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Quality attributes of high protein ice cream prepared by incorporation of whey protein isolate

Suchismita Roy[a], Shaik Abdul Hussain[b,*], Writdhama G. Prasad[a], Yogesh Khetra[a]

[a] M Tech Scholar, Dairy Technology Division, ICAR-National Dairy Research Institute, Karnal-132001 (Haryana), India
[b] Scientist, Dairy Technology Division, ICAR-National Dairy Research Institute, Karnal-132001 (Haryana), India

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Keywords:
Ice cream, Rheology, Particle size distribution, Meting profile, Whey protein isolate, High protein. Abbreviations: PDCAAS - Protein Digestibility Corrected Amino Acid Score DIAAS - Digestible Indispensable Amino Acid Score, SMP - Skim Milk Powder, WPI - Whey Protein Isolate, WPC – Whey Protein Concentrate, MPC – Milk Protein Concentrate

ABSTRACT

Protein is an important nutrient and plays various vital functions in our body. Its deficiency has been related to different disorders and diseases. Considering the huge demand of ice cream, it was used in the present study for protein supplementation. Whey protein isolate was used for increasing the protein level of buffalo milk ice cream to 6 and 8 and 10%. Increasing the protein content decreased the flow behaviour index (from 0.86 to 0.57), increased the consistency coefficient (from 0.18 Pa.s^n to 4.22 Pa.s^n) of ice cream mix. With increasing the protein content from 4 to 10% (w/w), loss modulus (at 100 Hz) values increased from 10.9 to 34.3 Pa and the storage modulus (at 100 Hz) of mixes increased from 7.25 to 32.7 Pa. Ice cream samples had decreased overrun (from 94.9% to 33.9%), increased instrumental hardness (from 13.60 N to 47.66 N) and melting rate (0.24 to 0.74 g/mL) with increasing protein content. Particle size distribution revealed that control sample (4% protein) had distinct peaks of casein micelles, individual and clumps of fat globules, while no such distinct peaks were observed in case of experimental samples. Sensory attributes viz. meltdown score and body and texture score were decreased with an increase in the protein concentration.

1. Introduction

Protein is an important macro-nutrient and required for the constitution of tissues and regulation of various physiological functions in our body. Protein plays an important role in our body's growth and development, and also provides an energy of about 4 Kcal per gram. The daily requirement of protein is 54 g/day for men of 65 kg (ICMR NIN, 2020). High quality protein intake is particularly important in case of vegetarian people as about 60% of protein is derived from cereals and pulses, both of which has low digestibility and bioavailability in comparison to the protein from animal sources (Swaminathan, Vaz, & Kurpad, 2012). About 375 million of the global population are vegetarian, particularly 10 % of people in Europe, 4 % men and 7 % women in the United States, and 31 % people in India are vegetarian (Expo, 2015). In addition, millions of people consume cassava and maize as staple foods, which are also poor sources of essential amino acids (Nuss & Tanumihardjo, 2011). As per Indian Council of Medical Research (a premier autonomous organization for health and well-being), an adult should consume about 0.8–1 gm protein per kg body weight for a healthy life; however, the actual value of protein consumption is close to 0.6 g per kg ideal body weight in the > 18 years age Indians (India's Protein Paradox Study, 2020). This emphasizes on the need to supplement superior quality protein in the high consumer demand vegetarian foods. Among the different dietary protein sources, whey protein (PDCAAS value of 1.00 and DIAAS value of 1.09) finds a prominent place for protein supplementation and improvement in the health status. Functional attributes of whey proteins, viz., anti-oxidative, protection against cardiovascular diseases, immunity enhancement, anti-microbial, anti-inflammatory, etc. has been well documented (Patel, 2015). Further, they are considered as 'Gold standard of protein' for professional sport persons for development and maintenance of 'strong well-built' muscle. Whey proteins are also classified as 'Fast proteins' because of their easy digestion, efficient absorption and ability to provide quick nourishment to muscles (Kumar, Chauhan, Shinde, Subramanian, & Nadanasabapathi, 2018).

Ice cream is a popular dairy dessert consumed by almost all segment of population. As per a recent report ice cream had a global market size of US $ 62 billion in 2019 and has an expected compounded annual growth rate of 5% (Imarcgroup, 2020). Because of its huge consumer demand, ice cream has been supplemented with different herbs (Granato et al., 2018), pre- and pro-biotics (Parussolo et al., 2017) and micronutrients (Tipchuwong, Chatraporn, Ngamchuachit, & Tansawat, 2017). As per Spence, Navarra, & Youssef (2019), ice cream could also be explored as an effective vehicle for providing nutrition to the elderly people (aged > 60 years) in hospitals and old peoples' facilities. These reports substantiate the idea that ice cream could be explored as an effective vehicle for protein supplementation to address the widespread protein malnu-

* Corresponding author.
E-mail addresses: suchismitaroy93@gmail.com (S. Roy), abdulndri2006@gmail.com (S.A. Hussain), wgprasad.ndri@gmail.com (W.G. Prasad), yogeshndri@gmail.com (Y. Khetra).

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trition. However, protein supplementation in ice cream could not be achieved by simply increasing the skimmed milk powder (SMP) level (principal source of milk protein in the conventional ice cream) in its formulation. Preliminary work done at our lab revealed that an unacceptable product with very low overrun and too hard body was obtained while using SMP to reach the protein content of 8% because of increased total solids and viscosity of the ice cream mix (data not reported). Protein plays an important role in the structure development of ice cream by its emulsification, whipping, and water-holding capacity. Generally, ice cream contains about 4% protein and this level is optimum as far as the sensory quality of the product is concerned. Increasing the protein content beyond this level has been reported to cause detrimental change in the quality attributes of ice cream. Patel, Baer, & Acharya (2006) increased the protein content in ice cream up to 7 % level using milk protein concentrate (MPC) 70 and reported that the product had excessive hardness and lower overrun. Similar results were reported by Daw & Hartel (2015), who used different milk protein sources viz., skimmed milk powder, whey protein isolate and MPC 85 to increase the protein content in ice cream. The authors observed that increasing the protein content of ice cream using different protein sources affected the partial coalescence of fat globules and melt-down rates thus resulted in ice cream with higher hardness and overrun. Among the studies mentioned above, all had SMP as an ingredient in the ice cream formulation. The SMP contains about 45-50 % lactose, which increases the freezing point depression and affects the related quality attributes of ice cream such as melt down rate, etc. In a study conducted on rheological properties of lactose hydrolyzed (~98 %) ice cream mix by Matak et al. (2003), the authors reported that lactose reduction resulted into concomitant decrease in the hardness of the ice cream. Similarly, Abbasi & Saeedabadian (2015) reported a decrease in hardness and melting resistance in the ice cream samples prepared using lactose hydrolysed milk. Considering the above information, there appears an opportunity to increase the protein content in ice cream by substituting the entire SMP with WPI, which will not only increase the protein content of ice cream but also decrease the susceptibility towards increased hardness in the sample. Hence, the present work was undertaken with an aim of supplementing protein content in the conventionally available ice cream using WPI, which could be a potential source of protein supplementation in vegetarian diets.

2. Materials and methods

2.1. Materials

Fresh chilled raw buffalo milk and cream were collected from Experimental Dairy, ICAR-National Dairy Research Institute, Karnal. The fat and SNF content of raw buffalo milk varied between 7-7.5% and 9.5-10.5% and fat content of cream ranged from 65-70%. Skimmed milk powder (SMP) was procured from Modern Dairy Ltd., Karnal. SMP contained 1.5% fat, 95% MSNF and 3.5% moisture as per the manufacturer's instructions. White crystalline sugar (sucrose) was procured from a local market of Karnal. Whey protein isolate (WPI) manufactured by Davisco, USA (Brand name: BiPRO) was procured for use in ice cream as protein source. WPI had 87% protein on dry basis, 0.8% fat, 2.0% ash and 5% moisture. Stabilizer and emulsifier mix manufactured by Danisco (India) Pvt. Ltd., under the brand name of CREMODAN SAMPOORNA® was procured from the local market of Karnal. It was a mixture of guar gum, mono- and diglycerides of fatty acids, sorbitan esters of fatty acids and carrageenan. Polystyrene cups (100 mL) were procured from the local Karnal market for ice cream packaging.

2.2. Ice cream preparation

Control ice cream (containing 4 % protein and 10 % milk fat) was prepared by first calculating the required amount of buffalo milk, SMP, cream and other ingredients required to standardize the mix. All the liquid ingredients i.e. milk and cream in the required amount were precisely measured and blended in a steam kettle. Temperature of the contents was increased to 45°C and calculated amount of SMP was slowly added into the liquid blend along with continuous stirring. Stabilizer-emulsifier mix (CREMODAN SAMPOORNA®, Danisco, India, Pvt. Ltd.) (@ 0.15% of the mix) was dry-blended with half of the amount of total sugar (@15% of the mix) to prevent clump formation and to achieve proper dispersion of stabilizer - emulsifier mix. This dry blend was then added to the steam kettle when the temperature reached to 60-65°C, followed by addition of the remaining sugar. After proper mixing of the contents, ice cream mix was pasteurized at 80°C for 1 min and then was homogenised (140 kg/cm^2). The mix was them cooled to 4°C and aged for overnight. Vanilla essence (1 ml/lit mix) (International Flavours and Fragrances Ltd., Chennai, India) was added to the aged mix and then freezing was performed in a batch freezer (GOMA Engineering Pvt. Ltd., Mumbai, India) to -5°C followed by packaging in polystyrene cups. Hardening of ice cream was done at -20±2°C in still air hardening cabinets (Cellfrost India Pvt. Ltd., New Delhi, India) and then stored at -20±2°C.

For preparation of high protein ice cream samples (6, 8 and 10% protein containing samples), SMP was replaced with WPI as protein source. Ice cream mix was formulated using buffalo milk, cream, WPI, sugar and stabilizer - emulsifier mix to 10% fat and different protein levels (6, 8 and 10%). The calculated amount of WPI (required to maintain the desired protein level) was dispersed in 1/3rd of milk (at 40°C) using high shear mixer (8000 rpm) and then was mixed with the rest of the milk and cream. After this, sugar, stabilizer and emulsifiers were added in the similar manner as the control sample and same protocol as reported in case of control ice cream preparation was followed. Flow charts for control and high protein ice cream preparations and Ice cream mix formulation table were given as Supplementary files S1 and S2, respectively.

2.3. Analysis of experimental samples

2.3.1. Rheological attributes of ice cream mix

Rotational rheometer (MCR 52, Anton Paar, Austria) fitted with a cone (CP-75, 75 mm diameter, 1° angle) and plate assembly was used to study the rheological properties of the ice cream mix at 4°C. Aged ice cream mix was mixed thoroughly with a spatula and gently placed on the pre-cooled (4°C) rheometer plate. Apparent viscosity of ice cream mix was measured in the shear rate range of 1 to 100 sec^{-1} and was recorded in Centipoises (cP). Flow behavior index and consistency coefficients values were calculated from the flow curve results obtained from the rheometer. For frequency sweep test, the Linear Viscoelastic Range (LVR) of the ice cream mix was determined using amplitude sweep test. Amplitude sweep test (0.01-100% strain) was performed at a constant frequency of 1 Hz at 4°C. Frequency sweep measurements {storage modulus (G') and loss modulus (G'')} were determined as a function of frequency at 4°C temperature. The G' refers to the storage or elastic modulus which reflects elastic behavior of a material when it is deformed. The G'' refers to the loss or viscous modulus of a material which reflects flow of a material when deformed. It relates change in strain over time that occurs when the applied stress is removed. The frequency sweep measurements were performed within the LVR (1%) over frequencies 0.01 to 10 Hz (21 points) and strain level of 1%.

2.3.2. Particle size analysis

Particle size analysis of the ice cream samples was performed using Malvern Mastersizer 3000E using laser diffraction technology to determine the extent of fat de-emulsification and cluster formation as per the methodology provided by Roy, Hussain, Prasad, & Khetra (2021). Ice cream sample was diluted by using deionized water in 1:1 ratio. Drop by drop diluted ice cream sample was added to the dispersing water until the obscuration values of 14-16% were obtained. Deionized water (refractive index = 1.33) was used as the continuous phase and milk fat (refractive index = 1.44) was used as dispersed phase.

2.3.3. Overrun

Overrun of ice cream samples was observed as per the method provided by Bolliger, Kornbrust, Goff, Tharp, & Windhab (2000). The increase in volume of ice cream mix takes place when air is incorporated during the freezing process. Overrun (%) of the ice cream was determined using the formula:

$$\% \text{ Overrun} = \frac{W1 - W2}{W2} \text{ X } 100$$

Where, W_1= Weight of unit volume of ice cream mix; W_2= Weight of unit volume of ice cream.

2.3.4. Melting rate

Melting rate of ice cream was determined as per the method provided by Muse & Hartel (2004) with minor modifications. In brief, about 200 mL of sample was transferred on a wire mesh (9 holes/cm^2), which was placed on a pre-weighed measuring cylinder (400 mL capacity). The whole assembly was kept undisturbed at 25±1°C on a weighing machine. Weight of the melted samples which was collected in the measuring cylinder was recorded after an hour from the onset of the experiment and melting rate was determined by the formula:

$$\text{Melting rate (g/min)} = \frac{\text{Weight of the melted ice cream (g)}}{\text{Time (min)}}$$

2.3.5. Instrumental hardness

Instrumental hardness of the samples was measured as per the method provided by Sofjan & Hartel (2004) with major modifications. After dynamic freezing, the ice cream samples were carefully filled in the 100 mL cups. To avoid fluctuations during testing, the cups were filled in such way that no air pockets remained within the sample. Samples were then hardened by keeping them at -20±2°C temperature for minimum 48 h. After hardening, the instrumental hardness of the ice cream samples were measured using Texture Analyzer (Model TA.XT2i, Stable Micro Systems, UK) fitted with 25 kg load cell and combined with Texture Expert Exceed Software. Experiments to measure hardness were carried out at room temperature using Warner Bratzler Blade (7.5 cm width) assembly. To reduce any variations arising out from the temperature difference between the probe and ice cream sample, the probe was tempered at -20±2°C for half an hour before starting the experiment. For each sample, minimum six readings were taken to minimize experimental errors. Test conditions maintained for measuring the instrumental hardness were as follows: Test Mode and option= Measure Force in Compression; Pre-test Speed= 2 mm/s; Test Speed= 3.0 mm/s; Post-test Speed= 10 mm/s; Distance (Distance travelled by the blade from the surface of the sample= 15 mm). The sample height was around 35 mm and the percentage of sample height covered by the probe was ∼40%.

2.3.6. Sensory evaluation

Sensory analysis of the ice cream samples was conducted as per the procedure described by Roy, Hussain, Prasad, & Khetra (2021). In brief, for sensory evaluation, the ice cream samples were served in polystyrene cups (100 mL) to a panel of six judges having adequate knowledge about ice cream quality. The samples of ice cream were evaluated for various sensory attributes viz. colour and appearance (Maximum score 4), flavour (Maximum score 35), body and texture (Maximum score 55) and melting rate (Maximum score 6) characteristic using a descriptive sensory score card. Colour and appearance attribute was evaluated to determine the color uniformity in the sample. Flavour attribute was evaluated to check the clean vanilla flavour and presence of any off-flavour in the ice cream samples. Body and texture attribute was evaluated for gumminess, sogginess, fluffy body, sandiness, coarse/iciness, crumbly and weak body in the samples. Meltdown attribute was evaluated to observe the rapid or slow melting of ice cream during eating. On the descriptive sensory score card used, each sensory property further described into different attributes with definite scores viz. (a) colour and appearance included uniformity (4) and non-uniformity (1.5-3); (b) flavour included lacks fine flavour (5-9), unnatural flavour (4-7), whey (3-8) and salty (4-8); (c) body and texture included gummy (5-9), soggy (5-8), sandy (5-7), fluffy (5-7), crumbly (3-6), weak body (2-5) and coarse/icy (3-6); (d) meltdown included rapid meltdown (1-2) and slow meltdown (1-2).

2.3.7. Statistical analysis

The data obtained during the investigation was subjected to one-way ANOVA (analysis of variance) using Graph Pad Prism Statistics Software (Graph Pad Software, Inc., California, USA) and Microsoft Excel 2016. The ice cream sample prepared with SMP containing 4% protein was considered as control sample. The ice cream samples with increased protein content of 6, 8 and 10% protein prepared with WPI were compared with the control samples. The comparison of the mean values was done by Tukey's post hoc test. The significance was taken at 95% level of significance ($P < 0.05$). Experiments were carried out in triplicates.

3. Results and Discussion

3.1. Effect of WPI supplementation on rheological attributes of high protein ice cream mix

Viscosity could be defined as the resistance offered by a liquid to flow in response to an applied force. It is primarily associated with the internal friction between one part/layer of the liquid to slide over the other part/layer. Viscosity is an important factor affecting the quality attributes of ice cream. Stabilizers are included in the ice cream formulation so as to increase the mix viscosity and obtain a superior quality product. During freezing higher viscosity mixes are difficult to beat thus retards air incorporation; on the other hand, lower viscosity mixes are easy to whip but the film strength is not enough to sustain the air cells and the bubbles coalesce shortly. Addition of WPI to supplement the protein content in ice cream significantly ($p < 0.05$) affected the consistency coefficient and flow behaviour index of ice cream mix. Increasing the protein content decreased the flow behaviour index (from 0.86 to 0.57) and increased consistency coefficient (from 0.18 $Pa.s^n$ to 4.22 $Pa.s^n$) (Table 1). Consistency coefficient values of ice cream mixes increased with increasing the protein content. Control sample had the lowest consistency coefficient value and 10% protein containing mix had the highest value. Enhancing protein content might have restricted the water movement thus increasing the viscosity of mixes leading the higher consistency coefficient values. All the WPI added ice cream mix samples had lower flow behaviour index (0.57 to 0.75) than that of control (0.86), indicating that pseudoplastic behaviour became more prominent with an increase in the protein concentration. Calcium (present in the buffalo milk) and β-lactoglobulin (the principal component in WPI) could be associated with the increase in the viscosity of WPI supplemented ice cream samples. Heat treatment of WPI (95°C for 15 min) in presence of calcium leads to formation of linear polymers of whey protein resulted in increased viscosity of protein dispersions (Britten, 1997).

Goff, Kinsella, & Jordan (1989) reported that increasing the whey protein concentration in the ice cream mix lowers the interfacial tension at the oil/water interface and increases the mix viscosity. Similarly, Akalın, Karagözlü, & Ünal (2008) reported that addition of 4%

Table 1
Effect of protein concentration on flow behaviour index (n), consistency coefficient (k) and overrun of ice cream.

Protein concentration (% w/w)	Flow behaviour index	Consistency coefficient ($Pa.s^n$)	Overrun (%)
4 (Control)	0.86 ± 0.06^a	0.18 ± 0.07^a	94.9 ± 4.62^a
6	0.752 ± 0.01^b	0.37 ± 0.18^a	60.5 ± 1.01^b
8	0.68 ± 0.03^b	1.61 ± 0.44^b	44.3 ± 0.65^c
10	0.57 ± 0.04^c	4.22 ± 0.38^c	33.9 ± 2.82^c

Note: Values are expressed as mean ± standard error (n=3), Means with different superscripts (a, b, c) in a column differ significantly ($p < 0.05$) with each other.

WPI resulted in excessive thickening and gelation in ice cream thus led to sharp increase in ice cream mix viscosities. During the sample preparation, ice cream mix containing WPI was subjected to pasteurization at 80°C/1 min which would have caused whey protein denaturation thus resulted in increased viscosities in mixes with higher WPI levels. Denatured whey proteins possess increased water binding properties and had increased the mix viscosity.

With an increase in the protein content of mix from 4 to 10% (w/w), flow behaviour index values decreased from 0.854±0.05 to 0.571±0.03 and consistency coefficient values increased from 0.18±0.07 to 4.22±0.37, respectively. Our results are in agreement with Sanchez, Schmitt, Babak, & Hardy (1997) who reported that WPI (28.60 % w/w) solution had shear thinning behaviour, which the authors ascribed to the reversible molecular association of whey proteins along the plane of applied shear stress. Whey protein has been reported to possess the ability to interact with water and thus affect the viscosity. Significant ($p < 0.05$) differences observed among the different samples could be attributed to the difference in their whey protein concentration, which caused the difference in water binding among the samples and thus viscosity. Lim, Swanson, Ross, & Clark (2008) observed similar rheological behaviour with low-fat ice cream mixes upon increasing the protein content.

Loss modulus (G") indicates the viscous fluid behaviour and corresponds to the lost deformation energy. With increasing the protein content from 4 to 10% (w/w), G" (at 100 Hz) values increased from 10.9 to 34.3 Pa, respectively. Throughout the entire frequency range applied during the experiment i.e. 0.1-100 Hz, highest G" values were obtained for 10% protein containing sample (Fig 1). The storage modulus (G') indicates elastically stored deformation energy and solid like behaviour of the product. The value of G' (at 100 Hz) of mixes increased from 7.25 to 32.7 Pa as the protein content enhanced from 4 to 10% (Fig. 1). The G" values of all the ice cream mix samples were higher than G' over the entire frequency range tested i.e. 0.1 to 100 Hz (at 4°C). These results indicated that viscous behaviour was more prominent than the elastic component in all the ice cream mixes. The increase in both G' and G" with the frequency indicates strong frequency dependence of the mix. The increased storage modulus values with increased protein concentration shows higher hardness of the mix due to strong network formation of whey protein in the mix. Adapa, Dingeldein, Schmidt, & Herald (2000) prepared low fat ice cream with whey protein-based fat replacer and reported higher values of loss modulus than storage modulus in the frequency range of 0.1 to 10 rad/s. They concluded that the viscous component was more prominent than the elastic component when fat was replaced with whey protein based fat replacer. Saraiva et al. (2020) also reported that enhancing the protein content (3.25 to 7.44 %) using WPC (88.62% protein) resulted in higher G' and G" values. However, they observed that G' was higher than G" for whey protein supplemented high protein mixes which is contrary to our results. The differences in rheological behaviour observed in this study could be attributed compositional variations in the mix formulations and freezing process (liquid nitrogen freezing).

3.2. Effect of WPI supplementation on overrun of high protein ice cream

Overrun is an important criterion for selection of ingredients in ice cream preparation. Ice cream is sold on volume basis and an increase in the volume of ice cream from the mix is represented as % overrun. Hence, a higher overrun is preferable by the manufacturer because of increased profits. But, too high overrun leads to fluffy body and foamy texture; while too low overrun leads to soggy and heavy body of ice cream both of which is not liked by the consumers and may lower the sales of the product. Overrun values were found to be 94.9% in case of control (4% protein) sample, which decreased significantly ($p < 0.05$) to 60.5, 44.3 and 33.9% with an increase in the protein concentration to 6, 8 and 10%, respectively (Table 1). This could be attributed to the water binding and gelling ability of whey proteins, which in turn results into increased viscosity of the samples (Table 1). Control sample (4% protein) having flow behaviour index of 0.86 and consistency co-efficient of 0.18 had an overrun of 95%, while the sample containing 10% protein having flow behaviour index of 0.57 and consistence co-efficient of 4.22 had an overrun of 33.9%. Ice cream mix with higher viscosity requires high shearing action for agitation and air incorporation (Chang & Hartel, 2002). Further, increased viscosity causes difficulty in air distribution during the whipping process in the ice cream mix because of the strong matrix that increases the resistance to move the ice cream mix (Moeenfard & Tehrani, 2008). In the present study, dasher speed was kept constant so as to eliminate the effect of variable dasher speed on the overrun of different samples. Hence, the required amount of shearing action and agitation could not be imparted into the ice cream mix with respect to its increased viscosity. In addition to this, increasing viscosity of ice cream mix also tends to decrease the shearing effect of dasher during freezing, which results into lesser fat destabilization and clump formation (Fig. 4). Destabilized fat is reported to aid in foam stability by forming a layer around the air cell (Daw & Hartel, 2015).

3.3. Effect of WPI supplementation on instrumental hardness of high protein ice cream

Hardness (expressed in N) in case of ice cream is determined by measuring the amount of force required by a probe to penetrate the sample to a certain distance from the surface of ice cream. The hardness value of ice cream depends on several factors, among which formulation and overrun are the main factors affecting the hardness of the ice cream. Hardness of the ice cream samples was found to increase ($p < 0.05$) from 13.60 to 47.66 N upon increasing the protein content from 4 to 10% (Fig. 2). There was no significant difference ($p > 0.05$) between control and 6% protein containing ice cream sample. But as the protein percentage increased to 8 and 10%, hardness value increased significantly ($p < 0.05$). The observed results could be due to increased viscosity and decreased overrun in the samples with higher WPI content. Decrease in overrun implies higher amount of solids present in the same volume of sample, thereby resulting into increased hardness in the ice cream samples (Sofjan & Hartel, 2004). Further, the strong three-dimensional network formed by β-lactoglobulin (the principal protein fraction in WPI) could also have resulted into increased hardness in the ice cream samples by increasing the viscosity of the samples. Muse & Hartel (2004) reported that increased viscosity results into increase in the resistance to penetration by the probe during the hardness measurement of ice cream.

3.4. Effect of WPI supplementation on melting rate of high protein ice cream

Melting rate is an important physical property of ice cream which indicates the amount of ice cream melted per unit time. Melting rate is affected by several factors like ingredients, type, and level of stabilizer and emulsifier and overrun. Melting rate of ice cream samples with different levels of protein is provided in Fig. 3. It was observed that increasing the protein content resulted into a significant ($p < 0.05$) increase in the melting rate of ice cream. Control and 6% protein sample had a melting rate of 0.26 and 0.24g/min, while the sample containing 8 and 10% protein had significantly higher ($p < 0.05$) melting rate of 0.54 and 0.74g/min, respectively. Increase in melting rate could be attributed to differences in the extent of fat clumps formation and overrun in the samples. Clump of de-emulsified fat globules, formed during the freezing operation, forms a protective layer around the air cells and tends to impart foam stability and yield higher overrun. On the other hand, sample containing lesser amount of fat clumps has lower foam stability and overrun. Air has low thermal conductivity (0.024 W/m K at 0°C) and hence it acts as an insulator for heat penetration. For the same reason, ice cream samples having lesser over run (8 and 10% protein samples, Table 1) had fewer air cells, which resulted into higher heat transfer rates and hence rapid melt down rate was observed in these samples.

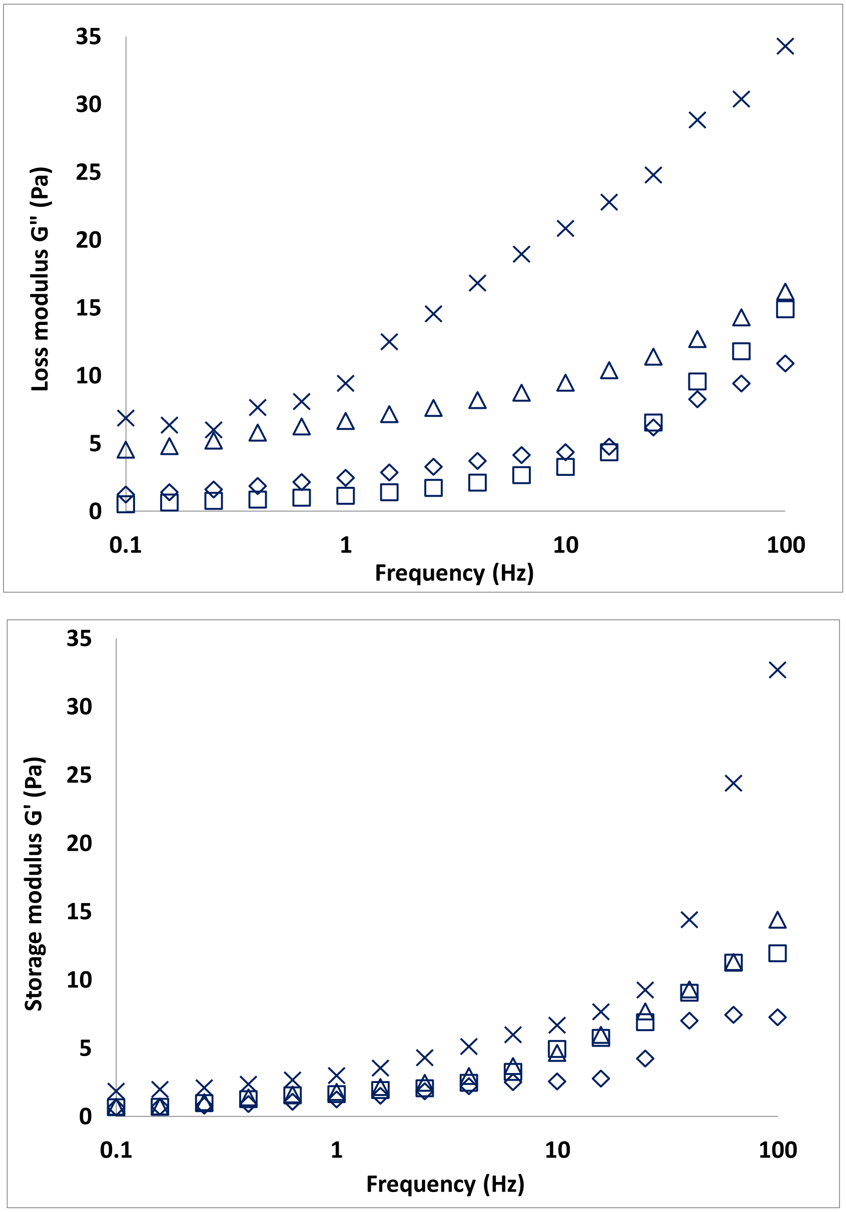

Fig. 1. Effect of protein content on loss modulus (G") and storage modulus (G') of ice cream mix.
Note: Ice cream sample with 4% (w/w) protein (◇); 6% (w/w) protein (□); 8% (w/w) protein (△); 10% (w/w) protein (×).

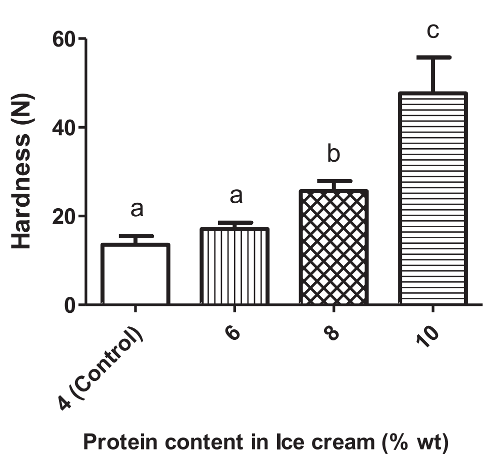

Fig. 2. Effect of protein content on instrumental hardness values of ice cream.
Note: Values are expressed as mean±standard error (n=3); mean values with different superscripts (a, b, c) differ significantly ($p < 0.05$) with each other.

Daw & Hartel (2015) also reported that increasing the protein content (4-8%) of ice cream led to higher melting rates.

3.5. Effect of WPI supplementation on fat globule size of high protein ice cream

As a result of the shearing action of the dasher, fat globule de-emulsification occurs during freezing of ice cream and the resultant fat globules form clusters. Ice cream added with different levels of WPI was evaluated for particle size distribution using Malvern Mastersizer 3000E (Malvern Instruments, UK) based upon laser diffraction technology. After homogenization, size of the fat globules in ice cream mix reduces to around 2-3 μm. During the aging process, emulsifier (being highly surface active than protein) replaces the protein adsorbed over the fat globule surface and decreases the interfacial tension. However, due to its low molecular weight and smaller size, emulsifier forms thin layer around the fat globules. This thin layer of emulsifier gets easily displaced from the fat globule surface by the scrapping action of the dasher during freezing operation (Goff & Jordan, 1989). This results into de-emulsification and partial coalescence of fat globules during the

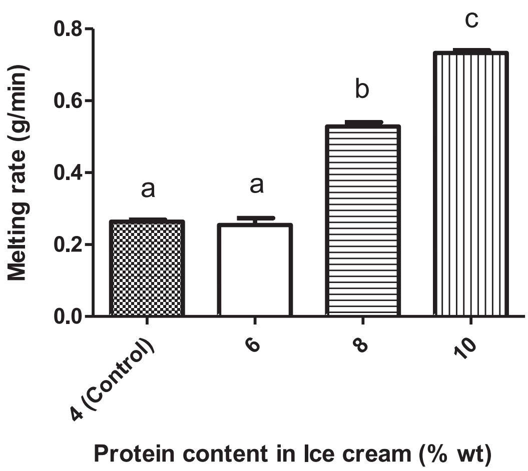

Fig. 3. Effect of protein content on melting rate of ice cream.
Note: Values are expressed as mean±standard error (n=3), mean values with different superscripts (a, b, c) differ significantly ($p < 0.05$) with each other.

freezing of ice cream mix. As a consequence, three peaks were obtained in control ice cream. The first peak (around 0.3-0.5µm) was of small casein micelles, the second peak (around 1µm) indicated the individual fat globules and the third peak (around 12 µm) indicated the size of the clusters of the fat globules produced during freezing (Fig. 4). Upon increasing the protein content, changes in the particle size distribution in the ice cream samples was observed. As like control (4% protein) sample, ice cream with 6% protein also had 3 peaks, of which two were (observed at 0.3-0.4 and 1-3 µm) similar to that of control sample, but the third peak (of fat globule clumps) was of smaller size (5-7 µm), indicating that less amount of fat de-emulsification and fat globules clump formation occurred in that sample. On the other hand, sample having 8 and 10% protein had only one peak (0.9-1.0 µm), which comprised of casein micelles along with individual and clumps of fat globules, as the peak super-imposed all the other peaks (of casein micelles, individual and clumps of fat globules) in both the samples. Also, upon comparison of the particle size distribution in different ice cream samples, it was observed that 6% protein sample had wide particle size distribution than that of 8 and 10 % protein ice cream samples. Wider peak might have included individual as well as clumps of fat globules, while narrow peak could be only of the individual fat globules. On the other hand, control sample had relatively distinct and narrow size distribution of individual and clumps of fat globules. This indicated that increasing the protein content resulted into decreased fat globule destabilization and clump formation. Goff, Kinsella, & Jordan (1989) reported that fat destabilization during freezing is greatly influenced by the composition of the ice cream mix (specifically the protein content). Absence of such clumps of fat globules in case of 8 and 10 % protein samples could have been due to higher protein content. Higher protein content resulted into higher adsorption of protein layer over fat globule surface, which would have acted as a protective layer for fat globules during freezing operation and hence lesser fat destabilization and clumping was observed.

D90 values indicate the maximum size limit of the 90% of the droplets present in the sample. The D90 values further supported the particle size distribution curves obtained by different ice cream samples. Control ice cream (4% protein) had significantly ($p < 0.05$) higher D90 value (15.95±0.63) than the remaining high protein counter parts indicating greater fat demulsification. However, non-significant differences were observed between D90 values of 8% (6.15±0.05) and 10% (5.97±0.07) protein containing ice cream samples and the same was evident from the particle size distribution curves (both were overlapped). These D90 values also proved that increasing the protein content decreased the fat emulsification rate in ice cream and thus resulted in bigger size droplets.

3.6. Effect of WPI supplementation on sensory attributes of high protein ice cream

The sensory analysis of ice cream samples was performed using a descriptive sensory score card (attached as Supplementary file S3) for flavour, colour and appearance, meltdown and body and texture attributes of the product. Non-significant ($p > 0.05$) changes were observed between all these four ice cream samples for colour and appearance scores. The flavour score of 8 and 10% protein ice cream samples was significantly ($p < 0.05$) lower than control and 6% protein samples (Fig. 5). This could be attributed to the characteristic 'whey' flavour of WPI thus led to decrease in flavour scores when used above certain concentration.

As the protein content of ice cream samples increased, body and texture score and meltdown score were decreased. Non-significant ($p > 0.05$) differences were observed between control (4% protein) and 6% protein containing samples for body and texture and meltdown

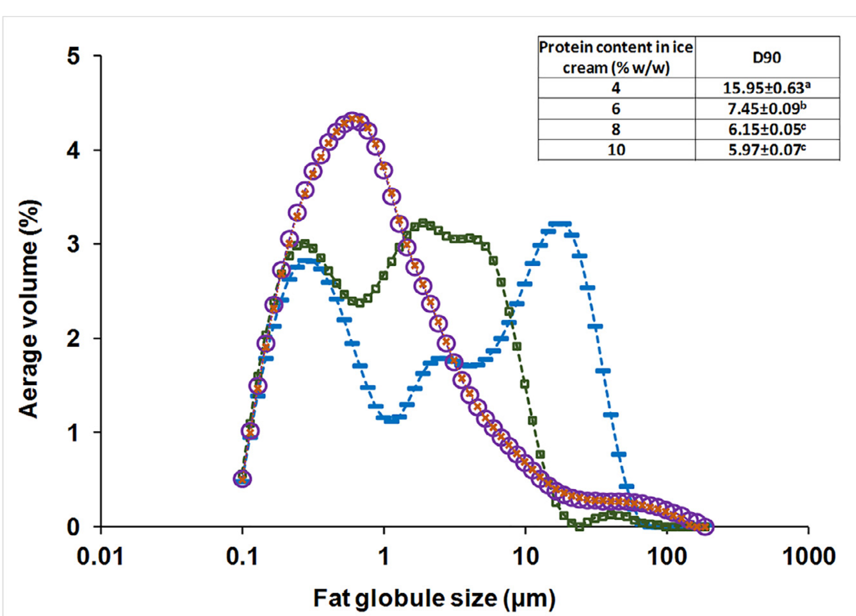

Fig. 4. Effect of protein content on particle size distribution and D90 (inset) value of ice cream.
Note: Values are expressed as mean±standard error (n=3); mean D90 values with different superscripts (a, b, c) differ significantly ($p < 0.05$) with each other. Ice cream sample with 4% (w/w) protein (Control) (-); 6% (w/w) protein (-□-); 8% (w/w) protein (-⊖-); 10% (w/w) protein (✕).

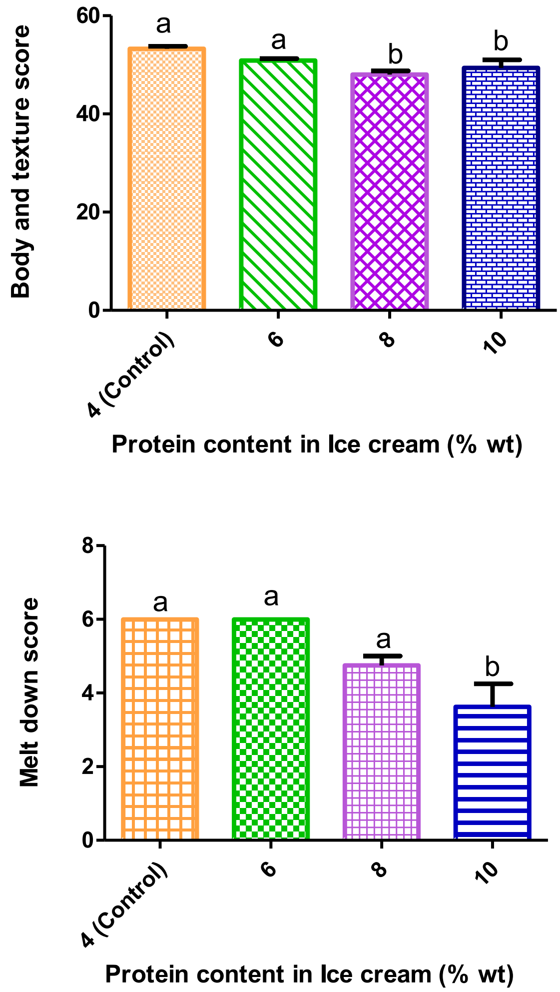

Fig. 5. Effect of protein content on sensory body & texture score and meltdown score of ice cream.
Note: Values are expressed as mean±standard error (n=3); mean values with different superscripts (a, b, c) differ significantly (p < 0.05) with each other.

scores. However, due to higher hardness values, ice cream samples having 8 and 10% protein level obtained lesser scores. The sensory panellists mentioned about fast meltdown of ice cream samples containing 8 and 10% protein. They also observed sogginess in the ice cream samples. These could be attributed to the higher viscosity of mix thus reduced its whipping ability and enhanced the hardness of ice cream samples.

4. Conclusion

Supplementation of WPI resulted into significant changes in the quality attributes of ice cream. Increasing the protein content increased the apparent viscosity and shear thinning behaviour of the ice cream mix. Particle size distribution indicated that increasing the protein content decreased de-emulsification of fat globule and their clump formation. Lower overrun and higher instrumental hardness values were obtained for samples containing higher levels (8-10%) of protein. Sensory evaluation revealed that body and textural attributes were significantly affected upon increase in the protein content, but the samples were not totally un-acceptable. The study reveals that variation in stabilizer and emulsifier blend needs to be explored to improve the sensory acceptability of high protein ice cream.

Conflict of interest

Authors have no conflict of interest with respect to this manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2021.100029.

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