Optimization of Sugar Reduction in the Formulation of Set-type Yogurt Using Pure Lactic Acid Bacterial Culture

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ABSTRACT

Background: Reducing the sugar content of processed products has been claimed to be one of the most efficient strategies for decreasing sugar intake. To investigate what level of sugar reduction is accepted in set-type yogurt, we conducted a hedonic test focusing on the sensory attributes and assessed the nutritional quality of the yogurt.

Methods: We prepared a total of 12 set-type yogurt samples using four sugar concentrations (viz. 0, 4, 5 and 12%). The starter culture was used as a 1:1 ratio mixture of Streptococcus thermophilus and Lactobacillus bulgaricus. The sensorial, chemical and microbiological analyses were performed.

Result: From the investigation, yogurt containing at least 4 and 5% added sugar was found acceptable based on overall sensory score. On the hedonic scale, yogurt containing 12% sugar was more often described as too sweet compared with yogurt containing 4 and/or 5% sugar. On the other hand, the sweetness and aroma intensity for yogurt containing 0% sugar was judged as too low. The 4 and 5% added sugar yogurt was recorded highest with protein content 4.35 and 4.53%, respectively. The initial total viable count (TVC) of the yogurt made was found highest in 4 and 5% sugar yogurt each with $5 \log_{10} \text{cfu/mL}$. These results indicate that consumers would accept set-type yogurts with 4 to 5% added sugar instead of 12 to 15%, but 0 to 3% sugar would be too low.

Key words: Consumer acceptance, Pure starter culture, Sugar reduction, Yogurt.

INTRODUCTION

For years, yogurt is one of the most popular fermented dairy products which have a wide acceptance worldwide whereas it’s nutritional and health benefits are well known. According to the Code of Federal Regulations of the United States Food and Drug Administration (FDA), yogurt can be defined as a food produced by culturing one or more of the optional dairy ingredients namely cream, milk, partially skim milk, used alone or in combination with a characteristic bacterial culture that contains lactic acid-producing bacteria, Lactobacillus bulgaricus and Streptococcus thermophilus. Yogurt should contain at least 3.25% of milk fat and 8.25% of SNF with a titratable acidity of not less than 0.9%, expressed as lactic acid. Traditionally, yogurt is made of cow, water buffalo and goat and sheep milk. However, yogurt is perceived as a healthy food because of the presence of live and active cultures (Popa and Ustunol, 2011) and this positive image should not be harmed by high levels of added sugar or by the use of artificial ingredients such as sweeteners. So far because of the growing health consciousness of the consumer, there is a demand for sugar reduction in yogurt (Chollet et al., 2013).

However, human beings have an inherent preference for sweet taste due to its pertinence as a natural cue for edibility and energy-rich foods (Birch, 1999). Progress in food technology made it possible to add sweeteners to foods to impart sweet taste and increase the pleasure of eating, which has made sugar one of the most important hidden sources of calories (Yebra-Biurrun, 2005). It has been estimated that daily sugar consumption worldwide is equivalent to 500 calories (Lustig et al., 2012). A growing body of evidence has associated excessive sugar intake with several negative health conditions, such as obesity and diabetes (Caballero, 2013; Morenga et al., 2013). In this context, it is necessary to develop strategies to reduce sugar consumption. A large proportion of processed foods contain high added sugar concentrations, especially those targeted at children and adolescents (Havey, 2010; Lustig et al., 2012). Therefore, one of the possible strategies for decreasing sugar consumption in a short time is reducing the sugar content of processed products (Mac Gregor and Hashem, 2014).

Strategies aimed at reducing sugar in food products should focus on minimizing changes in their sensory characteristics, as most consumers are not willing to compromise on sensory and hedonic aspects of products for their healthfulness (Civille and Oftedal, 2012). For this reason, it has been recommended to slowly and gradually...
reduce the sugar content of products, without changing their sensory characteristics, so that people become accustomed to sugar-reduced products (Mac Gregor and Hashem, 2014). Thus, the main objective of the research work was to study the effect of sugar reduction on the quality of set-type yogurt using pure lactic acid bacterial culture.

**MATERIALS AND METHODS**

The experiment was carried out at the Laboratory of Dairy Microbiology and Biotechnology, Department of Dairy Science, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh.

**Starter culture**

The starter culture was used as a 1:1 ratio mixture of *Streptococcus thermophilus* M140-2 and *Lactobacillus bulgaricus* M240-5 obtained from the Laboratory of Dairy Microbiology and Biotechnology, Department of Dairy Science, BAU. The starter culture was maintained in sterilized reconstituted skimmed milk (Himedia, India; 12 g/100 mL) by sub culturing once in a fortnight for attaining high activity.

**Experimental design**

Preliminary trials were conducted to determine the effects of different sugar levels to adopt a low-sugar concentration starting from 1% cane sugar. The study was designed based on the results of the preliminary trials, where 6 levels of sugar from 1 to 6% were used to prepare yogurt samples. Based on the sensory results of the trained panel, the best formulations were the yogurt prepared with 4 and 5% sugar. Finally, four sucrose concentrations (0, 4, 5 and 12% added sugar) were selected and a total of 12 samples were made (3 samples from each sugar concentration). The concentration of 12% added sugar was chosen because it corresponds to the average sugar concentration for yogurt in most parts of the world. The sugar-free (0%) plain yogurt was used for the comparison.

**Preparation of yogurt**

Fresh raw cow milk was collected from the BAU Dairy Farm. Set-type probiotic yogurt was prepared by using whole cow milk containing 4.0% (w/v) milk fat. In brief, milk was heated to boiling temperature to reduce the volume of milk by approximately up to 20%. Then the milk was divided into 4 equal portions. After that 0%, 4%, 5% and 12% sugar (Fresh, Dhaka-Bangladesh) was added in each part of the milk batch. The mixture was stirred gently. Afterward, the temperature of the milk was reduced to 40°C followed by inoculating each batch of the mixture with 2% formerly prepared starter culture. The mixture was nicely stirred and then poured in 50 mL labeled plastic cups. The samples were incubated at 37°C for 5-6 h while the complete coagulation occurred after that the samples were taken out from incubator and stored in the refrigerator (4°C). To optimize the formula the trials were conducted triplicate.

**Chemical analysis**

The following physic-chemical parameters were assessed viz. pH, acidity, moisture, total solids, fat, protein, sugar and ash. Fat content was determined by the Babcock method, total solids and moisture content were determined by the oven drying method at 105°C for 24 h and ash content was determined by igniting the dried samples (total solids) at 550°C for 5-6 hours in an electric muffle furnace according to AOAC method (AOAC, 2000). Protein was determined by the micro Kjeldahl method as per the instruction of the International Dairy Federation (AOAC, 2000). Titratable acidity, expressed as a percentage of lactic acid, was determined by mixing 10 g of yogurt with 20 mL of distilled water and titrating with 0.1 N NaOH using a phenolphthalein indicator to an end-point of faint pink color. The pH value was measured using a digital portable pH meter (Hanna, Romania). Total sugar (lactose + sucrose) was determined by the calculation method.

**Sensory characteristics**

Sensory evaluation of the samples was carried out according to the method modified from Martin-Diana et al. (2003). The panel group consisted of 8 experienced academicians from the Department of Dairy Science, BAU, Mymensingh, Bangladesh. Sensory evaluation consisting of color and appearance, aroma, taste, texture and overall acceptability were based on 5-point hedonic scales (1: dislike extremely; 5: like extremely). Panelists were also perceived unnatural characteristics by following a scorecard including for aroma viz. bitter, cooked, foreign, too high or low acid, or oxidized, among others, for texture viz. gel-like, grainy, coarse, too firm, or too weak, among others) and for color and appearance viz. whey-off or shrunken abnormal color. Each sample was scored individually and the samples were presented to the panelists in the individual plastic containers. Yogurts, coded with the treated level of sugar used, were randomly presented to the panel group at each session. Water was also presented to rinse their palate between samples. Panelists evaluated all of the samples after storage for 1 to 7 days at 4°C.

**Microbiological analysis**

Total viable count (Log cfu/mL) was performed as a microbiological assay in the selected blends by standard plate count method according to Vanderzant and Splittstoeser, (1992). In short, plate count agar and sterile saline of 0.9% NaCl (w/v) were prepared. The samples (1 mL) were transferred into 9 mL serially diluted (up to 10^6) sterile saline, then put into the plate and poured agar (10-15 mL) and allowed for solidification. After solidified, the plates were incubated at 32°C for 48 h. The colonies were enumerated having within 30-300 colonies on the plate. In all cases, duplicate-counting plates were prepared for appropriate dilutions.

**Statistical analysis**

The data regarding physico-chemical and sensory evaluation were statistically analysed using ANOVA to
determine if statistical difference existed at (P ≤ 0.05) and the Tukey test was used for means comparison. All the analysis was performed using Minitab version 17 (State College, PA, Minitab, Inc.).

RESULTS AND DISCUSSION

Effect of sugar reduction on sensory attributes of set-type yogurt

Statistical analysis showed that all the sensory attributes viz. color and appearance, aroma, taste, body and texture and overall acceptability were significantly different (p ≤ 0.05) among the samples (Table 1). The average color and appearance score was found highest (4.70) for yogurt prepared with 5% sugar which is next to close to 4% sugar and the lowest score (≈ 3.90) observed for both 0 and 12% sugar yogurt. The aroma value for 4 and 5% sugar yogurt was found approximately 1.4 points higher compared to plain yogurt (sugar-free). The yogurt prepared with 5 and 4% sugar were observed for similar taste score (4.75 and 4.85, respectively). On the contrary, 12% of sugar yogurt scored lower than plain yogurt. The body and texture score for yogurt prepared with 4, 5 and 12% sugar was reported for approximately identical scores 4.67, 4.70 and 4.40, respectively. However, the highest overall acceptability score was recorded for the yogurt prepared with both 4 and 5% sugar (4.66 and 4.73, respectively) and the lowest score (3.90) recorded for 12% sugar yogurt.

The variations in sensorial scores of yogurt samples usually depend on the types of milk, starter culture and manufacturing process involved (Younus, 1998). However, more than a few studies reported that a higher concentration of sugar is expected to lead to increasing preference (Barnes et al., 1991; Chollet et al., 2013; Johansen et al., 2010). The panelists perceived the yogurt with greater amounts of sugar as being sweeter, less sour and more aromatic. For consumers, increasing sucrose concentrations caused a shift toward the upper end of the hedonic scale for sweetness and aroma (Kålväinen et al., 2003). For flavored yogurts, the largest percentage of consumers perceived the yogurt with 10% sugar as “too sweet”. The strawberry yogurt containing 7% sugar was perceived as “just about right” in terms of sweetness. These results show that even if there is a significantly higher overall acceptability for yogurt with 10% sugar, which corresponds to commercial samples, many consumers rate the sweetness intensity of this sugar concentration as too high. This inconsistency is not astonishing in consumer science. As reported by Köster (2009), consumer decisions are, most of the time, unconscious. By asking a question they normally never ask themselves, consumers develop an analytical attitude where they want to conform to the social or cultural convention or to demonstrate that they are reasonable, instead of acting spontaneously. Indeed, sweetness acceptability varies among consumers (Bayarrí et al., 2011). In other few studies, key drivers for consumer preferences for dairy sweetmats, drinkable yogurts and dairy beverages were natural flavor or aroma and sweet taste (Thompson et al., 2007; Begum et al., 2019; Begum et al., 2019 and 2020). Furthermore, Finnish consumers who consider convenience, familiarity, mood and price to be important factors in their food choices found sweeter yogurt to be more pleasant (Pohjanheimo and Sandell, 2009). It was also suggested that some people are willing to consume yogurt that is less sweet when they appreciate natural ingredients and avoid additives (Pohjanheimo and Sandell, 2009). In a study in Norway, consumers who liked the less sweet yogurts were also those with a more positive attitude toward the healthy aspects (Endrizzi et al., 2011). However, yogurts containing 7% sugar were also liked, which is in line with a study conducted in the United States with 99 panelists, where strawberry yogurt containing 7% sucrose was also judged as acceptable (Bayarrí et al., 2011). These previous findings supported our results obtained that 4 to 5% added sugar can be used to manufacture set-type yogurt with promising consumer acceptability.

Effect of sugar reduction on physicochemical analysis

The results of the physicochemical analysis are depicted in Table 2. In the current experiment it was found that the average acidity and pH content of yogurt prepared with different sugar levels was found non-significant (p = 0.137). The mean percentage of acidity (0.9%) and pH (4.45) was found among the entire samples. Amna et al. (2008) found that plain yogurt had a titratable acidity of 0.14 to 0.38%. Sarker et al. (1996) reported that misty Dahi had a titratable acidity of 0.14 to 0.38%. Jeoun et al. (1995) reported that misty Dahi had a titratable acidity of 0.14 to 0.38%. Sarker et al. (1996) reported that misty Dahi had a titratable acidity of 0.36 to 1.17%. However, a mixed culture of *Lactobacillus mesenteroides* and *Streptococcus thermophilus* produced yogurt with the desirable acidity concentration as too high. This inconsistency is not astonishing in consumer science. As reported by Köster (2009), consumer decisions are, most of the time, unconscious. By asking a question they normally never ask themselves, consumers develop an analytical attitude where they want to conform to the social or cultural convention or to demonstrate that they are reasonable, instead of acting spontaneously. Indeed, sweetness acceptability varies among consumers (Bayarrí et al., 2011). In other few studies, key drivers for consumer preferences for dairy sweetmats, drinkable yogurts and dairy beverages were natural flavor or aroma and sweet taste (Thompson et al., 2007; Begum et al., 2019; Begum et al., 2019 and 2020). Furthermore, Finnish consumers who consider convenience, familiarity, mood and price to be important factors in their food choices found sweeter yogurt to be more pleasant (Pohjanheimo and Sandell, 2009). It was also suggested that some people are willing to consume yogurt that is less sweet when they appreciate natural ingredients and avoid additives (Pohjanheimo and Sandell, 2009). In a study in Norway, consumers who liked the less sweet yogurts were also those with a more positive attitude toward the healthy aspects (Endrizzi et al., 2011). However, yogurts containing 7% sugar were also liked, which is in line with a study conducted in the United States with 99 panelists, where strawberry yogurt containing 7% sucrose was also judged as acceptable (Bayarrí et al., 2011). These previous findings supported our results obtained that 4 to 5% added sugar can be used to manufacture set-type yogurt with promising consumer acceptability.

Table 1: Effect of different level of sugars on sensory characteristics of yogurt.

| Parameters                | Level of sugar | p-value |
|---------------------------|----------------|---------|
|                           | 0%             | 4%      | 5%      | 12%     |         |
| Color and appearance      | 3.90 ± 0.15    | 4.65 ± 0.12 | 4.70 ± 0.14 | 3.92 ± 0.55 | 0.006   |
| Aroma                     | 3.30 ± 0.20    | 4.57 ± 0.17 | 4.70 ± 0.14 | 4.07 ± 0.12 | 0.001   |
| Taste                     | 3.90 ± 0.15    | 4.75 ± 0.10 | 4.85 ± 0.05 | 3.82 ± 0.55 | 0.001   |
| Body and texture          | 3.90 ± 0.16    | 4.67 ± 0.17 | 4.70 ± 0.16 | 4.40 ± 0.27 | 0.000   |
| Overall acceptability     | 4.36 ± 0.11    | 4.66 ± 0.11 | 4.73 ± 0.09 | 3.90 ± 0.37 | 0.000   |

Mean ± SD. Means with different superscript in the same row differ significantly (p ≤ 0.05).
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Table 2: Effect of different level of sugar on physicochemical properties of set-type yogurt.

| Parameters                  | Level of sugar | p - value |
|-----------------------------|----------------|-----------|
|                             | 0%             | 4%        | 5%        | 12%       |
| Acidity (%)                 | 0.91 ± 0.04    | 0.92 ± 0.03 | 0.91 ± 0.05 | 0.89 ± 0.04 | 0.137 |
| pH                          | 4.40 ± 0.32    | 4.37 ± 0.36 | 4.43 ± 0.32 | 4.63 ± 0.20 | 0.733 |
| Total Solids (%)            | 16.10 ± 0.75   | 16.92 ± 0.65 | 17.43 ± 0.41 | 25.57 ± 1.73 | 0.000 |
| SNF (%)                     | 7.25 ± 0.02    | 11.32 ± 0.08 | 11.57 ± 0.07 | 20.50 ± 0.48 | 0.000 |
| Fat (%)                     | 5.83 ± 0.76    | 5.60 ± 0.57  | 5.86 ± 0.41  | 5.57 ± 0.17  | 0.302 |
| Protein (%)                 | 4.25 ± 0.50    | 4.35 ± 0.12  | 4.53 ± 0.05  | 4.23 ± 0.35  | 0.021 |
| Total sugar (Lactose + sucrose) % | 2.30 ± 0.11 | 6.24 ± 0.08  | 6.20 ± 0.08  | 15.35 ± 0.81 | 0.000 |
| Ash (%)                     | 0.70 ± 0.05    | 0.73 ± 0.06  | 0.84 ± 0.05  | 0.92 ± 0.03  | 0.003 |

Mean ± SD. Means with different superscript in the same row differ significantly (p ≤ 0.05).

hand, Fellows (1991) reported that the pH of good quality yogurt is 4.5 which nearly agrees with the present findings (mean pH 4.40). The pH content of the present experiment also agrees with the findings of Islam et al. (2010).

In the current experiment, it was found that the TS content of yogurt prepared with different sugar levels was significantly differed (p<0.05). The highest total solids content (25.57%) was found in 12% sugar added. Variation in the TS content of yogurt could be attributed due to the addition of sucrose (Ghosh and Rajorhia, 1987). They also stated that the TS content of plain market Dahi varied from 26.92 to 43.04% with an average value of 34.64%. According to Ray and Srivasan (1972) concentration of milk during heat treatment could increase the total solids content of Dahi. The SNF content of yogurt prepared with different sugar levels was differed significantly (p<0.05). The highest SNF content (20.50%) was found 12% sugar yogurt. Usually, the SNF content of sweet yogurt ranges from 18.30 to 23.06% (Gonc and Okter, 1973). The SNF content of the present experiment agrees with the findings of Islam et al., (2010) and Akter et al., (2010) who reported the value within the range of 11 to 20% and 19 to 25%, respectively. It was observed that the fat content was found statistically non-significant (p>0.05). The fat content of yogurt samples was studied by different workers. Ghosh and Rajorhia (1987) found that fat percent of plain misty Dahi ranged from 4.3 to 8.8% with an average of 3.78%. Sarker and Sannabhadti (1992) found that the fat content of Dahi samples of different areas of West Bengal ranged from 1.1 to 11.5 % with an average of 5.14%. This finding was found similar to our study where obtained 5.7% fat content. The reason behind it may be attributed to the volume reduction of the milk during yogurt preparation.

Statistical analysis showed that there was a significant (p<0.05) difference within the protein content of different types of yogurt. The highest protein was found in 4 and 5% sugar added samples each with 4.35 and 4.53%, respectively. Generally, the protein content of the yogurt becomes higher than the protein content of raw milk. This was mainly due to the reduction of volume of milk due to heat treatment and at the same time due to microbial protein synthesis as well (Hassan and Amjad, 2010). In this regard, Amna et al. (2008) found that the protein content of yogurt was 3.57% which is close to our present findings. Rangappa and Aenaya (1974) reported that good quality yogurt contains around 3.2 to 3.4 % of protein. The protein content of the present experiment agrees with the findings of Desai et al. (1994) and Islam et al. (2010). Milk composition varies from species to species or individual to individual animals or day to day that was used for yogurt making, so protein content might be a little variation among the different products made. The total sugar content indicated that there was a highly significant difference (P=0.000) within the yogurt samples. The amount of sugar added is largely dependent on the consumer’s demand. In some areas, consumers more prefer sweetened Dahi (made by using mixed starter culture) whereas those in other areas prefer otherwise. Of the sample studied, 12% sample had the highest total sugar (15.35%) content due to higher amounts of sucrose added. From this study, it was noted that the average ash content of yogurt significantly varied (p = 0.003) among the yogurt samples. The Ash content of plain yogurt was 0.83% (Desai and Aenaya, 1974) that is supported our findings (0.70%). The ash content of the present experiments also agrees with the findings of Islam et al. (2010) who reported ash content in the range of 0.7 to 0.8%.

Effect of sugar reduction on starter culture viability

In this experiment, it was found that the total viable count (TVC) of yogurt samples varied considerably (p < 0.05). As shown in Fig. 1, the total viable count (TVC) of yogurt samples varied considerably (p < 0.05). The highest mean TVC (5 log cfu/mL) of yogurt samples was found 12% sugar added. Variation in the TVC content might be a little variation among the different products made. The total sugar content indicated that there was a highly significant difference (P=0.000) within the yogurt samples. The amount of sugar added is largely dependent on the consumer’s demand. In some areas, consumers more prefer sweetened Dahi (made by using mixed starter culture) whereas those in other areas prefer otherwise. Of the sample studied, 12% sample had the highest total sugar (15.35%) content due to higher amounts of sucrose added. From this study, it was noted that the average ash content of yogurt significantly varied (p = 0.003) among the yogurt samples. The Ash content of plain yogurt was 0.83% (Desai et al., 1994) that is supported our findings (0.70%). The ash content of the present experiments also agrees with the findings of Islam et al. (2010) who reported ash content in the range of 0.7 to 0.8%.
log10cfu/mL in industrial yogurts which is in the agreement of the current study (Beheshtipour et al., 2012; Rad et al., 2019). It is worth to mention that the formulated set-type yogurt contained the recommended level of bacterial count to be considered as a functional food.

CONCLUSION
Several diseases are associated with high consumption of sugar; therefore, a low intake of this macronutrient is strongly recommended. However, the sensory properties of a sugar-reduced product have to be acceptable to the consumer. The results of the current study indicate that consumers would accept set-type yogurt with 4 to 5% added sugar instead of 12% added sugar. Based on the hedonic scale, yogurt containing 12% sugar was more often described as too sweet compared with yogurt containing 4 to 5% sugar. By following the research finding, it is possible to reduce sugar consumption by more than 50% when considering functional food intake. Further research aiming at determining difference thresholds in sweet taste could contribute to the development of strategies for gradual sugar reduction in processed products. Therefore, we recommend to the dairy industry to reduce the level of sugar in set-type yogurts.

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