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Quality Evaluation of Processed Cheese Made From Milk of Ewes Fed Diets Supplemented With *Moringa Oleifera* or *Echniacea Purpurea*

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Abstract

Ewe's diets supplementation with dried *Moringa Oleifera* or *Echniacea Purpurea* leaves impact on the nutritional characteristics of the processed cheese was studied. Nine lactating ewes were randomly divided into three groups of three animals each, using a completely random design. The control group (C) was fed the basic diet consisting of 30% CFM and 70% berseem. The second and third groups were fed control diet + 15 g dried leaves of *Moringa Oleifera* (MOR) or *Echinacea Purpurea* (ECH) for each kg dry matter of the control diet, respectively. After four weeks, the collected milk from each group was pooled and made into processed cheese. It was noted that the supplemented diets with MOR and ECH increased ($P < 0.05$) the conjugated linoleic acid (CLA) and decreased ($P > 0.05$) the n-6/n-3 fatty acid ratio in the processed cheese. All additives decreased ($P > 0.05$) the total saturated fatty acids (TSFA) but improve ($P > 0.05$) the unsaturated fatty acids (TUFA). Physical properties of processed cheese were not affected by MOR and ECH addation. Addition of healthy herbal plants (MOR and ECH) to ewe's diets not only enhances the health and animal productivity, but also improves the chemical composition and nutritional value of the processed cheese made from the treated ewe's milk.

Keywords: Ewe’s milk; Processed cheese quality; Fatty acids profile; Sensory properties.

Introduction

Processed cheese is produced by chopping natural cheese curds with different degrees of maturity and mixing them with emulsifiers under hot conditions with ambient pressure, until a homogeneous mass is obtained [1]. Processed cheese has many technical advantages over natural cheese, such as resistance to separation when cooking (meltability), uniform appearance, distinct physical behavior and a much longer shelf life [2]. It is also noted that processed cheese can be produced at a lower cost for producers and consumers alike than traditional cheese making. This, in turn, enables lower distribution costs, more stable supplies, and a much faster production time compared to conventional cheeses. Dairy products from goat and sheep's milk can provide a profitable alternative to bovine's milk products due to their special texture, taste, and health characteristics [3-4].

Ewe milk has an almost double concentration of fat and protein, and of course a higher energy value than cow's milk [3]. Additionally, ovine milk is a more suitable source for cheese production compared to cow's milk as milk of ewe has a lower colloidal stability [4]. Therefore, the nutritional properties of ewe's milk make it ideal for the manufacture various types of cheese [5]. However, ewe milk fat contains a high percentage of total saturated fatty acids (TSFA), which is not desirable because it accompanies an increase in the content of some fatty acids (C12: 0, C14: 0, C16: 0) that are associated with an increase in the level of cholesterol in the blood, which in turn is linked to an increased risk of heart disease and blood

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clots [6]. Several studies have indicated that the diet of ruminants is one of the most important factors affecting the milk fat quality [7-9]. Therefore, the ever-increasing consumer consciousness and the need to respond to the growing demand for healthy foods has prompted animal nutrition experts to revise dairy animal feeds in proportion to nutritious and healthy dairy production[10].

The main objective of dairy sheep feeding is to maintain a high yield of milk with good healthy specification and feeds are generally selected in practice according to this goal [11]. Some previous studies reported that, the supplemented diets with Moringa oleifera or Echinacea purpurea dried leaves showed superior positive effect on milk components and milk fatty acid profile of lactating ewes [12-13]. These diets have proven able to modify the milk fatty acids, obtaining a reduction of the total solid fatty acids content along with an increase of the monounsaturated and polyunsaturated fatty acids content [14]. Moreover, increasing the concentration of both n-3 and conjugated linoleic acids (CLA) is particularly beneficial for human health [15]. However, changes in the milk fatty acid profile may have a clear effect on the physical and processing properties of milk and dairy products [16]. It was hypothesized in this study that supplementing ewe's diet with *Moringa Oleifera* or *Echniacea Purpurea* will affect the concentration and enhancement of milk fatty acids and alter the nutritional quality of processed cheese.

Experimental:

Animals and Feeding

Nine Rhamani lactating ewes in the second and fourth lactation seasons with an average live weight of 45 ± 0.5 kg were divided into three equal groups. The entire experimental period was 12 weeks. They were assigned at random to the three dietary treatments. Animals were fed individual on (1) basal diet consists of 30% concentrate feed mixture (CFM): 70% berseem clover on dry matter basis (control); (2) basal diet + 15g/DM Kg *Moringa oleifera* dried leaves powder (MOR) and (3) basal diet +15g/DM Kg *Echinacea purpurea* dried leaves powder (ECH). Ewes were fed dry matter according to 4% of their body weight changed continuously according to animal weight changes. The chemical analysis of CFM and berseem is shown in Table (1). The ewes were fed individually on CFM once per day at 8:00 am, while fresh berseem was provided at 10.00 and 16.00 hours. Drinking water was available at all times. Dry matter, crude protein, crude fiber, ash, and ether extract of feed were analyzed according to methods of AOAC [17].

Table 1: Chemical analysis (%on DM basis) of concentrate feed mixture and berseem clover of experimental rations

Item	CFM	Berseem clover
Dry matter	92.31	16.3
Organic matter	90.85	88.6
Ash	9.15	11.4
Crud protein	14.19	12.7
Ether extract	4.05	2.6
Crude fiber	14.37	28.5
Nitrogen-free-extract	58.24	44.8
Neutral detergent fiber (NDF)	34.54	47.31
Acid detergent fiber (ADF)	11.70	32.53

CFM: Concentrate feed mixture consisted of 35% yellow corn, 25 % wheat bran, 23% decorticated cotton seed meal, 15% rice bran, 1.5% ground limestone and 0.5% Mineral and vitamin mix.

Sampling and Analysis of Milk

The ewes were hand milked twice a day at 8.00 am and 4.00 pm during the last three days of each month of the trial period. Milk samples were collected directly from each animal after morning and evening milking, and milk yield was recorded daily. Milk samples were mixed with respect to the amount of milk produced from morning and evening milking. Samples of milk were analyzed for fat, protein, lactose and total solids using infrared spectrophotometry (Bentley Instruments, Chaska, MN, USA) according to AOAC [17] procedures. The solids not fat content was calculated by difference and the milk ash content was calculated after heating in a muffle at 550o C for 16 hours.

Cheese base production

The pooled ewes' milk from each treatment at the end of each month of the experiment period was used in the manufacture of the processed cheese. Pasteurization of milk at a temperature of 72 ° C for 15 second then rapid cooling of the milk to a temperature of 39 ° C. Adding a yogurt starter at 1% and rennet at a ratio of 2.5 g to every 100 kg of milk. Then it is incubated at 39 ° C for a period of 60-40 minutes, so that coagulation occurs. The curd was putted in a piece of cloth and pressed until the filtration of whey ceases. The obtained cheese base was cooled down in ice water and stored at 6°C until processing. Three different experimental batches of processed cheese were produced as follows control, MOR and ECH of the cheese base. The formulations of processed cheese blends are presented in Table (2).

Table 2: Chemical composition (%) of the cheese base used in manufacture of processed cheese

Items	Cheese base		
	Control	MOR	ECH
Total solids	32.51	34.72	34.00
Fat	14.30	14.78	14.62
Protein	14.24	15.10	14.63
pH	5.30	5.21	5.24

MOR = *Moringa oleifera*, ECH = *Echinacea purpurea*

Processed cheese spread production

Cheese base was milled during mixing at a constant rate of 300 rpm for 15 min at 85-90˚C and placed into the processing batch type kettle, a pilot machine. The cheese base was heated using direct injection of steam at a pressure of 1.5 bar. The emulsifying salt (K2211) was added at the following the optimum level as calculated by the relative casein according to Meyer [2]. The hot processed cheese of each treatment was hand-packed into airtight glass containers, then rapidly cooled and stored at 7 ° C for 3 months. Control and experimental processed cheese spreads were produced in three replicates in which cheese base obtained from different experiments were used. The different formulations of processed cheese are shown in Table (3).

Table 3: Formulation of the different blends (%) used for manufacturing of processed cheese

Items	Control	MOR	ECH
Cheese base	88.89	88.89	88.89
Salt	1.50	1.50	1.50
Starch	0.50	0.50	0.50
Emulsifying salts	2.22	2.22	2.22
Water	6.89	6.89	6.89
Total	100	100	100

MOR = *Moringa oleifera*, ECH = *Echinacea purpurea*

Physical analysis

Colour parameter using a Hunter colorimeter model D2s A-2 (Hunter Assoc. Lab. Inc. Va, USA) according to the directive of user manual. The instrument was first standardized employing a reference with a white surface. As in the Hunter L, a and b tally where, L: the value represents darkness from black (0) to white (100), a: value represents color ranging from red (+) to green (-), and b: value represents yellow (+) to blue (-). Melting test was determined by Olson and Price [18]. Penetrometer reading was measured using a penetrometer (Kochler Instrument Co. Inc., USA) as characterized by Gupta and Reuter [19]. The penetration depth was recorded in units of 1/10 mm, and in general the greater in the depth of penetration the weaker in the body of cheese. Oil separation index of cheese was calculated according to Thomas [20]. In general, physical analyzes were done on cheese samples in 4 stages which are fresh and at the end of each month during storage

Chemical analysis of processed cheese

Processed cheese samples were analyzed for total solids, total protein, fat, ash and moisture according to the AOAC [17]. Value of pH was measured by using a digital pH meter (M4 1150 USA). Processed cheese fatty acids were determined using methyl esters prepared by base-catalyzed methanolysis of the glycerides (KOH in methanol) according to International Standards [21]. Fatty acids methyl esters were separated using a Cp-Sil 88 fused-silica capillary column on a Perkin-Elmer chromatograph (model 8420, Beaconsfield, Perkin Elmer, Beaconsfield, UK) equipped with a flame ionization prospector.

Descriptive sensory analysis of processed cheese

The processed cheese products were evaluated for appearance, consistency, flavor, and general acceptance by a committee of nine members (6 males and 3 females; between 30 and 46 years of age). Prior to sensory evaluation, cheese samples were kept at 18 ° C overnight. The test order was randomly selected, in order to assist the judges in describing the defects. The grading paper included a list of appearance, consistency, and suggested defects.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS [22]. Data of milk composition, cheese properties, cheese fatty acid profile and sensory analysis were analyzed as a complete random design where treatment was the main source of variation using the following model:

$$Yij = \mu + Ti + Eij$$

Where, Yij is the parameter under analysis of the ij milk samples or processed cheese samples, µ is the overall mean, Ti is the effect due to treatment on the parameter under analysis and eij is the experimental error for ij on the observation. Treatment means were separated using Duncan’s multiple range test [23].

Results and Discussion

Milk components

The milk protein and solid not fat contents (SNF) were increased ($P<0.05$) with MOR and ECH treatments compared with control. It was also noticed that milk fat and total solids (TS) increased ($P<0.05$) with MOR compared to other treatments. It was also noted that protein and milk ash were not affected ($P > 0.05$) by any of the different treatments **(Table 4)**. Supplementing the ewes' diet with Moringa or Echinacea caused an increase in both fat and milk protein compared with control group. Previous research has found that using of Moringa to goats feed [13, 15] or Echinacea to ewes feed [12] improved milk composition which concurs with our findings. The improvement in milk components is due to improved microbial protein synthesis and total volatile fatty acids concertation in rumen [12,24], as well as improvement in rumen fermentation [25].

Table 4: Milk composition (%) of lactating ewes fed a diet supplemented with MOR or ECH which was used in the manufacture of processed cheese.

Item	Control	MOR	ECH	SEM	P-value
Fat	4.04^b	4.41^a	4.14^{ab}	0.06	0.002
Lactose	4.08	4.31	4.18	0.09	0.423
Protein	4.11^c	4.47^a	4.36^b	0.08	0.009
Total solids	12.94^b	13.93^a	13.41^b	0.11	0.040
Solid not fat	8.9^c	9.52^a	9.27^b	0.07	0.003
Ash	0.71	0.74	0.73	0.06	0.089

a, b = means with different lowercase superscripts in a row differ significantly (P < 0.05).
MOR = *Moringa oleifera*, ECH = *Echinacea purpura*.

Processed cheese composition

The cheese contents of total solid, fat, and protein of the samples under test are in the range of recognized standard for the processed cheese components (Table 5). There was no difference (P > 0.05) in the chemical composition of processed cheese made of different treatments.

Table 5: Chemical composition (%) of processed cheese manufacture from the milk of treated ewes

Item	Control	MOR	ECH	SEM	P-value
Total solids	40.86	40.85	40.87	2.61	0.521
Fat	20.50	20.50	20.50	1.32	0.094
Protein	12.33	12.30	12.35	1.81	0.152
Lactose	3.75	3.70	3.75	0.18	0.223
Ash	3.60	3.69	3.67	0.22	0.068
Ph	5.77	5.76	5.76	0.41	0.192

MOR = *Moringa oleifera*, ECH = *Echinacea purpurea*

Fatty acids profile in processed cheese

MOR group achieved an increase (P<0.05) in both C15:1, C16:1, C18:0, C18:1, C18:2 cis9 trans11, C18:2 trans7cis9, C18:2 trans10cis12, C18:4, total conjugated linoleic acid (CLA) and the atherogenicity index over the control group. While ECH group had increased (P<0.05) both C14:1, C16:1, C18:0, C18:1, C18:2 trans7cis9, C18:2 trans10cis12, C18:4 and total CLA and the atherogenicity index compared with control group. Control group was increased (P<0.05) in C15:0, C20:0 compared with MOR and ECH groups but, it was increased (P<0.05) in C17:0 compared with ECH group. It is worth noting that the MOR and ECH treatments increased (P> 0.05) total unsaturated fatty acids (TUSFA), and on the other hand, caused a decrease (P> 0.05) total saturated fatty acids (TSFA) and also the ratio N6 / N3 compared to control (Table 6).

Table 6: Fatty acids profile (g/100 g total fatty acids) of processed cheese witch manufacture from the milk of lactating ewes fed a diet supplemented with MOR or ECH.

Item	Control	MOR	ECH	SEM	P-value
C6:0	1.23	1.34	1.21	0.21	0.177
C8:0	1.67	1.56	1.74	0.27	0.156
C10:0	6.88	6.52	6.46	1.20	0.641
C12:0	3.67	3.46	3.27	0.82	0.128
C14:0	10.80	10.00	10.04	1.94	0.751
C14:1,2	0.16^b	0.18^b	0.27^a	0.041	0.037
C15:0	2.05^a	1.35^b	1.14^c	0.031	0.024
C15:1,6	0.23^b	0.27^a	0.25^{ab}	0.037	0.041
C16:0	27.50	26.01	25.63	4.32	0.129
C16:1,7	1.43	1.43	1.36	0.28	0.411
C16:1,9	0.32^b	0.44^a	0.46^a	0.073	0.029
C17:0	1.67^a	1.45^{ab}	1.05^b	0.16	0.047
C16:3,4	0.26	0.25	0.24	0.061	0.381
C18:0	15.99^c	18.96^b	19.20^a	0.98	0.024
C18:1.4	21.80	21.93	23.11	1.74	0.154
C18:1,7	0.25	0.28	0.26	0.073	0.181
C18:1,5	0.25^c	0.50^a	0.34^b	0.061	0.012
C18:2 *cis9, trans11*	0.51^b	0.57^a	0.52^b	0.044	0.027
C18:2 *trans7,cis9*	0.26^c	0.44^a	0.32^b	0.023	0.010
C18:2 *trans10,cis12*	0.16^c	0.22^b	0.27^a	0.035	0.004
C18:3N3	0.30	0.33	0.31	0.073	0.165
C18:3N6	1.21	1.10	1.15	0.236	0.491
C18:4,3	0.41^c	0.47^b	0.51^a	0.057	0.001
C20:0	0.35^a	0.27^b	0.26^b	0.038	0.020
TSFA	72.15	71. 74	71.81	5.24	0.507
TUFA	27.85	28.76	29.19	2.78	0.662
MUSFA	24.74	25.38	25.87	2.16	0.310
PUSFA	3.11	3.38	3.32	0.450	0.149
Total CLA	0.93^c	1.23^a	1.11^b	0.09	0.002
N6/N3	4.03	3.33	3.70	0.71	0.371
Atherogenicity index	2.67^a	2.41^b	2.36^b	0.021	0.001

a, b, c = means with different lowercase superscripts in a row differ significantly (*P* < 0.05). MOR = *Moringa oleifera*, ECH = *Echinacea purpurea*. TSFA= total saturated fatty acids, TUSFA= total unsaturated fatty acids, MUSFA= mono unsaturated fatty acids, PUSFA= poly unsaturated fatty acids, N6/N3= omega 6 fatty acids/ omega 3 fatty acids CLA= conjugated linoleic acid. Atherogenicity index = (C12:0+4 × C14:0 + C16:0) / Σ of UFA

In general, the fatty acid profile of milk is similar to that found in cheese made from the same milk, indicating that processing milk into cheese does not change the fatty acid composition [26]. Improvement in processed cheese fatty acids profile, especially conjugated linoleic acid (CLA) and USFA in the processed cheese were a result of the use of milk which had high levels of CLA and USFA in the manufacture of cheese. Similar findings have also been reported for cheese made from ewe's milk [26] and goat's milk [27, 28]. Supplemented ewe diet with MOR or ECH increased TUFA and CLA however; they decreased TSFA of milk compared with control diet [12]. Add sources rich in PUFA such as herbaceous plants on the ruminant diets cause inhibition to the novo synthesis of fatty acids in milk and compete for esterification with short-chain fatty acids synthesized in the mammary gland, thus reducing the total SFA content [29]. Another reason for the low TSFA concentration is the inhibitory effect of trans-18 isomers produced during biohydrogenation on de novo synthesis of TSFA [30].

In this study, the addition of MOR or ECH reduced the concentrations of some fatty acids have negative consequences for human health such as C12:0, C14:0 and C16:0 [31]. however, increasing the concentrations of C18: 1, C18: 2 and C18: 3 in processed cheese as a result of the treatments could significantly improve the plasma lipoprotein profile [32]. Moreover, it is responsible for increased blood plasma total cholesterol concentrations [33] as well as low density lipoprotein plasma levels [34]. Conjugated linoleic acid is formed in milk by two ways either from bio-hydrogenation in the rumen of linoleic acid [35] or from the endogenous synthesis from vaccenic acid in the mammary gland [36]. Therefore, it is possible to increase the concentration of milk CLA by adding some nutritional supplement to the ruminant diets which in turn improves rumen fermentation [13, 28]. Also, the increase in unsaturated fatty acids over saturated fatty acids led to a significant decrease in the Atherognicity index (AI), which is considered a healthy sign of the fat in cheese [37].

Physical Properties of processed Cheese

There was no difference (P> 0.05) between the different experimental treatments in the penetrometer reading, the oil separation index, and the melting index of the processed cheese (Table7). The penetrometer reading and melting index also showed a gradual decrease during cold storage to the end of 3 months with all treatments. Conversely, the oil separation index was gradually increased in all treatments during cold storage (7 ° C) until the end of 3 months.

The colour properties of the processed cheese were not affected by the treatments, and there were no significant differences between the treatments (Table 8). The physical properties of the processed cheese give an indication of the quality of the processed cheese and whether it is affected by the different treatments or not. The absence of any significant differences between treatments in Physical properties and Color parameters, this means that the physical properties of the cheese were not affected by the different treatments. In previous studies, found that the physical properties of processed cheese made from goat milk-fed with some additives were not affected [27, 28]. The penetrometer reading is inversely related to hardness. Therefore, the hardness of the cheese has increased due to the decrease in its moisture content during storage; this finding was confirmed with several researchers who found an inverse relationship between cheese hardness and moisture content [19, 28].

Table 7: Physical properties of processed cheese manufactured from the milk of treated ewes, fresh and during cold storage

Physical properties	Storage period	Con	MOR	ECH	SEM
Penetro Meter reading (mm)	Fresh	176	175	175	6.2
	1	175	173	174	4.3
	2	170	164	168	3.2
	3	166	164	165	4.4
Oil separation Index	Fresh	20.0	20.0	20.0	1.3
	1	21.6	22.0	22.0	2.1
	2	25.0	25.6	25.3	2.7
	3	26.3	28.0	27.3	1.8
Melting index (mm)	Fresh	161	163	160	4.3
	1	153	155	151	3.8
	2	149	152	148	2.9
	3	145	142	140	4.6
pH	Fresh	5.77	5.76	5.76	0.66
	1	5.76	5.76	5.75	0.68
	2	5.72	5.73	5.74	0.74
	3	5.71	5.70	5.71	0.61

Con= Control, MOR = Moringa oleifera, ECH = Echinacea purpurea

Table 8: Colour properties of processed cheese manufactured from the milk of treated ewes, fresh and during cold storage

Colour parameters	Storage period	Control	MOR	ECH	SEM
L	Fresh	87.80	87.75	87.73	2.2
	1	87.51	87.55	87.50	2.5
	2	87.20	87.31	87.41	2.1
	3	87.11	87.11	87.05	2.4
a	Fresh	-2.12	-2.01	-2.05	0.92
	1	-2.22	-2.13	-2.21	0.87
	2	-2.34	-2.42	-2.44	0.84
	3	-2.44	-2.49	-2.16	0.88
b	Fresh	21.53	21.60	21.50	1.52
	1	22.00	22.11	22.19	1.81
	2	22.98	22.77	22.87	1.59
	3	23.03	23.15	23.08	1.88

L: lightness Black (0) to white (100), a: redness (+) to greenness (-), b: yellowness (+) to blueness (-). MOR = Moringa oleifera, ECH = Echinacea purpurea

The slight increase in the value of the oil separation index with a long storage time can be caused by a decrease in the pH value and the changes that have occurred in the soluble nitrogen contents [38]. Also, changes in the pH and soluble nitrogen content of processed cheese may be the cause of a tendency to decrease the cheese melting index over the period of storage. The results are consistent with that of Morsy et al. [28]

Sensory evaluation

With regard to the sensory properties of the processed cheese, which are of interest to the consumer, such as aroma, taste, consistency and appearance, there was no difference ($P > 0.05$) between the treatments (Table 9). Sensory analysis is an important tool for evaluating and enhancing these new cheese preparations. Consumer responses to the organoleptic properties of processed cheese, especially its appearance, aroma, flavor, taste, and texture, are important factors in determining the acceptance of new products. The sensory analysis of processed cheese depends on its chemical composition [39] and physical properties [40]. Based on the above, in this study, there were no significant differences between the treatments in the Sensory evaluation of cheese. These results were agreed with Morsy et al., [27, 28].

Table 9: Sensory evaluation of processed cheese manufactured from the milk of treated ewes

Sensory characteristics	Control (10)	MOR (10)	ECH (10)	SEM
Colour	8	8	8	0.93
Aroma	7	7	8	0.81
Consistency	8	7	7	0.95
Oiling off	6	6	5	1.01
Taste	9	9	9	1.04
Total	38	37	37	2.34

MOR = Moringa oleifera, ECH = Echinacea purpurea

Conclusions

The addition of Moringa or Echinacea in ewe rations could change the chemical composition of milk, but it clearly affected the fatty acid profile. Therefore, processed cheese manufactured by milk from ewes fed on a diet supplemented with Moringa or Echinacea contains a high content of unsaturated fatty acids and conjugated linoleic acid and low content of saturated fatty acids, thus reducing the atherogenicity index, which has a healthy nutritional effect on humans.

Conflicts of interest

“There are no conflicts to declare”.

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