To study the effect of chicory root extract on physico-chemical properties of synbiotic yoghurt-ice cream

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Abstract

The study was designed to examine the effect of addition of chicory root extract on physico-chemical properties of multifunctional synbiotic yoghurt-ice cream. The probiotic *Lactobacillus acidophilus* (NCDC-195) cells were used in free as well as encapsulated form and chicory root extract was used as a prebiotic in three proportions (0, 3 and 6 % w/w) in this experimental study. Higher concentrations of chicory root (3 % and 6 %) significantly increased the overrun, first dripping time and complete melting time in all samples containing encapsulated cells ($p<0.05$). Supplementing with free/encapsulated form did not significantly ($p<0.05$) influence the acidity value of the samples. A significant difference ($p<0.05$) was observed in thiobarbituric acid (TBA) values too. The major component of chicory root extract in synbiotic yoghurt ice cream inhibits fat oxidation during storage, due to its anti-oxidant properties.

Keywords: Encapsulation, chicory root, yoghurt, ice cream, synbiotic

Introduction

Yogurt-ice cream is a fermented frozen dairy dessert having the physical characteristics of ice cream with sensory and nutritional properties of fermented milk at the same time. It is an excellent source of calcium and proteins having enhanced storage stability and shelf life than milk and other dairy products. It may be produced either by direct acidification, or indirect acidification method or blending [1]. Out of these, the indirect acidification technique, which involves production of ice cream mix and plain yoghurt individually and then blending them in appropriate proportions, has been found to be feasible at a commercial scale. The recent years have witnessed an increase in the consumption of fermented milk and related products by incorporation of probiotic species [2]. Probiotics are the living organisms which when administered in adequate amounts, confers health benefit to the host [3]. They impart therapeutic effects and many health promoting benefits, for instance, alleviation of lactose intolerance, anticarcinogenic and antimutagenic effects, stimulation of immune system, prevention of cancers, relieving diarrhoeal diseases, hypercholesterolaemia, promotes calcium absorption, synthesis of vitamins etc [4, 5]. Prebiotics are the non-digestible fiber compounds that stimulate the growth and/or activity of probiotics in the gastrointestinal tract. These must be able to resist digestion and absorption process and be accessed only by the beneficial microorganisms in human colon [6]. These organisms selectively utilize prebiotics as their sole carbon source resulting in an increase in their growth and number.

Chicory (*Cichorium intybus* L.) is a herb belonging to the *Asteraceae* family and may be used in the forms of flowers, leaves and roots. Inulin is the principle non-digestible food compound present in chicory root which is a polymer of fructose with β (2-1) glycosidic linkage, that nourishes the probiotic bacteria [7]. Inulin is similar to fructo-oligosachharides (FOS) but they differ in their chemical structure. FOS chains of molecules are shorter than inulin chains. It has been reported that inulin may be used as an ingredient for fat and sugar replacement-as a low calorie bulking agent and as a texturizing agent [8]. A food product containing properties of both, probiotic and prebiotic is called a synbiotic which offers prophylactic management of gastro-intestinal defects [9, 10].

It has been well documented by International Dairy Federation (1991) [11], that a minimum of 107 live cells must be consumed per gram of the product and the therapeutic effects of these cells depends on their viability after consumption [31]. Supplementation of yoghurt ice-cream with encapsulated probiotic bacteria and prebiotics may provide additional health benefits to human beings. The process of encapsulation of probiotic cells within alginate based microcarriers provides a relatively new approach to enhance their survival and viable count during frozen storage. Alginate is cheap, non-toxic, easy to handle, and has been used in many applications as matrices for release or immobilization [12]. Shori [13] and Kumar et al. [7] have also reported the survival

of probiotic cells in frozen yoghurts because of encapsulation. It seems that with attention to the alginate in stabilizing of ice cream, it can be a good vehicle for probiotic delivery in ice cream. There is a few literature on the yoghurt ice cream supplementation with encapsulated cells and chicory root extract as the prebiotic and fat replacer. This study has been conducted to improve commercial value of ice-cream yoghurt. The objective of present study is to evaluate the physico-chemical properties (first dripping time, complete melting time, viscosity, titratable acidity, overrun and TBA value) of synbiotic yoghurt-ice cream by addition of chicory root extract.

Materials and Methods

Preparation of bacterial culture:

Freeze-dried probiotic yoghurt cultures namely, *Streptococcus salivarius* ssp. *thermophilus* NCDC-074 (*S. thermophilus*) and *Lactobacillus delbrueckii* ssp. *bulgaricus* NCDC-009 (L. bulgaricus) and pure freeze-dried probiotic culture of *Lactobacillus acidophilus* NCDC-195 (*L. acidophilus*) were purchased from National Dairy Research Institute, Karnal, Haryana, India. Yoghurt bacteria were inoculated into 10 mL of skim milk and inoculated milk was incubated at 42°C for 8 hours. Probiotic culture was inoculated into 10 mL MRS broth (HiMedia Laboratories Pvt. Ltd. Mumbai, India) and incubated at 37°C for 24 hours under aerobic conditions to obtain a cell density of about 107 colony forming units per mL (cfu/mL). Further, the culture was transferred into 95 mL of MRS broth and incubated under the same conditions. Cells were harvested by centrifugation at 8,000 rpm (3,578 × g) for 10 minutes and after that the supernatant was discarded. Furthermore, cell pellet was re-suspended in peptone saline (1 g/L peptone, 8.5 g/L NaCl) and centrifuged again under the same conditions. Then washed cells were re-suspended in a total of 10 mL peptone saline and stored at 4°C until usage. Fresh cells suspension was prepared for encapsulation. Encapsulation of L. acidophilus was done using emulsion method [14].

Preparation of encapsulated cells:

Sodium alginate microcapsules of probiotic cells were made using a gelation technique [15, 4]. 18 g of sodium alginate solution (10g/L) was mixed with 1 g washed bacterial suspension. The mixture was subsequently emulsified in 100 g vegetable oil containing 5 g/L Tween 80 using a magnetic stirrer at ~900 rpm for 20 minutes. Gelation was initiated by adding 32 mL of an emulsion containing Na+ ions (60 g vegetable oil, 5 g/L Tween 80 and 62.5 mM NaCl). The alginate microcapsules were formed during continuous stirring for 20 min. The beads were allowed to stand for 30 min for gelification and then rinsed with and subsequently kept in sterile 0.1 % (w/w) peptone solution at 4 °C.

Treatments:

Six treatment combinations i.e. three samples of 0, 3 and 6 % chicory root extract without encapsulated bacteria (designated as T_1, T_2 and T_3 respectively) and three samples of 0, 3 and 6 % chicory root with encapsulated bacterial cells (designated as A_1, A_2 and A_3 respectively) were analyzed. The experiments were conducted in triplicates.

Product preparation:

Multifunctional synbiotic yoghurt-ice cream was prepared as a combination of yoghurt and ice cream mix. Yoghurt was prepared from standardized milk (4.5 % fat and 8.5 % solid not fat). It was pasteurized (80 °C, 20 min), cooled to 45 °C, inoculated with 2 % (w/v) of a commercial yoghurt culture (mixed flora 1:1 of *L. bulgaricus* and *S. thermophilus*) and incubated at 45°C for 4 hours. For ice cream mix production, standardized milk with the addition of 12 % (w/w) white sugar (sucrose), 0.5 % stabilizer (w/w) and 0.3 % (w/w) emulsifier, was prepared. 0, 3 and 6 % chicory root extract (in various batches) was added to this and the mixture was heated to 45 °C and homogenized at 1000 rpm/60±1°C by using a micro tissue homogenizer (Capacity 10 LPH, Ralliwolf Limited, Ahmednagar, India) until no clumps were present. α-monoglyceride (E471) (Sigma-Aldrich, London, UK) was used as a emulsifier and guar gum was utilized as the stabilizer. The mixture was then pasteurized at 80°C for 5 minutes and rapidly cooled to about 10°C and then finally yoghurt was homogenized at 200-300 megapascals at 30-40°C and blended with the ice cream premix (2:3). The yoghurt-ice cream mix was standardized to 10 % fat with the total solid content of 36 % by addition of skim milk powder and cream, with continuing agitation at 42°C. The yoghurt-ice cream mixture was split into one litre batches, each of which was supplemented with free or encapsulated form at a rate of 3 % (w/v). The mixtures were aerated and frozen by a self-contained freezing unit and stored in 100 mL cups at -18°C.Treatments were made in triplicates.

Analysis of physico-chemical properties:

The viscosities of all yoghurt ice-cream samples were determined at 4˚C using a digital Brookfield Viscometer, Model DV-II (Brookfield Engineering Laboratories, Stoughton, MA, USA) as given by the method of Akin et al. [17]. The first dripping time and the complete melting time of different treatment combinations were measured using standard protocols [18, 19]. 50 g of each of the samples were placed in a Buchner funnel on the top of a flask for 15 min and was allowed to melt at room temperature (24±1°C). The values obtained were recorded with a digital timer. Results were expressed as time (in sec). The titratable acidity of multifunctional synbiotic yoghurt-ice cream samples was determined by back titration method [20]. The overrun of the final product was determined by using the following equation [17]:

$$Overrun = \frac{Weight\ of\ unit\ mix - Weight\ of\ equal\ volume\ of\ yoghurt\ ice{-}cream}{Weight\ of\ equal\ volume\ of\ yoghurt\ ice{-}cream}$$

Oxidation products (i.e. malondialdehyde) were analyzed spectrophotometrically using the TBA test [21]. TBA test is based on the malondialdehyde (secondary product of lipid oxidation) reaction with TBA reagent to obtain a red/pink pigment (chromogen), which results from the condensation of two molecules of TBA with one molecule of malondialdehyde and the probable elimination of two molecules of water with absorbance at 532 nm. The TBA reagent was prepared immediately before use by mixing equal volumes of freshly prepared 0.025M TBA (neutralized with NaOH) and 2M H_3PO_4/2M citric acid. Reactions were terminated by pipetting 5.0 mL of yogurt sample containing microcapsules into a glass centrifuge tube and mixed throughly with 2.5 mL TBA reagent. The mixture was heated immediately in a boiling water bath for exactly 10 min, and then cooled on ice. Then 10 mL cyclohexanone and 1 mL of 4M ammonium sulfate were added and centrifuged at 2,490 x g for 5 min at room temperature. The orange red cyclohexanone supernatant was decanted and its absorbance at 532 nm was measured spectrophotometically in a 1cm light path. Average absorbance ×100 as the TBA values is reported with the result normalized per g of sample. All measurements were run in triplicate.

Statistical analysis

All statistical analysis were performed using SPSS statistical software program version 16 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range method at ($p<0.05$) was used for analysis of multiple comparisons between means. All analyses were done in triplicate.

Result and Discussion

Physico-chemical characteristics:

Several factors such as pH, titratable acidity, presence of various solutes,

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Table 1: Physico-chemical properties of multifunctional synbiotic yoghurt-ice cream

Treatments		First Dripping Time (sec)	Complete Melting time (sec)	Viscosity (cP)	Titrable acidity (%)	Overrun (%)
Free probiotic cells	T_1	456.25±9.28^{a}	2028.20±4.99^{ab}	6254 ± 7^{b}	0.465±0.013^{bc}	34.121 ± 0.090^{ab}
	T_2	458.25±8.18^{a}	2028.20±4.79^{ab}	6258 ± 64^{ab}	0.473±0.010^{b}	34.128± 0.112^{a}
	T_3	455.00±6.53^{a}	2032.80±4.99^{b}	6290 ± 46^{b}	0.503±0.015b^{c}	35.020 ± 0.125^{ab}
Encapsulated cells	A_1	452.00±8.04^{a}	2025.00±2.45^{ab}	8112 ± 26^{b}	0.478±0.017^{bcd}	36.03 ± 0.190^{ab}
	A_2	454.75±8.62^{a}	2027.80±7.41^{ab}	8139 ± 46^{b}	0.483±0.010^{bcd}	36.76 ± 0.294^{ab}
	A_3	459.50±10.66^{a}	2037.80±4.57^{ab}	8201 ± 46^{b}	0.510±0.018^{cd}	37.097 ± 0.995^{ab}

T_1, T_2 , T_3 : Treatment samples containing 0, 3, 6 % chicory root extract and unencapsulated probiotics.
A_1, A_2 , A_3: Treatment samples containing 0, 3, 6 % chicory root extract and encapsulated probiotics
Results presented as a mean ± pooled standard deviation of the mean. Different small letter superscripts depict the statistical difference within a column, $P < 0.05$ between means for different batch

temperature, encapsulation etc. have known to affect the viability of probiotics in fermented dairy products [22]. The first dripping time, complete melting time, overrun values, viscosity and titratable acidity values of all treatment combinations in synbiotic yoghurt-ice cream are mentioned in Table 1. Analysis of all these characteristics in ice cream yoghurt revealed significant differences among the varying proportions of chicory root extract ($p<0.05$). The samples supplemented with the prebiotic compound in higher concentration, had higher viscosity as compared to others. The viscosity was even more pronounced for samples having encapsulated probiotic cells. This means that, in addition to chicory root extract, the increase in viscosity may also be attributed to sodium alginate used for encapsulation. Similar results have been shown by Guler-Akin et al. [23]. This may be due to the high water binding capacity of alginate microcapsules as well as inulin present in chicory root extract.

Higher concentration of chicory root (6 %) increased the overrun percentage in samples ranging from 37.42-39.35. Overrun depends on the amount of air incorporated in the yoghurt ice-cream and also the amount of total solids present in it. Marshall and Arbuckle [29] also reported that the percentage of overrun for ice cream is usually between 30 and 60 % depending on total solids used in the formulation. During aeration, air/serum interface formed due to air incorporation is initially stabilized against immediate coalescence by adsorption of proteins from the aqueous phase to the interface [4]. With continuous aeration, the bubbles break down into smaller bubbles. The stability of these bubbles probably increased because of the adsorption of inulin molecules present in chicory root onto the air/serum interface along with proteins. This would have led to a higher proportion of air phase in the final product which was measured as a higher overrun. The addition of chicory root extract showed an increased value of overrun in the samples and this increase was more prominent in the case of encapsulated bacterial cells. Similar results have been obtained where addition of prebiotic has also increased the overrun in ice-creams [24, 25].

First dripping time is the time for the ice cream at room temperature to begin dripping. The first dripping time as well as the complete melting time increased with increasing concentrations of chicory root extract. Also, the values of first dripping time and the melting time showed significant difference ($p<0.05$). The values are shown in Table 1. This may be because of the presence of inulin in chicory root, which has water binding ability, that the water molecules become immobilized and are not able to move freely among other molecules of the mix. An increase may also be attributed to the presence of sugars, emuslifiers as well as stabilizers in the final product. Similar results have been observed by El-Nagar et al. [26] who reported that adding inulin up to 5 % level significantly slowed down the melting rate of yoghurt-ice cream. A resistance to melting was also observed in the ice creams where guar gum and sodium alginate amount was found in the mixture [18]. However, Li et al. [27] reported that higher the concentration of the water solute, the lower is the freezing point and the faster is the melting rate. Therefore, the samples that are high in solids and fat melt faster than do the samples that are low in solids and fat. Table 1 depicts the variations in the titratable acidity (% lactic acid) profile of synbiotic yogurt-ice cream samples.

The difference in titratable acidity of yoghurt-ice cream samples with free/encapsulated cells as well as varying proportions of chicory root extract was non-significant ($p<0.05$). There was a gradual increase in the all the samples of yoghurt ice cream having free as well as encapsulated probiotic cells. It may be concluded the addition of chicory root extract in samples may have stimulated the metabolic activities of probiotic bacteria and enhanced the acidity values. Similar results have been reported by Guler-Akin et al. [23] where probiotic yoghurt ice cream is supplemented with carob extract and whey powder. Conversely, another study reported that the addition of ginger juice resulted in a progressive decrease in the acidity [28].

Another objective of the present study was to determine the extent of TBA value. Secondary product of lipid oxidation i.e. malondialdehyde was used as an oxidation marker in samples during storage up to 60 days. TBA reagent when reacts with malondialdehyde, resulting from degradation of hydroperoxide, forms a pink color complex called chromogen which has absorbance at 532 nm. Table 2 shows the results of the TBA analysis of the multifunctional synbiotic yoghurt-ice cream over 2 months of storage. There was slow increase in TBA values for all the treatments. It is generally considered that fat oxidation increases with increase in storage time and is also accelerated by lower pH values [29]. In the present study, however, there was a slow increase in TBA values for all samples ($p<0.05$) examined during storage at -18±1°C. The free and encapsulated cells of probiotic with higher content of chicory root treated samples showed lower TBA value simultaneously. Marshall [30] reported that lactic acid bacteria inhibited the promotion of fat oxidation of milk products such as yoghurt. It means the dried chicory root extract produced better nourishment of the probiotic bacteria [7] and gives lower fat oxidation in the product. In addition to this, the bacterial counts of the prepared multifunctional synbiotic yoghurt-ice cream containing *L. bulgaricus* and *S. thermophilus* along with dried chicory root extract, chosen as the source of prebiotic with dietary fibre, were studied from day 0 to 60 days during storage at -18°C. The viable counts of *L. bulgaricus* significantly ($p<0.05$) improved in multifunctional synbiotic yoghurt-ice cream

Table 2: Average concentrations of malondialdehyde (mg/kg) in the yoghurt-ice cream during the storage period

Treatments		0 Days	15 Days	30 Days	45 Days	60 Days
Free cells	T_1	0.24±0 .01^{ab}	0.26±0.01^{de}	0.30±0.02^{fg}	0.34±0.01^{e}	0.38±0.01^{ef}
	T_2	0.22±0.01^{a}	0.21±0.01^{a}	0.24±0.01^{abc}	0.28±0.01^{bcd}	0.35±0.01^{bc}
	T_3	0.23±0.02^{ab}	0.21±0.01^{a}	0.22±0.02^{a}	0.26±0.01^{a}	0.3133.01^{a}
Encapsulated cells	A_1	0.24±0.01^{ab}	0.28±0.02^{ef}	0.30±0.02^{ef}	0.34±0.01^{e}	0.40±0.01^{f}
	A_2	0.23±0.02^{ab}	0.21±0.02^{ab}	0.25±0.01^{bc}	0.28±0.02^{abc}	0.34±0.02^{b}
	A_3	0.23±0.02^{ab}	0.22±0.02^{abc}	0.24±0.02^{ab}	0.27±0.01^{ab}	0.35±0.01^{bc}

T_1, T_2 , T_3 : Treatment samples containing 0, 3, 6 % chicory root extract and unencapsulated probiotics
A_1, A_2 , A_3: Treatment samples containing 0, 3, 6 % chicory root extract and encapsulated probiotics
Results presented as a mean ± pooled standard deviation of the mean. Different small letter superscripts depict the statistical difference within a column, $P < 0.05$ between means for different batches

with supplementation of 6 % dried chicory root extract on 0 day. The count of *L. bulgaricus* in the chicory treated multifunctional synbiotic yoghurt-ice cream samples were found to be enhanced to 0.30 log cycle up to 30 days and then reduced gradually during storage.

Conclusion

The study demonstrated the effect of incorporating chicory root extract on physico-chemical properties of yoghurt ice-cream with encapsulated probiotic and therefore it enhances the therapeutic value of the product. Higher concentrations of chicory root (3 % and 6 %) significantly increased the overrun, first dripping time and complete melting time in all samples containing encapsulated cells. The results also indicate that components of chicory root extract in synbiotic yoghurt ice cream inhibit fat oxidation during storage, due to its anti-oxidant properties". The TBA values of synbiotic yoghurt ice cream with chicory root extract were found to be low, due to inhibition of oxidation via probiotic bacteria. Six percent of chicory root extract samples examined lower TBA value during 60 days of storage study. Overall, it is concluded that the addition of 6 % dried chicory root extract was most suitable in encapsulated synbiotic yoghurt ice cream.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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