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Physicochemical, Microbiological and Sensory Characteristics of White Cheese Made by Adding *Moringa Oleifera* Seeds Extract

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Abstract

The aim of this study is to investigate physicochemical and sensory properties of cheese made by adding *Moringa* seeds extract (MSE) as coagulant. Cheese samples were processed by adding 2, 3 and 4% MSE. The results of proximate analysis indicated that moisture content was 40.8, 45.8 and 42.4%, while protein content 16.50, 16, 84 and 16.97 in cheese produced by addition of 2, 3 and 4% MSE, respectively. Statistically, significant differences (p<0.005) were found in both chemical components. The fat content (25.53, 25.72 and 25.92%) in concentrations 2, 3 and 4%, respectively. On the other hand, the highest fat content was found in cheese sample made by adding 4% MSE. The ash content increased by an increase of the concentration of MSE, and the highest value was found in cheese made by addition of 4% MSE (0.82%). Titratable acidity %, pH and The total solids (T.S%) of cheese increased significantly (p<0.005) by an increase of MSE. The mineral content of cheese was statistically affected (p<0.005) by addition of MSE. The microbiological analysis revealed that *Colifrom* and *Salmonella* were not detected in processed cheese. The sensory evaluation indicated that all cheese samples were accepted by the panelists with preference to the cheese prepared by 4% MSE. The study recommends using other parts of *Moringa Oliefera* in cheese coagulation and the effect of storage conditions on the quality of the cheese must be further investigated.

Keywords: Cheese; *Moringa oleifera* seeds; Physicochemical characteristics; Microbiological characteristics; Sensory characteristics.

1. Introduction

Cheese is made in almost every country of the world and there exist more than 2000 varieties of cheese while may be classified into different groups, e.g. ripened and unripened cheese, cheese with low or high fat content and cheese with soft or hard consistency [1]. In the last decades have witnessed by the population explosion that lead to an increased demand for cheese production and consumption. Together with that the price of calf rennet was greatly increased, along with a reduced supply of natural calf rennet .In addition to that, the use of animal rennet is limited for religious reasons, safety reasons (bovine spongiform encephalopathy), dietary reasons (vegetarianism), or being against genetically engineered foods. All these reasons have necessitated the search for new protease with high specific milk – clotting activity and low general proteolytic activity to be used as a rennet substitute. Accordingly, much research interest has been directed towards discovering a milk-clotting enzyme which would satisfactory replace calf rennet in cheese manufacturing. Microbial rennet produced by genetically engineered bacteria have proven to be suitable substitutes for animal rennet, but increasing attention has been directed towards natural rennet extracted from plants [2].

*Moringa Oleifera*is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan and it is an important crop in India, Ethiopia, the Philippines and the Sudan. All parts of this rapidly-growing tree are edible have long been consumed by the ancient Romans, Greeks and Egyptians. It is the most nutrient-rich plant yet discovered as it contains all the essential nutritional elements that are essential for livestock and human beings, Many parts of this miracle tree have been used in various applications such as food for overcoming malnutrition, feed for weight gain andmilk production, medicine for the treatment and/or prevention of many diseases,and environment for water clarification. Despite the wide spread and huge uses of *Moringa oleifera*, it uses as a source of milk clotting enzymes is scarce [3]. The *Moringa* tree is grown mainly in semiarid, tropical, and subtropical areas, corresponding in the United States to USDA hardiness zones 9 and 10. In Sudan was cultivation in north of Sudan. It tolerates a wide range of soil conditions, but prefers a neutral to slightly acidic (pH 6.3 to 7.0), well-drained sandy or loamy soil. In waterlogged soil, the roots have a tendency to rot. *Moringa* is a sun- and heat-loving plant, thus does

not tolerate freezing or frost. *Moringa* is particularly suitable for dry regions, as it can be grown using rainwater without expensive irrigation techniques [3]. It's considered as one of the most useful trees in the world because almost all parts of this plant can be used as in food, in medicines and for industrial purposes [4]. In many countries, there are huge efforts to spread the use and cultivation of M. oleifera, since it is a significant source of fats, proteins, beta-carotene, vitamin C, iron, potassium, and other nutrients with low toxicity of seeds and leaves [5].

Moringa oleifera seeds extract are rich in proteases having specific clotting activity 200 times higher than that of flower extract. Approving to these results, it is concluded that the seed extract of M. oleifera generates suitable milk clotting activity for cheese making [6, 7] proved that Moringa oleifera seed extracts used to prepare cottage cheese with increasing in the yield of cheese. On the same line, sensorial properties including texture, appearance, flavour, taste and colour of the produced cheeses were improved by used Moringa oleifera seeds as coagulant [8]. The present study was to determine the effect on the quality of cheese produced by addition of Moringa seeds extract (MSE) as a coagulant.

2. Materials and Methods

2.1. Materials

Fresh whole cow milk was obtained from the Wad Elmagdob farm. Milk was transported to the factory in the Wad Elmagdob Gezira State, Sudan. *Moringa* seeds were collected from a local market in Wad Medani city, Gezira State, Sudan, it was transported to the laboratory and then to the factory in Wad Elmagdob Gezira State, Sudan.

2.2. Methods

2.2.1. Preparation of *Moringa* Seed Extract

Moringa seeds were thoroughly sun dried and then ground into powder using grinder(Hone) the powder had been sieved with a wire mesh Then 5grams of *Moringa* powder were put in four solutions Sodium acetate (pH=5), 5% Sodium chloride in distilled water, Distilled water, Sodium acetate and 5% sodium chloride, sodium acetate was the optimum one of them in clotting of milk, then they were put in stirrer for 4 hours then filtrated through a gauze and then it has been undergone to cold centrifugation (12000rpm, 20 min) [9].

2.2.2. Cheese Manufacture

Eight liters of cow milk were taken (two liter for each sample) in stainless steel container and heated to 60°C for 30minute. After pasteurization the milk was cooled to 34°C. Rennet powder was added to milk at 34°C. The salt was added at 2% (w/v) and then mixed with the milk. Four types of cheese were prepared; in the first type rennet powder was added, in the second type, Moringa seeds extract was added to the milk at level of 2%, in third type Moringa seeds extract was added at the level of 3. % and in the fourth type Moringa seeds extract was added at the level of 4%. After the addition of the rennet and Moringa seeds extract, the milk was put in the incubator about one hour for coagulation. The curd was poured into small clean wooden molds lined with cheesecloth and pressed overnight. The curd was then cut into small cubes and put in the whey water for ripening three days .The manufactured cheese samples was packaged in packaging, sampled, stored with whey at 4C°for 15 days for further analysis [10].

2.2.3. Chemical Analysis of Raw Milk

The various chemical analyses, which included moisture, total solids, fat, protein, lactose, ash, titratable acidity and pH of raw milk, were determined by using Milk Scanner instrumental model (EXPRESS PLUS).

2.2.4. Microbiological Analysis for the Processed Cheese

2.2.4.1. Preparation of Serial Dilution

Ten grams samples of cheese type were homogenized with 90 ml of distilled water by shaking for several minutes, from this suspension; 1 ml was taken from the dilution and transferred to another tube to make serial dilution up to 10^{-6}

2.2.4.2. Total Bacterial Count

The total viable count per ml of sample was obtained by pour-plating suitable in triplicates on plate Count Ager (Oxoid) following the method of APHA [11]. Incubation was accomplished at 37C° for48 hours. Plates containing between 30-300 Colonies were counted as colony forming units (c.f.u) of the sample.

2.2.4.3. Yeast and Mould Count

Yeast and mould were enumerated according to Marshall [12] using Potato Dextrose Ager (PDA). The plates were incubated at 25C° for 3-5 days, plates containing between 30-300 colonies were counted as colony forming units (c.f.u).

2.2.4.4. Coliform Bacterial Count

Coliform bacterial count was determined according to Araujo, *et al.* [13] using Mac Conkey broth. The tubes were incubated at 37C° for 48 hour. Positive tubes gave gas in Durham tubes. Then the positive tubes were sub

cultured into EC broth medium and then incubated at 44°C for 24 hours to determine the coli form bacteria, the tube showing any amount of gas production were considered positive.

2.3.4.5. Detection of Salmonella

Hundred ml of samples were incubated at 37°C for 24 hours. Then 10 ml were drawn aseptically and added to 100 ml Selenited Broth. The broth was incubated at 37°C for 24hours then with a lapful streaking was done on dried Bismuth Sulphite agar plates. The plates were then incubated at 37°C for 72 hours.

Black metallic sheen discrete colonies indicated the presence of *Salmonella*. A confirmatory test was carried out by taking a discrete black [14].

2.3.5. Physicochemical Characteristics Determination

pH value was measured using electric pH meter model 501 according to AOAC [15]. pH meter was used to measure the pH of cheese sample, 3-gram cheese were weighed and crushed with 10 ml water in porcelain mortar. This suspension was poured into small glass beaker. The pH and temperature probes were suspended in the liquid until the pH meter indicated a stable reading.

The titratable acidity (TA) of cheese was determined by AOAC [16] was used to determine the titratable acidity, where 3-gram cheese were weighed and crushed with 10 ml water in porcelain mortar. This solution was transferred into an Erlenmeyer flask 5 drops phenolphthalein were added and titrated with 0.1 N NaOH to the first permanent color change to pink.

$$\text{Acidity (lactic acid \%)} = \text{Titer} \times 0.1$$

Where:

0.1 N of NaOH

The total solid (TS) were determined by AOAC [17], heating 5gram of sample in oven at 100°C for 3 hours .Total solid were calculated by formula:

$$\text{Total solid (\%)} = \frac{\text{weight of residue after drying}}{\text{Weight of sample}} \times 100$$

2.3.6. Determination of Minerals

Determination of Sodium (Na+), calcium(Ca+) and potassium(K+) concentrations were accomplished by a flame photometer (model corning,400) according to AOAC [17]. In which different concentrations (20, 40, 60, 80,100 ppm) were prepared from stock solution of Na, Ca, and K. Then by using the flame photometer the reading taken and a graph was made. The sample was prepared by weighing 5gm of ash then the sample dissolved in distilled water and 0.1 N HCl was added to make 1000 ml. about 10ml were then taken and diluted to 100ml, and then 5ml were taken and diluted to 100ml to give 100 ppm.

2.3.7. Sensory Evaluation

White cheese samples were subjected to sensory evaluation using (10) panelists, the panelists were asked to assess each sample for color, appearance, flavor, texture and overall acceptability a 9 point hedonic scale with 1 as the extremely bad and 9 the excellent. All analysis took place in a room free from disturbing noises, and in which fresh air was circulation conditions were equalized for all the tests. The order of presentation for samples was randomized and the samples were given codes before being tested [18].

2.3.8. Statistical Analysis

Statistical analysis was done using Statistical Package for Social Studies Software Complete Randomized Design was used to estimate chemical, microbiological and sensory characteristics of the white cheese.

3. Results and Discussion

3.1. Chemical Composition of Raw Milk Sample used for Processing of White Cheese

The chemical compositions of raw milk sample used for production of white cheese are presented in Table (1). The moisture content of raw milk (83%). This result was in agreements with that reported by Salih, *et al.* [19], who found moisture content was 83%.

The total solids (T.S) content of raw milk (12.20%). This result was in agreements with that reported by Abdul, *et al.* [20] which was 12.16%, whereas higher than reported by Saha and Ara [21] which was11.50%.

Fat content of raw milk (3.97%) was in close agreement with that reported by Salih, *et al.* [19], who found fat content was 3.70 %.

The protein content of raw milk (3.30%), this result was close agreement to that reported by Salih, *et al.* [19] and Abdul, *et al.* [20] which were .3.39 %.,3.47%. respectively ,whereas lower than that reported by Saha and Ara [21] which was 4.14%.

The lactose content (4.00%) was agreement to the lactose content of raw milk as reported by Salih, *et al.* [19] and Fawaz, *et al.* [22], which were 4.92% and 4.97% respectively.

Table (1) also, shows that the ash content of raw milk (2.30%). This result agreed with that reported by Salih, *et al.* [19] andFawaz, *et al.* [22], who reported a value of 2.89% and.2.88%, respectively.

The titratable acidity of raw milk (0.26 %). The titratable acidity value of raw milk was higher with titratable acidity reported by Salih, *et al.* [19] and Abdul, *et al.* [20] which were 0.16% and 0.19%, respectively.

The data presented in Table (1) also, showed that the pH value of raw milk 6.50% this value was in close agreement to that reported by Sulieman, *et al.* [23], who found a pH value of 6.50% in raw milk.

Table-1. Physicochemical composition of raw milk used in cheese processing

Parameter	Raw milk
Moisture (%)	83.00±0.04
Total Solid (%)	12.20±0.03
Fat % (%)	3.97±0.06
Protein (%)	3.30±0.09
Lactose (%)	4.00±0.3
Ash (%)	2.30±0.01
Acidity (%)	0.26±0.07
pH	6.50±0.03

3.2. Physicochemical Composition of Processed Cheese

The physicochemical compositions of processed cheese are presented in Table (2). The value of moisture content in control was (48.4%) this value higher than other found in cheese with 2, 3, 4% *Moringa* seeds extract which were 40.8% , 45.8%, 42.4%,respectively. Statistically, significant differences (p<0.005) in moisture content of different concentrations of *Moringa* seeds extract in cheese samples were found. This result was in agreement with those reported by Zakaria, *et al.* [24] which was, (49.49 %.)

The data presented in Table (2) also, showed that the protein content of cheese sample was (16.12%) in control and 16.50, 16.84, 16.97 % in 2, 3 and 4%, respectively. The highest protein content recorded in cheese sample produced by 4% *Moringa* extract which was (16.97 %), while the lowest one in control sample was (16.12%). These values were higher than that reported by Fawaz, *et al.* [22] which was 14.60%. Statistically, significant differences (p<0.005) in protein content of different concentrations of *Moringa* seeds extract in cheese samples were found.

The fat content in control sample was 25.44%, this value was lower than that found in cheese sample processed by *moringa* seeds extract with added 2, 3, 4% which were 25.53, 25.72, 25.92 %, respectively. These values lower than that reported by Sulieman, *et al.* [23], which was 29.83% in control sample. Non-significant variation (P < 0.005) between cheese samples in fat content was found.

The ash content in control was 0.72% this value lower than other cheese sample processed by different concentration of *Moringa* seeds extract 2,3,4% which were 0.75, 0.78, 0.82%, respectively. The highest ash content was recorded in cheese sample produced by 4% Moringa seeds extract which was 0.82% and lowest one in control sample . These values lower than reported by Salma, *et al.* [25], which was 2.8% .Statistically, significant variation (P < 0.005) between cheese sample in ash content.

The pH value in Table (2) control sample was(6.15) ,while in other cheese samples with 2,3,4% *Moringa* seeds extract which were 6.51, 6.55, 6.57, respectively. did not show significant different (p>0.005) effect on the pH value in the samples. These results were in close agreement with those reported by Salma, *et al.* [25], which was (6.75%).

The data presented in Table (2) also, showed that the values of Titratable acidity (lactic acid %) was 1.28 in control sample this value lower than found in cheese sample processed by different levels of *Moringa* seeds extract 2,3,4% which were 1.24, 1.22 , 1.20%,respectively. These result higher when compared to Sulieman, *et al.* [23], which was0.96%statistically, significant different (P < 0.005) between cheese sample in titratable acidity.

The total solids (T.S) content of cheese sample were 60.04, 62.4, 63.84% and 59.04% in control 2, 3 and 4%, respectively. Increased significantly (p<0.005). The highest total solids content was (63.04 %) recorded in cheese sample produced by 4% of *Moringa* seeds extract, while the lowest one in control sample which was (59.04%). These result in line with those found by Fawaz, *et al.* [22] which was 60.54 in control sample.

Table-2. Physicochemical composition (%) of processed cheese using *Moringa oliefera* seeds extract

Parameters	Control sample	*Moringa oleifera%*		
		2%	3%	4%
Moisture%	48.4 ± 1.08^{a}	40.8 ± 1.08^{c}	45.8 ± 1.08^{b}	42.4 ± 1.08^{b}
Protein%	16.12 ± 0.55^{c}	16.50 ± 0.55^{b}	16.84 ± 0.55^{b}	16.97 ± 0.55^{a}
Fat %	25.44 ± 0.17^{b}	25.53 ± 0.17^{b}	25.72 ± 0.17^{ab}	25.92 ± 0.17^{a}
Ash%	0.70 ± 0.08^{c}	0.75 ± 0.08^{c}	0.78 ± 0.08^{b}	0.82 ± 0.08^{a}
pH	6.15 ± 0.054^{b}	6.51 ± 0.054^{a}	6.55 ± 0.054^{a}	6.57 ± 0.054^{a}
Titratable acidity (%)	1.28 ± 0.18^{a}	1.24 ± 0.18^{a}	1.22 ± 0.18^{a}	1.20 ± 0.18^{a}
Total Solids%	59.04 ± 1.37^{d}	60.4 ± 1.37^{c}	62.84 ± 1.37^{b}	63.04 ± 1.37^{a}

Mean values ± standard deviation having different superscript letter(s) in each row differs significantly (p<0.005)

3.3. Minerals Content (mg/100g) of Processed Cheese

The mineral contents of control sample and different concentration of processed cheese by using *moringa* seeds extract are shown in Table (3). In processed cheese sample the concentrations of Sodium (Na) the highest one was recorded in cheese sample produced by 4% of *moringa* seeds extract which was 74.35 mg/100 g and while the lowest one in control sample which was 67.33mg/100 g . These results in line with those found by Ghada, *et al.* [26] which was 76.82mg/100g in control sample. Statistically, significant different ($P < 0.005$) between cheese sample in concentrations of Sodium.

The data presented in Table (3) also, showed that the concentrations of potassium (K) the highest one in cheese sample produced by 4% of *moringa* seeds extract which was 50.02 mg/100 g and while the lowest one in control sample which was 37.67 mg/100 g. These results higher than reported by Ghada, *et al.* [26] which was 30.45mg/100 g in control sample. Statistically, significant different ($P < 0.005$) between cheese sample in concentrations of potassium.

The concentrations of Calcium (Ca), the highest one in cheese sample produced by 4% of *moringa* extract which was 370.00 mg/100 g and while the lowest one in control sample which was 357.67 mg/100 g. These results were in line with those found by Ghada, *et al.* [26] which was 372.30mg/100 g in control sample. Statistically, significant different ($P < 0.005$) between cheese sample in concentrations of Calcium.

Table-3. Minerals content (mg/100g) of processed cheese

Element	Control sample	*Morina oleifera* %		
		2%	3%	4%
Sodium(mg/100g)	67.33 ± 0.203^{c}	71.33 ± 0.203^{b}	72.69 ± 0.203^{ab}	74.35 ± 0.203^{a}
Potassium(mg/100g)	37.67 ± 0.33^{d}	45.76 ± 0.33^{c}	47.00 ± 0.33^{b}	50.02 ± 0.33^{a}
Calcium(mg/100g)	357.67 ± 1.52^{c}	341.00 ± 1.52^{d}	361.00 ± 1.52^{b}	370.00 ± 1.52^{a}

Mean values ± standard deviation having different superscript letter(s) in each row differs significantly ($p<0.005$)

3.4. Microbial Load (c. f. u/ml) of Processed Cheese

Table (4) shows the microbiological characteristics of different cheeses. The total bacterial count (TBC) of control sample which was 5×10^{3}cfu/ml. while in processed cheese by using *moringa* seeds extract which were $10.67x10^{3}$, $16x10^{3}$, $20x10^{3}$ cfu/ml in 2, 3 ,4 ,respectively. The highest total bacterial count was recorded in cheese sample produced by 4% of *moringa* seeds extract and while the lowest one in control sample .These results were lower than reported by Sulieman, *et al.* [27], which was $15x10^{6}$ cfu/ml in control sample. Statistically, significant different ($P < 0.005$) between cheese samples in total bacterial count.

Table (4) showed that the yeast and moulds count in all cheese samples. In control sample which was $7.67x10^{2}$cfu/ml ,while in processed cheese by different concentration of *moringa* extract 2, 3, 4% which were $7.50x10^{2}$, $7.10x10^{2}$, $6x10^{2}$ cfu/ml in 2,3,4% ,respectively. The highest yeast and moulds count was recorded in control sample, while the lowest one in cheese sample produced by 4% of *moringa* seeds extract. These results lower than found by Sulieman, *et al.* [27] which were $8x10^{2}$ in control sample. Statistical analysis showed that there were $P < 0.005$) in yeasts and moulds.

Colifrom bacteria count not detected in control sample and other cheese samples by different concentration of *moringa* seeds extract. due to good pasteurization of milk, while in previous studies there is growth *Coli from* bacteria was detected which was 3.25×10^{3}cfu /ml in Fawaz, *et al.* [22].

Significant differences at *Salmonella* count not detected in all cheese samples due to good pasteurization of milk and the processing was done under controlled conditions ,while in previous studies there is detected growth of *Salmonella* was which was $12x\ 10^{2}$cfu /ml in Ghada, *et al.* [26].

Table-4. Microbial load (c. f. u/ml) of processed cheese using *Moringa Oliefera* seeds extract

Parameters	Control sample	*Moringa Oleifera %*		
		2%	3%	4%
Total bacteria count	$5\times10^{3}\pm0.12^{d}$	$10.67x10^{3}\pm0.11^{c}$	$11x10^{3}\pm0.16^{b}$	$16\times10^{3}\pm0.12^{a}$
Moulds and yeasts	$7.67x10^{2}\pm0.13^{a}$	$7.50x10^{2}\pm0.09^{a}$	$7.10x10^{2}\pm0.13^{b}$	$6x10^{2}\pm0.14^{c}$
Coliform	ND	ND	ND	ND
Salmonella	ND	ND	ND	ND

Mean values ± standard deviation having different superscript letter(s) in each row differs significantly ($p<0.005$); ND: not detected

3.5. Sensory Evaluation of Processed Cheese

Sensory evaluation of the white cheese samples by using *moringa* seeds extract are presented in (Table 5). comments given by the panelists showed preference for a product which has good on the color ,flavor, taste ,texture and overall acceptability were significantly different ($p<0.005$) among cheese samples .The highest color score in control sample while the lowest one was recorded in cheese sample with 4% *moringa* extract.

The additions of different concentration of *moringa* seeds extract affected the flavor of white cheese samples. The highest flavor scores were obtained in cheese sample with 4% *moringa* extract and the lowest one in control sample , with significant differences ($P\geq0.005$).

Taste of white cheese samples, the highest scores was obtained in 3% *moringa* extract, while the lowest one in 2% *moringa* seeds extract, with significant differences ($P\geq0.005$).

The effect of *moringa* seeds extract on the texture of cheese samples .The highest texture score of white cheese samples was recorded in 4 % *moringa* seeds extract and control ,while the lowest one in 2% *moringa* seeds extract, with significant differences ($P \geq 0.005$).

White cheese sample with 4% *moringa* seeds extract had the highest overall consumer acceptability scores and the lowest one in white cheese samples with 2% *moringa* seeds extract, with significant differences ($P \geq 0.005$).

Table-5. Effect of using *Moringa Oliefera* seeds extracton consumer acceptability (Mean ± SE) of white cheese samples (n = 10)

Treatment	Color	Flavor	Taste	Texture	Overall acceptability
control	8.80^{a}	6.30^{c}	7.80^{b}	8.60^{ab}	7.80^{b}
Mo 2 %	7.40^{b}	7.40^{b}	6.70^{a}	6.50^{c}	6.70^{a}
Mo 3%	7.00^{d}	8.40^{ab}	8.90^{a}	7.50^{b}	7.40^{c}
Mo 4%	6.90^{c}	9.00^{a}	7.80^{b}	8.50^{ab}	8.50^{ab}

n = Total number of panelists. **Mo**:*Moring Oliefera*

4. Conclusion

Understanding the effects of *Moringa* seeds extract, on the microbial and biochemical properties as well as on consumer acceptability, is essential in improving traditional fermented milk products, and use it as an alternative for the calf rennet in cheese production,

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