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Full Length Research Paper

Physical and microbial properties of fruit flavoured fermented cowmilk and soy milk (yoghurt-like) under different temperature of storage.

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Quest for affordable shelf stable and acceptable fermented milk from non- dairy source informed this study, in which soymilk and dairy milk were processed into fermented drink (yoghurt-like). Water from fermented maize (maize steep water) was used as starter source while natural extracts of orange , pineapple, grape and banana were employed as flavors. The flavored fermented milk samples were physically observed and microbiologically evaluated for 17 days. Samples stored at ambient lasted for 24 hours. Change in color and flavor were observed by Day 2 and Day 4 at ambient (± 27^{0}C) and refrigerated temperature (±4^{O}C) respectively. Total aerobic count and Lactic acid bacteria count of most of the samples increased by Day 4 at refrigerated temperature followed by a decrease from Day 10. Staphylococcus count was nil at freezing temperature in all the fermented milk samples. Freezing drastically reduce the microbial load of all the fermented milk (yoghurt-like) samples.

Keywords: Cowmilk, Soymilk, fermented milk, yoghurt-like, ambient, refrigerated, freezing temperature.

INTRODUCTION

Yoghurt is the Turkish word for milk that has been curdled with lactic starter (Fias Co. Farm 2006). Yoghurt is a probiotic product. Probiotic product contains live active micro-organisms which upon ingestion in sufficient number excert health benefits beyond the inherent basic nutrition (Guarner and Shaafsma, 1998) Yoghurt consumption has increased due to its health benefit (Wood, 1992).

Milk is produced by a number of animals for human consumption, though commercial wise, source from cow is the most popular.

Yoghurt is traditionally produced from cowmilk. Goatmilk has also been found as a good raw material for yoghurt processing as it compared well with cowmilk in terms of nutrients composition (Ohiokpehai, 2003; Obatolu, et al., 2007).

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Due to continuous increase in population and inadequate supply of animal protein leading to malnutrition in developing countries, many research work have been geared towards finding alternative protein sources from legumes (Siddhuraju *et al.*, 1996; Nsofor and Maduako, 1992). Soymilk yoghurt serves as a very good alternative to the expensive cowmilk yoghurt (Nsofor and Maduako, 1992; Ashaye *et al*., 2001; Jimoh and Kolapo, 2007; Osundahunsi *et al*., 2007; Farinde *et al*., 2008; Farinde *et al*., 2009)

Soymilk has a characteristic beany flavour and this off-flavour has often made it less acceptable than cow milk, but this has reportedly been reduced by lactic acid fermentation (Mital *et al*., 1974; Pithang *et al*., 1980). Lee *et al*. (1990) reported the health benefit of lactic acid fermentation of soymilk to include reduced level of cholesterol. Chang *et al*. (2005) also reported that intake of fermented soymilk improves the ecosystem intestinal tract by increasing the amount of probiotics. Various processing methods have been developed to reduce

syneresis in soy-yoghurt and improve its acceptability (Jimoh and Kolapo, 2007; Lee, 1990; Moor, 1985 and Collins, 1991).

The commercially available yoghurt is flavored with synthetic flavors such as vanilla, strawberry, chocolate, etc. Nowadays, some industries add fruits in form of fruit preserves, canned fruits, frozen fruits and miscellaneous fruit products (Tiamime and Robinson 1985) Natural fruits are known to be rich in vitamins and minerals which subsequently fortify cowmilk and soymilk when they are added to them as flavors. The possibility of fresh fruit as flavor in yoghurt processing for cost reduction and micronutrient fortification can be the focus of the research. This study therefore aimed at monitoring the physical and microbial changes in fresh fruit flavored yoghurt from cowmilk and soybean milk stored under different temperature.

MATERIALS AND METHODS

Fresh cowmilk was obtained at a local dairy farm via Omi-Adio, Ibadan, Nigeria. Soybean (*Glycine max*) was purchased at Apata market, Ibadan. Fruits (oranges, pineapple, banana and grape) were also purchased at fruit market, Ibadan, Nigeria. Maize steep water (starter) was water on top of fermented maize paste (omi ogi). The yoghurt samples were processed using the method described by Muhammad and Abubakar, 2004; Farinde *et al*., 2008 with slight modification. The cowmilk was pasteurized at 65°C ± 3°C for 30 minutes in water bath. Soymilk was boiled at 100°C for 20 minutes. Milk from both sources were cooled down to 50°C ± 2°C. Milk samples (5000mls) were inoculated with 500mls of maize steep water (10:1). The inoculated milk was incubated in a tight fitted warmer and placed in a warm place to ferment for 12 hours and 8 hours respectively for cowmilk and soymilk during which curds were formed. The curd from each type of milk was blended separately using mechanical blender (Magic Blender Petunjuk Nakai, Japan). Blend from each type of milk was dispensed into five sterile containers. Fruit juice (orange, pineapple, banana and grape) was added to the blend of cowmilk and soymilk in four different containers respectively while the fifth container contain the control. The milk and the fruit juice were mixed in ratio 4:1 i.e. 1000mls of milk + 250mls of fruit juice. The mixture were filled into sterile bottles, coded and labeled as follows:

PSY – Plain soymilk yoghurt-like
SYO – Soymilk yoghurt-like flavored with orange
SYP – Soymilk yoghurt-like flavored with pineapple
SYG – Soymilk yoghurt-like flavored with grape
SYB – Soymilk yoghurt-like flavored with banana
PCY – Plain cowmilk yoghurt-like
CYO – Plain cowmilk yoghurt-like flavored with orange
CYP – Cowmilk yoghurt-like flavored with pineapple
CYG – Cowmilk yoghurt-like flavored with grape
CYB – Cowmilk yoghurt-like flavored with banana.

Storage

The yoghurt samples were stored at ambient temperature (27°C ± 2^0C), refrigerated temperature (4 ± 2 °C) and freezing temperature (– 18°C) for 17 days.

Physical Observation

The samples were physically observed using visual appearance, taste and smell characteristics to determine their wholesomeness on day 0 to day 2 at ambient and day 0 to day 8 at refrigerated and freezing temperature respectively.

Microbial Determination

Microbial count of the fermented milk samples was determined using the method used by Jimoh and Kolapo (2007).Sample (0.1ml) of the appropriate dilution was plated out on nutrient agar, manRogsa and Sharpe medium, and manitol salt agar for determination of Total viable count, Lactic acid bacteria count and *Staphylococcus* count respectively. The plates were incubated at 35^0C for 48 hours and colony forming unit per ml sample (cfu/ml) was estimated.

Statistical Analysis

Data were analyzed using SPSS. Experimental design adopted was10 x 4 x 3 factorial experiment ,ie number of samples were 10, number of days of storage were 4 (0, 4, 10 and 17), and temperature of storage were 3 (ambient, refrigeration and freezing) respectively.

RESULTS AND DISCUSSION

Physical Observations:

The physical observations of the flavored and unflavored yoghurt-like samples from both cowmilk and soymilk showed spoilage by 48 hours storage at ambient temperature (Table 1). Change in color from creamy to brownish and change in taste and flavor were observed in all the stored yoghurt-like samples at refrigerated temperature by Day 4 (Table 2). The reason for this might be due to the fact that no preservative was added, It has been reported by many authors that physical properties of yoghurt are affected by the milk composition, processing condition, type of heat treatment applied, acidity, coagulum handling and presence of stabilizer (Nielson 1975; Rasic and Kurman 1978; Parnell et al. 1986). All the yoghurt-like samples retained their color with no whey separation at freezing temperature till Day 10 (Table 2) This is in line with the finding of Ashaye *et al*., (2001) in which the shelf life of soy-yoghurt in freezing temperature (– 18°C) was longer than those stored at refrigerated and ambient temperature.

Microbial Count

Total aerobic count of all the stored yoghurt-like samples decreased with days of storage and temperature of storage. Total aerobic count was nil in soymilk yoghurt flavored with orange stored at freezing temperature on Day 17 (Table 3). Freezing drastically reduce the total aerobic count in all the stored yoghurt samples (Table 3). The highest total aerobic count was recorded in cowmilk yoghurt-like flavored with banana stored at ambient

Table 1. Physical observation of flavored fermented milk (cowmilk yoghurt-like and soymilk yoghurt-like) during storage Day 0 – Day 2.

Storage Days	**Day 0**	**Day 1**			**Day 2**		
Storage temperature	**Ambient**	**Ambient**	**Refrigerated**	**Freezing**	**Ambient**	**Refrigerated**	**Freezing**
PCY	Whitish wholesome	Whitish, whey not separated	Whitish, whey not separated	Whitish, whey not separated	Whitish, whey not separated, smell spoilt	Whitish, whey not separated, not spoilt	Whitish ,whey not separated, not spoilt
CYO	Creamy white, wholesome	Creamy white, whey not separated, not spoilt	Creamy white, whey not separated, not spoilt	Creamy white, whey not separated, not spoilt	Creamy, whey separated, smell spoilt	Creamy, whey separated, not spoilt	Creamy white, whey not separated, not spoilt
CYP	Whitish wholesome	Whitish, whey not separated, not spoilt	Whitish, whey not separated, not spoilt	Creamy, whey separated, not spoilt	Creamy, whey separated, smell spoilt	Creamy, whey separated, not spoilt	Creamy, whey not separated, no spoilt
CYG	Whitish wholesome	Whitish, whey not separated, not spoilt	Whitish, whey not separated, not spoilt	Whitish, whey not separated, not spoilt	Creamy, watery, whey separated, smell spoilt	Creamy, whey not separated, not spoilt	Creamy, whey not separated, not spoilt
CYB	Creamy wholesome	Creamy, whey not separated, not spoilt	Creamy, whey not separated, not spoilt	Creamy, whey not separated, not spoilt	Yellowish, watery whey not separated, brownish mold on the surface spoilt	Creamy, whey not separated, not spoilt	Creamy, whey not separated, not spoilt
SYO	Creamywhite wholesome	Creamy white, whey not separated, not spoilt	Creamy white, whey not separated, not spoilt	Creamy white, whey not separated, not spoilt	Creamy white, whey not separated, smell spoilt	Creamy white, whey not separated, not spoilt	Creamy white, whey not separated, not spoilt
SYP	Creamywhite wholesome	Creamy white, whey separated, not spoilt	Creamy white, whey separated, not spoilt	Creamy white, whey separated, not spoilt	Creamy white, whey separated, smell spoilt	Creamy white, whey separated, not spoilt	Creamy white, whey separated, not spoilt

Table 1 continue

SYG	Creamy white, wholesome	Creamy white, whey not separated, not spoilt	Creamy white, whey not separated, not spoilt	Creamy white, whey not separated, not spoilt	Creamy white, whey not separated, smell spoilt	Creamy white, whey not separated, not spoilt	Creamy white, whey not separated, not spoilt
SYB	Creamy, wholesome	Creamy, whey not separated, not Spoilt	Creamy, whey not separated, not spoilt	Creamy, whey not separated, not spoilt	Turning brownish not separated, mold on the surface, smell spoilt	Creamy, whey not separated, not spoilt	Creamy, whey not separated, not spoilt
PSY	Creamy, wholesome	Creamy, whey not separated, not Spoilt	Creamy, whey not separated, not spoilt	Creamy, whey not separated, not spoilt	Creamy, whey not separated, smell spoilt	Creamy, whey not separated, not spoilt	Creamy, whey not separated, not spoilt

Table 2. Physical observation of flavoured fermented milk (cowmilk yoghurt-like and soymilk yoghurt-like) during storage (Day 3, Day 4, Day 10 and Day 17

Sample	**Day 3 Refrigerated**	**Freezing**	**Day 4 Refrigerated**	**Freezing**	**Day 10 Refrigerated**	**Freezing**	**Day 17 Refrigerated**	**Freezing**
SYO	Creamy, whey not separated	Creamy, whey not separated, not spoilt	Turning brownish whey not separated, not spoilt	Creamy, whey not separated, not spoilt	N. D.	Creamy, whey not separated, smell not spoilt	N. D.	Creamy, whey separated, not spoilt
SYP	Brownish, whey separated, not spoilt	Creamy, whey separated, smell not spoilt	Yellowish, whey separated, smell spoilt	Creamy, whey separated, smell not spoilt	N. D.	Creamy, whey Separated, smell not spoilt	N. D.	Creamy, whey separated, smell not spoilt
SYG	Creamy, whey not separated, smell not spoilt	Creamy ,whey not separated, smell not spoilt	Creamy, whey not separated, smell not spoilt	Creamy, whey not separated, smell not spoilt	N. D.	Creamy, whey separated, smell not spoilt	N. D.	Creamy, whey separated, smell not spoilt
SYB	Creamy, whey separated, smell not spoilt	Creamy, whey not separated, smell not spoilt	Brownish, whey not separated, smell not spoilt	Creamy, whey not separated, Smell not spoilt	N. D.	Creamy, whey not separated, smell not spoilt	N. D.	Whitish, whey separated, smell not spoilt
PSY	Creamy white, whey separated, smell not spoilt	Creamy white, whey separated, smell not spoilt	Brownish, whey Separated, smell spoilt	Creamy white, Whey not separated, Smell not spoilt	N. D.	Creamy white, whey separated, smell not spoilt	N. D.	Creamy white, whey separated, smell not spoilt

Table 2. continue

Days of storage	Day 3		Day 4		Day 10		Day 17	
Sample	Refrigerated	Freezing	Refrigerated	Freezing	Refrigerated	Freezing	Refrigerated	Freezing
PCY	Creamy white ,whey separated, smell not spoilt	Creamy white, whey separated, smell not spoilt	Brownish, whey separated, smell spoilt	Creamy, whey separated smell not spoilt	N.D	Creamy white, whey separated, smell not spoilt	N.D	Creamy white, whey separated, smell not spoilt
CYO	Creamy, whey not separated, smell not spoilt	Creamy, whey not separated, smell not spoilt	Yellowish, whey separated, smell spoilt	Creamy, whey separated, smell not spoilt	N.D	Creamy, whey separated, smell not spoilt	N.D	Creamy, whey separated, smell not spoilt
CYP	Creamy whey separated, smell not spoilt	Creamy, whey separated, smell not spoilt	Brownish, whey separated, smell spoilt	Creamy, whey separated, smell not spoilt	N.D	Creamy, whey separated, smell spoilt	N.D	N.D
CYG	Whitish, whey not separated, smell not spoilt	Whitish, whey not separated smell not spoilt	Yellowish, whey separated, smell spoilt	Whitish, whey separated, smell not spoilt	N.D	Whitish, whey separated, smell not spoilt	N.D	Whitish, whey separated smell not spoilt
CYB	Creamy, whey separated, not spoilt	Creamy, whey not separated, not spoilt	Yellowish, whey separated, spoilt	Creamy, whey not separated, not spoilt	N.D	Creamy, whey not separated, smell not spoilt	N.D	Creamy, whey not separated, not spoilt

N.D. = Not Done

Table 3. Total aerobic count (TAC) of yoghurt samples at storage (cfu/ml)

Days of Storage	**Day 0**	**Day 4**		**Day 10**		**Day 17**	
Temperature of Storage Samples	**Ambient**	**Refrigerated**	**Freezing**	**Refrigerated**	**Freezing**	**Refrigerated**	**Freezing**
SYO	10.5 ± 1.2	11.5± 0.1	10.0 ± 0	10.3 ± 0.1	10.4 ± 0.1	6.0 ± 0.1	0 ± 0
SYP	2.2 ± 0.5	6.5± 0.4	2.2 ± 0.1	6.1 ± 0.1	1.7 ± 0.2	6.3 ± 1.1	1.1 ± 0.2
SYG	8.0 ± 0.7	9.8± 0.7	2.7 ± 0.5	7.1 ± 0.2	1.1 ± 0.3	7.0 ± 1.0	1.0 ± 0.4
SYB	7.2±0	7.9± 0.6	5.2 ± 0.3	6.1 ± 0.4	2.2 ± 0.2	6.1 ± 0.4	2.0 ± 0.1
PSY	20.3 ± 1.1	11.5± 0.9	5.6 ± 0.3	6.5 ± 0.4	5.3 ± 0.4	4.1 ± 0.7	2.1± 0.1

Table 3 continue

CYO	20.2 ± 0.7	21.5 ±.0.1	3.0 ± 0.5	12.7 ± 0	2.2 ± 0.1	10.2 ± 0.1	1.4 ± 0.2
CYP	21.2 ± 1.1	21.7 ± 0.1	5.2 ± 0.4	13.7 ± 1.0	9.4 ± 0.3	16.4 ± 0.4	4.5 ± 0.1
CYG	10.0 ± 1.1	9.8 ± 0.8	6.3 ± 1.1	9.6 ± 0.8	6.3 ± 0.1	10.0 ± 0.1	5.5 ± 0.8
CYB	32.5 ± 0.6	36.3 ± 1.2	6.5 ± 0.3	33.3 ± 0.2	5.5 ± 0.3	12.5 ± 0.2	8.2 ± 0.5
PCY	14.9 ± 0.1	19.9 ± 1.1	11.5 ± 0.4	16.5 ± 0.3	8.3 ± 0.2	10.1 ± 0.3	2.3 ± 0.2

Values represent the means ± standard error of three replicates of each plate count.

Table 4. Lactic Acid Bacteria Count of yoghurt-like samples at storage (cfu/ml)

Days of Storage	**Day 0**	**Day 4**		**Day 10**		**Day 17**	
Temperature of Storage Samples	**Ambient**	**Refrigerated**	**Freezing**	**Refrigerated**	**Freezing**	**Refrigerated**	**Freezing**
SYO	15.0 ± 1.5	15.5 ± 1.2	4.4 ± 0.9	14.3 ± 0.3	4.4 ± 0.1	12.4 ± 0.1	4.1 ± 0.8
SYP	11.3 ± 0.2	15.5 ± 1.2	10.1 ± 0.1	11.2 ± 0.1	4.7 ± 0.4	10.2 ± 1.0	4.2 ± 0.2
SYG	15.0 ± 1.2	12.3 ± 0.1	7.1 ± 0.5	15.2 ± 0.4	5.9 ± 0.3	12.6 ± 0.2	4.2 ± 0.1
SYB	12.3 ± 1.2	22.0 ± 1.1	4.6 ± 0.1	18.3 ± 0.2	6.0 ± 0.1	11.3 ± 0.2	5.6 ± 0.1
PSY	16.1 ± 1.2	16.5 ± 1.0	1.1 ± 0.1	15.5 ± 0.3	6.1 ± 0.1	18.5 ± 0.1	5.0± 0.3
CYO	19.4 ± 1.2	22.6 ±.1.1	3.0 ± 0.5	12.7 ± 0	2.2 ± 0.1	10.2 ± 0.1	1.4 ± 0.2
CYP	20.0 ± 1.1	22.1 ± 1.0	8.5 ± 0.2	20.6 ± 1.0	7.1± 0.4	18.2 ± 0.2	6.9 ± 0.4
CYG	19.2 ± 0.1	19.6 ± 1.1	5.5 ± 0.7	18.8 ± 0.2	4.1 ± 0.2	20.4 ± 0.4	5.8 ± 0.2
CYB	25.2 ± 0.6	23.1 ± 0.1	8.7 ± 0.1	22.1 ± 1.2	7.2 ± 0.4	22.2 ± 0.2	7.2 ± 0.4
PCY	26.0 ± 0.1	24.2 ± 0.8	11.5 ± 0.1	24.2± 0.2	7.1 ± 0.1	22.0 ± 0.1	7.2 ± 0.1

Values represent the mean ± standard error of three replicates of each plate count.

Table 5. *Staphylococcus* Count of yoghurt-like samples at storage (cfu/ml)

Days of Storage	**Day 0**	**Day 4**		**Day 10**		**Day 17**	
Temperature of Storage	**Ambient**	**Refrigerated**	**Freezing**	**Refrigerated**	**Freezing**	**Refrigerated**	**Freezing**
SYO	1.0 ± 0.2	1.8 ± 0.2	0 ± 0	1.0 ± 0.1	0 ± 0	1.0±0	0 ± 0
SYP	0±0.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 5. continue

SYG	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
SYB	1.1 ± 03	1.3 ± 0	1.1 ± 0	1.1 ± 0.2	0 ± 0	1.2 ± 0.1	0 ± 0
PSY	0 ± 0	0 ± 0	1.0 ± 0.1	0 ± 0	1.0 ± 0	0 ± 0	0 ± 0
CYO	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
CYP	0 ± 0	1.0 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
CYG	1.1 ± 0.7	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.1	0 ± 0	1.0±0	0 ± 0
CYB	1.1 ± 0.2	1.1 ± 0.2	0 ± 0	1.3 ± 0.1	1.1 ± 0.1	0 ± 0	0 ± 0
PCY	0 ± 0.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Values represent the means ± standard error of three replicates of each plate count.

Table 6. ANOVA Table (Mean Square) showing the interactive effect of Sample, Day and Temperature on the microbial count of the yoghurt-like samples

Source	**df**	***Staphylococcus* Count**	**Total aerobic Count**	**Lactic acid bacteria Count**
Sample	9	2.966*	393.779*	181.626*
Day	2	0.979*	470.222*	32.989*
Temperature	1	5.618*	1684.836*	6421.333*
S x D	18	0.366	68.413NS	10.595*
S x T	9	0.897*	294.880*	55.634*
D x T	2	0.037NS	129.199NS	11.827*
S x D x T	18	0.250*	87.815NS	12.768*
Error	140	0.013	57.711	0.378
Total	209			

S = Sample
D = Day
T = Temperature
* = Significant at P<0.05
NS = Not Significant

temperature 32.5 ± 0.6 cfu/ml (Table 3). Plain cowmilk yoghurt-like recorded the highest lactic acid bacteria count 26.0 ± 0.1 cfu/ml on Day 0 at ambient temperature and this was reduced to 7.2 ± 0.1cfu/ml on Day 17 at freezing temperature (Table 4). *Staphylococcus* count was present in soymilk yoghurt-like samples flavored with orange and banana and cowmilk yoghurt-like samples flavored with grape and banana (SYO, SYB, CYG and CYB) on Day 0 at ambient, 1.1± 0.2, 1.1± 0.3, 1.1± 0.7, and 1.1±0.2 respectively. (Table 5). Presence of *Staphylococcus* could be that the fruit added especially banana was contaminated since none of the plain yoghurt-like sample recorded staphylococcus count, Banana flavored yoghurt-like sample (from both cowmilk and soymilk) recorded presence of *Staphylococcus.* However, the *Staphylococcus* count in these samples was very negligible, although the Bulletin of the Ministry of Agriculture (Codex Alimentarius, 1998) stated that no *Staphylococcus* is allowed in final milk product.

The result of the interactive effect of Sample, day and temperature on the microbial count of the yoghurt-like samples showed a significant ($P<0.05$) interactive effect on *Staphylococcus* and Lactic acid bacteria count, while a non significant ($P<0.05$) interactive effect was shown for Total aerobic count (Table 6).

CONCLUSION

Soymilk and cowmilk can be fermented into yoghurt using maize steep water as starter and flavored with natural fruit juice. The flavored milk could only be stored at ambient temperature for 1 day and at refrigerated temperature for 4 days without any change in physical and visual appearance.

The result of the microbial determination showed that fruits addition if not sterile before use would contaminate the yoghurt as the plain fermented milk samples (both cowmilk and soymilk) were free of *Staphylococcus* contamination.

Freezing drastically reduce the microbial load of the flavored and unflavored yoghurt samples at storage hence it is recommended that yoghurt either from cowmilk or soymilk is best stored at freezing condition.

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