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Survival of *Listeria monocytogenes*, and other food spoilage microbes in vacuum packaged West African soft cheese 'wara'

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Abstract

'Wara' soft cheese is traditionally produced in Nigeria and has a poor microbial quality. This study assessed the survivability of *Listeria monocytogenes* and other food spoilage microbes (enterobacteriacea, molds and yeasts) in vacuum packaged soft cheese treated independently with *Carica papaya* (Vcpc), *Terminalia cattapa* (Vtcc) crude extracts, nisin (Vnc), and the combination of these three treatments (V+3) stored at 15°C and 28°C for a three week storage period. Vacuum packaging did not suppress *Listeria monocytogenes*, and there were no significant differences in the *L. monocytogenes* counts throughout the storage weeks (P>0.05). The enterobacteriacea counts were suppressed to undetectable levels at 15°C storage temperature by the third week of storage in all treatments except the Vnc and V+3. Molds and yeasts were undetectable in all treatments throughout the storage weeks. 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 enterobacteriacea, molds and yeasts. Food technologists developing industrialized 'wara should consider including these extracts and vacuum packaging in their production. Therefore, their use in extension of the shelf-life of soft cheese is recommended.

Key words: Microbial- quality, *Carica papaya, Terminalia cattapa,* nisin, soft cheese

Résumé

Wara fromage à pâte molle est traditionnellement produit au Nigeria et a une faible qualité microbienne. Cette étude a évalué la capacité de survie de Listeria monocytogenes et d'autres microbes de dégradation des aliments (les entérobactéries, les moisissures et les levures) des fromages à pâte molle emballées traités individuellement avec du Carica papaya (VCPC), de la Terminalia cattapa (VTCC) des extraits bruts, de la nisine (VNC), et de la combinaison de ces trois traitements (V +3) stockés à 15 ° C et 28 ° C pendant une période de trois semaines de stockage. L'emballage sous vide n'a pas supprimé la bactérie Listeria monocytogènes et il n'y avait pas de différences significatives dans les comptes de L. monocytogènes durant toutes ces semaines de stockage (P> 0,05). Les entérobactéries ont été réduites à des niveaux indétectables à la température de stockage 15 ° C à la troisième semaine de stockage dans tous les traitements sauf le Vnc et V +3. Les moisissures et les levures ont été indétectables dans tous les traitements tout au long des semaines de stockage. De différences significatives se sont produites dans la numération microbienne aux deux températures de stockage et semaine de stockage (P <0,05). On peut donc conclure de ce travail que l'emballage sous vide et l'ajout d'extraits bruts (Carica papaya, Terminalia cattapa) à l'entreposage du fromage à pâte molle peut supprimer les entérobactéries, les moisissures et les levures. Les technologues alimentaires du fromage industrialisé en développement devraient envisager d'inclure ces extraits et l'emballage vide dans leur production. Par conséquent, leur utilisation dans le prolongement de la durée de conservation des fromages à pâte molle est recommandée.

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Introduction

West African soft cheese 'wara' is unripened and has a poor microbial quality. 'Wara' has an average short shelf-life of 2-3 days. Deterioration in cheese is primarily caused by microbial growth of gram-negative psychrotrophic bacteria such as *Pseudomonas, Proteus*, and *Aeromonas*, which results in undesirable off-flavors, pigment formation, or slimy curd [1, 2, 3]. Growth of yeasts and molds, such as *Geotrichum, Penicillium, Mucor,* and *Alternaria,* also cause spoilage of flavor, texture, and appearance [4]. *Listeria monocytogenes* 'a gram-positive bacterium' has also been implicated with food spoilage and food-borne illness. A mortality of 27% of listeriosis was reported in Northern Nigeria by Onyemelukwe *et al.* [5]. Sixl *et al.* [6] reported 28.7% prevalence using serological method of diagnosis. Onyemelukwe and Lawande [7] gave the first report on the occurrence of *Listeria* meningitis in a neonate

and the mother in Nigeria. An outbreak of listeriosis in a herd of cattle associated with stillbirth, abortion, nervous signs and death has been reported in Nigeria by Akpavie and Ikheloa [8].

Food items identified as sources of infection include soft cheeses such as Mexican soft cheese [9], coleslaw [10], prepared salads [11] and paté [12]. Report has shown an excess of 10 000 cells/g of *L. monocytogenes* in some samples of soft cheeses and paté (Committee on the Microbiological Safety of Food, [13]). Adeyemi *et al.*, [14] have given the list of incriminated Pathogens reported in unripened soft cheese 'wara' to include: *Staphylococcus aureus, Bacillus cereus, Clotridium perfrigens, Clotridium botulinum, Brucella abortus, Escherichia coli, Listeria monocytogenes* and *Salmonella spp.* [15, 14].

The short shelf life of soft cheese requires the appropriate preservation techniques to prevent its spoilage by extending its shelf life to meet market demands. Some of the modern technologies employed include modified atmospheric packaging, use of protective cultures, use of bacteriocins and other culture products and enzymes [16]. These methods and others may be used synergistically to further improve the microbial safety 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 [17]. Attempts have been made in the recent past to include starter cultures or various preservatives such as propionic acid, sodium benzoate, and ascorbic acid in the production of wara [18,19]. Some of these preservatives have been shown to be effective in inhibiting mesophilic and psychotrophic bacteria as well as coliforms. However, these preservatives may not be easily accessible to the local cheese processors in West Africa.

Vacuum packaging and modified atmospheric packaging have been used for the preservation of many products including cheese [20,21,22]. These methods reduce the oxygen level which retards browning and spoilage and maintain fresh appearance [23,24]. In the case of meat, 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 [25].

The antimicrobial properties of *Carica papaya* and *Terminalia catappa* have been widely reported [26, 27, 28, 29]. Nisin 'a bacteriocin' produced by lactic acid bacteria has also been shown to inhibit *Listeria monocytogenes* in cottage cheese stored at 4°C [30]. To our knowledge no work on the use of natural sources of medicinal plants in 'wara' cheese preservation has been done in this environment. This work is therefore aimed at evaluating the microbial quality of 'wara' cheese through the synergistic actions of plant extracts and nisin under vacuum packaging.

Materials and methods

Preparation of L. monocytogenes inoculum.

Five different strains of *L. monocytogenes* were grown and subcultured on modified oxford agar base (MOX) supplemented with modified oxford antibiotic supplement (acrifla vine, nalidixic acid and cycloheximide) (Becton, Dickinson and Company). The inoculated plates were incubated at 37°C for 24 h. Subsequently a colony of each culture w plicate to ensure accuracy transferred into 10 ml tryptic soy broth (TSB), respectively. The inoculated broth was incubated at the same temperatures as described above. Following the incubation, the absorbance of each culture was taken using a spectrophotometer (Pharmacia Biotech) at a wavelength of 600 nm. The broth cultures were then serially diluted and appropriate dilutions were plated on MOX agar base with antibiotic supplements (acriflavine, nalidixic acid and cycloheximide) (Becton, Dickinson and company). One ml of each broth culture was placed into a sterile test tube to make a 5 ml mixture of *L. monocytogenes.*

Preparation of milk for wara processing

Four liters of 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 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 600g of soft ripened cheese ready for treatments.

Preparation of crude extracts

Terminalia cattappa (2.38gm) was grinded to powder and extracted in 10ml of 70% ethanol. *Carica papaya* (4.5g) was also grinded and extracted in 20ml ethanol to obtain between 0.2 – 0.25gm/ml concentration. After extraction the mixture was sieved and the resulting liquid was concentrated in a rotavapour .

Treatment of samples for storage

Cheese from the above process was divided aseptically into 10g pieces. Then 6 sample groups of 8 from these pieces were then subjected to the following treatments. 1. Two cheese pieces spiked with 10^4 cfu/ml of *Listeria monocytogenes* 2. Two cheese pieces treated with Crude extract of *C. papaya* stored in vacuum package. 3. Two cheese pieces treated with *T. cattapa 4.* Two cheese pieces treated with nisin 5. Two cheese pieces treated with combination of (2, 3 and 4). 6. An untreated control. One of each pair treatment, each one with its replicate was stored at 28°C while the other was stored at 15 °C (refrigeration) for a 3-week storage period.

Microbiological sampling

Sampling was done on every week for a 3-week 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 Modified oxoid agar with antibiotic supplements for enumeration of *Listeria monocytogenes*, MacConkey agar for *Enterobacteriacea* counts and potato dextrose agar for yeasts and molds counts. Plates for *Listeria monocytogenes* counts, *Enterobactericea* , molds and yeasts were incubated aerobically at 37°C for 18hours.

Table 1: Survival of *Listeria monocytogenes in* Vacuum Packaged Soft Cheese along a 3weeks Storage period

Storage weeks	Storage temperatures 28°C	15°C
1st	6.36a	5.53a
2nd	6.65a	6.09a
3rd	6.15a	5.82a
1st	5.59a	5.83a
2nd	5.05a	5.92a
3rd	4.93a	5.68a

Numbers with similar alphabets showed no significant differences (p>0.05)

Table 2: Microbial counts and pH of vacuum packaged lemon juice cheese treated with *Carica papaya* and *Terminali cattapa* crude extracts, and $

Storage Temperature	Vcpc 28°C	Vcpc 15°C	Vtcc 28°C	Vtcc 15°C	Vnc 28°C	Vnc 15°C	V+3c 28°C	V+3 15°C	Vc (cont.) 28°C	Vc (cont.) 15°C
Enterobactericea										
Day 1	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a
1stwk	3.92cb	<1.00a	3.72c	3.81cb	4.53a	4.64a	3.92cb	3.99b	5.46a	3.63b
2nwk	2.64d	2.24e	2.75d	2.94d	7.47a	6.62b	3.78c	3.6c	<1.00a	<1.00a
3rdwk	4.26e	<1.00a	2.85f	<1.00a	8.27a	8.16b	5.4c	4.49d	<1.00a	<1.00a
Molds/yeast										
Day 1	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a
1stwk	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	1.35a
2ndwk	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a
3rdwk	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a	<1.00a
PH										
1stwk	5.72b	5.64c	5.6d	5.49e	4.94g	5.08f	5.76a	5.74b	5.65b	5.65b
2ndwk	5.48c	5.7b	4.91d	5.71b	4.57e	4.92d	5.46c	5.81a	5.59cb	5.65b
3rdwk	5.46b	5.45b	4.62d	5.48b	5.1c	4.61d	5.48b	5.85a	5.23a	5.66a
									4.84d	5.7c

Numbers with similar alphabets showed no significant differences (p>0.05)

Numbers with different alphabets showed significant differences (p<0.05)

$=Nisin' (a bacteriocin-like substance) used was extracted from pure cultures of Lactococuss lactis after boiling cultures at 100°C for 25 minutes and its inhibitory effect observed on pure cultures of L. monocytogenes and E. coli O157:H7

Vcpc=vacuum packaged Carica papaya treated cheese[a]

Vtcc=vacuum packaged Terminali cattapa treated cheese[b]

Vnc=vacuum packaged nisin treated cheese

V+3=vacuum packaged cheese treated with a, b and c

Con. C= vacuum packaged cheese with no treatment

Statistical analysis

The study was performed in two replicates, each with appropriate duplications. All microbiological data were transformed into Log_{10} CFU/ml or Log_{10} CFU/g before comparison of means. Analysis of data was accomplished using the Fisher's least significant difference of means of bacterial populations calculated with the General Linear Model (GLM) procedure of SAS based on a 95% confidence level.

Results

The result showed that the growth of *Listeria monocytogenes* was not inhibited in vacuum packaged lemon juice soft cheese. Higher counts were recorded in the 2nd week at both temperatures (i.e. 28 and 15°C). There were no significant differences in *L. monocytogenes* counts and pH recorded at 28°C and 15°C throughout the storage periods (i.e. P>0.05) (Table 1).

There were significant differences in the enterobacteriacea counts of the vacuum packaged lemon juice cheese treated with *Carica papaya* (Vcpc), *Terminalia cattapa* (Vtcc) crude extracts, nisin (Vnc), and the combination of the three (V+3) at 15 and 28°C respectively.

The enterobacteriacea were better inhibited as lower counts were obtained compared with what was observed in both aerobes and anaerobes (Table 2). The enterobacteriacea counts were undetectable (<1.0logcfu/ml) in Vcpc and Vtcc at 15°C storage temperature in the 1st and 3rd week of storage respectively. Enterobacteriacea counts showed no significant differences (P>0.05) in Vcpc (28°C), and Vtcc (15°C) in the 1st and 2nd week respectively. However, there were significant differences in counts of all the treatments in the 3rd storage week (P<0.05).

Mold and yeast were undetectable throughout the storage period and at the two storage temperatures for all treatments. There were no significant counts in all the treatments throughout the storage periods (P>0.05).

The pH of the cheese in the different storage medium also varied significantly with the storage week and temperature. Decreases in pH from 5.72 and 5.76 to 5.46 and 5.48 were observed in Vcpc and V+3 respectively at 28°C storage temperature. Also a decrease occurred from 5.64 and 5.08 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°C and 15°C respectively.

Discussion

The inability of vacuum package to prevent the growth of *L. monocytogenes* in this report was consistent with Whitley *et al* [31] who also demonstrated the growth of *L. monocytogenes* in modified atmospheric package.

The higher enterobacteriacea counts throughout storage weeks when compared with molds and yeasts 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 [32]. The fluctuation in the total aerobic counts in different storage time could be due to variations in the oxygen content of the cheese samples. The lower enterobacteriacea counts reported sometimes along the storage week is as a result of gradual oxygen depletion within the cheese; being used up by the microbes. Whitley *et al.* [31] also gave a similar report on decline in total viable bacterial counts of a mature stilton cheese during storage under reduced oxygen.

The reduction in the enterobacteriacea and yeast/mould could be as a result of the modification of cheese environment by carbon (iv) oxide produced from cellular respiration of the microbes. The inhibitory effects of carbon (iv) oxide on bacteria were earlier reported [33, 16]. 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°C and 28°C storage temperatures are due to changes 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 [34].

The severe inhibition of yeasts and molds in all treatments as observed in this work, was due to O_2 deplated by the vacuum packaging process. This is consistent with the reports on severe inhibition of yeast by vacuum packaging [35].

The inability of nisin to suppress enterobacteriacea despite it 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* [36]. The slight drop in pH recorded as a result of addition of nisin was not

sufficient to suppress the microbial growth. However, in an earlier study, Adetunji *et al.* [37] reported suppression in microbial loads of raw milk due to decrease pH following the addition of lemon juice.

The effectiveness of vacuum packaged soft cheese treated with *Carica papaya* and *Terminalia cattapa* leaf extracts in cheese preservation is both time and temperature dependent. Vacuum packaged lemon cheese treated with *Carica papaya* (Vcpc) or *Terminalia cattapa* (Vtcc) suppress enterobacteriacea counts to undetectable level at 15°C in the 3rd week of storage. This result may have been due to the antimicrobial properties which these plant extracts possess [29]. However further studies are needed to ascertain the reason for the inability of the combination of treatments tc suppress enterobacteriacea in the cheese.

It is evident in this study that lower temperature storage with the addition of either of the two leave extracts will give an improved microbial quality. It is recommended that modern technologies should be introduced into 'wara' cheese processing to completely evacuate the air bubbles that might be retained within the cheese as this can limit the packaging success.

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