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Characterization and Comparison of Soy Milk and Buffalo Milk Based Synbiotic Product

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Today consumers prefer foods that not only provide basic nutrition but also contain compounds that confer health benefits. Among the functional food, synbiotic products are showing a promising future. Soy based synbiotic products using different probiotics: A1B and MgA (lactobacillus strains) and L.rhamanosus ATCC 9595 and prebiotics: lactulose, sunfibre and inulin were prepared and its antiradical activities along with other characteristics were studied. The study also aimed to determine the most suitable prebiotics and probiotics. Furthermore, the prebiotics and the organisms were also cultured in buffalo milk to compare its properties with synbiotic soy yoghurt. Synbiotic soy yoghurt showed highest anti-radical activity with % inhibition at the range of 41-45 % and the one with probiotic L.rhamnosus and prebiotic inulin was found to be the most suitable product.

Keywords: Synbiotic, Probiotic, Prebiotic, Inulin, Anti-radical

Introduction

Soy milk is very nutritive: it's a good source of high quality proteins, isoflavones and B-vitamins. It is free of milk sugar (lactose) and is a good choice for people who are lactose intolerant. Consumers in western countries consume soymilk mainly as an important replacer of cow milk due to lactose intolerance or allergic reaction to cow's milk, and as a low cost source of good quality protein and energy (Rosenthal et al., 2003; Lui, 1997; Kwok and Niranjan, 1995; Kanthamani et al., 1978). Soy milk has antiradical activity, through the isoflavones, which acts as an effective reducing tool for oxidative degradation of DNA, prevention of premature aging and the emergence of diseases like Alzheimer's (Hsieh et al., 2009). Fermented soymilk have numerous advantages over nonfermented one (Chow, 2002). Fermentation may reduce flatulence, destroy undesirable pathogens, improve product flavor and reduce beany flavor, give new textures, and, when un-pasteurized, protect those who have eaten it from intestinal infections, and help replenish the intestinal flora (Trindade et al., 2001). Lactic acid fermentation has been reported as a means to reduce beany flavors. Consequently, soy milk based yoghurts offer a considerable appeal for a growing segment of consumers with certain dietary and health concern. In addition, it has several nutritional advantages over cow milk yoghurt such as, reduced levels of cholesterol, of saturated fat and free of lactose (Pyo and Song, 2009).

A synbiotic is a supplement that contains both a prebiotic and a probiotic that work together to improve the "friendly flora" of the human intestine. The main reason for using a synbiotic is that a true probiotic, without its prebiotic food, does not survive well in the digestive system. To enhance viability, not only on the shelf but also in the colon, the product must allow for much greater attachment and growth rate of the healthy bacteria in order to minimize the growth of harmful bacteria. Without the necessary food source for the probiotic, it will have a greater intolerance for oxygen, low pH, and temperature. In addition, the probiotic will have to compete against other bacteria that will take over if its specific food source is not available (Verma and Palanchoke, 2007) . Therefore, a "synbiotic" product (probiotic + prebiotic = synbiotic) makes for a better choice.

The objective of this study was to develop soy based synbiotic yoghurt with good acceptability using different probiotics and prebiotics, to determine the most suitable probiotic and prebiotic as well as compare the synbiotic soy yoghurt with buffalo milk based synbiotic yoghurt and finally evaluate its different properties specifically anti-radical activity.

Materials and Methods

Prebiotics (Microorganisms) and Probiotics

Lactobacillus rhamnosus ATCC 9595, A1B and MgA (lactobacillus strains) were used for experiment. A1B and MgA strains cultures were obtained from National

Institute of Science and Technology (NIST), Nepal. All three strains were sub cultured in MRS agar media containing 1% calcium carbonate. Inulin, Lactulose and Sunfibre were used as prebiotics.

Preparation of soymilk

Soy milk was prepared in the laboratory using white soybean variety. Soybean soaked in water for 16 hr was decapitated and boiled in 1% sodium bicarbonate for 5 min and subsequently washed with water. It was then grinded along with warm water (7:1 ratio) and the resulting soymilk was filtered through cheese cloth. It was finally sterilized by autoclaving and stored in refrigerator at 4°C.

Soy milk/buffalo milk based synbiotic and probiotic products preparation

Soymilk/Buffalo milk was heated at 110-112° C and poured into culture tubes into which 1% prebiotics (inulin, lactulose and sunfibre) was added. The strains (L.rhamnosus, A1B and MgA)were then inoculated with 6 log CFU/ml into it, stirred thoroughly and incubated at 37°C for 24 hr to obtain synbiotic and probiotic yoghurts. Furthermore, different properties of thus prepared synbiotic soymilk and buffalo milk were studied and compared with each other as well as with *Juju dhau* (king curd from Bhaktapur) and probiotic curd from DDC.

Twenty four different synbiotic and probiotic soy yoghurt and buffalo yoghurt prepared in our laboratory are listed below.

A. *Lactobacillus rhamnosus* with 1% inulin for both soy milk and buffalo milk
B. *Lactobacillus rhamnosus* with 1% lactulose for both soy milk and buffalo milk
C. *Lactobacillus rhamnosus* with 1% sunfibre for both soy milk and buffalo milk
D. *Lactobacillus rhamnosus* for probiotic soy milk and probiotic buffalo milk
E. A1B strain with 1% inulin for both soy milk and buffalo milk
F. A1B strain with 1% lactulose for both soy milk and buffalo milk
G. A1B strain with 1% sunfibre for both soy milk and buffalo milk
H. A1B strain for probiotic soy milk and probiotic buffalo milk
I. MgA strain with 1% inulin for both soy milk and buffalo milk
J. MgA strain with 1% lactulose for both soy milk and buffalo milk
K. MgA strain with 1% sunfibre for both soy milk and buffalo milk
L. MgA strain for probiotic soy milk and probiotic buffalo milk

Biochemical changes during fermentation of Synbiotic and Probiotic Products

pH and Titratable acidity

pH measurements were carried out at room temperature (27°C) by means of a digital pH meter(HANNA instruments pH211 Microprocessor pH meter) (AOAC, 2005), 24 hours from incubation, after 5 days and after 10 days from incubation.

Titratable acidity was measured by titration with 0.1 N sodium hydroxide solutions and using 1% ethanol solution of phenolphthalein as indicator (Agrarwala and Sharma, 1961). It was also measured 24 hours from incubation, after 5 days and after 10 days from incubation.

Total soluble solids (TSS)

TSS was determined by using refractometer (portable refractometer, model: FG 103, Brix 0-32%; Comecta S. A.) (Daniel 2010) in terms of degrees Brix for all soy and buffalo yoghurt samples along with *Juju dhau* (king curd) and prebiotic yoghurt from DDC.

Viability of Probiotic organisms present in synbiotic and probiotic yoghurts

MRS agar containing 1% calcium carbonate was used for propagation of the lactobacilli strains in the samples. Standard Plate Count (SPC) method (Eaton et al., 1960) was used. After incubation, the inoculated plates having 30 to 300 colonies were considered for counting and expressed as log CFU/ml of soymilk.

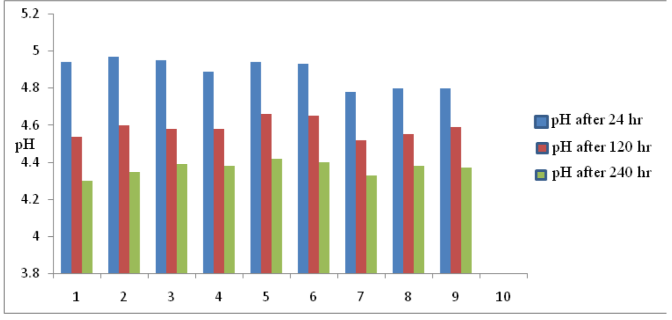
Anti-radical activity

1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) is free radical but stable. DPPH solution is initially violet in color which fades when antioxidants donate hydrogen. The change in color is monitored by spectrophotometer. DPPH free radical scavenging activity was calculated on the basis of the method described by Molyneux (2004).

Result and Discussion

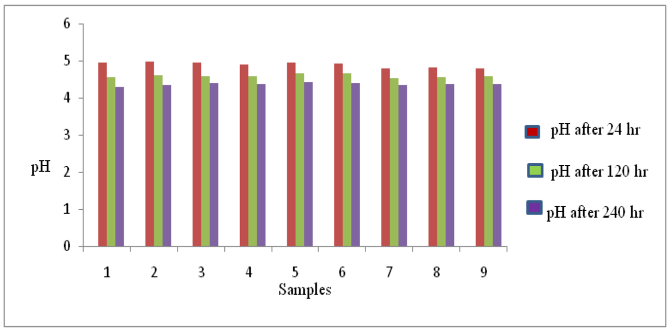
Change in pH

The change in pH was determined after 1 day, 5 days and the 10 days of fermentation. The pH showed decreasing trend with the progression of fermentation (Fig 1, Fig2 and Fig 3). Synbiotic soy yoghurt with lactulose as prebiotic and *L.rhamnosus* as probiotic showed decreases in pH (4.94 to 4.30) slightly more than other synbiotic soy yoghurts. Similarly pH of synbiotic buffalo yoghurt with prebiotic lactulose and probiotic *L.rhamnosus* decreased (4.51 to 4.12) slightly more than other products. The pH of probiotic soy yoghurt and probiotic buffalo yoghurt both with *L.rhamnosus* showed decrease in pH from 5.20 to 4.85 and 4.82 to 4.57 respectively which was slightly more than other similar probiotic soy yoghurt and buffalo yoghurt. The pH of *Juju dhau* decreased from 4.50 to 4.21 and that of probiotic yoghurt (DDC) from 4.49 to 4.25. The pH of the product with *L.rhamnosus, A1B* strain and MgA was almost similar for prebiotic lactulose but different from sunfibre and inulin. Inulin containing yoghurt was found to exhibit low pH when compared with samples containing lactulose and sunfibre. Production of lactic acid in the samples was found to be influenced by the prebiotic used. The lowest difference in pH was found around 0.24 in probiotic yoghurt (DDC) which resembles very close to probiotic buffalo yoghurt with *L rhamnosus.*



Fermentation Time – Days

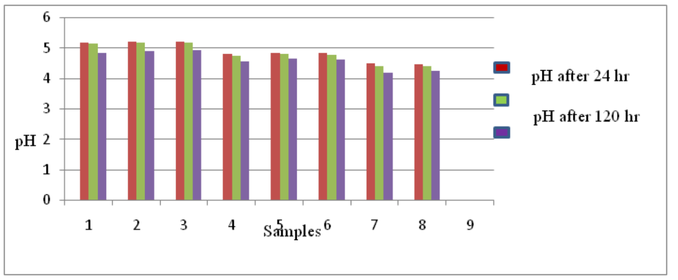
Figure 1. pH of Soymilk based Synbiotic Product



Fermentation Time – Days

Figure 2. pH of Buffalo Milk based Synbiotic Product

For Figure 1 and 2:
1: Lactulose + *L.rhamnosus* 6: Sun Fibre + MgA strain
2: Lactulose + A1B strain 7: Inulin + *L.rhamnosus*
3: Lactulose + MgA strain 8: Inulin + A1B strain
4: Sun Fibre + *L.rhamnosus* 9: Inulin + MgA strain
5: Sun Fibre + A1B strain



Fermentation Time – Days

Figure 3. pH of Soymilk and Buffalo Milk based Probiotic product, King Curd and Probiotic Yoghurt (DDC)

1: Soymilk + *L.rhamnosus* 5: Buffalo milk + A1B strain
2: Soymilk + A1B strain 6: Buffalo milk + MgA strain
3: Soymilk + MgA strain 7: King Curd
4: Buffalo milk+*L.rhamnosus* 8: Probiotic Yoghurt (DDC)

Change in Titratable Acidity

The titratable acidity of soy based synbiotic yoghurt with *L.rhamnosus* as probiotic and inulin as prebiotic showed highest acidity with 0.315 to 0.360% and the one with MgA strain as probiotic and lactulose as prebiotic showed lowest acidity with 0.279 to 0.321% (Fig 4). Similarly, Buffalo based synbiotic yoghurt with *L.rhamnosus* as probiotic and lactulose as prebiotic showed highest production of lactic acid with change in titratable acidity from 0.315% to 0.380% after 10 days while the one with prebiotic sunfibre and probiotic MgA strain showed lowest value with increase in titratable acidity from 0.288 % to 0.335% (Fig 5). For both probiotic soy yoghurt and probiotic buffalo yoghurt product with *L.rhamnosus* showed highest acid production with change in titratable acidity from 0.270 % to 0.327% and 0.297 % to 0.332% respectively among the different probiotic yoghurts (Fig 6). *Juju dhau* showed acidity of 0.360 to 0.416% and probiotic yoghurt (DDC) had acidity of 0.342 to 0.408%.

Result showed that *L.rhamnosus* produced more lactic acid than MgA and A1B strain either by addition of prebiotics or without addition of prebiotics. Titratable acidity depends on the amount of carbohydrate contained in the yoghurt samples, lactose content in buffalo milk is high thus buffalo based yoghurts showed high titratable acidity compare to soy yoghurts.

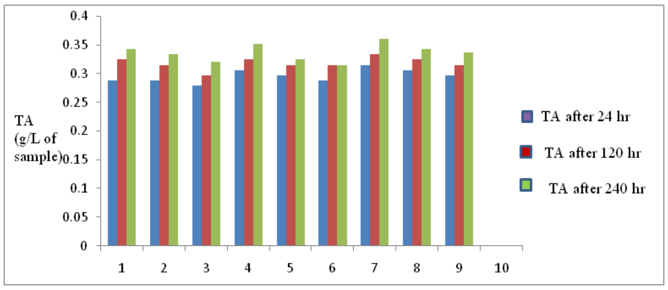


Figure 4. Titratable acidity (TA) of Soymilk based Synbiotic Product

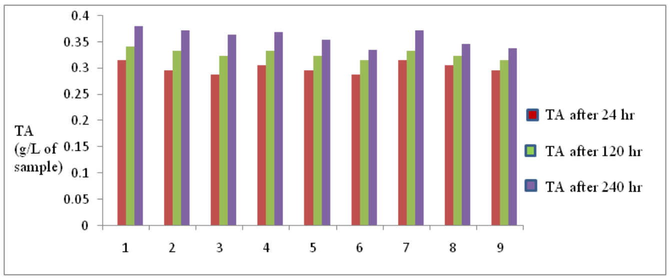


Figure 5. Titratable acidity (TA) of Buffalo Milk based Synbiotic Product

For Fig 4 and 5:
1: Lactulose + *L.rhamnosus* 6: Sun Fibre + MgA strain
2: Lactulose + A1B strain 7: Inulin + *L.rhamnosus*
3: Lactulose + MgA strain 8: Inulin + A1B strain
4: Sun Fibre + *L.rhamnosus* 9: Inulin + MgA strain
5: Sun Fibre + A1B strain

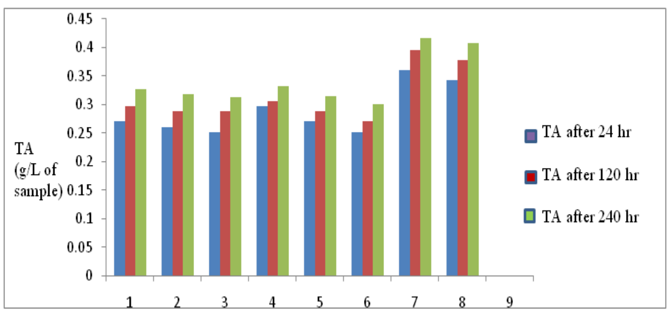


Figure 6. Titratable acidity (TA) of Soymilk, Buffalo Milk based probiotic product, King Curd and Probiotic yoghurt (DDC)

1: Soymilk + *L.rhamnosus* 5: Buffalo milk + A1B strain
2: Soymilk + A1B strain 6: Buffalo milk + MgA strain
3: Soymilk + MgA strain 7: King Curd
4: Buffalo milk + *L.rhamnosus* 8: Probiotic Yoghurt (DDC)

Total Soluble Solid

Figure 7 Shows that Buffalo milk based synbiotic product containing lactulose as prebiotic and A1B strain as probiotic and the one with sunfibre as prebiotic and *L.rhamnosus* as probiotic had the highest solid content around 11.4% However, the Synbiotic soy yoghurt containing lactulose as prebiotic and *L.rhamnosus* as probiotic, sunfibre as prebiotic and MgA as probiotic and inulin as prebiotic and *L.rhamnosus* as probiotic was found to contain solid content around 6.2%. Similarly, probiotic soy yoghurt with *L.rhamnosus* as the probiotic showed the lowest solid content of 2.4 % .among all other probiotic soy products. Similarly, total solid content of probiotic buffalo yoghurt containing *L.rhamnosus* was found highest with value 8.2.The total soluble solid content of *Juju dhau* and Probiotic yoghurt from DDC was found to be 15.4 and 9.6 respectively.

From the observations of all the yoghurt samples, it was found that *Juju dhau* had the highest total solid content; which might be due to the addition of sugar at higher concentration (1 kg sugar in 20 litres of milk) compared to other yoghurts in which no sugar was added. Also, buffalo yoghurt had higher solid content than the soy yoghurt which may be due to presence of lactose in buffalo milk and other carbohydrates which is absent or present in considerable fewer amounts in soy milk.

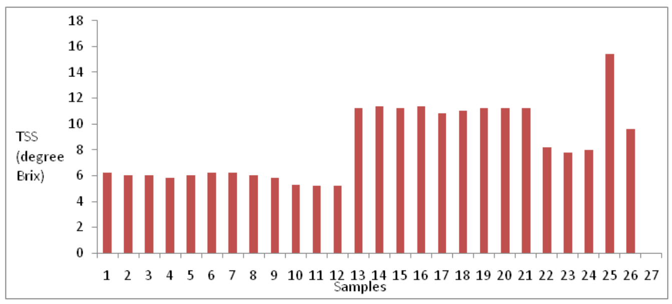


Figure 7. Total Soluble Solid (^{0}Brix) of different products

Soy Milk: 1: Lactulose + *L.rhamnosus,* 2: Lactulose + A1B strain, 3: Lactulose + MgA strain, 4: Sun Fibre + *L.rhamnosus,* 5: Sun Fibre + A1B strain, 6: Sun Fibre + MgA strain, 7: Inulin + *L.rhamnosus,* S8: Inulin + A1B strain, 9: Inulin + MgA strain
Buffalo Milk: 10: Lactulose + *L.rhamnosus,* 11: Lactulose + A1B strain,12: Lactulose + MgA strain, 13: Sun Fibre + *L.rhamnosus,* 14: Sun Fibre + A1B strain, 15: Sun Fibre + MgA strain, 16: Inulin + *L.rhamnosus,*17: Inulin + A1B strain, 18: Inulin + MgA strain
Probiotics: 19: Soymilk + *L.rhamnosus,* 20: Soymilk + A1B strain, 21: Soymilk + MgA strain 22: Buffalo milk + *L.rhamnosus,* 23: Buffalo milk + A1B strain, 24: Buffalo milk + MgA strain, 25: King Curd, 26: Probiotic Yoghurt (DDC)

Viability of Probiotic Organisms present in different products

The viability of different probiotic strains present in all the samples, after 1 day, 5 days, 10 days and 15 days of fermentation was deetermined. Clear zones of colonies were observed in MRS media. According to our observation (Fig 8), probiotic strain present in both buffalo based and soy based synbiotic product maintained 8 logs cfu/ml till 15 days from incubation. Similar result was observed in case of *Juju dhau* (8 logs CFU/ml for 15 days). The stability of probiotics present in the probiotic yoghurt from DDC decreased with the number of days i.e. by a log cycle from 8.46 logs CFU/ml to 7.27 logs CFU/ml in 10 days, showing viability till 5th day from incubation. This decrease may be due to the low survivability rate of the probiotics used in the yoghurt. The viability of the probiotic soy yoghurt and the probiotic buffalo yoghurt was reduced after 10th day where as the viability of the synbiotic soy yoghurt, synbiotic buffalo yoghurt and *Juju Dhau* were seen to be stable till the 10th and 15th day.

So, it can be inferred that the presence of prebiotics in synbiotic yoghurt enhanced the viability of the probiotic strain present in it because of their ability to be fermented by the lactobacilli strains. In the presence of inulin, sunfibre and lactulose, cultures showed better retention of viability (8.0 log cfu/ml) in comparison to that of the yoghurts without prebiotics.

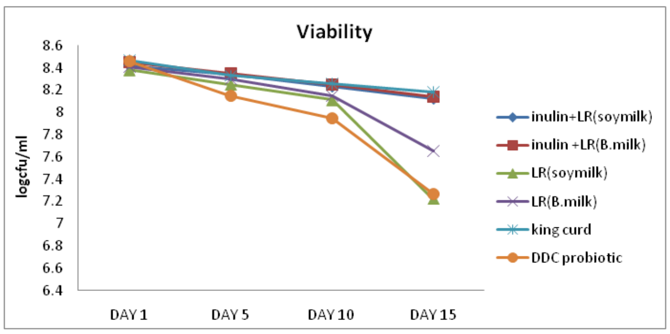


Figure 8. Viability of Probiotics in different products

- Inulin+LR (soymilk) represents all soymilk based synbiotic products
- Inulin+LR (B.milk) represents all buffalo milk based synbiotic products
- LR (soymilk) represents all probiotic soy yoghurts
- LR (B.milk) represents all probiotic buffalo yoghurts
- King curd represents *Juju dhau*
- DDC probiotic represents DDC probiotic yoghurts

Anti-radical Activity

Figure 9 depicts that the concentration of BHT showed 50% inhibition of DPPH which is 0.1 mM. While comparing the value obtained from the samples with BHT, the inhibition shown by soymilk based synbiotic products was at the range of 41-45 %. Similarly, the inhibition shown by the buffalo milk based synbiotic products, probiotic buffalo yoghurts, *Juju dhau* and probiotic yoghurt (DDC) was within the range of 9.5-12 %. Among all the samples the highest inhibition was shown by probiotic soy yoghurts at the range of 68-70 % and soy yoghurts containing *L.rhamnosus* as probiotic (Table 1).

So, we can conclude that the antioxidant activity was higher in soy based products than in buffalo milk based products. Soybean or soy based products contain high amount of antioxidants than buffalo milk based products due to the presence of isoflavones (Daidzein, genistein) in soy products which acts as antioxidants and are responsible for antioxidant activity.BHT was used as standard antioxidant and solutions with different concentrations of BHT was prepared in order to compare the absorbance of the BHT solutions with the samples and hence on the basis of the relation between absorbance and different concentrations of the BHT solutions, the amount of the antioxidants in the samples was determined (Table 10).

Table 1 shows the effect of percentage inhibition in various yoghurt products prepared by using probiotics strains (L.rhamnosus, A1B and MgA) in combination with prebiotics either by using soymilk or buffalo milk as a substrate. The addition of prebiotics (lactulose, sunfibre, and inulin) in soymilk irrespective of the probiotics used in the study did not show significant difference (40.43 – 44.81%) inhibition. Similarly, the synbiotic yoghurt prepared by using buffalo milk and various prebiotics and various probiotic strains as explained above was found between (9.84 – 11.57%) inhibitions.

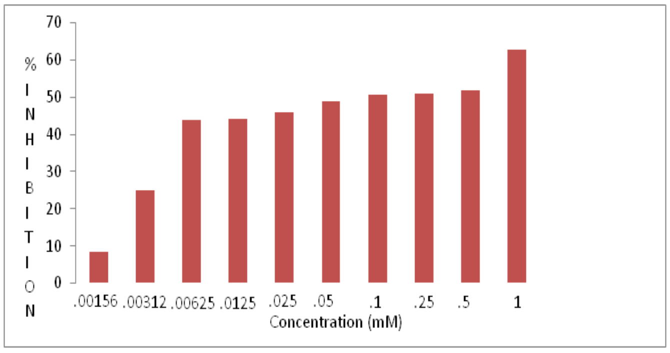


Figure 9. Relation between % inhibition and concentrations of BHT

Table1. Percentage Inhibition of Antioxidant against DPPH

Sample	Prebiotic	Probiotic	% inhibition
Soymilk	Lactulose	*L.rhamnosus*	44.41
		A1B strain	42.82
		MgA strain	43.08
	Sunfibre	*L.rhamnosus*	44.81
		A1B strain	41.62
		MgA strain	40.43
	Inulin	*L.rhamnosus*	41.22
		A1B strain	43.22
		MgA strain	43.75
Buffalo milk	Lactulose	*L.rhamnosus*	10.10
		A1B strain	9.84
		MgA strain	10.37
	Sunfibre	*L.rhamnosus*	9.84
		A1B strain	10.77
		MgA strain	10.10
	Inulin	*L.rhamnosus*	11.70
		A1B strain	11.17
		MgA strain	11.57
Soymilk		*L.rhamnosus*	69.14
		A1B strain	68.75
		MgA strain	68.08
Buffalo milk		*L.rhamnosus*	9.70
		A1B strain	9.97
		MgA strain	9.84
Juju dhau			10.23
Probiotic (DDC)			9.84

Conclusion

Based on the comparison of the characteristics of all yoghurts along with their antiradical activity, it was found that synbiotic soy youghurt with *L.rhamnosus* as probiotic and inulin as prebiotic was the most suitable product in all respect. Hence, probiotic and prebiotic can be used in appropriate combination to make a synbiotic product with health benefits due to the presence of high concentration of isoflavones which was evident from higher anti-radical activity. Moreover, the stability of our synbiotic soy yoghurt was higher than the probiotic yoghurt available in the local market of Nepal.. Also, synbiotic yoghurt prepared from buffalo milk can be as good as *Juju dhau*.

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