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Research Article

Characterization of Free Fatty Acids Contents of Sudanese White Cheese during Storage

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ABSTRACT

This study was conducted in the Dairy Technology Laboratory of College of Animal Production Science and Technology at Sudan University of Science and Technology and the Central lab of Khartoum University to characterize the fatty acids of Sudanese white cheese during storage period. Fresh whole cow's milk (30 liters) was used to made white cheese. The process cheese was stored for 28 days (0, 7, 14, 21 and 28 intervals). Fatty acids profiles were analyzed using GC (Gas Chromatography) for each storage period interval. One way ANOVA was used for statistical analysis. The Results showed that 12 organic fatty acids were recorded during the storage period of Sudanese White cheese capric (C10), lauric (C12) myristic, cis10-pentadcanoic, pentadcanoic, palmitic, palmitolic, cis10-heptadecanoic, heptadcanoic, linoleidacid, oleic and steric) The results were revealed that Oleic acid area % was gradually increased from day 7 until day 21 then thereafter decreased at day 28.Also the results showed that at day 28 of storage the concentrations of most fatty acids of the white cheese decreased .Also the results revealed that there were no significant differences' during storage periods in area % of all fatty acids except pentadecanoic acid($p \leq 0.01$), no significant differences' during storage periods of all fatty acids concentrations were observed exceptcis10 pentadecanoicacid ($p \leq 0.05$).

Keywords: white cheese, fatty acids, lipolysis, storage

1. INTRODUCTION

White cheese is the most popular type of cheese produced in Sudan, locally known as (Gibna Bayda). It is a pickled soft cheese that is stored under anaerobic conditions in air tight containers filled with whey [1]. The manufacture of *Gibna Bayda* which is a soft cheese variety is much simpler than that of semi-hard cheese or hard cheese types. The skills required for making Gibna Bayda is relatively small [2]. Cheese making is aimed to make milk preservation attractive and durable. Its shelf life varies from few days to several years [3]**.** Gibna Bayda is defined as a soft white cheese made in Sudan. It is similar to Domiati Cheese which made in Egypt, starter is not used. The storage life of this cheese maybe more than one year, and about one Kilogram of cheese will be obtained from seven litters of milk [4].

In Sudan cheese processing is a major preservation method of surplus milk in rural areas especially during rainy season when plenty of milk is available. The product is an important nutrient for humans especially under conditions where other animal proteins are not available [5]. White cheese (Gibna Bayda) is a unique among pickled cheese varieties in that high concentrations of sodium chloride are added to the milk before renneting. When manufactured from raw milk no starter culture is added, the natural lactic acid bacteria present in the raw milk carry the fermentation process required for the cheese maturation. The cheese is usually left over night to drain the whey with or without pressing. Ripening takes place while the cheese was submerged in whey [2]. Its appearance, texture and flavour closely resemble the Greek Feta [6].

Cheese maturation includes chemicals, physical and biological changes. Mature cheese processes include glycolysis, proteolysis and lipolysis [7]. It is widely accepted that several free fatty acids contribute directly to the final flavour characteristics of many types of cheese or indirectly by serving as precursors of such aroma components as ketones [8]; however ,the relation between total free fatty acid composition and final cheese is still not completely established [9]. Furthermore, the relative proportions of free fatty acids in cheese are expected to depend on the seasonal composition of the milk (which in turn results from the animals' feed and physiological condition) and on the relative activity of lipases in the cheese [9].

FFA contributes to cheese flavor and served as precursors for a variety of other compounds such as alcohols, esters, aldehydes, ketones and lactones [10]. Lipolysis is one of the

major biochemical changes that contribute to flavor development during the cheese ripening, together with proteolysis [11].Analysis of the short and medium-chain FFA profile has been suggested as an index for characterizing cheeses over the ripening period [12]. FFA is the major contributor to the development of the characteristic flavour in some cheese varieties, e.g. hard Italian and blue type cheeses [13].

The main FFA observed in the raw and pasteurized milk Örgü cheeses during ripening were palmitic, oleic, myristic, steric and capric acids, representing together approximately 82% of total FFA content. Butyric (C4), caproic (C6), caprylic (C8), capric (C10) and lauric (C12) acids contents of both RMC and PMC increased during ripening. However, myristic (C14), palmitic (C16), stearic (C18), oleic (C18:1) and linoleic (C18:2) acids contents of RMC remained constant (P>0.05) during storage [14].The objective of this study was to characterize the fatty acids (FA) contents of white cheese during storage

2. MATERIAL AND METHODS

2.1. Location of the study

The study was conducted at laboratory of milk technology, College of Animal Production Science and Technology, Sudan University of Science and Technology.

2.2. Materials

Fresh whole milk (30 liters) was brought from local farms at Hillat Kuku; Khartoum North. Rennet powder was obtained from chr. Hanens lab. Denemark. Clean and fine sodium chloride was purchased from local market.

2.3. Experimental Design

In this study complete randomized design (CRD) was used, white soft cheese was made from raw cow's milk and stored for 30 days. Fatty acid analysis was made at 7 days intervals.

2.4. Cheese making method

Sudanese white soft cheese (Gibna Beyda) was made as described by Osman [2].Thirty lit of raw cow's milk was heated to 45°c and then yoghurt starter culture was added at the rate of 2% of the milk. Rennet powder (1gL45kg milk) was dissolved in little amount of water and added to milk at 40c.The milk was left until coagulation occurred. Then the mixture was left to develop a curd. After coagulation the curd was cut by stainless steel kitchen knife and left for 30 minutes to separate the whey, and then 4% table salt was added with stirring for 10 minutes. The curd was transferred to a clean wooden moulds lined with clean cloth then pressed using weight over night. In the next day the cheese was weighed and cut into small cubes(5x5x5cm) then each 250 grams of cheese were transferred into plastic cans in triplicates and the drained whey was boiled for five minutes and used for cheese preservation therefore the cheese stored at room temperature for 0,7,15,21 and 28 days. Fatty acids composition was analyzed for the cheese samples at the specified intervals.

2.5. Chemical analysis;

Fat contents of the milk were determined according to Official Methods of Analysis [15].

2.6. Free fatty acids analysis

The cheese samples were analyzed for free fatty acids (FFAs) on 1st, 7th, 14th, 21th and 28th days. Fat was extracted from cheese samples as described by [16], 10 gm of cheese sample was weighed, 37.5 ml of a mixture chloroform and methanol (1:2) was added, vortex for 10-15 min,then12.5 ml of chloroform with mixing for 1 min. was added with 12.5 ml distilled water with mixing another min before centrifugations, after centrifugation the lower phase was collected through the protein disk for large volume of liquid, the liquid phase was centrifuged to allow the formation of the two liquid phase and then evaporated, and the precipitate was weighed, and ethylated according to the procedure of Christie [17]. Fatty acids methyl esters were analyzed using GC (Thermo Quest) equipped with flame ionization detector (FID), and fitted with a fused silica capillary column (DB-U6554753H, 30 m, 0.25 mm, 0.25um). Injector and detector temperature was 250°C. The initial oven temperature was 150°C for 1.0 min, and then increased to 300°C at 4°C/min. The final temperature was maintained for 38.5 min. A standard fatty acid mixture containing 37 fatty acids was used to provide standard retention times. Fatty acids were identified by comparing their retention times with those of fatty acids in standard samples. An auto system Thermo quest GC-MS, equipped with flame ionization detector (FID) was used to analyze FFAs of cheese samples. The carrier gas was helium at 2 ml min-1. Injection of 1 μL sample was applied with a split ratio of 1:0 into the injector.

2.7. Statistical analysis

All statistical calculations were performed using Statistical Package for Social Studies [18] to analyze the data by ONE WAY ANOVA. Least Significant Difference (LSD) was used to show the effect of storage period on fatty acids profiles of white soft cheese.

3. RESULTS

Results in Table 1 show fatty acids composition (area %) of Sudanese white cheese during storage. The results indicated that there were no significant differences in all fatty acids contents during storage periods except cis10- pentadecanoic

Table 1: The effect of storage periods on area% of fatty acids of Sudanese white cheese

Means with different superscript in the same column are significantly different at (p≤ 0.01).

Fatty acids Concentration	Storage Period (days)					
	0	7	14	21	28	Sig.
Capric	4.59±0.08	5.10±0.90	4.76±0.61	3.90±0.26	3.63±0.30	NS
Lauric	5.69±0.29	5.37±0.39	5.17±0.30	4.72±0.51	4.69±1.03	NS
Myristic	17.31±0.52	17.89±0.86	16.47±0.23	16.48±0.61	15.20±1.20	NS
cis10pentadecanoic	0.38±0.06[b]	0.31±0.13[b]	0.34±0.3[b]	11.69±0.1[a]	11.56±0.37[a]	**
Pentadecanoic	0.56±0.09	0.59±0.17	0.80±0.27	0.60±0.04	0.46±0.03	NS
Palmatic	0.48±0.06	0.46±0.11	0.41±0.02	0.44±0.03	0.38±0.01	NS
Palmitoleic	1.63±0.01	1.74±0.23	1.48±0.08	1.46±0.03	2.13±0.79	NS
cis10heptadec	17.25±3.45	15.57±1.39	16.54±8.16	11.69±0.10	11.56±3.68	NS
Heptadecanoic	17.99±1.86	19.77±3.30	17.19±8.82	23.11±0.58	24.02±2.26	NS
Linoleinic acid	0.26±0.03	0.29±0.01	0.26±0.05	0.28±0.01	0.25±0.04	NS
Oleic	8.56±1.17	13.43±1.56	19.42±1.36	19.53±2.42	17.84±1.83	NS
Stearicacid	8.04±1.04	8.02±1.89	9.44±0.30	10.03±0.93	8.20±2.43	NS

*Sig.: Significant, NS: Not significant;**: significant at probability (P<0.05)*

Table 2: The effect of storage periods on concentration (%) of fatty acids of Sudanese white cheese

Fatty acids Conc.	Storage Period (days)					
	0	7	14	21	28	Sig.
Capric	1.32±0.67	.97±0.23	3.42±2.61	3.08±1.03	1.29±0.07	NS
Lauric	1.44±0.64	0.98±0.48	3.25±2.32	3.32±0.99	1.34±0.07	NS
Myristic	3.66±1.71	2.61±0.95	8.42±5.54	7.16±0.12	4.03±0.27	NS
cis10pentadcennoic	0.09±0.03	0.05±0.00	0.21±0.15	0.23±0.09	0.10±0.01	NS
Pentadecanic	0.10±0.03[dce]	0.07±0.01[ec]	0.34±0.13[a]	0.33±0.11[ba]	0.11±0.01[c]	*
Palmatic	0.51±0.25	0.36±0.01	1.13±0.81	1.26±0.48	0.85±0.43	NS
Palmitoleic	0.12±0.04	0.08±0.01	0.25±0.16	0.33±0.15	0.12±0.01	NS
cis10heptadec	1.08±0.33	0.72±0.35	3.07±3.10	2.15±0.84	0.97±0.44	NS
Heptadecanoic	1.13±0.66	0.81±0.21	2.28±0.64	3.87±1.68	1.82±0.10	NS
Linoleicacid	0.05±0.01	0.04±0.01	0.12±0.10	0.15±0.07	0.06±000	NS
Oleic	0.45±0.53	0.72±0.37	3.63±2.62	4.25±2.17	1.68±0.07	NS
Stearicacid	2.28±1.37	1.60±0.98	6.32±4.38	7.88±3.80	2.78±0.45	NS

Sig: Significant; NS: Not significant;: significant at probability (p≤0.05); a, b, c: means with different super script in the same column are significantly different at (p≤ 0.05)*

acid. In this study, Heptadecanoic and Myristic acids (area %) were the major saturated fatty acid (SFA) in white soft cheese samples at day zero. While the highest unsaturated fatty acids (area%) in the cheese samples was cis10heptadecanoic (17.25±3.45) at day 0. The results indicated that at day 7 the lowest levels of area % were 0.38±0.06 and 0.48±0.06 recorded for cis10pentadcennoic and palmitic, respectively. As the storage period progressed still pentadecanoic and palmatic acids were the lowest fatty acids (area %) until the end of the storage period. It is observed that Oleic acid area % was gradually increased from day 7 until day 21 then thereafter decreased at day 28.The Saturated fatty acids of the white cheese samples at the end of storage period (day 28) showed decrease in area % while most of the unsaturated fatty acids of the cheese samples revealed an increase in area % at the end of storage period (Table2). The area % of Heptadecanoic and Oleic (24.02±2.26 +17.84±1.83) acids were represented the major fatty acids at day 28 in the white cheese samples.

Results in Table 2 showed the effect of storage period on fatty acids concentration of Sudanese white cheese during storage. The results indicated that there were no significant differences of all fatty acids contents during storage periods

except Pentadecanoic acid .The lowest levels of concentrations were 0.09±0.03; 0.05±0.01 and 0.10±0.03 % at day 0 recorded for cis10pentadecannoic, Linoleic acid, and Pentadecanoic acids, respectively. Whilst the highest concentrations at day 0 were 17.99±1.86, 17.31±0.52 and 17.25±3.45% registered for heptadecanoic, myristic and cis10heptadec acids, respectively.

After 28 days of storage the concentrations of most fatty acids of the white cheese decreased except cis10pentadcennoic, palmitoleic, Heptadecanoic and Oleic were increased during storage. The highest concentrations were recorded for Heptadecanoic (24.02±2.26%) and oleic (17.84±1.83%) acids at day 28. Whereas, the lowest levels of concentrations were registered for Linoleicacid, Palmatic and Pentadecanoic acids respectively. Therefore, It's observed that at day 7 all fatty acids of the white decrease in concentration except oleic acids. However at day 14 all fatty acids showed an increase in concentration.

Results in Table 3 show the effect of storage period on fatty acids (retention time) of Sudanese white cheese during storage. The results indicated that there were no significant variations in retention time of all fatty acids during storage.

Table 3: The effect of storage periods on retention time (minutes) of fatty acids of Sudanese white cheese

Fatty acid	Storage period (Days)					Sig.
	0	7	14	21	28	
Capric	10.22±0.09	10.21±0.03	10.14±0.07	10.17±0.04	10.31±0.04	
Lauric	14.25±0.05	14.22±0.01	14.21±0.00	14.21±0.00	14.22±0.00	
Myristic	18.18±0.13	18.19±0.06	17.94±0.22	18.04±0.08	18.15±0.01	
Cis10pen	19.98±0.06	19.96±0.03	19.83±0.12	19.81±0.05	19.97±0.01	
Penta	20.11±0.06	20.11±0.08	19.98±0.12	19.96±0.11	20.1±0.01	
Palmatoliec	21.72±0.16	21.76±0.06	21.43±0.27	21.38±0.19	21.64±0.01	NS
Palmatic	21.85±0.18	21.89±0.06	21.52±0.28	21.32±0.00	21.67±0.08	
Cishep	21.95±0.13	21.86±0.07	21.71±0.48	21.54±0.21	21.84±0.03	
Hepta	22.08±0.13	22.07±0.05	21.89±0.14	21.66±0.19	21.96±0.05	
Linoleic	23.91±0.07	23.91±0.02	23.77±0.13	23.75±0.09	23.90±0.00	
Oleic	25.29±0.22	25.44±0.08	25.02±0.32	24.95±0.23	25.28±0.01	
Stearic	25.82±0.04	25.81±0.03	25.58±0.29	25.55±0.08	25.79±0.00	

4. DISCUSSION

Fatty acids profiles of white soft cheese were studied during 28 days of storage. There was highest level of the area % (19.53±2.42) of oleic acid (Table 1) at day 21 these results were similar to those of [1] who stated that oleic acid content was the major total unsaturated fatty acid (19.39–31.89%) of cheese samples during storage. In the present study, linoleic acid was the primary PUFA in cheese, Linoleic acid percent was 0.29%, these results were not similar to those of Gokhan Zengin [20] who reported that the area% of Linoleic acid was (2.3)%, these oscillations may be due to the treatment of the milk at70°c. The area % of capric acid (Table1) was 3.90% at 21st day; these results were in accordance to those of Gokhan Zengin [20], who mentioned that the area% of capric acid was 2.26 % from cheese made of milk heated at 70 °C. The area% of lauric and myristic acids were about 5.69 %, 17.89% respectively, these results were higher than those reported by [21], and the area % of luaric and myristic acids were 2.93% and 10.8%, respectively. The reason of higher area percent could be due to use of raw milk that may be subjected to further lipolytic actions by microflora. The area % of palmatic acid was (0.48±0.06) at day 0, these results were not agreed to those of [20] who found that area % of palmatic acid was (37.07) %, these variations may be due to the treatment of milk for cheese making at70 °c. The area % of palmitolic acid was (2.13±0.79) at 28th day, these results were in line to Gokhan Zengin [20] who reported that the area% of Palmatoliec was (2.84±0.21).

Results in Table 2, revealed that there were no significant differences between concentrations of all fatty acids of white cheese during storage periods except pentadecanoic acid ($p<0.05$), this result was similar to those of [15], who stated that the total FFA content of Örgü cheese made from raw milk (RMC) remained constant until the day 30, but then it increased significantly ($p<0.05$). In the present study, myristic acid was the major saturated fatty acid (SFA) in cheese samples. Analysis of variance of all such data indicated that the concentration of each free fatty acid changed during ripening. Among the saturated fatty acids, the most abundant in the cheeses investigated were myristic, stearic, lauric and capric

acids. These results were not agreed with those of [19], who reported that the most abundant SFA was palmitic acid, followed by myristic acid and stearic acid in some traditional Turkish cheeses. The FFA profile in cheese at the day of manufacture showed that the Linoleic acid was at lower concentrations. These observations agree with those by [21] for ovine milk cheeses. Therefore, the highest fold increased were observed for myristic acid (8.42±5.54%) was at day 14 ,this could be due to the fact that lipases (originating mainly in milk and microorganisms) involved in cheese ripening. This result was not similar to those of [14] who demonstrated that, the highest fold increases were observed for myristic (5.2%) acid after day 35. The fold increased for oleic, linoleic, and lauric acids were 4.25, 0.15 and 3.32 % respectively at day 21, These results were not coincided to those by [14] who reported that the highest fold increases were observed for oleic, linoleic and lauric acids (2.5,2.3 and 1.8%,respectively) after 35 days. The amounts of all individual FFAs increased at different pattern during ripening period (Table 2), so the FFA composition of cheese samples varied considerably over the ripening period of 28 days. The concentrations of capric, linoleic and linolenic acids did not increase significantly until 21 days of ripening. Therefore, the significant differences' were observed for pentadecanoic acid ($p<0.05$) during storage periods. The highest rate of hydrolysis for capric acid was between day 0 and day28 and they ranged (1.32±0.67%) to (1.29±0.07%), this result was similar to those of [22] who reported that he highest rate of hydrolysis for capric acids was between day 1 and 30 and they ranged from 0.5 to 1.6 mg/100 g cheese. The differences in the free fatty acid concentrations found by various authors may be related, either to the techniques used for the distillation and extraction and type of cheese, or the formation of acetic acid, due to lactate fermentation. The amounts of palmitic acid was (0.51±0.25%) at day 0 and (1.26±0.48%) at day 21, these results were similar to those of [22], who founded that the amount of palmitic acid was (0.7±0.1%) at day10 and (1.2±0.3) at day20. The concentration of stearic acid was (7.88±3.8) at day 21, these results were in the line to [22], who found that the concentration of stearic acid was (12.2±0.1%) at 20th day. The concentrations of heptadecanoic acids (C17:O) in Sudanese white cheese did not changed statistically during ripening, these results agree with the those of Gomez [23] and Najera [24].

5. CONCLUSION

The higher relative increase of all fatty acids concentration were determined at day 14 except palmitolic, heptadecanoic and stearic acids, also the concentration of all fatty acids decreased at day 21. Palmitic (C16:0), and oleic acids (C18:1) were the most abundant FFA in Sudanese white cheese, also the area % of Heptadecanoic and Oleic acids were represented the major fatty acids at day 28 in the white cheese samples. There were no variation of area % between all fatty acids during storage periods except pentadecanoicacid and there were no variation in the concentrations of all fatty acids during storage periods except cis10pentadecanoic acid.

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