

MICROBIOLOGICAL CHARACTERISTICS OF SUDANESE WHITE SOFT CHEESE MADE WITH DIFFERENT LEVELS OF CASSAVA POWDER (*MANIHOT ESCULENTA*)

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ABSTRACT: This study aimed to investigate the microbiological quality of Sudanese White soft Cheese made from different levels of Cassava powder (*Manihot esculenta*). One hundred and twenty (120) liters of fresh cow's full cream milk were used for making cheese in this study. Four treatments were carried out; The first treatment is the control in which cow's cheese milk had left free without any additive, While in the second (Cw1), third (Cw2) and fourth (Cw3) treatments 0.5%, 0.75% and 1% of cassava powder were added respectively to cheese milk before pasteurization then white cheese was made and stored at room temperature for 90days. Microbiological evaluation was done for the cheese samples at 0, 30, 60 and 90 days intervals. Statistical analysis showed that cassava powder significantly ($p < 0.05$) affected the Total viable bacteria count, Lactobacilli, Streptococci, Yeast and Moulds and Staphylococcus aureus while there were no significance ($p < 0.05$) effect on the Coliforms count. The results also showed that the storage period had significance effect ($p < 0.05$) on all the characteristics mentioned above.

INTRODUCTION

Cheese is known as a complete nutritious food product and excellent source of many key nutrients, suitable for any ages. It is rich in protein and minerals ([Pantaleao et al., 1990](#)). According to [Scott, \(1986\)](#) cheese can be defined as the solid food made from the milk of cows, goat, sheep and other mammals, and ore compact and has a longer shelf life than the milk from which it is made.

The dominate cheese manufactured in Sudan is Gebna Bayda, which is the local name for white cheese, and the most popular type ([Khateeb, 1997](#)). Sudanese white soft cheese traditionally manufactured in different areas of Sudan. The cheese may be consumed fresh but more commonly after maturation in salt brine or salted whey ([Abdalla and Abdel Razig, 1997](#)).

Sudanese White cheese is widely consumed by people of all socioeconomic classes; most of it is made in houses and some private farm. Sudanese white cheese is delivered to the market immediately after processing, under inadequate conditions, poor handling technique, inappropriate packaging materials and lack of adequate storage facilities, however, essential dairy products including cheese must be safe, acceptable and meet consumer's satisfaction ([Khalifa, 1989](#); [Ibrahim, 2003](#)). As a result, cheese production must be protected from pathogenic and spoilage microorganisms, as well as from decaying both on the sites of production and consumption ([Scott, 1986](#)). The organisms may find their way into cheese as a result of environmental contamination during processing and packaging. The quality of cheese depends on a variety of factors among which raw milk composition, technological process parameters, bacteria species, storage, transportation and delivery conditions ([Rotaru et al., 2008](#)).

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The Cassava crop is originated from the American tropics ([William, 1989](#)), it's very essential crop for food security in the humid and sub humid tropics of Africa ([Thottappiliy, 1992](#)). There are many varieties of cassava and it is classified as sweet or bitter cassava ([Ravindran, 1991](#)). For the making of cassava flour, the fresh roots are peeled, washed, and cut into large or small slabs and allowed to dry under the sun; the dry slabs can be milled to produce grayish white flour which can be used for producing many type of food ([John and Sons, 1978](#)).

MATERIALS AND METHODS

The present study was conducted during 2013 at the Laboratory of Dairy Science and Technology Department, College of Animal Production Sciences and Technology, Sudan University of Science and Technology. In this Study four treatments were carried out as follows: First treatment is a control in which fresh cow's full cream milk cheese had no additive. In the second (Cw1), third (Cw2) and fourth (Cw3) treatments 0.5, 0.75 and 1% of cassava powder were added respectively to the fresh cow's full cream milk before pasteurization.

2.1. Materials

One hundred twenty liters (120 liters) of fresh cow's full cream milk were purchase from a private farm at Khartoum North. Cassava roots were brought from Konyo-Konyo market at Juba and then were cut into small pieces and dried under the sun light for 1-3 days, then grinded to a fine powder (flour) before added to the milk. A fine commercial Salt (Sodium Chloride) was purchased from the local market. Rennet powder of one gram per 50 liters of milk was obtained from Hassan El-said center for veterinary services at Hellat Kuku Khartoum North–Bahary. Calcium Chloride Powder was purchased from Lab line International Company, Khartoum – Sudan. Commercial starter (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) was purchase from local markets. The Triplicate Sterile plastic buckets of (500 gram) were brought from Hala Plastic Factory–Omdurman.

2.2. Cheese manufacturing

Cheese was manufacture according to the method described by [Ibrahim, \(2003\)](#) with some modifications. One hundred and twenty liters (120 liters) of fresh clean cow's full cream milk was divided into four equal volumes and kept into four separate tanks .The first volume was left free without any additive of cassava powder, while in the other three volumes cassava powder was added at the rates of 0.5, 0.75 and 1% to the milk respectively. The different milk samples were pasteurized at 72°C for 1 minute. The milk samples were then transferred into stainless steel containers for cheese manufacture and then cooled to 42°C. Commercial starter (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) in the ratio of 1:1% concentrate was added at the level of 1 % (W/v). The milk was stirred gently for 15 minutes to avoid creaming before renneting. Rennet powder (1 gram/50 liters) was dissolved in 50 ml of distilled water and added to the milk at 40°C. Fine Calcium chloride was added at the levels of 0.02 % immediately. Milk was then stirred for 20 minutes and then left undisturbed for 3 hours to develop curd. The curd was cut into small cubes (2.5x2.5x2 cm). After draining, salt at 2% (w/v) was mixed with the curds. The curd was poured into small clean wooden molds lined with cheese cloth and pressed by 30 kg weight overnight. The next day, brine solution was prepared by adding salt to the collected whey (8 % w/v), and pasteurized at 72°C for 1 minutes and cooled to 40°C. The pressed cheese was cut into small cubes and then transferred to the Triplicate Sterile plastic buckets containers filled with whey. The Containers were sealed and stored at room temperature (38±2) for 90 days. Chemical evaluations of cheese samples were carried out at 0, 30, 60 and 90 days interval.

2.3. Microbiological examination

Total viable Bacterial Count (TBC) was determined according to [Ramakant, \(2006\)](#) using standard plate count agar and the plates. Total lactobacilli were determined according to [Frank et al., \(1992\)](#) using MRS agar medium. M17 agar medium (Merck-15108) was used to determine streptococci count according to [Oksuztepe et al., \(2005\)](#). Yeasts and moulds were enumerated according to [Marshall, \(1993\)](#) using Potato Dextrose Agar (PDA). Coli form bacteria was determined according to [Marshall, \(1993\)](#) using MacConkey broth. Mannitol Salt Agar was used for enumeration of *Staphylococcus aureus* count according to [Rayman et al., \(1988\)](#).

2.4. Statistical analysis

Statistical analysis was done by using [SPSS, \(1998\)](#) program (version 17). General Linear models were used to estimate the effect of storage periods, Cassava powder on the microbiological characteristics of cow's milk white soft cheese. Least Significance Difference (LSD) was used for mean separation between the treatments. The level of Significance (0.05) was used in this Study.

RESULTS

The average microbiological characteristics of the pasteurized milk used for cheese making is presented in Table 1.

3.1. Effect of the storage period on the Microbiological characteristics of the cow's white soft cheese

Results in Table 2 show the main effect of storage period on the Microbiological quality of the cow's white soft cheese. All the characteristics under investigation total viable bacteria, Lactobacilli, Streptococci, Yeast and moulds, Coli forms and *Staphylococcus aureus* were significantly ($p < 0.05$) affected by the storage period.

The results in Table 2 indicated that the Total viable bacteria of the cheese was affected significantly ($p < 0.05$) by the storage period. It was increased from 6.51 ± 0.35 cfu/ml at the day zero to 7.93 ± 0.06 cfu/ml at day 60 and then decreased to 6.58 ± 0.41 cfu/ml at the 90 of the storage period.

Data in Table 2 show that the Lactobacilli of the cow's milk cheese was affected significantly ($p < 0.05$) by the storage period. It was decreased from 5.00 ± 0.40 cfu/ml at the day zero to 3.34 ± 0.33 cfu/ml at the day 90 of the storage.

The results (Table 2) showed that Streptococci count in the cheese was affected significantly ($p < 0.05$) by the storage period. It was decreased from 5.00 ± 0.36 cfu/ml at the day zero to 2.65 ± 0.09 cfu/ml at the day 90.

It was observed (Table 2) that Yeast and moulds of the cheese was significantly ($p < 0.05$) increased from 4.76 ± 0.07 cfu/ml at the day zero to 6.66 ± 0.09 cfu/ml at the day 90.

It was clear (Table 2) that the Coliforms of the cheese decreased significantly ($p < 0.05$) from 5.00 ± 0.15 cfu/ml at the day zero to 2.29 ± 0.06 cfu/ml at the day 60 and it was not detected at the day 90 of the storage period.

The results (Table 2) presented that the *Staphylococcus aureus* of the cow's milk cheese was affected significantly ($p < 0.05$) by the storage period. It was decreased from 4.83 ± 0.05 cfu/ml at the day zero to 3.84 ± 0.08 cfu/ml at the day 90 of the storage period.

Table1: Microbiological analysis of the pasteurized milk used in the study

Total viable bacteria cfu/ml	Coliforms cfu/ml	Lactic acid bacteria cfu/ml	<i>Staphylococcus aureus</i> cfu/ml	Yeast and moulds cfu/ml
3.0×10^3	-ve(no growth)	- ve(no growth)	-ve (no growth)	-ve(no growth)

cfu= Colony forming unit

Table 2: Effect of the Storage period on Microbiological characteristics of white cheese:

Storage Period	Microbiological quality					
	Total viable bacteria (cfu)	Lactobacillus (cfu)	Streptococci (cfu)	Yeast and moulds (cfu)	Coiforms (cfu)	Staphylococcus aureus (cfu)
Day 0	6.51 ± 0.35^d	5.00 ± 0.40^a	5.00 ± 0.36^a	4.76 ± 0.07^d	5.00 ± 0.15^a	4.83 ± 0.05^a
Day 30	7.61 ± 0.45^b	4.09 ± 0.34^b	3.99 ± 4.49^b	5.13 ± 0.34^c	3.31 ± 0.06^b	4.77 ± 0.05^b
Day 60	7.93 ± 0.06^a	3.71 ± 0.13^c	3.81 ± 0.31^c	5.83 ± 0.12^b	2.29 ± 0.06^c	4.63 ± 0.05^c
Day 90	6.58 ± 0.41^c	3.34 ± 0.33^d	2.65 ± 0.09^d	6.66 ± 0.09^a	ND	3.84 ± 0.08^d
LS	***	***	***	***	***	***

- Mean values bearing different superscripts within columns are significantly different ($P < 0.05$)

- LS= Levels of significance

3.2. Effect of different levels of Cassava powder on Microbiological characteristics of white cheese

Results in Table 3 illustrated the effects of different levels of Cassava powder on the microbiological characteristics of the white cheese. Total viable bacteria, Lactobacilli, Streptococci, Yeast and moulds, and *Staphylococcus aureus* significantly ($p < 0.05$) affected by the different levels of cassava

powder, while there were no significance difference ($p < 0.05$) in Coli forms counts due to addition of Cassava powder.

The results in Table 3 indicated that there were significance difference ($p < 0.05$) in Total viable bacteria count between all treatments. The highest Total viable bacteria count 7.35 ± 0.57 cfu/ml was found in the Cow's milk cheese with 0.5 % Cassava (Cw1) while the lowest one 6.63 ± 0.82 cfu/ml was recorded in the Cow's milk cheese without Cassava.

The results obtained in table 3 raveled that there were significance difference ($p < 0.05$) in the Lactobacillus count of the cow's milk cheese between all the treatments. The highest Lactobacilli count 4.25 ± 1.09 cfu/ml was recorded in the cow's milk cheese without Cassava (Control), while the lowest one 3.95 ± 0.52 cfu/ml was in the Cow's milk cheese with 0.75 % Cassava (Cw2).

Streptococci count (Table 3) were affected significantly ($p < 0.05$) by the different levels of Cassava powder. The highest Streptococci 4.27 ± 1.16 cfu/ml was recorded in the Cow's milk cheese without Cassava (control) while the lowest one 3.63 ± 0.79 cfu/ml was found in the cow's cheese milk with 0.5 % Cassava (Cw1).

Table 3: Effect of different levels of cassava powder on Microbiological characteristics of white cheese:

Treatments	Microbiological quality					
	Total viable bacteria (cfu)	Lactobacillus (cfu)	Streptococci (cfu)	Yeast & moulds (cfu)	Coiforms (cfu)	Staphylococcus aureus (cfu)
Control	6.63 ± 0.82^d	4.25 ± 1.09^a	4.27 ± 1.16^a	5.75 ± 0.67^a	2.64 ± 1.78	4.54 ± 0.44^a
Cw1	7.35 ± 0.57^a	3.97 ± 0.50^b	3.63 ± 0.79^d	5.50 ± 0.77^d	2.65 ± 1.87	4.50 ± 0.36^d
Cw2	7.32 ± 0.60^c	3.95 ± 0.52^d	3.76 ± 0.75^c	5.54 ± 0.81^c	2.65 ± 1.88	4.50 ± 0.44^c
Cw3	7.33 ± 0.60^b	3.96 ± 0.50^c	3.79 ± 0.76^b	5.60 ± 0.77^b	2.66 ± 1.88	4.53 ± 0.41^b
LS	***	***	***	***	NS	**

Mean values bearing different superscripts within rows are significantly different ($P < 0.05$).

Cfu=Colony forming units

Cw1=Cheese with 0.5 % Cassava

Cw2= Cheese with 0.75 % Cassava

Cw3=Cheese with 1 % Cassava

The results (Table 3) demonstrated that there were significance difference ($p < 0.05$) in the Yeast and moulds count of the cow's milk cheese between all treatments. The highest Yeast and moulds count 5.75 ± 0.67 was recorded in the cow's milk cheese without Cassava (Control), while the lowest one 5.50 ± 0.77 was found in the cow's milk cheese with 0.5 % Cassava (Cw1). It was clear from the results (Table 3) that there were no significance difference ($p < 0.05$) in the Coli forms count between all treatments. The highest Coliforms count 2.66 ± 1.88 was found in the Cow's milk cheese with 1 % Cassava (Cw3) while the lowest Coliforms count 2.64 ± 1.78 cfu/ml was found in the Cow's milk cheese without Cassava (Control). The results (Table 3) indicated that there were significance difference ($p < 0.05$) in the *Staphylococcus aureus*. The highest *Staphylococcus aureus* 4.54 ± 0.44 cfu/ml was found in the cow's milk cheese without cassava (Control) while the lowest one 4.50 ± 0.36 cfu/ml was in the cow's milk cheese with 0.5 % Cassava (Cw1).

DISCUSSION

4.1. Effects of Storage period and different levels of cassava powder on Microbiological characteristics of white cheese

Total viable Bacteria increased significantly with the storage period from the day zero up to day 60 and then decreased at the day 90 of the storage period. Similar result were obtained by [Muna et al., \(2013\)](#) who studied the total bacteria count of Sudanese white soft cheese stored in five different containers (metal tin, metal tin lined with polyethylene bags, plastic, plastic lined with polyethylene bags and metal gallon) they found that the total bacteria count increased from the day zero up to the day 75 of the storage period and then decreased from the day 90 up to the day 180 in some containers and no bacteria was detected from the day 120 up to the day 180 of the storage period in the other containers. Our results were in agreement with the results of [Ur-Rehman et al., \(2000\)](#) and [Ceylan et al., \(2003\)](#) who found that the total bacteria of the cheese increase during the storage period due to microbial count of raw milk. However, our results were not in accordance with those of [Sana et al., \(2011\)](#) who stated that total viable bacteria count decreased with the storage period from the day 1 up to day 90 in white cheese made from *Solanum dubium* extract and rennet. Similar decreased in total viable bacteria count was obtained by [Barakat, \(2009\)](#) who studies the total viable bacteria count of Domiati cheese made with the cells of *Lactobacillus reuteri* and he found that the log of total bacteria count was 8.9 ± 0.029 , 8.76 ± 0.066 , 8.72 ± 0.014 and 8.59 ± 0.020 cfu/ml at

the day zero, 30, 60, and 90 of the storage period respectively. The lactobacilli decreased significantly with the storage period. These results coincide the results that obtained by [Sana et al., \(2011\)](#) who found the lactobacillus of white cheese made from *Solanum dubium* extract and rennet decreased with the storage period from the day 1 up to the end of the storage period (90) days. These results was also in line with that found by [Sousa and Malcata, \(1997\)](#) who reported that lower lactobacilli count was obtained for cheese manufactured with plant rennet until 28 days of the ripening. The streptococci count decreased significantly during the storage period. Similar results were obtained by [Sana et al., \(2011\)](#) who found that the streptococci of white cheese made from *Solanum dubium* extract and rennet decreased from the day 1 up to the day 90 of the storage period. Yeast and mold increased significantly with the storage period. These findings support the results obtained by [Muna et al., \(2013\)](#) who studies the yeast and mould counts of Sudanese white soft cheese stored in five different containers, they found that the yeast and moulds increased from the day zero up to the day 75 of the storage period. The constant increase of moulds and yeasts during storage might be due to the fact that yeasts and moulds counts could metabolize lactic acid and lower pH value ([Turkoglu et al., 2003](#)). However [Nour El-Diam and El-Zubeir, \(2007\)](#) found that the heat treatment and processing improve the cheese quality via reducing the counts of yeasts and moulds. The coliforms count decrease significantly with the storage period. Our results coincide the results that obtained by [Muna et al., \(2013\)](#) who studies the coliform count of Sudanese white soft cheese stored in five different containers, they found that the coliforms decreased from the day Zero up to the day 180 of the storage period and not detected in some containers from the day 120 of the storage period. Our results also was in line with the results of [Houghtby et al., \(1992\)](#) who realize similar decrease of coliforms in Sudanese white soft cheese stored in two different containers (anti-acid cans and plastic containers) the coliforms decreases with the storage period till the day 60, and then were not detected in the cheese kept in anti-acid cans at day 120 till the end of the storage period at day 240. Whereas the cheese stored in plastic containers showed reduced counts (0.49 ± 0.08 log MPN/ml) at day 120 then completely disappeared in days 180 to 240. *Staphylococcus aureus* decreased significantly with the storage period. Our results was in agreement with that found by [Barakat et al., \(2009\)](#) who studies the staphylococcus aureus of Domiati cheese made with the Cells of *Lactobacillus reuteri* and he found that the log of staphylococcus aureus was 4.51 ± 0.095 , 4.78 ± 0.016 , 3.84 ± 0.078 and 3.42 ± 0.085 on the day zero, 30, 60 and 90 of the storage period. Our results was in disagreement with the results that found by [Muna et al., \(2013\)](#) who studies the yeast and mould counts of Sudanese white soft cheese stored in five different containers they found that *Staphylococcus* is detected only in the day zero of the storage period. *Staphylococcus aureus* was detected at day zero as 2.8×10^5 then it disappeared after 60 days storage, their results was supported by the results of [El-Owni and Hamid, \(2009\)](#) who stated that *Staphylococcus aureus* count in Sudanese white cheese was detected at zero time before storage and completely disappeared after 60 days of storage. [Ali and Galal, \(2000\)](#) attributed the disappearance of *Staphylococcus aureus* to the increase acidity of the cheese and the high salt level. These presences of *Staphylococcus aureus* in the cheese from the day zero up to the end of storage period might be due to the poor sanitary conditions and contamination of cheese during processing and storage.

CONCLUSION

It would be concluded that The Sudanese white soft cheese without Cassava (control) obtained the maximum values for the, Lactobacilli, Streptococci, Yeast and Molds, and *Staphylococcus aureus*, while the Maximum Total viable bacteria count was obtained by Cheese made with 0.5 % Cassava and the maximum Coliform was obtained by Cheese made from 1 % Cassava Powder.

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