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Homogenization of milk and its effect on sensory and physico-chemical properties of yoghurt

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This study was designed to evaluate the chemical composition, sensory properties and microbial load of differently homogenized milk for yoghurt-making. Milk was homogenized with a hand whisker (HW), pressure sprayer (PS) and high-speed mixer (HM) while the control was not homogenized (NH) prior to yoghurt-making. Samples were stored in a refrigerator for 10 days at 4°C and thereafter examined for microbial counts using pour plate technique. Results show that homogenization had no significant ($p > 0.05$) influence on taste and flavor of yoghurt. The chemical composition of the yoghurt samples in all the treatment groups were improved as the days in storage progressed. The highest total viable count (TVC), coliform and fungal counts were obtained with NH while PS recorded the least counts.

Key words: Chemical composition, sensory properties, microbial load, homogenizer and yoghurt.

INTRODUCTION

Yoghurt is one of the most popular fermented dairy products widely consumed all over the world and its consumption has increased considerably since the 1970s to the present decade (Deeth and Tamine, 1981; Hassan and Amjad, 2010) due to its perceived health benefits. Ayebo and Shahani (1980) reported that fermented dairy products are more nutritious than the milk from which they are made. Furthermore, the higher nutritional value of these products has been attributed to the increased production of certain nutrients and to the pre-hydrolysis of the major milk components by lactic starter cultures, rendering them more digestible (Hewitt and Bancroft, 1985; Bystron and Molenda, 2004). In general, the overall properties of yoghurt, such as acidity level, free fatty acid production, production of aroma compounds (diacetyle, acetaldehyde and acetoin) as well as the sensory profile and nutritional value, are important traits of the product (Lee and Lucey, 2010). The production of yoghurt entails many processes including standardization and homogenization of milk. This study was designed to evaluate the effect of different homogenization methods on the microbial, physico-chemical and sensory properties of yoghurt.

MATERIALS AND METHODS

Milk sampling and transportation

Fresh cow milk was collected from lactating White Fulani cows. The cows were milked between 06.00 and 07.00 h by hand milking procedure in hygienic conditions. The milk was thoroughly mixed with a ladle spoon for about three (3) minutes. The milk collected in

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plastic containers was ice-packed in a cooler and immediately transported to the laboratory for pH determination, yoghurt making and chemical analysis.

Preparation of yoghurt samples

The fresh milk obtained was clarified to remove dirt, debris and udder tissues using a clean cheese cloth. Thereafter, the milk was pre-heated to 50-60°C and 10% skimmed milk was added. The whole milk was then divided into four equal portions (treatments). Thus, not homogenized (NH) served as the control and hand whisker (HW), pressure sprayer (PS) and high-speed mixer (HM) were homogenized with a whisker (Rudong Jiahua Food Machinery Co., China), pressure sprayer (Hymatic Agro, New Delhi, India) and a high-speed mixer (10,000 to 13,000 rpm; Qlink^R Shangai, China), respectively. The different milk portions were then pasteurized at 85°C for 20 min and sucrose was added at 6% inclusion level. The milk was allowed to cool to 40 to 45°C and then inoculated with a mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (yoghurt starters) and then allowed to ferment in an incubator set at 42°C till a firm coagulum was formed. After incubation, the yoghurt so formed was cooled to 4-5°C, stirred and then filled into small transparent cups (covered) with labels. The labeled cups were then placed into larger plastic containers and kept under refrigerated conditions at 4°C until further analyses.

pH determination

The pH of fresh filtered milk and laboratory prepared yoghurt were obtained using a digital pH meter (PHS – 3C, TBT, Jiangsu, China). The pH meter was calibrated with buffer standards of pH 4 and pH 10 prior to use. 50mL each of the samples was placed in a beaker, the probe of the pH meter was inserted and pH value was recorded. The probe was rinsed thoroughly with distilled water before use on other samples.

Determination of titratable acidity (TTA)

The titratable acidity (TTA) of the fresh milk and freshly prepared yoghurt were determined using 0.1 M NaOH and phenolphthalein indicator according to the procedures of AOAC (2005).

Ash content determination

The ash content of milk and yoghurt samples was determined at 550°C according to AOAC (2005). The ash content was expressed as the inorganic residue left as a percentage of the total weight of milk and yoghurt incinerated.

Total solids

The weight of the residue obtained from moisture content analysis was expressed as percentage total solids using the formula below:

$$\text{Total solids (\%)} = \frac{\text{(Weight of dish + Dry yoghurt) - (Weight of dish)}}{\text{Weight of the sample}} \times 100$$

Chemical analysis

The fresh milk and the yoghurt samples prepared were studied for dry matter, fat, protein, lactose while ascorbic acid, calcium, iron and phosphate using atomic absorption spectrophotometer. All measurements were in triplicates.

Microbiological analysis

The pour plate technique (Adegoke, 2000) was used for the microbiological examination of fresh milk and the various yoghurt samples as described below.

Preparation of media

Nutrient agar (NA)

28 g of powdered commercially prepared nutrient agar was accurately weighed into clean, dry 1L flask and 1000 ml of distilled water was added and placed inside water bath (Classic Equipment, Mumbai, India) set at about 90°C to allow the agar to dissolve. The dissolved agar was then distributed into MacCartney bottles and placed inside autoclave (Systec GmBh, Germany) set at 121°C for 15 min.

MacConkey agar (MCCA)

55 g of MacConkey Agar (Sigma-Aldrich) was accurately weighed and 1000 mL of distilled water added and boiled to dissolve the agar. The dissolved agar was then distributed into MacCartney bottles and autoclaved as for nutrient agar.

Potato dextrose agar (PDA)

39 g of PDA (BD Worldwide) was accurately weighed and 1000mL of distilled water added and bring to boil to dissolve the agar. The dissolved agar was then distributed into MacCartney bottles and autoclaved as for Nutrient Agar.

Serial dilution/pouring of plates

9 mL of distilled water was pipette into clean test tubes and plugged with cotton wool and wrapped with aluminum foil. This was then sterilized in autoclave at 121°C for 15 min.

1 mL each of the samples (milk/yoghurt) was measured into a clean test tube containing 9 mL of sterile distilled water and serially diluted until a dilution factor of 10^{-5} was achieved and 1 ml of the last dilution factor plate out into sterile plates. The media was poured individually; that is, NA, MCCA and PDA into separate plates and each was duplicated.

The plate for total viable count (NA) and coliform counts (MCCA) were allowed to cool and set and incubated invertedly at 37°C for 48 h. However, the plates for fungal counts (PDA) were inverted and incubated at 28 - 30°C for 72 h.

Sensory evaluation

The fresh yoghurt produced in the different treatment groups was subjected to evaluation. It was assessed for colour, taste, texture, flavour and overall acceptability. A total of sixty (60) respondents who were familiar with the taste of yoghurt were provided with the score cards comprising a 9-point hedonic scale (Larmond, 1977). The hedonic scale ranged from 1 (dislike extremely) to 9 (like extremely).

Statistical analysis

The data collected were subjected to statistical analysis in a completely randomized design using ANOVA procedure of SAS (1999)

Table 1. Chemical composition of White Fulani cow milk used for the study.

Parameter	Mean
Total solids (%)	15.00
Protein (%)	3.90
Fat (%)	4.70
Ash (%)	1.20
Lactose (%)	4.80
pH	6.60
Titratable acidity (%)	0.18
Ca^{2+} (mg/100 g)	130.00
Fe^{2+} (mg/100 g)	0.60
PO_4^{3-} (mg/100 g)	80.00
Ascorbic acid (mg/100 g)	1.10

*Each value is a mean of 3 determinations.

Table 2. Chemical composition of yoghurt produced with different homogenizers at Day 0.

Parameter	Homogeniser type				
	NH	HW	PS	HM	±SEM
TS (%)	17.97^a	14.77^b	18.87^a	14.97^b	0.59
Fat (%)	3.87^a	3.51^c	3.59^b	3.59^b	0.04
Protein (%)	3.69	3.90	3.68	3.67	0.07
Lactose (%)	4.29^c	4.50^a	4.49^a	4.39^b	0.03
Ash (%)	1.63^b	2.01^a	1.03^c	2.04^a	0.13
TTA (%)	0.92^a	0.96^a	0.96^a	0.88^b	0.01
pH	5.10^a	4.44^b	4.61^b	5.07^a	0.09

*Each value is a mean of 3 determinations; [abc]means with different superscripts along the same row are significant (p< 0.05); SEM=standard error of mean; TS = total solids; TTA = titratable acidity; NH = not homogenized; HW = hand whisker; PS = pressure sprayer; HM = high-speed mixer.

Table 3. Chemical composition of yoghurt produced with different homogenizers at Day 5 of storage.

Parameter	Homogenizer type				
	NH	HW	PS	HM	±SEM
TS (%)	19.08^b	19.89^b	21.30^a	16.83^c	0.51
Fat (%)	3.93^a	3.57^c	3.66^b	3.64^b	0.04
Protein (%)	3.83^{ab}	3.88^a	3.92^a	3.77^b	0.02
Lactose (%)	4.46^{ab}	4.52^b	4.59^a	4.43^c	0.02
Ash (%)	2.09^b	2.13^b	2.20^a	2.09^b	0.01
TTA (%)	0.97^b	1.00^b	1.10^a	0.93^b	0.02
pH	5.07^a	4.92^b	4.99^b	5.33^a	0.05

*Each value is a mean of 3 determinations; [abc]means with different superscripts along the same row are significant (p< 0.05); SEM = standard error of mean; TS = total solids; TTA = titratable acidity; NH = not homogenized; HW = hand whisker; PS = pressure sprayer; HM = high-speed mixer.

Table 4. Chemical composition of yoghurt produced with different homogenizers at Day 10 of storage.

Parameter	Homogenizer type				
	NH	HW	PS	HM	±SEM
TS (%)	17.32^c	18.63^b	19.32^a	16.82^c	0.30
Fat (%)	3.96^a	3.63^c	3.73^b	3.73^b	0.04
Protein (%)	3.89^b	3.97^a	3.82^b	3.84^b	0.02
Lactose (%)	4.55^b	4.64^b	4.76^a	4.51^b	0.03
Ash (%)	2.21^b	2.31^a	2.32^a	2.23^b	0.02
TTA (%)	1.05^{bc}	1.12^{ab}	1.17^a	0.99^c	0.08
pH	5.59^a	5.36^b	5.36^c	5.62^a	0.53

*Each value is a mean of 3 determinations; [abc]means with different superscripts along the same row are significant (p< 0.05); SEM = standard error of mean; TS = total solids; TTA =titratable acidity; NH = not homogenized; HW = hand whisker; PS = pressure sprayer; HM = high-speed mixer.

version 8. Treatment means were separated by Duncan multiple range test of the same package.

RESULTS

Chemical composition of milk

Chemical composition of milk samples are presented in Table 1. Table 2 shows chemical composition of yoghurt produced with different homogenizers at day 0. Table 3 shows chemical composition of yoghurt produced with different homogenizers at day 5 of storage. Chemical composition of yoghurt produced with different homogenizers at day 10 of storage is presented in Table 4. Table 5 shows mean scores of sensory properties of yoghurt samples produced from different homogenizers and stored at refrigeration temperature of 4°C.

Day 0

The colour, taste, flavour, texture and overall acceptability (OA) mean scores for yoghurt produced using different homogenizers at day 0 are as shown in Table 5. There was no significant (p>0.05) difference recorded for all the parameters measured. However, NH (control) had the best mean scores for taste, flavour and overall acceptability. Furthermore, best colour.

Day 5

The type of homogenizer used had significant (p<0.05) influence on the colour, texture and overall acceptability of yoghurt stored for 5 days under refrigeration temperature. Yoghurt homogenized with HM had the highest scores for all the parameters measured except colour. There were significant differences (p<0.05) in colour scores across the treatments, with PS giving the

Table 5. Mean scores of sensory properties of yoghurt samples produced with different homogenizers and stored at refrigeration temperature of 4°C.

Storage (day)	Homogenizer (type)	Colour	Taste	Flavour	Texture	OA
0	NH	6.85	7.50	7.70	6.85	7.23
	HW	6.90	6.45	6.95	6.90	6.80
	PS	7.15	6.60	6.45	6.45	6.66
	HM	7.65	7.00	6.90	7.00	7.14
SEM		0.15	0.18	0.15	0.17	0.16
5	NH	7.25[ab]	6.75	6.75	6.90[b]	6.91[b]
	HW	7.10[b]	6.75	6.85	6.65[b]	6.84[b]
	PS	7.75[a]	7.10	6.90	7.40[ab]	7.29[ab]
	HM	7.70[ab]	7.45	7.30	7.80[a]	7.56[a]
SEM		0.11	0.15	0.14	0.15	0.12
10	NH	7.60	7.60	7.60	7.10[b]	7.48
	HW	7.80	7.60	7.20	7.00[b]	7.40
	PS	8.10	7.20	7.10	7.70[ab]	7.53
	HM	8.20	7.90	7.30	8.00[a]	7.85
SEM		0.12	0.13	0.13	0.15	0.10

*Each value is a mean of 10 determinations; [ab]means with different superscripts within a column on storage are significant ($p<0.05$); OA = overall acceptability; NH =not homogenized; HW = hand whisker; PS = pressure sprayer; HM = high-speed mixer.

highest score and HW the least. Overall acceptability also varied significantly ($p<0.05$) across the treatments with HM having the highest acceptability score and HW the least.

Day 10

Except for texture, there were no significant differences ($p>0.05$) in sensory properties of yoghurt when milk was not homogenized or homogenized with HW, PS or HM. At day 10 of storage, texture of yoghurt was significantly ($p<0.05$) affected by type of homogenizer. The highest texture score was recorded for PS and least for HW. Although overall acceptability of yoghurts with differently homogenized milk did not differ significantly, the highest overall acceptability score (7.85) was recorded for HM.

Generally, taste and flavour were not significantly ($p>0.05$) influenced from day 0-10 in all the treatment groups. Furthermore, all the sensory parameters evaluated were improved as the days in storage progressed (Table 5).

Table 6 shows the microbial load of fresh milk and yoghurt produced with different homogenizers and stored at refrigeration temperature.

DISCUSSION

The total solids content of milk fell within the range 11.1 to 16.8% as reported by Adeneye et al. (1970) and Onatola (2004). The fat content (4.7%) also agreed with the range of 3.0-8.2% (Adeneye et al., 1970; Ogunsiji, 1974; Onatola, 2004). The White Fulani cow is traditionally a low milk yielder (Adeneye et al., 1970; Olaloku et al., 1971; Olaloku, 1972). The fat content of its milk is supposed to be high because of the inverse relationship between milk yield and the butterfat (Schmidt, 1971; Bath et al., 1978; Belewu, 2006). The low fat content observed for White Fulani cow's milk in the present study did not follow such expectation. This may be attributed to the season at which the milk was collected (during rainy season). There is increase in milk yield during this time. It is a well known fact that milk yield is inversely proportional to the fat content of milk. The crude protein (CP) content (3.9%) was within the range of 3.3 to 4.8% (Adeneye et al., 1970), 3.4 to 4.2 (Ogunsiji, 1974) and 2.9 to 5.0 (O'Mahony, 1988). The CP content (3.9%) was lower than that of fat (4.7%) as earlier reported by Laben (1963) and Williamson and Payne (1978).

The ash value (1.2%) obtained in the present study is above the range (0.5-0.8%) reported by Basu et al. *(*1962), Olaloku (1972), Ogunsiji (1974) and Adebowale (1976). The average pH of milk used for this study was 6.6. This suggested that the milk was normal. A range of 6.5-6.7 was considered to be normal pH for cow milk (O'Mahony, 1988). pH 6.3 and 6.5 had been reported for

Table 6. Microbial load of fresh milk and yoghurt produced with different homogenizers and stored at refrigeration temperature of 4^0C.

Storage (day)	Homogenizer (type)	TVC (cfu/g)	Coliform count (cfu/g)	Fungal Count (cfu/g)g
	Fresh milk	3.2×10^5	7.5×10^5	6.5×10^5
0	NH	8.0×10^3	1.0×10^3	Nil
	HW	5.0×10^3	1.0×10^3	Nil
	PS	3.0×10^3	Nil	Nil
	HM	4.0×10^3	Nil	Nil
5	NH	8.0×10^3	1.0×10^3	Nil
	HW	6.0×10^3	3.0×10^3	Nil
	PS	3.0×10^3	Nil	Nil
	HM	6.0×10^3	Nil	Nil
10	NH	1.4×10^4	4.0×10^3	1.0×10^3
	HW	9.0×10^3	5.0×10^3	1.0×10^3
	PS	5.0×10^3	3.0×10^3	1.0×10^3
	HM	8.0×10^3	2.0×10^3	1.0×10^3

NH = Not homogenized; HW = hand whisker; PS = pressure sprayer; HM = high-speed mixer; TVC = total viable count.

White Fulani cow's milk (Afolabi, 1991; Onatola, 2004). These lower values indicated that the milk used by these authors was acidic probably as a result of activity of lactic acid bacteria or presence of colostrum. pH values higher than 6.7 are always associated with mastitic milk. The titratable acidity (TTA) of 0.18% obtained in this study was higher than 0.14% reported by Aworh and Akinniyi (1989). TTA is normally expressed as percentage lactic acid (Lee, 1985). The milk used for this experiment was ice-packed in a gallon to prevent lactose breakdown to lactic acid and to prevent multiplication of lactic acid producing bacteria.

Homogenization is known to break fat globules down increasing surface area for ingredients and fermentative bacteria to act. Homogenized milk is whiter in colour with better taste, flavour and texture. This suggests the high nutritive and best sensory scores obtained with HM and PS. The least nutritive and sensory scores obtained with HW as compared with HM and PS is due to the lower force or pressure applied with the whisker and probably due to inadequate timing of homogenizing.

Fresh milk had the highest microbial load (Table 6). This suggests that homogenization reduced the TVC, coliform and fungal counts of the yoghurt produced. Milk homogenization has been reported to reduce the microbial load of milk since the microbes tends to adhere to fat globules and are lost in the homogenizer (Eckles et al., 1951). The nil coliform and fungal counts in HM and PS on the day of manufacture may probably be due to the shear force applied by the high-speed mixing and the high pressure of the pressure sprayer. The appearance of coliforms and fungi in the latter days of storage might possibly be as a result of contamination which provided conducive environment for the growth of the microbes.

Conflict of Interests

The authors did not declare any conflict of interests.

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