

Full Paper

EFFECT OF PRESERVATIVES AND STORAGE CONDITIONS ON MICROORGANISMS IN NIGERIAN UNRIPENED CHEESE (WARA)

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ABSTRACT

Nigerian unripened cheese is a highly perishable food. This study investigated the preservative effect of ginger and sorbic acid on the quality of unripened cheese. The cheese sample was produced and treated with 5 % ginger, 2.5 % ginger plus 0.05 % sorbic acid and 0.1 % sorbic acid and kept at ambient and refrigeration temperatures. Microorganisms were enumerated and the microorganisms were identified using standard methods. Sensory assessment was also carried out on the samples. The results showed that microbial load was generally lower in samples stored at refrigeration temperature than those at ambient temperature. Lactobacillus delbrueckii, Lactobacillus acidophilus, Leuconostoc mesenteroides, Streptococcus thermophilus, Bacillus pumilus, Saccharomyces cerevisiae, Saccharomyces lactis, Torulopsis versatalis, Aspergillus spp and Penicillium spp were isolated during storage. A sample with 0.1% sorbic acid had the highest score for its taste, colour, texture, aroma and overall acceptability. The addition of ginger powder to the cheese had a negative influence on the sensory properties. The findings therefore recommend the use of 2.5% ginger with 0.05% sorbic acid in the preservation of unripened cheese.

Keywords: Cheese, Lactobacillus, ginger, sorbic acid, yeast

1. INTRODUCTION

Cheese is produced by curdling milk, removing the whey, and pressing the curd. The desired flavour of cheese is achieved by ripening, curing, or ageing the curd obtained (Raheem *et al.*, 2009; Oladele, 2017). The composition, texture, taste, flavours, and appearance of

cheese is unique. These characteristics can be attributed to protein coagulation and metabolism by proteolytic enzymes present in the coagulants used. Cheese contains a larger amount of nutrients than milk because of the low moisture content. Temperature, percentage of acid, calcium content of the milk and absence of antibiotics are very important factors to consider in cheese production (Adewumi and Akinloye, 2014).

Nigerian cheese, colloquially called wara, is majorly produced by the Fulani tribe from the Northern part of the country. (Ogundiwin, 1978). They are a nomadic tribe that lacks basic facilities such as refrigerators to preserve excess milk obtained from cows. Their women therefore process excess milk into soft cheese, which is compact and easy to transport. This cheese is mainly kept in its whey inside the calabash or other containers at room temperature. It is highly perishable under this condition with a shelf life of 2 - 3 days (Adetunji and Babalobi, 2011; Orhevba, 2016). The detection of E. coli, Acinetobacter, Alcaligenes, Flavobacterium, Micrococcus species, and Staphylococcus aureus in wara may be due to faecal-oral route transmission (Uzeh et al., 2006). The main purpose for adding chemical preservatives is to inhibit the growth and activity of microorganisms (Anu et al., 2010). According to Belewu et al. (2005), other chemical preservatives that have been employed in the preservation of cheese asides sorbic acid include sodium benzoate and propionic acid.

Many consumers are no more interested in foods with chemical additives because of their negative effects on health and the environment. This has led to an increase in the production of food with natural preservatives with beneficial effects to the consumers (WHO, 2002). Spices such as ginger and garlic or their extracts have been used in the preservation of certain foods to reduce reliance on chemical preservatives. Ginger has been used in Asia for thousands of years because of its antibacterial properties (Weil, 2005). Likewise, Belewu *et al.* (2005) reported the effectiveness of ginger in the preservation of cheese.

In this study, sorbic acid (0.05 % and 0.1 %) was used below its maximum permissible limit (0.2 %) in cheese (Codex, 2010). The study investigated the microbial population and isolated microorganisms associated with Nigerian soft cheese preserved with sorbic acid and/or ginger at ambient and refrigeration storage conditions.



2. METHODOLOGY

Raw milk and ginger bulbs were obtained from Alakowe in Ile-Ife. Sorbic acid was obtained from Sigma-Aldrich Chemical Company, St. Louis, Missouri, USA.

2.1. Production of ginger powder

The ginger root was prepared by washing 1000g of ginger bulbs thoroughly under running water. They were then peeled and aseptically cut into small pieces using a sterile knife on a workbench. After this the ginger bulbs were dehydrated in a cabinet dryer at 60°C for 8 hours to reduce the moisture content and then the dried ginger bulbs were milled in the blender to obtain ginger powder (Guler and Mutlu, 2005).

2.2. Production of unripened cheese

Unripened cheese was produced using the method of Adetunji and Salawu (2011). Calotropis procera leaves were ground in blender, filtered with muslin cloth and the extract was stored in the refrigerator. Fresh cow milk was divided into four portions (A, B, C and D). Five percent (5%) ginger was added to sample A, 2.5 % ginger and 0.05% sorbic acid was added to sample B, 0.1% sorbic acid was added to sample C and no preservative was added to sample D (which served as the control). The milk was heated to 95 °C, the extract was added and the mixture was left for 15 min until the curd separated from the whey. The curd obtained was moulded using a cone shaped raffia basket and allowed to drain for 5 - 8 min to give it its desired shape (the cone shape). The curd was covered with whey and stored in sterile bottles at ambient (i.e. 28 \pm 2 °C) and refrigeration (i.e. 4 \pm 2 °C) temperatures.

2.3. Enumeration of microorganisms

Each sample (5 g) was homogenised with sterile peptone water (45 ml) in a Colworth Stomacher. The representative dilution obtained from serial dilution was dispensed into sterile petri dishes. Molten agar was poured on the suspension and was swirled gently clockwise and anticlockwise to ensure even distribution of growth. Nutrient Agar (NA, Lab M, United Kingdom) was added for determination of the total aerobic count and Potato Dextrose Agar (PDA, Lab M, United Kingdom) with the addition of 1% streptomycin to prevent the growth of bacteria for yeast and mould. Each petri dish was incubated in an inverted position in a Gallenkamp incubator for the total aerobic microorganism at 35 ± 2 °C for 24 hours and yeast and mould count at 25 ± 2 °C for 72 to 105 hours (Harrigan, 1998). The plates were examined for growth, colonies were counted, and the results obtained were recorded in colony forming units per gram (Cfu/g). The streaking method was used for obtaining pure isolates. Each pure isolate was maintained on a suitable agar slant stored in a refrigerator at 4 ± 2 °C for identification.

2.4. Identification of microbial isolates

Bacterial isolates were identified using cultural characteristics. Biochemical tests such as production of catalase, oxidase and indole, production of ammonia from peptone, fermentation of sugar, CO_2 production from glucose, and methyl red test were also carried out (Malomo *et al.*, 2018). Yeast isolates were identified using the colony characteristics. The shape of cell, cell size,

characteristics of buds, and arrangement of cell were viewed under microscope. Assimilation of carbon and nitrate were observed on yeast assimilation agar (Barnett et al., 2000, Malomo et al., 2018). Identification of mould was based on visual observation and microscopic examination of the colour of the colony on the media, colour of mycelium, and texture. In addition, features such as hyphae (septate or non-septate), characteristics of spore head (size, shape and arrangement), mode of reproduction, and presence of special structures such as foot cell or rhizoids were also evaluated with the aid of a microscope (Leica DM500 Model 13613210) (Harrigan and McCance, 1976; Harrigan, 1998; Malomo et al., 2018).

2.5. Sensory analysis

Freshly prepared cheese was coded and presented to fifteen judges, each of whom was familiar with cheese. The panelists were each presented with 10g of each *wara* sample on a white plate. The panelists analysed the sample for colour, taste, flavour and overall acceptability using a 9 -point Hedonic scale where 1 was the lowest score and 9 was the highest (Yangilar and Yildiz, 2017).

3. RESULTS AND DISCUSSION

3.1. Total aerobic count

There was no viable count at the beginning of storage (freshly produced cheese samples) probably due to the heat treatment the samples were subjected to during production (Table 1). Samples stored at ambient had higher counts (4.11 - 6.30 log Cfu/g) than samples stored at refrigeration temperature (4.04 - 5.83 log Cfu/g) from week one to week three. Most microbial activities are inhibited at lower temperatures (Prescott et al., 1999; Adeniran et al., 2016), thus limiting the exponential phase of the organisms present in the cheese. The cheese samples without preservatives had the highest bacterial count (5.63 - 6.30 log Cfu/g) while the cheese sample treated with 0.01% sorbic acid had the lowest count (3.98 - 5.36 log Cfu/g). Cheese treated with 2.5 % ginger and 0.05 % sorbic acid had lower microbial count (4.04 - 5.46 log Cfu/g) than samples with 5% ginger (5.63 - 6.30 log Cfu/g). Belewu et al. (2005) also reported that ginger extract reduced the microbial load of cheese. This can be attributed to the presence of phenolic compounds in ginger, which possess antimicrobial properties (Schulick, 1993; Belewu et al. (2005); Olaniran et al. (2015)). There was no significant difference (p > 0.05) between the total aerobic counts of samples preserved with 5 % ginger and 2.5 % ginger plus 0.05% sorbic acid at week one but there was a slight difference from week one to week two. The synergistic effect of both ginger and sorbic acid also caused a notable reduction (p > 0.05) in total aerobic count of the sample. Samples stored at refrigeration temperature had lower counts than samples stored at ambient temperature. It has been reported that ginger also reduced the bacterial population of yoghurt and soft cheese (Gutle and Mutlu, 2005; Belewu, 2005).

3.2. Total fungal count

The freshly produced treated cheese samples had no fungal growth. There was generally no microbial count at the beginning of storage (Table 2). The microbial count



Table 1: Total aerobic count of preserved unripened cheese during storage (log

Camples	Week					
Samples	0	1	2	3		
Refrigerated storage						
A	Nil	4.08 ± 0.05 ^b	5.26 ± 0.05 ^b	5.58 ± 0.08 ^{ab}		
В	Nil	4.04 ± 0.06 ^b	5.11 ± 0.05^{b}	5.43 ± 0.03^{b}		
C	Nil	3.98 ± 0.07^{c}	4.85 ± 0.05^{c}	5.08 ± 0.02^{c}		
D	Nil	5.63 ± 0.06^a	5.83 ± 0.20^a	5.79 ± 0.08a		
Ambient storage						
A	Nil	4.35 ± 0.80 ^b	5.45 ± 0.06 ^b	5.63 ± 0.03 ^b		
В	Nil	4.26 ± 0.04 ^b	5.26 ± 0.10^{bc}	5.46 ± 0.04 ^{bc}		
C	Nil	4.11 ± 0.03bc	5.00 ± 0.05^{c}	5.36 ± 0.05^{c}		
D	Nil	5.73 ± 0.07 ^a	6.30 ± 0.10^a	6.23 ± 0.07 ^a		
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There was no significant difference (p < 0.05) in the values with identical superscript in rows. A - Cheese plus 5% ginger; B - Cheese plus 2.5 % ginger and 0.05 % sorbic acid; C - Cheese plus 0.1% sorbic acid; D - without any preservative

was generally higher in cheese samples without preservative than samples treated with either ginger and / or sorbic acid. The sample treated with 0.1% sorbic acid had the lowest fungi count (2.81 - 3.21 log Cfu/g) throughout storage, followed by the sample treated with both ginger (2.5%) and sorbic acid (0.05 %). A significant difference (p < 0.05) existed between the samples stored at both ambient and refrigeration temperatures in the third week of storage. The use of ginger and / or sorbic acid generally reduced the total fungi count of the cheese during storage. This is an indication of the antifungal activities of the preservatives used: ginger and sorbic acid. The samples stored at refrigeration temperature had lower counts throughout storage. Adepoju et al. (2012) also reported the effectiveness of sorbic acid against yeast and mould during the storage of fura de nunu. Accordingly, the study by Arekemase and Babashola (2019) also reported the effectiveness of ginger in minimizing fungal growth in soymilk during storage.

Table 2: Yeast and mould count of preserved unripened cheese during storage $(\log C f u/g)$

Camples	Week				
Samples	0	1	2	3	
Refrigerated storage					
A	Nil	3.85 ± 0.05b	4.26 ± 0.04b	4.30 ± 0.05c	
В	Nil	$3.20 \pm 0.03c$	4.20 ± 0.06b	4.52 ± 0.04b	
C	Nil	2.81 ± 0.04d	$3.00 \pm 0.06c$	$3.09 \pm 0.00d$	
D	Nil	4.54 ± 0.06a	5.12 ± 0.02a	$5.23 \pm 0.02a$	
Ambient storage					
A	Nil	3.90 ± 0.05b	4.34 ± 0.06c	4.66 ± 0.06c	
В	Nil	4.10 ± 0.04b	$4.72 \pm 0.07b$	$5.00 \pm 0.05b$	
C	Nil	$3.00 \pm 0.07 d$	$3.12 \pm 0.02d$	$3.21 \pm 0.03d$	
D	Nil	4.66 ± 0.04a	$5.83 \pm 0.03a$	6.16 ± 0.11a	

There was no significant difference (p < 0.05) in the values with identical superscript in rows. A - Cheese plus 5% ginger; B - Cheese plus 2.5 % ginger and 0.05 % sorbic acid; C - Cheese plus 0.1% sorbic acid; D - without any preservative

3.3. Bacteria isolated from unripened cheese during storage

Lactobacillus delbrueckii, Leuconostoc mesenteroides, Streptococcus thermophilus, Lactobacillus acidophilus, and Bacillus pumilus were isolated from the cheese samples during storage (Table 3). These organisms were isolated in the samples from the first week to the third week. Lactic acid bacteria were dominant during the storage of cheese. Lactic acid bacteria are prominent in the fermentation of food (Beuchat, 1995; Mohammed et al., 2017). These microorganisms are important in the conversion of sugar into organic acids and other substances. These substances improve the sensory and keeping qualities of the fermented foods. Pathogenic organisms are also inhibited because their ability to produce bacteriocins (Aguirre and Collins, 1993; Adepoju et al., 2016).

Table 3: Bacteria isolated from unripened cheese during storage

Tests			Bacterial		
	I	II	III	IV	V
Morphological	Rods	Cocci	Cocci	Rod	Rod
characteristics					
Colour of colony	Cream	Cream	Cream	Cream	Transparent
Gram reaction	+	+	+	+	+
Catalase	-	-	-	-	+
reaction					
Presence of	-	-	-	-	+
endospore					
Growth at:					
15°C	-	+	-	-	N
45°C	+	+	+	+	N
Production of	-	+	-	-	N
CO_2					
Production of	N	N	N	N	+
ammonia from					
peptone:					
Production of	N	N	N	N	-
H_2S					
Indole test	N	N	N	N	+
Voges Proskauer	N	N	N	N	+
Sugars fermented:					
Glucose	+	+	+	+	+
Galactose	+	+	+	+	+
Lactose	+	+	+	+	+
Fructose	-	+	+	-	+
Arabinose	-	+	-	+	-
Trehalose	+	+	-	-	+
Salicin	+	+	-	+	+
Sucrose	+	+	-	+	+
Maltose	+	+	-	+	+
Raffinose	-	+	+	+	+
Mannitol	-	-	-	-	-
Dextran	-	+	-	-	N
production					
Probable	Lactob	Leucono	Streptoc	Lactoba	Bacillus
identity of	acillus	stocmes	occus	cillus	pumilus
Organism	delbrue	enteroid	thermop	acidophi	ı
	ckii	es	hiles	lus	
+ = Positive;	-= neg			not dete	

negative; N = not determined

3.4. Fungi isolated from unripened cheese during

The yeasts isolated from unripened cheese during storage were Saccharomyces cerevisiae, Saccharomyces lactis, and Torulopsis versatalis. Saccharomyces were isolated from weeks 1 to 3 in all samples while T. versatalis was isolated in the sample without preservative and the sample preserved with 5 % ginger at both ambient and refrigeration storages. Aspergillus spp and Penicilllum spp (Table 4 and Table 5) were isolated in samples stored at ambient temperature from week 2 to week 3.

3.5. Shelf-life studies

The samples with 5% ginger powder had lower microbial counts than the control samples, the samples



with 2.5 % ginger and 0.05 % had lower microbial growth than samples with 5% ginger powder while cheese with 0.1% sorbic acid had the lowest count after storage. It was

Table. 4.: Yeasts isolated from unripened cheese during storage

Test	Yeast				
Test .	I	II	III		
Morphology:					
Pellicle	-	-	-		
Colour	White	Cream	Cream		
Shape	Ovoid	Ovoid	Ovoid		
Reproduction	Budding	Budding	Budding		
Sugars fermented:					
Lactose	-	+	-		
Galactose	+	+	+		
Sucrose	+	+	+		
Maltose	+	-	+		
Raffinose	+	+	+		
Glucose	+	+	+		
Sugars assimilated:					
Lactose	-	+	+		
Maltose	+	+	+		
Sucrose	+	+	+		
Raffinose	-	+	-		
Galactose	+	+	+		
Glucose	+	+	+		
Nitrate	-	-	+		
assimilated					
Probably identity	Saccharomyces	Saccharomyces	Torulopsis		
of organism	cerevisiae	lactis	versatalis		

+ = Positive; - = negative

Table 5: Mould identified during storage of unripened cheese

Isolate	Colour	Hyphae	Arrangement of	Probable
			spores	microorganism
1	Green	Septate	Arranged on Conidiophore	Aspergillus spp
2	Green	Septate	Arranged on septate Conidiophore	Penicilllum spp

observed that the refrigerated samples in general had lower counts when compared with the samples stored at ambient temperature. This showed that sorbic acid, ginger and refrigeration extended the shelf life of the products for three weeks of storage. All samples stored at refrigeration temperature remained stable and retained their fresh aroma while samples stored at ambient temperature showed a change in aroma from week 2 to the end of the storage time. An offensive odour was perceived in the without preservative stored at ambient temperature by week 3. There was no pathogenic organism in the samples throughout storage. This may be due to the occurrence of bacteriocin producing lactic acid bacteria (Aguirre and Collins, 1993; Adepoju et al., 2016). Findings of Belewu et al. (2005) showed that the keeping quality of unripened cheese increased by fifteen days, which is also similar to the findings obtained from this study.

3.6. Sensory assessment of unripened cheese

The cheese sample without preservatives had the highest score for taste, flavour, colour and overall acceptability (Table 6). However, the scores for the cheese sample without preservative were not significantly different (p \times 0.05) from scores recorded for the sample with 0.1 % sorbic acid. This is an indication that 0.1%

addition of sorbic acid had no significant influence on all the sensory properties of the cheese samples. Samples treated with 2.5% ginger and 0.05% sorbic acid had significantly lower scores than samples preserved with 0.1% sorbic acid. This observation showed that increase in ginger led to decrease in the acceptability of cheese. The addition of the ginger extract to cheese probably negatively impacted the colour, taste and flavour, and hence the overall acceptability of the samples as assessed by the judges.

Table. 6. Sensory evaluation of the unripened cheese samples

_	, ,			
Sensory Attributes	A	В	С	D
Colour	2.40c	5.80b	8.40a	8.67a
Taste	3.47c	4.87b	8.07a	8.33a
Flavor	2.53c	5.73b	8.40a	8.40a
Overall acceptability	2.40c	2.93b	8.53a	8.53a

There was no significant difference (p < 0.05) in the values with identical superscript in rows. A - Cheese plus 5% ginger; B - Cheese plus 2.5 % ginger and 0.05 % sorbic acid; C - Cheese plus 0.1% sorbic acid; D - without any preservative

4. CONCLUSION

Preservation of unripened cheese with ginger with or without sorbic acid significantly reduced the microbial load during the period of storage. The decrease was highest in samples treated with 0.1 % sorbic acid and lowest in the unpreserved sample. The decrease was also lower in the samples preserved with 2.5 % ginger and 0.05% sorbic acid than samples preserved with 5% ginger Refrigerated samples had better keeping quality in terms of microbial load, type of microorganisms, colour, texture and aroma than samples kept at ambient temperature.

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