Nutritional Quality, Sensory Evaluation, Phytochemicals Analyses and In-Vitro Antioxidant Activity of the Newly Developed Soy Ice Cream

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Abstract: The aim of the study was to develop the soy milk based ice cream and analysis of proximate, minerals, sensory evaluation, phytochemicals and in - vitro antioxidant activity of developed product and compared with traditional market brand ice cream. The compositions of developed ice cream are soy milk 65%, sugar 27%, oil 7%, salt 0.2 % and carboxy methyl cellulose 0.3%. It was found that 100 grams of soy ice cream has 57.1 ± 1.37 % moisture, $6.98\pm.04$% fats, 5.47 ± 0.45% protein, 29.33 ± 1.46% carbohydrate, 0.011 ± 0.001% fibre, 0.51 ± 0.02% ash and $27.4^{\dagger}0 \pm 0.61$% sucrose. The protein content is significantly ($p< 0.01$) higher and fat content is significantly ($p< 0.01$) lower in soya ice cream than traditional ice cream. Analysis for minerals and heavy metals content was carried out using the Atomic Absorption Spectrophotometer (AAS). 100 gm of soy ice cream contain 25.59 ± 0.43 mg calcium, 5.46 ± 0.96 mg iron, 11.16 ± 0.48 mg magnesium, 0.80 ± 0.07 mg zinc, 17.35 ± 0.70 mg potassium, 86.43 ± 0.71mg sodium 0.24 ± 0.01mg manganese, and 0.56 ± 0.03 mg copper. Heavy metals were not present in developed product. Sensory attributes of this product is very much acceptable. Phytochemicals and in- vitro antioxidant models studied were the total antioxidant capacity by phosphomolybdenum method, FRAP assay , 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and reducing power done by spectrophotometrically. The contents of phenolics, flavonoids and tannin are 23.69 ± 0.45 mg tannic acid equivalent, 14.57 ± 0.45mg catechin equivalent and 3.81 ± 027 mg catechin equivalent in 100 gm sample respectively. Flavonoids and tannin were not present in traditional ice cream. The total antioxidant capacity is 505.60 ± 9.77 mg ascorbic acid equivalent in phosphomolybdenum method and 62.13 ± 1.05 μ mol ascorbic acid/100 gm fresh soy ice cream in FRAP assay and IC_{50} of soy ice cream in DPPH assay is 156.24 mg/ml whereas IC 50 of standard ascorbic acid in DPPH assay is 5.69 µg/ml but traditional ice cream has no DPPH free radical scavenging activity. The IC_{50} in reducing power assay of soya ice cream, traditional ice – cream and standard ascorbic acid were 197.51 ± 0.83 mg, 228.98 ± 1.74 mg (fresh weight) and 31.12 ± 0.13 mg respectively. Total anti oxidant activity and phytochemicals are significantly ($p< 0.001$ and $p< 0.01$) higher in soya ice cream than the traditional ice cream. The experiments repeated three times in all cases. The findings are soya ice cream is better than traditional ice cream.

Keywords: Soy ice cream, Proximate, Minerals, Antioxidant, Phytochemicals and $IC_{50.}$

I. INTRODUCTION

Ice-cream is a frozen mixture of milk components, sweeteners, stabilisers, flavourings and other ingredients. Ice cream ingredients, especially milk fat and milk solid, are used for product classification in accordance with legislation (Wiwat, 2012). There are many kind of ice cream are present in the world. But vegetarians and health enthusiasts have known for years that foods rich in soy protein offer a good alternative to meat, poultry, and other animal-based products. As consumers have pursued healthier lifestyles in recent years, consumption of soy foods has risen steadily, encouraged by scientific studies showing health benefits from these products. There are many soy-based frozen desserts in the market, but there is no evidence of a commercial frozen dessert formulated with

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soy. Because the non-dairy frozen desserts lack the milk-fat, which makes the product creamier that are often related with soy-based frozen desserts.

Soybeans are the most important food source of isoflavones, which have been associated with beneficial health effects in humans, including prevention of cancer, cardiovascular diseases, osteoporosis, and relief of menopausal symptoms (Messina and Lane, 2007). There are 12 isoflavones in soybeans and soy products, 3 free aglycones (genistein, daidzein, and glycitein) and their respective glucosidic, malonyl, and acetylglucosidic conjugates (Murphy *et al.*,). In addition, soya milk is a liquid extract of soya bean, a good dietary source containing almost all components of soya bean which are beneficial to health, such as peptide and protein, lectin, trypsin inhibitor, dietary fibre, oligosaccharide, phytin, saponin, isoflavone, linoleic acid, linolenic acid, lecithin, tocopherol, plant sterol, vitamin K and minerals (Wiwat, 2012).

Plants consumed by humans may contain many different phenolic compounds and soya is special one from these plants. Dietary phenolics have antioxidative and possible anticarcinogenic activities. A popular belief is that dietary phenolics are anticarcinogens because they are antioxidants, but direct evidence supporting this supposition is lacking (Yang, 2001). Phenolics may inhibit carcinogenesis by affecting the molecular events in the initiation, promotion, and progression stages. The bioavailability of the dietary phenolics has been discussed extensively, because the tissue levels of the effective compounds determine the biological activity. Epidemiological studies concerning consumption of phenolics and human cancer risk suggest the protective effects of certain food items and phenolics, but more studies are needed to reach clear-cut conclusions (Takahashi, 2005). Several approaches have been undertaken to include more soya bean in the diet for better health of the people and new soya recipes have been developed.

The aim of the study was to develop new soya ice cream and determination of proximate, minerals, sensory evaluation, phytochemicals and in-vitro antioxidant activity of soy-ice cream and compare with traditional ice cream available in Bangladesh.

II. MATERIALS AND METHODS

2.1. Chemicals

Chemicals are collected from Merck Germany, sigma Aldrich, BDH UK, Merck India, etc. and all chemicals are analytical grade.

2.2. Samples

Traditional ice cream 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 by heating. The ingredients of soya ice cream are Soya milk 65.0%, Sugar 25 .0%, Oil 5.0%, Salt 0.2% and CMC 0.3%. Color and flavor are added in adequate amount. The mixture was pasteurized, homogenized and held overnight in a refrigerator before freezing, together with vigorous agitation until it formed a semi-solid consistency. The ice cream was then packaged and placed into a freezer for hardening and storing (Wiwat, 2008).

2.3. Extraction of the Sample

A total of near about 0.5-1.0 g of soy ice cream was extracted by vortex, mechanical shaking for four hours and finally sonication for 20 minutes with 50 ml methanol. Methanol extract was obtained by filtering the mixture through Whatman No. 1 filter paper and the supernatant was used in the experiment. The extraction repeated three times.

2.4. Proximate and Other Chemical Analysis

Determination of Moisture Content

The method described by Pearson (1999) 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 Pearson (1999). 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 Pearson (1999) was used. The nitrogen content was multiplied by 6.25 (conversion factor) to obtain the percentage protein for soya ice-cream and 6.38 for traditional ice-cream 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 (Pearson, 1999). 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 Pearson (1999). 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.

2.5. Carbohydrate Estimation

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

2.6. 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.

2.7. Determination of Sucrose

Sucrose was determined by the copper reduction method (Pearson, 1999).Extraction of sugar was carried out by 50 % alcohol and clearing agents were lead acetate and di -potassium oxalate. Inversion of the sugar was done by using hydrochloric acid and then mild heating in water bath. Finally dilute to mark, filter and determine the sucrose by Lane and Eynone's method using standard Fehling solution. Sucrose % was determined by following formula.

Sucrose % = (TI-BI) X 0.95

Where BI is % of reducing sugars before inversion and TI is % of reducing sugars after inversion.

2.8. Determination of Lactose

Lactose was determined by the copper reduction method (Pearson, 1999). 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.

2.9. Total Solid Content

Total solid content of the both ice cream were determined gravimetrically by drying a sample to constant weight in an oven at 105ºC. Ice cream samples were 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 (Method 33.2.44; 990.20, AOAC 2006).

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

2.10. Minerals and Heavy Metal Determination

Minerals were determining according to Pearson's, 1999 .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.

2.11. Sensory Evaluation

The sensory evaluation of the 'soya ice cream' was conducted at the director meeting room in presence of our honorable director, IFST. The experiment conducted by quality control section chief, IFST according to Isong *et al.*, 2013. Scientists from the institute of food science and technology (IFST) were randomly selected to evaluate the likeness of soya ice cream. The nine hedonic scale where nine was the highest and one the lowest scores was employed. The rating scale for the degree of likeness were as follows: 9 - like extremely; 8 - like very much, 7 - like moderately; 6 - like slightly, 5 - neither like nor dislike, 4 - dislike slightly, 3 - dislike moderately; 2 - dislike very much, and 1 - dislike extremely. 'Soya ice cream' was presented to the judges in white plastic plates.

Tap water was provided for the judges to rinse their mouth in between evaluation. The judges evaluated "soya ice cream' for appearance color, texture, flavor, softness, taste and overall acceptance.

2.12. Phytochemicals and In-Vitro Antioxidant Activity Analysis

Total Phenolic Content (TPC)

The amount of total phenol content can be determined by Folin-Ciocateu reagent method (McDonald *et al.*, 2001). 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) are mixed and incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate was added and further incubated for 30 min at room temperature and absorbance measured at 760 nm spectrophotometrically (Specord 205, double beam). Tannic acid (Wolfe *et al.*, 2003 was used as positive controls. The total phenolic content is expressed in terms of standard equivalent (mgg-1 of extracted compound).

2.13. Total Flavanoid Determination

Total flavonoid content was determined by Aluminium chloride method (Chang *et al.*, 2002) using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). 5 min after adding 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically (Specord 205, double beam).Total flavonoid content was calculated as catechin (mg/100g) using the following equation based on the calibration curve: $y = 574.4x - 2.171$, $R2 = 0.992$, where x was the absorbance and y was the catechin concentration.

2.14. Tannin Content Determination

Quantitative estimation of tannin was carried out using the modified vanillin– HCl method (Abdelseed *et al.*, 2011). Vanillin reagent (0.5%, 5 ml) was added to the extract (1 ml) and the absorbance of the color developed after 20 minutes at $30^{o}C$ was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg 100 g-1) which gives a colour intensity equivalent to that given by tannin after correcting for blank. Tannin content was calculated as catechin (mg/100g) using the following equation based on the calibration curve: $y = 1298x +2.954$, $R2 = 0.996$, where x was the absorbance and y was the catechin concentration.

2.15. Determination of Total Antioxidant Capacity

The determination of total antioxidant activity was done as per the phosphomolybdenum method with some modifications (Alakh *et al.,* 2011). The basic principle of the assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate Mo (V) complex at acidic pH. 0.3 ml extract was combined with a mixture of 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were then capped and incubated at 95 °C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the solution was then measured at 695 nm against blank. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the mg of equivalents of ascorbic acid.

2.16. Determination of Ferric Reducing Antioxidant Power Assay (FRAP)

FRAP assay was carried out according to the method of Maizura *et al.* (2011) with some modification. FRAP reagent was prepared from acetate buffer (1.6 g sodium acetate and 8 ml acetic acid make up to 500 ml) (pH 3.6), 10 mM TPTZ solution in 40 mM HCL and 20 mM iron (III) chloride solution in proportion of 10:1:1 (v/v) respectively. The FRAP reagent was prepared fresh daily and was warmed to 37oC in oven prior to use. A total of 200 µl samples extract were added to 4 ml of the FRAP reagent and mixed well. The absorbance was measured at 593 nm using

using UV-visible spectrophotometer (Specord 205, double beam). Samples were measured in three replicates. Standard curve of ascorbic acid (125 μmol, 250 μmol, 500 μmol, 750 μmol and 1000 μmol) was prepared using the similar procedure. The results were expressed as μmol ascorbic acid /g extract sample.

2.17. Free Radical Scavenging Activity

1,1_-Diphenyl- _-picryl-hydrazyl radical scavenging (DPPH) Assay. The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the nonradical form DPPH-H (Blois, 1958). This transformation results in a color change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple color is monitored at 517 nm. The free radical scavenging activity can be measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1-diphenyl-2-picryl-hydrazyl by the method of McCune and Johns (2002). The reaction mixture (3.0 ml) consists of 1.0 ml of DPPH in methanol (0.3 mM), 1.0 ml of the extract and 1.0 ml of methanol. It is incubated for 10 min in dark, and then the absorbance is measured at 517 nm. In this assay, the positive controls can be ascorbic acid (Blois, 1958)

The percentage of inhibition can be calculated using the formula:

Inhibition (%) = (A0 – A1 / A0) × 100

Where; A0 is the absorbance of control and A1 is the absorbance of test.

2.18. Reducing power (RP)

The reducing power can be determined by JAYANTHI *et al.*, 2011 method. Various concentrations of the plant extracts in corresponding solvents were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power. IC 50 is concentration of extract or standard to require absorbance is 0.50.

2.19. 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.

III. RESULTS AND DISCUSSION

3.1. Proximate and Other Chemical Analyses

The composition of developed ice cream is soy milk 65%, sugar 27%, oil 7%, salt 0.2 % and carboxy methyl cellulose 0.3%. It was found that 100 grams of soy ice cream has 57.1±1.37 % moisture, 6.98±.04% fats, 5.47±0.45% protein, 29.33±1.46% carbohydrate, 0.011±0.001% fibre, 0.51±0.02% ash, 42.90±1.37% total solid and 27.40 ± 0.61% sucrose. The energy is also calculated and 204±5.67 k calorie was found per 100 gm sample. On the other hand traditional market brand of ice cream contain 60.45±0.51 % moisture, 13.02±0.68% fats, 2.26±0.06% protein, 23.68±0.61% carbohydrate, 0.009±0.001 % fibre, 0.92±0.03% ash, 39.55±0.51 % total solid and 20.37±1.29% sucrose (Table 1). Lactose is not present in soya ice cream but the content in traditional ice cream is 4.37±0.13% (Table 1).

Table1. *Proximate and other chemical analysis (means ± SD, n = 3)*

Test parameters	Soya ice cream	Traditional ice cream (Market brand)
Moisture %	57.1±1.37	60.45±0.51 NS
Ash%	0.50±0.02	0.92±0.03*
protein%	5.47±0.45	2.26±0.06*
Fat%	6.98±0.04	13.02±0.68*
Crude fibre%	0.011±.001	0.009±0.001 NS
Carbohydrate %	29.93±1.46	23.68±0.61*

Sucrose %	27.40±0.61	20.37±1.29*
Lactose %	Absent	4.37±0.13
Total solid %	42.90±1.37	39.55±0.51 NS
Calorie (Kcal/100 gm)	204.45±5.67	220.94±3.89 NS

*Degree of freedom is 4 in all cases; * P < 0.01 significant when compared to market brand traditional ice cream group versus soy ice cream group (Student's t- test).*

NS = Non significant

Approximately 65-75% of people worldwide have decreased intestinal lactase levels, which may lead to lactose intolerance and difficulty digesting dairy products (Vesa *et al.*, 2000 and Suarez *et al.*, 1998). Lactose malabsorption occurs when lactose, the primary sugar in dairy products, is not completely digested and absorbed in the small bowel. Lactase, the enzyme required to hydrolyze lactose for intestinal absorption, is found primarily in tips of the villi in the jejunum (McCray, 2003).This soy product is lactose free and

3.2. Mineral Analyses of Soy Ice Cream

The mineral content of soybeans, determined as ash, is about five percent. When soybeans are processed, most of the mineral constituents go with the meal and few with the oil. The major mineral constituents are potassium, calcium and magnesium. The minor constituents comprise trace elements of nutritional importance, such as iron, zinc, copper etc.

Our soya ice cream contain remarkable amount of minerals and that are essential for our health. 100 gm of soy ice cream contain 25.59±0.43 mg calcium, 5.46±0.96 mg iron, 11.16±0.48 mg magnesium, 0.80±0.07 mg zinc, 17.35 ±0.70 mg potassium, 86.43±0.71mg sodium 0.24±0.01mg manganese, and 0.56±0.03 mg copper (Figure 1). Heavy metals such as arsenic, lead, chromium, stenus and cadmium are not present in developing product.
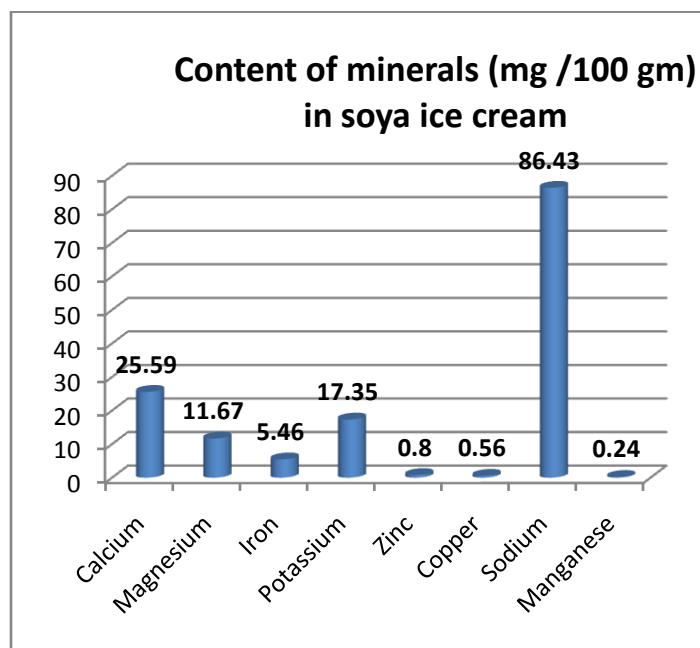

Figure1. *Minerals content in soya ice cream per 100 gm.*

3.3. Sensory Evaluation

The sensory evaluation showed that the product is very much acceptable (final comment from quality control section chief). Color, flavor and taste are like very much and texture and softness are like moderately.

3.4. Phytochemicals Analyses

The contents of phenolics, flavonoids and tannin are 23.69±0.45 mg tannic acid equivalent, 14.57±0.45mg catechin equivalent and 3.81±027 mg catechin equivalent in 100 gm soya ice cream sample respectively (Table 2) but

traditional ice cream contain very much low amount of total phenolics that are significantly ($p<0.001$) lower than soya ice cream. But flavonoids and tannin were not present in traditional ice c ream that is collected from local market.

Table2. *Content of phytochemicals in soy ice cream and traditional ice cream (mg/100 gm fresh weight).*

| Names of phytochemicals	Content (means ± SD) in soy ice cream.	Content (means ± SD) in traditional ice cream
Total phenolics	23.69±0.45	5.74±0.47*
Flavonoids	14.57±0.45	Absent
Tannins	3.81±0.27	Absent

*Degree of freedom is 4 in all cases; * P < 0.001 significant when compared to market brand traditional ice cream group versus soy ice cream group (Student's t- test). So Soy ice cream is better than traditional ice cream.*

3.5. Determination of Total Antioxidant Capacity

The total antioxidant capacity in the methanolic extracts of soy ice cream was determined using the linear regression equation ($y=55.45x+0.636$, $r2 = 0.997$ and where x is absorbance and y is ascorbic acid concentration in microgram) of the calibration curve (Figure 3) and was expressed as ascorbic acid equivalent. The total antioxidant capacity of the soya ice cream is 505.61 ±9.770109 mg Ascorbic acid equivalent/100g but the total antioxidant capacity of the traditional ice cream is 351.1033 ± 4.211666 mg Ascorbic acid equivalent/100g (Figure 2) and the capacity of soy ice cream is significantly ($p<0.01$) higher than traditional ice cream.
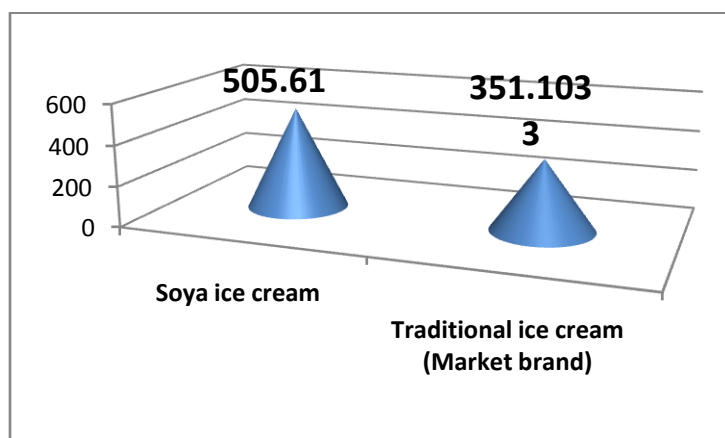
Figure2. *Graphical presentation of total antioxidant content in soya ice cream and traditional ice cream.*
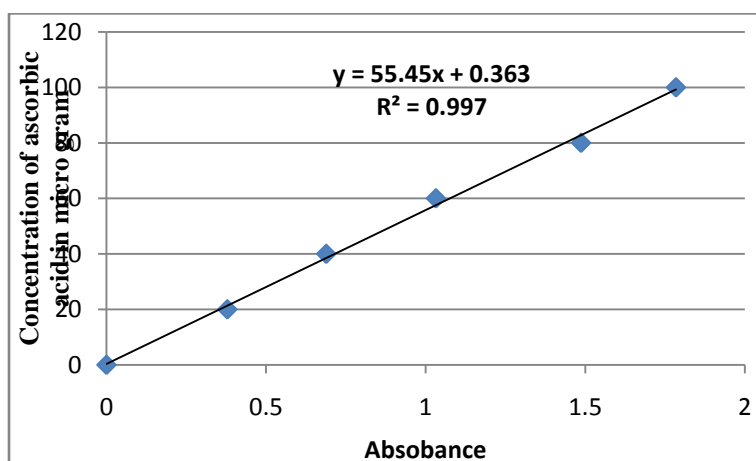
Figure3. *Standard calibration curve of ascorbic acid for total antioxidant determination*

3.6. Determination of Ferric Reducing Antioxidant Power Assay (FRAP)

In this study, the antioxidant activity is also determined on the basis of the ability of antioxidant in these ice cream extracts to reduce ferric (III) iron to ferrous (II) iron in FRAP reagent (Alothman *et al*., 2009; Wong *et al*., 2006).

Generally, FRAP assay was used due to its simplicity and reproducibility. The standard curve of FRAP assay was shown in figure 4. The antioxidant activity of ice cream extracts were 62.13 ± 1.05 μ mol ascorbic acid/100 gm fresh soy ice cream and 33.67 ± 2.08 μ mol ascorbic acid/100 gm traditional ice cream (n=3). Soy ice-cream has significantly higher antioxidant activity (p<0.01) than traditional ice cream. The ability to reduce Fe (III) may be attributed from hydrogen donation from phenolic compounds which is also related to presence of reductant agent. The higher absorbance of the reaction mixture indicated greater reducing power. The reducing properties are generally associated with the presence of different reductants. The antioxidant action of reductants is based on the breaking of the free radical chain by donating a hydrogen atom. Reductones also react with certain precursors of peroxide, thus preventing peroxide formation. The reductive power of different fractions of long bean extract and gallic acid may be the reason for their antioxidant activity (Emynur Shafekh *et al.*, 2012).
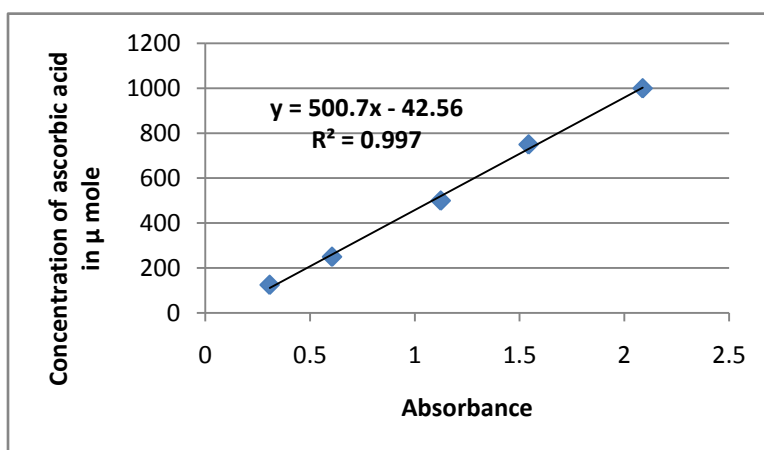

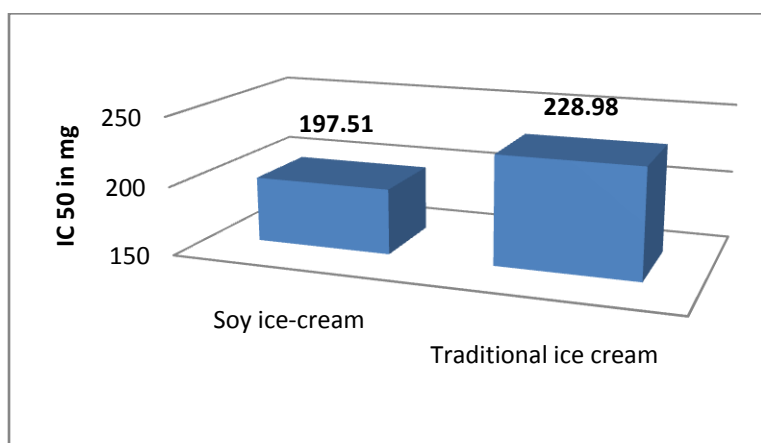**Figure4.** *Standard curve of FRAP assay*

Figure5. *Comparative reducing power of soy ice cream and traditional ice cream*

3.7. DPPH Free Radical Scavenging Assay

The IC 50 value soya ice cream in DPPH free radical scavenging method is 156.24 mg/ml (Table 4) and standard ascorbic acid is 5.69 microgram/ml (Table 3) but traditional ice cream has no DPPH free radical scavenging activity.

Radical scavenging activities are very important due to the deleterious role of free radicals in foods and in biological systems. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end-point. DPPH radical scavenging methods are common spectrophotometric procedure for determining the antioxidant capacities of component.

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances (O¨ zcelik *et al*., 2003). In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow coloured diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants (Oyaizu, 1986). DPPH

is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997).

With this method it was possible to determine the antiradical power of an antioxidant by measuring of a decrease in the absorbance of DPPH_ at 517 nm. Resulting a color change from purple to yellow, the absorbance decreased when the DPPH_ was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH_ molecule. In the radical form, this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.

A simple method utilizing the stable 2,2-diphenyl- 1-picrylhydrazyl (DPPH) radical has been developed to determine the antioxidant activity of natural products. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm.

Table3. *% of inhibition of DPPH free radical scavenging activity and IC 50 of ascorbic acid*

Concentration in µg	% of Inhibition	Linear regression equation [ascorbic acid concentration (µg) vs. % of inhibition]	IC 50 in µg/ml
5	66.99±2.13		
10	83.52±1.29		5.69
15	91.83±0.88	Y=3.368x + 30.85	
20	97.50±0.73		
25	97.92±0.44		

Table4. *% of inhibition of DPPH free radical scavenging activity and IC 50 of soya ice cream*

Concentration in mg	% of Inhibition	Linear regression equation [ice cream concentration (mg) vs. % of inhibition]	IC 50 in mg/ml
9.34	4.44±0.09		
18.68	7.95±0.17		
28.01	9.76±0.51	Y=0.314x + 0.942	156.24
37.35	11.75±0.11		
46.69	15.81±0.13		

3.8. Reducing power (RP)

The reducing power of methanolic extracts of ice-cream was found to be correlated with increasing absorbance (at 700 nm) as compared with ascorbic acid, a known antioxidant. The IC $_{50}$ of ascorbic acid is 31.12±0.13 (n=3) micro gram and the IC $_{50}$ of soya ice cream and traditional ice – cream were 197.51±0.83 and 228.98±1.74 mg (fresh weight) respectively. IC $_{50}$ of soya ice cream is significantly (p<0.01) lower than traditional ice cream. Lower IC $_{50}$ indicates the higher antioxidant activity. So soya ice cream has better reducing power than traditional market brand ice cream. Polyphenols in the soy ice-cream extracts appear to function as good electron and hydrogen atom donors and therefore should be capable of converting free radicals to more stable products. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay *et al.*, 2003). Soy ice-cream with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995).

Only soy milk was used in this soy product and soy milk contains good amounts of protein, polysaccharides and indigestible fibre, unsaturated fat and lecithin, vitamins and minerals, as well as bioactive organic molecules including polyphenols, such as phenolic acids, isoflavones, tannins and saponins (Anderson, 2000). The contents of phenolics, flavonoids and tannin are 23.69±0.45 mg tannic acid equivalent, 14.57±0.45mg catechin equivalent and 3.81±027 mg catechin equivalent in 100 gm soy ice cream respectively (Table 2). These phytochemicals may added from soyabean during the development of soya ice cream because soya bean contain secondary metabolites, such as, isoflavones, phyto-sterols, lecithins, saponins and many more (Ajay , 2011). Polyphenolic compounds have an important role in stabilizing lipid oxidation and areassociated with antioxidant activity (Cook, and Samman, 1996). Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) (Rice-Evans *et al.*, 1997). Phenolics acid possesses diverse biological activities, for instance, antiulcer, anti- inflammatory, antioxidant (Silva, 2007), cytotoxic and antitumor, antispasmodic, and antidepressant activities (Ghasemzadeh, 2010). We hope that this soya product may possess the above biological activities in human body when taking.

This product is a good source of flavonoid (Table 2) and flavonoids present in food of plant origin are also potential antioxidants (Satheeshkumar, 2011). The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for freeradical generation (Benavente-Garcia, 1997). Depending on their structure, flavonoids are able to scavenge practically all known ROS. The many pharmacological effects of phenolic compounds and flavonoids are linked to their ability to act as strong antioxidants and free radical scavengers, to chelate metals, and to interactwith enzymes, adenosine receptors, and biomembranes. It was reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds (Sathyaprabha, 2011).

Tannin is also found in this product and they show also antioxidant activity ().Several health benefits have been recognized for the intake of tannins and some epidemiological associations with the decreased frequency of chronic diseases have been established (Serrano, 2009).

IV. CONCLUSION

Lactose free and antioxidant based this non dairy soy ice cream provide a wide range of nutrients to lead a healthy and active life of human. 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 large amount of soybean is cultivated in rural area of Bangladesh .Soybean is one of the good sources of high quality oil and protein can play an important role in solving the malnutrition problem of Bangladesh. Low income people can easily fill up their protein demand by consuming this soya product because cost of this product is will be lower than traditional ice cream in local market. As per we know soya ice cream industry is not present in Bangladesh. So this project may help the industrialist to develop this new soya product by using local soybean. We concluded that lactose free, low fat and low carbohydrate containing this soya product may provide the protein, minerals, phytochemicals and antioxidant for the different physiological activities of human body.

V. ACKNOWLEDGEMENTS

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