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Evaluation of the nutritional quality and microbiological analysis of newly developed soya cheese

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Abstract

The aim of this study was to determine the chemical and microbiological analysis of soya cheese and compare with cow milk cheese and raw soya. Fat content is lower and protein content is higher in soya cheese than cow milk cheese. Moisture, ash, fat, total carbohydrate, total energy, pH, total chlorides and Total solids are significantly higher in cow milk cheese than soya cheese. Cheese yield, total solid yield and protein yield are 135%, 51.18% and 72.33% respectively. Pepsin digestibility of soya cheese is significantly ($p < 0.001$) higher than cow milk cheese. Minerals were analyzed by atomic absorption spectrophotometer and showed that most of the minerals are significantly higher in soya cheese than cow milk cheese but calcium is higher in cow milk cheese. Vitamin content were also analyzed by UPLC-MS-MS and the concentration of water soluble vitamins in 100 gm cheese are 2.423333 ± 0.05 µg thiamine, 5.063333 ± 0.020 µg riboflavin, 11.28 ± 0.35 µg niacin, 1.323333 ± 0.05 µg pantothenic acid, 212.3723 ± 9.80 µg Folic acid and 63.48 ± 3.98 µg biotin on dry basis of soy cheese. The contents of vitamin in cow milk cheese were also analyzed. The effect of processing on the losses or gain of minerals and vitamin were also calculated and showed that most of the minerals and vitamin losses and a few minerals were gain after processing. Standard plate count, total fungus, total coliform and Salmonella were tested by using APHA standard and no significant amount of microorganisms were found in the product. Besides with other nutrients this soya product contains highly digestible protein, minerals, B vitamin, low fat and low carbohydrate that are valuable for human health.

Introduction

Cheese is a popular food product. Cheese making started out as an accidental curdling of milk. Cheese is a nutrient-dense food. Cheese provides a high concentration of nutrients relative to its energy content. The nutritional composition of cheese depends on the type of milk used and the manufacturing and ripening procedures. Therefore, cheese contains relatively small amounts of the water soluble constituents (whey proteins, lactose, and water-soluble vitamins), which partition mainly into the whey. None of the milk components is fully retained in cheese and new substances may be added, notably salt (Ercan, 2009).

The continuous increase in population and inadequate supply of protein has inadvertently increased the occurrence of malnutrition in developing countries (Siddhuraju *et al*., 1996). However, in order to meet the protein demands in developing countries, where animal protein is also grossly inadequate and relatively expensive, research effort is geared towards finding alternative sources of protein from legume seeds. It must be stressed that for the selective few that are able to afford animal milk, there is always an increasing concern about its fat and cholesterol contents. This factor has made vegetable milk to become an alternative source of milk. In this regard, soymilk has been recognized as being nutritionally helpful. For instance, soy cheese (a product from soymilk) accords advantages in terms of nutrition and health, since it contains no cholesterol or lactose and only small quantities of saturated fatty acids; in addition to its advantage of low cost. The basic reason for purposely processing milk into cheese is to preserve a perishable food and to convert it into a stable and storable product. It also expands the variety of food.

The objective of our study was comparative analysis of the proximate, lactose, salt, total chlorides, acidity, pH, total solid, cheese yield, total solid yield, protein yield, minerals, heavy metals, B vitamins and microbiological data of the soy cheese and cow milk cheese and effect of processing gain or loss of

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minerals and B – vitamin in soya cheese.

Materials and Methods

Samples

Cow milk cheese was collected from local market (Sapno super store, Dhanmondi, Dhaka-1205) in capital city Dhaka. Soya was collected and cleaned. Soya milk was developed after dehulling the soya and trypsin inhibitor destroyed. Fresh cheese was developed by coagulating the soya milk and finally developed mature cheese by using the solution of citric acid, sodium chloride and heating in oven for appropriate time. After these steps, cheese was preserved in refrigerator for analysis.

Proximate and other chemical analysis

Determination of moisture content

The method described by Kirk and Sawyer (1991) was used. Moisture content was determined as the loss in weight due to evaporation from sample at a temperature of 105°C.

Determination of ash

This was determined according to the method described by Kric and Sawyer (1991). The crucible with sample was gently heated on the Bunsen flame until smoke ceased, and then transferred into a muffle furnace where it was burnt at 600°C to white ashes. The crucible and its contents were then removed and placed in a desiccator to cool after which it was weighed to a constant weight and calculated the amount of ash content.

Determination of crude protein by Kjeldahl Method

The method described by Kirk and Sawyer (1991) was used. The nitrogen content was multiplied by 6.25 (conversion factor) to obtain the percentage protein for soya cheese and 6.38 for cow milk cheese respectively. The procedure was carried out in three stages: digestion, distillation and titration.

Determination of fat

Determination of fat was carried out by Werner-Schmid process (Kirk and Sawyer, 1991). Proteins are digested with conc. hydrochloric acid. Liberated fat is extracted with alcohol, ethyl ether and petroleum ether. Ethers are evaporated and residue left behind is weighed to calculate the fat content.

Determination of crude fibre content

The crude fibre content was carried out using the method described by Kirk and Sawyer (1991). 2-4 g of sample was defatted. The defatted sample was boiled under reflux for 30 min with 200 ml (1.25%) H_2SO_4. It was further filtered and washed with boiling water until the washing was no longer acidic. The residue was boiled in a round bottom flask with 200 ml (1.25%) NaOH for another 30 min filtered and washed with boiling water until the washing was no longer alkaline. The residue was scraped into a previously weighed crucible and dried at 100°C. It was left in a desiccators to cool and weighed. It was thereafter incinerated in a muffle furnace at about 600°C, left in a desiccator to cool and then weighed and calculated the crude fibre.

Carbohydrate estimation

Carbohydrate content was calculated by subtraction of the sum of moisture, protein, fat, crude fibre and ash contents.

Total energy (calorific value) determination

The energy value was calculated using the Atwater factor method [(9 x fat) + (4 x carbohydrate) + (4 x protein)] as described by Eneche (1991); Chinma and Igyor (2007) and Nwabueze (2007). The proportion of protein, fat and carbohydrate were multiplied by their physiological fuel values of 4, 9 and 4 kcal, respectively and the sum of the product was taken.

Determination of lactose

Lactose was determined by the copper reduction method Kirk and Sawyer (1991). Weight the sample into 250ml volumetric flask, dilute with hot water and allow standing for 30 minute. Cool and add 4 ml carrez I solution, mix and 4 ml carrez II solution. Finally dilute to mark, filter and determine the lactose by Lane and Eynone's method using standard Fehling solution.

Total salt

The salt content of samples was determined by Mohr method by using 5% potassium chromate and titrates with 0.1M silver nitrate and finally using the following formula Kirk and Sawyer (1991).

$$1 \text{ ml } 0.1M\ AgNO_3 = 0.005844 \text{ g NaCl}$$

Total chlorides

Total chlorides were determined according to Ranganna (2007) by titrating with 0.1N silver nitrate and applying the following formula:

$$\text{Total chloride \%} = ((\text{Sample titre- Blank titre}) \times N \text{ of } AgNO_3 \times 3.546) / \text{Weigh of sample}$$

Total solid content

Total solid content of the cheeses were determined

gravimetrically by drying a sample to constant weight in an oven at 105ºC according to the AOAC (2006). Cheese sample (3 g) was crushed with 20 g sea sand and glass stick in pre dried weighing dish. The difference in weight before and after drying for 4-5 hours at 105ºC gives the results of total solid content.

Total solid content (%) = [(Total solid content of cheese (g) / cheese (g))] ×100

The pH and titratable acidity

The pH of the cheese was determined by mixing cheese and distilled water in the ratio of 1:1 in stomacher and then results were measured with a pH meter (Rehman and Fox, 2002). For determination of titratable acidity, 10 g cheese was weighed and crushed with 105 ml water (40ºC) in porcelain mortar. This solution was filtered and 25 ml of filtered solution was used for titration. Three drops of phenolphthalein were added and titrated with 0.1N NaOH until the first permanent pink color (Ercan, 2009).

% lactic acid = (0.1N NaOH amount (ml) x 0.009 x 100) / Cheese amount (g)

Yield calculation of soya cheese

The yield of cheese was calculated based on cheese obtained and the soybean used. The yield calculated by following ways (Andika *et al*., 2011).

Yield of fresh cheese % = (weight of fresh cheese (g)/ weight of soybean (g)) x 100%

Yield of cheese solid % = (Weight of dried cheese (g)/ weight of dried soybean (g)) x100%

Yield of protein (%) = (Weight of cheese protein (g)/ weight of soybean protein (g)) x 100%

In vitro protein digestibility (IVPD) by pepsin

The *in vitro* protein digestibility was carried out according to the method of Maliwal (1983) as described by Sulieman *et al.* (2008) with a minor modification. A known weight of the sample containing 16 mg nitrogen was taken in triplicate and digested with 1mg pepsin in 15 ml of 0.1M HCl at 37^{o}C for 3 h. The reaction was stopped by addition of 15ml of 10% trichloroacetic acid (TCA), the mixture was then filtered quantitatively through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-Kjeldahl method. Digestibility was obtained by using the following equation:

$$\text{Protein digestibility \%} = \frac{\text{N in supernatant - enzyme N}}{\text{N in sample}} \text{ X } 100$$

Minerals and heavy metal determination

Minerals were determining according to Kirk and Sawyer (1991). Weight the sample and ash was prepared in muffle furnace. The stock solution was prepared by using hydrochloric acid and then minerals and heavy metals were determined by using the Atomic Absorption Spectrophotometer (AAS), model: Thermo scientific, ICE 3000 series.

Determination of B vitamins by UPLC-MS-MS

Water soluble vitamins were determined according to Shanaz *et al.* (2009) and Evelyn (2010) by UPLC-MS-MS (model: Thermo scientific, ultimate 3000) with some modification.

Materials

Formic acid (BDH, Anala R) and methanol (BDH, Anala R) were used. Vitamin standard were purchased from Sigma-Aldrich. Acetic acid (BDH, Anal R), HCl (reagent grade) and water (deionized) were used.

LC condition

LC System: Acquity UPLC system, Column: Acquity UPLC C 18, 2.1 X 50 mm, 1.8 µm, Column temperature: 40°C, Sample temperature: 4°C, Flow rate: 0.4 ml/min. Mobile Phase A: 0.1% formic acid in water, Mobile Phase B: 0.1% formic acid in ACN, Total runtime: 5.0 min, Injection Volume is 10.0 micro liters, full loop.

MS condition

MS System: Xevo TQ MS, Ionization: ESI Positive, Capillary Voltage: 1.0 KV, Source Temperature: 130°C, Desolvation Temperature: 450°C, Desolvation gas : 900 L/hr, Acquisition: Multiple reaction monitoring (MRM) with RADAR full scan, Collision gas: Argon at $3.5x10^{-3}$ mhb.

Preparation of standard solution

The standards (each) were freshly prepared by dissolving into acetic acid and methanol. The contents were diluted to volume with water. Prior to injecting into the liquid chromatograph, the solution was filtered through 0.45 micrometer membrane filter.

Preparation of sample solution

The sample was refluxed for 15 minutes on boiling water bath using 0.1N hydrochloric acid and water.

Table 1. The MRM transitions, cone voltages, and collision energies for the analyzed compounds, along with expected retention times

Analyte	Parent (m/z)	Dau 1/Dau 2 (m/z)	CV (V)	CE 1/ CE 2 (eV)	RT (min)
Ascorbic acid,C	177.0	141.0	16	8	0.37
Thiamine, B1	265.2	122.0	18	16	0.41
Pyridoxal, B6	168.0	150.0	14	14	0.64
Nicotonic acid, B3	124.0	80.2	34	20	0.51
Pantothenic acid, B5	220.1	90.0	20	14	2.73
Folic acid, B9	442.2	295.1	18	16	2.99
Biotin, B7	245.1	227.0	20	14	3.10
Riboflavin, B2	377.2	243.1	36	24	3.15

The content was centrifuged and the supernatant was filtered through filter paper followed by 0.45 µm membrane filter before inject into LC system.

Acquisition and processing method

Data were acquired using MassLynx software, v.4.1 and processed using TargetLynx Application manager. IntelliStart Technology was used to automatically develop fully optimized MRM acquisition methods for the vitamin compounds targated in this analysis. Two MRM transitions were optimized for each vitamin compound. The dwell times for the transitions were automatically optimized to give a minimum of 12 point across each chromatographic peak for reproducible quantitation. The MRM transitions, cone voltages, and collision energies for the analyzed compounds, along with expected retention times, are shown in table 1.

Microbiological analysis

Microbiological analysis was done according to Shakir *et al.* (2009). Isolation and enumeration of bacteria were done by observing growth in selective media. For Standard Plate Count portion of cheese were diluted as 1:10 using sterile phosphate buffer which were subsequently diluted with the same as needed and then enumerated for total viable count using nutrient agar. Since this is a onetime study, 3-6 samples were taken and surface plates were made in triplicates in appropriate selective media. Bacterial isolation was performed by pour plate method and fungal isolation was performed by spread plate method. Both bacterial and fungal enumerations were expressed as colony forming units (cfu) per gm. In all the cases counts were made up to 48 hours.

Total coliforms were detected by MPN procedure according to standard method (APHA, 1985). Presence of faecal coliforms were determined using Brilliant Green Lactose Bile broth (44.50ºC for 48 hours), followed by confirmation of gas positive tubes using Eosin methylene Blue agar. Inoculated plates were incubated at requisite time temperature combinations (FAO, 1917; FDA, 2001).

For detecting the presence of *Salmonella* 25 g of cheese were preenriched in Lactose Broth and incubated for 24 hours, then one loopful Lactose Broth was transferred to Selenite Broth and after incubation at 37°C for 24 hours one loopful from Selenite Broth were streaked on Bismuth Sulfite Agar. After incubation for 24 hours positive characteristics colonies were biochemically confirmed the presence of *Salmonella.* In all cases for confirmation of the pathogens typical colonies were identified on the basis of cultural, microscopic analysis and biochemical characteristics. All the media were obtained from Himedia Laboratories Limited, India.

Statistical analysis

The significance of difference between means was determined by student's t test where the values of $p < 0.05$ were considered significant and those of $p < 0.01$ & $P < 0.001$ were highly significant. Calculated value of t was determined by using software.

Results and Discussion

Proximate and other chemical analysis

Results proximate and other chemical analysis are shown in table 2. Moisture, ash, fat, total carbohydrate, total energy, pH, total chlorides and Total solids are significantly higher in cow milk cheese than soya cheese on the other hand protein is slightly higher in soya cheese on fresh basis. Fat is dramatically higher in cow milk cheese. The public is now fully aware of the risks in relation to atherosclerosis and other diseases. Total fat intake may influence some of the major risk factors for coronary heart disease, particularly through its impact on obesity and type II diabetes. Recent studies have shown that a high-fat meal may also impair vaso-activity and transiently impair endothelial function (Metha and Swinburn, 2001). Fat intake is a much smaller contributor to coronary heart disease than the type of fat (Kennel, 1986). So people may reduce the risk of animal fat by consuming this soya product.

This soya product contains 58.95% protein on dry basis. Daily consumption of 25 g soy protein (6.5 g of soy protein per serving) may lower LDL cholesterol in individuals who have high cholesterol and who also adhere to a low-fat diet (Nicolas and Lawrence, 2007). The animal data on hypocholesterolemic effects of soy proteins have been confirmed by numerous human studies. Although statistically significant, the decreases are only 6-12% of total cholesterol. A possible mechanism of the cholesterol-lowering effect of soy protein is its ability to modulate LDL receptor levels in the liver (Mendel and David, 2001).

In recent studies, soy protein contributed to the control of hyperglycemia and reduced body weight,

Table 2. Proximate and other chemical analysis (means ± SD)

Parameters	Soya cheese	Cow milk cheese
Moisture %	65.43±0.76	45.24±0.83**
Ash %	3.52±0.17	4.21±0.02*
Protein %	20.38±0.53	20.19±0.93 NS
Fat %	3.21±0.29	16.63±0.34***
Crude fibre %	ND	ND
Total carbohydrate %	7.47±0.78	13.73±1.96*
Lactose %	ND	0.05±0.005
Energy (kcal)/100 gm	140±3.40	285.35±1.59***
Acidity (as lactic acid)%	0.56±0.04	0.67±0.02 NS
pH	4.11±0.03	4.82±0.015***
Salt %	1.67±0.1	2.20±0.15 NS
Total chlorides %	0.03±.002	0.05±0.001*
Total solid %	34.57±0.76	54.76±0.83**
in vitro protein digestibility %	55.11±0.81	46.63±0.71***

Degree of freedom is 4 in all cases; * P < 0.05, *** P < 0.001 and ** P < 0.01 significant when compared to cow milk cheese group versus soy cheese (Student's t- test).
NS = Non significant
ND= Not detected

Table 3. The concentrations of Minerals and heavy metals in Cheese and comparison with cow milk cheese in mg/100 g (means ± SD)

Minerals / Heavy metals	Cheese (Fresh weight)	Cheese (Dry weight)	Raw soy (Dry weight)	Cow milk cheese (Dry weight)
Calcium	190.82±0.95	621.91±3.33	255.135±7.09**	1284.30±4.68***
Magnesium	55.165±0.09	179.745±0.30	276.5±4.94*	78.17±1.06**
Iron	1.915±0.007	6.24±0.02	13.42±1.24 NS	2.42±0.02**
Potassium	53.36525±1.7	173.86±5.52	1103.225±3.10	156.58±4.33*
Zinc	1.8646±1.74	9.315±1.11	8.16±0.15NS	6.14±0.35 NS
Copper	0.73±0.01	2.375±0.04	2.29±0.21NS	0.25±0.03*
Sodium	619.4549±1.62	2018.405±5.29	30.235±3.52***	1239.34±18.09*
Manganese	0.7887±0.03	2.555±0.12	2.68±0.002	0.09±0.007
Cadmium	ND	ND	ND	ND
Arsenic	ND	ND	ND	ND
Chromium	ND	ND	ND	ND
Lead	ND	ND	ND	ND

Degree of freedom is 2 in all cases; * P < 0.05, *** P < 0.001 and ** P < 0.01 significant when compared to cow milk cheese group versus soy cheese & soy cheese versus raw soya in dry basis (Student's t- test).
NS = Non significant
ND= Not detected

hyperlipidemia, and hyperinsulinemia (Bhathena and Velasquez, 2002). These characteristics may be useful to both non diabetic and diabetic persons in the control of obesity and blood sugar. The U.S. Food and Drug Administration (FDA) have approved the use of health claims on product labels about the role of soy protein in reducing the risk of coronary heart disease (Mendel and David, 2001). This soya product contains 58.95% protein on dry basis and this protein may provide above physiological activities for human body. Crude fiber was not found in both soya cheese and cow milk cheese. Lactose is present in cow milk cheese but not in soya cheese.

Yield calculation of soya cheese

Cheese yield, total solid yield and protein yield are 135%, 51.18% and 72.33% respectively.

In vitro protein digestibility (IVPD) by pepsin

Protein digestibility is one of the important factors that determine protein quality. It is mainly related to the release and availability of amino acids for absorption in the small intestine. Pepsin digestibility of soya cheese and cow milk cheese were 55.11 ± 0.81and 46.63 ± 0.71 respectively (Table 3).The digestibility of protein is significantly ($p < 0.001$) higher in soya cheese than cow milk cheese. The higher value of digestibility indicates the good quality of protein. So we may conclude that the protein quality is good in soya cheese than cow milk cheese.

Minerals and heavy metals

Minerals and trace elements represent less than one-half of one percent of the total nutrients we consume every day, and yet without them, our bodies would be unable to efficiently use the carbohydrates, proteins, and fats in our diet. Minerals play an essential role in the body. Many vitamins and enzymes need a mineral cofactor for proper function.

Minerals and heavy metals are analyzed by atomic absorption spectrophotometer and showed that 190.82 ± 0.95 mg calcium, 55.165 ± 0.091 mg magnesium, 1.915 ± 0.007 mg irons, 53.36525 ± 1.7 mg potassium, 1.8646 ± 1.747119 mg zinc, 0.73 ± 0.01 mg copper, 619.4549±1.62 mg sodium and 0.7887 ± 0.03 mg Manganese are present in 100 gm fresh soy cheese and 621.91 ± 3.33 mg calcium, 179.745 ± 0.30 mg magnesium, 6.24 ± 0.02 mg irons, 173.86 ± 5.52 mg potassium, 9.315 ± 1.11 mg zinc, 2.375 ± 0.04 mg copper, 2018.405 ± 5.29 mg sodium and 2.555 ± 0.12 mg Manganese are present in 100 gm dry soy cheese (table 3).The contents of minerals in dry cow milk cheese and raw soya are also sown in table 3. Heavy metals (cadmium, chromium, lead and arsenic) were not detected in the sample, raw soya and cow milk cheese.

Cheese is a rich source of bioavailable calcium. The health aspect of cheese mainly focuses on the role played by this specific mineral, but at the same time, specific roles played by other ingredients such as protein, bioactive peptides, and lipids cannot be ignored. Calcium is highly significantly ($p < 0.001$) in cow milk cheese and we got only calcium that is higher in cow milk cheese but soy cheese also contain remarkable amount of calcium. The role of calcium in bone health is well documented and recognized (Heaney, 2000). As discussed above, cheese can provide a significant amount of calcium, especially in the diet of lactose-intolerant individuals. Kato *et al.* (2002) could even show, in their experiments with rats, that milk calcium taken with cheese is even better absorbed than milk calcium taken without cheese. And as a consequence, the bone mineral density of the rats fed milk calcium with cheese was significantly higher than in the control group.

Sodium is significantly ($p < 0.05$) higher in soya cheese than cow milk cheese due to soaking of soya cheese in sodium salt with citric acid. Sodium has some adverse effect to the hypertensive patients. In

Table 4. The concentrations of B vitamin in cheese and comparison with cow milk cheese in µg/100 g (means ± SD)

Vitamins	Cheese (Fresh weight)	Soya cheese (Dry weight)	Raw soy (Dry weight)	Cow milk cheese (Dry weight)
Vitamin B1	0.743333±0.02	2.423333±0.05	821.73333±27.34*	32.7733±4.09*
Vitamin B2	1.55±0.01	5.063333±0.02	217.7±30.43**	261.0233±24.40*
Vitamin B3	3.463333±0.10	11.28±0.35	2870.267±117.05*	ND
Pantothenic acid	0.406667±0.02	1.323333±0.05	3562.767±33.35*	33.90±4.02*
Folic acid	15.14333±3.05	212.3723±9.80	82.26±2.08**	ND
D Biotin	19.47333±1.23	63.48±3.98	64.98633±3.54 NS	60.9133±9.71 NS

Degree of freedom is 4 in all cases; *P < 0.01 significant when compared to dry soya cheese group versus cow milk cheese and * P < 0.001 and ** P < 0.01 significant compared to row soya bean group versus dry cheese (Student's t- test). NS = Non significant

addition to raising the blood pressure dietary salt is responsible for several other harmful effects. The most important are a number which, though independent of the arterial pressure, also harm the cardiovascular system. A high salt intake increases the mass of the left ventricle, thickens and stiffens conduit arteries and thickens and narrows resistance arteries, including the coronary and renal arteries. It also increases the number of strokes, the severity of cardiac failure and the tendency for platelets to aggregate. In renal disease, a high salt intake accelerates the rate of renal functional deterioration. Apart from its effect on the cardiovascular system dietary salt has an effect on calcium and bone metabolism, which underlies the finding that in postmenopausal women salt intake controls bone density of the upper femur and pelvis. Dietary salt controls the incidence of carcinoma of the stomach and there is some evidence which suggests that salt is associated with the severity of asthma in male asthmatic subjects (Wardener and Macgregor, 2000). So this product should taken cautiously by hypertensive patients.

Magnesium and iron are also significantly ($p < 0.01$) higher in soya cheese than cow milk cheese. Magnesium is essential for the formation and maintenance of healthy bones and teeth where 70 percent of the body's magnesium is found (Ilich and Kerstetter, 2000). It is involved in the metabolism of carbohydrates and amino acids, and plays an important role in neuromuscular contractions. It is also an activator of hundreds of enzymes essential to life.

Other minerals such as copper, manganese and potassium are significantly ($p < 0.05$) higher in soya cheese than cow milk cheese in Bangladesh and zinc is also higher in soya cheese but not significant (Table 3). We concluded that these mineral is not destroyed during the development of soya cheese. Copper is essential for enzymes that help to synthesise collagen. Also, copper is a critical component of the enzyme superoxide dismutase (SOD), an important antioxidant in cell cytoplasm, and acts as a catalyst in the formation of hemoglobin (Harris, 2000). Zinc is a component of hundreds of enzymes. It is

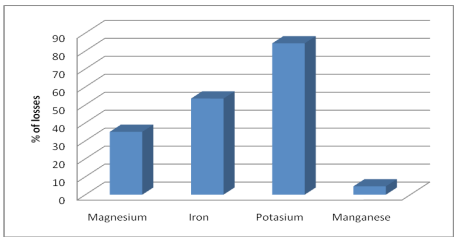

Figure 1. % Of minerals loss in cheese when compared to raw soya

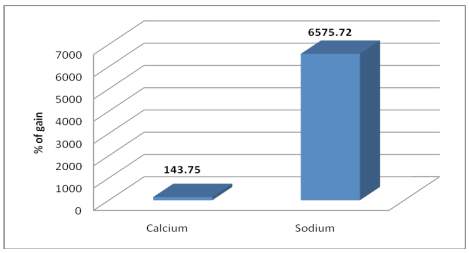

Figure 2. % Of minerals gain in cheese when compared to raw soya

associated with enzymes involved in carbohydrate, fat, and protein metabolism, as well as DNA and RNA replication. Zinc functions as an antioxidant, aids in maintaining healthy bone structure development (Saltman and Strause, 1993), maintains healthy immune functions, maintains healthy vision, and supports normal foetal growth (Simmer *et al*., 1991).

Heavy metals like mercury, arsenic, lead and chromium are also analyzed by atomic absorption spectrophotometer and they are not present in this product and cow milk cheese. The percentage of losses of magnesium, iron, Potassium, and manganese is 34.99, 53.50, 84.24, and 4.66 respectively (Figure 1). So we concluded that these minerals are losses due to boiling of soy milk or drainage of raw material with water or due to dehulling of raw soya. Again the content of calcium and sodium are gain after processing (Figure 2) of raw soya. This may be occurs because of using calcium chloride and sodium chloride during the processing of cheese from raw soya.

B vitamins

Water soluble vitamin analysis data of the two samples are shown in Table 4. Vitamins are analyzed by UPLC-MS-MS and the concentration of water soluble vitamins in 100 gm cheese are 2.423333 ± 0.05 µg thiamine, 5.063333 ± 0.02 µg riboflavin, 11.28 ± 0.35 µg niacin, 1.323333 ± 0.05 µg pantothenic acid, 212.3723 ± 9.80 µg Folic acid and 63.48 ± 3.98 µg biotin on dry basis of soy cheese (Table 4). The content of vitamins in fresh cheese, raw soya and dry cow milk cheese also in table 4. Vitamin B3 and folic acid was not found in cow milk cheese and other vitamin were found in cow milk cheese and that were significantly ($p < 0.01$, $n = 3$) higher in cow milk

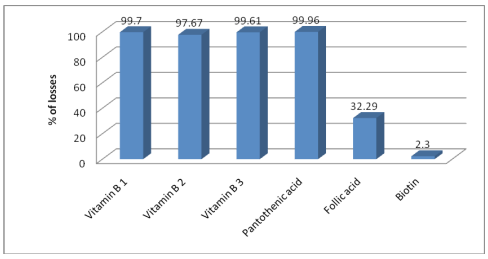

Figure 3. Losses of vitamin after processing when compared to raw soya

cheese than soya cheese.

The amount of biotin in soya cheese is 63.48 ± 3.98 µg (n = 3) per 100 gm dry cheese. Biotin is an essential micronutrient for normal cellular function, growth, and development. Biotin deficiency leads to pathologic, dermatologic, and neurocutaneous manifestations in skin and its appendages. Biotin is involved in important metabolic pathways such as gluconeogenesis, fatty acid synthesis, and amino acid catabolism. Biotin regulates the catabolic enzyme propionyl-CoA carboxylase at the posttranscriptional level whereas the holo-carboxylase synthetase is regulated at the transcriptional level. It is well documented that biotin deficiencies in humans cause pathologic changes in the skin and its appendages such as desquamative dermatitis and alopecia (Franziska *et al.*, 2003). Biotin therapy rapidly reverses the skin and hair abnormalities in the human congenital disorders and in exogenous biotin deficiency in humans (Tsao and Kien, 2002). In addition, pharmacologic doses of biotin have been shown to improve the quality of nails and hair in humans in the absence of apparent biotin deficiency. Likewise, pantothenic acid and its derivatives are beneficial in the maintenance of healthy skin and for cellular wound healing processes (Ebner *et al*., 2002)

Prodanov *et al*. (2004) have studied the effects of soaking in different solutions, and cooking, on thiamine, riboflavin and niacin contents of legumes. The authors have proved that vitamin losses depend on conditions in which technological process is conducted. The vitamin losses can reach 51–61% for thiamine, 66% for riboflavin and 61–78% for niacin in different kinds of legume. The losses of water soluble vitamins in cheese is 99.70% thiamine, 97.67% riboflavin, 99.61% B 3, 99.96% pantothenic acid, 2.30% biotin and 32.29% folic acid (Figure 3). This is due to the processing of raw soya. We may conclude by this study that cooking, coagulating and dehulling also losses B vitamins during processing of soya cheese from soya.

Microbiological analysis

Microbial count of cheese was also evaluated. The product was tested for several times in two week intervals for two month. Standard plate count, total fungus and total coliform, Salmonella were tested. Total coliform and Salmonella were absent in first two month after manufacturing and Standard plate count and total fungus were <10 CFU/gm. From the microbiological point of view the product is acceptable in quality because no significant amount of microorganisms were found in the product within two month.

Conclusion

Humans need a wide range of nutrients to lead a healthy and active life. The required nutrients for different physiological groups can only be derived from a well balanced diet. Components of the diet must be chosen judiciously to provide all the nutrients to meet the human requirements in proper proportions for the different physiological activities. Now soybean is cultivated in rural area of Bangladesh. Soybean (*Glycine max*) is one of the good sources of high quality oil and protein can play an important role in solving the malnutrition problem of Bangladesh. Soybean is a crop which can produce high quality and highest quantity of protein (seed contains 38.04% proteins according to this study). Low income people can easily fill up their protein demand by consuming this soya cheese because cost of soya cheese is will be lower than cow milk cheese (total soya cheese yield is 135%, table 4). As per we know soya cheese industry is not present in Bangladesh. So this project may help the industrialist to develop this new soya product by using cultivated soya. We concluded that lactose free, low fat and low carbohydrate containing this soya product may provide the minerals, vitamin and highly digestible protein for the different physiological activities.

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