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Effect of Papaya Juice, Stabilizer and Storage Duration on Physical, Microbiological and Acceptability of Yoghurt

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Abstract

This study was carried out to improve the physicochemical, nutritional and functional value of cow's milk yoghurt by the addition of papaya (Carica papaya) juice and gelatine at different levels. Yoghurt samples with added fruit juice were compared with the control (without fruit juice) for physical, chemical and sensory attributes. The physico-chemical properties of the papaya- fruit yoghurt were significantly different ($p < 0.05$) from the control. Control yoghurt sample showed significantly higher values for pH and lowest for lactic acid bacterial (LAB) count as compared to papaya juice added yoghurt samples. The addition of papaya juice significantly increased ($p < 0.05$) the total solids, protein, ash, total carotene and phenolics contents of yoghurt samples. There was no significant difference ($p > 0.05$) in the fat, carbohydrate, energy and total soluble solid contents. Sensory evaluations using a hedonic test showed that all yoghurt samples were acceptable. Overall, the syneresis of the yoghurt samples increased with decreasing pH throughout storage at 4 °C.

Keywords: Functional Food, papaya, physicochemical, sensory characteristics, Yoghurt

Introduction

In Ethiopia, a significant proportion of milk is consumed in the form of 'Ergo' were the fermentation process is usually natural (Ashenafi, 1994; Gonfa *et al.*, 2001). Yoghurt production is a well-controlled process that utilizes pure cultures of lactic acid bacteria (LAB) which are responsible for the fermentation process. Yoghurt is a healthy and delicious food due to its high nutritive and therapeutic value (Perdigon *et al.*, 2002) and generally considered as a safer product, well known and popular worldwide (Munzu *et al.*, 2004).

The associations of fruits with cultured dairy products have created healthy perceptions in the consumers mind. Papaya fruit provides health benefits because it is sources of vitamins, minerals, antioxidants and dietary fibers (Cho *et al.*, 2004). It has nutritional, medicinal, pharma-cological and a therapeutic effect on the human body (Krishna *et al.*, 2008). Milk is rich in protein and fat but it is deficient in vitamins and fiber. Thus, blending milk with papaya juice for yoghurt would produce a nutritionally rich functional food.

Gelatin enhance the viscosity, influence texture, creaminess and mouth feel as well as help to prevent separation of whey from yoghurt (Fiszman and Salvador, 1999). Considering the above, the production of functional yoghurt with papaya juice is an alternative for increasing the market of health conscious consumers and may contribute to an increase in vitamins and mineral consumption. Previous works carried out did not consider the inclusion of fruits such as papaya as a source of vitamins and minerals to enrich milk products. Therefore, the objectives of this work were to prepare yoghurt from cow's milk added with papaya juice and gelatin and to evaluate physical, microbiological and sensory properties as a function of storage time to increase the nutritional quality and acceptability of the yoghurt.

Materials and Methods

The experiment was conducted in the Food Science and Dairy laboratory of Haramaya University. Fresh whole cow's milk was collected from Haramaya University dairy farm. Fresh papaya was purchased directly from farmer's field. The milk and papaya juice were analyzed for proximate compositions .Gelatin (with 240 Blooms manufactured in Brazil by Bake Mate) was purchased from the supermarket, Addis Ababa. Freeze-dried yoghurt starter culture (YC-X11 CHR HANSEN), was purchased from chemical supplier (Yomex- Import and Export, Addis Ababa).

Yoghurt production

Prior to yoghurt preparation all equipment used for yoghurt manufacturing were sterilized in autoclave after thoroughly washing them. A freeze-dried yoghurt starter culture was used to inoculate fresh milk 1lt, which had been heated at 90 ^{0}C for 30 min. and immediately cold to 45 ^{0}C. The inoculated milk was incubated at 43 ^{0}C until pH 4.6 was attained, then stored overnight at 4 ^{0}C and then used in the yoghurt preparation (Brabandere and Baerdemaeker, 1999). The resulting 12 different yoghurt formulations were further divided into three each coded screw capped glass jars and a temperature of 43 ^{0}C was maintained throughout the incubation period until the pH of the control yoghurt reached 4.7. The yoghurt samples were transferred to a refrigerator at 4 ^{0}C, then all the yoghurt samples were stored at the same temperature for 21 days during which yoghurt samples were taken at (1, 7, 14 and 21 days) for analysis. The samples were taken out and left at room temperature prior to analyses. The samples were then homogenized to obtain a uniform mixture for further analysis. Microbiological, titratable acidity, pH, and syneresis were measured on the day after fermentation (day 1) and weekly at different storage intervals (7, 14 and 21 days) thereafter until day 21 (Brabandere and Baerdemaeker, 1999).

Physical analysis

The papaya juice and fresh milk samples were analysed for moisture by oven - drying method, crude fat using the Mojonnier method, crude protein using the Kjeldahl method, and ash by furnace- drying. All of these parameters were determine based on the procedures as described in A.O.A.C. (2005). The total soluble solid content was determined with a refractometer (Hanna H1 96801, USA) and the carbohydrate and calorie values calculated. The pH of the samples was measured by a pH meter and titratable acidity (TA) of both yoghurt and milk was determined using the method described by Marth (1978), where 9 g of sample was weighed into a 100 ml wide mouth flask and in the case of yoghurt, 20 ml of fresh distilled water was added before titrating against a 0.1 N NaOH after adding 3-5 drops of 1 % phenolphthalein solution. The titaration was stopped when a persistent (30 sec.) faint pink color was observed. The titre obtained was used to calculate the titratable acidity content expressed as percentage of lactic acid.

The firmness analysis was performed with a texture analyser, TA-XT2 model using an Expert Software version 1.05 (Stable Micro Systems, Surrey, UK). The analytical method was modified from an aluminium cylinder probe P36R with a diameter of 35 mm. A compression strain of 60% was used with a 5 kg load cell at a speed of 5 mms^{-1}. The firmness of the yoghurt samples was then measured (Ng *et al.*, 2012).

Syneresis of the homogenised yoghurt was determined by spreading evenly 20 ml each of the yoghurt sample in a Buchner funnel (laced with a Whatman number 1 filter paper) placed in an Erlenmeyer flask and attached to a vacuum pump (Fisher Scientific, FB 70155) to vacuum-filter for 10 minutes. The collected residue was weighed and percent syneresis was calculated by dividing the weight of the residue by the initial sample weight multiplied by 100 (Ng et al., 2012).

Treatments and microbiological analysis

Yoghurt sample for microbiological analysis was prepared according to the method described by Marth (1978), from thoroughly mixed sample, 11 g of powdered yoghurt was weighed in wide mouth container and mixed with 99 ml of peptone water (40 ^{0}C) using a shaker (Grant GL, 5400, England) until a homogenous dispersion (10 min) was obtained. The diluted yoghurt (1:10) was then directly used for YMC and CC, whereas a serial dilution of up to 10^{-7} using peptone water was prepared for LAB count.

The treatment combinations used were three levels of gelatin (0.5, 0.6 and 0.7), four levels of papaya juice (0, 10, 15, and 20) both on a weight by weight percent basis and four storage durations of seven days interval (1, 7, 14 and 21), laid out in complete bock design in three replications. Fixed quantities of cane sugar (4% w/w) and yoghurt starter (0.03% w/w) were added to all the treatments combination as fermentation starter and energy provider respectively for the growth of LAB bacteria.

Total LAB count

Total LAB count was carried out as described by Keating and White (1990). Hansen's Yoghurt Agar (HYA) was used to support the growth of yoghurt bacteria. Triplicates of appropriate decimal dilutions (10^{-5}, 10^{-6} and10^{-7}) of 1 ml was poured on sterile petri plates and 15 – 20 ml Hansen's Yoghurt Agar (HYA) was added and then incubated at 37 ^{0}C for 72 h. The total number of LAB per gram of yoghurt was obtained by multiplying the number of colony forming on the plate with respective dilution factor and then converted into logarithmic form and expressed in $\log_{10}$ Colony Forming Unit (cfu) g^{-1} of yogurt.

Coliform count

Coliform count (CC) was determined using violet red bile agar (VRBA) method described by Marth (1978). 1 ml of the diluted yoghurt (1:10) was poured on three sterile petri plate to which 15–20 ml of VRBA was added and the plates were incubated at 30^{0}C for 24 hours. Typical dark red colonies (> 0.5 mm in diameter) were considered as coli forms and counts were reported as counts per gram of yoghurt.

Yeast and mould counts

Yeast and mould count (YMC) was carried out by incubating 1 ml of the diluted yoghurt (1:10) by the pour plate method using Acidified Potato Dextrose Agar (APDA) medium after incubating it at 25^{0}C for 5 days. Yeast and mould count per gram of yoghurt was reported as described by McClements and Keogh (1995).

Sensory evaluation

Twenty-five panellists (13 males and 12 females from Haramaya University) were purposely selected for sensory evaluation. A hedonic form with a 9-point scale was given to each panel. The yoghurt samples were served in randomised order in small cups coded with three random digits. The hedonic form scales ranged from 1, representing 'dislike very much', to 9, representing 'like very much'. The sensory parameters used were color, appearance, body and texture, flavor and over all acceptability as described by Ng et al., (2012).

Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) using SPSS version 17.0 for Windows. The Duncan's multiple range tests was used for means separation where significant differences exist between treatment means at 5 % level.

Results

Chemical analysis of milk and papaya juice

Proximate Composition of milk and papaya used for yoghurt production was analyzed before use. Moisture, total solid, fat, protein, ash, acidity, pH, and solid-non-fat (SNF) were determined. Results of Proximate Composition of milk and papaya are shown in Table 1 and Table 2. The results were more or less similar to earlier studies, (O'Mahony, 1988; Emun, 2012; Galindo-Estrella *et al.*, 2009).

Table 1. Proximate Composition of milk used for yoghurt production

Parameter	Mean ±SD
Total Solids (%w/w)	11.56 ±0.01
Moisture (%w/w)	88.45±0.05
Solid-not-fat (%w/w)	8.06±0.01
Protein (%w/w)	3.0 5±0.03
Fat (%w/w)	3.61±0.15
Ash (%w/w)	0.71 ±0.04
Titratable acidity (%w/w lactic acid)	0.13±0.01
pH	6.62±0.05

Values are means± standard deviation of triplicate sample, expressed on fresh milk weight basis.

Table 2. Proximate composition of papaya juice used for yoghurt production

Parameters	Mean ± SD
Total solid (%,w/w)	19.60±0.12
Moisture (% w/w)	80.40 ±0.07
Total soluble solids (^{0}Brix)	8.80±0.2 0
crude protein (% w/w)	6.20±0.15
Total carotenoid (mg/Kg)	68.25±10.9
Total sugar (% w/w)	7.38 ±1.66
Total phenols (mg GAE/K g)	53.70 ±3.20
Reducing Power(mg AAE/Kg)	541.60±0.99
Titratable acidity (as citric acid % w/w)	0.23±0.02
pH	4.50±0.10

Values are mean ± standard deviation of triplicate samples, expressed on 100g of fresh juice in weight basis

Physical and Microbiological Properties of Papaya Yoghurt

pH and titratable acidity

During the storage time, the pH of yoghurt ranged from 4.43±0.02 to 4.15±0.01, 4.43±0.09 to 4.20±0.05, 4.49±0.02 to 4.22±0.03 and 4.55±0.03 to 4.25±0.03 for yoghurts with 20%, 15% and, 10% papaya juice and plain yoghurt (control), respectively. Storage study result (Table 3) showed TA of yoghurt was significantly ($p<0.05$) increased as storage time and papaya juice level increased. Yoghurt samples with papaya juice had significantly ($p<0.05$) higher TA values at each storage days compared with the control. The results showed that the acidity tends to increase in all yoghurt treatments within 21 days of storage. Moreover, the rate of TA in papaya juice supplemented yoghurts was higher compared with the control.

Table 3 Effect of papaya juice levels and storage days on pH of yoghurt samples

Storage-D	Papaya juice levels (%,w/w)			
	0	10	15	20
1	4.55±0.03[a]	4.49±0.02[b]	4.43±0.09[c]	4.43±0.02[c]
7	4.46±0.07[bc]	4.35±0.05[d]	4.28±0.06[e]	4.25±0.06[ef]
14	4.29±0.05[e]	4.26±0.05[ef]	4.24±0.03[efg]	4.18±0.05[hi]
21	4.25±0.03[efg]	4.22±0.03[fgh]	4.20±0.05[gh]	4.15±0.01[i]

Storage-D=Storage Days. Means in a column or row with different superscript letters are different (p<0.05).

The mean TA of yoghurt was in the range from 0.643± 0.043% to 1.056 ± 0.018% lactic acid throughout the storage time for papaya supplemented yoghurt and 0.630± 0.034 to 1.021± 0.039 for the control (Table 4).

Table 4. Effect of storage time and papaya juice on TA of yoghurt samples

Storage day	Papaya juice (%, w/w)			
	0	10	15	20
1	0.630±0.034[g]	0.643±0.043[g]	0.680±0.020[fg]	0.710±0.019[ef]
7	0.683±0.043[fg]	0.750±0.082[de]	0.760±0.089[de]	0.800±0.094[cd]
14	0.847±0.081[c]	0.910±0.065[b]	0.927±0.043[b]	0.960±0.035[b]
21	1.021±0.039[a]	1.010±0.019[a]	1.020±0.024[a]	1.056±0.018[a]

Different superscripted letters in each column indicates significant difference between the samples at a level of 0.05 (P<0.05). The values given were Lactic acid%. Values were given as average ± standard deviation

Syneresis and Firmness

The papaya yoghurt was significantly lower syneresis and more firmness than the control. The papaya juice had the most influence on the textural quality of the yoghurt. This result could be due to the pectin component of the papaya juice reinforcing gelatine, which tended to produce resistance to the structural deformation of the yoghurt. It was speculated that the addition of the fruit juices to the yoghurt might increase syneresis and affect the strength of the internal bonds formed in the food. Syneresis significantly (P ≤ 0.05) decreased as the amount of gelatin level increased from 0.5% to 0.6%, but the mean value between 0.6% and 0.7% gelatin levels was not significant (P ≥ 0.05). Moreover, papaya juice addition also significantly (P ≤ 0.05) decreased syneresis value compared to plain yoghurt (control) of day 1 samples (Table 5). The syneresis of yoghurt at each papaya juice level decreased at the highest gelatin level and these differences were significant (P ≤ 0.05) at (0.07%) level. At 0.6% gelatin addition, the syneresis of yoghurt samples significantly (P ≤ 0.05) decreased as the amount of papaya juice increased. Addition of papaya juice in yoghurt samples at 0.5% gelatin level resulted in significant ($p \leq 0.05$) decrease in syneresis as compared to the control but the difference in syneresis value between yoghurt samples with 10%, 15% and 20% papaya juice was not statistically different ($P \leq 0.05$). Similarly, yoghurt samples with 0.7% stabilizer level showed a significant decrease ($P \leq 0.05$) in syneresis with increasing papaya juice addition except that there was no significant difference in syneresis between yoghurt samples with 10% and 15% papaya juice.

Table 5. Syneresis of yoghurt samples during storage days at 4 ^{0}C

		Syneresis (%, w/w) (mean±SD) Papaya juice levels (%, w/w)			
Gelatin level	Storage days	0	10	15	20
0.5	1	17.00±1.00^{abc}	16.00±1.00^{abc}	16.00±1.00^{abc}	16.00±2.00^{abc}
0.5	**7**	16.00±0.00^{abc}	15.83±1.44^{abc}	15.67±0.58^{abc}	15.60±0.05^{abc}
0.5	14	16.67±2.08^{abc}	16.50±0.50^{abc}	16.33±1.53^{abc}	16.33±2.08^{abc}
0.5	21	18.33±2.08^{c}	18.00±0.00^{bc}	17.33±0.58^{abc}	17.33±2.52^{abc}
0.6	1	16.05±0.95^{abc}	15.72±0.25^{abc}	15.57±0.02^{abc}	15.49±0.01^{ab}
0.6	**7**	15.55±1.00^{abc}	15.45±0.10^{ab}	15.40±0.20^{ab}	15.33±5.03^{ab}
0.6	14	16.23±0.72^{abc}	15.92±0.14^{abc}	15.90±0.31^{abc}	15.72±0.16^{abc}
0.6	21	17.00±0.00^{abc}	16.67±0.29^{abc}	16.60±0.00^{abc}	16.55±0.01^{abc}
0.7	1	15.43±0.07^{ab}	15.31±0.30^{ab}	15.30±0.05^{ab}	15.15±0.10^{a}
0.7	**7**	15.33±4.16^{ab}	15.17±0.76^{a}	15.00±2.00^{a}	15.00±1.00^{a}
0.7	14	15.68±0.08^{abc}	15.67±2.52^{abc}	15.52±0.05^{ab}	15.50±0.05^{ab}
0.7	21	16.53±0.06^{abc}	16.52±0.03^{abc}	16.50±0.10^{abc}	16.35±0.30^{abc}

Different superscripted letters in each column or row indicates significant difference between the samples at ($P<0.05$).

Generally, at day 1, yoghurt sample with 20% papaya juice had significantly ($P \leq 0.05$) lower syneresis values compared to the control at each gelatin level. This could be related to the high soluble solid and fiber content of papaya juice which increases the solid content of yoghurt. One of the common reasons for the occurrence of whey on cultured products was low solids content (Lucey, 2004). Moreover, increased gelatin and papaya juice levels synergized in lowering syneresis of yoghurt samples and the lowest syneresis values were observed at 20% papaya juice and 0.7% gelatin level.

The syneresis of yoghurt samples (Table 5) was not influenced by storage time. Moreover, the three-way interaction effect of storage time, gelatin and papaya juice addition at the seventh day with 0.07% gelatin level had significant ($p<0.05$) effect on syneresis of yoghurt samples. The overall result of syneresis was in the range of 18.33±2.00% to 15.00 ± 1.00%, the minimum syneresis value was recorded at the 7th day of storage time for all treatments and then it gradually increased throughout the storage time until the 21st day.

Microbiological Analysis

LAB count

The results showed that LAB was the dominant micro-flora (population similar to that of total viable counts (TVC) because of the addition of starter cultures. Microbiological analysis on the first day of the yoghurt showed that the total LAB count was significantly ($P\leq0.05$) increased by the amount of papaya juice addition in the yoghurt (Table 6) but main effect of gelatin addition and the interaction effect of papaya juice and gelatin addition were not significant. Yoghurt samples with papaya juice at all level had significantly ($P\leq0.05$) higher mean LAB count compared to the (control) without papaya juice. The 20% papaya juice supplemented yoghurt had the highest mean LAB count (8.15±0.13 log cfu/g) and the lowest (7.81±0.07 log cfu/g) was found in yoghurt without papaya juice (control).

Storage time and its interaction with amount of papaya juice significantly ($p \leq 0.05$) affected the LAB count of yoghurt samples. However, gelatin addition and any of its interactions did not significantly ($p \geq 0.05$) affect the LAB count of yoghurt samples. The LAB count in all samples with papaya juice were significantly ($p\leq0.05$) higher than the control up to the 14th day of storage time. Their population during storage remained unchanged, and only at the end, there had been a slight reduction (from 8.20 log10 cfu/g to 6.08 log10 cfu/g) (Table 6). The minimum LAB is obtained in yoghurt supplemented with 20% papaya juice and stored for 21 days. The mean LAB count on day 21 was 6.85, 6.67, 6.25 and 6.08 logs cfu/g for yoghurt samples without papaya juice (control), with 10% papaya juice, 15% papaya juice, and 20% papaya juice, respectively.

Generally, during storage time, the LAB count of all yoghurt samples increased until day 7 then started to decline for the remaining storage period.

Table 6. Effect of papaya juice and gelatin level on LAB count of yoghurt during storage

		Log cfu/g			
Gelatin level	Storage days	Papaya juice level (%,w/w)			
		0	10	15	20
0.5	1	7.81±0.07^{de}	7.89±0.06^{de}	8.06±0.12^{abcd}	8.15±0.13^{abc}
0.5	7	7.85±0.05^{de}	7.92±0.10^{cde}	8.15±0.05^{abc}	8.20±0.05^{ab}
0.5	14	7.56±0.02^{i}	7.58±0.13^{hi}	7.68±0.03^{fghi}	7.75±0.02^{fghi}
0.5	21	7.05±0.05^{jk}	6.93±0.12^{k}	6.83±0.29^{k}	6.32±0.16^{no}
0.6	1	7.83±0.03^{de}	7.87±0.08^{de}	7.92±0.11^{bcde}	8.03±0.15^{abcd}
0.6	7	7.85±0.05^{defg}	7.92±0.10^{cde}	8.03±0.15^{abcd}	8.20±0.05^{a}
0.6	14	7.20±0.10^{j}	7.55±0.05^{i}	7.67±0.08^{fghi}	7.75±0.22^{fghi}
0.6	21	6.93±0.12^{k}	6.69±0.19^{mn}	6.50±0.00^{mn}	6.33±0.29^{no}
0.7	1	7.82±0.08^{de}	7.84±0.09^{de}	7.86±0.05^{de}	8.07±0.12^{abcd}
0.7	7	7.84±0.13^{efgh}	7.90±0.06^{cde}	8.17±0.15^{abc}	8.27±0.08^{a}
0.7	14	7.23±0.15^{j}	7.60±0.05^{ghi}	7.68±0.08^{fghi}	7.77±0.03^{efghi}
0.7	21	6.85±0.31^{k}	6.67±0.29^{no}	6.25±0.22^{po}	6.08±0.08^{p}

Different superscripted letters in each column or row indicates significant difference between the samples at a level of (P<0.05). Values were given as average ± standard deviation

Yeast and mould and coliform counts

Yeast and mould count (YMC) and coliform count (CC) was below 10 cfu/ gram (i.e., below the detection limit) throughout the storage time in all treatments. This could be attributed to the high hygienic conditions followed in the laboratory that prevented post- production contamination. Yoghurt made using only pure culture showed no growth of yeast or mould up to 4 days of storage as compared to that produced with carry- over culture (old yoghurt culture or yoghurt culture from previous batches) (Yaygin and Kilie, 1990). It is recommended that yoghurt should have a total yeast and mould count of < 10 cfu/g (Mostert and Jooste, 2002).

Sensory analysis

The sensory analysis of the yoghurt samples were shown in (Table 7). The result showed that addition of papaya juice significantly (p<0.5) affected the score of yoghurt samples by the consumer panelists for appearance, color, body and texture and overall acceptability. Panelists' rating for appearance and color was similar for all yoghurt samples but yoghurt with 10% papaya juice was rated significantly (p< 0.5) higher than the (control) without papaya juice. Addition of up to 15% papaya juice received significantly higher mean score for overall acceptability, which was in the range of 6.20 to 7.55 (i.e., in the range between like and like very much). However, addition of 20% papaya juice decreased the ratings to 4.45 to 5.30, which was between dislike slightly and like slightly (Table 7).

Table 7. Effect of papaya and gelatin level on consumer acceptability of yoghurt

Parameters	Gelatin (%,w/v)	Papaya juice (%,w/v)			
		0	10	15	20
Body & Texture	0.5	6.55 ± 1.91^{b}	7.6 ± 1.23^{a}	7.25 ± 1.42^{a}	5.85 ± 2.50^{c}
	0.6	6.05 ± 1.57^{b}	7.30 ± 1.08^{a}	6.40 ± 1.39^{b}	5.05 ± 2.61^{c}
	0.7	6.30 ± 1.66^{b}	7.15 ± 1.14^{a}	5.90 ± 1.62^{a}	5.15 ± 2.32^{c}
Appearance^{NS}	0.5	5.85±1.18	6.55±1.67	6.55±1.54	6.80±1.28
	0.6	5.85±1.66	6.35±1.73	6.55±1.19	6.80±1.20
	0.7	5.50±1.73	6.25±2.05	6.25±1.45	6.65±1.39
Color^{NS}	0.5	5.90 ± 1.55^{c}	6.20 ± 1.32^{c}	6.10 ± 1.92^{c}	6.05 ± 1.85^{c}
	0.6	6.90 ± 1.59^{b}	6.20 ± 2.24^{c}	7.20 ± 1.67^{b}	7.05 ± 1.46^{b}
	0.7	6.20 ± 1.36^{c}	7.15 ± 1.31^{b}	6.70 ± 1.17^{c}	6.80 ± 1.24^{c}
Flavor	0.5	6.48 ± 1.12^{ab}	6.52 ± 1.32^{a}	6.56 ± 1.27^{a}	6.37 ± 1.43^{b}
	0.6	6.37 ± 1.55^{b}	6.36 ± 1.44^{b}	6.27 ± 1.28^{b}	6.17 ± 1.49^{c}
	0.7	6.20 ± 1.18^{bc}	6.30 ± 1.05^{b}	6.17 ± 1.50^{c}	6.12 ± 2.05^{c}
Overall acceptability	0.5	7.00 ± 1.08^{ab}	7.55 ± 0.76^{a}	7.05 ± 1.05^{a}	5.30 ± 1.75^{c}
	0.6	6.45 ± 1.79^{b}	7.50 ± 1.05^{a}	7.05 ± 1.00^{a}	5.30 ± 1.66^{c}
	0.7	6.20 ± 1.91^{b}	7.35 ± 0.93^{a}	6.40 ± 1.27^{a}	4.45 ± 2.01^{d}

Values are mean ± standard deviation of 20 panelist judgments on 9-point hedonic scale. NS=Non-Significant

Yoghurt samples with 10% papaya juice received higher mean scores for overall acceptance than yoghurt samples with other all papaya juice levels but the difference in mean scores between yoghurt samples without papaya juice (control) was not significant. For all sensory attributes, yoghurt samples with 10% papaya juice had significantly higher mean hedonic scores than the control (without papaya juice), except for appearance in which case both samples received similar scores

Discussion

The presence of milk sugar (carbon source) and milk protein (nitrogen source) in the rich total solid medium of milk encourages the bacterial strain to grow rapidly (Lourens-Hattingh and Viljoen, 2001). The two species of bacteria transform lactose into lactic acid, acetaldehyde, diacetyl, and formic acid. The accumulation of all these fermentation products increased the acid production during fermentation. The liberation of lactic acids reflected the high metabolic activity of the lactic acid bacteria. The rapidly increased acidity in yoghurt prepared with papaya juice was due to its lower buffering capacity and higher content of non-protein nitrogen and vitamins which were needed for fast growing microorganisms (Salvador and Fiszman, 2004).

The generally recommended minimum acidity for yoghurt is pH 4.6 or lower (Frye, 2006) whereas codex standard for yoghurt starter should have had a minimum acid of 0.6%. However, in the present study the acidity of all the yoghurt formulations were above the minimum recommended limit. In general, the pH values of all samples decreased while TA increased during the storage time, and this was the result of lactic acid fermentation by yoghurt bacteria. The TA values were similar to the results obtained by (O'neil et al., 1997) who reported an increase during storage. In fact, syneresis increased markedly at lower pH, where the elastic deformation of the gel network occurred (Tamime and Robinson, 1999). The gel interaction network was weakened and eventually ruptured which reduced the water holding capacity of yoghurt structure. The yoghurt was kept at a cool temperature (4°C) to avoid bond relaxation at high temperature which might accelerate the syneresis. The cooling would cause the protein casein to become more swollen. Moreover, moisture loss would occur at a low temperature and subsequently decreased the occurrence of syneresis. Moreover, addition of gelatin and papaya juice as stabilizer to prevent syneresis is cost-effective method compared to other techniques e.g. milk solids fortification. It could be concluded that syneresis increased during storage time (Salvador and Fiszman, 2004).

The result of syneresis was in agreement with the result of (Ayar et al., 2006). It was observed that a similar trend of change in syneresis of yoghurt during storage time in which syneresis decreased until 14th day of storage but it then increased until day 28 of storage time (Vahedi et al., 2008). Syneresis reduction can be related to absorption of unbound water by gelatin gel network and further reduction in syneresis until day 7 could be due to increased hydration of gelatin during the cold storage. However, increased syneresis was observed after day 7 over the rest of the storage time which could be associated to increased acidity of yoghurt samples due to acid production by the starter culture. Pointed out that yoghurt syneresis was stimulated by increased acidity (Tamime and Robinson 1986). Yogurt samples containing papaya had lower syneresis compared to the control but statistically not significant ($P \geq 0.05$) (Table 4). The syneresis values were similar to the results of (Mishra, 2004) reported that syneresis of yoghurt increased due to the addition of papaya juice, which was not in agreement with the result of the current study. However, in this research the addition of gelatin and papaya juice greatly reduced the degree of syneresis until 7 days of storage times.

As explained by (Hickson *et al*., 2002), the ability of a gel to exhibit syneresis, viscosity, rigidity and elasticity will be affected by the types of protein, the temperature and time of heating, the protein concentration, and the ionic strength. Several studies of milk gel interaction and its rheological properties have previously been reported by (McClements and Keogh, 1995; Oh et al., 2007; O'Kennedy and Kelly, 2000). Various polysaccharides such as xanthan gum, wheat starch, gelatine and locust bean gum can be used in yoghurt for higher shear consistency and viscosity (Lucey et al., 1998). A combination of pectin and sugar in the presence of acid contributed to the gelling properties of milk and subsequently affected its texture (Dennapa et al., 2006). An appropriate thermal process was applied during the sample preparation to denature the enzymes papain found in papaya juice to avoid the hydrolytic digestion of milk protein. Proteolytic enzymes would have interfered with the interaction between milk casein and whey protein to form the milk-clotting structure.

The TA values of yoghurt observed in the present study were lower than values reported by (Bakirei and Kavaz, 2008) which ranged from 0.90 to 1.07%. pH and titratable acidity measurements in yogurt samples were important because acidification was the key mechanism during yogurt fermentation (Brabandere and Baerdemaeker, 1999). The declining of pH during fermentation was due to the proto-cooperative action of two strain of bacteria *S. thermophilus* and *L.bulgaricus* (Brabandere and Baerdemaeker, 1999).

Growth of *S. thermophilus* could be inhibited at pH 4.4 to 4.2 but *L. bulgaricus* could grow until a pH of 3.5 to 3.8 (Glass and Bishop, 2007). The drying of the product over time had also a significant effect on viable counts of organisms. However, throughout the storage time, yoghurt samples supplemented with papaya juice had a total LAB count of > 6 log cfu/g that is the minimum limit recommended by National Yoghurt Association (NYA) for yoghurt bacteria. The health benefit of yoghurt has been known for hundreds of years. The presence of a large number of live and active bacterial cells and/or metabolites formed during yoghurt fermentation has beneficial effects on human health (Lucey et al., 1998). Yoghurt containing health-promoting bacteria is an important segment of the functional food market. Potential benefits include: prevention of osteoporosis and hypertension, improvement of intestinal health, modulation of the immune response, reduced risk of cancer, reduced risk of heart diseases, control of serum cholesterol level and improved tolerance to milk sugar (lactose) (Karagozlu et al., 1990).

The increased in LAB count in yoghurt samples with higher papaya juice level could be due to higher total solids content compared to the control. Growth and activity of starter bacteria improved in samples with higher amounts of total solids (Mahdian and Tehrani, 2007). The LAB count result of day 1 yoghurt samples was in agreement with (Keating and White, 1990) who reported that LAB count of yoghurt to be in the range of 7.7 to 8.6 logs cfu/g. Many reports showed that (Bari et al, 2006; Robinson and Itsaranuwat, 2006) similar trend in the growth pattern of yoghurt bacteria during storage time. The relatively higher average pH value of yoghurt on day 1 (4.48±0.05), might have allowed the continuation of yoghurt bacterial growth until day 7. However, after day 7, the further reduction in pH (i.e., from 4.33± 0.08 at day 7 to 4.20± 0.03 on day 21) might have inhibited their growth, because starter bacteria *streptococcus thermophilus* was less acid tolerant than *lactobacillus bulgaricus*.

Microbiological quality control of fermented milk products is of primary importance in ensuring food safety as well as conforming to the existing standards and/or regulations in the country of sale. The main factors for yeast and mould growth as well as for coliforms in yoghurt production are microbiological quality of any ingredients that are introduced into the yoghurt after heat treatment (90 ^{0}C for 30 min) of the base milk, i.e. starter culture and flavorings; the hygiene of the operator; cleanliness of the working environment and equipment used (Tamime and Robinson, 1999).

Most of the scores given by consumers for appearance, texture, flavor and overall acceptability for each treatment were concentrated between 5.50 (like slightly) and 7.50 (like very much), on the liked part of the scale for all the treatments. However, despite the unfamiliarity of the consumers to papaya supplemented yoghurt, participants found the sensory attributes of yoghurt supplemented with papaya juice to be very acceptable. This could be explained by (Berridge, 1996) the familiarity of consumers for the individual ingredients, i.e., papaya and milk (Huotilainen et al., 2006). In this study, yoghurt containing 10% papaya juice at all gelatin level had overall acceptability compared to all the other treatments.

Conclusion and Recommendation

Based on the study result, functional and nutritional values of yogurt and fermented dairy products were enhanced by adding papaya fruit. The physical and microbiological characteristics of the yogurts remained unaffected. The added functional yogurt value appeals to a wide variety of consumers; therefore has the potential to increase sales of yogurt in the market. Generally, the study indicated the possibility of producing papaya juice supplemented yoghurt commercially under small or large-scale condition. Even though the results of the present investigation were of practical value, however, further research is required in selecting different papaya varieties with higher carotenoids and phenolic content for antioxidant and sensory properties of yoghurt throughout storage time.

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