Evaluation of Yoghurt Flavored with Natural Fruit Juices

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Abstract

Yoghurt is a good source of essential nutrients in human diet and its demand is increasing when flavored with different fruit juices. The aim of this study was to evaluate physico-chemical properties, microbial quality and overall acceptability of fruit-flavored yogurts and develop most preferred combination. Flavored yogurt was prepared with mango and papaya juices each added to yoghurt at the rate of 10, 15, 20, and 25%. The result of this study indicated that addition of fruit juices increased titratable acidity and decreased protein, fat, ash and total solid contents of the flavored yoghurt. The lactic acid bacteria count was significantly higher in plain yoghurt; however, the total aerobic mesophilic bacteria, coliform and yeast and mold counts were higher in fruit flavored yoghurt. Yoghurt incorporated with 15% of mango juice was most preferred in sensory evaluation as compared to the other treatments. Results of the current study confirmed that addition of fruit juice to the yoghurt significantly improved the sensorial acceptability of yoghurt.

Introduction

Yoghurt is a dairy product obtained from the lactic acid fermentation of milk. It is one of the most popular fermented milk products in the world (Willey *et al.*, 2008). Yoghurt can be good source of essential nutrients and minerals in the human diet. It could significantly contribute to the recommended daily requirements for calcium and magnesium to maintain the physiological process in human beings (Sanchez *et al.*, 2000).

There is an increasing demand of yoghurt flavored with fruits and introduction of various fruit-flavored yoghurts has significantly contributed to its consumption (Shahani, 1993). Incorporation of fruits also reflects the healthy image of yoghurt. In line with this, Bardale *et al.* (1986) have reported that the addition of fruit flavors, fruit purees and flavor extracts enhances versatility of taste, color, and texture for the consumer. Sensory appeal is also one of the essential strategies associated with market success of fermented products like yoghurt. The popularity of yoghurt as a food component has been linked to its sensory characteristics (Routray and Mishra, 2011). The key to the increase in sales of yoghurt is a continuous evaluation and modification of the product to match consumer expectations (Hugunin, 1999). Hence, flavoring yoghurt with fruits such as papaya and mango, which are not only easily available and mostly consumed fruits in high amount, but also rich in β- carotene, lycopene, phenol, anti-oxidants and minerals, appears to be important. However, researches in the area developing fruit based yoghurt have not yet been done in Ethiopia. Therefore, the aim of this study was to evaluate the nutritional quality and acceptability of yogurts flavored with mango and papaya juices based on sensory, physico-chemical and microbial properties.

Materials and Methods

Raw materials

Two types of fruits (papaya and mango) were used to develop fruit flavored yoghurt. Fresh cow milk was collected from dairy farm of Holetta Agricultural Research Center, Ethiopia. Ripened fruits and yoghurt containing 3% of *Stereptococcus thermophilus* and *Lactobacillus acidophilus* were purchased from local supermarket and transported to Holetta Agricultural Research Center Dairy laboratory.

Preparation of fruit juice and fruit flavored yoghurt

Mango and Papaya fruits were washed and peeled by clean knife. Fruit juices were extracted and homogenized by juice maker and homogenizer (POLYTRON, Switzerland). Both mango and papaya juices were filtered by clean cheesecloth and kept in a refrigerator (4°C) in a sterilized glass bottle. Yoghurt was prepared according to International Dairy Federation's yoghurt manufacture procedures (IDF, 1988). Fresh cow milk was used for yoghurt production. Briefly, the milk was filtered to remove foreign matters and pasteurized at 72^{o}C for 15 sec to reduce the population of pathogenic microorganisms. Then, the milk was cooled to 42^{o}C, 3% of the starter cultures were added in to the pasteurized milk, and mango and papaya juices were added to the yoghurt at the rate of 10, 15, 20 and 25%. Then, cup of fruit flavored yoghurt and plain yoghurt were incubated at 42^{o}C until complete coagulation of yoghurt for 5-6 hr. After complete coagulation, all treatments were cooled in the refrigerator at 4^{o}C until sensory evaluation, physio-chemical and microbial analysis was carried out.

Sensory evaluation of fruit flavored yoghurt

Fruit flavored yoghurts were subjected to sensory evaluation by ten semi-trained panelists. The panelists comprised of both females and males who had previous experience in milk and milk products evaluation. Fruit flavored yoghurt was evaluated based on color, flavor, taste, odor and overall acceptability. The evaluation was done at room temperature in Holetta Dairy Research Laboratory. Each panelist scored samples independently and recorded the values on the basis of five point hedonic scale (1= dislike very much, 2= dislike moderately, 3= neither dislike nor like, 4= like moderately and 5= like very much). This scale was used to rank both plain and fruit flavored yoghurts. Panelists were served water and unsalted crackers to clean their mouths before tasting each sample (Bodyfelt *et al*., 1988).

Physico-chemical analysis of fruit flavored yoghurt

Titratable acidity (expressed as % lactic acid) of flavored yoghurt was determined by titration with 0.1 N sodium hydroxide solution; using phenolphthalein as indicator according to the procedure of O'Connor (1994). Total solids of freshly prepared flavored yoghurt and plain yoghurt samples were measured by oven drying at 105°C until steady weight was achieved (approximately overnight) (O'Connor, 1994). PH of the samples was measured by using portable pH meter (pH-016, China), inserting a pH probe into homogenized yoghurt samples, according to the procedure developed by Akpakpunam and Safa-Dedeh, (1995). Percentage of ash in the samples was measured using total solid dried in the muffle furnace by igniting at 550°C for 5 hr (Michael and Frank, 2004). Gerber method according to the procedures developed by O' Connor (1994), while protein contents were determined by using AOAC (2005) measured fat content of samples.

Microbial analysis

Total aerobic mesophilic bacteria count

A total aerobic mesophilic bacterial count was done according to the procedure of FAO (1997), using plate count agar (Oxoid, CM 0325, and UK). One ml of yogurt sample was homogenized using vortex mixer (VM-300, Taiwan) with 9 ml sterile peptone water (0.1%) to obtain first dilution. One ml of the sample from a selected dilution was pour-plated in duplicate and incubated for 48±2 h at 35°C. The enumeration of bacteria was performed using digital colony counter and the result was expressed as colony forming units per ml (CFU/ml).

Total coliform count

Total coliform count was done according to the procedure developed by Michael and Frank (2004). One ml sample was taken from appropriate serial dilutions and plated on violet red bile agar (VRBA, Oxoid, CM 0107) in duplicate, and incubated at 32°C for 24 hr. The enumeration of total coliform was performed using digital colony counter and the result was expressed as colony forming units per ml (CFU/ml).

Yeast and mould count

Yeast and mould count was carried out according to the procedure of FAO (1997). Homogenized samples were serially diluted by adding 1 ml of sample into 9 ml of peptone water (Oxoid, CM0009). Potato Dextrose Agar (PDA, Himedia, M096) media was used and antibiotics (Streptomycin and Chloramphenicol) were added to inhibit the growth of bacteria. One ml of homogenized sample was taken and plated on the PDA media in duplicate. The enumeration of yeast and mould was performed and the result was expressed as colony forming units per ml (CFU/ml).

Lactic acid bacteria count

Lactic acid bacterial count was done according to the Standard Methods for the Examination of Dairy Products (Michael and Frank, 2004), using lactobacillus MRS agar (Himedia, M641). One ml of each yogurt sample was homogenized with 9 ml sterile peptone water using vortex mixer (VM-300, Taiwan) (Oxoid, CM0009). Then, 1 ml of the homogenized sample from appropriate serial dilutions was poured-plated on the melted MRS agar in duplicate. It was then incubated in anaerobic jars at 35°C±2 for 48 hr. Colonies of lactic acid bacteria were counted and expressed as colony forming units per ml (CFU/ml).

Statistical analysis

Physico-chemical and microbial data were analyzed using the general linear model (GLM) procedure of the Statistical Analysis System (SAS) and means were compared using Duncan's Multiple Range Test (SAS Inc., Cary, USA) version 9. The microbial count results were log transformed before subjected to statistical analysis. Sensory data was analyzed using Kruskal -Wallis test of the SPSS statistical package program (SPSS, Inc., Chicago, IL, USA) version 20.

Results and Discussion

Physico-chemical properties

Titratable acidity and pH

As shon in Table 1, the effect of treatments on pH and titratable acidity was highly significant (P<0.001). Addition of fruit juice significantly decreased the pH of yoghurt samples. However, titratable acidity increased with increasing fruit percentage. Similar observation has been reported by Roy *et al.*(2015) who indicated that the acidity of yoghurt increased with increases in banana, papaya and watermelon juice percentage. In the present study, it was observed that 25% mango flavored yoghurt had the highest titratable acidity, but the lowest pH (3.92) (Table 1).

Fat and protein contents

Fat and protein contents of plain and fruit flavored yoghurts are presented in Table 1**.** The effect of fruit on yoghurt fat and protein contents was highly significant (P<0.001). The results indicated that percentage of fat and protein in yoghurt samples was highly influenced by addition of fruit juice, where the values of both parameters decreased with increasing proportion of fruit juice. This might be attributed to lower protein and fat contents of fruits as compared to milk. This finding agrees with the work of Roy *et al.* (2015) who reported that the fat and protein content of yoghurt decreased with increases in percentage of banana, papaya and watermelon pulp juices. Hossain et al. (2012) who found that the fat and protein content of fruit yoghurt decreased with increasing proportion of strawberry, orange and grape juices have also reported similar finding.

Total solids and ash

Addition of fruit juice resulted significant differences (P<0.05) in total solid and ash contents of yoghurt. The content of total solids of fruit flavored yoghurt decreased with increasing percentage of fruit juice, probably because of lower fat and protein content in fruits (Table 1). Hossain et al. (2012) who found that total solid content decreased with increasing fruit juice percentage have reported similar observation. The result of the present study indicated that the highest (16.57%) total solid content of yoghurt was recorded for the control sample, while the mean values for fruit flavored yoghurt (25% mango and 25% papaya) were nearly similar (Table 1). Ash content of fruit flavored yoghurt also decreased with increases in fruit juice percentage. This result was in agreement with the findings of Roy *et al.* (2015) who reported that ash content in papaya and watermelon yogurts decreased with the increase in juice concentration. This might be due to the lower content of ash in mango and papaya fruits as compared to cow milk. The highest (0.67%) ash content was recorded for plain yoghurt (control sample) and the lowest (0.63%) was for fruit flavored yoghurt containing 25% mango (Table 1).

Table 1. Physico-chemical properties of fruit flavored yoghurts

Treatment	pH	Titratable acidity (%)	Fat (%)	Protein (%)	Total solid (%)	Ash (%)
C	4.30±0.07[a]	0.64± 0.00[g]	4.05±0.07[a]	3.69 ± 0.00[a]	16.570±.00[a]	0.67± 0.00[a]
M1	4.25±0.04[a]	0.74±0.01[e]	3.95± 0.07[a]	3.360±.02[d]	16.08 ± 0.2[b]	0.66±0.00[ab]
M2	4.01± 0.01[bc]	0.78 ±0.00[c]	3.80±0.00[b]	3.38± 0.00[cd]	16.06 ± 0.01[b]	0.65±0.07[b]
M3	3.93±0.01[cde]	0.81 ± 0.01[b]	3.65± 0.07[cd]	3.34±0.01[de]	15.20± 0.07[c]	0.63±0.00[c]
M4	3.92±0.01[e]	0.85± 0.01[a]	3.5± 0.00[e]	3.29 ± 0.01[f]	14.64± 0.08[e]	0.63±0.00[c]
P1	4.180 ±07[ab]	0.68 ± 0.01[f]	3.80± 0.00[bc]	3.54±0.05[b]	16.05±0.00 [b]	0.67±0.00[a]
P2	4.09 ±0.01[bc]	0.76 ±0.01[d]	3.75± 0.07[cd]	3.43±0.03[c]	15.03±0.00[cd]	0.66±0.01[ab]
P3	4.06± 0.09[bcd]	0.77± 0.01[b]	3.55± 0.07[cd]	3.39±0.01[cd]	14.82±0.08[de]	0.66±0.00[ab]
P4	4.010±0.9[cde]	0.80 ± 0.01[b]	3.55± 0.07[ef]	3.30±0.00[ef]	14.85±0.07[de]	0.65±0.01[b]

Means with different superscripts within a column are significantly different (P < 0.05); C: control (plain yoghurt), M1= 10% Mango + 90% yoghurt, M2= 15% Mango + 85% yoghurt, M3= 20% mango + 80% yoghurt, M4= 25% mango + 75% yoghurt, P1= 10%Papaya + 90 yoghurt, P2= 15% Papaya + 85% yoghurt, P3= 20% of Mango + 80% yoghurt, P4 =25% papaya + 75% yoghurt.

Microbial quality of fruit flavored yoghurts

The microbial quality of plain and fruit flavored yoghurt samples are presented in Table 2. Lactic acid bacteria, total aerobic mesophilic bacteria, total coliform and yeast and mould counts were significantly (P<0.05) affected by the type of fruit and juice percentage used in yoghurt. The lowest total coliform (3.65 log_{10} cfu/ml) and yeast and mould counts (5.78 log_{10} cfu/ml) were observed for plain yoghurt sample. Although there was no significante difference (P<0.05) between 25% mango and 25% papaya flavored yoghurts, the highest total aerobic mesophilic bacteria and total coliform counts were recorded for yoghurt containing 25% mango (Table 2). Total mesophilic bacteria, total coliform and yeast and mould counts increased with increasing juice percentage in fruit flavored yoghurt samples. This might be due to increased level of the non-pasteurized fruit juices with lower microbial load. Con et al. (1996) who found that yeast and mould counts increased when the proportion of fruit flavor increased in yoghurt have reported similar results. Similarly, Tarakci and Kucukoner (2003) have also stated that total aerobic mesophilic bacteria and yeast and mould counts in plain sample were lower than yoghurt samples containing fruit juice. The results of the present study indicated that, among the samples, yoghurt containing 10% mango exhibited the least microbial quality for total aerobic mesophilic bacteria (7.85 log_{10} cfu/ml), total coliform (4.24 log_{10} cfu/ml) and yeast and mould count (6.21 log_{10} cfu/ml). This might be due to the low percentage of mango with lower microbial load as compared to those samples with more than 10% mango juice.

The result of the present study also indicated that the highest (7.22 log_{10} cfu/ml) lactic acid bacteria count was recorded for the control sample, while the lowest (7.03 log_{10} cfu/ml) value was for yoghurt containing 25% mango juice. Lactic acid bacteria count of fruit flavored yoghurt decreased with increases in fruit juice percentage (Table 2). The finding of this study was in agreement with the report of Prescott *et al.* (2005), indicating that lactic acid bacteria grow optimally under slightly acidic condition when the pH is between 4.5 and 6.4. In addition to the pH value, concentration of lactose in fruit flavored yoghurt samples might have also influenced the growth of lactic acid bacteria.

Table 2. Microbial characteristics of fruit flavored yoghurt (log_{10} cfu/ml)

Treatment	Total bacteria	Total coliform	Yeast and mould	Lactic acid bacteria
C	7.86±0.05^{b}	3.65±0.02^{e}	5.78±0.03^{c}	7.22 ±0.00^{a}
M1	7.85±0.06^{b}	4.24±0.01^{d}	6.21±0.06^{ab}	7.16±0.00^{b}
M2	7.89±0.01^{b}	4.63±0.03^{b}	6.27±0.00^{ab}	7.08±0.01^{de}
M3	8.16±0.00^{a}	4.84±0.01^{a}	6.28±0.02^{ab}	7.05±0.02^{de}
M4	8.22±0.01^{a}	4.84±0.04^{a}	6.32±0.02^{a}	7.03±0.00^{e}
P1	8.17±0.00^{a}	4.37±0.01^{c}	6.03±0.01^{b}	7.14±0.01^{bc}
P2	8.18±0.00^{a}	4.82±0.12^{a}	6.18±0.01^{ab}	7.09±0.06^{cd}
P3	8.18±0.01^{a}	4.79±0.01^{a}	6.19±0.01^{ab}	7.06±0.02^{de}
P4	8.20±0.01^{a}	4.78±0.01^{a}	6.23±0.00^{ab}	7.03±0.07^{de}

Means with different superscripts within a column are significantly different (P < 0.05); C: control (plain yoghurt), M1= 10% Mango + 90% yoghurt, M2= 15% Mango + 85% yoghurt, M3= 20% mango + 80% yoghurt, M4= 25% mango + 75% yoghurt, P1= 10%Papaya + 90 yoghurt, P2= 15% Papaya + 85% yoghurt, P3= 20% of Mango + 80% yoghurt, P4 =25% papaya + 75% yoghurt.

Sensory evaluation

There was highly significant differences (P<0.01) between 10% of fruit juice yoghurts and the plain for color, odor, taste and overall acceptability. The color of plain yoghurt (C), 10% mango (M1) and 10% papaya (P1) were ranked as 38.7, 53.2 and 20.4% respectively. The highest rank values of taste, odor and overall acceptability were recorded for the M1, while the lowest values were for the plain yoghurt. Similarly, the result indicated that the highest and lowest values of flavor were observed for 10% mango yoghurt and plain yoghurt (control), respectively, while P1 had intermediate values between the two for all variables (Table 3). These results were more or less similar to the findings of Madhu *et al*. (2012).

Differences in sensory attributes were also significant for yoghurts incorporated with 15% mango (M2), 15% papaya (P2) and plain yoghurt. Fruit flavored yoghurt with 15% mango scored highest values for color (68.6), odor (76.5), taste (69.4), sourness (60.3), flavor (77.7) and overall acceptability (78.3) followed by P2. Although 15% papaya juice flavored yoghurt (P2) was less preferred by panelist, it had higher rank values for all sensory attributes as compared to plain yoghurt (Table 3). This result was in agreement with the work of Roy *et al*. (2015), who reported that yoghurt with 15% of papaya juice is mostly acceptable and recommendable for large scale production. Furthermore, similar findings have also been reported by Abdallah and Mohamed (2017), who suggested that mangoes are more suitable to use as flavoring material in yoghurt manufacturing. The current study also indicated that all sensory attributes (color, flavor, taste, sourness and overall acceptability) of yoghurt become optimal when 15% mango juice is added. The same is true for 15% of papaya juice, which resulted in acceptable level of improvement for sensory attributes (Table 3) and nutritional quality of yoghurt (Table 1).

Score values for color, odor, flavor and overall acceptability of yoghurt flavored with 20% mango (M3) or papaya (P3) were lower than M2 and P2. There were highly significant differences ($P < 0.01$) between plain and 20% fruit flavored yoghurt for color, odor, taste, flavor and overall acceptability. The highest values for taste (72.2) and other attributes were recorded for 20% mango flavored yoghurt (M3) (Table 3). This result was in agreement with the finding of Dessie *et al.* (1994), who have reported that addition of fruit juice improved the color and texture of yoghurt. In general, the results of the present study indicate that addition of fruit juice with appropriate properties has improved sensory attributes of yoghurt. On the other hand, except for color, lowest values of sensory attributes (odor, sourness, taste, flavor and overall acceptability) were recorded for plain yoghurt (Table 3). In line with this, Dessie *et al.*(1994) have reported that smell and taste scores of mango and pineapple flavored yoghurt were higher than that of the control plain yoghurt. The taste of mango juice yoghurt (M3) was found to be better as compared to papaya juice yoghurt (P3) and plain yoghurt. This might be attributed to more carbohydrate content of the former as compared to the later treatments.

Differences between 25% mango (M4) or papaya (P4) flavored and plain yoghurt (C) were significant ($P < 0.05$) for color, odor, sourness and overall acceptability and highly significant ($P < 0.01$) for taste and flavor (Table 3). M4 exhibited the highest values for color (53.1), odor (37.6), taste (44.8) and flavor (42.5), while the lowest score values for odor (18.4), taste (25.1), flavor (27.6) and overall acceptability (20.2). The values of color (31.9) and sourness (19.0) were also lowest for P4 and M4 (Table 3). On the other hand, P4 was superior over M4 in sourness and overall acceptability. Nevertheless, the improvement in flavor and, thus, overall acceptance of fruit based yoghurts could be attributed to the improved taste and odor and their interaction.

Table 3. Organoleptic evaluation of fruit flavored yoghurt

Sensory attribute	10% fruit flavored yoghurt				15% fruit flavored yoghurt				20%fruit favored yoghurt				25% fruit flavored yoghurt			
	C	M1	P1	LS	C	M2	P2	LS	C	M3	P3	LS	C	M4	P4	LS
Color	38.7	53.2	20.4	**	38.7	68.6	46.0	**	38.7	65.5	32.0	**	38.7	53.1	31.9	*
Odor	18.4	55.8	43.5	***	18.4	76.5	46.2	***	18.4	56.4	41.8	***	18.4	37.6	33.3	*
Sourness	37.0	47.2	39.6	NS	37.0	60.3	52.1	*	37.0	57.4	47.8	*	37.0	19.0	49.2	*
Taste	25.1	44.8	30.9	**	25.1	69.4	35.2	***	25.1	72.2	43.4	**	25.1	44.8	32.4	**
Flavor	27.6	42.5	29.9	*	27.6	77.7	34.4	***	27.6	61.1	45.5	**	27.6	42.5	34.5	**
Overall acceptability	20.2	51.9	44.8	**	20.2	78.3	39.3	***	20.2	58.1	36.9	***	20.2	44.8	45.2	*

*Note: C= control (plain yoghurt), M1= 10% Mango + 90% yoghurt, P1= 10%Papaya + 90 yoghurt, M2= 15% Mango + 85% yoghurt, P2= 15% Papaya + 85% yoghurt, M3, 20% mango + 80% yoghurt, P3= 20% of Mango + 80% yoghurt and Papaya, M4= 25% mango +75% yoghurt, P4 =25% papaya +75% yoghurt, LS=Level of significance, * Significant at P< 0.05, ** Significant at P< 0.01, *** Significant at P< 0.001, NS: Not Significant.*

Conclusion

Results of the present study revealed that blending yoghurt with optimum proportion of different fruit juices would improve its sensorial acceptability and chemical properties. Hence, it was concluded that yoghurt flavored with 15% mango juice had high preference by consumers and, thus, can contribute to food diversity as well as to both food and nutrition security in the country.

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