Effects of Packaging, Treatments, and Storage Conditions on the Survivability of Aerobes and Anaerobes in Vacuum Packaged ‘Wara’ a Soft White Cheese

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Abstract: This study assessed total aerobes and anaerobes in vacuum packaged ‘wara’ a West African soft white cheese processed with lemon juice as coagulant. The cheese was treated with Carica papaya (Vcpc), Terminalia cattapa (Vtcc) crude extracts, nisin (Vnc), and the combination of these three treatments (V+3) and stored at 15 and 28ºC for a three weeks storage period. The total aerobes and anaerobes were suppressed to an undetectable level (<1.00 logcfu/mL) in Vcpc at 15ºC in the first week of storage. In Vtcc the total aerobes which was initially 6.24 logcfu/mL in the first week of storage was suppressed to 3.16 log cfu/mL by the 3rd week of storage. Inhibition of microbes was not apparent in Vnc and V+3. Significant differences occurred in the microbial count at the two storage temperatures and storage weeks (p<0.05). It can therefore be concluded from this work that Vacuum packaging and addition of crude extracts (Carica papaya, Terminalia cattapa) in soft cheese storage can suppress total aerobes and anaerobes) Therefore their use in extension of the shelf-life of soft cheese is recommended.

Key words: Aerobes, anaerobes, Carica papaya, Terminalia cattapa, nisin, soft cheese

INTRODUCTION

The number of microorganisms found on produce can vary according to a number of factors including product quality, product characteristics, and normal microflora of the product, handling, processing, storage, distribution and retail display (Gonzalez-Fandos et al., 2000). Microbial growth is influenced by the physiology of the minimal processed product, as well as pre-harvest management, pre- and post-processing treatments and application of an appropriate packaging (Watada, 1997). The utmost aim of preservation is to prevent food spoilage of easily perishable products by extending their shelf-life to meet market demands. Some of the modern technologies employed include modified atmosphere packaging, use of protective cultures, use of bacteriocins and other culture products and enzymes (Gould, 1996).

Wara an unripened soft cheese has an average shelf-life of 2-3 days when stored in whey at ambient temperature (approximately 28ºC) (Adegoske et al., 1992; Belewu et al., 2005). Attempts have 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 (Belewu et al., 2005; Aworh and Egounlely, 1985). 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. Therefore, the need for improvement on the methods of preservation of the soft cheese in this study.

Vacuum packaging and modified atmospheric packaging have been used for the preservation of many products: fruits, vegetables, cheese among others (Kader et al., 1989; Gorny, 1997; Pintado and Malcata, 2000). These methods reduce the oxygen level which retards browning and spoilage and maintain fresh appearance (Ray, 2000). However, it can cause off-flavour and flavour loss (Cameron and Smyth, 1997). In the case of meats, up to 10-20% carbon (iv) oxide may develop within 4 hours and the concentration may ultimately reach 30% from the respiratory activities of the aerobic biota (Gardner et al., 1967).

These methods and others may be used synergistically to further obtain higher microbial quality of the preserved products. One of such is the inactivation of spores of Bacillus cereus in cheese by high hydrostatic pressure with the addition of nisin or lysozyme (Lopez-Pedemonte et al., 2003).

Carica papaya a milk coagulant (Veringa, 1961) has also been used for medicinal purposes (Tang et al., 1976; Lal et al., 1976; Kermanshai et al., 2003). Antihelmintic and antibacterial effect of extracts of Carica papaya has been demonstrated (Adebiyi and Adaikan, 2005). Terminalia cattapa is also of high medicinal value. It has been shown to have anticarcinogenic potential, aphrodisiac activity and also used in the treatment of sickle cell disorders (Ratnasooriya and Dharmasi, 2000). The wound healing potential of some species of Terminalia grown in Mali and the antimicrobial activity of extracts Terminalia cattapa root have been described (Diallo et al., 2002; Pawar and Pal, 2002).
Nisin is a bacteriocin produced by Lactococcus lactis sub-specie lactis. It has been shown to inhibit Listeria monocytogenes in cottage cheese stored at 4°C (Benkerroum and Sandine, 1988). It was also observed that bacteriocin (nisin) delayed the growth of psychrotrophic organisms that cause cheese spoilage. Non-sterilized cottage cheese samples without nisin spoiled 1 week earlier than those with nisin (Benkerroum and Sandine, 1988).

This work assesses total aerobes and anaerobes in vacuum packaged lemon juice cheese treated with two indigenous plants Carica papaya and Terminalia catappa crude extracts and the bacteriocin-like substance extracted from Lactococcus lactis.

**MATERIALS AND METHODS**

**Preparation of the Nisin:** A bacteriocin(nisin) producing Lactococcus lactis strain was isolated from unpasteurized raw milk according to Barrow and Feltham (1993). Test for bacteriocin production was done according to Aslim et al. (2005) with slight modification. 24 h fresh culture of Lactococcus lactis strains showing inhibitory qualities against test (Listeria monocytogenes and E. coli) (laboratory stock cultures from food) bacteria was grown from raw milk according to Barrow and Feltham (1993). Test strains showing inhibitory qualities against test (Listeria monocytogenes and E. coli) (laboratory stock cultures from food) bacteria was grown against test (Listeria monocytogenes and E. coli) (laboratory stock cultures from food) bacteria was grown on M17 agar and inoculated into tryptose soy broth and incubated at 37°C for 24 h. This was then incubated at 37°C for 24 h. The 24 h broth culture was then centrifuged at 3000 rpm for 5 min. The supernatant was decanted into sterile test tubes, adjusted to pH 6.5-7.0 with NaOH (40G/1000 mL) to remove organic acid effect. H2O2 was neutralized by addition of catalase from bovine liver at 200 μ/mL. The mixture of the supernatant of Lactococcus lactis culture, NaOH and catalase was filtered and sterilized with a 0.2 μm Millipore filter membrane. Inhibitory effect of free bacteriocin (nisin) on test bacteria was then determined by agar well diffusion method using the filtrate from the mixture and the supernatant alone. Mueller Hinton II agar plates were punched with 8 mm diameter sterile clubs. The bottom of each well was covered with viscous agar which was allowed to solidify to prevent spreading of the mixture and supernatant through the base. The wells were then filled with 20 μL of the mixture and filtrate of supernatant alone thereafter was refrigerated for 2 h to allow diffusion of test substrate, followed by incubation for 24 h at 37°C. The diameter of inhibition was then measured with meter ruler. A zone of inhibition of 18-20 mm was reported against the two foodborne pathogens.

**Preparation of milk for wara processing:** Four liters of 12% fat 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 is stored at 4°C until use. This milk was put in a sterile pot for ‘wara’ soft cheese processing. The milk described above was heated to approximately 50°C in about 30-40 min and stirred gently during heating using magnetic stir bars. Freshly squeezed lemon juice (49.5 mL) was added to the warm milk, and the milk and lemon juice mixture was heated with intermittent stirring until it reached the boiling point. The milk with added lemon juice was kept at the boiling point until it coagulated and the separation of 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. Yield from this process was 600 g of soft ripened cheese ready for treatment with crude extracts.

**Preparation of crude extracts:** Terminalia catappa (2.38 g) was grinded to powder and extracted in 10 mL of 70% ethanol. Carica papaya (4.5 g) was also grinded and extracted in 20 mL ethanol to obtain between 0.2-0.25 g/mL concentration. After extraction the mixture was sieved and the resulting liquid was concentrated in a rotavapour (Drissa et al., 2002).

**Sample preparation, packaging and storage:** Wara cheese from the above process was divided aseptically into 60 10 g pieces. Then five sample groups of 8 from these pieces were then subjected to treatment by drenching with 1 mL.

- Each of 1. Terminalia catappa seed extracts 2. Carica papaya seed extracts 3. Nisin 4. Treatment with all 3 (T. catappa and C. papaya extracts and nisin) 5. Untreated control. All cheese samples after treatment were stored in vacuum packaged bags. One of each pair treatment was stored at 28°C while the other pair was stored at 15°C for a 3 week storage period.

**Microbiological sampling:** Sampling was done on every week for a 3 weeks storage period. A separate sample was used representing the various weeks of storage on each sampling day. The pH of the samples was taken using a VWR scientific model 1800 electrode pH meter. The samples were then homogenized within each bag with 10mls of 0.1% peptone buffer with Seward Stomacher Lab. System on each sampling day. Serial dilution was made with 0.1% peptone water for ease of counts. Appropriate dilutions were then surface plated on tryptose soy agar for total aerobic bacteria, and anaerobic bacteria counts. Plates for aerobic bacteria were incubated aerobically at 37°C for 18hours while plates for anaerobic bacteria were incubated anaerobically at 37°C in a GasPak system for 18 h. Discrete colonies were counted using a darkfield colony counter. Counts were expressed in cfu/mL and then converted to log10 cfu/mL.
RESULTS

There were significant differences in the total aerobes and anaerobes of the vacuum packaged lemon juice cheese treated with *Carica papaya* (Vcpc), *Terminalia cattapa* (Vtcc) crude extracts, nisin (Vnc), the combination of the three (V+3), and the control experiment (Con.C) at 15 and 28°C, respectively (Table 1). At 28°C storage temperature, the total aerobes in the 1st, 2nd and 3rd week were 7.61, 7.59, and 5.46 log CFU/mL in Vcpc, 6.68, 6.48, and 4.84 log CFU/mL in Vtcc, 7.11, 5.04, and 4.23 log CFU/mL in Vnc, respectively. The corresponding values at 15°C storage temperature were 4.73, 4.61, and 3.16. The total aerobes were undetectable (< 1.00) in Vcpc, Vtcc, and Vnc at 15°C storage temperature. However, significant reductions from 7.39-4.84, 9.55-7.14 and 7.41-6.73 log CFU/mL were obtained in Vtcc, Vnc, and V+3 respectively at 28°C storage temperature in the 1st and 2nd week of storage. A sharp increase in anaerobes from 4.84-5.7, 7.14-10.82 and 6.73-7.75 log CFU/mL was observed in Vtcc, Vnc, and V+3 respectively at 28°C storage temperature in the 3rd storage week. Counts were undetectable in Vcpc in the 1st storage week at 15°C storage temperature but increased rapidly to 5.28 log CFU/mL in the 3rd week. The total anaerobes which were 5.92 and 6.83 log CFU/mL in the 1st storage week decreased to 5.7 and 6.49 log CFU/mL in Vtcc and V+3 respectively in the 3rd week at 15°C storage temperature. However, counts increased from 4.64 and 2.81 log CFU/mL in the 1st storage week to 10.5 and 4.68 log CFU/mL in the 3rd week in Vnc and Con.C, respectively (Table 1).

The pH of the cheese in the different storage medium also varied significantly with the storage week and temperature. Decrease in pH from 5.71, 5.76, and 5.80 to 4.64 and 4.73, respectively for Vcpc, Vtcc, Vnc, V+3 and Con.C at 28°C storage temperature. Also a decrease occurred from 5.64 and 5.07 to 5.45 and 4.61 in Vcpc and Vnc respectively at 15°C storage temperature in the 1st to 3rd week of storage. On the other hand, an increase in pH from 4.94 and 5.74 to 5.1 and 5.85 was obtained in Vnc and V+3 at 28 and 15°C, respectively (Table 1).

**DISCUSSION**

The higher total aerobic counts in the weeks 1 and 2 in this study compared to anaerobes observed in this study could be due to the retention of some oxygen within the soft cheese for cellular respiration by the oxygen dependent aerobic microbes. There have been reports on the correlation between oxygen content and microbial growth (Gould, 1989). The fluctuation in the total aerobic
counts in different storage time could due to variations in the oxygen content of the cheese samples. The lower aerobic bacterial counts reported sometimes along the storage week could be as a result of gradual oxygen depletion within the cheese; being used up by the microbes. Similar report on decline in total viable bacterial counts of soft cheese during storage under reduced oxygen has been given (Whitley et al., 2000).

The inhibition of bacteria in anaerobic conditions has also been reported (Farber, 1999; Church and Parsons, 1995). The sharp increase in anaerobes in nisin treated samples is expected because of increase in growth of anaerobes like lactic acid bacteria.

The significant differences recorded in the microbial loads at 15 and 28°C storage temperatures are due to change in the respiration rate influenced by temperature changes. Higher temperature could favour the respiration rate of microbes in the vacuum packaged cheese thus favouring the microbial growth. This report corroborates with the report that the respiration rates of carrots increased by about 5-fold when temperature increased from 0°C to 10°C, and the increase in microbial population was about 100-fold greater at 10°C than at 0°C (Izumi and Watada, 2004). Increase antimicrobial efficacy of carbon iv oxide modified atmosphere packaging of food at reduced temperature has been reported (Gonzalez-Aguilar et al., 2004).

The inability of nisin substance to suppress microbial growth despite its well known antimicrobial abilities could be due to its limited spectrum of activity. Earlier reports also showed the differences in efficacy of bacteriocins produced by various strains of Lactococcus spp. (Adetunji and Adegoke, 2007). The lower pH recorded as a result of addition of nisin was inimical to the microbial growth probably due to reduced efficacy.

Vacuum packaged lemon juice cheese treated with Carica papaya (Vcpc) and Terminalia cattapa (Vtcc) crude extract preserved cheese better than when only vacuum packaging was used but their effectiveness in cheese preservation is both time and temperature dependent. The combination of the two plants crude extracts and nisin to preserve the cheese did not improve the microbial quality of cheese. Similar to this report in an earlier study inhibition of enterobacteriacea was not evident in vacuum packaged cheese treated with Carica papaya and Terminalia cattapa crude extract (Adetunji, 2008). It is therefore evident in this study that much lower temperature with the addition of the two vegetable extracts can be suggested to give a much more lower microbial count. It is recommended that modern technologies should be employed in West African countries soft cheese processing to completely evacuate the air bubbles that might be retained within the cheese as this can limit the packaging success. The use of purified plant extracts and reduction in water activity can be introduced in future research.

**CONCLUSION**

Arising from this study storage of soft cheese at lowered temperature with the addition of the two vegetable extracts will give lower microbial count.

**REFERENCES**


