Physico-chemical analysis of calcium enriched herbal ice-cream

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Introduction
Ice cream is a frozen dairy food made by freezing a pasteurized mix with agitation to incorporate air and to ensure uniformity of consistency. The mix is composed of a combination of milk products, sugar, and stabilizer/emulsifier all of the edible material (Marshall et al. 2003). Ice-cream is a complete food in which constituents are completely digested which makes a desirable food for children and persons who need to put on weight (Arbuckle, 1996). Nowadays, consumers are becoming health conscious and the demand for dietetic/health foods has been increasing. There has been considerable interest in extending the use of herbs in dairy foods, fruits juice-based products and pharmaceuticals (Singh, 2010). Herbs can be incorporated into ice cream and have the potential for preventive and remedial purposes. Herbal ice cream is gaining wider popularity over normal ice cream due to its functional, nutritional and pharmacological activities (Kumar et al. 2013). Medicinal properties of herbal ice cream are antiseptic, anti-microbial, anti-viral and antidiabetic. Ice cream is a widely enjoyed palatable food product that provides a useful source of dietary calcium in the diet and can be fortified to provide additional amounts without changing flavor and color. India is known for its traditionally well-practiced knowledge of herbal medicines. In spite of having a large number of medicinal plants and well-practiced knowledge of herbal medicines, the share of India in the global market is not up to the mark (Rajkumar and Singh, 2009).

Bacopa monniera is an Ayurvedic medicine, clinically used for memory enhancing, epilepsy, insomnia and as a mild sedative (Chunekar, 1960). Bacopa monniera is considered as a nerve tonic in Indian traditional medicine (Chopra et al. 1969). It was used in traditional medicine to treat various nervous disorders, as a brain tonic to enhance memory, learning, improves concentration and to provide relief to patients with anxiety. It is also used in digestive complaints, for skin disorders and as an antiepileptic, antipyretic, and analgesic (Satyavati et al. 1976). High Performance Liquid Chromatography (HPLC) (Baig et al. 2019 a and Haratifar et al. 2014) and methods based on UV detection have been developed for the determination of bacosides in B. monniera (Shrikumar et al. 2004). Calcium, an important
mineral component of the human diet and chiefly available in milk and milk products, seafood, legumes, and some vegetables. Over the past years, the economic burden of osteoporosis is increasing, the clinical implications of calcium deficiency are being better recognized, and deficiency of vitamin D (important for calcium absorption) was being documented in tropical countries. Calcium absorption from dairy products can be easily done by the intestine compared to calcium from vegetables and cereals (Weaver et al. 1999). Dairy products like ice cream, desserts, cottage cheese, cream cheese, yogurt, yogurt drinks, and sour cream can be added with calcium due to their higher bioavailability (Gerstner, 2002).

Texture is an important factor as it influences how a sample of ice cream reacts within a person’s mouth. The resistance of ice cream to the mechanical forces imparted by the tongue, upper palate and teeth will dictate the overall perception of ice cream texture (Yilsay et al. 2006). A coarse texture is most frequently cited defect in ice cream (Marshall and Arbuckle, 1996). As this defect becomes pronounced, a gritty or icy mouthfeel is followed by a relatively cold sensation in the mouth caused by excessively large ice crystals. To achieve small initial ice crystals, the ice cream mix must be rapidly subcooled to the point of maximal nucleation rate (Hartel, 1996). This allows the greatest number of ice crystals to form and the least amount of ice crystal growth in the freezer. Upon extrusion from the freezer, ice cream must immediately be hardened to minimize recrystallization. The temperature and rate of hardening determine the final ice crystal size and the physical and sensory properties of the product (Sutton and Bracey, 1996). The texture defect icy is eminent as ice crystals grow throughout storage. The present study aims at the utilization of ice-cream as a carrier of calcium (calcium gluconate) and Brahmi (ethanol extraction) for added nutritional benefits to the consumers with objectives like sensory, textural characters and microbial quality were found out.

Materials and Methods

The skim milk and cream were procured from KV ASU Dairy Plant, Mannuthy used for product preparation. Food grade calcium gluconate (CG) was procured from Nice chemicals Ltd, Cochin. Ice cream mix was prepared using a batch freezer according to Marshall et al. (2003).

Preparation of calcium enriched herbal ice cream

Preparation of calcium enriched herbal ice cream was done according to Baig et al. (2019 b). Skim milk (0.5% Fat, 8.7% SNF) and cream (40% Fat, 5% SNF) was taken and standardized to 10% fat and 11% SNF (Solid non fat), subjected to pasteurization (80°C/25 sec). After pasteurization, product was cooled to 60°C and dry ingredients including CG (2.2g/L mix), sugar (15%) and stabilizers/emulsifiers (0.5%) were added. BME (100 mg/L) was added to the ice cream mix. Thorough mixing was done by using egg beater and then subjected to two-stage homogenization at 60°C (13.8MPa and 3.45 MPa). After homogenization, the ice cream mix was kept for aging at 4°C/24 hrs. Then the ice cream mix was frozen and incorporation of air was done by batch freezer at -4°C/7 min. The product so obtained was filled in polystyrene cups and placed in hardening room for hardening (-24°C 6 h). Finally, the product was taken from the hardening room and stored in a freezer at -15°C. Response surface methodology (RSM) was used to optimize the levels of two ingredients (CG and BME) according to Baig et al. (2019 b). Central Composite Rotatable Design (CCRD) using two 8 variables and five responses comprising of sensory attributes was used for computation of optimized solution. All the responses fitted well into the quadratic equation with R²>0.60. The optimum levels of CG and BME are 217.34 mg/100 mL and 10 mg/100 mL for preparation of experimental ice cream. Flow chart of product preparation is shown in Fig 1.

Sensory evaluation of calcium enriched herbal ice cream

The sensory characteristics of the ice cream were judged by 10 panelists according to the method modified from Bodyfelt et al. (1998). Judges were selected based on their availability and willingness to participate in the study. Hardened ice cream samples were tested at a serving temperature of -10°C. Freshly calcium enriched herbal ice cream prepared was evaluated for its sensory characteristics such as color, flavor, body, and texture, melting property and overall acceptability scores based on 9-point Hedonic scale (Amerine et al. 1965). Panelists were asked to note any defects or undesirable characteristics. Physicochemical and sensory analyses were carried out 2 weeks after the production of ice cream samples.

Textural analysis

Various textural characteristics such as hardness, cohesiveness, springiness, gumminess, and chewiness were measured for sample ice cream and for control ice cream, using Stable Microsystems TA HD Plus textural analyzer, fitted with 25 kg load cell. Experiments were carried out using a cylindrical probe (10 mm diameter) combined with Exponent lite Texture Expert Software. The ice cream samples were carefully filled up in 100 ml ice cream cup so that no air pockets remained within the sample. It was then stored for minimum 24 hrs at -21 ± 2°C temperature for hardening. The probe was kept at -21°C for 30 min before starting the experiment in order to reduce variations due to the temperature difference between the probe and ice cream sample. The experiments were carried out after maintaining room temperature (20°C). Ice cream cup was kept for 5 min and then hardness was measured (6 readings minimum). The hardness was determined as the peak compression force during penetration (Fig 2)

Following the conditions were employed while testing the texture of ice cream samples:
Sample: Ice cream has taken in a polystyrene cup

Compression: Ice cream samples were compressed to 75 percent of its height

Load cell: P/5 10 mm diameter stainless-steel cylindrical probe

Probe speed: A probe speed of 1 mm/sec during the test and 2 mm/sec for pre- and post-test was used throughout the study

Testing temperature: Ice cream sample is taken at -20°C tempered to -10°C.

Interpretation of texture profile parameters from texture profile curve: A typical force-deformation curve for double cycled compression is given in Fig. 2

Hardness - Maximum force recorded during the first compression cycle (g)

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**Fig 1.** Flow chart of preparation of calcium enriched herbal ice cream
Springiness - Width of the downstroke in curve A₂ (mm)
Gumminess - Hardness x Cohesiveness (g)
Chewiness - Gumminess x Springiness (g.mm)

Colour

Colour measurement was done by using color Flex (Hunter Associates Laboratory, Inc., Reston VA, USA) color measurement system equipped with dual beam xenon flash lamp and universal software. The instrument was calibrated prior to sample measurements with standard black and white tiles as prescribed by the supplier. The results were represented by the L* a*, and b* notation. It is a 3-D color presentation method in which L* is the lightness of color and equals 0 for black and 100 white. The a* represents the amount of red (0 to 60) or green (0 to 60) while b* represents the yellowness (0 to 60) or blueness (0 to -60).

Microbiological quality

Total colony count of ice cream sample was estimated by pour plate technique, as described in IS 2802: 1964 Coliform count of each ice cream sample was estimated by IS2802:1964.

Melting rate (g/min)

According to Cruz et al. (2009), the melting time of ice cream is related to its stability after overrun and indicates the extent of the stabilization and partial coalescence of fat. Furthermore, an increase in coalesced fat provides greater resistance to flow of the liquid phase resulting in slower melting (Muse & Hartel, 2004). Melting rate is a selective term and it depends upon the melting temperature and time. The melting rate was determined by the following procedure: ice cream sample of known weight was placed on a wire mesh, which was placed on a pre-weighed measuring cylinder (100 ml) with a glass funnel (10 cm dia.). The whole assembly was kept undisturbed at 25±1°C for 45 min. The weight of the melted samples collected in the measuring cylinder was noted and the melting rate was determined by the formula given below:

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\text{Melting rate (g/min)} = \frac{\text{Weight of melted ice cream (g)}}{\text{time (min)}} \times 100
\]

The process optimization of calcium enriched herbal ice cream was carried out by using Central Composite Response Design (CCRD) of Response Surface Methodology (RSM). CG and Bacopa monniera extract were two variables selected for this purpose and responses noted were sensory, color, hardness and melting quality. The data generated during the study were analyzed statistically using Design expert version 8.0.7.1. ANOVA was applied to the data of sensor score of ice cream with different level of ingredients.

Results and Discussion

Sensory characteristics

The sensory profile of traditional ice cream (control) was compared with two coded samples (A and B) of CEHI prepared from the same lot of ice cream mix, were assessed by a panel of judges using the principle of triangle test. The obtained results were presented in table 1 and Fig 3. The sensory response to the ice cream samples was affected by the variation in calcium level and BME level. The sensory attribute of the textural appearance corresponded to whether an ice cream sample cut smoothly or crumbled by using a spoon. Similarly, graininess was related to whether the surface had a typical appearance on the product or the granular icy appearance. Although these attributes are similar, the sensory panel found differences (P < 0.05) for the CEHI sample with a control sample when scoring the samples for appearance. Panelists found crumbled during cutting or scooping CEHI sample. The appearance term, air holes, described whether or not discrete air pockets were observed as the panelist scraped the surface of the ice cream with a spoon. Although all of the samples had the same amount of air incorporated into them (90% overrun), the panelists scored the higher for CEHI samples as having more...

Table 1 Sensory attributes of calcium enriched herbal ice cream compared to control ice cream

| Sensory attributes | Type of ice cream | Chi-square value |
|-------------------|------------------|-----------------|
|                   | Traditional ice cream | A            | B            |                  |
| Appearance        | 8.8±0.08 a        | 7.61±0.06 a     | 7.69±0.04 a  | 5.25**          |
| Flavor            | 8.75±0.08 b       | 7.3±0.06 a      | 7.2±0.06 ac  | 7.40**          |
| Body and texture  | 8.62±0.07 a       | 8.33±0.07 a     | 8.40±0.06 a  | ns              |
| Overall acceptability | 8.6±0.07 c      | 7.72±0.06 ac    | 7.65±0.07 a  | 6.30**          |

Figures are mean ± standard error of three replications. **-Significant at one percent level (p<0.01), ns- non-significant (p>0.05) a
b mean scores with the same superscript within rows did not differ from each other, A and B–calcium enriched herbal ice cream
of these visible, discrete air pockets than the control samples (Table 1). The appearance term, stickiness, referred to the adhesion of the product to itself. The scores for the appearance attributes of stickiness and glossiness increased in CEHI compared to control ice cream. Pinto (2006) also noted that calcium imparts smooth texture to the product thereby resulting in enhanced glossiness of the ice cream. All of the ice cream samples were formulated to have relative sweetness values of 15 [contained the sweetness equivalent of 15% sucrose (wt/wt)]. Correspondingly, no significant (P > 0.05) differences in sweetness were observed as the calcium content of the ice cream was increased from 145 to 165 mg/100ml. Singh et al. (2008) have reported that there is no difference in the control yogurt and CG enriched fruit yogurt up to 260 mg/100 mL when compared to four different characteristics viz., flavor, appearance and overall acceptability. The flavor scores were lower for CEHI samples (P < 0.05) as the BME imparts a bitter taste. Similar findings reported by Russo and Borrille, (2005) that Increasing the BME adversely affected the flavor of the product because of the presence of phenolics, which are bitter in taste and thereby decrease the flavor score. BME concentration had significantly reduced overall acceptability, affected the sweetness of the product because of

Fig 2. Force versus time x speed curve from the compression test

Fig 3 Sensory attributes of calcium enriched herbal ice cream from two lots of samples compared to control ice cream

Table 2 Color values of calcium enriched herbal ice cream compared to control ice cream

| Color value | CEHI    | Control ice cream | t-value |
|-------------|---------|-------------------|---------|
| L           | 69.4±0.1| 89.1±0.4          | 18.5**  |
| a           | 24.5±0.6| 0.8±0.2           | 21.4**  |
| b           | 5.6±0.3 | 7.8±0.1           | ns      |

Figures are mean ± standard error of three replications, **-Significant at one percent level (p<0.01), ns- non-significant (p>0.05) of these visible, discrete air pockets than the control samples (Table 1). The appearance term, stickiness, referred to the adhesion of the product to itself. The scores for the appearance attributes of stickiness and glossiness increased in CEHI compared to control ice cream. Pinto (2006) also noted that calcium imparts smooth texture to the product thereby resulting in enhanced glossiness of the ice cream. All of the ice cream samples were formulated to have relative sweetness values of 15 [contained the sweetness equivalent of 15% sucrose (wt/wt)]. Correspondingly, no significant (P > 0.05) differences in sweetness were observed as the calcium content of the ice cream was increased from 145 to 165 mg/100ml. Singh et al. (2008) have reported that there is no difference in the control yogurt and CG enriched fruit yogurt up to 260 mg/100 mL when compared to four different characteristics viz., flavor, appearance and overall acceptability. The flavor scores were lower for CEHI samples (P < 0.05) as the BME imparts a bitter taste. Similar findings reported by Russo and Borrille, (2005) that Increasing the BME adversely affected the flavor of the product because of the presence of phenolics, which are bitter in taste and thereby decrease the flavor score. BME concentration had significantly reduced overall acceptability, affected the sweetness of the product because of
the increased perceived bitterness and astringency owing to the presence of phenolics in the extract. The developed calcium enriched herbal ice cream has shown overall acceptability score of 7.5 (Like moderately) which is a well indication of the higher organoleptic quality for frozen products. Though the developed product had bitter taste due to the frozen condition makes it to accept the product moderately. The sensory analysis not only was more sensitive to the textural differences between ice cream samples but also distinguished ice creams based on flavor, which is the final indicator of ice cream quality. The perception of sweetness was affected by the increase in CG content; however it couldn’t mask the bitter flavour from BME.

**Effect of calcium and BME on the color of ice cream**

The color of the CEHI sample decreased in whiteness (P < 0.05) compared to the control sample. Correspondingly, CEHI samples were less red and greener due to BME, as reflected by increasing a* values (Table 2). According to Baig et al. (2019 b), BME contains phenolics which imparted a bitter taste and green color to the ice cream. The b* value is higher in CEHI samples which corresponded to the sample being more yellow and less blue (Table 2). The obtained results were shown in Fig 4.

**Hardness and melting rate**

Ice cream with desirable melting quality begins to show definite melting within 15-20 mins. When kept at room temperature and melted product should flow readily and form a homogenous fluid with the appearance like that of the unfrozen mix (Marshal et al. 2003). The textural attributes of the sample were compared with that of control ice cream. Structure development in ice cream often is attributed to the macromolecules present in the ice cream mix-such as milk fat, protein, and complex carbohydrates. The strengthening of the protein network produces a uniform and stable emulsion and reduces the formation of ice crystals during storage (El-Nagar et al. 2002). The hardness of experimental ice cream and control ice cream are 42 N and 30 N (Table 3) respectively and had shown a significant difference (p < 0.01). According to Yonis et al. (2013) hardness of CG fortified banana yogurt shown a significant increase of hardness compared with the control. Due to the interaction between calcium salt and

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**Table 3** Hardness and melting rate of calcium enriched herbal ice cream compared to control ice cream

| Property       | CEHI          | Control ice cream | t-value |
|----------------|---------------|-------------------|---------|
| Hardness (N)   | 42.76±0.87    | 30.47±1.03        | 9.65**  |
| Melting rate (g/min) | 0.75±1.60    | 0.56±1.40         | 5.94**  |

Figures are the Mean ± Standard Error of six replications, **-Significant at one percent level (p<0.01), ns- non-significant (p>0.05)

**Table 4** Microbial count of calcium enriched herbal ice cream compared with control ice cream

| Parameter       | Calcium enriched herbal ice cream (log CFU/g) | Control ice cream (log CFU/g) | t-value |
|-----------------|---------------------------------------------|-----------------------------|---------|
| Viable count    | 11.848±0.02                                 | 11.854±0.03                  | 0.532ns |
| Coliform count  | Nil                                         | Nil                         | -       |

Figures are the Mean ± Standard Error of six replications, ns- non-significant (p>0.05)
protein matrix present in the ice cream mix, it makes ice cream slight harder than usual. Melting rate of experimental ice cream and control ice cream are 0.75 and 0.56 g/min (Table 3) respectively and had shown a significant difference \((p<0.01)\). Calcium had a negative effect on the melting rate. On increasing calcium in ice cream, the melting rate decreased. Similar findings of the effect of calcium on melting quality of ice cream have been reported Marshal, (2003). The obtained results were shown in Fig 4 and Fig 5.

**Microbial Quality**

The microbiological quality of the Calcium enriched herbal ice cream was analyzed by enumerating the viable cells and coliform results are presented in Table 4. In the present study viable count of calcium enriched herbal ice cream (Table 4) had shown no significant difference when compared to control ice cream \((p>0.05)\). There was no detection for yeasts and mold for calcium-enriched herbal ice cream and the control. These results indicate that the manufacture of calcium enriched herbal ice cream was carried out under proper hygienic conditions, resulting in the elimination of the contamination with such undesirable bacteria. According to Younus et al. (2002) absence of coliform bacteria in fresh control and CG fortified banana stirred yogurt was due to pasteurization of premix prior to incubation.

**Conclusions**

Sensory parameters play a major role in determining the acceptability of a food product as well as the ultimate purchase decision of consumers. In this study, it was found that CG is suitable for enrichment of CG without any stabilizer for
neutralizing calcium salt. BME alone imparted light green color and bitter taste to ice cream without altering the body characters. Beyond the level (10 mg/100 g) BME had shown lower acceptability in terms of color, flavor and overall acceptability. The effect of CG at had shown no negative effect on melting rate and improved the glossiness of the product. In this newly developed product, additional calcium fortification was done to meet the 30% RDA of calcium and reduce the chances of calcium deficiency diseases.

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