Journal of Food Protection, Vol. 79, No. 1, 2016, Pages 169–173
doi:10.4315/0362-028X.JFP-15-136

Research Note

Safety of Street-Vended Soy Wara in Nigeria

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MS 15-136: Received 24 March 2015/Accepted 17 June 2015

ABSTRACT

Soy *wara* is a common ready-to-eat food whose production and sale are currently unregulated. Microbiological sampling indicated that 21% of the samples had standard plate counts exceeding 100,000 CFU/g, and 14% had *Staphylococcus aureus* counts higher than 100,000 CFU/g. The occurrence of *S. aureus* at these levels can result in food poisoning. *Listeria monocytogenes* was isolated in 14.4% of the samples, although the counts were generally low, typically <1,000 CFU/g. Although counts of *L. monocytogenes* were low, immunocompromised individuals and children may particularly be at risk of listeriosis. All samples showed low counts of *Bacillus cereus* (<10,000 CFU/g). *Escherichia coli* and *Salmonella enterica* were detected in 5.6 and 2.2% of all samples, respectively, indicating fecal contamination and possible links to gastroenteritis and enteric fever. Fungal counts were variable, ranging from 6.0×10^3 to 2.0×10^4 CFU/g, with *Alternaria* spp., *Fusarium* spp., and *Rhizopus* spp. being the predominant species. Aluminum content was as high as 0.776 mg of Al per g in soy wara processed with alum. Significantly higher aluminum contents were observed in alum-processed soy wara compared with those processed with lime or *ogi* (an acid-fermented gruel of either maize [*Zea mays*], sorghum [*Sorghum bicolor*], or millet [*Pennisetum glaucum*]) ($P < 0.05$). These results indicate the need to improve personal hygiene and environmental sanitation in the production and preparation of soy wara, and further studies are warranted for the implication of the accumulation of aluminum.

Soy *wara* is a Nigerian curdled soy milk product. Wara is a local name for Nigerian cheese-like products, owing to the product's similarity in appearance with the curdled milk product, wara. Soy wara was reportedly introduced in Nigeria by a Japanese researcher, Dr. O. Nakayama, who worked as part of a scientific exchange program sponsored by the Japan International Cooperation Agency *(20)*. The researcher developed methods to make tofu by using cheap local coagulants. In Asia, calcium sulfate or bittern (magnesium chloride) is used, but these chemicals are either too expensive or not readily available in Nigeria, especially in rural areas *(20)*.

Nakayama is credited with a tofu-making procedure with the same coagulant (juice extract from bombom leaves) that housewives used for making the indigenous wara. He also found that other coagulants, such as lime or lemon juice, worked well. Once exposed to the process, Nigerian tofu makers discovered several other agents that could serve as coagulants.

Wara is produced by boiling fresh cow's milk and adding a small quantity of *Calotropis procera* leaves, the vegetable equivalent of rennet, resulting in the curdling of milk. After this step, the whey is decanted, and the resulting soft mass is cut into small sizes and consumed raw or fried. Soy wara, on the other hand, is prepared by first soaking soybeans in water, after which it is wet milled and sieved. The resulting liquid is boiled, and as it froths, the choice coagulant is added. The coagulants used in curdling fresh, hot soy milk include the juice of *Calotropis procera*; an aqueous solution of calcium chloride; and an aqueous solution of lime, alum, or steeped wastewater made from *ogi,* an acid-fermented gruel of either maize (*Zea mays*), sorghum (*Sorghum bicolor*), or millet (*Pennisetum glaucum*) *(20)*. The curdled soy milk is poured (while still hot) into a muslin bag and pressed to expel the liquid portion. It is also cut into small sizes and consumed raw or fried. The procedures are summarized in Figure 1.

The use of soybean (*Glycine max*) for making wara is due to its perceived nutritional quality and relatively low cost. It is estimated to contain up to 40% protein compared with a protein content of 1 to 5.6% in most animal milk *(6)*. The potentially immense benefits of soybeans have stimulated their use in indigenous diets, including soy wara in Nigeria *(18)*.

The shelf life of these two products is variable, depending on size, ambient temperature, and whether they are fresh or fried. The degree of frying also contributes to shelf stability. The average shelf life is about 2 to 4 days for the fried forms of soy wara and wara, respectively. The shelf life is considerably shorter for the fresh products. Soy wara is most often sold in its fried form alone or immersed in soup. Currently, the production of this snack is still largely a traditional art that is often associated with poor hygiene and inconsistent quality.

Foodborne illness constitutes a major problem in Nigeria, according to the World Health Organization data.

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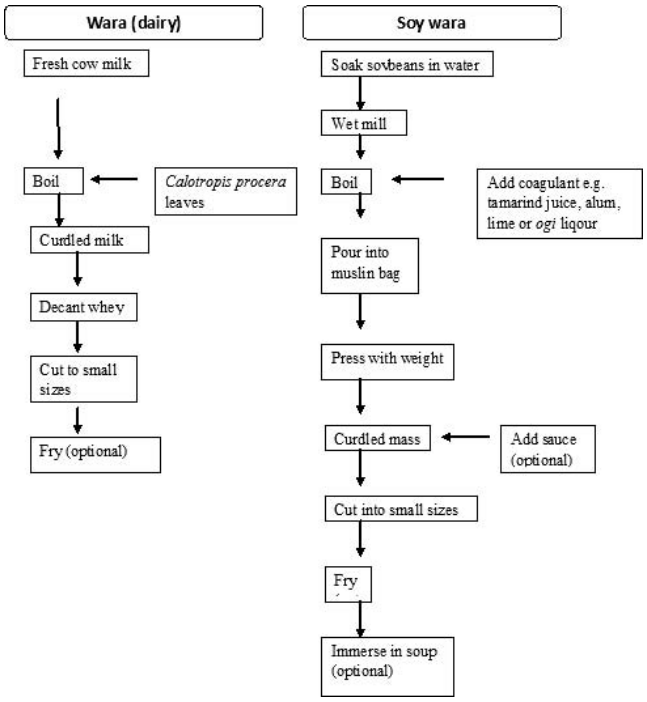

FIGURE 1. *Flow chart for the production of dairy and soy wara. Horizontal lines show concurrent procedures.*

The direct cost from foodborne illness is 17 to 25% of the total costs from all illness in Nigeria *(21)*. It is also estimated that about 200,000 deaths occur yearly from foodborne pathogens in Nigeria *(22)*. Although the health risks of ingestion of aluminum from food sources is considered low, cereals and cereal products, vegetables, beverages, and certain infant formulas appear to be the main contributors to the dietary aluminum exposure *(3, 5)*. Toxicities due to aluminum are currently uncertain, but there are a number of risks that have been investigated, including nervous disorders, such as Alzheimer's disease, bone abnormalities, and hematopoiesis *(15)*.

Information on the microbiological quality of soy wara is sparse, and findings appear equivocal (*9, 14*). To date, we are also unaware of any study on aluminum content of soy wara. Presently, there is no detailed microbiological study on soy wara or the implications of using alum as a coagulant during the production of soy wara. Therefore, we assessed the microbiological safety, as well as the aluminum content due to the use of alum as a coagulant by some producers.

MATERIALS AND METHODS

Sample collection. Soy wara samples were obtained from different locations in Gwagwalada Area Council in Nigeria's capital, Abuja. Six different locations with a high number of vendors were chosen. Ninety samples (15 samples from each location), produced by using different coagulants, were purchased. All samples were collected in sterile plastic containers and analyzed within an hour of collection.

Determination of pH. To determine pH, we used a pH meter (Jenway, Staffordshire, UK), according to the manufacturer's instructions.

Microbiological analysis: enumeration of bacteria and fungi. Samples of soy wara were aseptically cut into pieces with flame-sterilized scissors. Twenty-five grams of soy wara samples were homogenized with 225 ml of 0.1% peptone water in a laboratory blender (National, Guangdong, China) at high speed for 2 min. For each sample, the resultant homogenate was also serially diluted, and 0.1 ml of suitable dilutions was spread plated in triplicate on different media. All media, except otherwise stated, were from Oxoid, Basingstoke, UK. Plate count agar was used for aerobic plate counts, MacConkey agar for coliforms, Oxford *Listeria* agar for listeriae, Baird Parker agar for staphylococci, *Bacillus cereus* agar for *Bacillus cereus,* and Sabouraud dextrose agar for fungi.

All inoculated plates were incubated at their optimum temperatures to obtain viable bacterial and fungal counts. Colonies were counted at the expiration of incubation period by using the colony counter (Gallenkamp, England, UK) and counts were expressed as CFU per gram of sample. Characteristic discrete colonies on the different media were isolated and purified by repeated subculturing on nutrient agar. Pure cultures were stored on agar slants at 4°C, and the isolates were identified by using macroscopic, cultural, and biochemical tests.

Microbiological analysis: detection of specific pathogens. To detect *Escherichia coli* O157:H7, 25 g of the soy wara sample was macerated in 225 ml of modified tryptic soy broth supplemented with novobiocin (Oxoid) and incubated at 41.5°C for 6 and 22 h and plated on sorbitol MacConkey agar supplemented with cefexime tellurite (Oxoid). For *Salmonella,* 25 g was also macerated in lactose broth and preenriched at 36°C for 20 h, and aliquots were plated on *Salmonella Shigella* agar. For *Listeria* spp., 25 g was macerated in *Listeria* enrichment broth supplemented with *Listeria* selective enrichment supplement (Oxoid), and aliquots were streaked on Oxford *Listeria* agar.

Microbiological analysis: identification of isolates. After Gram staining, characteristic colonies that resulted from the different media were subjected to biochemical tests. For *Salmonella,* triple sugar iron agar slants were used. For *E. coli,* IMViC test were used for identification. The confirmatory test for *Staphylococcus aureus* was a positive coagulase test, while for *Listeria monocytogenes,* a positive CAMP test and nonproduction of acid from xylose were confirmatory. Fungal isolates were grown as slide cultures and identified according to Samson and van Reenen-Hoekstra *(19)*.

Determination of aluminum by using Eriochrome black T as an indicator. Determination of aluminum was done as described by Dubenskaya and Levitskaya *(11)*. Briefly, 5 g of each sample was measured and placed in a beaker containing 25 ml of nitric acid and boiled until no bubbles were noticed. Each digested solution was filtered with paper and diluted to 100 ml. Finally, EDTA solution of 0.01 M and zinc sulfate solution of 0.01 M were used for titration after pH adjustment by using ammonia and incorporation of indicator.

Statistical analysis. Log-transformed aerobic mesophile, staphylococci, and fungi counts were analyzed by using analysis of variance (ANOVA) with IBM SPSS statistical software, version 19 (IBM Corp., New York, NY). One-way ANOVA was also used to analyze Al concentration and pH values of the different samples. Means were separated by using Duncan's test, when significant differences were observed.

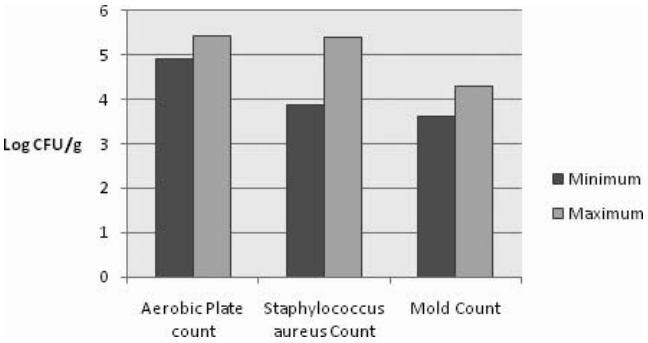

FIGURE 2. *Microbiological counts of soy wara sold on streets in Abuja, Nigeria.*

RESULTS

In soy wara, minimum and maximum aerobic plate counts were 7.8×10^4 (4.9 log) and 2.7×10^5 (5.4 log) CFU/g, respectively (Fig. 2). *S. aureus* counts were between 7.7×10^3 (3.9 log) and 2.4×10^5 (5.4 log) CFU/g, whereas fungi counts were between 4.0×10^3 (3.6 log) and 2.0×10^4 (4.3 log) CFU/g. Most of the fungal isolates belonged to the genera *Alternaria, Fusarium,* and *Rhizopus*.

Coliform counts, *B. cereus* counts, and listeriae counts were all less than 1,000 CFU/g (data not shown). The aerobic counts of 6 of 30 samples representing 20% of soy wara processed by using alum exceeded the acceptable aerobic count. All coliform counts, *B. cereus* counts, and *Listeria* counts were within acceptable ranges, while 4 (13%) of 30 staphylococcal counts exceeded acceptable limits (data not shown). Statistically, there were no significant differences in the numbers of organisms on the samples processed by using different coagulants ($P < 0.05$).

The overall prevalences of *L. monocytogenes, Salmonella enterica,* and *E. coli* (non-O157:H7) were 13 (14.4%), 2 (2.2%), and 5 (5.6%), respectively (Table 1).

Statistical analysis showed that the concentration of aluminum in soy wara processed with alum was significantly different from soy wara processed with ogi or lime ($P < 0.05$). Similarly, the pH value of soy wara processed with alum was significantly different from soy wara processed with ogi or lime ($P < 0.05$; Table 1).

DISCUSSION

The vended soy wara at the different locations had particularly high counts with respect to total aerobic, staphylococcal, and fungal counts. These were similar irrespective of locations or processing methods. Although the maximum and minimum counts were different by up to 1 log between groups processed by using different coagulants (Fig. 2), these differences were not significant ($P > 0.05$). The areas in which these samples are sold are usually highly populated and often dusty, which may be partly responsible for the high microbial numbers. The samples could also have been contaminated due to exposure and handling by intended buyers. Usually, potential purchasers handle the wara while negotiating a price and may not purchase that piece of wara, meaning there could be extended time periods for contamination and growth under ambient temperatures. There may also be opportunity for growth after the purchase has been made, as purchased samples may either be eaten straight away or taken home and shared among family or friends. Counts of coliforms, *B. cereus*, and listeriae were all below 1,000 CFU/g. The trend was also similar for all samples, irrespective of the type of coagulant used or sample location. The low counts of *B. cereus* are indicative of relatively low risk of *B. cereus* diarrhea or emetic disease because existing literature incriminates food containing more than 10^3 *B. cereus* organisms per g *(13)*. Our study on *Listeria* did not show counts exceeding 1.0×10^3 CFU/g. *L. monocytogenes* was, however, detected in 13 (14.4%) of 90 samples. Although, *L. monocytogenes* has been associated with ready-to-eat foods, outcomes depend on a number of factors, including age and immune status *(2)*. Empirical evidence indicate large numbers of *L. monocytogenes* between 1.9×10^5 and 1.6×10^9 CFU are required for gastrointestinal listeriosis *(12, 16)*. From our study, it appears the risk of listeriosis is minimal for this product. *S. aureus* counts were comparatively high compared with other gram-positive pathogens, and as many as 13 (14%) of 90 samples had counts exceeding 10^5 CFU/g. Counts above 10^5 CFU/g imply significant risk of *S. aureus* enterotoxin production and its sequel of food poisoning *(1)*. Although *S. aureus* food poisoning is usually self-limiting, the implications can be serious as seen by the U.S. Department of Agriculture data indicating 1,210 deaths and a cost of $1.2 billion *(7)*. High staphylococci counts are suggestive of postproduction contamination. Apart from spores, any organism present on the seed before processing and on the milled mash would have been removed by the high temperatures during boiling and frying. The organisms encountered could have been transferred during storage, negotiation, and other postproduction activities.

TABLE 1. *Occurrence of pathogens, indicator of fecal contamination, aluminum content, and pH value*[a]

Coagulant used for wara	No. (%)				Al concn (mg/g ± SD)	pH ± SD
	L. monocytogenes	*Salmonella enterica*	*E. coli* O157:H7	*E. coli* as indicator		
Alum	3	2	0	2	0.568 ± 0.09 A	7.058 ± 0.06 A
Ogi	4	0	0	0	0.073 ± 0.03 B	6.840 ± 0.11 B
Lime	6	0	0	3	0.070 ± 0.03 B	6.846 ± 0.02 B
Total no. (%)	13 (14.4)	2 (2.2)	0 (0)	5 (5.6)		

[a] $n = 90$. Means with different letters are significantly different ($P < 0.05$).

E. coli was present in about 6% of samples, indicating fecal contamination. *Salmonella enterica* serovar Typhi was detected in 2 (2.2%) of the samples. Enteric fever results from infection with *Salmonella* enterica Typhi and *Salmonella enterica* subsp. serovar Paratyphi A, B, and C. It is associated with poor sanitation and lack of access to safe food and clean water *(8)*. The detection of *E. coli* indicated fecal contamination, and the risks are further confirmed by our isolation of *Salmonella enterica*. Enteric fever affects an estimated 20 million people annually worldwide and causes approximately 200,000 deaths *(8)*.

Two of the mold genera frequently isolated, namely, *Alternaria* spp. and *Fusarium* spp., are associated with mycotoxin production *(4, 17)*. More detailed studies focusing on mycotoxin detection and quantification are required to assess the risks that may be associated with the consumption of soy wara.

The relatively high microbial counts of *S. aureus* and molds and contrasting low counts of coliforms may likely indicate an unfavorable water activity for most gram negatives, which, in general, requires higher water activity than gram positives and fungi *(10)*.

In addition to assess the microbiological risk, the aluminum content of soy wara made with alum and other coagulants was determined to evaluate possible aluminum toxicity. For adults, a limit of 1 mg of aluminum per kg of body weight per week is recommended *(3)*. This study did not encompass the consumption pattern; hence, extrapolations could not be made on actual risk to consumers based on observed values of aluminum in the samples. Notably, most artisanal soy wara is made by using stainless steel and iron pots for processing and frying. The use aluminum of pots should be discouraged to further minimize the concentration of aluminum in the product.

A number of measures in combination can be used to improve the microbiological quality of soy wara, as well as its shelf life. The traditional form of processing, which involves high temperatures, such as boiling and frying, are known to reduce microbial growth. This can be used in conjunction with increased acidity by using organic acids and reduction in water activity by increasing solutes, such as salt or sugar. However, these measures need to be carefully evaluated to avoid loss of sensory appeal. Refrigeration can also be used, but three important factors are critical. Most parts of Nigeria suffer chronic shortages of electricity that may preclude this form of preservation. The second factor is the frequent occurrence of *L. monocytogenes,* which is psychrotrophic, and growth may continue under refrigeration temperatures, though this can be controlled through food-grade antimicrobials, such as nisin. The third is to ensure that the microbiological quality of ingredients and wara are good prior to employing refrigeration or other preservation measures because cooking and lower water activity will make a difference for lowering bacterial counts but may not affect staphylococcal enterotoxin if it is produced already.

Consumer health needs to be safeguarded by continuous health education training for producers and sellers, which should include regular and correct washing of hands with potable water and soap, environmental hygiene, packaging, and creation of awareness on the potential toxicity associated with the use of alum. Monitoring the microbiologic quality of ready-to-eat foods should be done regularly, particularly for homemade and unregulated products. For now, an outright ban on these products is neither pragmatic nor enforceable taking into account the prevailing socioeconomic indices, including high unemployment rates. This product is undoubtedly a cheap source of high-quality protein and potentially a healthy food, if proper measures are taken.

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