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Physiochemical composition and microbial load of some branded yoghurt sold in Addis Ababa and Bishoftu city, Ethiopia

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Abstract

Study were carry out to determined physiochemical and microbial quality analysis for different seven brand yoghurt were collected and analyzed for Bishoftu and Addis Ababa city. For this analysis each brand of yoghurt were collected three times to reduce error. The range of total bacterial count and coliform count were $1.99X10^6$ to $2.50X10^9$ cfu/ml and $2.59X10^2$ to $1.12X10^4$ cfu/ml respectively. In current study total yeast and mould counts were ranged from $1.11X10^2$ to $4.93X10^2$ cfu/ml. Protein and lactose percentage of yoghurt brands range from 4.3% to 4.7% and 4.1% to 4.7%. Protein and lactose percentage of all yoghurt brands was not significantly different at p>0.05. In addition, fats and total solids percentage, titratable acidity of yoghurt also drastically diverse at P>0.05. Occurrence of high total bacterial count, coliform and yeast and mould in yoghurt has impact on health of the consumer. Due to this and other factor it needs special issue from concerning body to solve the problem.

Keywords: Physiochemical, microbial, brand yoghurt, Coliform, milk.

Introduction

Yoghurt is a fermented dairy product and partially semisolid food made from milk. Yoghurt is a popular dairy product and its sensory evaluation has great effect on consumer acceptability[1]. From the oldest fermented milk product, yoghurt is one which is widely consumed by large segments of our population either as a part of diet or as a refreshing beverage[2].

Yoghurt preparation process posses the change of the milk sugar to lactic acid, acetic acid, CO_2, acetaldehyde, diacetyl etc. This change was happened due to presence of starter culture which contains *Streptococcus thermophilus* and *Lactobacillus bulgaricus*[3]. Two bacterial species have symbiotic relationship. At combination level they produce acid rapid than the single strain culture[4]. To get required flavor characteristic of yoghurt different type spice and fermenting bacteria were used. In addition to this yoghurt has a great role in therapeutic activity. Amount of culture added where depend on strength and activity of starter culture, commonly yoghurt producer adds 2-4% of starter culture.

There are different brands of yoghurt produced industrially in Bishoftu and Addis Ababa city is one of its major market place where it is commonly sold.

Poorly produced and handed yoghurt has its' negative effect on health of consumer. This study was considered to evaluate physiochemical and microbiological value of seven popularly sold yoghurts in Bishoftu and Addis Ababa city, Ethiopia.

Materials and methods

Study area: The study were done around Bishoftu and Addis Ababa city which located at altitude of 1,920 and 2,388 meters above sea level and Latitude 8,7346N, 39.9985E and 8.9806 N, 38.7578E respectively. Number of population lived in each city is 171,115 and 2,757,729 respectively. Average annual rainfall and temperature in Bishoftu and Addis Ababa city is 18.7°C, 892 millimeter and 16.3°C, 1089 millimeter respectively.

Sample collection: This study was continuously done starting from April, 2019 up to August, and 2019. Yoghurt samples were collected from seven milk processing companies those have largest market share. Each processing company was involved in manufacturing and distribution of yoghurt.

Fifty six samples of set yoghurt were collected from both study area. Immediately after collection samples were transported to Hawassa University using Ice box 4°C and cool down to 4/5 degree Celsius until required. Finally this samples were analyzed for it physiochemical composition and microbiological analysis with 1st, 3rd, 7th and 9th of its production date.

Analysis of yoghurt Samples: Experimental plan: Samples represent seven different manufacturers with different Yoghurt brands (YB1, YB2, YB3, YB4, YB5, YB6 and YB7). Samples were taken two times to reduce error. Each sample was analyzed for its physiochemical composition and microbiological analysis.

Chemical analysis; Yoghurt total solid percentage was calculated based on the technique of AOAC[5]. To determine protein content of yoghurt kjeldahal method was used[6]. In addition fat percentage was calculated by Gerber technique and total solid according to AOAC[5]. PH value of yoghurt was determined by desktop PH meter and titrable acidity was determined titrating Sodium hydroxide solution.

Microbiological Analysis: For Microbial test all media were organized based on manufacturer information. For total bacteria analysis plate count agar were used[7]. MacConkey agar was used to analysis Coliform bacterial count[8] and Potato dextrose agar were used to decide Yeast and Mould in the yoghurt[9]. Every equipments used for analysis was boiled in Autoclave with a temperature of 121°C for 15 minute[10].

Measurement of yoghurt fat content: Fat content of yoghurt samples were analyzed according to Gerber Method. For this analysis Sulphuric acid and amyl alcohol were used. 10ml of sulphuric acid added to clean and dry butrometre then 10.75ml of yoghurt were added. Next to yoghurt 1ml of amyl alcohol were carefully added and clean and dry tip of butrometre mouth. Finally butrometre were closed by rubber stopper and carefully mixed by shaker. Butrometre were putted in the water bath for 4/5 minute. Tubes with sample were highly circulated at 1100 revolutions per minute (rpm) for four or five minutes. After four or five minutes tube with sample were put in hot water (65ºC) for similar time to above. Finally amount of result was recorded from butrometre by pushing up stopper.

Measurement of Protein content: It amount in yoghurt was calculated based on Kjeldahal techniques[5]. According to this technique, eleven (11) grams of yoghurt and twenty five (25) milliliter of concentrated sulphuric acid (H_2SO_4) in sequence mixed followed by addition of two Kjeldahal tablets of copper sulphate ($CuSO_4$). Due to digestion of the mixture by heater the solution were develop a separate layer within three hours. Materials for solution collection were gone to cold. Yoghurt diluted in hundred (100) milliliter of distilled water using hundred (100) milliliter of volumetric flask. From diluted sample five (5) milliliter were neutralized by ten (10) milliliter of forty (40) percent sodium hydroxide (NaOH) and the neutralized solution was then distilled. Using conical flask which contain twenty five (25) milliliter of four (4) percent H_3BO_3 in addition three droplet of color indicator was added. Activities was stopped only when attend required amount. Finally it was disconnected and distillation was continued until red color obtained.

The protein percentage was estimated by the formula of:

$$\text{Nitrogen (\%)} = \frac{TX0.1X0.014X20}{Weight\ of\ Sample} \text{X100}$$

Protein (%) = amount of Nitrogen x6.38

Where: T: Titration figure (milliliter), 0.1: Normality of Hydrochloric acid, 0.014: Atomic weight of nitrogen/1000
20: Dilution factor

Yoghurt solids percentage (TS%): Solid percentage of yoghurt was done according to the tailored method of AOAC[5] as follows: Using aluminum dish three (3) grams of total solid were weighed. The dish was heated on a steam bath for 10 minutes. To remove all moisture dish was heated within 100ºC for specific hour (3hr). The dish refrigerated by using desiccators and took its kilogram as soon as possible. To get less than 0.1 milligram reading difference between two dishes heating, cooling and weighing were repeated. Finally total solids (TS) of yoghurt were estimated by the following equation:

$$\text{TS \%} = \frac{W2}{W1} X100$$

Where: W1: Sample weight before drying, W2: Sample weight after drying

Titratable acidity: The titratable acidity of yoghurt was determined by the following method[6].

In clean beaker nine (9) grams of yoghurt sample were weighed and mixed. Finally a droplet of color indicator were added and titration was continued until required color obtained.

$$\text{Acidity (\%)} = \frac{(ml\ NaOH)x\ (N\ NaOH)x9}{Weight\ of\ Sample}$$

Where: NaOH: Sodium hydroxide, 9: exchange factor for lactic acid.

PH testing: PH meter is used to measure PH value of yoghurt (H. Jurgons Co. Beremen, L Puls Munchen 15). This PH value indicated whether the yoghurt is acidic of basic. For this value determination titration was begun after calibrating with pH 4, 10 and 7 buffers.

Microbiological examination: Preparation of media: Solid media: Violet red bile agar (VRBA): Violet red bile agar was required for bacterial growth. To prepare one liter 41.53gram of powder was dissolved in distilled water. The mixture was boiled to dissolve the medium completely. During dissolving prolong heating and autoclaving is not allowed, due to its effect on medium efficiency.

Acidified potato dextrose agar (APDA): Potato dextrose agar was used for growth of Yeast and Mould. To prepare one liter 39 gram of potato dextrose agar powder was required. After mixed, boiling was continued for complete dissolving. Then sterilized at temperature of 121°C for 15 minutes and refrigerated to forty six degree Celsius. Finally adequate $C_4H_6O_6$ were added.

Growing techniques: Pouring techniques were used to add media on petridish. This techniques only used for growth of total Bacterial Count and Coliforms. For Yeast and Mould growth surfaces plating techniques were used [9].

Preparation of sample dilutions: Well mixed yoghurt sample were prepared by adding eleven (11) gram of yoghurt to ninety nine (99) milliliter of sterile distilled water at 40-45^{o}C in flask. After this it was well mixed by automatic mixer to got homogenous solution to make 10-1 dilution. One milliter of dilution was taken from 10-1 and added to 9 milliliter sterile distilled water. Similarly serial dilution was continued to 10-1, 10-2, 10-3, 10-4, 10-5, 10-6, 10-7, and 10-8. For growth one (1) milliliter of dilution from each was taken from each dilution and transferred to petridish. Then prepared media was poured and well mixed incubated in an inverted position. Finally colonies grown on each petridish were counted by colony[7].

Examination of bacteria: Coliform count: The medium (VRBA) can enumerate this bacteria cell[8]. This bacterium was incubated and grown at 32^{o}C for 24 hours. Among grown bacterial cell only dim red colonies were counted.

Yeasts and moulds count: To count Yeast and Mould acidified potato dextrose agar was used[8]. Using this media plate was incubated at 25^{o}C for at least 5 to 7 days.

Escherichia coli testing: Brilliant green broth (Oxoid) in three test tube which is fitted with Durham tubes were pipetting 1ml and incubated at 45°C for 48 hours to find out E. coli then tubes with gas foam in the Durham tubes were marked on MacConkey agar (Oxoid) plate and incubated at 37°C for 24 hours.

After this colonies were tested for indole production. Indole positive colonies were produce pink color on MacConkey, which indicate the presence of E.coli for further confirmation and characterization by PCR and antibiotic susceptibility testing E.coli was maintained at −20°C in 20% glycerol brain heart infusion broth. For positive controlling Escherichia coli ATCC was used.

Identification of Salmonella S. aureus: Mannitol salt agar (Oxoid) plate was used for identification of Salmonella S.aureus. Sample were carefully spread on the agar and incubated at 37°C for 24 hours. Positive colonies were identified by Staphylase Test Kit (Oxoid). Positive colony form yellow color cocci in clump, oxidase negative, and coagulase and catalase positive and which produced clots and kept at −20°C in 20% glycerol brain heart infusion broth for additional studies. For positive controlling S. aureus ATCC was used.

Statistical analyses: Statistically this research data were analyzed by analysis of variance (ANOVA). General linear model (GLM) procedure of the statistical Analyses systems (SAS) were used decide the quality of plain yoghurt.

The samples were analyzed to determine the outcome of brand on total solids, fat, protein, titratable acidity, Total Bacterial count, coliform count and yeasts and moulds count. Means were alienated using Duncan Multiple Range Test with $P \leq 0.05$.

Results and discussion

Compositional Analysis of Yoghurt: As shown on (Table-1) physiochemical composition of set yoghurt of seven diverse brands were illustrated. As described on the (Table-1) Fat and total solid proportion of yoghurt brand were drastically diverse at $p<0.05$. Protein and lactose percentage of all yoghurt brands was not significantly different at $p>0.05$. Protein and lactose proportion of yoghurt brands range from 4.3% to 4.7% and 4.1% to 4.7%.

In addition, fats and total solids percentage, titratable acidity of yoghurt also significantly different at $P>0.05$. Fat content of yoghurt brand (YB) 5 and 6 (5.5±0.34% and 5.5±0.36%) were higher as comparing to other brands. Total solid content of yoghurt brand (YB) 7 (15.6±0.45%) was higher as comparative to others.

Microbial and compositional quality of set yoghurt varies due to milk processing company intrinsic and extrinsic factors during processing and division. Total bacterial counts of two milk processing companies were extremely higher than the rest. Two milk processing companies' yoghurt samples were positive for Escherichia coli, due to hygienic raw milk production and handling problem. Those two companies raw milk were contaminated with fecal.

PH meter is a material that used to test acidity or alkalinity of the yoghurt. The current yoghurt pH range is $4.6 \pm 0.13 – 4.8 \pm 0.61$. The minimum international acceptable yoghurt pH standard is 4.4[13,14]. This international standard show that PH value of milk products were slowly decrease after processed into different milk products. The value of milk PH were found in the range of 6.7-4.3. The values for titratable acidity were in the range of 0.38 to 1.22 [11]. This result was agreed with the current research result.

Microbial quality of yoghurt: Total bacterial and coliform count of seven yoghurt brands result were drastically different at $P<0.05$. Yeast and Mould analysis was not radically by brand. However, the highest value of Yeast and Mould was found at yoghurt brand 4 which were $4.93x10^2$. In all yoghurt brands Salmonella S. aurus bacteria screening result were zero. However, Escherichial coli bacteria positive in yoghurt brand (YB) 5 and 7.

Total Bacterial Count: The yoghurt samples were collected from different milk processing companies located in Bishoftu and Addis Ababa city. Total Bacterial Count Result was fault within the range of $1.99x10^6$ to $2.50x10^9$ cfu/ml. Sample collected from both cities had high quantity of total bacterial count. The higher initial Total Bacterial Count values for this study might be due to the raw milk production and handling problem, processing hygiene problem, storage and distribution channel problem.

Table-1: Physiochemical quality (Mean ± SD) of different brand of yoghurt.

parameter	Measure	YB1	YB2	YB3	YB4	YB5	YB6	YB7	P value
Fat	Mean±SD	5.1±0.32^{b}	4.9±0.43^{a}	5.4±0.61^{c}	5.0±0.40^{b}	5.5±0.34^{c}	5.5±0.36^{c}	5.3±0.53^{b}	0.03
Protein	Mean±SD	4.5±0.33^{a}	4.3±023^{a}	4.7±0.30^{a}	4.6±0.32^{a}	4.4±0.22^{a}	4.7±0.53^{a}	4.5±0.42^{a}	0.2
Lactose	Mean±SD	4.1±0.55^{a}	4.4±0.21^{a}	4.6±0.54^{a}	4.3±0.60^{a}	4.7±0.33^{a}	4.5±0.11^{a}	4.6±0.27^{a}	0.08
Total solid	Mean±SD	14.7±0.62^{a}	14.6±0.43^{a}	14.7±0.64	14.9±0.31^{b}	14.6±0.34^{a}	14.7±0.65^{a}	15.6±0.45^{b}	0.007
PH value	Mean±SD	4.8±0.61^{b}	4.67±0.43^{a}	4.6±0.28^{a}	4.67±0.53^{a}	4.73±033^{a}	4.8±075^{b}	4.6±0.13^{a}	0.004
Titratable acidity	Mean±SD	0.63±0.06^{a}	0.62±0.04^{a}	0.67±0.01	0.73±0.03^{b}	0.70±0.03^{b}	0.69±0.07^{a}	0.76±0.12^{b}	0.01

Total bacterial count result of YB6, YB7 and YB4 were higher than the other. This bacteria were significantly different between the samples ($p<0.05$). Among all the samples YB6 had the highest count of total bacterial count. Among all samples analyzed for Escherichia coli only 2(28)% samples were positive. Opposite to this Salmonella sp. were not present in all samples.

Total Bacterial Count Result of this study was fault within the range of $1.99x10^{6}$ to $2.50x10^{9}$. G.M.M. Total bacterial count in yoghurt were within the range of $3.0x10^{3}$ to $10.5x10^{4}$ [16].

Bacteria including coliforms and fungi were analyzed from the yoghurt samples. More bacteria were obtained from the samples than fungi. The high viable counts may be attributed by using of infected packaging materials, production in an unhygienic environment, bad sanitary condition, and contaminated water.

Total Coliform Counts (TCC): Total coliform counts found in yoghurt samples were ranged from $2.59x10^{2}$ to $1.12x10^{4}$cfu/ml. The highest quantity was found in yoghurt of YB7 and lowest quantity was found in YB3 (Table-2). The studied result reflected highly poor hygienic conditions and improper sanitation during raw milk production, handling, manufacturing of yoghurt and distribution.

The microorganisms screened from the samples were *Escherichia coli* but *Salmonella S. aurus was* zero. In some Yoghurt samples *Escherichia coli* bacteria were found[17,3]. The presence of *Escherichia coli* in the samples could be as a result of poor processing, handling and packaging, since they are often found on the outer surface of the body[18].

The YB7 sample had a relatively high mean coliform count (1.12×10^{4} cfu/ml), which is closely in line with the value ($2.5x10^{4}$ cfu/ml) obtained[19]. In yoghurt samples Coliform count bacteria were found in concentration of $4.25x10^{7}$ cfu/ml[20]. As comparing to this outcome, current study result of coliform count bacteria was low. In all yoghurt samples coliform growth was observed. The result of this study is a pointer to indicate the health status of the yoghurt samples which inform the concerning body to check the yoghurt quality in different countries[21,22].

On the other hand, the high coliform growth observed from the YB7 sample could be a indication of its direct containment with fecal materials or contamination at display and storage outlets, indicating the poor hygienic condition.

Coliform bacteria were not found in sample analyzed[23,11,12,2]. Inversely, it was found in all samples of the present study. In principle pasteurized milk coliform bacteria cannot be found more than 5cfu/ml. High coliform bacteria presence indicate poor quality of milk[23].

Presences of Escherichia coli in yoghurt were due to fecal contamination of milk samples[15]. However, Escherichia Coli were absent in all yoghurt samples analyzed[24]. Fecal contamination is one of the main factors for occurrence coliform bacteria in yoghurt. High quantity of these bacteria in yoghurt was due to presence of poor milk hygienic and handling problem.

Salmonella S. aurus was not detected in any of the samples[12]. Similarly this study Results also obtained the same finding. Absence of these bacteria in this yoghurt samples were due to good milk handing starting from producer up to processors. The product has good quality and save when its' count result were not exceed 10cfu/ml[13]. Research done in Khartoum indicated that there is no salmonella species found in yoghurt samples[15]. For accelerating the bacterial contamination of yoghurt and the post manufacturing contamination the main factors are, unclean hands of worker, poor quality of milk, unhygienic conditions of manufacturing unit, inferior quality of material used and water supplied for washing the utensils[25]. Whereas Yoghurt produced and available in Bishoftu and Addis Ababa city market were also found contaminated with E.coli. E.coli is one of coliform bacteria which contaminate yoghurt[26]. Although E.coli is frequently occurred organisms in milk and its products, the incidence of the species of E. coli in milk and milk products were insignificant because E. coli normally is a ubiquitous organism[27]. Presence of pathogenic stain of E.coli is danger for health of consumers.

Table-2: Total bacteria, Coliform, Fungus, Escherichia coli and Salmonella S.aurus analysis (Mean ± SD) result of set yoghurt.

Yoghurt Brand	Total bacterial count (cfu/ml)	Coliform count (cfu/ml)	Yeast and mould (cfu/ml)	Presence of Escherichia coli	Salmonella S. aurus
YB1	$2.13X10^{6\,a}$	$1.82X10^{3\,b}$	$3.2X10^{2\,a}$	Negative	Negative
YB2	$2.97X10^{6\,a}$	$9.08X10^{2\,a}$	$1.11X10^{2\,a}$	Negative	Negative
YB3	$1.92X10^{7\,a}$	$2.59X10^{2\,a}$	$1,56X10^{2\,a}$	Negative	Negative
YB4	$2.14X10^{8\,b}$	$6.91X10^{2\,a}$	$4.93X10^{2\,a}$	Negative	Negative
YB5	$1.99X10^{6\,a}$	$3.28X10^{2\,a}$	$3.36X10^{2\,a}$	Positive	Negative
YB6	$2.50X10^{9\,c}$	$1.64X10^{3\,b}$	$3.63X10^{2\,a}$	Negative	Negative
YB7	$1.98X10^{8\,b}$	$1.12X10^{4\,b}$	$2.98X10^{2\,a}$	Positive	Negative
P value	0.002	0.006	0.08		

Total Yeast and Mold Counts: Total yeast and mould counts in yoghurt samples were ranged from $1.11x10^2$ to $4.93x10^2$ ^{cfu}/ml. The lowest amount was found in YB2 and the highest amount of result was found in YB4 (Table-2). There is no a significant difference ($p>0.05$) between yoghurt the brands. Presence of yeasts or moulds in yogurt is also indicative for poor sanitary practices in manufacturing or packaging.

In the present study Yeast and Mould results was found within the range of $1.11x10^2$ to $4.93x10^2$. Presence of yeasts or moulds in yogurt is indicative of poor sanitary practices in manufacturing, handling or packaging. Yoghurt can be free from Yeast and Mould developments. However, in this study samples were contaminated by Yeast and Mould, which is higher than the standard.

Therefore, the producers should be aware with those producing Yoghurt. As moulds are widely distributed as environmental contaminants of air, water soil, they are responsible for spoilage of drinking yogurt which causes economical losses. The public health importance of mould has been emphasized as certain types of moulds produce mycotoxins which implicated in liver cancer. The high count of mould and yeast in all examined samples may be due to any of the following reasons: high count in raw materials; ineffective processing methods, ineffective sanitizing methods or faulty storage of the products. Therefore, sanitary control measures should be adapted to dairy processing plants by application of HACCP system on processing, packaging, storage and distribution of such products.

Conclusion

In Ethiopia milk processing companies produce and distribute yoghurt in different size and type. Different brand of yoghurt produced in Bishoftu and Addis Ababa city were analyzed for its physiochemical composition and microbial quality. High amount of total bacterial count, Coliform, Yeast and Mould were found the result which is higher than the recommended. Total bacterial and Coliform count were significantly different between the brand. However, Yeast and Mould were not significantly different between the brands. In addition to microbial quality Protein and lactose content of yoghurt was not significantly different at $p>0.05$. Whereas Fat and total solid content was significantly different at $p<0.05$. High amount of microbial content was due to raw milk quality problem, hygienic problem during processing and distribution time. In brand of yoghurt Escherichia coli bacteria was observed and Salmonella S. aurus were absence from the samples. Number of total bacterial count, coliform count and Yeast and mould count result of this research is higher than national standard count. Generally Stakeholders including food regulatory agencies, yoghurt producers, vendors and customers should pay critical attention to the health quality of commercial yoghurts in order to control the health danger of poorly produced commercial yoghurt to the public. To achieve this feat, training and retraining of staff of yoghurt Production Company's workers as well as public awareness through print and electronic media should be giving urgent priority.

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