



Effect of flaxseed (*Linum usitatissimum*) and soybean (*Glycine max*) oils in Egyptian lactating buffalo and cow diets on the milk and soft cheese quality

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ABSTRACT. Produce and compare soft cheese with potential benefits of human health from Egyptian buffalo's and cow's milk was studied. Eight Egyptian lactating buffalos and cows were fed a total mixed ration supplemented with either 0% oil (CD), 2% flaxseed oil (DFO), 2% soybean oil (DSO), or 2% of their mixture (1:1, DFSO) according to a double 4 x 4 Latin Square design. Milk yield was similar between buffalo's diets but was higher in cows fed a DFO, DSO or DFSD resulting in 11.15, 8.21% or 8.97% increases compared with the control diet, respectively. Milk composition was not significantly affected in both buffalos and cows fed diets. The DFO, DSO or DFSD displayed decreased short-chain fatty acids, especially DSO and DFSD (3.73 and 3.33%, respectively) when compared to CD for buffalo milk (6.32%). The DSO and DFSD were more effective for increasing unsaturated fatty acids followed by the DFSD in buffalo's milk fat (42.31 and 41.90 %), whereas DFO and DFSD were more effective in cow's milk fat (39.67 and 39.84%), respectively. DFO, DSO or DFSD had no significant effect on the yield, composition and sensory properties of resultant soft cheese compared to the CD for both lactating cows and buffalos. During storage, a diet rich in unsaturated fatty acids enhances protein proteolysis and antioxidant activity of soft cheese during storage compared to the CD especially for soft cheese produced from buffalo's milk.

Keywords: flaxseed oil; soybean oil; milk yield; fatty acids profile; antioxidant activity, soft cheese properties.

Received on March 28, 2019.

Accepted on July 31, 2019.

Introduction

The past decade, two main strategies, genetic improvement and animal feeding has seen a renewed importance to improving the milk production with potential beneficial effects on human health. The application of animals feeding strategies may lead to rapid and marked improvement of milk FA profile, however, these improvements are not permanent, and disappearing when feeding regimen is interrupted (Conte, Serra, & Mele, 2017). The quality and composition of milk fat are influenced by numerous interacting dietary factors, including quality and composition of pasture, the proportion of concentrate to forage, the fatty acids (FA) composition of dietary lipids, and the rumen inertness and digestibility of fat supplements (Wadhvani & McMahon, 2012). Milk FA composition can be modified by feeding strategies that influence the pattern of fat precursors that the mammary gland removed from blood for fat synthesis. Polyunsaturated lipid source used in the diet of dairy ruminant has been extensively adopted in order to modify the milk FA composition toward a more desirable profile for human health (Corradini et al., 2013; Corradini et al., 2014). The FA profile of milk can be changed dramatically through altering the feeding regimen and lipid supplementation (Abo El-Nor & Khattab, 2012; Ye et al., 2009). Feeding plant oil with high concentrations of unsaturated FA (e.g., soybean and flaxseed oils) are the most common sources of lipids used in animal feeding and provide polyunsaturated FA to enhancing nutrient utilization and animal productivity (Hernandez et al., 2017; Lerma-Reyes et al., 2018; Matloup et al., 2017). Feeding oil seeds and oils rich in polyunsaturated FA resulted in the production of milk rich in total polyunsaturated FA such as n-3 FA contents, conjugated linoleic acid (CLA), linoleic acid, and linolenic acid. Flaxseed and soybean oil are excellent sources of C18:3 and C18:2, which has been found to be expressed in milk and have health benefits

for human (Caroprese et al., 2010). In addition, flaxseed oil contains approximately 60% C18:3 and supplementation in a lactation diet should increase milk CLA through ruminal biohydrogenation to C18:1 trans-11 (Thanh & Suksombat, 2015). Furthermore, milk efficiency for cheese making is related to the milk fat and protein contents, which in turn could affect milk renneting properties (Todaro, Scatassa, & Giaccone, 2005). Cheese prepared using milk rich in total polyunsaturated FA present a number of desirable properties to many consumers (Albenzio & Santillo, 2011). Dietary oils supplementation was hypothesized that the inclusion of soybean oil and/or flaxseed oil in dairy animal diet, with a moderate F/C ratio will potentially increase the CLA and n-3 unsaturated FA content which results in a milk FA profile more beneficial to human health, with no reduction in either milk fat content or soft cheese prepared.

The objective of this study was to compare the effect of supplementing the diet of Egyptian lactating buffalos and cows with flaxseed oil, soybean oil separately and their combination on the milk components and milk FA profile as well as the physicochemical of pro-health soft cheese produced during storage at $5\pm 2^{\circ}\text{C}$ for two months.

Material and Methods

Crud flaxseed (*Linum usitatissimum*) and soybean (*Glycine max*) oils were purchased from local market, Cairo Egypt. Starter cultures (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*) were obtained from Chr. Hansen's Lab., A/S Copenhagen, Denmark. 1-diphenyl-2-picrylhydrazyl (DPPH^{*}) was obtained from Sigma/Aldrich (St. Louis, MO., USA). Methanol anhydrous (HPLC grade) was obtained from Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leics, LE11 5RG, UK. All other chemicals used in the present study were analytical grade.

This study was conducted at a private experimental farm in Om Dinar, Embaba, Giza, Egypt during January to April 2018. Eight early Egyptian lactating buffalos and cows of 3-4 years with average 515 ± 26 and 420 ± 20 Kg BW, respectively, were assigned randomly into 4 groups of 2 animals each using 4×4 Latin Square design with 28 days interval periods. All animals were housed in a tie-stall barn and individually fed according to National Research Council (NRC, 2001) recommendations with free access to drinking water. The basal diet was feed to the animals which contained per kg, as dry matter basis, 600 g of Egyptian berseem clover (*Trifolium alexandrinum*) and 400 g of concentrates feed mixture (Table 1). As recommended by the results of previous research (Morsy et al., 2015; Ye et al., 2009) and to prevent negative effect of feeding high levels of plant oil (e.g., decreased feed intake and fiber digestion), animals were fed the basal diet (control) or the basal diet supplemented at 2% of daily feed intake with crude soybean oil, crude flaxseed oil, or crude soybean oil plus crude flaxseed oil (1:1 v/v). The oils were stored at room temperature and mixed manually with the diets once daily and fed individually two times a day at 07:00 and 18:00 h in two equal portions.

Table 1. Ingredient and chemical composition of total mixed ration of experimental lactating buffaloes and cows (DM basis).

	Berseem	CFM*	TMR**
Roughage : Concentrate ratio	0.6	0.4	1.0
Chemical composition (g 100 g ⁻¹ DM)			
Dry matter	91.2	91.4	91.3
Ash	31.6	14.9	24.9
Organic matter	88.4	85.2	87.1
Crude protein	12.3	15.9	13.7
Ether extract	2.89	5.04	3.75
Crude fiber	2.66	18.12	8.84
Nitrogen free extract	39.0	46.1	41.8

CFM, concentrate feed mixture consisted of crushed yellow corn, cotton seed meal, wheat bran, calcium carbonate, minerals and vitamins and common salt at rate of 50:25:20:2:2:1, respectively; TMR, total mixed ration

Buffalos or cows were milked by hand twice a day at 7:00 and 18 h during the last three days (i.e. days 26 to 28) of each experimental period. Milk samples were collected during morning and evening times from each animal to record the milk yield. The sample of each animal represented mixed samples of constant percentage of the evening and morning yield. Milk samples were analyzed for total solids, fat, protein, lactose, and ash by Bentley150 infrared milk analyzer (Bentley Instruments, Chaska, MN, USA), and then frozen at -20°C until soft cheese manufacture.

The collected milk from each group was pooled and standardized to casein/fat ratio of 0.85. All milk batches were heated to 75°C for 30 Sec., followed cool to 38°C, inoculated with starter culture at the rate of 1% (w/w) and held for 30 min. Salt (4% w/w) was added to milk and appropriate amount of rennet was added to achieve coagulation in ~120 min. After coagulation, curd was cut and transferred to mould and left to rest overnight at room temperature. Cheese blocks were cut, weighted and cheese yield was calculated as the weight of finished cheese divided by the weight of milk used. The resultant soft cheese samples were divided into two parts; the first part was analyzed at fresh and the second part was separately pickled in a brine solution (5% NaCl) and stored at $5 \pm 2^\circ\text{C}$ for two months.

Total solids, fat, total nitrogen and ash content of soft cheese were determined according to Association Official Analytical Chemist (AOAC, 2005). The protein content was obtained by multiplying the percentage of TN by 6.38. Microprocessor pH-meter (HANNA Instruments, pH 211, Italy) was used for measuring the pH value of cheese samples. Water soluble nitrogen (WSN) was determined according to a method proposed by Alizadeh, Hamedi, and Khosroshahi (2006) as indices of cheese proteolysis with some modifications. Based on this method, 20 g of cheese samples were stirred with 100 ml distilled water (40°C) in a Mixer (Heidolph No. 50 111, Type RZRI, Germany) at speed setting 10 for 5 min. The extract was filtered through Whatman no. 42 filter paper and the filtrate was used for determination of WSN.

The fatty acid methyl ester was prepared according to the method of AOAC (2005). Fatty acid methyl esters were injected into HP 6890 series GC apparatus provided with a DB-23 column (60 m x 0.32 mm x 25 μm). Carrier gas was N_2 with flow rate 2.2 mL/min, splitting ratio of 1:50. The injector temperature was 250°C and that of Flame Ionization Detector (FID) was 300°C. The temperature setting was as follows: 150°C to 210°C min^{-1} and then held at 210 C for 25 min. peaks were identified by comparing the retention times obtained with stander methyl esters.

Antioxidant activity of cheese samples was estimated using a stable DPPH radical (DPPH *) assay according to Brand-Williams, Cuvelier, and Berset (1995). Briefly, 10 g of cheese sample were mixed with 10 mL distilled water and the cheese mixtures were incubated at 40°C for 1h, after which the cheese extracts were separated by centrifugation at 4000 $\times\text{g}$ for 5 min. 200 μL of cheese samples extract were distributed into different test tubes and then 3.9 mL of a DPPH solution (25 mg DPPH L^{-1} methanol) was added to each tube. After incubation for 30 min in the dark at room temperature the absorbance was recorded at 517 nm. A control solution, without a tested extract, was prepared in the same manner as the assay mixture. The degree of de-colorization indicates the radical-scavenging efficiency of the extract. The DPPH radical-scavenging activity was calculated using the following formula:

$$\text{DPPH Radical scavenging activity(\%)} = [(1 - A_1/A_0) * 100]$$

A_0 is the absorbance of the control (DPPH solution), and A_1 is the absorbance of the sample.

The texture profile analysis (TPA) method is an instrumental texture measurement using double bite compression and was developed to imitate the compressing action of molar teeth during food mastication. TPA of soft cheese was measured with a multi-test 1-d texture analyzer (mecmesin limited, Slinfold, West Sussex, UK). Experiments were carried out by a compression test that generated a plot of force (N) versus time (sec). Samples were double compressed at a compression speed of 2 cm/min G. The analysis was carried out at 10 EC. Hardness (N), cohesiveness and gumminess (N) were calculated from the obtained TPA according to the definition given by the International Dairy Federation [IDF] (1992).

Cheese samples were judged by a panel taste of the staff members of the Dairy Department, National Research Center, Egypt. Nine-point hedonic scale, ranging from like extremely (9) through like or dislike (5) to dislike extremely (1), was used to score the cheese samples as described by Wadhvani and McMahon (2012).

Statistical analysis was performed using the GLM procedure with SAS software (Statistical Analysis Software [SAS], 2004). Duncan's multiple comparison procedure was used to compare the means. A probability of $p \leq 0.05$ was used to establish statistical significance.

Results and discussion

Milk yield and composition of Egyptian lactating buffaloes and cows fed a diet supplemented with flaxseed oil (DFO), soybean oil (DSO) or flaxseed plus soybean oils (DFSO) compared with control diet are shown in Table 2. DFO, DSO or DFSO had an appositive effect on the milk yield of cows ($p < 0.05$) resulting

in 11.15, 8.21 or 8.97% increases compared with cows received the control diet, respectively. Such an effect has been found by Dai, Wang, and Zhu (2011) and Antonacci, Gagliostro, Cano, and Bernal (2017) in cows fed a diet supplemented with different plant oils. However, there was no difference in milk yield of lactating buffalo fed a DFO, DSO or DFSO ($p > 0.05$) compared with the control diet. These results are agreement with many researchers as well (Vafa, Naserian, Moussavi, Valizadeh, & Mesgaran, 2012). Milk fat, protein, lactose and ash contents were not significantly affected in both buffalos and cows fed diets ($p > 0.05$). Also, a DFO, DSO or DFSO caused a slight improve in the fat and proteins contents, and hence improve the total solids content of the resultant milk ($p > 0.05$). The pH value, lactose and ash contents did not affect in both buffalos and cows fed diets. Caroprese et al. (2010) have reported the milk yield and composition could depend on several factors such as the breed, genetic variation, health, environment, management practices, and diet as well as the source of fat and the level of supplementation. These results indicated that the type of diet does not make a significant change in milk composition as much as the amount of milk production.

Table 2. Milk yield and composition of lactating buffaloes and cows fed a diet supplemented with flaxseed oil and soybean oil separately and in combination.

Animals diets	Milk yield (kg d ⁻¹)	Milk composition					
		pH	Total solids	Fat	Protein	Lactose	Ash
Buffalo's milk							
CD	8.31 ^{AB} ±0.14	6.69 ^A ±0.11	17.54 ^A ±0.56	6.64 ^A ±0.26	4.09 ^A ±0.34	5.71 ^A ±0.46	0.93 ^A ±0.03
DFO	8.55 ^A ±0.29	6.68 ^A ±0.06	17.92 ^A ±1.68	6.92 ^A ±0.67	4.25 ^A ±0.41	5.82 ^A ±0.72	0.95 ^A ±0.09
DSO	8.17 ^{AB} ±0.24	6.74 ^A ±0.08	18.21 ^A ±1.48	7.04 ^A ±0.19	4.45 ^A ±0.77	5.90 ^A ±0.56	0.94 ^A ±0.11
DFSO	8.25 ^{AB} ±0.18	6.71 ^A ±0.07	17.72 ^A ±1.21	6.72 ^A ±0.20	4.23 ^A ±0.63	5.90 ^A ±0.54	0.96 ^A ±0.11
Cow's milk							
CD	7.80 ^B ±0.35	6.57 ^A ±0.02	12.92 ^B ±0.87	3.41 ^B ±0.85	3.54 ^B ±0.23	5.09 ^A ±0.23	0.88 ^A ±0.05
DFO	8.67 ^A ±0.30	6.60 ^A ±0.03	13.69 ^B ±1.09	4.07 ^B ±0.69	3.64 ^B ±0.15	5.11 ^A ±0.30	0.87 ^A ±0.29
DSO	8.44 ^A ±0.25	6.58 ^A ±0.02	13.62 ^B ±0.94	4.08 ^B ±1.13	3.53 ^B ±0.12	5.05 ^A ±0.26	0.86 ^A ±0.05
DFSO	8.50 ^A ±0.28	6.58 ^A ±0.06	13.27 ^B ±1.00	3.95 ^B ±1.07	3.66 ^B ±0.11	5.06 ^A ±0.12	0.86 ^A ±0.04

Means (±SD, n=4) with the same letters are not significantly different ($p < 0.05$); CD, control diet; DFO, diet supplemented with 2% flaxseed oil; DSO, diet supplemented with 2% soybean oil; DFSO; diet supplemented with 2% flaxseed and soybean oils.

Table 3 present the main groups of fatty acids composition of milk fat produced from lactating buffaloes and cows fed a DFO, DSO or DFSO. Buffalo's milk fat was higher in short chain fatty acids (SCFA), and long chain fatty acids (LCFA) but lower in medium chain fatty acids (MCFA) than cow's milk fat, the difference in unsaturated fatty acids USFA and USFA/SFA ratio was not clear. Penchev, Ilieva, Ivanova, and Kalev (2016) have reported the buffalo milk displayed significantly lower contents of C8:0-C14:0 and higher of C18:1, total SFAs being lower compared to the cow milk. However, supplementing the diet of both cows and buffalos with flaxseed oil, soybean oil or their mixture decreased short chain- (SCFA), medium chain- (MCFA), and saturated fatty acids (SFA), and increased long chain (LCFA) and unsaturated fatty acids (USFA) as well as USFA/SFA ratio. The DSO was more effective for increasing USFA and USFA/SFA ratio followed by the DFSO in buffalo's milk fat whereas DFO and DFSO were more effective in cow's milk fat compared with the control diet. The USFA representing an increase of 24.25 or 23.05% in milk fat of buffalos fed DSO or DFSO whereas the increase was 12.73 and 13.21% in milk fat of cows fed DFO or DFSO, respectively. These results are compatible with Caroprese et al. (2010) in milk fat when crushed flaxseed supplements were used. Milk's fatty acids are entirely related with the ruminant's diet; including the feed and intake levels as well as accumulation of USFAs, especially oil plants that are rich in linolenic acid (Vargas-Bello-Pérez & Garnsworthy, 2013).

Before soft cheese manufacture, milk produced from lactating buffaloes and cows fed on the DFO, DSO or DFSO was standardized to casein/fat ratio of 0.85. As shown in Table 4, cheese yield was significantly affected by the type of milk and milk composition ($p < 0.05$). Cheese yield was significantly higher in buffalo's milk than cow's milk, due to total solids, protein and fat contents are high in buffalo's milk (Rasheed, Qazi, Ahmed, Durrani, & Azmat, 2016). Feeding on the DFO, DSO or DFSO caused a slight increase ($p > 0.05$) in cheese yield compared with the control diet. Cheese yield was the highest in milk produced from animals fed on a DSO, while was the lowest in that produced from animals fed on the control diet; the difference was not significant ($p > 0.05$). Cheese yield of buffalo's milk was in the range reported by Hamad (2015). Also, there was no significant difference in cheese composition produced from buffalo's milk and cow's milk ($p > 0.05$). Total solids, proteins, fat, ash and salt of resultant soft cheese ranged between

36.47 to 38.75, 12.22 to 13.99, 15.57 to 16.55, 4.11 to 4.29 and 3.36 to 3.64%, respectively. Total solids, proteins, and fat percentage lie in the range reported by other researchers as well Ismail, Eltahra, Ammar, and Eid (2011).

Table 3. The main groups of milk fatty acids profile of lactating buffaloes and cows fed a diet supplemented with flaxseed oil and soybean oil separately and in combination.

Fatty acid groups	Milk fat treatments, %			
	CD	DFO	DSO	DFSO
Buffalo's milk fat				
SCFA (C ₄₋₈)	6.32	5.60	3.73	3.33
MCFA (C ₁₀₋₁₄)	12.29	9.83	9.64	9.43
LCFA (C ₁₅₋₁₈)	81.34	84.51	86.59	87.20
SFA	65.90	61.87	57.65	58.06
USFA	34.05	38.07	42.31	41.90
USFA/SFAs(%)	0.52	0.62	0.73	0.72
Cow's milk fat				
SCFA (C ₄₋₈)	5.44	4.70	4.88	4.25
MCFA (C ₁₀₋₁₄)	14.92	13.77	14.45	13.78
LCFA (C ₁₅₋₂₂)	79.43	81.48	80.67	82.02
SFA	64.65	60.28	61.58	60.21
USFA	35.19	39.67	38.42	39.84
USFA/SFA (%)	0.54	0.66	0.62	0.66

CD, control diet; DFO, diet supplemented with 2% flaxseed oil; DSO, diet supplemented with 2% soybean oil; DFSO; diet supplemented with 2% flaxseed and soybean oils; SCFA, short chain fatty acids; MCFA, medium chain fatty acids; LCFA, long chain fatty acids; SFA, saturated fatty acids; USFA, unsaturated fatty acids.

Table 4. Yield and composition of soft cheese made from the milk of lactating buffaloes and cows fed a diet supplemented with flaxseed oil and soybean oil separately and in combination.

Animal's diets	Cheese yield (%)	Soft cheese composition, %				
		Total solids	Fat	Protein	Ash	Salt
Cheese buffalo's milk						
CD	28.46 ^{AB} ±1.91	37.40 ^A ±1.83	16.55 ^A ±0.85	12.57 ^A ±1.93	4.32 ^A ±0.38	3.56 ^A ±0.27
DFO	29.99 ^A ±3.29	38.75 ^A ±1.34	16.25 ^A ±1.23	13.99 ^A ±0.70	4.27 ^A ±0.20	3.44 ^A ±0.24
DSO	31.09 ^A ±3.10	37.26 ^A ±1.47	16.28 ^A ±0.47	13.87 ^A ±0.09	4.32 ^A ±0.18	3.64 ^A ±0.22
DFSO	30.24 ^A ±3.26	36.78 ^A ±1.91	15.89 ^A ±0.95	13.94 ^A ±1.93	4.32 ^A ±0.27	3.50 ^A ±0.19
Cheese cow's milk						
CD	24.83 ^C ±1.41	37.29 ^A ±1.47	16.25 ^A ±1.35	12.26 ^A ±0.47	4.29 ^A ±0.42	3.51 ^A ±0.30
DFO	25.21 ^C ±1.91	36.71 ^A ±1.89	15.83 ^A ±0.65	12.98 ^A ±0.72	4.25 ^A ±0.35	3.48 ^A ±0.25
DSO	25.96 ^{BC} ±1.98	37.85 ^A ±0.97	16.87 ^A ±0.74	12.22 ^A ±1.39	4.11 ^A ±0.11	3.36 ^A ±0.17
DFSO	25.19 ^{2C} ±2.31	36.47 ^A ±0.66	15.57 ^A ±0.85	12.39 ^A ±0.54	4.26 ^A ±0.18	3.41 ^A ±0.32

Means (±SD, n=4) with the same letters are not significantly different (p < 0.05); CD, control diet; DFO, diet supplemented with 2% flaxseed oil; DSO, diet supplemented with 2% soybean oil; DFSO; diet supplemented with 2% flaxseed and soybean oils

Figure 1 shows the changes in pH of the soft cheese made from the milk of lactating buffaloes and cows fed on the DFO, DSO or DFSO during storage at 5 ± 2°C for 2 months. The pH value of cheese had no significant affected (p > 0.05) by the type of milk or the feeding diet at different storage periods. Both buffalo's (Figure 1A) and cow's (Figure 1B) soft cheese showed a significant decrease in pH (p < 0.05) during storage period (2 months). The range of pH decrease was 1.61 to 1.71 units in buffalo's cheese and was 1.60 to 1.74 units in cow's cheese indicated that no significant difference in the pH decrease (pH > 0.05) during storage between buffalo's and cow's cheese (Effat, Mabrouk, Sadek, Hussein, & Magdoub, 2012; Farrag, El-Sheikh, Fouad, Sayed, & Abd El-Aziz, 2019). The decrease in pH may be due to the convert of residual lactose in cheese to lactic acid and free fatty acid which had developed in the cheese at the end of the storage period (Yerlikaya & Ozer, 2014).

Cheese proteolysis, as measured by water soluble nitrogen/total nitrogen (WSN/TN ratio), of soft cheese is depicted in Figure 2A and B. Buffalo's soft cheese exhibited a higher WSN/TN ratio than cow's soft cheese; the difference was significant only at month 2 (p < 0.05). The increase of WSN/TN ratio in fresh buffalo's soft cheese may be due to the whey proteins in buffalo's milk is more resistant to heat treatment than that in cows' milk. During the storage period, soft cheese samples showed a continued increase in proteolysis being significant (p < 0.05) at month 1 and 2 for cow's cheese whereas for buffalo's cheese the changes were significant at month 2 (p < 0.05). Similar trends were observed by Ismail et al. (2011), Abd El-Aziz,

Mohamed, Seleet, and Abd El-Gawad (2015) and Todaro et al. (2017) they reported that storage cheese showed higher solubility than fresh cheese. The increase in proteolysis could be due to the proteolytic activity of the starter bacteria, non-starter bacteria and residual rennet. Several chemical and biochemical reactions can occur during cheese ripening such as degradation of the casein matrix of the curd to a range of peptides, which is followed by degradation to free amino acids (Sgarbi et al., 2013). However, the DFO, DSO or DFSO had no significant effect ($p > 0.05$) on the cheese proteolysis during the cold storage period. Such an effect was found in both brined Tallaga cheese and that made from high CLA milk with slight differences between the two treatments (Abd El-Salam et al., 2011).

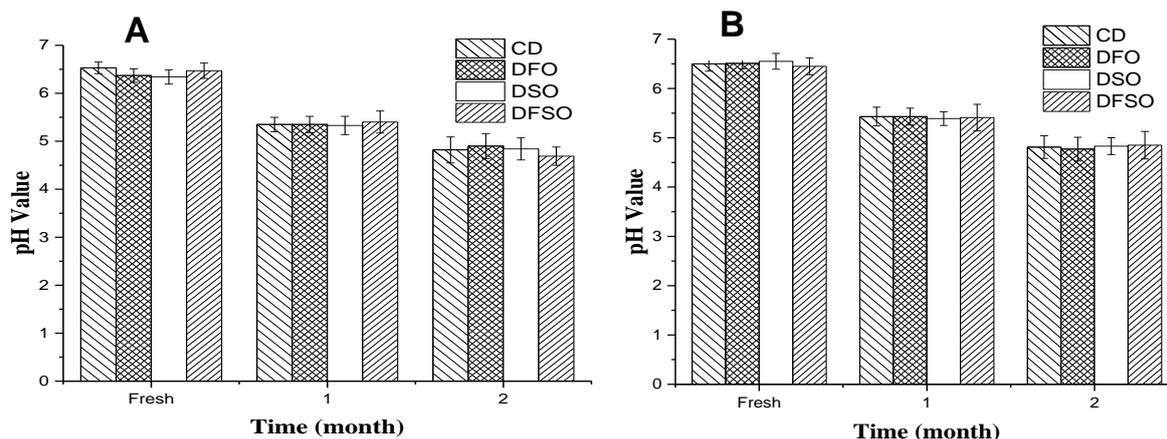


Figure 1. The pH of soft cheese made from milk of lactating buffaloes (A) and cows (B) fed a diet supplemented with flaxseed oil and soybean oil separately and in combination during storage at $5\pm 2^{\circ}\text{C}$ for 60 days. CD, control diet; DFO, diet supplemented with 2% flaxseed oil; DSO, diet supplemented with 2% soybean oil; DFSO; diet supplemented with 2% flaxseed and soybean oils

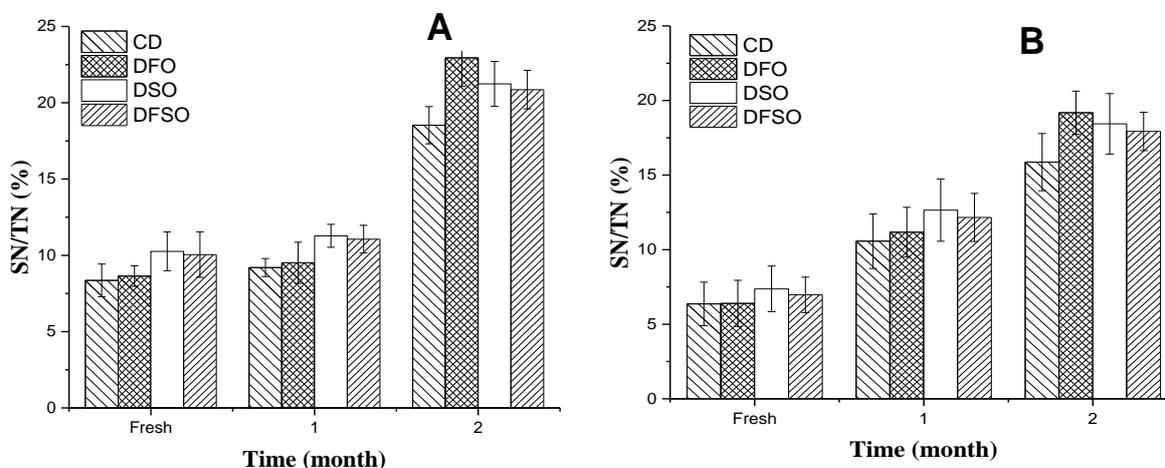


Figure 2. Proteolysis of soft cheese made from milk of lactating buffaloes (A) and cows (B) fed a diet supplemented with flaxseed oil and soybean oil separately and in combination during storage at $5\pm 2^{\circ}\text{C}$ for 2 months. CD, control diet; DFO, diet supplemented with 2% flaxseed oil; DSO, diet supplemented with 2% soybean oil; DFSO; diet supplemented with 2% flaxseed and soybean oils.

The antioxidant activity, as measured by DPPH[•] radical scavenging activity, was similar to the trend of WSN/TN ratio (Figure 3A and B). Buffalo's cheese had significantly higher ($p < 0.05$) antioxidant activity against DPPH[•] than cow's cheese at fresh, and month 1 and 2. The antioxidant activity significantly increased ($p < 0.05$) as cheese proteolysis increase. Statistically, antioxidant activity was highly positively correlated with the WSN/TN ratio ($r^2 = 0.75$). These results are similar to the observation of Shazly et al. (2019), who found that the high antioxidant capacity of both bovine and buffalo casein related to the degree of hydrolysis. Wu et al. (2012) reported that the antioxidant capacity of peptides is affected by different parameters including protein source, type of protease, the degree of hydrolysis, and amino acid constituents and sequences. In addition, the DPPH[•] radical scavenging activity of soft cheese made from the milk of lactating animals fed on the DFO, DSO or DFSO was slightly higher ($p > 0.05$) than those made from the milk

of lactating animals fed on the control diet. The increase in the DPPH• radical scavenging activity might be due to increasing the antioxidant components in milk fat produce from of lactating animals fed on the DFO, DSO or DFSSO such as vitamins A and E.

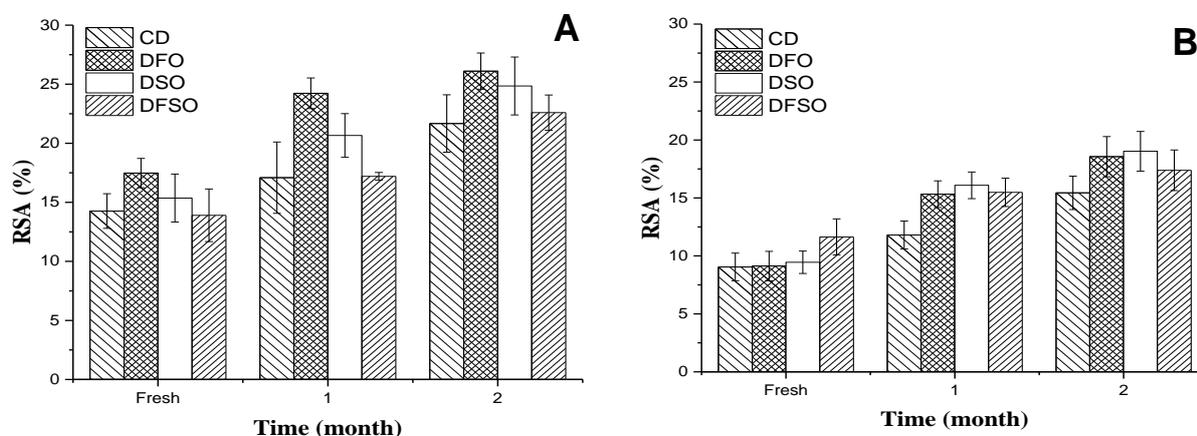


Figure 3. Antioxidant activity of soft cheese made from milk of lactating buffaloes (A) and cows (B) fed a diet supplemented with flaxseed oil and soybean oil separately and in combination during storage at $5\pm 2^{\circ}\text{C}$ for 2 months. CD, control diet; DFO, diet supplemented with 2% flaxseed oil; DSO, diet supplemented with 2% soybean oil; DFSSO, diet supplemented with 2% flaxseed and soybean oils.

Texture attributes of soft cheese made from buffalo's milk and cow's milk during storage at $5\pm 2^{\circ}\text{C}$ for 2 months are presented in Table 5. On day 1, buffalo's soft cheese characterized by high hardness (N) and gumminess compared with cow's soft cheese; the difference was not significant ($p > 0.05$). The hardness and gumminess of buffalo's soft cheese were ranged between 8.25 to 7.55 and 5.53 to 4.97 but ranged between 7.03 to 6.29 and 4.76 to 4.13 for cow's soft cheese. During storage, all soft cheese samples showed a continued decrease in hardness and gumminess being significant only ($p < 0.05$) after 2 months for buffalo's cheese. Negative correlations were observed between hardness and SN/TN ratio ($r^2 = -0.73$) as well as gumminess and SN/TN ratio ($r^2 = -0.79$). Similarly, Dimitreli, Exarhopoulos, Antoniou, Zotos, and Bampidis (2017) found the SN/TN ratio increased during storage resulting in decreased hardness, cohesiveness, springiness and elasticity. The changes in texture attributes were also found in Feta cheese made from UF cow's milk and soymilk blend (Rohani, Mortazavi, & Tehrani, 2010). The decrease in cheese hardness due to the extended proteolysis that decreases the surface area occupied by the protein fraction in cheese microstructure, leading to the decrease of the force bearing component in cheese texture (Khosrowshahi, Madadlou, zadeh Mousavi, & Emam-Djomeh, 2006). Fathollahi, Hesari, Azadmard, and Oustan (2010) found that proteolysis can contribute to textural softening during ripening of UF white cheese. However, the type of milk and storage period had no significant effect on the cheese cohesiveness ($p > 0.05$).

The hedonic scale evaluation of sensory attributes of soft cheese during storage at $5 \pm 2^{\circ}\text{C}$ for 2 months is shown in Table 6. There was no significant difference in sensory attributes ($p > 0.05$) including appearance, flavor and body & texture among all cheese samples during different storage periods. Both of fresh buffalo's and fresh cow's cheese characterized with white color, milk order, salty taste, soft body and grittiness during mastication. After 2 months, the flavor and texture scores were higher than the fresh cheese and those after 1 month storage; the changes not significant ($p > 0.05$). Cheese samples more softness and solubility as well as more odor of cheese flavor compared to fresh cheese samples. During storage, the breakdown of fat and protein by the activity of microbial and residual rennet more gives a smooth texture and produced more flavoring compounds in cheese (Papetti & Carelli, 2013). The improving in cheese flavor and texture during storage was also observed by Rohani et al. (2010) and Todaro et al. (2017). Rohani et al. (2010) reported that the sensory properties of UF-Feta cheese did not significantly change, whereas the flavor and texture scores at month 3 were higher than that after two months of storage. Also, the type of feeding had no significant effect on the sensory attributes of soft cheese.

Table 5. Texture attributes of soft cheese made from milk of lactating buffaloes and cows fed a diet supplemented with flaxseed oil and soybean oil separately and in combination oils during storage at 5±2°C for 2 months.

Texture attributes	Storage period (month)	Cheese treatments				
		CD	DFO	DSO	DFSO	
Hardness (N)	CBM	Fresh	7.55 ^a ± 1.12	8.25 ^a ± 0.77	7.82 ^a ± 1.69	7.64 ^a ± 1.62
		1	6.95 ^{ab} ± 0.95	7.11 ^{ab} ± 1.10	6.80 ^{ab} ± 0.80	6.90 ^{ab} ± 0.60
		2	5.48 ^b ± 0.80	6.15 ^b ± 0.65	5.51 ^b ± 0.81	5.55 ^b ± 0.82
	CCM	Fresh	6.29 ^{ab} ± 0.84	7.03 ^{ab} ± 1.14	6.53 ^{ab} ± 1.28	6.76 ^{ab} ± 0.83
		1	6.32 ^{ab} ± 0.99	6.88 ^{ab} ± 0.94	6.78 ^{ab} ± 0.84	6.60 ^{ab} ± 1.18
		2	5.03 ^b ± 0.63	5.40 ^b ± 0.76	5.11 ^b ± 1.01	5.16 ^b ± 1.21
Cohesiveness	CBM	Fresh	0.68 ^a ± 0.03	0.67 ^a ± 0.13	0.66 ^a ± 0.08	0.65 ^a ± 0.24
		1	0.66 ^a ± 0.11	0.63 ^a ± 0.02	0.62 ^a ± 0.06	0.68 ^a ± 0.04
		2	0.55 ^a ± 0.17	0.61 ^a ± 0.08	0.53 ^a ± 0.12	0.57 ^a ± 0.10
	CCM	Fresh	0.66 ^a ± 0.29	0.67 ^a ± 0.12	0.63 ^a ± 0.23	0.64 ^a ± 0.25
		1	0.62 ^a ± 0.14	0.65 ^a ± 0.12	0.55 ^a ± 0.08	0.57 ^a ± 0.11
		2	0.66 ^a ± 0.13	0.67 ^a ± 0.04	0.62 ^a ± 0.22	0.63 ^a ± 0.15
Gumminess (N)	CBM	Fresh	5.13 ^a ± 1.22	5.53 ^a ± 0.66	5.16 ^a ± 0.87	4.97 ^a ± 1.59
		1	4.59 ^{ab} ± 0.15	4.48 ^{ab} ± 1.09	4.22 ^{ab} ± 1.02	4.69 ^{ab} ± 0.78
		2	2.90 ^b ± 0.84	3.75 ^b ± 0.67	2.81 ^b ± 0.82	3.05 ^b ± 0.54
	CCM	Fresh	4.15 ^{ab} ± 1.21	4.76 ^{ab} ± 0.77	4.36 ^{ab} ± 1.06	4.33 ^{ab} ± 0.29
		1	3.89 ^{ab} ± 1.27	4.47 ^{ab} ± 1.33	3.58 ^{ab} ± 1.26	3.79 ^{ab} ± 1.09
		2	3.32 ^{ab} ± 1.07	3.52 ^b ± 0.52	3.17 ^b ± 0.92	3.20 ^{ab} ± 0.43

Means (±SD, n=4) with the same letters are not significantly different ($p \leq 0.05$); CBM, cheese buffalo's milk; CCM, cheese cow's milk; CD, control diet; DFO, diet supplemented with 2% flaxseed oil; DSO, diet supplemented with 2% soybean oil; DFSO; diet supplemented with 2% flaxseed and soybean oils.

Table 6. Sensory attributes of soft cheese made from milk of lactating buffaloes and cows fed a diet supplemented with flaxseed oil and soybean oil separately and in combination during storage at 5 ± 2°C for 2 months.

Sensory attributes	Storage period (month)	Animal's diets				
		CD	DFO	DSO	DFSO	
Appearance	CBM	Fresh	8.00 ^A ± 0.81	7.86 ^A ± 0.69	8.14 ^A ± 0.71	8.19 ^A ± 0.75
		1	7.78 ^A ± 1.03	8.00 ^A ± 0.75	7.88 ^A ± 0.86	8.15 ^A ± 0.53
		2	7.89 ^A ± 0.57	8.00 ^A ± 0.59	8.25 ^A ± 0.50	8.25 ^A ± 0.92
	CCM	Fresh	7.41 ^A ± 0.94	7.35 ^A ± 0.86	7.70 ^A ± 0.59	7.71 ^A ± 0.58
		1	7.33 ^A ± 0.52	7.33 ^A ± 0.82	7.17 ^A ± 1.16	7.22 ^A ± 1.16
		2	7.22 ^A ± 0.97	7.22 ^A ± 0.97	7.56 ^A ± 0.52	7.55 ^A ± 0.53
Body & texture	CBM	Fresh	7.76 ^A ± 0.69	7.61 ^A ± 0.48	8.02 ^A ± 0.53	8.00 ^A ± 1.00
		1	7.67 ^A ± 0.64	7.55 ^A ± 1.03	7.68 ^A ± 0.83	7.68 ^A ± 0.53
		2	8.21 ^A ± 0.45	8.10 ^A ± 0.86	8.43 ^A ± 0.67	8.32 ^A ± 0.52
	CCM	Fresh	7.39 ^A ± 0.92	7.52 ^A ± 1.12	7.46 ^A ± 0.62	7.47 ^A ± 0.62
		1	7.53 ^A ± 0.72	7.43 ^A ± 0.82	7.33 ^A ± 0.82	7.41 ^A ± 0.82
		2	7.91 ^A ± 0.92	7.94 ^A ± 0.70	8.23 ^A ± 0.71	8.23 ^A ± 0.71
Flavor	CBM	Fresh	7.55 ^A ± 0.82	7.66 ^A ± 0.70	7.90 ^A ± 0.53	7.79 ^A ± 0.49
		1	7.63 ^A ± 0.91	7.55 ^A ± 0.71	7.70 ^A ± 0.92	7.67 ^A ± 0.83
		2	8.22 ^A ± 0.45	8.15 ^A ± 0.67	8.54 ^A ± 0.87	8.33 ^A ± 0.72
	CCM	Fresh	7.26 ^A ± 1.14	7.41 ^A ± 1.12	7.58 ^A ± 0.87	7.58 ^A ± 0.87
		1	7.00 ^A ± 0.89	7.17 ^A ± 0.75	7.66 ^A ± 0.82	7.67 ^A ± 0.86
		2	8.03 ^A ± 0.87	8.12 ^A ± 0.37	8.23 ^A ± 1.00	8.33 ^A ± 1.00

Means (±SD, n=4) with the same letters are not significantly different ($p \leq 0.05$); CBM, cheese buffalo's milk; CCM, cheese cow's milk; CD, control diet; DFO, diet supplemented with 2% flaxseed oil; DSO, diet supplemented with 2% soybean oil; DFSO; diet supplemented with 2% flaxseed and soybean oils.

Conclusion

The inclusion of flaxseed and/or soybean oils in the diet of lactating dairy animals may modify the milk quality and its products. Increased USFA and decreased SFA in both milks suggest a positive role of a DFO, DSO and DFSO in improving the potential benefits of human health. The DFO and DSO giving rise to a soft cheese rich in antioxidant compounds, with a higher concentration of USFA of interest to humans, and without significant alterations of the sensory and texture properties of soft cheese. The DFO and DSO had a positive effect on the cheese proteolysis and antioxidant activity during cold storage.

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