

## Effect of *L. helveticus* and Buttermilk Powder on Quality Characteristics of Reduced Fat Ras Cheese

Abdel-Hamid, M. ; E. M. Hamad and E. A. Romeih

Department of Dairy Science, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt



### ABSTRACT

There is an increased consumer demand on the consumption of low fat cheese. Reduction of fat associates with defects in quality and consumer acceptability of the resultant cheese. The present study was designed to improve flavor and texture of reduced fat Ras cheese (50% fat reduction) by using *Lactobacillus helveticus* (LH) and buttermilk powder (BMP). Reduced-fat Ras cheese was made by using LH, LH+0.5% BMP or LH+1% BMP. For comparison reduced-fat and full-fat Ras cheeses without LH or BM were also studied. The chemical, physicochemical and proteolysis indices of all cheese treatments were measured throughout ripening for 90 days. The results show that addition of 0.5% and 1% BMP increased significantly ( $P < 0.05$ ) the moisture content, whereas salt on moisture (S/M %) was significantly ( $P < 0.05$ ) decreased in reduced-fat cheese when compared to reduced-fat control treatment. These results of moisture and S/M% were associated with improved proteolysis in these cheese treatments, which resulted in an improvement in the flavor and texture of the reduced fat Ras cheese. This impact was more pronounced with the addition of 1% BM than 0.5% BM. This trend of results was in accordance with that of scanning electron microscope analysis. Addition of BMP resulting in a more compact and dense cheese structure accompanied by regularly agglomerated protein aggregates. The sensory evaluation revealed possibility of improving consumer acceptability of reduced fat Ras cheese with the addition of 1% BM in the presence of LH as adjunct culture.

**Keywords:** Reduced fat Ras Cheese – Buttermilk – *L. helveticus*–Microstructure - Reverse-phase HPLC profile.

### INTRODUCTION

During the last decades, consumers become more aware about the relationship between diet and health. The higher consumption of dietary fat was associated with chronic diseases like obesity and coronary heart diseases (Miller and Rolls, 1996). Therefore, the demand on reduced or low fat food and dairy products were increased. Consequently, the demand of low fat cheese around the world have been significantly increased (Mistry, 2001). Fat is affecting significantly the texture, flavour, and consumer acceptability of cheese. This is the reason of why low-fat cheeses produced by conventional methods have sensory and texture defects (Banks, 2004). Therefore, many studies have been conducted to overcome the defects of low fat cheeses.

Ras cheese is one of the most popular cheeses in Egypt (Abou-Donia 2002). It is a hard cheese that is similar to a Greek type cheese named Kefalotyri (Hofi et al. 1970). This cheese type can be produced from cow's milk alone or mixed with buffalo's milk (Awad et al., 2003). In addition, the demand of low fat cheeses is also increased in Egypt. Therefore, the Egyptian's cheese industry is facing a demand to develop reduced or low fat cheeses with improved organoleptic and quality properties.

Flavor of cheese is an important determinant for consumer acceptability of reduced or low fat cheese. Proteolysis in cheese after manufacture and during ripening is affecting significantly the texture and flavor of Ras cheese (Awad et al., 2007). The non-starter lactic acid bacteria like *Lactobacillus* ssp. is considered as an important part of the flavor formation in cheese (Beresford et al., 2001). Recently, the adjunct culture of selected *Lactobacillus* ssp. Improved sensory profiling attributes of low fat cheese (Skeie et al., 2013). In a previous study, *Lactobacillus helveticus* was used as adjunct culture to improve the flavor of typical Ras

cheese due to its proteolytic activity in cheese (Awad et al., 2007).

Buttermilk is a by-product of butter manufacturing. It is rich in phospholipids that come from fat globule membrane during manufacturing of butter. The phospholipid content was found to be 8.5 times higher in buttermilk than in skimmed milk (Morin et al., 2008). Previously, the components of fat globule membrane from buttermilk showed many beneficial health effects (Dewettinck et al., 2008). Besides its nutritional benefits, buttermilk has been used to improve functional properties of different cheeses (Govindasamy-Lucey et al., 2006 & Morin et al., 2008). Recently, the addition of buttermilk powder improved texture and quality characteristics of low fat cheddar cheese (Romeih et al., 2012).

The aim of the present work was to use of both adjunct culture (*L. helveticus*) and buttermilk powder to improve flavor and texture of reduced fat Ras cheese. Chemical composition, Reverse-phase HPLC profile of the water-soluble fraction, and organoleptic properties of cheese after 90 days of ripening were measured. In addition, scanning electron microscope was used to study the micro-structural characteristics of the resultant ripened Ras cheese.

### MATERIALS AND METHODS

#### Milk, culture, rennet and buttermilk powder

Fresh cow milk was obtained from the Dairy Unit of Faculty of Agriculture, Cairo University and processed at the pilot laboratory of Dairy Science Department. A quantity of whole milk was kept as control (see experimental design and cheese making), and the remaining amount of the milk was subjected to separation (at 45°C) up to ~50% fat reduction.

A freeze-dried yoghurt culture (YO-MIX™) consists of *Str. thermophilus* and *Lb. bulgaricus* was obtained from Danisco, 75017 Paris, France. The culture was reactivated and grown at 43°C for 7 h

followed by refrigeration overnight before cheese manufacture. The activated starter cultures were added to cheese milks at a level of 1% (v/v). *Lactobacillus helveticus* CNRZ53 "LH", which was added to cheese as adjunct starter was obtained in MRS broth medium from Cairo Microbiological Resources Centre (MIRCEN), Ain Shams University, which was activated and sub-cultured for 12 h at 32°C in re-constituted skim milk (12.5% TS) at least twice before use, and then was added to the respective cheese milk treatments at a level of 0.5% (v/v), simultaneously with the main culture.

Commercial liquid calf rennet (0.125 N) obtained from the Dairy Unit of Faculty of Agriculture, Cairo University was used. A commercially available sweet buttermilk powder "BM" (Barry Farm Foods Co., Wapakoneta, Ohio 45895, USA) was used in this study. The chemical composition of BM was 34.3% protein, 5.8% fat, 3.4% moisture, 49.1% lactose and 7.4% ash. The BM was added to raw reduced-fat milk of the respective treatments at concentration of 0.5% and 1% (w/w).

#### **Experimental design and cheese making and ripening**

The experimental design was performed to compare full-fat and reduced-fat Ras cheeses as controls (without addition of LH or BM and coded as FF and LF treatments, respectively) with three different reduced-fat Ras cheeses consisted of the following treatment: reduced-fat with addition of LH, reduced-fat with addition of LH + 0.5% BM and reduced-fat with addition of LH + 1% BM; where are represented in codes LF-LH, LF-0.5%BM-LH and LF-1%BM-LH, respectively. Five cheese vats were made in three replicate blocks (i.e. cheese making days).

Before each cheese making session and prior to milk heat treatment, BM was added to raw reduced-fat milk according to their proposed treatments and stirred for 14 min or until no lumps were visible to ensure fully dissolved powder. All cheese milks were heat treated at 75°C/15s in double-walled stainless-steel vats followed by rapid cooling to 32°C, and inoculated with the cultures. The milk was ripened at 32°C until acidity developed to 0.19%, then the appropriate amount of rennet was added to clot the milk in about 40 min. Thereafter, the curd was cut vertically and horizontally into cubes using 0.5 inch knives. The curd was stirred and heated gradually to 45°C in 15min, and held at this temperature until the whey acidity reached 0.14%. About one third of the whey was drained off and commercial salt was added at the rate of 2%. After 15min, the whey was completely drained off and the curd was cooled. The curd was then molded and pressed for 24h. The cheese was turned over every day and rubbed with dry salt for 5d. The resultant cheese were then coated with plastic films and ripened at 13 ± 2°C and 80 ± 5% relative humidity for 90 days.

#### **Chemical analysis**

Moisture and titratable acidity (TA %) were determined according to AOAC (1990). Cheese dry matter (DM) was calculated by subtracting moisture content from 100. The cheese milk total solids were

determined using the moisture analyzer (Mettler Toledo Model HR73, Switzerland). The fat content in milk and cheese samples was determined by the Gerber method as described by Ling (1963), whereas NaCl content was determined according to IDF standard methods (17A: 1972).

The total nitrogen content (TN %) was measured by the Kjeldahl method (International Dairy Federation (IDF), 1993). Total protein content was calculated by multiplying the TN % by 6.38. The water-soluble fraction (WSN) was prepared essentially as described by Romeih et al. (2002), Using 20 g grated cheese with 100 ml H<sub>2</sub>O. Water-soluble N content of the cheese extract was determined by the Kjeldahl method using 10 ml of cheese extracts. The water content of the extracted cheese was taken into account when calculations of the N content of cheese extract were made; the water soluble nitrogen was expressed as a percentage of total nitrogen (WSN/TN). All samples were analyzed in duplicate. The water-soluble fraction (WSN) used in the chemical analysis was also employed for reverse phase RP-HPLC analysis, essentially as described by Michaelidou et al. (1998), to compare the casein degradation profile in the five different types of cheeses.

#### **Scanning Electron Microscopy (SEM)**

Small cubic samples from the center of the Ras cheese blocks (approximately 3 x 3 mm) were prepared using a surgical blade. The protein network of the cheese cubes was fixed overnight in 4% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 6.8. The samples were washed several times in 0.1 M sodium cacodylate buffer (pH 6.8) at 15 min intervals, and then the fat was fixed in 2% (w/v) osmium tetroxide (OsO<sub>4</sub>) in 0.1 M sodium cacodylate for 1-2 h. The cheese samples were re-washed several times in 0.1 M sodium cacodylate buffer at 15 min intervals, followed by dehydration in increasing concentrations of aqueous ethanol solutions (25%, 50%, 75%, 90% and 100%, 15 min in each). Samples were then dried to critical point using CO<sub>2</sub> in a Critical Point Dryer (Polaron, Waterford, England), and mounted on aluminum SEM stubs, sputter-coated with gold (Spi module sputter coater, spi supplies division of structure probe.). Samples were examined at 25 KV through scanning electron microscope (JEOL-jsm 5200, Faco Europe Sarl, 84120 Pertuis, France) and magnification of 1500x.

#### **Sensory evaluation**

Organoleptic assessment of the cheeses after 90 d of ripening was performed as described by Volikakis et al. (2004) using a seven graduated scale and reference substances to define the scale by five-member panel of the Department's staff selected on the basis of interest and experience in sensory evaluation of cheese products. The panel was asked to evaluate the coded samples of the five different types of cheese (all of the same batch) using a score from 1 to 7 on a numerical equal-interval scale assigned for each of the following attributes: appearance (color and surface holes), body and texture (hardness and crumbliness), elasticity, taste (flavor and saltiness) and total impression. Preference scale (very undesirable, undesirable, a little undesirable,

neutral, a little desirable, desirable and very desirable) was used for scoring total impression. Panel members were also instructed to report any defects of sensory characteristics for the cheese samples (e.g. bitter, rancid, spicy flavor, mottled appearance). Cheese samples were tempered by holding at ambient temperature ( $20 \pm 5^\circ\text{C}$ ) and then presented to the panelists in a random order for testing.

**Statistical analysis**

Statistical analysis was performed by Minitab® 16 (MINITAB Inc., State College, PA, USA), using the general linear model (GLM) and Tukey’s test for pair wise comparison in analysis of variance.

The factorial design was made with three factors for chemical analysis, Replicate batches (3 levels), cheese treatment (5 levels) and ripening time (5 levels), whereas for sensory analysis was two factors: Replicate batches (3 levels), cheese treatment (5 levels). Each of the combined factors had two replication for the chemical parameters and five replications as five panelists for sensory evaluation.

**RESULTS AND DISCUSSION**

The chemical composition and titratable acidity (TA) of cheese milk is presented in Table (1).

**Table 1. Chemical composition and titratable acidity (TA, % as lactic acid) of milk used in the manufacture of Ras-cheeses**

	Full-fat milk	reduced-fat milk
Fat %	3.35 ± 0.15	1.65 ± 0.06
Protein %	3.32 ± 0.10	3.41 ± 0.14
Total solids %	12.27 ± 0.28	10.17 ± 0.19
TA%	0.17 ± 0.02	0.17 ± 0.01

Values are means of double measurements of 3 batches ± standard deviation.

**Chemical and physicochemical properties of Ras cheese**

Table (2) is showing chemical composition of Ras cheese when fresh and during 90 days of ripening. As can be noticed, moisture of reduced fat cheese control (LF) was increased significantly ( $P<0.05$ ) with the addition of 0.5&1% buttermilk (BM) and *L. helveticus* (LH). In other studies, addition of buttermilk was associated with reduction of dry matter of the final low fat Norvegia (Skeie *et al.*, 2013) and low fat cheddar cheeses (Romeih *et al.*, 2012). The reduced fat cheese made with the addition of LH without BM did not show similar significant ( $P>0.05$ ) increase in moisture content. Generally, Moisture content of all cheeses was reduced significantly ( $P<0.05$ ) all over the ripening period (Table 2). Similarly, Abdel Baky *et al.* (1986) found that *L. helveticus* did not show any significant effect on the moisture content of the resultant Ras cheese.

There was no significant effect ( $P>0.05$ ) of all treatments on the fat content when compared with LF cheese (Table 2). When fat was calculated as a ratio to the dry matter (F/DM), buttermilk treatments (0.5 & 1 %

with the addition of LH) showed a significant increase ( $P<0.05$ ) in F/DM values compared with the LF cheese. Both fat and F/DM contents increased significantly ( $P<0.05$ ) during ripening period. Similarly, Abdel Baky *et al.* (1986) found that *L. helveticus* did not show any significant effect on the fat content of the resultant cheese.

Table (2) is showing the results of total nitrogen (TN). It can be noticed that only the reduced fat treatment that containing 1% BM and LH was having a significant higher TN content ( $P<0.05$ ) when compared to the LF cheese. The TN content was increased gradually during storage period ( $P<0.05$ ).

The salt content of all cheeses are presented in Table (2). Addition of BM (with LH) was associated with a decreased salt content ( $P<0.05$ ). Similarly, the addition of BM was showing the same trend when the ratio of salt to moisture (SM %) was calculated. Addition of BM (0.5 & 1%) and LH showed a significant decrease ( $P<0.05$ ) in both salt content and SM% during ripening period. These results are in line with those found by Abdel Baky *et al.* (1986) who found that *L. helveticus* did not show any significant effect on the salt content of the resultant cheese.

**Table 2. Effect of cheese treatment and ripening time on gross composition of Ras-cheeses, as indicated by General Linear Model analysis.**

Factors		Moisture %	Fat %	*F/DM %	TN %	Salt %	SM %
Cheese treatment (n = 15)	FF	37.0 <sup>D</sup>	31.2 <sup>A</sup>	49.5 <sup>A</sup>	3.7 <sup>C</sup>	1.3 <sup>AB</sup>	3.5 <sup>A</sup>
	LF	38.1 <sup>C</sup>	17.2 <sup>B</sup>	27.7 <sup>D</sup>	3.8 <sup>B</sup>	1.3 <sup>A</sup>	3.6 <sup>A</sup>
	LF-LH	37.5 <sup>CD</sup>	17.5 <sup>B</sup>	27.9 <sup>D</sup>	3.9 <sup>AB</sup>	1.3 <sup>AB</sup>	3.5 <sup>A</sup>
	LF-0.5%BM-LH	40.7 <sup>B</sup>	17.1 <sup>C</sup>	28.6 <sup>C</sup>	3.8 <sup>AB</sup>	1.2 <sup>BC</sup>	3.0 <sup>B</sup>
	LF-1%BM-LH	41.7 <sup>A</sup>	17.0 <sup>C</sup>	29.3 <sup>B</sup>	3.9 <sup>A</sup>	1.2 <sup>C</sup>	2.8 <sup>C</sup>
SE		0.702	0.264	0.231	0.061	0.088	0.166
Ripening time (n = 15)	Fresh	42.4 <sup>A</sup>	18.3 <sup>E</sup>	31.6 <sup>E</sup>	3.6 <sup>E</sup>	1.1 <sup>C</sup>	2.6 <sup>E</sup>
	15 d	41.0 <sup>B</sup>	19.2 <sup>D</sup>	32.3 <sup>D</sup>	3.7 <sup>D</sup>	1.2 <sup>C</sup>	2.8 <sup>D</sup>
	30 d	39.4 <sup>C</sup>	19.9 <sup>C</sup>	32.7 <sup>C</sup>	3.8 <sup>C</sup>	1.2 <sup>C</sup>	3.2 <sup>C</sup>
	60 d	37.0 <sup>D</sup>	20.9 <sup>B</sup>	33.0 <sup>B</sup>	3.9 <sup>B</sup>	1.3 <sup>B</sup>	3.7 <sup>B</sup>
	90 d	35.2 <sup>E</sup>	21.7 <sup>A</sup>	33.4 <sup>A</sup>	4.1 <sup>A</sup>	1.5 <sup>A</sup>	4.2 <sup>A</sup>
SE		0.507	1.50	2.264	0.022	0.024	0.084

SE: Standard Error

\*Abbreviations are as follows: F/DM- fat in dry matter basis, TN-total nitrogen, SM-salt in moisture basis.

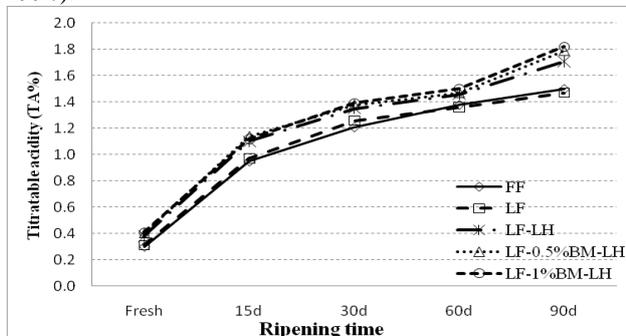
Means with different superscripts in the same column are significantly different ( $P<0.05$ ).

Values are means of n=15 determinations.

FF: full fat control, LF: reduced fat control, LF-LH: reduced fat with addition of LH, LF-0.5%BM-LH: reduced fat with addition of LH and 0.5% BM, LF-1%BM-LH: reduced fat with addition of LH and 1% BM.

The percentages of titratable acidity (TA %) of Ras cheese treatments are illustrated in Fig. (1). The TA% of all cheese treatments was increasing during ripening period. It can be noticed that the TA% of both full fat control cheese (FF) and LF was showing a similar trend during ripening. The TA% was tended to be higher than that of control cheeses by the addition of either LH or LH+BM (0.5 & 1%). At the end of storage, the TA% of Ras cheese that contains LH+BM (0.5 &

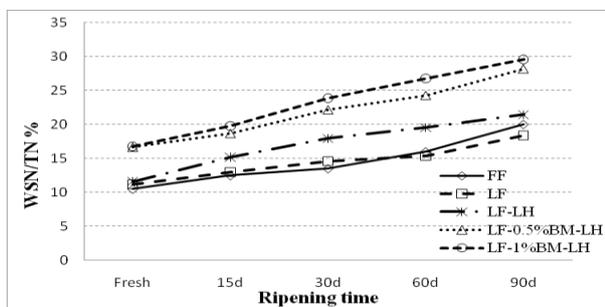
1%) was showing the highest values (1.79 and 1.82, respectively,  $P < 0.05$  vs LF). Similar effect of LH on the TA% of the resultant cheese was reported earlier by Kebary et al. (1996). The chemical composition and TA% of all cheese treatments in the present study when fresh and during ripening were in agreement with those reported earlier for Ras cheese (Awad et al., 2007; Abou-Donia, 2002; Awad et al., 2003; Kebary et al., 1996).



**Fig. (1). Effect of *L. helveticus* (LH) and buttermilk powder (BM) on titratable acidity (TA %) of Ras cheese treatments during ripening.**

**Water-soluble nitrogen (% of TN) of Ras cheese during ripening:**

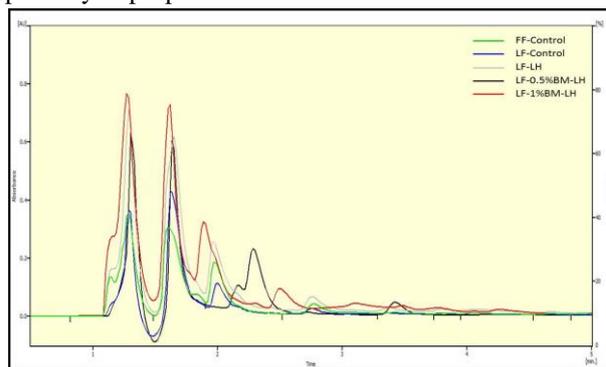
Water-soluble nitrogen (WSN) as a percentage of total nitrogen (TN) is presented in Fig. (2). Due to the fact that WSN is an indicator of proteolysis in cheese, it increased in all Ras cheese treatments during ripening period. It is obvious from Fig. (2) that cheese treatments containing LH+BM (0.5 and 1%) were showing the highest WSN% when compared to the LF control (59 and 77% increase vs LF, respectively,  $P < 0.05$ ). Although the WSN of LH treatment did not differ significantly from that of LF, it showed an increase tendency ( $P > 0.05$ ). All of these results indicated more proteolysis in Ras cheese with the addition of BM. This could be due to the lower SM% in cheese made with the addition of 1% BM which did not suppress activity of proteolytic enzymes (Kebary et al., 1996). Moreover, addition of *L. Helveticus* to Ras cheese was associated with a significant increase ( $P < 0.05$ ) in the formation of water soluble nitrogen of the resultant cheese that could be due to the ability of LH culture to produce more peptidases and proteinases (Abdel Baky et al., 1986).



**Fig. 2. Effect of *L. helveticus* (LH) and buttermilk powder (BM) on water-soluble nitrogen (as % of TN) of Ras cheese treatments during ripening.**

**Reverse-phase HPLC profile of the water-soluble fraction**

RP-HPLC Chromatograms of the water soluble extracts of five Ras cheese treatments at 90 d are shown in Fig. 3. Three major peaks with retention time of 10-25 min were presented in all cheese treatments. According to their retention times, these peaks contain hydrophilic peptides. There was small difference of the concentration of peptides in the major peaks between FF-control and LF-control cheeses. The concentration of peptides in the major peaks was enhanced with the addition of *L. helveticus* to LF-Control cheese due to the proteolytic activity of LH strain (Luoma et al., 2001). Ong and Shah (2008) reported that addition of *L. acidophilus* L10 and *L. helveticus* H100 as adjunct culture to Cheddar cheese improved the proteolytic activity. The peptide concentration of the major peaks for LF-0.5%BM-LH and LF-1%BM-LH treatments were higher than those in the other treatments. In addition, there was a new peak appeared in LF-1%BM-LH profile with retention time 25 min. This result can be explained by the addition of BM that enhanced the proteolytic activity in LF-LH cheese by holding moisture in the matrix of cheese (Table 2). This result is in agreement with Govindasamy-Lucey et al., (2006) who found that using buttermilk in manufacture of pizza cheese enhanced the proteolysis properties.



**Fig.3. Reverse-phase HPLC profile of the water-soluble fraction of Ras-cheese treatments after 90 days of ripening.**

**Sensory evaluation of Ras cheese:**

The sensory attributes of Ras cheeses are shown in Table (3). It is obvious that the addition of either LH alone or combined with 0.5 or 1% BM improved sensory attributes of the resultant cheeses when compared with LF control cheese. The addition of LH+BM (1%) showed body & texture and elasticity that were similar to that of FF control ( $p > 0.05$ ) as shown in Table (3). In addition, the same treatment showed values of appearance and taste that were very near to those of FF control. These results revealed that addition of 1% BM and using LH as starter bacteria could improve the sensory properties of reduced fat Ras cheese. Presence of LH that increased WSN in cheese could improve formation of flavor in reduced fat cheese. These results are in accordance with those found by (Awad et al., 2007; Abou-Donia, 2002; Awad et al., 2003; Kebary et al., 1996). In this context, Romeih et al

(2012) reported that addition of BM improved sensory attributes of low fat cheddar cheese through softening of texture and reduction of hardness of the resultant cheese. Similarly, the texture of low fat Norvegia cheese was improved by supplementation with buttermilk (Skeie *et al.*, 2013). Some investigators suggested that

nitrogenous compounds (like free amino acids) could be converted through specific metabolic pathway into volatile fatty acids that work as a flavor precursor (Abdel Baky *et al.*, 1986).

**Table 3. Sensory attributes ratings (scores 1 to 7) of the 90-d ripened Ras-cheeses as indicated by General Linear Model analysis.**

Factors		Appearance	Body & Texture	Elasticity	Taste	Overall impression
Cheese treatment (n = 15)	FF	6.33 <sup>A</sup>	4.67 <sup>A</sup>	5.67 <sup>A</sup>	6.33 <sup>A</sup>	6.33 <sup>A</sup>
	LF	3.67 <sup>B</sup>	2.33 <sup>B</sup>	3.00 <sup>C</sup>	3.33 <sup>C</sup>	3.33 <sup>D</sup>
	LF-LH	4.33 <sup>B</sup>	2.66 <sup>B</sup>	3.67 <sup>B</sup>	4.00 <sup>C</sup>	4.00 <sup>C</sup>
	LF-0.5%BM-LH	5.67 <sup>A</sup>	4.33 <sup>A</sup>	5.33 <sup>A</sup>	5.00 <sup>B</sup>	5.67 <sup>B</sup>
	LF-1%BM-LH	6.00 <sup>A</sup>	4.67 <sup>A</sup>	5.67 <sup>A</sup>	5.33 <sup>B</sup>	6.00 <sup>A,B</sup>
SE		0.607	0.513	0.459	0.562	0.397

SE: Standard Error

Means with different superscripts in the same column are significantly different ( $P < 0.05$ ).

Values are means of n=15 determinations.

FF: full fat control, LF: reduced fat control, LF-LH: reduced fat with addition of LH, LF-0.5%BM-LH: reduced fat with addition of LH and 0.5% BM, LF-1%BM-LH: reduced fat with addition of LH and 1% BM.

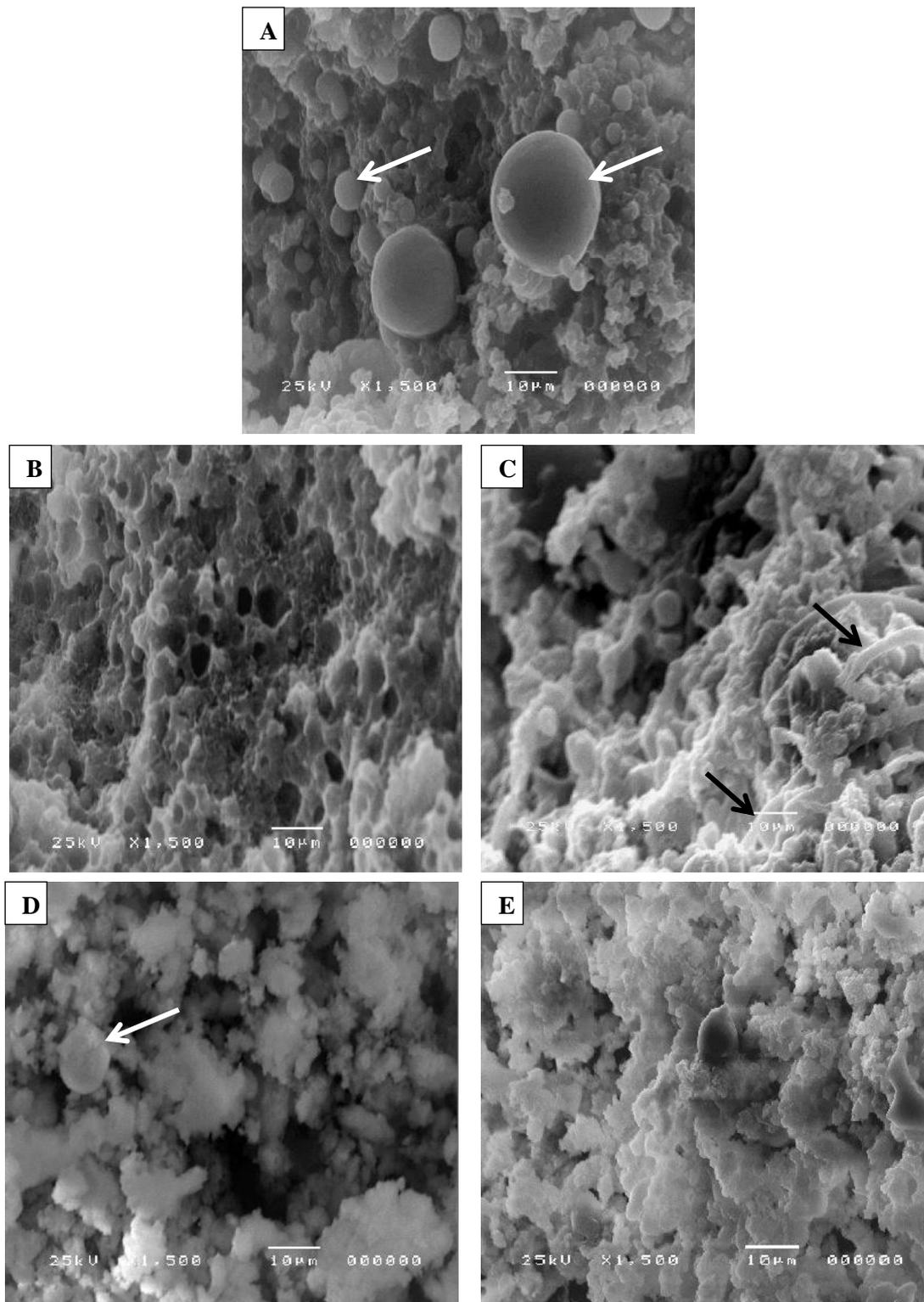
### Microstructure of Ras cheese

The SEM micrographs of the reduced-fat Ras cheeses ripened for 90 d are shown in Fig. 4. The protein matrix (gray area) formed a continuous phase permeated by an amorphous system of voids (black areas), and spherical fat globules made the spatial dimensions of these images. As these micrographs show, an obvious variation in the cheese microstructure was obtained between full-fat and reduced-fat Ras cheeses as control treatments without addition of *L. helveticus* and/or BMP. An extremely porous, open and coarse structure was obtained in reduced-fat Ras cheese (Fig. 4B), whereas a continuous phase of protein aggregate network characterized by a more compacted and dense structure accompanied by less voids was revealed in the full-fat Ras cheese where the spherical fat globules of different sizes (pointed with white arrows) were obviously dispersed and scattered throughout the protein matrices. This result is in parallel with that of Lobato-Calleros, *et al.* (2007) and Liu *et al.* (2008).

As it might be seen in Fig. 4C, the protein network appeared to be made up from rather densely aggregated protein particles where the adjunct lactobacilli rods (pointed with black arrows) were embedded and dispersed uniformly throughout the protein matrix. Despite the fact that uniform protein content was achieved in reduced-fat cheese treatments (Table 1), cheeses with addition of BMP and/or *L. helveticus* characterised by a compact fusion and a dense structure. The higher cheese acidity obtained in LF-LH, LF-0.5%BMP-LH and LF-1%BMP-LH cheeses (Fig. 1) as a function of adjunct lactobacilli addition may contribute to the induced protein aggregation and compacted structure of these reduced-fat Ras cheeses. In

this context, Ong *et al.* (2012) stated that the microstructure of curd made at lower pH had a denser and more compact structure than the microstructure observed in samples at a higher pH, and that the protein micelles has different rate and altered mechanism of aggregation as a result of acidity changes.

The manifested microstructure in Fig. 4D and 4E clearly revealed that addition of BMP to reduced-fat cheese treatments promoted regularly aggregated protein matrices and interconnectivity of the network accompanied by irregularly clustered protein folds. The relatively high levels of fused proteins and the increased agglomerated clusters obtained by addition of BMP were most probably attributed to its high levels of milk fat globule membrane components (MFGM) components as well as total protein content (Table 2). This finding is in agreement with those of Lopez *et al.* (2007) and Romeih *et al.* (2012). Morin *et al.* (2008) reported that MFGM fragments may physically be entrapped within the paracasein network, which in turn could induce direct interactions with casein by folding casein micelles inside reconstituted aggregates. Moreover, it has been reported that MFGM fragments could induce direct physical and chemical interactions with casein (CN) by folding CN micelles inside reconstituted aggregates (Ong *et al.*, 2010). A closer observation of the microstructure details in these micrographs revealed that the impact of BMP on Ras cheese microstructure characteristics was more pronounced and further intensified by increasing BMP addition as illustrated in SEM micrographs of LF-1%BMP-LH compared to LF-0.5%BMP-LH treatments (Fig. 4E and 4D, respectively). This trend of result is in close agreement with the result of Mistry *et al.* (1996) for reduced-fat cheddar cheese made with ultrafiltered sweet buttermilk.



. 4. Scanning electron micrographs (1500 x) of Ras-cheese treatment; (A) full-fat control cheese, (B) reduced-fat control cheese, (C) reduced-fat + *L. helveticus*cheese, (D) reduced-fat + BM 0.5% + *L. helveticus*cheese and (E) reduced-fat + BM 1% + *L. helveticus*cheese. Scale bar is 10 µm.

## CONCLUSION

The present study was conducted to improve both flavor and texture of reduced fat Ras cheese (a common Egyptian hard cheese). Addition of *L. helveticus* as adjunct culture improved proteolysis,

which in turn improved flavor of the final reduced fat Ras cheese. Addition of buttermilk resulted in more softer Ras cheese texture. The obtained results revealed that reduced fat Ras cheese could be produced by addition of 1% buttermilk in the presence of *L. helveticus* as adjunct culture with improved quality

characteristics that are so close to those of full fat Ras cheese.

## REFERENCES

- Abdel Baky, A. A., El-Neshawy A. A., Rabie A. M. and Ashour M. M. (1986) Heat shocked lactobacilli for accelerating flavour development of Ras cheese. *Food Chemistry*, 21:301-313.
- Abou-Donia, S. A. (2002). Recent developments in Ras cheese research: a review. *Egyptian Journal of Dairy Science* 30,155–166.
- AOAC (1990). Association of official analytical chemists. Official methods of analysis (15th ed.) .Association of Anal. Chem. INC., Suite 400, 2200, Wilson Boulevard, Arlington, Virginia22201, USA.
- Awad S., Ahmed N., and El Soda M. (2007).Evaluation of isolated starter lactic acid bacteria in Ras cheese ripening and flavour development. *Food Chemistry*, 104:1192–1199.
- Awad, S., El-Attar, A., Ayad, A. H., and El-Soda, M. (2003).Characterisation of Egyptian Ras cheese. 1. Sensory evaluation, rheological, physico-chemical, properties and microbiological analysis. *Egyptian Journal of Dairy Science*, 31: 289–303.
- Banks, J. M. (2004). The technology of low-fat cheese manufacture. *International Journal of Dairy Technology*, 57, 199-207.
- Beresford, T. P., Fitzsimons N.A., Brennan N.L., and Cogan T.M. (2001).Recent advances in cheese microbiology. *International Dairy Journal*, 11:259–274.
- Dewettinck, K., Rombaut, R., Thienpont, N., Le, T. T., Messens, K., and Van-Camp, J. (2008).Nutritional and technological aspects of milk fat globule membrane material. *International Dairy Journal*, 18, 436e457.
- Govindasamy-Lucey, S., Lin, T., Jaeggi, J.J., Johnson, M.E., and Lucey, J.A.(2006). Influence of condensed sweet cream buttermilk on the manufacture, yield, and functionality of pizza cheese. *Journal of Dairy Science*, 89,454-467.
- IDF/FIL International Dairy Federation (1972). Determination of chloride content of cheese.*IDF Standard 17A*, Brussels, Belgium.
- IDF/FIL International Dairy Federation (1993). Determination of nitrogen content (Kjeldahl method). *IDF Standard 20B*, Brussels, Belgium.
- Kebary, K. M. K., Khader, A. E., Zedan, A. N., and Mahmoud, S. F. (1996). Accelerated ripening of low fat Ras cheese byattenuated lactobacilli cells. *Food Research International*, 29(8):705-713.
- Ling, E. R. (1963). A text book of dairy chemistry.Vol. 2 (3<sup>rd</sup>ed.). Chapman & Hall (eds.), London.
- Liu, H., Xu, X. M. and Guo, S. D. (2008). Comparison of full-fat and low-fat cheese analogues with or without pectin gel through microstructure, texture, rheology, thermal and sensory analysis. *International Journal of Food Science and Technology*, 43, 1581-1592.
- Lobato-Calleros, C., Reyes-Hernandez, J., Beristain, C. I., Homelas-Uribe, Y., Sanches-Garcia, J. E. and Vemon-Carter, E. J. (2007). Microstructure and texture of white fresh cheese made with canola oil and whey protein concentrate in partial or total replacement of milk fat. *Food Research International*, 40, 529-537.
- Lopez, C., Camier, B.andGassi, J. Y. (2007). Development of the milk fat microstructure during the manufacture and ripening of Emmental cheese observed by confocal laser scanning microscopy. *International Dairy Journal*, 17, 235–247.
- Luoma, S., Peltoniemi, K. and Joutsjoki, V. (2001). Expression of six peptidases from *Lactobacillus helveticus* in *Lactococcus lactis*. *Appl Environ Microbiol*, 67, 1232–1238.
- Michaelidou, A., Alichanidis, E., Urlaub, H., Polychroniadou, A. and Zerfridis, G. K. (1998).Isolation and identification of some major water-soluble peptides in Feta cheese. *Journal of Dairy Science*, 81,3109–3116.
- Miller, D. L. and Rolls, B. J. (1996). Implications of fat reduction in the diet. In *Handbook of Fat Replacers*, pp. 27 – 45. Roller S, Jones S A eds. Florida: CRC Press.
- Mistry, V. V. (2001). Low fat cheese technology. *Journal of Dairy Science*, 11: 413–422.
- Mistry, V. V., Metzger, L. E., and Maubois, J. L. (1996). Use of ultrafiltered sweet buttermilk in the manufacture of reduced fat Cheddar cheese. *Journal of Dairy Science*, 79, 1137–1145.
- Morin, P., Pouliot, Y., and Britten, M. (2008). Effect of buttermilk made from creamwith different heat treatment histories on properties of rennet gels and model cheeses. *Journal of Dairy Science*, 91, 871-882.
- Ong, L. and Shah, N.P. (2008). Influence of Probiotic *Lactobacillus acidophilus* and *L.helveticus* on Proteolysis, Organic Acid Profiles, and ACE-Inhibitory Activity of cheddar Cheeses Ripened at 4, 8, and 12 °C. *Journal of Food Science*, 73: M111–M120.
- Ong, L., Dagastine, R. R., Kentish, S. E. and Gras, S. L. (2010). The effect of milk processing on the microstructure of the milk fat globule and rennet induced gel observed using confocal laser scanning microscopy. *Journal of Food Science*, 75, 135-145.
- Romeih E. A., Moe K. M., and Skeie S. (2012).The influence of fat globule membrane material on the microstructure of low-fat Cheddar cheese. *International Dairy Journal*, 26: 66-72.
- Romeih, E. A., Michaelidou, A., Biliaderis, C. G. and Zerfiridis, G. K. (2002). Low-fat white-brined cheese made from bovine milk and two commercial fat mimetics: chemical, physical and sensory attributes. *International Dairy Journal*, 12, 525-540.
- Skeie S., Alseth G.M., Østlie H., Abrahamsen R.K., Johansen A.G., and Øyaas, J. (2013). Improvement of the quality of low fat cheese using a two step strategy. *International Dairy Journal*, 33 (2):153–162.
- Volikakis, P., Biliaderis, C., Vamvakas, C. and Zerfiridis, G. K. (2004). “Effects of a commercial oat-b-glucan concentrate on the chemical, physico-chemical and sensory attributes of a low-fat white-brined cheese product”. *Food Research International*, 37, 83-94.

تأثير استخدام *L. helveticus* واللبن الخض المجفف علي جودة الجبن الراس منخفض الدسم  
محمود عبد الحميد ، عصام محمد حمد و ايهاب روميح  
قسم الألبان - كلية الزراعة - جامعة القاهرة

في الاونة الاخيرة زاد اقبال المستهلكين علي تناول الجبن منخفض الدسم. ويؤدي خفض الدهن إلي ظهور عيوب في الجبن ويقلل من قابليته للاستهلاك. وقد صممت هذه الدراسة بهدف تحسين نكهة وقوام الجبن الراس منخفض الدهن (خفض ٥٠ %) وذلك باستخدام بكتريا (*L. helveticus* ( LH) واللبن الخض المجفف. تم تصنيع جبن راس منخفض الدسم باستخدام LH ، LH + ٠.٥ % لبن خض مجفف ، LH + ١ % لبن خض مجفف. كما تم تصنيع جبن راس كامل الدسم وجبن راس منخفض الدسم للمقارنة (كونترول). تم تقدير الخواص الكيمائية والطبيعية الكيمائية ودلائل التحلل البروتيني في كل المعاملات علي مدار ٩٠ يوم من التسوية. وقد أظهرت النتائج أن اضافة اللبن الخض بنسبة ٠.٥ % و ١ % أدت الي زيادة معنوية في محتوى الرطوبة بالجبن وانخفاض معنوي في محتوى الملح/الرطوبة مقارنة بالجبن الكونترول منخفض الدهن مما أدى إلي تحسن معدل التحلل البروتيني في هذه المعاملات وبالتالي تحسن قوام ونكهة الجبن لهذه المعاملات. وكان التأثير أكثر وضوحاً في حالة اضافة ١ % لبن خض مجفف. أظهرت نتائج الميكروسكوب الالكتروني أن إضافة اللبن الخض المجفف أدت الي ظهور تركيب بنائي دقيق أكثر تماسكا وكثافة مع ظهور تكتلات بروتينية مندمجة في هذا التركيب البنائي. وقد اشارات نتائج التقييم الحسي ان استخدام LH مع اضافة اللبن الخض يمكن ان تحسن من قبول الجبن الراس منخفض الدسم لدي المستهلكين.