Original Research Article

Proximate Constituents and Microbiological Characterization of Natural Cheese Enhanced with Ascorbic Acid

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Abstract: Twenty litres of milk was obtained from lactating Bunaji cows and clarified and then divided into fifteen parts of one litre such that each treatment had three replicates. Each part (1 L) was heated to a temperature of 50˚C with intermittent stirring. Thereafter, 20 g of coagulant (*Calotropis procera*) leaves juice was added and immediately, ascorbic acid was added at varying levels of 0 mg (T1), 50 mg (T2), 100 mg (T3), 150 mg (T4) and 200 mg (T5) and allowed to form curd. The whey was drained off to obtain the curd and refrigerated for fourteen (14) days. The cheeses obtained were investigated for proximate and microbial qualities at days 1, 7 and 14. Storage effect showed significant (p<0.05) reduction in carbohydrate and increase in moisture contents of the cheese. Treatment 1 had the highest moisture (35.52%) and ash (3.03%) values than cheeses enhanced with ascorbic acid. Treatment 4 was superior in fat (28.48%) and protein (22.73%) while carbohydrate concentration was at maximum (21.61%) in treatment 5. The highest Total viable bacteria count (TVBC), coliform count (TVCC), Lactobacillus count (TVLC), mold and yeast count (TVMYC) were quantified as 131.60×10^2, 63.10×10^2, 54.93×10^2 and 25.67×10^2 cfu respectively at day 14. Treatment influence showed significant (P<0.05) difference with treatment 3 having the highest TVBC (141.11×10^2 cfu) and TVCC (67.94×10^2 cfu) values respectively. Treatment and storage effects showed that T3 had the highest TVBC (147.33×10^2 cfu) and TVCC (74.50×10^2 cfu) at day 14. The TVMYC (26.83×10^2 cfu) was superior in T3 at day 1, while the TVLC was at its peak (61.67×10^2 cfu) in T4 at day 14. Isolated bacteria were *Micrococcus lactis, Lactobacillus spp, Bacillus subtilis,* and the mold and yeast were *Fusarium solani, Candida albican* and *Saccharomyces cerevisiae.* Conclusively, due to the higher concentration of carbohydrate in the refrigerated ascorbic acid enhanced cheese, it could serve as a source of energy for human, however, it should be consumed within 7days of production.

Keywords: Ascorbic acid, wara, wholesomeness, nutritional quality, enhancement.

INTRODUCTION

Cheese is one of the products obtained from milk processing. It is concentrated in nutrients and it is made by the coagulation of casein, the major milk protein by enzymatic or acidic reactions. Cheese provides a useful service in extending the shelf life of a valuable human foodstuff-milk (Alalade and Adeneye, 2006) and making them available throughout the year. Natural cheese is cheese produced directly from milk without undergoing any ripening process. Cheese have been found to be contaminated with both spoilage and pathogenic organisms due to unhygienic production processes. Microorganisms can have negative effects, causing spoilage (e.g *Pseudomonas*, *Clostridium*, *Bacillus* and other spore-forming microbes), as well as disease (e.g. *Listeria, Salmonella, Escherichia coli, Campylobacter* and mycotoxin producing fungi) (Quigle *et al.,* 2013). Undesirable bacteria that can cause spoilage of dairy products include Gram-negative psychrotrophs, coliforms and lactic acid bacteria (Willey *et al.,* 2008; Oyeleke, 2009; Yabaya and Idris, 2012). Milk is usually fortified with vitamins and minerals it lacks but attention is not directed to fortifying dairy products such as cheese (Ibhaze *et al.*, 2020). Ascorbic acid (vitamin C) is a water-soluble nutrient present in some foods. In the body, it acts as an antioxidant and helps to boost the immune system.

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Apart from the fact that vitamin C is not largely produced in milk it has been found that vitamin C content in milk is also reduced during heating process (pasteurization) which makes it incapable to supply enough vitamin C that is required in the body at different stages of human life (McGuire and Kathy 2017). Therefore, there is need to improve the vitamin C content in dairy products such as cheese in order to enhance the nutritional benefit to the consumer. This study therefore aims at examining the nutrient constituents and microbial characterization of cheese enhanced with ascorbic acid.

MATERIALS AND METHODS
Study site
The study was carried out at the Nutrition Laboratory in the Department of Animal Production and Health, Federal University of Technology Akure, Ondo State, Nigeria. Akure is located on longitude 4.944055 °E and 5.82864 °E, and latitude 7.491780 °N with annual rainfall ranging between 1300 mm and 1650 mm average maximum and minimum daily temperature of 38 °C and 27 °C respectively (Daniel, 2015).

Experimental materials
Twenty liters (20 L) of fresh whole milk from White Fulani cows was obtained from cattle herdsmen in Akure, Ondo State, Nigeria. The food grade vitamin C was purchased at a reputable store in Lagos and the Sodom apple leaves were obtained from the Fulani camp at Benin garage, Akure, Ondo State.

Production of cheese (*Wara*)
The raw milk (20 L) obtained was clarified and divided into fifteen (15) parts of 1000 ml (1L) each. Each part was heated to a temperature of 50°C inside an aluminum pot on a low intensity burner (regulated cooking gas) with intermittent stirring. Thereafter, 20 g of the *Calotropis procera* leave was squeezed directly into the milk and allowed to coagulate and food grade Vitamin C was added at varying levels of 0, 50, 100, 150 and 200 mg/L of milk in three replicates. Heating of milk continued until total coagulation was achieved. The curd was obtained by pouring the coagulated milk into a 1mm sieve to drain the whey and draining of the whey lasted for 45 minutes. Thereafter, samples were kept in the refrigerator for 14 days and subjected to proximate and microbiological evaluations at days 1, 7 and 14.

Proximate constituents determination
The fat content was determined by Gerber method, protein content using Kjeldahl method according to methods described by AOAC (1990). Also, moisture content was determined by gravimetric method and ash were determined according to the methods of AOAC (1990). The total carbohydrate was calculated by difference as 100- (moisture +ash +protein +fat).

Microbiological investigation
The samples were examined for total viable bacteria, coliform bacteria, moulds and yeast, and *Lactobacillus* count. The media used were in a dehydrated form and prepared according to the manufacturer's instructions. Eleven grams (11 g) of each cheese sample was added to 99 ml of sterile distilled water in a flask and shaken well to make 10^{-1} dilution. Further decimal dilutions were prepared in sterile distilled water. Total viable bacteria were enumerated by pour plate method using standard plate count agar (Houghtby *et al.,* 1992) and the plates were incubated at 37°C for 48 hours. MacConkey agar was used for the enumeration of coliform bacteria and the plates were incubated at 37°C for 24 hours (Christen *et al.,* 1992). The total count of yeasts and moulds were determined according to Frank *et al.*, (1992) using yeast extract agar, and the plates were incubated at 25°C for 5 days. Total Lactobacillus count was determined using Man Rogosa Sharpe (MRS, Oxoid), in anaerobic media at 37 °C for 72 hours (Halkman, 2005). Isolated colonies after incubation were pure cultures. Pure cultures were identified based on their colonial morphology, Gram reaction, and biochemical tests including indole production, citrate utilization, urease activity, growth on Kligler iron agar (KIA) fermentation of glucose, lactose, and maltose, motility, oxidase production, starch hydrolysis, catalase production, casein hydrolysis, and the MR-VP test, according to protocols described by (Forbes *et al.,* 2007, Cheesbrough, 2011).

Statistical Analysis
Statistical analysis was done using the Statistical Analysis Systems (SAS, ver. 9). General Linear Models (GLM) was used to determine the effect of treatment and storage on the proximate constituents and microbiological quality of cheese. Means were separated using least significant difference at P<0.05.

RESULTS
Presented in Table 1 are the proximate constituents of cheese enriched with ascorbic acid at 1, 7 and 14 days storage periods. Storage period had significant (P<0.05) effect on moisture and carbohydrate contents. Moisture content increased from 32.32% at day 1 to 34. 64% at day 14 while carbohydrate value decreased from 16.65 - 14.67%. Treatment effect showed significant (P<0.05) difference as moisture (35.52%) and ash (3.03%) contents were superior in T1. Treatment T4 had the highest fat and protein contents of 28.48 and 22.73% respectively while carbohydrate value was at maximum of 21.61 % in T5. The interaction between storage periods and treatments had significant effect (P<0.05) on the parameters. Treatment 1 had the maximum moisture value of 36.90% at day 14 and highest ash content (3.12%) at day 1. Treatment 4 showed superiority in fat content of 28.67% at day 7 of storage. Optimum protein value (27.85%) was observed in treatment 2 at 14 days

of storage while the carbohydrate concentration was highest (17.67%) in T5 at day 1 of storage. Table 2 revealed the effects of storage period and treatments on the microbial analysis of the ascorbic acid enriched cheese. The highest Total viable bacteria count (TVBC) of 131.60×10^2 cfu was recorded at 14 days storage period while 120.57×10^2 cfu and 126.60×10^2 cfu were recorded at 7 and 14 days respectively. At 14 days storage period, the highest Total viable coliform count (TVCC) and Total viable lactobacillus count (TVLC) were recorded as 63.10×10^2 cfu and 54.93×10^2 cfu respectively while 25.67×10^2 cfu was quantified in day 1 for Total viable yeast and mold count (TVMYC). Treatment influence showed significant ($P<0.05$) difference with treatment 3 having the highest TVBC

$(141.11 \times 10^2$ cfu) and TVCC (67.94×10^2 cfu) values respectively. The TVMYC was highest (20.83×10^2 cfu) in T2 while the TVLC value (44.33×10^2 cfu) was at its peak in T5. Treatment and storage effects showed that T3 had the highest TVBC (147.33×10^2 cfu) and TVCC (74.50×10^2 cfu) at day 14. The TVMYC (26.83×10^2 cfu) was superior in T3 at day 1 of storage while the TVLC was at its peak (61.67×10^2 cfu) in T4 at day 14. Shown in Table 4 are the bacteria isolated from the cheese samples which are; *Micrococcus lactis, Lactobacillus spp, Bacillus subtilis, Staphylococcus epidemidis, Enterobacter spp, Bacillus cereus* and the fungi isolated are; *Candida krusei, Fusarium solani, Candida albican* and *Saccharomyces cerevisiae*.

Table 1: Proximate composition (%) at different storage periods of cheese enhanced with ascorbic acids

Parameters	Moisture	Ash	Fat	Protein	Carbohydrate
Storage Period (Days)					
1	32.32 ± 0.68^b	2.41 ± 0.18	26.53 ± 0.40	22.09 ± 0.36	16.65 ± 0.27^a
7	34.34 ± 0.69^a	2.25 ± 0.19	26.52 ± 0.40	21.99 ± 0.35	14.90 ± 0.28^b
14	34.64 ± 0.69^a	2.21 ± 0.19	26.49 ± 0.40	21.99 ± 0.35	14.67 ± 0.28^b
P value	0.040	0.690	0.990	0.990	0.001
Treatments					
T1	35.52 ± 0.63^a	3.03 ± 0.30^a	26.41 ± 0.16^{bc}	21.09 ± 0.42^b	13.95 ± 0.35^d
T2	31.90 ± 0.83^b	2.11 ± 0.24^{bc}	27.44 ± 0.18^{ab}	22.72 ± 0.44^a	15.83 ± 0.30^{bc}
T3	34.35 ± 1.19^a	1.55 ± 0.13^c	25.83 ± 0.71^c	22.26 ± 0.55^{ab}	16.01 ± 0.46^b
T4	31.47 ± 0.48^b	2.11 ± 0.11^{bc}	28.48 ± 0.22^a	22.73 ± 0.40^a	15.21 ± 0.23^{bc}
T5	30.08 ± 0.83^c	2.60 ± 0.20^{ab}	24.40 ± 0.43^d	21.31 ± 0.31^b	21.61 ± 0.28^a
P value	0.001	0.001	0.001	0.030	0.001
Treatments * Storage Period					
T1*1	32.77 ± 0.88de	3.12 ± 0.54^a	26.52 ± 0.32^b	21.31 ± 0.78^{ab}	16.28 ± 0.23^b
T1*7	36.80 ± 0.70^a	2.68 ± 0.57^b	26.15 ± 0.57^b	20.57 ± 0.77^{bc}	13.80 ± 0.42^e
T1*14	36.90 ± 0.70^a	2.98 ± 0.57^b	26.35 ± 0.57^b	20.98 ± 0.77^{bc}	12.79 ± 0.42^f
T2*1	30.91 ± 1.69^d	2.23 ± 0.47^{de}	27.52 ± 0.32^{ab}	22.77 ± 0.83^b	16.57 ± 0.57^b
T2*7	32.40 ± 1.40^{de}	2.04 ± 0.44^e	27.40 ± 0.35^{ab}	27.70 ± 0.81^a	10.46 ± 0.47^g
T2*14	34.40 ± 1.40^c	2.23 ± 0.44^e	27.64 ± 0.35^{ab}	27.85 ± 0.81^a	7.88 ± 0.47^h
T3*1	33.48 ± 2.29^b	1.65 ± 0.26^f	25.83 ± 1.31^b	22.30 ± 1.02^b	16.74 ± 0.93^b
T3*7	34.79 ± 2.11^c	1.50 ± 0.24^f	25.93 ± 1.31^b	22.25 ± 1.01^b	15.53 ± 0.76^c
T3*14	34.89 ± 2.11^c	1.70 ± 0.24^f	25.63 ± 1.31^b	22.45 ± 1.01^b	15.33 ± 0.76^c
T4*1	30.55 ± 0.75^g	2.29 ± 0.19^{de}	28.48 ± 0.41^a	22.79 ± 0.72^b	15.89 ± 0.43^c
T4*7	31.43 ± 0.88^{ef}	2.06 ± 0.19^e	28.67 ± 0.40^a	22.70 ± 0.74^b	15.14 ± 0.31^c
T4*14	31.93 ± 0.88^{ef}	2.02 ± 0.19^e	28.48 ± 0.40^a	22.76 ± 0.74^b	14.81 ± 0.31^d
T5*1	33.88 ± 1.41^d	2.76 ± 0.31^c	24.40 ± 0.80^c	21.29 ± 0.59^{ab}	17.67 ± 0.44^a
T5*7	35.68 ± 1.53^b	2.52 ± 0.39^d	24.40 ± 0.80^c	21.32 ± 0.57^{ab}	16.08 ± 0.35^b
T5*14	35.87 ± 1.53^b	2.84 ± 0.39^c	24.95 ± 0.80^c	21.76 ± 0.57^{ab}	14.58 ± 0.35^d
P value	0.030	0.050	0.020	0.990	0.05

[abc-f]*Means along the same column with different superscripts are significantly (P<0.05) different. T1=unfortified cheese (no ascorbic acid), T2=Cheese fortified with 50g of ascorbic acid, T3= Cheese fortified with 100g of ascorbic acid, T4= Cheese fortified with 150g of ascorbic acid, T5= Cheese fortified with 200g of ascorbic acid.*

Table 2: Microbial load count at different storage periods of cheese enhanced with ascorbic acid

Parameters	TVBC (CFU/g)$\times 10^2$	TVCC (CFU/g) $\times 10^2$	TVMYC (CFU/g)$\times 10^2$	TVLC (CFU/g) $\times 10^2$
Storage Period (Days)				
1	120.57 ± 7.65^c	52.40 ± 5.05^b	10.47 ± 0.91^c	24.63 ± 1.07^c
7	126.60 ± 7.31^b	54.57 ± 3.88^b	19.53 ± 0.64^b	35.33 ± 1.22^b
14	131.60 ± 7.29^a	63.10 ± 3.96^a	25.67 ± 0.68^a	54.93 ± 1.37^a
P value	0.570	0.050	0.001	0.001
Treatments				
T1	106.06 ± 8.53^d	65.33 ± 4.30^a	16.33 ± 2.05^c	31.67 ± 2.98^c
T2	138.28 ± 1.57^{ab}	66.78 ± 1.65^a	20.83 ± 1.15^a	36.78 ± 2.83^b
T3	141.11 ± 8.03^a	67.94 ± 4.49^a	19.33 ± 1.71^{ab}	41.89 ± 3.49^a
T4	111.33 ± 10.33^c	49.22 ± 8.26^b	16.94 ± 2.04^c	44.33 ± 3.49^a
T5	134.50 ± 12.90^{ab}	34.17 ± 2.34^c	19.33 ± 1.68^{ab}	36.83 ± 3.49^b
P value	0.030	0.001	0.001	0.001
Treatments* Storage Period				
T1*1	100.00 ± 15.84^l	56.00 ± 6.75^{fg}	24.83 ± 2.32^{bc}	19.00 ± 1.59^n
T1*7	107.17 ± 15.57^k	67.50 ± 7.70^{cd}	17.33 ± 1.71^{ef}	29.00 ± 1.59^j
T1*14	111.00 ± 15.37^{ij}	72.50 ± 7.44^b	16.83 ± 1.42^g	47.00 ± 2.21^e
T2*1	132.67 ± 1.20^f	60.00 ± 1.91^e	25.67 ± 0.76^b	25.33 ± 2.06^l
T2*7	138.50 ± 2.22^d	67.67 ± 2.43^{cd}	21.67 ± 0.76^d	34.67 ± 2.47^h
T2*14	143.67 ± 2.56^b	72.67 ± 1.33^b	15.17 ± 1.05^h	50.33 ± 2.75^d
T3*1	135.50 ± 14.72^d	60.83 ± 8.29^e	26.83 ± 0.40^a	27.17 ± 2.21^k
T3*7	140.50 ± 13.80^c	68.50 ± 7.68^c	20.00 ± 0.77^d	38.83 ± 2.37^g
T3*14	147.33 ± 15.37^a	74.50 ± 7.71^a	11.17 ± 2.07^i	59.67 ± 2.35^b
T4*1	104.00 ± 20.29^l	57.83 ± 21.23^f	25.00 ± 2.42^b	29.67 ± 2.50^j
T4*7	112.50 ± 18.67^i	34.33 ± 5.54^j	17.67 ± 1.71^{ef}	41.67 ± 2.80^f
T4*14	117.50 ± 17.58^h	55.50 ± 12.15^{gh}	18.17 ± 2.29^e	61.67 ± 2.35^a
T5*1	130.67 ± 24.12^g	27.33 ± 3.09^k	26.00 ± 0.86^a	22.00 ± 1.06^m
T5*7	134.33 ± 23.84^e	34.83 ± 4.00^j	21.00 ± 1.29^d	32.50 ± 0.89^i
T5*14	138.50 ± 23.25^d	40.33 ± 3.64^i	11.00 ± 1.77^g	56.00 ± 0.86^c
P value	1.000	0.630	0.540	0.530

[ab]*Means along the same column with different superscripts are significantly (P<0.05) different. T1=unfortified cheese (no ascorbic acid), T2=Cheese fortified with 50g of ascorbic acid, T3= Cheese fortified with 100g of ascorbic acid, T4= Cheese fortified with 150g of ascorbic acid, T5= Cheese fortified with 200g of ascorbic acid, TVBC= Total viable bacteria count, TVCC= Total viable coliform count, TVYMC= Total viable yeast and mould count, TVLC= Total viable lactobacillus count.*

Table 3: Characterization of bacteria in cheese samples

Treatment	GRAM STAIN	INDOLE	SHAPE	SPORE	NITRATE	H$_2$S PRODUCTION	VOGES PROSKAUER	CITRATE UTILIZATION	METHYL - RED	OXIDASE TEST	GLUCOSE	MANNITOL	MALTOSE	SUCROSE	LACTOSE	MOTILITY	PIGMENT OF ORGANISM ON N.A	NAMES OF ORGANISM TESTED
T1	+	Cocci	–	–	+	–	–	–	–	–	+	+	+	+	–	–	Citron yellow	*Micrococcus lactis*
	+	Cocci in chain	–	–	+	–	–	–	–	–	+	+	+	+g	+g	–		*Lactobacillus spp*
	+	Long rod	+	–	+	–	+	–	–	–	+	+	+	+	–	+	White spreading	*Bacillus subtilis*
T2	+	Long rod	+	–	+	–	+	–	–	–	+	+	+	+	–	+	White spreading	*Bacillus subtilis*

																		Organisms
	+	Cocci	_	_	+	_	+	_	_	_	+	+	+	+	_	_		*Staphylococcus epidemidis*
	+	Cocci in chain	_	_	+	_	_	_	_	+	+	+	+g	+g	_			*Lactobacillus spp*
T3	+	Cocci in chain	_	_	+	_	_	_	_	+	+	+	+g	+g	_			*Lactobacillus spp*
	+	Cocci	_	_	+	_	_	_	_	+	+	+	+	_	_		Citron yellow	*Micrococcus lactis*
	_	Rod	_	_	_	+	_	_	_	+	+	+	+	+	+		Creamy	*Enterobacter spp*
T4	+	Long rod	+	_	+	_	+	_	_	_	+	+	+	+	_	+	White spreading	*Bacillus subtilis*
	+	Cocci	_	_	+	_	+	_	_	_	+	+	+	+	_	_		*Staphylococcus epidemidis*
	+	Long rod	_	_	+	_	_	_	_	+	+	+	+	+	_			*Lactobacillus casie*
T5	+	Cocci	_	_	+	_	_	_	_	+	+	+	+	_	_		Citron yellow	*Micrococcus lactis*
	+	Cocci in chain	_	_	+	_	_	_	_	+	+	+	+g	+g	_			*Lactobacillus spp*
	+	Long rod	+	_	+	_	+	_	_	_	+	+	+	+	_	+	White spreading	*Bacillus subtilis*

T1=unfortified cheese (no ascorbic acid), T2=Cheese fortified with 50g of ascorbic acid, T3= Cheese fortified with 100g of ascorbic acid, T4= Cheese fortified with 150g of ascorbic acid, T5= Cheese fortified with 200g of ascorbic acid.

Table 4: Isolated mold and yeast (fungi) from cheese samples

Treatment	Organisms
T1	*Aspergillus niger, Candida albican, Saccharomyces cerevisiae, C. krusei*
T2	*Fusarium solani, Saccharomyces cerevisiae, Mucor hiemalis, Candida albican*
T3	*Saccharomyces cerevisiae, Candida albican*
T4	*Saccharomyces cerevisiae, Fusarium solani*
T5	*Saccharomyces cerevisiae, Fusarium solani*

T1=unfortified cheese (no ascorbic acid), T2=Cheese fortified with 50g of ascorbic acid, T3= Cheese fortified with 100g of ascorbic acid, T4= Cheese fortified with 150g of ascorbic acid, T5= Cheese fortified with 200g of ascorbic acid.

DISCUSSION
Proximate constituents
Moisture content is used to assess the storability of a product and provides a measure of the water content (Aremu *et al.,* 2006). The increase in moisture content observed in this study as storage progressed may be attributed to the gain of moisture within the storage container during refrigeration. However, Ashaye *et al.* (2006) reported that moisture content is dependent on the duration allowed for whey expulsion. Piska and Štětina (2004) reported moisture content in processed cheese in the range of 51.0-64.21% which is higher than the values obtained in this investigation.

Fat content found is comparable to 29.22-29.50% reported by Suleiman, et al in processed cheese sold in Khartoum market and the findings of Kwak *et al*. (2002) who reported fat content of 29.0 – 33.0% in processed cheese made from cheddar cheese with different types and ratios of emulsifiers. Protein concentration found in this study is higher than the values (19.6 – 22.6%) opined by Acharya and Mistry (2005) of pasteurized processed cheese made with condensed or ultrafiltered milk and 12.82-17.56%

reported by Razig and Yousif (2010) for processed cheese made with groundnut milk. Also, the protein content of the cheese obtained was within the range of 16-23.26% reported by Nuser (2001) and Ceylan *et al.,* (2003) for fresh white cheese (salty cheese made from unpasteurized sheep or cow milk) and Orgu cheese (a type of Turkish braided cheese).

The ash content obtained is in tandem with the values reported by Kwak *et al*. (2002) who reported ash content of 1.33 – 4.33% and higher than 0.83% reported by Ogunlade *et al*., (2017) who studied the use of different coagulants in cheese production. The storage effect which showed a reduction in carbohydrate as the storage period increased could be that the lactic acid bacteria present in the cheese continued their fermentative activity thereby causing a reduction in the carbohydrate concentration as the storage period progressed. However, treatment and storage effects indicated increase in carbohydrate concentration as storage progressed. The carbohydrate contents of cheese obtained in this study is higher than 10.45 % reported by Ogunlade *et al*., (2017). The reduction in carbohydrate as the storage period increased could be that the lactic acid bacteria present in the cheese

continued their fermentative activity thereby causing a reduction in the carbohydrate concentration as the storage period progressed. The carbohydrate contents of the cheese in this study is higher than 10.45 % reported by Ogunlade *et al.*, (2017).

Microbial Analysis

Microorganisms are known to associate with milk because of its nutritional values. One of the most important factors which affect the quality of cheese is microbiological flora. The microflora of cheeses results from the microorganisms in the milk used in production and post -production. The number and type of microorganisms that make up the microflora varies due to the chemical and physical properties (Çetinkaya and ÖZ, 2019).

The highest TVBC obtained is lower than the report of Alalade and Adeneye (2006) who reported 472.72×10^5 CFU/g. The TVCC and TVMYC results obtained are at variance with the report of Osman *et al.* (2009) who reported values of 0.28 - 4.04 and 1.74 – 2.65×10^2 CFU/g for TVCC and TVMYC respectively. ElDiam and ElZubeir (2006) reported values of total bacteria (6.0×10^6 – 6.5×10^7 cfu/gm), yeasts and moulds (3.6×10^4 – 6.5×10^5 cfu/gm) in processed cheese made from Sudanese white cheese. Results obtained are in agreement with the findings of Aly *et al.*, (1995) who reported that the total bacterial count of processed cheese never exceeded 600 cfu/gm regardless of storage temperature or degree of substitution of mature cheese with ultrafiltered retentates. *Lactobacillus spp*. was the most abundant throughout the period of storage. The prevalence of LAB during fermentation could decrease pathogenic bacteria due to acid environment caused by their production of lactic acid or bacteriocins *Lactobacillus,* a lactic acid bacterium, is an important species in food fermentation and is considered beneficial to human health. It has been shown to have antimicrobial and antifungal activity, with a bacteriostatic mode of action (Ahmadova *et al.,* 2013). The prevalent fungi observed in this study are *Candida spp, Saccharomyces cerevisiae* and *Fusarium solani*. However, results obtained from this study are within the tolerable level recommended for human consumption by Center for Food Safety (CFS, 2014).

CONCLUSION

Conclusively, the production of cheese enhanced with ascorbic acid revealed improved nutritional quality and the microbiological characteristics showed wholesomeness and safety for human consumption due to their low microbial load.

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