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Physicochemical properties of SPI, inulin and stevia enriched ice-cream

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Abstract
In recent years consumers are directed their interest toward lowering fat and calorie content in food products and producing the healthier and therapeutic food products. In the present study Reduced fat, low calorie and protein rich Ice Cream was prepared by using soy protein isolates, inulin and stevia extract powder. The chemical and physical properties of Ice Cream samples were analyzed including fat, protein, carbohydrate, ash, moisture, total solid, titratable acidity, pH, overrun, viscosity, calorie value and melting rate. All chemical and physical properties of optimized Ice Cream was found significantly (P<0.05) different from the control sample except pH.

Keywords: Soy protein isolates (SPI), inulin, stevia, ice cream, physicochemical properties

Introduction

Ice cream has composite food structure with a dissolve phase. It consists of three main structural components (air bubbles, ice crystals and emulsified fat globules) which absorbed in a continuous liquid phase (unfrozen water with dissolved sugar, proteins and hydrocolloids) (Marshall *et al*., 2003; Clarke, 2004) [25, 10-11]. Characteristically, it contains 10–14% fat (Patel *et al*. 2010) [30], provide about 180–250 kcal/100 g.

Consumers have directed their demands to the food manufacturer for the development of new products with health promoting features. Nutritionists also recommended reducing the dietary animal fat (Kucukoner and Haque 2003) [23].

Soybeans contains many kind of nutrients includes proteins, vitamins, oligosaccharides, dietary fiber and trace minerals. Form the point of composition soybean has 40% protein, 20% oil contents, and 15% dietary fiber, 15% saccharides, and 10% others (Kim *et al*., 2005). It is also known as excellent source of high quality proteins and carbohydrates but free from lactose and cholesterol. Soybeans are considering an absolute source of protein for vegetarian population due to the high quality proteins (Romanchik-Cerpovicz *et al*. 2011) [34]. A range of soybean proteins are utilized in human foods such as flours, infant formulas, protein concentrates, protein isolates and textured fibers. Due to the positive effects like lowering of plasma cholesterol, prevention of cancer, diabetes, obesity and protection against bowel and kidney disease consumption of soy foods is increases. Soy protein isolate (SPI) is a kind of plant protein (90% protein content). Generally it is used as food supplement. SPI has many affirmative attributes for example gelling, swelling, foaming and solubility at the same time it has some negative features like low viscosity and limited mechanical strength (Nishinari *et al*. 2014) [29]. Emulsifying and gelling are very common properties of SPI which are utilized in the development of foodstuffs (Totosaus *et al*. 2002; Hua *et al*. 2003) [40, 21].

Milk fat plays an important role in the development of color and body and texture in the dairy products. In milk products and non-fat ice cream several defects such as deficiency of flavor, weak body and poor texture occurred due to the reduction in fat content (Haque and Ji 2003) [20]. Fat replacers can be used to solve some physical and sensory problems arising from fat reduction. The fat in ice creams can be reduced by partial replacement with lower energy ingredients (Sandoval-Castilla *et al*. 2004) [36]. Inulin is a linear non digestible polysaccharide of b-(2-1) linked fructose residues with a terminal glucose unit. As a soluble dietary fiber and sugar or fat replacer inulin can be utilizes in food industries and as a soluble dietary fiber in pharmaceutical industries. It is claimed that inulin have anticancer and immune-enhancing properties in particulate form (Barclay *et al*. 2010) [8]. Apart from its nutritional benefits, in the development of new foods for scientific properties it is used as sugar replacer or as fat replacer and texture modifier (Meyer *et al*. 2011) [27]. Fat mimetic properties of the inulin have been credited to its capability to bind water molecules and form a particle gel network (Franck 2002) [17]. In low fat or fat free foodstuffs like confectionery, chocolate, cheese and ice cream predominantly inulin is appropriate for fat replacement, since it gives gelling capacity and good body, fat creamy form, texture and mouth feels. Moreover, in aerated dairy desserts, ice cream, table spreads and sauces it improves the stability of foams and emulsions. Successful fat replacement using inulin was reported by Murphy (2001) [28] in most water-based foods. An increase in the viscosity and improvement in sensory properties was reported by Schaller-Povolny and Smith (1999) [37] in reduced fat ice cream containing inulin and also decrease in the freezing point. Effect of inulin and sugar levels on the physical and sensory characteristics in probiotic ice cream was studied by Akin *et al*. (2007) [5].

In the ice cream preparation an ample variety of sweeteners has been used. Most common and frequently sucrose is used in the ice cream manufacturing due to both economic and rheology reasons. On the other hand, due to high glycemic index (GI) sucrose has a lot of disadvantages. Because it is interrelated with, metabolic syndrome, diabetes mellitus (DM), obesity, hypertension, ischemic heart diseases and dental caries (Marshall and Arbuckle, 1996; Aliha *et al*., 2013) [32, 6]. Stevia is a short & shrubby plant which is grown in Amambi mountainous area of Brazil & Paraguay. It is a natural sweetener with 250 – 300 times sweeter as compared to sucrose. It is a good alternative of sugar for patients suffering from DM and other sedentary life related diseases due to the very low calorie content (Curry and Roberts, 2008) [12]. In addition to its sweetness properties, stevia prevents diabetes, decreases weight, prevents tooth decay, increases digestion etc (Rao and Giri 2009) [33].

In the dissolve phase of Ice Cream fat is a very essential component which plays an important role in melting, air stabilization and ice crystals formation (Chung *et al*., 2003; Clarke, 2004; Granger *et al*., 2005) [9, 10-1]. Dispersed and emulsified fat globules avoid coalescence and forming a film around air bubbles to stabilizing the system during aging (Chung *et al*., 2003) [9]. Fractional coalescence of ice crystals or protein which is also related to fat induces flocculation and affects ice cream texture (Flores and Goff, 1999) [16].

In ice cream manufacturing process, a huge number of physicochemical changes occurred during aging and air incorporation which are favored by the proteins and emulsifiers. This will stabilizing both emulsion and produced foam. The capability of the ingredients to act together with each other sustains the physical and sensory properties of the frozen ice cream base during and after the frozen process, cold-chain storage and lastly, when ice cream is consumed (Pintory Totosaus, 2013) [31].

Materials and methods

Preparation of Ice Cream

Ice cream mix was prepared by standardization of milk fat at 6% and 10% for control and experimental ice cream. All other ingredients such as skimmed milk powder, sugar, emulsifier and stabilizers were added. Another pre-mix for experimental ice cream was also prepared by adding non-dairy based ingredients including soy protein isolates, inulin and stevia in the required quantity and mix it thoroughly to dissolve these ingredients. Both the control and experimental ice cream mix was pasteurized at 82 ºC for 20 sec. and homogenized at 2000 PSI separately. Ice cream mix was then cooled to room temperature and transferred to the aging vat for overnight aging at 4 ºC. Freezing was performed to whipping the ice cream mix using continuous ice cream freezer (Akshar Industries Pvt. Ltd. India) at -5 ºC temperature with constant speed.

Physicochemical analysis of the ice cream

Ice cream samples were subjected to the physicochemical analysis. Each sample was analyzed in triplicates to overcome the error. Moisture content of the ice cream samples were observed by the method suggested by AOAC (1995). 5 g of ice cream sample was weight accurately into an aluminum dish and heated the sample in an oven maintained at ±105 ºC for 4 hours. The dish was transferred to desiccator and ahead cooling, weighed. Moisture content was calculated as under:

$$\text{Moisture (\%)} = \left(\frac{W2 - W1}{W1 - W}\right) \times 100$$

Where, W is the weight of empty dish, W_1 is the weight of dish with sample and W_2 is the final weight of dish in gram.

Total solid content was measured by gravimetric method described by AOAC (1990) following formula was used to determine the total solid:

% Dry matter (Total solid) = 100- percentage of moisture content

Fat percentage was measured by gerber method by using cream butyrometer. Protein content of the ice cream samples was evaluated through kjeldahl method described in AOAC (1990) Protein per cent was calculated by following formula:

Nitrogen per cent = (Sample titer – Blank titer) x Normality of HCl x 14.01 x 100.

Protein (%) = Nitrogen % × 6.38

Carbohydrate percentage was analyzed by using difference method (FAO, 1998). Total carbohydrate was calculated by the following formula:

Total Carbohydrate = 100- (Weight in grams [fat + protein + moisture + ash] in 100 g of food)

Ash content was evaluated by method described in AOAC (1995). pH of the ice cream was measured by digital pH meter (Systronic India Ltd.). Titratable acidity was determined by back titration method similar to the method suggested by BIS (1989). The titratable acidity was calculated as:

$$\text{Titratable acidity (\%)} = \frac{9\ AN}{W}$$

Where, A is volume of N/10 sodium hydroxide used, N is normality of sodium hydroxide and W is weight of milk sample in gram.

Calorie value was estimated by IKA oxygen bomb calorie meter (IKA India Pvt. Ltd.) viscosity was observed by the method suggested by Metwelly (2007) using Brookfield viscometer Model DV2T; Brookfield Engineering Laboratories, Stoughtm, MA, USA) by using LV spindle number 3 at 30 rpm. Overrun of ice cream was calculated using the method suggested by Marshall and Arbuckle (1996) [32]. Overrun percentage was calculated using the following formula

$$\text{Overrun (\%)} = \left(\frac{M2 - M1}{M1}\right) \times 100$$

Where, M_1 is the weight of ice cream mix; M_2 is the weight of ice cream had the same volume as the mix.

Melting rate was evaluated as method described by Sakurai *et al.* (1996) [35]. Melting rate was obtained using the following equation:

$$\text{Melting rate (\%)} = \left(\frac{A1 - A2}{A1}\right) \times 100$$

Where, A_1 and A_2 are the weight of initial sample (50 g) and melted sample respectively

Statistical Analysis
All data were calculated on the basis of three independent experiments. Data are expressed as Mean ± SE and the two sample independent t-test was used to know the suitability. The t-statistics and its p-value were calculated using the IBM SPSS version 16 to find the significance difference of the sensory attributes for both control and optimized Ice Cream samples.

Result and Discussion
Chemical properties
It is evident from the Table 1 that the proteins content of optimized and control Ice Cream were 7.64 percent and 3.89 percent respectively. Optimized Ice cream contains significantly (P<0.05) higher percentage of protein as compare to the control Ice Cream. The percentage increase in protein content of optimized Ice Cream was due to the substitution of skimmed milk powder with soy protein isolate in the pre-mix. Similar findings were observed by Pereira *et al.* (2011) [13] the higher protein concentration in the Ice Cream samples were related to the increase in the substitution level of the skimmed milk powder by soy extract. This was also in agreement with the study of Dervisoglu *et al.* (2005) [14] who had reported that the if non-fat dried milk (NFDM) was replaced by soy protein concentrate the nitrogen content of the Ice Cream samples incorporated with SPC was higher than the control sample it was due to the higher protein content of the SPC.

Fat content of the optimized Ice cream was found 6.6 percent whereas in control Ice Cream it was observed as 10.30 percent. There were no significant changes were observed in the fat percentage of both control and optimized Ice Cream samples due to the fat percent of control and optimized Ice Cream were standardized at the level of 10 percent and 6 percent respectively and also utilization of non-fat milk powder and soy protein isolate. Similar finding was observed by Dervisoglu *et al.* (2005) and Pereira *et al.* (2011) [13, 14] they studied that the fat content of the control and other samples were not changed significantly due to the utilization of non-fat forms of both dry milk and soy concentrate.

Table 1: Chemical property of Control and Optimized Ice cream

Chemical Parameters	Control	Optimized Ice cream	p value
	Mean ± SE	Mean ± SE	
Fat	10.3 ± 0.25	6.6 ± 0.02	0.002
Protein	3.89 ± 0.24	7.64 ± 0.31	0.0003
Carbohydrate	17.50 ± 0.94	11.90 ± 0.70	0.004
Ash	1.4 ± 0.02	1.1 ± 0.01	0.0002
Moisture	66.91 ± 1.63	72.76 ± 1.62	0.032
Total Solid	34.09 ± 1.16	27.24 ± 0.29	0.004
Titratable Acidity	0.14 ± 0.01	0.17 ± 0.01	0.060
pH	6.45 ± 0.12	6.57 ± 0.03	0.195

Table 1 shows the carbohydrate content of the control and optimized Ice Cream. It is observed, that the percentage carbohydrate content of control Ice Cream is 17.50 percent whereas it was found 11.90 percent in optimized Ice Cream. It is confirmed that the carbohydrate content of the optimized Ice Cream was significantly (P<0.05) different from the control Ice Cream. The percentage decrease of carbohydrate content in optimized Ice Cream could be due to the complete replacement of sugar with the stevia. In the same way Giri *et al.* (2014); Singh *et al.* (2017) [19, 38] observed that the carbohydrate content of the kulfi decreased with increased level of replacement of sugar with stevia.

Ash is the solid leftovers of fire. Particularly, ash refers to the all non-gaseous and non-aqueous residue that stay behind after something is burned. The ash content of both control and optimized Ice Creams were depicts in Table 1. The percentage ash content of control Ice Cream was 1.4 which is significantly (P<0.05) different from the optimized Ice Cream as it contains 1.1 percent. The percentage decrease of ash content for the optimized Ice Cream as compare to control Ice Cream could be due to the decreasing the SNF content. Complete replacement of sugar with stevia and replacement of SPI with skimmed milk powder decreases the SNF content of the Ice Cream Sample. Similar findings were observed by Kumar *et al.* (2010) they studied that if paneer samples incorporated with SPI, this samples were contains higher amount of ash than control sample due to increased SNF content. The present study is in agreement with opposite manner of the above study. It was again in agreement with Dervisoglu *et al.* (2005) [14] according to their findings when SPC was substituted with NFDM in the ice cream formulation than the increase in the SPC ratio significantly decreased the ash value of the Ice Cream Sample. Reason behind It, the total solid content of the NFDM is higher than the SPC. Ultimately the ash content of the NFDM is higher than the SPC.

Moisture is refers to the presence of liquid in trace amount in any foodstuffs. It affects the weight, texture, appearance, taste, and shelf-life of food products. It can be seen from the Table 1, that the moisture content of control Ice Cream was 66.91 percent and It was found in optimized Ice Cream was 72.76 percent. The amount of moisture content in optimized Ice Cream was significantly (P<0.05) higher than the control Ice Cream it could be due to the low content of fat, total solid and complete replacement of sugar with stevia of the Ice

Cream sample. Giri *et al*. (2014) [19] reported that the when the sugar was replaced with stevia in manufacturing of kulfi the total solid content were decreased due to the lower levels of sugar. So the moisture percentage increased proportionally in the kulfi samples. Similarly Kumar *et al*. (2010) also reported that moisture content increased with decreased in fat content of the paneer.

The total solid content includes both suspended solid and dissolved salts in the foodstuffs. It is revealed from Table 1, that the total solid content obtained in control Ice Cream was 34.09 whereas in optimized Ice Cream it was obtained as 27.24 percent. The percent content of total solid was significantly (P<0.05) different in optimized Ice Cream as compare to the control Ice Cream; it was due to the use of higher amount of milk and no use of sugar in optimized Ice Cream. Dervisoglu *et al.* (2005) [14] reported that the Total solid contents of the ice cream samples incorporated with 3 and 4.5% SPC were significantly lower than the control and the ice creams. This finding was expected that SPC was substituted for NFDM in the ice cream formula and the total solid content of the NFDM is higher than the SPC.

Titratable acidity is an estimate of the total acid present in the Ice Cream. It determines the keeping quality of the foodstuffs. The values of titratable acidity can be seen from Table 1 for control and optimized Ice creams were 0.14 and 0.17 percent. Titratable acidity of optimized Ice Cream was non-significantly (P>0.05) different as compare to the control Ice Cream. The higher value of titratable acidity is due to the addition of soy protein Isolate, Inulin and stevia. Dervisoglu *et al.* (2005) [14] reported that the SPC addition had a negative effect on the titratable acidity values of all samples as expected

pH is the scale of acidity from 0-14 and it gives quantitative measure of the acidity or basicity of any liquid solution. It is observed from the Table 1, that the pH value of control Ice Cream is lower (6.45) as compare to the optimized Ice Cream (6.57). The pH value of control and optimized Ice Cream was non-significantly (P>0.05) differing from each other. Higher pH value of optimized Ice Cream was due to the addition of SPI, Inulin and stevia. Similar finding was observed by Dervisoglu *et al.* (2005) [14] according to their study ratio of SPC increased to substitute the NFDM significantly (p<0.05) increased the pH of the all samples. This result was expected because the pH value of the SPC (pH 7.0) is higher than the average pH value of the control ice cream (pH 6.3). Pereira *et al*. (2011) [13] reported that the pH value of the Ice Cream mix was rises as the substitution level of skimmed milk with soy extract increased. The increase in pH value is due to the higher pH of the soy extract as compared to the skimmed milk powder.

Physical properties

Overrun refers to the percentage expansion of Ice Cream by amount of air pushed into the Ice Cream during the freezing process. In general overrun is percentage increase in volume of Ice Cream greater than the amount of mix used to produce that Ice Cream. It can be observed from Table 8, that the mean values of percentage overrun for control and optimized Ice Creams were 72.71 percent and 61.29 percent respectively. Optimized Ice Cream has significantly (P<0.05) high overrun percentage compare to the control Ice Cream. It could be due to the addition of Inulin, stevia and SPI in the Ice Cream pre-mix. Akalin *et al*. (2008) [2] found that the highest overrun was obtained in reduced fat Ice Cream sample with Inulin whereas the lowest overrun was observed in low fat Ice Cream with WPI. Adapa *et al*. (2000) [1] reported that carbohydrate based fat replacers had low overrun because of the higher viscous component. High viscous system does not favor foaming capacity. Further they also reported that the similar fat content with protein based fat replacer had also low overrun value because protein based fat replacer did not increase the whipping ability. Dervisoglu *et al.* (2005) [14] reported that the addition of SPC significantly lowered the overrun percentage of the samples. Sutar *et al.* (2010) [39] prepared soy Ice Cream using soy milk and found that the overrun of the soy Ice Cream was much lower than the traditional Ice Cream. It could be due to the poor whipping ability of the soy proteins.

Table 2: Physical properties of control and optimized Ice cream

Physical properties	Control	Optimized Ice cream	p value
	Mean ± SE	Mean ± SE	
Overrun (%)	72.41 ± 0.78	61.29 ± 0.70	0.0002
Calorie value (Kcal)	177.37 ± 1.38	139.67 ± 0.14	0.0006
Viscosity (Cp)	1260 ± 24.58	1662 ± 26.90	0.0001

Calorie is a unit that measures energy. It is usually used to measure the energy content of food and beverages. Generally calorie is defined as the amount of heat required at a pressure of 1 standard atmosphere to raise the temperature of 1 gram of water to 1 ºC. Calorie value of optimized Ice Cream was significantly lower than the control Ice Cream. Table 2 shows the calorie value as 177.37 and 139.67 Kcal for control and optimized Ice Cream respectively. Calorie value of optimized Ice Cream was significantly (P<0.05) different from the control Ice Cream. The lower calorie value of optimized Ice Cream could be due to the reducing the fat percent and complete replacement of sugar with stevia. Stevia is a natural sweetener and it has very low calorie value than other sweetener (Curry and Roberts, 2008) [12]. Similar findings were observed by Alizadeh *et al.* (2014) [7] who had reported that the complete replacement of sugar with stevia resulted in significant reduction in calorie value. Giri *et al*. (2014) [19] also reported that the calorie value of kulfi samples treated with stevia extract powder was lower than the control sample. It was due to the replacement of sugar with stevia because sugar is a major contributor to the calorie value in kulfi.

Viscosity is a measure of a fluid’s resistance to flow. It describes the internal fraction of a moving fluid. In general, viscosity denotes opposition to flow. It was observed from the above table that the viscosity of optimized and control Ice Cream was 1260 and 1662 centipoises. The viscosity of optimized Ice Cream was significantly higher than the control Ice Cream. Higher viscosity of optimized Ice Cream could be due to the addition of SPI and inulin which gives thickness to the Ice Cream. Akalin and Erisir (2008) [2] confirmed that the probiotic Ice Cream added with oligofructose and Inulin performed higher viscosity value. The higher apparent viscosity resulted due to the higher molecular weight of the Inulin and interaction between milk proteins present in the system and Inulin. It can also be explained by the interactions of the dietary fiber and liquid components of the probiotic ice cream mix. Akesowan (2009) [4] reported that the addition of SPI to Ice Cream mixes significantly increases the viscosity of the Ice Cream sample. Similarly Friedeck *et al*. (2003) [18] reported that the higher viscosity value was achieved for SPI incorporated Ice Cream used to substitute the NFDM. Pereira *et al*. (2011) [13] also reported the when the skimmed milk powder substituted by soy extract in the Ice Cream mix it influenced the consistency coefficient. Higher levels of substitution indicate higher consistency coefficient (*K*) value. It is an informative parameter of the viscous properties of Ice Cream mix. Higher *K* value means that the Ice Cream mix is more viscous.

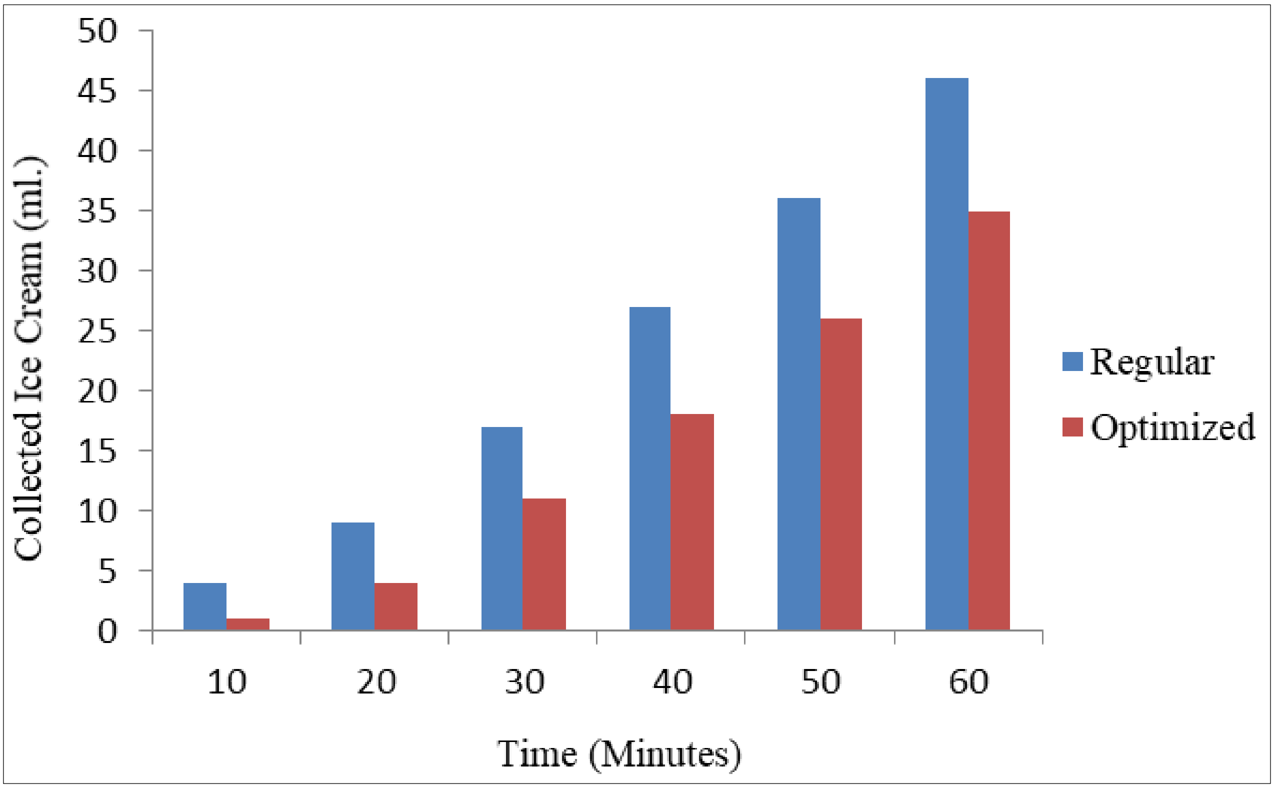

Fig 1: Melting rate of control and optimized Ice Cream

Melting is a process to transform a solid into a liquid when heat up. This occurs when the internal energy of the solid increases, usually by the application of heat or pressure, which raise the substances temperature to the melting point. The figure 1 shows the relationship between amounts of collected Ice Cream with the increment of time interval. From the graph 1 it is evident that the melting rate of optimized Ice Cream was lower than the control Ice Cream. It could be due to the replacement of skimmed milk powder with SPI and addition of the Inulin. Correspondingly Akalin and Erisir (2008) [2] reported that the melting property of the low fat probiotic Ice Cream was improved by the using oligofructose and Inulin. They found that the most remarkable improvement in melting value was observed in product containing Inulin. Akesowan (2009) [4] reported that the SPI substituted Ice Cream was showed lowest melting compare to the control Ice Cream which showed faster melting. This result indicated that the due to the liquid binding capacity of the SPI it forms a stable gel network which immobilized the free movement of the water molecules among other molecules of the mix. Similarly Dervisoglu *et al*. (2005) [14] studied that melting rate of the Ice Cream sample added with SPC becomes slower and prevented from melting. It could be due to that SPC contains high percentage of protein which makes the Ice Cream mix more viscous than the NFDM. This was in also agreement of the work of El-Nagar *et al*. (2002) [15] who reported that due to the water binding capacity of inulin, it may act as a stabilizer and reduced the free movement of water molecules, consequential in the decline of melting characteristics of ice cream products.

Conclusion

In the current study from the above findings it was concluded that the manufacturing of reduced fat, low calorie and protein rich Ice Cream by means of the partial substitution of skimmed milk powder with SPI, fat by inulin and replacement of sugar with stevia extract powder significantly (P<0.05) affect the chemical and physical properties except pH. These ingredients can be best utilized in a limited quantity to produce Ice Cream with quantitatively high protein, low fat and low calorie than the control ice cream which made it therapeutic product for the health conscious people and suffering from the diabetes, obesity etc.

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