

Physicochemical, Biochemical and Microbiological Changes of Sudanese White Soft Cheese (jibna- beida) during Ripening

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Abstract:

Cheeses receive high attention since they are important food items in the Sudanese meal. Sudanese white soft cheese (Jibna-Beida) is a traditional product and little information is available about its manufacture.

In this study the main objective was to study the physicochemical, biochemical and microbiological changes of Sudanese soft white cheese (Jibna-Beida) during ripening. Different samples were subjected to physicochemical, biochemical and microbiological analysis.

Total solids (TS), Soluble Nitrogen (SN), Titratable Acidity (TA), Free Amino Acids (FAAs) and Free Fatty Acids (FFAs) in all samples investigated increased during the ripening period (60 days), while fat, protein and pH decreased.

*Other than the added starter culture (1:1 Combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*), *Bacillus plantarum*, *Lactobacillus lactis*, *Lactobacillus brevis*, *Streptococcus lactis*, *Enterococcus faecalis*, *Lactococcus lactis* subsp. *Lactis*,*

Pediococcus pentosaceus, Pediococcus acidilactici and Pediococcus halophilus were detected and enumerated.

Key words: Sudanese soft white cheese, jibna-beida physicochemical, biochemical, and microbiological

1. INTRODUCTION

Numerous fermented food products owe their production and characteristics to the fermentative activities of microorganisms. Many foods such as ripened cheeses, pickles are preserved products and hence their shelf life is extended considerably compared to the raw materials from which they are made. In addition to their shelf stability, all fermented foods have aroma and flavor characteristics that have resulted directly or indirectly from the fermenting organisms. From all these indications, no other single group or category of foods or food products are as important as such products. In addition fermented foods have their role in the nutritional well-being of consumers throughout the world.

Cheese making in Africa is largely dictated by tradition and the availability of milk supplies (O'Connor, 1993). Cheese making is one of the most important methods for preserving the majority of milk in dry hot countries.

There are many reasons for the increased consumption of cheese due to changes in the eating habits, personal preference and products cost. In some countries, soft fresh cheese constitutes a large proportion of total cheese consumption (Robinson, 1985).

In Sudan jibna-beida (Sudanese white soft cheese) is the most available cheese to the general public on the market. There is little doubt that the technology of making "Jibna-beida" has been introduced into Sudan from Egypt or through Egypt from the Mediterranean countries, such as Greece (Dirar, 1993). Jibna-beida is a pickled type of cheese that is stored in airtight containers filled with salted whey (Kur, 1992). In Sudan, it is manufactured as a soft pickled ripened cheese, produced from raw or pasteurized milk (Osman, 1987).

The cheese is white colored with close texture, generally consumed fresh or matured for a period of several months (Ahmed, 1985). White cheese production, in Sudan, is based mainly in small modern dairies and family plants which is often resulted in different compositions and poor hygienic quality (Ali, 1984).

Cheese as a fermented milk product is a complex and dynamic system that contains microbiologically defined and undefined highly variable starter flora. The diversity of the micro flora and hence the different enzyme systems involved in cheese ripening, add to the complexity of the process (Davis, 1987).

The particular flavour and the typical organoleptic properties of cheese are associated with specific attributes of raw milk. Environmental micro flora may also have a role in the fermentation process and the quality of cheese (Corroler *et al*, 1998).

Major biochemical changes take place during cheese ripening such as fermentation of lactose, degradation of proteins, hydrolysis of lipids and production of volatile aroma compounds. Proteolysis is the most complex and important phenomenon during cheese ripening and it plays a major role in internal bacterially ripened cheeses (Fox *et al.*, 1993). During proteolysis, proteins are converted from insoluble to soluble forms and thus broken down to proteose-peptones, polypeptides, amino acids and ammonia (Ling, 1963). This reaction is caused mainly by the added rennin, the coagulant indigenous milk proteinases (especially plasmin) and proteinases and peptidases from starter and non-starter bacteria (Fox *et al.*, 2000). Enzymes from starter bacteria seem to play a major role in cheese ripening and in the development of cheese flavour (Martley & Crow, 1993). However, insufficient flavour and texture defects are observed in cheeses made with non-specific starter culture (Karakus & Alperden, 1995). Peterson *et al*, (1990) reported that to effectively determine the contribution of particular adjunct strains in cheese flavour development, the Non-starter lactic acid bacteria (NSLAB) must be correctly identified.

2. MATERIALS AND METHODS

2.1. Cheese-making

Fresh cows' milk (about 200 kg) was obtained early in the morning from Khartoum university farm. Milk was transferred into stainless steel containers for cheese manufacture. The milk was pasteurized and cooled to 42 °C. Starter (1% of 1:1 Combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) was then added. 6 % Salt was stirred in. The milk was stirred gently to avoid creaming for 15 min before renneting. Rennet tables (1 tablet/50kg) were added to large area of the milk surface and stirred for few minutes. Milk was then stirred for 20 min and left at the coagulation temperature of (40°C) for 3 hours. After the completion of curdling, the curd was cut into about two centimeter cubes with an ordinary stainless steel knife to allow the separation of whey. The curd cubes were then allowed to release whey before being transferred to the cheese wooden moulds and wrapped with cheese cloth. The curd was kept for about 45 minutes for whey to drain. Much of the whey was collected into a clean container. The edges of the cloth were overlapped firmly in the wooden frame. Then a wooden cover was put on top of the curd inside the wooden mould and weights were put on top of the wooden cover to press the curd. Suitable weights were put on the moulds cover for about 24 hours. The formed curd was then cut into small pieces and stored in the salted whey at room temperature (35-37°C).

2.2. Assays for physicochemical and biochemical Parameters:

Total solids, Fat content and Titratable acidity (TA) were determined by (AOAC) methods (1990), Total nitrogen (TN) and soluble nitrogen (SN) were determined using the Kjeldahl method according to Ling (1963). pH was done by Newlander and Atherton (1964), Free Amino Acids (FAA) was determined according to FIL-IDF (1982). Amino acid concentrations of individual free amino acids were determined using amino acid analyzer (Beckman model 6300 amino acid analyzer equipped with a Beckman model P-N 338052 Na⁺ cation-exchange column 12-0.4 cm). Fatty acids methyl esters were prepared according to the (ISO, 2002). Fatty acids methyl esters were separated by Gas Liquid Chromatography, (O.D. stainless steel 10 % carbowax 20 M on Chromosorb W.A.W 80-100 mesh fitted with Konik 3000C).

2.3. Microbiological analysis

Microorganisms were enumerated in cheese samples during ripening (0 and 60 days). Cheese samples were emulsified in sterile 2% (w/v) trisodium citrate, serially diluted in sterile saline solution and plated in duplicate on the MRS agar at pH 5.8, incubated anaerobically at 30°C and 37°C for 48 h. After incubation, randomly selected colonies were purified by two subsequent subcultures and then submitted to microscopic examination, Gram staining, oxidase test, catalase test, production of gas from d-glucose, growth in 6.5% (w/v) NaCl, growth at 10°C and 45°C. Representative colonies were isolated Purified and identified with the conventional methods (Kiss, 1984). Biochemical tests described by Barrow and Feltham, (1993) were used for the identification of bacteria.

3. RESULTS AND DISCUSSION

The total solids (TS), soluble nitrogen (SN), titratable acidity (TA), in all the cheese samples investigated increased during the ripening period (60 days), while the fat, protein and pH decreased (**Table 1**).

The acid curdling affected the TS during ripening as expected in both samples with added starter. These results agreed with the findings of Celik et al, (2002) who reported that the TS content increased significantly for all types of cheese with ripening possibly because of salt diffusion into the cheese.

Abd El-Salam, (1987) reported that the total nitrogen content of cheese decreased gradually, while soluble nitrogen fractions increased continuously during storage, indicating continuous proteolysis. The transfer of degradation products to the brine by diffusion explains the decrease in total nitrogen during storage.

The increase of Titratable Acidity (TA) % in these samples was attributed to the high load of micro flora in the raw milk used in manufacturing. This result was in agreement with, Goncu and Alpkent (2005), who used starter culture in the processing of white cheese from raw milk which seemed to increase the TA %. During cheese ripening, the TA increased in all types of cheese. This may be attributed to lactic acid bacteria present in raw milk. Similar findings were obtained by, Marth and Steele, (2001).

Abdalla, (1992) reported that the decrease in fat was due to leakage of some fat into brine solution. Also the results agreed with the Nofal et al., (1981) who reported that the decrease in fat content of cheese could be attributed to the increased actions of microorganisms at low salt concentration.

The pH values of cheese samples decreased gradually during ripening as expected. McSweeney & Fox, (1993) found that the pH of cheese is influenced by the growth of both starter and non-starter lactic acid bacteria in raw and pasteurized milk cheeses.

Table 1. Cheese Properties at day 1 and 60 of storage period

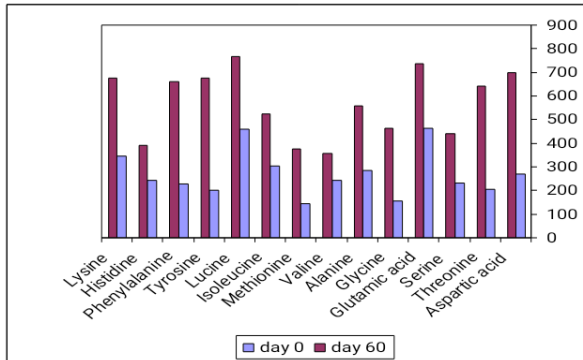
Properties	Day 1	Day 60
Total solids %	51.51	61.88
Fat content (in-dry matter) %	20.00	13.93
Titrateable Acidity (as lactic acid)	0.34	2.20
Crude Protein content %	29.67	17.85
Soluble Nitrogen %	0.49	1.03
pH	5.50	1.83

The increase in the Free Amino Acids (FAAs) during ripening of the cheese samples is shown in **Figure 1**. The dominant FAAs in zero time of storage were Aspartic acid, Threonine Glutamic acid, Alanine, Isoleucine, Leucine and Lysine. By the end of ripening, Phenylalanine and Tyrosine showed the highest increase. Beresford *et al.*, (1998) reported that the FAA increased during ripening and Aspartic acid, Glutamic acid, Alanine, Isoleucine, Leucine, Lysine and Threonine were the principal FAAs in samples at zero time of ripening. Other workers (Michaelidou et al, 2003) found that Leucine, Glutamic acid, Valine and Lysine were the major FAAs in Feta cheese made from cows' milk. These FAAs were also dominant in 60-day old Turkish White-brined cheese made from pasteurized cows' milk (Ucuncu, 1981).

Protein breakdown evolving short peptides and free amino acids is of the major concern in cheese flavour and taste. Since proteolytic enzymes are highly specific, any alteration in peptide chain structure and amino acids sequence in the chain might produce bitter taste and bad flavour during cheese ripening. This is why the need of proper use of the added enzyme and the starter cultures that

will produce the required taste and flavour of cheese according to its type are of high importance to the cheese manufacturers.

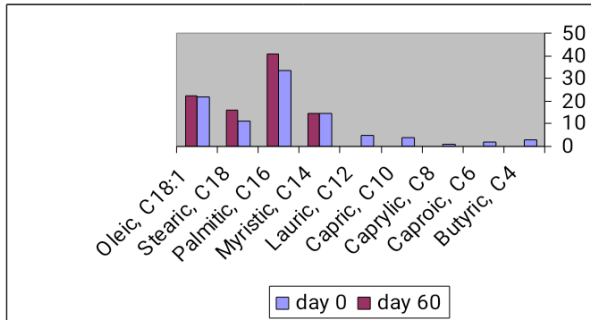
Fig. 1: Changes of Free Amino acids (FAAs) (mg/100 g DM) in cheeses samples during ripening



In **Figure 2** the Free Fatty Acids (FFAs) in cheese samples during ripening were shown. Myristic, Palmitic, Stearic and Oleic were the principal FFAs in cheese samples at zero and at the end of the ripening period. The total FFA contents were higher than those reported for other pickled cheese varieties, such as feta (Abd El-Salam *et al.* 1993), Teleme (Mallatou *et al.* 2003) and Iranian brined cheese (Azarnia *et al.* 1997). This could be due to the type of milk used and to the type of bacterial load involved. The total FFA in these white cheeses would contribute significantly to the flavour and sensory characteristics of the cheeses produced. Similar results were reported in Domiati cheese by Abd El Salam *et al.* (1993). According to the relative proportions of free fatty acids; palmitic (C16:0) and stearic (C18:1) acids were the dominant free fatty acids in Turkish White cheese. In Feta cheese, the principal free fatty acids were acetic and palmitic acids in control experimental cheeses (Alichanidis *et al.*, 1984) and all experimental cheeses (Katsiari *et al.*, 2000).

As for the proteins and fatty acids types and prevalence they contribute significantly to the flavour of the cheeses involved. It's known that short chain fatty acids are the main contributors to milk products flavour including cheeses. Hence, fatty acids breakdown should be monitored carefully by choosing the right starter cultures in order to achieve the right flavour.

Fig. 2: Changes of free fatty acids (mg/100 g DM) in cheeses samples during ripening



The identification of species isolated from cheese samples was shown in **Table (2) and Figure (3)**. The Results showed that *Lctococcus lactis sp lactis* represented (33.3%), while *Streptococcus Lactis*, *Enterococcus faecalis*, *Pediococcus acidilactici* and *Pediococcus pentosaceus* showed (16.7%) of the samples at zero storage respectively.

However the bacterial isolates from samples at the end of ripening were identified as *Lctococcus lactis sp lactis* (40 %), *Enterococcus faecalis*, *Pediococcus acidilactici* and *Pediococcus pentosaceus* (20%). *Streptococcus Lactis* was not detected. *Streptococcus lactis* was only detected at zero time in cheese samples thus producing lactic acid from lactose.

These results are typical for microorganisms isolated from semi-hard cheese and comparable to those reported for other artisanal cheeses originated in Portugal (Tavaria & Malcata, 1998), Spain (Fontecha et al., 1990).

Results showed that the *Lactococcus Lactis ssp. Lactis* was found predominating in cheese samples. Smit et al, (2005) found that the enzymes from *Lc lactis* starter cultures were shown to degrade caseins which led to the formation of key flavour components that contributed to the sensory perception in cheese. Uysal (1996) used a lactic culture (1:1 mixture of *Lactococcus lactis ss. lactis* and *Lc. lactis sp. cremoris*) at two levels in the manufacture of Turkish White cheese and analyzed the cheese during 90 days of storage. The results revealed that while the acidity, water-soluble nitrogen content and ripening index values of the white cheese increased during storage,

the pH and the level of total nitrogen in the cheese decreased. He added that the level of proteolysis in the cheese increased as the level of starter culture used was increased.

Production of lactic acid from lactose, in combination with short- and medium-chain fatty acids produced by *Streptococcus lactis* and *Streptococcus cremoris*, is the cause of acid flavours in raw and pasteurized milk. Because of the ubiquitous nature of these bacteria and their ability to inhabit different types of environments, most milk is unintentionally inoculated with these organisms immediately after milking or during processing. If the milk is not cooled to 4.4°C or below, it will eventually develop an acid flavour as a result of proliferation of these bacteria and their conversion of lactose to lactic acid (Bassette et al, 1986).

Enterococcus faecalis predominated during the ripening period in cheese samples. Tzanetakis and Litopoulou-Tzanetaki, (1992) reported that Enterococci (e.g. *E. faecalis* and *E. durans*) had been found in high numbers in fresh feta cheese. *Enterococcus faecalis* is found in many cheeses and is thought to have a positive influence on cheese flavour (Franz, et al., 1999).

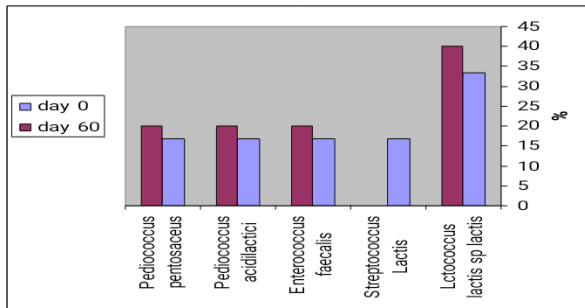
Pediococcus pentosaceus and *P. acidilactici* were detected and predominated in the cheese samples.

P. acidilactici and *P. pentosaceus* were present as secondary flora in different types of cheese (Vafopoulou et al, 1999). Tzanetakis et al., (1991) reported that *P. pentosaceus* improved the flavour of the soft Greek cheese, Teleme, which was made with yogurt starter cultures.

Table 2 Identification of dominant LAB species found – number of isolates in cheese samples

Species	Day 0%	Day 60%
<i>Lctococcus lactis sp lactis</i>	33.3	40
<i>Streptococcus Lactis</i>	16.7	0
<i>Enterococcus faecalis</i>	16.7	20
<i>Pediococcus acidilactici</i>	16.7	20
<i>Pediococcus pentosaceus</i>	16.7	20

Figure 3: Identification of dominant LAB species found – number of isolates in cheese samples



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