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Effect of Temperature and Modified Vacuum Packaging on Microbial Quality of Wara A West African Soft Cheese

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ABSTRACT
This study introduced vacuum packaging into wara a West African soft cheese storage.Wara was vacuum packaged and stored in whey. Samples were taken during 5 and 21 day storage period at 15 and 28°C to determine populations of total aerobes(TA), anaerobes, Enterobacteriacea, psychrotrophs, as well as molds and yeasts(M/Y) in Log_{10} CFU g^{-1}. TA increased from 2.25 and <1.00 to 7.67-8.16 and 5.82-8.33 respectively for *Calotropis procera* processed cheese (CPPC) and Lemon Processed Cheese(LPC) stored in whey at 28 and 15°C during the 5 day storage. Enterobacteriacea were undetectable (<1.00) during the 5 day storage at both temperatures. Anaerobes increased from 2.43 for CPPC and undetectable levels for LPC on 1day of storage to 6.91-8.68 and 5.67-9.01, respectively at 15 and 28°C storage in whey. Population s of M/Y remained undetectable until the 5d when the M/Y increased to 6.16-8.04 and 4.82-7.8, respectively for the CPPC and LPC at 28 and 15°C storage temperatures. In vacuum packaged cheese TA increased from 2.25 and <1.00 to 5.45-6.80 and 4.73-6.45, respectively for CPPC and LPC stored in whey at 28 and 15°C during the 21 day storage. Enterobacteriacea and M/Y were undetectable at the 1 day and at the end of 21 day storage at both temperatures. Anaerobes increased from 2.43 for CPPC and undetectable levels for LPC on 1 day of storage to 4.68-6.76 and 4.8-6.24, respectively at 15 and 28°C at the end of 21 day storage. The study suggests vacuum packaging can be introduced into "wara" storage to further reduce the microbial population.

Key words: Wara cheese, *Calotropis procera*, lemon juice, vacuum packaging, spoilage microorganisms

INTRODUCTION
In Nigeria appropriately 90% of the dairy cattle belong to the Fulani pastoralist who process surplus fresh milk to various nutritious milk products such as nono (sour milk) kindirmo (local yoghurt), manshanu (local butter), and wara (West African soft cheese). Wara is an un-ripened cheese consumed in several parts of West Africa. The cheese is prepared by coagulating fresh cow milk with the rennet extract of Sodom apple (*Calotropis procera*) or pawpaw (*Carica papaya*). The preferred coagulant is from *C. procera* because the cheese

made with this coagulant has a sweeter flavor then that of the cheese made with the other coagulant. The ingredient in the leaves of *C. procera* useful for cheese production is calotropin, an enzyme that curdles milk proteins (Belewu and Aina, 2000). The extract of *C. procera* has also been shown to have some antimicrobial and antifungal properties, respectively by Ahmed *et al.* (2006) and Hassan *et al.* (2006).

Wara has an average shelf life of 2-3 day when stored in whey at ambient temperature (approximately 28°C) (Adegoke *et al.*, 1992; Umoh and Solomon, 2001; Belewu *et al.*, 2005) although its storage at lower temperatures had only a little effect on the microbial counts (Adetunji and Chen, 2009) and the concern in refrigerated food is still listeriosis and botulism. Some kinds of *Clostridium botulinum* grow at refrigeration temperature (Garcia *et al.*, 1987). The wara cheese is usually dip fried in vegetable oil near the end of its shelf life in order to extend the shelf life. Attempts has been made in the recent past to include starter cultures or various preservatives such as propionic acid, sodium benzoate, and sorbic acid in the production of wara (Aworh and Egounlety, 1985; Joseph and Akinyosoye, 1997; Sanni and Onilude, 1999; Belewu *et al.*, 2005). Some of these preservatives have been shown to be effective in inhibiting mesophilic and psychrotrophic bacteria as well as coliforms. However, these preservatives may not be easily accessible to the local cheese processors in West Africa.

Previous laboratory studies have confirmed the presence of pathogenic organisms like *Staphylococcus auerus* and *Listeria monocytogenes* in milk and milk products processed in Nigeria including ice-cream, fermented milk and local butter (Adetunji *et al.*, 2003).

Leave extract of *C. procera* has been shown to introduce the microorganisms naturally associated with them into wara cheese (Adetunji and Chen, 2009). In this study vacuum packaging was introduced into to the storage of wara in order to improve the microbial quality and shelf life of wara cheese.

MATERIALS AND METHODS

Study site: This study was conducted between January to June 2006 at the Department of Food Science and Technology, The University of Georgia, Griffin GA, USA.

Preparation of milk for wara processing: Pasteurized whole milk was purchased from a store at Griffin Georgia. The milk was maintained at 4°C in a cooler and transported to the laboratory where it was stored at 4°C until use. Three liters of this milk each was put in two sterile pots for wara soft cheese processing (Fig. 1).

Samples, packaging and storage: Cheese from the above processing was divided into 16 pieces of 10 g each and divided into two equal groups for 28 and 4°C storage. Four in each group was inserted aseptically into a vacuum bag and sealed with a vacuum machine. The remaining four in each group is then aseptically inserted into sterile nylon bags which are not vacuum packaged; these four served as the control at each storage temperature. The two groups were then stored at the respective temperatures. Another group of cheese was stored in whey at the same storage temperatures as the vacuum packaged cheese.

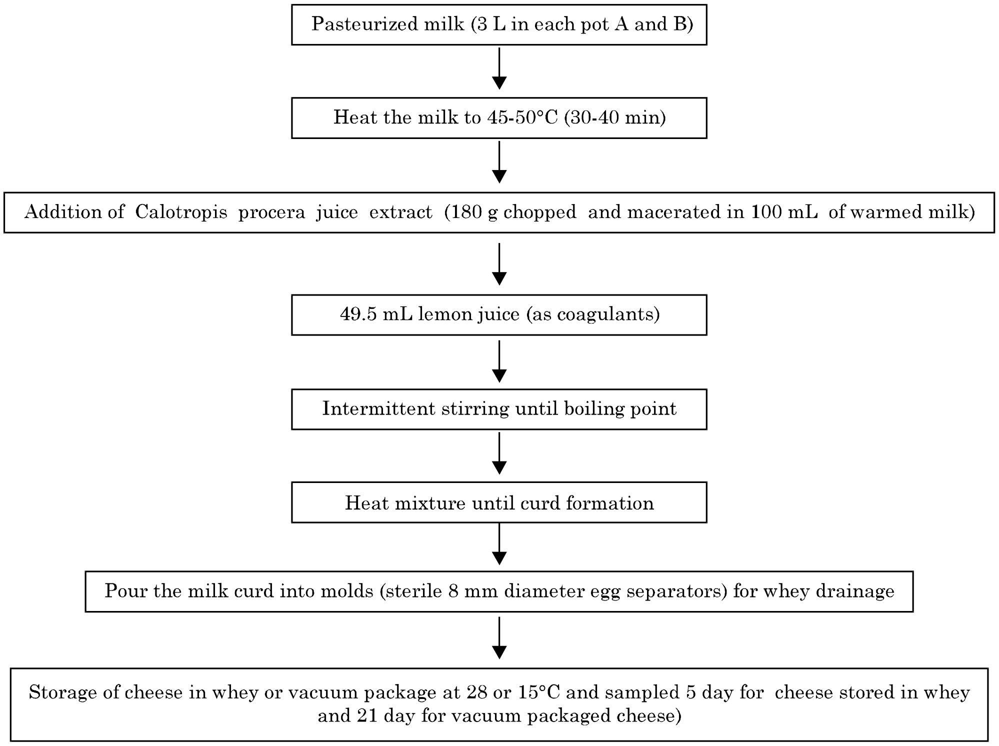

Fig. 1: Flow chart of wara processing

Microbiological sampling: Sampling was done every other day for a period of 5 days for cheese stored in whey and 21 day storage for vacuum packaged cheese. At each sampling day, one bag each of vacuum packaged cheese, one nylon packaged cheese and whey cheese containing 10 g cheese each was used. The pH was taken using a VWR scientific model 1800 electrode pH meter.

The samples were then homogenized within the bags with 10 mL of phosphate buffered saline with Seward Stomacher Lab System on the first sampling day. On subsequent sampling days cheese was homogenized with 20 mL of phosphate buffered saline. Serial dilution was made with 0.1% peptone water for ease of counts. Appropriate dilutions were surface plated on MacConkey agar (Becton, Dickinson and Company) for Enterobacteriaceae counts, potato dextrose agar (PDA) for yeast and molds counts, tryptose soy agar (tsa) for total aerobic bacteria, psychrotrophic and anaerobic bacteria counts. Plates for Eenterobacteriacea, molds and yeast and aerobic bacteria were incubated aerobically at 37°C while psychrotrophic bacteria plates were incubated at 6-7°C for 5-7 days. Plates for anaerobic bacteria were incubated anaerobically at 37°C in a GasPak system.

Statistical analysis: The study was performed in two replicates, each with appropriate duplications. All microbiological data were transformed into Log_{10} CFU mL^{-1} or Log_{10} CFU g before comparison of means. Analysis of data was accomplished using the Fisher's least significant difference (Glimm *et al*., 1997) of means of bacterial populations calculated with the General Linear Model (GLM) procedure of SAS based on a 95% confidence level (SAS, 2000).

RESULTS AND DISCUSSION

In wara cheese stored in whey, general the population of total aerobes, anaerobes, molds and yeasts counts increased with storage days except on two occasions in whey stored wara cheese.

Table 1: Microbial counts and pH of *Calotropis procera* and Lemon juice cheese stored in whey

Cheese type	C.P cheese 28°C	C.P cheese 15°C	Lemon juice cheese 28°C	Lemon juice cheese 15 °C
Total aerobes				
D1	2.25a	2.25a	<1.00b	<1.00b
D2	7.04 a	3.34 b	6.45a	<1.00c
D3	7.25a	4.88b	7.19a	<1.00d
D4	8.79a	6.59c	8.04b	<1.00d
D5	8.16a	7.67a	8.33a	5.82
Enterobactericea				
D1	<1.00a	<1.00a	<1.00a	<1.00a
D2	<1.00a	<1.00a	<1.00a	<1.00a
D3	<1.00a	<1.00a	<1.00a	<1.00a
D4	<1.00a	<1.00a	<1.00a	<1.00a
D5	<1.00a	<1.00a	<1.00a	<1.00a
Anaerobes				
D1	2.43a	2.43a	<1.00b	<1.00b
D2	7.13a	4.35c	5.57b	<1.00d
D3	7.1a	4.88b	7.08a	<1.00c
D4	9.28a	6.61b	8.52a	<1.00c
D5	8.68a	6.91b	9.01a	5.067c
Molds and yeast				
D1	<1.00a	<1.00a	<1.00a	<1.00a
D2	<1.00a	<1.00a	<1.00a	<1.00a
D3	<1.00a	<1.00a	<1.00a	<1.00a
D4	<1.00a	<1.00a	<1.00a	<1.00a
D5	8.04a	6.16b	7.8a	4.82c
PH				
D1	6.14a	6.14a	5.65b	5.65b
D2	6.23b	6.39a	5.57d	5.69c
D3	6.17b	6.37a	5.53d	5.65b
D4	5.58b	6.35a	5.56b	5.64b
D5	5.49c	6.34a	5.55cb	5.16b

C.P = *Calotropis procera* cheese ; D= storage days, Values followed by same letters in a same row are not significantly different with respect to storage temperature.

Enterobacteriacea counts were undetectable through out storage and pH dropped with extension of storage period in whey stored cheese (Table 1). In vacuum packaged wara cheese molds and yeast counts which were initially undetectable became detectable by the 4 to the 7 day of storage and then remained undetectable to the end of the 21 day storage period. Enterobacteriacea counts were undetectable throughout storage except on few occasions on days 7 and 11 of storage. pH which decreased from 5.65-6.14 on day 1 to 4.97-6.22 on day 11 also decreased from 5.23- 6.26 on day 14 to 4.84- 6.17 on day 21 (Table 2). A significance difference occurred between storage temperatures and *C. procera* and Lemon juice processed cheese.

Some types of whey cheeses are supposed to be consumed within a short time upon manufacture (e.g., Ricotta, Requeijão and Manouri), whereas others bear a longer shelf life (e.g., Gjetost, Mysost and Myzithra). Whey cheeses are significantly different from one another in terms of chemical composition, which is mainly due to variations in the source and type of whey, as well as to the processing practices followed (Pintado *et al.*, 2001).

Table 2: Microbial counts and pH of vacuum packaged *Calotropis procera* cheese and lemon juice cheese for 21 day storage at 28 and 15°C

Cheese type Storage temp.	C.P cheese 28°C	C.P cheese 15°C	Lemon juice cheese28°C	Lemon juice cheese 15°C
Total aerobes				
D1	2.25a	2.25a	<1.00b	<1.00b
D4	6.07a	<1.00c	5.82a	2.43b
D7	8.48a	<1.00c	7.50b	1.00c
D11	7.25c	8.18b	10.73a	6.35d
D14	5.98a	6.70a	7.24a	6.60a
D18	6.03ba	6.64a	5.24b	5.3b
D21	5.45b	6.80a	6.45a	4.73b
Anaerobes				
D1	2.43a	2.43a	<1.00b	<1.00b
D4	5.67a	3.46b	5.79a	2.91b
D7	9.32a	3.9c	7.65b	2.81d
D11	8.24b	7.71b	9.95a	8.38b
D14	6.72ba	7.71a	7.52a	6.0b
D18	6.29a	6.11a	5.45a	5.23a
D21	4.8b	6.76a	6.24a	4.68b
Molds/yeast				
D1	<1.00a	<1.00a	<1.00a	<1.00a
D4	7.39b	4.24c	8.06a	4.15c
D7	3.6b	<1.00c	5.46a	3.63b
D11	<1.00a	<1.00a	<1.00a	<1.00a
D14	<1.00a	<1.00a	<1.00a	<1.00a
D18	<1.00a	<1.00a	<1.00a	<1.00a
D21	<1.00a	<1.00a	<1.00a	<1.00a
Enterobactericea				
D1	<1.00a	<1.00a	<1.00a	<1.00a
D4	<1.00a	<1.00a	<1.00a	<1.00a
D7	2.76a	2.40a	<1.00b	1.35b
D11	1.28a	<1.00a	<1.00a	<1.00a
D14	<1.00a	<1.00a	<1.00a	<1.00a
D18	<1.00a	<1.00a	<1.00a	<1.00a
D21	<1.00a	<1.00a	<1.00a	<1.00a
PH				
D1	6.14a	6.14a	5.65b	5.65b
D4	5.6d	6.54a	5.79b	5.7c
D7	5.49c	6.52a	5.59cb	5.65b
D11	5.24c	6.22a	4.97d	5.63b
D14	5.7a	6.26a	5.23a	5.66a
D18	5.9a	5.97a	4.49b	5.7a
D21	6.71a	5.92b	4.84d	5.7c

CP = vaccum Calotropis cheese; Values followed by same letters in a same row are not significantly different with respect to storage temperature($p<0.05$).

Generally the populations of total aerobes and anaerobes, increased with extension of storage time at 15 and 28°C and the effect of storage temperature on total aerobes and anerobes was significant ($p<0.05$) in this study. The populations of total aerobes and anaerobes increased more rapidly in wara cheese stored at 28°C as compared to 15°C. The populations of these two groups of microorganisms were significantly different in cheese samples stored at 28°C compared to the samples stored at 15°C ($p<0.05$). This is probably because the temperature of 28°C is more close to the optimal growth temperatures of these organisms. Lower storage temperatures such as 15°C slow down microbial metabolic activities therefore, retarding the proliferation of microbial cells (Jay, 2000). Enterobacteriacea were undectected throughout storage and molds and yeast counts were undetec until the 5 day of storage in whey cheese. This probably due to a low level contamination of of these microbes in the raw milk used for processing. The slight decrease in pH along the storage days is likely due to increase in the number acid–producing bacteria.

The effect of vacuum packaging was greatest on molds and yeast and enterobactericea counts which were undetec by the end of 21 day storage. Similar report were made in Requeijao a traditional Portuguese whey cheese which was packaged under vacuum, Yeasts were severely inhibited. Packaging under vacuum decreased yeast growth rates and also reduced the populations attained at the end of the exponential phase of growth, resulting in a shelf-life extension of the Stracchino cheese of over 28 days (Sarais *et al.*, 1996). A similar reduction in coliform counts along storage days of cheese has earlier been reported (Alalade and Adeneye, 2006). This report is similar to entero bacteriacea counts, which were undetec in this study. Similarly an extension in shelf-life was reported in vacuum packaged gas flushed bananas (Chauhan *et al.*, 2006). The inhibition of microbes in vacuum packaged cheese is because air is evacuated from gas-impermeable pouches followed by sealing (Jay, 2000). But the count of total aerobes despite the vacuum packaging is an indication that wara is porous and still has some air stored within its matter thereby encouraging multiplication of aerobes. The presence of anaerobes throughout the storage is expected because the wara was stored in an anaerobic condition. These findings are particularly important in terms of improving wara and other dairy products. Moisture content and pH of whey cheeses are usually high and favor microorganism growth (molds, yeasts, lactic acid bacteria and Enterobacteriaceae accounting for the dominant microflora in these cheeses). A positive correlation was earlier reported between coliform bacterial count and moisture content (Alalade and Adeneye, 2007). The use of lemon juice as a sanitizer and bacteriocidal agent against pathogens has been reported (Sengun and Karapinar, 2005; Sengun and Karapinar, 2004) this explains the reason for the significant difference in all counts between the *Calotropis procera* and lemon juice processed cheese.

CONCLUSION

Our findings elucidated the limitation of shelf-life duration in ‘wara’ cheese in a developing country (Nigeria). Overall our data indicate that vacuum packaging suppressed molds, yeast and enterobacteriacea. The use of lemon juice in wara processing also suppressed all the microbes in this study. This study suggests that ‘wara’ cheese (as well as other whey cheeses and dairy products) could be vacuum packaged to reduce the risks of food poisoning bacteria and extend the shelf-life of these products.

Adequate packaging of whey cheeses should be provided, and legislation should be prepared to fix standard characteristics of each type of whey cheese.

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