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Microbial Features and Qualitative Detection of Adulteration along with Physicochemical Characteristics of Sweetened Yoghurt

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAR designed the study and wrote the manuscript. Authors MAR, AH and MI conducted the experiment and analyzed the data. Authors MSH and RH critically revised the manuscript. All authors read and approved final manuscript.

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ABSTRACT

Background and Objective: Food adulteration is of major public health concern worldwide. The food safety situation in Bangladesh is at an alarming stage due to food adulteration, microbiological contamination and fraudulence. Consumption of milk and milk product are increasing because of their health benefit effect. However, milk and milk products are being currently adulterated by substituting ingredients with cheaper materials. The aim of this study was the qualitative detection of adulterants along with physico-chemical and microbial features of sweetened yoghurt.
Methodology: Sixteen sweetened yoghurt samples (Ten locally available and six commercial brands) were collected from different area in Bangladesh. All the sweetened yoghurt samples were subjected to urea, starch, ammonium sulphate, hydrogen peroxide analyses. Physico-chemical analysis of sweetened yoghurt was also done. Moreover, yoghurt samples were also microbiologically assessed.

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Results: Acidity content of all sweetened yoghurt was found to be ranges from 0.66 ±.05% to 0.77±0.05 which was lower than the standard level of (0.8 to1.2). In both cases (Commercial) branded and (Local) unbranded yoghurt had lower moisture content and higher total solid content compared to standard level (13.5%). Those measurements indicated that sweetened yoghurt samples were adulterated with starch. Meanwhile, both branded and unbranded sweetened yoghurt samples had fat contents ranging between 1.56 ±0.09% to 2.63 ±0.2% which were below the standard level (3%). On the other hand, protein content ranged between 3.71±0.02% to 4.33±0.15%, which was higher than standard level of 3.2%. These results revealed that all sweetened yoghurt samples were highly adulterated with urea, starch and ammonium sulphate. From the microbiological point of view, all sweetened yoghurt samples had good quality. For instance, *L. bulgaricus* and *S. thermophilus* were found to be under acceptable range, and there were no pathogenic coliform bacteria were found in any of the samples.
Conclusion: Results obtained from this study might help the consumers to be more aware when they purchase yoghurt from local market.

Keywords: Sweetened yogurt; adulteration; physico-chemical characteristic; microbiological quality.

1. INTRODUCTION

Sweetened Yoghurt is the most demanding and mouthwatering fermented milk product available in Bangladesh. People devour sweetened yoghurt either as a part of appetizing dish or as a refreshing food item. Nutritive value of sweetened yoghurt is very good. It is easily digestible due to pre-digested nutrients used by bacterial starter cultures [1]. In Bangladesh and some other countries use fresh whole milk from cow, buffalo, goat or mixing powdered milk with whole milk for preparing sweetened yoghurt. It was established that best grade of sweetened yoghurt rely on the presence of Streptococc*i* and *Lactobacilli* in the ratio of 1:1 [2]. Yoghurt is beneficial to health. For instance, it cures intestinal disease like constipation, diarrhoea and dysentery [3]. Sweetened yoghurt is also being found to be easily digestible than normal milk. In the intestinal tract, unfavorable conditions created by the acid fermenting bacteria and lactose in milk prevent the growth of putrefying bacteria thus preventing the formation of gas, a condition known as autointoxication. Sweetened Yoghurt is also known to lower blood cholesterol level [4]. Unfortunately, adulteration of yoghurt is increasing day by day throughout the world. The reasons behind yoghurt adulteration include using cheaper ingredients to get more cost benefit, demand and supply gap, perishable nature of yoghurt, and lack of suitable detection tests [5]. The aim for yoghurt fraud is economical, but it has a strong effect on public health [6] Adulterated yoghurt is detrimental to health due to presence of various toxic elements and, lack of various nutrients which are essential for adequate growth and evolution of human body [7]. The quality of sweetened yoghurt is remarkably worse in Bangladesh due to the absence of adequate market observation, insufficient law enforcement and lack of proper awareness of the consumers. Quantitative detections of adulterants are complex and diverse than qualitative detection as it is chemical reactions based. In Bangladesh, most of the local vendor sells yoghurt in open markets and keep at room temperature without covering the yoghurt. On the other hand, some sellers of the city areas keep their products in refrigerators for prolong storage. The variation of temperature and unhygienic condition are responsible for the deterioration of both chemical and microbial quality of yoghurt. The main focus of this study was to assess the sweetened yoghurt of different branded (Commercial) and unbranded (Local) in terms of chemical and microbiological quality. In addition, qualitative detection of adulteration of yoghurt was also done.

2. MATERIALS AND METHODS

This research experiment was carried out at Food Safety and Quality Analysis Division, Institute of Food and Radiation Biology, Savar, Dhaka.

2.1 Experimental Design

The study was divided into three parts. First part included the chemical tests of yoghurt. While, second part included qualitative detection of adulterants and finally third part included the microbiological examination. Data obtained from these analyses were analyzed statistically by using Microsoft excel and SPSS software.

2.2 Sample Collection

Ten samples (S1, S2, S3, S4, S5, S6, S7, S8, S9, S10) of unbranded yoghurt and six samples (P, I, S, B, M, R) of branded yoghurt (commercial) were collected. These samples were transported to laboratory under very hygienic condition and in ice box to maintain the temperature at 4°C. Subsequently, samples were stored in refrigerator around at 4°C for further analysis.

2.3 Physicochemical Analyses

Moisture, total solids (TS) and ash content of the different type sweetened yoghurt samples were determined according to the method of AOAC [8]. While, acidity content was determined by titration with 0.1N sodium hydroxide solution using the procedure described by Aggarwala and Sharma [9]. Protein content was measured by the Kjeldahl method as recommended by the International Dairy Federation (IDF) [10]. Finally, Gerber method was used to determine fat content.

2.4 Qualitative Detection of Adulterants

Qualitative detection of adulterants in sweetened yoghurt was determined using color based chemical reactions of the Food Safety & Standard Authority of India (FSSAI) manuals for milk and milk products [11].

2.5 Microbiological Test

Total *Streptococcus thermophilus* counts (M17), total *Lactobacillus bulgaricus* counts (MRS) and total coliform counts were determined by standard plate count method as described by Coppuccino and Sherman [12]. In brief, ten grams of each sample was taken separately in a 90-ml conical flask containing sterile normal saline water (0.9% of NaCl) and homogenized properly. This gave a 10^{-1} dilution. Serial dilutions of $10^{-1}$to 10^{-6} dilution was then made for microbiological assessment. Sterilized media (20 ml) was poured in sterile petridish and allowed to cool down. About 50 ml sterilized lactose solution (10%) was added and mixed well (the lactose can be exchanged for other carbohydrates e.g. glucose, resulting in GM17 medium) during M17 media preparation. After solidification of the plates, each set of THC, TLBC and TCC, were marked and 0.1 µl amounts of desired serial diluted samples placed on the surface of plates and spread continuously, the plates were left to dry. All microbiological procedures were carried out under laminar air flow to maintain an aseptic condition. After inoculation, M17 media and *Lactobacillus* plates were incubated at 37°C temperature for 48 hours. After incubation, colonies of the all plates were counted. Counts of ranges between 30-300 were reckoned with while counts above 300 were regarded as too numerous to count.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Analysis

The physico-chemical analysis shows the extent of adulteration in yoghurt production and deterioration of its components.

3.1.1 Acidity (%)

The percentage of acidity of unbranded (Local)sweetened yoghurt was found to be ranges from 0.66 ±0.05% to 0.72±0.05% and branded(Commercial)sweetened yoghurt was ranged from 0.71 ±0.05% to 0.77±0.05% (Table 1 and Table 2). Acidity content of branded sample (S) was found to be the highest followed by others and the lowest acidity was found in unbranded sample (S6).The difference of acidity content of different yoghurt samples might be due to use of different quantity of bacterial culture, processing condition and preservation method. In branded yoghurt, acidity content was slightly increased (Table 2) because of using starch powder. All the samples had acidity below 1% which is in line with the results as demonstrated by Castaneda et al. [13]. The Bureau of Indian Standards has recommended an acidity range of 0.8% to 1.2% for sweet yoghurt. It is evident that the acidity of all yoghurt samples fell below the acidity range from the Bureau of Indian Standards recommended.

3.1.2 Moisture (%)

The moisture content of unbranded sweetened yoghurt sample was found to be ranged from 65.9 ±0.52% to 71.69 ±0.58% and branded sweetened yoghurt sample was ranged from 71.68 ±0.10% to 75.07 ±0.04% (Tables 1 and 2). The highest moisture content was found in branded sweetened yogurt sample (M). While, unbranded sample (S1) had the lowest moisture content. Moisture content might vary due to the difference in milk collected from a different breed of cows and different incubation technique. Previously, it was reported that a good flavored

Table 1. Physico-chemical composition of local (unbranded) sweetened yoghurt

Sample	Acidity (%)	Moisture (%)	Total solid (%)	Ash (%)	Fat (%)	Protein (%)
S1	0.72±0.05	65.9 ±0.52	34.1±0.52	0.54 ±0.02	1.83 ±0.05	4.3±0.15
S2	0.69 ±0.05	70.13±0.05	29.86 ±0.05	0.57±0.02	2.3 ±0.2	4.2±0.05
S3	0.70 ±0.03	68.05±0.04	31.04 ±0.04	0.51 ±0.05	1.4 ±0.2	4.03±0.11
S4	0.68 ±0.00	68.66±0.2	32.33 ±0.18	0.64 0.05	1.8±0.1	3.82±0.02
S5	0.69 ±0.01	71.69 ±0.58	28.97±0.01	0.57±0.05	2.16 ±0.11	4.33±0.15
S6	0.66 ±0.05	67.57 ±0.24	32.43±0.24	0.66±0.01	2.63 ±0.2	3.73±0.05
S7	0.67 ±0.05	68.76 ±0.29	31.24 ±0.29	0.71 ±0.05	1.86±0.07	4.2 ±0.05
S8	0.71 ±0.05	68.31 ±0.40	31.66 ±0.37	0.63±0.01	1.64 ±0.11	4.13 ±0.02
S9	0.70 ±0.05	67.06 ±0.15	32.93 ±0.15	0.56±0.05	2.11 ±0.02	3.9 ±0.08
S10	0.69 ±0.05	70.13 ±0.05	29.86 ±0.05	0.62 ±0.05	1.56 ±0.09	3.8±0.086

Data represented as Mean± Standard deviation of three sample

Table 2. Physico-chemical composition of commercial (branded) sweetened yoghurt

Sample	Acidity (%)	Moisture (%)	Total solid (%)	Ash (%)	Fat (%)	Protein (%)
I	0.74±0.02	73.1 ±0.36	26.9 ±0.34	0.67 ±0.05	2.59 ±0.1	3.93±0.02
P	0.72 ±0.13	72.36 ±0.45	27.63 ±0.45	0.73 ±0.01	2.34 ±0.06	3.76±0.02
B	0.73 ±0.05	74.31 ±0.48	25.68 ±0.48	0.69 ±0.011	1.8±0.02	4.13 ±0.02
R	0.71 ±0.05	72.24 ±0.30	27.76 ±0.30	0.71 ±0.05	1.81±0.1	3.88 ±0.057
M	0.73±0.00	75.07 ±0.04	24.92 ±0.04	0.67±0.05	1.57 ±0.03	4.04±0.05
S	0.77±0.05	71.68 ±0.10	28.31 ±0.10	0.68 ±0.05	1.81±0.02	3.71±0.02

Data represented as Mean± Standard deviation of three sample

and sweetened yoghurt contained up to 86.5% or less moisture [14]. All the samples in this study had moisture content lower than the range according to the Bureau of Indian Standards recommended (86.5%). So, all are acceptable.

3.1.3 Total solid (%)

The total solid content of unbranded sweetened yoghurt and branded yoghurt are shown in Table 1 and Table 2, respectively. The highest content of total solid was found in unbranded sample (S1) and the lowest content found in branded sample (M). The variation of total solid content depends on a solid percentage of milk used in yoghurt production. Adding starch may be another way to increase total solid. In case of yoghurt milk which has not been subjected to standardization, there could be too much variation as found in total solid content of market yoghurt samples. This data also agrees with the previous observation of Younsh [15].

3.1.4 Ash (%)

The result of ash content of different sweetened yoghurt samples is presented in Tables 1 and 2 respectively. Branded sweetened yoghurt sample (P) contained the highest amount of ash compare to others sample. While, unbranded yoghurt sample (S3) found with the lowest amount of ash content. It was also found that there was wide variation in case of ash content among different yoghurt samples. Ash content variation in yoghurt samples might be due to defects in standardization of milk, difference in concentration of milk and adulteration as well.

3.1.5 Fat (%)

The results obtained in this study showed that fat percentage in unbranded samples and branded sample varied between 1.4 ±0.2% and 2.63 ±0.2% (Tables 1 and 2). Previously, it has been demonstrated that fat percentage of sweetened yoghurt found in Bangladesh ranged from 3.00 to 4.75% [16]. The reason behind lower level of fat content found in this study, might be due to preparation of yoghurt from skim milk. In addition, the variation in fat content between different yoghurt samples could be due to lack of quality control or standardization of milk used for yoghurt production and added adulterants such as starch as well.

3.1.6 Protein (%)

Both unbranded yoghurt and branded sweetened yoghurt were recorded a high amount of protein

ranging from 3.71± 0.02% to 4.33± 0.152% as presented in Tables 1 and 2 respectively. All samples in this study had higher protein values above the recommended value (3.2%) of the Bureau of Indian Standards recommended. The reason for the high protein values found in yoghurt could be due to the use of stabilizer and standardized milk during preparation and might be due to the addition of urea and ammonium sulphate.

3.2 Adulteration Analysis

In this study, unbranded and branded yoghurt samples were subjected to analysis for adulteration test.

Unbranded (Local) yoghurt was found to contain starch (33%), urea (29%), ammonium sulphate (19%) and hydrogen peroxide (19%) as shown in Fig. 1. In contrast, branded (Commercial) sample was found that contained starch (29%), urea (14%), ammonium sulphate (36%) and hydrogen peroxide (21%) (Fig. 2).

Commercial urea is usually added to milk to increase non-protein nitrogen content, which in turns increase crude protein of yoghurt [17]. The density of diluted milk and crude nitrogen was increased by addition of Ammonium sulphate. Hydrogen peroxide is used as a preservative for the long-time storage because of yoghurt is perishable [18]. In this study, most of the yoghurt samples was adulterated by various adulterants (starch, urea, ammonium sulphate and hydrogen peroxide) which may have a negative impact on health. High amount of starch in yoghurt may cause diarrhea due to the effects of undigested starch in colon, and excessive accumulation of starch in the body which could be fatal for diabetic patients. Ammonium sulphate and peroxides in yoghurt can cause gastro-intestinal complications, which can lead to gastritis and inflammation of the intestine [19]. Urea present in yoghurt may cause overburdens the kidneys as they have to do more work to filter out the urea content from the body [20].

3.3 Microbiological Quality

Yoghurt is conventionally produced using a culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. These bacterial species are, measures of the quality of yoghurt. For this reason, this study examined these two types of bacterial species. While, coliform bacterial species was examined as indicator organism to identified pathogenic fecal contamination. The highest *Lactobacillus* spp. Count was found in sample (S3) and the lowest count was found sample as (S10) (8.75×10^4 cfu/gm, and 2.5×10^2 cfu/gm respectively).*Lactobacillus* count was highest count in sample (1) (7.3×10^4cfu/gm) and the lowest in sample (S7) (1.1×10^3 cfu/gm). This result is supported by Adeyl (1998) report, where the value of *L. bulgaricus* and *S. thermophilus* ranged between 2.20×10^6 cfu/gm and 1.70×10^6 cfu/gm, respectively. The NYA has established standards for probiotics yoghurt to contain minimum of 100 million cultures per gram during manufacture of sweetened yoghurt [21].

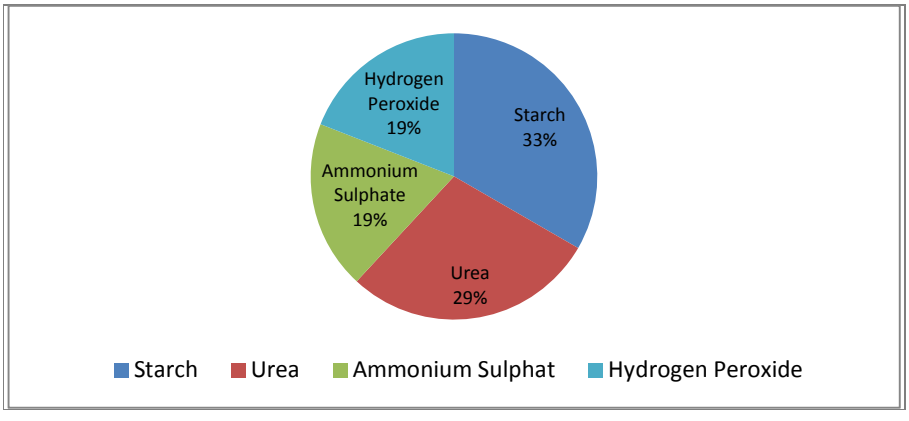

Fig. 1. Presence of adulterants in local sweetened yoghurt

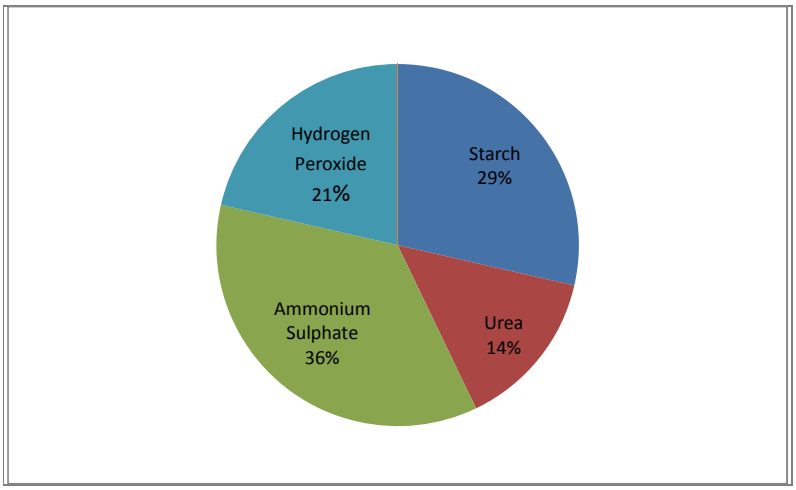

Fig. 2. Presence of adulterants in commercial sweetened yoghurt

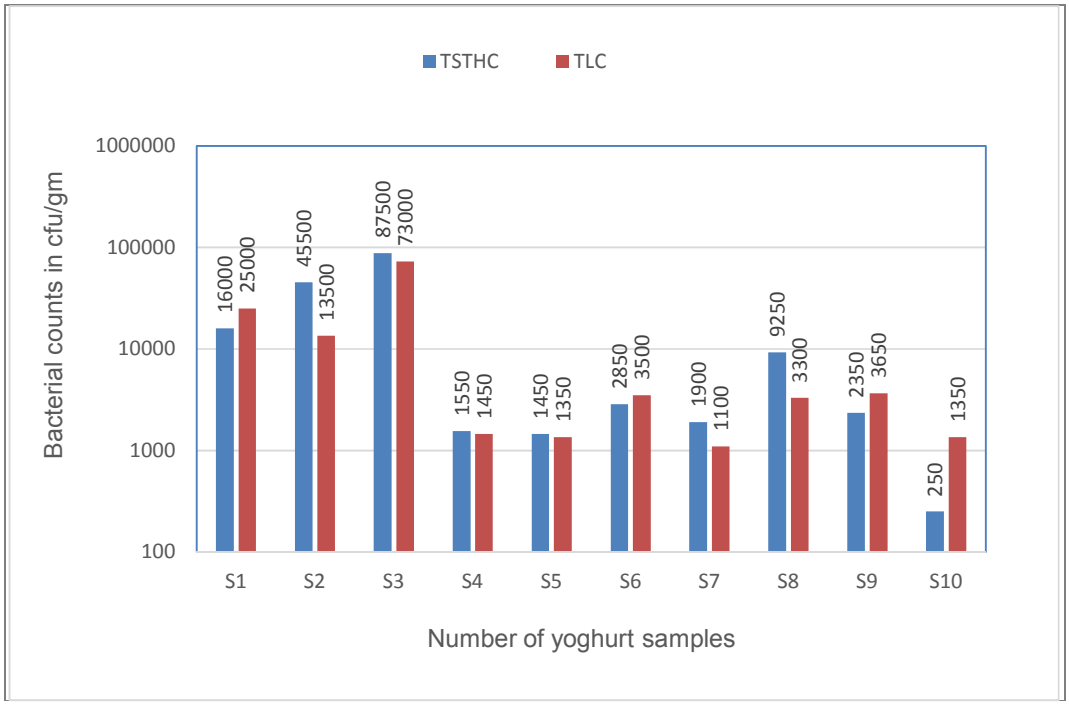

Fig. 3. Bacterial loads of locally (unbranded) available yoghurt samples in Bangladesh

Each gram of frozen yoghurt contained 10 million cultures. The standard acceptable bacterial culture should be at least 1 × 106 in yoghurt [22]. The results of the microbial count in this study were under acceptable range. In case of pathogenic coliform bacterial count, there were no coliform bacteria present in any locally available samples tested.

The commercial yoghurts are very popular especially for school children. This study examined six commercial yoghurts. The highest *Lactobacillus* count was found in sample B (5.7 x 10^3 cfu/gm), and the lowest *Lactobacillus* count in sample S(2.5 x 102 cfu/gm) (Fig. 4). Mean while, the highest S. thermophilus count was found in sample (S1) and the lowest count found

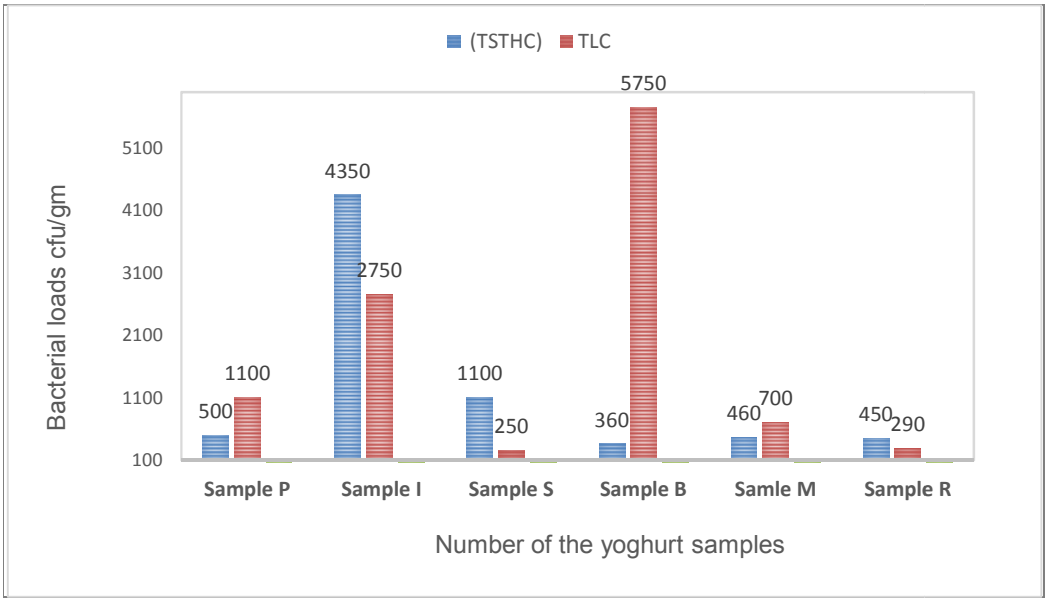

Fig. 4. Bacterial loads of commercially available yoghurt samples in Bangladesh

in sample (B). The *S. thermophiles* count in commercial yogurt sample was observable lower than locally available yoghurt samples except sample (I), which is close to local yoghurt sample and match the acceptable range. But in case of *Lactobacillus* spp. counts were found to be drastically lower than local samples except sample B, which had similar result with local sample. These data revealed that local yoghurts were better than commercial yoghurt in terms of starter culture content because the amount of culture in the local yoghurt were higher than those of the commercial yoghurt, an indication of local yoghurt as good probiotics food.

4. CONCLUSION

The present study concludes that the maximum number of sweetened yoghurt were adulterated by urea, ammonium sulphate and starch which in turn increased total solid content and crude protein content but decreased fat content. Presence of starch, urea and ammonium sulphate were made the yoghurt inferior quality and hazardous to health. In addition, the low number of *L. bulgaricus* and *S. thermophilus* bacteria in the starter culture revealed that starter culture uniformity should be maintained to get superior quality of sweetened yoghurt.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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