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Improvement of physicochemical and probiotic properties of frozen yoghurt by incorporating inulin and guar gum

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Abstract

Frozen yoghurt is considered as a healthy alternative for ice cream due to its probiotic properties. Inulin and guar gum are well known soluble dietary fibers used as gelling and stabilizing agents in food processing along with their prebiotic properties. However, not that much of investigations have been performed to evaluate their applications in frozen yogurts, particularly their combination effect on physicochemical, probiotic and sensory properties of frozen yogurts. Individual (2.7% inulin and 0.3% guar gum) and combined (2.7% inulin with 0.3% guar gum) effects of inulin and guar gum on pH, titratable acidity, melting rate, overrun, survival ability of probiotics and sensory attributes of frozen yoghurt were investigated in freezing (-18 0C) storage for a period of 21 days. During the frozen storage, a reduction of pH and an increment of acidity were noticed for all the tested samples. This was more in samples treated with both dietary fibers. The overrun rate was increased in the presence of inulin and guar gum where guar gum had a greater impact. In freezing storage, probiotic bacteria, *Bifidobacterium* (BB-12) and *Lactobacillus acidophilus* (LA-5) showed greater survivability in the presences of inulin and guar gum. This was more prominenet when both dietary fibers were added. Frozen yoghurt with combination of soluble dietary fiber (0.3 % guar gum and 2.7 % inulin) had the most appealing sensory characteristics. It is evident from this study that inulin can be combined with guar gum appropriately to enhance quality attributes of frozen yoghurt including probiotic properties.

Key Words: *Frozen yoghurt1, guar gum2, inulin3, physicochemical properties4, probiotics5*

Introductıon

Frozen yoghurt has combining characteristics of yoghurt (acidic taste) and ice cream (cooling effect) (Tamime and Robinson, 2007). This fermented diary product contains probiotic bacteria and thus is considered as a health beneficial alternative dairy product for ice cream. The environment of frozen yoghurt is not favorable for the existence of bacteria due to the fact that certain stress related environment factors such as pH, acidity, redox potential, freezing, oxygen, sugar concentration and osmotic effects, hydrogen peroxide, antagonistic impact of co-cultures, and mechanical shearing can cause damage to the bacterial cell (Mohammadi, 2011). So, it is a challenge to maintain the viability of bacteria in frozen conditions to get potential benefits.

Inulin is a soluble dietary fiber consisting of non-digestible carbohydrate called fructans that occurs mainly in chicory roots. The wider applicability of inulin in food industry is attributed to its unique functionalities like gel-forming ability, fat replacing ability, limited solubility in water, thermal stability in short food processing steps, hygroscopic and act as humectants, caloric value, low glycemic index, and prebiotic properties (Soiab *et al*., 2016). It has been revealed that the addition of soluble dietary fibers like inulin and guar gum had a protective effect on probiotic bacteria in frozen yoghurt (Isik *et al*., 2011). In addition, fiber incorporation modified and improved the texture, sensory characteristics and shelf-life of frozen yoghurt (Rezaei et al., 2014). They further reported that the addition of inulin remarkably improved the viability of Lactobacillus acidophilus and Bifidobacterium lactis. Working on different ice cream formulations, Aykan et al., (2008) found that inulin provided better flavor and textural properties of ice cream. Guar gum also possesses excellent gelling thickening and emulsifying properties and thus is a widely used food hydrocolloids in food industry (Gupta and Variyar 2018). Rezaei et al., (2011) reported that guar gum significantly affect the viscosity, overrun and melting rate of frozen yoghurt. Soluble dietary fibers like inulin, guar gum are universally agreed-upon prebiotics (Carlson, 2018). However, little is known on their impact on frozen yoghurt, particularly their combine effects on probiotic, physicochemical and sensory attributes in frozen yoghurt.

The present work focused to develop probiotic frozen yoghurt by incorporating inulin and guar gum aiming to determine the individual and combined effects of these soluble dietary fibers on physicochemical, probiotic and sensory attributes of frozen yoghurt.

Materials and methods

Frozen yoghurt preparation

Preparation of yoghurt

Standardized milk with 2.5 % fat was heated up to 70 ^{0}C and then sugar, gelatin, Highland full cream milk powder with 40 % fat was added and mixed until it dissolved well. The mixture was pasteurized at 95^{0}C for 5 minutes. After that, the mixture was cooled to 43^{0}C and inoculated with probiotic yoghurt culture. Then the prepared mixture was incubated at 43^{0}C for 3 hours until the pH become 5.

Preparation frozen yoghurt base mix

Frozen yoghurt base mix was prepared by mixing skim milk, cream, sugar, glucose syrup according to the recipe given by the Milco Pvt Limited. Then mix was heated to 90 ºC for 15 seconds for pasteurization.

Preparation of frozen yoghurt

Frozen yoghurt was prepared by mixing 70 % of yoghurt with 30 % of frozen yoghurt base mix and this mix was kept in refrigerator (at 7^{0}C) for 15 hours for aging. Laboratory home ice cream maker machine was used for the target overrun of 80 percent to prepare frozen yoghurt. The frozen yoghurt was then packed in cartons and hardened and then stored at -18 ^{0}C for 21 days.

Inulin and guar gum treatment

Inulin and guar gum were added to the frozen yoghurt base mix. Four treatments were tested as T1 (only 3% inulin), T2 (only 0.3% guar gum), T3 (2.7% inulin and 0.3 % guar gum) and control (with no dietary fibers).

Determination of overrun

A fixed volume of the aged pre-mix and its volume expansion after frozen yoghurt preparation (freezing step) were measured and the overrun was calculated using the below equation.

$$\text{Overrun} = \frac{\text{(Volume of frozen yoghurt - Volume of mix used)}}{\text{(Volume of mix used)}} \times 100$$

Determination of titratable acidity pH

The acidity level of yoghurt sample was measured as the amount of lactic acid according to AOAC, (2000) by titrating with 0.1% NaOH in the presence of two drops of phenolphthalein and pH was measured in melted frozen yoghurt samples according to AOAC method, (2000) by using a pH meter (PH850-DP portable pH meter).

Melting Rate

The method described by Muzammil *et al*., (2015) was employed to estimate the melting rate. Frozen yoghurt (50 g) was placed on a sieve (0.5 mm) set on top of a beaker at 25 °C. The melted frozen yoghurt was collected in the beaker. The time of first dripping was noted and the weight of melted frozen yoghurt and the weight of the residual sample were obtained after 30 min. The melting rate was calculated by dividing the melted sample over the time (g/min). Melting rate was calculated after one week of frozen storage.

Microbiological properties

Enumeration of probiotic LABs were carried out to measure the bacterial survivability during frozen storage. The plate count technique on selective media (MRS and M17) was used after inoculation (starting level, 0 day) and after 21 days of frozen storage at -18 ^{0}C. Each sample was prepared following the methods provided by the International Dairy Federation (IDF, 2003). Enumeration of *Streptococcus* species was done on M17 agar by aerobic incubation at 37 ^{0}C for 48-72 h. Enumeration of *Bacillus* species was carried out on MRS agar after being adjusted to pH 5.4 by anaerobic incubation at 37 ^{0}C for 48-72 h as described by Almeida *et al.*, (2008).

Sensory properties

The sensory characteristics were measured using 15 trained and non-trained panelists for day 21 samples. The scores were allocated according to 5-point hedonic scale and the acidity, smoothness, yoghurt flavor, sweetness, freshness of samples was evaluated. Samples were ranked according to the overall acceptability.

Statistical analysis

Data were analyzed using SPSS univariate, Duncan's mean separation and LSD multiple comparison. Graphs were illustrated using Microsoft Excel 2010. The sensory evaluation data were analyzed in non-parametric Freidman test using SPSS statistics 26.0 version.

Results and discussion

pH and Titratable acidity

The change of titratable acidity and pH of frozen yoghurt samples are shown in Table 1. The acidity of all the tested samples was increased during the storage time while reducing the pH. The drop of pH is comparatively more prominent in samples treated only with inulin whereas the development of acidity (0.10) appeared to be the highest in sample treated with both dietary fibers at the end of storage time. Control sample showed the lowest development of acidity during frozen storage (0.083). According to the literature (Żbikowska et al., 2020, Rezaei et al., 2014, Aryana et al., 2007), the acidity is increased during the storage due to the lactic acid production by lactic acid bacteria utilizing prebiotics and thus high acid production shown in combined dietary fiber treated sample could imply that the use of both inulin and guar gum support the greater frozen storage survival and growth of probiotic bacteria. By performing the preliminary consumer preference and acceptance tests, some studies have

reported that consumers are most preferred the frozen yoghurts with pH range 4.3 to 5.3 and even in some cases 4.0 to 5.7 (Ionna *et al*., 1990, Isik *et al*., 2011). The pH range of the all the frozen yoghurt samples tested was in the consumer acceptable range.

Table 01:. pH and titratable acidity of frozen yoghurt incorporated with inulin and guar gum during frozen storage

	pH	
	Day 0	Day 21
Control	4.950±0.01a	4.817±0.005b (0.133
T1	4.970±0.01a	4.787±0.015b (0.183
T2	4.973±0.005a	4.830±0.01b (0.143
T3	4.980±0.01a	4.830±0.02b (0.150
	Titratable Acidity	
Control	0.677±0.005a	0.760±0.01b (0.0830
T1	0.677±0.005a	0.770±0.01b (0.093
T2	0.673±0.005a	0.767±0.005b (0.94
T3	0.667±0.005a	0.767±0.005b (0.10

Means in the same raw indicated by different letters are significantly different ($P < 0.05$)

Overrun and melting rate

The addition of inulin and guar gum increased the overrun of the frozen yoghurt where guar gum had much greater impact than inulin and when inulin was combined with guar gum the resulting overrun was higher than their individual impact (Table 2). The higher the overrun the lower the melting rate in frozen yoghurt. According to Sofjan and Hartel, (2004) higher overrun made ice creams with smaller initial air cell, due to the higher shear stresses during processing. Further they mentioned that lower overruns produced harder ice cream than that of higher overrun (120%) but they melted rapidly. It has been found the rate of overrun is increased in the presence of inulin in frozen yoghurt (Rezaei et al., 2014). Several studies observed the reduction of melting rate of frozen yoghurts and ice cream due to the added inulin (Xavier and Ramana 2021, Isik et al., 2011). Guar gum is commonly used in stabilizing ice cream and as a thickener in food applications due to its extremely high viscosity enhancement even at a very low concentration (≤1% w/v) in aqueous solutions (Gupta and Variyar 2018, Jagdish et al., 2015). Rezaei et al., (2011) also observed that gums like guar gum significantly alters the viscosity, melting rate and rate of overrun in frozen yoghurt.

Table 02: Melting rate and overrun in the inulin and guar gum added frozen yoghurt

	Melting rate (g/min)	**Overrun %**
Control	0.7300±0.010a	54.0967±1.035c
Treatment 1	0.7044±0.015b	56.0100±0.555b
Treatment 2	0.5156±0.005c	79.1733±0.259a
Treatment 3	0.5056±0.005c	79.2633±1.223a

Mean values with different letters shown in columns are significantly different ($P < 0.05$).As reported by Tamime and Robinson, (2007) overrun of frozen yoghurt should be in between the range of 50-60 % to consider as soft frozen yoghurt and 70-80 % overrun for hard frozen yoghurt. Each frozen yoghurt samples tested in this study falls within the above ranges in which control and Treatment 1 ranged 50-60 % while Treatment 2 and 3 ranged 70-80 %. According to Muse and Hartel, (2004) factors affecting on the melting rate of ice cream include destabilization of fat, the size of ice crystal and the consistency coefficient of the mix. As Xavier and Raman, (2021) reported, high water absorption capacity of soluble dietary fibers could cause a significant increase in melting time of ice cream. They further mentioned that soluble fibers enhanced the serum concentration of ice cream mixture resulting in increased viscosity and reduced melting rate. Dwórznicka et al., (2022) reported that the properties of ice cream produced with combination of soluble dietary fibers, especially, guar gum with another dietary fiber showed positive results in shortening of melting time. This claim is further confirmed from the results we observed in present study. The decreased meltdown rate offers ice cream with better melting resistance.

Probiotic bacteria count

Table 03: Colony forming units of probiotic bacteria (Lactobacillus acidophilus, Bifidobacterium, Streptococcus thermophilus) in inulin and guar gum added frozen yoghurt

Treatment	N	Day 0 1011 (cfu/g)	Day 21 108 (cfu/g)
Control	3	6.00 ±1.00a	09.00±1.00c
Treatment 1	3	7.00 ±1.00a	60.00±10.00b
Treatment 2	3	6.33 ±0.57a	66.66±5.77b
Treatment 3	3	6.66 ±1.52a	390.00±10.00a

Means in the same column indicated by different letters are significantly different ($p < 0.05$).

Probiotic counts in all freshly prepared samples were not significantly different (Table 03). However, from all the tested samples a significant reduction of probiotic counts was observed at the end of the storage time but to a different extent. Decomposition of bacteria counts could attribute to cold shock and osmotic pressure caused by freezing (Magariños et al., 2007). Rezaei et al., (2014) have also reported the reduction of number of lactic acid bacteria during cool storage. The highest remaining count after the 21 days of frozen storage was exhibited from treatment 3 (0.3 % guar gum+2.7 % inulin) followed by treatment 1 and 2 and the lowest from control sample. These results suggest that the inulin and guar gum significantly improve the survivability of probiotic bacteria in unfavorable environment existed in frozen storage and this is more in samples treated with both inulin and guar gum indicating that combine effect of inulin and guar gum provides a more favorable environment for probiotic bacteria than their individual counterparts. Inulin is one of the most commonly used prebiotics in dairy products (Żbikowska et al., 2020, Soukoulis et al., 2009). The decline rate of probiotic bacteria counts in inulin-containing frozen yoghurt samples showed lesser than the control sample (Rezaei et al., 2014, Isik et al., 2011). The recommended dose to receive the health benefits of the probiotic organisms, especially Bifidobacterium should be 106 cfu/g (FAO-WHO, 2003). In the present study, the total colony count of probiotic bacteria (Bifidobacterium and Lactobacillus acidophilus) remained after the frozen storage was higher than the above recommended value, particularly when both inulin and guar gum were added together. This is confirmed by the finding of Waitzberg et al., (2012) who found positive results when using prebiotic inulin- hydrolyzed guar gum mixture on the composition of intestinal microbiota. Further, they have mentioned that the combination of inulin and guar gum had an additional protective effect on gut microbiota by decreasing the number of pathological bacteria of the Clostridium genera.

Sensory analysis

Sensory characteristics, the taste, aroma, texture or color are the key quality attributes that play a crucial impact on consumer attraction. Keeping the appropriate sensory characteristics during the storage of frozen yoghurt is one of the most important things need to be maintained. Figure 1 , shows the results of Friedman's test analysis done for the different sensory attributes of 21 days stored samples. Acidity, smoothness, yoghurt flavor and sweetness were not significantly different among all the tested samples at the end of freezing storage time (21 days at -18 °C). But combination of inulin with guar gum had slightly higher sensory values of smoothness, sweetness and freshness than other treatments. The lowest sensory values of acidity and sweetness were noticed from control sample.

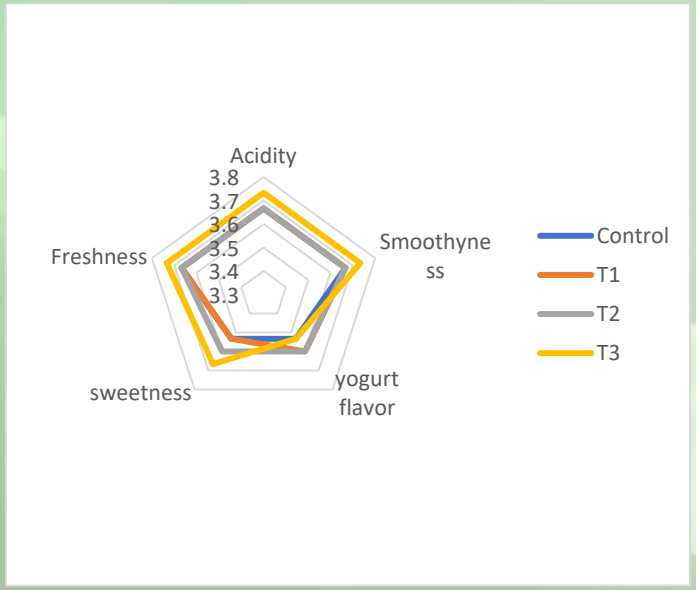

Figure 01: Sensory properties of frozen yoghurt prepared with inulin and guar gum at the end of day 21.

Dietary fiber can provide a wide range of functionalities to food especially in dairy products. They can modify and improve the texture and sensory characteristics of foods through their water-binding and gel-forming ability, fat mimetic, anti-sticking, anticlumping, texturizing and thickening ability (Soukoulis et al., 2009, Yangilar, 2013, Rezaei et al., 2014). According to Isik et al., (2011), except for the foamy melting attribute, adding inulin makes no significant difference in any of the sensory characteristics between tested frozen yoghurt samples. Akin, (2005) reported that inulin addition in (1% to 2%) had no impact on sensory attributes of frozen yoghurt. In contrast, Rezaei et al., (2014) mentioned that inulin improved the flavor, texture and total acceptance of frozen yoghurt. Guar gum is added to various dairy products due to its number of important functional properties (Gupta and Variyar, 2018). It has been reported that 0.2% of guar gum is sufficient to enhance the overall consumer acceptability of ice cream (Jagdish et al., 2015). The present study revealed that the inulin (2.7%) and guar gum (0.3%) together improved the yoghurt sweetness, freshness and smoothness during frozen storage showing combination effect has more impact on sensory attributes than individual effect of inulin and guar gum.

Conclusion

Inulin and guar gum significantly and positively alter probiotic, physicochemical and consumer acceptable qualities of frozen yoghurt where the combination effect of inulin and guar gum has more impact on the above properties of frozen yoghurt than their individual counterparts. It can be concluded that the soluble dietary fiber, inulin and guar gum in combination can be used as prebiotics in frozen yoghurts to improve probiotic bacteria viability with high consumer acceptable organoleptic properties. Further studies are required to optimize the combination effect of inulin and guar gum in a view of producing more heath beneficial and consumer acceptable frozen yoghurt.

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