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Research Article

Quality of White Cheese Made Using *Moringa oleifera* Leaf Extract

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Abstract

The aim of this study was to evaluate the stability and efficiency of Sudanese white cheese made with *Moringa oleifera* leaves extract (MOLE), using rennet enzyme as control and to evaluate the effect of *Moringa oleifera* leaves extract on cheese yield, physiochemical, minerals content, microbial analysis and sensory characteristics on processed white cheese and to determine the shelf-life after six months of storage, and the quality of white cheese products. Refined coagulants such as pure calf rennet (enzyme) are still unavailable in most rural areas of Sudan where most of the cheese is produced. The effect of (MOLE) on physiochemical analysis (protein, fat, ash, T.S and pH) values decreased at storage periods (1st, 2nd, 3rd and 4th), while moisture content and acidity increased at storage periods. Minerals content (Ca, Na, K) were significantly ($P<0.05$) affected by the extract and control on storage period. Minerals content were higher in white cheese produced with MOLE than control cheese. Microbiological analysis (*T. coliform, E. coli,* Staphylococcus, Salmonella, Spore formers bacteria and Brucella rings) were not detected on cheese with MOLE and control sample, while (Total bacterial, Yeast and moulds, Lactic acid bacteria) were detected with normal account. Sensory characteristics were significant differences ($P<0.05$) of cheese samples with MOLE and control sample, control sample (7.00±0.47) was higher value in overall acceptability but cheese samples with MOLE was higher on texture (6.10±0.74) than control samples (3.10±0.35). Cheese production with MOLE is very nutritious for consumption because it has high content on fat, protein and minerals. In addition, MOLE is less expensive than other coagulant to manufacture white cheese.

Keywords: White cheese, *Moringa oleifera,* sensory characteristics, microbiological analysis, ripening.

Introduction

Cheese making as means of preserving is the most important constituents of milk in highly concentrated form are in vogue all over the world. It provides a palatable milk product of high nutrition value which can be kept fresh for a long time (Osman, 2009). Cheese is an economical source of milk protein. It is rich in calcium, vitamins, nourishing and easily digestible food (Nir, 2004). Cheese making is aimed to make milk preservation attractive and durable. Its shelf-life varies from few days to several years (Walstra *et al*., 1999). Cheese as the solid food made from the milk of cows, goats, sheep and other mammals and it is lighter weight, more compact and has a longer shelf-life than the milk from which it is made (Smith, 2005). The contributions of cheese as source of proteins, calories, minerals and some vitamins are vital to the development of a good health (Talib*et al*., 2009). In Sudan cheese processing is a major preservation method of surplus milk in rural areas especially during rainy seasons when plenty of milk is available. The product is an important nutrient for humans especially under conditions where other animal proteins are not available (Kosikowski, 1982). El-Owni and Hamid (2007) stated that the most popular type of cheese produced in Sudan is the white cheese locally known as Gibna Bayda. It is generally consumed fresh or matured for a period of several months. It's made from full fat raw milk; high concentrations of sodium chloride are added before rennet method (Osman, 1987). Raheem (2006) added that Gibna Bayda is white cheese made in Sudan. It is similar to Domiati cheese made in Egypt. Starter is not used, and the storage life of the cheese may be more than one year. In the latter part of the last century, cheese consumption increased while the availability of calf rennet decreased, which led to rennet shortages and subsequent price increases. In addition, more restrictive ethical concerns associated with production of such animal rennet led to a search for suitable rennet substitutes for cheese making. Several proteases from animal, microbial and plant sources were investigated as likely substitutes and have been reviewed (Fox *et al*., 2000). Plant extracts have been used as milk coagulants in cheese making since ancient times.

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Cheeses made with vegetable coagulant can be found mainly in Mediterranean, West African, and southern European countries (Shah *et al*., 2014). *Moringa oleifera* (*Moringa oleifera* Lam.) belongs to the family Moringaceae which is a single genus family of shrubs and trees cultivated across the whole of the tropical belt and used for a variety of purposes (Jahn, 1986). Moringa is native to the Indian subcontinent and has become naturalized in the tropical and subtropical areas around the world. The plant thrives best under the tropical insular climate. It can grow well in the humid tropics or hot dry lands and can survive in less fertile soils and it is also little affected by drought (Anwar *et al*., 2007). It is considered as one of the World's most useful trees, as almost every part of the Moringa tree can be used for food, medication and industrial purposes (Khalafalla *et al*., 2010). People useits leaves, flowers and fresh pods as vegetables, whileothers use it as livestock feed (Anjorin*et al*., 2010). Thistree has the potential to improve nutrition, boost foodsecurity and foster rural development (Hsu, 2006). Mostpeople in South Africa, however, are not aware of the potential benefits of Moringa. Studies from other countries indicate that the leaves have immense nutritional value such as vitamins, minerals and amino acids (Anwar *et al*., 2007). Casinolytic and milk-clotting activities from *Moringa oleifera* flowers extracts has been explored. The extract has been used to precipitate milk protein in presence of ammonium sulphate (Pontual *et al*., 2012). The limited supply of pure calf rennet enzyme and its high price for cheese industry have necessitated research to come up with an alternative milk coagulant. Refined coagulants such as pure calf rennet enzyme is still not available in most rural areas of Sudan where most of the cheese is produced. On the other hand, countries that export pure calfrennet are now retaining the enzyme for their own uses; and export porcine or its mixture with microbial or calf rennet to other countries. Considering the above facts this study was aimed with the following objectives:

1. To evaluate the effect of *Moringa oleifera* leaves extract on chemical, physicochemical, microbial and sensory characteristics at processed white cheese.
2. To determine the shelf-life and the quality of white cheese products.

Materials and methods

Materials: Fresh cow's full cream milk (80 L) was obtained from Department of Animal production Shambat farm College of Agricultural Studies. Milk was filtered and divided into 4 equal volumes of (20 L) each and kept into separate 4 containers. *Moringa oleifera* leaves were obtained from Salah Abdoon at Moringa Research and Development Group Farm, Khartoum North. The extraction procedure is described below. A fine commercial salt (Sodium chloride) was purchased from local markets.

Powder rennet was obtained from veterinary pharmacy in Hillat Koko (Copenhagen, Denmark). Calcium chloride powder of good quality was bringing from Lab of chemistry in Department of food science and technology in Agricultural College.

Cheese processing: Milk was purchased and transported to college of Agricultural in Sudan University for Science and Technology in Shambat Department of food science and technology for processing into white cheese (Jibna Beyda). In this study two treatments were carried out. First treatment is a control in which fresh cow's full cream milk was processed into cheese without additive. In the second (M. leave) treatments of Moringa extraction powder were added respectively to the fresh cow's full cream milk before pasteurization. Extraction method was determined according to El-Siddig *et al.,* (2018). White cheese was manufactured according to the method described by (El-Hag *et al*, 2013) with some modifications.

Procedure:

Using (GMP) Good Manufacture Practice on cheese processing: The white cheese was made by heating the milk to 50°C, calcium chloride (2%) was added to milk then added enzyme extraction solution at 45°C. enzyme powder (0.3 g for rennet and 3gm for leaves extraction) was dissolved in 10 ml distilled water and then added to milk at 45°C. This was stirred with milk for 5 min and left to coagulate. After coagulation the curd was cut into approximately 2 cm cubes with ordinary stainless steel knives to allow whey separation. The curds were transferred to wooden molds (50×50×20 cm) tined with cheese cloth. The curd was pressed for about 45 minutes for whey drainage. The whey was collected in a clean plastic receptacle. A flat wooden cover (49×49×2 cm) was put on top of the curd inside the wooden frame, and weights of about 15 kg were put on top of the wooden cover to press the curds. The weights were left there overnight. The weights, wooden cover, and the cloth were then removed of the pressed curd. The curd was cut into small blocks, and put into plastic container, and then the whey was salted at the rate of (7% w/w). The salted whey was pasteurized at 72°C then cooled to 40°C and putted on it. 250 grams of these blocks were transferred to the lab for analysis before packaging. After packaging the container was stored at room temperature (36 ±1°C).

Cheese yield percentage and Coagulation time of white cheese using Moringa oleifera leaf extract: The cheese yield was determined according to Paolo *et al*. (2008); Walstra *et al*. (1999) and Abdel Moneim *et al*. (2012) as follows:

$$\text{Yield} = \frac{\text{weight of cheese}}{\text{weight of sample}} \text{ X } 100$$

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Coagulation time (h) of white cheese samples were measure according to the method of Berridge (1952).

Shelf-life of cheese: Sudanese white cheese samples storage for 6 months. The analysis (Physiochemical microbiological and sensory evaluation) done at zero time and after two, four, and six months.

Physiochemical analysis: The physiochemical composition of cheese samples (Control-Moringa leaves cheese) was determined as follows:
The moisture content, total solids content of milk, and cheese and titratable acidity of the milkand cheese was determined according to the modified AOAC method (1990). Fat content was determined by Gerber method, protein content was determined by Kjeldahl method, Ash content and pH of cheese and milk determined according to AOAC (2009). The concentration of lactose was determined according to Rynne *et al*. (2007).

Calcium, sodium and potassium determination: Calcium, sodium and potassium contents of the samples were determined by Atomic Absorption Spectrometer according to Perkin Elmer (1994) and AOAC (2009). Two grams of cheese was maintained in muffle furnace at 550°C for 4 h. Samples were cooled and 10 mL of 3N HCL was added. Covered with watch glass and boiled gently for 10 minutes. Then cooled, filtered, diluted to volume (100m) with distilled water and taken for determination of sodium (Na) potassium (K) and for the determination of calcium 1 mL of 1% lanthanum chloride was added to the final dilution. Minerals content was calculated as follows:

$$\text{Mg mineral/100 mg sample} = \frac{\text{volume used mg / L X 100}}{\text{1000 x wt. of sample}}$$

Microbiological analysis: The microbiological analyses of milk and cheese samples were determined as follows:
The Coliforms count was performed according to Christen *et al*., (1992) and Marshall, (1993) using MaConkey agar media. The *E. coli* count was performed according to (William and Dennis, 1998) using MaConkey agar media and Eosin Methylene Blue agar (EMB) for identification. The count of Staphylococcus aureus was performed according to (Christen *et al*., 1992 and Marshall, 1993) using Mannitol salt agar. The Lactobacillus count was performed according to (Murray *et al*., 1995) using Mann-Rogosa Sharpe (MRS). Potato dextrose agar medium was used for the enumeration of yeasts and moulds count according to Frank *et al*., (1992) and Marshall (1993).The presence of Salmonella was performed according to (Harrigan, 1976) using Bismuth sulphite agar media for identification. The MRS agar medium (oxoid) will be used for the enumeration of lactic acid bacteria according to Frank*et al*. (1993).

Spore formers bacteria in white cheese samples were determined according to (Meer *et al*., 1991). Brucella ring test in milk and white cheese will be determined according to Cruickshank *et al*. (1975).

Sensory analysis: White cheese samples were subjected to sensory evaluation using 12 trained panelists (Narvhus *et al*., 1998). Cheese samples were assessed by panelists for color, flavor, texture and overall acceptability.

Statistical analysis: Two sample-paired Tests were performed to examine significant differences between two types of cheese. The effect of storage periods on stability of *Moringa oleifera* leave extract for each specific parameter was assessed by One-way ANOVA. Least Significance Difference (LSD) was used for mean separation between treatments. The level of Significance ($P<0.05$) was used in this study.

Results and discussion

Effect of coagulation time on the yield of White cheese samples: Table 1 showed the effect of coagulation time on the yield (%) of white cheese samples. The results showed that there was a significant difference in the yield (%) of cheese produced by control (rennet) (14.61± 0.0833%) and *Moringa oleifera* leaves extract (12.05±0.04%). The results of control cheese had higher yield of cheese compared to that from *Moringa oleifera* leaf extract. Abdul-Rahman (2013) stated that high content of moisture in cheese directly affect the yield percentage, since moisture considers one of the fundamental factors affecting the increase or decrease of yield percentage. He concluded that the yield of cheese made using safflower (12.73%) enzyme extract was lower than that made using calf rennet (13.8%). On the other hand, the results indicated that significant differences were found in coagulation time of rennet and *Moringa oleifera* leaves extract (1.333±0.0289 and 3.333±0.0289 h). These results of coagulation time of *Moringa oleifera* extracts is less than which reported with Abdelrahim (2017) who stated that coagulation time of sunflower *Helianthus annuus* enzyme cheese was 3.60 ± 0.27 h.

Effect of storage periods on the physiochemical characteristics of the Sudanese white soft cheese: Results in Table 2 illustrated the effects of *Moringa oleifera* extract and control sample on physicochemical characteristics of soft white cheese at storage periods. The results indicated that there were significance differences ($P<0.05$) in the moisture content of the samples. The data showed that highest moisture content (48.30±0.265%) was in the control sample (renin) at 4th month while the lowest one (38.03±0.15%) was recorded in leaf extract at first month. The moisture content is increase on enzyme extraction and control at storage periods.

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Table 1. Effect of coagulation time on the yield of white soft cheese.

Parameters	Type of cheese	
	Control	Leaf extract
Yield (%)	14.61 ± 0.08^{a}	12.05 ± 0.04^{c}
Coagulation time (h)	1.333 ± 0.03^{d}	3.333 ± 0.03^{b}

Values are mean ± SD; Means carrying different superscript letter in the same row are significantly different at P‹0.05.

Table 2. Effect of physiochemical characteristics on cheese samples at storage periods.

Parameters	Storage period (months)	Cheese samples	
		Control	Leaf extract
Moisture%	1	$46.87 \pm 0.15^{a}_{a}$	$38.03 \pm 0.15^{c}_{b}$
	2	$47.03 \pm 0.47^{a}_{a}$	$39.20 \pm 0.10^{c}_{b}$
	3	$49.03 \pm 0.38^{a}_{a}$	$40.70 \pm 0.2^{b}_{b}$
	4	$48.30 \pm 0.27^{a}_{a}$	$43.00 \pm 1.31^{b}_{b}$
Protein%	1	$26.27 \pm 0.15^{d}_{d}$	$29.77 \pm 0.06^{a}_{a}$
	2	$25.40 \pm 0.2^{d}_{d}$	$29.77 \pm 0.12^{a}_{a}$
	3	$25.23 \pm 0.15^{d}_{c}$	$29.57 \pm 0.15^{a}_{a}$
	4	$24.43 \pm 0.21^{d}_{d}$	$28.07 \pm 0.31^{b}_{a}$
Fat%	1	$22.43 \pm 0.15^{d}_{c}$	$25.47 \pm 0.15^{c}_{b}$
	2	$21.50 \pm 0.10^{d}_{c}$	$24.93 \pm 0.15^{c}_{b}$
	3	$20.27 \pm 0.12^{d}_{c}$	$24.33 \pm 0.06^{c}_{b}$
	4	$22.20 \pm 0.10^{d}_{d}$	$23.93 \pm 0.06^{c}_{c}$
Lactose	1	00.00 ± 0.00	00.00 ± 0.00
	2	00.00 ± 0.00	00.00 ± 0.00
	3	00.00 ± 0.00	00.00 ± 0.00
	4	00.00 ± 0.00	00.00 ± 0.00

Values are mean ± SD; Means carrying different superscript letter in the same column are not significantly different at P‹0.05 using DMRT.

Table 3. Effect of physiochemical characteristics on cheese samples at storage periods.

Parameters	Storage period (months)	Cheese samples	
		Control	Leaf extract
Ash	1	$5.87 \pm 0.12^{a}_{c}$	$6.93 \pm 0.06^{a}_{b}$
	2	$5.67 \pm 0.06^{a}_{d}$	$6.73 \pm 0.12^{a}_{b}$
	3	$5.47 \pm 0.06^{b}_{c}$	$6.20 \pm 0.10^{b}_{b}$
	4	$5.40 \pm 0.10^{b}_{b}$	$6.03 \pm 0.06^{b}_{a}$
Total solids	1	$53.73 \pm 0.12^{d}_{d}$	$60.93 \pm 0.15^{a}_{b}$
	2	$52.70 \pm 0.10^{d}_{d}$	$60.17 \pm 0.06^{b}_{b}$
	3	$51.93 \pm 0.15^{d}_{d}$	$59.17 \pm 0.12^{c}_{b}$
	4	$50.80 \pm 0.10^{d}_{c}$	$56.33 \pm 0.21^{c}_{c}$
pH	1	$5.50 \pm 0.05^{a}_{a}$	$5.13 \pm 0.03^{b}_{a}$
	2	$5.27 \pm 0.02^{a}_{a}$	$5.06 \pm 0.02^{b}_{b}$
	3	$5.11 \pm 0.01^{b}_{a}$	$5.01 \pm 0.01^{c}_{a}$
	4	$4.92 \pm 0.03^{c}_{a}$	$4.81 \pm 0.01^{d}_{c}$
Acidity	1	$1.25 \pm 0.02^{c}_{c}$	$1.32 \pm 0.02^{b}_{b}$
	2	$1.29 \pm 0.02^{c}_{d}$	$1.35 \pm 0.01^{a}_{b}$
	3	$1.31 \pm 0.01^{b}_{c}$	$1.37 \pm 0.15^{a}_{b}$
	4	$1.37 \pm 0.01^{a}_{b}$	$1.38 \pm 0.01^{a}_{b}$

Values are mean ± SD; Means carrying different superscript letter in the same column are not significantly different at P‹0.05 using DMRT.

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The storage periods significantly (P<0.05) affected the protein content of the white cheese (Table 2). The protein content decreased at the storage periods (at 1st, 2nd, 3rd and 4th week). The highest value was recorded in the leaf extract (29.77±0.06%) at first week while the lowest protein content (24.43±0.21%) was recorded on control sample (renin) at 4th week. Samples were significantly different (P<0.05) values at protein content. Abdalla (1992) and Nuser (2001) reported that the decrease of total solids contents during storage period was attributed to the degradation of total protein, dissolution of salt and fat in to pickling solution or absorption of pickling whey by curd. The results indicated that there were significance differences (P<0.05) in fat contents of the cheese samples (leaves and control sample) (Table 2). The highest fat (29.77±0.06%) was recorded in the leaves sample at 1st storage period (month), while the lowest value (22.20±0.1%) was found in the control sample at 4th month. Ramzi and Ahmed (2013) stated that fat contents of the cheese with 1% cassava were significantly different (P<0.05). The highest fat (21.28±1.57%) was recorded in the cheese with 1% cassava while the lowest value (20.25±1.84%) was found in the control cheese. Fat contents of the cheese samples decreased with the storage period. The decrease in fat contents could be explained by the degradation of the total fat, dissolution of salt and fat into the pickling solution or absorption of whey by curd (Nasur, 2001). The results in (Table 2) showed that there is no significance differences (P<0.05) at sample and storage periods. Lactose was absent at 2nd, 3rd and 4th storage period (00.00±0.0). These results were very lower than as estimated by Mortada *et al.* (2013) where he stated that lactose percentage was 6.9% on Sudanese white cheese. Results in Table 2 illustrated the effects of *Moringa oleifera* extract and control sample on physicochemical characteristics of soft white cheese at storage periods. The results indicated that there were significance differences (P<0.05) in the moisture content of the samples. The data showed that highest moisture content (48.30±0.265%) was in the control sample (rennin) at 4th month while the lowest one (38.03±0.15%) was recorded in leaves extraction at first month. The moisture content is increase on enzyme extraction and control at storage periods.

Table 3 findings indicated that there were few significance difference (P>0.05) in the ash contents between all samples (leaves and control). The highest ash (6.93±0.06%) was in the leaves sample in the 1st month; however, the lowest value (5.40±0.10%) was in control sample at 4th storage periods. Ash content of the cheese samples in this study decreased with the levels of storage periods at cheese samples. Similar result was found by Krishnaswamy *et al.* (1961) who found that ash content was decreased at storage periods. Osman (2009) stated that ash content was decreased on cheese made with *Solanum dubium* at storage period. Total solids contents of white soft cheese with leaves extract was significantly (P<0.01) higher than cheese made with control (rennin) enzyme (Table 3). Total solids values decrease at storage periods. The value of total solids was highest in leaves sample (60.93±0.15%) at 1st storage period and lowest in control sample (50.80±0.1) in 4th storage period. These results were higher compared to those made with *Helianthus annuus* 35.87±2.70% (Abdalrahim, 2017). Bilal (2000) and Hamid (2005) reported that the total solids content of the white soft cheese increased during storage period. Abdalla and Mohamed (2009) found significant decrease of total solids content of Sudanese white cheese with the advancement of storage period (45 d). The pH of the cheese samples was significantly (P<0.05) affected by the different samples of cheese made with leaves extract and control. The highest value (5.50±0.05) of pH was scored by the control sample while the lowest one (4.81±0.01) was scored by the leaf sample (Table 3). The pH values is significant different on samples at storage period (P<0.05). Also the pH values were decreased at storage periods at 1st, 2nd, 3rd and 4th period. Biradar*et al.* (2012) reported that pH decreased with the levels of soybean milk at storage period. Statistical analysis revealed that it had few significant effect (P<0.05) on titratable acidity of white soft cheese samples (leaves and control). The lowest value in control (1.253±0.02%) at 1st storage period compared to highest value in cheese produced with leaves extract (1.38 ±0.01%) at 4th storage period. However, Abu-Zeid (1994) found that higher acidity value in cheese made with vegetable rennet from *Sonchus olerceus* L. that the longer coagulation time of vegetable rennet possibly favored microbial growth and consequently, a higher acidity was reached in curd from vegetable rennet.

The effects of extract on minerals contents of cheese at storage periods: Data in Table 4 illustrated the effects of extract on minerals contents of cheese at storage periods. The results indicated that significant variation (P<0.05) was found in calcium (Ca) content of all cheese samples. Also results showed that there were significance differences (P<0.05) at storage periods of cheese samples. The highest Ca content (13.76±0.01 mg/L) was on leaves sample at 1st storage periods, while the lowest one (10.23±0.01 mg/L) was found in control cheese at 4th storage period. Calcium contents of the cheese samples decreased gradually throughout the storage period. Abdalla *et al.* (2013) showed that calcium of the Sudanese white soft cheese decreased with the storage from day zero up to day 180. Abdel Razig (2000) stated that the calcium content of the white cheese decreased as the storage time progressed.

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Table 4. Effect of mineral contents on cheese samples at storage periods.

Parameters	Storage period (months)	Cheese samples	
		Control	Leaf extract
Ca	1	$11.42 \pm 0.01^{c}_{c}$	$13.76 \pm 0.01^{a}_{a}$
	2	$11.51 \pm 0.01^{c}_{c}$	$13.41 \pm 0.01^{a}_{a}$
	3	10.60 ± 0.01^{d}c	12.62 ± 0.01^{b}a
	4	$10.23 \pm 0.01^{d}_{b}$	$11.55 \pm 0.01^{c}_{a}$
Na	1	$54.54 \pm 0.01^{b}_{c}$	$56.91 \pm 0.01^{a}_{a}$
	2	$52.82 \pm 0.01^{c}_{c}$	$54.71 \pm 0.01^{b}_{a}$
	3	$51.21 \pm 0.01^{d}_{c}$	$53.85 \pm 0.01^{b}_{a}$
	4	$50.53 \pm 0.01^{d}_{d}$	$52.62 \pm 0.01^{c}_{b}$
K	1	$10.22 \pm 0.01^{d}_{b}$	$11.87 \pm 0.01^{a}_{a}$
	2	$10.21 \pm 0.01^{d}_{c}$	$11.11 \pm 0.01^{c}_{a}$
	3	$10.13 \pm 0.01^{d}_{c}$	$10.85 \pm 0.01^{a}_{a}$
	4	$10.11 \pm 0.01^{d}_{c}$	$10.69 \pm 0.01^{b}_{a}$

Values are mean ± SD; Means carrying different superscript letter in the same column are not significantly different at P‹0.05 using DMRT.

Results in Table 4 is the sodium content of the cheese samples, it clearly showed that sodium content was significantly (P<0.05) affected by the different extract and storage period. Sodium content decreased at storage period. The lowest sodium content was recorded (50.53±0.00577 mg/L) by control sample, but the highest value of sodium content was recorded (56.91±0.00577 mg/L) at 1st storage period in leaf sample. These results were different from values observed by Abdel Razig (2000) who stated that the sodium content of braided cheese increased significantly (P≤0.05) with storage time. Abdalla *et al.* (2013) showed that sodium contents of the Sudanese white cheese decreased with storage period. Table 4 showed that the Potassium content significantly (P<0.05) affected in different extracts and during storage period, it increased at 1st, 2nd and 3rd but it decreased at 4th storage period. The highest value of potassium content (11.87±0.01 mg/L) was reported at 1st storage period in the cheese with leaf extract sample, while the lowest one (10.11±0.00577 mg/L) was reported at 4th storage period in the control cheese sample. All cheese samples increased at 4th storage period. This increase could be due to fact that moringa contains significance amount of potassium. Al-Tahir *et al.* (2014) used three levels of salt concentration to manufacture a Sudanese cheese and stated that K increased with the levels of salt concentration. On the other hand, Gonzalez *et al.* (2009) studied the minerals contents of the cheese during 6 months of ripening and found that Ca ranged from 4.49 to 40.38 g/kg, P ranged from 3.35 to 6.03 g/kg, Na ranged from 2.76 to 13.92 g/kg and K ranged from 0.62 to 2.17 g/kg.

Effect of storage period on Microbiological quality of white cheese: Results in Table 5 shows the effect of storage period on microbiological quality of the white cheese of different extracts.

Total viable bacterial count (TBC): Result in Table 5 illustrates changes in TBC of the different cheese samples during storage. TBC at different cheese sample during storage period had few significant (p<0.05). Total viable bacterial count of cheese significantly increased with increase in storage time. The highest count of TBC (5.85±0.61) was recorded in control sample at 4th storage period, while the lowest count of TBC (3.63±0.46) on leaf sample was recorded at 1st storage period. Ramzi and Ahmed, (2013) reported that TBC at 4th storage period was (6.58±0.41). In another study, Nour-El-Daim and El-Zubeir (2006) showed that the storage periods showed significant differences (p<0.05) in total bacterial counts in Sudanese white cheese.

Yeast and Molds: Table 5 showed that yeasts and molds count significantly (P<0.05) affected by storage period, it increased at storage period from 1st to 4th at all samples. The lowest yeasts and molds count (3.63±0.46 log cfu/g) was recorded at 1st storage in the leaf sample, while the highest one (5.68±0.29 log cfu/g) was recorded at 4th storage period in control cheese sample. Cheese samples were significantly (P<0.05) different at yeast and molds count. Ramzi and Ahmed (2013) showed that the highest count of yeast and molds were 6.73±0.05 log cfu/g when the lowest count of yeast and molds were 4.71±0.06 log cfu/g on white cheese samples.

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Table 5. Microbiological analysis of cheese samples at storage periods.

Parameters	Storage period (months)	Cheese samples	
		Control	Leaf extract
Total Bacterial (Log cfu/mL)	1	$3.87 \pm 0.35^{d}_{a}$	$3.63 \pm 0.46^{d}_{b}$
	2	$4.81 \pm 0.40^{c}_{a}$	$4.62 \pm 0.61^{c}_{c}$
	3	$4.91 \pm 0.23^{b}_{a}$	$4.69 \pm 0.69^{b}_{d}$
	4	$5.85 \pm 0.58^{a}_{a}$	$5.74 \pm 0.29^{a}_{c}$
Yeast and molds (Log cfu/mL)	1	$3.54 \pm 0.17^{d}_{d}$	$2.80 \pm 0.31^{c}_{b}$
	2	$3.62 \pm 0.35^{c}_{c}$	$3.66 \pm 0.46^{b}_{b}$
	3	$4.85 \pm 0.61^{a}_{a}$	$3.81 \pm 0.35^{d}_{d}$
	4	$5.68 \pm 0.29^{b}_{a}$	$4.66 \pm 0.23^{a}_{c}$
Lactic acid bacteria (Log cfu/mL)	1	$1.59 \pm 0.06^{d}_{b}$	$1.50 \pm 0.17^{d}_{d}$
	2	$2.71 \pm 0.35^{c}_{a}$	$2.67 \pm 0.31^{c}_{c}$
	3	$2.77 \pm 0.52^{b}_{a}$	$2.74 \pm 0.12^{b}_{c}$
	4	$4.85 \pm 0.46^{a}_{a}$	$3.63 \pm 0.35^{a}_{c}$

Values are mean ± SD; Means carrying different superscript letter in the same column are not significantly different at P<0.05 using DMRT.

El-owni and Hamid (2009) attributed the high yeasts count of white cheese to its high acidity. The constant increase of yeasts and molds during storage might be due to the fact that yeasts and molds counts could metabolize lactic acid and lower pH value (Turkoglu *et al*., 2003).

***Lactic acid bacteria*:** Table 5 showed significant (P<0.05) differences on Lactic acid bacteria count on cheese samples. The highest value on Lactic acid bacteria count was 4.85±0.46 log cfu/g on control sample at the 4th storage, while the lowest value was 1.50±0.173 log cfu/g at leaves on 1st storage period. The Lactic acid counts increased significantly at samples throughout the storage period. Lactic acid bacteria are essential for fermentation and are acceptable in very large numbers mainly in natural cheese. Kheir *et al*. (2011) found that lactic acid counts of Sudanese white cheese decreased at storage period.

E. coli count: Table 5 showed *E. coli* count was not detected in all the cheese samples throughout the storage period. Abdelrahim (2017) stated that *E. coli* count was not detected in all white cheese samples ripening with *H. annuus* enzyme. Warsama *et al*. (2006) reported that the presence of *E. coli* in raw milk and the absent after the milk pasteurized and throughout cheese processing and storage period may be due to good hygienic conditions.

Staphylococcus aureus count: *Staphylococcus aureus* count was not detected in all the cheese samples throughout the storage period showed in Table 5. Abdalla *et al*. (2013) found that Staphylococcus is detected only in the day zero of the storage period. *Staphylococcus aureus* was detected at day zero then; it disappeared after 60 d of storage. The presence 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.

T. coliforms: Table 5 showed that *T. coliform* count was not detected in all the cheese samples throughout the storage period. Abdelrahim (2017) stated that lowest count of *T. coliform* at storage period was 3.97±2.41 Log cfu/mL on Sudanese white cheese. Also Abdel Razig and Babiker (2009) showed that coliforms was not found in all Sudanese white cheese samples during the storage of cheese using lemon orange and grapefruit juices, as coagulants.

Salmonella: Salmonella count was not detected in all the cheese samples Table 5 throughout the storage periods. Hamid and El-Owni (2007) decided that Salmonella count was not detected on cheese sample throughout the storage period. Warsama *et al*. (2006) found some of salmonella spp. in some samples of Sudanese white cheese. Amran and Abass (2011) reported that pathogenic flora such as salmonella detected in some cheese samples and disappeared at the end of storage period. Results in Table 5 showed Spore forming bacteria were not detected in all the cheese samples throughout the storage periods. Sadiq *et al*. (2016) showed that total spore former count was not detected in processed cheese. The obtained results are lower than those reported by Eldiam and Elzubeir (2006).

Brucella rings: Brucella rings test was not detected in all the cheese samples throughout the storage period. Moslemi *et al*. (2018) found that 25% of cheese samples were infected with *Brucella* spp., it seems the pasteurization methods are not effective for destruction of this pathogen. Therefore, reducing the possibility of being infected by this pathogen by using accurate molecular detection techniques like real-time PCR should be consider (Yaran *et al*., 2016). In this study, *T. coliform*, *E. coli*, *Staphylococcus*, *Salmonella*, Spore forming bacteria and Brucella rings were not detected.

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Table 6. Effect of coagulants types on sensory characteristics of cheese samples.

Cheese	Sensory characteristics				
	Appearance	Flavor	Taste	Texture	Overall acceptance
Control	7.30 ± 0.68a	6.90 ± 0.32a	6.90 ± 0.57a	3.10± 0.35c	7.00 ± 0.47a
Leaf extract	5.70 ± 1.06b	6.10 ±0.74b	5.10 ± 0.32a	6.10 ± 0.74b	6.20 ± 0.42b

Fig. 1. Cheese samples.

Cheese of Moringa oleifera leaf extract

Control- Sudanese white cheese

Effect of coagulant on Sensory characteristics of white soft cheese: Results in Table 6 shows the effect of coagulant type on sensory characteristics of white cheese. All sensory characteristics of white soft cheese illustrated significant (P<0.05) differences in cheese made with the partially purified *Moringa oleifera* leaf extract compared to rennet one (Fig. 1). The results were not in accordance with Abdul-Rahman (2013) who observed no significant differences in sensory characteristics between rennet and safflower cheese. In this study, the appearance of the cheese samples was significantly (P≤0.05) affected (Table 6). Control cheese is higher on appearance score (7.30±0.68) than leaf extract sample (6.20±0.42). Felfoul *et al*. (2016) showed the appearance score of control cheese is highest than cheese with olive oil.

The flavour of cheese were not significantly (P<0.05) affected by the type of extract of *Moringa oleifera* but it had slight significant between control and *Moringa oleifera* extract (Table 6). Cheese made with rennet had the highest scores (6.90±0.32) while cheese made with the purified *Moringa oleifera* leaf extract was less than the control in flavour scores (5.70±1.06). Our findings were similar to Galan *et al*. (2012) who revealed significant differences in flavour in ewe's cheese using rennet calf and plant coagulant from cardoon (*Cynara cardunculus*). The taste of the white cheese samples was significant (P<0.05) on coagulants types. Whereas, the best preferences for taste were obtained from control (rennet) was 6.90±0.57, and leaves were 6.10±0.74. These results were in accordance with those of Hamid (2014) who showed that the best value for the taste were obtained from enzymatic (rennet), competitive than acidic (citric) cheese. The texture of white soft cheese was significantly different (P<0.05) in cheese samples made with the partially purified *Moringa oleifera* leaf extract compared to that made with rennet (control). *Moringa oleifera* leaf extract were higher on texture score (5.10±0.32) than that made with rennet (3.10±0.32). These results were similar with that reported by Abd-El-Raheem (2017) who found that texture of white soft cheese was higher in cheese made with the partially purified *Helianthus annuus* compared to that made with rennet. Kheir *et al*. (2011) concluded significant differences in texture of white soft cheese samples. In Table 6 over all acceptability had a few differences (P<0.05) of cheese samples. In general cheese made with rennet (control) recorded higher overall acceptability (7.00±0.47) flowed by that made with *Moringa oleifera* leaf extract. Cheese made with leaf extract was lowest in score (6.10±0.74). Ramzi and Ahmed (2013) stated that control cheese obtained the highest acceptability than the other cheese with 0.5, 0.75, and 1% Cassava respectively.

Conclusion

From this study, we can be conclude that MOLE can be used to production Sudanese white cheese since this production of cheese has more neutrinos, less microbial count and also less expensive.

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