

Full Length Research Paper

Effect of temperature and salt on the quality of waragashi cheese during storage in Benin Republic

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The effect of the storage temperature on the physico-chemical and microbiological composition of waragashi cheese was investigated. For this study, three batch waragashi cheese productions from 10 L of fresh cow milk were investigated. Salted waragashi cheese (7%) was stored for four weeks at -2, 6, and 25°C temperatures and was compared to untreated cheese. Fresh waragashi cheese contained 69.7% moisture, 30.3% total solids, 0.1% acidity (d.b), 11.4% fat (d.b), 7.5 N texture, and a pH of 6.8. At the end of 4 weeks storage, the moisture, the fat, the pH and the texture of salted waragashi cheese at 25°C were 40.09%, 11.45%, 5.0, and 9.97 N, respectively. Significant ($p < 0.05$) variations were found in texture between samples of stored waragashi at -2 and 6°C. However, salted waragashi cheese at low temperature showed similar physico-chemical and microbiological characteristics to that recommended in various norms. At the same temperature, microbial count of stored waragashi cheese did not change considerably, thus showing the efficiency of temperature and salt on storage of waragashi cheese.

Key words: Waragashi cheese, low temperature storage, texture, chemical composition, microbiological quality.

INTRODUCTION

Approximately, one third of the world's milk production is used for cheese manufacturing to conserve the most desirable milk components such as casein, fat, calcium and phosphorus (Kashtanova, 2010). Fresh milk is considered as a perfect food owing to its nutrition, diversity of flavors and texture, and good taste (Fox and Kelly, 2006). In developing countries, due to the lack of accurate conservation methods of the fresh cow's milk, attempts of technological approaches were developed to transform cow's milk into added value products (Dossou et al., 2006). The production of cheese provides a useful service by extending the shelf life of a valuable human

foodstuff-milk.

In Benin, the production of local cheese called waragashi (Peulh cheese) is an alternative, because it increases shelf life of the milk and facilitates its handling and improves profits from its production. Waragashi cheese is widely consumed in rural as well as suburban and urban zones. Lack of standard processing methods explains the variations observed in the quality of waragashi cheese in various production zones (Turkoglu et al., 1987; Belewu, 2001). Indeed, waragashi cheese is an excellent source of protein, fat and minerals (calcium, iron, and phosphorous), vitamins, and essential amino acids, thus making this cheese an important food in the diet for both young and old men. It is often consumed as substitution to meat and fish in various dishes (Kees, 1996). In spite of its economic and nutritional importance,

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it is difficult to preserve waragashi cheese for a long time. Common traditional methods applied for preservation did not extend efficiently its shelf life.

With a view to increasing the shelf life of waragashi cheese, a study on the preservation of waragashi cheese by evaluating the effect of drying and vacuum packaging was investigated (Sacramento, 2008). Results showed that waragashi cheese can be conserved for two months; however, the cost of the product makes the application of developed process difficult in economical point of view. A previous work in Nigeria on waragashi cheese conservation focused on the effect of short-term frozen storage on the chemical composition and coliform microflora (Alalade and Adeneye, 2006).

Although, frozen storage treatment was applied in this study, this had no adverse effect on the chemical composition of waragashi cheese. Moreover, there is a lack of physical parameters data of preserved cheese product. Besides the above techniques used to conserve waragashi cheese, other methods, such as chemical processing and recently, application of plant extracts have been experimented (Egounlety, 1982; Kèkè et al., 2009). However, the taste of produced cheeses was significantly affected.

It is well known, that storing foods under cold temperature, preserve the shelf life of the products as well as color, flavor, and nutritive value (Kuo and Gunasekaran, 2003). Commercially, produced cheeses are frozen and stored to decrease the rate of the spoilage and to prolong the shelf life during marketing (Fontecha et al., 1996). In spite of the popularity of waragashi cheese in West Africa areas, few studies on effect of cold temperature on the texture of waragashi cheese were investigated. The present work aims to evaluate the effect of temperature and salt on physical properties and microbiological characteristics of waragashi cheese during four weeks storage.

MATERIALS AND METHODS

Preparation and storage of waragashi cheese

The milk used for waragashi preparation was obtained from a Peulh camp at Abomey-Calavi. Collected cow's milk was stored immediately in isothermal box containing ice in order to suppress the increase of microorganism population. This study used three batches of equal quantity of 10 L of milk for the preparation of waragashi cheese according to the process developed by Dossou et al. (2006). The *Sorghum vulgare* panicle was purchased from the Godomey market and was used to color waragashi cheese. The effect of salt to increase the shelf life of waragashi cheese was also tested. Produced waragashi cheese from each batch production was divided into two parts, the main one (cheese A) and salted waragashi cheese (7%) (Cheese B). The two types of waragashi cheese were preserved at three temperatures -2°C for AG and BG, 6°C for AR and BR and 25°C for BE for four weeks. In total, 15 waragashi cheese samples were analyzed for physico-chemical and microbiological analysis during storage. Analysis was carried out at intervals of 0, 1, 2, 3, and 4 weeks storage.

Physico-chemical analysis

Total solids content and acidity were determined according to the methods described by AOAC (1990). Cheese samples were analyzed for pH values by using a digital pH-meter (HANNA HI 98129). The fat was extracted from cheese in a Soxhlet extractor with petroleum ether. The fat content was gravimetrically measured after the removal of the solvent by the rotary evaporation under a vacuum. All physico-chemical analysis tests were conducted in triplicate.

Physical analysis

The texture of all waragashi cheese samples were tested during storage and was determined using Stevens LFRA Texture Analyzer. All analyses were done in triplicate.

Microbiological analysis of milk and various types of cheeses

Microbiological analyses were achieved both on the milk and waragashi cheese for evaluation of the total bacterial count, the lactic bacterial, the coliform bacterial count, the yeast and the mould. The plate count agar was used for the total bacterial count. The Man Rogosa Sharp was used for the lactic bacterial, the malt extract agar was used for the yeast and the mould and the violet red bile agar was used for coliform bacteria counts. All microbiological analyses samples of 25 g were taken from the cheese and transferred into 225 ml of the peptone water and were homogenized. From the initial dilution, appropriate decimal dilutions were prepared and aliquots were plated in duplicate on various mediums.

Total bacterial count

One milliliter of each dilution was placed into each box after homogenization. Then, 15 to 20 ml of the plate count agar were smoothly added and kept at $45 \pm 0.5^\circ\text{C}$. After the solidification, the box was turned over and incubated it at 30°C for 72 h.

Lactic bacterial count

One milliliter of each dilution was sowed into each Man Rogosa Sharpe box. The incubation was done at 30°C for 3 days.

Yeast and mould count

The malt extract agar was changed selectively with the lactic acid at 10% after sterilization at 121°C for 15 min and 2 ml from 100 ml of the sterile malt extract agar were used. The incubation was done at 25°C for 3 days.

Coliform bacteria counts

From the decimal dilution taken from 10^{-3} to 10^{-1} , 1 ml in two boxes was carried aseptically twice. Then, about 20 ml of the violet red bile agar were added in each box and were first melted and cooled at $45 \pm 1^\circ\text{C}$. The mixture was homogenized by a circular movement. A range of box is incubated at 37°C during 24 to 48 h for detected the total coliform and a second range is incubated at

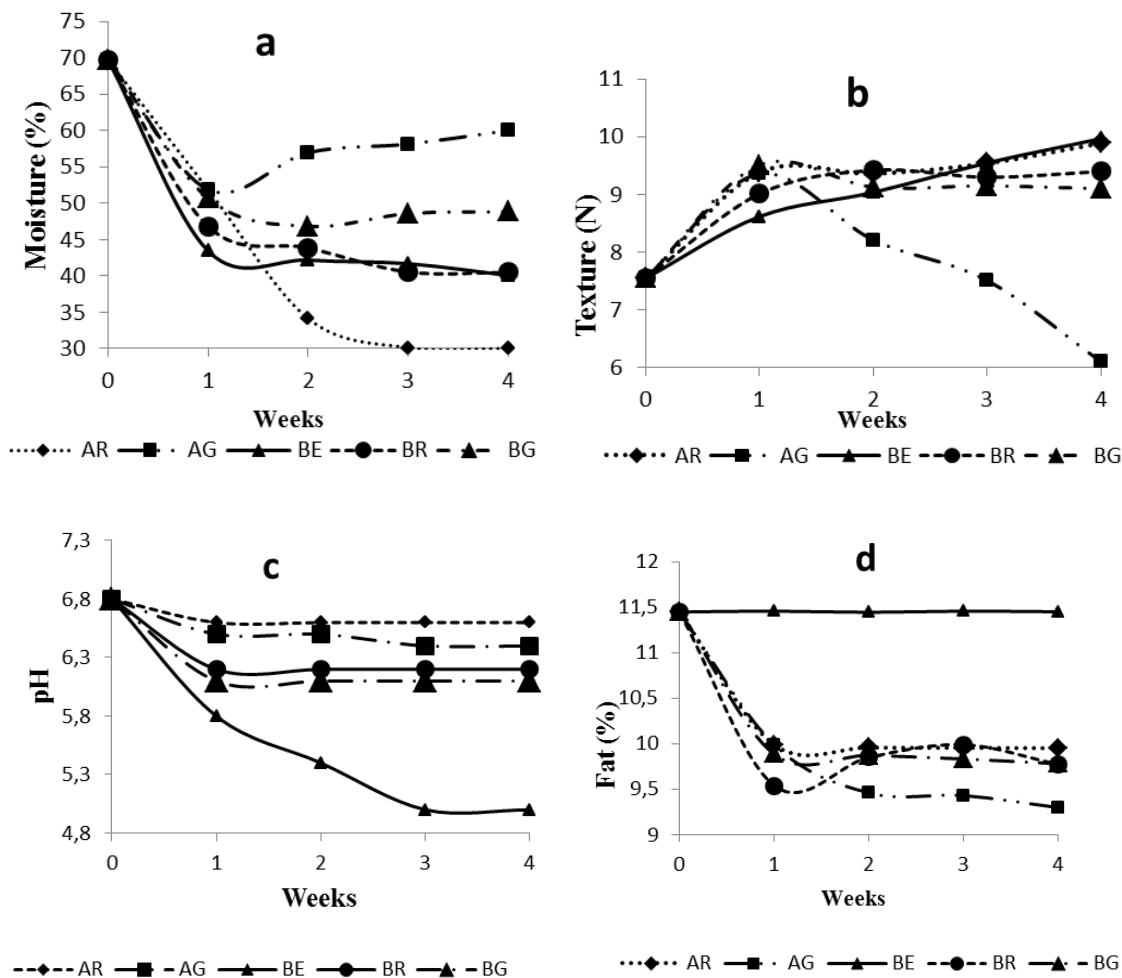


Figure 1. Variation of moisture, texture, pH, and fat of waragashi preserved during four weeks.

44°C for 24 to 48 h for detected fecal coliforms. Boxes incubated at 44°C were recovered and the germ which they contained was sowed in eosin-methylene blue (EMB), and then incubated at 37°C for 24 h in order to detect *Escherichia coli*.

Statistical analysis

The data obtained from these studies were analyzed using Statistical Analysis Software (SAS) and SYSTAT 5.05. The statistical analyses carried out were mean and standard deviation and analysis of variance (ANOVA) (Ogbeibu, 2005).

RESULTS

Physico-chemical characteristics of waragashi cheese during storage

Figure 1 shows changes in moisture, pH, and fat contents of waragashi cheese samples during four weeks of storage. The moisture content of salted and stored waragashi cheese at ambient temperature (waragashi cheese BE) varied from 69.68 to 40.09% and those from

refrigerated sample (waragashi cheese BR) varied from 69.68 to 40.50%, while frozen sample (Waragashi cheese BG) varied from 69.68 to 48.85% (Figure 1a). As observed during experimentation, ambient temperature waragashi cheese samples were completely spoiled. Therefore, data on these samples were not considered.

On the other hand, moisture content of refrigerated waragashi cheese (Waragashi AR) varied from 69.68 to 30.05% and those from deep freezing (Waragashi cheese AG) varied from 69.68 to 60.01% (Figure 1a). As clearly shown in Figure 1a, waragashi cheese (BE) as Waragashi cheese (BR, BG, and AG), lost progressively their moisture and reached a minimum of 46.80, 50.67, and 51.88% respectively during the first week of storage, while waragashi cheese AR reached a lower amount of 34.15% after the second week. Moreover, a decrease of fat content (Figure 1d) was observed with all refrigerated and frozen waragashi cheese during the four weeks storage. The texture of stored waragashi cheese was also investigated. From the results of Figure 1b, an increase of ambient salted sample texture was observed during the four weeks storage.

Table 1. Microbiological quality of milk and waragashi cheese produced.

Germ	CFU/ml	
	Milk	Waragashi
Whiteside test	-	*
Blue methylene test	-	*
Total bacterial count	2.5×10^4	$>3.10^2$
Total coliforms count	1.1×10^3	$<10^2$
Faecal coliform count	6.1×10^1	*
<i>E. coli</i> count	$<10^1$	$<10^1$
Yeasts count	*	2.1×10^2
Moulds count	*	1.2×10^2
Enterobacterial count	*	10^2

*Absence of criterion; †Disregarded.

Table 2. Microbiological quality of Waragashi cheese during four weeks storage (CFU/g).

Germ	Total germ	Yeast	Mould	Lactobacilli	Coliform bacterial
Waragashi A	$<10^{3a}$	2.1×10^{2a}	1.2×10^{2a}	2×10^{2a}	10^2a
Waragashi AR	4.2×10^{3b}	4.7×10^{2b}	1.8×10^{2a}	1.5×10^{3b}	3.5×10^{2b}
Waragashi AG	$<3 \times 10^{2c}$	2.1×10^{2a}	1.2×10^{2a}	2×10^{2a}	10^{2a}
Waragashi BE	1.5×10^{3d}	10^{2c}	10^{2b}	10^{2c}	$<10^{1c}$
Waragashi BR	2×10^{2e}	2×10^{1d}	4×10^{1c}	4.6×10^{1d}	$<10^{1c}$
Waragashi BG	$<10^{2f}$	10^{1e}	2×10^{1d}	10^{1e}	$<10^{1c}$

The mean values followed by same letter in the same column are not significantly different ($p < 0.05$).

Microbiological quality of milk and fresh waragashi cheese

Table 1 shows the microbiological quality of the raw material as milk and produced waragashi cheese. The average total bacterial count, total coliforms count and faecal coliform count for milk were determined and were, respectively, 2.5×10^4 , 1.1×10^3 and 6.1×10^3 CFU/ml, while value for *E. coli* count was inferior to 10^1 CFU/ml. The total bacterial count was lower than 10^5 CFU/ml and was in concordance with (AFNOR, 1976) criteria's. However, the value of total bacterial count founded was slightly superior to that advisable for commercialized milks by (ISN, 1988). Moreover, the obtained negative results from the both tests of white side and blue methylene may confirm the good quality of the milk for the production of waragashi cheese.

Microbial characteristic of waragashi cheese during storage

The values of total germ, yeast, mould, lactobacilli, and coliform bacterial of waragashi cheese at the first day were respectively: 10^3 , 2.1×10^2 , 1.2×10^2 , 2×10^2 and 10^2 CFU/g (Table 1). After four weeks of refrigeration, these values were 4.2×10^3 , 4.7×10^2 , 1.8×10^2 , 1.5×10^3

and 3.5×10^2 CFU/g and were more important ($p < 0.05$) to those of ambient salted samples (BE) (Table 2).

DISCUSSION

It is well known that during storage of cheese, biochemical changes such as glycolysis, proteolysis, and lipolysis take place by modifying the composition of the product and enhancing the characteristics features to cheese, especially the texture and flavor. As shown in Figure 1, changes in moisture, pH, texture, and fat during four weeks storage of waragashi cheese were observed. In terms of moisture content of waragashi cheese at the day of production, the obtained average water content (70%) was similar to those found by (Alalade and Adeneye, 2007).

However, this result agree less with those of several authors (Egounley et al., 1994; Uzeh et al., 2006; Aissi et al., 2009) who found respectively 55.68, 59.99, and 59.92% as averages of moisture. The observed variations may be due to the variability in the process of waragashi cheese production and the conditions of handling and storage of the cheeses (Aissi et al., 2009). The water concentrated in frozen waragashi cheese (AG) as observed in Figure 1a, may increase the volume of cheese and explain the sharply decrease of the texture from the first week to fourth period of storage (Figure 1b).

The decrease of texture is physically expressed by friability of waragashi cheese as reported in several works in the literature. Diefes et al. (1993) suggested that local dehydration of proteins and ice crystal formation in cheese during freezing and frozen storage might cause breaks in the protein structures that allow small fat globules to contact each other and form granules. Kuo and Gunasekaran (2003) reported that extended frozen storage might result in a more extensive breakdown of the cheese structure due to recrystallization of melted ice crystals. After thawing, the proteins are unable to fully rebind water; therefore, water is less confined to the protein matrix, leading to a more porous protein matrix in frozen-stored samples (Kuo and Gunasekaran, 2003). Nevertheless, as shown in Figure 1 b, salted waragashi cheese (BG) under frozen storage became firm (7.547 to 9.1 N). In this case, salt reduced water content of cheese by decreasing the frozen water below -2°C .

Generally, the pH of the stored waragashi cheese in cold temperature decreased slowly from 6.8 ± 0.1 to 6.5 ± 0.1 , while the salted waragashi cheese at 25°C showed the lowest acidity after four weeks of conservation (Figure 1c). This observation was also reported by Egunlety (1982), Sacramento (2008), and Kèkè et al. (2009). It was also observed that waragashi cheese under ambient temperature undergoes considerable and undesirable chemical changes after the second day of storage (Ashaye et al., 2006). These changes are caused by increased activity of the resident lactic bacterial and adventitious microorganisms. There is therefore the need to use agents such as salts for the prolongation of shelf life of cheese, as experimented in the present work. In other works, natural acidifying agents, namely, white vinegar, lemon juice, and grapefruit juice were successfully tested (Abdel-Razig and AlGamry, 2009). As found, the cheese samples stored at room temperature had a shelf life of 30 days, while that stored at cold temperature were still better till the end of storage of 60 days.

Changes in fat content of stored waragashi were also observed (Figure 1d). The decrease of fat content with cold waragashi cheese may probably be attributed to lipolytic activities of microorganisms inherent in waragashi cheese (Alalade and Adeneye, 2007). As reported in another work, Loney and Bassette (1970) observed an increase in fatty acid concentration during cheese storage.

The positive effect of the treatment of waragashi cheese during ambient temperature storage was clearly observed in Figure 1d. As obtained, the rate of fat concentration under ambient temperature storage with salted waragashi cheese (BE) is till almost unchanged. In contrast to the available norms used for the enumeration of total bacterial count for milk, there is at this time no norm for waragashi cheese. As shown in Table 1, the produced waragashi cheese showed lower total bacterial count and total coliforms than that detected in the milk.

This finding confirms the destruction of the micro-organism by heat. On the other hand, the count of yeasts and moulds in waragashi cheese were higher to that recommended by the norms from AFNOR (1976), which maintain the count of such microorganism at the level inferior than 10 CFU/ml (Guirau and Galzy, 1980).

For both refrigerated and frozen samples, significant increase of germs was not observed, as reported also by Foucaud (2006) on refrigerated sea products. The decrease of temperature inhibited the development of most germs, as observed in this work during storage of waragashi.

In terms of coliform bacterial count, the salted frozen samples (BG) and ambient stored samples (BE) showed the lowest values (< 10 CFU/g) and are in agreement with those found by Kuo and Gunasekaran (2003) which confirmed the elimination of some bacteria by salt.

Conclusion

From the results of this study, the effect of the storage temperature (-2 and 6°C) and salt do not only preserve texture of waragashi cheese, but also preserve it from the multiplication of microorganisms. However, frozen storage affected the texture of waragashi cheese, although its microbial quality is acceptable. Because of the cost of energy in development countries, further work needs to be carried out for the accurate storage of waragashi cheese in rural areas.

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