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PHYSICO-CHEMICAL, SENSORY AND MICROBIOLOGICAL QUALITIES OF HOMEMADE YOGURT USING COMMERCIAL STARTER CULTURE

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ABSTRACT

This research was initiated with the objectives of evaluating the physico-chemical, sensory and microbiological qualities of four homemade yogurt treatments. Fresh morning milk was collected from Andassa Livestock Research Center farm, thoroughly mixed, transported to Bahir Dar University for yogurt preparation. The milk samples were homogenized at 800rpm for three minutes and filled in to coded cups of 300ml capacity. The four yogurt treatments were pasteurized milk inoculated with starter culture containing thermophilic yogurt culture (YoFlex®) at 42oc (T1), pasteurized milk inoculated with plain yogurt at 42oc (T2), pasteurized milk inoculated with plain yogurt at room temperature (T3) and unpasteurized milk inoculated with plain yogurt at room temperature (T4). The treatments were subjected to physico-chemical and microbial count laboratory analysis and sensory evaluation, on the 1st, 7th, 14th and 21st day of storage. The analysis was done in duplicate and the design was a 4*4*2 factorial design. The results obtained showed that the mean moisture content of the treatment yogurt ranged between 86.52 and 88.26%, without showing significant difference between each other. The entire treatment yogurt seems to have a relatively long shelf-life because of the high acidity value of 4.3-4.5 recorded. Mean titratable acidity level of the yogurt ranged from 0.86 to 0.94 compare to the mean titrable acidity level of 0.67 - 0.95 recorded for good quality traditional yogurt in the area. The total solid content of the yogurt treatments ranged between 6.8 and 7.3. Relatively higher fat content of 9.88-10% was obtained from both yogurt treatments T1 and T2. The result of microbiological analysis indicated that the analysis ranged from <0.1 – 11420 (*10^2 cfu/ml) of Coliforms and 0 – 834 (*10^2 cfu/ml) of yeast and mold count. All the yogurt treatments studied fit into acceptable range set for good quality yogurt throughout the storage periods. Based on the panellist's sensory evaluation the yogurt treatment T4 ranked first in acceptance followed by T1. Testing different types of commercial starters in the area of, affordability and accessibility in the market seems to be the future direction of research.

Keywords: Microbiological, Pasteurized, Sensory, Starter Culture and Yogurt.

INTRODUCTION

Dairy products constitute an important group of foods; they are known to contain energy and such nutrients as protein, fat, carbohydrate, and calcium. They also make an important contribution to vitamin intake (Igbabul *et al.,* 2014). The consumption of milk and a dairy product is common in the world. Of these, yogurt is a coagulated milk product obtained by lactic acid fermentation through the action of Lactobacillus bulgaricus and Streptococcus thermophilus (Bourlioux and Pochart, 1988), and is a popular product throughout the world (Mohammed and Mohamed, 2017). According to FDA (2013) yogurt is defined as a food produced by culturing one or more of dairy ingredients i.e. cream, milk, and skim milk, used alone or in combination with a characteristic bacterial culture that contains lactic acid producing bacteria (Tamime, 2006). Yoghurt is a valuable healthy food for both infants and elderly persons. For children, it is a balance source of protein, fats, carbohydrates, and minerals. For senior citizens who usually have more sensitive colons or whose intestines have run out of lactase, yoghurt is also a valuable food (Igbabul *et al.,* 2014). A number of human studies have clearly demonstrated that yoghurt containing viable bacteria (Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus) improved lactose digestion and eliminated symptoms of lactose intolerance, thus these cultures clearly fulfill the current concept of probiotics (Mohammed and Mohamed, 2017). Regular consumption of yogurt is thought to be beneficial in the strengthening of the immune system, improvement in lactose digestion, blood glucose management (Yaday *et al.,* 2007) and the reduction of constipation, diarrhea, colon cancer, inflammatory bowel disease and allergies (Adolfsson *et al.,* 2004). The beneficial health effects of yogurt have partly linked to the proteolysis products, produced during fermentation and storage in particular, a group of peptides can lower the blood pressure in hypertensive patients (FitzGerald *et al.,* 2004). During the manufacturing process of industrial/commercial fermented dairy products, such as yogurt, pasteurization of the milk is a prerequisite process-step before inoculation of the milk with fermentative starter cultures. This process destroys pathogenic and spoilage micro flora from the products (Tamime, 2006) making the fermented milk safer for consumers. In principle, worldwide, there is no any difference between manufacturing of homemade and factory-made yogurt (Akin and Rice, 1994). The shelf-life of yoghurt is 1 day at 25 to 30°C and 5 days at 7°C or 10 days at 4°C according to the Turkish standards for plain yoghurt (Hayaloglu *et al.,* 2007). The easiest and least expensive way of obtaining a starter culture is to purchase plain yogurt at a grocery store/super markets. It should be plain– no fruit added. To maintain a culture, save a small portion of yogurt (1 cup is enough for a 1-gallon batch) to use as a starter culture for the next batch (Hutkins *et al.,* 2006). Based on FAO (2005, dry season synthesis report), in Ethiopia, 28.6 million liters of milk per year was lost at the farm level. This covers 1.3 to 6.4% of the value of available milk at the farm level and the total value of post-harvest milk losses per year amounted to 14.2 million US dollars. For both on-farm level and in the market chain, milk losses are largely in form of spoilage,

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spillage, and "forced home consumption" (including by calves and humans) over and above normal household consumption (Tezira *et al.,* 2005). The biggest challenge for this type of milk loss for smallholders in Ethiopia is to prevent the milk from spoilage during fasting periods; and distance to milk cooperatives and cities. This situation warrants the production of market yogurt with safe procedures. In this regard, this article was initiated to address the following objectives.

Objective

- To evaluate the physico-chemical, and microbiological qualities of homemade yogurt using different commercial starter culture.
- To evaluate the sensory parameters of homemade yogurt using different commercial starter culture

MATERIALS AND METHODS

Preparation of Homemade Yogurt

Adequate volume of fresh morning cow's milk was collected from Andassa Livestock Research Center dairy farm. The milk collected from different lactating animals, was thoroughly mixed and transported to Bahir Dar University using stainless still milk container. Yogurt was prepared according to the procedure suggested by Hutkins, (2006). Soon after arrival at the university, the milk was homogenized at 800rpm for three minutes and the homogenized milk was filled in to coded cups each with 300ml capacity. The experiment was made by four treatments, four analysis intervals and the analysis were done in duplicate (4*4*2 factorial design). The milk was pasteurized at 80^{o}c for 30 minutes and cooled down to 45^{o}c. A total of four experimental treatments presented below were prepared through inoculation of the milk samples with either starter culture (containing thermophilic yogurt culture (YoFlex®) (17mg for 300ml milk) or plain yogurt (2% i.e. 6ml) at 42^{o}c or at room temperature until coagulation occur. The treatment yogurts were stored in refrigerator at 4^{o}c. These were subjected to chemical and microbiological quality analysis on the 1st, 7th, 14th and 21st days.

Experimental treatments

- Treatment 1. Pasteurized milk inoculated with starter culture at 42^{o}c
- Treatment 2. Pasteurized milk inoculated with plain yogurt at 42^{o}c
- Treatment 3. Pasteurized milk inoculated with plain yogurt at room temperature
- Treatment 4. Un pasteurized milk inoculated with plain yogurt at room temperature

Chemical and Microbial Analyses

The experimental treatments (Yogurt) were subjected to the determination of pH, moisture, titrable acidity, total solid, fat and protein contents, using the procedure suggested by AOAC (2005). Microbiological analysis of the treatment yogurt was done for coli forms, yeast and molds according to the Compendium methods for the microbiological examination of foods as suggested by APHA, (2001). For determination of coli form count (CC) and yeast and mold count (YMC) a respective Brilliant Green Bile broth and potato distress agar were used to develop a culture for bacterial growth. One gram of the yoghurt samples was dissolved in 9 mL of sterilized distilled water and serial dilution was made from 10^{-2} to 10^{-5} rate. All parameters were analyzed in duplicates. The analysis was conducted in collaboration with Bahir Dar University Faculty of Chemical and Food Engineering.

Determination of titrable acidity: NaOH as a base (titrant) and a color indicator (phenolphthalein) was used to determine the end point of the acid-base reaction. And the following formula was used to calculate the total acidity.

$$Titratable\ acididty = \frac{Volume\ of\ titrant\ x\ N\ x\ 90}{Weight\ of\ sample\ x\ 1000} \text{x } 100$$

Where; N = normality of titrant; 90 = Equivalent weight for lactic acid

Determination of fat percentage: to determine the fat percentage in yogurt, 10ml sulfuric acid (98% concentration) was used for dilution of 11.3gram sample; and 1ml amyl alcohol was used to separate the fat. All the ingredients were shacked well to avoid the yogurt droplet. Then the solution was kept in water bath (65-70^{o}C) for 5 minutes. Then the solution was centrifuge under 1000 RPM for 4 minutes and it was so wait for 3 minutes in water bath (65-70^{o}c).

pH measurement: pH meter was used to direct measure of the pH of yogurt samples.

Sensory Evaluation

The sensory evaluation was done in order to check the texture, flavor, taste and overall acceptability of the treatment yogurt by four untrained panelists. The Panels were from Andassa Livestock Research Center, Woreta ATVET College and Bahir Dar University staffs and the treatment samples were randomly presented at room temperature. The panelists were given a five Likert scale values (1 = excellent; 2 = good; 3 = acceptable; 4 = bad; 5 = insupportable) aimed at testing, appearance/color, texture/smell, flavor/test, and overall acceptance of the fresh coded samples of the treatment yogurt, stored for 1, 7, 14 and 21 days. Panelists were asked to keep the yoghurt in the mouth for 12 seconds before scoring for flavor/test parameter. Water was used for rinsing mouth between samples (International Dairy Federation, IDF, 2002). For 14th day analysis, taste was not conducted due to fasting. The characteristics of the sensory evaluations considered for:

- **Appearance/color:** involves the filling and the surface of the product, color, visible purity, presence of foreign matters, spots of mold, and seepage/leakage of whey and phase separation.
- **Texture/smell:** the evaluation involves thickness, stickiness and coarseness. Evaluation can be made by blending the product with a (black) spoon before evaluating the sample in the mouth.
- **Flavor/test:** The evaluation of flavor is made by smelling and tasting the product.

Statistical Analysis

The data obtained were computed as mean and analyzed by Analysis of Variance (ANOVA) of SPSS. Likert scale values for sensory evaluation were analyzed by index method. Index was computed with the principle of weighted average according to the following formula.

Index = $R_n*C_1+R_n-1*C_2\ldots. R_1*C_n/\sum R_n*C_1+ R_{n-1}*C_2\ldots.R_1$

Where;

R_n = Value given for the least ranked level (example if the least rank is 5th rank, then R_{n-5}, R_{n-1}=4 and … R_1= 1)
C_n = Counts of the least ranked level (in the above example, the count of the 5th rank = C_n, and the counts of the 1st rank = C_1)

RESULT AND DISCUSSION

Physico-Chemical Property

Moisture percentage

The results of the mean value for chemical properties of the treatment yogurt were presented in Table 1 and 2. According to the results presented in Table 2, the moisture content of the treatment yogurt ranged between 86.52 and 88.26%, without showing significant difference between each other. Numerically lower moisture content of 86.52% was recorded from the treatment yogurt containing pasteurized milk inoculated with starter culture at 42^{o}c (T1). The moisture content of the current study indicated a decreasing trend from day one to 14. A moisture content of 78.2 – 87.1% was reported by (Joseph and Joy, 2011) for yogurt samples collected from market in Nigeria.

pH content

The current result showed that lower pH of 4.22 (T1) to higher value of 4.53 (T2) was reported in the current study. The pH value of the current study was in line with the report of Joseph and Joy (2011) which was 3.7 – 4.33. The treatment yogurt seems to have a relatively long shelf-life because of the high acidity value ranging between 4.3 and 4.5 as reported by Lourens-Hattingh and Viljoen (2002).

Titrable acidity

Titratable acidity is an approximation of the total acidity in a substance. It determines how much of a base (NaOH) is required to neutralize an acid. The base, also known as the titrant, is of known concentration. Comparable mean Titrable acidity level of 0.85 and 0.86 was recorded from T1 and T2, respectively. Titrable acidity for Ergo (yogurt) collected from small scale, large scale and research center was reported to be 0.85, 0.67 and 0.95, respectively (Zelalem and Bernard, 2006). Considerably lower titrable acidity content was reported to be 0.22 – 0.5% (Joseph and Joy, 2011) in yogurt samples collected from market. While considerably higher titrable acidity (1.22) was reported by Silva and Rathnayaka (2014).

Total solid

As shown in Table 2, the total solid (TSS) recorded from the treatment yogurts followed the same trend as that of titrable acid. Mean total solid of 6.8 was recorded from T1 and T2. Higher level of total solid (12.9 – 21.8) (Joseph and Joy, 2011), and 22.89 (Silva and Rathnayaka, 2014) was reported for market collected yogurt samples.

Fat and protein percentage

Based on the US recommendations for adults, Yogurt (plain yogurt from whole milk) is 81% water, 9% protein, 5% fat, and 4% carbohydrates, including 4% sugars (Nielsen, 2003). Most of the proteins in yogurt (80%) are caseins and the remaining 20% are Whey protein. These proteins had different health benefits; Casein increases absorption of minerals like calcium and phosphorus and promotes lower blood pressure while whey protein promotes weight loss and lower blood pressure. The amount of fat in yogurt depends on the type of milk it's made from; and the fat content can range from 0.4% in nonfat yogurt to 3.3% or more in full-fat yogurt (Atli Arnarson, 2019). In the current study, the fat content ranges from 7.23 to 10%. Lower fat content (1.88 – 4.0%) for yogurt samples collected from market in Nigeria was reported (Joseph and Joy, 2011). An average fat content of 3.59 was reported by Silva and Rathnayaka (2014). Protein content of the current study ranges from 2.85 (T1) to 3.03% (T2). An average protein percentage of 4.36 was reported from samples collected in different supermarkets (Silva and Rathnayaka, 2014). According to USDA (2001), yoghurt with less than 0.5% fat content should be labeled as" not fat yoghurt", those with fat content within the range of 0.5-2.0% before the addition of bulky should be labeled "low fat yoghurt" and those with fat content above 3.25% should be labeled "yoghurt". Based on this classification, the yogurt made for this activity was labeled as yogurt.

Table 1. Physico-chemical properties of yogurt across different analysis days

Analysis day	Moisture %	pH	Titrable acidity	TSS	Fat %	Protein %
Day 1	87.75	4.44	0.91	7.71	7.85	2.86
Day 7	87.53	4.24	0.78	6.60	8.44	2.97
Day 14	86.37	4.68	0.94	6.69	9.50	3.03
Day 21	87.84	4.23	0.92	7.21	9.50	2.85

Table 2. Physico-chemical properties of yogurt across different treatment groups

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Moisture %	86.52	87.17	88.26	87.53
pH	4.22	4.54	4.43	4.41
Titrable acidity	0.86	0.85	0.94	0.92
TSS	6.8	6.8	7.3	7.3
Fat %	9.88	10.00	8.19	7.23
Protein %	2.85	3.03	2.90	2.92

Microbial Properties of yogurt

The results of the microbial quality of the treatment yogurt were presented in Table 3. The microbiological analysis indicated that the result of the analysis ranged from <0.1 - 11420 (*10^2 cfu/ml) for Coliforms and 0 – 834 (*10^2 cfu/ml) for yeast and mold count. Values ranging from 0.25 to 0.37 cfu/ml for coliform count was reported by Zelalem Yilma (2014) in Jimma town. Mean value of 8 cfu/ml was also reported by the same author. The coliform count was decreased across the working days.

The result showed that T1 had lower coli form and yeast and mold count which might be due to the use of commercial starter culture. The coli form count was decreased across the working days which might be due to the absence of hand and other materials contaminations. Serhan (1999), suggested that satisfactory yogurts contain more than l08 cfu ml^{-1} of the starter organisms, <1 coli form cfu/ml, <1 mold cfu/ml and <10 yeasts cfu/ml (fruit-containing yogurts may contain up to 100 yeasts ml^{-1} and remain of satisfactory quality). Based on this, T1, T3 and T4 had satisfy the settled values in different days of analysis.

Sensory Evaluation

The results of the sensory quality (appearance, texture, and flavor) are crucial for consumer acceptance. The sensory evaluations of the treatment yogurt were presented in Table 4. According to Yonca and Mary (2013), the understanding and measuring of the sensory properties of dairy products is important. Based on the panelists sensory evaluation result, T4 ranked first in overall acceptance followed by T1; this might be attributed to unpasteurized milk resembles the natural appearance and flavor of homemade yogurt done traditionally. The sensory evaluation was done based on the standards set by Quality guidelines for USDA specifications for yogurt as indicated in Yonca and Mary (2013).

Table 3. Microbial properties of yogurt across different analysis days and treatments

Analysis days	Coli form (*10² cfu/ml)	Yeast and Mold (*10² cfu/ml)	Treatments	Coliform (*10² cfu/ml)	Yeast and Mold (*10² cfu/ml)
Day 1	7668.13	416	Treatment 1	0.7	0.031
Day 7	2402.78	26.85	Treatment 2	5548.75	38.06
Day 14	269.53	40.68	Treatment 3	2517.5	260.25
Day 21	395.65	44.44	Treatment 4	2669.13	229.63

Table 4. Sensory evaluation index value (rank) between treatments across analysis days

Day	Treatments	Appearance/color	Texture/smell	Flavor/test	Overall acceptance
1	Treatment 1	0.263 (1)	0.263 (1)	0.222 (4)	0.25 (2)
	Treatment 2	0.225 (4)	0.258 (2)	0.274 (1)	0.241 (4)
	Treatment 3	0.227 (3)	0.257 (3)	0.272 (2)	0.242 (3)
	Treatment 4	0.258 (2)	0.225 (4)	0.258 (3)	0.258 (1)
7	Treatment 1	0.261 (1)	0.246 (1)	0.230 (4)	0.261 (1)
	Treatment 2	0.253 (2)	0.24 (3)	0.253 (3)	0.253 (2)
	Treatment 3	0.25 (3)	0.220 (4)	0.279 (1)	0.25 (3)
	Treatment 4	0.230 (4)	0.246 (1)	0.276 (2)	0.246 (4)
14	Treatment 1	0.5 (1)	0.5 (1)	Due to fasting test was not conducted	
	Treatment 2	0.466 (2)	0.533 (2)		
	Treatment 3	0.466 (2)	0.533 (2)		
	Treatment 4	0.461 (4)	0.538 (4)		
21	Treatment 1	0.235 (4)	0.294 (1)	0.235 (3)	0.235 (4)
	Treatment 2	0.263 (1)	0.263 (2)	0.210 (4)	0.263 (1)
	Treatment 3	0.25 (3)	0.25 (3)	0.25 (2)	0.25 (3)
	Treatment 4	0.263 (1)	0.210 (4)	0.263 (1)	0.263 (1)

CONCLUSION

Market yogurt formation with safe procedure increases the well-beingness and healthy life style of the consumers, helps in reduction of wastage of milk and provides job opportunities for cooperatives, youths and women found in and around urban areas. Market price of fresh milk is very low during Christian Orthodox fasting times. During this occasion preparation of yogurt in a safe and standard way is mandatory. Even though there was no standard set for yogurt in Ethiopia, the treatments used in this study had satisfy the standards set for yogurt in different countries like USA. Based on the organoleptic analysis, yogurt storage after 14 days had lowered its quality in relation to its texture and test; and from this it is best to use the yogurt before 14 days kept in refrigerator. The results obtained indicated that using commercial starter can help small scale milk producers and cooperatives to form standard yogurt for sale and use. The yogurt treatment prepared from pasteurized milk inoculated with starter culture at 42oc (T1) can be widely demonstrated for cooperatives and small-scale dairy producers. Based on these facts, the following recommendation were amended.

- Further study on different types of commercial starters in terms of affordability and accessibility in the market and identification of the active bacteria in market yogurts should be done.

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