus casei YIT9018. The cell wall fragments which are composed of polysaccharides (cell wall polysaccharides) were found to be active agents.

Allergy: Probiotic supplementation with either *Lactobacillus* GG or *Bifidobacterium lactis* Bb-12 in hydrolyzed whey formula resulted in significant improvement of the skin condition along with a decrease in markers of systemic allergic inflammation as compared to the infants receiving unsupplemented formula (Isolauri, 2003). The mechanism behind relief from allergy in infants with atopic eczema and in infants with challenge-proven cow milk allergy may be attributed to one of the various listed in Table 2.

Conclusion

Owing to different possible health benefits in human by consuming probiotics or food containing lactic acid bacteria, Bifidobacteria and yeast, a vast array of works are being done. Recently not only the development on probiotic mechanism of known species but also the searches of alternative and new strains have gained popularity. For the newly entering probiotic species the in vitro test on different probiotic selection criteria postulated in this review is designated as basic criteria. In some cases, the probiotic activity could be enhanced in conjunction with fermented milk, although, milk is not only the source to harbor probiotic strains. Lately cereal based fermented foods appeared as a substitute of milk based medium. Also, the strains from vegetables and cereals have gained attention after several in vitro tests for proposed health benefits for well known probiotics.

Studies documenting probiotic effects by in vitro and in vivo studies in animal model for several organisms have been an old practice. However, those studies can't be denied and the proposed health benefits in human can be only fully accepted when extensive studies are carried out in humans. The most compelling evidence for probiotic efficacy after several studies in human is in the areas of anti-diarrheal effects and improved digestion of lactose in lactose intolerant cases. Also several lines of research in animal models on colon cancer, breast cancer including human study on cancer recurrence of

bladder suggests probiotic bacteria may be able to mediate this effect. Human data on anti-hypertensive effects, cholesterol lowering, urogenital health, inhibition of *H. pylori*, immune function are not comprehensive enough to be considered definitive effect on human, although several positive data are available. Modulation of these activities to deal with the mechanism by which probiotic effects are achieved needs further extensive research on human cases. Epidemiological studies in large scale have been suggested for the effect that regular consumption of probiotic bacteria may have in generally healthy populations.

Table 2: Potential and established effects of probiotic bacteria and their postulated mechanisms

Health Benefits	*Postulated mechanism*	*Reference*
Aid in lactose digestion	Bacterial lactase hydrolyses lactose	Kolars et.al., 1984
Resistance to enteric pathogens	Promotion of intestinal IgA immune response	Malin et al.,1996
	Colonization resistance	Heyman & Ménard, 2002
	Alteration of intestinal conditions to be less favorable for pathogenicity (pH, short chain fatty acids, bacteriocins)	Heyman & Ménard, 2002
	Alteration of toxin binding sites	Sanders & in't Veld, 1999
	Influence on gut flora populations	Sanders & in't Veld, 1999
	Up regulation of intestinal mucin production, interfering with pathogen attachment to intestinal epithelial cells	Salminen & Isolauri, 1996
Anti-colon cancer effect	Mutagen binding	Rowald & Grasso, 1975
	Host mediated immune stimulation	Sekine et al., 1985
	Carcinogen degradation	Hirayama & Rafter, 1999
	Suppress the growth of bacteria that converts procarcinogens into carcinogens	De Roos & Katan, 2000
	Inhibition of carcinogen-producing enzymes of colonic microbes immune response	Sanders & in't Veld, 1999
	Influence on secondary bile salt concentration	Sanders & in't Veld, 1999
Small bowel bacterial overgrowth	Influence on activity of overgrowth flora, decreasing toxic metabolite production	Sanders & in't Veld, 1999
	Alteration of intestinal conditions to be less favorable to overgrowth flora activities of populations	Ouwehand et al, 2002
Immune system modulation	Strengthening of non-specific defense against infections and tumors	McNaught & MacFie, 2001
	Macrophase stimulation and increase in Kupffer cells	Kaur et al., 2002
	Adjuvant effect in antigen-specific immune responses	Sanders & in't Veld, 1999
	Enhancement of secretory IgA production	Sanders, 1999
Allergy	Prevention of antigen translocation into blood stream	Sanders & in't Veld, 1999
	Degradation/structural modification of enteral antigens	Isolauri, 2003
	Enhanced host defense maturation and stimulation, generation of anti-inflammatory cytokines	Isolauri, 2003
Blood lipids, heart disease	Assimilation of cholesterol within bacterial cell	Gilliland et al., 1985
	Increased excretion of bile salts due to deconjugation by bile salt hydrolase	DeSmet et al., 1994
	Anti oxidative effect	Sanders & in't Veld, 1999
Antihypertensive effect	Peptidase action on milk protein yields tripeptides which inhibit angiotensin-1 concerting enzyme	Hata et al., 1996
	Cell wall component act as angiotensin concerting enzyme inhibitors	Sawada et al., 1990
Urogenital infections	Adhesion to urinary and vaginal tract cells	Sanders & in't Veld, 1999
	Colonize resistance	Sanders & in't Veld, 1999
	Inhibitor production (H2O2, biosurfactants)	Hiller et al., 1992
Infection caused by *Helicobacter pylori*	Production of inhibitors of *H. pylori* (lactic acids and others)	Heyman & Ménard, 2002
Hepatic encephalopathy	Inhibition of urease-producing gut flora	Sanders & in't Veld, 1999

Reference

Adachi S. (1992). Lactic acid bacteria and the control of tumours. In: The Lactic Acid Bacteria in Health and Disease, Wood B.J.B. (eds) Elsevier applied Science, London, Vol. 1, pp. 233-261

Aiba Y., Suzuki N., Kabir A.M.A. Takagi A. and Koga Y. (1998). Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a gnotobiotics murine model. The American Journal of Gastroenterology 93: 2097-2101

Anderson J.W. & Gilliland S.E. (1999). Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on

serum cholesterol in hypocholesterolemic humans. Journal of American College of Nutrition, 18: 43-50

Collins J. K., Thornton G., & Sullivan G.O. (1998). Selection of probiotic strains for human applications. International Dairy Journal, 8:487-490

Corzo G., & Gilliland S. E. (1999). Bile salt hydrolase activity of three strains of *Lactobacillus acidophilus*. Journal of Dairy Science, 82, 472–480

Cruce P., S. and Goulet J. (2001). Improving probiotic survival rates. Food Technology, 55 (10):36-42

de Roos N.M. & Katan M.B. (2000). Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998, American Journal of Clinical Nutrition, 71: 405–411

DeSmet I., Van Hoorde L., De Saeyer N., Van de Woestyne M. and Verstraete W. (1994). In vitro study of bile salt hydrolase (BSH) activity of BSH isogenic *Lactobacillus plantarum* 80 strains and estimation of cholesterol lowering through enhanced BSH activity. Microbial Ecology in Health and Disease, 7: 315-329

du Toit M., Franz C.M.A.P., Dicks L.M.T., Schillinger U., Haberer P., Warlies B., Ahrens F., Holzapfel W.H. (1998). Characterization and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. International Journal of Food Microbiology, 40, 93–104.

Fernandes C.F., Chandan R.C., Shahani K.M.(1992). Fermented dairy products and health. In: The Lactic Acid Bacteria Vol. 1 The Lactic Acid Bacteria in Health and Disease. Brian J.B. Wood (eds) Elsevier Applied Science, London, Vol. 1.

Fukushima M. & Nakano M., (1996). Effects of mixture of organisms, *Lactobacillus acidophilus* or *Streptococcus faecalis* on cholesterol metabolism in rats fed on a fat- and cholesterol-enriched diet. British Journal of Nutrition, 76: 857-867

Fuller R. (1989). Probiotics in man and animals. Journal of Applied Bacteriology, 66(5): 365-378

Fuller R. (1992). History and development of probiotics. In: Probiotics - The scientific Basis. Fuller, R. (eds) Chapman & Hall, London, pp. 1-9

Gilliland S.E., Nelson C.R., Maxwell C. (1985). Assimilation of cholesterol by *L. acidophilus*. Applied and Environmental Microbiology, 49: 377– 81

Grant C. & Salminen S. (1998). The potential of *Propionibacterium* spp. as probiotics. In: Lactic acid bacteria, microbiological and functional aspects. Salminen S. & von Wright A. (eds), Marcel Dekker Inc, New York, pp. 588-603

Guslandi M., Mezzi G., Sorghi M. & Testoni P.A. (2000). *Saccharomyces boulardii* in maintenance treatment of Crohn's disease. Digestive Diseases Sciences, 45:1462-1464

Hallen A., Jarstrand C. and Pahlson C. (1992). Treatment of bacterial vagionosis with lactobacilli. Sexually Transmitted Diseases, 19: 146-148

Hata Y., Yamamoto M. Ohni M., Nakajima K., Nakamura Y. and Takano T. (1996). A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects. American Journal of Clinical Nutrition, 64: 767-771

Havenaar R. & Huis in't Veld J. H. J. (1992). Probiotics: A General View. In: The Lactic Acid Bacteria- The Lactic Acid Bacteria in Health and Disease. Brian J.B. Wood (eds), Elsevier Applied Science, London, Vol. 1, pp. 155

Heyman M. and Ménard S. (2002). Probiotic microorganisms: how they affect intestinal pathophysiology. CMLS, Cellular and Molecular Life Science, 59: 1151-1165

Hilliam M. (2000). Functional food: How big is the market? World of Food Ingredients, 12:50-53

Hillier S.L., Krohn M.A., Klebanoff S.J. & Eschenbach D.A. (1992). The relationship of hydrogen peroxide-producing lactobacilli to bacterial vaginosis and genital microflora in pregnant Woman. Obstetrics Gynecology, 79: 369-373

Hirayama K & Rafter J. (1999). The role of lactic acid bacteria in colon cancer prevention: mechanistic considerations. Antonie van Leeuwenhoek., 76:391-394

Holzapfel W.H., Schillinger U. (2002). Introduction to pre- and probiotics. Food Research International, 35, 109–116

Isolauri E. (2003). Probiotics in the treatment and prevention of allergies. Monatsschrift Kinderheilkunde Suppl. 1. 151: S27-S30

Jacobsen CN., Nielsen VR., Hayford AE., Møller PL., Michaelsen KF., Pærregaard A., Sandstrom B., Tvede M., Jakobsen M. (1999) Screening of probiotic activities of forty-seven strains of lactobacillus spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. Applied and environmental microbiology, 65:4949–4956.

Jiang T., Mustapham A., Savaiano D.A. (1996). Improvement of lactose digestion in humans by ingestion of unfermented milk containing *Bifidobacterium longum*. Journal of Dairy Science, 79: 750-757

Kalantzopoulos G. (1997). Fermented products with probiotic qualities. Anaerobe, 3:185-190

Kato I., Endo K. & Yokokura T. (1994). Effects of oral administration of *Lactobacillus casei* on antitumor response induced by tumor resection in mice. International Journal of Immunopharmacology, 16:29-36

Kaur I.P., Chopra K. & Saini A. (2002). Probiotics: potential pharmaceutical applications. European Journal of Pharmaceutical Sciences, 15: 1–9

Kitazawa H., Tomioka Y., Matsumura K., Aso H., Mizugaki M., Otoh T. and Yamaguchi T. (1994). Expression of mRNA encoding IFN± in macrophases stimulated with *Lactobacillus gasseri*. FEMS Microbiology Letters, 120: 315-322

Kolars J.C., Lewitt M.D., Aouji M. & Savaiano D.A. (1984). Yogurt- an autodigesting source of lactose. The New England Journal of Medicine, 310: 1-3

Kruis W., Schutz E., Fric P., Fixa B., judmaier G., & Stolte M. (1997). Double-blind comparision of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. Alimentary Pharmacology Therapeutics, 11: 853-858

Krishnakumar V. and Gordon I.R. (2001). Probiotics: Challenges and opportunities. Dairy Industries International, 66(2): 38-40

Lankaputra W.E.V. and Shah N.P. (1998). Antimutagenic properties of probiotic bacteria and of organic acids. Mutation Research, 397: 169-182

Ling W. H., Korpela R., Mykkänen H., Salminen S. and Hänninen O. (1994). *Lactobacillus* strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy human female adults. Journal of Nutrition, 124: 18-23

Link A.H., Rochat F., Saudan K.Y., Mignot O. & Aeschlimann J.M. (1994). Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunology and Medical Microbiology, 10: 55-63

LipiDski T., Jones C., Lemercinier X., Kowal A.K., Strus M., Rybka J., Gamian A., Heczko P.B. (2003). Structural analysis of the Lactobacillus rhamnosus strain KL37C exopolysaccharide. Carbohydrate Research, 338: 605–609

Liu S. Q., Pritchard G. C., Hardman M. J. and Pilone G. J. (1994). Citrulline production and ethyl carbamate (urethane) precursor formation from arginine degradation by wine lactic bacteria Leuconostoc and Lactobacillus buchneri. American Journal of Enology and Viticulture, 45: 235-242.

Malin M., Suomalainen H., Saselin M. and Isolauri E. (1996). Promotion of IgA immune response in patients with crohn's disease by oral bacteriotherapy with *Lactobacillus* GG. Annales of Nutrition and. Metabolism, 40:137-145

Malchow H.A. (1997). Crohn's disease and *Echerichia coli*. Journal of Clinical Gastroenterology, 25: 653-658

Marteau P., Flourie P., Chastang C., Desjeux J.F. & Rambaud J.C. (1990). Effect of the microbial lactase (EC 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose: an in vivo study in lactase-deficient humans. British Journal of Nutrition, 64: 71-79

Martini M.C., Kukielka D., Savaiano D.A. (1991). Lactose digestion from yogurt: Influence of a meal and additional lactose. American Journal of Clinical Nutrition, 53: 4253-4258

McFarland L.V., Surawicz C.M., Greenberg R.N., Fekety R., Elmer G.W, Moyer K.A., Melcjer S.A., Bowen K.E., Cox J.L., Noorani Z., Harrington G., Rubin M. and Greenwald D. (1994). A randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. The Journal of the American Medical Association, 271(24): 1913-1918

McNaught C.E., MacFie J. (2001). Probiotics in clinical practice: a critical review of the evidence. Nutrition Research, 21: 343–353

Metchnikoff E. (1908). The prolongation of life. Optimistic Studies. G.P. Putnam's Sons, New York

Mikelsaar M., Mandar R., Sepp E., (1998). Lactic acid microflora in the human microbial ecosystem and its development. In: Salminen, S., von Wright, A. (eds.) Lactic Acid Bacteria, Microbiology and Functional Aspects, 2nd ed. Marcel Dekker, New York, pp. 211–253

Mishra V. and Prasad D.N. (2005). Application of in vitro method for selection of *Lactobacillus casei* strains as potential probiotics. International Journal of Food Microbiology, 103: 109– 115

Molin G. (2001). Probiotics in foods not containing milk or milk constituents, with special reference to *Lactobacillus plantarum* 299v. American Journal of Clinical Nutrition, 73 (suppl): 380S–5S

Naidu A.S., Bidlack W.R. & Clemens R.A. (1999). Probiotic spectra of lactic acid bacteria (LB). Critical Reviews in Food Science and Nutrition, 38(1): 13-126

Nikoskelainen S., Salminen S., Bylund G., Ouwehand A.C., (2001). Characterization of the properties of human and dairy-derived probiotics for prevention of infectious diseases in fish. Applied and Environmental Microbiology, 67: 2430–2435

Ouwehand A. C., Salminen S. & Isolauri E. (2002). Probiotics: an overview of beneficial effects. Antonie van Leeuwenhoek, 82: 279-289

Prasad, J., Gill, H., Smart, J. & Gopal, P.K. (1998) Selection and characterization of Lactobacillus and Bifidobacterium strains for use as probiotics. International Dairy Journal, 8: 993-1002

Pedrosa M.C., Golner B.B., Goldin B.R., Barakat S., Dallal G.E. and Russel R.M. (1995). Survival of yogurt-containing organisms and Lactobacillus gasseri (ADH) and their effect on bacterial enzyme actibity in the gastrointestinal tract of healthy and hypochlorhydric elderly subjects. American Journal of Clinical Nutrition, 61: 353-359

Perdigon G., de Macias N.M.E., Alvarez S., Oliver G. and de Ruuiz Holgado, P.A.A. (1990). Prevention of gastrointestinal infection using immunobiological methods with milk femenmted with Lactobacillus casei and Lactobacillus acidophilus. The Journal of Dairy Research, 57: 255-264

Pochain M. (2000). The effect of probiotics on *Clostridium difficile* diarrhea. American Journal of Gastroenterology, 95: S11-S13

Pool-Zobel B.L., Neudecker C., Domizlaf I., Ji S., Schillinger U., Rumney C., Moretti M., Vilarini I., Scassellati-Sforzolini R. and Rowland I., (1996). *Lactobacillus* and *Bifidobacterium*-mediated antigenotoxicity in the colon of rats. Nutrition and Cancer, 26: 365-380

Portier A., Boyaka N. P., Bougoudogo F., Dubarry M., Huneau J.F., Tomé D., Dodin A., and Cost M. (1993). Fermented milks and increased antibody responses against cholera in

mice. International Journal of Immunotherapy, IX: 217-224

Rafter J. (2002). Lactic acid bacteria and cancer: mechanistic perspective. British Journal of Nutrition, 88: Suppl. 1, S89–S94

Reddy B.S., Rivenson A. (1993). Inhibitory effect of *Bifidobacterium longum* on colon, mammary and liver carcinogenesis induced by 2-amino-3-methylimidazo(4,5)quinoline, a food mutagen. Cancer Research, 53: 3914-3918

Rollan G., Lorca G.L. Font de Valdez G. (2003). Arginine catabolism and acid tolerance response in *Lactobacillus reuteri* isolated from sourdough. Food Microbiology, 20: 313–319.

Rowald I.R., Grasso P. (1975). Degradation of N-nitrosamines by intestinal bacteria. Applied Microbiology, 29: 7-12

Saavedra L., Taranto M.P., Sesma F., de Valdez G.F. (2003). Homemade traditional cheeses for the isolation of probiotic Enterococcus faecium strains. International Journal of Food Microbiology, 88: 241– 245

Salminen S. & von Wright A. (1998). Current probiotics safety assured? Microbial Ecology in Health and Disease, 10: 68-77

Salminen S., Deighton M., Benno Y., & Gorbach S. (1998). Lactic acid bacteria in health and disease. In: Lactic Acid Bacteria: Microbiology and Functional Aspects. S. Salminen, & A. von Wright (eds) Marcel Dekker, New York

Salminen S, Isolauri E. (1996). Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges. Antonie von Leeuwenhoek., 70: 347–58

Sanders M.E. & in't Veld J.H. (1999). Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. Antonie van Leeuwenhoek., 76: 293-315

Sanders M.E., (1999). Probiotics. Food Technology, 53 (11): 67-77

Satonaka K., Ohashi K., Nohmi T., Yamamoto T., Abe S., Uchida K. and Yamaguchi H. (1996). Prophylactic effect of Enterococcus faecalis FK-23 preparation on experimental Candidiasis in mice. Microbiology and Immunology, 40: 217-222

Sekine K., Toida T., Saito M., Kuboyama M., Kawashima T. & Hashimoto Y., (1985). A new morphologically characterized cell wall preparation (whole peptidoglycan) from Bifidobacterium infantis with a higher efficacy on the regression of an established tumor in mice. Cancer Research, 45: 1300-1307

Shalev E., Battino S., Weiner E., Colodher R. and Keness Y. (1996). Ingestion of yoghurt containing Lactobacillus acidophilus compared to pasteurized yoghurt as prophylaxis for recurrent candidal vaginitis and bacterial vaginosis. Archives of Family Medicine, 5: 593-596

Shermak M.A., Saavedra J.M., Jackson T.L., Huang S.S., Bayless T.M. & Perman J.A. (1995). Effect of yogurt on symptoms and kinetics of hydrogen production in lactose-malabsorbing children. American Journal of Clinical Nutrition, 62: 1003-1006

Shrestha H. & Rati E.T. (2003). Defined microbial starter formulation for the production of poko-A traditional fermented food product of Nepal. Food Biotechnology, 17(1): 15-25

Sindhu C.S., Khetarpaul N. (2003). Effect of feeding probiotic fermented indigenous food mixture on serum cholesterol levels in mice. Nutrition Research, 23: 1071-1080

Sreekumar O. & Hosono A. (2000). Immediate effect of Lactobacillus acidophilus on the intestinal flora and fecal enzymes of rats and the in vitro inhibition if Escherichia coli in coculture. Journal of Dairy Science, 83: 931-939

Tanaka H., Doesburg K., Iwanaki T., Mierau I. (1999). Screening of Lactic Acid Bacteria for Bile Salt Hydrolase Activity. Journal of Dairy Science, 82: 2530-2535

Taranto M.P., Medici M., Perdigon G., Ruiz Holgado A. P. and Valdez G. F. (2000). Effect of *Lactobacillus reuteri* on the Prevention of Hypercholesterolemia in Mice. Journal of Dairy Science, 83: 401–403

Tejada-Simon M.V., Lee J.H., Ustunol Z., and Pestka J.J. (1999). Ingestion of yogurt containing Lactobacillus acidophilus and Bifidobacterium to potentiate immunoglobulin A responses to cholera toxin in mice. Journal of Dairy Science, 82: 649–660

Tonon T. and Lonvaud-Funel A. (2002). Arginine metabolism by wine Lactobacilli isolated from wine. Food Microbiology, 19: 451-461

Tuomola E.M., Ouwehand A.C., Salminen S.J. (1999). Human ileostomy glycoproteins as a model for small intestinal mucus to investigate adhesion of probiotics. Letters in Applied Microbiology, 28: 159–163

Vinderola C.G. & Reinheimer J.A. (2003). Lactic acid starter and probiotic bacteria: a comparative "in vitro" study of probiotic characteristics and biological barrier resistance. Food Research International., 36: 895-904

Whitfield, C., & Valvano, M. A. (1993). Biosynthesis and expression of cell-surfaces polysaccharides in gram-negative bacteria. Advances in Microbiology and Physiology, 35, 135–146.

Yeung P.S.M., Canom R., Tong P.S. & Sanders M.E. (1999). Comparision of API, 16S rDNA sequencing and fatty acid analysis as methods to speculate commercial probiotic bacteria. Journal of Dairy Science, 82: 9, abstract #D22.

Yong-Shi B & Ben-Heng G. (2003). Studies on cholesterol-reducing by Lactic Acid Bacteria. In: Proceedings I, The Fifth International Conference of Food Science and Technology (ICFOST), Oct 22-24, Wuxi, P.R. China, pp 278-281

Zamfir M, Callewaert R., Cornea P.C., Savu L., Vatafu I. and De Vuyst. (1999). Purification and characterisation of a bacteriocin produced by Lactobacillus acidophilus IBB 801. Journal of Applied Microbiology, 87(6): 923–31

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Chitosan: A Functional Biopolymer for Food and Pharmaceutical

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Chitosan is an N-deacetylated derivative of chitin, which is a natural polysaccharide abundantly found in crustaceans, insects and fungi. The commercial grade of chitosan has degree of deacetylation in the range of 70-90% and molecular weight 1x10⁵ KDa. However, the degree of deacetylation and molecular weights greatly affects on physical, chemical and biological properties of chitosan. Chitosan exhibits a broad-spectrum antimicrobial activity due to its functional amino group at C-2 position of glucosamine residue, which is cationic in nature. It exhibits an anticholestrerolemic effect due to its ability to bind dietary lipids and also interrupts the enterohepatic bile acid circulation. Similar mechanism of dietary fat binding effect of chitosan could also be helpful to control obesity in human. Several studies with cells on tissues and animals indicate that chitosan could promote wound healing, increase immune response, possesses antitumor and hypouricemic activities and so on. Since, chitosan is a low toxic compound as well as affirmed for GRAS by US-FDA, it has been gaining popularity as an important bio-polysaccharide for food and pharmaceutical applications.

Keywords: Chitin; Chitosan; Food additive; Pharmaceutical application; Safety

Introduction

Chitin was discovered by French scientist Henri Braconnot in 1811 (Brine, 1984). In 1823, Odier found the same compound in the cuticle of insects and named as 'chitin'. In 1859, Rouget published his finding that a 'modified chitin' could be prepared by treating chitin with boiling concentrated solution of potassium hydroxide in water. This modified chitin was renamed as 'chitosan' by Hoppe-Seiler in 1894 (Winterowd and Sandford, 1995).To date, several hundred of research papers have already been published dealing with chitin and chitosan.

Chitin is most abundant natural marine polysaccharide, chiefly found on the shells of crustacean e.g. crabs and shrimps, the cuticles of insects and spider and the cell walls of fungi (Stevens, 1999, Kittur, *et al*, 2002, New, *et al*.2002). It is also found in massive amount in marine organism especially in plankton (Stevens, 1999). Structurally, it is a biopolymer of N-acetyl-D-glucosamine (N-acetyl-2-amino-2 deoxy-D-glucopyranose) units linked by ²-D (1'!4) linkage (Ravikumar, 2000, Kubota, *et al*., 2000, Kittur, *et al*., 2002). Chitosan is obtained from chitin by N-deacetylation with an alkaline treatment and composed of 2-amino-2-deoxy-D-glucopyranose (D-glucosamine, GlcN) units (Kubota, *et al*., 2000, Kittur, *et al*, 2002). Chitosan also can be produced by fungal fermentation using *Gongronella butleri* USDB0201 (New, *et al*., 2002). Most of commercial grade of chitosan contain 75-95% glucosamine and 5-25% N-acetylglucosamine units (Winterowd & Sandaford, 1995).The molecular structure of chitin and chitosan is given in Fig 1

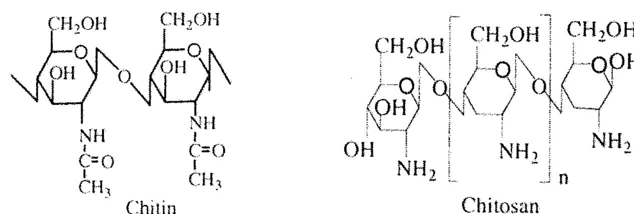

Fig 1: Molecular structure of Chitin and Chitosan

Viscometry is a simple and rapid method for the determination of molecular weight, where the constants ± and K in the Mark-Houwink equation have been determined in 0.1 M acetic acid and 0.2 M sodium chloride solution. The intrinsic viscosity is expressed as: [·] = KM ± = $1.81 \times 10^{-3} M^{0.93}$. Furthermore, converting chitin into chitosan lowers the molecular weight, changes the degree of deacetylation.The average molecular weight of chitin is 1.03×10^6 to 2.5×10^6 KDa but chitosan has in the range of 1×10^5 KDa (Ravikumar, 2000).However various industrial chitosan exhibits different average molecular weights and physical properties.

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Source of Chitin and Chitosan

Chitin is found naturally on the shells of crustacean, cuticles of insects and cell wall of fungi (Stevens, 1999, New, *et al*., 2002). Shahidi, *et al* (1992) reported that the chitin content in shell fish discarded waste was 17 % for shrimp and varied from 22.3 to 32.3% for crab segments. The yield of chitosan from chitin ranged between 72.25 to 77% (Shahidi, *et al*., 1992). The chitin content of selected crustacean, insects, molluscan organs, and fungi is given in Table 1.

Manufacturing of chitosan

Kyowa oil and fat company of Japan established the first chitin and chitosan production facility in 1971. Most of chitin and chitosan production plants have been located near shellfish processing operation and the polymers are isolated commercially from crab, shrimp, prown and krill shell waste. This arrangement also solves a waste disposal problem for

Table 1: Chitin content of selected crustacean, insects, molluscan organs, and fungi.

Type	Chitin %
Crustacean	
Paralithodes (King crab)	35[b]
Callinectes (blue crab)	14 [a]
Pleuroncodes (red crab)	1.3-1.8[b]
Crangon (shrimp)	5.8[b]
	69.1[c]
Alaskan shrimp	28[d]
Lepas (barnacles)	58.3[c]
Insects	
Periplaneta (cockroach)	2.0[d]
	18.4[c]
Blatella (cockroach)	10 [b]
	35[c]
Colcoptera (beetle)	5-15[b]
	27-35[c]
Pieris (sulfur butterfly)	64[c]
Grasshopper	2-4[a]
	20[c]
Bombyx (silkworm)	44.2[c]
Molluscan Organs	
Clamshell	6.1
Oyster shell	3.6
Squid,skeletalpen	41.0
Fungi	
Aspergillus niger	42.0[e]
Penicillium notatum	18.5[e]
P. chrysogenium	20.1[e]
S. cerevisiae	2.9[e]

[a]Wet body weight; [b]Dry body weight; [c]Organic weight of cuticle; [d]Total dry weight of cuticle
[e]Dry weight of the cell wall. Source: Knorr, (1984).

the shellfish processing plants(Winterowd & Sandford,1995). Chitosan is commercially produced from chitin by deacetylation process (Trzcinski, *et al.*, 2002).The general flow sheet for production of chitosan is shown in Fig 2.

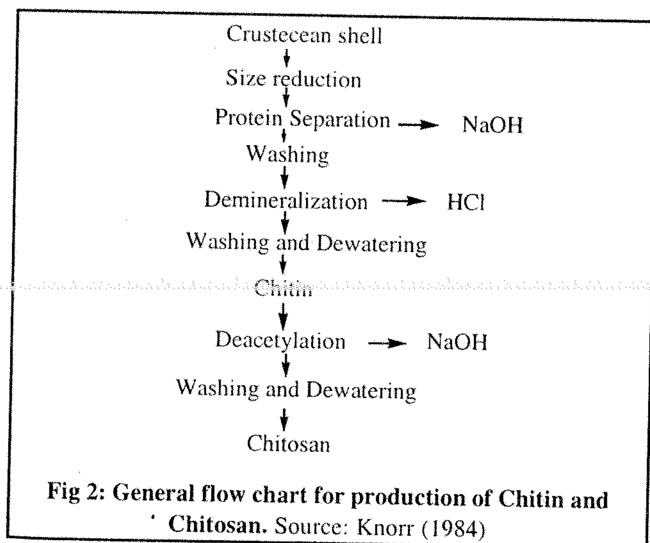

Fig 2: General flow chart for production of Chitin and Chitosan. Source: Knorr (1984)

Deacetylation

Deacetylation is the treatment of chitin with an alkali so that the acetamide groups are hydrolyzed to give free amino groups. This hydrolysis is usually carried out with a 40% of a sodium hydroxide or potassium hydroxide at elevated temperature under heterogeneous conditions using chitin flakes or powder (Kurita, 1992).Their functional properties such as solubility, viscosity are greatly varied by the degree of acetylation and molecular weight (Kennedy, 1988).The sample with 45 to 55 % deacetylated were found to be water soluble, where as those with above 60% or below 40% deacetylation were naturally insoluble in water (Kurita, 1992). The commercial grade of chitosan have a degree of deacetylation is usually 70-90% (Onsøyen, 1990).

Commercial quality standard

The chemical compositions of the chitosan may vary due to the processing conditions and market requirements. According to Bioshell Inc. the specification of chitosan is given in the Table 2.

Table 2 : Sepcification of Chitosan

Specification	Level
Moisture content %	6-10
Ash content %	0.50
Protein %	0.10 %
Degree of Deacetylation %	77-83
Viscosity range (cps)	100-800
Bulk density	0.20/cc
Chloride %	0.50
Sodium %	0.25
SiO_2 (ppm)	400
Total heavy metals (ppm)	<5
Salmonella or Coliforms	Absence
TPC	<10colonies/g

Source: Reference (Kaye.1985)

Applications

Food application

Chitosan has been proposed as a texturing, emulsifying, foaming, gelling, coating, water binding and fat binding agent for a number of foods and beverages (Jiang & Li, 2001). Chitosan is a useful emulsifier that yields a stable water-in-oil-in-water (W/O/W) multiple emulsions without adding any surfactant because of different degree of deacetylation.Chitosan with high degree of deacetylation (DD) promotes the formation of O/W emulsion, whereas those of lower DD promotes the emulsification of water inside the oil droplets. Emulsion viscosity, emulsion stability, aging are proportional to the concentration of chitosan (Rodriguez, *et al.*, 2002).

Chitosan can also be used on coating or treatment of fruits like apple, strawberries, table grape, tomato, bell pepper, cucumber (Ghaouth, *et al*, 1991, Choi, *et al*, 2002, Romanazzi, et al, 2002). At the concentration of 1-2% chitosan coating improves the quality of fruits; reduce respiration rate, weight loss, delayed increase in PPO (Polyphenol Oxidase Activity), change in color and decay of fruit during storage (Ghaouth, *et al*, 1991, Choi, *et al*, 2002). A study showed that 6mg/ml chitosan inhibited the growth of *Botrytis cinerea* and *Botrytis cinerea* fungi by 95.5% and 71.5% respectively in PDA media (Romanazzi, *et al.*, 2002). Furthermore raising the concentration of chitosan coating enhanced the beneficial effects of chitosan in post harvest treatment of fruit (Jiang, & Li, 2001).

Furthermore, in meat products slightly inhibition (1-2 log cfu/g) of TPC growth in refrigerated beef patties has been reported in the presence of 1% chitosan (Roller, 2001). It is reportedly used as a preservative in foods in Japan such as Kamaboko, Noodles, Soy sauce, Chinese cabbage, Sardines, Cookies, Vinegar, Potato chips (Roller, & Covill, 2000, Rodrigufz, *et al.*, 2003).

Bio-packaging material

The concept of edible biopackaging has evolved by many scientists for food preservation (Mau-Chang, *et al.*, 1996, Arvanitoyannis, *et al*, 1998, Coma, *et al.*, 2002). The antimicrobial film is an active biopackaging that release the preservative action on food surface because of positive charged residue in chitosan, which can inhibits several pathogenic microorganism like *Listeria monocytogenes*, *Penicillium notatum* and *Rhodotorula rubra* (Mau-Chang, *et al.*, 1996, Coma, *et al.*, 2002). Highly deacetylated chitosan could be used as edible or biodegradable food packaging (Coma, *et al.*, 2002) .However, the film would be more suitable for those foods which have relatively high moisture content (Mau-Chang, *et al.*, 1996).

Antimicrobial action

Chitosan *in vitro* has shown antimicrobial activities in different studies against several bacteria, fungi and virus (Kennedy, 1988, Cuero, 1999, Rodriguer, 2003).The antibacterial mechanism of chitosan is generally considered due to the amino group at the C-2 position of the glucosamine residue, which is cationic in nature in acidic condition.Chitosan has been reported to bind a range of heavy metals and trace elements. Antibacterial activities of chitosan derivative can also be closely related to the formation of hydrophobic micro area. Its amphiphilic structure provides a structure affinity between the cell wall of the bacteria and the chitosan derivative (Xie, *et al.*, 2002).Furthermore, inhibitory action against nutrient permeation, reaction with bacterial teichoic acids, polyelectrolyte complexes could be the vital causes for antimicrobial action of Chitoasan(Cuero, 1999). Some antibacterial activities of chitosan are given in Table 3.

Antibacterial activity of chitosan may vary depending on their molecular weights and specific bacterium. Lower molecular weight chitosan was more effective than higher molecular weight (Hong Kyon No, *et al.*, 2002a). Chitosan generally showed higher antimicrobial activity on gram positive bacteria rather than on gram negative in the presence of 0.1% chitosan .It also inversely affected by pH (pH 4.5-5.9) higher activity at low pH value (Hong Kyon No, *et al.*, 2002b).

Table 3 : Antibacterial activity of Chitosan

Bacteria	MIC (ppm)
Agrobacterium tumefaciens	100
Bacillus cereus	1000
Corinebacterium michiganence	10
Erwinia spp.	500
Erwinia carotovora subspp.	200
E.coli	20
Klebsiella pneumoniae	700
Micrococcus luteus	20
Pseudomonas fluorescens	500
Staphylococcus aureus	20
Xanthomonas compestris	500

Source: Seo, *et al.*, (1992).

Hypercholesterolemia

Chitosan and its derivatives have exhibited hypocholesterolemic activities on several animals as well as on human experiments (Sugano, *et al.*, 1992, Ikedo, *et al*, 1993, Koide, 1998, Stevens, 1999). Chitosan was administered to 8 adult males in the form of biscuit over a study period of 2 weeks when chitosan was given in the diet (3-6 g/day) the total serum cholesterol levels were significantly decreased from 188mg/dl to 177mg/dl, whereas the serum HDL –cholesterol levels were significantly increased (51mg/dl to 56mg/dl) as compared with the level for each of them before ingestion (Koide, 1998, Muzzarelli, 1999). Dietary chitosan was effective to reduce cholesterol by 52% in the chitosan fed mice following 20 weeks of treatment. Likewise, blood glucose cholesterol and triglycerides were significantly decreased in chitosan treated normal mice. Chitosan was therefore proposed for treatment of non insulin-dependent diabetes mellitus (Muzzarelli, 1999). In other study, the result indicated that the partially hydrolyzed chitosan were more effective to decrease the serum and especially liver cholesterol levels in rats fed with a cholesterol enriched diet at level of 2% (Sugano, *et al.*, 1992).The cholesterol lowering capability of chitosan has been attributed to physicochemical properties such as degree of deacetylation, pH and viscosity (Trautwein, *et al*, 1997) (Table 4). That's why some vinegar product containing chitosan are manufactured and sold in Japan because of their cholesterol lowering property (Rodrigufz, *et al.*, 2003). Effects of partial hydrolyzed chitosan on serum and liver cholesterol levels of rats are given in the Table 4.

Chitosan can easily dissolves in acidic fluid of stomach and forms micelles with cholesterol in alkaline fluids at the upper part of the intestine resulting in an inhibition of both absorption of dietary cholesterol and the circulation of cholic

Table 4 : Effects of partial hydrolyzed chitosan on serum and liver cholesterol levels of rats.

Groups	Molecular Weight	Degree of Deacetylation	Serum cholesterol (mg/dl)	Liver cholesterol (mg/dl)
Cellulose	-	-	166±21	34±1.2[a]
Chitosan no 1.	$3x10^5$	82.4	128±17	22.0±0.9[b]
Chitosan no 3.	$7x 10^3$	78.6	123±7	17.1±2.4[c]
Chitosan no 4.	$6x10^2$	97.4	151±19	29.2±2.0[b]

Mean± SE of 5 rats. [a, b, c] values in the same column not sharing a common letter are significantly different at $p<0.05$ (Trautwein, *et al.*, 1997)

acid to liver. Cholic acid is extensively synthesized from blood cholesterol into liver resulting in a decreased of cholesterol levels in the blood. The micelles are digested away by chitinase and chitosanase secreted from intestinal organs in the large intestine and an excess amount of bile acids and sterols is excreted as free forms through feces without absorption (Hirano, and Akiyama, 1995).

Control of obesity

Chitosan has shown positive effect on obesity in human as well as in animal experiments (Kanauchi, *et al.*, 1995, Muzzarelli, 1999). In one study involved 1000 volunteers with a body mass index (kgm^{-2}) (BMI) over 25 for 12 weeks. In the course of this study it was realized that some subjects didn't respond to the chitosan treatment but responders reduced their weight significantly (Muzzarelli, 1999). In fact, the ingested chitosan is dissolved in stomach by gastric acid, which is mixed with dietary fat to form a chitosan fat complex thereafter subsequently makes a gel in the small intestine and this gel-entrapped the dietary fat, which is finally excreted through the feces. This will be a helpful ingredient to control the calorie intake and prevent from obesity.

Wound and burn healing therapy

Wound healing consists of a complex series of biochemical processes regulated by humoral factors and anti-inflammatory mediators resulting in rebuilding of tissue and protection against infection (Muzzarelli, *et al.*, 1999). Chitin and chitosan may facilitate wound healing by stimulating granulation tissue formation or repithelization (Koide, 1998). However lower molecular weight (LMW) chitosan has been reported as most effective at 99% confidence level. The admixture of silver sulfadiazine with LMW chitosan may be beneficial since the survival rate increased from 82 to 95% at the 95% confidence level (Allan, *et al.*, 1984). Infact, chitosan can form a tough water absorbent biocompatible film over the burn and it provides a cool and pleasant soothing effect when applied to the burn patients and also allows to excellent oxygen permeability, adsorbs water and naturally degraded by body enzymes that's why need not to be removed from wound after wound healing (Allan, *et al.*, 1984). An analgesic effect of chitosan is due to the absorption of proton ions released in the inflammatory site (Okamoto, *et al.*, 2002)

Besides, chitosan are useful for immune stimulation, hypouricemic effect, antioxidant, dental care, making ideal contact lens, antitumor action, prevention of celiac disease and so on (Weiner, 1992, Allan, *et al.*, 1994, Muzzarelli, 1996, Koide, 1998, Xue, *et al.*,1998, Kittur, *et al.*, 2002).

Toxicity

An early study pertaining to the toxicity of chitosan was carried out in 1968 by Arai *et al.* and LD_{50} of 10g/kg was reported indicating a lack of acute oral toxicity. Mice were fed with a high level of chitosan for 19 days and evaluated for survival, liver weight and kidney weights. Mice fed 17.9 g of chitosan/kg of body mass /day were all survived but exhibited an increase in liver weight/body weight ratio and a decreased in kidney weight /body weight ratio. At a dosage of 21g of chitosan /kg of body mass/day; 40% of the mice were died (Winterowd, & Sandford, (1995). Minami, *et al.*, (1996) reported that the chitosan above 150 mg/kg body weight treated subcutaneously induced hemorrhagic lethal pneumonia to dogs; however, 200 mg/kg of body weight chitosan caused no physiological or hematological response in cats, mice and cows (Minami, *et al.*, 1996).

Continuous and massive intake of chitosan with sodium ascorbate affected on the mineral and fat-soluble vitamins status on rats (Denchi *et al.*, 1995, Koide, 1998). In another experiment, excess dose 3.6-4.2g/kg body weight for 189 days caused to decrease hen's appetite and egg –laying capacity (Hirano, *et al.*, 1990). Mitu, (1997) reported that an acute toxicity test (LD_{50}) was reported in over 15g/kg orally in rats, over 10g/kg subcutaneously in mice, 5.2g/kg intraperitoneally in mice and 3g/kg in rats (Minami, *et al.*, 1996). From animal experiment it has been suggested that long term oral intake of chitosan especially in high amount may caused to deleterious effect on growth (Koide, 1998).

Legal and safety measure

Although, the US-FDA approved chitosan as a food additive in animal feed in 1983 (<0.1%), it has recently been affirmed as GRAS by the US-FDA in 2001 (McCurdy, 1992, Rodrigufz, *et al.*, 2003). It is also approved for use in medicinal and pharmaceutical applications in Europe and USA as well as for controlling obesity in human subject in Italy and already approved as a food additive in Korea and Japan (Roller, 2000, Hong Kyon No, *et al.*, 2002b).

Chitosan has been used safely over 30 years. The chemical structure of chitin and chitosan suggests a low order of toxicity (Weiner, 1992); however, its effectiveness can be increased by taking Vit-C with it. A person who has any kind of shellfish allergies or in pregnant or breast feeding situation should not

take chitosan. It would be better to see physician before taking it (Ravikumar, 2000).

Conclusion

Chitosan is obtained by N-deacetylation of chitin, which is chiefly found in shells of different crustaceans, cuticles of insects and even in cell wall of fungi. The degree of deacetylation greatly effects on physical properties of chitosan such as solubility, molecular weight and other functional properties etc. Normally, a commercial grade of chitosan has a degree of acetylation in the range of 70-90 % and average molecular weight of $1x10^5$ KDa.

Because of its cationicity chitosan possessed a great number of applications particularly in food preservations e.g. antibacterial, in making an edible biopackaging, texturizer, emulsifying, foaming, gelling, fruit coating agent, water and fat binding for several food materials, etc. Additionally, it has been proved that chitosan is an effective dietary source for reducing hypercholesteromic effect, blood sugar, control obesity, effective analgesic as well as wound healing agent. Therefore, it has become a very important bio-polysaccharide for foods and biomedical applications.

Chitosan has been considered as a very low toxic food additive though; a long term oral intake in high dose may cause deleterious effects on growth, loss of appetite and egg laying capacity of hen as well as affect on the status of mineral and fat soluble vitamins and even hemorrhagic lethal death *in vivo*. Likewise, the use of chitosan may not be suitable to pregnant, breast feeding women and person who has allergy with shellfish. In fact, further studies in real food systems and further validated clinical trials on human subjects are necessary to assure fully in the commercial applications of chitosan as a food additive and bio-medical material.

References:

Allan G.G., *et al.*, (1984).Biomedical applications of chitin and chitosan.In: *Chitin, chitosan and related enzymes*, ed., Zikakis J.P., Academic Press Inc.Orlando.p 119-134.

Arvanitoyannis, I.S., et al (1998).Chitosan and gelation based edible films state diagrams, mechanical and permeation properties, *Carbohydrate Polymers*, 37,371-382.

Brine, C.J. (1984).Introduction: Chitin accomplishments and perspectives.In: *Chitin and Chitosan and related enzymes*; ed.J.P.Zikakis, Academic Press Inc.Orlando, NewYork, p xvii.

Choi,W.Y., *et al.*, (2002).Wettablility of chitosan coating solution on 'Fuji' Apple skin., *J.of Food Science*, 67(7),2668-2672.

Coma V., *et al.*, (2002).Edible antimicrobial films based on chitosan matrix, *J. of Food Science*,67(3) 1162-1168.

Cuero, R.G. (1999).Antimicrobial action of exogenous chitosan.In: *Chitin and chitinases*, ed., Muzzarelli R.A.A., Jolles P.Birkhanser Verlag, Basal, Boston, Berlin.p 315-333.

Denchi K., et al (1995).Continuous and massive intake of chitosan affects mineral & fat soluble vitamin status on rats food in a high fat diet. *Bioscience Biotechnology Biochemistry*, 59(7), 1211-1216.

Ghaouth, A.E. *et al.*, (1991).Chitosan coating effects on storability and quality of fresh Strawberries, *J.of Food Science*, 56(6), 1618-1620.

Hirano S. & Akiyama Y. (1995).Absence of a Hypocholesterolemic action of chitosan on high serum cholesterol Rabbits.*J.of Science and Food Agriculture*, 69, 91-94.

Hirano S. *et al.*, (1990).Chitosan as an ingredient for domestic animal feeds, *J. ofAgricultural Food Chemistry*, 38, 1214-1217.

Hong Kyon No, *et al.*, (2002a).Antibacterial activities of chitosans and chitosan oligomers with different molecular weights on spoilage bacteria isolated from Tofu. *J. of Food Science*, 67(4)1511-1514.

Hong Kyon No, *et al.*, (2002b).Antimicrobial activity of chitosans and chitosan oligomers with different molecular weight, *International Journal of Food Microbiology*,74,65-72.

Ikedo I., *et al.*, (1993).Effects of chitosan Hydrolysates on lipid absorption and on serum and liver lipids concentration in rats.*J. of Agriculture and Food Chemistry*,41,431-435.

Jiang, Y., Li Y. (2001).Effects of chitosan coating on post harvest life and quality of longon fruits, *J. of Food Chemistry*, 73,139-143.

Kanauchi O. *et al.*, (1995).Mechanism for the inhibition of fat digestion by chitosan and for the synergistic effect of ascorbate. *Bioscience, Biotechnology, Biochemistry*, 59(5), 786-790

Kaye, R. (1985).Chitosan markets and quality go hand-in-hand.In: *Biotechnology of marine polysaccharides*; ed. Colwell R.R.Hemisphere Publishing Corporation. Washington, New York, London.p-334-342.

Kennedy, J.F. (1988).*Carbohydrate chemistry* .Oxford science Publications.p597-632.

Kittur, F.S. *et al.*, (2002) Characterization of chitin, chitosan and carboxymethyl derivatives by differential scanning calorimetry.*Carbohydrate Polymers*.49, 185-193.

Knorr.D, (1984).Use of Chitinous polymers in Food, *Food Technology*,38(1),85-96.

Koide, S.S. (1998).Chitin chitosan: Properties, benefits and risks. *Nutrition Research*, 18(6), 1091-1101.

Kubota N. *et al.*, (2000).a simple preparation of half –N-acetylated chitosan highly soluble in water and aqueous organic solvents .*Carbohydrate Research*.324, 268-274.

Kurita, K. (1992).Chemical modifications of chitin and chitosan .In: *advances in chitin and chitosan;* eds., Sandford, P.A., Zikakis, J.P.Elsevier Applied Science.London, NewYork.

Mau-Chang, C., *et al.*, (1996).Antimicrobial and physicochemical properties of methyl cellulose and chitosan films containing preservatives, *J. of Food Processing and Preservation*, 20,379-390.

McCurdy J.D. (1992).FDA & the use of chitin and chitosan derivatives.In: *Advances in chitin and chitosan*, eds., Brine C.J., Sandford P.A., Zikakis J.P. Elsevier Applied Science.London, New York, p 659-662.

Minami S., *et al.*, (1996). Chitosan inducing hemorrhagic pneumonia in dogs .*Carbohydrate Polymers*, 29,241-246.

Muzzarelli R.A.A. (1996).Chitosan –based dietary foods. *Carbohydrate Polymers*.29, 309-316.

Muzzarelli R.A.A., *et al.*, (1999).Biochemistry, histology and clinical uses of chitins and chitosans in wound healing.In: *Chitin and chitinases*, ed., Jolles P. and Muzzarelli R.A.A.Birkhanser Verlag, Basel, Switzerland, p-251-264.

New, N. *et al.*, (2002).Production of fungal chitosan by solid state and submerged fermentation. *Carbohydrate Polymers* 49,235-237.

Okamoto Y., Kawakami, K., Miyatake, K., Morimoto, M., Shigemasa, Y. & Minami, S. (2002).Analgesic effects of chitin and chitosan.*Carbohydrate polymers*.49, 249-252.

Onsøyen, E. *et al.*, (1990).Metal recovery using chitosan.*J. of Chimical Technology, Biotechnology*,49,395-404.

Ravikumar, R.N.V. (2000).Chitin and Chitosan for versatile Applications Homepage. Website: http://www.saccharomics.com/

Rodriguer M.S. (2003).Antimicrobial action of chitosan against spoilage organisms in precooked pizza.*J. of Food Science*, 68(1),271-274.

Rodriguez, M.S. *et al.*, (2002).Emulsification capacity of chitosan.7*Carbohydrate Polymers*, 48,271-276.

Rodrigufz M.S. *et al.*, (2003).Relationship between astringency & chitosan–saliva solutions turbidity at different pH. *J. Food Sci.* 68 (2), 665-667.

Roller, S., Covill N. (2000).The antimicrobial properties of chitosan in Mayonnaise and Mayonnaise based shrimp salads.*J. Food Protection*, 63(2), 202-209.

Roller, S., *et al.*, (2001).Novel combinations of chitosan, carnosine and sulfite for the preservation of chilled pork sausages. *Meat Science*.62, 165-177.

Romanazzi, G., *et al.*, (2002).Effects of pre-and post harvest chitosan treatments to control storage grey mold of table Grapes., *J. Food Science*, 67(5),1862-1867.

Seo H., Mitsuhashi K. & Tanibe H. (1992).Antibacterial and antifungal fiber blended by chitosan .In: *Advances in chitin and chitosan*; eds.Brine C.J., Sandford P.A., Zikakis J.P.Elsevier Applied Science, London, NewYork.p 34-40.

Shahidi, F. and Synowiecki J. (1992).Quality and compositional characteristics of New foundland shellfish processing discards.In: *Advances in chitin and chitosan*; eds.Brine C.J., Sandford P.A., Zikakis J.P.Elsevier Applied Science, London, NewYork.p617-626.

Stevens, M.P. (1999).Polymer chemistry an introduction. Oxford University Press.NewYork, Oxford.p- 491.

Sugano M., *et al.*, (1992).Hypocholesterolemic activity of partially hydrolyzed chitosans in rats. In: *Advances in chitin and chitosan*; eds.Brine C.J., Sandford P.A., Zikakis J.P.Elsevier Applied Science, London, NewYork.p 472-478.

Trautwein E.A., *et al.*, (1997).Cholesterol lowering and Gallstone preventing action of chitosan with different degrees of deacetylation in hamsters fed cholesterol rich diets. *Nutrition Research*, 17(6), 1053-1065.

Trzcinski, S. *et al.*, (2002).Comparative studies on molecular chain parameters of chitosans and polydiallyl dimethyl ammonium chloride) the stiffness ²-parameter and the temperature coefficient of intrinsic viscosity. *Carbohydrate Polymers*, 48,171-178.

Weiner M.L. (1992). An overview of the regulatory status and of the safety of chitin and chitosan as food and pharmaceutical ingredients .In: *Advances in Chitin and Chitosan*.eds. Brine C.J., Sandford P.A., Zikakis J.P., Elsevier Applied Science, London, & NewYork,p 663-672.

Winterowd J.G. and Sandford P.A., (1995).Chitin and Chitosan.In: *Food polysaccharides and their applications*, ed., Stephen A.M.,Marcel Dekker Inc.NewYork,Basel,Hong Kong,p441462.

Xie W., *et al.*, (2002).Preparation and antibacterial activities of water –soluble chitosan derivative, *Carbohydrate Polymers*, 50, 35-40.

Xue C., *et al.*, (1998). Antioxidative activity of several marine polysaccharides evaluated in a phosphatidylcholine-liposomal suspension and organic solvents, *Bioscience, Biotechnology, Biochemistry*,62(2),206-209.

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Mycotoxins in Milk and Milk Products: A Review

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Certain fungi produce chemical substances that cause toxic symptoms when food containing them is ingested by man or animals. These compounds are referred to as mycotoxins. These are ubiquitous and may contaminate dairy products by mould growing on them (e.g., Sterigmatocystin), or by the carry-over of mycotoxins occurring in animal feedstuffs ingested by dairy cattle (e.g., aflatoxin M_1). Aflatoxin M_1 often occurs in milk when aflatoxin B_1 containing feeds are fed to milch animal. Due to the fact that processing of milk does not decrease the aflatoxin M_1 content, it occurs in various dairy products. Aflatoxin M_1 suspected to be carcinogenic, B_1 is a presumed human hepatocarcinogen. The most fundamental way to tackle the problem of mycotoxin contamination of dairy products is to prevent fungal growth on the dairy products or in the case of carry-over of mycotoxins in the crop before, during and after harvest.

Keywords: Mycotoxin, Aflatoxin M_1, Dairy products, Breast milk

Introduction

Thousands of natural toxins exists that have known or potential adverse health effects in humans and animals. The diet contains at least 10,000 times more 'natural toxins' exist compared with 'synthetic toxins' (such as pesticides or environmental chemicals), natural toxins probably pose the greater threat to human and animal health (Ames, 1983). One large group of natural toxins are nearly universal contaminants to food and feed i.e., the mycotoxins, the toxic secondary metabolites produced by fungi. The word mycotoxin is derived from the Greek words 'mykes' meaning Fungus and 'toxicum' meaning poison or toxin. Thus the literal meaning is fungus poisoning.

Pre- or post harvest contamination of various food crops by mycotoxigenic fungi is a common problem; approximately 25% of the world's food supply is contaminated by mycotoxins annually (Anonymous, 1989) or likely to be high (Coulombe, 1993), although actual resultant economical loss to agriculture is difficult to determine with accuracy. The severity of mycotoxins contamination in agricultural commodities varies yearly, however excessive moisture in the field and in storage, temperature extremes, humidity, drought, variations in harvesting practices, and insect infestation are major environmental factors.

Aflatoxin (AF) is the most thoroughly studied mycotoxin. AF is the collective term used for a group of highly toxic secondary metabolic products by three common molds, *Aspergillus flavus, A. flavus* subsp. *parasiticus* (Lie & Marth, 1968); and *A. nomius* (Kurtzman et al., 1987). They easily occur on feeds and foods during growth, harvest, or storage that support mold growth (Galvano et al., 1996). Fungal growth is influenced by factors such as substrate composition, substrate moisture, relative humidity, temperature, and the presence of other competing microorganisms (Ellis et al., 1991). As AF is carcinogenic, teratogenic and mutagenic to animals and humans, contamination of feed and food is a current problem (Piva et al., 1995).

Aflatoxin M_1 (AFM_1) is considered a milk toxin because AFM_1 is a major metabolite found in milk of lactating animals and women exposed to dietary AFB_1. In addition to milk, AFM_1 can be excreted to urine. Both milk and urinary AFM_1 have been used as biomarker of aflatoxin exposure (Zhu et al., 1987). The acute toxicity of AFM_1 differs little from that of AFB_1 (Pong & Wogan, 1971), and it is both mutagenic and carcinogenic.

Structures

The aflatoxins have closely related structures and form a unique group of highly oxygenated heterocyclic compounds. All toxin forms have a coumarin nucleus used to a difurano moiety and contain either a pentanone ring or a 6 membered lactone. The two major forms of aflatoxin were named aflatoxin B and G (blue and green) after the colour of their fluorescence under long wave UV. This intense fluorescence forms the basis of most assay techniques for aflatoxin. Different forms of aflatoxin, varying in structure is shown in Fig. 1.

Mycotoxins in dairy products

The presence of mycotoxins in dairy products may be the result of:

Direct contaminations: Direct fungal growth in the diary product which may result in the formation of mycotoxins. Different types of mycotoxin producing organisms are widely

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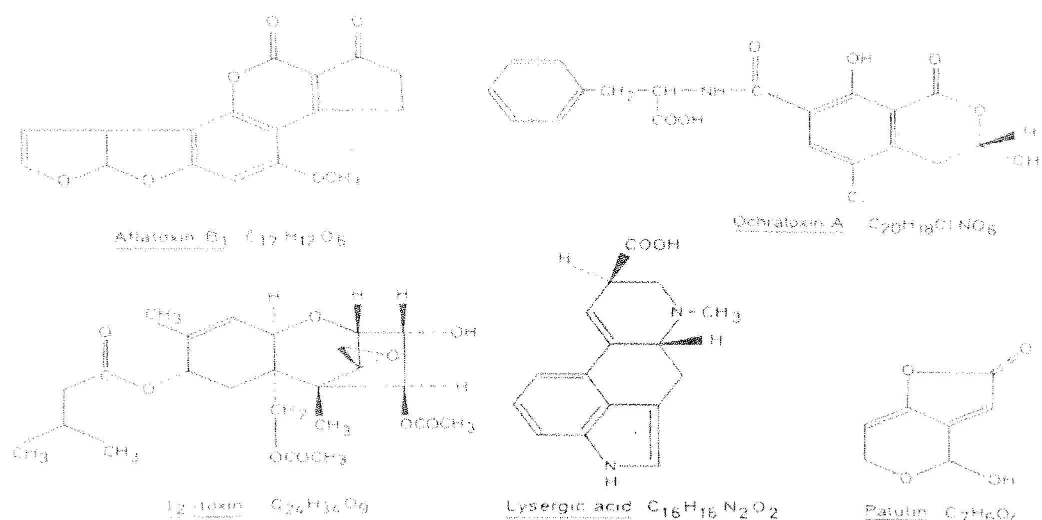


Fig. 1 Chemical structures of some mycotoxins

distributed in soil and plant materials especially in warm, damp climate.

Indirect contaminations: The contamination of the milk caused by contamination of the feed stuffs consumed by the milch animal. Allcroft & Carnaghan (1963) discovered that intake of aflatoxin B_1 contaminated feed to the cows leads to the creation of a toxic factor in the milk few hours after injection. The major sources of contamination are grains and grain products, oil cakes, fish meals, etc.

Health hazard by Aflatoxins

The acute diseases caused by AF are referred to as Aflatoxicoses. AFB_1 is the most potent of the group. The potency in decreasing order is $B_1>G_1>B_2>G_2$ (Arora et al, 1995). The intensity of toxicity of aflatoxin directly depends on dose, animal species, breed, age, and the nature of feed. Prolonged exposure to low levels of AF results in carcinogenic responses and liver tumours in a number of animals. The AF reacts with nucleoproteins and nucleic acids and ultimately effect protein synthesis and cellular integrity. DNA and RNA synthesis also will be inhibited.

Hsieh (1982) conducted a chronic study with two groups of fifty male fisher rats which were continuously fed diets containing 50 ¼g M_1/kg and 50 ¼g B_1/kg respectively, for 18 months. At the end of this period, multi-hepatocarcinomas were found in B_1 group; where as no evidence of neoplasm was found in any of the M_1-group.

Aflatoxin in blood and other parts of the human body and transportation of aflatoxin from diet of the lactating mother to infants has also been reported (Hendricks, 1984). The case of outbreak of primary liver cancer in man in Czechoslovakia is reported as 27 to 34 patients out of 38 were affected with viral hepatitis (van Ronslury et al., 1985). More than 100 people have died in India (Krishnamachari et al., 1975) in 1974 due to the outbreak of aflatoxin poisoning.

In DFTQC (2004/05), 9 samples of skimmed milk were analyzed for Aflatoxin B_1 and B_2, but none of the samples were traced positive.

Dhand et al., (2001) have found fungal contamination in 112 milk and milk products having 23 ´10^2 to 26 ´ 10^5 cfu/ml in milk sample; 28 ´ 10^2 to 15 ´ 10^6 cfu/g in khoa; 300 to 115 ´ 10^5 cfu/g in burfi; and 13 ´ 10^2 to 63 ´ 10^6 cfu/g in paneer and *Aspergillus flavus* was the most predominant fungus in 28.57% of the sample.

AflatoxinB_1

Among 150 different mycotoxins known at present, AFB_1 had generated the greatest concern and has stimulated the most research effort because of its extreme toxicity and its widespread occurrence in staple foods and feeds such as peanuts, corn, cotton seed etc. For this reason, AFB_1 currently is the only mycotoxin that is regulated by the FDA. In foods, the current "action level" (the concentration above which the commodity is condemned) is 20 ppb of total AF. The action level for the AFB_1 metabolite AFM_1 in milk is 0.5 ppb. Other regulatory guidelines for AFB_1 include 20 ppb in corn for dairy cows, 300 ppb in corn for finishing beef cattle and swine, and 100 ppb for breeding stock (Ezzell, 1988).

AFB_1 at 0.4 ppb fed over 14 months resulted in a 14% incidence of hepatocellular carcinomas (HCC) in rainbow trout, the animal species known to be most sensitive to the carcinogenic effects of this mycotoxin (Lee et al., 1968). By contrast, tumor incidence was only 5% during a similar time in Fischer rats exposed to 5 ppm of AFB_1 (Wogan et al., 1974).

Stability and Occurrence of Aflatoxin M_1 in Milk

Mammals ingest aflatoxin B_1 (AFB_1) contaminated diets, eliminate into milk with its principal metabolite known as "milk toxin" or aflatoxin M_1 (AFM_1) as shown in Fig. 2.

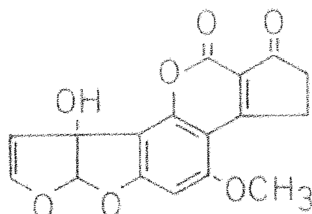


Fig. 2. Chemical structure of aflatoxin M_1.

AFB_1 is metabolized by the hepatic microsomal mixed-function oxidase system, but it also can undergo several metabolic conversions depending on species (Masri et al., 1974). AFM_1 is as toxic as AFB_1 to rats (Pong & Wogan, 1971), rainbow trout (Sinnhuber et al., 1970).
Since milk has the greatest demonstrated potential for introducing AF residues from edible animal tissues into the human diet and also the main nutrient for infants and children, occurrence of AFM_1 in commercially available milk and dairy product is a concern.

Excretion of AFM_1 into milk has been shown to account for 1 to 4% (Van Egmond, 1983); 0.01 to 4% (Patterson et al., 1980) of ingested AFB_1. Due to the potent carcinogenicity of AFM_1, most countries regulate both AFB_1 in dairy cattle feed and the AFM_1 level in milk. The presence of AFM_1 in milk is a potential threat to the health of consumers of dairy products. Growing children are more sensitive than adults and vulnerable, as milk is one of their main sources of nutrients. The commission of the European communities (1998) has set a maximum admissible level of 0.05 ¼g/kg for AFM_1 in raw milk, milk for the manufacture of milk-based products and heat-treated milk. Whereas, the action level established by the U.S. FDA (1996) is 0.5 ppb in whole, low fat, and skim milk. Both instances prohibit mixing of contaminated products with good quality products in order to reach the set admitted level. Use of products with excess aflatoxin levels as an ingredient for the manufacture of other foodstuffs is also prohibited. Furthermore, detoxification of products by chemical treatments is not allowed in the European Union.

To reduce the risk most of the developed countries have regulated the maximum permissible levels of AFB_1 in foods and feeds as well as the levels of AFM_1 in milk and milk products. However, the matter of the legal limits is questionable. Currently the limits are highly variable (Table1), depending on the degree of development and economic involvement of the countries in setting regulatory limits: according to Pohland & Yess (1992), regulatory limits seem to be a practical compromise between the need to have carcinogen- free commodities and the economic consequences of setting regulatory limits. For example, the Swiss legislation (the most restrictive in the world) forbids the use of peanuts, perhaps the most frequently AFB_1-contaminated feed, in dairy cow feeding: would it be possible in countries which are big groundnut producers?

The only two countries which consider the additive amounts of some aflatoxins in regulatory limits for milk and milk products are Swiss (10 ng of B_1 plus M_1 per kg of infant formula) and Argentina (500 ng of B_1, B_2, G_1, G_2 and M_1 combined per kg of milk) (Table 1).

Survey by the Thai Department of Medical Science (anonymous, 1994) reported that 32% of milk samples were contaminated with AFM_1. AFM_1 level in raw milk ranged from 0.004 to 0.34 ppb, in pasteurized milk from 0.008 to 0.21 ppb, and UHT milk from 0.005 to 0.67. Similarly, Saitanu (1997) has studied the milk sample in same country and found that over 90% of milk samples were contaminated with AFM_1, and 18% of them contained over 0.5 ppb. The level of AFM_1 contamination were consistent throughout the year, rather than occurring in the summer season as in the United States. This may be due to the tropical climate where temperature and humidity are optimum for mold growth and aflatoxin development in all season.

These results suggest that bulk milk samples contained relatively lower level of AFM_1 than the milk samples collected from the individual animals from the same area during the same period.

The results on AFM_1 content in the cow and buffalo milk samples indicate that on an average the cow milk contained relatively higher level of AFM_1 than the buffalo milk samples. This is observed to be true both for the samples collected from the individual animals and the bulk milk samples. Relatively lower levels of AFM_1 recorded in the buffalo milk samples in this study could be explained on the basis of the fact that the conversion process of AFB_1 to AFM_1 through the hydroxylation process appears to be different in different species.

Yadagiri and Tupule (1975), while studying the metabolism of aflatoxin in-vitro in the liver of farm animals reported that the efficiency of conversion of AFB_1 to AFM_1 by the liver slices of sheep, goat, cow and buffalo was 17.10, 16.00, 8.20 and 5.50 percent respectively, suggesting that the conversion efficiency of liver of buffalo was least than the other species studied.

Effect of Processing on AFM_1

Milk is highly variable product that rapidly loses it homogeneity and spoils if untreated. Since milk may be processed in numerous ways, the effects of storage and processing on stability and distribution of AFM_1 are of great concern.
The incidence of AFM_1 contamination is often higher in raw farm milk than in commercial milk, because of the dilution effect of processed milk in commercial level (Visconti, et al.,

Table 1. Regulatory limits for AFM_1

Country	Milk (ng/L)	Infant formula (ng/kg)	Cheese (ng/kg)
Argentina	500[a]	100	
Austria	50	10	250
Brazil	500	10	
France	200		
Germany	50	10	
Italy	50	50	
Netherlands	50	50	200
Switzerland	50	10[b]	250
US FDA	500		

[a] Sum of B_1, B_2, G_1, G_2, M_1
[b] Sum of B_1 and M_1.
(Source: Galvano et al. (1996))

1985). For the same reason high AFM_1 contamination levels in commercial milk seldom occur.

The frequent occurrence of aflatoxin M_1 in milk has raised the question: what happens if such contaminated milk is processed in the normal manner by the diary industry? There have been several investigations into this subject.

Cooling

Kiermeier & Meshaley (1977) observed that detectable AFM_1 decreases by 11 to 25% after 3 days at 5°C, 40% after 4 days at 0°C and 80% after 6 days at 0°C, whereas, Mcknney et al. (1973) revealed that freezing at -18°C for 30 days resulted in an apparent loss of 14%, with 85% lost after 53 days. This shows that time factor plays important role concerning cold treatment.

Pasteurization

As regards the effect of pasteurization, contrasting data on AFM_1 thermoresistance arise from studies revealing no reduction of AFM_1 under various conditions, or widely variable effects. AFM_1 content does not significantly reduce by heat treatments (Galvano et al., 1996), or by pasteurization or in roller drying (Allcroft & Carnaghan, 1963). Wiseman & Marth (1983) reported that AFM_1 recovery could be affected by the influence of heat treatment on milk proteins and solubility of salts.

Cream separation

AFM_1 distribution in milk is not homogeneous, cream separation can affect AFM_1 distribution since 80 % is partitioned in the skim milk portion because of AFM_1 binding to casein. According to van Egmond & Paulsch (1986) the behaviour of AFM_1 in processes which involve fat separation may be explained by its semipolar character, leading to a predominance in the nonfat fraction.

Different dairy products

Milk naturally contaminated with M_1 at level of 1.57 ¼g/kg was processed for different diary products viz. in pasteurized milk, sterilized milk, yoghurt and cheese were prepared and their the M_1 level was found as 1.58 ¼g/kg in pasteurized milk, 1.50 ¼g/kg in sterilized milk, 1.73 ¼g/kg in yoghurt, 0.93 ¼g/kg in whey and 6.54 ¼g/kg in cheese (van Egmond et al., 1977). The aflatoxin M_1 content of milk is not reduced by heat treatments like pasteurization and sterilization, nor was any reduction noticed in the M_1 content during the preparation of yoghurt and cheese. It is of interest to note that the M_1 concentration in cheese was about four times higher than in the milk from which it was prepared. Accordingly, the M_1 concentration in the whey was proportionally lower. Furthermore the various yoghurt cultures and cheese- starters did not influence the M_1 content.

Processing of milk does not destroy aflatoxin M_1 an observation which is of considerable practical importance. It gives rise to the question: What possibilities, then, exist to eliminate M_1 from milk?

Seasonal effect

Climatic factors can account for AFM_1 occurrence, especially in countries with wide regional climatic variability. According to several authors, a seasonal effect influences AFM_1 occurrence. Some authors reported a higher incidence of AFM_1 contamination during cold seasons that hot ones (Kiemeier, 1973), because in winter cows are fed with greater amounts of compound feeds, whereas during spring and summer forage, roughage, and pasture are widely available. Piva et al. (1987), in Italy observed lower AFM_1 occurrence and contamination level in southern Italy than in central and northern Italy. Galvano et al. (1998) found that a clear seasonal effect on the level of contamination. Milk samples collected in the period November to April (16.84 ± 2.59) showed a contamination level ca. four times as high as samples collected in the period May to October (4.19 ± 0.52). Furthermore, they have found all the samples collected in the hottest month (August) were negative for AFM_1.

Vittani (1987), detected AFM_1 in the spring and summer period lower by a factor of 1.5 to 3.7 than those in the winter period. Heeschen et al. (1990) indicated late summer as the period of lowest AFM_1, contamination.

Feed effect

Ali & Sayed (1993) studied buffalo feed and found that 86% source of aflatoxin contamination was by cotton seed after oil extraction having 260 to 360 ppb of aflatoxin, whereas the bread and gram skins have only 50 and 40 to 65 ppb respectively and dry grass and wheat bran were found to be free from aflatoxins.

Seasonal effects on occurrence of AFM_1 in milk are likely to be related to feed type, since in winter cows are fed on greater amounts of compound feeds, whereas during spring and

summer forage, roughage, and pasture are widely available. However, since climatic conditions, agricultural systems, and feeding conditions are closely interrelated, it is not easy to distinguish their roles in influencing AFM_1 incidence and contamination levels. Observations over long periods and surveys on the occurrence of AFB_1 in feeds should provide additional information.

Stability and occurrence of AFM_1 in Yoghurt

Several authors reported no influence in yogurt manufacture on AFM_1 content (van Egmond & Paulsch, 1986; Blanco et al., 1993). Van Egmond et al. (1977) observed no reduction of AFM_1 in yoghurt stored for 7 days at 7°C. El Deeb et al. (1992) observed that enzymic, microbial, and particularly acid coagulation caused degradation of AFM_1 in buffalo milk.

AF can cause some negative effects on its nutritive properties. El Deeb et al. (1992) observed some negative effects of AFM_1 on *Lactobacillus bulgaricus* (cell wall thickening and shortening of cell chain length) and *Streptococcus thermophilus* (cell wall thickening and cell shape changing from coccoid to oval).

Elimination of aflatoxin M_1

Prevention of Aspergillus infection in foods and feeds is the most desirable method of reducing AFB_1, although AFB_1 contamination often is unavoidable, even with the best agricultural practices. So, attention should be directed to the prevention of aflatoxin formation in agricultural commodities, which are fundamentally a problem of good agricultural practice and the curtailment of fungal growth in storage.
Some methods involve early identification and segregation of grossly contaminated kernels of corn or peanuts use of electronic devices to identify and to reject grains that exhibit fluorescence that is due to AFB_1. Although these methods have been useful for peanuts, they have not been practical for decontaminating corn and cottonseed (Park et al., 1986).

Chemical treatment

Applebaum et al. (1982a, 1982b) have investigated several possibilities to eliminate or inactivate AFM_1 in milk. One method involves chemical treatment with potassium sulphite; another used hydrogen peroxide in combination with riboflavin and a third method used bentonite to adsorb the toxin. Although M_1 seems to be reduced by 45-98%, these methods are not really applicable for the dairy industry, at least not at this moment, since as yet nothing is known about the biological safety and the nutritional and functional properties of the treated products; moreover, the costs of the processes may be considerable.

AFM_1 in human breast milk

Humans ingest AF-contaminated foods eliminate variable amounts of the toxins in body fluids or accumulate them in the tissues. The occurrence of AF in human tissues or fluids, is a current problem in either tropical or subtropical countries much more than in cold ones, although Dragsted et al.(1988) found some aflatoxin like substances in urine samples of Danish people, which were related to consumption of beer, dairy products, and meat.

Wild et al. (1987) detected the occurrence of AFM_1 in human milk samples collected in Sudan, Ghana, and Zimbabwe, whereas no AFM_1 was detected in human milk samples from France. An indirect assessment of widespread exposure to AF- contaminated food produced and consumed in tropical and subtropical countries was provided by Wilkinson et al. (1993). They found a much higher level of AF metabolites in sera of Nigerian and Nepalese people compared to sera of people in the UK.

Indications of serious prenatal health hazards to infants due to the exposure to AF have been reported. Wilkinson et al. (1993) have reported that AF can cross the human placental membrane and may be concentrated by the developing fetoplacental unit.

Saad et al. (1995) have concluded that, although the interaction between dietary AF intake and exposure of the mother, fetus, and newborn infants is very complex, depending on the physiological status of the mother and on food composition, the presence of AFB_1 and its metabolites in human blood and breast milk presents serious health hazards. Although it may be difficult, it would be desirable, at least during pregnancy and the lactation period, for mothers to ingest low-content AF or AF-free foods to avoid the undesirable transfer of AF metabolites to the fetus or infant.

Conclusion

Currently the regulatory limits are widely variable and there has been little scientific basis in their setting. Efforts should be made in attempting to provide further and extensive scientific information on human health hazards related to low-level long term AF exposure and to standardize the already existing regulatory limits for AF.

Since it is generally assumed that neither storage nor processing determine reduction of AFM_1 content, further information on possible AFM_1 concentration following milk processing should be furnished. The occurrence of AF and their metabolites in human breast milk is of great concern especially in tropical and subtropical countries.

The high incidence and concentration of fungi in general and toxigenic. *A. flavus* in particular is of great significance for diary industry and public health authorities. So, strong quality control measures should be taken at all levels.

References

Ali R. & Sayeed, S.A. (1993). Effect of aflatoxin-contaminated feeds on buffalo milk. Indian Food Indistry,12 (2): 38-40

Allcroft, R. & Carnaghan, R.B.A., (1963). Groundnut toxicity: An examination for toxin in human food products from animals fed toxic groundnut meal. Vet. Rec. 75:259-263.

Ames B.N. (1983). Dietary carcinogens and anticarcinogens. Science (Washington) 221: 1256

Anonymous (1989). Mycotoxins : Economic and health risks. Task force report no 116. Council for agricultural science and technology, page 21 and 43, Ames, IA

Anonymous (1994). The level of aflatoxin Mun $_1$ in milk from the lower part of the northeastern part of Thailand, p 110. In: Annual report 1993. Thailand ministry of public health, Department of medical science

Applebaum R.S. & Marth E.H. (1982a). Inactivation of aflatoxin M_1 in milk using hydrogen peroxide and hydrogen peroxide plus riboflavin or lactoperoxidase. *J. Food Prot*, 45:557-560

Applebaum R.S. & Marth E.H. (1982b). Use of sulphite or bentonite to eliminate aflatoxin M_1 from naturally contaminated raw whole milk. Z. Lebensm. Unters. Fosch., 174:303-305

Arora S., Bajaj, V.K. & Raim T. (1995). Aflatoxins in dairy products. I. dairyman. 47(6):13-18

Blanco J.L., Carrion B.A., Liria N., Diaz S., Garcia M.E., Domingues L. & Suarez G. (1993). Behavior of aflatoxin during manufacture and storage of yogurt. Milchwissenschaft 48:385-387

Colombe R.A. (1993). Biological action of mycotoxins. *J. Dairy Sci.* 76:880-891

Coulombe R.A. (1991). Mycotoxins and phytoalexins - p 113 Sharma R.P. & Salunkhe D.K. (eds), CRC press, Boca Raton, Florida

DFTQC (2004/05). Annual bulletin, Department of Food Technology and Quality Control, Ministry of Agriculture and Cooperatives. Nepal Government.

Dhand N.K., Joshi D.V. & Jand S.K. (2001). Fungal contaminants of milk and milk products and their toxigenicity. *Indian Vet.* J. 78:956-957

Dragsted L.O., Bull I. & Autrup H. (1988). Substances with affinity to a monoclonal aflatoxin B_1 antibody in Danish urine samples. Food Chem. Toxicol. 26:233-242

El Deeb S.A., Zaki N., Shoukry Y.M.R. & Kheadr E.E. (1992). Effect of some technological processes on stability and distribution of aflatoxin M_1 in milk, Egypt. *J. Food Sci* (Suppl.) 20:29-42.

Ellis W.O., Smith J.P. & Simpson B.K. (1991). Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection, and method of control. *Crit. Rev. Food Sci. Nutr* 30:403-439

Ezzell C. (1988). Aflatoxin contamination of US corn. Nature (London) 335:757

Galvano F., Galofaro V. & Galvano G. (1996). Occurrence and stability of Aflatoxin M1 in milk and milk products: A worldwide review. *J. of Food Protection*, 59 (10):1079-1090

Galvano F., Galofaro V., Angelis A.D., Galvano M., Bognanno M. & Galvano G. (1998). Study of the occurrence of Aflatoxin M_1 in diary products marketed in Italy. *J. of Food Protection*, 61 (6):738-741.

Heeschen W.H., Bluthgen A.H. & Hahn G. (1990). Aflatoxin M_1 in pasteurized market milk, p131 Proceedings of the 23rd International Dairy Congress, Montreal, Canada

Hendricks R.G. (1984). Transactions of the Royal Society of Tropical Medicine and Hygiene 78, 427. In: Ali R. & Sayeed S.A. (1993). Effect of Aflatoxin contaminated feeds on Buffalo milk. Indian Food Industry, 12 (2):38-40

Hsieh D.P.H. (1982). The mutagenicity and carcinogenicity of mycotoxins. Proceedings V. International Symposium on Mycotoxins and Phycotoxins. Vienna, Austria, September, 228-231

Kiermeier F. & Meshaley R. (1977). Einfluss der molkereitech nischen behandiung der rohmilich auf des aflatoxin M1. Gehalt derdaraus hergestellten produkte, Z, Lebensm. Unters, Forsch.164:183-187. In: Galvano et al. (1996) *J. of Food Protection*, 59(10):1079-1090

Kiermeier F. (1973). Aflatoxin residues in fluid milk, Pure Appl. Microbiol. 35:271-273

Krishnamachari K.A., Bhat V.R., Nagarajan R.V. & Tilak T.B.G. (1975). Hepatitis due to aflatoxin. Lancet I, 1061-1065

Kurtzman, C.P., Horn B.W., & Hesseltine C.W. (1987). *Aspergillus nomius*, a new aflatoxin producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. Antonie Leeuwenhoek J. Microbiol. 53:147-158.

Lee D.J., Wales J.H., Ayresand J.L. & Sinnhuber R.O. (1968). Synergism between cyclopropenoid fatty acids and chemical carcinogens in rainbow trout (*Salmo gairdneri*). Cancer Res. 28:2312

Lie, J.L., & Marth, E.H., (1968). Aflatoxin formation by *Aspergillus flavus* and *Aspergillus parasiticus* in a casein substrate at different pH values. J. Dairy Sci. 51: 1743-1743.

Masri M.S., Booth A.N. & Hsieh D.P.H. (1974). Comparative metabolic conversion of aflatoxin B_1 in aflatoxin M_1 & Q_1. Life Sci. 15:203-209

McKinney J.D., Cavanaugh G.C., Bell J.T., Bell A.S., Hoversland A.S., Nelson D.M., Pearson J. & Selkirk R.J. (1973). Effects of ammoniation on aflatoxins in rations fed lactating cows. *J. Amer. Oil Chem. Soc.*50:79-84

Park D.L., Lee L.S., Price R.L. & Pohland A.E. (1986). Review of the decontamination of aflatoxins by ammoniation: current status and regulation. *J. Offic. Anal. Chem*. 71:685

Patterson D.S.P., Glancy E.M. & Robert B.A. (1980). The carry over of aflatoxin M_1 into the milk of cows fed rations containing a low concentration of aflatoxin B_1. Food Cosmet. Toxicol. 18:35-37

Piva G., Galvano F. Pietri A. & Piva A. (1995). Detoxification methods of aflatoxin A review. Nutr. Res. 15: 689-715

Piva G., Pietri A., Galazzi L., & Curto O. (1987). Aflatoxin M_1 occurrence in dairy products marketed in Italy. Food Addit. Contam. 5: 133-139

Pohland A.E. & Yess N.J. (1992). Food contaminants: Scientific and public health implications. Proc. Nutr. Soc. Aust. 17: 1-12

Pong R.S. & Wogan G.N. (1971). Toxicity and biochemical and line structural effects of synthetic aflatoxin M_1 and B_1 in rat liver. *J. Nat. Cancer Inst.* 47: 585-590

Saad A.M., Abdelgadir A.M. & Moss M.O. (1995). Exposure of infants to aflatoxin M_1 from mothers' breast milk in Abu Dhabi, UAE. Food Addit. Contam. 12:255-261

Saitanu K. (1997). Incidence of aflatoxin M_1 in Thai milk products. *J. of Food Protection*, 60 (8): 1010-1012

Sinnhuber R.O., Lee D.J., Wales J.H., Landers M.K. & Keyl A.C. (1970). Aflatoxin M_1 a potent carcinogen for rainbow trout. Fed. Proc. 29:568

The commission of the European Communities (1998). Commission regulation (EC) No 1525/98 of 16 July 1998 amending Regulation (EC) No 194/97 of 31 January 1997 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Communities 201:43-46.

U.S. Food and Drug Administration (1996). Section 527.400 whole milk, low fat milk, skim milk-aflatoxin M_1 (CPG 7106.10). In: FDA compliance policy guides August 1996 ed. U.S. Food and Drug Administration, Washington, D. C. p.219

Van Egmond H.P. & Paulsch W.E. (1986). Mycotoxins in milk and milk product, *Netherlands Milk Dairy J.*, 40: 175-188

Van Egmond H.P. (1983). Mycotoxins in dairy products, Food Chemistry, Applied science publishers ltd. England 11:289-307

Van Egmond H.P., Paulsch W.E., Veringa H.A. & Schuller P.L. (1977). The effect of processing on the aflatoxin M_1 content of milk and milk products. Arch. Inst. Pasteur Tunis 54:381-390

Van Ronslury S.J., Van Der watt J.J., Vincent T.J. & Purchases I.F. (1985). British J. of cancer 51:713. In: Ali R. & Sayeed, S.A., (1993). Effect of Aflatoxin contaminated feeds on Buffalo milk. Indian Food Industry, 12 (2):38-40

Visconti A., Bottalico A., & Solfrizzo M. (1985). Aflatoxin M_1 in milk in southern Italy. Mycotoxic Res, 1:71-75

Vittani M. (1987). Sulla presenza diaflatossina M_1 in latti pastorizzatie in latti dietetici in polvere, Latte 12:1103-1107.

Wild C.P., Pionneau F.A., Montesano R., Mutiro C.F. & Chetsanga C.J. (1987). Aflatoxin detected in human breast milk by immunoassay. *Int. J. Cancer* 40:328-333

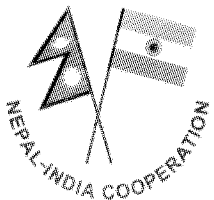
Wilkinson A.P., Denning D.W., Lee H. A., Ward C.M. & Morgan M.R.A. (1993). Analysis of human sera for aflatoxin. In: Food and cancer prevention: chemical and biological aspects. Waldron K.W., Johnson I.T. & Fenwick G.R.J. (ed.), Royal Society of Chemistry, Cambridge, pp97-105

Wiseman D.W. & Marth E.H. (1983). Heat and acid stability of aflatoxin M_1 in naturally and artificially contaminated milk. Milchwis senschaft 38:464-466

Wogan G.N, Paglialunga S. & Newberne P.M. (1974). Carcinogenic effects of low dietary levels of aflatoxin B_1 in rats. Food Cosmet. Toxicol. 12:681

Yadagiri B. & Tulpule P. G. (1975). Metabolism of aflatoxin *in vitro* in the liver of farm animals. *Indian J. Dairy Sci.* 28:108-109

Zhu J.Q., Zhang L.S., Hu X., Xiao Y., Chen J.S., Xu Y.C., Fremy J. & Chu E.S. (1987). Correlation of dietary aflatoxin B_1 levels with excretion of aflatoxin M_1 in human urine. Cancer Res. 47:1848-1852


NEPAL-INDIA COOPERATION
नेपाल-भारत सहयोग
नेपाल सरकार-भारत सरकारको आयोडिनको
कमीबाट हुने विकृति निवारण योजना

आयोडिनयुक्त नुन

आयो नुन
नेपाल भारत सहयोग

आयो नुन
औषधिको गुन
दुई बालबालिका चिन्ह अंकित
आयोडिनयुक्त पाकेटका नुन खानाले :
बालबालिकाको शारीरिक तथा मानसिक विकासको साथै पढाई लेखाई तथा खेलकुदमा समेत तेज हुन्छन् ।
I.Q.Level मा वृद्धि हुन्छ ।
बच्चाहरु बहिरा, लाटा-लठेउरा, बामपुड्के, डेढो, आदि हुदैनन् ।
आयोडिनयुक्त नून घाम र पानीले असर नपार्ने सुरक्षा ठाउँमा, बिर्को बन्द भएको बट्टामा राख्नु पर्दछ ।
आयोडिनको कमीबाट हुने विकृति हटाउन दुई बालबालिका चिन्ह अंकित प्याकेटको आयो, शक्ति, तेज र भानु ब्राण्डका नून मात्र प्रयोग गरौ ।
आयोडिन जीवनभर एक चम्चा भए पुग्छ तर एकै पटक नभै प्रत्येक दिन थोरै थोरै खानु पर्छ ।
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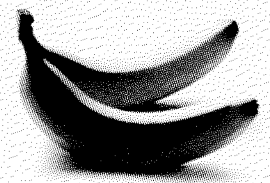

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चपाउँदा नरम अनि fresh fruit को feeling
sujalgum हो यो साह्रै refreshing
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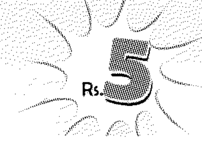

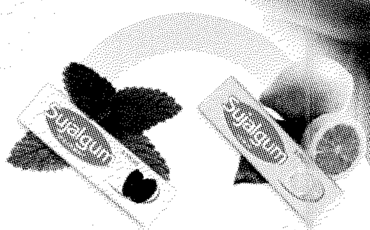

Mint र Lemon मा
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Nisin - A Major Bacteriocin of Lactic Acid Bacteria

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Nisin is one of the major bacteriocin among the bacteriocins produced by lactic acid bacteria. Nisin producing organism has been found to be Lactococcus lactis subsp lactic, formerly known as streptococcus lactis. Nisin shows its inhibition spectrum especially to all gram-positive bacterial strains that are responsible for food spoilage and food borne diseases showing its effective action to elongate the shelf life of processed foods as well as to prevent some of the food borne illness. Food and beverages such as cheese and other dairy products, meat products, canned vegetable products and alcoholic beverages are known as the major applicable food products. Nisin is being effectively applied in various food products as a natural food preservative in more than 40 countries.

Keywords: Nisin; Natural food preservative; Dairy products; Meat products; Canned products; Alcoholic beverages.

Introduction

Nisin is one of the major bacteriocin among the bacteriocins produced by lactic acid bacteria. It is being known historically with antimicrobial effects to the food spoilage microorganisms and food borne pathogens showing its beneficial role in food preservation and food safety (Vuyst and Vandamme-1994 a, b, Ouwehand-1998). This article describes the bacteriocin Nisin and its preservatives action on various food products.

Nisin, which has been known for about 5 decades, is a lanthionine containing bacteriocin produced by Lactococcus lactis subsp lactic strain formerly streptococcus lactis. Recently Lactococcus lactis subsp lactis ATCC 11454 is reported as a producer organism for Nisin (Vuyst-1994, Hurst-1983, Frank and Month-1983, Mishra and Lambert-1996.

Nisin is a heat stable pentacyclic lantibiotic displaying a broad spectrum of activity towards almost all gram positive bacteria. Five Nisin compounds A, B, C, D and E were described that differ in amino acid composition and biological activity. Nisin A is most active and appears to be the commercial compounds. Nisin was isolated, characterized and named by Mattick and Hirsch in 1947 (Mishra and Lambert-1996, Daridson and Hoover-1993)

Nisin innovation for food application

Rogers and Whitter in 1928 in England were the first investigators to show that certain lactococcal strains can inhibit the growth of other lactic acid bacteria (Fowler and Gasson-1991) Whitehead and Riddet in 1933 found that storage of milk caused a marked delay in and development by starter cultures used during Cheddar Cheese. (Chandan-1982) Hunter and Whitehead in 1994 in Newzeland found that the slow acid development during Cheese manufacture was actually due to the starter cultures themselves. In 1943 Shattock and Mattick identified the strains as *Lactic streptococci* of serological group N. Mattick and Hirsch in 1994 concentrated the inhibitory compound isolated by Meanwell in 1943 to test its antagonist activity. They found that it was inhibitory towards several pathogenic bacteria and this called it an antibiotic (Frank and Mouth-1983, Tamine and Robinson-1999). They coined the name "Nisin" derived from the phrase group N inhibitory substances i.e. an antibiotic produced by streptococci of lance fields serological group N or Lactic streptococci, now called Lactococci (Vuyst and Vandame-1994, Huyst-1983, Frank and Mouth-1983)

In 1951, Hirsch and Grinstead conducted detailed taxonomic tests of 21 inhibitory cultures, all of which were found to be Lactococcus lactis. Mattick and Hirsch in 1947 helped to characterize the nature and properties of the inhibitory substances from Lactococcus lactis and led to the name Nisin. Nisin was recognized as being a polypeptide or small protein and the structure proposed by Gross and Morell in 1967 for a polypeptide containing 34 amino acids residues with a molecular weight of 3510 (Davidson and Hoover-1993, Fowler and Gasson-1991)

Nisin is of particular interest because it was the first antibiotic compound to be used on a commercial scale in food industry. The first commercial Nisin preparation was marketed in England in 1953. In 1983, Nisin was added to the positive list of food additives. Now Nisin use is authorized in more than 40 countries. (Davidson and Hoover-1993, Fowler and Gasson-1991, Deschel-1993)

Characteristic property of Nisin

Physicochemical property

Nisin possesses an amphiphilic character with a cluster of bulky hydrophobic residues at the N-terminus and hydrophilic ones at the C-terminus. The distribution of polar and apolar residues over the molecular surface of Nisin may be of

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relevance with respect to its mode of action and biological activity. It doesn't contain aromatic amino acid so it shows no absorption at 260-280 nm. (Davidson and Hoover-1993, Vyust and Vandame-1994b)The solubility, stability and biological activity of Nisin are highly pH dependent. They drop sharply and continually as the pH is increased. *Solubility* of Nisin ranged from 57 mg/ml at pH 2.0 to about 1.5 mg/ml at pH 6.0, it dropped further to 0.25 mg/ml at pH 8.5 and then it leveled off (Vyust and Vandame-1994b). The Nisin molecule is acidic in nature and exhibits greater *stability* under acid conditions as shown in Table 1. Refrigerated storage of

Table 1: Effect of pH on the retention of Nisin (as Nisaplin) activity in buffer heated at 121° C/15 min

PH	*% Retention of Nisin activity.*
3.0	100
4.0	71
5.0	35
6.0	14.5
7.0	0.5

(Source: Broughton-1990)

Nisin for months gives no detectable chemical or biolog cal changes. (Brougton-1990, Davidson and Hoover-1993)
The reversible conformational and charge effects that Nisin undergoes at low pH may reflect its higher *biological activity.* Irreversible inactivation of Nisin above pH 7.0 even at room temperature is as a result of a combination of denaturation and intramolecular chemical modifications. (Fowler and Gasson-1991, Ching and Hancock-2000)

Biochemical properties

Nisin is inactivated by α-chymotrypsin, pancreatin and subtilopeptide but not by carboxypeptidase A, elastase erepsin, pepsin and trypsin. Nisin is stable at treatment of 115.6 ° C at pH 2.0. At the same temperature, 40% and 90% loss of activity occurred at pH 5 and 6.8 respectively. Less inactivation occurred in complex media (milk, microbiological broth) due to its protective effect. Heat resistance of 121° C for 15 min has been reported for Nisin. Nisin remains stable for years in the dry form but in foods, its activity is generally lost (Fox-1993, Hurst-1983)

Mode of action of Nisin

The unusual amino acids might be responsible for the important functional properties of Nisin molecule i.e. acid tolerance, thermo-stability and a specific bactericidal mode of action (very reactive double bond). Nisin is a cationic polypeptide, which acts as a surface-active cationic detergent. The first step in nisin inactivation of a microorganism is adsorption. Nisin is adsorbed to sensitive vegetative cells while resistant cells did not adsorb the compound. The primary site of action of Nisin is the cell membrane resulting in disruption of the cytoplasmic membrane and resultant release of cytoplasmic material, energy depletion, proton motive force dissipation and ultimately death. The rings in nisin are essential for the antimicrobial function of the molecule i.e. to kill bacteria via the formation of pores in cell membranes. (Lindstrom et al.-2001, Brenkink et al.-1998, Wiedemann et al.-2001)

Cells, which had been made resistant, did not absorb Nisin. The adsorption by sensitive cells is highly pH dependent peaking at pH 6.5; 69% at pH 5.5 and only 43% at pH 4.5. Lipid II has found for greatly enhancing the pore forming abilities of Nisin. It was discovered that Nisin could bind and form pores in bacterial membranes via a specific interaction with the lipid II molecule. In membranes of eubacteria, lipid II provides the building blocks for the cell wall synthesis. The activity of Nisin in pore formation is given in Fig 1 (Wiedemann et al.-2001)

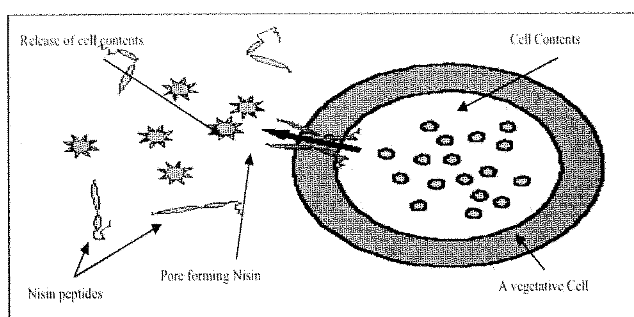

Fig 1: Mode of action of Nisin on Pore formation and subsequent release of cell contents.
(Source : Brenkink et al-1998)

The leakage assay demonstrates that addition of only little lipid II makes Nisin 1000 times more specific for pore formation. The sensitivity of vegetative cells to Nisin is given in Table 2. At higher concentration (1500IU/ml instead of 150 IU/ml), Nisin can inhibit peptidoglycan synthesis (Hurst-1983)
Nisin is believed to inhibit the germination process at the stage of pre-emergent swelling of spore. Heat injured spores have increased sensitivity to Nisin. In spores, the cytoplasmic membrane is apparently destroyed immediately after germination

Measurement of Nisin activity:

The lowest amount of Nisin required to inhibit the test organism Streptococcus agalactiae was originally referred to as the reading unit (RU) because the original work on Nisin was done at the University of Reading in England.(Vuyst and Vandamme-1994b)

The international unit (IU) of Nisin activity has been defined as the activity contained in 1 microgram of this international reference preparation, which is identical to the sole commercial Nisin preparation-Nisaplin. Nisaplin is produces with the activity standardized at the same level of 1×10^6 IU/gm whereas 1 gm pure Nisin contains 40×10^6 IU/gm. A biological activity of 40 IU hence corresponds to 1 microgram of pure Nisin (Davidson and Hoover-1993).

Nisin to control food borne pathogens

Sensitivity to Listeria monocytogenes

Table 2: Sensitivity of vegetative cells to Nisin

Organism	MIC (IU/ml)
Bacillus	2-4
Clostridium	0.25-80
Corynebacterium	4-120
Mycobacterium tuberculosis	100-500
Nisseria	2-50
Staphylococcus aureus	100

Note: MIC-minimum inhibitory concentration.
(Source: Hurst-1983)

Listeria monocytogenes is a gram positive, non-spore forming short rod that is motile with petitrichum flagella. It has become the model target bacterium in many studies of food preservatives. In particular the ability of Listeria monocytogenes to grow at refrigerated temperature, its propensity for chilled meats and dairy foods, and its gram positive nature are all the factors that have spurred the many investigations addressing its sensitive to Nisin (Daeschel-1993, Fox-1993).

An antagonistic assay and well diffusion assay shows that lactococcus lactis ssp lactics 11454 and L. lactis ssp lactis SIK83 (nisin producer) against eight strains of L. monocytogenes. Both inhibited all eight strains. The combined antimicrobial effect of nisin and a listeriophage against Listeria monocytogenes in broth (not in buffer or on raw beef) was reported (Davidson and Hoover-1993, Fowler and Gasson-1991).

The sensitivity of nine strains of Listeria to Nisin as well as the minimum inhibitory concentration of Nisin to completely inhibit the growth of these strains was determined. All strains tested were variably sensitive to Nisin and different MIC values were obtained ranging from 740 to 1.18×10^5 IU/ml in trypticase soy agar (TSA) and from 1.85 to 3.36×10^3 IU/ml in MRS agar. This is given in Table 3 (Benkersoum and Sandine-1988).

The increased awareness of food borne listeria has stimulated study of the antilisterial effect of nisin. It has demonstrated that Nisin is active against Listeria monocytogenes and studied in cottage cheese showed no survivors of a 0.35x106 cells / gm inoculum after 24 hrs at 37 and 4°C when Nisin added at a concentration of 2.55 mg/gm.(Amoako-1999)

Table 3: MIC of Nisin on Listeria monocytogenes ATCC 7644

Strain	MIC (IU/ml)	
	TSA	MRS
L. monocytogenes 7644 K	1.48×10^4	37
L. monocytogenes 35152	1.48×10^4	3.7
L. monocytogenes V7 type 1a	1.18×10^5	3.36×10^3
L. monocytogenes Scott A type 4b	1.18×10^4	1.65×10^3

Note: MIC determined on TSA (pH 7.3) and MRS agar (at pH 6.8)
(Source: Benkerkoun and Sardii-1988)

The synergistic effect of lactoperoxidase and Nisin on the behaviour of Listeria monocytogenes ATCC 15513 in Skim milk was reported. The best combination were found to add Nisin and to (initial time) followed by the lactoperoxidase addition after 4 hr, when maximum inhibitory effect of Nisin was reached (Boussonl et al.-2000).

Sensitivity to Clostridium botulinum:

Nisin has acticlostridial activity. Hurse in 1972 cited Clostridium botulinum as one of themore nisin resistance species among the clostridia. Scott and Taylor in 1981 studied the nisin sensitivity of Clostrisium botulinum according to inoculum level. Nisin level, pH and strains. They found resistance to nisin to be a greatest in type A strain followed by type B and then type E. Type 56A was most resistance to the two type A strain tested.(Daeschel-1993)

Spores of Clostridium bifermentum, clostridium sporogenes and clostridium butyricum were more sensitive than vegetative cells. (Daeschel-1993) Nisin level required to inhibit C. botulinum spore outgrowth in brain heart infusion broth inoculated with 10^3 spores per ml is given in Table 4.
Maximum concentration required to inhibit the organism in brain heart infusion were 200, 80, 20 microgram/ml (8000, 3200 and 800 IU/ml) for types A, B and E respectively. The concentration required to inhibit the Clostridium botulinum in cooked meat medium (CMM) was beyond the highest concentration tested for type A (> 200 microgram/ml) and B (> 80 microgram/ml), the higher levels required in CMM was due to binding of Nisin by meat particles. C. sporogeses was inhibited in a meat system (pork) by 5-75 microgram /ml (200-300 IU/ml) Nisin at pH 6.5-6.6 (Davidson and Hoover-1993)

Nisin Application in Food

The stimulus for investigating Nisin use for food preservation was presented in the period of the late 1940's and early 1950's when clostridial spoilage of low acid natural cheeses became a serious problem. In 1951, Hirsch et.al. successfully used a Nisin producing culture to control this effect in Gruyere cheese. About this time, Aplin and Barrett Ltd experienced up to 30% of clostridial spoilage in processed cheese products made at their Westbury factory in southwest England. The problem was dramatically reduced and then eliminated with the space of 3 months by the inclusion of Nisin-containing milk cultures in the product mix. With this success and call for remedial action from cheese processors in several countries, the company developed a dry milk based product with a Nisin content of 0.2 mg/g. By further research and development, the company had by 1959, substantially improved the potency of Nisin to 25 mg/g to form the basis of the commercial Nisin concentrate, which is used worldwide for food presentation up to the present time. First the LAB is indispensable in the production of certain foods and beverages where their fermentation properties endow a myriad of desirable and unique sensory attributes to foods. Second the

preservation properties of LAB when used as fermentation against in food were historically and still an important means

Table 4: Sensitivity (MIC) of Nisin to Clostridium botulinum spore

Strain	Nisin Level	
	IU/ml	Ppm
Type 56 A	5000	125
Type 69A	5000	125
Type 113 B	1500	37.5
Type 213 B	1000	25
Type EB	500	12.5
Type E	100	2.5

(Source: Hurst-1983)

of food preservations. Two relatively recent factors accelerate interest in LAB bacteriocin are the increasing incidence and detections of food borne diseases and emerging consumer resistant to highly processed foods (Broughton-1990a, Broughton-1990b, Fowler and Gasson-1991).

Cheese products

The first uses of Nisin producing cultures were reported for the control of Clostridial spoilage of matured cheeses. At this time, some disadvantages were also associated such as it interferes with the development of bacteria responsible for cheese ripening, production of bland cheese, eye formation in cheese and other abnormalities and slow ripening. To overcome these disadvantages, Lipinska in 1977 followed the approach of training the known important bacteria for cheese making such as Lactic streptococci, Lactobacilli and propionic bacteria to grow in presence of nisin. Successful experiments with these trained strains in combination with nisin producing culture were conducted in Poland and the USSR. When the principle was applied to Gouda manufacturer, 90% of the cheese produced was of high quality. (March and Steel-1998, Fowler and Gasson-1991)

The environment of processed cheese can be most favorable for the growth of Clostridia. Clostridial spore number may be as high as 1000 per gram in good quality matured cheese. [10] In pasteurized process cheese (in which 250 IU/g added Nisin, pH 5.6-6.0 and moisture 54-58%) at 20, 25 and 30 ° C; the retention of Nisin was about 90%, 60% and 40% respectively after 30 weeks (Davidson and Hoover-1993)

Fowler in 1971 found the minimum Nisin concentration considered to be effective against putrefactive clostridia was 0.25 –0.625 mg/kg and for butyric clostridia was 0.175 mg/kg in cheese (spore number < 10/gm). As a general rule in cheese making the Nisin level needs to be doubled for each 10 fold increased in spore numbers. Somers and Taylor in 1987 showed that Nisin at 500-10,000 IU/gm was effective in delaying or preventing growth and toxin production by Clostridium botulinum type A and B in pasteurized process spread. Higher levels of Nisin were required in cheese with high moisture and low levels of salt and phosphates. Taking all these factors into consideration, the effective range on Nisin additive level in cheese processing is 3.75 – 12.5 mg/kg. (Fowler and Gasson-1991)

Other milk products

In warm climate (where there may be inadequate refrigeration and refrigerated transport), there is an interest in extending the shelf life of pasteurized whole milk. [10]. It has been seen that a relatively low concentration of Nisin (eg. 0.5-10 mg/lit) can extend the shelf life by more than 6 days at 15 ° C or by 2 days at 20 ° C. Reduction of both processing temperature and time also in the presence of Nisin produced a spoilage free product with improved quality and vitamin retention. (Daeschel-1993)

The experiment about the effect of Nisin on keeping quality of reduced heat-treated milk was carried out. The effectiveness on the combination of reduced heat-treated Nisin and low storage temperature against gram-positive spore forming bacteria suggesting the potential use of Nisin in extended shelf life products. A study on the effect of Nisin and high temperature pasteurization on the shelf life of whole milk has been done. Addition of Nisin to milk prior to pasteurization provides an opportunity to achieve extended shelf life in region with poor refrigeration.

Canned vegetable products

Nisin has been recommended for use in canned vegetable products to prevent the outgrowth of Clostridium botulinum when less severe sterilization condition are designed or required. Regulation in UK require that Nisin treated low acid canned foods receive a minimum F_0 (121°C) of 3 min to destroy Clostridium botulinum spore (Davidson and Hoover-1993)

The ability of Nisin to control spoilage of canned tomato juice at pH 4.5 was studied by porretta et.al in 1968. The tomato juice was infected with 100,000 clostridium pasteurianum spores per ml prior to filling into 0.5 kg cans and subsequent heat treatmentat 100°C for 40 minute. When stored at 30°C for 6 months, 99% control cans exhibited clostridial spoilage where the spoilage rate was 6% with Nisin added at 0.25 mg/kg and no spoilage occurred with nisin added at 1.25 and 2.5 mg/Kg (Fowler and Gasson-1991)
Numerious studied have been made of the use of Nisin to control thermophilic spoilage in canned peas. Gillespy in 1957 reported on the effect of Nisin treatment on thermophilic spoilage of peas from 6 canners in England. No spoilage occurred in 266 incubated cans treated with Nisin at 2.5 mg/kg and 3 out of 261 spoiled when the concentration was 0.5 mg/kg. In the absence of Nisin, 39 cans out of 266 spoiled on incubation at 55°C. Canned products in which Nisin has been shown to be of value included beans, tomato sauce, tomato juice, soups, evaporated milk, mushrooms, corn, chowmein and peas (Brenkink et al-1998, Davidson and Hoover-1993).

Meat products

Reports on Nisin use in this product area have indicated variable success in presentation performance, depending mainly on the type of target bacteria and nature of meat products. Gola in 1962, included Nisin in the gelatine for canning of large hams (4kg) and found that an addition level of 0.012 mg/kg of can contents substantially reduced the bacterial content on incubation at 38-40oC over a period of 7 days. Caserio et.al. in 1981 found that nisin at 0.005 mg/Kg in combination with a reduced nitrite level of 75 mg/kg effectively controlled development of Clostridium prefrengence in wurstel sausages during an incubation period upto 38 days at 20°C. In 1983, Rayman et.al. found that nisin at 200-300 mg/kg in combination with nitrite at 60 mg/kg prevented the outgrowth of Clostridium botulinum spores in pork slurries when pH value was below 5.5 but failed to do so at higher pH value. Colderon et.al. in 1985 treated vacuum packaged bacon with different levels of Nisin (6.25, 12.5 or 18.75 mg/kg) in combination with nitrite at 50 mg/kg measured by suppression of Lactic acid bacteria at 30° C, Nisin levels of 12.5 and 18.75 mg/kg extended shelf life by 1 day. At 5° C, a Nisin level of 18.75 mg/kg extended shelf life by 1 week. (Davidson and Hoover-1993, Fowler and Gasson-1993).

It has been recommended that 400 mg Nisin per Kg in conjugation with 0.1% sorbic acid and 2.5% (w/w) polyphosphate retarded the spoilage of sausage at 5°C. Nisin (150-200 mg/kg) has been suggested as an adjunct to nitrite in cured meat for purpose of preventing the growth of clostridia. But the inhibition of growth of nonproteolytic clostridium botulinum type B in Sous vide cooked meat product was reported not to be achieved by Nisin, rather thermal processing was effective for it (Lindstrom et al-2001)

Alcoholic beverages

The application of Nisin in beer has been the particular subject of study and a series of reports have described the evaluation of Nisin application applied in beer fermentation and in cleansing of contaminated pitching yeasts. Nisin has been used potentially in brewing. Depending on the amount used and the strains of bacteria involved, Nisin either kills LAB or inhibits their growth. The inhibition of beer spoilage lactic acid bacteria by Nisin was assessed. 149 strains beer spoilage bacteria had been screened using a well test assay for sensitivity to the food preservative- Nisin. Nisin inhibited growth of 92% of the gm positive strains, predominantly lactic acid bacteria of the genera Lactobacillus and Pediococcus. In contrast, all 32 gram negative strains tested, except 3 flavobacter strains, were nisin resistant. In addition non of the brewing yeasts showed Nisin sensitivity. Therefore Nisin has potential applicaton in preventing spoilage of wort or beer(Ogden 1986, Ogen and Waites 1985, Oglan and Tubb-1985)

The subject of undesirable lactic acid bacteria in wine has been studied by Radler in 1990. Leuconostoc and Pediococcus strains in acidified grape must were found to be very sensitive to nisin at levels of 0.2 mg/lit and lower. Nisin showed no adverse effect on 6-yeast strain used forwine fermentation. In simulated wine fermentation, Nisin was added at 2.5-25 mg/lit to grape must plus yeast extracts and then simultaneously inoculated with wine yeast, L. casie, C. brevis, and Leuconostoc oenos. In the presence of Nisin neither C. brevis nor C. oenes was detectable after the 3rd day of fermentation. L. casei grew in the presence of Nisin at 2.5 mg/lit but not at the higher level. The presence of nisin did not adversely affect the taste of wine. It was concluded that the use of Nisin could permit a reduction in the SO_2 treatment of wines when malolactic fermentation is not desirable. (Fowler and Gasson-1991)

Other application

An experiment on the use of Nisin in a edible packaging has been carried out. Edible cellulose film made with hydroxypropyl methyl cellulose (HPMC) has proven to be inadequate moisture barriers. To improve its water vapour barrier properties, different hydrophobic compounds were incorporated into the HPMC matrix. Some fatty acids and derivatives were included into the film forming solution prior to film formation. Stearic acid was chosen because of its high capacity to reduce significantly the water vapor transmition rate. Antimicrobial activity of edible HPMC film was obtained by the incorporation of Nisin into the film forming solution. The inhibition activity of Nisin was tested and observed for the inhibition of Listeria innocula and staphylococcus aureus.(Come et al-2001)

Toxicity and Legal status of Nisin:

FAO/WHO experts committee recommended that use of Nisin, as a food "is considered acceptable, the unconditional average daily intake being 0-33,000 IU/Kg body weight." There are about 40×10^6 IU in 1 gm of pure Nisin (Broad and Govld-1991).

Up to 400 units /gm of food are usually recommended for food preparations, that is about 10 ppm. The average daily intake (33,000 IU) is just less than 1 mg. An acceptable daily intake for 70 Kg person would then be about 60 mg of pure Nisin (Fowler and Gasson-1990).

At present time, it is positively allowed in various products in 45 countries including UK and USA. Table 5 gives a brief information about the Nisin application for food in different countries.

Conclusion

Lactic acid bacteria have been known to be a paramount importance in food for their role in the inhibition of growth of food spoilage bacteria not only by the production of various organic acids, alcohols and related compounds but also by the productions of growth inhibitory substances known as bacteriocin (Ouwehand-1998, Frank and Mouth-1983). Nisin

is a heat stable pentacyclic lantibiotic bacteriocin displaying a broad spectrum of activity towards almost all gram-positive bacteria (Vuyst-1994, Fowler and Gasson-1991). The development of Nisin use for food preservation had been started about 5 decades back when clostridial spoilage of low acid natural cheese became a serious problem. After this a tremendous research work has been carried out regarding food preservation and food safety application to Nisin. The outcome of these researches has shown its beneficial role as a natural food preservative to the foods and beverages especially to dairy products, meat products, canned products and alcoholic beverages (Ogden and Tubb-1985, Coma et al-2001). The sensitivity of Nisin has shown to be greatly influenced by the composition of food and the resistive nature of the target microorganisms to it. Recently, tremendous works is being carried out to enhance the bacteriocin activity of Nisin which may lead to bring the new era in food preservation and food safety issues in the near future. Nisin is now being used as a permitted food preservative in more than 40 countries (broughton-1990, Broad and Gauld-1991)

Table 5: Permissible level of Nisin in different countries with respect to various Food

Country	Food permitted	Max limit (IU/gm food)	Country	Food permitted	Max limit (IU/gm)
Abu Dhabi	Dairy products and Canned vegetables	No limit	Malaysia	Canned foods and cheese	No limit
Argentine	Processed cheese	500	Malta	Same as UK	No limit
Australia	Cheese and canned tomato products	No limit	Mexico	Permitted additive	500
Bahrain	Dairy products and canned vegetables	No limit	Netherlands	Cheese Cheese powder	500 800
Belgium	Cheese	100	New Zealand	Processed cheese	500
Bolivia	Use not prohibited in foods	No limit	Philippines	Processed Cheese	4000
Bulgaria	Cheese	200 mg/kg	Poland	Cheese	4000
Chile	Food products	4000	Portugal	Cheese	500
Colombia	Processed cheese	500	Qatar	Milk and its products	No limit
Costa Rica	Cheese products	No limit	Saudi Arabia	Dairy products	500
Cyprus	Dairy product and Canned vegetables	No limit	Singapore	Cheese Canned foods	No limit
Czechoslovákia	Bakery products, Dairy products, canned vegetables	500	South Africa	Cheese	500
Dubai	Milk product and canned vegetables	No limit	Spain	Cheese	500
Egypt	Processed cheese	500	Sweden	Cheese	500
Eire	Processed cheese	500	Taiwan	Cheese	1000
Finland	Process cheese	480	Thailand	Cheese	4000
France	Processed cheese	No limit	Trinidad	Canned foods, cheese and cream	No limit
Gibraltar	Same as UK	No limit	Turkey	Cheese	4000
Hong Kong	Same as UK	No limit	UK	Canned foods, cloned cream	No limit
India	Cheese	1000	USA	Cheese	10,000
Italy	Cheese, vegetables and confectionary	500 100	Uruguay	Processed cheese	4,000
Jordan	Process cheese	500	USSR	Cheese Canned vegetables	8,000 4,000
Kuwait	Process cheese	4000			

(Source: Broughton-1990)

References

Amoako E.B., Ablett R.F., Harris J., and Broughton J.D. (1999). Combined effect of nisin and moderate heat on destruction of listeria monocytogenes in cold palk Lobster Meat. *Journal of food protection.* 64 (1): 46-50

Benkerroum N. and Sandine W.E. (1988). Inhibiory action of nisin against Listeria monocytogenes. *J. Dairy Science.* 71:3237-3245.

Board R.G. and Gould G.W. (1991). Future prospects. In *Food preservatives.* Edited by Ressell N.J. and Gould G.W. Blackie and Sons Ltd. USA 267-283.

Boussoul N., Mathieu F., Junelles A.M.R. and Milliere J.B. (2000). Effects of combination of lactoperoxidase system and nisin on the behaviour of listeria monocytogenes ATCC 15313 in skim milk. *International Journal of food microbiology.* 61: 169-175.

Breukink E.B., Kraaij C.V., Dalen A.V., Damel R.A., Seben R.J., Kruijff B.D. and Kuipers O.P. (1998). The orientation of nisin in membranes. *Biochemistry* 37: 8153-8162.

Broughton J.D. (1990). Nisin and its application as a food preservatives. *Journal of the society of Dairy Technology.* 43(3): 73-75.

Broughton J.D. (1990). Nisin and its uses as a Food preservative. *Food Technology.* 100-117.

Chandan R.C. (1982). *Prescott and Dunns Industrial Microbiology. 4th Edition.* Edited by Reed G. Avi Publishing Company Inc. 113-184.

Chung W. and Hancock R.E.W. (2000). Action of Lysozyme and nisin mixtures against lactic acid bacteria. *International Journal of Food Microbiology.* 60: 25-32.

Coma V., Sebti I, Pardon P. Deschamps A. and Chavant F.H. (2001). Antimicrobial edible packaging based on cellulosic ethers, Fatty acids and nisin incorporation to inhibit Listeria innocua and staphylococcus aureus. *Journal of Food Protection.* 64 (4) : 470-475.

Daeschel M.A. (1993). Applications and Introduction of bacteriocins from LAB in foods and beverages. In *Bacteriocins of Lactic acid bacteria.* Ed. By Hoover D.G. and Steenson L.R.. Academic press Inc California. 63-91.

Davidson P..M. and Hoover D.G. (1993). Antimicrobial components from lactic acid bacteria. In *Lactic acid bacteria.* Ed by Salminens and Wright A.V. Marcel Dekker Inc.

Dodd H.M. and Gasson M.J. (1994). Bacteriocins of Lactic acid bacteria. In *Genetics and Biotechnology of lactic acid bacteria* Ed. By. Gasson M.J. and Vos W.M.D. Blackie Academic and Professional .224-245.

Fowler G.G. and Gasson M.J (1991), Antibiotic-nisin. In *Food preservatives* Ed. By Russel N.J. and Gould G.W. Blackie and Sons.Ltd USA 135-151.

Fox P.F. (1993). *Cheese: Chemistry, physics and Microbiology. Vol 1. General Aspects, Second Edition.* Chapman and Hall. 228-231.

Frank J.F. and Month E.H. (1983). Fermentation. In *Fundamentals of dairy Technology.* Third Edition Ed. By. Wong N.P., Jenness R. Keeney M. and Marth E. Van Nostrand Reinhold Company 694-711.

Hurst A. (1983). Nisin and other inhibitory substances from lactic acid bacteria. In *Antimicrobials in foods.* Ed. By Branen A.L. and Davidson P.M. Marcel Dekker Inc. 327-351.

Lancini G. and Lorenzetti R. (1993). Biotechnology of antibiotics and other bioactive microbial metabolites. PlenumPress, New york. 124-127.

Lindstrom M., Mokkila M., Skytta E., Trees E.H., Lahteenmaki L., Hielm S., Ahvenainen R. and Korkeala H. (2001). Inhibition of growth of nonproteolytic Clostridium botulinum Type B in Sous Vide Cooked Meat Products is achieved by using thermal processing but not nisin. *Journal of food protection* 64(6). 838-844.

Marth E.H. and Steel J.C. (1998). *Applied Dairy Microbiology.*Marcel Dekker Inc. 100-103

Mishra C. and Lambert J. (1996). Production of antimicrobial substances by probiotics. *Asia pacific J.Chem. Nutr.* (5): 20-24 (internet information).

Ogden K. (1986). Nisin : A bacteriocin with a potential use in Brewing. *J. Inst. Brew.* 92:379-383.

Ogden K. and Tubb R.S. (1985). Inhibition of beer spoilage lactic acid bacteria by nisin. *J. Inst. Brew.* 91:390-392.

Ogen K. and Waites M.J. (1985). The action of nisin on beer spoilage lactic acid bacteria. *J.Inst. Brew.* 92:463-467.

Ouwehand A.C. (1998). Antimicrobial components from Lactic acid bacteria. In *Lactic acid bacteria-Microbiology and fundamental aspects. Second edition*, Revised and Exp[anded. Ed. By Salminensis and Wright A.V. Marcel Dekker Inc, Newyork. 139-155.

Tamime A.Y. and Robinson RC. (1999). *Youhurt Science and Tecnology. Second Edition.* Woodhead Publishing Limited. 50-51.

Vuyst L.D. (1994). Bacteriocins produced by Lactococcus lactis strains. In *Bacteriocin of lactic acid bacteria-Microbiology genetics and applications.* Edited by Vuyst L.D. and Vandamme E.J. Blackie Academic and Professional, Great Britain. 143-150.

Vuyst L.D. and Vandamme E.J. (1994a). Antimicrobial potential of Lactic acid bacteria. In *Bacteriocin of lactic acid bacteria- Microbiology genetics and applications.* Edited by Vuyst L.D

Vuyst L.D. and Vandamme E.J. (1994b). Nisin-a lantibiotic produced by lactococcus lastis subsp lactic: Properties, biosynthesis, fermentation and applications. In *Bacteriocin of lactic acid bacteria- Microbiology genetics and applications.* Edited by Vuyst L.D. and Vandamme E.J. Blackie Academic and Professional, Great Britain. 151-221.

Wiedemann I., Breckink E., Kraaij C.V., Kuipers o.p., Bierbaum. G. Krauijff B. D. and Sahl H.G. (2001). Specific Binding of Nisin to the peptidoglycan precursors Lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. In *Journal of biological chemistry* 276 (1): 1772-1779.

Wirjantoro T. I., Lewis M.J., Grandison A.S., Williams G.C. and Broughton J.D. (2001). The effect of nisin on the keeping quality of reduced heat treated milks. *Journal food pretection* 64(2): 213-219.

Wirjantoro T.I. and Lewis M.J. (1996). Effect of nisin and high temperature pasteurization on shelflife of whole milk. *Journal of society of Dairy Technology.* 49(4): 99-102.

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Risk Analysis : An Unavoidable Scientific Process for Assuring Safety in Food Chain

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This article highlights the importance of Risk analysis and emphasizes the need for implementing assurance of food safety. A comparative review in risk analysis has been made in terms of assessing various approaches used in several recent risk assessments modalities for addressing pertinent issues that a developing countries faces during its implementation and quality assurance endeavors.

Keywords: Risk, Hazard, Risk assessment, ADI, MRL, ALARA.

Introduction

Risk analysis has evolved over the last decade in the Codex Alimentarius Commission (CAC) as an important tool in safety assurance. Since the Uruguay Round Trade Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) entered into force in 1995, the significance of risk analysis has increased dramatically. In 1991, a Joint FAO/WHO Conference on Food Standards and Chemicals in Foods and Food Trade, convened in Rome, Italy, emphasized importance of scientific committees for providing evaluations based on sound science and risk assessment principles. Further, this conference recommended that FAO and WHO take necessary steps to enhance awareness of these principles. Later on, FAO and WHO convened a series of three expert consultations to address the three essential components of Risk analysis in terms of risk assessment, risk management and risk communication (FAO/WHO. 2004).

Risk analysis is a structured process for determining the risks associated with any type of hazard which are basically comprised of biological, chemical, or physical—in a food. The primary objective is to make characterization of the nature and likelihood of risk arising from human exposure to the above hazardous agents in the environment. The characterization of risk embodies both qualitative and quantitative information which are associated with a certain degree of scientific uncertainty (Rietjens M.C.M. 2004). The risk analysis is a challenging task for most of the developing world because of paucity of required human resources and the expertise in the field of toxicology, epidemiology, and the effective surveillance mechanism. The capacity building is a felt-need particularly to develop scientific basis for setting standards, guidelines, and reliable trading practices. This is also essential in putting up national views and position for establishing standards for our indigenous products or to modify existing ones particularly from the perspective of contaminants, and food additives. This paper focuses on familiarizing the concept of risk analysis process, for food professionals to undertake the upcoming challenges in the WTO scenario.

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Definitions of Hazards, Toxicity and Risk by Codex

Hazard: a biological, chemical or physical agent or condition, with the potential to cause an adverse health effect.

Toxicity: a capacity of the chemical substance to cause injury. It is principally a dose related phenomenon.

Risk: a function of the probability (P) of the occurrence of an adverse health effect and the severity (S) of the effect arising from hazards.

Therefore, Risk = f (P, S) or, Risk=P.S. Thus risk is defined as a function of probability (P) of incidence multiplied by the severity (S) of the effect.

Definition of risk (by others)

Producers: Risk= f (P, S, Controllability)
Consumers: Risk= f (P, S, Perception)
Policy Makers: Risk = f (P, S, Controllability, Trade barriers). (Bouwmeester Hans 2005).

According to FDA/CFSAN (2002), the risk analysis process comprises of three steps i.e Risk assessment, Risk management, and Risk communication

Risk assessment helps to organize scientific information and to characterize the nature and likelihood of harm to the public. It helps to identify and define uncertainties, identify data gaps, and identify what assumptions must be made. Thus, risk assessment provides the decision-maker a scientific basis with a level of confidence in that decision. *Risk management* is the process of weighing policy alternatives in light of the results of the risk assessment and selecting and implementing appropriate control options. The goal of risk management is to protect the public health by controlling risks as effectively as possible through the selection and implementation of appropriate measures. *Risk communication* is the exchange of information and opinion on risk among risk assessors, risk managers, and other interested parties, including the general public. These three components are interrelated among themselves in such a way that nothing could be avoided while conducting risk analysis

Importance of Risk Analysis

Food safety has always been an important issue, and currently it is high on the political agenda of many countries. More than ever before, there is strong consumer awareness of food quality and safety, and this continues to increase. New risks and challenges are emerging as a result of changes in the methods of food production at the farm and processing levels. (Knotttners O., 2005). Further challenges arise from the emergence and re-emergence of food-borne pathogens. Consumption patterns and consumer demands regarding such issues as the variety and shelf-life of foods, as well as the preservation techniques used, are changing. International trade in food has also increased the risk of infectious agents being disseminated from the original point of production to locations thousands of kilometers away. The consequence leads to an increased risk to human health. There is a need for an integrated approach to food safety. This involves careful monitoring at each step of the food chain and linking this with the human health outcome (FAO/WHO. 2004).

The development of the risk analysis process has however provided us with a tool to make this possible. Risk assessment, as a tool, can be used for through examination of a food production system, for enhancing capacity in determining strengths and weaknesses in terms of physical chemical and biological control which will eventually provide an estimate of the risk to the consumer, based on an available system or as a result of simulated changes to a system. Therefore, as a tool, risk assessment has the potential to allow the user develop targeted and effective risk management strategies (Luning P.A. *et.al.* 2002). The scope of the risk assessment is dependent on problem faced by risk management system. Traditionally, risk assessment has been viewed simply as the means to provide an estimate of risk. However, it can serve much broader applications such as estimating the relative value of different hygiene interventions in reducing risks to consumers.

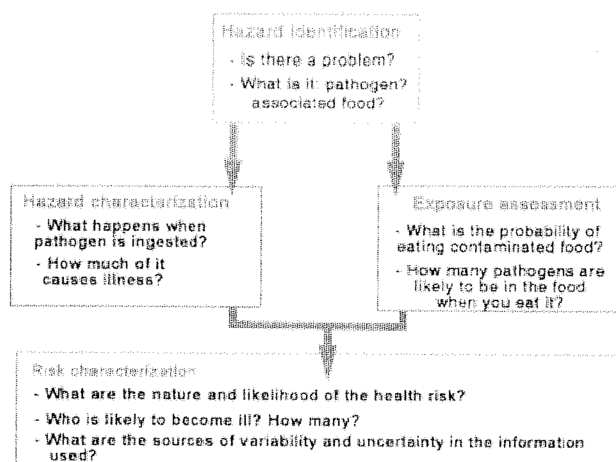

Fig. 1 Systematic structure of Risk Assessment steps. (Source: FDA/CFSAN 2002)

Risk Analysis Process

There are five very distinct steps in the risk analysis process. The definitions for these steps differ slightly depending on whether it is about nutritional risk assessment or food safety risk assessment (FDA/CFSAN 2002). The generally accepted framework separates risk assessments into four components: hazard identification, exposure assessment, dose-response assessment and risk characterization (Figure 1). These components are used in a five-step process to format scientific data in a computer compatible manner to answer key risk management questions (Buchanan, R.L. 1997).

Step 1. Statement of the problem. The risk analysis process starts with risk evaluation, which is the first step of the risk management component that defines the reasons that the risk assessment is required (FAO/WHO, 2005). Therefore, it is the responsibility of the risk managers to develop and provide risk assessors with the questions to be answered and the key assumptions that define the scope of the work. The problem should be apprehended during the planning stage of the assessment which will be refined by both the risk assessors and risk managers for the relevance of the stated assessment. Initial identification of food safety problems and issues comes under the functioning of risk management. Relevant information on food safety will be gathered from various sources e.g. regulators, public health institutions, the food industry, academician, and interested consumer groups. This initial risk management function is usually carried out in conjunction with risk assessors. The information leading to the identification of a food safety problem may be provided by a single stakeholder or may be the result of collaboration among many stakeholders.

FAO/WHO (2001) emphasizes that a detailed elaboration of a risk profile is essential for effective risk management program. A risk profile should place an issue within a food safety context. Also it should provide as much information as possible to mitigate further action. Establishment of a risk profile will help to clarify risk management questions and goals. A risk profile may include the following features:

- Administrative information, including initial description/statement of the issue
- Description of the commodity (may include distribution, marketplace), disease agent, outcome of exposure; food safety scenario (i.e. particular processing, handling, consumption profile)
- Values potentially at risk (human health, may in some situations include economic values, cultural, aesthetic)
- Distribution of risk, i.e. who are the producers of the risk, who benefits from the risk situation, who bears the risk.
- What characteristics of the commodity/agent might affect risk management options?
- What are known risk management characteristics of the risk producer and of the risk bearer?
- What are the current risk management practices relevant to the issue?

- What are the public perceptions of the risk?
- Familiarity and substantial equivalence
- International agreements, if any, those affect the risk issue.

Step 2. Hazard identification. This step includes collection, organization, and evaluation of all information pertaining to a physical, chemical and biological hazard associated with the foods and the adverse outcome (illness or death) resulted from the consumption of contaminated foods (Amor Ben Kaouther 2005).

Step 3. Exposure assessment. The purpose of the exposure assessment is to provide an estimate of the levels of the pathogen and chemicals consumed. This includes the probability that the pathogen or chemicals will be present in the commodity, the levels of these in the food consumed and the impact of food handling, processing and storage conditions on the overall potential exposure. It can be divided into two parts

Route and site of exposure: It may be through gastrointestinal tract (oral), Lungs (Inhalation), Skin (Dermal), Injections (for drugs).

Duration and frequency of exposure

Acute: refers to injury induced by short exposure (e.g. single exposure) to relatively high doses (or concentrations), <24 hour single dose

Sub acute: 1 month or less, repeated exposure

Sub chronic: 1-3 months, 10% of life span

Chronic: refers to injury induced by long exposure (e.g. life time exposure) to relatively low doses (or concentrations), >3months, 80 90% of life span

Effect varies with dose and exposure regime (FAO/ WHO. 2005).

Step 4. Dose-response assessment (or hazard characterization). In this step, the relationship between the exposure level (dose) and frequency of illness or other adverse effect (response) is estimated that is risk evaluation is done. The severity of the health effect also must be considered. This often includes the challenge of attempting to extrapolate data acquired from clinical trials, as well as from animal models to humans (Anonymous 2005).

Qualitative principles : Some of the terms must be answered to facilitate the assessment process. Is the target organ is the most sensitive organ? Is it reversible or irreversible effect? Is it local or systemic effect? Local means at site of contact and Systemic means of absorption and distribution from entry point to distant site target organ Central nervous System (CNS), liver, Kidney (FDA/CFSAN 2002).

Quantitative Principles: Dose response relationship is characterized by the following equation.
LD50 = Lethal dose 50%, NOAEL = No Observed Adverse Effect Level.

Safety levels can be denoted as ADI or TDI (Acceptable or Tolerable Daily intake). But in case of Geno-toxic carcinogenic chemicals there is no ADI or TDI. Thus, first we have to know what compound is it. Acceptable daily intakes are defined for avoidable contaminants like additives, pesticides whereas Tolerable daily intake for unavoidable environmental contaminants likes dioxins, mycotoxins, heavy metals etc. The scale of effect of these compounds is different on each individual. Some shows very sensitive towards low level of dose while some shows high resistance even on exposure to high doses (Figure 2). Thus some safety factors are included while calculating ADI of those compounds.

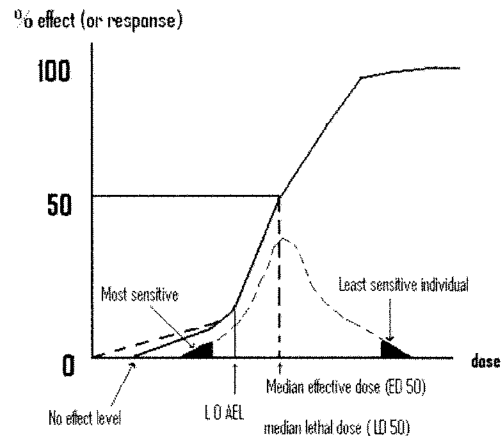

Fig. 2 Dose response relationship

ADI = NOAEL x 1/10 x 1/10 x other safety factors. (1/10 = Safety factors).
Other safety factors are for quality of data and type of exposure etc.
In case of unavoidable contaminants adverse effect cannot always be avoided. Thus terms used are TDI (for consumer), MRL (Maximum Residual Limit), or ALARA (As low as reasonably achievable) product. Bouwmeester (2005) have proposed some process for safety assessments of veterinary medicines, pesticides and environmental contaminants. (Fig 3, 4 and 5 respectively)

Step 5. Risk characterization. This step combines the findings of the prior steps to determine the likelihood of the adverse outcome from exposure to the pathogen (i.e., consumption of a food). In this step, the exposure and dose response assessments are integrated to mathematically express the probability of the effect on public health. An important part of this step is to determine the degree of uncertainty in

relation to the results and to distinguish this from the variation that is inherent in any biological system. The results of the risk characterization are generally interpreted by the risk assessors so that they effectively communicate the information needed by the risk manager. (FAO/WHO. 2001). There are two types of involuntary risk chemical compounds.

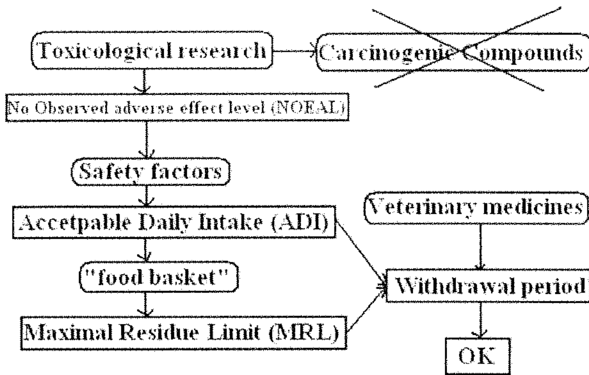

Fig 3. Safety assessment of veterinary medicines

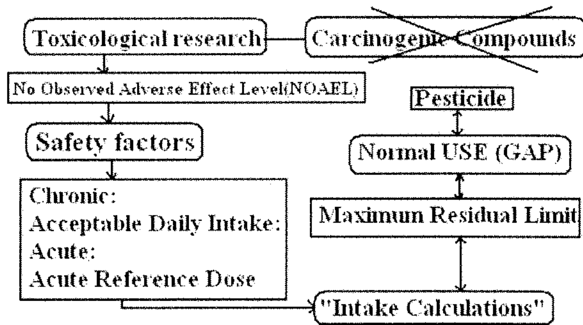

Fig 4. Safety assessment of Pesticides

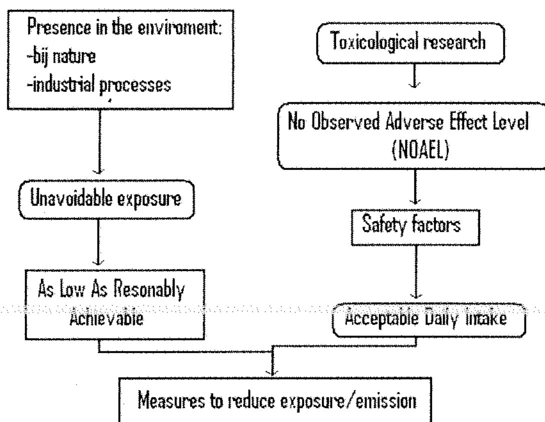

Fig 5. Safety assessment of (Environmental) Contaminants

Non-genotoxic compounds. A NOAEL and ADI or TDI can be established.

Risk characterization for man, Quotient method:

$$\text{Margin of Safety (MOS)} = \frac{\text{Total estimated daily intake (EDI)}}{\text{Acceptable daily intake (ADI)}} \text{ should be} < 1$$

Genotoxic carcinogenic compounds.

If, compounds found are characterized under avoidable contaminants, then the compounds cannot be used, but for unavoidable contaminants, ALARA (As low as Reasonably Achievable) or VSD (Virtual Safe Dose) must be established. The dose must be defined with acceptable cancer risk. Such that, 1 per 10^6: Consumers, 1per 10^5: high level consumers, 1 per 10^4: industrial workers

Important steps to ensure food safety in absence of Risk analysis

- Management of pesticide residues in agro-produces and record keeping of pesticide used must be encouraged. Types of pesticide, date, amount, type of food material treated etc are needed to be recorded in a log book. This will help to determine future treatments strategies in order to control the pest and safe food. Uses of overdose of pesticides have no use in increasing the quality of crops rather it will effect the environment and humane health. (Luning P.A. *et.al.* 2002).
- Use of Integrated Pest Management (IPM) is an effective method to Control pest in farm. IPM helps to recognize all the problems that pests and their control can cause and take all necessary preventive actions. This will not only substitute the risk of using dangerous pesticides but will also increase productivity and quality of the crops. This leads to enhances safety and increase profit.
- Encourage the cold chain operation especially for perishable food products in the entire food chain. This will decrease the rate of unfavorable biochemical reactions and will maintain the quality of the product.
- Implement Good Manufacturing Practice (GMP), Good Agricultural Practice (GAP), Good Veterinary Practice (GVP) and Good Laboratory Practice (GLP) in each step of production. Implementation of Hazard Analysis Critical Control Points (HACCP) in the production process will ensure safety and quality of foods (Amor Ben Kaouther 2005).
- Encourage use of minimal processing techniques in food handling, processing and preservation. Avoid the use of excess chemical additives in food and food products. Encourage the use of physical preservation techniques rather than the use of chemical preservatives.
- Avoid exposure to hazardous chemical substances for a long time. Use safety measures while dealing with such chemicals.

References

Amor Ben Kaouther (2005). Food Microbiology and Hygiene, IAC, Wageningen.

Anonymous (2005), Food Safety and Agricultural Health Standards, Poverty Reduction and Economic Management Trade Unit, and Agriculture and Rural Development Department, World Bank.

Buchanan, R.L. (1997), National Advisory Committee on Microbial Criteria for Foods "Principles of Risk Assessment for Illnesses Causes by Foodborne Biological Agents." *J. Food Protection* 60(11), pp. 1417-1419.

Bouwmeester Hans (2005), Risk Analysis, Institute of Food Safety, Wageningen University.

Codex: Risk Analysis topics: a) Proposed Draft Working Principles for Risk Analysis; b) The Application of Risk Analysis in the Elaboration of Codex Standards and c) Consideration of the Development of Working Principles for Risk Analysis to be Applied by Governments; Available at; ftp://ftp.fao.org/codex/allinorm03/A103 33e.pdf; also: proposed draft working principles for risk analysis: CL 2002/39-GP of July 2002.

FAO/WHO. (1999). Expert Consultations on Risk Assessment of Microbiological Hazards in Foods (JEMRA). Available at: http://www.who.int/fsf/Micro/report30April01.pdf and http://www.who.int/fsf.Micro.reportcv.pdf

FAO/WHO. (2001). Hazard identification, exposure assessment and hazard characterization of Compylobacter spp. In broiler chickens and Vibro spp. In seafood, Joint FAO/WHO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods, WHO Headquarters, Geneva, Switzerland.

FAO/WHO. (2005). Food Safety Risk Analysis, Part II, Case Studies, Food and Agricultural Organisation of the United Nations (FAO), World Health Organization (WHO).

FAO/WHO. (2004). Nutrition and Consumer protection, FAO/WHO Expert Meeting on the Development of Practical Risk Management Strategies Based on Microbiological Risk Assessment Outputs, Available at: http://www.fao.org/ag/agn/jemra/riskassessment en.stm

Knotttners O., (2005), Quality assurance and application of food safety systems in the supply chain, Principles of food legislation, IAC, Wageningen.

Luning P.A., Marcelis W.J. and Jogen W.M.F. (2002), Food Quality Management a techno-managerial approach, Wageningen Pers, Wageningen, The Netherlands.

Rietjens Ivonne M.C.M., (2004), Introduction to toxicology and Risk assessment, Food Toxicology, Wageningen Pers, Wageningen, The Netherlands.

U.S. Food and Drug Administration/Center for Food Safety and Applied Nutrition (FDA/CFSAN) 2002. "Initiation and Conduct of All 'Major' Risk Assessments within a Risk Analysis Fromework". Available at: http://www.cfsan.fda.gov/dms/rafw-toc.html

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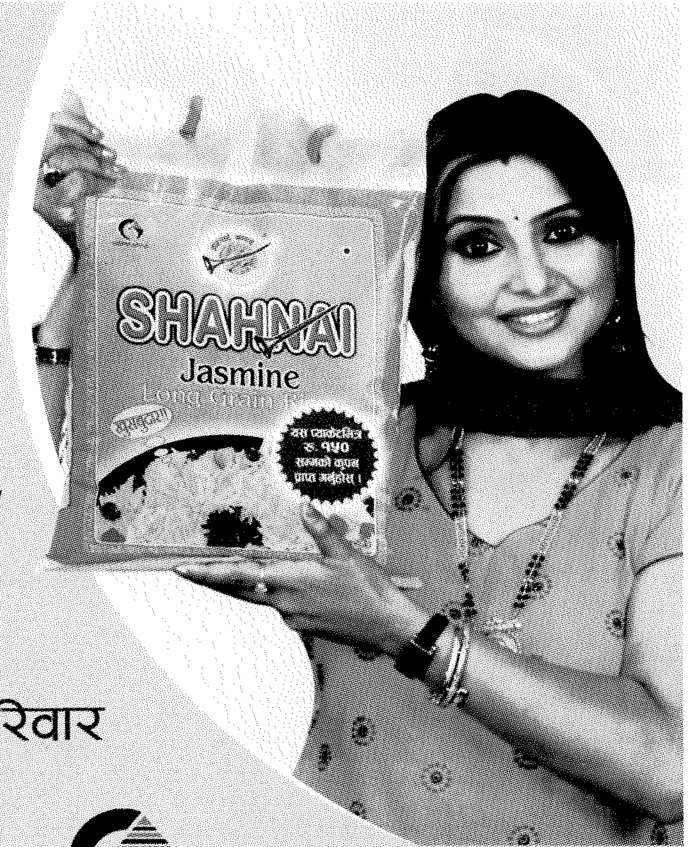

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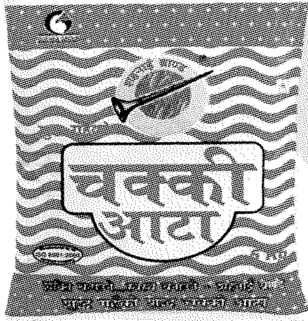

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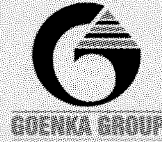

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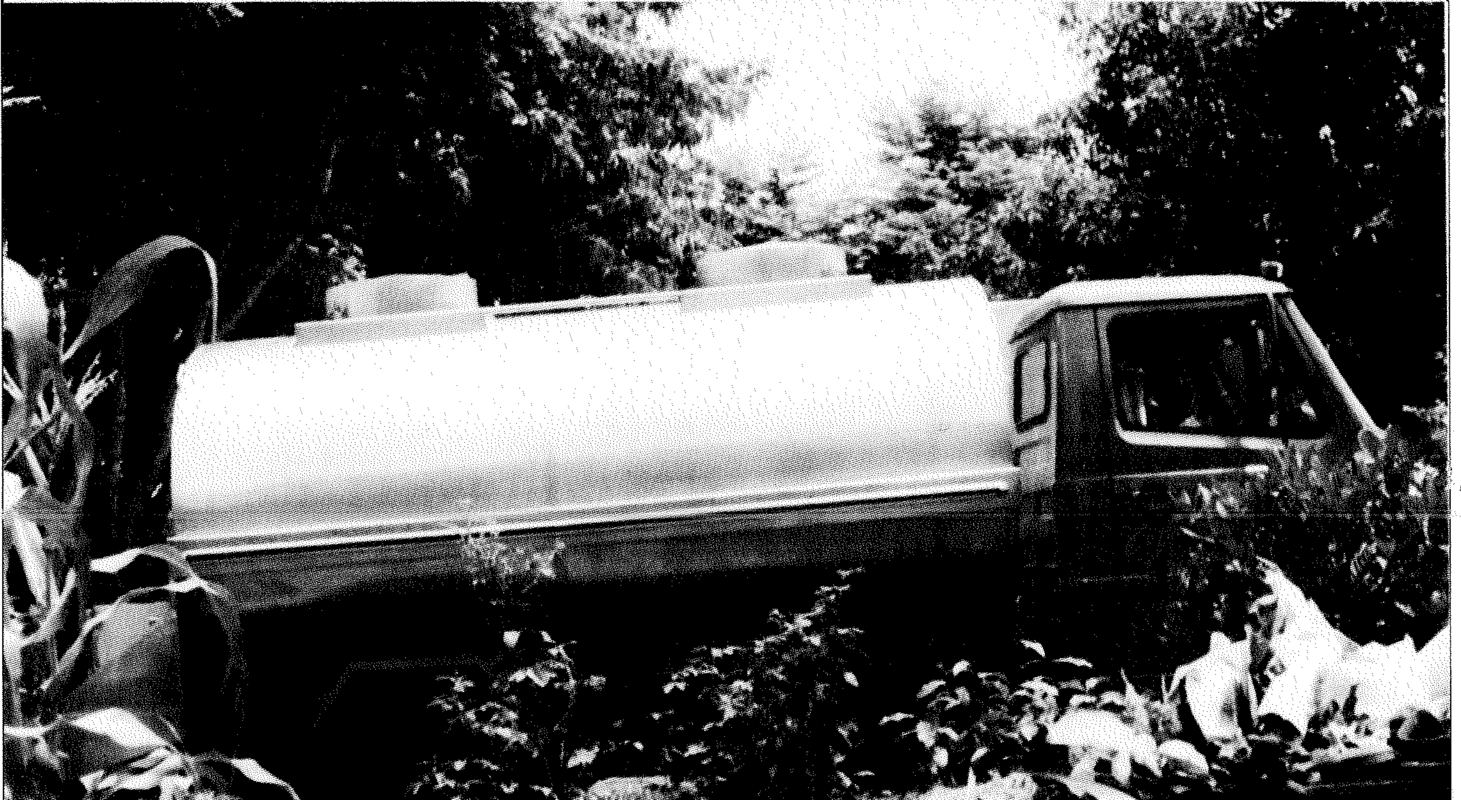

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Morbidity Profile and Prevalence of Anemia in Indian Women from a Rural Coastal Community

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Morbidity profile and prevalence of anemia was studied in a group of the rural women residing in a coastal region. A total of 316 women, aged between 18 to 55 years, were interviewed to collect background information. The prevalence and incidence of morbidity was computed from a fortnightly household survey over a period of one year. The weights and heights of the women were measured and BMI computed. Blood samples were used for determination of hemoglobin. Dietary iron was estimated from the 24 hr dietary recall collected through a dietary interview and a food frequency questionnaire. Results indicated that frequently occurring ailments were cold, cough and headache. Based on body mass index, 30.7% of women exhibited chronic energy deficiency (CED), 57.8% were normal and 11.5%, obese. Majority of the women (49.4%) were moderately anemic, and 42.1% were mildly anemic. The nutritional status of women or the extent of anemia did not influence morbidity profile. Dietary intake of iron was very low and was found to be <30mg/day in women suffering with anemia. Consumption of hem iron from fish did not seem to improve the iron status. All correlation coefficients between the selected socio demographic characteristics and nutritional adequacy, hemoglobin, and total morbidity were non significant.

Keywords: Body mass index, Chronic energy deficiency, Dietary iron, Hem iron, Nutritional adequacy, Socio demographic characteristics.

Introduction

The importance of anemia as a major public health problem throughout the world is widely recognized. Iron deficiency anemia is a global nutritional problem with about 40% of the world's population being anemic. The prevalence is extraordinarily high among all age groups and specially in developing countries with figures being four times that of developed countries. Current estimates of anemia for developing countries are, for pregnant women, 56%, for school children 53%, for preschool children 42% and for men 33%. Asia has the highest rates of anemia in the world. In India, the prevalence of anemia is 50% in women and 74% in small children. During last trimester of pregnancy the incidence could be as high as 70%. It is present even in men to a large extent (NFHS, 2000).

The consequences of iron deficiency anemia are manifolds, and much of it is reversible, with better access to and absorption of iron. In infancy and childhood, iron deficiency results in impaired motor development and coordination, impaired language development and scholastic achievement, psychological and behavioral effects and decreased physical activity. In adults there is fatigue, decreased physical work efficiency and earning capacity. It has important functional consequences for females of childbearing age. Anemia may impair memory in adolescent girls (Bruner et al, 1996). Iron deficiency reduces work efficiency in non-pregnant women (Li et al, 1994). Pregnant women with low iron stores may be at higher risk of iron deficiency resulting in increased maternal and fetal morbidity and mortality (Ramakrishnan, 2001). The main characteristic of the third and final stage of iron deficiency is a reduction in the concentration of hemoglobin in the RBC (Golder et al, 2001).

Inadequate dietary intake and poor bioavailability of iron from food are considered as prime etiological factors of anemia. A very high incidence of iron deficiency has been attributed basically to poor diets and then to the presence of many inhibitors of iron absorption in foods. The situation is also compounded due to excessive worm infestation due to unhygienic surroundings. Despite implementation of large-scale nutritional intervention/prophylaxis programs iron deficiency anemia still continues to be a public health problem in most of the developing countries (Allen & Sabel, 2001).

In Indian context, the consumption of vegetarian and non vegetarian diets makes a difference. Since the absorption of iron from non haem sources is very low, it can be expected that population on animal foods or haem iron may have lesser incidence of anemia. The population belonging to coastal areas tend to have rice and fish as staple food thus increasing the intake of haem iron. It was interesting to examine the iron status of women from such an area. In this study, the prevalence and incidence of morbidity and the extent of anemia were studied in the rural women residing in a coastal Karnataka region of India.

Methodology

Study area - The study area was a town located on the Arabian sea coast in Karnataka State of India called 'Honavara'. The population of this town is distributed in 93 villages, out of which 10 were selected for the study based on their distance

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from the town, the total distance being 3-35 km from center of the town. Five villages were selected between 3 and 20 km and another five between 20 and 35km. In certain instances, if a selected village was beyond reach, then another village, which was easier to reach in terms of approachability was included for the data collection. The list of households was taken from the administrative office of the town. One respondent from every third house of the village was selected for interviewing.

Background information - A total of 316 women, aged between 18 to 55 years, non pregnant and non lactating during the study period, one from each household were interviewed on an individual basis. Background information was collected with the help of a detailed questionnaire regarding age, education, family size and number of working members.

Morbidity Profile - A schedule was drawn up to collect data on morbidity and the women were observed and interviewed over a period of 12 months at fortnightly intervals. The physical examination of the women was carried out with the help of the heath worker in the area. Absence and presence of various illnesses was determined with the help of a checklist. The prevalence and incidence of morbidity was computed from a household interview survey covering 316 households. Prevalence rate (PR) and incidence rates were computed as per the WHO (1993). Duration was considered as sum of period suffered from an illness during the study period of one year.

Nutritional status - The weights of the women were measured whilst they were dressed in light clothes. For weight measurements the personal weighing scale was employed. The heights of the women were measured with a non-stretchable tape. Weight was recorded to the nearest 50g and height to the nearest 0.1 cm. The BMI was computed with the help of anthropometric measurements. For classification of the body mass index (BMI) the definition used by WHO/FAO (Shetty & James, 1994 ; Latham, 1997) was used to identify women who were underweight and overweight. Blood samples were collected for determination of haemoglobin. The estimation was carried out by Cyanmethemoglobin method (Demaeyer et al., 1989), using Drabkin's reagent. Women were classified into grades of anemia based on WHO standards.

Dietary intake of iron - Dietary iron was estimated from the 24 hr dietary recall collected through a dietary interview and a food frequency questionnaire (Christopher and Basiotis, 1992). One complete 24-hour recall of food intake was carried out on each woman. To help the women remember everything that had been consumed, they were asked to report systematically on their intake of food and beverages. For this purpose, common household utensils such as cups, glasses, spoons and ladles of various sizes were used. The recipes for common dishes were collected and entered as ingredients. In addition, the weights of foods consumed were recorded on a digital balance carried by the investigator. This helped in determining exact amount of foods consumed. The estimated nutrient contents were computed from the food composition tables (Gopalan, 1996) and compared with recommended dietary allowances for Indians to determine adequacy and contribution of iron from hem and non hem sources (ICMR, 1998). Prevalence of anemia was analyzed in relation to nutritional status and intake of dietary iron. The morbidity pattern was compared with hemoglobin levels and nutritional grades. For statistical analyses, SPSS package version 10 was used.

Results and Discussion

The results of the study are presented in Tables 1-6. The morbidity profile of women given in Table 1 shows that frequently occurring ailment were cold, cough and headache, sometimes accompanied by fever. The next two problems frequently encountered were body pain and stomach disorders. The incidence of other illnesses such as stomach pain, throat pain, toothache, ear pain, and vomiting was low. General health problems were for the most part one- time occurrences. However, the women continued with normal chores and were restricted to bed only in case of severe infections. The duration computed as mean days of morbidity suffered by subjects as highest for fever. For rest of the morbidities, it ranged between 1.0 to 2.1 days. In a similar study Ravindran (1998) reported that out of 351 subjects included, 150 women had a health problem during a six month duration. The prevalence rate for all health problems was 43% and the incidence rate, 68%. The single most important cause of morbidity was non-specific fever (27%). Nineteen percent of the women complained of severe backache or joint pains that restricted them to bed, while respiratory infection, diarrhea, eye infections and injuries related to work and to domestic violence were reported at lesser frequent intervals.

Table. 1. Prevalence, incidence and duration of morbidity in women

Illnesses	Percent Incidence Rate	Percent prevalence rate	Duration (Mean days)
Cold	4.46	33.86	1.6
Cough	3.61	27.53	1.6
Throat pain	0.76	6.96	1.3
Fever	2.51	21.84	4.3
Head ache	3.93	30.06	1.6
Nausea	0.16	1.27	1.5
Vomiting	0.53	4.75	1.3
Stomach / abdominal pain	1.19	9.49	1.5
Diarrhoea	0.13	1.58	1.0
Ear pain	0.55	5.06	1.3
Skin rash	0.16	1.90	1.0
Itching	0.45	3.48	1.5
Body pain	0.79	9.49	2.1
Tooth ache	0.84	6.01	1.9
Any other	1.03	12.34	2.0

For the purpose of further analysis of the morbidities in association with nutritional grades and anemia, following categories were used.

- Fever with or without headache
- Acute respiratory infections – cold, cough, sore throat
- Gastro intestinal disorders – Diarrhea, vomiting and abdominal pain
- Other infections.

Table 2 present the distribution of morbidities based on nutritional grades of women. Based on body mass index, 30.7% of women exhibited chronic energy deficiency (CED), 57.8% were normal, whereas 11.5% were obese. According to the survey by NFHS (2000), which represents the national averages, the mean BMI for women in India is 20.3. More than 1/3rd (36%) of the women have a BMI below 18.5, indicating a high prevalence of nutritional deficiency. Nutritional problems are particularly serious for rural women and illiterate women from low socio economic groups. When the prevalence of morbidity is considered as such, fewer women statistical analyses, SPSS package version 10 was used.

Table. 2. Distribution of morbidity based on nutritional grades

Grades of nutrition based on BMI	N	Morbidities ARI	Fever	GID	Others
Chronic energy deficiency	96 (30.7)	42 (43.8)	40 (41.7)	14 (14.6)	14 (14.6)
Normal	181 (57.8)	77 (42.5)	79 (43.6)	23 (12.7)	23 (12.7)
Obese	36 (11.5)	15 (41.7)	12 (33.3)	7 (19.4)	7 (19.4)
X^2=2.723, P=0.843, DF=6					

(Values in parenthesis indicate percentages)

CED and obese category were observed to be morbid, but when percentage of morbidity is taken, there were no statistically significant differences in the prevalence of infections between three categories of women.

Distribution of morbidity based on anemia is given in Table 3. There were no cases of severe anemia in women of this region of Karnataka. Majority of the women (49.4%) were moderately anemic, and 42.1% were mildly anemic. Small number of women had normal hemoglobin levels. Distribution of morbidity based on anemia showed some differences. More number of women with moderate anemia suffered with ARI and fever and other infections. Women with mild anemia had higher incidence of GID. Women with normal hemoglobin levels had less morbidity of all types. However, statistically difference were not significant.

Table. 3. Distribution of morbidity based on anemia

Grades of Anemia	N	Morbidities ARI	Fever	GID	Others
Moderate	156 (49.4)	70 (49.3)	72 (46.2)	19 (12.2)	41 (26.3)
Mild	133 (42.1)	55 (41.4)	50 (37.8)	24 (18.0)	28 (21.1)
Normal	27 (8.5)	9 (33.3)	11 (40.7)	1 (3.7)	5 (18.5)

(Values in parenthesis indicate percentages)

The percent distribution of subjects in different grades of anemia based on nutritional grades is compiled in Table 4. It can be noted that women in all categories were suffering from either moderate or mild forms of anemia irrespective of their nutritional grades. This also indicates that anemia was not related to their BMI status but may be due to their low dietary intake of iron.

Table 4: Percent distribution of subjects in different grades of anemia based on nutritional grades

Nutritional Grades	N	Grades of Anemia Moderate	Mild	Normal
Chronic energy deficiency	96	47 (49.0)	40 (41.7)	9 (9.3)
Normal	181	89 (49.2)	77 (42.5)	15 (8.3)
Obese	36	19 (52.8)	15 (41.7)	2 (5.6)
N	313	155	132	26
X^2=0.562, P=0.967, DF=4				

(Values in parenthesis indicate percentages)

The results of the 24-hour recalls of the women concerning their iron intake are listed in Table 5 and compared with the RDAs for Indians. The mean and the median daily intake of dietary iron were found to be 13 mg/day and 10.3mg / day, providing 43% and 34% of the RDA. This indicates that a low iron intake was one of the causes of iron deficiency anemia. Majority of women with moderate and mild anemia were consuming <30mg of iron per day whereas all women with higher haemoglobin levels were taking >30 mg iron per day, the differences being significant. In the present study haem iron was in the form of fish, which was consumed 4 to 5 times a week. However the portion size of fish was inadequate in helping meet the RDA for iron. Golder et al (2000) also indicated in their study of the Maldivian women, that the coastal diet where fish is part of the meal, yields high iron availability, because in fish 15 % of the iron is absorbed. But despite fish consumption the iron intake was low in the present study. The findings in the present study are similar to those seen in the Mexico, where 35.2% of the non pregnant women had low iron stores and 38.4% had anemia (low Hb). The median daily intake of iron was 16mg and all iron consumed (>98.9%) was in the non-heme form (Jeffrey et al., 2002),

Table: 5. Anemia and iron intake

Grades of Anemia	N	Iron intake <30 mg/day	Iron intake >30 mg/day
Moderate	156	148 (94.9)	8 (5.1)
Mild	133	128 (96.2)	5 (3.8)
Normal	27	-	27 (100)
N	316	276 (87.3)	40 (12.7)
X^2=203.827, P=0.001, DF=2			

(Values in parenthesis indicate percentages)

In this study the coastal women from Honavar taluka consumed haem iron available from fish. The non-haem iron was coming from ragi (*Eleucine coracana*, a millet with fair amounts of iron but low bioavailability due to presence of tannins, phytates and fiber). The absorption of non-haem iron is also strongly influenced by other constituents in the meal. These include ascorbic acid, and non- nutritional factors, like parasites and infections, which exert a negative influence on iron status in the meal. The mean and median intakes of ascorbic acid in the current study were 27.0 mg/day and 24.2 mg/day which corresponded to 67 % of the RDA. The primary problems with regard to the iron status of the women in Honavar taluka, therefore, were low dietary iron mainly in the form of non-haem iron, which was compounded by lower ascorbic acid intakes.

The data collected was also analyzed for associative factors for nutritional adequacy, haemoglobin and total morbidity and the results are given in Table 6. All correlation coefficients between the selected socio demographic characteristics and nutritional adequacy, hemoglobin, and total morbidity were non significant.

Table 6: Correlations between selected socio-demographic variables and nutritional adequacy, anemia and total morbidity.

Variables	Nutritional Adequacy	Anemia	Total morbidity
Age	r = .048 p = .400	r = .001, p = .989	r = .005, p = .927
Education	r = .022, p = .692	r = .034, p = .542	r =- -.022, p = .702
Family size	r =- .047, p = .402	r = .037 p = .527	r = .003, p = .952
Number of Working Members	r = .020, p = .718	r = -.040, p = .484	r = .014 p = .800

It can be concluded that all the subjects of the study suffered with various morbidities irrespective of their nutritional status or presence of anemia. The dietary intake of iron was low and an important causative factor for anemia. The consumption of fish in the study population did not seem to increase the iron status due to low amount taken.

References

Allen L. Sabel J.C. (2001). Prevalence of nutritional anaemia. In : *Nutritional Anaemia*. U. Ramakrishnan (ed.). pp 7-23. CRC Press : New York.

Bruner A.B. Joffe A. Duggar A.K. Casella J.F. & Brandt J. (1996). Randomized study of cognitive effects of iron supplementation in non anemic iron deficient adolescent girls. *Lancet* 348: 992-996.

Christofar S.P. & Basiotis P.P. (1992). Dietary intakes and selected characteristics of women aged 19-50 years and their children aged 1-5 years by reported perception of food sufficiency. *Journal of Nutrition Education* 24: 53-58.

De Maeyer E. & Tegman A. (1985). The prevalence of anemia in the world. *Word Health Statistics Quarterly* 38: 302-316.

De Maeyer E. (1989). Preventing and controlling Iron deficiency anemia through primary health care. World Health Organization, Geneva.

Golder M.A. Erhardt J.G. Schebaum V. Saheed M. Biesalski H.K. & Furst P. (2001). Dietary intake and nutritional status of women and preschool children in the Republic of Maldives. *Public Health Nutrition* 4: 773-780.

Gopalan C. Shastry B.V.R. Balasubramanium S.C. Narasinga Rao B.S. Deosthale Y.G. & Pant K.C. (1996). Nutritive value of Indian Foods. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.

ICMR (1998). Recommended Dietary Allowances for Indians. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.

Jeffery R.B. Lindsay H.A. Black A.K. De Mata M. Pelto G.H. (2002). 24hr periods diet and iron status of non pregnant women in rural central Mexico. *American Journal of Clinical Nutrition* 76: 156-164.

Latham M.C. (1997). Human nutrition in the developing world. Food and Nutrition Series, pp. 147-55. Food and Agricultural Organization, Rome.

Li R. Chen X. Yan H. Deurenberg P. Garby L. & Hautvast J. (1994). Functional consequences of iron supplementation in iron deficient female cotton mill workers in Beijing, China. *American Journal of Clinical Nutrition* 59: 908-913.

NFHS (2000). National Family Health Survey (NFHS-2). International Institute for Population Sciences (IIPS) and ORC Macro, 1998-99. Mumbai.

Ramakrishnan U. (2001). Nutritional Anemias. CRC Press, New York.

Ravindran T.K.S. (1998). Women's Health in a rural poor population in Tamil Nadu. In: Women's Health in India: Risk and Vulnerability. D.M. Gupta C.L. Chen N.T. Krishnan (eds) pp 76-81. Oxford University Press. Delhi.

Shetty P.S. & James W.P.T. (1994) Body mass index: A measure of CED in Adults. FAO Food and Nutrition paper No 56. Food and Agriculture Organization, Rome.

WHO (1993) Measuring health and disease in basic epidemiology. R. Beaglehole & R. Bontia (eds.) pp. 13-30. World Health Organization, Geneva.

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Effect of Lactic Acid on Spray Drying Behavior of Acid-Whey and Study of their Glass Transition Temperature

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Spray drying of acid-whey is difficult as lactic acid in whey causes stickiness inside the dryer and during storage of the powder. This study examined effect of lactic acid on glass transition and stickiness properties of lactose-lactic acid mixtures (a model for acid-whey). Lactose solutions with various levels of lactic acid (0 to 12%, g lactic acid/100g lactose, 6 levels) were spray dried at an inlet and outlet temperatures of 180°C and 80°C, respectively. The amorphous nature of the spray-dried powder was confirmed by X-ray diffraction. Spray drying at low concentration of lactic acid was smooth with free flowing lactose powders but increasing acid concentration (>4.2%) started to affect recovery with wall deposition and agglomeration of powder. No product was recovered when lactic acid concentration reached at (~12%). Glass transition temperature of products found to decrease with increasing lactic acid concentration in lactose powder for example, 86°C and 63°C for lactose with 0% and 9.6% lactic acid, respectively. Sorption studies showed the higher lactic acid render lactose powder more hygroscopic. Crystallization and caking of lactose powders occurred even at low humidity when the concentration of the lactic acid was high.

Keywords: Acid-whey, Spray drying, Stickiness, Glass transition temperature,

Introduction

Lactose in milk powders exists as an amorphous solid that can undergo a glassy-to-rubbery transition when held at temperature higher than glass transition temperature or T_g. The amorphous solids like lactose if processed or stored at temperatures higher than their T_g tend to become soft and sticky leading to crystallization and caking that results in poor product quality, lower yield during processing, operation problems and difficulty in handling and storage of the product.

Lactose (dry) has relatively high T_g of 97 to 116°C (Hill *et al.*, 1998; Roos & Karel, 1990; Haque & Roos, 2004; Shrestha *et al.*, 2005) and unlikely to become sticky or caked under ambient condition. However, small molecules and water have less T_g values, diffuse slowly in glassy matrix and decrease the molecular mobility and viscosity (and T_g) of the materials such as lactose they are part of. Considering very low T_g value of water (-135°C) (Levine and Slade, 1986), T_g value of a food powder is a function of the moisture content (Berlin *et al.*, 1973; Levine & Slade, 1986; Jouppila & Roos, 1994a & 1994b). Organic acids such as citric acid, malic acid and tartaric acid in fruit juices and lactic acid in dairy products are some of the low molecular weight compounds that have potentially T_g lowering effect on sugar-based products (Bhandari *et al.*, 1997). Lactose is hygroscopic and absorbs water at higher humidity resulting in plasticisation and lowering of T_g and crystallization temperature (T_{cr}) (Roos & Karel, 1992).

A significant portion of milk produced in Australia is utilized for cheese production. Australia produced 374,599 tonnes of cheese and 105,225 tonnes of whey powder in the year 2004/2005 (Dairy Australia, 2006). Whey produced during cheese production has long been considered as a by-product and was first dumped as a waste. Considering the microbial pollution it generates, whey is now spray-dried and formulated into varieties of whey products, dairy ingredients with useful functional and nutritional properties. Spray drying of 'sweet whey' derived from Cheddar cheese is easy whereas the 'acid-whey', derived from cultured milk such as cottage and mozzarella cheese, with a pH of about 4.7 (acidity as high as 14%, dry basis) (Kessler, 1981), is difficult and at times impossible.

Lactic acid with T_g of -60°C (Maltini *et al*, 1967) and residual moisture severely depresses the T_g value of acid-whey (Bhandari & Hartel, 2005). Considering difficulty of spray drying acid-whey, most of the cheese industries in Australia dispose it into the farmlands, use for animal feed or simply discard into waste treatments. One possible way of spray drying of acid-whey is neutralizing the lactic acid with a caustic soda or other bases followed by mixing with a milk solid (mostly skim milk powder), concentrating and finally spray drying. There is, however, still a possibility of neutralized end product becoming sticky (personal communication with dairy industries), therefore, very few dairy industries bother to process acid-whey. There is very little scientific literature available on the effect of lactic acid on the spray drying behavior of acid-whey. Introducing a glass transition temperature concept into this field of research will enhance understanding of the problems and the process. This study examined the effect of lactic acid on the spray drying behavior of lactose, simulating acid-whey condition. The glass transition and crystallization temperature of lactose-lactic acid

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mixtures also measured to relate stickiness properties of acid-whey.

Materials and Methods

Materials

Edible grade lactose, á-lactose monohydrate, was purchased from Murray Goulburn Cooperative Co. Ltd., Melbourne, Australia. Lactic acid (88% concentration, 1.2 g/mL) was obtained from Chem-Supply, Gillman, South Australia.

Spray drying

Anhydro Lab 1 spray-dryer (Copenhagen, Denmark) with a rotary atomizer was used for spray drying of all the products. The speed of the atomizer was set at 20,000 rpm. The flow rate of the feed solution was 14-18 mL/min to achieve an inlet temperature of 180 to 185°C and outlet temperature of 80 to 85°C. The temperature of the feed was maintained at about 50°C. The room temperature during spray drying was around 20°C/°16°C (dry bulb/wet bulb). The product was collected in an insulated glass bottle connected at the end of the cyclone collector.

The basic composition of acid-whey is given in the Table 1.

Table 1 Composition of rennet (sweet) whey and acid-whey[1]

Ingredients	Rennet whey[2]	Acid-whey[3]
Water, %	93–94	94–95
Density, %	1.026 (15°C)	1.025 - 1.026 (15°C)
Fat, %	up to 0.8 (12.3)	Traces
Protein, %	up to 0.9 (13.8)	up to 0.9 (16.4)
Lactose, %	4.5–5 (69.2–76.9)	3.8–4.4 (69.1–80.0)
Lactic acid, %	Traces	up to 0.8 (14.5)
Minerals (ash), %	0.5–0.7 (7.7–10.8)	0.7–0.8 (12.7–14.5)
pH value	6.2–6.6	4.5–4.7

[1]Original table from Kessler, 1981. Dry basis calculation is done by authors.
[2]Values in parenthesis indicate % dry basis based on 6.5% total solid.
[3]Values in parenthesis indicate % dry basis based on 5.5% total solid.

It shows that the acid-whey contains up to 0.8% lactic acid on wet basis (or up to 15% lactic acid on dry basis). To study the effect of acidity on spray drying of acid-whey, initially various amounts of lactic acid were added to the lactose solution: 0 or lactose only, 2.4, 4.2, 6.0, 9.6 and 12.0% (g lactic acid/100g lactose). For all trials, 250 g of lactose was added in 1000 mL of water (with known level of lactic acid) previously heated to 50°C and stirred by magnetic stirrer. The pH of the lactose-lactic acid solutions was also measured before spray drying. The lactic acid content in spray-dried lactose-lactic acid powders was also determined by titrating against the standard 0.1N NaOH, using phenolphthalein as an indicator. The product was collected in a pre-weighed, insulated glass bottle connected at the end of cyclone collector.

The amounts of the powder collected in glass bottle (cyclone recovery) and by manual sweeping walls of spray dryer and switching the fan on to collect the powder from the cyclone (sweep recovery) were also calculated.

Total recovery was calculated by adding cyclone and sweep recovery. Powder recovery was chosen as a measure of spray drying performance as it is easily measured with reproducible results (Bhandari *et al.*, 1997). These powders were immediately vacuum packed in Cryovac®plastic bags and stored in a dry chamber containing silica gel.

Glass transition temperature (T_g) analysis

Differential Scanning Calorimetry or DSC (Pyris 1 equipped with Intracooler II, Perkin Elmer 7, CT, USA) was used to determine the glass transition temperature (T_g) and crystallization temperature (T_{cr}) of all spray-dried powders. The purge gas used was dry nitrogen (20 mL/min). The onset, midpoint and endpoint values for T_g of samples were calculated from the DSC thermogram. Indium and zinc (Perkin Elmer standards) were used for temperature and heat flow calibration. An empty aluminium pan was used as a reference. About 5-10 mg samples were scanned in hermetically sealed 50 µL DSC aluminium pans (Perkin Elmers). All analyses were done in triplicate. The rate of thermal scanning was mostly in the following order:

1) Isothermal at -20°C for 1 minute,
2) Heat scanning from -20°C to temperature just over T_g at 10°C/min,
3) Cooling rapidly -20°C at 50°C/min, and
4) Heat scanning from -20°C to 200°C.

A heating rate of 10°C/min. was chosen as a standard based on the literature. A second scanning of sample was used in this method to reduce the enthalpy relaxation of the amorphous powder which appears in the first scan, thereby enhancing the accuracy of T_g measurement on the DSC thermogram. The transfer of samples from the container to the DSC pan was done in a sealed 'Dry box' containing silica gel with regular N_2 flushing, to avoid moisture absorption by the sample.

Sorption isotherm studies

The spray-dried samples were dried overnight at 50°C in a vacuum oven followed by further drying in vacuum desiccators over P_2O_5 for two days. To make sure the powders were fully dry, these were further analysed for water activity and residual moisture content. About 2 g of amorphous powders, in triplicates, were transferred into the pre-weighed plastic cups with a screw cap and placed in evacuated desiccators over P_2O_5 (0%RH) and different saturated salt solutions of LiCl, CH_3COOH, $MgCl_2$, K_2CO_3 and $Mg(NO_3)_2$ with respective relative humidity of 11.4%, 23.1%, 33.2%, 44.1% and 52.9% giving a_w of 0.01 x %RH (Labuza *et al.*, 1985). The samples were stored for 7 days at 23-24°C in an air-conditioned room (temperature about 23°C). The samples

after equilibrium was reached were tightly closed with the screw cap, weighed and stored in a dry glass chamber containing silica gel until further analysis for DSC and X-ray diffraction. The moisture content of each sample was measured and the mean value of triplicates was calculated, as grams of water per 100g of dry sample.

Assessment of physical properties of powders

The particle size and size distribution of the spray-dried powders was measured using a Malvern Laser Diffraction Particle Size Analyser with a 100 mm lens, Malvern Mastersizer B, from Malvern Instruments Co. Worcestshire, UK. Isopropanol was used as a dispersing medium for all powders. Mechanical stirring was applied to ensure better dispersion and particle distribution. The measurements were done in triplicates. The volume-surface mean diameter (Sauter mean diameter) and the most frequent diameter (average size of 90% of all particles), were calculated from the size distribution data.

X-ray diffraction patterns of selected samples were determined between 8 to 25° on a 2-theta scale using an automated Diffractometer to obtain information on amorphousness (or crystallinity).

The viscosity of the solutions was also measured before spray drying using Brookfield Model DV-II+ Viscometer (Stoughton, MA 02072, USA). It was measured using spindle size 2 at 100 rpm, at 50°C and room temperature (25°C) in a 250 ml beaker.

The moisture content of the freshly spray-dried powders was measured by AOAC method 927.05 (AOAC, 1990). Water activity of the powders was measured by using AquaLab 3 Water Activity Meter (Decagon Devices, Inc., Pullman, USA) at 25°C.

Results and Discussions

Spray drying behaviour and powder recovery

The recovery of lactose/lactic acid mixtures during spray drying is given in Table 2. It shows that at low lactic acid concentration (up to 4.2%, g lactic acid/100g solid or about 1%, g lactic acid/100mL lactose solution), lactose powders were free flowing and had a reasonably good cyclone recovery (52%). Higher lactic acid concentrations (above 4.2%) found to affect the recovery of the product as more powder deposited on the wall of the drier, particularly on the conical part of the spray dryer. These powders, however, could be recovered by manual sweeping which means the total recovery remained high. With further increase in the lactic acid concentration, the powders in the cyclone collector started to stick together forming sticky and flaky masses. When the acid concentration reached 12%, no powder was recovered (except thick sticky mass) as most of the spray-dried lactose was firmly deposited on the spray dryer wall.

Table 2 Recoveries of powders during spray drying of lactose/lactic acid system

Samples[1]	% Product recovery[2]	Powder characteristics
Lactose only	79 (88)	No deposition on wall, free flowing powders
Lactose with 2.4% LA	77 (93)	No deposition on wall, free flowing powders
Lactose with 4.2% LA	52 (90)	Slight deposition on wall, free flowing powders
Lactose with 6.0% LA	44 (95)	More deposition on wall, free flowing powders with slight agglomeration
Lactose with 9.6% LA	41 (87)	More deposition on wall, free flowing powders with slight agglomeration and flaking
Lactose with 12% LA	No recovery	No powder. Very sticky mass deposited on the wall and cyclone collector

[1]Based on weight of lactic acid added to 220 g lactose (in 880 g water)
[2]Product recovered in cyclone collector. Values in parenthesis are total recovery i.e. cyclone + sweep recovery

The result showed that a low level of lactic acid (below 4.2%) does not significantly affect the spray drying behaviour and powder characteristics of the lactose or whey but the actual problem starts when the lactic acid content reaches about 5%. Acid-whey has a pH of about 4.7 and titratable acidity 0.8% (up to 14% dry basis) (Kessler, 1981). Lactic acid is highly hygroscopic, exists in liquid state at room temperature (melting point 17°C), and has a very low T_g at -60°C (Maltini *et al.*, 1967). The product containing a significant amount of lactic acid, therefore, will be hygroscopic and sticky. The effect of having high lactic acid in the product is as bad as having high water content from the point of view of stickiness. This makes the drying of acid-whey very difficult. The presence of moisture in the powder further reduces the T_g of the lactose thereby further aggravating the stickiness. For example, the lactose solution with 12% acidity could no longer be dried and gave very thick paste (hardened) product. It took a great deal of time and effort to clean the spray dryer wall as it was coated with a thick layer of acidified lactose solution (or paste).

Considering lower boiling point (122°C) of lactic acid, some degree of evaporation of lactic acid was expected during spray drying at 180°C. However, the lactic acid content of the spray-

dried lactose-lactic acid powders was very close to the content prior to spray drying (results not shown). It is likely that lactic acid becomes encapsulated in the lactose system, as does flavour in many spray-dried foods, and is retained in the powder.

The pH of lactose, 2.4, 4.2, 6.0 and 9.6% lactic acid containing lactose powders were 5.9, 2.71, 2.58, 2.57 and 2.35 respectively. The result showed that the pH value starts to plateau after lactic acid concentration reached concentration.

Physio-chemical properties

The viscosity of lactose solution was 22 m.Pa.s and 8.8 m.Pa.s at 25°C °C and 50°C respectively. Analysis of viscosity of lactose solutions with various lactic acid concentrations showed little change in viscosity. It indicated that there is no measurable effect of lactic acid in viscosity of the lactose or whey solution. The particle size diameter (Sauter mean) of spray-dried lactose was 18 µM. The particle size diameter of lactose powders with added lactic acid could not be accurately determined due to the highly hygroscopic and subsequent sticky nature of the powder. These particles tend to stick together forming small clumps. The repeatability of the measurement was low as the sizes of the particles were varied, from fine to various sized clumps, although appear as free flowing. Table 3 shows the moisture content, water activity and colour profile of the acidified lactose powders. There was no drastic change in the colour characteristics of these powders with increase in acid concentration. The lightness (L) value seemed to increase slightly with acidity. In fact the powder with higher acidity appeared to be fluffy white that was easily distinguishable from the spray-dried lactose powder. There was a slight decrease in 'a' value and increase in 'b' value suggesting increased greenness and yellowness due to increased acidity in lactose. It is likely that high lactic acid content in lactose might have induced some hydrolysis of lactose leading to formation of glucose and galactose and subsequent caramellization.

The moisture content and water activity of lactose with added lactic acid, from cyclone and sweep, is given in Table 3. As expected, the products from sweep had a lower moisture and water activity than the ones from the cyclone. A longer stay in the drying chamber at higher temperatures might have removed water, resulting in drier powders. Some crystallisation of lactose is also possible in this powder. Due to dryness, the powders collected from the sweeping were less sticky and free flowing compared to their cyclone counterparts.

Table 3 Moisture content and colour characteristics of acidified lactose[1, 2]

% Lactic acid	%, Moisture, (a_w) [from cyclone]	% Moisture, (a_w) [from sweep]	Colour characteristics		
			'L'	'a'	'b'
0	2.3±0.1 (0.15)	1.3±0.0 (0.11)	95.7±0.8	-1.7±0.32	6.2±0.8
2.4	2.1±0.1 (0.14)	0.9±0.0 (0.10)	97.8±0.4	-1.5±0.18	5.2±0.5
4.2	4.2±0.2 (0.15)	1.3±0.1 (0.07)	98.4±0.2	-2.3±0.6	7.3±0.1
6.0	2.6±0.3 (0.14)	0.9±0.0 (0.07)	98.1±0.1	-2.1±0.54	7.2±1.0
9.6	2.1±0.0 (0.15)	1.2±0.0 (0.07)	NA[3]	NA	NA

[1]Mean values±standard deviation (of duplicate samples)
[2]The values in parenthesis is water activity at 24.6°C.
[3]Color not measured.

Glass transition temperature and crystallization temperature

The T_g value of spray-dried lactose was 83°C (Table 4), which is well below the generally reported values of 97 to 116°C (for dry lactose) (Hill *et al.*, 1998; Jouppila and Roos, 1994a; Roos and Karel, 1990; Shrestha et al., 2005). However, the lactose had 2.3% moisture content. The plasticisation effect of water on lactose is responsible for the low T_g value in spray-dried lactose. Lactose with added lactic acid was found to have lower T_g values than lactose. There was a clear trend of decreasing T_g values with increasing lactic acid concentration in the acidified lactose samples (Figure 1). The present result also shows the plasticising effect of lactic acid on the T_g value of lactose (Table 4).

Table 4 Glass transition temperature of lactose-lactic acid system[1]

%Lactic acid	Moisture content	Glass transition temperature (T_g), °C			Crystallization temperature (T_{cr}), °C		
		Onset	Midpoint	Endset	Onset	Midpoint	Endset
0	2.3	80.5±1.1	83.2±1.5	86.0±1.9	138.6±1.0	140.8±0.9	142.4±1.1
2.4	2.1	74.7±1.7	78.1±1.6	81.3±1.8	131.3±1.3	132.4±1.2	133.9±1.3
4.2	4.2	70.5±2.3	73.3±1.6	76.2±1.9	124.7±1.2	126.4±0.9	128.1±1.2
6.0	2.6	63.3±2.4	66.6±2.3	69.4±2.5	111.0±3.7	113.4±3.6	116.6±3.2
9.6	2.1	59.6±3.0	61.4±2.1	63.0±1.3	96.3±0.8	98.0±0.9	100.9±1.3
9.6[2]	1.2	73.0±3.1	77.0±2.1	80.8±1.6	128.3±2.1	130.9±2.1	134.2±1.7

[1]Mean values±standard deviation (of four replicates)
[2]Sample from sweep recovery

Table 4 shows that the T_g value of lactose with 9.6% lactic acid collected in the cyclone and from sweep were 61.4°C and 77°C, respectively. Obviously, the powder collected inside the spray drying chamber had the lower moisture content (1.2%) and consequently the lower T_g value compared to the one collected in the cyclone (2.1%). The powder collected from inside the spray dryer was smooth and non sticky whereas the one in the cyclone had already started caking (in small flakes). Based on recovery results, a tentative diagram showing the effect of glass transition temperature and difficulty in drying due to lactic acid content of lactose is given in Figure 1.

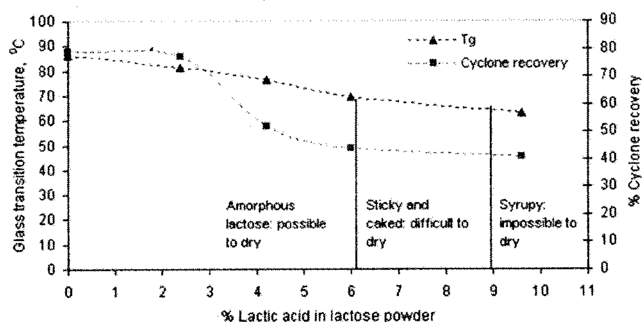

Figure 1 Relationship between lactic acid percentage in lactose, cyclone recovery and glass transition temperature.

Lactic acid is highly hygroscopic in nature and has a low melting point of 17°C (MSDS, 2004). Amorphous lactose is also hygroscopic in nature and the presence of lactic acid rendered it more susceptible to moisture absorption. The handling of high lactic acid containing lactose was difficult under normal room conditions (20°C and 60 %RH) as it readily absorbs the moisture and becomes sticky followed by caking and subsequent minute crystal formation. It was noticed that by the time the powder was transferred from the container to the DSC pan, weighed and covered, some sample absorbed enough moisture to liquidify the lactose particles. There was a significant variation in T_g values of the triplicates, probably due to uneven moisture levels in the sample. It posed a lot of problem in our initial studies with lactose-lactic acid powders and some tests had to be abandoned. To minimize the moisture absorption from surrounding, a low humidity chamber (RH<20%) was built which can be flushed with dry Nitrogen gas, (named as DRY BOX,). It certainly reduced moisture sorption from surrounding but the lactose-lactic powders were still so hygroscopic that moisture sorption from surrounding was inevitable.

The instant crystallization temperature of lactose measured using the DSC was 140.8°C at the given moisture content (Table 4). Literature data on crystallization temperature (T_{cr}) of dry lactose vary with the researchers, depending on the 'dryness' of the lactose, DSC scanning rate and type of DSC pan (sealed/puncture) (Haque and Roos, 2004). Roos and Karel (1991) reported a lactose crystallization temperature of 167°C. As with T_g values, lactic acid was found to depress the T_{cr} value of lactose. The difference in temperature between T_{cr} and T_g decreased with increased lactic acid levels in powder *e.g.*, 54.3 to 34.6°C when the lactic acid concentration increased from 2.4 to 9.6 %.

Sorption isotherm of the powder

Figure 2 shows the sorption isotherm of spray-dried lactose and lactose acidified with 2.4 to 9.6% lactic acid, held at water activities from 0 to 0.441 for 7 days at room temperature. Lactose exhibited a typical sorption isotherm curve with steep curve and no signs of commencement of crystallization at a_w e" 0.332. After maximum absorption of water, lactose molecules start to re-align and form crystals, resulting in release of water from the crystal matrix (which is absorbed by the saturated salt solution).

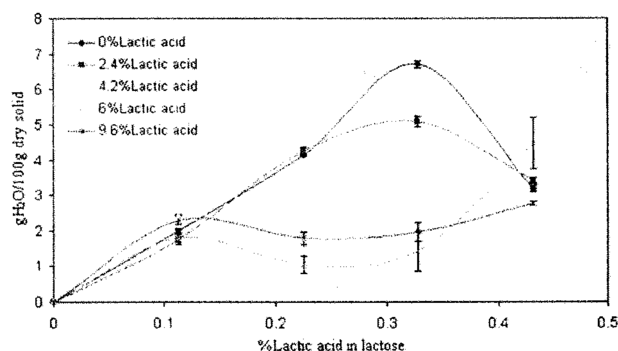

Figure 2 Sorption isotherm of lactose-lactic acid system sorbed at 24°C for 7 days.

The crystalline structure of lactose was further confirmed by X-ray diffraction analysis (Figure 3). Increasing lactic acid content in lactose powder to 4.2% and higher shifted crystallization at lower water activities e.g., a_w e" 0.114. Further humidification of these samples for one more week resulted in more water absorbed by the powders and shifting of crystallization point to lower water activities, particularly at higher acidity (results not shown). The moisture absorption by lactose was less uniform when the storage time increased. This may be due to variable level of crystallisation of lactose in the samples.

Lactic acid along with water will form compatible mixture with lactose and will have plasticising effect on lactose molecules. Therefore, in the presence of lactic acid, the mobility of lactose molecules will increases as a result of lower T_g value, as a consequence, the crystallization of lactose will occur at lower water activity. The sorption studies showed that even if acid-whey is successfully spray-dried, it is likely to be sticky during handling, transport and storage. Therefore, extreme care is needed while packaging and storage of the acid-whey powder as slight increase in %RH (particularly

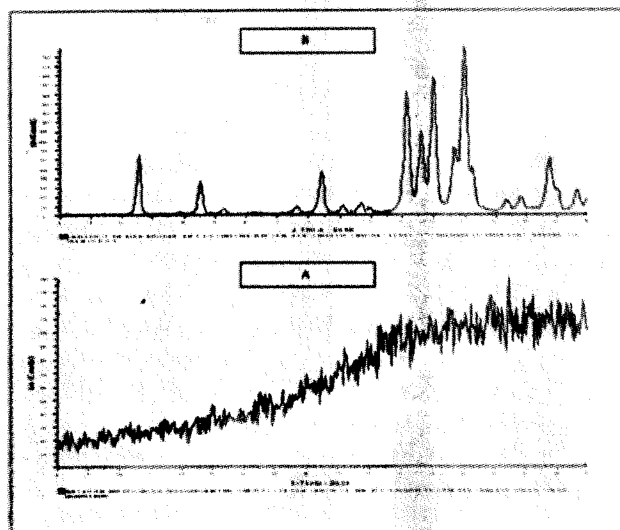

Figure 3. X-ray diffraction pattern of spray dried lactose humidified at aw 0.80 (A, amorphous structure) and aw 0.441 (B, crystalline structure).

above 30%) and temperature will induce crystal formation in powder.

Conclusions

Spray drying of acid-whey is difficult because of an increase in lactic acid concentration will reduce the glass transition temperature significantly. The upper limit for spray drying of acid-whey can be 5% lactic acid (in dry basis). No product could be recovered when lactic acid concentration in lactose solution reached to 12%. Lactic acid renders the product more hygroscopic. Even if lactose-lactic acid solution or acid-whey is spray-dried successfully, it will absorb moisture very quickly if the surrounding environment has a high % relative humidity. The critical water activity to induce crystallisation of lactose is also reduced by the presence of lactic acid. This means that the caking of the powder can occur at much lower moisture content due to presence of lactic acid.

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References

AOAC International (1990). *Official Methods of Analysis of AOAC*, AOAC International, Gaithersburg, MD.

Berlin, E., Anderson, B. A. & Pallansch, M. J. (1973). Water sorption by dried dairy products stabilized with carboxymethyl cellulose. *Journal of Dairy Science*, 53: 1339-1344.

Bhandari, B. R., Datta, N. & Howes, T. (1997). Problems associated with spray drying of sugar-rich foods. *Drying Technology*, 15 (2): 671-684.

Dairy Australia (2006). Production and Manufactured Dairy Products of 05/06 by Products. http://www.dairyaustralia.com.au/Content/Markets_and_Trade/Australian_Dairy_Industry/Manufactured_products/ProductionSummary/CheeseProductionByType.xls.

Haque, M. K. & Roos, Y. H. (2004). Water plasticization and crystallization of lactose in spray-dried lactose/protein mixtures. *Journal of Food Science*, 69: 23-29.

Hill, V. L., Craig, D. Q. M. & Feely, L. C. (1998). Characterization of spray-dried lactose using modulated differential scanning calorimetry. *International Journal of Pharmaceutics*, 161: 95-107.

Jouppila, K. & Roos, Y. (1994a). Water sorption and time-dependent phenomena of milk powders. *Journal of Dairy Science*, 77: 1798-1808.

Jouppila, K. & Roos, Y. (1994b). Glass transition and crystallization in milk powder. *Journal of Dairy Science*, 77: 2907-2915.

Jouppila, K., Kansikas, J. & Roos, Y. H. (1997). Glass transition water plasticization, and lactose crystallization in Skim Milk Powder. *Journal of Dairy Science*, 80: 3152-3160.

Kessler, H. G. (1981). *Food Engineering and Dairy Technology*, Verlag A. Kessler, Munich, Germany.

Labuza, T. P., Kaanane, A. & Chen J. Y. (1985). Effect of temperature on the moisture sorption isotherms and water activiy shift of two dehydrated foods. *Journal of Food Science*, 50: 385-391.

Levine, H. & Slade, L., (1986). A polymer physico-chemical approach to the study of commercial starch hydrolysis products (SHPs). *Carbohydrate Polymers*, 6: 213.

Maltini, E. Anese, M. & Shtylla, I. (1967). State diagram of some organic acid-water systems of interested in food. *Cryo-Letters*, 18 (5): 263.

MSDS (2004) *MSDS for Lactic acid*, http://www.jtbaker.com/msds/englishhtmL/ l0522.htm

Roos, Y. & Karel, M. (1991). Plasticizing effect of water on thermal-behaviour and crystallization of amorphous food models. *Journal of Food Science*, *56* (1): 38-43.

Roos, Y. & Karel, M. (1992) Crystallization of amorphous lactose. *Journal of Food Science* 57: 775-777.

Roos, Y. H. & Karel, M. (1990). Differential Scanning Calorimetry study of phase transition affecting the quality of dehydrated materials. *Biotechnology Progress*, 6: 159-163.

Shrestha, A. K., Adhikari, B., Howes, T. & Bhandari, B. (2005). Glass transition and crystallization behaviour of lactose in spray-dried mixtures of hydrolysed whey protein and lactose, In *Proceeding of 4th Asia Pacific Drying Conference*, 13-15 December, 2005, Kolkata, India (pp. 1118-1129).

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Effect of Microbial Transglutaminase and Sodium Caseinate on Mechanical Properties of Batter Gel as Dependent on Cooking Method

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The effect of three cooking methods (water bath, retort and microwave) on the binding and textural properties of pork gel processed with (2%) or without sodium caseinate in the presence (0.25% or 0.5%) or absence (control) of microbial transglutaminase (MTGase) was investigated. The MTGase and sodium caseinate treatment increased ($P<0.0001$) textural and binding properties (e.g., hardness, fracturability, gumminess, gel strength and breaking force) as measured by compression and punch tests. Microwave cooked mixed gels had the lowest hardness and fracturability, however highest springiness and cohesiveness than retort and water bath cooked samples. The result suggests that addition of sodium caseinate and MTGase influence the binding and textural properties but the effectiveness of the MTGase treatment depended on the cooking methods.

Keywords: MTGase, sodium caseinate, cooking method, binding, textural

Introduction

Meat is a complex food with a highly structural nutritional composition. It becomes edible and more digestible when it is subjected to cooking. Generally speaking, cooking is one of the most important factors influencing the quality of meat products due to series of chemical and physical reactions, as cooking changes certain texture and flavours. In addition, non-meat ingredients derived from a variety of plant and animal sources are commonly used in comminuted meat products to modify the functional properties of the products, including emulsification, water and fat binding capacity, texture and appearance (Whiting, 1988). Sodium caseinate alone or combined with other ingredients, have been widely used in a variety of comminuted meat product (Pietrasik & Jarmoluk, 2003; Kilic, 2003; Yusof & Babji, 1996; Marco, Navarro & Flores, 2004) to form gels and retain water and to provide a desirable texture. The effectiveness of the different functional ingredients, however, can vary greatly with the cooking methods, specially the rate of heating. Physical properties, such as breaking force and strength, of resultant gels are influenced by heating rate and final gelling temperatures (Foegeding et al., 1986; Xiong & Brekke, 1991).

The most dramatic changes in meat during heating, such as shrinkage, tissue hardening, juice release, and discolouration, are caused by the changes in muscle protein denaturation (Bowers et al., 1987). During meat cooking, the time temperature relationship affects the resulting tenderness strongly, as well as several other quality factors such as juiciness, colour and flavour (Prestat et al., 2002). Similarly, cross-linking of food proteins can influence many properties of food (Dickinson, 1997), including texture viscosity, solubility, emulsification and gelling properties (Kuraishi et al., 2001). Transglutaminase is a protein cross-linking enzyme, which catalyses the acyl-transfer reaction between the ã-carboxyamide group of peptide bound glutamine residues and various primary amines (Motoki & Seguro, 1998). Therefore, the objective of this study was to examine the combined effect of sodium caseinate and microbial transglutaminase on binding and textural properties of pork gel as a function of cooking methods.

Materials and methods

Materials

Post rigor pork leg meat (semimembranous muscles) was purchased from a local market 24-36 h post mortem (pH 5.9). The pork was trimmed of visible fat and connective tissue, and then ground in a laboratory grinder (JCW 6 Shanghai Instrument Company LTD) through a plate with 3 mm diameter orifices. The ground meat was portioned, vacuum packaged and frozen at -20°C until product formulation. The ingredients used in the homogenate formulations included sodium chloride, sodium nitrite, sodium ascorbate, sodium tripolyphosphate. All the ingredients were of analytical grade (Sinopharm Chemical Reagent Co Ltd, Shanghai, P. R. China). Sodium caseinate was provided by Hualing Milk Products Group, Gansu Province, P.R.China (Protein min 90 % dry basis).

Microbial transglutaminase (MTGase) (Yiming Fine Chemical Plant, Gensi, Jiangsu, P.R.China) was a mixture containing 90, 9, and 1 % maltodextrin, sodium caseinate and microbial transglutaminase, respectively (activity of 65 u/g). Enzymatic activity was measured by the hydroxamate procedure (Folk & Cole, 1966) with CBZ (Carbobenzoxy)-L-glutaminylglycine (Sigma-Aldrich Chemie, GmbH Germany). The enzyme concentration is reported in the present study as the commercial concentration.

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Protein analysis

The protein concentration of the ground pork was determined by the AOAC (1997) procedures (928.08). Nitrogen values were converted to protein using a conversion factor of 6.25. The protein content of the pork meat was 21.7 %.

Preparation of pork batter gel

Pork batter gel was prepared by the method of Pietrasik & Li-Chan (2002a) Before processing meat was tempered at 4°C and meat protein content was adjusted to a constant level of 10 % with added ice & water (50:50) in all formulations. Composition of pork batter formulations are shown in Table 1. Sodium caseinate was hydrated with chilled brine for 30 min before mixing with other ingredients. Treatments (300g each) were prepared by mixing ground meat and other ingredients for 15 seconds on high speed food blender (model DS-1 Shanghai Instrument Company LTD, P.R.China). The levels of sodium chloride, tripoly phosphate, nitrite and ascorbate were 2.5, 0.2, 0.01 and 0.05 %, respectively. Concentrations of MTGase in separate formulations were MTGase free as control, 0.25 and 0.5 %.

Table 1 Composition (% by weight) of the pork batter formulations

Batter	Meat	Sodium caseinate	MTGase	Ice & Water	Others*
	46.08	-	Control	51.16	2.76
No 1	46.08	-	0.25	50.91	2.76
	46.08	-	0.5	50.66	2.76
	46.08	2	Control	49.16	2.76
No 2	46.08	2	0.25	48.91	2.76
	46.08	2	0.5	48.66	2.76

*0.01, 0.05, 0.2 & 2.5 % sodium nitrite, sodium ascorbate, sodium tripolyphosphate and sodium chloride, respectively.

Immediately after homogenate preparation, the batter was stuffed in 30mm×120mm poly-vinyl casings (Yurun Meat Company, Nanjing, P. R. China). The casings were tightly closed and allowed to stand overnight in a cold room at 4 °C. The separate homogenate samples were then cooked in three heating methods, isothermally at 90°C in a water bath for 15 min or autoclaved at 121°C for 15 min (pressure 1.05MPa) or cooked in house hold Microwave oven (model Galanz WD 800T with an adjusted electric output power at medium and operating frequency 2450 MHz (85°C)) for 15 min and thereafter cooled down in ice water until a core temperature of 20°C was reached. The gel samples were stored at 4 °C until analyzed. Three replicate pork batter gels were prepared and three measurements were taken from each replicate.

Mechanical properties

Gel samples (25 mm diameter & 30 mm height) were equilibrated to room temperature for 30 min in a plastic bag to avoid dehydration before the mechanical properties were measured. The mechanical properties were determined using a TA-XT2i Stable Micro Systems Texturometer (England). The texture expert version 1.2 software was used to collect and process the data.

Punch test

The puncture test was performed, compressing samples to 75% of the initial height using a compression speed of 60 mm min^{-1} and a cylinder probe (P/20) with 1.2 cm diameter. The breaking force (g), deformation (cm) and gel strength (g × cm) for each treatment were measured. Nine samples were analyzed for each treatment.

Texture profile analysis

Texture profile analysis (TPA) (Bourne, 1978) was performed using aluminium cylindrical probe (P/50) with 50 mm diameter. Gel samples were compressed twice to 25% of their original height at a constant cross head speed of 60 mm min^{-1}. The TPA parameters, namely hardness [peak force on first compression (g)], springiness [distance the sample recovered after the first compression (mm)], fracturability [first bite, the force required to produce the first fracture (g)], cohesiveness [ratio of the active work done under the second force-displacement curve to that done under the first compression curve (dimensionless)], gumminess (hardness × cohesiveness) and chewiness [hardness × cohesiveness × springiness (g×mm)] were computed.

Statistical analysis

The experimental design was a complete randomized 2×3×3 factorial with two batter formulations (with or without sodium caseinate), three MTGase levels (MTGase free as control, 0.25 and 0.5 %) and three cooking methods (Water bath, Retort and Microwave). Reported data are means of nine measurements, three replicates with triplicate measurements. Data were analyzed by analysis of variance (ANOVA) using the generalized linear model procedure of the SAS system for windows version 8.1. When ANOVA showed significant treatment effects ($P<0.05$), mean separation was done by using the Duncan test.

Results and discussion

Punch test

The mechanical properties of heat induced gels as measured by punch test, increased with increasing MTGase level (control to 0.5%) and sodium caseinate level (0 to 2%) shown

in Figure 1. Under the same cooking treatment, addition of MTGase and sodium caseinate exhibited higher breaking force, deformation and gel strength. Retort cooked gel samples showed lower breaking force (708.8 g) and gel strength (655 g × cm) than water bath and microwave cooked samples.

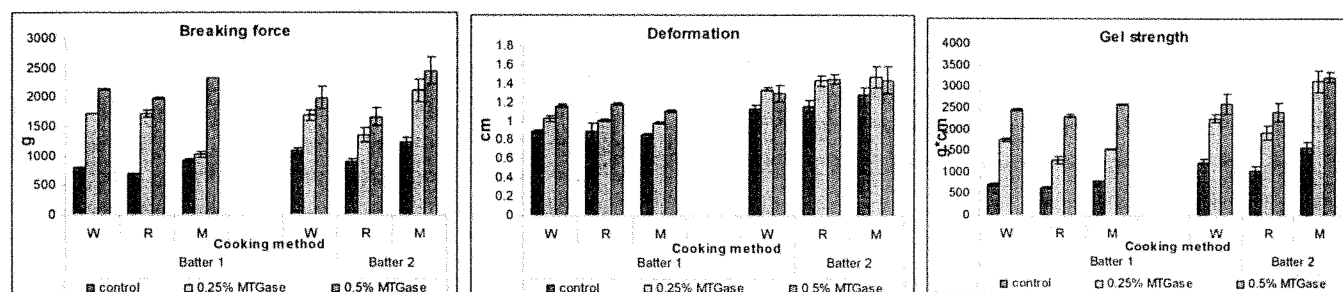

Fig 1. Effect of MTGase level and cooking methods on punch test parameters of batter gel processed with (batter 2) or without (batter 1) sodium caseinate (W= water bath, R= retort, M= microwave). Bars show standard deviation.

Pressurization may cause denaturation and/or aggregation (Cheah & Ledward, 1996), which could limit heat gelation in meat batters so that samples are less hard (Carballo et al., 1996). The disruption of myofibrillar proteins is mainly responsible for the tenderizing effect and responsible for the heat-induced aggregation involved in gelling and binding mechanism (Bouton et al., 1977). All the punch test parameters had a coefficient of determination (R2) higher than 0.90 and analysis of variance (Table 2) demonstrated that addition of sodium caseinate, MTGase and cooking method had significant effects (P<0.0001) on all test parameters. Samples without caseinate, the breaking force was in the range of 708 to 2331 g. The gel strength varied from 722 to 2595 g × cm wh.le sodium caseinate added batter, the breaking force and gel strength varied from 903 to 2461 g and 1051 to 3232 g × cm, respectively. In addition significant interaction between type of batter (with or without sodium caseinate), MTGase and cooking method occurred.

Table 2. Mean values of the punch test properties of pork batter gel as influenced by sodium caseinate, MTGase and cooking methods.

	Breaking force (g) $R^2 = 0.9435$	Deformation (cm) $R^2 = 0.9363$	Gel strength (g*cm) $R^2 = 0.9372$
Batter (A)			
No 1 (without SC)	1387.29[a]	1.11[a]	1555.97[a]
No 2 (with SC)	1614.19[b]	1.33[b]	2160.21[b]
P-Value	P<0.0001	P<0.0001	P<0.0001
MTGase (B) %			
control	947.60[a]	1.13[a]	1076.60[a]
0.25	1607.40[b]	1.26[b]	2065.10[b]
0.5	1947.23[c]	1.27[b]	2432.57[c]
P-Value	P<0.0001	P<0.0001	P<0.0001
Cooking method (C)			
Water bath (W)	1575.16[a]	1.14[a]	1841.94[a]
Retort (R)	1241.92[b]	1.33[b]	1676.01[b]
Microwave (M)	1685.15[c]	1.19[c]	2053.31[c]
P-Value	P<0.0001	P<0.0001	P<0.0001
Interaction P-Value			
A×B	P<0.01	P<0.001	P<0.05
A×C	P<0.001	P<0.0001	P<0.0001
B×C	P<0.0001	P<0.001	P<0.0001
A×B×C	P<0.0001	P<0.0001	P<0.0001

[abc] different letters in the same column are significantly different (P<0.05) at treatment level. Ns= not significant (SC = Sodium caseinate)

Texture profile analysis

Texture properties of pork batter were affected by sodium caseinate, MTGase and cooking method. However, a different range of values was obtained by the type of cooking method used in same batter formulation with or without MTGase levels. Fig 2. shows the results of texture profile analysis on both batter samples in different MTGase and cooking method and Table 3 indicates the mean values of the textural properties of pork batter as influenced by sodium caseinate, MTGase and cooking method. Batter formulations containing MTGase showed significant (P<0.0001) increase in hardness, fracturability, cohesiveness, chewiness and gumminess. Cooking method also had significant influence (P<0.0001) on hardness, (P<0.01) gumminess, chewiness, springiness and cohesiveness parameters of batter gels. However, heating media did not produce any significant effect on fracturability. In addition, for all of these textural parameters except few combinations, significant interactions between batter type, MTGase and cooking method occurred.

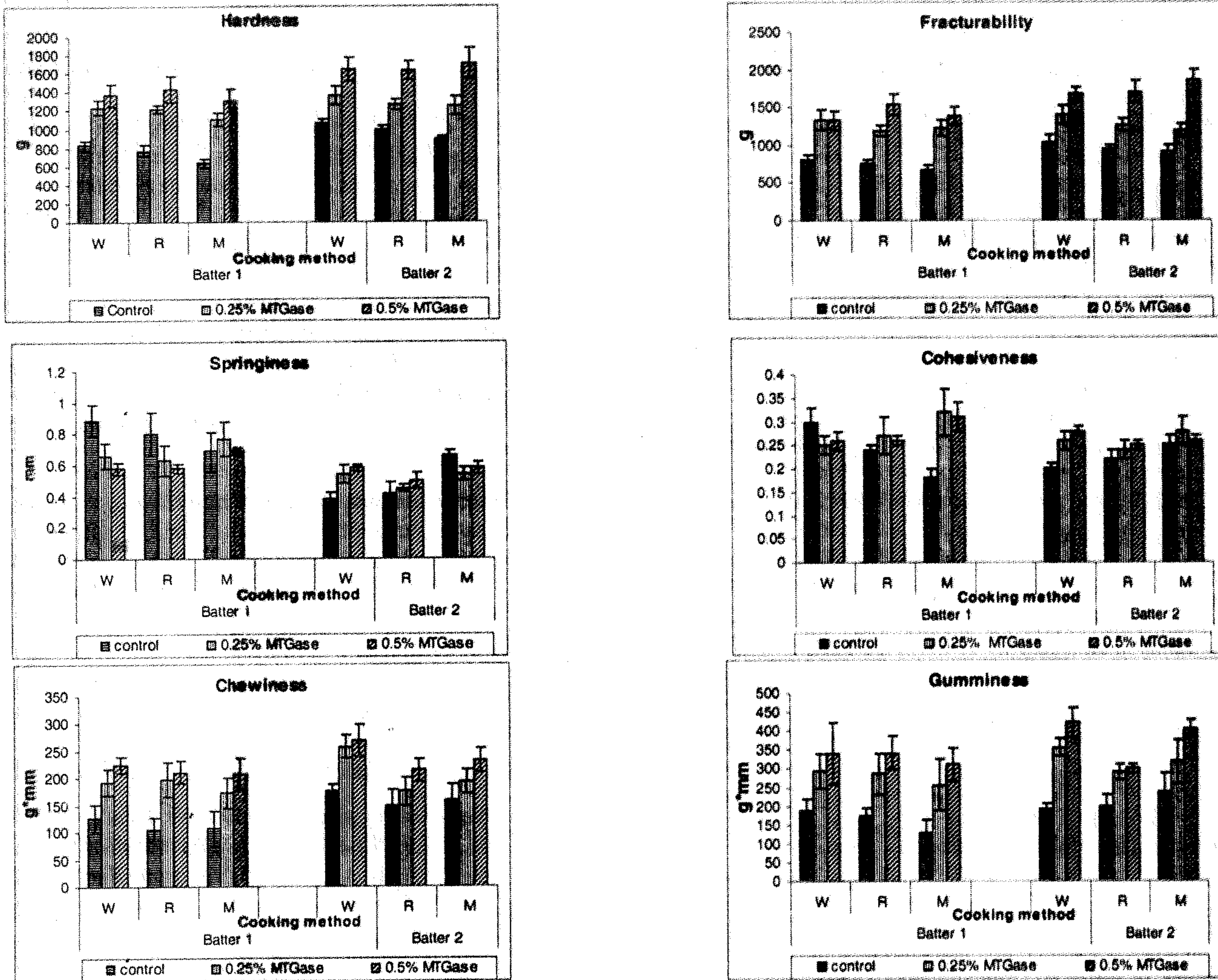

Fig 2. Effect of MTGase level and cooking methods on texture profile parameters of batter gel processed with (batter 2) or without (batter 1) sodium caseinate (W= water bath, R= retort, M= microwave). Bars show standard deviation.

The springiness represents the elastic properties of the batter. The higher the springiness, the more elastic or rubbery characteristics the product will exhibit. The microwave cooked samples exhibited higher springiness as compared to the retort and water bath cooked samples. Pietrasik and Li-Chan (2002b) reported that gels processed with increasing MTGase level exhibited higher hardness and chewiness in relation to ones processed without or with lower levels of MTGase. Hammer (1998) also reported that finely comminuted sausages containing 0.2% MTGase were harder and firmer in comparison to sausages produced without MTGase. Our results also support the findings of the above authors. Based on texture profile analysis results, Killic (2003) demonstrated that a döner kebab made with sodium caseinate and MTGase had higher hardness and chewiness. Our results showed that hardness value was increased with addition of MTGase for all samples. If the hardness value is high the product is tough. If the hardness value is low, the product is

Table 3. Mean values of the texture profile parameters of pork batter gel as influenced by sodium caseinate, MTGase and cooking methods.

	Hardness (g) R^2=0.9115	Fracturability (g) R^2 = 0.8751	Springiness (mm) R^2 =0 .8243	Chewiness (g×mm) R^2 =0.8576	Cohesiveness R^2 =0.7623	Gumminess (g) R^2 = 0.8478
Batter (A)						
No 1 (without SC)	1101.92[a]	1130.28[a]	0.70[a]	177.61[a]	0.27[a]	265.14[a]
No 2 (with SC)	1309.88[b]	1330.47[b]	0.52[b]	185.31[a]	0.25[b]	301.85[b]
P-Value	P<0.0001	P<0.0001	P<0.0001	Ns	P<0.001	P<0.0001
MTGase (B) %						
control	864.33[a]	852.37[a]	0.64[a]	127.14[a]	0.23[a]	197.18[a]
0.25	1238.12[b]	1265.44[b]	0.60[ab]	198.54[b]	0.27[b]	316.68[b]
0.5	1515.26[c]	1573.30[c]	0.59[b]	218.72[c]	0.27[b]	336.63[b]
P-Value	P<0.0001	P<0.0001	P<0.01	P<0.0001	P<0.0001	P<0.0001
Cooking method (C)						
Water bath (N)	1250.87[a]	1257.28[a]	0.60[a]	199.81[a]	0.26[a]	309.41[a]
Retort (P)	1218.63[a]	1230.26[a]	0.56[a]	165.38[b]	0.25[b]	264.97[b]
Microwave (M)	1148.22[b]	1203.57[a]	0.66[b]	179.20[b]	0.27[a]	276.12[b]
P-Value	P<0.0001	Ns	P<0.01	P<0.01	P<0.01	P<0.01
Interaction P-Value						
A×B	P<0.001	P<0.0001	P<0.0001	P<0.001	Ns	P<0.001
A×C	Ns	Ns	P<0.01	Ns	Ns	Ns
B×C	P<0.05	P<0.0001	Ns	Ns	P<0.0001	Ns
A×B×C	Ns	Ns	P<0.0001	P<0.0001	P<0.0001	P<0.0001

[abc] different letters in the same column are significantly different (P<0.05) at treatment level. Ns = not significant (SC = Sodium caseinate)

soggy (Laycock et al., 2003). When the temperature of the cooking medium was above 85°C the average hardness was always high, regardless of processing time, even for small pieces of meat (Califana et al., 1997).

Conclusion

The results of this research indicate that addition of sodium caseinate and MTGase level is critical in ensuring binding and textural characteristics of processed batter gels. In addition different cooking method also had detrimental effect on mechanical and textural properties. Results showed that the addition of sodium caseinate was good for non-muscle protein substrate for the MTGase due to high degree of cross-linking to myosin. The results suggested that MTGase treatment influenced the mechanical properties of batter gels; however the effectiveness of the enzyme treatment dependent on the cooking methods.

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References

AOAC (1997). *Official methods of analysis* (17th Ed) Washington, DC, USA: Association of Official Analytical Chemists.

Bourne, M. C. (1978). Texture profile analysis. *Food Technology, 32*(7), 62-66,72.

Bouton, P.E., Ford, A.L., Harris, P.V., Macfarlane, J.J. & O'shea, J.M. (1977). Pressure-heat treatment of post rigor muscle: Effects on tendeness. *Journal of Food Science, 42*(1), 132-135.

Bowers, J. A., Craig, J. A., Kropf, D. H. & Tucker, T. J. (1987). Flavour, colour and other characteristics of beef longissimus muscle heated to seven internal temperatures between 55° and 85°C. *Journal of Food Science, 52*(3), 533-536.

Califano, A. N., Bertola, N. C., Berilaqua, A. E. & Zaritzky, N. E. (1997). Effect of processing conditions on the hardness of cooked beef. *Journal of Food Engineering, 34*(1), 41-54.

Carballo, J., Fernandez, P. and Jimenez-Colmenero, F. (1996). Texture of uncooked and cooked low and high fat meat batters as affected by high hydrostatic pressure. Journal of Agricultural Food Chemistry, 44(7), 1624-1625.

Cheah, P.B. & ledward, D.A. (1996). High pressure effects on lipid oxidation in minced pork. *Meat Science, 43*(2), 123-134.

Dickinson, E. (1997). Enzymic cross-linking as a tool for food colloid rheology control and interfacial stabilization. *Trends in Food Science & Technology, 8*(10), 334-339.

Foegeding, E. A., Allen, C. E. & Dayton, W. R. (1986). Effects of heating rate on thermally formed myosin, fibrinogen and albumin gels. *Journal of Food Science, 51*(1), 104-108.

Folk, J. E. & Cole, P. W. (1966). Mechanism of action of guinea pig liver transglutaminase. *Journal of Biological Chemistry, 241*(23), 5518-5525.

Hammer, G. F. (1998). Microbial transglutaminase and diphosphate in finely comminuted cooked sausage. *Fleischwirtschaft, 78*, 1155-1156, 1159-1162, 1186.

Killic, B. (2003). Effect of microbial transglutaminase and sodium caseinate on quality of chicken doner kebab. *Meat Science, 63*(3), 417-421.

Kuraishi, C., Yamazaki, K. & Susa, Y. (2001). Transglutaminase: Its utilization in the food industry. *Food Review International, 17*(2), 221-246.

Laycock, L., Piyasena, P. & Mittal, G. S. (2003). Radio frequency cooking of ground comminuted and muscle meat products. *Meat Science, 65*(3), 959-965.

Marco, A., Navarro, J. L. & Flores, M. (2004). Volatile compounds of dry-fermented sausages as affected by solid-phase microextraction (SPME). *Food Chemistry, 84*(4), 633-641.

Motoki, M. & Seguro, K. (1998). Transglutaminase and its use for food processing. *Trends in Food Science & Technology, 9*(5), 204-210.

Pietrasik, Z. & Jarmoluk, A. (2003). Effect of sodium caseinate and k-carrageenan on binding and textural properties of pork muscle gels enhanced by microbial transglutaminase addition. *Food Research International, 36*(3), 285-294.

Pietrasik, Z. & Li-Chan, E. C. Y. (2002a). Binding and textural properties of beef gels as affected by protein, k-carrageenan and microbial transglutaminase addition. *Food Research International, 35*(1), 91-98.

Pietrasik, Z. and Li-Chan, E. C. Y. (2002b). Response surface methodology study on the effects of salt, microbial transglutaminase and heating temperature on pork batter gel properties. *Food Research International, 35*(3), 387-396.

Prestat, C., Jensen, J., Mckeith, F. K. & Brewer, M. C. (2002). Cooking method and end point temperature effects on sensory and colour characteristics of pumped pork loin chops. *Meat Science, 60*(4), 395-400.

Whiting, R. C. (1988). Ingredients and processing factors that control muscle protein functionality. *Food Technology, 42*(4), 104-110.

Xiong, Y. L. & Brekke, C. J. (1991). Protein extractability and thermally induced gelation properties of myofibrils isolated from pre-and post rigor chicken muscles. *Journal of Food Science, 56*(1), 210-215.

Yusof, S. C. & Babji, A. S. (1996). Effect of non-meat proteins, soy protein isolate and sodium caseinate, on the textural properties of chicken bologna. *International Journal of Food Science and Nutrition, 47*(4), 323-329.

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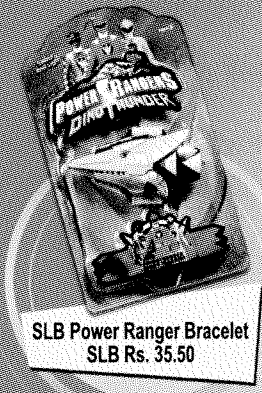

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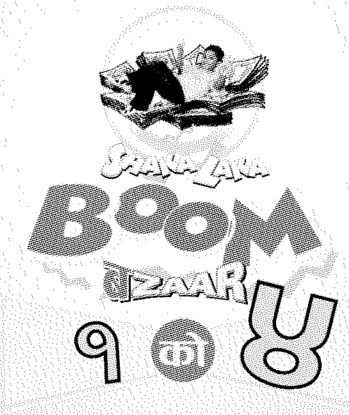

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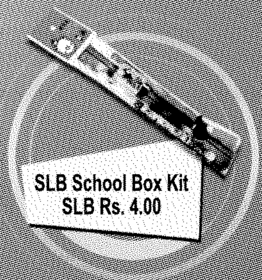
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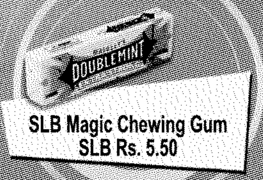

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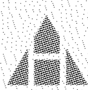
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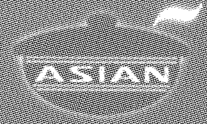

वडा दशैं तथा
शुभ दिपावली २०६३

उत्सव का रंगहरु हाम्रो घर आँगनमा छाउन थाले ।
यस शुभ घडी संगै यहाँको जीवनमा सुस्वास्थ्य, शान्ति एवं
समृद्धिको आगमन होस् ।

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Assessment of Oxalate and Phytate levels and Bioavailability of Calcium and Zinc in *Masyaura*

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Masyaura is a legume based traditional fermented food product of Nepal prepared from blackgram dhal and colocosia tuber. Oxalate and phytate levels in Masyaura were monitored during preparation by different fermentation methods. Calcium and zinc bioavailability were also evaluated. Results indicated that the oxalate and phytate levels reduced during fermentation and ranged 61.8-87.9 and 109.0-161.4 mg/100g dry mater from the initial value of 99.8 and 199.9 mg/100g dry matter respectively. The ranges of Oxalate: Calcium, Phytate: Zinc, Calcium: Phytate and [Calcium] [Phytate]: [Zinc] Molar Ratio in Masyaura ranged from 0.16-0.32, 3.23-4.83, 12.8-25.4 and 0.14 to 0.16 respectively. The result indicated that bioavailability of calcium and zinc is better in Masyaura product. Raw ingredients especially colocosia tuber showed lower level of calcium and zinc bioavailability. Fermentation reduced the oxalate and phytate levels in Masyaura which leads to improve the bioavailability of essential minerals like calcium and zinc.

Keywords: *Masyaura*, Oxalate, Phytate, Mineral bioavailability, Fermented food, Nepal

Introduction

Masyaura is an important legume based fermented food of Nepal has been used by all classes of people as a substitute for meat (Karki, 1986). It is usually prepared in cottage or home scale level and used as an adjunct in curry. The raw materials for the preparation of *Masyaura* are legumes especially blackgram and greengram and vegetables like colocosia tuber, ashgourd, raddish etc. *Masyaura* technology is also a good method of preserving the perishable vegetables (Gajurel & Baidya, 1979).

Masyaura similar to Indian *Wari* are friable, brittle and spongy dried balls 2-5 cm diameter. Once it is prepared, the dried balls are stored for future use. At the time of cooking, it is mixed with curry to make soup and served with rice as side dish (Gajurel & Baidya, 1979). Little information is available in the literature on nutritional, biochemical, nutritional (Karki, 1986; Gajurel & Baidya, 1979, Dahal et al., 2003a) and antinutritional (Dahal et al, 2003b) aspects of *Masyaura*. Antinutritional constituents are of major concerns for the bioavailability characteristics of nutrients like minerals. *Masyaura* product showed the major concern on heat stable phytate and oxalates (Dahal et al, 2003b) as those constituents are not easily removed by general cooking method like most of the enzyme inhibitors. Oxalates in tubers may either be a cause or a contributor as to the acridity, which causes irritation and swelling of mouth and throat. Phytate is widespread in roots and tubers (Libert and Franceschi-1987). Oxalates and phytates are well known antinutrients of plant foods and they are associated with a decrease in bioavailability of nutritionally significant mineral elements. These organic substances can bind essential minerals to form insoluble or indigestible complexes in the lumen of intestinal tracts, thereby preventing their absorption. In this study, oxalate and phytate levels were examined during the fermentation of *Masyaura* since such information is lacking. The effects of those antinutrients of the bioavailability of calcium and zinc were also studied by calculating respective Ox:Ca, Phy:Zn, Ca:Phy and [Ca][Phy]/[Zn] molar ratios.

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Materials and Methods

Blackgram (*Phaseolus mungo*) dhal was collected from local market of Kathmandu, Nepal and Colocosia (*Colocosia esculanta*) tuber was purchased from the local market of Wuxi, China.

Microbial Cultures: *Pediococcus pentosaceous* 1.2695 (ATCC 33316) and *Saccharomyces cerevisiae* As. 2.399 strains were collected from the China General Microbiological Culture Collection centre (CGMCCC), Beijing, P.R. China. Supplier's instruction was followed for the cultivation of the cultures. *Pediococcus pentasaceous* was grown in MRS medium, transferred to the sterile vial with 20% sterile glycerol and preserved at -70°C as the method given by Parton & Willis, 1990 and subcultured as per the method given by Valdez, 2001. *Saccharomyces cerevisiae* was stored at 4° C.

Preparation of Masyaura:

Traditional method: Cleaned split blackgram dhal was washed thoroughly with water to remove husk and foreign matter and soaked for 16h in water. The soaked dhal was lightly hand washed in tape water to remove husk and ground into a thick paste (Moisture around 70%) using a wet grinder. The colocosia tuber was washed peeled and finally wet grinded to get thick paste (Moisture of colocosia paste was maintained about 70% with colocosia powder. Colocosia tuber was sliced blanched at 70°C for 5 min dried at 50°C and was grinded to get powder). The blackgram dhal paste and colocosia paste was then mixed in a ratio of 1:1. The dough was made into

small lumps weighing 20-30g each distributed 1-2 inches apart on steel trays between lumps, left to ferment overnight at ambient temperature. The spongy textured balls are then sun dried for 5 days (Room Temp.20°C, Humidity 70%).

Controlled fermentation and mechanical drying technique: Dough was prepared and distributed on steel trays similar to traditional method. Trays were then subjected for fermentation at three different temperatures of 20, 30 and 40° C separately (Relative humidity 80-90%). Trays were removed after 12, 24, 48 and 72 h of fermentation and dried at 50°C for 12-16 h.

Culture Inoculation and Fermentation:

Culture Inoculation: a) *Pediococcus Pentosaceus: Pediococcus Pentosaceus* was grown in MRS broth for 3 days at 37°C, centrifuged at 10,000 rpm at 4°C for 20 min. Supernant was discarded and the pellet was washed twice using phosphate buffer (20 mM, pH 7.0). Same buffer was used to dissolve the bacterial cells (20% of the original medium), vortexed. The number of cells in the suspension was determined using the linear standard curve (Standard curve was previously plotted after maintaining the OD_{540} of the same suspension at 0.2, 0.4, 0.6, 0.8, and 1.0 with the corresponding number of the cells in the respective suspension as determined by the plate count method after the appropriate dilution of the different set of suspensions. (b) *Saccharomyces cerevisiae*: *Saccharomyces cerevisiae* was grown on wort medium for 2 days at 30°C and centrifuged at 5,000 rpm for 20 min. Supernant was discarded and the pellet was dissolved in sterile physiological saline [0.9% (w/v) NaCl] using 20% of the original medium and vortexed. The suspension was used to count the yeast cells under microscope on Thoma counting chamber. [Inoculum size for *Pediococcus pentosaceus* was used at 10^7 cells/g and for *Saccharomyces cerevisiae* was 10^4/g separately on dough. Inoculum size was used on basic background of the natural dough. (Lactic counts of 7.4 log CFU/g and yeast and mold count of 3.7 logs CFU/g) (Dahal et al., 2003a)]

- *Fermentation:* Dough distribution on tray: Dough inoculated with both microorganisms were distributed on trays separately making spherical balls of about 20-30 gms and with a spacing of about 1 cm between the balls. Trays were subjected for fermentation at 30° C. Trays were then removed after 24, 48 and 72 h of fermentation (Relative humidity: 80-90%).
- *Drying*: Trays were then dried at 50°C for 12-16 h.

The dried product was packed and stored in sealed polythene bags at ambient temperature. The dried *Masyaura* was powdered in a grinder to pass through 60 mesh sieve and used for chemical analysis.

Moisture was estimated by the standard AOAC (1990) method. The samples were dry ashed (Ranganna-1995) and ash solution was used for estimation of *Calcium and Zinc* in Atomic absorption spectrophotometer (Varian Company, USA). Phosphorous was determined according to Ranganna (1995) using molybdic acid to form a phosphomolybdate complex which was then reduced with amino napho sulphonic acid to form the complex molybdenum blue which was measured calorimetrically at 650 nm using spectrophotometer. *Oxalates:* Total and soluble oxalate (as oxalic acid) present in the samples were estimated according to the method described by Wilson et.al (1982). Extraction by 3N HCL (for total oxalate) and by water (for soluble oxalate) was done. The results were calculated as mg of oxalic acid per 100 gm of the sample. 1 ml of 0.01N KMNO4 = 0.045 mg of Oxalic acid. Insoluble oxalate was calculated subtracting soluble oxalate from total oxalate. *Phytate Phosphorous*: Phytate phosphorous was determined as the precipitate analysis method given by Thompson and Erdman (1982). Non-phytate phosphorous was calculated by subtracting phytate phosphorous from total phosphorous.

Mineral Bioavailability: Calcium and Zinc bioavailability was interpreted with the calculated [Oxalate]/[Calcium], [Phytate]/[Zinc], [Calcium]/[Phytate] and [Calcium][Phytate]/[Zinc] molar ratios. [Oxalate]/[Calcium] molar ratio was calculated as per the method of Davis-1979. [Phytate]/[Zinc] was calculated as per the method of Turnlund, et al-1984. [Calcium]/[Phytate] was calculated as Oladimeji et al -2000 and [Calcium][Phytate]/[Zinc] was calculated as per the method given by Davies and Warrington-1996.

Result and Discussion

Changes in oxalate levels during fermentation of *Masyaura*

Oxalates level was monitored during the fermentation of *Masyaura* by different methods (Table 1). Raw ingredients Blackgram dhal and colocosia tuber were also analyzed. Blackgram dhal and colocosia tuber were found to have oxalate level of 54.0 and 156.0 mg/100g, db out of which 55% and 77.1% were insoluble oxalate respectively. Initial dough made by mixing blackgram dhal paste with colocosia paste was of 99.8 mg/100g total oxalate. Sun dried *Masyaura* showed the value of 61.8mg/100g oxalate showing about 40% reduction during sun drying of *Masyaura* (Table 1).

Oxalate level was found to be constantly decreased during fermentation. Controlled fermented *Masyaura* at 24 h showed the value of 83.2 as compared to the initial value of 99.8 mg/100g on dry basis. Bacteria fermented and Yeast fermented samples at 24 h of fermentation period showed the value of 87.9 and 64.7 respectively. Longer the fermentation time, the reduction in the oxalate level was also higher in all type of fermentation conditions (Table 1). Moreover, yeast fermentation was found more pronounced for the reduction of the oxalate level. Another aspect observed during fermentation of *Masyaura* is that soluble oxalate level was increased with concomitant reduction in total oxalate level

Table 1: Oxalate levels* in *Masyaura* prepared by different fermentation methods

Sample		Oxalate	Soluble Oxalate	Insoluble oxalate (%)
Blackgram dhal		54.0 ± 8.4	24.3 ± 1.51	55.0
Colocosia tuber		156.0 ± 18.6	35.7 ± 0.80	77.1
		Oxalate level during sun-drying		
Initial Dough		**99.8**^{a} ± 3.3	24.7 ± 1.21	75.3
Sun dried *Masyaura*		**61.8**^{b} ± 0.6	27.9 ± 1.99	54.9
		Oxalate level during controlled fermentation.		
	ID	99.8^{a} ± 3.3	24.7 ± 1.21	75.3
Controlled	0h	96.5^{a} ± 3.7	27.7 ± 0.97	71.3
fermented	24h	**83.2**^{b} ± 3.8	35.8 ± 0.49	57.3
Masyaura	48h	77.6^{b} ± 7.0	38.7 ± 1.80	50.1
	72h	64.8^{c} ± 4.4	33.5 ± 1.18	48.3
		Oxalate level during bacterial fermentation.		
Bacterial	ID	99.8^{a} ± 3.3	24.7 ± 1.21	75.3
fermented	24h	**87.9**^{b} ± 2.1	39.3 ± 1.56	55.3
Masyaura	48h	83.0^{b} ± 2.0	37.8 ± 0.45	54.5
	72h	72.6^{c} ± 1.0	41.6 ± 0.66	42.7
		Oxalate level during yeast fermentation.		
Yeast fermented	ID	99.8^{a} ± 3.3	24.7 ± 1.21	75.3
Masyaura.	24h	**64.7**^{b} ± 3.4	36.7 ± 0.92	45.3
	48h	45.2^{c} ± 3.3	28.5 ± 1.64	36.9
	72h	37.8^{c} ± 2.0	20.4 ± 0.47	46.0

- * Oxalate levels in mg/100g on dry weight basis. Values are the mean of duplicates ± S.D.
- Means having the different letters (a, b, c, d, e) in the same column indicates the significant (P=0.05) difference.

(Table 1). Initial level 24.7% of soluble oxalate in dough was found to be increased to 45.1% in case of sun drying process. Controlled fermented sample at 24 h showed soluble oxalate level of 42.7% of total oxalate. Similarly bacteria and yeast fermented samples at 24h showed the value of 44.7 and 54.7%. This might be due to the release of soluble oxalate during fermentation by the action of enzymes produced by microorganisms that cleaved the insoluble oxalate.

In tropical root crops, calcium oxalate is present as fine needles like crystals or raphids. The occurrence of these crystals has been considered as either the cause of or a contributor to the acridity, which initiates irritation and swelling of mouth and throat (Holloway et. al., 1989) Oxalic acid and its salts can have deleterious effects on human nutrition and health particularly by decreasing the calcium absorption and aiding the formation of kidney stones. Currently kidney stone patients are advised to limit their intake of foods with total intake of oxalate not exceeding in 50-60 mg/day (Massey et al-2001). However the lethal dose of oxalic acid for healthy man varies from 2-30g at once depending on a variety of factors (Libert and Franceschi-1987) Boiling and steaming of yam reduced the oxalate levels from 154 mg/100g edible part to 65 and 131 mg/100mg respectively. Total oxalate concentration was ranged from 43 to 156 mg/100 g fresh weight (Doyle et al, 1994). Oxalate content of locust bean (unfermented) from 0.21 mg/100g was found to be reduced to 0.12 mg/100g (by 42.9%) while making *Dadawawa* was also reported (Reddy & Peterson 1994).

Changes in phytate levels during fermentation of *Masyaura*

Phytate level was monitored during the fermentation of *Masyaura* (Table 2). Blackgram dhal contained 306 mg of phosphorous in 100g dry basis out of which phytate phosphorous was 72.8%. Colocosia tuber contained about 87.5% of phytate phosphorous in 259 mg /100g of total phosphorous.

During sun-drying the level of phytate phosphorous decreased from 200 to 109 mg/100g. Phytate level was also found to be constantly decreased during fermentation. Controlled fermented *Masyaura* at 24 h showed the value of 161 as compared to the initial value of 200 mg/100g on dry basis. Bacteria fermented and Yeast fermented samples at 24 h of fermentation period showed the value of 157 and 132 respectively. Longer the fermentation time, the reduction in the phytate level was also higher in all type of fermentation conditions (Table 2). Moreover, yeast fermentation was found more pronounced for the reduction of the phytate level.

Phytic acid markedly decreases calcium bioavailability and forms ca-phytate complexes which inhibit the absorption of Fe and Zinc. Phytic acid intake of 4-9mg/100g dry matter is said to decrease iron absorption by 4-5 fold in humans (Hurrel et al., 1992). Phytates have also been implicated in decreasing protein digestibility by forming complexes and also by interfering with enzyme such as pepsin and trypsin (Reddy & Peterson., 1994). Phytic acid affects the digestibility of starch and protein. Phytate will reduce the bioavailability of dietary minerals and have adverse effects on digestion of starch and proteins.

Microorganisms associated with *Idli* fermentation reduce the phytic acid and content of the substrate (Tamang, 1998). Most of the phytate was hydrolyzed during fermentation white bread and free phosphorous increased simultaneously (Reddy &

Table 2: Phosphorous (P), Phytate P and Non-phytate P levels* in *Masyaura* prepared by different methods

Sample		Phosphorous	Phytate P	Non-Phytate P (% of Total P)	% Phytate P hydrolyzed.
Blackgram dhal		306 ± 5	223 ± 10	27.2	---
Colocosia tuber		259 ± 4	227 ± 10	12.5	---
		Phytate level during sun-drying			
Initial Dough		274 ± 4	**200^{a}** ± 9	27.0	0
Sun dried *Masyaura*		268 ± 5	**109^{b}** ± 4	59.3	**45.5**
		Phytate level during controlled fermentation.			
	ID	274 ± 4	200^{a} ± 9	27.0	0
Controlled fermented	0h	218 ± 11	180^{b} ± 9	17.5	10.2
Masyaura	24h	269 ± 6	**161^{c}** ± 4	39.9	**19.3**
	48h	225 ± 4	136^{d} ± 9	39.6	32.1
	72h	216 ± 4	116^{e} ± 4	46.3	41.9
		Phytate level during bacterial fermentation.			
Bacterial fermented	ID	274 ± 4	200^{a} ± 9	27.0	0
Masyaura	24h	249 ± 8	**157^{b}** ± 10	36.9	**21.5**
	48h	238 ± 8	151^{b} ± 10	36.3	24.2
	72h	227 ± 6	129^{c} ± 5	43.5	35.7
		Phytate level during yeast fermentation.			
Yeast fermented	ID	274 ± 4	200^{a} ± 9	27.0	0
Masyaura.	24h	243 ± 4	**132^{b}** ± 10	45.8	**34.1**
	48h	244 ± 8	124^{bc} ± 5	49.2	38.1
	72h	234 ± 5	111^{c} ± 4.	52.7	44.6

- * Phytate levels in mg/100g on dry weight basis. Note: Values are the mean of duplicates ± S.D.
- Means having the different letters (a, b, c, d, e) in the same column indicates the significant (P=0.05) difference.

Peterson 1994). Reduction in phytate level from 0.52 to 0.22 g/100g (hydrolyzed upto 57.7%) in brown breads and from 0.10 to 0.01 g/100g (hydrolyzed by 90%) in white breads (Reddy & Peterson 1994).

Phytate content of *Rabadi* (a fermented pearl millet food) during fermentation was reported from 480mg/100g for unfermented to 350 mg/100g showing the hydrolysis of 27.1% (Reddy & Peterson 1994) Similarly phytate content of soybean reduced from 1.41 to 0.80% (38.5% hydrolysis) while preparing *Tempeh.* Phytate content of peanut presscake from 1.36% was reduced to 0.05 % (by 96.3%) and 0.7% (by 48.5%) to *Oncom* as fermented by *Rhizopus oligosporous* and *Neurospora spp* respectively. (Reddy & Peterson 1994) Phytate content of unfermented *Dokla*- an Indian fermented food from 2.13mg/g to 0.06 mg/100g after fermentation (Hydrolyzed by 97.2%) was observed. Similarly unfermented *Khaman*- an Indian fermented food from 4.40 mg/g to 1.84 mg/g (by 58.2%) and *Idli* from 8.87 to 5.32 mg/g (by 40%) were reported (Reddy & Peterson 1994)

The destruction of phytate in three types of wheat flour named *Bazari, Sangak* and *Tanak* in Iran (by yeast fermentation) to reduce (at 6 h fermentation) from 120, 165 and 190 to 25, 70 and 90 mg/100g respectively (Liener 1987). Phytate reduction in rye, wheat and whole wheat breads has been reported as influenced by the amount of yeast used in fermentation as well as fermentation time. Increasing the amount of yeast and time of fermentation did significantly reduce phytate levels in all products (Mega, 1982). Phytase isolated from *P. Vulgaris* had an optimum pH for activity of 5.2. Phytase is thermostable (optimum activity 50°C), also at 25°C, the enzyme was stable over a pH range of 3.5 to 7.8 which could be also advantageous in food processing (Mega, 1982).

Calcium and Zinc Bioavailability in *Masyaura*

Based on the sensory characteristics, one best scored controlled fermented *Masyaura* (30°C/24h/80-90%RH), *Pediococcus pentasaceus* inoculated *Masyaura* (30°C/24h/ 80-90%RH) and another *Saccharomyces cerevisiae* inoculated best scored *Masyaura* (30°C/24h/80-90%RH) along with sun-dried *Masyaura* were analyzed along with its raw ingredients. The molar ratios for oxalate, calcium, Zinc and Phytate were calculated to evaluate the effects of elevated levels of oxalate and phytates in the bioavailability of dietary minerals (Table 3). The calculated values are compared with the reported critical values, which are presented in Fig 1.

The importance of oxalate contents of an individual plant product is limiting total dietary calcium availability is of significant when the molar ratio of oxalate: calcium is greater than one. Under these circumstances, the oxalate has potential to complex, not only to the calcium contained in plant but also that derived from the other food sources. A high level ratio of oxalate: calcium in the diet may cause the chronic calcium deficiency (Davis, 1979).

In this investigation, *Masyaura* samples showed the ratio of oxalate: calcium in the range of 0.16 to 0.32 while blackgram dhal showed the ratio of 0.22 and second raw ingredients colocosia showed the highest ratio of 0.73 among all tested

Table 3: Concentration of Calcium, Zinc and calculated Ox: Ca, Phy: Zn, Ca: Phy and [Ca][Phy]:[Zn] molar ratios (Mol/Kg) of *Masyaura* and its raw ingredients

Samples	Ca*	Zn*	Ox:Ca	Phy:Zn	Ca:Phy	[Ca][Phy]:[Zn]
BGD	111 ± 2.7	3.74 ± 0.01	0.22	5.88	8.21	0.16
CS	95 ± 0.6	1.71 ± 0.01	0.73	12.82	6.91	0.31
SDM	168 ±1.4	3.35 ± 0.04	0.16	3.23	25.4	0.14
CFM	128 ±1.1	3.13 ± 0.01	0.29	4.83	13.1	0.16
BIM	122 ±1.3	3.90 ± 0.02	0.32	3.97	12.8	0.12
YIM	122 ±0.7	3.68 ± 0.1	0.24	3.54	15.3	0.11

[BGD: Blackgram Dhal, CS: Colocosia Tuber, SDM: Sun-dried *Masyaura*, CFM: Controlled Fermented *Masyaura*, BIM: Bacteria Inoculated *Masyaura*, YIM: Yeast Inoculated *Masyaura*]

* Results are the mean of duplicates ± S.D. and are expressed as mg/100g on dry basis.

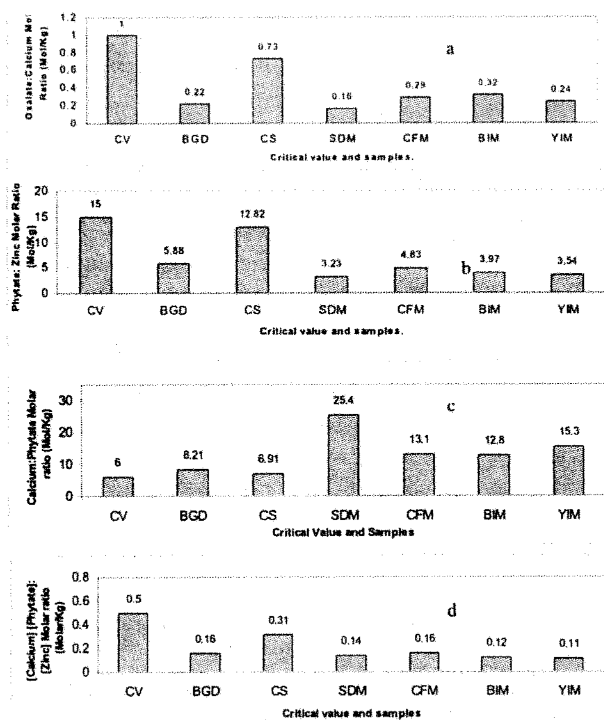

Fig 1: Oxalate: Calcium (a), Phytate: Zinc (b), Calcium: Phytate (c) and [Calcium] [Phytate]: [Zinc] (d) Molar Ratio of raw ingredients and *Masyaura* samples as compared to critical value

[CV: Critical Value, BGD: Blackgram Dhal, CS: Colocosia Tuber, SDM: Sun-dried *Masyaura*, CFM: Controlled Fermented *Masyaura*, BIM: Bacteria Inoculated *Masyaura*, YIM: Yeast Inoculated *Masyaura*]

samples. However the ratio was found to be less than one. This result indicated that oxalate levels in raw materials used during the preparation of *Masyaura* in this investigation may not affect the bioavailability of dietary calcium. Further the fermented *Masyaura* samples are still better than its raw ingredients for the bioavailability of dietary calcium. Moreover choice of colocosia variety might be the major concern during preparation of *Masyaura* product concerning the bioavailability of dietary calcium.

The importance of foodstuffs as a source of dietary zinc depends on both zinc content and level of other constituent in the diet that affect zinc bioavailability. Zinc deficiency has been shown to be the cause of dwarfism and hypogonadism among adolescents from the lowest class of Egypt. Phytate may reduce the bioavailability of dietary zinc by forming insoluble minerals chelates at a physiological pH (Obserleas, 1983). Zinc has been described as the essential mineral most adversely affected by phytate and the phytate to zinc molar ratio has been proposed as an indicator of zinc bioavailability. In human studies, phytate: zinc molar ratios of 15:1 have been associated with reduced zinc bioavailability (Trunlund et al, 1984).

In this study, the phy: zinc ratios of *Masyaura* ranged from 3.23 to 4.83. Blackgram dhal showed the value of 5.80 and colocosia tuber 12.82. Colocosia tuber showed the higher values of phy: zinc among samples tested however the value is slightly lower than the critical value of 15. The result indicated that zinc may be more available in *Masyaura* samples as compared to its raw ingredients. Variety of colocosia may be the major concern on the bioavailability of zinc.

Phytic acid markedly decrease calcium bioavailability and ca: phy molar ratio has been proposed as an indicator of calcium bioavailability. The critical molar ratio of ca:phy is reported to be 6:1 (Olaolimeji et al-2000). The molar ratio of ca: phy obtained in *Masyaura* samples were ranged from 12.8 to 25.4, blackgram dhal showed the value of 8.21 and the colocosia tuber of 6.91. The ratio is lower in case of colocosia tuber. The results clearly showed that bioavailability of calcium may be in the order of *Masyaura* >Blackgram dhal>Colocosia. Reduction in phytate level during fermentation (Table 2) have influenced in increased calcium bioavailability in *Masyaura* products.

The values of [Ca][Phy]/[Zn] are given in Table 3. The values of molar ratios [Ca][Phy]/[Zn] ranged from 0.11 to 0.16 in *Masyaura* samples, blackgram dhal showed the value of 0.16 and the colocosia tuber showed the value of 0.31. Davies and Warrington-1986 indicated that the ratio of [Ca][Phy]/[Zn] is a better predictor of zinc bioavailability and if the values were greater than 0.5 mol/Kg, there would be interference with the availability of zinc. In this study the ratio were found less than 0.5 in *Masyaura* and its raw ingredients. However colocosia tuber showed the higher values among samples tested. This is to say that although zinc availability may not be adversely affected but might be lower in case of Colocosia.

Fermented *Masyaura* samples showed the better calcium and zinc bioavailability characteristics.

It has been reported that high levels of antinutrients such as oxalates and phytates are known to be very poisonous to humans. High level of oxalates could be partly responsible for the acrid taste and for causing the inflammation (Ekpendeme at al-2000). It appears that food containing higher level of oxalate is unsuitable for consumption by those who are suffering from oxalate kidney stones.

Conclusion

Our results showed that colocosia tuber has higher level of antinutrients especially oxalate and phytate levels and consequently the lower level of mineral bioavailability especially for calcium and zinc as compared to the *Masyaura* and blackgram samples. Fermentation could be recommended before consumption for the better mineral bioavailability.

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References

AOAC (1990). Official Methods of Analysis, 15th Edn, Association of Official Analytical Chemists Inc, Arlington, VA, USA.

Dahal N.R., Qi L. and Swamylingappa B.(2003a) Assessment of Antinutritional and Digestible characteristics of *Masyaura*-A Traditional Fermented Food of Nepal. The 5th International Conference on Food science and Technology (ICFST) Proceedings-II, Wuxi, P.R. China: 246-251

Dahal N.R., Rao E. R. and Swamylingappa B. (2003b). Biochemical and Nutritional Evaluation of *Masyaura*-A legume based Traditional Savory of Nepal. J. Food Sci. Technol. 40(1), 17-22.

Davis N.T. (1979). Anti-nutritional factor affecting mineral utilization. Proceedings of Nutrition Society 38, 121-127.

Davis N.T. and Warrington S. (1986). The phytic acid, mineral, trace elements, protein and moisture content of UK Asian Immigrant Foods. Human Nutrition and Applied Nutrition, 40A. 49-59.

Doyle M.K., Steinhart C.E., Cochkane B.A. (1994). Food Safety-1994. Food Research Institute, University of Wisconsia, Madison

Ekpendeme V.A., Bassey A.N. and Ekaete U.E. (2000). Mineral and antinutrients in fluted pumpkin (Telfairia occidentalis Hook). Food Chemistry, 70:235:240.

Gajurel C. and Baidya K. (1979). *Masyaura* Technology. In Traditional Technology of Nepal (In Nepalese). Trivuwan University, Kathmandu, Nepal pp 238-242.

Holloway W.D., Argall M.E., Jealous W.T., Lee J.A. and Bradbury J.H. (1989). Organic acid and ca-oxalate in tropical root crops. J. of Agric Food Chem. 37, 337-341.

Hurel R.F., Juillert M.A., Reddy M.B, Lynch S.R., Dassenko S.A. and Cook J.D. (1992). Soy protein, phytate and iron absorption in humans. American J.Clinical Nutr. 56:573-578

Karki T (1986). Some Nepalese Traditional Foods and Beverages. In Traditional Foods, some products and technologies. Central Food Technological Research Institute, Mysore, India, pp 84-98.

Libert B. and Franceschi V.R. (1987). Oxalate in crop plants. Journal of Agric. Food Chem. 35: 926-938.

Liener IE (1987) Detoxifying enzymes. In Food Biotechnology I. Ed. by King R.D. and Cheethman P.S.J. Elsevier Applied Sci Publishers Ltd. Pp 249-271.

Massey L.K., Palmer R.G. and Hower H.T. (2001). Oxalate content of soyabean seeds (Glycine Max: Leguminosae), soya foods and other edible legumes. J. Agric. Food Chem. 49:4262-4266.

Mega J.A. (1982). Phytate: It's Chemistry, Occurrence, Food interactions, Nutritional considerations and Method of analysis. J. of Agric Food Chem. 31(1): 1-7

Oberles D. (1983). Phytate content in cereals and legumes and methods of determination. Cereal Food World, 28: 352-357.

Oladimeji MO, Akindahuns AA and Okafor A.F. (2000). Investigation of bioavailability of Zinc and Calcium from some tropical tubers. Nahrung, 44, 136-137.

Ranganna S. (1995) Handbook of analysis and quality control for fruits and vegetable products. Tata McGraw Hill New Delhi.

Reddy N.R. and Peterson M.D. (1994). Reduction in antinutritional and toxic components in plant foods by fermentation. In Food Research International 27:281-290.

Tamang J.P (1998). Role of microorganism in Traditional Fermented Foods. Indian Food Industry 17(3):162-167.

Thompson D.B. and Erdman J.W. (1982) Determination of Phytic acid in soybean. J. Food Sci. 47: 513-517

Trunlund JR, King JC, Keyes WR, Gong B and Michel MC (1984). A stable isotope study of Zinc absorption in Young men: Effect of phytate and alpha-cellulose. American Journal of Clinical Nutrition. 40; 1071-1077.

Wilson C.W., Shaw P.E. and Knight R.J. (1982). Analysis of oxalic acid in Carambola (Averhoa Carambola L) and Spinach by HPLC. J. Agric. Food Chem. 30:1106-1108

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Detection Threshold and Flavour Potentiating Effect of Monosodium Glutamate

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Monosodium glutamate (MSG) is a salt from orient origin used to enhance the taste quality of food. However, other ethnic groups are unfamiliar with the taste of MSG. The objective of study was to investigate the taste response of Indian population to MSG for threshold level, synergestic effect of MSG on other basic tastes and effect of added MSG on sensory attributes of a fried food product prepared with or without spice. The results revealed that the taste response of MSG were distributed over the entire intensity scale for all concentrations tested unlike other basic tastes wherein they increased in a specific order. MSG was found to have synergestic effect on the taste quality of sugar and salt. MSG incorporated to the product at four different levels affected the texture and taste significantly. These responses were not found when the products were prepared with a spice, namely, omum. Omum masked the flavour potentiating effect of MSG. Overall the products scored good to excellent for all sensory attributes on the grading scale. The choice profiling of products indicated overall higher acceptance of MSG incorporated products for aroma, saltiness and taste. This was true for products with or without spice. The products with highest levels of MSG were distinctly different from the others in sensory attributes. It can be concluded from the above results that MSG influenced the taste responses even in population unfamiliar with the taste or use of MSG.

Keywords: Threshold tests, Synergistic effect, Choice profiling, Sensory attributes

Introduction

Monosodium glutamate (MSG) is recognized as a powerful flavour potentiator with a unique basic taste termed as 'Umami'. The taste response to umami is different from any other known basic taste recognized or established conventionally. A flavour potentiator or enhancer is a substance added to food to enhance its original taste and flavour (Yamaguchi, 1987). Glutamate is the most commonly used additive throughout the world. Glutamic acid is one of the most abundantly occurring amino acids in nature and an important component of proteins. It imparts an intrinsic taste which is expressed as unique and distinct. It corresponds to Japanese of 'umami', which indicates deliciousness. Foods such as tomato, cheese, mushrooms etc. contain high levels of glutamate and are used for their flavoring quality. Free glutamate is effective as food flavour. MSG is added to many prepared and processed foods to enhance flavour (Mallick, 1999).

MSG when tasted as such is not very pleasant, but the pleasantness score increases tremendously when it is added to a food product. However, according to Yamaguchi and Takahashi (1984) an optimum concentration exists for MSG to added foods beyond which it does not contribute to the flavour. MSG is also known to have synergistic effect with other basic tastes which is also apparent only at certain concentrations. It also has a carry over effect, which can modify the responses for other basic tastes (Amerine et al, 1965).

MSG as a food additive is used mostly by the orient population and other ethnic groups are not familiar with its taste. Indian traditional foods do not use MSG at all. Hence, it was thought worthwhile to explore the response of Indian population to basic taste of MSG to identify detection thresholds, to study its synergistic effect on two basic tastes and try out the sensory responses to MSG incorporated products. The present study was planned with the objective of investigating the flavour potentiating effect of MSG using Indian population as panel members.

Materials and Methods

The various food ingredients used for the study were refined wheat flour (*Tritricum aestivum*), refined sunflower (*Helianthus annuus*) oil, omum (*Trachyspermum ammi*), salt and sugar. These were purchased from local market. Commercially available food grade monosodium glutamate (MSG) was also procured. Citric acid and caffeine were from SD Fine Chemicals Co. Mumbai India. Clean packaged drinking water of a single brand was used for sensory analysis experiments.

Selection of panel members

The panel members enrolled for the sensory analysis were postgraduate students of the institution between 22-25 years of age. None of them were habitual smokers and drinkers and did not use MSG for everyday cooking. However, they were familiar with oriental foods which use MSG as one of the essential ingredients. They were familiar with sensory analysis techniques but were not used to the taste of MSG. The number of panel for each test varied between 54-56.

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Sensory evaluation

The actual experiment was planned in three phases. Phase I was based on threshold testing for basic tastes utilizing standard techniques. The results of these were used to determine the detection and identification threshold of MSG. In Phase II, the detection threshold of MSG were added to salt and sugar solutions which in turn were derived from the results of Phase I to study the synergistic or potentiating effect of MSG. In Phase III MSG was added to an Indian traditional product at different levels with or without a spice to evaluate its effect on the sensory attributes of product.

Threshold tests

For identification and detection thresholds of basic tastes, solutions of sugar, salt, citric acid, caffeine and MSG were prepared in increasing concentration and presented to panel members with an intensity scale on a score card according to standard procedures (Amerine et al, 1965; Jellinek, 1964). MSG solutions were placed at the end to eliminate the possibility of 'carry over' or an 'after taste' effect on other basic tastes.

Synergistic effect of MSG

Two basic tastes were selected to study the synergistic effect of MSG namely salt and sugar. Sugar and salt were used at 1.5% and 0.15% concentration respectively along with MSG. The detection level of MSG was 0.013% which formed the midpoint of concentration along with one higher (0.015%) and lower (0.011%) level of MSG. These were presented to panel members with a score card. The control solution with no MSG was the reference point, based on which they were asked to identify other solutions with different levels of MSG but same level of sugar or salt as either similar or different. If found different, they had to indicate the intensity as high or low (ISI, 1972). The tests for sugar and salt were conducted on different days.

Effect of incorporating MSG on sensory attributes of product

For this experiment, an Indian traditional deep fried product used as snack was selected namely "Diamond cuts". Diamond cuts are prepared with refined wheat flour, salt, spices and oil. The proportion of ingredients used was as follows, refined wheat flour, 87g, refined sunflower oil for shortening 15 ml, omum 1g, salt 2 g, and oil for deep frying. Refined wheat flour was sieved, mixed well with heated and cooled sunflower oil, and salt and kneaded to a tight dough with water. The dough was rolled over a board with a rolling pin to a flat sheet of 4.0mm thickness. These were cut into diamond shapes of approximately 1" and fried in refined sunflower oil at 173°C±1°C till the product attained a golden yellow colour. MSG was incorporated into this product at four levels viz, 0.025, 0.05, 0.075 and 0.1% levels. Another set of product was also made similarly incorporating 1g of omum into each one of the variations including control. These were subjected to sensory analysis using a 10 point grading scale as presented in the score card given in Table 1 (Gridgeman, 1967). In addition to scoring the panelists were also requested to express their opinion regarding the product using choice profiling. Choice profiling is a new method of sensory analysis, wherein untrained panel expresses their opinion regarding the attributes of products using descriptive quality (Cordello, 1998).

Table 1. Score card for sensory evaluation of products

Attributes	Control	B	C	D	E
Color					
Texture					
Aroma					
Taste					
Presence of After taste Yes/No					

Attributes	Choice profiles	B	C	D	E
Aroma	Not pleasing **(NP)**				
	Pleasing **(P)**				
	Excellent **(E)**				
Taste	Not appealing **(NA)**				
	Appealing **(A)**				
	Excellent **(E)**				
Saltiness	Low **(L)**				
	Similar **(S)**				
	High **(H)**				
After taste	Low **(L)**				
	High **(H)**				
	Very high **(VH)**				

You are given ***'Diamond cuts'*** prepared using different flavouring ingredients. Evaluate them for quality attributes according to the grade description given below and give your choice profile.

Grade description

Quality grade description	**Score**
Excellent	9 -10
Good	6 - 8
Fair	4 - 5
Poor	1- 3

Choice profiling (Fill in the code mentioned against the quality attribute in comparison to control sample).

Statistical analysis

The sensory analysis data was tabulated and subjected to statistical analysis. Mean±SD and ANOVA were used to determine significant differences in sensory attributes of different products. The data for choice profiling was analyzed for frequency distribution using chi-square.

Results and Discussion

The results of threshold tests are summarized in Table 2. A liking for sweet taste is said to be inherent for human beings. Since the human offspring first familiarizes with sweet taste through milk, the liking for sweetness is said to be natural. At lowest concentration of 0.5%, majority of the respondents (28) could not identify the taste quality and graded it as taste of pure water. A smaller number (19) graded it as different

from water but taste quality 'not identifiable'. At 1.0% concentration, a majority could identify the taste quality of sugar solution as different from water but 'not identifiable' (10) or 'weak' (16). At next higher concentration of 1.5% the responses were divided between three categories of grading as X, 1 and 2 representing 'identifiable', 'weak' and 'medium' taste. At this concentration a majority of panelists could distinguish the taste quality. At 2% level of sugar concentration, 22 of the responses were as 'medium' followed by 12 responses for 'weak taste'. At 2.5% this response remained same for medium but 19 had termed the taste as strong. At higher concentration of 3 % sugar solution the responses were termed as either strong (15 panelists), very strong (16 panel members) or extremely strong (12 panel members).

For the salt solution, at lowest concentration, most of the panelists could not recognize the taste of salt solution. At the next concentration it was recognized as either different from water, 'very weak' or 'weak'. At 0.15% concentration majority were able to recognize the taste as 'weak taste'. Similarly maximum response were obtained for 0.2% concentration as 'medium', 0.25% concentration as 'strong' and 0.3% concentration as 'very strong'. All the responses in each category did follow a distribution wherein some of them were spread below or above the median at each level of concentration. The responses were definite and progressively increased from weaker to stronger category with each increasing concentration. The detention threshold of salt solution could be said to be at 0.15% wherein a majority identified the taste as weak taste.

For the basis taste representing sour, citric acid was used to prepare solution ranging from 0.01% to 0.11%. Similar to the taste response of sugar and salt at the lowest concentration, majority of the respondents identified the taste as bland. At 0.03% concentration, a sizeable number (24) could say that the taste was different from water but could not identify it. But at the same time, 10 respondents recognized it as 'very weak' taste and 13 as 'weak' taste. 5 of the respondents also termed it as 'medium'. These results show that sour taste was easily recognizable at lower concentration in comparison to the taste of sweet and salt. This is possible because sour taste is less often encountered in foods when compared with sugar and salt, hence the respondents were more sensitive to the taste of citric acid. At 0.05% concentration, the responses were spread in the range of 'very weak' to 'extremely strong' taste categories. A majority of responses were distributed between the intensity scaling of X, 1 and 2. As the concentration of citric acid was increased to 0.07%, this distribution was found to be between the intensity scaling of 1, 2 and 3. At further increase, the distribution further scaled up to higher taste response. At the maximum concentration of 0.11%, a majority found the solution to be 'strong', 'very strong' or 'extremely strong'. Alt all levels of concentration respondents were more sensitive to the taste of sour than to the taste of either sugar or salt.

Table 2. Responses for threshold tests for basic tastes

Concentration (%)	0	?	X	1	2	3	4	5
Sugar								
0.5	28	19	5	4	-	-	-	-
1.0	4	21	10	16	4	1	-	-
1.5	-	4	15	16	17	3	1	-
2.0	-	2	9	12	22	11	-	-
2.5	-	1	2	3	22	19	8	1
3.0	-	1	2	4	6	15	16	12
Salt								
0.05	24	17	4	9	2	-	-	-
0.1	-	15	14	15	9	2	1	-
0.15	1	4	12	19	9	10	1	-
0.2	-	-	2	16	22	8	7	1
0.25	-	-	1	2	19	22	9	3
0.30	-	-	-	1	6	17	23	9
Citric acid								
0.01	38	17	-	1	-	-	-	-
0.03	4	24	10	13	5	-	-	-
0.05	-	-	18	14	15	6	2	1
0.07	-	-	4	15	19	16	1	1
0.09	-	-	-	4	16	18	16	2
0.11	-	-	-	1	5	17	19	14
Caffeine								
0.01	31	16	7	2	-	-	-	-
0.03	13	22	9	10	2	-	-	-
0.05	4	5	20	13	12	2	-	-
0.07	-	1	4	20	13	11	6	1
0.09	-	-	-	2	20	17	13	4
0.11	-	2	-	2	2	20	16	14
MSG								
0.007	36	20	-	-	-	-	-	-
0.009	14	29	7	2	4	-	-	-
0.011	14	11	16	14	1	-	-	-
0.013	8	7	6	13	11	9	2	-
0.015	12	5	4	6	18	9	2	-
0.017	10	4	3	7	4	21	5	2

Grading scale: 0 – none or taste of pure water, ? – different from water but taste quality not identifiable, X – Threshold, very weak taste, 1 – weak, 2 – medium – 3 – strong, 4 – very strong, 5 - extremely strong.

For the taste of bitter, at the initial two concentration of 0.01 and 0.03%, 16 and 22 respondents recognized the taste as different from water but were not able to recognize it. A majority were able to recognize it at 0.05% concentration as 'very weak' taste. The responses for higher concentrations followed a pattern similar to taste of citric acid wherein they progressively increased on the intensity scaling. At highest concentration the solution were termed as 'strong', 'very strong' and 'extremely strong'. Here also, the respondents were found to be more sensitive to the taste of caffeine or bitter and responses were in the higher category of intensity scaling.

MSG was placed at the end to avoid any carry over effect it may have on the other taste responses. The responses obtained for MSG were distinctly different from any other tastes observed. At lowest concentration of 0.007%, a majority could

not identify the taste quality, the number of respondents being 36. Strangely, even at increased concentrations up to highest level tested, there were respondents (number ranging from 8-14) in all levels of concentration, who termed the solution as bland, indicating that the taste of MSG was totally unrecognizable at least by some of the respondents. At all levels of concentration the responses were widely distributed over a wide range. At 0.009%, 29 of the panelists termed the taste to be different from water. At 0.011 and 0.013% concentration, 16 and 13 respondents identified the taste as 'very weak' and 'weak' respectively. At 0.015% concentration, 18 found the taste to be 'medium' and at 0.017% concentration, 21 found the taste to be 'strong'. Only a negligible number found the taste to be 'very strong' or 'extremely strong' at highest concentration. This shows that the respondents were not able to recognize the taste of MSG very easily. They did not find it to be strong basic taste and were not sensitive to its taste. It might be noted that in majority of studies, the detection threshold of MSG was found to be 0.011-0.013% (Filer and Stegink, 1994) and even in present study, there were found to be detection and identification threshold for MSG despite the respondents not being familiar with this taste.

Synergistic effect of MSG with salt and sugar

Basically MSG is recognized for its flavour potentiating effect. Hence it can increase the intensity of other basic taste. The synergistic effect of MSG with two common basic tastes namely sweet and salt was tested. The results are presented in Figures 1 and 2. For testing the synergistic effect of MSG with sweet, the detection threshold of sugar, as evident from the results of threshold taste was selected and presented along with 3 added levels of MSG. These levels of MSG were also selected from the results of threshold testing. The respondents were asked to identify the taste quality of solution as similar to A or not similar to 'A'. ('A' was the control solution). If it was different, they had to indicate whether it's intensity was low or high. With 0.011% of MSG, 33 respondents found the sugar solutions to be higher in intensity, whereas 16 found it to be lower in intensity. At the next concentration of sugar solution with 0.013% MSG only a slight change was observed with 37 terming the solution to be high. But at 0.015% MSG with sugar, almost 50 panelists found the sugar solution to be tasting sweeter, clearly indicating that MSG does have a synergistic effect on sweet taste. The differences between the responses were found to be significant ($X^{2\ (4df)}$17.216**). However, even for the potentiating effect, a minimum concentration is required, which in this case was 0.015%. It has been stated by Maga (1983) that even for synergestic effect of MSG on other basic tastes, an optimum concentration exists, below or above which the impact becomes dilute.

Similar testing was also conducted with salt and MSG solution. Here the detention threshold of salt selected from the earlier experiment was 0.15% to which three levels of MSG as stated in methodology were added. Salt solution with no added MSG served as controls. At lower level of salt and MSG, i.e 0.011%, 14 of the respondents could not find the solution as different but 24 termed it as having a higher intensity of salt. At higher level of MSG, (0.013%) the difference was easily pointed out and 37 of them termed it to be high. But at 0.015% concentration, almost all the panelists (51) identified the solution to be higher in intensity in comparison to control. The differences were found to be extremely significant on application of chi square test ($X^{2(4df)}$36.607***). These results further confirm that MSG has a synergistic effect on the taste quality of salt

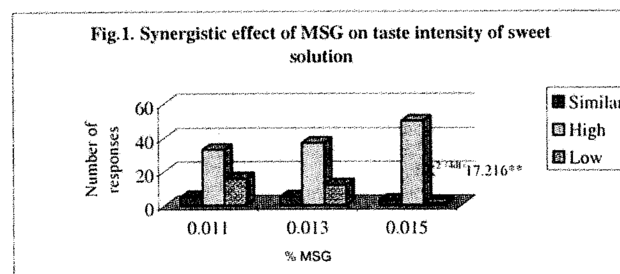

Fig.1. Synergistic effect of MSG on taste intensity of sweet solution

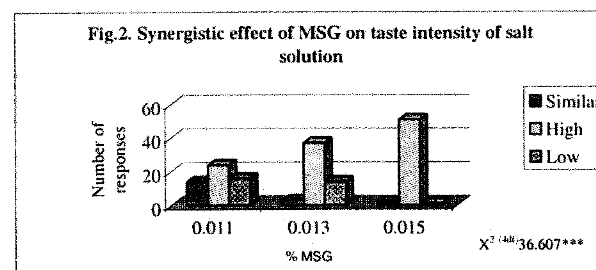

Fig.2. Synergistic effect of MSG on taste intensity of salt solution

Effect of incorporating MSG on taste quality of product

In the final experiment MSG was incorporated to a product to study its sensory attributes. The sensory scores of products are presented in Table 3. In general all the products scored good to excellent for all sensory attributes. For the quality of colour, the scores ranged from 7.74 to 8.23. For the quality of texture, the control product showed highest with 8.28 followed by product with 0.05% MSG at 8.00, 0.075% MSG at 7.89, 0.025% MSG at 7.77 and with 0.01% MSG at 7.07. The scores for aroma were between 7.65–8.25. For the quality of taste, product with highest level of MSG i.e. 0.1% scored highest at 8.53 followed by product with 0.075% MSG at 8.33. This indicates that incorporation of MSG at higher level improved the taste quality of products tremendously. It is been stated that the concentration of MSG for altering taste sensation is a significant factor in determining the taste response to MSG added products (Maga, 1983). The results of sensory analysis were subjected to ANOVA and significant differences were found in the qualities of texture and aroma ($P<0.001$). It can be concluded that MSG influenced the sensory scores of the products. The panelists were also asked to respond to the presence of after taste in the product and the results showed that almost all products had a certain degree of after taste. It was higher in products incorporated with higher level of MSG.

Table 3. Effect of incorporation of MSG on sensory scores* of products

Products	Colour	Texture	Aroma	Taste	Presence of after taste
Product 1 - Without spice					
A. Control	8.053	8.280	8.245	7.803	-
	± 1.007	± 0.959	± 1.326	± 1.624	
B. + MSG 0.025%	7.929	7.771	7.912	7.666	35
	± 1.099	± 1.195	± 1.65	± 1.539	
C. + MSG 0.05%	8.175	8.000	7.894	7.754	52
	± 0.984	± 1.052	± 1.484	± 1.228	
D. + MSG 0.075%	8.228	7.894	7.789	8.333	53
	± 1.085	± 1.227	± 1.729	± 1.300	
E. + MSG 0.01%	7.736	7.07	7.649	8.526	54
	±1.727	± 1.907	± 1.747	± 1.615	
F Ratio	1.5181	6.6421	1.09	5.8972	-
P Value	0.1969ns	0.00004***	0.3617ns	0.001***	-
Product 2 - With omum					
A. Control	8.037	8.462	8.518	7.962	-
	± 1.258	± 0.884	± 0.863	± 1.589	
B. + MSG 0.025%	8.2037	8.185	8.000	8.000	29
	± 0.876	± 0.912	± 1.453	± 1.228	
C. + MSG 0.05%	8.296	8.129	7.870	7.925	41
	± 1.075	±1.064	± 1.332	± 1.179	
D. + MSG 0.075%	8.407	8.148	7.944	8.166	47
	± 1.267	± 1.352	±1.485	± 1.328	
E. + MSG 0.01%	8.407	8.056	8.037	8.315	48
	± 1.236	± 1.294	± 1.373	± 1.564	
F Ratio	0.9870	1.0560	2.0326	0.7445	-
P Value	0.41506ns	0.3787ns	0.0901ns	0.5623ns	-

* : Mean ± SD of responses of 54 panelists.
ns : Not significant.
*** : P≤0.0001.

The next set of products were prepared by adding 1% of omum along with MSG. This was done to study the effect of adding MSG to a product with spice, since a strongly flavored spice can mask the flavour of MSG. As evident from the data, all the products prepared incorporating the spice were given high scores representing the grading from good to excellent . For the quality of colour, the scores ranged from 8.04 to 8.41. The spiced products scored higher than controls. For the quality of texture also, the scores were between 8.05 to 8.46 representing excellent on the scale. Slightly differential scores were obtained for the quality of aroma, wherein the control was rated high with 8.52 and the products with MSG were between 7.87 to 8.04 scores.

The taste quality was equally rated high with 7.93 to 8.32 scores. The product with maximum level of MSG (0.1%) scored highest for taste. When the data were subjected to statistical analysis using analysis of variance, there was no significant difference in the quality attribute of the products evaluated. These results show that on addition of spice to a MSG containing product, there could be a masking of MSG flavour or MSG may not have a potentiating effect with this particular spice. A majority of panelist found presence of after taste. It was lesser in products with lower levels of MSG but higher in products with higher levels of MSG.

The products were also subjected to choice profiling. Choice profiling assista in using descriptive quality profile analysis with untrained panel. For the quality of aroma, most of the responses were in the category of pleasing and excellent with very minor differences among products. Products with higher level of MSG were put into the excellent category for aroma in comparison with products with lower levels of MSG. For the quality of taste very few found the products to be not appealing. Most of the responses were in the category of appealing for all products and the products with 0.1% MSG were found to be excellent for taste quality by majority of respondents. For the feel of saltiness the product with 0.25% MSG was scored as low. Some of the respondents also found the saltiness to be similar, but for the product with 0.075 and 0.1% MSG, many respondents found the saltiness to be high. This indicates the synergistic effect of MSG with salt and confirms our earlier finding of threshold tests. The presence of after taste was least in the product with less MSG (0.025%). It was found to be higher in the next set of product and very high with highest level of MSG. The differences between the responses for different products for sensory attributes were found to be significant.

The choice profiling for next set of products prepared with spice shown in Table 4 indicated similar responses for aroma as seen for first set of products. Aroma was found to be pleasing by majority and between products the differences were not significant. Taste was appealing for the products with first three levels of MSG and was found to be excellent for the product with 0.01% of MSG. This indicates that MSG enhanced the taste quality of product at 0.1% level. Though the effect could be seen even with lower levels, it was more marked with higher level of MSG. For saltiness, the responses were similar to first set of products, wherein the saltiness was found to be more as the level of MSG increased. The after taste was recognized by a large number of panelists with high

level of MSG and was least with low level of MSG. The differences were statistically significant.

The overall results of choice profiling can be summarized as follows – for the quality of taste and aroma, MSG incorporated products were accepted very well. Saltiness was found to be more in product with higher level of MSG indicating a flavour potentiation effect of MSG and incorporation of MSG definitely lead to presence of aftertaste in the product.

From the results of sensory analysis of MSG incorporated products. It can be concluded that MSG definitely improves the sensory quality of deep fried traditional product chosen in the present study. However, to decide which level of MSG is optimum, data need to be collected with a wider range of MSG levels to bring out the differences. In the present study, the effects wee more marked at 0.1% level and much lesser at a lower level of incorporation.

Table 4 Responses of panelists for choice profiling tests

Attribute	Product 1 (Level of MSG incorporation)				Product 2 (Level of MSG incorporation)			
	0.025%	0.05%	0.075%	0.01%	0.025%	0.05%	0.075%	0.01%
Aroma								
Not Pleasing	9	9	5	14	11	7	7	7
Pleasing	43	42	36	30	35	42	38	38
Excellent	5	6	16	13	8	5	9	9
$X^{2\ (6df)}$	15.886*				3.534ns			
Taste								
Not Appealing	7	5	2	3	4	7	6	3
Appealing	43	44	39	16	42	42	39	15
Excellent	7	8	16	38	8	5	9	36
$X^{2\ (6df)}$	54.248***				59.973***			
Saltiness								
Low	24	6	5	5	18	10	8	0
Similar	26	39	20	9	32	37	23	8
High	7	12	32	43	4	7	23	46
$X^{2\ (6df)}$	82.626***				93.162***			
After taste								
Low	37	19	13	6	42	26	10	2
High	18	38	38	15	11	27	38	9
Very high	2	0	6	36	1	1	6	43
$X^{2\ (6df)}$	122.783***				170.965***			

ns : Not significant.
* : $P \leq 0.05$.
*** : $P \leq 0.0001$.

References

Amerine M.A., Pangborn R.M. & Roessler E.B. (1965). Principles of sensory evaluation of food. Academic press, New York.

Cardello A.V. (1998). Perception of Food Quality. *in the book*, Food Storage Stability. (Ed.) Taub, I A. and Singh, R.P. CRC press, New York.

Filer L.J. & Stegink L.D. (1994). A Report of the Proceedings of an MSG workshop held Aug. 1991. *CRC Critical Reviews in Food Science and Nutrition.* 34:159-174.

Gridgeman N.T. (1967). Sensory assessment in quality control. *in the book* Quality control in the food industry. Ed. Herschdoerfer S.M. Volume 1, Academic Press, London. 235-283.

ISI. (1972). Indian standard guide for sensory evaluation of foods, Part 2. Methods and evaluation cards. Indian Standard Institution. New Delhi. 28.

Jellinek G. (1964). Introduction to and critical review of modern methods of sensory analysis with special emphasis on descriptive sensory analysis. *The Indian Journal of Nutrition and Dietetics.* 1:219 –259.

Maga J.A. (1983). Flavour potentiators. *Critical Reviews in Food Science and Nutrition.* 18: 231-312.

Mallick H.N. (1999). Glutamate as food additive. Proceedings of the symposium on safety and usefulness of glutamate as a flavour enhancer: Current status of the knowledge. Eds. Prakash V. Lonsane B.K., Prabhakar J.V. & Srihari B.R. Central Food Technological Research Institute, Mysore, pp 32-46.

Yamaguchi S. and Takahashi C. (1984). Hedonic functions of monosodium glutamate and four basic taste substances used at various concentrations levels in single and complex systems. *Agricultural Biological Chemistry.* 48:1077-1081.

Yamaguchi S. (1987). Fundamental properties of umami in human taste sensation. Umami: A basic taste. Ed. Kawamura Y. & Kare M.R. Marcel Dekker. New York. 41-93.

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Effects of Processing and Frozen Storage on the Retention of Ascorbic Acid and β-Carotene Content of Selected Vegetables

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Ascorbic acid (AA) and β-carotene content of processed and frozen stored vegetables were evaluated to determine the retention of the vitamins in frozen vegetables in comparison to fresh ones. These summer vegetables namely French beans (Phaseolus vulgaris L.), Okra (Abelmoschus esculentus Moench) and Capsicum (capsicum annuum L.) were cut, branched, frozen by Individual Quick Freezing (IQF) technique and stored at -20 to -25°C. Vitamin content was determined for fresh, frozen and frozen stored vegetables after two, four and six months. Blanching prior to freezing resulted in a significant ($P \leq 0.05$) reduction in AA (14.3 to 26.2 %) and -carotene (18.6 to 30.0 %) in the vegetables. However, no significant reduction was observed in quick freezing and different periods of frozen storage. Quick freezing resulted in 67.8 to 80.4 % retention in AA and 69.5 to 73.0 % in β-carotene content. Hence, quick freezing is a suitable method of preventing AA and β-carotene losses in vegetables.

Keywords: Ascorbic acid, β-carotene, Blanching, vegetables, IQF.

Introduction

The health benefits of vegetables are well recognized by nutritional and medical communities but the per capita intake of vegetables in India is far below recommendations. Antioxidant vitamins i.e. ascorbic acid (AA) and b-carotene are present in good amount in the vegetables. b-carotene has also been identified as a potential anticarcinogen.

Freezing serves the purpose of preservation, convenience and nutrition. It is recognised as the method of preservation of perishables like fruits and vegetables as the quality of frozen vegetables is very close to the parent fresh ones. Freezing in most cases is considered to be superior to dehydration and thermal processing. It provides an environment which inhibits microbial growth and generally retards chemical reaction rate, hence prevents discolouration, off-flavour and oxidation of ascorbic acid (Pruthi, 1989). AA is routinely used as an index to measure processing effects on nutrient retention because of its liability (Vanderslice *et al*, 1990).

There is demand for high quality, fresh, shelf-stable and ready-to-use vegetables for dual income group families as well as for retail fast food outlets and restaurants. There is a tremendous scope for frozen vegetables in India for export and domestic consumption. India has the necessary potential for developing vegetable processing. The present investigation was undertaken to study the retention of AA and -carotene content in French beans, okra and capsicum during processing and frozen storage.

Materials and Methods

Two summer vegetables namely French beans (*Phaseolus vulgaris* L.) and capsicum (*Capsicum annuum* L.) were purchased from the local market and okra (*Abelmoschus esculentus* Moench.) of variety - Shagun was procured directly from the farm, at their peak maturity storage. All the vegetables were processed at PAGRO Foods Ltd., Fatehgarh Sahib, Punjab, India in the month of July. French bean pods were cut into 1.0 and 6.0 cm long pieces. Small as well as large okra were selected. The larger pods were cut into rings and smaller pods were slitted length wise in the centre. Capsicum was cut into halves and seeds and stems were removed. Different combinations of time and temperature were evaluated to find out the optimum combination for blanching process. French beans and okra were blanched at 95°C for 2 minutes and capsicum was blanched at 85°C for 2 minutes prior to freezing.

The vegetables were brought to –40°C temperature in a short period of 3-6 minutes and were stored at –20 to –25°C. The samples of fresh vegetables, immediately after blanching and freezing ; after two, four and six months of frozen storage were analysed for AA and β-carotene contents. The AA was estimated colorimetrically using the method given by AOVC (1996). β-carotene was determined by the method of Rao (1967). A minimum of three replications of each sample were analysed. The analysis of variance was used to interpret the data statistically.

Results and Discussion

Ascorbic Acid: The AA content of fresh, blanched and frozen vegetables has been shown in table 1. The AA content of French beans having 6.0 cm cut and okra trimmed was higher than that of the French beans 1.0 cm and okra rings throughout the processing and frozen storage period of six months.

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Table 1 Effect of processing and frozen storage on retention of ascorbic acid content (mg/100g, fresh weight basis) of the vegetables

Vegetable	Fresh	Blanched	Frozen storage period, months				F^1-ratio	F^2-ratio	F^3-ratio
			0	2	4	6			
French bean 1.0cm	25.2	19.6	19.5	19.0	18.6	18.0	29.39* (1.607)	4.48 ^{NS}	46.55** (1.076)
French bean 6.0cm	26.0	21.3	21.2	21.2	20.8	20.1	7.83^{NS}	1.21^{NS}	7.69* (3.274)
t-value	0.39 ^{NS}	4.71*	6.01*	2.2 ^{NS}	4.12^{NS}	3.90 ^{NS}			
Okra rings	15.0	12.6	12.5	12.3	12.1	11.8	20.03* (1.428)	2.74 ^{NS}	20.52** (0.882)
Okra trimmed	15.3	13.1	13.0	12.7	12.4	12.3	18.78* (1.355)	1.08 ^{NS}	18.87** (0.882)
t-value	0.59 ^{NS}	3.54 ^{NS}	0.93 ^{NS}	1.41 ^{NS}	1.34 ^{NS}	1.77 ^{NS}			
Capsicum halves	145.0	107.0	106.4	103.4	100.8	98.3	281.55** (5.952)	13.86* (3.667)	269.09** (3.669)

F^1-ratio for fresh, blanched and frozen (0 month) samples; F^2-ratio for frozen stored samples; F^3 overall F-ratio NS – Non-significant, * - significant at 5% level; ** - significant at 1% level Figures in parenthesis indicate C.D. values

This can be due to smaller surface area of vegetables in contact with water during blanching. A linear decrease (P £ 0.05) in AA retention was found in all the three vegetables throughout processing and frozen storage. Water blanching prior to freezing significantly decreased the AA content (14.3 to 26.2%) of all the vegetables. The freezing process caused little reduction in the AA content (0.46 to 0.79%) of the vegetables. There was a non-significant reduction in AA content i.e. 5.1 to 7.6% during frozen storage period of six months (Table 2).

Thus, it was the blanching not freezing process that resulted in AA loss of vegetables. The losses of AA during blanching may be due to leaching as it is water soluble vitamin. Further ascorbic acid is readily oxidised by heat under alkaline or neutral conditions. 26 mg/100 g AA is fresh French beans and 66 per cent loss after five minutes of blanching in boiling water was reported (Kaur and Kapoor, 2001).

Table 2: Losses (%, fresh weight basis) of ascorbic acid content during processing and frozen storage.

Vegetable	Blanching	Freezing	Six months frozen storage	Overall loss
French bean 1.0cm	22.2	0.6	7.5	28.5
French bean 6.0cm	18.0	0.46	5.1	22.6
Okra rings	16.0	0.79	5.6	21.3
Okra trimmed	14.3	0.76	5.4	19.6
Capsicum halves	26.2	0.56	7.6	32.2

There was 23.8 per cent loss of AA in bell peppers after 4.5 minutes of blanching in boiling water (Ramesh *et al*, 2002). Further a loss of less than 10% loss of AA after frozen storage period of 10 months was observed (Favell, 1997). The loss of AA content in the present study is comparable to reported data. Pruthi (1999) observed no loss of AA during 9 months storage at –40^{0}C. The AA loss can be reduced if blanching is carried out with steam instead by storing the product at much lower temperature of –40^{0}C, where no loss is expected due to oxidation. But both steam blanching and maintenance of the temperature at –40^{0}C is not economical.

β-carotene: The β-carotene concentration of fresh, blanched and frozen stored vegetables has been shown in Table 3. The β-carotene content of French beans having 6.0 cm cut (125.0 to 91.3 /mg/100g) and okra trimmed (50.0 to 36.5mg/100g) was higher than that of the French beans 1.0 cm (123 to 86.0mg/100g) and okra rings (49.0 to 35.2 mg/100g) throughout the processing and frozen storage period of six months.

This can be attributed to smaller surface area exposed to air and heat. It was observed from the data that the losses of b-carotene from vegetables were large during blanching and relatively small during frozen storage. Table 4 shows that water blanching before freezing significantly (P £ 0.05 and 0.01) decreased the β-carotene content (18.6 to 30.0%) of all the vegetables. The minimum reduction i.e. 0.50 to 1.0% was observed during freezing process. A linear decrease in the level of β-carotene during the frozen storage period was exhibited (8.2 to 12.2%). The data shows that the major β-carotene loss was due to the blanching process as carotenoids are susceptible to oxidative loss caused by

Table 3: Effect of processing and frozen storage on retention of -carotene content (µg/100g, fresh weight basis) of the vegetables

Vegetable	Fresh	Blanched	Frozen storage period, months				F^1-ratio	F^2-ratio	F^3-ratio
			0	2	4	6			
French bean 1.0cm	123.0	95.9	95.3	92.8	89.9	86.0	65.78** (8.811)	5.78^{NS}	49.03** (6.516)
French bean 6.0cm	125.0	100.0	99.5	96.8	94.3	91.3	26.30* (12.841)	0.81 ^{NS}	4.49* (10.251)
t-value	0.47 ^{NS}	1.28 ^{NS}	1.54 ^{NS}	1.17 ^{NS}	1.93 ^{NS}	2.27 ^{NS}			
Okra rings	49.0	39.5	39.3	38.1	36.7	35.2	19.45* (5.677)	0.98^{NS}	19.97** (3.770)
Okra trimmed	50.0	40.7	40.5	39.3	37.9	36.5	10.23* (7.664)	1.24^{NS}	9.56** (5.341)
t-value	0.36 ^{NS}	1.27 ^{NS}	0.57 ^{NS}	0.80 ^{NS}	0.79 ^{NS}	0.70 ^{NS}			
Capsicum halves	435.0	348.0	344.5	334.0	318.0	302.4	319.34** (10.07)	20.46** (7.69)	331.24** (9.419)

F^1-ratio for fresh, blanched and frozen (o month) samples; F^2-ratio for frozen stored samples; F^3 overall F-ratio
NS – Non-significant
* - significant at 5% level; ** - significant at 1% level
Figures in parenthesis indicate C.D. values

Table 4: Losses (%, fresh weight basis) of -carotene content during processing and frozen storage.

Vegetable	Blanching	Freezing	Six months frozen storage	Overall losses
French bean 1.0cm	22.0	0.6	9.7	30.0
French bean 6.0cm	20.0	0.5	8.2	27.0
Okra rings	19.4	0.5	10.4	28.1
Okra trimmed	18.6	0.5	9.8	27.0
Capsicum halves	30.0	1.0	12.2	30.5

heat and light. Vegetables after steam blanching and quick freezing retained over 70% of their β-carotene during prolonged storage (Howard *et al*, 1999). There was a loss of 50.3% of total carotenoids in carrots and French beans during 5 minutes water blanching (Kaur and Kapoor, 2001). A significant decrease of the b-carotene in carrots during frozen storage period of 10 months was observed. The losses observed in the present study could be different due to the difference in time, temperature and medium of blanching.

The results concluded that in spite of 19.6 to 32.2% losses in AA and 27.0 to 30.5% losses in b-carotene, on fresh weight basis, vegetables retained about 67.8 to 80.4% and 69.5 to 73%, AA and b-carotene, respectively after processing and frozen storage period of six months. The major losses of AA and b-carotene contents were during blanching process than freezing or frozen storage. The frozen vegetables may be better sources of AA and β-carotene than their fresh counterparts as the analysed vegetables are consumed in cooked form which results in greater losses when compared to blanching. Further, it is not unusual for fresh vegetables to be in transit under variable conditions of heat, cold and humidity for approximately 7 to 14 days and under these conditions, nutrients are rapidly lost due to respiration. But when vegetables are processed by commercial freezing, these are picked and frozen within hours of harvest. Hence, individual quick freezing is a suitable technique of preserving AA and β-carotene contents of vegetables taken directly from the fields, with no sign of deterioration in their physical quality. In addition to this, these can be an important source of minerals and fibre and will be available throughout the year for healthy balanced diets.

References

AOVC. 1996. *Methods of Vitamin Assay* (3rd edn). pp 306-12. Association of Vitamin Chemists, Inc., Inter-Science Publishers, New York.

Favell D J. 1997. A comparison of the vitamin C content of fresh and frozen vegetables. *Fd Chem* 62 59-64.

Howard L A, Wong A D, Perry K, Klein B P. 1999. b-carotene and ascorbic acid retention in fresh and processed vegetables. *J Fd Sci* 64: 929-30.

Kaur C and Kapoor H C. 2001. Effect of different blanching methods on the physico- chemical qualities of frozen French beans and carrot. *J Fd Sci* 38: 65-67.

Pruthi J S. 1989. Marketing of quick frozen foods: present status, problems and prospects. *Indian Fd Packer* 43: 111-26.

Pruthi J S. 1999. *Quick freezing preservation of foods – principle, practices, R&D needs Vol.II Foods of Plant Origin* pp 23-42. Allied Publishers Ltd., New Delhi.

Ramesh M N, Wolf W, Tevini D, Bognar A. 2002. Microwave blanching of vegetables. *J Fd Sci* 67: 390-98.

Rao CN. 1967. True vitamin A value of some vegetables. *Ind J Nutr Dietet* 4 : 10-16.

Vanderslice J T, Higgs D J, Hayes J M, Block G. 1990. Ascorbic acid and hydroascorbic acid content of foods-as-eaten. *J Food Comp and Anal* 3: 106-111

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Peroxidase Activity, Chlorophylls and Antioxidant Profile of Two Leaf Vegetables (*Solanum nigrum* L. and *Amaranthus cruentus* L.) under Six Pre-treatment Methods before Cooking

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The study evaluated the effects of six pretreatment methods before cooking on the peroxidase activity, chlorophyll and antioxidant profile of Amaranthus cruentus L. and Solanum nigrum L. The six pretreatments used were: chopped only (raw sample) (Coded M1); chopped and dried at 50 ^{0}C for 5 hours(Coded M2); chopped and squeezed in water (at room temperature) (Coded M3); chopped and soaked in salt-treated water (approx. 20 g NaCl per L of water) for 15 mins, then squeeze (Coded M4), chopped and soaked in warm water (approx. 60 ^{0}C), then cool and squeeze (Coded M5); and chopped and soaked in boiling water (100 ^{0}C), then cool and squeeze (Coded M6). The main effect of vegetable type and the main effect of pretreatment methods have significant effects ($P \leq 0.05$) on the parameters measured while the interaction of vegetable type and pretreatment methods have no significant effect on the parameters measured. Statistical analyses ($p \leq 0.05$) showed that chlorophyll "a" and "b" occur in ratio 3:1 in the two vegetables irrespective of the pretreatment imposed. Peroxidase activity test showed that A. cruentus, irrespective of the pretreatment imposed showed no peroxidase activity while S. nigrum showed high peroxidase activity for all the pretreatments except for M6. Results showed that there was significantly ($P \leq 0.05$) higher content of carotenoids in A. cruentus compared to S. nigrum, while the total phenolics, total flavonoids and total tannins contents were higher in S. nigrum compared to A. cruentus, irrespective of the pretreatment method used. For the two vegetables the percentage losses in total carotenoids, phenolics, flavonoids and total tannins at M6 compared to M1 were 53.3-60.5, 55.6-57.1, 62.4-63.6 and 66.1-73.5%, respectively. There was a sharp drop in the carotenoids, phenolics, flavonoids and tannins contents of the two vegetables at M4 and M6, with both treatments having closely similar values for each parameter.

Keywords: Vegetables, Pretreatment methods, Chlorophyll, Peroxidase activity, anti-oxidants.

Introduction

Amaranthus cruentus L., family Amaranthaceae, and *Solanum nigrum* L., family Solanaceae, are two important leaf vegetables that are available in the market and widely eaten in Africa and different parts of Asia. The nutritional and medicinal importances of these two important vegetables have been known to science for a long time. According to Amin *et al.* (2005) overwhelming scientific data, from epidemiological studies, indicate that diets rich in fruit, vegetables and grains are associated with a lower risk of several degenerative diseases, such as cancer (Steinmetz & Potter, 1996) and cardiovascular diseases (Rimm *et al.*, 1996). Amin et al. (2006) also stated that this association is often attributed to different antioxidant components, such as vitamin C, vitamin E, carotenoids, lycopenes, polyphenols and other phytochemicals.

Most leaf vegetables are passed through one pretreatment or the other before the actual cooking and there are reasons attached to the type of pretreatment that is used at any point in time. A study by Adebooye *et al.* (2001) showed that consumers of leaf vegetables in certain parts of Africa subject specific leaf vegetables to certain pretreatments before cooking to achieve the following objectives: (a.) to soften the tissue for specific soup preparations (b.) to remove bitter factor from vegetables that have bitter leaves (c.) to make the vegetable suitable for some traditional soups and (d.) to preserve the vegetable in dry form for ready use during the off-season.

Applications of some pretreatments on leaf vegetables have been shown to have significant effect on food value. For example, Price *et al* (1997) reported 82% loss of phenolic compounds to cooking water after leaf vegetable was blanched for 15 mins. Also, Papetti *et al.* (2002) reported a decrease in total antioxidant values when vegetable juices were cooked at 102 ^{0}C for 10 mins while the work of Gill *et al.* (1999) showed that boiling of fresh cut spinach for 10 minutes released 50-60% of its antioxidant compounds into the cooking water. Boiling processes at above 95^{0}C had been shown to decompose the antioxidant components of vegetables (Hunter and Fletcher, 2002). Other reports including that of Yadav and Sehgal (1995), Chu *et al.* (2000), Amin *et al.*(2004) and Amin *et al.* (2006) have shown that cooking has a reducing effect on the antioxidant components of leaf vegetables.

A study by Adebooye *et al* (2001) identified five pretreatment methods that are given to vegetables in certain parts of Africa before the actual cooking: (a.) chop and cook directly (b.)

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chop the leaves and dry before cooking. This method is used to preserve the vegetables in dry form for use during the off-season. (c.) chop the leaves and squeeze in water (at room temperature) to soften the tissue. (d.) chop the leaves and soak in salt (NaCl) treated water for 15 mins, then squeeze to improve palatability. (e.) chop the leaves and soak in warm water (approx. 60 ^{0}C) for 5 mins, then cool and squeeze to soften the tissue. (f.) chop the leaves and soak in boiling water (100 ^{0}C) for 5 mins, thereafter cool and squeeze to soften the tissue. Each of these methods has specific culinary application in different soup preparation in different parts of Africa. Generally these above mentioned pretreatments are used on *A. cruentus* and *S. nigrum* depending on the type of soup to be prepared but their effects on the nutraceutical qualities of the vegetables have not been investigated, especially with respect to peroxidase activity, total phenolics, total flavonoids, total carotenoids, tannins and chlorophyll profile. Therefore this study was designed to investigate the effect of six pretreatment methods on the antioxidant profile, peroxidase activity and chlorophyll contents of *A. cruentus* and *S. nigrum*.

Materials and Methods

Collection of samples

Amaranthus cruentus (variety: Pusa Early Bunching) and *Solanum nigrum* (variety: All Green) were identified on farmers farms where organic agriculture is practised and chemicals are not applied, in Mysore, Karnataka State, India. Eighteen (18) kilograms of each vegetable was purchased from the farmers and the samples were immediately taken to the Fruit and Vegetable Department Analytical Laboratory, Central Food Technological Research Institute, Mysore, India for analyses. The samples were carefully washed under running tap water to remove soil particles and other contaminants. Thereafter, the samples were spread evenly on perforated large aluminum trays for air-drying to remove water droplets.

Experimental Design

The laboratory study was a factorial arrangement laid out in a randomized complete block design (RCBD). The two vegetables served as Factor A while the six pretreatment methods and control served as Factor B. Each 18 kg fresh vegetable was divided into six sub-samples of 3 kg each and each of the 3 kg sub-samples was further sub-divided into three sub-sub-samples of 1 kg each which eventually served as three parallel replicates for each pretreatment. On each 1 kg sub-sub-sample, we applied the following pretreatment methods to mimic the traditional methods of pretreatments in certain parts of Africa. The pretreatments were applied in parallel triplicate:

a. Chopped only (raw sample) (Coded M1).
b. Chopped and dried at 50 ^{0}C for 5 hours(Coded M2)
c. Chopped and squeezed in water (at room temperature) (Coded M3)
d. Chopped and soaked in salt-treated water (approx. 20 g NaCl/L of water) for 15 mins, then squeeze (Coded M4) .
e. Chopped and soaked in warm water (approx. 60 ^{0}C), then cool and squeeze (Coded M5).
f. Chopped and soaked in boiling water (100 ^{0}C), then cool and squeeze (Coded M6).

It must be noted that for treatments M3, M4, M5 and M6, 1 kg of vegetable sample was treated with 4 L of water. After each treatment, the water was drained and discarded while the vegetable alone was prepared for chemical analyses.

Reagents

The (±) catechin, tannic acid, Folin-Denis reagent, Folin-Ciocalteu reagent, guaiacol (o-methoxyphenol) were obtained from Sigma-Aldrich Chemie Gmbh, Germany. Other chemicals were of analytical grade and obtained from Qualigens Fine Chemicals, India.

Parameters determination

Total phenolics

To 250 mg fresh sample was added 50 ml double distilled water in triplicate and the mixture was ground in a mortar. The mixture was transferred to a rotary shaker (Remi Instruments, India) for 12 hours to ensure full extraction. The mixture was thereafter filtered and the filtrate was made up to 50 ml. Total phenolics were determined by the Folin-Ciocalteu method. Two hundred microliters of diluted filtrate were added to 1 ml of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 800 μl of sodium carbonate (75 g/l) were added. After 2 h of incubation at room temperature, the absorbances at 765 nm were measured. Gallic acid (0–100 mg/l) was used for calibration of a standard curve. The results were expressed as gallic acid equivalents (GAE)/100g fresh weight of plant material.

Total carotenoids and Chlorophylls profile

For the extraction of chlorophylls and carotenoids the method of Mackinney (1941) was used. About 5 mg fresh sample for each treatment was weighed in triplicate and extracted in 50 ml of 80:20 v/v acetone using pestle and mortar. The extraction was repeated until a colourless residue was obtained and the mixture was filtered. The extraction procedures of these pigments were carried out under dim light and in a glassware wrapped with aluminium foil. The filtrates (extracts) were made up to 50mL with acetone. One ml of the extract was taken and diluted to 10 ml using 80:20 v/v acetone and thereafter the concentration of carotenoids was measured at 440 nm, chlorophyll "a" at 663 nm, and chlorophyll "b" at 645nm using a UV-Visible spectrophotometer Model UV 1601 Version 2.40 (Shimadzu, Japan).

Tannins determination

The method described by Pearson (1976), with slight modification, was used for the determination of tannin content

of samples. Extraction of tannin in the samples, in triplicate, was achieved by grinding 250 g of fresh sample in 50 ml distilled water in pestle and mortar. The mixture was transferred to a rotary shaker (Remi Instruments, India) for 12 hours to ensure full extraction. Thereafter the mixture was filtered and the filtrate (extract) made up to 50 ml. One milliliter of the extract or 1ml of standard tannic acid (0.01 g/l tannic acid) was measured into different test tubes and 1ml of Folin–Denis reagent was added to each flask followed by 2.5 ml of saturated sodium carbonate solution. The solutions were made up to 10 ml mark with distilled water and shaken to mix properly. Thereafter mixtures were incubated at room temperature (approx. 30 ^{0}C) for 30 min. The absorption of these solutions were measured against that of the reagent blank (containing 1 ml distilled water in place of extract or standard tannic acid solution) in a UV-Visible spectrophotometer Model UV 1601 Version 2.40 (Shimadzu, Japan) at 760 nm wavelength.

Total flavonoids determination

Extraction of flavonoid in the samples, in triplicate, was achieved by grinding 5 g of fresh sample in 50 ml distilled water in pestle and mortar. The mixture was transferred to a rotary shaker (Remi Instruments, India) for 12 hours to ensure full extraction. Thereafter the mixture was filtered and the filtrate (extract) made up to 50 ml. The aluminum chloride colourimetric assay as described by Marinova et al. (2005) was used. Briefly, 1 ml of extracts or standard solution of catechin (20, 40, 60, 80 and 100 mg/ L) was added to test tubes containing 4 ml of double distilled water. To the mixture was added 0.3 ml 5% NaNO2. After 5 mins, 0.3 10% AlCl3 was added. Immediately, 2 ml 1M NaOH was added and the total volume was made up to 10 ml with double distilled water. The solution was mixed thoroughly and the absorbances of both the samples, blank and standard were read at 510 nm using UV-Visible spectrophotometer Model UV 1601 Version 2.40 (Shimadzu, Japan). Total flavonoids content was expressed as mg catechin equivalents (CE/100 g fresh weight).

Test for peroxidase activity

To 2 ml of the water extract, in triplicate, was added 20 ml double distilled water in a test tube. To the mixture was added 1 ml 0.5% ethanolic guaiacol (o-methoxylphenol) without mixing. Immediately, 1 ml 0.08% H2O2 was added without mixing. The blank was prepared by adding 2 ml of sample water extract to 22 ml double distilled water.

The mixtures were left for 2 mins and development of any color was observed. Development of any colour within 2 mins indicates high peroxidase activity and it is coded positive (+ ve) while no change in colour indicates no peroxidase activity and it is coded (- ve).

Statistical analyses

Considering the two vegetables as Factor A and the six pretreatment methods as Factor B, all replicated three times, the data obtained were subjected to analyses of variance (ANOVA) using the standard methods of Steele and Torrie (1980)

Results and discussion

The analyses of variance (ANOVA) as presented in Table 1 showed that the main effect of vegetable type and the main effect of pretreatment methods have significant effects ($P \leq 0.05$) on the parameters measured while the interaction of vegetable type and pretreatment methods have no significant effect on the parameters measured.

Table 1. ANOVA for effects of vegetable type and pretreatment methods on nutraceutical parameters

Source	Chlorophyll a	Chlorophyll b	Carotenoid	Flavonoids	Tannins	Phenolics
Vegetable (V)	*	*	*	*	*	*
Pretretment (M)	*	*	*	*	*	*
Interaction (V×M)	ns	ns	ns	ns	ns	ns

ns, * non significant or significant at $P \leq 0.05$ respectively.

Table 2 showed that the chlorophyll "a" and "b" contents of *S. nigrum* were significantly higher than that of *A. cruentus* irrespective of the pretreatment imposed. An interesting finding in this study is that the ratio of chlorophyll "a" to "b" was 3:1 for both vegetables irrespective of the pretreatment used. In many plants, earlier report (Wolf, 1956) showed that chlorophyll "a" composed 62.5-75.4% of the total chlorophyll in 25 autumn plant species. However, research studies are yet to clarify what the exact health significance is of this chlorophyll "a" to chlorophyll "b" ratio. Although research is still at its infancy, chlorophyllin, a water-soluble derivative of chlorophyll has been used clinically in various capacities, including as an aid to wound healing and that when administered during carcinogen exposure, chlorophyllin is a potent antimutagen and anticarcinogen (Dashwood, 1997). Thus, Egner *et al.* (2001) suggested prophylactic interventions with chlorophyllin or supplementation of diets with foods rich in chlorophylls (especially leaf vegetables) as practical means to prevent the development of hepatocellular carcinoma or other environmentally induced cancers.

There was no significant difference in the chlorophyll "a" and "b" contents of the two vegetables at M1, M2 and M3. However, in *S. nigrum*, there were 27.6, 48.2 and 57.9% losses of chlorophyll "a" in M4, M5 and M6 pretreatments compared

TABLE 2 . Effect of vegetable type and pretreatment method on chlorophyll "a" , "b" and peroxidase activity+

Sourcce		Chlorophyll (mg/100g fresh weight) "a"	"b"	"a" : "b"	Peroxidase activity
A. cruentus	M1	132.0	43.7	3 : 1	-ve
	M2	131.3ns	43.0ns	3 : 1	-ve
	M3	128.4ns	42.1ns	3 : 1	-ve
	M4	102.1*	34.1*	3 : 1	-ve
	M5	62.6*	20.8*	3 : 1	-ve
	M6	53.0*	18.0*	3 : 1	-ve
S. nigrum	M1	165.8	55.3	3 : 1	+ve
	M2	162.6ns	53.1ns	3 : 1	+ve
	M3	157.8ns	53.2ns	3 : 1	+ve
	M4	120.1*	38.7*	3 : 1	+ve
	M5	85.4*	28.4*	3 : 1	+ve
	M6	69.8*	24.0*	3 : 1	-ve

ns, *, : non-significant, or significant at $P \leq 0.05$, respectively, Least Squares Means analysis.
+All values are means of paralle triplicate analyses.

to M1, while in *A. cruentus* the losses were 30.0, 48.6 and 56.6%, respectively. The chlorophyll "b" contents of the two vegetables at M4, M5 and M6 followed similar trends as chlorophyll "a". In a study of the effect of cooking on squash, green beans, peas, leek, broccoli and spinach, Turkmen (2005) reported that chlorophylls were retained to 19–100%, depending on the vegetable type and cooking method while pheophytins increased in all vegetables after cooking. Highest chlorophyll *a* and chlorophyll *b* losses were observed in boiled leek while microwaved peas and boiled peas retained the most chlorophyll *a* and chlorophyll *b*, respectively. When green vegetables are cooked and/or exposed to acid, studies have shown that the magnesium gets removed from the center of this ring structure and replaced by an atom of hydrogen, therefore the chlorophyll "a" gets turned into a molecule called pheophytin "a", and the chlorophyll "b" gets turned into pheophytin "b". The pheophytin "a" provides a green-gray color while the pheophytin "b" provides an olive-green color. This probably accounted for the decline in the chlorophylls contents under pretreatments M4, M5 and M6.

Peroxidase activity test showed that *A. cruentus,* irrespective of the pretreatment imposed, showed no peroxidase activity while *S. nigrum* showed high peroxidase activity for all the pretreatments except for M6. The inactivation of peroxidase activity at M6 for *S. nigrum* is attributable to the high temperature (100 ^{0}C) of the water used for the pretreatment. This result confirmed earlier observation made on the two vegetables because experience has shown that *A. cruentus* could be stored in cool corners of homes for at least three days after harvesting without showing any serious sign of deterioration while *S. nigrum* starts to shown sign of loss of "greenness" and quality as early as 5 hours after harvesting, the storage medium notwithstanding. The negative peroxidase activity in *A. cruentus* could be attributed to genotype. Earlier reports by Lin and Brewer (2005) showed that blanch treatments by steaming and by boiling water reduced peroxidase activity by 97% compared with controls (unblanched) in peas.

Table 3 shows the effect of pretreatment methods on the total carotenoids, total phenolics, total flavonoids and total tannins contents of *A. cruentus* and *S. nigrum*. Results showed that there was significantly ($P \leq 0.05$) higher content of carotenoids in *A. cruentus* compared to *S. nigrum*, while the total phenolics, total flavonoids and total tannins contents were higher in *S. nigrum* compared to *A. cruentus*, irrespective of the pretreatment method used. For each vegetable, the total carotenoids for M1, M2 and M3 did not differ significantly. However, when compared with M1 in *A. cruentus*, the losses in total carotenoids due to pretreatments were 38.0, 36.4 and 53.3% for M4, M5 and M6, respectively while in *S. nigrum;* the losses were 25.0, 48.5 and 60.5%, respectively. In the M1 samples, there were 23.5, 60.1 and 12.2% more flavonoids, tannins and phenolics in *S. nigrum* compared to *A. cruentus*. This trend was observed even among the different pretreatments used. There was a sharp drop in the carotenoids, phenolics, flavonoids and tannins contents of the two vegetables at M4 and M6, with both treatments having closely similar values for each parameter. The sharp drop at M4 was suspected to be as a result of two important factors, first, the osmotic effect of the salt solution on the protoplasmic fluid of the vegetable cell. In this case, it was suspected the protoplasmic fluid (containing carotenoids, phenolics, flavonoids and tannins) of lower concentration was released to the salt solution of higher concentration, especially when squeezing was involved, thereby leaving the protoplasm placid and empty. Second, the salt solution might have undergone some chemical reactions with the carotenoids, phenolics, flavonoids and tannins contents, thereby converting them to some other products just as it has been observed in chlorophyll by Turkmen *et al* (2005). The observation on M6 was thought to be due to the high temperature (100 ^{0}C) of the water used for the pretreatment. Papetti *et al.* (2002) reported a decrease in total antioxidant values when vegetable juices were cooked at 102 ^{0}C for 10 mins while the work of Gill *et al.* (1999) showed that boiling of fresh cut spinach for 10 minutes released 50-60% of its antioxidant compounds into the cooking water. Boiling processes at above 95^{0}C had been shown to decompose the antioxidant components of vegetables (Hunter and Fletcher, 2002). Other reports including that of Yadav and Sehgal (1995), Chu *et al.* (2000), Amin *et al.*(2004) and Amin *et al.* (2006) have shown that cooking has a reducing effect on the antioxidant components of leaf vegetables.

It can be concluded from the results of this study that pretreatments M1 (raw vegetable) and M2 (chop and dry) retained substantial amounts of chlorophylls, carotenoids, phenolics, flavonoids and tannins, while salt water (M4) and boiling water (M6) have profound eroding effects on these valuable components of nutritional significance. Since

Table 3 . Effect of vegetable type and pretreatment methods on total carotenoids, phenolics, flavonoids and tannins contents⁺

Source		Carotenoids (mg/100 g)	Phenolics (GAE/100 g fresh weight)	Flavonoids (CE/100 g fresh weight)	Tannins contents mg/100g
A. cruentus	M1	24.2	61.8	49.4	20.4
	M2	23.8ns	60.4ns	47.0ns	18.4ns
	M3	21.2ns	50.7*	39.7*	11.3*
	M4	15.0*	28.1*	20.1*	6.1*
	M5	15.4ns	37.4*	30.2*	8.6*
	M6	11.3*	27.4*	18.6*	5.4*
S. nigrum	M1	20.0	70.4	64.3	50.7
	M2	19.4ns	68.4ns	61.2ns	47.2ns
	M3	18.7ns	59.4*	46.0*	37.9*
	M4	15.0*	31.2*	26.1*	18.9*
	M5	10.3*	44.0*	35.2*	25.0*
	M6	7.9*	30.2*	23.4*	17.2*

ns, *, : non-significant, or significant at $P \leq 0.05$, respectively, Least Squares Means analysis.
⁺All values are means of parallel triplicate analyses

pretreatment M2 (chop and dry) is used to preserve the vegetables for a long period of time, there is the need to study the effect of long time storage of vegetables under this method on chlorophylls carotenoids, phenolics, flavonoids and tannins contents. The peroxidase activity was high in *S. nigrum* indicating its fast deterioration and fast loss of quality.

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References

Adebooye, O.C., F.M.D. Ogbe and J.F. Bamidele (2001) Ethnobotanical studies and utilization of the indigenous leaf vegetables of southwest Nigeria. Technical Report. United Nations University/Institute for Natural Resources in Africa, Accra, Ghana. 64pp.

Amin, I., Zamaliah, M. M., & Chin, W. F. (2004). Total antioxidantactivity and phenolic content in selected vegetables. *Food Chemistry* 87 (4): 581–586.

Amin, I., Y. Norazaidah and K.I. Emmy Hainida (2006) Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species *Food Chemistry* 94 (1) 47-52

Chu, Y. H., Chang, C. L., & Hsu, H. F. (2000). Flavonoidseveral vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture*. 80: 561–566.

Dashwood, R.(1997) Chlorophylls as anticarcinogens. *International Journal of Oncology* 10:721-727.

Egner , P. A., J. Wang, Y. Zhu, B. Zhang, Y. Wu, Q. Zhang, G. Qian, S. Kuang, S. J. Gange, L. P. Jacobson, K. J. Helzlsouer, G. S. Bailey, J. D. Groopman and T. W. Kensler (2001) Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk for liver cancer. *Proceedings of the National Academy of Sciences* 98(25):14601-14606

Gil, M. I., Ferreres, F., & Thoma´s-Barbera´n, F. A. (1999). Effect ofpostharvest storage and processing on the antioxidant constituents(flavonoids and vitamin C) of fresh-cut spinach. *Journal of Agricultural and Food Chemistry*. 47: 2213–2217.

Hunter, K. J., & Fletcher, J. M. (2002). The antioxidant activity andcomposition of fresh, frozen, jarred and canned vegetables. *Innovative Food Science and Emerging Technology*. 3: 399–406.

Lin S. and M.S. Brewer (2005) Effects of blanching method on the quality characteristics of frozen peas. *Journal of Food Quality* 28 (4) Page 350-360

Mackinney, G. (1941) Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry* 140: 315pp.

Marinova, D., F. Ribarova and M. Atanassova (2005) Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy* 40 (3): 255-260

Papetti, A., Daglia, M., & Gazzani, G. (2002). Anti and pro-oxidant water soluble activity of *Chicorium* genus vegetables and effect of thermal treatment. *Journal of Agricultural and Food Chemistry*. 50: 4696–4704.

Pearson, D. (1976). The chemical analysis of foods (7th edn.). London: Churchill Livingstone. 572pp

Price, K. R., Bacon, J. R., & Rhodes, M. J. C. (1997). Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*). *Journal of Agricultural and Food Chemistry* 45: 938–942

Rimm, E. B., Ascherio, A., Grovannucci, E., Spielgelman, D., Stampfer, M. J., & Willett, W. C. (1996). Vegetable, fruit and cereal fiber intake and risk of coronory heart disease among men. *Journal of the American Medical Association*. 275: 447–451.

Steele S.G.D and J.H. Torrie (1980) Principles and procedures of statistics. McGraw Hill Publishers, New York. 489 pp.

Turkmen, N., E. S. Poyrazoglu, F. Sari & Y. S. Velioglu (2005) Effects of cooking methods on chlorophylls, pheophytins and colour of selected green vegetables. *International Journal of Food Science and Technology* (In Press) doi:10.1111/j.1365-2621.2005.01061.x

Wolf, F. T. (1956) Changes in Chlorophylls A and B in Autumn Leaves *American Journal of Botany*. 43 (9): 714-718

Yadav, S. K., & Sehgal, S. (1995). Effect of home processing on ascorbic acid and b-carotene content of spinach and amaranth leaves. *Plant Foods for Human Nutrition*. 47: 125–131.

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Study on Moisture Sorption Behavior of CTC Black Tea

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Moisture sorption isotherms of CTC black tea (BOP grade) at different temperatures (25°, 35° and 45°C) using Wink's weight equilibrium method were studied. Effects of tea moisture content on the tannin and caffeine content in hot water extractives and the cup quality of the tea stored for two months at ambient condition (25-30 °C, 65-85 % relative humidity) were evaluated. Results indicated that sorption isotherms were of type III and no intersection of isotherm was observed as a characteristic of low sugar containing products, downward shifting of isotherm on increasing temperature occurred. Oswin and Halsey models were best fitted to the sorption data. Monolayer moisture content as determined by BET and GAB models were found to be 0.05, 0.046, and 0.038; and 0.049, 0.044 and 0.041(kg water/kg dry matter) at 25°C, 35°C and 45°C respectively The range of water activity for monolayer moisture was found to be 0.2-0.4.Teas moisture content of up to 9 % (db) had no significant effect($p>0.05$) on the caffeine content in the hot water extractives of stored for two months at ambient condition while tannin content and cup quality were seriously affected by moisture content at 7 % and onward.

Keywords CTC, Orthodox, Water activity, Sorption isotherm, EMC, Sensory evaluation

Introduction

Tea is the most popular and one of the oldest known beverages in the world. Tea, in the context used by most consumers, is a beverage consisting of an infusion of the processed and dried leaves of the tea plant, *Camellia sinensis*. The tea drinking habit gradually spread from Asia along the trade routes of Asia minor and was introduced to Europe by Dutch traders during the 17th century (Vernam and Sutherland, 1994).

The processing stages of tea are unsophisticated, but bear directly on the quality of the made tea. The difference between finished tea products are due to composition, physical properties of the fresh leaves, manufacturing process used and the degree of fermentation. According to fermentation of tea leaves, there are black tea, green tea and oolong tea. Black tea is fermented whereas oolong tea is semi fermented and green tea is non-fermented. Black tea manufacturing process involves withering, rolling, fermentation, firing (drying) and grading. Black tea may further be subdivided into Orthodox and CTC tea (Considine and Considine, 1982).

Tea being a very hygroscopic, its ability to remain in good condition is highly dependent on its moisture content. So, the main technical requirement for packaging of tea is the provision of an adequate moisture barrier to protect mold growth and to loss of aroma by evaporation. As a food product dries, both moisture content and water activity changes. At any given relative humidity of air used for drying, there is equilibrium moisture content (EMC) with the product. At this point, the activity of water in the air is the same as that in the product .The relationship between the EMC in the food and the relative humidity of air is important in drying process.

Whenever water content of a food is plotted against the water activity at constant temperature, a sigmoid curve usually results.

This curve is known as the sorption isotherm for that product. Brunauer *et al.* (1940) classified sorption isotherm of material into five general types. However, it has been found that the nature of this relationship depends on whether the food product is being dried or allowed to pick up moisture. Isotherms for different foods vary both in the shape of the curve and in the water present at each relative humidity (Heldman and Harter, 1998).

According to Labuza and Rahman (1999), the need for reliable sorption data has always useful in the formulation of shelf stable food products. Temperature has a significant effect on sorption isotherm and its knowledge is essential for the efficient design and operation of several processing operation and storage. The self-life of packaged food involves the reactivity of the food and the capacity of the package to protect the food from these influences.

Hence, to predict the self-life of a particular packaged food, properties of the food product and package are important. The permeability of the package is the most important property to be considered. So, the knowledge of sorption is of great importance in food processing (Gal, 1983).

This study was undertaken to determine the EMC of tea at different temperature and relative humidity, to determine sorption isotherm at different temperature and to evaluate the suitability of various mathematical model for fitting the isotherm and to study the effect of moisture content on the extraction of tannin and caffeine and cup quality.

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Materials and Methods

The sample (CTC black tea, BOP grade) was collected directly from factory (Himalaya Goodricke, Damak, Nepal).

Determination of sorption isotherm

The sorption method employed was the gravimetric method, based on the use of Sulphuric acid to maintain a fixed relative humidity.

The relative humidity employing different concentrations of Sulphuric acid was in the ranges from 10 to 90 % and duplicate samples of about 2 g (tea powder) were placed on petri plate inside desiccators and were placed in thermal chamber (maintaining temperatures at 25°, 35°, and 45°C) respectively. EMC was determined by Wink's weight equilibrium method as described by Ranganna, 1994.

Fitting of sorption data to different models

Different models as Literature were fitted to the moisture isotherm data, using Nonlinear Regression Analysis of the Statistical Analysis System. This utilizes Gauss-Newton method to find the least square estimates of model parameters.

Effect of tea moisture on the caffeine, tannin content and cup quality of liquor

To determine the effects of moisture content on the extraction of caffeine and tannin in liquor and cup quality, four samples of about 20 g each were placed in desiccators, 2 in desiccators of 10% and 2 in 60 % relative humidity and subjected to room temperature to make samples having moisture content 3, 5, 7 and 9 % (db) and were stored for 2 months by packing in air tight dried glass container under ambient condition (25°-30° C, 65-85 % relative humidity). *Chemical Analysis*

Moisture determination was carried out by hot air oven method (Ranganna, 1994) and caffeine and tannin were estimated as described by Sathe (1999).

Sensory evaluation

Sensory evaluation of brew made from 3,5,7 and 9% moisture content(db) tea samples was carried out by using 9 points hedonic test (Ranganna, 1994).The brew was evaluated by tea tasters at Himalayan Goodricke tea estate.

Data analysis

All experiments were performed in triplicates and numerical datas were analyzed by ANOVA. Means were compared by LSD method at 5 % level of significance (Gomez and Gomez, 1984).

Results and discussion

Sorption isotherm of CTC black tea at different temperatures

The tea samples were analyzed for EMC at different relative humidity (i.e, 10, 20, 30, 50, 60, 70, 80 and 90 %) at different temperature (25, 35, and 45 °C) .The EMC data for the tea at different relative humidity and temperature are shown in the form of isotherm in Fig.1.

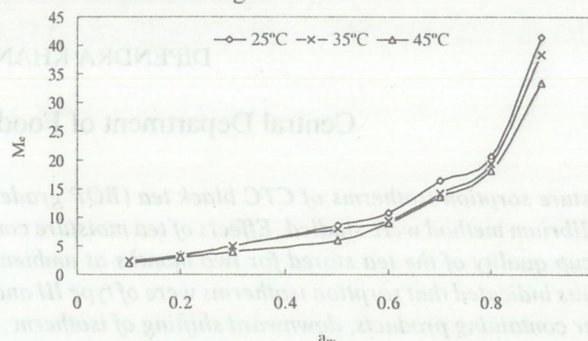

Fig. 1 Sorption isotherm of CTC black tea at different temperatures

Each value represents the mean of two replication for each water activity. These are sigmoid in shape and belong to the type III isotherm, according to the classification of Brunauer *et al.* (1940). The time required for reaching at EMC was shorter at high temperature and equilibrium moisture content was obtained somewhat early in lower relative humidity than in high relative humidity. The time taken to reach for EMC at 25°C was 29days, at 35°C was 23 days and at 45°C was 17 days In the curve, we can see that there is slight increase in moisture content with decreasing temperature. The graph also shows an increase in EMC with increasing water activity at constant temperature. This affirmed that as moisture content decreases, moisture in foods tends to show a lower vapor pressure changing with environmental humidity. There was a consistent shift to the right due to increase in temperature at given moisture content. This shift plays an important role in the stability of stored food at different temperature and given relative humidity. At all temperature, the stability of the product becomes critical at relative humidity above 70 %. This was due to the increase in rate of reaction in the product as a result of microbial and enzymatic activities.

Fitting of sorption data to different models

Various mathematical models were used to fit the sorption data for black tea. The models of interest and importance were BET, GAB, Oswin and Hasley. The equations were fitted using non linear regression analysis and coefficients of models were calculated. Coefficients of regression were determined and the best fitting model was suggested.

Oswin model

This is a mathematical series expansion for sigmoid shaped curves and may be written as:

$$M_e = a\left(\frac{a_w}{1-a_w}\right)^n$$

where, a and n are constants.

This equation was used to determine the value of a and n which are given in Table 1.

Table 1 Values of constants and coefficients of regression for Oswin model.

Parameters	Temperatures(°C)		
	25	35	45
a	0.0895	0.0824	0.074
n	0.6485	0.6398	0.64569
R2	0.990	0.989	0.986

When the data were plotted as ln M_e versus ln $(a_w/1-a_w)$ (Fig. 2), a straight line was obtained. The fit of the data to Oswin model was quite good as seen from the regression coefficient

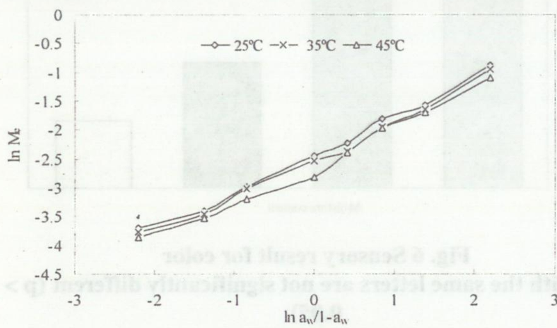

Fig. 2 Oswin plot of experimental data at different temperatures

BET model

The BET equation can be written as:

$$\frac{a_w}{M_e(1-a_w)} = \left[\frac{C-1}{M_m C}\right] a_w + \frac{1}{M_m C}$$

where, M_m = BET monolayer

C= Temperature dependence for sorption excess enthalpy.

This equation was used to determine the values of M_m and C (Table 2).

Table 2 Values of constants and coefficients of regression for BET model.

Parameters	Temperatures(°C)		
	25	35	45
Mm	0.050	0.046	0.038
C	5.843	6.365	7.394
R2	0.956	0.941	0.963

Since BET model is valid over water activity upto 0.5,so data from 0.1 to 0.5 were used.

When the data were plotted as a_w/M_e $(1-a_w)$ versus a_w (Fig. 3), a straight line was obtained. The fit of the data to BET model was satisfactory as seen form the regression coefficient.

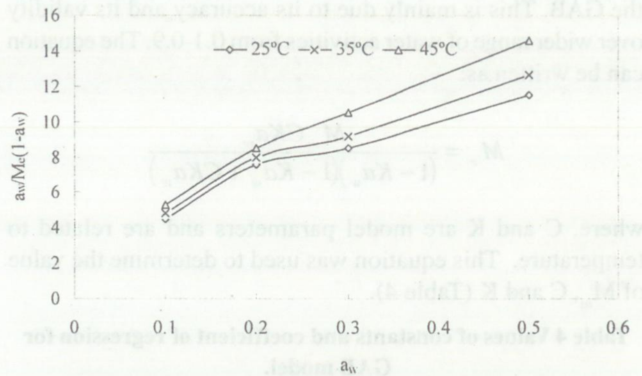

Fig. 3 BET plot of experimental data at different temperatures

Halsey model

This model is also widely used to represent the sorption isotherm of foods and can be written as:

$a_w = \exp\left(-\frac{a}{RT}\right) M_e^{\ b}$ where, a and b are constants parameters.

This equation was used to determine value of a and b (Table 3).

Table 3 Values of constants and coefficients of regression for Halsey model.

Parameters	Temperatures(°C)		
	25	35	45
a	0.043RT	0.038RT	0.035RT
b	-1.094	-1.107	-1.096
R^2	0.992	0.993	0.993

When the data were plotted as ln M_e versus ln(-ln a_w) (Fig. 4), a straight line was obtained. The fit of the data to Halsey model was not satisfactory as seen form the regression coefficient.

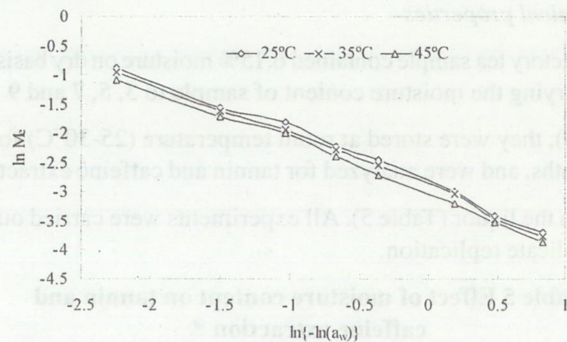

Fig. 4 Halsey plot of experimental data at different temperatures

GAB model

The GAB isotherm was developed by Guggenheim, Anderson and De Boer. In recent years, the most widely accepted and represented model for sorption isotherm for foods has been

the GAB. This is mainly due to its accuracy and its validity over wider range of water activities from 0.1-0.9. The equation can be written as:

$$M_e = \frac{M_m C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)}$$

where, C and K are model parameters and are related to temperature. This equation was used to determine the value of M_m, C and K (Table 4).

Table 4 Values of constants and coefficient of regression for GAB model.

Parameters	Temperatures (°C)		
	25	35	45
C	6.17	7.01	9.45
Mm	0.049	0.044	0.041
K	0.98	1.00	0.94
R2	0.920	0.937	0.923

When the data were plotted a_w versus a_w/M_e (Fig. 5), a parabolic curve was obtained. The fit of the data to GAB model was satisfactory as seen form the regression coefficient. It should be noted that M_m values obtained by GAB model decreased with increasing temperature.

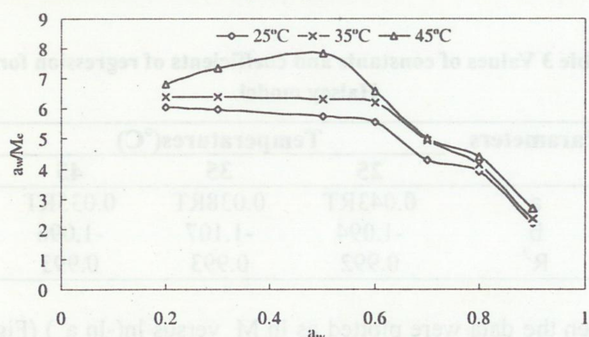

Fig. 5 GAB plot of experimental data at different temperatures

Chemical properties

The factory tea sample contained 6.15% moisture on dry basis. By varying the moisture content of sample to 3, 5, 7 and 9 % (db), they were stored at room temperature (25-30 °C) for 2 months, and were analyzed for tannin and caffeine extraction in the liquor (Table 5). All experiments were carried out in triplicate replication.

Table 5 Effect of moisture content on tannin and caffeine extraction *

Constituents	Moisture content (% db)			
	3	5	7	9
Tannin (%)	13.40[a]	13.50[a]	12.36[b]	12.06[b]
Caffeine (%)	2.79[a]	2.70[a]	2.63[a]	2.59[a]

*Values are the means of three replications. Means followed by the same superscripts are not significantly different at p=0.05

Effects of moisture content on tannin extraction in the brew was significant ($p<0.05$), where as it was insignificant for caffeine extractability. It was found that tannin extractability was affected by moisture content e" 7 %.

Sensory evaluation

Cup quality of the samples having different moisture (i.e., 3, 5, 7 and 9 % db) was carried out after 2 months of storage at room temperature. It was seen that, brews of sample having 3 and 5 % moisture (db) were significantly superior ($p<0.05$) in terms of color (Fig. 6), infusion (Fig. 7), appearance (Fig 8) and flavor (Fig. 9).

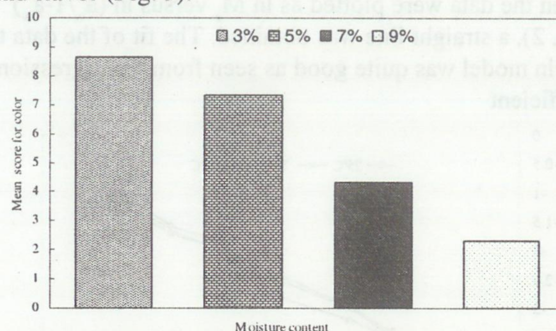

Fig. 6 Sensory result for color
Bars with the same letters are not significantly different (p > 0.05)

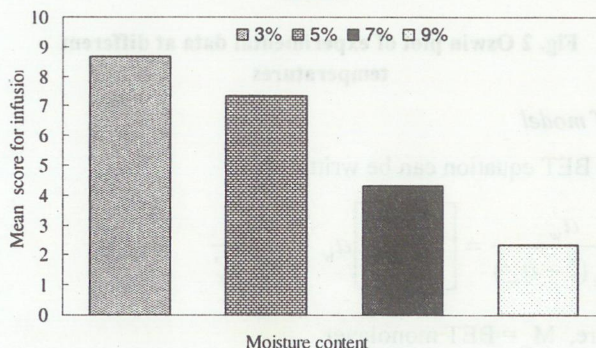

Fig. 7 Sensory result for infusion
Bars with the same letters are not significantly different (p > 0.05)

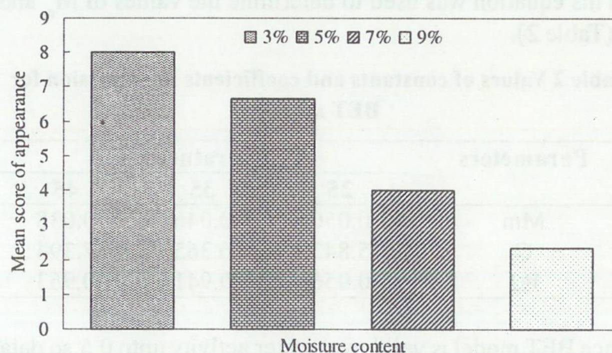

Fig. 8 Sensory result for appearance
Bars with the same letters are not significantly different (p >0.05)

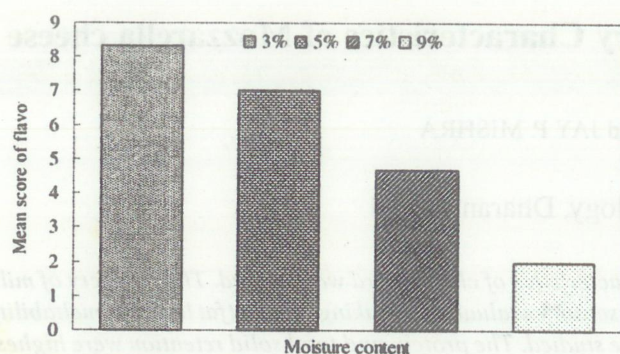

Fig. 9 Sensory result for flavor
Bars with the same letters are not significantly different ($p > 0.05$)

The mean sensory scores for color and infusion were 8.33, 7.33, 4.66 and 2.33 and 8.66, 7.33, 4.33 and 2.33 for tea samples having 3, 5, 7 and 9 % moisture content (db) respectively. Similarly, the appearance and flavor scores were 8.00, 6.66,4.00 and 2.33 and 8.33, 7.00, 4.66 and 2.00 for 3, 5, 7 and 9 % moisture content (db) tea samples.

All the sensory attributes between 3,5 % and 7,9 % moisture content (db) tea samples were not significantly different. The sensory attributes were seriously impaired when the tea moisture content was e" 7 %.Critical moisture content (CMC) for CTC tea based on tannin and caffeine contents in the hot water extractives and cup quality could be regarded as <7 %.

Conclusions

According to Brunauer classification, the moisture sorption isotherm of CTC tea was found to be of type III.Of the various models evaluated, Oswin and Halsey were best fitted to the sorption data. The quality of tea significantly impaired at and above 7 % moisture levels. The critical moisture content (CMC) based on tannin extraction and cup quality lies below 7 %.

References

Brunauer,S., Deming, L. S,W. E. & Teller, E. (1940).On a theory of the van der walls absorption of gases, *Am. Chem. Soc. J.*,62 (3),1723, [In:Hand Book of Food Preservation, Rahman, M.S.,1999(ed), Marcel Dekker, Inc., New York].

Gal, S. (1983).Necessary of sorption isotherm evaluation. In:Physical Properties of food, Jowitt, R.,Escher, F. ,Hallstrom, B., Meffert, H. F. T. Spicss, W. E. L., & Vos, G., 1983 (ed), Applied Science Publishers, London.

Gomez, A. K.&Gomez, A.A.(1984).Statistical Procedure for Agriculture Reasearch,2nd,Wiley-Interscience Publ., London

Heldman, D. R. & Harter, R.W. (1998). Principles of Food Processing, 17-180, An Aspen Publ.

Labuza T. P. & Rahman, M. S. (1999).Water activity and food preservation. In: Hand Book of Food Preservation, Rahman, M. S., 1999 (ed), Marcel Dekker Inc., New York, pp 166-168

Ranganna, S. (1994).Hand Book of Analysis and Quality Control for Fruit and Vegetable Products, 2nd ed.,Tata McGraw Hill, New Delhi

Sathe, A.Y (1999). An Introduction to Food Analysis, New Age Intl. (P) Ltd, Publisher, New Delhi

Vernam, A.H. & Sutherland, J.P. (1994).Beverages: Technology, Chemistry and Microbiology, 1st ed., Chapman and Hall, London

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Effect of pH on Physico-chemical and Sensory Characteristics of Mozzarella cheese

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The effect of pH 5.0, 5.2, 5.4 on Physico-chemical and sensory characteristics of cheese curd was studied. The recovery of milk constituents in cheese, sensory quality (flavor, body and appearance), sensory evaluation of baking quality (fat leakage, meltability and flavor) and stretchability of the resultant mozzarella cheese were studied. The protein and total solid retention were highest in mozzarella cheese stretched at pH 5.0. Whereas, the fat retention was highest at pH 5.0 and 5.2. The fat leakage, meltability and stretchability of cheese stretched at pH 5.0 were significantly ($p<0.05$) higher. The average sensory score and baking quality of the cheese stretched at pH 5.0 were highest. Hence sensory quality, baking quality, fat leakage, meltability, stretchability, protein retention, fat retention, total solid retention of cheese stretched at pH 5.0 gave significantly better result. Whereas, the retention in moisture, ash and yield of cheese stretched at pH 5.4 gave the better result.

Keywords: Cow milk, Fat, Physico-chemical qualities, Protein, Total solids.

Introduction

In Nepal production of cheese started for about 46 years (1953-54) ago when a public sector cheese factory was established over the Langtang mountain range. The pioneers of the cheese involved in Nepal were Warner Schulthes a Swiss Nationale and Senior specialist from FAO, Gauri Prasad Sharma (Vaidya and Sardana, 1994).

Nepal produced about 350 MT cheese in 2000 (Adhikari *et al.*, 2001). In 1994, Nepalese cheese generated a foreign exchange of $525,000 (Colavito, 1994). The statistics may not be so impressive but is nevertheless encouraging. The demand for cheese is steadily increasing. At a very conservative estimate, the annual requirement of cheese in Nepal is around 800 MT. As of now Nepal has meeting the demand for cheese by importing them from foreign countries. Such cheeses are sold at price about 5-10 times higher than the price for Nepalese cheese (Adhikari *et al.*, 2001).

The important varieties of cheese produced in Nepal are yak cheese, Kanchan cheese, mozzarella like cheese and processed cheese. Yak and cow milk cheeses are produced by both Dairy Development Corporation (DDC) and private sectors. The substantial percentage of private yak cheese is of lower quality than DDC yak cheese in terms of taste, storage qualities, and texture and health safety. This is due to private producers' inexperience, shortcuts in production that save resources, difficulty in obtaining quality inputs and poor credits facilities that make investment cost high (Colavito,1994).

Its melting and stretching characteristics are highly appreciated in manufacture of pizza, where it is a key ingredient. (Ghosh and Kulkurani, 1996). True mozzarella cheese is made from the milk of buffalo. Today, what is called mozzarella is a cow's milk adaptation of the original process. Distinction between true mozzarella and that from cow's milk is fading (internet).The cheese is mostly used in cooking, especially on pizzas. The flavors should have adequate melting properties, i.e. soften on heating, become smooth and flow. In Nepal, true mozzarella (soft type) is produced from cow's milk. It is also prepared from a mixture of cow and buffalo milk. It is produced by some of private cheese industries. It is mainly consumed by hotels for making pizzas. Although the cheese is very popular, due to various reasons, the quality of cheese does not remain consistent (Acharya *et al.*, 2001).

The objective of present work is to prepare mozzarella cheese at different pH conditions and to study its physicochemical qualities viz. sensory qualities (flavor, body and appearance), sensory evaluation of baking quality (fat leakage, meltability and flavor), stretchability and retention of different milk constituents and yields.

Materials and methods

Materials

Cow milk was locally collected. The rennet powder (NATURENTM STAMIX 1150 CHR.HANSEN, DENMARK, Force minimum guaranteed: 1070 IMCU/g, Guaranteed min. strength at expiry date: 1070 IMCU/g, Instruction to use: 2.5-6 g for 100lt. milk Composition: 50% chymosin and 50% pepsin) was used. Lactic acid bacteria cultures (Freeze- dried lactic culture for direct vat set (DVS). TCC-20, Thermophilic culture blend , CHR.HANSEN) was used. pH meter, water bath, cooking vessel and globes were used for the experiment.

Methods

The basic mozzarella cheese making procedure employed with some modification was that of Danish soft mozzarella (Acharya *et al.*, 2001). The milk was locally collected and was filtered with muslin cloth .The milk was pasteurized at temperature 63±1°C for 30 minutes. The milk was then cooled down to 38°C. The freeze dried thermophilic starters DVS

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were inoculate into sterile milk and incubated at temperature 41±1°C for 3-4 hours. Then the culture was kept in refrigeration temperature. After the milk has attained the 38°C, starters @ 2% were added into the vessel and mixed well. After 30-40 minutes when the pH of the milk has attained to 6.3 the renneting are done @ 2.5g/100lt. milk, as water solution in the vessel. From the vessel the renneted milk was poured into six beakers. Throughout the setting period the temperature was maintained constant i.e. 38°C and left undisturbed. The coagulum was cut into cubes resembling maize grains with a sharp flat knife. The curd cubes are stirred gently for 5 minutes. Then temperature of the curd and the whey was raised slowly over a water bath up to 41°C.The whey in each beaker was separated by filtering through the muslin cloth .the cloth was pressed to remove the whey from the curd. The curd was quantitatively transferred to vessel containing hot water (83-85°C) stretched/plasticized and molded by hands. The cheese was removed from the hot water and then dipped into chilled water for two hours. The cheese was removed from the chilled water and kept in the refrigerator for draining out the water for 6 -8 hours. Then the cheeses were used for further analysis.

Chemical and physicochemical analyses of milk and cheese

Fat in milk was determined by Gerber method, total solids by lactometer and moisture of cheese by oven drying as per the procedure given in DDC (1989). Total protein in milk was determined by formal titration. The pH of milk was determined directly with a pH meter by immersing the electrode. Ash in milk and cheese was determined by muffle furnace method as per (Egan *et al.,* 1981). Protein in cheese was determined by determining the total nitrogen by the Micro-kjeldahl method (Ranganna, 1999) and conversion factor used was 6.38 (Egan *et al.,* 1981).

The meltability and fat leakage of cheese was determined by the method (Ranganna, 1999). It was expressed as ratio of increase in area of cylindrical base of cheese on exposure a 140°C for 15 minutes. The increase in area was measured with the help of traced graph paper. The fat leakage was determined from the same discs of cheese used for meltability measurement. The stretchability of cheese was evaluated on a 9-point hedonic rating scale. Sensory evaluation of fresh mozzarella cheese were evaluated by a selected panel of judge on a 9-point Hedonic rating scale for appearance, flavor and body (Ranganna, 1999).

Sensory evaluation of baking quality of cheese

About 100 g of shredded cheese was topped on each prebaked pizza loaf, without any vegetable filling and transferred to an oven maintained at 150°C and kept for about 15-20 min, to allow melting, till the shreds fused uniformly. The pizzas were served to 9 panelists, who evaluated them for fat leakage, meltability and flavor on 9-point hedonic rating scale (Ranganna, 1999).

Statistical data analysis

The experiment was conducted in triplicates. The data were analyzed by Genstat programming (Genwin version 532-2) at 5 % level of significance. The means were compared using LSD (Lawes Agricultural Trust, 1995).

Results and discussion

The chemical composition of milk, mozzarella cheese and its yield

The chemical composition of milk was 4.8, 4.3, 14.9, 85.1, and 0.71 respectively for fat, protein, total solid, moisture content and ash content. The average chemical compositions of protein and fat were 23.8, 21.1, 19.1 and 28.9, 26.4, 23.4, respectively, for mozzarella cheese stretched at pH 5.0, 5.2, 5.4. The average chemical composition of moisture content and ash were 45.7, 51.1, 55.7 and 0.82, 0.86, 0.89, respectively, for mozzarella cheese stretched at pH 5.0, 5.2, 5.4. Similarly, the average chemical composition of total solids and yield were 53.7, 53.2, 52.0 and 14.8, 16.2, 17.5, respectively, for mozzarella cheese stretched at pH 5.0, 5.2, 5.4. The total yield was higher at pH 5.4 and lower at pH 5.0.

Effect of different pH on stretching/plasticizing of cheese curd and retention of milk constituents in resultant mozzarella cheese.

The average retention (%) of protein and fat were 82.46, 79.19, 77.85 and 89.32, 90.18, 85.35 respectively for the resultant mozzarella cheese in which the cheese curd was stretched/plasticized at pH 5.0, 5.2 and 5.4. Similarly retention of total solid, moisture content and ash were 53.75, 53.27, 52.04 and 45.73, 51.12, 55.71 and 17.14, 19.67, 21.94 respectively for the resultant mozzarella cheese in which the cheese curd was stretched/plasticized at pH 5.0, 5.2, 5.4 as shown in Fig. 1.

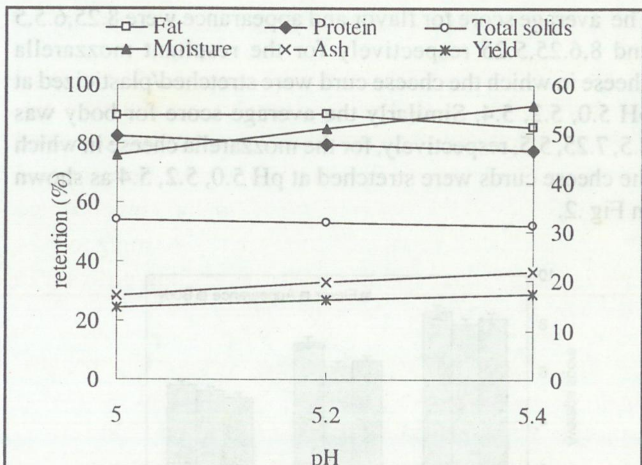

Fig. 1 Effect of different pH on the retention (%) of different milk constituents in Mozzarella cheese.

Statistical analysis showed that the stretching of cheese curd at different pH (5.0, 5.2, 5.4) has the significant effect ($p<0.05$) on the retention of the milk component (protein, fat, ash,

moisture content and total solids). This signifies that the retention of the milk component in the resultant mozzarella cheese was affected by stretching /plasticizing of cheese curd at different pH.

The averages scores of retention of protein at pH 5.2 and pH 5.4 were not significant different. However the averages scores for protein retention was highest at pH 5.0. This might be due to the aggregation and contraction of the *p*-casein gel matrix when the cheese curd subjected a plasticization process at this pH. The average scores of fat retention at pH 5.5 and pH 5.2 were not significantly ($p>0.05$) differ each other. However, the average scores for fat retention were significantly highest at pH 5.0 and pH 5.2 and lowest at pH 5.4. The lower retention of fat might be due to coagulum formed does not reach a certain degree of firmness. So, there will be loss of fat in whey (Irvine and Hill, 1985). The average scores of milk component retention in mozzarella cheese stretched / plasticized at different pH were significantly different with each other for total solid, ash and moisture content. The observed results showed that the total solid retention in resultant mozzarella cheese were highest at stretching of curd cheese at pH 5.0. This might be due to relatively higher retention of protein and fat in the resultant mozzarella cheese. The ash retention in the resultant mozzarella cheese was highest at stretching of the curd cheese at pH 5.4 whereas lowest at pH 5.0. The lower retention of ash might be due to conversion of dicalcium caseinate to monocalcium caseinate. The moisture content in the mozzarella was highest at stretching of curd cheese at pH 5.4 whereas moisture content was lowest at stretching of curd cheese at pH 5.0. The lowest retention of moisture might be due to expulsion of more whey from the curds by the continuous development of lactic acid.

Sensory quality of mozzarella cheese

The average score for flavor and appearance were 8.25,6.5,5 and 8,6.25,5.25 respectively for the resultant mozzarella cheese in which the cheese curd were stretched/plasticized at pH 5.0, 5.2, 5.4. Similarly the average score for body was 8.5, 7.25, 5.5, respectively, for the mozzarella cheese in which the cheese curds were stretched at pH 5.0, 5.2, 5.4 as shown in Fig .2.

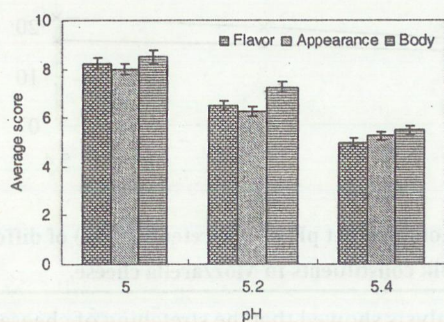

Fig. 2 Effect of pH on average score of sensory qualities of mozzarella cheese.

Statistical analysis showed that the stretching of curd at different pH had a significant effect ($p<0.05$) on the sensory quality of mozzarella cheese. The average scores of the body of mozzarella cheese stretched at pH 5.0 and pH 5.2 were not significantly different with each other. The average scores for body was highest a pH 5.0 and pH 5.2. The reasoned might be due to the cheese formed by stretching at pH 5.0 and 5.2 were more elastic and had good texture. The average scores of mozzarella cheese stretched at different pH were significantly ($p<0.05$) different from each other in flavor and appearance. The score for flavor and appearance were highest at mozzarella cheese stretched at pH 5.0.

Meltability, fat leakage and stretchability of mozzarella cheese

The average score of meltability, fat leakage and stretchability for the mozzarella cheese stretched at different pH is presented in Fig. 3. The average score of meltability, fat leakage and stretchability for the mozzarella cheese stretched at pH 5.0, 5.2, 5.4 were found to be 7.17, 5.15, 3.31; 12.42, 8.9, 5.3 and 8.5, 6.5, 5.0, respectively.

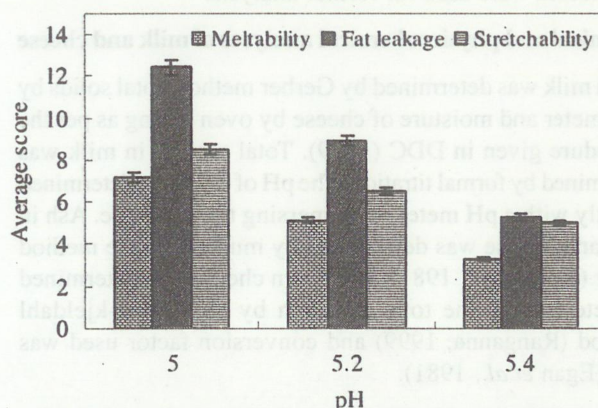

Fig. 3 Effect of different pH on the mean score of meltability, fat leakage and stretchability of mozzarella cheese.

The meltability, fat leakage and stretchability of the mozzarella cheese stretched a different pH were significantly differ ($p<0.05$).The average scores of the meltability, fat leakage, stretchability of the mozzarella cheese stretched at pH 5.0, 5.2, 5.4 were significantly ($p<0.05$) different with each other. The average score for meltability, fat leakage and stretchability were highest at pH 5.0. The highest meltability and fat leakage might be reasoned due to higher amount of fat present (Kindstedt, 1993). The highest stretchability of mozzarella cheese might be due to conversion of dicalcium paracaseinate to monocalcium paracaseinate (Shaw, 1986).

Sensory evaluation of baking quality of mozzarella cheese

The average score of sensory evaluation is represented in Fig. 4. The average scores for meltability and fat leakage were 7.67, 6.33,5 and 7.83, 6.2,5 respectively for mozzarella cheese stretched at pH 5.0, 5.2, 5.4. Similarly the average score for flavor were 8.25, 7.0, and 5.75, respectively, for the mozzarella cheese stretched at pH 5.0, 5.2, 5.4.

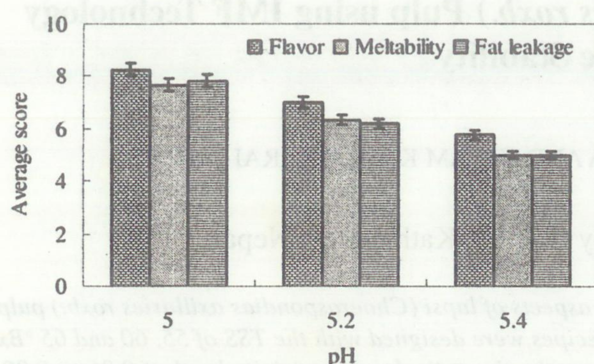

Fig. 4 Effect of different pH levels on the sensory qualities of mozzarella cheese.

Statistical analysis showed that sensory evaluation of baking quality (fat leakage, flavor and meltability) of mozzarella cheese stretched at different pH had a significant ($p<0.05$) effect on the baking quality. The average score of the fat leakage, flavor, and meltability were significantly different with each other. The average score for fat leakage, flavor and meltability were highest at pH 5.0.

Conclusions

The present work was carried out to study the effect of stretching the casein curd at different pH (pH 5.0, 5.2, 5.4) on the recovery of milk constituents (protein, fat, moisture content , ash, and total solid), sensory and baking quality, meltability, fat leakage and stretchability of the resultant mozzarella cheese.

Result showed that the stretching the cheese curd at different pH had a significant effect ($p<0.05$) on the recovery of milk constituents. The protein retention was highest in mozzarella cheese stretched at pH 5.0. Whereas, the average value of protein retention in mozzarella cheese stretched at pH 5.2 and pH 5.4 were not significantly ($p>0.05$) different. The fat retention was highest in mozzarella cheese stretched at pH 5.0 and pH 5.2 and lowest at pH 5.4. The total solid retention was highest in mozzarella cheese stretched at pH 5.0 and lowest at pH 5.4.The ash retention was highest in mozzarella cheese at pH 5.4 and lowest at pH 5.0. The moisture retention in mozzarella cheese stretched at pH 5.4 was highest and lowest at pH 5.0. The yield of cheese was highest at pH 5.4 and lowest at pH 5.0.

The fat leakage, meltability and stretchability of the mozzarella cheese stretched at different pH had a significant ($p<0.05$) effect. The fat leakage, meltability and stretchability of mozzarella cheese stretched at pH 5.0 was significantly highest.

The sensory quality (flavor, body and appearance) of mozzarella cheese stretched at different pH had a significantly ($p<0.05$) effected. The average score for sensory quality (flavor, body and appearance) of the mozzarella cheese stretched at pH 5.0 were highest.

The baking quality (fat leakage, stretchability and meltability) of mozzarella cheese stretched at different pH had a significant effect ($p<0.05$).The average score for baking quality (fat leakage, stretchability and meltability) of the mozzarella cheese stretched at pH 5.0 was highest. Hence, the sensory quality, baking quality, fat leakage, meltability, stretchability, protein retention, fat retention, total solid retention of mozzarella cheese stretched at pH 5.0 gave a better result. However, the retention of moisture, ash and yield of mozzarella cheese stretched at pH 5.4 gave the better result.

References

Acharya, P.P., Mishra, S.K. and Rai, B.K. (2001).Comparative study of the cheese making practice in Denmark and Nepal. Project work. Denmark.

Adhakari, I.P., Bajracharya, G.D. and Thapa, T.B. 2001. *Cheese Production in Nepal*, NDDB, DANIDA SUPPORT PROJECT, Harihar Bhawan, Lalitpur, Nepal.

Colavito, A.I.(1994).The yak cheese sector: A summary of preliminary findings. A paper submitted to dairy enterprise support component of the Agricultural technology system Project (HMG/NUSAID/N) for the yak cheese sector workshop.Nov.29,

Dairy Development Corporation (1989). *Quality Control Handbook,* Specification for milk Products., Nepal.

Egan, H., Kirk, R.S. and Sawyer, R. (1981). Pearson's Chemical Analysis of Foods, Churchill Livingstone, 499, 534,535

Ghosh, B.C. and Kulkarni, S. (1996). *Indian J. Food Sci Technol., In:* Low Cholesterol Mozzarella Cheese Technology Standardization, Vol. 33(6), 488-92

Internet:http://mozzarellahistory.search.com/3.html.Accessed Bhadra 15, 2062.

Irvine, D.M. and Hill, A.R. (1985).Cheese technology. *In:* "Compresive Biotechno-logy",vol.3(M.Moo-young, ed.-in-chief),1985, Pergamon press, Oxford, New York, Toronto, Sydney, Frankfurt, pp.523-446.

Kindstedt, P.S. and Guo, M.R. (1997).Recent development in science and Technology of Pizza cheese. *Aus J. Dairy.*, 52, 41-43.

Lawes Agricultural Trust, (1995). Rothamsted Experimental Station, PC/Windows 95, Genstat 5, 2nd Ed. (for windows), Library release 3[3] (PL 9)

Ranganna, S. (1999)."Handbook of analysis and Quality Control for Fruit and Vegetable",2nd edn.,Tata-McGraw Hill, New Delhi.

Shaw, M.B (1986). Modern cheese making method: soft cheese.*In* "Modern dairy technology" (R.K. Robinson, ed), pp: 20,159,167,170-71,206.

Vaidaya, R. and Sardana, V. (1994).Prospects of cheese products: Key to Poverty alleviation. Proceedings of the II National Conference on Science and Technology, June 8-11, Kathmandu, Nepal, pp.963-68.

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Preparation of Lapsi *(Choerospondias axillaries roxb.)* Pulp using IMF Technology and Study on Storage Stability

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The work was carried out to study the storage stability and food safety aspects of lapsi (Choerospondias axillaries roxb.) pulp stock prepared using Intermediate Moisture Food Technology. Three recipes were designed with the TSS of 55, 60 and 65 °Bx and the TSS/Acidity ratio of 20, 25 and 30 respectively so as to achieve the theoretical water activity level of 0.86 to 0.90. Further three treatments using no preservative, potassium sorbate (0.3%) as preservative and pasteurisation with hot filling were done to those recipes. All samples were found to be safe from the food poisoning organism Staphylococcus aureus. All preservative added and pasteurised samples as well as 65 °Bx sample with no preservative were stable up to 5 months storage and no Mold growth were observed. Mold observed after 2 month in 55°Bx sample and after 69th day in 60 °Bx sample with no preservative. Preservative added samples were faint in colour while pasteurised samples were dark due to browning reaction during heating. No preservative used sample was best in appearance. 65 °Bx with no preservative sample was good design but protection from air to prevent browning and use of sorbate to further extend shelf life was found necessary.

Keywords: Lapsi, IMF Technology, Storage stability, Food safety

Introduction

Lapsi is found in middle hills of Nepal at an elevation from 1300 to 1800 m. (Vaidya, 1994). These trees are 10-15 m. tall, graceful and deciduous and leave of odd-pinnate (Regmi, 1982). Tree bears fruit in a year during the month of November to January. A fruit lie between cylindrical and oval in shape and has an olive green coloured peel covering a white fleshy pulp, firmly attached all around the stone. The average size of matured fruit ranges from 1-1.5 inch in length and 0.75-1.9 inch in diameter (Shah, 1978). IMFs are characterized by a moisture content of around 15-50 % and an a_w between 0.60 and 0.85 by the use of additives such as glycerol, glycols, sorbitol, sucrose and so forth, as humectants and by their content of fungistats such as sorbate and benzoate.

The general water activity range of IMF products makes it unlikely that gram-negative will proliferate. This is true also for most gram-positive bacteria with the exception of cocci, some spore formers and lactobacilli.

Staphylococcus aureus is the only bacterium of public health importance that can grow at a_w values near 0.86. Staphylococci may be expected to exist at least in low numbers in any or all food products that are of animal origin or in those that are handled directly by humans unless heat-processing steps are applied to effect their destruction. (Jay, 2000).

With respect to mold in IMF systems, a large number of molds are capable of growth in the 0.80 range, and the shelf life of IM pet foods is generally limited by the growth of these organisms.

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In Nepal, mostly in Newari Kitchen lapsi is usually consumed either in the form of *chatani* or pickle as an appetizing agent after the end of traditional Newari *Bhoj*. Like wise, other traditions of Nepalese are also becoming habitual to use this seasonal fruits in their kitchen to prepare *chatani* and pickle by their own costumes. Regarding this fact in mind, the present study is aimed to develop a suitable appetizing product "lapsi pulp stock" that can be used for extended period of time so as to meet or satisfy the need of every household of Nepalese culture and tradition.

The objective of present work is to study the storage stability and food safety aspects of lapsi pulp stock prepared using IMF technology.

Materials and Methods

Lapsi was obtained form the local market. It was washed with water and sorted out the defective pieces and other contaminates. Then, it was boiled in water (1:1 ratio) until the skin broken down and then cooled to room temperature. The skin was removed and pulping was done. Stone was removed form pulp. Pulp was then, mixed with weighed amount of sugar, citric acid and salt to make the TSS of 55, 60, 65 °Bx. Then these samples were treated in three ways: (Fig. 1)

a. Kept in clean bottle and packed
b. Heat pasteurisation and hot filling in clean bottle, packaging and inverting for three minutes
c. Added 0.3% potassium sorbate, mixed and kept in bottle.

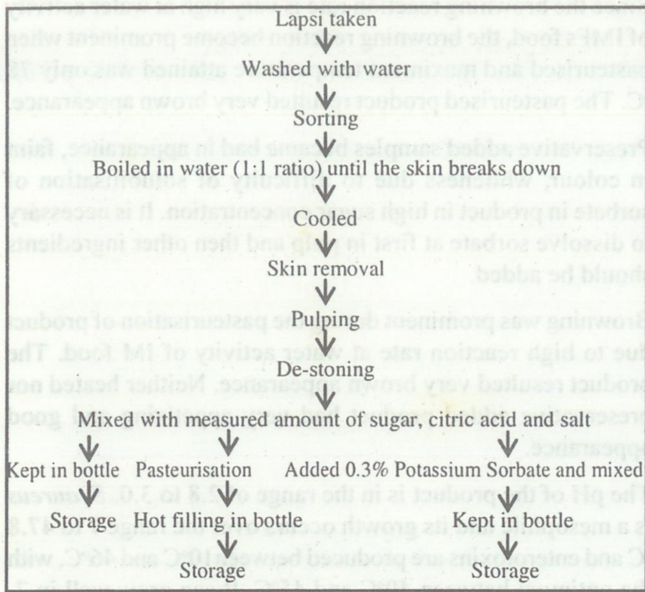

Fig. 1: Preparation of Lapsi Pulp

Treatment Matrix:

The treatment matrix may be drawn as:

Treatment 1. ***Recipe:*** Recipe 1 (55°Bx) (R_1)
Recipe 2 (60°Bx) (R_2)
Recipe 3 (65°Bx) (R_3)

Treatment 2. ***Preservative*** No preservative (N)
Potassium Sorbate (P)
Heat (H)

The Treatment matrix for this study is given in Table 1.

Table 1. Treatment matrix for the study

Treatments: -	Recipe		
Preservative	R_1	R_2	R_3
N	N R_1	N R_2	N R_3
P	P R_1	P R_2	P R_3
H	H R_1	H R_2	H R_3

Product Design and Recipe Calculation:

In general beverages, °Bx/Acidity ratio of 20 to 30 is considered best from consumer perception point of view. So, the product was designed in the same range. The sugar requirement was calculated to have the theoretical water activity of 0.86 to 0.92 ranges, since literature explains that sugar reduces water activity more than the theoretical value.

The formula for calculation of water activity is:

$$a_w = \frac{\text{Moles of solvent}}{\text{Moles of solute + Moles of solvent}}$$

The analysis report of pulp was:

°Bx	15%
Acidity	4.9%
Moisture	80.1%

The theoretical product design of lapsi stock is given in Table 2 to make it IM food. The °Bx/Acidity ratio has been kept in between 20 to 30 and theoretical water activity level has been kept in between 0.86 to 0.90.

Table 2. Theoretical Product design

Parameter	Recipe 1	Recipe 2	Recipe 3
°Bx	55	60	65
Acidity	2.8	2.5	2.28
a_w (Theoretical)	0.90	0.88	0.86

Recipes have been calculated using the principle of material balance so as to achieve the theoretical product design. Material balance was done for each component (Sugar, Salt, Acid and Total). The calculated recipes is given in Table 3.

Table 3. Calculated recipes of lapsi stock using material balance

Materials	Recipe 1	Recipe 2	Recipe 3
Pulp (gm)	550	482	424
Sugar (gm)	410	476	528
Salt (gm)	38	42	47
Acid (gm)	2	0.6	1.2
Total (gm)	1000	1000	1000

After material balance recipes were designed. Water activity of these three recipes has been calculated as in Table 4.

Table 4. Calculation of theoretical water activity level of different recipes

Recipe	Amount per Kg product								Calculated water activity
	Water		Sugar		Salt		Citric Acid		
	(gm)	(moles)	(gm)	(moles)	(gm)	(moles)	(gm)	(moles)	
1	440	24.44	493	1.44	38	0.67	27.2	0.39	0.907
2	386	21.42	548	1.6	42	0.74	24.6	0.35	0.888
3	339	18.83	592	1.73	47	0.83	22	0.31	0.867

Theoretically, water activity of the designed product is in the range of 0.86 to 0.90. Sugar reduces the water activity level more than the theoretical value (Jay, 2000). Hence, practically the water activity level of the product will be lesser than that calculated one. Therefore, the product will fall in the desired water activity level of IM foods. The product can be considered IM foods with sugar as major humectant.

Analytical methods

°Bx and pH were analysed using hand refractometer and pH meter respectively. Acidity as citric acid was calculated by

titration method and reducing sugar was analysed by Fehling's method.

Results and Discussion

The yield of different portion of lapsi is given in Table 5. The percentage yield of pulp, stone, and peel was found to be 41.4, 24 and 20 respectively. The remaining 14.6% was lost during boiling due to moisture loss and soluble solid loss in water.

Table 5. Percentage yield of different portion of lapsi

S.N.	Portion	Percentage yield
1.	Pulp	41.4
2.	Stone	24.0
3.	Peel	20.0

The analytical report of different recipes is given in Table 6. The report was very close to the theoretical product design.

Table 6. Analytical Report of different samples

Parameters	Recipe 1	Recipe 2	Recipe 3
°Bx	55	60	65
Acidity (as citric acid)	2.72	2.46	2.11
pH	2.89	2.93	2.96
Reducing sugar	8.67	7.30	6.15
°Bx/ acidity	20.22	24.39	30.80

Storage Study:

After one month storage:

- Mould count was negative in all recipes.
- *Staphylococcus aureus* count was negative for all recipes.

After two months storage:

- Mold growth occurred in NR_1 product
- No Mold growth in NR_3 product
- No Mold growth in all samples of heat treated as well as chemical treated product
- Mold growth started in NR_2 product after 69th day of preparation

After three months storage:

- Two colonies being observed in NR_2 product
- No Mold growth in NR_3 product.
- No Mold growth in all samples of heat-treated as well as chemical treated product.

After five months storage

- No Mold growth in all heat treated as well as chemical treated product
- No Mold growth in all NR_3 samples

After six month

- No mold growth in all chemical treated products.
- Mold growth in 66% of HR1 and no mold growth in HR2 and HR3
- No mold growth in all NR3 samples but brown ring at top with liquid exudates and slight off-flavour.

Since the browning reaction rate is very high at water activity of IMFs food, the browning reaction become prominent when pasteurised and maximum temperature attained was only 78 °C. The pasteurised product resulted very brown appearance.

Preservative added samples became bad in appearance, faint in colour, whiteness due to difficulty of solubilisation of sorbate in product in high sugar concentration. It is necessary to dissolve sorbate at first in pulp and then other ingredients should be added.

Browning was prominent during the pasteurisation of product due to high reaction rate at water activity of IM food. The product resulted very brown appearance. Neither heated nor preservative added product had very appetizing and good appearance.

The pH of the product is in the range of 2.8 to 3.0. *S. aureus* is a mesophile and its growth occurs over the range 7 to 47.8 °C and enterotoxins are produced between 10°C and 46°C, with the optimum between 40°C and 45°C. It can grow well in 7-10% salt concentrations and some strains can grow in 20 %. It can grow over the pH range of 4.0-9.8 but its optimum being in the range of 6-7 and a_w of 0.86 is generally recognized minimum value for growth. Growth was not observed in brain heart infusion (BHI) broth containing NaCl and sucrose as humectants either at pH 4.3, a_w of 0.85, or 8 °C or with a combination of pH < 5.5, 12°C and a_w of 0.90; and pH < 4.9, 12°C and a_w of 0.96 (Jay, 2000). Notermans and Hessvelman (1983) showed that growth of *S. aureus* is not observed at a_w 0.852 or temperature < 8°C or pH < 4.3 (Banwart, G.J. 2002). Barber and Deivbel (1972) showed that aerobically some strains of *S. aureus* produce enterotoxin at pH of 4.87 but anaerobically enterotoxin is found at pH 5.4. *S. aureus* grew and produced enterotroxin in precooked bacon stored aerobically either at 37°C and minimum a_w of 0.84 or at 20°C and an a_w of 0.89 by Lee, Silverman and Munsey(1981). Anaerobically the organism required a minimum a_w of 0.98 at 37°C and 0.94 at 20°C. Similarly, Mclean, Lilly and Alford (1968) showed that above 2% salt concentration, enterotoxin production decreases and becomes minimum above 4% salt concentration (Banwarat, G.J. 2002).

The pH range of the product is 2.8 to 3 and salt concentration is 3.8 to 4.7% with a_w of 0.86 to 0.91. The analysis of product also showed no growth of *S. aureus* in product during storage. Hence the product is safe from public health importance bacteria.

Mold growth was the problem for sample with no other hurdles. Storage study showed that Mold growth started after 2 months in 55°Bx product with no preservative that occurred after 10 weeks in 60°Bx with no preservative but not occurred in 65°Bx until five months storage. With respect to Mold in IMF systems, these products would be made quite stable if a_w were reduced to around 0.70, but a dry type product would then result. A large number of Molds are capable of growth in the 0.80 range, and the shelf life of IM foods is generally

limited by the growth of these organisms. Acott *et. al.* (1976) showed that growth of *Aspergillus niger* and *A. glaucus* occurred in 2 weeks in the a_w 0.85 formulation without inhibitors but did not occur until 25 weeks when potassium sorbate and calcium propionate were added (Jay, 2000).

Vacuum packaging may be another option for the preservation, since the major problem is Mold growth. Further research is necessary to study the effect of vacuum packaging and / or required amount of preservative required for self-stabilization. It can be assumed that product NR_3 is stable from Mold for further five months after opening the vacuum packed product.

Conclusions

Lapsi pulp stock can be prepared and stored safe with recipe of 65°Bx, and acidity of 2.11% using sugar as a major preservative for at least five months if hygienic conditions are maintained but use of preservative like sorbate and protection from air is necessary to prevent browning and to increase shelf life further. The product is safe from public health important microorganism.

The product can be used to prepare beverage by diluting with water and addition of spices as per requirements or may also be used to prepare other lapsi products such as candy since the sugar and pulp ratio is optimum for them as well. Again it can be used to prepare lapsi relish with the addition of spices as per requirement. The stock could be marketed for the lean period of lapsi in the market.

References

Banwart, G.J. (2002). Basic Food Microbiology. CBS Publication.

Food Composition Table, National Nutrition Programme, DFTQC / HMG, Nepal

Jay, J.M. (2000). Modern Food Microbiology, Sixth edition. An Aspen Publication. Gaithersburg, Maryland.

Regmi, P.R. (1982). An introduction to Nepalese Food plants. First edition. Royal Nepal Academy, Kathmandu.

Shah, D.J. (1978). Food research annual bulletin, Babarmahal, Kathmandu.

Vaidya, M. (1994). Study on the retention of Vitamin C on Lapsi Candy. Dissertation submitted to Central Campus of Technology, Dharan.

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Proximate, Mineral and Amino Acid Compositions of Common Legumes Consumed in Nepal

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The proximate composition, mineral constituents and amino acid profile of four legumes, Rahar (yellow lentils), Kalomas (Black lentils), Masoor (Red gram), and Moong (Green gram), which are most common in Nepali kitchen, were studied in order to evaluate their nutritional performance. Masoor had highest content of protein (25.5%), while Kalomas was good in minerals among the legumes studied. In general, macro-minerals: potassium, phosphorus, and calcium, and micro-minerals: cupper, iron and zinc, were found abundance in these legumes. Cystine and methionine were the first limiting amino acids.

Keywords: Legumes; Proximate composition; Minerals; Amino acids; Nutrition.

Introduction

'Dal-Bhat' is the main staple of Nepali people all over the country. Dal is the Nepali term for peas, beans, or lentils that have been split and often skinned, but the name is sometimes used for all lentils, peas, or beans, or to cooked dishes made with them. These pulses are the main dietary items for supplying trace elements and nutrients.

Grain legumes are used as pulse (dal), and are grown in both tropical and temperate regions of the globe. The legumes have been reported to contain adequate amounts of lysine, but are deficient in sulphur-containing amino acids such as methionine and cystine (Farzana and Khalil, 1999). Food composition varies and influenced by a number of factors such as, climate, soil, variety, transport, storage, and preparation (Greenfield and Southgate, 1992). These factors vary from one region to another and even within the same country. Since the chemical composition of crops varies with crop cultivars, soil and climatic conditions of the area, it is imperative to study the chemical composition of some important legumes (Rahar, Kalomas, Masoor, and Moong) prevalently consumed in Nepal. To the author's best of knowledge, there is no published report on the nutritional composition of these Nepali legumes. Here, we have analysed four legumes which are used in the normal dietary system in Nepal. This paper presents the results on their proximate, mineral and amino acids compositions in order to highlight their nutritional significance.

Materials and methods

Materials

Dried seeds of four legumes (Rahar, Kalomas, Masoor and Moong) were purchased from the local market of Kathamandu, Nepal. The seeds were dehulled and then ground to pass a 50-mesh screen. Powdered samples were preserved in air-tight bottles at room temperature.

Methods

The moisture content, ash, crude fat, crude protein, crude fiber was determined in accordance with the methods prescribed by Bhandari, Kasai and Kawabata (2003). Carbohydrate content was determined by difference while calorific values were obtained by the summation of multiplied mean values for protein, fat and carbohydrate by their respective Atwater factors 4, 9 and 4 (Udosen, 1995). All results for proximate composition are recorded on the basis of fresh weight of the uncooked sample as g/ 100 g fresh weight.

The minerals such as calcium, copper, iron, magnesium, manganese, sodium, potassium and zinc were analyzed after first wet-ashing according to the method prescribed by Onwuliri and Anekwe (1992) with an atomic absorption spectrophotometer (Model No. AA-6400 F, Shimadzu Corporation, Japan). Legumes flour sample (1 g) was digested by heating in a Kjeldahl flask with 16 N HNO_3 and 6 N $HClO_4$ mixtures for oxidation of carbonaceous matter. For each sample, a blank was prepared simultaneously by taking same amount of acids mixture. The mixture was heated slowly at first (at 300°C) until frothing ceases. Then temperature was raised to 600°C and heated until a clear solution was obtained. It was cooled and 10 ml HCl (1+1) was added and transferred to 50 ml volumetric flask. For Ca and Mg determinations, 10 ml 5% La solution was added. The volume was made up to 50 ml with HCl (1+1). The mineral contents of the samples were quantified against standard solutions of known concentrations, which were analyzed concurrently (Shimadzu, 1996). Phosphorus was estimated colorimetrically (UV-visible spectrophotometer, Model No: UV-1600, Shimadzu Corporation Japan), using potassium dihydrogen phosphate (Wako pure chemicals industries Ltd., Japan) as the standard

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(AOAC, 1980). All results for mineral composition are recorded on the basis of edible portion fresh weight of uncooked sample as mg/100 g fresh weight.

The amino acids were estimated following the methods previously described by Bhandari et al (2003). The contents of different amino acids were presented as g/100 g protein. The amino acids content of the legumes samples were compared with the FAO/WHO reference pattern (FAO/WHO, 1985) and whole egg protein (FAO, 1968).

Results and discussion

Proximate composition

The results in Table 1 show the comparative proximate composition of four legumes. The moisture and ash content of legumes were ranging 8.2-9.1% and 2.6-4.1% of fresh weight, respectively. The moisture and ash contents obtained for these legumes were similar to that of reported values for common legumes (Freefield and Southgate, 1992). The protein content in legumes was ranging from 23.2% to 25.5%. It is evident that Masoor contained the maximum amount of protein (25.5%), followed by Kalomas (24.1%). The higher protein content of Masoor indicated its nutritional superiority over the other legumes. Kalomas and Masoor showed the highest (4.1%) and lowest (2.6%) content of ash, respectively. Similarly, the crude fat content was high in Rahar (4.9%), followed by Kalomas (4.3%), Masoor (3.5%) and Moong (2.1%). These findings were in accordance with the others (Khalil, 1994; Radhuvanshi, Shukla and Sharma, 1994; Jood, Bishnoi and Sharma, 1998). The energy values of food can be determined either directly by bomb calorimeter or by calculations using various caloric conversion factors (Merrill and Watt, 1955). In this study, the Atwater factors (Udosen, 1995) were used in the calculation of energy value. The energy values were ranging from 364 to 378 kcal/100 g fresh weight.

Table 1: Proximate composition of four legumes consumed in Nepal*

Nutrients (g/100 g)	Rahar	Kalomas	Masoor	Moong
Moisture	8.5±0.03	9.1±0.05	8.9±0.04	8.2±0.05
Carbohydrate	59.5±0.92	58.4±0.51	59.5±0.65	63.0±0.81
Crude protein	23.9±0.21	24.1±0.07	25.5±0.06	23.2±0.02
Crude fat	4.9±0.01	4.3±0.04	3.5±0.04	2.1±0.02
Ash	3.2±0.02	4.1±0.03	2.6±0.04	3.5±0.02
Energy (kcal/100g)	378	369	372	364

* Values ar expressed as: Mean±SEM; n=3.

Mineral composition

The results of the mineral estimation of the legumes are presented in Table 2. There was wide variation in the content of individual minerals among these legumes. The results showed that potassium was the most abundant mineral, ranging from 974 to 1385 mg per 100 g legumes. Among the four legumes studied, Kalomas had the highest concentrations of potassium, magnesium and phosphorus. Sodium was found in an appreciable concentration in Moong. Rahar contained good amounts of calcium, zinc and copper. These results revealed that legumes may provide sufficient amounts of minerals to meet the human mineral requirement (USDA, 2006).

Table 2. Mineral composition of four legumes consumed in Nepal

Minerals (mg/100 g)	Rahar	Kalomas	Masoor	Moong
Sodium	112±2.8	102±3.7	89±1.8	125±1.9
Potassium	1250±3.5	1385±6.1	974±4.5	1050±8.8
Phosphorus	231±4.3	313±5.6	274±2.5	261±2.1
Calcium	207±2.5	177±3.2	129±4.4	125±2.5
Iron	3.5±0.14	2.9±0.14	3.7±0.18	2.6±0.03
Copper	12.2±0.14	9.1±0.14	10.1±0.07	10.0±0.28
Zinc	6.2±0.18	5.4±0.14	4.9±0.14	4.0±0.39
Manganese	2.1±0.07	1.9±0.02	1.7±0.02	2.4±0.01
Magnesium	4.4±0.02	4.9±0.07	4.2± 0.03	4.1±0.02

* Values ar expressed as: Mean±SEM; n=3.

Amino acid composition

Table 3 shows the amino acid compositions of four of legumes and whole egg protein. The amino acid composition of the four legumes indicated little variation in the contents of total essential and non-essential amino acids. However, significant variation existed in the individual amino acid contents, particularly for arginine, histidine and methionine. Among the four legumes, lysine, cystine and tyrosine were found to be rich in Moong, while phenylalanine and histidine were found in appreciable amounts in Kalomas. Rahar was found high in arginine, leucine and isoleucine. Glutamic acid and aspartic acid were found to be major non-essential amino acids in these legumes. These findings are in fair agreement with others (Hussain and Basahy, 1998; Bhatty, Gilani and Nagra, 2000). A comparison of amino acid compositions of these legumes with the FAO/WHO reference pattern of essential amino acids, and whole egg protein indicated that studied legumes are fairly good source of both essential and non-essential amino acids. In all legumes studied essential amino acids, histidine and lysine exceeded to those of whole egg protein. In general, the results indicated that essential amino acids except sulphur-containing were present in appreciable amounts in all the legumes tested.

Conclusion

Evaluation of nutritional charcteristics of these legumes in the current study revealed that studied legumes are promising sources of high protein, carbohydrate, energy, dietary minerals and essential amino acids. These legumes contained good quality of protein with comparatively well balance of amino acids. The essential amino acid profile compared well with the FAO/WHO recommended pattern except for methionine. Thus, it was concluded that studied legumes could serve as good sources of essential dietary nutrients in human nutrition. The nutritional significance of these legumes must be

Table 3. Amino acid composition of four legumes consumed in Nepal*

Amino acids (% of protein)	Rahar	Kalomas	Masoor	Moong	FAO/WHO pattern[a]	Whole egg protein[b]
Arginine	8.4	7.7	7.9	7.5		6.1
Histidine	3.0	3.2	2.6	2.5	1.9	2.4
Isoleucine	4.8	4.5	4.1	4.5	2.8	6.3
Leucine	8.8	7.6	7.8	7.4	6.6	8.8
Lysine	7.2	7.3	7.1	8.2	5.8	7.0
Methionine	1.1	2.2	0.8	1.1	2.5	3.4
Phenylalanine	5.6	5.5	5.0	5.2	6.3	5.7
Threonine	3.1	3.8	3.5	3.8	3.4	5.1
Tryptophan	ND	ND	ND	ND	1.1	1.7
Valine	4.6	5.1	4.9	5.0	3.5	6.9
Alanine	4.9	4.1	4.2	5.1		5.9
Aspartic acid	11.5	10.8	11.9	11.1		9.6
Cystine	0.6	0.5	0.9	1.4		5.9
Glutamic acid	16.3	17.1	19.5	17.6		12.7
Glysine	3.6	3.7	3.4	4.3		3.3
Proline	3.8	4.1	3.6	3.7		4.2
Serine	3.8	4.2	5.3	5.1		7.6
Tyrosine	2.9	3.0	3.1	3.5		4.2

* Values are expressed as Mean; n=3.
[a] FAO/WHO pattern (FAO/WHO, 1985); [b] Whole egg protein (FAO, 1968); ND= Not detected.

highlighted by mass media (press, radio and T.V.), workshops and seminars.

References

AOAC (1990). In Helrich K. (Ed.). *Official methods of analysis.* (15th ed.). Arlington, VA, USA.

Bhandari, M. R., Kasai, T. and Kawabata, J. (2003). Nutritional evaluation of wild yam (*Dioscorea* spp.) tubers of Nepal. *Food Chemistry*, 82(4): 619-623.

Bhatty, N., Gilani, A. H., and Nagra, S. A. (2000). Effect of cooking and supplementation on nutritional value of gram (*Cicer arietinum*). *Nutrition Research,* 20 : 297–307.

Deshpande, S.S. (1992).Food legumes in human nutrition: A personal perspective. *Reviews in Food Science and Nutrition,* 32: 333–363.

FAO (1968). *Amino acid composition of foods and biological data on proteins.* Rome, Italy: Food and Agricultural Organization of United Nations.

FAO/WHO (1985). Energy and protein requirements. Nutrition Report Series 724, Geneva.

Farzana, W., and Khalil, I. A. (1999). Protein quality of tropical food legumes. *Journal of Science and Technology,* 23:13–19.

Greenfield, H and Southgate, D.A.T. (1992). Food Composition Data. Production, Management and Use. Elsevier, London/New York.

Hussain, M. A., and Basahy, A.Y. (1998). Nutrient-composition and amino acid pattern of cowpea (*Vigna unguiculata* L.) grown in the Gizan area of Saudi Arabia. *International Journal of Food Science and Nutrition,* 49:117–124.

Jood, S., Bishnoi, S., and Sharma, A. (1998). Chemical analysis and physico-chemical properties of chickpea and lentil cultivars. *Nahrung,* 42:71–74.

Khalil, I.A. (1994). Nutritional yield and protein quality of lentil (*Lens culinaris* Med.) cultivars. *Microbiogie Aliments Nutrition,* 12:455–463.

Merrill, A. L., and Watt, B. K. (1955). *Energy Values of Foods. Basis and Derivation*; U.S Department of Agriculture, Handbook 74.

Onwuliri, V.A. and Anekwe, G.E. (1992). Proximate and elemental composition of *Bryophyllum pinnatum* (Lim). *Medical Science Research,* 20:103–104.

Raghuvanshi, R. S., Shukla, P., and Sharma, S. (1994). Nutritional quality and cooking time tests of lentil. *Indian Journal of Pulses Research,* 7(2):203–205.

Siddhuraju, P., Becker, K., and Makkar, H. P. S. (2000). Studies on the nutritional composition and anti-nutritional factors of three different germplasm seed materials of an under-utilized tropical legume, *Mucuna pruriens* Var. Utilis. *Journal of Agricultural and Food Chemistry,* 48 (12): 6048–6060.

Udosen, E. O. (1995). Proximate and mineral composition of some Nigerian vegetable. *Discovery and Innovation,* 7 4:383–386.

USDA (2006). http://www.everything2.org/index.pl?node_id=461038 (Citation date: March 10, 2006).

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Design and Construction of Solar Incubator in Nigeria

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Solar incubators have relative advantages over other incubators, particularly in tropical environment like Nigeria where solar radiation and intensity are relatively high. Efforts are geared at developing solar incubator for the Nigerian farmers. This paper presents the development of a laboratory scale solar incubator in Nigeria.

Key words: Incubator, Solar Energy, Temperature, Relative humidity, Poultry

Introduction

The high demand for poultry due to its cheap source of protein and high calorie requires regular supply of chicks within the shortest possible time. In a year, a hen can only lay and hatch about 30-40 eggs (Charnwit et al, 2003). These necessitated the development of incubators. Artificial incubation stimulates the natural incubating conditions (temperature and relative humidity) provided by the mother bird and have the capacity to hatch over 5000 eggs at once, on continuous basis (Obioha, 1992; Singh, 1990: Nico and Johan, 1990). It also has the capability of hatching varieties of poultry birds with higher commercial value such as the geese and ostrich eggs (Obioha, 1992).

Artificial incubation of poultry eggs is an ancient practice. The Chinese developed artificial incubation as early as 246 BC (Berry, 1998 and World Poultry, 1998a). Adewumi and Oduniyi (1999) classified incubators on basis of system capacity, source of energy and mode of air movement in the incubator. Various types of incubator available can also be broadly categorized into two (2) different groups, namely the small model and large model incubators (Olorunnisola and Ewemoje, 1998, Adewumi, 1998; Adewumi and Falayi, 1999).

The small model incubators have a capacity between 50 and 500 eggs (Adewumi and Falayi, 1999). They are usually called the flat-type or still air incubators. The large models have a capacity to handle above 500 to many thousands of eggs. They are referred to as cabinet or forced draught incubators (BMAFF, 1982). The source of energy during incubation could be electrical, biogas, solid fuel, fossil fuel or solar (Adewumi, 2004). Incubators are also classified by the nature of air movement, i.e. the nature of air circulation pattern. Free convection is usually adopted in table incubator. Large scale chick production involves the use of forced convection for proper air circulation and sources energy from an electricity supply.

Solar incubator is very effective where farmer have problem with electrical power supply (Charnwit et al, 2003). It is powered by the sun via solar collector, solar water heater or solar cell panel. Solar collector could be the flat plate or concentrator type. Solar water heater supplies heat to the incubating chamber, either directly from the hot water tank or from a battery. Solar cell panel converts solar energy into electrical energy and store it in battery. In case of absence of sunshine the battery can last for 3 days. Charnwit et al (2003) reported a solar hot water bath incubator powered by six solar cell panels (with storage device). It has a capacity for 720 eggs with hatching efficiency in the range of 60 to 70%, which is comparable with some electrical incubators.

The bird flu problem that is currently devastated the poultry industry in Nigeria calls for the use of incubators in order to accelerate the production of poultry as soon as the country recovers from the bird flu syndrome. Aside, the high cost of purchase, the cost of supply of energy for incubation is another challenge faces by the farmers in Nigeria. In the rural areas in Nigeria where agricultural production is predominantly practised, electricity supply is either not available or not in constant supply. Electrical incubator is not advisable in such areas (Adewumi and Oduniyi, 1999). Solar incubator is therefore a feasible option because it reduces the cost of energy supply and maintenance cost. It is easily adaptable to farmers. It has low production cost and no environmental pollution hazards are associated. Therefore the aim of the paper is to develop a solar incubator suitable for the rural communities in Nigeria.

Materials and Methods

Description of the Incubator

The incubator is made up of three (3) units namely the incubating unit, solar collector unit and the heat storage unit (Fig. 1).

The incubating unit is made of wood with a thickness of 0.02 m. It has an external dimension of 0.63 m x 0.43 m x 0.7 m. It consists of three egg setters fastened together. The egg setter is made up of three plastic crates, each could take 40 eggs and with a total capacity for 120 eggs. The egg setter rests on a turning mechanism. The door of the incubating unit has a glass inspection panel of dimension 0.21 m x 0.19 m, which aids the reading of the wet and dry bulb thermometers placed in the incubating unit and allow the visibility of the interior of the incubation unit without opening the door. A total of twelve holes, which serves as ventilation ports, each of diameter 0.08 m were perforated on the sides of the incubating chamber. On top of the incubating unit is a ventilation port of

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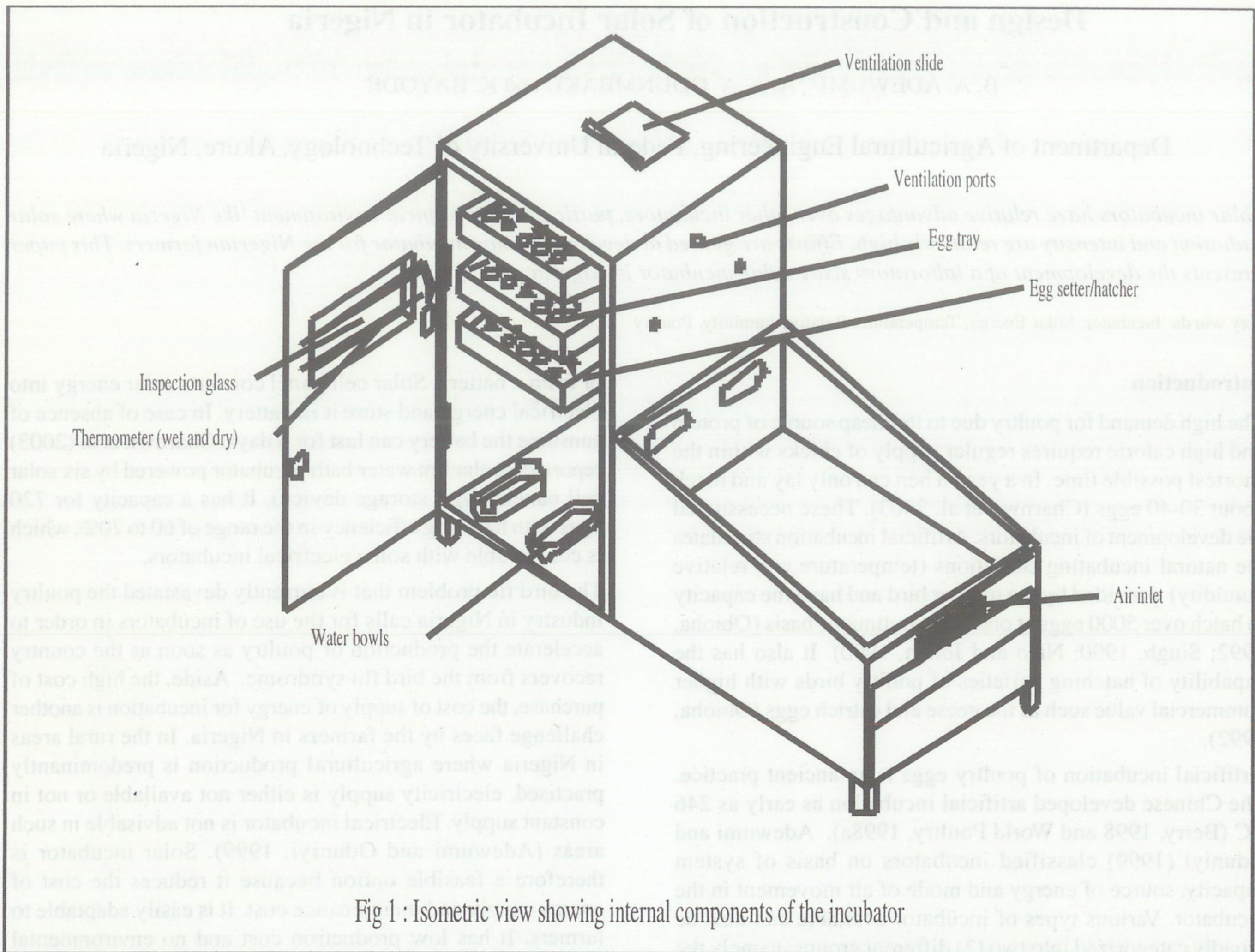

Fig 1 : Isometric view showing internal components of the incubator

dimension 0.08 m x 0.11 m with a slide cover to regulate the flow of air into and out of the incubation unit.The second unit is the solar collector (flat plate type), which harnesses and supplies the heat to the incubating chamber. It consists of a plain glass panel cover, an absorber and frame. The glass cover sheet allows the sun's rays to pass through to the black absorbing surface. The heat trapped and collected is transported by natural convection through a netted inlet. The surface is painted black to maximize radiant energy absorbed and minimize the loss of heat.

The third unit, heat storage unit, is located below the collector plate and made up of pebble bed. It occupies a space of 0.61 m x 0.41m x 0.18 m. Pebbles of diameter between 3 and 5 cm were used. Heat is released into the incubating chamber from the storage unit via a port. It serves as a heat reservoir at night or during raining periods, during periods of low solar radiation.

Design of the Incubator

The total heat requirement for incubation, angle of tilt of the collector and heat storage unit were considered in the design of the incubator.

The Total Heat Requirement

The total heat requirement of the incubator includes the heat energy required to raise the eggs temperature from ambient temperature to incubation temperature (Qe), the heat required to raise the air in the incubation unit from ambient temperature to incubation temperature (Qa), the heat losses to the wall of the incubating chamber (Qs) and the heat loss through natural ventilation (Qv) That is,

$$Qt = Qe + Qa + Qs + Qv \quad \text{.................} (1)$$

The basic conductive and convective heat transfer equations in equations 2 and 3 were utilized to obtain the respective heat requirements (Microsoft Encarta encyclopedia, 2004). The rate of heat loss by ventilation through the outlet parts is as expressed in equation 4 (Culpin, 1981).

$$Q_e = M_e C_e d_T \quad \text{.......................} (2)$$

$$q = KA (T_i - T_o)/L \quad \text{............ ...} (3)$$

$$q_v = V r (Ti - T_o) \quad \text{...................} (4)$$

Where,
Q_e = Conductive heat requirement by eggs (J)
M_e = Mass of eggs (kg)
C_e = Specific heat capacity of the incubating eggs (J/kg°C)
d_T = Temperature difference
q =Rate of convective heat requirement (W)
A = Surface area of incubating unit (m^2)
T_i = Temperature of the incubating unit (°C)
T_o = Temperature of the surrounding (°C)
L = Thickness of material (m)
K = Thermal conductivity (W/m°C)
q_v =Rate of heat loss (KW)
V = Ventilation rate (0.027m^3/min) for small incubator
r = Density of air at atmospheric pressure (kg/m^3)

The thermal conductivity of wood (K_w) is 0.12W/m°C and that of glass (K_g) is 0.8W/m°C (Norman, 1983). Egg has a C_e value of 2.32KJ/Kg°C (Adewumi, 1998). For an incubator with capacity for 120 eggs with average ambient temperature of incubation of 25.8°C (Weather Underground Inc, 2005) and the maximum incubating temperature of 39.5°C, a total of 517.89 KJ of heat is required.

Tilt Angle of the Collector

Proper orientation of solar collector can increase system payback up to 50% quicker than a haphazardly installed system. Generally solar collectors are tilted relative to the horizontal plane at an angle equal to the latitude ±15° and are oriented to face true south or north with ±20° (Microsoft Encarta Encyclopedia, 2004). The solar collector was tilted at an angle equal to Ondo state location which is on latitude 7.1° N plus 15° to optimize the harnessing of heat. The collectors were mounted vertically facing south. Collectors should face as true south as possible towards the solar radiation (true south is where the sun is at noon). If a choice of east or west is available it is usually best to orient the collectors to the west but if you are in an area where mornings are normally clear and afternoons are cloudy, it would be best to face the collector in a more eastern direction. Therefore, the solar collector angle of tilt of 22.1° was used.

Heat Storage Volume

Pebble can be used as a means of heat storage for night and early morning in the incubator. Other storage medium available include rock, water (or water-antifreeze mixture and phase-change chemical substances called Glauber's salt (Eckhoff and Okos, 1998). Pebble (rock) storage bed was used in this design because it is cheap and has a good heat transfer characteristic with air. The heat storage volume needed for incubator depends on four factors.

(1) Heating requirement of the heated area
(2) Days of storage reserve desired
(3) Temperature range over which heat is stored
(4) Type of storage material used.

The heat storage volume was calculated using the worksheet employed by Eckhoff and Oko's (1998) and the inner dimension of the heat storage unit was estimates to be 0.61m x 0.41 m x 0.20 m

Construction and Preliminary Testing of the Incubator

The incubator was constructed at the wood workshop of the Federal University of Technology, Akure, Nigeria. It was constructed with cheap and locally available materials at a cost of thirty thousand Naira (N30,000), about $240. It was thereafter subjected to preliminary test for 5 days in October, 2005. The environmental conditions (temperature and relative humidity) in the incubator were determined so as to be able to effect modifications. October was chosen for the test because it is one of the critical and determining months in Nigeria for a successful use of solar systems. Solar intensity and ambient temperatures are generally low, while relative humidity is generally high during October in Nigeria. It is envisaged that success during such periods could imply success for the other periods in the year.

The incubator was positioned on a plain ground at a location without obstacle that may hinder the solar radiation from getting into the incubator. The direction and the angle at which the sun rays are incident on the collector plate of the incubator was noted and adjusted to the required magnitude. 2-hourly readings of the wet and dry bulb temperatures and relative humidity were taken between 6.00 am (GMT 07.00) to 4.00 am (GMT 05.00) of the next day daily from 10th to 15th October, 2005. The wet bulb, dry bulb and relative humidity in the incubator were determined using the wet and dry bulb thermometers, and a psychometric chart.

Table 1 shows the meteorological data on September, 2005 typical of the situation during the period of the experiment. Fig 2 shows the collector ambient temperatures obtained during the test. Figs. 3 to 7 show the wet and dry bulb temperatures and relative humidity during the period of the experiment. The temperature in the incubator increased with the intensity of the sun. During the afternoons the collector and incubator temperature attained up to 41°C and 39 °C respectively (Table 2).

The temperatures in the incubator drop rapidly when the weather is cool mostly in the early hours of the morning and at night from 39°C to as low as 25°C. This shows that the incubator temperature depends greatly on the environmental conditions since the temperatures drop as the sun intensity decreases. Relative humidity records attained by the incubator throughout the period are within the recommended range favorable for incubation. Favorable temperatures were only attained during the day but not kept constant inside the incubator at nights. This is as a result of the weather conditions and probably the inability of the storage unit to effectively store and release the heat. The design of the storage unit shall however be improved upon before further tests can be conducted.

Conclusion

The use of mechanical systems has contributed in to upgrading the production level of agricultural produce. Locally developed technology has given the rural farmers to benefit in the utilization of mechanical system at affordable cost.

Table 1: Metrological data for Ondo state for six consecutive days of the experiment Updated WAT on September 12, 2005

Friday
Overcast. High: 75° F / 24° C Wind SW 4 mph / 7 km/h
Friday Night
Overcast. Low: 69° F / 21° C Wind SSW 4 mph / 7 km/h
Saturday
Clear. High: 78° F / 26° C Wind WSW 6 mph / 10 km/h
Saturday Night
Partly Cloudy. Low: 69° F / 21° C Wind SW 4 mph / 7 km/h
Sunday
Overcast. High: 80° F / 27° C Wind SW 4 mph / 7 km/h
Sunday Night
Overcast. Low: 68° F / 20° C Wind SW 4 mph / 7 km/h
Monday
Overcast. High: 78° F / 26° C Wind SW 6 mph / 10 km/h
Monday Night
Clear. Low: 69° F / 21° C Wind SSW 4 mph / 7 km/h
Tuesday
Clear. High: 82° F / 28° C Wind SSW 4 mph / 7 km/h
Tuesday Night
Overcast. Low: 69° F / 21° C Wind SSW 4 mph / 7 km/h
Wednesday
Overcast. High: 75° F / 24° C Wind SW 6 mph / 10 km/h
Wednesday Night
Scattered Clouds. Low: 69° F / 21° C Wind SSW 2 mph / 3 km/h

Source: Weather underground Inc (2005)

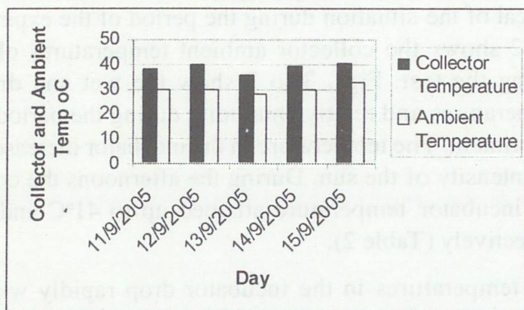

Fig 2: Collector and Ambient Temperature recorded

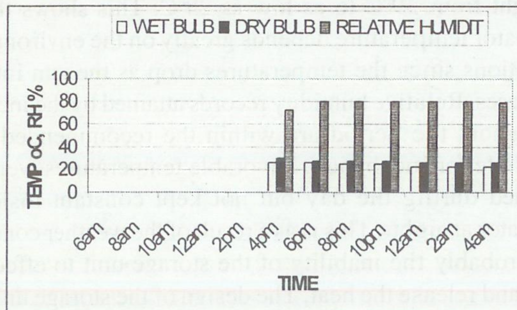

Fig. 3: Dry and wet bulb temperatures and relative humidity recorded during test on 10/10/2005

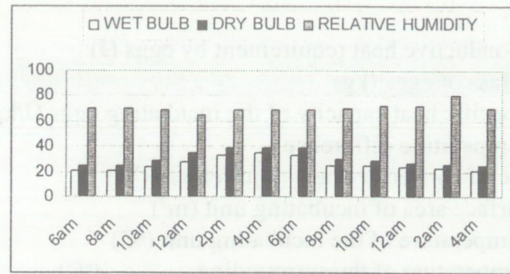

Fig. 4: Dry and wet bulb temperatures and relative humidity recorded during test on 11/10/2005

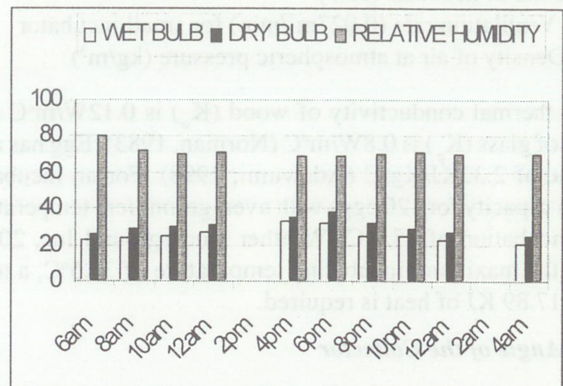

Fig. 5: Dry and wet bulb temperatures and relative humidity recorded during test on 12/10/2005

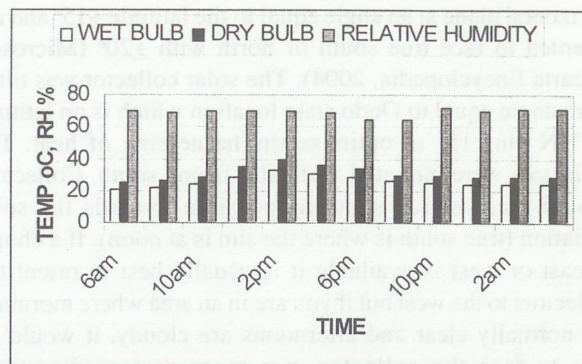

Fig. 6: Dry and wet bulb temperatures and relative humidity recorded during test on 13/10/2005

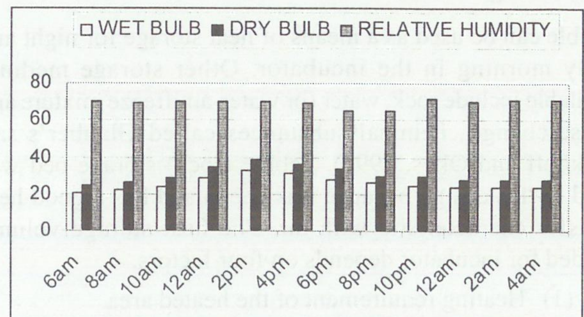

Fig. 7: Dry and wet bulb temperatures and relative humidity recorded during test on 14/10/2005

Table 2: Mean, Standard deviation (SD) and Range of Temperatures and Relative Humidity during Test

DAY	Statistical parameters	Wet bulb Temperature (°C)	Dry Bulb Temperature (°C)	Relative Humidity (%)	Remarks
10/10/2005	Range	22 - 25	26 - 30	72 - 80	Heavy rainfall all through the day
	Mean	24.29	27.43	78.71	
	SD	1.604	1.512	2.984	
11/10/2005	Range	21 - 35	25 - 39	65 - 80	Light showers in the morning
	Mean	26.083	30.667	70.417	
	SD	4.804	5.513	3.353	
12/10/2005	Range	23 - 33	25 - 38	69 - 80	Sunny day, no rain
	Mean	25.8	30.2	71.2	
	SD	3.882	4.002	3.225	
13/10/2005	Range	21 - 33	25 - 39	69 - 71	Sunny day
	Mean	25.9167	30.667	69.333	
	SD	3.475	4.1500	2.0950	
14/10/2005	Range	21 - 33	25 - 39	69 - 72	Sunny day but light showers of rain
	Mean	26.1	30.6	70.4	
	SD	2.228	4.949	0.966	

Improving and extending the solar incubator may help to alleviate poverty among the local poultry farmers in Nigeria.

References:

Adewumi, B.A (1998). Development of kerosene fueled free convention incubator. Transaction of Nigeria Society of Engineers. 33 (20):30-40.

Adewumi B.A. and Falayi, F.R. (1999). Performance evaluation and modification of kerosene fueled table incubator. Nigeria Journal of Animal Production.

Adewumi, B.A and Oduniyi, A.J. (1999) Design, fabrication and testing of charcoal fueled incubator. Journal of Applied Sciences. 2(1): 159-175.

Adewumi, B. A. (2004). Preliminary studies on AC/Dc hear source cum kerosene lantern for egg incubation. Nigerian journal of Animal Production 31 (1): 32 -39.

Berry, G.H. (1998). Artificial Incubation, Oklahoma Co-operative Extension Services Publications. OSU F-1800.

BMAFF, (1982). Incubation and hatchery practice reference book. No 148, Publication of the Ministry of Agriculture, Fishery and Food, United Kingdom, Great Britain

Charnwit U., Krissanapong K., Sirichai T., Mana A. (2003). Solar energy incubator, Energy Publications 4(11):23-24

Culpin, C. 1981. Farm Machinery, Granada Publishing Limited. Pp 359-361.

Eckhoff S. and Okos, M. (1998). Solar energy heating for home, farm and small business. Prudent University Cooperative Extension Service Publication.http://www.ces.pruduce.edu/extmedia/AE/AE-89.html.

Mauldin, J.M. and Buhr, R. J. (1996). Chick abnormality caused by hatchery conditions,

World Poultry, Misset International. 12 :(3) 40-41.

Microsoft Encarta encyclopedia, 2004. An article on solar energy, Category: Direct collection of solar energy. Microsoft corporation USA

Nico, V. W. and Johan, W. (1990). Hatching egg by hens and in an incubator. Published by Agromisa, Wagininga, Netherlands. Second edition. Pp 4-25.

Norman, C.H. (1983). Modern air conditioning practice. Mc Graw-Hill Books Company. Third edition. Pp 40-51.

Obioha, F.C. (1992). A guide to poultry production in the tropics. ACENA publishing, Nigeria. First edition. Pp 170-174.

Olorunnosola, A. O and Ewemoje, J. A. (1998) The development and evaluation of a flat type wooden incubator. Applied Tropical Agriculture 3(2): 114 – 119.

Singh, R.A. (1990). Poultry production. Kenya Publishing Company, New Delhi. Third

Edition. Pp 89-100.

Smith, T.W. (1998). Care and Incubation of hatchable eggs. www.msstate.edu/dept/poultry/hatch.htm.

Weather underground Inc, 2005 Weather underground, Ondo state, Nigeria. http://www.underground.com/global/regional/AF/temperature.html (Sept, 2005)

World Poultry (1998a). Baladi chickens are special creatures. World Poultry, Elsevier International 14(11): 30-31.

World Poultry, (1998b). Effective artificial incubation of Ostrich eggs. World Poultry Publishers 14(7):20-21.

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Evaluation of the Antioxidant Activity of Ocimum sp.

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Extracts of leaves of Ocimum gratissimum and O. basilicum were investigated for antioxidant activities. The proximate composition determination revealed that extracts of Ocimum sp. had a high crude protein content (20.18 and 20.15) respectively. The crude fiber and ash content were high and the physicochemical parameters of the extracts were found to be similar. About 11-14% Vitamin E was obtained from the extracts as Vitamin E is said to be the most abundant lipid soluble antioxidants. An evaluation of the antioxidant effectiveness of the two extracts were carried out and compared with the synthetic antioxidant (BHT). Extracts of the spice were found to exhibit effective antioxidant activities as the synthetic antioxidants except for the green pigment of the extract. O. basilicum exhibited an appreciable antioxidant effectiveness which is significantly equal to the activity of BHT at THE 5th and 10th days during the 20 days of storage.

Keywords: Ocimum gratissimum, O. basilicum, antioxidant, BHT, Vitamin E

Introduction

Utilization of spices as food additives has been an age long practice carried out by different people in many part of the world. Spices extractives, such as oleoresin of rosemary, can provide inhibition of oxidative rancidity and retard the development of "warned–over" flavor in some products. Thus, some spices not only provide flavor and aroma to food and retard microbial growth but are also beneficial in prevention of some off-flavor development (Giese, 1994).

Lipid peroxidation is a major cause of food quality deterioration during processing and storage (Shahidi and Naizk, 1995). Oxidation of lipid imitates other changes in food, which affects its nutritional quality, wholesomeness, safety, colour, flavor and texture (Pomeranz, 1991).

Antioxidants are known to protect food quality by delaying or inhibiting free radical oxidation of fats and oils and the resulting off flavour and colour (Buck, 1991). Although synthetic antioxidants like butylated hydroxytoluene, butylatedhydroxy anisole and propyl gallate are currently being used in food industries, concerns about the possible adduce effects of these and others have been documented (Adegoke et al., 1998).

Ocimum sp. belong to the plant family Labiates, which comprises of several species such as *O. gratissimum, O. basilicum O. americanus* and *O. tenuiflorum* (Tindall, 1983). The leaves from these various species have been put to various uses such as condiments, used for abdominal pains, as a tooth gargle, to regulate menstruation and cure prolapsed of the rectum (Chogo & Grank, 1981). Also the fragment leaves are also added to various dishes with a fishy or disagreeable smell. It occurs directly or indirectly for example in the development of ketch up, spices are ground, mixed and put in muslin bag which is dipped into the ketch up (Sethip Aggarual, 1950). There is dearth of literature on the antioxidant effectiveness of *Ocimum gratissimum* and *Ocimum basilicum*. Thus, the objective of this study was to evaluate the antioxidant properties of these spices that have been put to various uses.

Materials and Methods

Sample Collection and Preparation

Ocimum gratissimum and *O. basilicum* leaves were plucked from three different farms located around Federal University of Technology, Akure road in Akure. The freshly plucked lerics were identified at the Crop Soil and Pest Management Department FUTA. These leaves were sorted, washed under running tap water for 2 minutes, drained and dried at 45°C in the oven. The dried leaves were blended to obtain a fine powder and were sealed respectively in high density polyethylene film and stored until required for use.

Proximate analysis determination

Proximate composition of the spices were determined using the method of (AOAC, 1984).The spices were analyzed for percentage fat, moisture, protein, ash and carbohydrate contents. The moisture content and ash content were determined using A.O.A.C.(1984) procedure. The average of the triplicate readings gave the percentage moisture and ash contents respectively. The fat content, crude fiber and crude protein were all determined using (A.O.A.C,1984).All the analysis were carried out in triplicates. The carbohydrate content was determined by difference.

Determination of Physicochemical properties of *O.basilicum & O.gratissimum*

Some physicochemical properties of the spice extracts were determined. This include; the specific gravity, refractive index, pH peroxide value and iodine value. The specific gravity was determined as the weight of equal volume of oil to to the

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same volume of water. Refractive index was carried out using Abbe refractometer as described by Pearson (1976). The pH was determined using pH meter (Mettler 350). The peroxide value was determined using the method described by Pearson (1991). The iodine value was determined by Wijs method as described by Pearson (1991).

Extraction of antioxidant Anti-oxidant extraction were carried out using the method of Adegoke and Gopalakrishna, (1998) modified by extracting 25 g of finely ground spice with 250 ml Hexane in a Soxhlet extraction apparatus for 8 hours. This consists of a high recovery unit and the extraction rate of spice to solvent was 5:50, that is for every 5g of sample ; 50ml of solvent was used. To maximize antioxidant recovery ,the defatted spices were subjected to further extraction using fresh solvents. In order to reduce the extraction solvent in the antioxidant to permissible level, the antioxidant was desolventized. This was done by drying the antioxidant in an air oven at 50°C for 10 minutes. The crude extracts were evaporated to dryness and the percentage yield of the extracts was determined.

Application of the antioxidant

Three antioxidants were incorporated by direct addition into the groundnut oil (groundnut oil bought from the market) and were then thoroughly mixed to achieve a uniform dispersion- U.S. Food and Drug Administration regulations governing the use of antioxidants limits usage levels to 0.02% w/w of the lipid content of the food. The antioxidants inoculated into the groundnut oil sample include; extracts of O. basilicum, O. gratissimum and BHT. Hence, the minimum concentration of the antioxidant employed was 100 ppm while the maximum was 300 ppm. All the experiments were done in triplicates.

Assessment of the antioxidant activities

In order to assess the efficacy and potency of the spice extracts as antioxidants, the inoculated oil samples, and the control were subjected to accelerated stability test. This experiment was done at 63°C in an air light oven for a period of 20 days. The samples were shaken twice a day during storage and monitored for the peroxide value every 5 days. Decrease in the rate of formation of peroxides was taken as a measurement of the antioxidant activity of each extract (Adegoke *et al.*, 2003). The peroxide value, iodine value and refractive index were carried out using Pearson (1991).

Determination of Vitamin E content (Tocopherol)

Vitamin E was determined according to AOVC 1987 About 0.5g of sample (antioxidant extract) was weighed into a 100ml beaker .40ml of petroleum ether was added and shaken to ensure a uniform mixing. The mixture was filtered using whatman No 42 filter paper and made up to mark with petroleum ether in a 100ml volumetric flask. Standard Vitamin E (a- tocopherol) were prepared from range 25mg/ml-200mg/ml in petroleum ether and treated as sample. 2ml of sample and standard petroleum ether extracted were each treated with 10ml of the iron dipyridyl in glacial acetic acid and made up to 100ml volume with glacial acetic in 100ml volumetric flask. The absorbance of the standards were read on a spectronic 20 spectrophotometer at a wave length of 460nm.

Vitamin E in mg/100g was calculated using the formula;

Absorbance of sample × Gradient factor × Dilution factor / weight of sample

Results and Discussion

Proximate composition

The two extracts have low moisture contents but high crude protein contents. The result of the proximate composition of the extracts, Table 1, shows that there is no much difference in the values of moisture, protein and carbohydrate of the two samples. However, *O. gratissimum* was found to be considerably higher in total ash and crude fibre than *O. basilicum*. This could give higher digestion aiding ability over *O. basilicum* (Adejumo, 2000). The low moisture content obtained is similar to that observed by Adegoke *et al.*, (2003) where he obtained a moisture contents of 8.44% and 7.51% for *A. melegueta* and *X. aethiopica* respectively. Whereas, the protein content of *Ocimum* sp is higher (20.18% and 20.15%) as against 6.44% and 8.53% for *A. melegueta* and *X. aethiopics* (Adegoke *et al.*, 2003).

Table 1. The proximate composition of *O. gratissimum* and *O. basilicum*

Sample	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Crude Fibre (%)	CHO (%)
Ocimum gratissimum	8.11±0.10	11.78±0.09	20.18±0.09	15.62±0.07	8.70±0.09	35.61±0.05
O. basilicum	9.35±0.02	14.38±0.03	20.15±0.01	13.25±0.01	6.73±0.01	36.14±0.01

Physicochemical properties of the extracts

The result of the physicochemical properties of the two natural extracts which include, specific gravity, refractive index, pH, peroxide value and iodine value (Table 2), were found to be similar except for the iodine value which was found to be higher in the extracts, and O. gratissimum value was shown to be higher than that of the O. basilicum. The implication of this high iodine value is that the extracts have high unsaturated linkages and they could be more susceptible to oxidation (Potter, 1978).

Table 2: Chemical Values of *O.* gratissimum and *O. basilicum*

Parameters	O gratissimum	O.basilicum
Specific gravity	0.947±0.01	0.955±0.01
Refractive Index	1.43±0.01	1.44±0.01
pH	6.90±0.01	7.10±0.08
Peroxide value	5.80±0.07	7.10±0.09
Iodine value meq.kg	48.48±0.13	41.12±0.01

Vitamin E content and % yield of extract of the spices

The spices were found to contain 11-14% vitamin E (Table 3) , which shows that the spice extracts can act as strong antioxidant (Potter, 1978).Vitamin E is reported to favour the absorption of iron and play a role in maintaining stability of biological membranes (Potter, 1978).All tocopherols and tocotrienols when not esterified , have the ability to act as antioxidants; quench free radicals by donating the phenolic H and an electron. Naturally occurring tocopherols has been shown to contribute to the stability of highly unsaturated vegetable oils through their antioxidant activity (Fenema,1996).Due to its antioxidant activity, Vitamin E is able to spare carotene and vitamin A from oxidative destruction (Potter,1988).). The extracts were also found to contain 11-14% Vitamin E, which is the most abundant lipid soluble antioxidant and protects the lipid portions of the cell, especially cellular membranes (Kaur and Kapoor, 2001).

The antioxidant yield obtained from 10g of each spice are also shown in Table 3. *O.basilicum* had a higher yield than *O. gratissimum* but the two spices antioxidant yield is lower when compared with *Xylopia aethiopica* which had a higher yield of 44% (Adegoke *et al*., 2003).The higher percentage yield of *X. aethiopica* may be due to its higher fat content. From Table 3, the extraction yield showed a correlation with the fat contents, the more the fat content the more the extract yield. The fat content of any food may be considered to consist of the 'free' lipid and the' bound' lipid constituent. The 'free' lipid constituent is that which may be extracted by less polar solvent such as diethyl ether, hexane and petroleum spirit whereas the 'bound' constituents require more polar solvents such as alcohol for their extraction (Kirk & Sawyer,1991). In this work hexane was used for the extraction.

Table 3: Vitamin E content and % yield of extract of the spices.

Samples	Vit. E (mg / 100g)	% Yield of Extracts	% Fat
O. basilicum	10.29±0.01	14.69±0.01	14.38±0.01
O. gratissimum	13.34±0.01	11.26±0.01	11.78±0.01

Antioxidant activity of the spice

The antioxidant activities of the spice extracts were compared with the antioxidant activity of BHT at 100ppm and 300ppm inclusions respectively (Table 4&5).The control (groundnut oil without antioxidant) showed that the peroxide value of the oil increases with storage days. This shows that as storage days increase there is increas in the rate of oxidation of the unsaturated bonds. However, when the natural antioxidants (spice extracts), and the synthetic antioxidant (BHT) were added there were reductions in the peroxide values which were different significantly.

Table 4 Antioxidant activity of *O.basilicum, O. gratissimum* and BHT(100ppm)

Number of days	Control	BHT	O.basilicum	O.gratissimum
0	6.89±0.01	6.89±0.01	6.89±0.01	6.89±0.01
5	8.0±0.2[d]	2.86±0.15[a]	3.19±0.01[b]	4.29±0.01[c]
10	8.5±0.24[d]	3.06±0.11[a]	3.39±0.01[b]	4.49±0.01[c]
15	8.9±0.2[d]	3.10±0.02[a]	3.59±0.02[b]	4.79±0.01[c]
20	9.1±0.1[d]	3.13±0.11[a]	4.0±0.2[b]	5.10±0.1[c]

Each value represents the mean and standard deviation from three lots. a-c means within a column with a different letter are significantly different (P<0.05).

Table 5 Antioxidant activity of *O.basilicum, O. gratissimum* and BHT (300ppm)

Number of days	Control	BHT	O.basilicum	O.gratissimum
0	6.89±0.01	6.89±0.01	6.89±0.01	6.89±0.01
5	8.0±0.2[d]	2.03±0.05[a]	2.08±0.13[a]	3.13±0.15[b]
10	8.5±0.24[d]	2.20±0.1[a]	2.60±0.02[a]	3.60±0.05[b]
15	8.9±0.2[d]	2.20±0.1[a]	2.96±0.12[b]	3.90±0.1[c]
20	9.1±0.1[d]	2.30±0.1[a]	3.16±0.15[b]	4.2±0.1[c]

Each value represents the mean and standard deviation from three lots. a-c means within a column with a different letter are significantly different (P<0.05).

At 100ppm, the peroxide value of the control on the 0 day was (6.89±0.01) and it increased to 9.1±0.1) on the 20th day .The oil sample treated with BHT had a peroxide values (6.89± 0.15 and 3.13±0.11) on the 0 and 20 days respectively. However, the oil samples treated with spice extracts O.basilicum had values of (6.89 ±0.01 and 4.02 ±0.2) for 0-20 days respectively, while O.gratissimum treated oil had peroxide values reduced from (6.89 ±0.01 to 5.10 ±0.1).Though the peroxide value of the BHT treated oil was significantly different from the oil sample treated with the natural antioxidants these natural extracts could as well act as antioxidant.

At 300ppm inclusion however, there were no significant difference in the antioxidant activities of BHT and O.basilicum on the 5 th and 10th days (Table 5). This shows that if the natural antioxidants are added in higher concentrations they would be as effective as the synthetic antioxidants (Adegoke *et al*., 2003). But as the storage days

increases there is tendency for the natural antioxidant to loose its antioxidant ability due to evaporation because on the 15th and 20th days the BHT activity was found to be significantly different again(Table5). This work is in agreement with the research carried out by Wu *et al*.,1982) where they reported that naturally occurring compounds in rosemary extract exhibited antioxidant properties like BHA and equal or slightly less than BHT. The difference in antioxidant effectiveness of natural antioxidants could be as a result of plant species and the extraction method (Fernandez-Lopex *et al*.,2004).

Conclusion

This work entails the determination of the antioxidant activities of the *Ocitmum* species. The proximate composition revealed that the leaves are rich in protein and the Vitamin E content of the extracts are indications to their ability to serve as antioxidants. Thus, the oily, green aromatic extracts from *Ocimum* sp. possessed good antioxidant properties in oil except for the green colour of the extracts.

Reference:

Adegoke G.O., Kumar, M.V.; Gopalakrishna, A. G.; Varadaraj, M.C.; Sambacah, K; Lokesh, B.R. (1998). Journal of food Sci. Tech., 35: 283 – 298.

Adejumo, M.A. (2000). Effect of Varying Dehydration Temperature and Processing on the Organoleptic Properties of local Herbs use as flavour "Efirin". ND Project Submitted to the Dept. of Food Tech., Osun State Poly. Iree. Osun State Nig.

A.O.A.C. (1984). Official Methods of Analysis. 14th Edition. Association of Official Analytical Chemistry, Washington, D.C. USA.

A.O.V. C. (1987) Association of Vitamin Chemist, Methods of Vitamin Assay

Buck D.F. (1991). Antioxidants in food additives, users handbook. Academic, Glagow, p 5.

Chang, S.S; Matijasavic, B.O; Hisey, A.O.L. and Huang, C.L (1977). National Antioxidant from Rosemary and Sage. Journal of food Science. 42, 1102 – 1107.

Chipault, J.R; Mizuno G.R; Hawkins, J.M. and Lundberg, W.O. (1952). The antioxidant properties of natural spices. Food Research. 17: 46 – 55.

Chogo, J.B.; and Grank G. (1981). Chemical composition and biological activity of the Tanzanian plant O. suave. Journal of National products. 44, 308 – 311.

Fernandez-Lopex,J., Zhi, N., Aleson-Carbonell,L.,Perez-Alvarez, J.A., Kuri, V. (2004). Antioxidant and antibacteria activities of natural extracts: application in beef meatballs. Meat Science.69;371-380.

Fenema, O.R. (1996). Food Chemistry.3rd edition. Library of Congress Cataloging- in- Publication data

Giese, J. (1994). Spices and Seasoning blends. A taste for all season. Food Technology. 48(4); 87 – 98.

Kaur Charanjit and Kapoor Harish (2001). Antioxidants in fruits and vegetables – the millennium's health. International Journal of Food Sci. And Tech. 36 Pp. 711.

Kirk, R. and Sawyer, R. (1991). Pearson's composition and analysis of foods. Longman Publisher London. Pp 1- 650.

Pomesanz V. (1991). Functional Properties of Food Components. 2nd ed. Academic Sc. Diego. PP 16 – 20.

Patter, N.N (1978). Fats, oils and their Product. In: Food Science, 3rd edition, Avc Publishing Co-Inc. Westpart Connecticut Pp 499 – 500, 77.

Shahidi, F. and Naizk, M. (1995). Food phenolic sources, chemistry, effects and application. Technomic, Lancaster, Basel Pp 235 – 273.

Sethi, S. C. and Aggarual. J. S. (1950). Stabilization of edible fats by spices and Condiments. Nature 166, 518 – 519.

Tindall, H.D (1983). Vegetables in the Tropics. Macmillan Educational Publisher Ltd. Third Edition Pp 247 – 248.

Wu, T.W.; Lie, M.H.; Ho, C.T. and Chang, S.S: (1982). Elucidation of the chemical structure of natural antioxidants isolated from rosemary. Journal Amer. Oil Chem. Soc. 59: 339.

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A Study of Some Physical and Mechanical Properties of Coconut

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Mechanizations of coconut is essential to get maximum benefit of the nut. To this end, the physical and mechanical properties of coconuts are required. Laboratory experiments were carried out to determine these properties and parameters of dehusked coconuts. Major diameter ranged from 8.4 cm to 11.9 cm; minor diameter from 6.3 cm to 8.8 cm. Volume ranged from 350 cm³ to 680 cm; mass from 200 gm to 504 gm. Density ranged from 0.57 gm/cm to 0.79 gm/cm; surface area from 183.8 cm to 315.1 cm. Roundness and sphericity ranged from 0.52 to 0.92 and 0.61 to 0.92 respectively. Coefficient of static friction on galvanized iron and wood ranged from 0.36 to 0.46 and 0.49 to 0.69 respectively. Fracture force ranged from 0.3 kN to 5.7 kN while cracking force ranged from 0.5 kN to 12.3 kN. Regression models were developed for predicting these properties.

Keywords:- Physical and mechanical Properties, Coconut, Regression models, Prediction.

Introduction

The coconut (*cocos nucifera*.L) is a member of the palmae family, consisting one of the most important families of the monocotyledon. The coconut is the most extensively grown and used nut in the world and is the most important of all palms. It grows well on a loose sandy soil with high mean temperature ranging from 23.0C to 29.4C and an evenly distributed rainfall range of 152.4 cm to 203.2 cm. The first harvest in tall palms is usually taken 6 to 8 years after planting and takes another five to eight years to reach the stage of full productivity. The nut of the coconut palm attains full size when about 160 days. The shell begins to harden in about 220 days. It becomes fully hardened and the meat fully formed when the nut is 11 to 12 months old.

Since the seeds (nuts) are dispersed by water from continent to continent and island to island around the world, their native habitat has been a topic of controversy. The reason being that coconut can be carried by ocean currents and can establish themselves on open coasts without the aid of man even if such establishment is rare. However, most species are thought to have their origin in north western South America.

Coconut is grown for different purposes in various parts of the world. Its uses are legion. Its greatest multiplicity of uses is found on tropical islands in the Pacific and Asia where it provides food, drink, oil, medicine, fibre, timber, thatch, mats, fuel and domestic utensils. The oil is used for cooking, annointing the body, illumination, lubrication and soap making. Each and every part is useful to man in one way or the other. It is not surprising that it has been variously called "the tree of life", "the tree of heaven and mankind", "greatest provider in the tropics". The coconut fruit is a drupe with a thin epicarp overlying a thick fibrous mesocarp. Inside the mesocarp is the hard shell-like endocarp which inturn encloses the endosperm. The latter provides, as the fruit ripens, the white meat or copra rich in oil, and the watery milk of the central cavity. According to Asiedu (1989) the fruit consists of four parts: 35% husk; 12% shell; 38% meat and 15% water.

Enig (2000)reported that coconuts play a unique role in the diet of mankind because they are the source of important physiologically functional components ,and that these physiologically functional components are found in the fat part of whole coconut, in the fat part of desiccated coconut and in the extracted coconut oil. It was also reported (Van den Brekel ,1999) that clinical studies have shown that coconut oil has anti-microbial, and ant- viral properties and is now even being used in treating AIDS patients. Studies conducted in the Philippines recently showed that coconut oil does indeed reduce the viral loads in AIDS patients. Once mistakenly thought to be bad because of its saturated fat contents, coconut is now known to contain a unique form of saturated fat that actually helps to prevent heart disease, stroke, and hardening of the arteries. Another incredible fact about coconut oil is that even though it is a fat , it actually promotes weight loss. The reason is again because of the healthy medium chain fatty acids (Van den Brekel ,1999). These fatty acids do not circulate in the blood stream like other fats, but are sent directly to the liver where they are immediately converted into energy, just like carbohydrates. So the body uses the fat in coconut oil to produce energy, rather than be stored as body fat.

Therefore, in order to design equipment for handling, storing and processing of coconuts, its physical and mechanical properties need to be known. When these properties are known there is also the need to be able to predict properties of coconuts of the same variety under similar conditions. This may be done by the use of regression models. Presently, there is a dearth of knowledge in this area in Nigeria. The objectives of this study therefore was

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(i) to investigate some physical and mechanical properties of local variety of coconuts, relevant in its mechanization,

(ii) to develop models that can be used to predict these properties under similar conditions.

Materials and Methods

The coconuts that were used in this study were bought from local markets in Akure, Nigeria. They were mature, and dry. They were in various sizes and were kept in the laboratory for two weeks under room temperature to get them equilibrated with the environment before, measurements were taken of their properties. The moisture content of the shells was determined by the oven method and ranged from 6.5% to 8.2% (db).

For the measurement of physical axial dimensions, the largest dimension (L) second dimension (W) and smallest dimension (T) were measured with the aid of vernier calippers to 0.01 cm. At least 50 nuts were measured in each case. The geometric mean diameter D of a nut was calculated from the relationship (Screenarayanan et al. 1985):

$$D_{GM}\left(LWT\right)^{1/3} \quad (1)$$

According to Mohsenin (1970), the degree of sphericity, can be expressed as:

$$\varnothing = \frac{(LWT)^{1/3}}{L} \quad (2)$$

The equation was used to calculate the sphericity of coconuts in this study. The surface area, S was found by analogy with a sphere of geometric mean diameter using the following relationship given by Mc Cabie et al. (1986):

$$S = \pi D \quad (3)$$

The true density of the nut was determined by the liquid displacement method (Shephered and Bhardwaj, 1986; Deshpande and Ali, 1988). The nuts were coated with a very thin layer of epoxy resin adhesive (Aradite) in order to avoid any absorption of water during the experiment as suggested by Dutta et al. (1988). The adhesive was found to be insoluble in water, resistant to heat, humidity, solvents and acids. The increase in weight of the coconut due to the adhesive coating was negligible (less than 1.7%) and there was no change in weight of the nut even if it was kept submerged in distilled water for 1 hour.

To measure the fracture and cracking force of coconut, a Universal Testing Machine (SM 100, Model No CPI - 60) of 0.1 kN Calibrations was used. This method was similar to that reported by Manuwa (1998).

The static coefficient of friction for the nuts was determined against two structural materials namely galvanized iron and plywood. A coconut was made to lie in its natural resting position on an adjustable tilting plate. The plate was inclined gradually with a screw device until the nut just started to slide down and the angle of tilt was read from a graduated scale.

Data Analysis - Statistical models were developed to fit the data collected and several statistical procedures were used to test the performance of the models from this study.

These methods include: Coefficient of correlation which gives the degree of association (r), coefficient of determination, Rwhich evaluates the degree of association between data points and predicted values; the absolute mean difference (AMD) between measured and predicted values which evaluates the degree of coincidence; and the relative error (RE) between the measured and the predicted values: Mathematically,

$$AMD = \frac{1}{N}\sum_{i=1}^{N}|Mi - Pi| \quad (4)$$

$$RE = \frac{100}{N}\sum_{i=1}^{N}\frac{|Mi - Pi|}{Mi} \quad (5)$$

Where N = number of data points

M = the th measurement and

P = the th prediction.

Results and Discussion

A summary of the properties of cocnuts are presented in Table 1- 6.

Linear Dimensions

Average values of the three principal dimensions along orthogonal axes of the coconuts namely length, width and thickness determined in this study, and the range of values are presented in Table 1. Knowledge of axial dimensions of the nuts is important in the determination of aperture sizes in the design of handling equipment and machinery. Furthermore the geometric mean diameter of the axial dimensions is useful in determining the characteristic dimension for irregular shaped solids (Henderson and Pabis, 1962).

Geometric Mean Diameter

The average values of geometric mean diameter of coconut calculated are also presented in Table 1. The range of these values are also presented in the table.

Sphericity and Roundness

It is seen from Table 1 that the coconuts have mean values of sphericity ranging from 0.6171 to 0.9249 and an average value of 0.8078. The degree of roundness ranged from 0.5213 to 0.9232 with an average value of 0.7211 The observed mean sphericity is an indication that the coconut may be expected to roll easily, rather than slide, a property which is very important in the design of hoppers and conveyance systems for coconut handling equipment.

Table 1. Some Physical and Engineering Properties of Coconuts.

Properties	Average	Range
Major Diameter (cm)	10.3	8.4 - 11.9
Intermediate Diameter (cm)	9.0	8.2 - 10.4
Minor Diameter (cm)	7.9	6.3 - 8.8
Geometric Mean DIA (cm)	10.3	9.0 - 11.6
Volume (cm^3)	530	350 - 680
Mass (gm)	365	200 - 504
Density (gm/cm^3)	0.68	0.57- 0.79
Surface area (cm^2)	258	183 - 315
Roundness	0.72	0.52 - 0.92
Sphericity	0.80	0.61 - 0.92
Fracture force		
H H P (kN)	1.65	0.30 - 5.20
H V P (kN)	2.30	0.8 - 5.7
Cracking force		
H H P (kN)	2.44	0.5 - 7.4
H V P (kN)	4.14	1.6 - 12.3
Coefficient of Static friction on		
Galvanized Iron	0.41	0.36 - 0.46
Plywood, perpendicular to grain	0.63	0.52 - 0.69
Parallel to grain	0.60	0.49 0.68

Surface Area and Volume of Coconut

Surface area of the coconuts ranged from 183.88 cm to 315.1cm² with an average value of 258.1 cm. The volume of coconuts varied from 350.0 cmto 680 cmwith a mean value of 530.0 cm.These values are important in the design of appropriate bins and containers for handling of coconuts.

Mass and Density of Coconut

The mass of coconut varied from 200.00 gm to 504.00 gm with an average value of 365.7 gm. The density of coconut varied from 0.5714 to 0.7909 gm / cmwith an average value of 0.6822 gm/ cmThe mean density values can be very useful in computing product yield and throughput in processing machinery. It is also an indication (being less than 1gm/cm³) that the coconut even without its fibrous mesocarp would float in water, a property which is relevant in processing industries especially in washing and separation of denser and unwanted materials from the coconuts.

Fracture and Cracking Force

Loading In the hilum horizontal position (HHP), or transverse loading,the fracture force ranged from 0.3 to 5.2 kN with an average of 1.65 kN, while the cracking force ranged from 0.5 to 7.4 kN with an average of 2.44 kN. Loading In the hilum vertical position (HVP), or longitudinally, the fracture force ranged from 0.8 to 5.7 kN with an average of 2.30 kN, while the cracking force ranged from 1.6 to 12.3 kN with an average of 4.14 kN. It was observed that mature dry coconut offers greater resistance to fracture and cracking in the hilum vertical position than in the natural resting position..The values of these properties are very important in the desgn and development of coconut crackers which are necessary in the removal of coconut meat before further processing into coconut oil for example.

Coefficient of Static Friction

The values of coefficient of static friction were highest for plywood with the grain perpendicular to the direction of motion and lowest for galvanized iron with an average of 0.4148. The mean values for plywood were 0.638 and 0.607 for perpendicular and parallel to grain respectively. These values will be useful in hopper design for gravity flow since the angle of inclination of the hopper walls should be grater than the angle of repose to ensure continuous flow of the material.

Correlation of Weight and Size of Coconut

The weights and geometric means of the coconuts were correlated as shown in Table 2: The predicted weights are also shown and the residuals. The predictive model is shown as equation (6).

$$M = -1036.34 + 154.9\,D \qquad (R= 0.9467) \quad (6)$$

Table 2: Correlation between weight and size of coconut

Measured Weight (gm)	Measure Size (D_{Gm}) (cm)	Predicted Weight (gm)	Residual (gm)	%
340	9.0	368	-28.7	8.4
396	9.0	359	36.6	9.2
526	10.3	571	-45.6	8.7
634	10.9	655	-21.3	3.4
566	10.0	522	44.0	7.8
746	11.6	763	-17.7	2.4
700	11.0	667	32.3	4.6
620	10.5	590	29.8	4.8
720	11.3	714	5.9	0.8
400	9.5	435	-35.3	8.8
Mean 546	10.3	564	29.7*	5.89⁺

* = Absolute Mean Difference, AMD
\+ = Relative Error, RE

The absolute mean difference is 29.7 gm while the relative error is 5.89%.

Correlation of Surface Area and Weight

The weights of coconut were correlated with the surface area. A model was developed for prediction (Eq.7) while the predicted values and residuals are shown in Table 3.

$$S= 114.75 + 0.39M \qquad (R = 0.8112) \quad (7)$$

Table 3. Coconut Measured Weight, Surface area, Predicted Values and Residual

Measured weight (gm) M	Measured surface area (cm^2) S	predicted surface area (cm^2) S[1]	Residual (cm^2)	%
200	183	192	9.1	4.9
266	254	218	35.7	14.0
428	268	288	-13.6	5.1
435	257	284	27.2	10.6
477	313	301	12.1	3.9
504	315	311	3.2	1.0
430	304	282	21.3	7.0
370	247	259	-10.8	4.3
255	194	214	-20.4	10.5
300	240	232	8.8	3.6
Mean 366	258	258	16.22*	6.49 +

* = Absolute Mean Difference, AMD

\+ = Relative Error, RE

The absolute mean difference is 16.22 cm^2 with an error of prediction equal to 6.49%.

Correlation of Volume and Surface Area

The volume of the coconut was correlated with the surface area. There was good positive correlation (r=0.9506). A linear model fits the data points and can be used to make predictions:

$$V = -106.99 + 2.47\,S \qquad (R= 0.9038) \qquad (8)$$

The predicted values were compared with the measured values as shown in Table 4. The absolute mean difference is 11.55 cm while the prediction error is 4.54%.

Table 4. Coconut Measured Volume, Surface Area, Predicted Values and Residual

Measured Volume (cm^3)	Measured Surface area (cm^2)	Predicted Surface area (cm^2)	Residual (cm^2)	%
350	183	185	-1.38	0.7
450	254	225	28.81	11.3
600	268	286	-17.88	6.7
550	257	266	-8.52	3.3
680	313	318	-5.49	1.8
680	315	318	-3.79	1.2
620	304	294	9.62	3.2
540	248	262	-13.47	5.4
390	194	201	-7.29	3.8
440	240	221	19.25	8.0
Mean 530	258	258	11.55*	4.54 +

* AMD or Absolute Mean Difference

\+ RE or relative Error.

Correlation of Fracture and Cracking Force with Shell Thickness.

The variation of fracture force and cracking force with shell thickness is presented in Table 5 . The mean thickness of the shell of coconut under study was 4.62 mm. Models were developed to fit the data on Table 5 in two groups, namely: Hilum vertical position and hilum horizontal position. All the models have good positive correlations. The best models were intrinsically linear models: exponential and power models. The parameters of the models are presented in Table 6. With these models it is possible to predict the resistance of mature dry local variety coconut with reasonable degree of accuracy.

Table 5. Variation of Fracture and Cracking Force With Shell Thickness.

Shell thickness (mm)	Hilum Vertical Position		Hilum Horizontal Position	
	Fracture force (kN)	Cracking force (kN)	Fracture force (kN)	Cracking force (kN)
6.0	5.7	12.2	5.2	7.4
4.5	1.3	2.8	0.9	1.1
5.5	2.5	3.0	1.9	2.2
4.2	1.8	2.0	1.4	2.1
4.0	1.1	2.0	1.3	2.4
3.5	0.8	1.6	0.3	0.5
3.9	1.2	2.2	0.5	1.7
4.1	1.6	2.4	0.9	1.6
5.0	2.2	5.8	3.2	3.4
5.5	4.3	6.8	0.9	1.6
Mean 4.62	2.3	4.08	1.65	2.4

Table 6. Predictive Models Parameters for Fracture and Cracking force of Coconuts.

Parameters	Regression Models and Parameters			
	Hilum Vertical Position		Hilum Horizontal Position	
	Fracture force			
	Best fit (EXPFIT)	Linear fit	Best fit (PWRFIT)	Linear fit
r	0.9284	0.8855	0.7781	0.7329
slope	0.6589	1.3690	3.6601	1.3124
intercept	0.0927	-5.2701	0.0046	-4.4334
covariance	0.4550	1.1323	0.1155	0.9124
	Cracking Force			
	Best fit (EXPFIT)	Linear fit	Best fit (PWRFIT)	Linear fit
r	0.8922	0.8261	0.6953	0.6773
slope	0.7046	3.3172	2.7451	1.5652
intercept	0.1258	-11.2510	0.0298	-4.8312
covariance	0.4866	2.9142	0.0865	1.0824

The fracture or cracking force was regressed on the thickness of the shell after the models:

F = be (exponential fit) (9)

or F = bt (power fit) (10)

where b = intercept, = slope

Conclusions

From this study the following conclusion can be drawn::

(i) The geometric mean diameter of the coconuts variety varied from a minimum of 9.01 cm to a maximum of 11.62 c, with an average of 10.30 cm

(ii) The volume varied from 350 cm to 680 cm with a mean of 530 cm.

(iii) These properties have good positive correlations with one another: mass has correlation with geometric mean; surface area with mass; volume with surface area; fracture and cracking force with shell thickness.

(iv) Coconut offers greater resistance to compression when it is loaded in the hilum vertical position than in the hilum horizontal position.

(v) Regression models can be used to fit the data points of these properties so that Predictions can be made at other points within the range or scope of this study.

(vi) The errors arising from these models are sufficiently small thus confirming that the models are good for prediction.

References

Asiedu, J. J. (1989). Processing of Tropical Crops; Macmillan, Publ. London, pp266

Deshpande, S. D.; Ali, N (1988). Effect Of Harvest Moisture on Some Engineering Properties of Wheat. International Agro Physics, 4(1-2): 83 - 91

Dutta, S.K.; Nema, V.K.; Bhardwaj, R.K (1988). Physical properties of grains. Jour Agric. Eng. Res., 39:259 - 268.

Henderson ,S. M.and Pabis, S. (1962). Grain Drying Theory IV .The Effect of Air Flow Rate on Drying Index. Jour Agric. Eng. Res., 7(2): 85-89.

Manuwa, S.I. (1998). Fracture Resistance of Palm Nuts To Compressive Loading. NSAE Paper Held At Lagos Airport Hotel, Lagos, 9th - 12 th September, 1998.

Mc Cabie, W.L. Smith, S.C.; Harriott, P. (1986). Unit Operations of chemical engineering. N.Y. Mc graw-Hill Book Company.

Mohsenin, N.N. (1970). Physical Properties of Plant and animal Materials. New York: Gordon and Breach Science Publishers.

Screenarayanan, V.V.; Subramaniam, V.; Visvanathan, R. (1985). Physical and Thermal Properties of Soybean. Proceedings of Indian Society of Agricultural Engineers. 3:161-169.

Sharma, S.K.; Dubey, R.K.; techchandani, C.K. (1985).Engineering Properties of Black Gram, Soybean and Green Gram. Proceedings of Indian society of Agricultural Engineers, 3:181-185.

Shephered, E; Bhardwaj, R. K. (1986). Moisture-dependent Physical Properties of Pigeon Pea. Journal of Agricultural Engineering Research, 35: 227-237.

Enig, M. (1996). A new look at coconut oil. URL- http://.westonaprice.org/knowyourfata/coconut_oil.html

Van den Brekel , M. (1999). The health benefits of coconut oil. URL http:www.naturalhealthweb.com/articles/vandenbrekell.html

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The Moisture and Temperature Profiles of Cocoa Stored In Jute and Hessian Bags

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Environmental factors such as temperature and moisture are the most crucial factors limiting the distribution and abundance of fungi that contaminate stored food products. A comparative study of cocoa bean stored in Jute and Hessian bags was carried out to determine the moisture variation in the product so as to establish the best storage material for cocoa bean in a humid environment. Two storage materials (jute and Hessian bags) were used. Each stacked with 30kg of purchased grade one cocoa for six months. The temperature and relative humidity of the ambient air in the open laboratory were measure. The temperature and moisture content of the cocoa beans were also measured. The results showed that there was increase in temperature from the side of the two bags to the center of the bags with cocoa stored in jute bag having higher temperature than the Hessian bag. Fluctuations in the moisture content of cocoa bean and deterioration due to insect infestation and mould growth were experienced in cocoa stored in jute bag due to variations in ambient atmospheric humidity. Hessian bag will be a better storage material for cocoa in a humid environment while jute bag will only be suitable during the dry season.

Keywords : Storage, Jute, Hessian, Equilibrium Moisture content, Temperature and Humidity.

Introduction

The storage of cocoa beans in the tropics presents two potential problems; the development of mould and the spread of stored product pests. It is safe to store cocoa beans for two or three months but if the cocoa is to be stored for much longer period in the tropics, special precautions must be taken to ensure that the quality does not deteriorate. Prolonged storage in damp condition can lead to a rise in free fatty acid (Wood and Lass, 1989).

Nigeria is among many countries of world that is blessed with numerous resources among which agricultural resources play a major role and serve as a source of foreign exchange to the government and as a source of income to the farmers. It became imperative to find a better storage material for storing cocoa when the price and supply of the commodity become unstable as a result of infestation due to deterioration resulting from mould and insect infestation during storage. This study compares the effectiveness of jute and Hessian bags as storage materials for cocoa in a humid environment.

Materials and Method

The study in Akure is located within the humid region of Nigeria, at latitude 7⁰14ᴵN and longitude 5⁰08ᴵ. It lies in the rainforest zone with a mean annual rainfall of between 1300mm and 1600mm. The average temperature is 27.5⁰C, the relative humidity ranges between 85% and 95% during rainy season and less than 50% during the dry season.

Sampling Technique

Jute and Hessian bags were purchased and the diameter of the bags determined. The radius was divided into equal parts to find the depth at which thermocouples would be inserted.

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The method is similar to Igbeka (1987). For the jute bag, the radius was 25cm and the division was into five equal parts (5cm apart) while the radius was 20cm and divided into four equal parts for Hessian bag. Cocoa beans used for the experiment was well grade.

Moisture content determination

The moisture content was determined using the oven dry method at temperature of 105⁰C for 24hours as specified by Falayi (2000) to ensure complete dryness of the product.

Temperature and Humidity Measurement

Dry and wet bulb temperatures were measured by means of two electrical aspirated psychrometers with low-inertia platinum thermoresistors as sensors. The time constant of the thermoresistors was assumed to be less than 20s. The psychrometer was placed close to the bags and after measuring the dry and wet bulb air temperatures, the humidity was determined using psychometric chart. Temperatures at different depth of the bag was equally determined

Storage period and study Time

The cocoa beans were stored for a period of six months (December to May). The moisture content and temperature were measured once and thrice respectively every week of the study period since absorption of moisture and fluctuations of temperatures were assumed to be gradual in stored product. The collected data were subjected to the appropriate statistical analysis.

Results and Discussion

Variation in Moisture

The equilibrium moisture content of cocoa in Jute bag reduced from 7.8% to 6.5% during the initial storage period and thereafter the equilibrium moisture content increased to 8.9% towards the end of the storage period as shown in Fig.1.

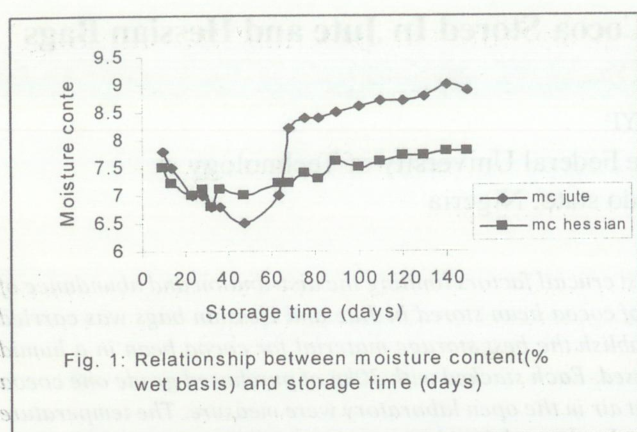

Fig. 1: Relationship between moisture content(% wet basis) and storage time (days)

The final equilibrium moisture content of the product was above the safe moisture content of cocoa. This fluctuation in moisture content could be said to be as a result of variations in the relative humidity of the ambient air as indicated in Fig 2.

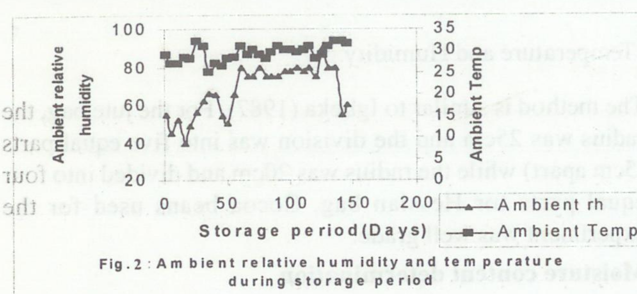

Fig.2 : Ambient relative humidity and temperature during storage period

A regression of equilibrium moisture content of cocoa in jute bag on ambient relative humidity shows correlation as shown in Fig.3.

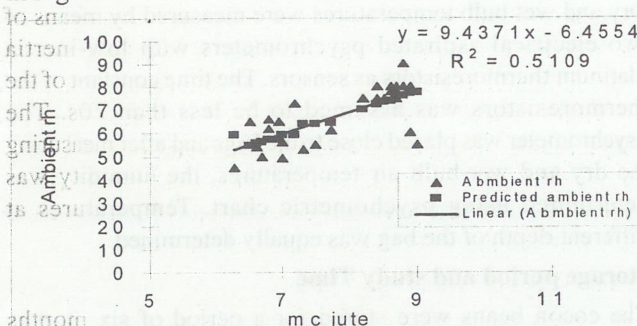

Fig 3 Equilibrium Moisture content of cocoa in Jute bag and ambient relative humidity

The product tends to attain equilibrium moisture content with the ambient air. The same trend of equilibrium moisture content variation was observed in Hessian bags but there was a slight decrease in the moisture content when compared to jute bag. The moisture content of cocoa in Hessian bag was within the safe moisture content throughout the period of storage which implied that the Hessian bag prevented the product from absorbing or losing moisture.

Physical examination of the product after the storage period showed that cocoa beans in the jute bag had been infested with insects and had started deteriorating due to mould growth.

Variation in Temperature

The temperature of cocoa at various depths in both bags were measured and compared. There was a slight increase in temperature towards the center of the bags. However temperature was higher in the center of jute bag than the center of Hessian bag throughout the storage period as shown in Fig 4.

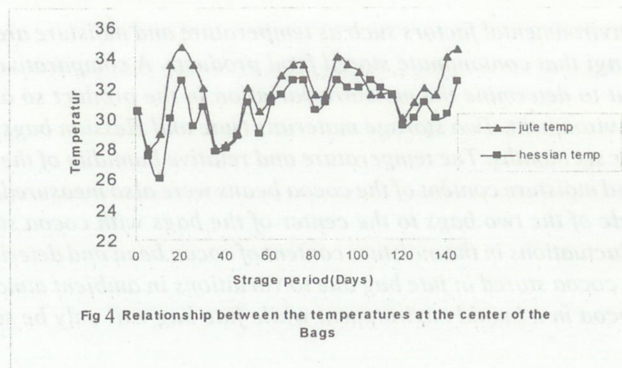

Fig 4 Relationship between the temperatures at the center of the Bags

Conclusions and Recommendation

The findings in this study can be used to predict the conditions of stored cocoa bean in a ware house if the initial moisture content and environmental conditions were known. In a dry environment, the jute bag would be a preferred as it would allow more aeration while in a humid environment; the Hessian bag would be a better storage material for cocoa bean. Cocoa stored in a high humidity environment particularly during raining season should be re-dried after 30days of storage when stored in jute bag otherwise Hessian bag would be a better storage material in order to prevent insect infestation and deterioration due to mould growth.

References

Abiola, S.S and O.O. Tewe (1986). Effect of Cocoa Husk on the Performance of Cockerels. Journal of Agricultural Science and Technology 12 (2): pg 131 – 134.

Aseidu, J.J (1989). Processing Tropical Crops. A Technology Approach. The Macmillan press limited. Pg 25-69.

Falayi F.R. (2000). Determination of Engineering Properties of Palm Kernel Shell. Unpublished MSc Project. University of Ibadan.

Igbeka J.C.(1987). Simulation of Moisture profile in Stored Gari. Journal of Food and Agriculture, (1): 2-9.

Pillaiyer, P (1988) – Rice post production Manual, Wiley Eastern Limited. Pp 230-264

Susan, M and Anne P. (1988) – Macmillan Tropical and Sub-tropical Foods. Macmillan Publishers pp 1991.

Wood G.A.R.(1975) – Cocoa Tropical Agricultural series. Longman Inc, New York. Pp12

Wood G.A.R. and Lass R.A (1989) – Cocoa Tropical Agricultural Series 4th edition, Longman and Scientific Technical. Pp 496-499

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Evaluation of Biscuits Enriched with *Tempe* and Soy Flour on Pre-School Children in Rural Area

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Tempe is the name popularly used for soy bean fermented product. Tempe has acceptable organoleptic quality, nutritional benefits, low cost and simple processing techniques and valuable food for all age groups, especially children, women and undernourished. The results of bio-assay shows that the gain in weight and height of children fed tempe biscuits is better than those children fed defatted soy flour biscuits. The study was conducted on 70 children from anganwadi centers. They were fed with the above biscuits and nutritional status was evaluated by recording their height and weight for a period of three months. The gain in weight and height of children fed tempe biscuits was better that those fed soy flour biscuits and classification of children according to Indian academy of Pediatrics showed a significant difference during initial months and end of the study period. Tempe should be thus encouraged for daily use as the cheapest source of protein and B complex vitamin in the country for solving protein calorie malnutrition besides other micronutrient deficiencies. Hence measures to popularize tempe technology should be undertaken by training programmes in both rural and urban area besides encouraging industrial manufacture on large scale for easy availability and consumers use and benefit.

Keywords: Tempe, Rhizopus oryzae, Beany flavour, Anganvadi centre, Beany flavour.

Introduction

The use of soybean with 40 per cent good quality protein and 20 per cent fat is limited in India due to its beany flavour. Tempe is the fermented soybean cake prepared with mold *Rhizopus oryzae*, has mushrooms organoleptic quality, nutritional benefits and it is a low cost simple processing technique and can be prepared at household levels. The introduction of low cost, nutritionally superior foods to the diet of the nutritionally at risk population groups would reduce malnutrition problems existing in India. The work regarding effect of feeding tempe to children is lacking this study intends to meet this gap. Hence, the present study was planned to introduce tempe biscuits and feed children to test the effect on health and growth of children in comparison with Defatted soy flour biscuits.

Methodology

Tempe was prepared using Maize grits and soy flour (Fig.1). Biscuits were standardized and acceptability was conducted on 10, 15, 20, 25 and 30 per cent levels of tempe flour and soy. Addition of 25 per cent of tempe to biscuits and 15 per cent for Defatted soy flour biscuits were best accepted and used for feeding trails.

The cookies were analyzed in the laboratory for the protein by micro Kjeldahl method and energy was estimated by bomb calorimeter (AOAC.1980). The cost of the ingredients used for the biscuits was noted from local market and the production cost of 1 kg biscuits were calculated accordingly including other essential charges (Table-1).

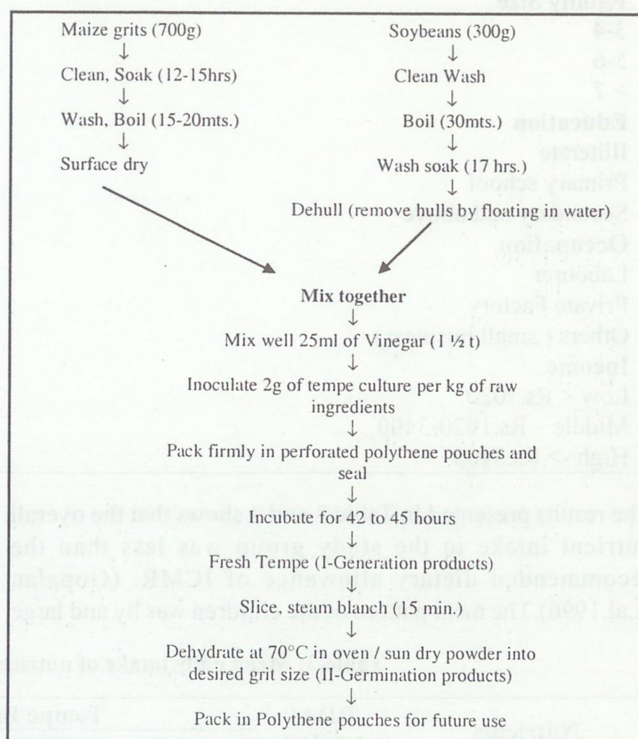

Fig. 1 Protocol for the Preparation Maize-Soy Tempe

Table-I: Nutritive value of Tempe and Soy blended biscuits (100g)

Products	Protein (g)	Energy (K.cal)	Cost/kg (Rs.)
Tempe biscuits	11	545	26.0
Soy flour biscuit	10	520	24.0

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A total of 70 children in the age group of 1 to 5 years were selected from five anganwadi centers of rural Bangalore. The children selected were from 1st, 2nd and 3rd degree malnutrition. A detailed schedule was prepared to assess the socio-economic status of the families. The children were grouped according to their age i.e. 1 to 3 years and 4 to 6 years and food intake was collected by 24 hours recall method (Thimayamma et.al.1987).

These children were divided into two groups. Thirty five children were fed tempe biscuits (Group-I) and other thirty five children were fed soy biscuits (Group-II). Each child was given 100g of biscuits (5 biscuits) which met 1/3rd of the requirement of RDA for a period of 3 months, 6 days in a week substituting the anganwadi diet. Anthropometric measurements were taken. Height and weight were recorded in the beginning (initial) and after each month for three months. The nutritional status of children was assessed according to Indian Academy of Pediatrics, weight for age classification. (Jelliffe1996). Statistical Analysis was done using t test to see the significance of the result.

Results and Discussion

From the Table 2 it is evident that there were 69 and 72 per cent of children from nuclear family and 31 and 28 per cent are joint family from both the groups. 54 and 56 per cent of the families are having 3 to 4 members per family. Mothers were in the age group between 18 to 25 years and majority of them were illiterates. 63 percent of them are labourers, 17 per cent work in private factory and 20 per cent in small business.

Table-2: Socio-economic profile of the study group

Variables	Tempe biscuits group		Soya biscuits group	
	n	%	n	%
Age of the Mother 18 to 25 years	35	100	35	100
Type of the family				
Joint	11	31.0	10	28.0
Nuclear	24	69.0	25	72.4
Family Size				
3-4	19	54.0	20	56.0
5-6	13	37.0	10	28.0
> 7	3	9.0	5	14.0
Education				
Illiterate	14	40.0	20	56.0
Primary school	15	43.0	10	28.0
Secondary and above	6	17.0	5	14.0
Occupation				
Labourer	22	63.0	25	72.4
Private Factory	6	17.0	5	14.0
Others (small business)	7	20.0	5	14.0
Income				
Low < Rs.1020	5	14.0	4	11.4
Middle – Rs.1020-3400	25	72.4	26	74.2
High -> Rs.3400	5	14.0	5	14.0

The results presented in Table 3 and 4 shows that the overall nutrient intake in the study group was less than the recommended dietary allowance of ICMR. (Gopalan et.al.1996).The meal pattern of the children was by and large on the lines of adult meal pattern. No special food was prepared for the children. The problem of deficient was not only in terms of calories and proteins, but also of minerals and vitamins among the children in both the groups.

Table-3: Mean daily intake of nutrients of the children (1 to 3 years) n = 35

Nutrients	RDA (ICMR)	Tempe Biscuits group Mean	±SD	Soy Biscuits group Mean	±SD
Protein (g)	23	17.1	1.2	16.5	1.54
Fat (g)	20	9.0	3.3	13.8	2.7
Energy (K.cal)	1240	895	163	924	159
Iron (mg)	12	8.4	1.1	8.1	1.7
Calcium (mg)	400	245.4	26.4	220.3	35.6
Vitamin-A (µg)	600	211.4	33.1	287.4	45.6
Thiamine (mg)	0.6	0.3	0.07	0.3	07
Riboflavin (mg)	0.7	0.4	0.07	0.3	0.06
Niacin (mg)	7.0	4.9	0.76	4.3	0.7

Table-4: Mean daily Intake of nutrients of the Children (4 To 6 Years) n = 35

Nutrients	RDA (ICMR)	Tempe Biscuits		Soy Biscuits	
		Mean	±SD	Mean	±SD
Protein (g)	30	21.6	2.2	20.7	2.3
Fat (g)	25	9.6	2.0	16.6	1.8
Energy (K.cal)	1690	1300	139	1316	170
Iron (mg)	18	9.0	1.3	13.4	1.0
Calcium (mg)	400	253.6	35.9	328.9	40.5
Vitamin-A (μg)	600	252.9	20.9	270.5	33.0
Thiamine (mg)	0.9	0.5	0.1	0.5	0.09
Riboflavin (mg)	1.0	0.6	0.09	0.6	1.1
Niacin (mg)	11	6.8	1.1	6.5	1.01

The effect of feeding children with tempe and soy flour biscuits on mean weight and height was compared. Table-5 depicts the cumulative monthly gain in weight and height of children. The mean weight gain of children at the end of 3rd month was 2.4 kgs for tempe biscuits where as it was 1.2 kg for soy biscuits. The mean increase in height at the end of 3rd month was 2.8 cms for children fed tempe biscuits as against 2.0 cms in children fed soy flour blended biscuits.

Table-5: Cumulative monthly mean Height and Weight gain of children fed Tempe and Soya flour biscuits n = 35 in each group

Product / period	Weight gain (kgs) Mean	±sd	Height gain (cms) Mean	±sd	't' value wt. (kg)	't' value ht. (cms)
Tempe Biscuits						
Initial	10.4 ±	1.4	76.2 ±	8.1		
1st month	10.8 ±	1.4	76.8 ±	8.2	5.01*	4.90*
2nd month	11.4 ±	1.4	77.9 ±	8.2	3.90*	3.60*
3rd month	12.0	1.4	79.0	8.3	4.12*	4.56*
Soy Flour Biscuits						
Initial	10.0	1.6	86.8	6.8	-	-
1st month	10.5	1.5	87.7	6.8	4.68*	3.52*
2nd month	10.9	1.6	88.0	6.7	1.26*	3.53*
3rd month	11.2	1.6	88.8	6.6	3.09*	3.95*

*Significant at 5%

Table 6 shows the classification of Children according to Indian Academy of Pediatrics (weight for age) into Normal, Grade-I, Grade-II and Grade-III. Initially there were 17 children in Grade-I, 17 in Grade-II and only 1 child in Grade-III for tempe biscuits and 14 in Grade-I, 16 in Grade-II and 5 in Grade-III for soy flour biscuits. At the end of feed test there were 16 normal, 15 in Grade-I, 4 in Grade-II and no child in Grade-III in the group fed tempe biscuits compared with 7 normal, 13 in Grade-I, 14 in Grade-II and in Grade-III in soy biscuits fed group. This shows that the tempe biscuits was more effective as 50 per cent of children came to normal category where as it was 20 per cent of children for soy biscuits.

Table-6: Classification of Children as per Indian Academy of Pediatrics (n = 35 in each group)

Period	Product	Normal	Grade I	Grade II	Grade III	X^2
Initial	Tempe Biscuit	-	17	17	1	16.99*
	Soya Biscuit	-	14	16	5	
First month	Tempe Biscuit	9	20	5	1	10.07*
	Soya Biscuit	-	16	17	2	
Second month	Tempe Biscuit	12	19	4	0	20.53*
	Soya Biscuit	2	14	18	1	
Third month	Tempe Biscuit	16	15	4	0	20.53*
	Soya Biscuit	7	13	14	1	
Cumulative Tempe Biscuit	Initial	-	17	17	1	119.17*
	Final	16	15	4	-	
Cumulative Soya Biscuits	Initial	-	14	16	5	27.34*
	Final	7(20)	13	14	1	

*Significant at 5% level

There was significant difference found between tempe and soy biscuits fed group at the end of 1st, 2nd and 3rd month for both the biscuits. The overall comparison shows that tempe is more effective for the recovery of malnutrition.

The data regarding the nutritional deficiency signs prevalent in children initially and after three months is presented in Table-7. Higher percentage of children from both the groups had prevalence of angular stomatitis, discoloured hair, bleeding of gums and bitot spots in the beginning of the experiment. At the end of the study period many children recovered from the deficiency symptoms. The overall comparison between the children fed tempe and soy flour biscuits showed that the recovery is better among the children fed tempe biscuits.

Table-7: Nutritional deficiency symptoms among children

Signs	Tempe Biscuits		Soya Biscuits	
	Initial (%)	Final (%)	Initial (%)	Final (%)
Hair spare	14.2	2.8	8.5	5.7
Discoloured hair	14.2	5.7	8.5	5.7
Bitot spots	5.7	2.8	8.5	5.7
Angular stomatitis	17.1	2.8	22.8	17.1
Gums bleeding	8.5	5.7	14.2	8.5
Kwashiorkar	8.5	2.8	22.8	11.4
Marasmus	5.7	2.8	17.1	8.5
Anaemia	28.5	14.2	37.1	28.5

Conclusion

Tempe with high rating in taste, protein quality, nutritional benefit and low cost processing technique is the most likely food product for popularization in India. Tempe prepared with balanced cereal, pulse and vegetable contribute for better nutritional status. Tempe should thus be encouraged for daily use as the cheapest source of protein and B-complex vitamins in the country. for combating malnutrition.

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References

AOAC (1984). Official methods of Analysis, 14th Edn, Association of official Analytical chemists, Washsington, DC. 20044.

Gopalan C., Sastri R.V.B. and Balasubramanian S.C. (1996). Nutritive value of Indian foods, NIN, ICMR, Off set press, ICMR, New Delhi.

Jelliffee D.B. (1996). Nutritional Anthropometry. The assessment of nutritional status of the community, WHO Press, Geneva, pp 50-80.

Thimayamma B.V.S. (1987). A Hand book of Schedules and Guidelines in Socio Economic and Diet Surveys, NIN, ICMR, Jamia- Osmania, Hyderabad, pp 40-42.

Snedecor G.W. and Cochran W.G. (1967). Statistical methods, 6th Edn, Oxford and IBH Publishing Co, Calcutta.

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Storage of Fresh White Button Mushroom in Cool Chamber

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White button mushroom (Agaricus bisporus) are highly perishable and needs immediate marketing or processing. Cool chamber is based on the principle of evaporating cooling system. The maximum temperature inside the cool chamber was 18⁰ C in the month of Jestha (May-June) and minimum temperature is 8⁰ C in the month of Poush (Dec-Jan) and Maximum ambient temperature of room is 25⁰ C and minimum is 12⁰C in Jestha (May-June) and Magh (Feb-March) respectively. Sound and clean mushrooms were kept in cool chamber for storage and found that there were no physiological losses in weight (PLW) during storage. Mushrooms in cool chamber were stored for 3 days in the month of Marga (Nov-Dec), Poush (Dec-Jan) and Magh (Jan-Feb), 2 days in the month of Kartik (Oct-Nov), Falgun (Feb-March) and Chitra (March-April) and one day in the month of Baisakh (April-May) and Jestha (May-June). Stored mushrooms were white in colour, no off flavor, no gills open and marketable. Physiological loss in weight of mushrooms in ambient conditions were 3.5 to 7%, highest in Jestha (May-June) and lowest in Poush (Dec-Jan) and mushrooms were dull in colour, off smell, slightly darker in stem and other parts of mushroom showed unmarketable within one or two days

Keywords: Cool Chamber, Button Mushroom, Storage, Temperature, Humidity

Introduction

Storage of fresh mushrooms is one of the major problems in the farmer level. The main purpose of storage of mushroom is to extend their shelf life and it also prevents the seasonal market gluts. Now a days, farmers are producing more mushrooms around the Kathmandu valley and main seasons of production of mushroom starts from the last week of Ashwin, Kartik, Marga and another season starts from last week of Magh, Falgun, Chaitra and sometimes goes up to first week of Jestha in farmers level (Mushroom Research Programme 2000). Mushrooms, like fruits and vegetables, are highly perishable and needs immediate processing or marketing. Freshly harvested mushroom can be kept for 24 hours in normal room temperature and kept for a week at refrigeration temperature (Bhal, N. 1984). It is reported that the mushroom covered with a PVC film loss of water and whiteness at a much slower rate than uncovered mushroom and such mushroom have a shelf life of 5-7 day at 15 -21⁰C during transportation (Garmley and Mac Canna - 1997). Fresh mushrooms can be preserved by controlled atmosphere storage, freeze-drying, steeping preservation methods (Bhal, N. 1984), but these methods are very expensive for the farmers. A cool chamber is a zero energy technology based on the principle of direct evaporative cooling system. Evaporation of water produces considerable cooling effect and the faster the evaporation the greater the cooling. Evaporative cooling occurs when air that is not already saturated with water vapor is blown across any wet surface. Thus an evaporative cooler consists of wet porous bed through which air is drawn, cooled and humidified by evaporation of water. The greatest importance of this low cost cooling technology lies in the fact that it does not require any electricity and the materials required to construct the cool chamber are available easily and cheaply. An unskilled person can install it. It has shown good performance to different fruits and vegetables for elongating the shelf life (Zero Energy cool chamber, IARI-1985). This study was focused on the performance of cool chamber for elongating the shelf life of fresh mushroom after harvesting.

Materials and methods

Construction of Cool Chamber

This study was carried out in Khumaltar Research complex by constructing a cool chamber. It is a box type design, which has an opening from the top (Fig 1). The size of cool chamber was 46"×36"×26"(L×B×H). The storage space was made of single layer of brick and sidewalls were made of double layer of bricks approximately 3 to 4" space between the brick. The gap was filled with sand. The top cover was made with gunny cloth in bamboo structure and given a thatch of straw for shading purpose. Before running the experiment, the brick wall, floor, sand used in cavity and top cover were completely wet by sprinkling with water till they were saturated. Once the cool chamber was completely wet, daily sprinkling of water was carried out (2-3 times daily) to maintain the temperature and RH. The thermometer and hygrometer were kept inside the chamber to record the temperature and RH. A thermometer was kept in the room to record the temperature of the room where the samples for control were kept. The temperature inside the chamber and room temperature were recorded three times a day (Morning at 10 AM, at 1 PM and 4 PM). The RH inside the cool chamber was maintained 85-90 % during the experiment.

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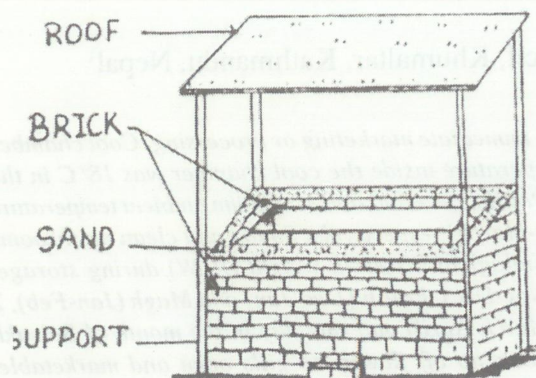

Fig. 1: Cool Chamber

Preparation of fresh mushroom (*Agaricus bispores*) for storage

Freshly harvested mushrooms were collected from the farmers of Imadole, Lalitpur and Bore Gaon, Kathmandu. Their mushrooms were trimmed to remove the roots of mushroom, removing the open gills mushroom i.e. mushroom were selected with gills covered by a tissue (veil) during experiment. Thus selected mushroom were washed with clean water, dipped 3 to 5 minutes in water containing Potassium Metabisulphite (2 g/lits), drained the water completely, packed 200 g to 500 g in a polythene bags, tight loosely with rubber band and kept in a cool chamber for storage and in room temperature for control. Only *Agaricus bisporus* (Gobre) button mushrooms were used during experiment.

Results and Discussion

The monthly average temperature inside the cool chamber and ambient temperature of room were given in table 1.

The maximum temperature inside the cool chamber was 18^{0}C and minimum was 8^{0}C in the month of Jestha and Magh respectively and the maximum ambient temperature of room was 25^{0}C and minimum was 12^{0}C in the month of Jestha and Magh respectively. The shelf life and Physiological loss in

Table 1. The monthly average temperature inside the cool chamber and ambient temperature in the room (1995, 1996, 1997)

Months	Temperature inside Cool Chamber ^{0}C	Ambient temperature of room ^{0}C
Kartik (Oct-Nov)	14	21.00
Marge (Nov-Dec)	12	18.00
Poush (Dec-Jan)	8	16.00
Magh (Jan-Feb)	8	12.00
Falgun (Feb-March)	12	20.00
Chaitra (MarchApril)	13	19.00
Baisakha (April-May)	16	23.00
Jestha (May-June)	18	25.00

weight (PLW) of mushrooms keeping in cool chamber are given in table 2. There was no PLW of mushroom in cool chamber during the storage. The self life of fresh mushroom in cool chamber was 3 days in the month of Marga, Poush and Magh , 2 days in the month of Kartik, Falgun and chaitra and 1 day in the month of Baisakha and Jestha where the temperature in cool chamber was also high as compared to other months. These mushrooms stored in cool chamber were white in colour, no off flavor, no gills open and marketable. The shelf life and PLW in ambient condition are given in table 3. The Physiological losses in weight in ambient condition were ranged 3.5 to 7%, highest in Jestha and lowest in Poush. Shelf life of fresh mushroom in ambient condition are less than 2 days in the month of Marga, Poush, Magh, 1 day in the month of Kartik, Falgun and Chaitra and less than 1 day in Baisakh and Jestha. But the mushrooms in ambient condition were dull in colour, little off smell, gills open and slightly darker in the stem and other parts of the mushroom. So the mushroom stored in ambient condition are not good for marketing. From the above results it is clear that the white button mushroom can be kept for 2 to 3 days in the cool chamber after harvesting and these stored mushrooms are marketable. So this cool chamber acts as a poor man refrigerator as it is cheap and used locally available materials for construction.

Table 2. Self-life and physiological loss in weight (PLW) of mushroom in cool chamber

Storage Month	Wt. of fresh Mushroom (g)	Loss in Weight (g)	% of PLW	Shelf Life, days	Remark
Kartik (Oct-Nov)	500	No loss	No loss	2	White,
Marg (Nov-Dec)	500	No loss	No loss	3	no smell,
Poush (Dec-Jan)	400	No loss	No loss	3	no gills open and
Magh (Jan-Feb)	350	No loss	No loss	3	marketable.
Falgun (Feb-March)	300	No loss	No loss	2	
Chaitra (March-April)	300	No loss	No loss	2	
Baisakha (April-May)	450	No loss	No loss	1	
Jestha (May-June)	350	No loss	No loss	1	

Table 3: Shelf life and physiological loss in weight (PLW) of mushroom in Ambient Condition.

Storage Month	Wt. of fresh Mushroom (g)	Loss in Weight (g)	% Of PLW	Shelf Life, days	Remark
Kartik (Oct-Nov)	500	25	5.0	1	Dull in colour, little off smell, slight dark in the stem & other ports of mushrooms, gills open and unmarketable.
Marge (Nov-Dec)	500	20	4.0	<2	
Poush (Dec-Jan)	400	15	3.5	<2	
Magh (Jan-Feb)	350	15	4.3	<2	
Falgun (FebMarch)	300	20	4.0	1	
Chaitra (March-April)	300	15	5.0	1	
Baisakha (April-May)	450	25	5.5	<1	
Jestha (May-June)	350	25	7.0	<1	

Conclusion

Cool Chamber is a Zero energy technology and has shown good performance to extend the shelf life of White button mushroom (Agaricus bisporus) for 2-3 days after harvest.

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References

Bahl, Nita, 1984, Handbook of mushrooms

Garmley, T R and C Mac Canna 1967, Irish journal of Agriculture Research, 6 (2): 255

S K Shrestha, D Dhakal, B Khadka, 2000, Simple method of cultivation of button mushroom (Agaricus bisporus), Plant Pathology Division, Mushroom Research Programme, Khumaltar, Lalitpur, Nepal

Zero Energy Cool Chamber, Research Bulletin, No.43, Indian Agricultural Research Institute, New Delhi, 1985

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Delta- endotoxin Immuno Cross-reactivity of Bacillus thuringiensis Isolates Collected from Khumbu Base camp of Mount Everest Region

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Bacillus thuringiensis strains were isolated from soil samples collected from Khumbu Base Camp of the Everest region and characterized by standard methods. Crystal protein (δ-endotoxin) was extracted from the crystal protein producing strains (46 from Phereche and 40 from Sagarmatha National Park) from stationary phase culture broth and tested for insect bioassay. Crystal proteins were further purified by Native-PAGE. Among ten randomly selected isolates, one isolate showed the highest insecticidal activity against Dipteron insects. Its crystal protein had molecular weight about 120-130KD (revealed by SDS-PAGE) and was used to produce polyclonal antibody in New Zealand's white rabbits. The presence of polyclonal antibody was confirmed by Ouchterlony double diffusion method. Indirect ELISA was optimized coating 6-8 ¼g crystal protein per well in microtitre plate. The optimal dilution of the polyclonal antibody was 1000 folds corresponding to OD_{450} = 0.045 for color observation. Of the total 86 crystal protein producing isolates, crystal proteins from 31 isolates (36.05%) were 25-30% cross-reactive, two groups of 6 isolates (6.97%) were 75-80% and 85-90% cross-reactive respectively, and 4 isolates (4.65%) were 80-85% cross-reactive with the polyclonal antisera. Only 3 isolates (3.49%) were more than 90% cross-reactive. The Discriminatory Index (D) of the Indirect ELISA was 0.92.

Keywords: Khumbu region, δ-endotoxin, Crystal protein, Insect-bioassay, Immunodiffusion, immunocross-reactivity.

Introduction

Microbial insecticides are especially valuable as their toxicity to non target animals and humans is extremely low compared to other commonly used chemical insecticides. They are safer for both the pesticide user and consumers of pesticide treated crops (Neppl, 2000). The soil bacterium *Bacillus thuringiensis* fulfills the requisites of a microbiological control agent against agricultural pest and vectors that cause massive crop destruction (Ben-Dov et al, 1999). The main target pest of *B. thuringiensis* insecticides include various Lepidoptera (butterfly), Diptera (flies and mosquitoes), and individual Coleopteran (Beatle) species and some strains kill off nematodes (Schnepf et al , 1998) where as *B. thuringiensis var. kurstaki* HD1 is highly potent strain due to its wide spread insecticidal properties (Dulmage, 1970).

Insect bioassay and rocket immunoelectrophoresis are currently used to detect and measure the levels of crystal proteins. Though the rocket immunoelectrophoresis is more sensitive than insect bioassay, it requires a considerable amount of antigen, at least 10 mg/ml (Wie et al, 1982). Immunodiffusion and ELISA are more practical and reliable methods. ELISA measures changes in enzyme activities proportional to the antigen or antibody concentrations. It is a highly versatile and sensitive analytical procedure for qualitative and quantitative determination of antibodies and almost any kind of antigens. The method discriminates different epitopes very efficiently provided that antibodies of high specificity and affinity are available. The detection limits of the assay may be well below 1 ng/ml (Perlmann and Perlmann, 2001). Hence ELISA can be effectively used to study cross reactivity of a given type of antigen. Identical antigens possess 100% crossreactivity with the given antisera and non identical ones don't show any degree of crossreactivity. Thus the diversity of the given antigen and hence organisms in a given complex population can be studied by their cross reactivities. In order to study crystal protein diversity of *B. thuringiensis* strains, Indirect ELISA procedure was optimized in this study.

Materials and methods

Soil sampling, isolation and biochemical characterization: Soil samples were collected from Sagarmatha National Park (SNP) and Phereche of Khumbu Base Camp of Everest region and were transported to **RLABB,** where the study was carried out from March 2005 to December 2005 in joint collaboration with Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal. *Bacillus thuringiensis* were isolated by acetate selection method (Travers et al, 1987). The isolated organisms were identified by standard microbiological techniques including colonial and morphological characteristics, and biochemical tests (Bergey's Manual, 1986).

Collection of Mosquito larvae: Mosquito (Dipteron) Larvae were collected from the ditches in local area of Bode, Bhaktapur, Nepal for insect bioassay. The larvae were identified as *Culex* spp. by zoologists at the Central

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Department of Zoology, Tribhuvan University, Kirtipur and bioassay was performed as described by Pang (1994).

Extraction and purification of crystal proteins: B. thuringiensis strains were incubated in Brain Heart Infusion broth (Calf brain infusion 200 g/l, beef heart infusion 250 g/l, protease peptone 10 g/l, dextrose 2 g/l, sodium chloride 5 g/l, disodium phosphate 2.5 g/l, final pH 7.4 ±0.2 at 25°C) and sterilized by autoclaving (15 lbs pressure 121°C, 15 min) at 30°C for 3-7 days till autolysis. Spores and crystals were separated by centrifugation (10,000 rpm, 20 min, 4 °C), and then washed four times with phosphate buffer (pH 7, 0.05M). The pellet was finally suspended overnight in carbonate buffer of pH 10.5 (0.05 M sodium carbonate, 0.01M β-mercaptoethanol, 1mM EDTA, 1mM PMSF) with constant shaking at 23-26°C, and centrifuged (10000 rpm, 20 min, 4 °C). Protein content in the supernatant was determined by Bradford assay (1976). In order to determine which proteins are responsible for the biological activities, they were electrophoresed under non denaturing conditions by Native PAGE (Blackshear, 1984) and the major bands were sliced , grinded in a minimum volume of phosphate-buffered saline (10 mM sodium phosphate, 150 mM NaCl, pH 7.2) , centrifuged (10000 rpm, 20 min, 4 °C) and concentrated using 20% TCA.

Insect bioassay and Molecular weight determination: For insect bioassay 10 larvae were taken in a jar containing 100 ml of sterilized water containing 0.3 ml of 5% Brewer's Yeast. Five ml of *B. thuringiensis* stationary phase culture was added and allowed to stand for 3 days. The number of deaths was recorded for one, two and three days. For the purified crystal protein bioassay, proteins (30μg/ml per assay) from each band were tested for insecticidal property as done by Pang (1994). Proteins from insect bioassay positive band was electrophoresed to determine molecular weight using molecular weight markers (lysozyme 14 KD, casein 22 KD, BSA 66KD) according to the method of Laemmli (1970).

Polyclonal antiserum production: Proteins from insect bioassay positive band was resuspended in saline to a concentration of 1.0 mg/ml. The suspension was emulsified in an equal volume of Freund's complete adjuvant (Difco, USA). A pair of New Zealand's white rabbit was injected with 500 μg of the emulsified proteins (1.25 ml) by the intramuscular route in hind limbs. The booster injections with incomplete adjuvant were given three times in 14 days interval. The animals were bled 7 days after third booster dose. Polyclonal antiserum was pooled and decomplemented by incubation at 56°C for 30 min. Aliquots of antiserum (0.1 to 0.5 ml) were stored at -20°C untill assayed

Immunodiffusion and ELISA: The presence of polyclonal antibody was confirmed in a 1% agarose gel (0.05 M phosphate buffer, pH 7.2) by the Ouchterlony method (Talwar and Gupta, 1997) where 50 μl undiluted and diluted (1:10,1:100, 1:1000,1:10000 dilutions) antisera was poured in a centre well surrounded by 5 wells in petri-plate. 50 μl crystal protein antigen preparations (1 mg/ml) from SNP and Phereche isolates were applied in the surrounding wells against antiserum. The petri-plate was incubated at 4°C for 72 hours in a moist chamber. Immuno cross-reactivity of *B. thuriengensis* crystal proteins was studied by indirect ELISA, where optimal dilutions of polyclonal antiserum and its second antibody (anti rabbit IgG conjugated with Horse Radish Peroxidase, Sigma, USA) was determined by chequerboard titration method (Trottier et al, 1972; Voller et al, 1976). To coat 96-well polystyrene microtitre plate, 100 μl crystal protein antigen (6μg) prepared in PBS (137 mM NaCl, 1.76 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.7 mM KCl; pH 7.2) was applied in each well, and incubated overnight at 4°C (Trottier et al, 1972). Uncoated protein was washed 3 times using washing buffer (0.05%Tween 20 in PBS). For blocking, 200μl of 2% BSA in PBS was applied in each well and allowed to stand for 1 hour at room temperature. Blocking was done twice. After 3 times washing, each well was incubated with 100 μl polyclonal antibody of different dilutions for 2 hours at room temperature, and unbound polyclonal antibody was washed 4 times. The microtitre plate was then incubated with 100 μl of second antibody of different dilutions per well at room temperature for 1 hour and washed 4 times. Finally it was incubated with 100 μl tetramethyl benzidine substrate solution (0.01% tetramethyl benzidine in citrate phosphate buffer of pH 4.9, 2μl of 30% H_2O_2), reaction was allowed proceed for half an hour, stopped adding 1N HCl and the intensity of color was read at 450 nm within 10 min in ELISA reader (Dynatech MR-250).

Calculation of discriminatory index value (D): D value was calculated according to the formula of Hunter and Gaston (1988).

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} x_j(x_j - 1)$$

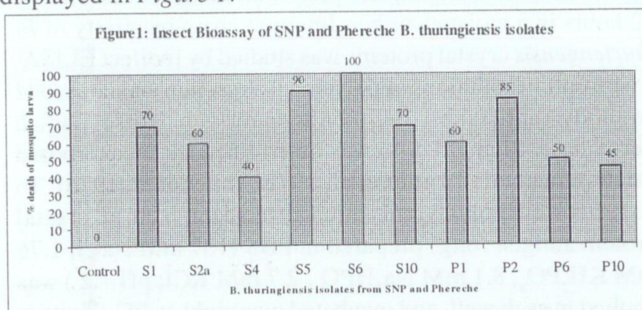
Where, N=Numbers of isolates;
S=Number of different polymorphic types

Results and discussion

Strain identification: Total 109 *B. thuringiensis* isolates were obtained from soil samples of Phereche and SNP. Sixty three and 46 isolates were obtained from 52 Phereche and 39 SNP soil samples respectively. All the isolates were Gram positive rods. Of the 109 total isolates, only 86 isolates (79.63%) were positive on crystal protein staining, which were further proceeded for immunological characterization (Bergey's Manual, 1986).

Molecular weight determination: Five bands were observed for S_6 preparation having molecular weight of 40, 58, 71, 83 and 107 KD in SDS-PAGE and a single band (Mol. Wt. - 120-130KD) was observed in Native PAGE. This indicates that S_6 crystal protein is composed of 5 different protein subunits. Similar result was observed by Drobniewski and Ellar (1989) and Pfannenstiel et al (1986) in their work on *B. thuringiensis*.

Insect bioassay: The result of insect bioassay of crystal protein from SNP and Phereche *B. thuriengensis* isolates is displayed in *Figure 1.*



For insect bioassay, cultures were grown to stationary phase which is suitable for the sporulation and production of crystal protein (Hunter and Gaston, 1988), and were used for the bioassay on mosquito larvae. Insecticidal activity of S_6 endotoxin (30µg/ml) partially purified from autolysed *B. thuringiensis* broth by alkaline solution method following purification in Native PAGE is found to be 100 % (10/10) efficient, and hence it was used for polyclonal antibody production. Purification by Native PAGE has also been reported by Pang (1994). These crystal proteins are solubilized in the alkaline environment of Lepidoptera larval midgut, and then processing by midgut proteases results in a relatively stable, mature toxin (Van Rie et al., 1990). Activated *cry* toxins have two known functions, receptor binding and ion channel activity. The activated toxin binds readily to specific receptors on the apical brush border of the midgut microvilli of susceptible insects. Binding is a two-stage process involving reversible and irreversible steps. The latter steps may involve a tight binding between the toxin and receptor, insertion of the toxin into the apical membrane, or both. It has been generally assumed that irreversible binding is exclusively associated with membrane insertion.P Soon after insertion, toxins made pores on membrane and enter to body system. As soon as they enter to body they stop feeding and alternately death of insects occurs due to blood poisoning (Schnepf et al, 1998).

Immunodiffusion and ELISA

Double immunodiffusion performed in agarose gel gives clearly visible precipitin bands against crystal proteins of *B. thuringiensis* isolates from both SNP and Phereche upto 1:100 dilution of polyclonal antisera tested.(*Fig. 2*). The method gives reproducible results to detect crystal protein antigen with 100% specificity. For performing ELISA (*Fig. 3*), first and second antibody of dilutions 1:1000 and 1:2000 were optimal respectively.

Well 1: Antiserum (50µl, 1:100 dilution)
Well 2: Negative control
Well 3: S_6 antigen (50 µl, 1 mg/ml)
Well 4: S_{2a} antigen (50 µl, 1 mg/ml)
Well 5: P_2 antigen (50 µl, 1 mg/ml)
Well 6: P_{10} antigen (50 µl, 1 mg/ml)

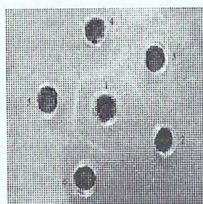


Fig2: Ouchterlony immunodiffusion

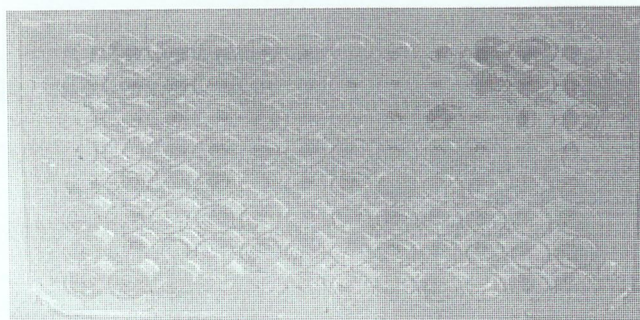


Fig 3: Immuno cross-reactivity study by ELISA

Below OD_{450} = 0.045, there was not clearly visible change in color to naked eyes. Trottier *et al* (1992) chose the optimal dilution of first and second antibodies difference between ELISA value with and without antigens with all other conditions remaining same. Between each ELISA step, plates were washed five times with a microtitre plate washer in order to prevent the false positive result (Trottier *et al*, 1992). The discriminatory index value, D (*Table 1*), was 0.92 (N=86, S=22). Hence the Indirect ELISA method divided all isolates into 13 groups with 92% confidence (Table-1). In which only 3 isolates (3.49%) were above 90% cross-reactive. The result

Table 1: Calculation of discriminatory index value (D)

Group of population	% Cross reactivity [a]	Isolates	N.
n_1	13.64	P_{12b}	1
n_2	15.91	P_{40},P_{41}	2
n_3	18.18	S_{39}	1
n_4	20.45	S_{16},S_{34},S_{38b},P_6,P_{12a},P_{15},P_{39},P_{46},P_{47}	9
n_5	22.27	S_{21},P_7,P_{21},P_{22},P_{43}	5
n_6	25.00	S_{35a},P_1,P_{28},P_{42},P_{50}	5
n_7	27.27	S_{17},S_{38a},P_3,P_5,P_{13},P_{18},P_{27},P_{52}	8
n_8	29.55	S_5,S_{13},S_{25},S_{26},S_{27a},S_{28a},S_{28b},P_9, P_{14},P_{17a},P_{26},P_{33},P_{34},P_{35},P_{37},P_{38},P_{44}	17
n_9	31.82	S_{14},S_{22},S_{24},S_{27b},P_{32},P_{36},P_{45}	7
n_{10}	34.09	S_8,P_{49},P_{51b}	3
n_{11}	36.36	S_4,S_9,S_{15b},S_{23},P_{24},P_{48a}	6
n_{12}	38.64	S_{30}	1
n_{13}	70.45	S_{15a}	1
n_{14}	77.27	S_1,S_{2a},S_3,S_{20},S_{35b},S_{41}	6
n_{15}	81.82	S_{32},P_{29}	2
n_{16}	84.09	S_{10},S_{33}	2
n_{17}	86.36	S_{31},P_{53}	2
n_{18}	88.64	S_{36},S_{37},P_{10},P_{30}	4
n_{19}	90.91	P_{31}	1
n_{20}	93.18	P_2	1
n_{21}	95.45	S_7	1
n_{22}	100.00	S_6	1
	Total Number (N)		**86**

N. Indicates the number of Isolates giving similar % of immuno cross- reactivity
S series: Isolates from SNP, P series: Isolates from Phereche

clearly shows that *B. thuringiensis* isolates from Khumbu Base Camp are highly diverse for their crystal protein antigenicity. The S_6 isolate showing potent insecticidal property tested against dipteron insects need to be studied further in larger trials so that it can have applicability to reduce the massive crop yield loss in this region.

Conclusion

Crystal protein from one strain of *B. thuringiensis* found in soil of Khumbu Base Camp of Mount Everest region showed highly effective insecticidal activity against dipteron insects. Further works on biological tests of this protein are encouraged.

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References

Neppl CC (2000). Managing Resistance to *Bacillus thuringiensis* Toxins. Environmental Studies University of Chicago.

Ben-Dov E, Wang Q, Zaritsky A, Manasherob R, Barak Z, Schneider B, Khamraev A, Baizhanov M, Glupov V, Margalith Y. Multiplex PCR screening to detect cry9 genes in *Bacillus thuringiensis* strains. *Appl Environ Microbiol* 1999; 65: 3714-6.

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR and Dean DH. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol Mol Biol Rev* 1998; 62: 775-806.

Dulmage HT Production of spore-delta-endotoxin complex by variants of *Bacillus thuringiensis* in two fermentation media. *J Invertebr Pathol* 1970; 16: 385-9.

Wie SI, Andrews R JR, Hammock B, Faust R.M and Bulla LA () Enzyme-Linked Immunosorbent Assays for Detection and Quantitation of the Entomocidal Parasporal Crystalline Protein of *Bacillus thuringiensis* subspp. *kurstaki and israelensist Appl Environ Microbiol*: 1982, 891-4.

Perlmann P and Perlmann H (2001) Enzyme-Linked Immunosorbent Assay ENCYCLOPEDIA OF LIFE SCIENCES 2001 *Nature Publishing Group*.

Travers RS, Martin PA and Reichelderfer CF Selective Process for Efficient Isolation of Soil *Bacillus* spp. *Appl Environ Microbiol* 1987; 53: 1263-6.

Bergey's Manual of Systematic Bacteriology, Volume 2, 1986).

Pang AS. Production of antibodies against *Bacillus thuringiensis* delta-endotoxin by injecting its plasmids. *Biochem Biophys Res Commun* 1994; 202: 1227-34.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem 1976;* 72: 248-54.

Blackshear PJ (1984). Systems for polyacrylamide gel electrophoresis. In Methods *in enzymology* (Jakoby WB eds.) vol 104: 237-255.

Laemmli, UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (London) 1970; 227:680-5.

Talwar GP and Gupta SK (1997). A hand book of practical and clinical immunology, second edition, volume 1, *CBS publishers and distributors*, Daryaganj, New Delhi.

Trottier YL, Wright PF and Lariviere S Optimization and standardization of an Enzyme-Linked Immunosorbent Assay protocol for serodiagnosis of *Actinobacillus pleuropneumoniae* serotype 5. *J Clin Microboil* 1992;30(1):46-53.

Voller A., Bidwell D and Bartlett A. (1976) Microplate enzyme immunoassays for the immunodiagnosis of virus infections Manual of clinical immunology. *American Society for Microbiology* p. 506-512.

Hunter PR and Gaston MA. Numerical Index of the Discriminatory Ability of Typing Systems: an Application of Simpson's Index of Diversity. *Journal of Clinical microbiology* 1988; 26: 2465-6.

Drobniewski FA and Ellar DJ. Purification and properties of a 28-kilodalton hemolytic and mosquitocidal protein toxin of *Bacillus thuringiensis* subsp. *darmstadiensis* 73-E10-2. *J Bacteriol* 1989;171: 3060-7.

Pfannenstiel MA, Couche GA, Ross EJ and Nickerson KW. Immunological relationships among proteins making up the *Bacillus thuringiensis* subsp. *israelensis* crystalline toxin. *Appl Environ Microbiol* 1986;52: 644-9.

Van Rie, J., S. Jansens, H. Ho¨fte, D. Degheele, and H. Van Mellaert. Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins. Appl. Environ. Microbiol 1990;56:1378-85.

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Prevalence of Vitamin A Deficiency and Iodine Deficiency Disorder in the Mid-Western Development Region of Nepal

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Nutritional Assessment including Vitamin A deficiency (VAD) and Iodine Deficiency Disorders (IDD) in preschool children and lactating and pregnant mother in the Midwestern Development Region of Nepal was carried out. The observed status showed that 4.8% VAD for 6-8 years of age children, 2.3% VAD for 8-10 years children, 4.1 % VAD for 10-12 Years children, 1.7 % VAD for 12-14 years children, 4.4% VAD for 14-16 years of children, 9.0% VAD for lactating mother and 7.6% VAD for pregnant mother are prevalent. Similarly the prevalence of IDD observed was 28.1, 32.2, 30.9, 28.9, 30.4, 41.3, and 37.8% respectively. These data showed that there is the severe malnutrition problem especially of VAD and IDD in the Mid-Western Development Region of the country.

Keywords: VAD, IDD, Preschool children, Lactation and Pregnant mother, Mid-western Development Region, Nepal

Introduction

Protein Energy Malnutrition (PEM) and Micronutrient Deficiencies mainly Vitamin A Deficiency (VAD), Iodine Deficiency Disorder (IDD) and Iron Deficiency Anemia (IDA) are the serious malnutrition problems in Nepal and the most vulnerable groups of these diseases are children and pregnant and nursing mothers (NMSS, 1998). The diversified agro climatic, geographic, ethnic and socio-economic condition of the Nation and educational status of the people are leading the countries overall nutritional situation. The number of epidemiological studies has been conducted to estimate the severity and distribution of these different nutritional problems over the past 25 years in Nepal. However integrated survey is still to be reviewed the overall nutrition and micronutrient status. This study aimed to assess the nutritional status of the preschool children and lactating and pregnant mothers in the Mid-Western Development Region of the Country since such information is lacking in the literature.

Materials and Methods

The study was conducted among the women of reproductive age, preschool age children and school age children residing in Banke district in the Midwestern Development Region of Nepal. The study population constituted of both male and Ès female. The households were selected by means of systematic sampling. In order to achieve the desired sample size, Banke district was divided into three clusters. From each cluster, Two VCDs were selected. Among the selected VCDs, only odd numbered Ward were taken to cover the sample size of 1200 households. Amongst these, one house was selected randomly. This house was the first household to be surveyed. Salt samples were collected in the ratio of 1:3 respondents from his/her house hold respectively. Structured questionnaire, clinical examination of goiter and VAD, estimation of iodine in salt constituted the data collection tools. A number of questions pertaining to ongoing malnutrition problems were included in the questionnaire and was administered for every respondent. Goiter was graded according to the recommendation of WHO/UNICEF/ICCIDD-1992 (Jelliffe-1989, Gibson-1990, WHO 1983). Estimation of Iodine in salt was done in the Regional Food Technology and Quality Control office, Nepalgunj.

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Results and Discussion

Basic Nutritional background Assessment of the population was conducted. The study showed that 36 % of people of the Mid-western Development Region of Nepal are illiterate, 44% household have very low (i.e. < 3000 NRs) monthly family income, 36% household have no access to home garden, 74% of population donot know the importance of micronutrient during pregnant and lactation. 82% household were familiar to the importance of Vitamin A, 91% household are receiving Vitamin A capsule in Kartic/Baisakh (December/May) month.

Similarly 66 % household were familiar to the importance of iodized salt, 82% household consuming iodized salt, 91% of household answered on the benefit of iodized salt to prevent goiter. 46% household consuming FODA (DHIKE or Block) salt. 73% of household salts contained less than 15 ppm of iodine. All FODA salt samples contained less than 15 ppm of iodine. 87% household did not use yellow fruit and green vegetable regularly in the diet to mother and child in past one week. 82% household had access to food grain. 89% household used traditional method for storage for food grain. 78% household had toilet facility in house.

Malnutrition (Weight according to Age) was 51%. Percentage of chronic malnutrition (Height according to Age) was 42% and % of severe malnutrition (Weight according to Height) was 8%. Malnutrition (Overnutrtition) according to arm circumstances was 8.5% whereas undernutrition was 64%. Prevalence of VAD observed in preschool children and Pregnant and Lactating mother is presented in Table 1and 2 respectively.

Table 1: Prevalence of VAD in preschool children in Mid-Western Development Region of Nepal

Prevalence of VAD	6-8 Yrs		8-10 Yrs		10-12 Yrs		12-14 Yrs		14-16 Yrs	
	M	F	M	F	M	F	M	F	M	F
Night Blindness	1 (0.5)	0 (0)	2 (1.1)	1 (1.0)	2 (1.5)	3 (3.4)	1 (0.6)	0 (0)	3 (2.5)	2 (4.9)
Short sightness	5 (2.4)	3 (1.7)	4 (2.1)	0 (0)	0 (0)	1 (1.1)	0 (0)	2 (2.7)	1 (0.8)	0 (0)
Long sightness	8 (3.7)	2 (1.1)	0 (0)	0 (0)	1 (0.7)	2 (2.3)	0 (0)	1 (1.4)	0 (0)	1 (2.4)
No defect	201 (93.4)	175 (97.2)	191 (97.0)	104 (99.0)	132 (97.8)	82 (93.2)	164 (99.4)	71 (95.9)	116 (96.7)	38 (92.7)
Total	215	180	197	105	135	88	165	74	120	41

Note: Values in the parenthesis indicates the percentage value[M: Male, F: Female]

Table 2: Prevalence of VAD in Lactating and Pregnant Mother in the Midwestern Development Region of Nepal

Prevalence of VAD	Lactating Mother	Pregnant mother
Night Blindness	12 (5.1)	8 (4.3)
Short sightness	7 (3.0)	2 (1.1)
Long sightness	2 (0.9)	4 (2.1)
No defect	214 (91.0)	171 (92.5)
Total	235	185

Note: Values in the parenthesis indicates the percentage value.

Table 1 shows that 4.8 % of 6-8 yrs of children are of VAD. Similarly 2.3, 4.1, 1.7 and 4.4 % of children of age 8-10 yrs, 10-12 yrs, 12-14 yrs and 14-16 yrs are found of VAD respectively. Prevalence of VAD for lactating and pregnant mother observed as 9.0 and 7.6 % respectively (Table 2).Similarly the prevalence of Goiter (TGR) observed was 28.1, 32.2, 30.9, 28.9 and 30.4 for 6-8, 8-10, 10-12, 12-14 and 14-16 years of children respectively (Table 3). The Goiter observed in lactating and pregnant mother were 41.3 and 37.8% respectively (Table 4). These data showed that there is severe malnutrition problem especially of VAD and IDD in the Mid-Western Development Region of the country.

Table 3: Prevalence of Goiter (IDD) in preschool children in Mid-Western Development Region of Nepal

Prevalence of Goiter	6-8 Yrs		8-10 Yrs		10-12 Yrs		12-14 Yrs		14-16 Yrs	
	M	F	M	F	M	F	M	F	M	F
Grade 0	156 (72.6)	128 (71.1)	135 (68.5)	70 (66.7)	94 (69.6)	60 (68.2)	119 (72.1)	51 (68.9)	82 (68.3)	30 (73.2)
Grade 1	58 (26.9)	52 (28.9)	62 (31.5)	33 (31.4)	41 (30.4)	24 (27.3)	40 (24.2)	20 (27.0)	36 (30.0)	10 (24.4)
Grade 2	1 (0.5)	0 (0)	0 (0)	2 (1.9)	0 (0)	4 (4.5)	6 (3.7)	3 (4.1)	2 (1.7)	1 (2.4)
TGR(Grade1 +2)	59 (27.4)	52 (28.9)	62 (31.5)	35 (33.3)	41 (30.4)	28 (31.8)	46 (27.9)	23 (31.1)	38 (31.7)	11 (26.8)
Total	215	180	197	105	135	88	165	74	120	41

Note: Values in the parenthesis indicates the percentage value. [M: Male, F: Female]

Table 4: Prevalence of Goiter in Lactating and Pregnant Mother in the Midwestern Development Region of Nepal

Prevalence of Goiter	Lactating Mother	Pregnant mother
Grade 0	138 (58.7)	115(62.2)
Grade 1	92 (39.1)	68 (36.8)
Grade 2	5(2.1)	2(1.2)
TGR(Grade 1+2)	97 (41.1)	70 (37.8)
Total	235	185

Note: Values in the parenthesis indicates the percentage value.

Conclusion and Recommendation

There is the serious malnutrition problem of Preschool children and Lactating and pregnant mother in the Mid Western Region of Nepal. Micronutrient Deficiency especially of Vitamin A Deficiency (VAD) and Iodine Deficiency Disorder (IDD) are of the major concern in that region. Health education, effective implementation of salt iodization act and other proper nutritional programmes is to be seriously implemented to eradicate the malnutrition problem in the Region.

Acknowledgement

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References

Gibson (1990). Rosalind S. Principles of Nutritional Assessment

Jelliffee (1989). D.B. Community Nutritional Assessment

NMSS (1988). Nepal Micronutrient Status Survey, HMG. Ministry of Health, UNICEF, WHO, MI.

WHO (1983). Measuring Change in Nutritional Status, Geneva

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Published papers

- Karki S. & Dahal K.P. (2005). Optimization of levels of ingredients and Drying Air Temperature in Development of Dehydrated Carrot Using Response Surface Methodology. *Journal of Food Science and Technology*, 43(1):17-26 *Books/ Approved methods*
- K.C. T.B. & Thapa T.N. (2002). Economic Aspects of Biotechnology, Cambridge University Press, Cambridge
- AOAC (1984). Official Methods of Analysis, 14th edn, Association of Official Analytical Chemists, Washington, DC

Chapter in edited books/book series/papers in symposium/souvenir

- Khatiwada A., Dangal T.R. & Rai T.P. (1995). Protein in Whey - Chemical, Physical and Functional Properties. In: Advances in Food and Nutrition Research. Kinsella J.E. (eds) Academic press, Newyork, pp 257-361
- Dahal N. R., Qi L. & Swamylingappa B. (2003). Assessment of the Antinutritional and Digestible Characteristics of *Masyaura*- A Traditional Food of Nepal. In: *Proceedings II, Fifth International Conference on Food Science and Technology (ICFOST)*, Oct 22-24, Wuxi, P.R. China, pp 246-251

Patents

Karki T.B. & Bhattarai U.K. (2004). Process for preparing algin/calcium gel-structured meat products. US Patent 2 5006 024

Thesis

Shah D.J. (1985). Production of Carrot Juice. Ph.D. Thesis, Central Campus of Technology, Dharan, Nepal

Papers presented at symposia but not published

Upadhyaya S, Shrestha H & Joshi S. (2004). Current Food Quality and Safety Issues in Nepal, Paper presented at 5th National Conference on Science and Technology, Royal Nepal Association of Science and Technology, Kathmandu, Nepal, 5-7 May

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News and Events

S.S. Fabrication in Nepal, Growing Towards Excellence.

Fabrication of Stainless Steel utensils and equipments does not have a longer history in this country. However some committed entrepreneurs have taken this challenge by remarkably improving their performances and have become capable to substitute imports of such goods in considerable extent. National Refrigerator Udhyog P. Ltd. situated at Naikap, Kathmandu is gaining excellence in manufacturing Milk Chilling Vats, Refrigerated S.S Showcase, Batch Pasteurizers, S.S. Ovens, Cheese vats and Storage Tanks etc suitable for different types of industries such as Dairies, Pharmaceuticals, Beverage industries, Fats and oil industries etc. Nepal Material Handling P. Ltd., situated in Nawalparasi, is another S.S. fabrication industry which is getting its popularity as a Fabricator of Road Milk Tanker.and Batch Pasteurizers required for Dairy industries. According to these Fabricators, Different dairies, Milk Producers Cooperatives and Milk handlers are using Milk chilling vats. Road Milk Tankers and Batch pasteurizers etc manufactured in Nepal and the import of such commodities has vastly decreased from past few years.

Stick Chewing gum Production, Started first time in Nepal.

Another category of confectionary product, **Stick Chewing gum**, has been started to produce first time in Nepal by Sujal Foods P. Ltd. Pokhara. Even though marketing of such product by importing from other countries like the USA, Korea, China etc was in practice for few years, Sujal Foods took the credit to become the first producer in the country and the second in the South Asian region. The company, already involved in introducing production of different confectionary categories in the country, claims its more than 30 years old legacy in manufacturing and marketing the confectionary products.

Automization of Conditioning in Arun Agro

Conditioning of wheat prior to milling is an important factor affecting the yield and quality of flour and other products. For achieving adequate conditioning in continuous process of wheat milling, industries have tried different systems. Amongst such systems a recent and advanced continuous conditioning system has been installed first time in Nepal by Arun Agro industries P. Ltd. Bhairahawa. The automatic system of conditioning, so called **Moisture Control Unit with Water Proportioning Unit-Made up of Buhler- Switzerland,** continuously measures the moisture content of wheat and corrects for any deviations during the continuous milling process. The company has claimed the improvement in its product yield and quality after installation of this system.

LP-System for Milk Preservation Approved

FAO, with support of the government of Sweden carried out fundamental studies on the natural Lacto Peroxidase System (LP-System) and developed method to preserve milk at an ambient temperature. After having good results from field and toxicological tests and other necessary processes, Codex Alimentarius Commission finally approved the method in 1991 for field implementation.

Although bovine milk contains 30 ppm Lactoperoxidase through the lactation period, it has no antibacterial effects of its own. But Lactoperoxidase combined with oxidized thiocyanate and hydrogen peroxide produces antibacterial compounds. Usually the thiocyanate content of milk is 4-5 ppm. In the approved LP-system, the content of Lactoperoxidase, thiocyanate and Hydrogen peroxide in milk should be >1 ppm, 15 ppm & 8-9 ppm respectivelly. Lactoperoxidase is already in sufficient quantity in milk, the thiocyanate and Hydrogen Peroxide content is maintained by adding Sodium thiocyanate (Na SCN- Available as Activator-1) and Sodium Percarbonate ($2Na_2CO_3H_2O_2$- Available as Activator-2). If used correctly, the LP- system improves the hygienic quality of raw milk and extends its shelf-life by several hours. To implement this system worldwide, Global Lactoperoxidase Programme has been developed by FAO, International Dairy Federation (IDF), WHO and Uppsala Agricultural University, Sweden and the **Manual on the Use of the LP- System in Milk Preservation** (M-26, ISBN-92-5-104254-3) has been issued by FAO in 1999. The manual contains explanations to this natural and harmless antibacterial system, its benefits and application.

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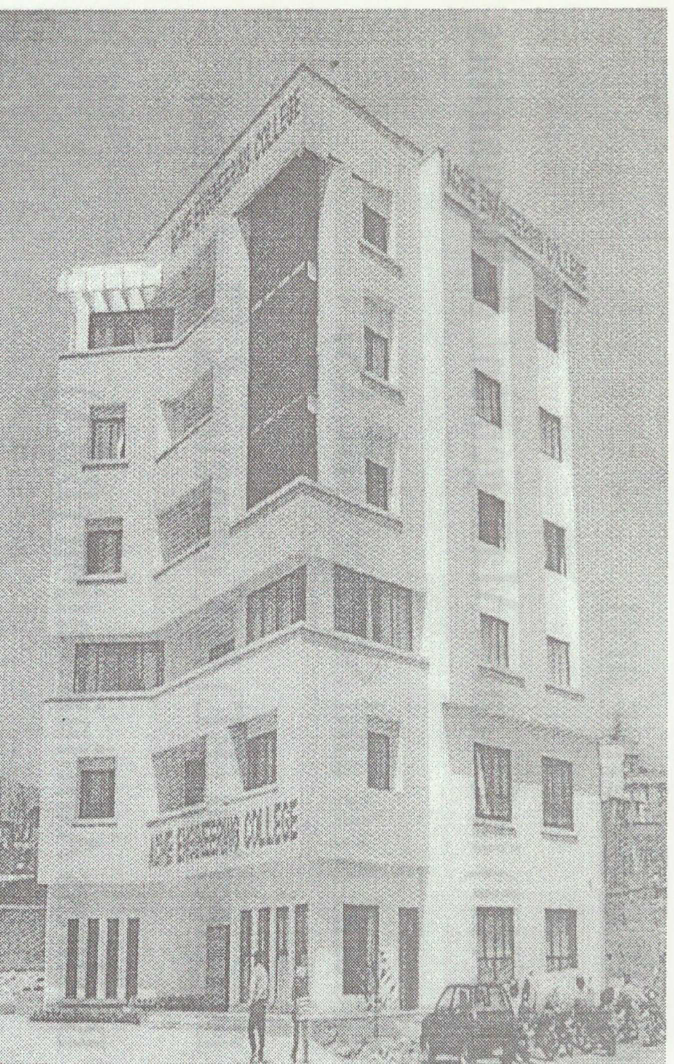

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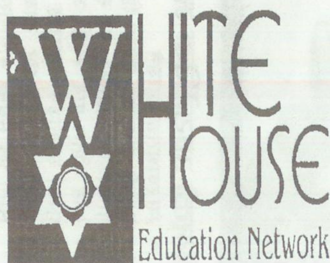

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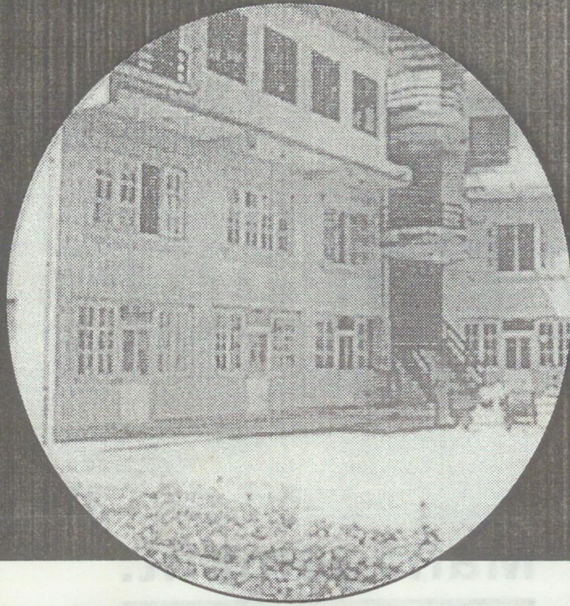

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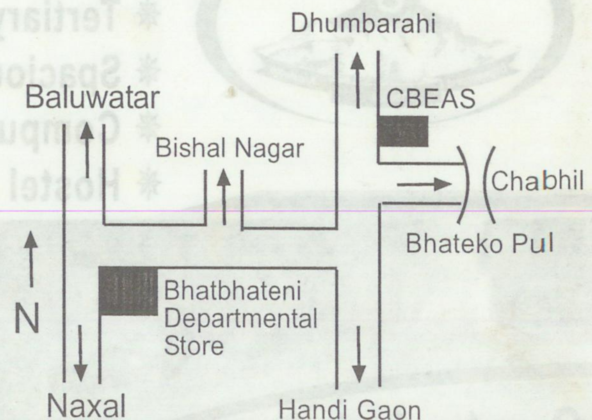

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