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Effect of Milk Fat Replacement with Vegetable Oil and/or Whey Protein Concentrate on Microstructure, Texture and Sensory Characteristics of Fresh Soft Cheese

Baraka A. Abd El-Salam

Department of Dairy Research, Food Technology Research Institute, Agricultural Research Center, Egypt

ABSTRACT

The microstructure, texture and sensory properties of control (full fat) and experimental fresh Tallaga cheeses produced by replacing milk fat with Sunflower Oil (SO) and/or Whey Protein Concentrate (WPC) were investigated. Scanning electron micrographs displayed WPC cheese (total replacement of milk fat with whey protein cheese) with a compact and close network. Also, SO cheese (total replacement of milk fat with sunflower oil) showed a compact network with small uniform oil droplets embedded in the protein matrix, but full fat cheese exhibited an open protein matrix containing milk fat globules of various sizes and forms. Textural tests showed that the WPC cheese was harder, more cohesive, gummier and chewier than those of other experimental cheeses. The cheese with 50% sunflower oil and 25% whey protein concentrate was more acceptable than other experimental cheeses and showed a similar texture and structure to those of fresh full fat Tallaga cheese.

Key words: Vegetable oil, whey protein concentrate, microstructure, texture, Tallaga cheese

INTRODUCTION

Fat consumption has been shown to be associated with an increased risk of obesity, atherosclerosis, coronary heart disease, elevated blood pressure and tissue injury diseases associated with lipid oxidation. This association has created an increased awareness and a dramatic increase in the demand and supply for, low-fat foods, including cheese varieties (Katsiari *et al.*, 2002). Milk fat contains over 70% saturated acyl groups and of these, laurate, myristate and palmitate are considered particularly atherogenic (Yu and Hammond, 2000a). The amount of fat intake is considered equally important to the balance of saturated to unsaturated fatty acids, so that diets abundant in mono and polyunsaturated fats are considered healthy (Tunick *et al.*, 1999). The production of reduced and low-fat cheese has significantly increased since 1980 (Koca and Metin, 2004). The fat in food carries much of its flavor and gives it a satisfying characteristic texture; removal of fat from cheese produces undesirable texture and appearance, altered rheological parameters, lack of flavor and poor keeping quality (Madadlou *et al.*, 2005). Most studies show that the partial reduction of Milk Fat (MF) modifies the microstructure and textural characteristics, because the protein matrix becomes more compact and texture more elastic, firm and chewy in the reduced-fat cheese compared to the full-fat cheese (Lobato-Calleros *et al.*, 2007).

Vegetable oils are a good source of healthy unsaturated fats and are generally cholesterol-free. Substitution of milk fat by vegetable oils in milk is an option for obtaining cheese with healthier saturated/unsaturated fat balance (Yu and Hammond, 2000b). However, incorporation of vegetable

oils alters the content, type and distribution of the fat droplets into the protein network, causing modifications in cheese microstructure and textural behavior (Lobato-Calleros *et al.*, 2002, 2003).

Whey Protein Concentrate (WPC) has been considered an interesting fat-substitute ingredient due to its functional and technological properties, as well as its nutritional properties since it contains high concentrations of bioactive proteins (Vidigal *et al.*, 2012). Whey protein functionality is associated with its composition and degree of denaturation (Lizarraga *et al.*, 2006). These provided a porous, less dense and fine protein network to the cheese and close textural characteristics compared to those of full-fat cheese. Also whey proteins possess excellent emulsifying properties (Dickinson, 1997).

In recent years, many studies have been related to low-fat Mozzarella, Cheddar, Kashkaval, Edam and Kefalograviera type cheeses (Kavas *et al.*, 2004). However, other cheeses with higher levels of consumption, such as low-fat soft cheeses, have got much less attention (Zalazar *et al.*, 2002). Tallaga cheese is a soft white cheese and manufactured by using a low salt percent.

The objective of this study was to determine the effects of milk fat replacement in cheese milk with sunflower oil and/or whey protein concentrate on microstructure, texture and sensory properties of Tallaga cheese to produce low-fat soft cheese healthy and with good microstructure, texture and sensory properties.

MATERIALS AND METHODS

Materials: The whole buffaloes' milk (7% fat) and skim buffalo's milk (0.1%) were obtained from Faculty of Agriculture, Cairo University, Egypt. Whey Protein Concentrate (WPC), was supplied by Dora Company in Turkey. The Sunflower oil was purchased from local markets. A commercial stabilizer/emulsifier blend named Lacta 815 (consists of gelatin, carrageenan and diglycerides) was obtained from Misr Food Additives Co., Egypt.

Fresh tallaga cheese-making: Cheese was made according to (Mehanna and Rashed, 1990) except, the milk wasn't treated with CO₂. Six treatments were produced: a control made from whole buffalo's milk containing 7% fat, an experimental cheeses made by replacing milk fat with WPC and/or Sunflower Oil (SO) and using Lacta 815 (0.5%) as an emulsifier. It was decided to substitute 1 g milk fat by 1 g oil, but 1 g milk fat by 0.42 g WPC in accordance to Lobato-Calleros *et al.* (2001, 2003). The fat replacement level for six cheese treatments with SO and/or WPC was illustrated in Table 1. Three replicates were carried out for each treatment.

Chemical analysis: Samples of different cheese treatments were analyzed for moisture, Ash and protein by methods of AOAC (2000). The pH on warm water macerates was determined using a digital pH-meter HANNA 213 (Italy). Fat content was determined by Soxhlet method according to AOAC (2000).

Table 1: Fat replacement level for cheese treatments with SO and/or WPC

Treatment	Milk fat replacement level with SO and/or WPC (%)
A (control)	0 (Full milk fat)
B	100 SO
C	100 WPC
D	25 SO+50 WPC
E	25 SO+25 WPC
F	50 SO+25 WPC

WPC: Whey protein concentrate, SO: Sunflower oil

Texture profile analysis: The evaluation of Texture Profile Analysis (TPA) was carried out using at least three samples for each treatment with Universal Testing Machine (Cometech, B type, Taiwan), provided with the software. Back extrusion cell with 35 mm diameter compression disc was used. Two cycles were applied at a constant crosshead velocity of 1 mm sec⁻¹ to 35% of sample depth then returned. From the resulting force-time curve the values for texture attributes, i.e., hardness, chewiness, cohesiveness, gumminess and springiness were calculated TPA graphic.

Microstructure analysis: Cheese samples were prepared for scanning electron microscopy following the method of Rahimi *et al.* (2007). Cheese samples were cut into approximately 5-6 mm³ cubes with a sharp razor and fixed in 2.5% glutaraldehyde (Merck) in 0.2 M sodium cacodylate buffer (pH 6.5) for 3 h, followed by post fixation in osmium tetroxide for 2 h. Cubes were then washed 6 times in distilled water (1 min each time), dehydrated in a graded (40, 55, 70, 85, 90 and 96%) series of ethanol for 30 min each, then dehydrated using Critical Point Dried instrument with liquid carbon dioxide. These pieces were mounted on copper stubs with double-sided adhesive tape, coated with gold using S150A Sputter Coater-Edwards-England. Samples were viewed in a scanning electron microscope (JXA-840A Electron probe Microanalyzer-JEOL-Japan).

Sensory evaluation: Sensory evaluation of cheese was carried out according to Katsiari and Voutsinas (1994) by a regular taste and texture panel of 20 staff members of the Dairy Department in Food Tech. Res. Institute. Panel members evaluated cheese for appearance, body and texture and flavor (odour and taste) by using a 10-point scale, with 1 being the worst and 10 the best quality. More importance was given to body and texture and to flavour than to appearance. Thus, the scores obtained for these two attributes were multiplied by 4 and 5, respectively. The total Score was obtained by adding the scores of the three attributes. An excellent cheese obtained a total score of 100.

Statistical analysis: The mean values and standard deviations were determined for each treatment. Differences between samples were determined by t-test and were considered to be significant when $p \leq 0.05$ (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Chemical composition: The composition of control and experimental cheeses are given in Table 2. It could be seen from the data that, the replacement of milk fat with SO and/or WPC affects the moisture, fat and protein contents in experimental cheeses. Moisture content in the cheeses was mainly influenced by Sunflower Oil (SO), Whey Protein Concentrate (WPC) and Milk Fat (MF). Water acts as a low viscosity lubricant between fat and casein and it occupies all the

Table 2: Chemical composition of control and experimental Tallaga cheeses

Samples	pH	Moisture (%)	Fat (%)	Fat/D.M.(%)	Protein (%)	Ash (%)
A	6.49 ^a	62.46 ^e	22.00 ^a	58.60 ^a	12.50 ^d	2.34 ^{ed}
B	6.47 ^a	65.66 ^c	19.20 ^c	55.90 ^b	11.80 ^f	2.26 ^f
C	6.50 ^a	72.00 ^a	00.20 ^f	0.73 ^f	20.79 ^a	2.92 ^a
D	6.48 ^a	68.74 ^b	09.38 ^e	30.00 ^e	13.61 ^b	2.76 ^b
E	6.45 ^a	63.54 ^d	18.70 ^d	52.90 ^d	13.17 ^c	2.45 ^c
F	6.46 ^a	62.26 ^{fb}	20.00 ^b	53.00 ^c	12.00 ^e	2.37 ^d
LSD at 0.05	0.0821	0.2108	0.3310	0.2981	0.1975	0.0491

A: Control ((Full milk fat), B: 100 SO, C: 100 WPC, D: 25 SO+50 WPC, E: 25 SO+25 WPC, F: 50 SO+25 WPC, *Means with the same letter in the same column are not significantly different

space between the fat and the casein (McMahon *et al.*, 1993). Increases in both MF and SO produce a higher fat droplet number in the cheese, producing a larger interruption of the protein network, giving way to more interstitial spaces. The increased moisture content of cheeses produced by fat replacer (WPC) indicated that curd syneresis was retarded during cheese making. It has been suggested that water can bind directly to fat replacers and the fat replacers can interfere with the shrinkage of the casein matrix. Hence, this lowers the driving force involved in removing water from curd particles (Koca and Metin, 2004).

The protein content of the cheeses was positively affected by SO, WPC and MF. While WPC contributes directly to the protein content of the cheese, the contribution of MF and SO to protein content must be indirect, as they are lipids in nature. When milk coagulates, the casein micelles aggregate into chains that eventually will all link together into mesh-like structure that envelope the fat globules, or even clusters of fat globules. Relatively large spaces exist in the network where the fat globules are present and act to interrupt the network. When the curd is cut, whey is removed and the mesh-like structure shrinks around the fat globules (Lobato-Calleros *et al.*, 2007). MF globules in cheeses reduce the degree of shrinking and expelling of whey carrying water-soluble components including proteins and peptides (Gunasekaran and Ak, 2003). Emulsifier contributes to higher emulsified sunflower oil retention in the cheese, so that the syneresis phenomenon is inhibited and less whey proteins are expelled from the cheese network.

The fat content of the cheeses was influenced by WPC, SO and MF. Increasing of WPC concentration in milk led to decrease the fat content in cheese. It is obvious that as the MF concentration in the formulation is higher, so is the fat content in the cheese. Emulsifier contributes to the formation, rapid stabilization and the retention of the oil droplets into the cheese protein matrix (Dickinson, 1992). The ash content was in the range from 2.26-2.92. Also, no significant difference between of the pH values of control and experimental cheeses.

Texture Profile Analysis (TPA): Texture Profile Analysis (TPA) is the most used imitative test to mimic the actions during chewing (Dinkci *et al.*, 2011). The texture, along with the flavor and appearance of the food are the main factors in the sensory acceptability of a food product (Bourne, 2004). According to Bourne (2003) the food texture is defined as that group of physical characteristics that arise from the structural elements of the food, are sensed primarily by the tactile perception, are related to the deformation, disintegration and flow of the food under a force and are measured objectively by functions of mass, time and distance.

The changes in TPA parameters (hardness, cohesiveness, springiness (as a primary parameters), gumminess and chewiness (as a secondary parameters) of the control and experimental cheeses are shown in Table 3. The results indicates that the replacement of MF with SO and/or WPC in fresh Tallaga cheese affected the parameters of hardness, cohesiveness, gumminess and chewiness but not affected springiness.

Table 3: Values of instrumental texture parameters for control and experimental tallaga cheeses

Samples	Hardness (N)	Cohesiveness	Gumminess (N)	Chewiness (N mm ⁻¹)	Springiness (mm)
A	2.510 ^{ad}	0.5220 ^{ed}	1.5290 ^d	1.1330 ^d	0.7600 ^a
B	2.350 ^f	0.4800 ^f	1.3070 ^f	0.9860 ^f	0.7060 ^a
C	8.040 ^a	0.6060 ^a	4.8750 ^a	3.6660 ^a	0.7760 ^a
D	5.440 ^b	0.5800 ^b	4.5510 ^b	3.4350 ^b	0.7590 ^a
E	3.240 ^c	0.5770 ^c	2.8380 ^c	2.1580 ^c	0.7750 ^a
F	2.650 ^d	0.5590 ^d	1.4210 ^{ed}	1.1040 ^{ed}	0.7410 ^a
LSD at 0.05	0.1421	0.0390	0.1081	0.0231	0.0721

A: Control ((Full milk fat), B: 100SO, C: 100 WPC, D: 25 SO+50 WPC, E: 25 SO+25 WPC, F: 50 SO+25 WPC, *Means with the same letter in the same column are not significantly different

Hardness is the force necessary to attain a given deformation. In sensory terms, it is the force necessary to compress cheese between the molar teeth (Chevanan *et al.*, 2006). The cheese containing 100% Sunflower Oil (SO) was softer than the full fat cheese. This is not surprising since fat breaks up the protein matrix and plays the role of lubricant to provide smoothness and a softer texture (Dinkci *et al.*, 2011). The highest value of hardness was recorded for 100% WPC treatment (C treatment). These results are probably due to the fact that WPC incorporation into the cheeses matrices (Drake *et al.*, 1996) yielded a higher protein/fat ratio that gave way to compact and continuous relatively large areas of protein matrix. This finding is in agreement with (Bryant *et al.*, 1995) who reported that the high protein densities in cheese are associated with high values of hardness. The 50 SO and 25 WPC treatment (F treatment) showed nearly similar hardness value to that of full fat cheese (A).

Cohesiveness is a measurement of the extent to which the cheese can be deformed before it ruptures. In sensory terms, it is the degree to which a substance is compressed between the teeth before it breaks. In cheese, cohesiveness is a measurement of the strength of the internal bonds of the protein mycelium (Chevanan *et al.*, 2006). The cohesiveness of the control cheese (full milk fat cheese) was much higher than its corresponding 100% Sunflower Oil (SO) treatment (B treatment). These differences might be due to the linkages by oil and milk fat which had varied casein intermolecular associations in the cheese paracasein network, the differences in the hydrophobic characteristics of the fat types used or the type of cheese and production techniques (Dinkci *et al.*, 2011). Also, increasing of milk fat replacement level with WPC led to increasing of cohesiveness value. Similar results were reported by Lobato-Calleros *et al.* (2007). They found that, the cohesiveness value was positive correlated with WPC level in soft cheese.

The gumminess (energy required to disintegrate a semi-solid food to a state ready for swallowing) and chewiness (it is a measurement of the energy required to masticate cheese into a uniform state before swallowing). In sensory terms, it is the energy required to disintegrate the cheese and to change it to a consistency suitable for swallowing, followed the same trend of hardness and cohesiveness and had the same differences of means between the cheese types. Also, it could be seen that, the milk fat replacement with SO and/or WPC had no significant effect on springiness (springiness is a measurement of the recovery of the original undeformed condition after the first compression force is removed during a TPA test). In sensory terms, it is the degree to which a product returns to its original shape once it is compressed between the teeth (Chevanan *et al.*, 2006). Zalazar *et al.* (2002) observed that the use of 2% Dairy-Lo (WPC) had no effect on the springiness values of low-fat cheese.

It could be observed from aforementioned results, the treatment containing 50 SO and 25 WPC (F treatment) showed closely resembled data to control cheese with regarded to textural characteristics.

Microstructure analysis: Scanning Electron Micrographs (SEM) of control and experimental fresh Tallaga cheeses are presented in Fig. 1. Control cheese (Fig. 1a) with full milk fat exhibited an open protein matrix containing milk fat globule voids of various sizes and forms. The holes in the protein matrix indicate the spaces occupied by fat globules. The complete substitution of milk fat by Sunflower Oil (SO) conferred noticeably different structural characteristics. The sunflower oil cheese (Fig. 1b) showed a compact network. The oil droplets were smaller, more uniform in size and embedded in the protein matrix in this cheese. This could be explained by the homogenization step during the production of sunflower oil cheese. The homogenization of cheese milk creates

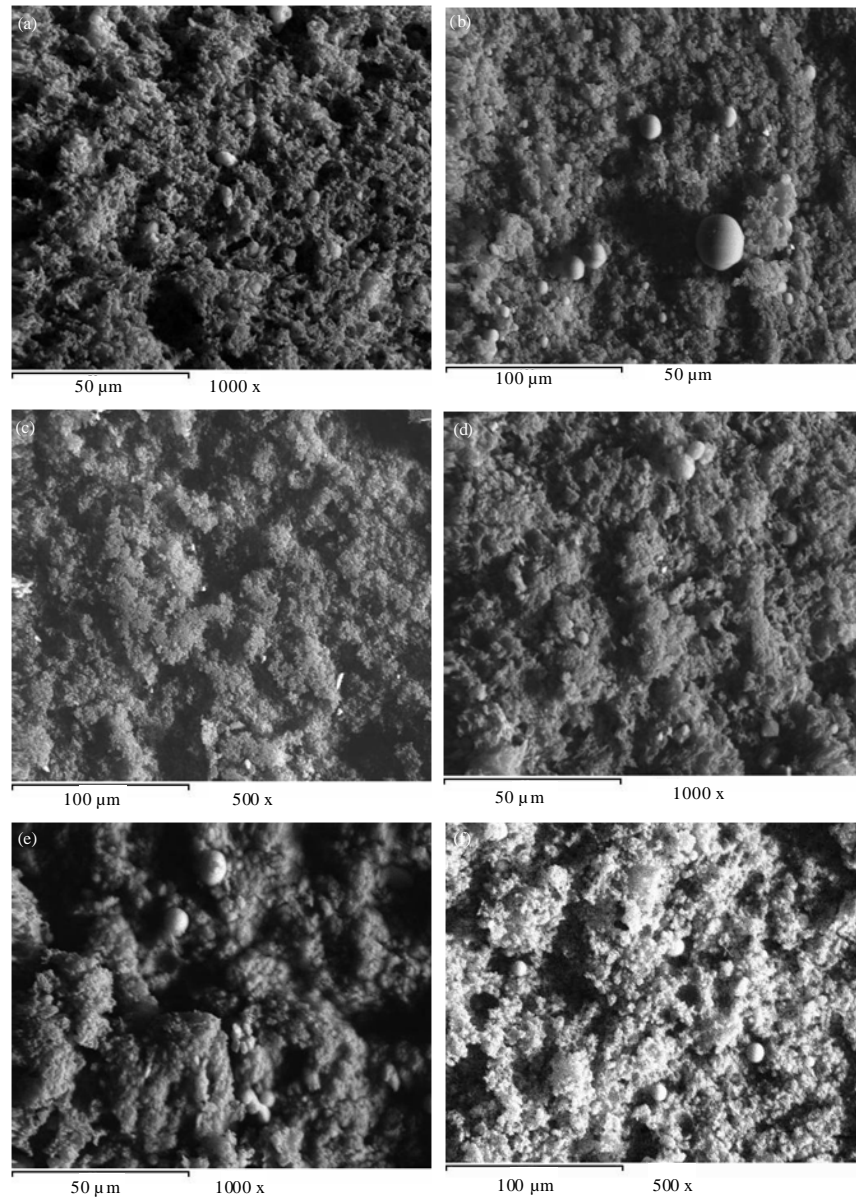


Fig. 1(a-f): Microstructure of fresh Tallaga cheese produced by replacing of milk fat with SO and/or WPC

smaller fat globules and disperses these more evenly in a more compact protein matrix (Everett and Auty, 2008). This is in agreement with the findings of Dinkci *et al.* (2011). The cheese with 100% WPC (Fig. 1c) showed compact protein network this explained by Lobato-Calleros *et al.* (2007), they reported that the milk whey proteins interfered with the casein chains linking, probably due to the formation of disulfide bonds between β -lactoglobulin molecules and k-casein micelles, as well as to hydrogen bonding between whey proteins and water incorporated into the WPC cheese protein matrix. Also, it can be seen that the protein was dense and rough.

Table 4: Effect of milk fat replacement with sunflower oil and/or whey protein concentrate on sensory properties of fresh Tallaga cheese

Samples	Flavour (50)	Body and texture (40)	Appearance (10)	Total score (100)
A	46.71 ^a	38.00 ^a	9.50 ^a	94.21 ^a
B	42.62 ^d	32.64 ^f	8.15 ^d	83.41 ^e
C	39.00 ^f	33.00 ^e	7.16 ^f	79.16 ^f
D	43.64 ^c	34.12 ^d	7.38 ^e	85.14 ^c
E	42.00 ^e	35.08 ^c	7.66 ^e	84.74 ^d
F	44.60 ^b	37.00 ^b	8.80 ^b	90.40 ^b
LSD at 0.05	0.3431	0.1321	0.1800	0.2811

A: Control ((Full milk fat), B: 100 SO, C: 100 WPC, D: 25 SO+50 WPC, E: 25 SO+25 WPC, F: 50 SO+25 WPC, *Means with the same letter in the same column are not significantly different

The cheese with 25% SO and 50% WPC exhibited (Fig. 1d) a compact, closed protein network with only a few voids originally occupied by fat globules. The cheese with 25% SO and 25%WPC (Fig. 1e) presented protein zones interrupted by relatively large MF globules, but also other smoother zones of higher protein density interrupted by few sunflower oil droplets. Finally, the cheese with 50% SO and 25% WPC (Fig. 1f) showed small void spaces originally occupied by oil droplets and homogeneity between milk fat and protein.

Sensory evaluation: The results of the sensory panel's assessment of control and experimental fresh Tallaga cheeses are given in Table 4. These data show that the flavor, body and texture, appearance and total score (over-all quality) were affected by milk fat replacement with SO and/or WPC. A significant difference was observed between the scores of all sensory properties of control (full milk fat) and experimental fresh Tallaga cheeses. The mean flavor, body, texture and appearance scores of control cheese were significantly higher than those of experimental cheeses. Also, It could be noticed that the cheese with 50% SO and 25% WPC received closer scores to control cheese. The cheese with 100% WPC had the lowest score of sensory properties compared to those of other experimental cheeses.

CONCLUSION

The milk fat replacement in Tallaga cheese production with sunflower oil and/or whey protein concentrate caused different structures. Also, textural tests showed significant differences between experimental cheeses. The cheese with 50% sunflower oil and 25% whey protein concentrate was more acceptable than other experimental cheeses and showed a similar texture and structure to those of fresh full fat Tallaga cheese.

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