

Effect of Types of Probiotic Bacteria on Physicochemical Properties of Sudanese White Soft Cheese

Alwaleed Dafalla ^{a*}, Kamal A. Abdel Razig ^b, Nagat A. Elrofaei ^c

^aDepartment of Biology, Faculty of Education and Science, AL-Zaeim AL-Azhari University, P.O. Box 1432
Khartoum North 13311, Sudan.

^bDepartment of Food Science and Technology, Faculty of Agriculture, AL-Zaeim AL-Azhari University, P.O. Box
1432 Khartoum North 13311, Sudan.

^cDepartment of Biotechnology, Faculty of Science, Technology Omdurman Islamic University, Sudan.

^aEmail: alwaleeddafalla@gmail.com

^bEmail: Kamalaabdel@gmail.com

Abstract

The study was conducted to assess the effect of three different types of probiotic bacteria (*Lactobacillus rhamnosus*, *Lactobacillus casei* and *Bifidobacterium bifidum*) on quality of Sudanese white soft cheese. The objectives of this study were to study the effect of three types of probiotic bacteria on the quality of Sudanese white soft cheese during different storage periods. The samples were subjected to physicochemical parameters, microbial examination. The result of physicochemical parameters showed increase in the yield. The highest yield obtained by cheese containing probiotic bacteria in comparing with control. The moisture content in all cheese samples decreased, while the weight loss, protein, fats and ash content increased during storage period ($p \leq 0.05$). The pH values decreased, while the titratable acidity increased and both parameters were affected significantly ($p \leq 0.05$) by the types of probiotic bacteria. The soluble nitrogen, formol ripening index, schilovich ripening index and total volatile fatty acids increased significantly during storage period, the highest values observed by samples containing probiotic bacteria comparing with control. The highest calcium, phosphorus, sodium, potassium, magnesium, iron was obtained by sample containing probiotic bacteria comparing with control.

Keywords: Probiotic bacteria, Sudanese white soft cheese, storage period.

* Corresponding author.

1. Introduction

Probiotic as a term is a relatively new word meaning for life and it is currently used to describe a group of bacteria when administered insufficient quantity, confer beneficial effects for humans and animal [1]. Probiotic bacteria are applied to balance disturbed intestinal microflora and important in the treatment of a wide range of human disorders including lactose intolerance, diarrhea, food allergies, intestinal infection, constipation gastroenteritis, hepatic, flatulence, colitis, gastric acidity, osteoporosis, high blood cholesterol and cancer [2, 3]. The most organisms used as probiotic belong to *bifidobacteria*, *Lactobacillus* and some *Enterococcus* spp. [4]. Lactic acid bacteria (LAB) and bifidobacteria are amongst the most important groups of microorganisms used in the food industry, used in the production of fermented products, such as yoghurts, cheese and pickled vegetables [5].

Bifidobacteria spp. beneficially affects human health by improving the balance of intestinal microflora and improving mucosal defenses against pathogens. Additional health benefits include enhanced immune response, reduction of serum cholesterol, vitamin synthesis, anti-carcinogenic activity, and anti-bacterial activity [6, 7]. *Lactobacillus casei* improvement of balance of intestinal microbiota and volatile fatty acid, antitumor action, stimulation of the immune system, and antimicrobial activity [8]. *Lactobacillus rhamnosus* benefits are reducing the activity of fecal enzymes such as β -glucuronidase and azoreductase which contribute to the risk of colon, mammary, and prostate cancer [9]. *Lactobacillus rhamnosus* increases the number and the activity of natural killer cells and exerts immunostimulating effects, including on fetuses [10]. Cheese is known as a complete nutritious food product and excellent source of many key nutrients, suitable for many ages. It is rich in protein and minerals such as calcium. There are different types of cheese: soft cheese, semi hard and hard cheese, the difference in these types is mainly due to water content or water activity and the methods and technology for cheese making [11].

The major traditional cheese types produced in Sudan include Gibna Bayda, Gibna Mudaffra and Mozarella and Romi cheese [12]. Cheese making in Sudan is the major preservation method for surplus milk in rural areas especially during rainy season when plenty of milk is available [13]. The Sudanese Gibna Bayda (white cheese) has a unique, very originated and traditional technology, and can be categorized as white-brined soft cheese [14]. White pickled cheese of the Sudan is a product traditionally made from raw milk to which salt (6-20%) has been added. White cheese is usually made from raw milk and without the use of starter culture [15].

The objective of this investigation is to study the effect of three types of probiotic bacteria (*lactobacillus rhamnosus*, *lactobacillus casei*, *Bifidobacteria bifidum*) on physiochemical of Sudanese white soft cheese.

2. Materials and Methods

Milk: Fresh cow's milk was obtained from Alarabia Company, Khartoum North, Sudan.

Clotting agent: Rennet tablets were obtained from (Chr.Hansens Lab., Copenhagen, Denmark).

probiotic bacteria: Freeze-dried of commercial strains cultures (*Bifidobacterium bifidum*, *Lactobacillus casei*, and *Lactobacillus rhamnosus*) were obtained from (Chr.Hansens Lab., Copenhagen, Denmark).

Salt and package: Fine powder salt (sodium chloride) and polyethylene packs were obtained from the local market in Khartoum North, Sudan.

2.1. Cheese manufacture

Sudanese white soft cheese was processed using fresh cow's milk according to the method of [15]. Cheese was manufacturing in faculty of agriculture lab, ALzaiem ALazhari University.

First milk was purified from foreign particles, then was pasteurized to 72°C for 1 minute, and cooled to 40°C. Milk was divided into four separate tanks (A, B, C, D) each one contained 10 L, (A) left as a control, 2g of probiotics bacteria (*Bifidobacterium bifidum*, *Lactobacillus casei* and *Lactobacillus rhamnosus*) were added to each tank (B, C, D) at 40°C respectively. Followed by the addition of CaCl₂ (0.02%) and rennet (0.2g). Milk was stirred for 5 minutes and then left undistributed to develop a curd. After the coagulated was completed, the curd was cut into cups with an ordinary stainless steel kitchen knife to allow whey separation. The curd was kept for about 20-30 minutes for whey drainage. The whey of each tank was collected and kept in a cold environment over night. The curd was transferred to clean wooden molds lined with cheese cloth and pressed with about 1 Kg over night. Next day the curd was removed from the molds, weighed and cut into rectangular of 160 gm each. 6% w/v NaCl was added to the collected whey, then pasteurizing at 72°C for 1 minute, and added to cheese curd into sterile plastic buckets. Cheese was stored at room temperature (38±2°C) for 60 days. Physico-chemicals, microbiological and organoleptic characteristics were carried out at 0,15,30,45 and 60 days intervals [15].

2.2. Physicochemical analysis:

Cheese samples were analyzed for titratable acidity, protein, fat, ash, according to [16]. The pH meter (model. A005673-3-5). Moisture content and soluble nitrogen were determined according to [17]. Formol and Schilovich ripening index According to [18]. Minerals Determined according to [19]. Total volatile fatty acids Determined according to [20]. Fatty acids contents were measured by GC-MS model (GC.MS-QP2010 Ultra, Japan).

2.3. Statistical analysis:

Data were analyzed as complete randomized design with three replicates using statistical analysis system program [21]. Means were separated using Duncan's Multiple Range Test.

3. Result and discussion:

The three types of probiotic bacteria (*Lactobacillus rhamnosus*, *Bifidobacterium bifidum* and *Lactobacillus casei*) effected the physiochemical properties of Sudanese white soft cheese manufactured from fresh cow's milk, for example titratable acidity increased with the addition of the probiotic bacteria, the highest TA (1.90%) was obtained by cheese samples containing *Lactobacillus rhamnosus*. The lowest (1.28%) by the control samples while the other samples ranked in an intermediate position ($p \leq 0.05$) as shown in table 1. This increase in titratable acidity may be due to the activity of proteinases and peptidases released from the experimental strains (probiotic strains), which resulted in higher proteolysis in cheese [22]. [23] observed that titratable acidity increased in all samples of Karish Cheese as affected of probiotic *Bifidobacterium bifidum* and

L.rhamnosus during days of storage. [24] said that titratable acidity increased in all samples of probiotic fresh white cheese with *L.rhamnosus* and *L.casei* during a storage period. Increase in acidity of Sudanese white soft cheese reported by [25] as probably due to growth of lactic acid bacteria in cheese.

Table 1: Effect of types of probiotic bacteria on titratable acidity (%) of white soft cheese during storage period.

Storage period (Days)	Types of probiotic bacteria			
	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
0	0.53 ^M ±0.05	0.60 ^L ±0.03	0.66 ^k ±0.07	0.63 ^{kL} ±0.06
15	0.79 ^J ±0.02	0.86 ⁱ ±0.11	0.97 ^s ±0.04	0.93 ^h ±0.12
30	0.83 ⁱ ±0.08	0.95 ^{gh} ±0.09	1.13 ^e ±0.02	1.11 ^{ef} ±0.03
45	1.00 ^f ±0.03	1.38 ^d ±0.06	1.70 ^b ±0.05	1.57 ^c ±0.04
60	1.28 ^d ±0.09	1.65 ^c ±0.12	1.90 ^a ±0.08	1.73 ^b ±0.11

- Mean ± SD. Having different superscript letters on columns and rows are significantly different (P≤0.05)

Also the three types of bacteria affected the protein content during the storage period. The highest (19.04%) was obtained by cheese samples containing *lactobacillus rhamnosus*. The lowest (18.75%) by the control samples, while the other samples ranked in an intermediate position (p≤0.05) as shown in Table 2. The protein content increased with the addition of the probiotic bacteria. [26] found that protein content increased in Dutch-type cheese and cheese-Like products with the addition of *L.rhamnosus*. [27] found that, the protein content of Sudanese semi braided cheese content increased during storage period due to the loss of moisture.

Table 3. Shows the effect of probiotic bacteria on fat content of the Sudanese white soft cheese during storage period. The highest (26.98%) fat content was obtained by the cheese samples containing *lactobacillus rhamnosus*. The lowest (25.70%) by the control samples, while the other samples ranked in an intermediate position (p≤0.05). The fat content increase in all cheese samples due to the loss of moisture content during storage period. The same trend of these results was reborted by [28, 22 ,29].

Table 4. Shows the effect of type of probiotic bacteria on ash content during storage period. The highest (4.70%) ash content obtained by the control samples. The lowest (3.00%) by the cheese samples containing *Lactobacillus rhamnosus*, while the other samples ranked in an intermediate position (p≤0.05). [30] found the ash content increased in Sudanese white soft cheese with starter culture. [31] conducted that ash content increased in soft cheese in Kariesh cheese made using probiotic bacteria. [15] reported that ash contents

increased in Sudanese white soft cheese "Gibna Beida" as storage time advanced.

Table 2: Effect of types of probiotic bacteria on protein content (%) of white soft cheese during storage period.

Storage period (Days)	Types of probiotic bacteria			
	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
0	16.72 ^m ±0.06	17.03 ^L ±0.09	17.16 ^{kl} ±0.08	16.91 ^m ±0.07
15	17.47 ^{kl} ±0.07	18.11 ^{Ji} ±0.05	18.24 ^{hi} ±0.03	17.86 ^k ±0.04
30	18.18 ⁱ ±0.05	18.43 ^s ±0.03	18.50 ^g ±0.03	18.37 ^h ±0.11
45	18.82 ^d ±0.09	18.82 ^d ±0.09	18.88 ^c ±0.06	18.69 ^f ±0.02
60	18.75 ^e ±0.04	18.94 ^b ±0.09	19.04 ^a ±0.04	18.88 ^c ±0.12

- Mean ± SD. Having different super script letters on columns and rows are significantly different (P≤0.05)

Table 3: Effect of types of probiotic bacteria on fat content (%) of white soft cheese during storage period.

Storage period (Days)	Types of probiotic bacteria			
	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
0	21.40 ^L ±0.02	22.92 ^k ±0.06	23.45 ^h ±0.03	22.30 ^J ±0.04
15	22.52 ^k ±0.01	23.83 ⁱ ±0.07	24.83 ^e ±0.05	23.63 ^{sh} ±0.08
30	23.00 ^J ±0.05	24.47 ^{gh} ±0.11	25.90 ^b ±0.09	24.80 ^e ±0.06
45	24.70 ^s ±0.07	25.30 ^f ±0.08	26.53 ^a ±0.12	25.82 ^c ±0.11
60	25.70 ^f ±0.04	26.00 ^d ±0.03	26.98 ^a ±0.13	25.97 ^{bc} ±0.02

- Mean ± SD. Having different superscript letters on columns and rows are significantly different (P≤0.05)

Table 4: Effect of types of probiotic bacteria on ash content (%) of white soft cheese during storage period.

Storage period (Days)	Types of probiotic bacteria			
	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
0	2.70 ^{hi} ±0.15	2.30 ^k ±0.09	1.00 ^l ±0.11	2.45 ^l ±0.13
15	3.00 ^s ±0.11	2.60 ⁱ ±0.19	1.40 ^j ±0.16	2.85 ^h ±0.09
30	3.90 ^c ±0.13	3.50 ^f ±0.08	2.10 ^g ±0.14	3.72 ^e ±0.12
45	4.45 ^{bc} ±0.12	4.00 ^d ±0.07	2.54 ^f ±0.15	4.20 ^{cd} ±0.16
60	4.70 ^a ±0.16	4.25 ^c ±0.17	3.00 ^d ±0.18	4.55 ^b ±0.19

- Mean ± SD. Having different superscript letters on columns and rows are significantly different (P≤0.05)

Also pH values effect by the three types of probiotic bacteria, the highest (4.05) pH value was obtained by the control samples. The lowest value (3.55) by the cheese samples containing *Lactobacillus rhamnosus* as shown in table 5. [32] conducted that pH decreased in Feta cheese made with a probiotic culture. Cheese making is based on application of LAB in the form of defined or undefined starter cultures that are expected to cause a rapid acidification of milk through the production of lactic acid, with the consequent decrease in pH values [33]. [34] stated that in Turkish Beyaz cheese supplemented with probiotic bacteria was lower in pH than the control without probiotic.

Table 5: Effect of types of probiotic bacteria on pH value of white soft cheese during storage period.

Storage period (Days)	Types of probiotic bacteria			
	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
0	5.50 ^a ±0.02	4.95 ^b ±0.06	4.73 ^{bc} ±0.07	4.48 ^{bc} ±0.05
15	5.10 ^a ±0.05	4.64 ^c ±0.03	4.28 ^c ±0.08	4.47 ^d ±0.12
30	4.58 ^d ±0.04	4.15 ^{ef} ±0.09	4.10 ^{gh} ±0.11	4.17 ^g ±0.06
45	4.22 ^f ±0.11	4.00 ^h ±0.02	3.69 ^{ij} ±0.05	3.88 ⁱ ±0.07
60	4.05 ^{gh} ±0.03	3.77 ^{ij} ±0.12	3.55 ^{jk} ±0.04	3.65 ^j ±0.08

- Mean ± SD. Having different superscript letters on columns and rows are significantly different (P≤0.05)

Moisture content effect by the types of probiotic bacteria during storage period. The highest (50.36%) moisture obtained by sample containing *Lactobacillus rhamnosus*. The lowest (47.34%) by the control samples. The highest values obtained at the beginning of the storage period while the lowest at the end ($p \leq 0.05$) as shown in table 6. Decreased in moisture might be due to the development of acidity, which leads to curd contraction that helps to expel the whey from the curd [35]. [36] pointed that moisture content of probiotic cheese supplemented with *Lactobacillus casei*, *Bifidobacterium lactis* all types of cheese revealed a natural loss of moisture upon 60 days of ripening. Results are in agreement with those reported by [37]. [38] reported that probiotic supplementation of Panele Cheese containing *L.rhamnosus* showed greater decreased in moisture.

Table 6: Effect of types of probiotic bacteria on moisture content (%) of white soft cheese during storage period.

Storage period (Days)	Types of probiotic bacteria			
	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
0	55.69 ^{de} ± 0.15	56.72 ^c ± 0.12	58.57 ^a ± 0.11	57.59 ^b ± 0.11
15	52.93 ^{fg} ± 0.19	54.66 ^f ± 0.16	55.97 ^d ± 0.15	55.13 ^e ± 0.15
30	49.81 ^{jk} ± 0.13	51.11 ^h ± 0.17	53.57 ^{fg} ± 0.14	52.85 ^g ± 0.16
45	48.1 ^{LM} ± 0.14	49.89 ^j ± 0.18	51.98 ^h ± 0.09	50.88 ⁱ ± 0.13
60	47.34 ^M ± 0.19	48.12 ^L ± 0.21	50.36 ⁱ ± 0.12	49.23 ^k ± 0.17

- Mean ± SD. Having different superscript letters on columns and rows are significantly different ($P \leq 0.05$)

Table 7. Shows the effect of types of probiotics on soluble nitrogen (SN) of Sudanese white soft cheese during storage period, the highest (0.78%) obtained by cheese samples containing *Lactobacillus rhamnosus*. The lowest (0.52%) by the control samples. Increased in SN of soft cheese could be due to the enzymes released by probiotic cultures during the pickling [39]. [23] observed that SN increased in all samples of Karish Cheese as affected by the probiotic *B.bifidum* and *L.rhamnosus* during days of storage. [40] reported that SN increased in probiotic white brined cheese with *L.casei* during the storage period. [41] enumerated that SN % increased with addition of *L. rhamnosus* in Kareish cheese during storage of the samples.

Also Formol Ripening Index (FRI) effect by the types of probiotic bacteria during storage period. The highest FRI (48.88mg/100g cheese) obtained by cheese samples containing *Lactobacillus rhamnosus*. The lowest (39.68mg/100g cheese) by the control samples. The lowest values obtained at the beginning of the storage period while the highest at the end ($p \leq 0.05$). As shown in table 8. These results could be attributed to the presence of proteolytic and lipolytic system of probiotic strains [42]. [43] stated that the salting method had a significant effect on FRI of Ras cheese. Increasing ripening index with the time is in agreement with that reported by [12, 44].

Table 7: Effect of types of probiotic bacteria on soluble nitrogen content (SN) (%) of white soft cheese during storage period.

Storage period (Days)	Types of probiotic bacteria			
	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
0	0.20 ^L ±0.06	0.30 ^I ±0.03	0.34 ^I ±0.04	0.32 ^J ±0.07
15	0.27 ^K ±0.01	0.37 ^H ±0.05	0.37 ^S ±0.02	0.38 ^H ±0.09
30	0.34 ^I ±0.08	0.43 ^{FG} ±0.07	0.43 ^E ±0.06	0.47 ^F ±0.05
45	0.42 ^{FG} ±0.04	0.61 ^D ±0.02	0.61 ^C ±0.08	0.63 ^{CD} ±0.11
60	0.52 ^E ±0.03	0.72 ^B ±0.08	0.78 ^{AB} ±0.05	0.77 ^{AB} ±0.04

• Mean ± SD. Having different superscript letters on columns and rows are significantly different (P≤0.05)

Table 8: Effect of types of probiotic bacteria on formol ripening index (FRI) (mg/100g cheese) of white soft cheese during storage period.

Storage period (Days)	Types of probiotic bacteria			
	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
0	15.08 ^P ±0.15	18.22 ^{NO} ±0.13	22.26 ^L ±0.13	19.00 ^N ±0.12
15	17.80 ^O ±0.16	20.78 ^M ±0.11	27.80 ^J ±0.23	24.34 ^K ±0.14
30	21.32 ^{LM} ±0.19	29.00 ^I ±0.12	32.39 ^S ±0.25	30.25 ^H ±0.42
45	31.80 ^{SH} ±0.18	36.68 ^F ±0.21	39.62 ^D ±0.16	38.00 ^E ±0.17
60	39.68 ^D ±0.17	42.00 ^C ±0.14	48.88 ^A ±0.19	46.92 ^B ±0.11

• Mean ± SD. Having different superscript letters on columns and rows are significantly different (P≤0.05)

Also Schilovich Ripening Index (SRI) effect by the three types of probiotic bacteria. The highest (60.32mg/100g cheese) SRI was obtained by cheese samples containing *Lactobacillus rhamnosus*. The lowest (49.83mg/100g cheese) by the control samples, table 9. [30] found the SRI content increased in Sudanese white soft cheese with starter culture. SRI increased from 18.80 to 31.33at the end of storage period. [22] stated that SRI of probiotic Domiati cheese supplemented with different levels of *L.rhamnosus* increased during ripening period. [45] conducted the increased of SRI during ripening of Swiss cheese affecting by adjunct cultures.

Table 9: Effect of types of probiotic bacteria on schilovich ripening index (SRI) (mg/100g cheese) of white soft cheese during storage period.

Storage period (Days)	Types of probiotic bacteria			
	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
0	23.89 ^o ±0.19	30.86 ^N ±0.18	34.80 ^L ±0.23	32.22 ^m ±0.25
15	30.53 ^N ±0.26	35.20 ^L ±0.16	40.20 ^J ±0.15	36.55 ^k ±0.21
30	42.37 ^U ±0.22	43.35 ^I ±0.14	46.17 ^{gh} ±0.24	45.16 ^h ±0.13
45	47.70 ^s ±0.11	54.56 ^c ±0.27	59.22 ^b ±0.19	56.70 ^d ±0.22
60	49.83 ^f ±0.17	56.55 ^a ±0.16	60.32 ^a ±0.14	57.98 ^e ±0.23

- Mean ± SD. Having different superscript letters on columns and rows are significantly different (P≤0.05)

In the minerals content as shows in Table 10. the three types of probiotic bacteria effect minerals in Sudanese white soft cheese as follows:

a- Calcium content: The highest calcium content (9.80 mg/l) was obtained by the cheese sample containing *L.rhamnosus*. The lowest (7.96 mg/l) by the control sample. [46] conducted the ionization yield of mineral compounds, mostly calcium, increases with a decrease in pH. [47] conducted that feeding rats of probiotic cheese resulted in increased calcium retention compared to control. [48] noticed that calcium content increased in white soft cheese (jibna-beida) prepared by using starter culture (LAB).

b- Phosphorous content: The highest phosphorous content (3.62 mg/l) was obtained by the cheese sample containing *L.rhamnosus*. The lowest (3.30 mg/l) by the control sample. [49] reported that phosphorous content increased in Dairy product with addition of *B.bifidum*. The addition of *L.rhamnosus* led to a minor increase (2%) in phosphorus availability. [46] conducted that the rate of changes in the pH of cheese was one of the key determinants of phosphorus availability. The ionization yield of mineral compounds increases with a decrease in pH.

c- Sodium content: The highest sodium content (57.80 mg/l) was obtained by sample containing *L.rhamnosus*. The lowest (56.51mg/l) by the control sample. [48] stated that sodium content increased in white soft cheese (jibna-beida) prepared by using starter culture (LAB). [50] observed that the sodium content of *B.bifidum* in some dairy products was 52mg/100g. [12] stated that the sodium content of braided cheese increased.

d- Pottasium content: The highest pottasium content (7.98 mg/l) was obtained by the cheese sample containing *L.rhamnosus*. The lowest (6.33 mg/l) by the control sample. [48] stated the pottasium content increased in white soft cheese (jibna-beida) prepared by using starter culture (LAB). The stimulating effect of probiotic cultures on the availability of mineral compounds in cheese can be attributed to intensified enzymatic conversion, mainly proteolysis and lipolysis. [51, 52, 53].

e- Magnesium content: The highest magnesium content (7.69 mg/l) was obtained by sample containing *L.rhamnosus*. The lowest (6.00 mg/l) by the cheese sample containing control. [54] conducted that milk magnesium content was high. [46] found that availability of magnesium from Dutch-type cheese with probiotics *L. rhamnosus*. The addition of probiotic culture resulted in increase in the availability of magnesium relative to control at 24, 20, and 10%, respectively. [55] stated the availability of magnesium from various ripe cheeses is also determined by proteolysis and lipolysis products.

f- Iron content: The highest iron content (1.35 mg/l) was obtained by the cheese sample containing *L.rhamnosus*. The lowest (1.18 mg/l) by the control sample. [50] stated that the amount of iron content in *B.bifidum* in some dairy product was 0.46mg. The stimulating effect of probiotic cultures on the availability of mineral compounds in cheese can be attributed to intensified enzymatic conversion, mainly proteolysis and lipolysis [51, 52, 53]. [56] reported that *B.animals* and *B.breve* dairy product appear to enhance micronutrient absorption (particularly iron) and bone development, but the effect appear to be highly dependent on the probiotic strain.

Table 10: Effect of types of probiotic bacteria on minerals contents (mg/L) of white soft cheese

Minerals	Types of probiotic bacteria				
	Mg/L	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
Calcium		7.96 ^c ±0.07	8.92 ^b ±0.09	9.80 ^a ±0.11	9.15 ^a ±0.05
Phosphorous		3.30 ^d ±0.04	3.42 ^c ±0.06	3.62 ^a ±0.05	3.55 ^b ±0.09
Sodium		56.51 ^b ±0.06	56.66 ^b ±0.03	57.80 ^a ±0.02	57.12 ^a ±0.04
Potassium		6.33 ^c ±0.07	6.68 ^b ±0.08	7.98 ^a ±0.04	6.72 ^{bc} ±0.05
Magnesium		6.00 ^d ±0.02	6.82 ^b ±0.04	7.69 ^a ±0.06	6.63 ^c ±0.08
Iron		1.18 ^c ±0.03	1.27 ^b ±0.02	1.35 ^a ±0.07	1.23 ^c ±0.06

• Mean ± SD. Having different superscript letters on rows are significantly different (P≤0.05)

The Total volatile fatty acids content (TVFA) effect by three types of probiotic bacteria during storage period. The highest (39.00 mls 0.1NaoH/100Kg cheese) TVFA was obtained by cheese samples containing *Lactobacillus rhamnosus*. The lowest (17.00 mls 0.1NaoH/100Kg cheese) by the control samples. as shown in table 11. [57] conducted the increased in TVFA of probiotic Ras cheese supplemented with different levels of *L.rhamnosus* during ripening. [58] found the TVFA increased in Edam cheese supplemented with *B.bifidum* as well as control during storage period. [59] stated that TVFA of cheese increased during the storage period from 23.00 (0.1 N ml NaOH/100 g cheese) at the beginning of the storage period to 50.00 (0.1 N ml NaOH/100 g cheese) at 90 days of storage in white soft cheese. [60] reported increase in TVFA in Domiati cheese effect of some lactic acid bacteria.

Table 11: Effect of types of probiotic bacteria on total volatile fatty acids (TVFA) (mls 0.1N NaoH/100kg cheese) of white soft cheese during storage period.

Storage period (Days)	Types of probiotic bacteria			
	Control	Bifidobacterium bifidum	Lactobacillus rhamnosus	Lactobacillus casei
0	10.20 ^P ±0.08	11.00 ^O ±0.04	14.03 ^L ±0.11	12.00 ^N ±0.03
15	11.22 ^O ±0.06	13.22 ^m ±0.07	18.00 ^I ±0.02	16.56 ^g ±0.12
30	14.28 ^L ±0.03	20.00 ^h ±0.05	25.20 ^f ±0.06	23.00 ^g ±0.07
45	15.00 ^{JK} ±0.09	29.08 ^e ±0.08	32.00 ^{cd} ±0.04	31.00 ^d ±0.11
60	17.00 ^{ij} ±0.05	33.17 ^c ±0.06	39.00 ^a ±0.07	37.00 ^b ±0.03

- Mean ± SD. Having different superscript letters on columns and rows are significantly different (P≤0.05)

3. Conclusion

The effect of probiotic bacteria (*L.rhamnosus*, *L.casei* and *B.bifidum*) on physicochemical properties of Sudanese white soft cheese made from cow's milk was investigated. Evaluations of physicochemical properties were carried out at 0,15,30,45 and 60 days interval.

- A significant (p≤0.05) increased in level of the titratable acidity during storage of cheese coupled with a concomitant decrease in pH values particularly by addition of probiotic bacteria.
- Major components of Sudanese white soft cheese such as fat and protein increased by the addition of probiotic bacteria during storage period.
- There was increased in SN, SN/TN, FRI, SRI and TVFA during storage period in all probiotic samples.
- Minerals content and fatty acids increased in all treated samples.

References

- [1] Kopp-Hoolihan, L. (2001). Prophylactic and Therapeutic Uses of Probiotics: A Review. *Journal of American Dietary Association*, 101(2): 229-241.
- [2] Lee, Y.K., Nomoto, K., Salminen, S and Gorbach, S.L. (1999). Hand book of probiotics. Jon Wiely and Sons, Inc., New York.
- [3] Pitino, I.; Randazzo, C. L.; Cross, K. L.; Parker, M. L.; Bisignano, C.; Wickham, M. S. J.; Mandalari, G. and Caggia, C. (2012). Survival of *Lactobacillus rhamnosus* strains inoculated in cheese matrix during simulated human digestion. *Food Microbiol*, 31:57–63.
- [4] Fooks, L.; Fuller, R. and Gibson, G. R. (1999). Prebiotics-probiotics and human gut microbiology. *International Dairy Journal*, 9(1): 53-61.
- [5] Venema, K. (2015). Health effect of pro and prebiotics: Utilization of sophisticated invitro Tools. In: Beneficial Microorganisms in Medical Health Applications; series: Microbiology Monographs, 28: 1-18.
- [6] Robinson, R. K. and Samona, A. (1992). Health aspect of Bifidus products: A review. *Int. J Food Sci Nutrition*, 43: 175-180.
- [7] Blanchette, L.; Roy, D. and Gauthier, S.F. (1996). Production of cottage cheese using dressing by bifidobacteria. *Journal of Dairy Science*, 78(7): 1421-1429.
- [8] Fujimoto, J.; Matsuki, T.; Sasmoto, M.; Tommii, Y. and Watanabe, K. (2008). Identification and quantification of *Lactobacillus casei* strain shirota in human faeces with strain specific primer derived from randomly amplified polymorphic DNA. *International Journal of food microbiology*, 126: 210-215.
- [9] Tanook, G. W.; Munro, K.; Harmsen, H.J.; Welling, G.w.; Smart, J. and Gopal, P.K. (2000). Analysis of faecal microflora of human subjects consuming a probiotic products containing *Lactobacillus rhamnosus* DR20. *Appl. Environ Microbiol*, 66: 2578-2588.
- [10] Ibrahim, F.; Ruvio, S.; Granlund, L.; Salminen, S.; Viitanen, M. and Ouwehand, A.C. (2010). Probiotics and immunosenescence: Cheese as a carrier. *FEMS Immunol. Med. Microbiol*, 59:53-59.
- [11] Pantaleao, A.; Moens, E. and O'Connor, C. (1990). The technology of traditional milk products in developing countries. *FAO Animal Production and Health*. FAO, Rome, Italy, 85: 333.
- [12] Abdel Razig, K.A. (2000). Quality attributes of Braided cheese "Mudaffra" as affected by level of salt and storage temperature. Ph.D. Thesis, University of Khartoum, Sudan.
- [13] El Owni, O. A. and Hamid, I. (2007). Production of white cheese (Gibna Bayda) in Zalingei area, West Darfur (Sudan). *Australian Journal of Basic and Applied Sciences*, 1: 756-762.
- [14] Robinson, R.K. (1983). Dairy microbiology. V. 2. The Microbiology of milk products. Elsevier Science Publishing Co., IN.
- [15] Abdel Razig, K.A. (1996). The production of white soft cheese from different sources. M.Sc. Thesis, University of Khartoum, Sudan.
- [16] AOAC. (1990). Official Methods of Analysis 15th edition. Association of Official Analytical Chemists (AOAC), Washington, D.C., U.S.A.
- [17] Ling, E.R. (1963). *Textbook of Dairy Chemistry*. Vol. 2 Chapman and Hall Ltd. London.
- [18] Abdel-Tawab, G.H. and Hofi, A.A. (1966). Testing cheese ripening, rapid chemical techniques. *Indian J. Dairy Sci*, 19: 39-41.

- [19] Perkin-Elmer. (1994). Analytical Methods for farance Atomic Absorption Spectrometry. Uberlingen, Germany, pp.332.
- [20] Kosikowski, F.V. (1982). Cheese and Fermented Milk Food. Edwards Brothers, Inc., Ann. Arbor., Michigan, USA.
- [21] SAS, 1997. SAS/STAT users Guid, Version 6.03 edition, Cary. NC: SAS Institute Inc., pp: 1028.
- [22] Kebary, K.K.; El-Shazly. H. A. and Youssef. I. T. (2015).Quality of probiotic Domiati cheese made by *Lactobacillus rhamnosus*. *Int. J. Curr. Microbiol. App. Sci*, 4(7): 647-656.
- [23] Mahmoud, S.F.; El-Halmouch, Y. and Montaser, M.M. (2013). Effect of probiotic bacteria on Karish Cheese production. *Life Sci J*, 10(2): 1279-1284.
- [24] Yerlikaya, O. and Ozer, E. (2014). Production of probiotic fresh white cheese using co-culture with *Streptococcus thermophilus*. *Food Sci. Technol*, Campinas, 34(3): 471-477.
- [25] Warsama, L.M.; El Zubeir, I.E.M. and El Owni, O.A.O. (2006). Composition and hygienic quality of Sudanese white cheese in Khartoum North markets (Sudan). *Int. J. Dairy Sci*, 1: 36- 43.
- [26] Cichosz, G.; Aljewicz, M. and Nalepa, B. (2014). Viability of the *Lactobacillus rhamnosus* HN001 probiotic strain in Swiss- and Dutch-Type cheese and Cheese-Like products, *Journal of Food Science*, 79 (6).
- [27] Abdel Razig, K.A.; Ahmed, R.A. and Mohamed, E.B. (2002). Ripening behavior of Sudanese braided cheese "Mudaffra": First international conference on biotechnology, application for arid Regions'. Pulpished by the Kuwait Institute for Science Research, (1):409-421.
- [28] El-Zayat, A.I. and Osman, M.M. (2001). The use of probiotics in Tallaga cheese. *Egypt. J. Dairy Sci*, 29: 99-106.
- [29] Kheir, E.S.; El Owni, O.A. and Abdalla. O.M. (2011). Comparison of Quality of Sudanese white cheese (Gibna bayda) Manufactured with Solanum dubium fruit exteract and rennet. *Pakistan J. Nutr.*, 10(2):106-111.
- [30] Abdalla, M.O.M. and Mohamed, S.N. (2009). Effect of storage period on chemical composition and sensory characteristics of vacuum packaged White soft cheese. *Pakistan Journal of Nutrition*, 8 (2): 145-147.
- [31] Fayed A.E.; Farahat A.M.; Metwally A.E.; Massoud M.S. and Emam A.Q. (2014). Health stimulating properties of the most popular soft cheese in Egypt Kariesh made using skimmed milk UF-retentate and probiotics. *Acta Sci. Pol., Technol. Aliment*, 13(4): 359-373.
- [32] Pappa, H. C. and Anifantakis, E. M. (2001). Effect of concentrated starter cultures on the proteolysis and organoleptic characteristics of Feta cheese. *Milchwissenschaft*, 56:325–329.
- [33] Briggiler-Marcó, M.; Capra, M.L.; Quiberoni, A.; Vinderola, G.; Reinheimer, J.A., and Hynes, E. (2007). Nonstarter *Lactobacillus* strains as adjunct cultures for cheese making: in vitro characterization and performance in two model cheeses. *J Dairy Sci*, 90: 4532-4542.
- [34] Kılıç, G. B.; Kuleşan, H.; Eralp, İ. and Karahan, A. G. (2009). Manufacture of Turkish Beyaz cheese added with probiotic strains. *LWT - Food Science and Technology*, 42: 1003-1008.
- [35] Effat, B.A.M.; Salem, M.M.E. and El-Shafei, K. (2001). Effect of using different starters on quality of Kareish cheese. *Egypt. J. Food Sci*, 29: 95-108.
- [36] Rodrigues, D.; Rocha-Santos, T.A.P.; Gomes, A.M., Goodfellow, B.J. and Freitas, A.C. (2012). Lipolysis in probiotic and synbiotic cheese: The influence of probiotic bacteria, prebiotic compounds and ripening time on free fatty acid profiles. *Food Chemistry*, 131:1414–1421.

- [37] Badawi, R.M.; Kebary, K.M.K. (1996). Accelerated ripening of cheese by partial lyzed Lactococci. *Menofiya J.Agric. Res.*, 21(1): 63-81.
- [38] Escobar, M.C.; Van Tassell, M.L.; Martinez-Bustos, F.; Singh, M.; Casta-o-Tostado, E.; Amaya-Llano, S.L. and Miller, M.J. (2012). Characterization of a Panela and fava bean starch. *Journal of Dairy Science*, 95(6): 2779-2787.
- [39] Shehata, A. E.; EL-Nawawy, M. A.; EL-Kenany, Y. M. and Aumara, I. E. (2001). Production of soft cheese with health benefits. In *Proc. 8th Egyptian conference Dairy Science and Technology*, pp. 635 – 651.
- [40] Dabevska-Kostoska, M.; Velickova, E.; Kuzmanova, S. and Winkelhausen, E. (2015). Traditional white Brined cheese as a delivery vehicle for probiotic bacterium *Lactobacillus casei*. *Macedonian Journal of Chemistry and Chemical Engineering*, 34(2): 343–350.
- [41] El-Shafei, K.; Abd El-Gawad, M. A.M.; Dabiza, N.; Sharaf, O. M. and Effat, B.A. (2008). A mixed culture of *propionibacterium ThoenII P-127*, *Lactobacillus rhamnosus* and *Lactobacillus planterium* as probiotic cultures in Kareish cheese. *Pol. J. Food Nutr. Sci*, 58(4): 433-441.
- [42] Bergamini, C.V.; Hynes, E.R.; Palma, S.B.; Sabbag, N.G.; Zalazar, C.A. (2009). Proteolytic activity of three probiotic strains in semi-hard cheese as single and mixed cultures: *Lactobacillus acidophilus*, *Lactobacillus paracasei* and *Bifidobacterium lactis*. *International Dairy Journal*, 19: 467–475.
- [43] El-Batawy, M.A.; El-Abd, M.M.; Younes, N.A. and El-tawel, H.S. (1992). Effect of salting method on the ripening of Ras cheese made from mixed goat's and cow's milk. *Egyptain J. Dairy Sci*, 20: 341-350.
- [44] Tarakci, Z. and Kucukoner, E. (2006). Changes onphysicochemical, lipolysis and proteolysis of vacuum packed Turkish Kashar cheese during ripening. *J. Central Europ. Agric*, 7: 459-464.
- [45] Chen, G.A.; Kocaoglu-Vunma, N.A.; Hasper, W.J. and Rodrigues Saona, L.E. (2009). Application of unframed micro spectroscopy and multivariate analysis for monitoring the effect of adjunct cultures during Swiss cheese ripening. *J. Dairy Sci.*, 92: 3575 3584.
- [46] Aljewicz, M.; Cichosz, G.; Nalepa, B. and Kowalska, M. (2014) Influence of Probiotics on proteolysis of Edam Cheese, *Food Technol. Biotechnol*, 52 (4): 439–447.
- [47] Klobukowski, J.; Modzelewska-Kapitula, M. and Komacki, K. (2009). Calcium Bioavailability from diets based on white cheese containing probiotics or synbiotics in short-time study rats. *Pak. J. nutr*, 8(7): 933-936.
- [48] Sidig, S.M.; Sulieman, A.E.; Salih, Z.A. and Abdelmuhsin, A.A. (2016). Quality characteristics of white soft cheese (jibna-beida) produced using camel milk and mixture of camel and cow milk. *International Journal of Food Science and Nutrition Engineering*. 6(3): 49-54.
- [49] Gabir, H.G. (2016). The effect of type of bifidobacterium on set yoghurt. PH.D. Thesis. Alzaiem Alazhari University. Sudan.
- [50] Akin, M.B.; Akin, M.S. and Kirmac, Z. (2007). Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. *Food Chem*, 104: 93–99.
- [51] Banasaz, M.; Norin, E., Holma, R. and Midtvedt, T. (2002). Increased enterocyte production in gnotobiotic rats mono-associated with *Lactobacillus rhamnosus* GG. *Appl. Environ. Microbiol*, 68: 3031–3034.
- [52] Lutz, T. and Scharrer, E. (1991). Effect of short-chain fatty acids on calcium absorption by the rat colon. *Exp. Physiol*, 76:615–618.

- [53] Scholz-Ahrens, K. E.; Peter, A.; Berit, M., and W. Petra. (2007). Prebiotics, probiotics and synbiotics affect mineral absorption, bone mineral content and bone structure. *J. Nutr.* 137: 838–846.
- [54] Hidioglou, M. and J. G. Proulx. (1982). Factors affecting the calcium, magnesium, and phosphorus content of beef cow milk. *Can. J. Comp. Med.*, 46:212–214.
- [55] Cross, K. J.; Huq, N. L.; Palamara, J. E.; Perich, J. W. and Reynolds E. C. (2005). Physicochemical characterization of casein phosphopeptide- amorphous calcium phosphate nanocomplexes. *J. Biol. Chem.*, 280:15362–15369.
- [56] Agustina, R.; Bovee-Oudenhoven, I.M.; Lukito, W.; Fahmida, U., van de Rest, O.; Zimmermann, M.B.; Firmansyah, A.; Wulanti, R., Albers, R. and van den Heuvel, E.G. (2013). Probiotics *Lactobacillus reuteri* DSM 17938 and *Lactobacillus casei* CRL 431 modestly increase growth, but not iron and zinc status, among Indonesian children aged 1-6 years. *J Nutr*, 143: 84–93.
- [57] Kebary, K.K.; Yousef, I.T.A.; El-Shazly, H.A.M. and Rajab, W.A.A. (2011). Quality of Ras cheese made by probiotic strain of *Lactobacillus rhamnosus*. *J. Food and Dairy Sci.*, Mansoura Univ, 2 (2): 69 – 78.
- [58] Sabikhi, L.; Sathish Kumar, M. H. and Mathur, B. N. (2014). *Bifidobacterium bifidum* in probiotic Edam cheese: influence on cheese ripening. *Food Sci Technol*, 51(12):3902–3909.
- [59] Suliman, A.H.Y.; Abdalla, M.I. and El Zubeir, I. E. M. (2013). Effect of level of milk fat on the compositional quality of Sudanese white cheese during storage, *Sky Journal of Food Science*, 2(1):1- 9.
- [60] El-Abd, M.M.; Abd El-Fattah, A.M.; Osman, S.G. and Abd El-Kader, R.S. (2003). Effect of some lactic acid bacteria on the properties of low salt Domiati cheese. *Egypt. J. Dairy Sci*, 31: 125-138.