

*Original article

MICROBIOLOGICAL QUALITY OF LOCAL SOYMILK: A PUBLIC HEALTH APPRAISAL

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SUMMARY

The ubiquity in the hawking of locally produced soymilk, packaged in different forms, was considered a public health concern. The attendant increase in the rate of soymilk consumption has encouraged low scale production of the milk under household condition with little or no regard to quality control measures. Accordingly, branded and unbranded soymilk samples were subjected to microbiological analyses to ascertain their hygienic standard of production. The soymilk samples were found to have pH in the range of pH 7.2 to 7.5. Screening for microbial contaminants revealed generally high bacterial and fungal counts of 2.9×10^7 cells/ml. to 1.02×10^8 cells/ml. and 3.5×10^7 to 2.13×10^8 cells/ml. respectively as well as high Most Probable Number (1.80^+) of coliform bacilli per 100ml of each sample. Regular contamination with *Escherichia coli*, other faecal coliforms and *Staphylococcus aureus* was detected in all the samples. In addition, *Pseudomonas aeruginosa*, other Gram-negative bacilli and streptobacilli were detected in most of the nylon packed (unbranded) samples. The fungal isolates were mainly *Aspergillus spp.*, *Penicillium spp* and *Fusarium*. The microbial population detected in terms of number and types reflected poor hygienic standard of production, constituting a public health hazard among the populace. There is a need to streamline soymilk production for proper monitoring and quality assurance.

Key Words: Microbiological quality, local soymilk, public health.

RESUME

*La non-régularisation dans la vente à la sauvette du lait de soja produit localement et enseché sous différentes formes est considérée comme un problème de santé publique. L'augmentation du taux de consommation du lait de soja a encouragé la production à basse échelle de lait domestiquement et sans respect des mesures de contrôle de la qualité. Respectivement d'échantillons de marques déposées et non - enregistrés de lait de soja étaient passés sous analyses microbiologiques pour déterminer leur niveau hygiénique de production. Des échantillons de lait de soja s'avaient avoir un pH de 7.2 à 7.5. En sélectionnant par contamination microbienne le taux de bactéries et de champignons s'avaient être de 2.9×10^7 cellules/ml - 1.02×10^8 cellules/ml et 3.5×10^7 - 2.13×10^8 cellules/ml respectivement, aussi le nombre élevé le plus probable (1.80^+) de bacille coliforme par 100ml de chaque échantillon. La contamination régulière de *Escherichia coli* (*E-coli*), d'autres coliformes fécaux et de staphylocoques *Aureus* était détectée dans tous les échantillons. De plus, *Pseudomonas aeruginosa*, d'autres bacilles de gramme négative et des streptocoques étaient détectés dans la plupart des échantillons enséchés (non- enregistrés). Les champignons isolés étaient principalement *Aspergillus sp*, *penicillium sp* et *Fusarium sp*. la population microbienne détectée en nombre et types reflète la pauvre standard d'Hygiène de production constituant un danger (obstacle) pour la santé publique. Il est d'intérêt de contrôler (régulariser) strictement la production du lait de soja et l'assurance de la qualité.*

Mots cle: Qualité microbiologiques, lait de soja locale, santé publique.

The nutrient composition of milk (Kon, 1972) sourced from cow or human breast, enabling its description as the nature's perfect food (Quinn, 1980) is also associated with soymilk (Mita and Stemkrain, 1975, IITA, 1989), an extract of soybean. This has therefore, encouraged the household production of soymilk, soy 'iru', soy 'ogi', soy meat, soy vegetable soup, soy 'gari' soy beans cake and others (IITA, 1989) for easy availability and at affordable prices. However, soybean is known to contain antinutritional components such as trypsin inhibitors, haemagglutinins, lipoxigenase, urease, phytic acid and others (Leiner, 1981). Besides, and just like cow milk, soymilk is an excellent medium for microbial growth (Sydner *et al.*, 1978; Frazier and Westhoff, 1988), making it a suitable vehicle for various food-borne diseases. Slimy curd is a spoilage pattern of soy milk that has been caused commonly by *Alkaligenes spp.*, *Proteus*, *Acinetobacter* and *Pseudomonas* (Olson and Mocquot, 1980). Staphylococcal food poisoning was reported by Hobbs (1955) from the consumption of soymilk contaminated with *S. aureus* which is known to produce potent enterotoxin (Meyrand *et al.*, 1998). Various moulds have also been reported (Bullerman, 1976). It is therefore, of paramount importance to subject soymilk production to a reasonable degree of hygiene. The presence of coliform bacteria in soymilk generally provides an index of the hygienic standard of soymilk and its keeping quality (Sara and Hilda, 1990). This study examines the hygienic status of the locally produced soymilk, through microbiological analyses.

MATERIALS AND METHODS

Soymilk samples

Ten soymilk samples were purchased on two occasions consecutively at 5 local markets in Ibadan. Of the total 20 samples, 6 were contained in plastic bottles (branded) while 14 were packaged in nylon sacs (unbranded). The branded samples were designated WS, BS and GHS to reflect their brand names while the unbranded were labeled N₁ to N₁₄.

Total viable counts

Each sample was diluted 1 in 10⁶ in Nutrient broth (OXOID) for bacterial counts and Sabouraud dextrose broth (OXOID) for fungal counts. From the dilution, duplicate pour-plates were prepared in Nutrient agar and Sabouraud dextrose agar for bacteria and fungi, respectively. The bacterial cultures were incubated at 37°C for 24 to 48hrs. and the fungal cultures at 28°C for 48hrs. to 5 days, both under aerobic condition. Bacteria and fungi were estimated as cells per ml. (c/ml.)

Bacterial isolates

Representative bacterial colonies on the plates for total viable count were Gram-stained and later subcultured into sterile Nutrient broth, 5ml. per test-tube. Isolated colonies resulting from plate cultures on Nutrient agar were subjected to conventional biochemical tests (Cowan, 1974) specifically to identify *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Fungal isolates

The fungal isolates observed on the total viable count plates were identified by their cultural characteristics such as colour, consistency and growth pattern of mycelia (Harrigan, 1976).

Presumptive coliform test (PCT)

This test gave an estimate of most probable number (MPN) of coliform bacilli per 100ml. of each soymilk sample. Every sample was diluted 1 in 100 in 200ml. of sterile distilled water and subjected to multiple tube technique of PCT as outlined by Lamikanra (1989). The bile-based medium used was MacConkey broth (DIFCO) in double- and single-strength concentrations appropriately. Tests that showed acid and gas production after 48hrs of incubation at 35°C were considered positive for coliform bacilli.

Faecal coliforms and Detection of *E. coli*.

All the positive tests in the PCT and an overnight broth culture of *E. coli* NCTC 97001, were subcultured in loop-full, each into MacConkey broth and incubated at 44°C to 45.5°C in an electrothermal water-bath for 48hrs. The subcultures that produced acid and gas as evidence of faecal coliforms (Harrigan, 1976) were streaked each on MacConkey agar plate and incubated at 37°C for 24hrs. Reddish pink colonies that stained Gram-negative rods as the *E. coli* control culture were subjected to Indole Methyl red Voges-Proskauer Citrate (IMViC) tests (Davis *et al.*, 1973) for differentiating the enteric coliforms. *E. coli* was detected as indole and methyl red positive but Voges-Proskauer and Citrate test negative.

pH determination

This was done for each undiluted sample on a pH meter (7020).

RESULTS

The twenty samples varied in pH from pH 7.2 to 7.5 (Table 1). The total viable counts varied from 2.9 x 10⁷ c/ml, to 1.02 x 10⁸ c/ml. For bacteria and 3.5 x 10⁷ c/ml. To 2.13 c/ml. For fungi. However, the bacterial counts were generally more than the fungal counts. Also, the branded soymilk samples gave lower counts of both microorganisms than the unbranded samples (Table 1). An MPN value of 180⁺ was recorded for each sample (Table 2) while the faecal coliform test signified faecal coliform contaminants from which *E. coli* was regularly isolated. In the bacterial identification, *E. coli* and *S. aureus* were common to all the samples. *Pseudomonas aeruginosa*, other unidentified Gram negative bacilli and streptobacilli were also detected in most of the nylon

Table 1
Summary of the pH and Total microbial viable counts of the soymilk samples

Sample code	PH of Sample	Plate count in cells/ml	
		Bacteria	Fungi
WS ₁	7.4	7.2 x 10 ⁷	3.5 x 10 ⁷
SWS ₂	7.25	7.4 x 10 ⁷	3.0 x 10 ⁷
GHS ₁	7.5	2.9 x 10 ⁷	6.9 x 10 ⁷
GHS ₂	7.3	3.5 x 10 ⁷	5.8 x 10 ⁷
BS ₁	7.2	5.7 x 10 ⁷	4.0 x 10 ⁷
BS ₂	7.3	5.3 x 10 ⁷	4.9 x 10 ⁷
N ₁	7.4	1.02 x 10 ⁸	4.2 x 10 ⁷
N ₂	7.3	9.3 x 10 ⁷	1.43 x 10 ⁸
N ₃	7.5	8.2 x 10 ⁷	2.13 x 10 ⁸

KEY: WS, GHS and BS = codes for branded Soymilk samples; N = nylon packed soymilk samples.

packed soymilk samples. The fungal isolates were mostly *Penicillium*, *Aspergillus* and *Fusarium* spp. in virtually all the samples.

Table 2
Outline of the Presumptive Coliform test

Volume of MacConkey broth	50ml (D/S)	10ml (D/S)	5ml (S/S)	
Volume of diluted sample (10 ⁻²)	50ml	10ml.	1ml.	
No. of tests	1	5	5	
SAMPLE:		No. of tests positive		MPN Estimate
WS _{1 & 2}	1	5	5	180 ⁺
GHS _{1 & 2}	1	5	5	180 ⁺
BS _{1 & 2}	1	5	5	180 ⁺
N ₁ - N ₁₄	1	5	5	180 ⁺

KEY: Most probable number of coliform bacilli per 100ml. of sample. D/S= Double strength; S/S = Single strength.

DISCUSSION

The pH 7.2 for soymilk (Davies, 1981) falls within the range obtained in this study, which incidentally favours bacterial growth. The general bacterial count and MPN obtained for every sample analysed exceeded the acceptable limit for both pasteurized milk (3 × 10⁴ c/ml and less than 10 coliforms) and ultra-high temperature treated milk (IDF, 1983). The detection of *E. coli* reflected a poor hygienic standard in the production (Sara and Hilda, 1990) of the soymilk samples tested, especially in view of the report of a food-borne pathogenic strain, *E. coli* 0157:H7 (Easton, 1997). Most strains of *S. aureus* are known to be pathogenic due mostly to the heat-stable enterotoxin (Stewart, 1974) they produce in direct relationship to their inoculum level (Meyrand *et al.*, 1998). Considering the notoriety of the resistance of *S. aureus* to methicillin, other penicillins and cephalosporins (Allen and Cowan, 1997; Davies, 1997; Adeleke and Odelola, 1997), its detection regularly in the soymilk samples analysed, poses a serious health hazard to the consumers. The likely presence of *Salmonella typhi*, the causative agent of typhoid fever, among the unidentified Gram-negative bacilli intensifies the health risk. The fungi detected in the soymilk samples are also known to produce mycotoxins such as aflatoxin (Harrigan, 1976).

Remarkably, the branded soymilk samples recorded generally lower bacterial viable counts than the unbranded samples. Thus, with adequate monitoring and adherence to quality control measures during production, acceptable standard is achievable in the production of soymilk. This poses a challenge to the National Agency for Food and Drug Administration Control (NAFDAC) in Nigeria to be alive to the function of food monitoring to avoid the hazardous cumulative effects of microbial contaminants in the human system.

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Received: May 1999

Accepted in final form: December 1999

