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MICROBIAL POPULATION DYNAMICS AND STORAGE-STABILITY OF SOYBEAN SUPPLEMENTED MAIZE ('SOY-KWOKA') PRODUCT SUBJECTED TO ACIDIFICATION AND ANTIOXIDANTS

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ABSTRACT

Soybean supplemented and unsupplemented maize products were subjected to acidification (pH 3.5) and incorporation of antioxidants: butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) at 0.01 and 0.05% w/w. Maximal total mesophilic counts (TMCs) of $\log_{10}$ 8.81 cfu were observed in samples neither acidified nor preserved with BHA/BHT (i.e. controls). But over 4-log cycle decrease in TMCs (i.e. from $\log_{10}$ 8.81 to $\log_{10}$ 4.32 cfu g^{-1}) occurred in samples acidified and preserved with 0.05% BHA/BHT. Lactic acid bacteria (LAB) population exhibited comparable trends as those of TMCs with a dramatic increase occurring after 72h of ambient storage of samples acidified and preserved with 0.01% BHA/BHT. Higher microbial counts were observed in soybean-supplemented ('soy-kwoka') products; being also less acidic. The storage-stability (aroma and firmness) and microbial safety potential of samples acidified and preserved with 0.05% antioxidants were enhanced.

KEY WORDS: Microbial dynamics, antioxidants, acidification, 'soy-kwoka'

INTRODUCTION

Maize products are of considerable value in various parts of the world (Capparelli and Mata 1975; Nyotu et al, 1986; Efiuvwevwere and Amadi 1992). 'Kwoka' is a traditional popular maize product in Southern Nigeria and known by different names depending on the locality. For example, it is called 'Ekoki' by the Ibibios, 'Asuruasu-oka' by the Ibos, 'Kwoka' by the Urhobos and "koga' in Cameroun.

The rapid deterioration of 'Kwoka' and similar maize products has been attributed to microbial contamination and proliferation (ICMSF 1980; Efiuvwevwere and Akoma, 1997). The high perishability hinders its production on a large scale.

Soybean-supplementation of maize products and their nutritional qualities have been investigated (Almeida - Dominguez et al., 1990). However, such supplementation has adverse microbial implications (Efiuvwevwere and Akoma 1997). The present work was therefore aimed at improving the microbial quality and storage-stability of both soybean - supplemented and unsupplemented 'Kwoka' using different antimicrobial agents alone or in combination with acidification. Filter-sterilised lime juice was also applied as a preservative agent. The overall objective was to minimise the microbial risks and extend the commercial potential of these products.

MATERIALS AND METHODS

Materials and Preparation of 'Soy-kwoka

The raw materials (maize, soybean, palm fruits and the other materials) used for the preparation of the products (soybean - supplemented and unsupplemented) were obtained from Mile 1 market, Port Harcourt.

The preparation of the products was carried out as previously described (Efiuvwevwere and Akoma 1997).

Addition of Antimicrobials to the Products

Following preparation of the slurry (Efiuvwevwere and Akoma 1997):

(i) Filter-sterilised (0.2µm Gelman, Ann Arbor, USA) lime juice (pH 2.5) was added (1:9 ratio) and the slurry acidified to pH 3.5 and mixed thoroughly.

(ii) A 0.01 or 0.05% (w/w) butylated hydroxyanisole or butylated hydroxytoluene was added and mixed as indicated above.

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Each group of the above sample treatments was dispensed into polyethylene bags for packaging and steamed for 50 min. at 70-76°C as generally practised (Efiuvwevwere and Akoma 1997).

Microbial evaluation

From each packaged sample type, 20g sample was obtained aseptically and homogenised with 180 ml sterile 0.1% peptone water using a stomacher (Laboratory Blender 400, Seward Medical, London). Further decimal dilutions (10^{-1} to 10^{-6}) were prepared and pour-plated (except otherwise indicated) using duplicate differential or non-selective culture media (Oxoid Ltd, England) for quantitative microbial evaluation as follows: tryptone soy agar incubated at 30°C for 24-48h for total mesophilic count (TMC); de-Man, Rogosa and Sharpe (MRS) agar incubated anaerobically (Gas Pak, BBL Becton Dickinson and Co., USA) at 30°C for 5 days for lactic acid bacteria population; Baird Parker agar incubated at 37°C for 24-48h for staphylococci count and tributyrin agar incubated at 30°C for 3 days for lipolytic microorganisms. The morphology of the colonies that developed was noted before enumeration (Speck 1984) and expressed as colony forming units (cfu g^{-1}). The cultural and biochemical characteristics as related to the qualitative microbial profile had earlier been reported (Efiuvwevwere and Akoma 1997).

Evaluation of Storage-Stability (Shelf-Life)

Different sample types were stored in display cabinet (31 ± 2°C) and withdrawn at different time intervals for organoleptic evaluation (aroma by "sniffing" and firmness by exertion of finger pressure to assess storage-stability/shelf-life). The samples were presented to the panelists individually at random and evaluated using a 9-point hedonic scale (where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely) as described by Larmond (1977).

Statistical Analysis

The obtained data were subjected to analysis of variance (ANOVA) and significant mean differences determined (Duncan 1955).

RESULTS AND DISCUSSION

The changes in total mesophilic counts (TMCs) of soybean supplemented and unsupplemented samples subjected to acidification (using lime juice) and antioxidants are shown in Fig. 1. Whereas the controls (i.e. samples neither acidified nor treated with antioxidants) showed maximal microbial populations, highly inhibitory growth patterns occurred in samples acidified

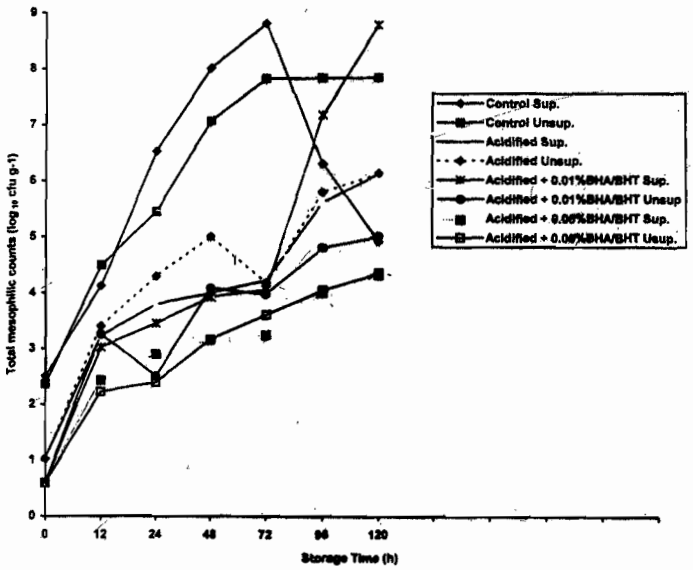

Figure 1. Changes in total mesophilic counts of soybean supplemented and unsupplemented samples subjected to acidification and antioxidants.

and treated with 0.05% BHA/BHT (Fig. 1). The TMCs of the latter increased to the peak of $\log_{10}$ 4.32 cfu on day 5 as compared with $\log_{10}$ 8.81 cfu on day 3 for controls (Fig. 1). The dramatic inhibitory impact exerted by the combination of acidification and antioxidants is probably attributable to synergistic effect since acidification alone induced less inhibition. However, the sharp decrease observed after 3 days in controls (Fig. 1) may be reminiscent of the effect of accumulation of toxic metabolic by-products and cell auto-catalytic phenomenon (Schlegel 1992). These growth kinetics observed in TMCs were also apparent in lactic acid bacteria (LAB) populations (Fig. 2) but the marked increase after 72h in soybean-supplemented-acidified samples containing 0.01% each of BHA and BHT clearly suggests

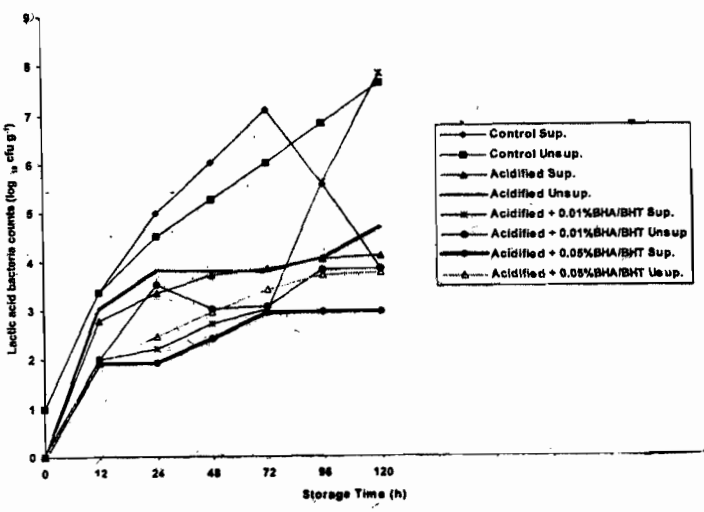

Figure 2. Dynamics of lactic acid bacteria population of soybean supplemented and unsupplemented samples acidified and treated with antioxidants.

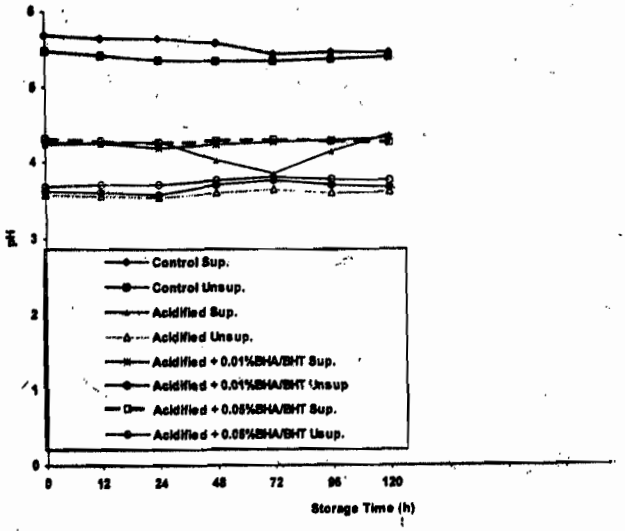

Figure 3. Changes in pH of soybean supplemented and unsupplemented samples acidified and treated with antioxidants.

ineffective antioxidant concentration. This also demonstrates that LAB are spoilage micro-organisms of 'soy-kwoka' in spite of the antioxidants (Fig. 2). Thus, these findings suggest that the effect of buffering capacity due to the soybean-supplementation may have influenced the LAB growth response (Fig. 2) (Jay 1996; Efiuvwevwere and Akoma 1997).

The level of acidity is a major controlling factor in microbial growth and behaviour, hence the upsurge in microbial population of samples having pH 5.35 to 5.68 in comparison with the low counts of samples having pH 4.38 and lower (Figures 1 and 3) corroborate the inhibitory effect of low pH (Gould 1989). In addition, increased buffering capacity of soybean supplemented products has been associated with more diverse microflora and higher microbial population (Jay 1996). Therefore, the remarkable increases in microbial populations of soybean-supplemented products in comparison with the controls may be partly ascribed to the slightly higher pH values (Figs 1-3). Such microbial increases in spite of minimal pH differences had been previously observed in soybean-supplemented products (Jay 1996; Efiuvwevwere and Akoma 1997).

BHA and BHT are primarily anti-auto-oxidative agents with some inhibitory action against Gram-negative and -positive bacteria but greater inhibition occurs with increase in concentration (Jay 1996). Thus, the sharp increase in TMCs after 72h in samples containing 0.01% (Fig. 1) is likely due to sub-optimal concentration and microbial recovery. Consequently, the importance of application of adequate and effective concentrations of antimicrobial agents in food systems is further established. It was also striking that Gram-positive bacteria (*Bacillus, Lactobacilli, Micrococous, Pediococcus* and *Staphylococus*) were more frequently isolated than the Gram-negative bacteria (*Enterobacter* and *Klebsiella)*, thus suggesting the greater phenolic antimicrobial and heat sensitivity of the latter organisms (Razavi-Rohani and Griffiths 1996; Efiuvwevwere and Akoma 1997). Among the Gram-positive bacteria isolated were staphylococci (including *Staphylococcus aureus*) with counts ranging from $\log_{10}$ 1.0 on day 0 to $\log_{10}$ 3.59 on day 5. However, the higher populations were observed in soybean-supplemented samples. Isolation of these organisms constitutes potential public health risks to consumers of these products especially as from day 4 of storage.

The storage-stability (shelf-life) based on changes in aroma and firmness of the samples as affected by acidification and the antioxidants is presented in Table I. In general, the sensory attribute of aroma was better preserved in unsupplemented acidified samples into which antioxidants were incorporated. The poor aroma observed in the soybean supplemented samples may be related to the adverse impact associated with soybean supplementation which may have resulted from increased fat and protein contents (data not shown). These changes could have led to increased buffering capacity, surface area (Jay 1996) and accelerated microbial activity.

Table I. Storage stability (aroma and firmness) of Soybean supplemented kwoka subjected to acidification and antioxidants

Storage time (days)	Aroma						Firmness					
	Control		Acidified + 0.01% BHA/BHT		Acidified + 0.05% BHA/BHT		Control		0.01% BHA/BHT		0.05% BHA/BHT	
	Suppl.	Unsuppl.	Suppl.	Unsuppl.	Suppl.	Unsuppl.	Suppl.	Unsuppl.	Suppl.	Unsuppl.	Suppl.	Unsuppl.
0	7.5±1.07	7.5±1.3	70±1.1	7.1±1.0	6.9±1.2	7.1±1.4	8,2±0.6	7.8±0.7	7,7±0.5	7.7±0.8	7.6±0.5	7.4±0
1	5.4±0.9	5.4±0.9	6.1±0.9	6.4±0.6	5.6±0.7	6.2±0.8	5.3±1.4	5.9±1.6	7.0±0.7	6.7±0.6	6.5±1.6	6.5±1
3*	1.1±0.2	1.1±0.2	1.0±0.0	1.2±0.4	1.1±0.3	1.4±0.5	3.0±1.0	3.8±1.2	2.9±0.8	2.8±1.0	2,6±0.7	3.4±0

Means (±SD) of 20 scores (i.e. 2 replicates) from 10-member panel

* Evaluation was discontinued after 3 days as a result of overt spoilage

Overall, soybean supplementation exacerbated the storage-stability of the products. Nevertheless, combination of acidification and antioxidants especially at 0.05% concentration enhanced the shelf-life. However, by day 3, all samples were rejected (Table I). Thus, suggesting the limitations of these treatments on both microbiological and sensory quality attributes. It has been asserted that the advantage of immediate acidification of foods (including fermentation processes) is the prevention of growth of pathogenic and spoilage micro-organisms, thereby enhancing the safety of the food product (Nout et al 1987). Evidently, similar benefits in relation to control of microbial population dynamics (Figs 1-2) through the use of a cheap acidulant (lime juice) have been shown from the present work.

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