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PRODUCTION OF YOGHURTS FROM COW AND SOY COMPOSITE MILK USING STARTER CULTURES FROM DIFFERENT SOURCES

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ABSTRACT
Objectives: Yorghurt quality, as affected by combining cow milk with soy milk and using starters from different sources, was investigated.
Methodology and Results: Premixes containing soy milk, cow milk and soymilk-cow milk (50:50) were used to produce yoghurt using commercially available starter cultures or cultures isolated from naturally fermenting soy milk and cow milk. Chemical and sensory characteristics of the yoghurts were evaluated. pH values of the yoghurt samples were between 4.10 and 4.94, titratable acidity varied between 0.15 - 0.33%, crude protein content was 3.50 - 12.40%, fat content between 0.13 - 2.67% and the total solid content was between 8.24 - 23.4%. Combining cow milk with soymilk significantly ($P<0.05$) increased the total solids, protein, ash, fat and carbohydrate contents of the yoghurt. Yoghurt from cow milk premixes was most preferred, while that from plain soymilk was least accepted. However, addition of cow milk to soymilk significantly ($P<0.05$) improved the sensory attributes of yoghurt produced from soymilk. Starter cultures isolated from cow milk gave better yoghurt irrespective of the starting premix.
Conclusion and application of findings: The results demonstrate that soy yoghurt adoption could be enhanced by premix modification and that production of soy yoghurt with acceptable quality needs a careful selection of starter culture. If soy yoghurt could become more acceptable it could help to fight malnutrition and hunger in developing countries.

Key words: Cow milk, soy milk, starter cultures, developing countries, nutrition

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Introduction

The different types of food groups that are traditionally included in meals are milk, meat, vegetables, fruits, bread and cereals. Unfortunately, milk and its products hardly feature among the major food items consumed in developing countries (Ene-Obong, 2001), especially in the diets of low income families which consist mainly of starchy staples. Animal sources of proteins which could be used to compliment the starchy foods are expensive and out of reach for low income families (Obatolu *et al.,* 2007). This poor state of access to nutritious food in developing countries is being addressed through efforts such as the Millennium Development Goals

(MDGs 1&2) that aim to reduce vulnerability to hunger by 2015.

Soybean (*Glycine max*) is one of the crops with huge potential to improve food security and reduce health related problems globally, with soymilk being one of the valuable products. Soymilk is a traditional oriental food beverage with increasing popularity worldwide (Hauman, 1984). However, some soy products have limited consumer acceptability due to undesirable off-flavours (Kanda *et al*. 1976; Pinthong *et al.* 1980).

Lactic acid fermentation reduces beany flavours and antinutritional factors in soybean products, and together with the addition of sweeteners it is possible to improve product acceptance (Pinthong *et al*. 1980; Buono *et al*. 1990). Recent reports indicate that soy yoghurt acceptability could be enhanced by the addition of various flavorings (Osundahunsi *et al.* 2007).

In developing countries, a more simplified and cost-effective possibility would be mixing cow and soy milk to produce yoghurt. However, this possibility has not yet been well investigated. Owing to soy yoghurt's potential immense health benefits, more research targeted at improving its acceptability should be undertaken. Tuitemwong & Tuitemwong (2003) reported that lactic acid bacteria (LAB) from different sources differ in their fermentation efficiencies. It is thought that poor choice of LAB may be responsible for the lack of the buttermilk-like aroma in soy yoghurt (Nsofor *et al*., 1992).

This study investigated the effect of combining cow milk and soymilk on the qualities of yoghurt produced using starter cultures from different sources.

Materials and Methods

Fresh cow milk was purchased from nomads at Bodija Market, Ibadan, Nigeria. It was transferred in a sterile container to the laboratory for the isolation of LAB. Soybean seeds (variety TGX-923-2E) were obtained from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan. Commercially available yoghurt cultures and other ingredients were purchased from local stores. Soy milk was produced by the method of Mital *et al*. as reported by Lee *et al*. (1990).

Isolation, characterization and identification of yoghurt starter cultures: Fresh cow and soy milk samples were left covered on the laboratory bench at ambient temperature (29±2°C) overnight. LAB were isolated from the naturally fermenting milk by serial dilution in 0.1% peptone water and poured into De Mann Rogosa Sharpe (MRS) agar. The isolates were purified by streak-plating on the same medium. Morphological characteristics such as cell shape, colour and arrangement were noted. Biochemical and physiological studies such as catalase and oxidase reaction, type of fermentation, production of ammonia from arginine, growth in 4% NaCl and sugar fermentation profiles were determined using standard methods (Harrigan & McCance, 1976; Gerhardt *et al.* 1981).The test results were used to identify the organisms by reference to Bergey's Manual of Systematic Bacteriology (Sneath *et al.* 1986).

Yoghurt production: Three yoghurt premixes were formulated to contain: (a) soy milk (b) cow milk (c) soy milk plus cow milk (50:50). Each premix also contained 3% sugar and 0.5% gelatin. Each of the three yoghurt premixes formulated was divided into three portions. Mixtures of premixes, sugar and gelatin were prepared, homogenized and pasteurized as described by Collins *et al.* (1991). The mixture was subsequently placed in a water bath to cool down to 43°C prior to inoculation with the starter cultures. The first portion of cooled mixtures of each of the three formulations was inoculated with 1% commercial yoghurt culture (50:50 mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) as described by Lee *et al*. (1990). A preculture of each of the two LAB isolates from soy milk (*Lactobacillus bulgaricus* and *Streptococcus thermophilus)* was prepared; and the mixed culture inoculated into the second portion of cooled mixture of each of the premixes at 5% final volume as described by Murti *et al*. (1992). Inoculation of the third portion of each of the yoghurt premixes formulation with starter culture obtained from cow milk was done using the second procedure described above. All the inoculated premixes were poured into plastic cups and incubated at 43°C to ferment for 12 hours. After incubation, the premixes were cooled in an ice bath, placed in a cabinet at 6±2°C until evaluation within 12 hours.

Analyses: Samples were analyzed for proximate composition using standard methods (AOAC, 1990). The pH was determined using a pH meter while acidity was measured as described by Olubamiwa *et al*. (2007). A 0.5 ml aliquot of a 1% solution of phenolphthalein in 95% alcohol was added to 10 ml of yoghurt sample. Acidity was measured by titrating the resulting mixture with 0.1N NaOH, expressed as g equivalent lactic acid/100g. All the determinations were carried out in triplicates and mean values were calculated.

Sensory evaluation: The yoghurt samples were kept at 6±2°C until evaluation. Members of the 20-member panel were regular yoghurt consumers consisting of students and staff of The Polytechnic, Ibadan. Evaluation was done on a nine point hedonic scale. Characteristics evaluated included flavour, colour, taste and overall acceptability.

Data analysis: Data were subjected to analysis of variance with a two-tailed P value of less than 0.05 considered to be statistically significant. Means with significant differences were separated using Duncan Multiple Range test on SPSS for Windows Verson 11.0.

Results

All the LAB isolates from naturally fermenting soy milk and cow milk were gram positive, catalase negative, indole negative, oxidase negative and produced acid from glucose and galactose (Table 1). Some of them fermented raffinose and sorbitol while the majority grew in 4% NaCl and at 45°C. Only *Lactobacillus plantarum* grew at 4°C. Ten out of twelve of these LAB were homofermentative.

Table 1: Morphological and biochemical characteristics of lactic acid bacterial isolates from naturally fermenting cow milk and soy milk.

Characteristics	Strains											
	1	2	3	4	5	*6	7	8	9	10	**11	**12
Gram reaction	+	+	+	+	+	+	+	+	+	+	+	+
Shape	R	R	R	C	R	R	R	R	R	R	R	R
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-
Spore staining	-	-	-	-	-	-	-	-	-	-	-	-
Growth in 4% NaCl	+	+	+	-	+	+	+	+	+	+	+	+
Growth at 45°C	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 4°C	-	-	+	-	-	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-
Fermentation type	Hm	Hm	Hm	Ht	Hm	Hm	Hm	Hm	Hm	Ht	Hm	Hm
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	_	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	-
Galactose	+	+	+	+	+	+	+	+	+	+	+	-
Maltose	+	+	+	-	+	+	+	+	-	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	-	+	+	-	-	-	-	-	-	+	+
Raffinose	-	-	+	+	-	+	-	-	-	-	+	-
Sorbitol	-	-	+	-	-	+	-	-	-	-	+	-
Xylose	-	-	-	-	-	-	-	+	-	-	-	-

*Isolate from cow milk only; **Isolates from soymilk only. Hm= Homofermentative; Ht=Heterofermentative; R=Rod; C=Coccus. Isolate identities are 1 = *Lactobacillus leichmannii*; 2 = *L. casei*; 3 = *L.plantarum;* 4 = *Streptococcus thermophilus;* 5 = *L.acidophilus;* 6 = *L.salivarus;* 7 = *L. delbrueckii*; 8 = *L. xylosus*; 9 = *L.bulgaricus*; 10 = *L. fermentum*; 11 = *L. coryniformis*; 12 = *L. homohiochii*.

The pH values of the yoghurt produced was between 4.10 and 4.94, titratable acidity (TA) varied between 0.15 - 0.33%, crude protein contents were between 3.50 - 12.40%, fat content ranged between 0.13 and 2.67% and total solid content was between 8.24 and 23.4% (Table 2).

Yoghurt from cow milk premixes was most preferred while yoghurt from plain soymilk was least accepted (Table 3). Addition of cow milk to soy milk significantly improved the sensory attributes of the yoghurt produced.

Discussion

Majority of the lactic acid bacteria that were isolated from the naturally fermenting cow milk and soy milk belonged to the genus *Lactobacillus.* Abdel-Moneim *et al.* (2006) isolated *Lactobacillus* constituting 74% of the LAB associated with garris (a Sudanese fermented camel's milk product). Lactobacillus strains from various sources such as the exterior of the udder, dairy utensils, dust, grass, cattle dung and feedstuffs have been reported to contaminate raw milk during milking (Sharpe, 1981; Teuber & Geize, 1981). Some strains of *Lactobacillus* that were isolated in this study are identical to those reported in many cultured African dairy products. Abdel-Moneim (2001) and Sulma *et al*. (1991) reported the occurrence of *L. fermentum* in Sudanese *robe* and *kisra,* (fermented dairy products), respectively. *L. plantarum* was also associated with fermented milk in Northern Tanzania and Cameroon (Jiwoua & Millier, 1990; Isono *et al*. 2001).

Table 2: Chemical composition of yoghurt produced from cow and soy milk.

Sample	pH	*Acidity	Moisture	Protein NX6.25	Fat	Ash	Carbohydrate	Total Solid
A	4.94^{a}	0.25^{b}	91.10^{a}	3.62^{c}	0.13^{c}	0.29^{d}	4.86^{d}	8.90^{d}
B	4.81^{a}	0.20^{c}	91.58^{a}	3.50^{c}	0.26^{c}	0.36^{c}	4.30^{d}	8.42^{d}
C	4.10^{b}	0.23^{bc}	91.76^{a}	3.14^{c}	0.24^{c}	0.27^{d}	4.59^{d}	8.24^{d}
D	4.23^{b}	0.33^{a}	76.60^{c}	12.40^{a}	2.20^{a}	0.80^{a}	8.00^{a}	23.4^{a}
E	4.12^{b}	0.20^{c}	78.18^{c}	12.13^{a}	2.67^{a}	0.9^{a}	6.11^{bc}	21.82^{a}
F	4.65^{a}	0.25^{b}	78.64^{c}	11.32^{a}	2.58^{a}	0.33^{c}	7.13^{ab}	21.36^{a}
G	4.85^{a}	0.28^{b}	83.00^{b}	8.63^{b}	0.98^{bc}	0.55^{b}	6.84^{b}	17.00^{b}
H	4.42^{ab}	0.28^{b}	85.12^{b}	7.36^{b}	1.45^{b}	0.54^{b}	5.52^{c}	14.88^{c}
I	4.49^{ab}	0.23^{bc}	84.10^{b}	8.28^{b}	1.53^{b}	0.49^{b}	5.59^{c}	15.90^{bc}

Values with different superscripts along columns are statistically significant (P<0.05). Samples A, B and C are yoghurts from soy milk fermented with commercial starter culture, starter from soymilk and starter from cow milk, respectively. Samples D, E and F are yoghurts from cow milk fermented with commercial yoghurt starter, starter from soymilk and starter from cow milk, respectively. Samples G, H and I are yoghurts from soy-cow milk (50:50) fermented with commercial yoghurt starter, starter from soymilk and starter from cow milk respectively.

Davis (1981) recommended lactic acid content of 0.1% in yoghurt, therefore the value reported in this study is considered to be satisfactory. The lactic acid content of the yoghurt developed in this study compares favorably with that between 0.17-1.16 % reported previously (Jimoh & Kolapo, 2007; Olubamiwa *et al*. 2007). Acidity of the different yoghurt samples was influenced by the starter culture used and the formulation of the premix. Lee *et al*. (1990) reported that acidity in yoghurt from cow milk was higher than that of yoghurt from soy milk. It was suggested that this might have been due to lower concentration of lactose in soy milk.

The proximate composition of soy yoghurts in our study is similar to those reported by Favaro *et al*. (2001), but the crude protein content of yoghurt obtained from cow milk is higher than the 4.9% reported by Lee *et al*. (1990). As expected, combining cow milk with soymilk for yoghurt production significantly (P<0.05) increased the total solid content of yoghurt obtained from such premix. Furthermore, the protein, ash, fat and carbohydrate contents of yoghurt developed through mixing milk were significantly increased (P<0.05).The significant increase (P<0.05) in the proximate contents of yoghurt obtained from the premixes containing cow and soy milk is likely to be due to contribution coming from the added cow milk. Yoghurt from premixes of cow and soy milk has had its value improved when compared with yoghurt from soy milk alone.

Table 3: Sensory evaluation of yoghurt from cow and soy milk.

Samples	Colour	Taste	Aroma	Consistency	Acceptability
A	4.30^{de}	4.70^{bcde}	4.40^{cd}	4.20^{bc}	4.40^{bcd}
B	5.00^{efg}	3.90^{cdef}	4.50^{cd}	4.15^{cd}	4.39^{cde}
C	6.70^{cde}	5.40^{bcde}	5.15^{cd}	6.35^{bcd}	5.90^{bcd}
D	6.30^{g}	6.50^{ef}	5.80^{de}	5.60^{d}	6.05^{de}
E	7.50^{fg}	6.20^{f}	6.15^{e}	6.30^{d}	6.54^{d}
F	7.50^{bcd}	6.45^{ab}	6.85^{ab}	6.85^{bcd}	6.91^{ab}
G	3.25^{cde}	2.75^{def}	2.90^{cd}	2.59^{bcd}	2.96^{bcd}
H	6.35^{g}	4.05^{ef}	4.70^{de}	5.65^{e}	5.19^{de}
I	6.50^{efg}	5.95^{g}	5.60^{de}	5.65^{e}	5.93^{de}

Within column values with different superscripts are statistically significant ($P<0.05$). Samples A, B and C are yoghurts from soy milk fermented with commercial starter culture, starter from soymilk and starter from cow milk, respectively. Samples D, E and F are yoghurts from cow milk fermented with commercial yoghurt starter, starter from soymilk and starter from cow milk, respectively. Samples G, H and I are yoghurts from soy-cow milk (50:50) fermented with commercial yoghurt starter, starter from soymilk and starter from cow milk respectively.

The results show that the use of starter cultures isolated from cow milk yields better yoghurt irrespective of the starting premix. The result of the sensory evaluation show that acceptance of soy yoghurt could be improved by adding cow milk.
The precarious state of food insecurity in most developing countries needs urgent attention and an innovative approach. Soy milk (and its products) could be an important resource for combating hunger and malnutrition in many developing countries. However, the sensory characteristics of soy milk and its products need to be improved to increase consumer appeal and acceptance.

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