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Comparative assessment of the effect of crude extracts of *Carica papaya* and *Terminalia cattapa*, and a bacteriocin on vacuum-packed West African soft cheese ('wara')

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This study evaluated the survival of Enterobacteriaceae, molds and yeasts in vacuum-packaged soft cheese treated independently with crude extracts of *Carica papaya* (Vcpc) and *Terminalia cattapa* (Vtcc), a bacteriocin (Vnc), and a combination of the three treatments (V+3). The cheese was then stored at 15 and 28°C, respectively, for three weeks. Enterobacteriacea were not detected in the Vcpc- and Vtcc-treated cheese at 15°C after three weeks of storage, while the yeasts and moulds were not detected in any of the treatments throughout the storage period. Inhibition of enterobacteriaceae was not apparent in Vnc- and V+3-treated cheese, but significant differences were observed regarding the microbial counts at the two storage temperatures and periods ($P < 0.05$). It can therefore be concluded that vacuum packaging and addition of crude extracts of *C. papaya* and *T. cattapa* to the soft cheese ('wara') can suppress growth of enterobacteriacea, molds and yeasts, thus leading to extension of the shelf-life of the soft cheese ('wara').

Key words: Enterobacteriaceae, molds, yeast, vacuum package, soft cheese, *Carica papaya*, *Terminalia cattapa*.

INTRODUCTION

West African soft cheese, 'wara', has a relatively short shelf-life of 2 - 3 days. Spoilage of soft cheese is mainly caused by Gram-negative psychrotrophic bacteria species such as *Pseudomonas* spp., *Proteus* spp. and *Aeromonas* spp., which result in undesirable off -flavors, pigment formation or slimy curd (Kosikowski and Brown, 1973; Rocklehurst and Lund, 1985, 1988). Growth of yeasts and molds, such as *Geotrichum* spp., *Penicillium* spp., *Mucor* spp. and *Alternaria* spp. have also been implicated in the spoilage and change in flavour, texture and appearance of cottage cheese (Chen and Hotchkiss, 1993). Other foods that have been implicated in food-borne infections include: Mexican soft cheese (Linnan et al., 1988), coleslaw (Schlech et al., 1983), prepared salads (Gellin and Broome, 1989) and paté (Morris and Ribeiro, 1989). The reports of Adetunji et al. (2003) and Adeyemi et al. (2003) also implicated food-borne pathogens such as *Stapthylococcus aureus*, *Bacillus cereus*, *Clotridium perfrigens*, *Clotridium botulinum*, *Brucella abortus*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp. In unripened soft cheese ('wara').

The use of bacteriocins, modified atmosphere packaging, protective cultures and enzymes has been employed to improve shelf-life of soft cheese (Jay, 2000). They may also be used synergistically to obtain a higher microbial quality of the preserved products. An example of synergy is the inactivation of spores of *Bacillus cereus* in cheese by high hydrostatic pressure with the addition of nisin or lysozyme (Lopez-Pedemonte, 2003). Attempts have been made previously to add starter cultures or preservatives such as propionic acid, sodium benzoate and sorbic acid in the production of 'wara' (Aworh and Egounlety, 1985; Belewu, 2005). Some of these preservatives have been shown to be effective in inhibiting mesophilic and psychrotrophic bacteria, as well as coliforms (Aworh and Egounlety, 1985; Belewu, 2005). Vacuum packaging and modified atmospheric packaging have also been used for the preservation of many products, including cheese (Kader et al., 1989; Gorny, 1997; Pintado and Malcata, 2000). These methods reduce the

oxygen level, which retard browning and spoilage, thereby maintaining the fresh appearance of the product (Jay, 2000). However, it can cause off-flavor and flavor loss (Cameron and Smyth, 1997). In the case of meats, up to 10 - 20% carbon (IV) oxide may develop within 4 h and the concentration may ultimately reach 30% from the respiratory activities of the aerobic biota (Gardner et al., 1967).

Carica papaya and *Terminalia catappa* have been widely reported to have antimicrobial properties (Lai et al., 1976; Tang et al., 1976; Pawar and Pal, 2002; Kermanshai et al., 2003) and nisin, a bacteriocin, produced by lactic acid bacteria has been shown to inhibit *Listeria monocytogenes* in cottage cheese stored at 4°C (Benkerroum, 1988). In this study, we report on the use of medicinal plant extracts, a bacteriocin and vacuum packaging as means to preserve and to improve the microbial quality of 'wara' cheese.

MATERIALS AND METHODS Preparation

of milk for 'wara' processing

Four liters of pasteurized whole milk was purchased from a store. The milk was maintained at 4°C in a cooler and transported to the laboratory where it was stored at 4°C until use. This milk was then put in a sterile pot for 'wara' soft cheese processing and heated to approximately 50°C for 30 - 40 min after which freshly-squeezed lemon juice (49.5 ml) was added to the warm milk. The milk and lemon juice mixture were heated to boiling with intermittent stirring. The mixture was kept at the boiling point until it coagulated and the separation of the curd and whey became visible. The milk pots were then removed from the heating source, and the curds and whey were ladled into sterile egg separators (8 mm in diameter), which facilitated whey drainage and gave the cheese its characteristic shape and size. The yield from this process was 600 g of soft ripened cheese that was ready for further treatments.

Preparation of crude plant extracts

Leaves of *T. cattappa* and *C. papaya* were harvested and dried in the sun for 4 weeks. *T. cattappa* (2.38 g) was milled and the powder was extracted in 10 ml of 70% ethanol. *C. papaya* (4.5 g) was ground and extracted in 20 ml of 70% ethanol to obtain a concentration of 0.2 - 0.25 mg/ml. After extraction the mixtures were sieved and the resulting liquid was concentrated in a rotavapour at 50°C for 72 h.

Treatment of cheese samples

The processed cheese samples were divided aseptically into 10 g portions and four sample groups of 8 from the cheese portions were subjected to the treatments indicated below in Table 1. A fifth group served as an untreated control.

One pair of each treatment was vacuum-packaged, using a vacuum packaging machine. This was an automated system that suctions out air completely from the gas-impermeable material (nylon bags), followed immediately by sealing. The samples were then stored at 28 and 15°C (refrigeration) for a 3 week storage period. All experiments were replicated.

Microbiological analysis

Sampling was done once every week over the 3 week storage period and separate samples were analyzed to represent various weeks of storage on each sampling day. The pH of the samples was determined using a VWR scientific model 1800 electrode pH meter. The samples were then homogenized within each bag with 10 ml of 0.1% peptone buffer on each sampling day using a Seward Stomacher. Aliquots of the serial dilutions were then made in sterile 0.1% peptone water, while appropriate dilutions were then surface plated onto MacConkey agar for the enterobacteriacea counts and onto potato dextrose agar for molds and yeasts counts. The culture plates for the enterobacteriacea and molds and yeasts counts were incubated aerobically at 37°C for 18 h. The study was performed in two replicates, each with appropriate duplicates.

Statistical analysis

All the microbiological data were transformed to $\log_{10}$ CFU/g before comparison of means. Analysis of data was accomplished using a five by two factorial design. Means of bacterial populations were compared using analysis of variance (ANOVA) and a post-hoc test procedure of SPSS (2003), based on a 95% confidence level.

RESULTS

There were significant differences in enterobacteriacea counts of the vacuum-packaged cheese treated with crude extracts of *C. papaya* (Vcpc) and *T. cattapa* (Vtcc), bacteriocin-treated cheese (Vnc) and a combination of the three (V+3) treatments at 15 and 28°C (Table 1). A significant difference was also observed between the two storage temperatures ($P < 0.05$). As shown in Table 1, the enterobacteriacea counts were undetectable (<1.0 $\log_{10}$ cfu/g) in cheese samples treated with crude extracts of *C. papaya* (Vcpc) and *T. cattapa* (Vtcc) at 15°C in the third week of storage, but the enterobacteriacea were detected in the bacteriocin-treated cheese samples. No significant differences ($P > 0.05$) were also observed in the enterobacteriacea counts in Vcpc (28°C) and Vtcc (28 and 15°C) cheese samples during the second week of storage. However, there were significant differences ($P < 0.05$) in the enterobacteriacea counts in all the treatments during the third week of storage at 28°C. Molds and yeasts were undetectable throughout the storage period at the two storage temperatures in all the treatments, while there was also no significant difference ($P > 0.05$) in the mold and yeast counts in all the treatments through-out the storage period.

The pH of the cheese samples in different storage medium varied significantly with temperature and in the first and second week of storage. A decrease in the pH from 5.72 and 5.76 to 5.46 and 5.48 was observed in Vcpc- and V+3- treated cheese samples, respectively, at 28°C. Similarly, a decrease occurred from 5.64 and 5.08 to 5.45 and 4.61 in Vcpc- and Vnc-treated cheese samples, respectively, at 15°C during the first and third week of storage. On the other hand, an increase in pH from 4.94 and 5.74 to 5.1 and 5.85 was observed in Vnc- and V+3-treated cheese sample at 28 and 15°C, respectively (Table 1).

DISCUSSION

'Wara', a West African soft cheese, is an indigenous fer-

Table 1. Microbial counts and pH of vacuum-packaged soft cheese treated with crude extracts of *Carica papaya* and *Terminali cattapa*, a bacteriocin and a combination of all three

Storage	Vcpc	Vcpc	Vtcc	Vtcc	Vnc	Vnc	V+3c	V+3	Vc (cont.)	Vc (cont.)
Temperature	28^{o}C	15^{o}C	28^{o}C	15^{o}C	28^{o}C	15 ^{o}C	28^{o}C	15^{o}C	28^{o}C	15^{o}C
Enterobacteriaceae										
Day 1	<1.00+0.00^{a}	<1.00+0.0^{a}	<1.00+0.0^{a}	<1.00+0.0^{a}	<1.00+0.0^{a}	<1.00+0.0^{a}	<1.00+0.0^{a}	<1.00+0.00^{a}	<1.00+0.00^{a}	<1.00+0.00^{a}
First week	3.92+0.005^{c}	<1.00+0.0^{e}	3.72+0.15^{d}	3.81+0.05^{b}	4.53+0.08^{b}	4.64+0.05^{b}	3.92+0.05^{c}	3.99+0.005^{c}	5.46+0.00^{a}	3.63+0.00^{d}
Second week	2.64+0.02^{g}	2.24+0.02^{e}	2.75+0.06^{g}	2.94+0.05^{g}	7.47+0.02^{a}	6.62+0.24^{b}	3.78+0.09^{c}	3.6+0.2^{c}	<1.00+0.00^{f}	<1.00+0.005
Third week	4.26+0.005^{e}	<1.00+0.0^{g}	2.85+0.05^{f}	<1.00a+0.0^{g}	8.27+0.03^{a}	8.16+0.03^{b}	5.4+0.005^{c}	4.49+0.005^{d}	<1.00+0.00^{g}	^{f}<1.00+0.00^{g}
Molds/yeast										
Day 1	<1.00+0.00^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}
First week	<1.00+0.00^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	1.35+0^{a}
Second week	<1.00+0.00^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}
Third week	<1.00+0.00^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	<1.00+0^{a}	**<**1.00+0^{a}	**<**1.00+0^{a}
pH										
Day1	5.75+0.00^{b}	5.75+0.00^{b}	5.75+0.00^{b}	5.75+0.00^{b}	5.75+0.00^{b}	5.75+0.00^{b}	5.75+0.00^{b}	5.75+0.00^{b}	5.65+0.00^{c}	5.65+0.00^{c}
First week	5.72+0.005^{d}	5.64+0.01^{c}	5.6+0.005^{d}	5.49+0.02^{e}	4.94+0.00^{g}	5.08+0.005	5.76+0.00^{a}	5.74+0.005^{b}	5.59+0.00^{d}	5.65+0.005^{c}
Second week	5.48+0.005^{d}	5.7+0.005^{b}	4.91+0.01^{f}	5.71+0.01^{b}	4.57+0.00^{g}	^{f}4.92+0.005	5.46+0.00^{d}	5.81+0.01^{a}	5.23+0.00^{e}	5.66+0.005^{c}
Third week	5.46+0.05^{c}	5.45+0.00^{c}	4.62+0.005^{f}	5.48+0.03^{c}	5.05+0.05^{d}	^{f}4.61+0.005^{f}	5.48+0.02^{c}	5.85+0.05^{a}	4.84+0.005^{e}	5.7+0.005^{b}

Numbers with similar alphabets showed no significant differences (P > 0.05) along the same row
Numbers with different alphabets showed significant differences (P < 0.05) along the same row
Vcpc = vacuum-packaged *Carica papaya* treated cheese^{a}
Vtcc = vacuum-packaged *Terminali cattapa* treated cheese^{b}
Vnc = vacuum-packaged bacteriocin-treated cheese (The bacteriocin-like substance used was extracted from pure cultures of *Lactococuss lactis* after boiling cultures at 100^{o}C for 25 min and its inhibitory effect observed on pure cultures of *L*. *monocytogenes* and *E*. *coli* O157:H7)
V+3 = vacuum-packaged cheese treated with *Carica papaya*, *Terminali cattapa* and bacteriocin
Vc = control vacuum-packaged cheese with no treatment

mented cow's milk product from Nigeria that is very popular among all the tribes of the country. However, it has a relatively short shelf-life due to the presence of some food-borne microbial flora, comprising of bacteria and fungi (Adetunji et al., 2003; Adeyemi et al., 2003). In this study, entero-bacteriaceae were enumerated since its members are involved in food spoilage, serve as indicators of fecal contamination of food products and some are food-borne pathogens.

Although fermentation of most Nigerian foods, such as 'wara', is widespread the production of such foods is still largely a traditional family art and is done under highly variable conditions. The method employed in the manufacture of fermented foods differs from one region to the other, because these processes are based on traditional systems, according to local custom, climate conditions, type of substrates used and process variations. In general, their fermentation takes place under conditions which the producers have found to be favourable for the appropriate growth and activity of the fermenting microorganisms (Achi, 2005). Consequently, food-borne bacteria have been detected in most of these indigenous fermented foods.

According to González-Aguilar et al. (2008), food preservation is critical for keeping the global food supply safe and available for consumers. Therefore, food scientists study production and processing to develop new technologies that improve the quality and quantity of healthy food products, since the main goal is to safely increase yields with effective quality control and to preserve the environment and fulfill consumer expectations.

The treatments evaluated for the control of the

enterobacteriacea, yeasts and molds present in wara' samples during the course of this study have indicated that there was a reduction in enterobacteriacea, yeast and mould counts in the 'wara' samples. This may be a result of the modification of the cheese environment due to the carbon (IV) oxide production during the cellular respiration of the associated microbes. The inhibitory effects of carbon (IV) oxide and oxygen depletion had also been reported earlier by Gould (1989), Farber (1991) and Whitley et al. (2000).

The significant differences recorded in the microbial loads of the 'wara' samples at 15 and 28°C storage temperatures can also be attributed to the changes in the respiration rates of the microorganisms, as influenced by temperature changes. This is supported by a report of Izumi and Watada (2004), who reported that the respiration rates of carrots increased by about five-fold when temperature increased from 0 to 10°C, and the increase in microbial population was also found to be a 100 fold greater at 10°C than at 0°C. The significant inhibition of yeasts and moulds in all the treatments, as observed in this study, was likely due to oxygen depletion due to the vacuum packaging process. This explanation is consistent with reports on severe inhibition of yeasts by vacuum packaging (Gonzalez-Aguilar et al., 2004).

The use of bacteriocins has been employed to improve the shelf-life of soft cheese (Jay, 2000). The inability of bacteriocins to suppress the growth of food-borne microorganisms associated with 'wara' in this study, despite its well known antimicrobial abilities, could be due to its limited spectrum of activity. Earlier reports also showed differences in the efficacy of bacteriocins produced by various strains of *Lactococcus* spp. (Adetunji and Adegoke, 2007). The lower pH recorded as a result of addition of the bacteriocin was not inimical to the microbial growth. The reason for this is not known, but it is probably due to insignificant changes along the storage days.

Antibacterial activities of extracts of *C. papaya* and *T. catappa* have been evaluated against some microorganisms and have been widely reported to have antimicrobial properties (Lail et al., 1976; Tang et al., 1976; Pawar and Pal, 2002; Kermanshai et al., 2003) against the test organisms. The effectiveness of vacuum-packaged 'wara' (soft cheese), treated with *C. papaya* and *T. cattapa* leaf extracts in this study, is both time- and temperature-dependent.

Pure extracts of these plants produced better results, but the combination of the two plants' crude extracts and the bacteriocin in preservation of the samples, however, did not improve the microbial quality of the cheese. Vacuum-packaged cheese treated with *C. papaya* (Vcpc) or *T. cattapa* (Vtcc) also suppressed the enterobacteriacea counts to an undetectable level at 15°C during the third week of storage. It is, therefore, evident from this study that lower temperature storage with the addition of either of the two leaf extracts will give an improved microbial quality.

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