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Study on Phenolic Compounds and Effect of Time-Temperature Treatment on Amylase Activities of High Hill Buckwheat in Nepal

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Two varieties of buckwheat, mithe, Fagopyrum esculentum and tite, Fagopyurum tataricum,were collected from Mustang district, Nepal, and their proximate composition was analyzed. Total flavonoids, Tannin and Total phenolics were significantly higher ($p<0.05$) in tite than in mithe buckwheat. Samples of Buckwheat were steeped in water for 12 h and germinated for 48, 72, 96, 120, and 144 h at eachtem perature of 15, 20, 23 and 27 ºC. The germination inhibited by stepwise drying from 50 to 80 ºC. Effects of germination time and buckwheat variety on amylase activity was studied.Germination time had a significant effect on amylase activity of buckwheat malts. Tite and mithe buckwheat were grown under four different temperatures at 15, 20, 23 and 27 ºC for 48, 72,96, 120 and 144 h. However, mithe buckwheat was not germinated at 27 ºC. The maximum amylase activity was observed at 23ºC for 96 h. At this temperature mithe and tite buckwheat had alpha and beta amylase activity 13.87 and 268.97 units/gdry malt and 13.18 and 275.96 units/g dry malt, respectively. Due to high amylase activity of tite buckwheat was selected and malted in bulk quantity.

Keywords: Germination, Amylase enzyme, *Mithe* and *Tite* buckwheat

Introduction

Buckwheat *(Fagopyrum* spp.) is considered as an under utilized crop, is of significant economic importance in several countries of the world (Campbell, 2003). The name buckwheat was either originated from the Anglo-Saxon words boc (beech) and whoet (wheat) or German words *Buch* meaning (false) and *Weizen (* wheat) ultimately considered false wheat (Baniya,1990). Ministry of Agriculture and Cooperatives (MoAC) has also collected data of the production of buckwheat in Nepal. Total buckwheat production in 2010/11 was recorded at 10,304 tons from 8841 hectares. Buckwheat productivity during the period was 0.85 tons per hectare.(http://www.myrepublica.com/portal/index.php?action=news_details&news_id=25966)

Buckwheat is an under-exploited, neglected crop and one of the minor food crops of Nepal. It occupies an important place in Nepalese agriculture and contributes greatly in food supply in remote areas. Common buckwheat *(F. esculentum)* and tartary buckwheat *(F. tataricum)* are two cultivated species and the other buckwheat *(F. cymosum)* is wild one. In the Western Himalayas, the natives prepare baked bread out of buckwheat flour, locally called *roti*. Apart from these uses, a thick porridge or paste in boiling water locally called *dhindo* is consumed. The tartary buckwheat in particular is also fermented, to prepare a local beer called *chang* or *pechuwi* or used for preparation of local wine and whisky in both China and Nepal (Joshi and Rana, 1992; Baniya, 1994). Phytochemicals are plant substances that may promote good health but are not essential for life (Insel *et al.,* 2004). According to the Zhao *et al*.(2001), Chinese tartary buckwheat is abundant in flavonoids as well as rich in starch and protein. Germination is to develop amylases, proteases and other endogenous hydrolytic enzymes (proteinases and peptidases) (Taylor, 1993).

Thus this study aims at investigating the phenolic compounds present in the raw buckwheat and the effect of time and temperature treatments on alpha and beta amylases.

Materials and Methods

Raw materials

The Nepalese buckwheat variety (common buckwheat or *mithe* and tartary buckwheat or *tite*) used in this research work were collected from Mustang District, Nepal.

Preparation of buckwheat malt

Seeds were screened and washed to remove impurities which were adhered with the grain. The cleaned seeds were then steeped in tap water for 12 h to increase the moisture content of the seed(42-46%).

Germination

The steeped buckwheat was spread over the tray and covered with moistened muslin cloth and kept for germination at 15, 20, 23 and 27 °C at RH 95±2% for 5 days. During germination, the grains were moistened by sprinkling water at 6 h interval and mixed gently in order to equalize temperature and aerate the mass.

The general procedure for the overall experimental details is given in Figure 1.

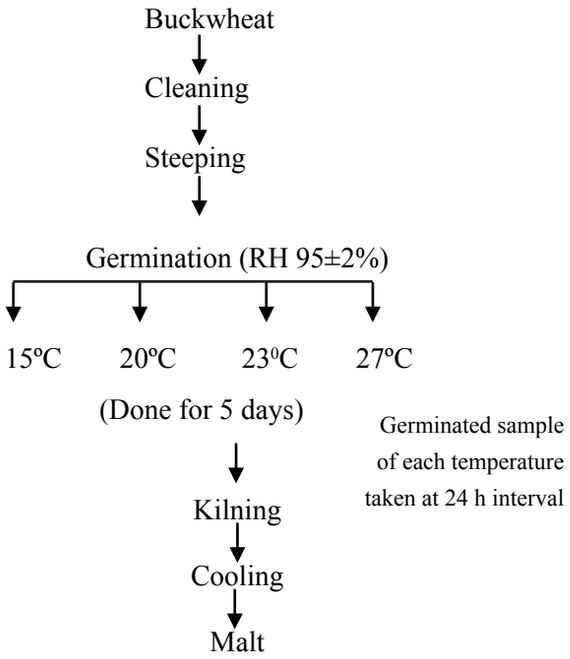

Figure 1. Flow diagram showing overall experimental procedure

Malt Kilning

The germinated buckwheat was successively dried into a cabinet drier at 50 °C, 63 °C, 73 °C and finally at 84 °C to the moisture contents of 24, 18, 12 and 4 % respectively. The grains were then rubbed and sprouts were removed with the help of screen. The malt then was packed in airtight plastic bag.

Proximate analysis

The moisture contents were determined as per AOAC (2005). Crude fat, crude protein, starch, ash content and crude fiber were determined as per Rangana (2001). Carbohydrate was determnined by difference.

Determination of Total phenols, Tannin content and Flavonoids

Total phenolics were determined as per Sadasivum and Manickam (1996).

Analysis of buckwheat malt

Determination of Enzyme activity

One gram of powdered sample was ground in a pestle and mortar with distilled water, volume made up to 50 mL, filtered through Whatman No.1 filter paper and the filtrate was used as enzyme source for determining alpha and beta amylases activity. Alpha and beta amylase activity were determined as per Mallik *et al*. (1980). Enzyme activity was expressed in terms of change in optical density at 620 nm in 1 mL of enzyme extract from 1% m/v per gm of dry matter per unit time.

Results and Discussion

Two local varieties *mithe* (*Fagopyrum esculentum;* or Common Buckwheat) and *tite (Fagopyrum tataricum,* or Tartary Buckwheat) buckwheat were collected from the Mustang district and proximate analysis was carried out at Central Campus of Technology, Dharan. Malts were prepared from the buckwheat germinated at different temperatures (15, 20, 23 and 27 ºC) and time (48, 72, 96,120 and 144 h). Alpha and beta amylase activities were determined and malt having highest alpha and beta amylase activity were selected. M*ithe* buckwheat was not germinated at 27 ^{0}C due to higher temperature.

Proximate composition of Common (mithe) and Tartary (tite) buckwheat

The proximate composition of *mithe and tite* buckwheat was carried out and the results are in the Table 1.

Table 1. Proximate composition of mithe and tite varieties of buckwheat

Parameter (%)	Mithe (% db)*	Tite (% db)*
Moisture content	11.81^a (0.081)	11.14^b(0.123)
Ash content	1.59^a (0.2)	2.059^a(0.251)
Crude fat	2.77^a (0.03)	2.09^b(0.025)
Crude protein	13.91^a (0.101)	15.76^b(0.26)
Carbohydrate	73.87^a (1.955)	72.85^b(2.081)
Crude fiber	7.85^a (0.404)	7.23^a(1.29)
Starch (g dextrose/ 100g)	50.03^a (2.02)	48.36^b(2.08)

Values are the means of triplicate determination. Figures in parentheses are the standard deviations. Means bearing similar superscripts are not significantly different ($p > 0.05$).

Ratan and Kothiyal (2011) reported that the protein of buckwheat is of excellent quality and is high in the essential amino acids e.g. lysine, unlike common cereals. Common buckwheat contains high nutritive substances (63% carbohydrate, 11.7% protein, 2.4% fat, 9.9% fiber, 11% water and 2% minerals).

According to the Cai and Corke (2004), buckwheat seed consists of about 59-74% carbohydrate and buckwheat grains also contain 0.65-0.76% reducing sugars, 0.79-1.16% oligosaccharides, and 0.1-0.2% non-starchy polysaccharides, 2.0-2.6% oil, 9.3-10.9% crude fiber along with 20-30% of which is soluble dietary fiber. Kiryluk *et al*. (2000) have also found crude protein content in hulled barley flour as high as 15.83% and the ash content of 2.19%. The high protein content might be due to the variation in the genetic material as well as agronomic and environmental conditions experienced by the tested material. Statistical analysis showed that the ash content and crude fiber were not significantly different ($p>0.05$) between *mithe and tite* whereas the moisture content, crude fat, crude protein, starch and carbohydrate were significantly different between *mithe* compared to *tite*. Wijngaard and Arendt (2006) reported that digestibility of protein is reduced if it contents tannins, phytic and protease inhibitors. Hence, malting may be one possibility for increasing protein digestibility while germination increases levels of essential fatty acids, minerals, and vitamins.

Chemical characteristics (total phenols, tannin and flavonoids content) of unmalted mithe and tite buckwheat

Table 2. Flavonoids, tannin content and total phenolics of unmalted mithe and tite buckwheat

Parameter	Mithe (db)	Tite (db)
Total flavonoids as rutin (mg %)	253.6^{a}(3.34)	424.4^{b}(4.54)
Tannin as tannic acid (mg %)	88.5^{a}(1.27)	109.2^{b}(1.1)
Total phenolics as gallic acid (mg %)	530.5^{a}(14)	828.1^{b}(13.13)

Values are the means of three determination.Figures in parentheses are the standard deviations. Means bearing similar superscripts are not significantly different ($p > 0.05$).

Statistical analysis showed that there were not significantly different ($p>0.05$) between different parameter of two samples. According to the Hodzic *et al.*(2009), the values of total phenolic compounds in extracts varied from 0.29-2.05 gallic acid/L of extracts at 20 ºC. According to the Sharma *et al.* (2011), the results revealed that the total phenolics(TP) content of tartary buckwheat was comparatively higher than the common buckwheat in all the three types of vegetables studied in the research. TP and total flavonoids (TF) content of the tartary buckwheat were varied and comparatively higher than those of the common variety. This variation in the TP and TF content in buckwheat seed could be due to the fact that exposure to natural light and the age of the buckwheat sprouts affects the phenolic and flavonoid composition of buckwheat (Kim *et al*., 2008). Wijngaard and Arendt (2006) reported that digestibility of protein is reduced if it contents tannins, phytic and protease inhibitors. Hence, malting may be one possibility for increasing protein digestibility and germination also increases the levels of essential fatty acids, minerals, and vitamins.

Effect of germination time and temperature on alpha and beta amylase activity of mithe buckwheat malt

Alpha amylase activities of *mithe* buckwheat malt prepared by germinating at different temperatures for different times are shown in Figure 1.

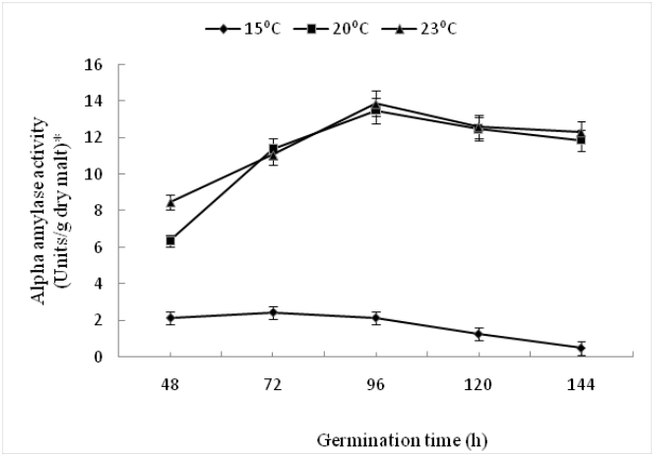

Figure 2. Alpha amylase activity of *mithe* buckwheat malts germinated at different time-temperatures

*One unit alpha amylase activity was defined as the unit change in OD per min under the experimental condition.

Alpha amylase activitties of *mithe* malt germinated at 15 ºC for 48, 72, 96, 120 and 144 h were found to be 2.14, 2.44, 2.06, 1.26, and 0.49 units/g dry malt, respectively. From Figure 2, it can be seen that alpha-amylase activity remained statistically constant up to 120 h and decreased afterwards during germination. Similarly, alpha amylase activities of buckwheat malt (var. *mithe*) germinated at 20 ºC for 48, 72, 96, 120 and 144 h were 6.3, 11.3, 13.48, 12.48, and 11.85 units/g dry malt respectively. Statistical analysis showed that germination time had a significant effect on alpha amylase activity of buckwheat malts. Alpha amylase activity increased with germination time up to 96 h (13.48 units/g drymalt) and decreased on further germination. Buckwheat malts germinated at 23 ºC had alpha-amylase activities of 8.43, 11.06, 13.87,

12.60 and 12.31 units/g dry malts for 48, 72, 96, 120, and 144 h of germination respectively. Alpha amylase activity was significantly affected ($p<0.05$) by germination time where it increased steadily upto 96 h (13.87 units/g dry malt) and then decreased during germination. Maximum alpha amylase production occurred at 96 h of germination at both 20 and 23 ºC and the values were not significantly different (13.68 ± 0.20 units/g dry malt). At germination temperature of 15 ºC, alpha amylase production was lesser and decreased with germination time.

Mithe buckwheat malt was prepared by germinating at 15, 20 and 23 ºC for 48, 72, 96, 120 and 144 h, their beta amylase activities determined and the results are presented in Figure 3.

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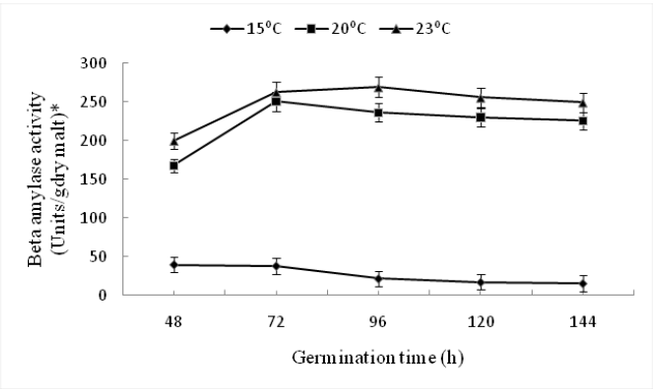

Figure 3. Beta amylase activity of mithe buckwheat malt

*One unit of beta amylase activity was defined as the production of 1 mg reducing sugar as maltose over 30 min of incubation under the experimental conditions.

Similarly, beta amylase activities of *mithe* malt germinated at 20 ºC for 48, 72, 96, 120 and 144 h were found to be 167.39, 250.39, 235.89, 229.94, and 225.38 units/g dry malt respectively. Beta amylase activity was affected by germination time and it reached at maximum value on 72 h (250.39 units/g dry malt) and then decreased on further germination. Beta amylase activities of *mithe* malt germinated at 23 ºC were 199.50, 262.54, 268.97, 255.49 and 249.21 units/g dry malt for 48, 72, 96, 120 and 144 h germination respectively. From Figure 3, it can be seen that beta amylase activity increased with increase in germination time and maximum value reached at 96 h of germination then decreased. Statistical analysis indicated that beta amylase activities of malts germinated for 72 and 96 were not different ($p>0.05$). Maximum values of beta amylase activity at 15 ºC for 48 h, 20 ºC for 72 h and 23 ºC for 96 h were found to be 32.42, 250.39 and 268.97 units/g dry malt respectively. Statistically it was found that the beta amylase activity was significantly different ($p< 0.05$) for all the variations

Effect of germination time and temperature on alpha and beta amylase activity of tite buckwheat malt

Tite buckwheat malt was prepared by germinating theat 15, 20, 23 and 27 ºC for, 48,72, 96, 120 and 144h and their alpha amylase activities determined and the results are presented in Figure 4.

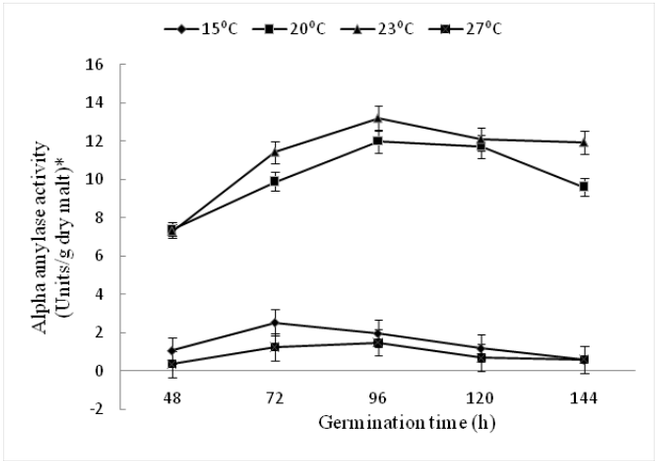

Figure 4. Alpha amylase activities of tite buckwheat malts germinated at different temperatures for different times.

*One unit Alphaamylase activity was defined as the unit change in OD per min under the experimental condition

Alpha amylase activities of *tite* malt germinated at 15 ºC for 48, 72, 96, 120 and 144 h were found to be 1.064, 2.528, 1.962, 1.184 and 0.590 units/g dry malt respectively. From Figure 4, it can be seen that alpha amylase activity was maximum in 72 h and decreased afterwards during germination. Similarly, alpha amylase activities of buckwheat malt (var. *tite*) germinated at 20 ºC for 48, 72, 96, 120 and 144 h were 7.395, 9.881, 11.988, 11.704 and 9.601 units/g dry malt respectively. Statistical analysis showed that germination time had a significant effect on alpha amylase activity of buckwheat malts. Alpha-amylase activity increased with germination time up to 96 h (11.988 units/g dry malt) and decreased on further germination. Alpha amylase activity was significantly affected ($p<0.05$) by germination time. Buckwheat maltsgerminated at 23 ºC had alpha amylase activities of 7.283, 11.411, 13.181, 12.080 and 11.918 units/g dry malts for 48, 72, 96, 120, and 144 h of germination respectively. The germination temperature is limited because of the hazard of mould growth. At temperatures above 24°C the grains started to moulder. Lower temperatures during germination can be compensated by prolonged duration of germination. The germination temperature was not changed, only the maximum temperature of 27 °C was reduced to a

maximum of 24 °C to avoid mouldering (Krahl *et al*., 2008).

Tite malt was prepared by germinating the *tite* buckwheat each at 15, 20, 23 and 27 ºC for 24, 48, 72, 96, 120 and 144 h their beta amylase activities determined and the results are presented in Figure 5.

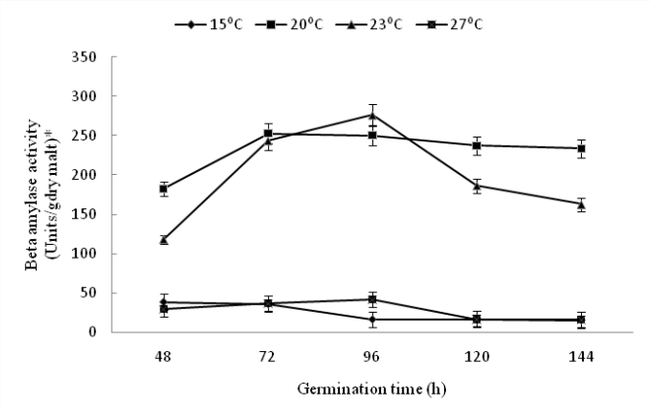

Figure 5. Beta amylase activity of *tite* buckwheat malt

*One unit of beta amylase activity was defined as the production of 1 mg reducing sugar as maltose over 30 min of incubation under the experimental conditions.

Beta amylase activities of *tite* buckwheat malt germinated at 15 ºC for 48, 72, 96, 120 and 144 h were found to be 38.36, 36.07, 15.81, 16.89 and 15.08 units/g dry malt respectively. Beta amylase activity decreased after 48 h and there was no significant different ($p>0.05$) in beta amylase activity at 15ºC between 48 and 72 h and between 96, 120 and144 h germination time. Similarly beta amylase activities of buckwheat malt (var. *tite*) germinated at 20 ºC for 48, 72, 96, 120 and 144 h were 182.28, 252.58, 250.54, 237.21 and 233.48 units/g dry malt respectively. Statistical analysis showed that germination time had a significant effect on beta amylase activity of buckwheat malts. Beta-amylase activity increased with germination time up to 72 h and decreased on further germination. Buckwheat malts germinated at 23 ºC had beta amylase activities of 117.66, 243.28, 275.96, 186.08 and 162.14 units/g dry malts for 48, 72, 96, 120, and 144 h of germination respectively. Beta amylase activity was significantly affected ($p<0.05$) by germination time where it increased steadily upto 96 h (13.87 units/g dry malt) and then decreased during germination. Mean value of beta amylase activity of *tite* buckwheat malt germinated at 27 ºC for 48, 72, 96, 120 and 144 h were found to be 29.58, 36.51, 41.18, 16.38 and 16 units/g dry matter respectively. ANOVA at 5% significance level showed that there was no significant different ($p>0.05$) in beta amylase activity at 27 ºC for 72, 96 and between 120 and144 h germination.

Conclusions

From the above obtained results it was found that protein and starch content of *tite* and *mithe* buckwheat were 15.76 % (db) & 48.36 g dextrose/ 100 g and 13.91 % (db) & 50.03 g dextrose/ 100 g respectively. This lied under the range of commercial barley. Total flavonoids , tannin and total phenolics contents of unmalted *tite* buckwheat were higher than that of *mithe* buckwheat. *Tite* and *mithe* buckwheat were grown under four different temperatures at 15, 20, 23 and 27 ºC for 48, 72, 96, 120 and 144 h. But at 27 ºC due to higher temperature the *mithe* buckwheat was not germinated sufficiently as well theirwas also growth of mould. The maximum amylase activity was observed at 23 ºC for 96 h. At this temperature *tite* and *mithe* buckwheat had alpha and beta amylase activity 13.87 & 268.97 units/g dry malt and 13.181 and 275.96units/g dry malt respectively.

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