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Physico-chemical, Sensory and Microbiological Characteristics of Strawberry Flavored Milk under Refrigerated Storage

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ABSTRACT

Background: In this study, physico-chemical, sensory and microbial characteristics of three formulated strawberry flavored milk samples were investigated under 14 days of storage at refrigeration (4°C) temperature.

Methods: pH, acidity, specific gravity, TSS, fat, protein, sensory attributes and microbial quality were investigated.

Result: Physicochemical properties varied from pH (5.47 to 6.37), acidity (0.15 to 0.19%), TSS (15 to 16°Brix), specific gravity (1.05 to 1.063), fat (1.87 to 2.07%) and protein (3.33 to 3.40%) under the storage conditions. The results of sensory parameters ranged color (3.27 to 7.93), flavor (3.27 to 7.93), taste (3.40 to 7.73) and overall acceptability (3.13 to 8.0). The total plate count (TPC) in strawberry flavored milk ranged from 3.67×10^3 to 24.67×10^3 cfu/ml. Sample 3 almost retained the highest values considering pH, acidity, specific gravity, fat and protein with few exceptions as compare to sample 1 and sample 2 which were not significantly different ($p\leq0.05$). Sample 2 obtained the highest score for color, flavor, taste and overall acceptability which was significantly different ($p\leq0.05$) from other sample 1 and sample 3. Although the TPC value lowest found in sample 1 but there was no significant different ($p\leq0.05$) with sample 2 and sample 3. Overall, sample 2 had more reliable results till the 7 days of refrigeration storage, followed by sample 3 and sample 1.

Key words: Acidity, Fat, Protein, Strawberry flavored milk, Total plate count.

INTRODUCTION

Milk is a crucial part of the healthy human diet starting from the birth. Wide varieties of essential nutrients are found in milk and dairy products for the growth and maintenance of the human body. Milk and dairy products rich sources of casein, whey proteins, immunoglobulins, conjugated linoleic acid, lactose and minor oligosaccharides such as prebiotics, calcium, phosphorous, vitamin D and riboflavin. It's also contained various probiotics bacteria as the main nutrients for promoting health and maintenance of the human body process (Özer and Kirmaci, 2010). Milk is enabled for maintaining good health, keeping bones strong and providing necessary nutrients during the development of children. It is also considered one of the main sources of protein for a certain community (Bermúdez-Aguirre *et al.* 2010). Furthermore, milk proteins such as caseins, a-lactalbumin, b-lactoglobulin are considered to be the major source of bioactive peptides which have functional properties like antithrombotic, antihypertensive, antimicrobial, antioxidant, antipyretic, immunomodulatory and anti-carcinogenic activities resulting they contribute more in maintaining consumer's health and well-being (Park and Nam, 2015).

Milk based beverages are the most acceptable food items for consumers in the functional food market (Özer and Kirmaci, 2010). Milk beverages includes flavored milk, fortified milks with bioactive compounds such as sterols, fish oils, fibers, *etc*., vitamins and minerals (Jelen, 2009). Flavored milk is an unfermented dairy product containing some flavors such as chocolate, cacao powder, fruits (banana, orange, strawberry) and sugar, often enriched with vitamins and calcium. In several countries such as the United States and India, school meal plans comprises flavored milks/drinks due to their high nutritional values (Guneser *et al.* 2019). Flavored milk-based beverages with different flavors have attracted the attention of consumers who are in search of a different flavor. To satisfy consumers' needs and preference as well as consumers' health, the present study was conducted. The objectives of the study were to standardize the formulation of strawberry flavored milk and assess the quality parameters as well as evaluate the consumer acceptance under 14 days of refrigeration storage.

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MATERIALS AND METHODS

Materials

Sample collection and sample preparation

The raw materials were purchased from the local market,

Dhaka, Bangladesh. After that, all the raw materials were brought to the Laboratory of Food Engineering, NPI University of Bangladesh and weighed according to the formulation. Raw milk was stored at 4°C temperature. The weighed raw ingredients were stored in polyethylene bag for further use.

Chemical and reagent

Milk, sugar, Strawberry concentrate, Solid Milk Powder, stabilizer, water, Strawberry color, Strawberry flavor, sulfuric acid, Amyl Alcohol, copper sulfate, sodium sulfate, NaOH, boric acid, HCL, phenolphthalein, Agar *etc*. were purchased from local market of Dhaka, Bangladesh.

Methods

Preparation of strawberry flavored milk

Firstly, Solid milk powder (SMP) was mixed with hot water. Then added to it with raw milk. After that, sugar combined with stabilizer, color with little hot water and strawberry flavor added to the mixture and mixed properly. Then the mixture was pasteurized at 71°C for 30 minutes. Finally, the mixture was filled into the bottle and stored at 4°C or below (Table 1 and Fig 1).

Determination of pH

The pH of strawberry flavored milk was measured by a pH meter (Hanna, HI 8417) calibrated using buffers of pH 7.0 and 4.0.

Determination of acidity

Acidity of strawberry flavored milk was determined according to the method reported by Teka (2013) with few modifications. The titratable acidity was calculated using the following equation.

$$\% \text{ Acidity (Lactic acidity)} = \frac{\text{ml NaOH} \times 0.1\text{N NaOH} \times 0.090}{\text{Volume of Sample}} \times 100$$

Determination of Total Soluble Solid (TSS)

The TSS (°Brix) of the strawberry flavored milk were determined by using a handheld refractometer (Model: HT119-ATC).

Determination of specific gravity

Specific gravity of strawberry flavored milk was determined by pycnometer according to the modified method of Javaid *et al.* (2009). Specific gravity of the sample was calculated using the following formula:

$$\text{Specific gravity (at 20°C)} = \frac{\text{Weight of sample}}{\text{Weight of distilled water}}$$

Determination of fat

Gerber method was used for the determination of fat as reported by Javaid *et al.* (2009) with slight modification.

Determination of protein

Protein of strawberry flavored milk was determined using Micro-Kjeldhal method as reported by Javaid *et al.* (2009) with few modifications. The nitrogen % was calculated using following formula and [Protein Factor=6.38, dairy products]:

$$\text{Nitrogen (\%)} = \frac{1.4 \times \text{HCl sample} - \text{HCl blank} \times \text{Normality of HCl}}{\text{Weight of sample taken}} \times 100$$

$$\% \text{ Protein} = \text{Nitrogen (\%)} \times \text{Protein Factor}$$

Sensory evaluation

Strawberry flavored milk samples were evaluated organoleptically for color, flavor, taste and overall acceptability, according to the Hedonic 9-point scale (9-like extremely to 1- dislike extremely) as reported by Roy *et al.* (2018). Randomly, the samples were presented to the panel members at least 15 peoples.

Microbial analysis

The total plate count of strawberry flavored milk was determined according to the method reported by Rana *et al.* (2020) with few modifications. 10^{-1},10^{-2},10^{-3},10^{-4},10^{-5} and 10^{-6} dilutions of the strawberry flavored milk samples were prepared. Then 0.1 ml of aliquot was taken from each dilution and spread on the agar plates incubated at 37°C for 48 hours. The total plate count (TPC) of samples were calculated using the following formula:

$$\text{Total plate count} \left(\frac{\text{CFU}}{\text{ml}}\right) = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{ml aliquot taken}}$$

Statistical analysis

Data were analyzed statistically using the statistical software R (windows version 2.13.1). All results were measures triplicate. Results were expressed as mean values with standard deviation (±SD). DMRT (Duncan Multiple Range Test) was performed to evaluate the significance of difference between mean values at p level of 5%.

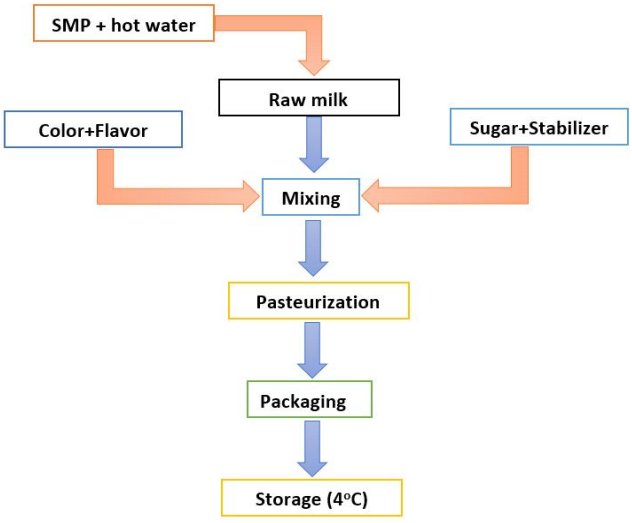


Fig 1: Flow diagram of strawberry flavored milk.

RESULTS AND DISCUSSION

Physico-chemical properties

Table 2 shows the physico-chemical characteristics of strawberry flavored milk under refrigerated condition with 14 days of storage. The pH value of strawberry flavored milk ranged from 5.47 to 6.37 whole storage period for all samples. The highest pH found in sample 3 was 6.37 and lowest found in sample 2 during storage period. This values were supported the work of (Kamble *et al.* 2019) who found the piper betel flavored milk pH ranged from 6.27 to 6.55. Our obtained values were closely related the findings of (Dhande *et al.* 2020) reported the pH value of lemongrass flavored milk between 6.33 to 6.46. The pH of carrot flavored milk beverage ranged 6.44 to 6.69 (Grewal and Sharma, 2005) was higher than our obtained values. It is observed that, the pH value of strawberry flavored milk for all sample followed downward trend after increasing the storage time which is significantly different. Increase in acidity of the product might be due to the variation of pH values under the storage. The decrease in pH might be due to slight growth of micro-organisms and their metabolites (Hassan *et al.* 2015). However, the acidity of the strawberry milk was ranged between 0.15 to 0.19% during the period of storage. At the beginning, sample 2 retained the lowest acidity 0.15% which is not significantly different with sample 1 and sample 2 whereas highest acidity was 0.19% for all sample found at the end of the storage. Our work is closely related with the results of Dhande *et al.* (2020) who observed the acidity of wheatgrass flavored milk ranged from 0.16 to 0.18%. The acidity of strawberry flavored milk comparable with the work of Dalim *et al.* (2012) who found the chikoo flavored milk beverage had 0.18% acidity and higher acidity than same work of banana flavored milk beverage was 0.20%. Our obtained values of acidity of strawberry flavored milk has lower the work of Hassan *et al.* (2015) who reported the acidity of fruit flavored milk acidity ranged from 0.20 to 0.28% in the 7days of storage. Increase acidity the results of decrease pH values for all sample were found in our investigation. The acidity increase results of growth of microorganism during storage or conversion of milk lactose into lactic acid by bacteria (Hassan *et al.* 2015). The total soluble solid (TSS) in strawberry flavored milk ranged from 15 to 16^{o} Brix in the entire storage period. Although TSS of prepared milk was almost similar till the last day of storage, initially it was lowest in sample 3 and highest in sample 1. The sample 1 and sample 2 significantly different with the sample 3 throughout the storage period.

Furthermore, the specific gravity of straw berry flavored milk contained ranged from 1.05 to 1.063 under the refrigerated storage. Overall storage duration, sample 3 found higher specific gravity whereas lower in sample 2. Our results supported by the findings of Dalim *et al*. (2012) who revealed that, the specific gravity of Chikoo and banana flavored milk beverage was 1.061 and 1.063 respectively. Our observed data was lower than the work of Kamble *et al.* (2019) who found the piper betel flavored milk had specific gravity between 1.066 to 1.074. It is clear that, the end of 7 days of storage there was no significance different found among the samples except that the sample 2 was significantly different from sample 1 and sample 3 after 14 days of storage.

The fat content of strawberry flavored milk is shown in Table 1. The fat content of strawberry flavored milk ranged from 1.87 to 2.07% during the storage. At the very beginning, the sample 1 retained the higher fat% and lower found in sample 2 and sample 3 after 14 days of storage. Our

Table 1: Formulation of strawberry flavored milk.

Sample	Milk (%)	Sugar (%)	Milk gel (%)	SMP (%)	Strawberry concentrate (%)	Strawberry color (%)	Strawberry flavor (%)	Water (%)
1	79.9178	8.9719	0.2130	2.5129	5.3274	0.0013	0.0402	3.0155
2	80.7470	8.0265	0.2153	2.5392	5.3831	0.0013	0.0406	3.0470
3	81.4231	7.2565	0.2171	2.5604	5.4281	0.0014	0.0409	3.0725

Table 2: Physico-chemical characteristics of strawberry flavored milk under refrigerated condition with 14 days of storage.

Storage period day (s)	Sample	pH	Acidity (%)	TSS (^{o}Brix)	Specific gravity	Fat (%)	Protein (%)
0	1	$^{A}6.33\pm0.06^{a}$	$^{A}0.16\pm0^{c}$	$^{A}16.0\pm0^{a}$	$^{AB}1.06\pm0^{a}$	$^{A}2.07\pm0.06^{a}$	$^{A}3.33\pm0.06^{a}$
	2	$^{A}6.33\pm0.06^{a}$	$^{A}0.15\pm0.0^{c}$	$^{A}15.67\pm0.58^{a}$	$^{A}1.053\pm0.01^{a}$	$^{A}1.97\pm0.06^{a}$	$^{A}3.37\pm0.06^{a}$
	3	$^{A}6.37\pm0.06^{a}$	$^{A}0.16\pm0.0^{b}$	$^{B}15.0\pm0^{a}$	$^{A}1.063\pm0.01^{a}$	$^{A}2.03\pm0.06^{a}$	$^{A}3.40\pm0^{a}$
7	1	$^{B}6.03\pm0.06^{b}$	$^{A}0.17\pm0^{b}$	$^{A}16.0\pm0^{a}$	$^{A}1.06\pm0^{a}$	$^{A}1.97\pm0.06^{ab}$	$^{A}3.33\pm0.06^{a}$
	2	$^{A}6.07\pm0.06^{b}$	$^{A}0.16\pm0.0^{b}$	$^{A}15.67\pm0.58^{a}$	$^{A}1.053\pm0.01^{a}$	$^{A}1.97\pm0.06^{a}$	$^{A}3.37\pm0.06^{a}$
	3	$^{B}6.07\pm0.06^{b}$	$^{A}0.17\pm0.0^{b}$	$^{B}15.0\pm0^{a}$	$^{A}1.053\pm0.01^{a}$	$^{A}1.93\pm0.06^{ab}$	$^{A}3.37\pm0.06^{a}$
14	1	$^{A}5.47\pm0.12^{c}$	$^{A}0.19\pm0^{a}$	$^{A}16\pm0^{a}$	$^{A}1.06\pm0^{a}$	$^{A}1.93\pm0.06^{b}$	$^{A}3.33\pm0.06^{a}$
	2	$^{A}5.47\pm0.06^{c}$	$^{A}0.19\pm0.19^{a}$	$^{A}15.67\pm0.58^{a}$	$^{B}1.05\pm0^{a}$	$^{A}1.87\pm0.06^{a}$	$^{A}3.33\pm0.06^{a}$
	3	$^{A}5.50\pm0.10^{c}$	$^{A}0.19\pm0.01^{a}$	$^{B}15\pm0^{b}$	$^{A}1.056\pm0.01^{a}$	$^{A}1.87\pm0.06^{b}$	$^{A}3.37\pm0.82^{a}$

$^{A-C}$Means followed by different superscript letter of alphabets in each column significantly different (P≤0.05) within the sample in same day.

$^{a-c}$Means followed by different superscript letter of alphabets in each column significantly different(P≤0.05) within the same sample every 7 days.

obtained value was lower than the fat content found in lemongrass flavored milk was ranged from 3.05 to 3.17% (Dhande *et al.* 2020). Our findings were almost similar with work of Palthur *et al.* (2014b) observed fat content of 2.05% when milk was prepared by using ginger juice. Palthur *et al.* (2014a) obtained 2.16% fat in different dietetic herbal milk which was little higher than our findings. It is observed that, increasing the time duration decreasing the fat content for all sample. A significance difference was found for sample 1 and sample 3 after 14 days of storage except sample 2. Changes in fat content it might be due to addition of sugar. Shelke (2008) reported that fat content of rose, vanilla, cardamom, strawberry, kesar, pineapple and mango flavored milks slightly decreased as compared with original milk. This was due to increase in volume of final flavored milk because of addition of sugar.

The protein content of strawberry flavored milk ranged from 3.33 to 3.40% during the storage periods. Primarily, higher protein content found in the sample 3 (3.40%) and lower in sample 1 (3.33%). Kamble *et al.* (2019) found the protein content in Piper betel flavored milk between 3.34 to 3.65% these data were supported to our observation. Our demonstration was a bit lower with the work of Dalim *et al.* (2012) who showed that average protein content of chikoo flavored milk was 3.56%. The protein content ranged from 3.20 to 3.31 found by Dhande *et al.* (2020) was lower than our findings. There is no significant difference found among the samples under storage. The protein variation may be due to the addition of flavoring agent with milk (Repate *et al.* 2010).

Sensory characteristics

Sensory characteristics of strawberry flavored milk under storage condition is shown in Table 3. Here sensory attributes like color, flavor, taste and overall acceptability were evaluated under 14 days of refrigerated storage at 7 days' interval period. At the very beginning, the sample 2 found the highest score for color (7.93) which is significantly different with sample 1 and sample 2. Lowest color value (3.27) found sample 3 after 14 days of storage which is significantly different with other samples during the entire storage period. At 0 day of storage, the higher score found for sample 2 which was significantly different as compare to sample 1 and sample 3. Sample 3 retained the lower score

Table 3: Sensory characteristics of strawberry flavored milk under storage condition.

Storage period day(s)	Sample	Color	Flavor	Taste	Overall acceptability
0	1	$^{B}6.47\pm0.99^{a}$	$^{B}6.47\pm0.83^{a}$	$^{B}6.47\pm1.13^{a}$	$^{B}6.67\pm0.82^{a}$
	2	$^{A}7.93\pm0.80^{a}$	$^{A}7.93\pm0.80^{a}$	$^{A}7.73\pm0.80^{a}$	$^{A}8.00\pm0.76^{a}$
	3	$^{B}6.40\pm0.63^{a}$	$^{B}6.47\pm0.99^{a}$	$^{B}6.80\pm1.08^{a}$	$^{B}6.33\pm0.72^{a}$
7	1	$^{B}6.07\pm0.80^{a}$	$^{B}6.6\pm0.74^{a}$	$^{B}6.60\pm1.06^{a}$	$^{B}6.60\pm0.99^{a}$
	2	$^{A}7.33\pm0.72^{b}$	$^{A}7.47\pm0.74^{a}$	$^{A}7.40\pm0.63^{a}$	$^{A}7.60\pm0.74^{a}$
	3	$^{B}5.67\pm0.72^{b}$	$^{C}5.73\pm0.70^{b}$	$^{B}6.13\pm0.83^{a}$	$^{C}5.87\pm0.74^{a}$
14	1	$^{A}3.87\pm0.87^{b}$	$^{A}4.07\pm0.59^{b}$	$^{A}4.20\pm0.77^{b}$	$^{A}4.07\pm0.70^{b}$
	2	$^{AB}3.67\pm0.62^{c}$	$^{A}3.93\pm0.70^{b}$	$^{A}4.13\pm0.92^{b}$	$^{A}3.87\pm0.74^{b}$
	3	$^{B}3.27\pm0.88^{c}$	$^{B}3.27\pm0.80^{c}$	$^{B}3.40\pm0.83^{b}$	$^{B}3.13\pm0.74^{b}$

$^{A-C}$Means followed by different superscript letter of alphabets in each column significantly different (P≤0.05) within the sample in same day.
$^{a-c}$Means followed by different superscript letter of alphabets in each column significantly different (P≤0.05) within the same sample every 7 days.

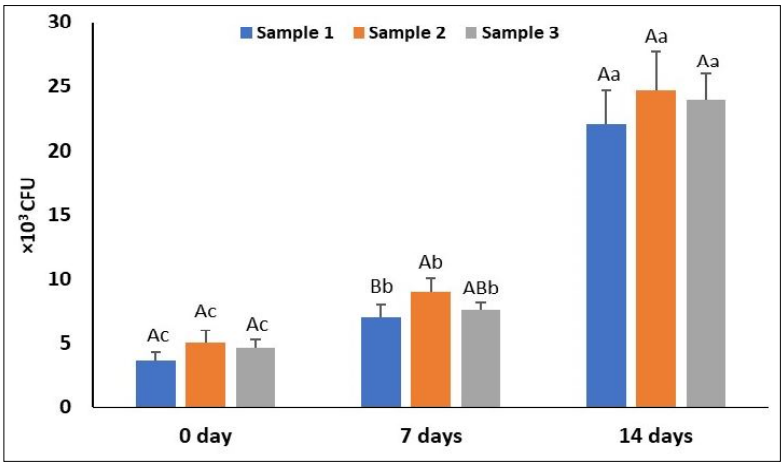


Fig 2: Microbial quality of strawberry flavored milk under refrigerated storage.
$^{A-C}$Means followed by different superscript letters significantly different (P≤0.05) within the samples in same day.
$^{a-c}$Means followed by different superscript letters significantly different (P≤0.05) within the same sample every 7 days.

for flavor after end of the storage periods. On the other hand, highest taste score (7.73) found by the sample 2 which had significance difference with sample 1 and sample 3. Reduced score of taste (3.40) found by the sample 3 at 14 days of storage. Furthermore, sample 2 retained the highest value (8.00) for overall acceptability compare to sample 1 and sample 3 had significant different with the same. The lowest score (3.13) possesses by the sample 3 after end of the storage. It has been clearly observed that, the consumer acceptance parameters for all samples followed by the decreasing trend after increasing the day. Among all the samples, sample 1 found highest score considering color, flavor and taste as well as overall acceptability which is significantly different from other two samples.

Microbial count

Fig 2 shows the microbial count of strawberry flavored milk under refrigerated storage. Total plate count (TPC) found ranged from 3.67×10^3 to 24.67×10^3 CFU throughout the storage period. Sample 2 found lowest 3.67×10^3CFU at 0 day and highest 24.67×10^3 found in 14 days of storage. Our obtained value was higher than the work of Grewal and Sharma,(2005) who found the TPC in carrot flavored milk ranged from 2.0×10^3CFU to 4.0×10^3CFU. Herbal flavored milk was contained TPC 15CFU/ml reported by Palthur *et al.* (2014b) which was very lower than that our work. Jamal *et al.* (2019) reported that the total viable count in mango flavored milk (50 to 1.8×10^2CFU/ml) and chocolate flavored milk (70 to 1.8×10^2CFU) were lower than that our findings. However, it was clearly known that, initially the value for all samples was lowest but end of the storage period observed highest value and this is very expected. Although the sample 2 found higher TPC as compare to other but in the same day there was no significant different ($P\leq0.05$) found except 7 days' data.

CONCLUSION

In this work, Physico-chemical characteristics and sensory acceptance of strawberry flavored milk declined with the increase in time. With few exceptions, sample 3 had highest results of pH, acidity, specific gravity, fat and protein as compare to other samples which were not significantly different ($p\leq0.05$). Sample 2 showed the highest sensory score. TPC value lowest found in sample 1 but there was no significant different ($p\leq0.05$) with sample 2 and sample 3. Overall opinion, sample 2 had better outcomes than the other sample.

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