

Microbiological Assessment and Prevalence of Food Borne Pathogens in Street Vended Wara - Nigerian White Cheese

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Abstract Fifty white cheese (wara) samples were collected from ten local cheese hawkwer/vendors within Abeokuta, Nigeria to determine the microbial characteristics and the prevalence of some common food borne pathogen. The total aerobic plate counts (TPC) ranged from 6.8 to 9.5 log cfu/g; Lactic acid bacteria counts ranged from 2.8 to 6.9 log cfu/g while yeasts and mould counts ranged from 2.8 to 6.6 log cfu/g. Significant ($p < 0.05$) variations were found between vendors and microbial population. The mean *S. aureus* count was 6.5 log cfu/g *Salmonella*, Presumptive *E. coli* and *Bacillus cereus* counts were 0.3, 2.4 and 3.3 log cfu/g respectively. *B. cereus* was found in 78%, *Staphylococcus aureus* in 100%, presumptive *E. coli* in 56% and *Salmonella* spp in 6% of the 50 wara cheese samples tested. None of the samples analyzed contained *Vibrio cholerae*. The results indicate that wara cheese presents a potential hazard for public health; and necessary precaution will have to be taken to improve the sanitary practices and cheese manufacturing technique.

Keywords: cheese, wara, food-borne, pathogen, street vended

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1. Introduction

Wara is a Nigerian soft, white, unsalted and unripened cheese usually processed from cow milk by the Fulani tribes who are mainly cattle rearers in Nigeria. Wara making is thought to have started with the Fulanis but as a result of their nomadic lifestyle, it has spread to other parts including Kwara, Oyo, Ogun, Ondo and the Benin republic (Raheem, 2006). Wara is made from unpasteurized, unfermented whole cow milk by coagulation of the milk with the juice extract of *Calotropis procera*. Wara cheese is an excellent source of protein, fat and minerals such as calcium, iron and phosphorus, vitamins and essential amino acids and therefore is an important food in the diet of both young and old people. It also provides an ideal vehicle for preserving the valuable nutrients in milk and making them available throughout the year (O'Connor, 1993).

Presently, the wara hawked on the street in Abeokuta, Nigeria is produced at dairy farms on a small scale using raw milk from cows and traditional techniques passed down from generation to generation (O'Connor, 1993; Raheem, 2006).

Microorganisms are widely distributed in the products of animal origin. All foods possess a finite risk of

microbiological contamination. The highest risk factors include foods of animal origin and foods consumed without prior cooking (Roberts, 1990). Wara cheese is one of those kinds of products. Microbial contamination of cheese may originate from various sources during cheese production. At the same time, pathogenic organisms may also be transferred to food by food handlers either directly or by cross-contamination (Temelli *et al.*, 2006; Brito *et al.*, 2008).

There have been outbreaks of infection associated with the consumption of cheese, and the predominant organisms responsible have included *Salmonella*, *Listeria monocytogenes*, verocytotoxin producing *Escherichia coli* (VTEC), and *Staphylococcus aureus* (De Buyser *et al.*, 2001; Callon *et al.*, 2008). Several outbreaks of food-borne illnesses associated with milk and dairy products indisputably revealed that cheeses, particularly made from unpasteurized or improperly pasteurized milk are main sources of Salmonellosis, Staphylococcal food poisoning and possibly *E. coli* infections (Doyle, 1991; IFST, 1998; Meng and Doyle, 1998).

Detailed investigations have demonstrated that the sources of contamination in cheese were raw milk, inadequately pasteurized milk, or post-pasteurization contamination with organisms originally derived from raw milk or from manufacturing environments.

The microbiological quality of *wara* cheese hawked on the street is of interest from a public health point of view since it is produced from raw milk, usually in small farm where basic facilities are absent, under poor hygienic practices, marketed by vendors who lack basic education on food safety and consumed raw without further processing. In addition, several articles have implicated street vended ready to eat food as positive vectors of food borne illnesses (Mosupye and von Holy, 1999; Edema and Omemu, 2004; Omemu, *et al.*, 2005; Omemu and Aderoju, 2008).

The present study was undertaken to investigate the safety and quality of traditional ready-to-eat *wara* vended on the street in Abeokuta, Nigeria.

2. Materials and Methods

2.1. Sampling Procedure

Fifty ready-to-eat *wara* samples were randomly collected from 10 typical street vendors at a taxi park, university campus and a market within central Abeokuta. Three independent replicate surveys of each vendor were conducted.

Approximately 250 g of each sample was collected using the vendors serving utensils, placed into sterile container, transported on ice to the laboratory and analyzed upon arrival.

2.2. Microbiological Analysis

Ten gram (10 g) of each *wara* cheese sample was homogenized with 90 mL of 0.1% w/v buffered peptone water (BPW; Merck, Germany) in a stomacher (Laboratory Blender, Seward, UK) for 60 s at room temperature. Serial decimal dilutions in 0.1% BPW were made and duplicate 1 mL or 0.1 mL samples of appropriate dilutions were poured or spread on agar plates.

Total aerobic plate counts (TPC) were enumerated on Plate Count Agar (Difco, USA) using the pour-plate method and incubated at $30 \pm 1^\circ\text{C}$ for 72 h. Lactic acid bacteria were enumerated on De Man–Rogosa–Sharpe (MRS) agar (Oxoid, UK) incubated at $30 \pm 1^\circ\text{C}$ for 72 h, following the pour-plate method. Coliform group bacteria were enumerated on Violet Red Bile Dextrose agar (Difco, USA). The plates were overlaid prior to incubation at $37 \pm 1^\circ\text{C}$ for 24 h. Yeast and mould counts were enumerated on Saboraud Dextrose Agar (SDA) incubated at $25 \pm 1^\circ\text{C}$ for 5 days.

Incidence of food-borne pathogens: For the enumeration of *B. cereus*, 1 ml of the 10^{-1} dilution of each sample was plated onto Polymyxin-Pyruvate-Egg Yolk-Mannitol-Bromothymol blue Agar (PEMBA) plates (Oxoid) which were air-dried and incubated at 37°C for 24 to 48 hours. For the enrichment procedure, 10 ml of the 10^{-1} dilution was added to 90 ml Cooked Meat Medium (Oxoid) and incubated at 37°C for 48 hours (ICMSF, 1978). A loopful of the culture was then streaked onto PEMBA plates which were incubated 37°C for 48 hours. Peacock blue colonies with blue zones were subjected to appropriate biochemical tests (ISO, 1987).

Presumptive *E. coli* were enumerated in Tryptone Bile X-glucuronide (TBX) medium (Oxoid CM 945). Incubation was at 30°C for 4 h then 18 h at 44°C . The

number of blue/green colonies on the plates were then evaluated (Bridson, 1998).

S. aureus counts was done on Baird–Parker’s medium (Oxoid CM 275). Plates were surface inoculated and incubated at 37°C for 24–48h. The typical *S. aureus* colonies (black, convex, shiny with narrow white entire margin surrounded by clear zone) were counted and tested for coagulase reaction (Oxoid Staphytest Plus DR 850 M) (Bridson, 1998).

For the detection of *Salmonella* spp., 10 g of the sample was mixed with 90 ml Buffered Peptone Water (Oxoid), homogenized for two minutes and incubated at 37°C for 16 to 20 hours. The pre-enrichment broth culture (10ml) was added to 90ml of Tetrathionate Brilliant Green Broth (Oxoid) and 90ml of Selenite Cystein Broth (Oxoid) which were then incubated for 24 to 48 hours at 43°C and 37°C , respectively. A loopful of each enrichment broth culture was streaked onto Brilliant Green Agar (Oxoid) and Xylose-Lysine-Desoxycholate Agar plates (Oxoid). The plates were then incubated at 37°C for 24 to 48 hours (Arvanitidou *et al.*, 1998). Appropriate biochemical tests were carried out on suspect colonies (ISO, 1993).

To determine the presence of *Vibrio* spp., 10g of each sample was mixed with Alkaline Peptone Water (90ml) (Oxoid), homogenised for two minutes and incubated at 37°C for 24 hours. A loopful of each enrichment culture was streaked onto a Thiosulphate Citrate Bile Salts Sucrose Agar plate (Oxoid) which was incubated at 37°C for 24 to 48 hours (ICMSF, 1996). Suspect colonies were subjected to appropriate biochemical tests.

2.3. Statistical Analysis

The count of colony-forming units (CFU) was transformed to logarithms ($\log \text{CFU/g}$) prior to statistical treatment. The statistical analysis of the results was done by ANOVA using SPSS 11. The least significant difference ($P < 0.05$) of data is reported.

3. Results

Microbial Contents of *wara* cheese Samples: The microbial contents of the sampled cheese are presented in Table I. No significant differences ($p \geq 0.05$) were observed between the 3 independent surveys for corresponding count types obtained from each vendor.

The average total bacterial counts (TPC) of the cheese samples ranged from 6.8 for vendor B to 9.5 $\log \text{CFU/g}$ for vendor I. Analysis of variance tests (ANOVA) indicated significant statistical differences ($P \leq 0.05$) in the mean values of TPC obtained from the food vendors. Least significance difference (LSD) test used to compare the means indicated that the mean TPC observed for vendor B was significantly different ($P < 0.05$) from mean TPC obtained for vendors H, I and J.

The lowest LAB count was 2.8 $\log \text{CFU/g}$ while the highest was 6.9 $\log \text{CFU/g}$. Significant ($p < 0.05$) differences were found in counts from different cheese vendors. Coliform counts of cheese samples ranged between 3.2 MPN/ml for vendor B to 4.8 $\log \text{MPN/ml}$ for vendor I. The yeasts and mould counts ranged from 2.8 $\log \text{CFU/g}$ for vendor B to 6.6 $\log \text{CFU/g}$ for Vendor I.

The highest TPC, LAB count, yeasts and mould counts were observed for vendor I while the least was observed

with vendor B. High positive correlation (99% confidence level) was observed between microbial counts and different cheese vendors.

The mean counts of some food borne pathogens in *wara* cheese samples are presented in Table II. *Bacillus cereus* mean count was 3.3 log CFU/g with a range of 1.9 to 5.7 log CFU/g. *Staphylococcus aureus* ranged from 3.5-7.4 log CFU/g with a mean of 6.5 log CFU/g. The *Salmonella* counts ranged from 0.0 to 0.7 log CFU/g. No *Vibrio cholerae* was observed in any of the 50 cheese samples analyzed.

Figure 1 summarized the prevalence and frequency of occurrence of some food borne pathogens in the samples analyzed. The frequency of occurrence presented showed that *B. cereus* was found in 78%, *Staphylococcus aureus* in 100%, presumptive *E. coli* in 56% and *Salmonella* spp in 6% of the 50 *wara* cheese samples tested. None of the samples analyzed contained *Vibrio cholerae*.

4. Discussion

The high TPC observed in some *wara* samples in this study agrees with the findings of Warsama *et al.*, (2006). The high total bacterial count in this study might be attributed to the use of low quality milk. According to Raheem (2006), the microbial quality of raw milk is crucial for the production of any high quality dairy food. It could also be as result of unsanitary conditions by the producers and vendors during processing and handling of the cheese. Mishandling and disregard of hygienic measures on the part of the food vendors may enable pathogens to come into contact with foods and in some cases to survive and multiply in sufficient numbers to cause illness in the consumer (Omemu and Aderoju, 2008).

The high coliform counts in the cheese samples were probably due to production of milk and cheese under poor conditions. According to international standards (Cyelan *et al.*, 2003, Warsama, 2006), white cheese should not contain more than 100 CFU/ml coliforms bacteria (Law, 1999)

The high counts of *S. aureus* observed in this study is similar to the findings of Tekinsen and Ozdemir (2006), who found *S. aureus* counts ranging from 2.48–7.15 in 50 samples of Turkish Van otlu (Herb) cheese analyzed. This may be due partly to the use of raw milk and manufacturing process carried out under unhygienic conditions.

The occurrence of more than 10^2 /g *S. aureus* in cheese samples may also indicate the use of milk from infected (clinical and subclinical mastitic) udder, which is frequently associated with this organism and/or an extensive contamination by personnel possibly involved in milk production and cheese making as human beings are common carriers (Hobbs and Gilbert, 1979).

Lactic acid bacteria are essential for fermentation and are acceptable in very large numbers mainly in natural cheese (Dolci *et al.*, 2008). The presence of *Salmonella* sp in some of the cheese samples analyzed is contradictory to the results of Turantas *et al.* (1989) who did not find *Salmonella* spp. in 38 white cheese samples studied.

The occurrence of *S. aureus* and presumptive *E. coli* in more than 50% of the samples reveals an extensive deficiency of satisfactory sanitary practices during milk

production and cheese manufacturing and/or post production handling of the *wara* cheese. *S. aureus* and *E. coli* in cheese are frequently used as indicators of hygienic quality and show lack of microbiological safety (IFST, 1998). Some strains of these organisms are known to cause diseases in humans. Therefore they are undesirable in high numbers.

In conclusion, due to the presence of some pathogenic bacteria in *wara* cheese sampled, there is a need to develop the production technique through adequate heat treatment, application of Good Manufacturing Practices (GMP) during processing and also improvements in hygienic practices during and after processing.

Table 1. Microbiological contents of *wara* cheese samples vended within Abeokuta metropolis in Nigeria

Vendors	TPC* (log CFU/g)	LAB counts* (log CFU/g)	Coliform count* (log CFU/ml)	Yeasts and mould count* (log CFU/g)
A	7.0	6.4	3.4	3.8
B	6.8	6.9	3.2	2.8
C	7.9	4.6	3.5	4.1
D	8.7	5.8	3.7	4.8
E	7.6	4.8	4.0	4.5
F	8.3	3.2	3.2	6.1
G	8.7	4.4	3.9	4.5
H	9.0	3.0	4.3	5.8
I	9.5	2.8	4.8	6.6
J	9.1	2.8	4.6	6.4

TPC= Total Aerobic Plate Count

LAB= Lactic Acid Bacteria

*mean of triplicate determinations

Table 2. Mean counts of food borne pathogens in samples of *wara* cheese vended within Abeokuta metropolis in Ogun state

Pathogen	n	Counts of microorganisms (log CFU/g)	
		Mean	Range
<i>Bacillus cereus</i>	50	3.3	1.9 - 5.7
<i>Salmonella</i> spp	50	0.3	0.00 - 0.7
<i>Staphylococcus aureus</i>	50	6.5	3.5 - 7.4
Presumptive <i>E. coli</i>	50	2.4	0.0 -3.8
<i>Vibrio cholerae</i>	50	0.0	0.0

n- number of samples tested

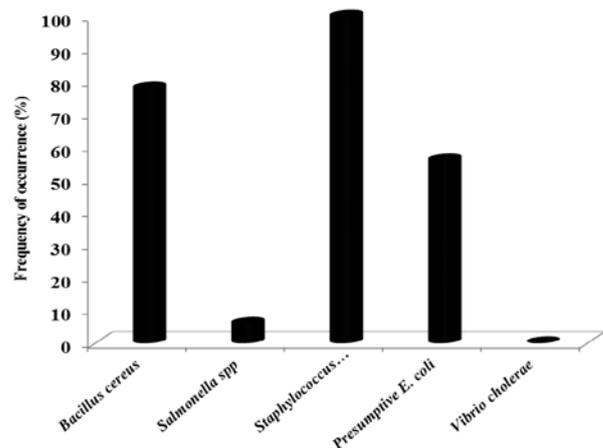


Figure 1. Prevalence and frequency of occurrence of food borne pathogens in samples of *wara* cheese vended within Abeokuta metropolis in Ogun state

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