Influence of Milk Treatment and Adjunct Culture on Quality of Ras Cheese

Awad, S.A., Nor El-Hoda, A. Mohammed & El Soda, M.A.
Dairy Sci. & Technol. Dept., Fac. of Agric., Alex. Univ., Alexandria, Egypt

ABSTRACT

Classical techniques used to improve milk's shelf life and safety are based on heat treatments, like pasteurization and sterilization. Those techniques modify some physic-chemical properties of milk, for example its coagulation by rennet. Microfiltration constitutes an alternative to heat treatment to reduce the presence of bacteria and improve the microbiological safety of dairy products without modifying the physic-chemical properties of milk. In this study, the effect of microfiltration (MF) and pasteurization on proteolysis, lipolysis and flavor development in Ras cheese during 4 months of ripening were studied. Mixtures of adjunct cultures, isolated from artisanal dairy products, have been also evaluated in experimental Ras cheese for flavor development. In the first trial, raw skim milk was microfiltered and then the protein/ fat ratio was standardized using pasteurized cream. The pasteurized milk with same protein/ fat ratio was also used in the second trial. Milk treatment "microfiltration or pasteurization" affect in the cheese making procedure for amount of added chymosin and cooking time. The chemical composition of cheeses seems to be affected by milk treatment "microfiltration or pasteurization" rather than the culture types. The moisture content was higher and the pH was lower in pasteurized milk cheeses than in microfiltrated milk cheeses at day one of manufacture. Proteolysis and lipolysis during cheese ripening were lower in microfiltrated milk cheeses comparing to pasteurized milk cheeses. Very significant variations in free amino acids, free fatty acids and sensory evaluation have been found among the cultures used in Ras cheesemaking. In general, microfiltrated milk cheeses received higher score in body & texture and lower score in flavor comparing with pasteurized milk cheeses.

Key words: Ras cheese, microfiltration, pasteurization, proteolysis

INTRODUCTION

Before heat treatment was introduced, milk was a source of infection, as it is a perfect growth medium for microorganisms. Diseases such as tuberculosis and typhus were sometimes spread by milk. Fortunately, all common pathogenic organisms likely to occur in milk are killed by relatively mild heat treatment, which has only a very slight effect on the physical and chemical properties of milk. The most resistant organism is thermoduric bacteria, which can survive pasteurization conditions, and spore formers, which produce the heat- and desiccation-resistant structures known as spores (Bramley & McKinnon 1990).

Membrane filtration processes are not new in the cheese industry. Ultrafiltration of milk prior to cheesemaking has been widely used (Cheryan, 1998). It markedly increases cheese yield by retaining whey proteins in the cheese but it has been shown to cause textural and flavor defects in Cheddar and Mozzarella cheeses (Jameson and Lelievre 1996), largely due to the retention of whey proteins (Bech, 1993). On the other hand, microfiltration (MF) of skim milk prior to cheesemaking offers the advantage that milk serum proteins permeate the membrane and hence they are not retained in the cheese, but the MF is not expected to increase cheese yield (Papadatos *et al*., 2003).

Microfiltration is the passage of product under relatively low pressure (approximately 1 bar) through a semipermeable membrane with pore sizes ranging from 0.2 to 5 μm (Olesen & Jensen 1989). As bacteria generally range from 1 to 3 μm, under some circumstances MF should be able to completely remove bacteria from the fluid permeate. MF might provide a lower temperature option, and thus, a less pronounced cooked flavor than pasteurize processing for extended shelf-life dairy products and no calcium chloride should be added in MF milk for cheese making.

Microfiltration has been shown to be effective in reducing the number of bacteria in skim milk (Kelly & Tuohy 1997). Reduction in total bacteria of 2.8 logs (Hoffmann *et al*., 1996) has been reported for the Bactocatch MF process. Microfiltration utilizing a 1.4-μm membrane provides complete

removal of somatic cells from skim milk (Giffel & van der Horst 2004). Olesen & Jensen (1989) found that the initial content of *Bacillus cereus* spores in milk had a significant effect on the content of spores in the microfiltered milk.

McSweeney *et al*. (1993) found no differences between Cheddar cheese made from pasteurized or microfiltered milk. However, Beuvier *et al*. (1997) concluded that microfiltration reduced the total amount of bacteria more effectively than pasteurization and those facultative heterofermentative lactobacilli grew slower in cheese from pasteurized milk. The observed sensory differences in the cheeses were attributable to the various treatments. Skeie & Ardo (2000) showed that cheeses made from raw, pasteurized and microfiltered milk influenced the profiles of free amino acids in a Gouda type cheese.

Since microfiltration reduces microorganisms more effectively than pasteurization (Kelly & Tuohy 1997), the lactobacilli associated with good quality cheese would probably be removed also. Adjunct cultures are nonstarter lactic acid bacteria, consisting mainly of lactobacilli, which are used in addition to a standard starter to improve and to enhance the flavor of cheese (El Soda *et al*., 2000). Non-starter lactic acid bacteria (NSLAB) have been isolated from traditional Egyptian dairy products (El-Soda *et al*., 2003), and the influence of theses bacteria on the quality of Ras cheese has been investigated (Awad *et al*., 2007), among several species evaluated, *Lactobacillus helveticus*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus delbrueckii* subsp *lactis* and *Enterococcus faecium* were associated with good quality Ras cheese. The objective of the present study was to investigate the effect of milk treatment (pasteurization, microfiltration) and the addition different concentrations of adjunct culture on Ras cheese quality. The chemical composition, microbiological and biochemical changes during the cheese ripening were measured, in particular the interaction between biochemical changed during ripening and sensory evaluation.

MATERIALS AND METHODS

Starter cultures

Commercial lactic culture (DVS R707, Chr. Hansen's Lab., Denmark), contains *Lactococcus lactis* subsp *lactis* and *Lactococcus lactis* subsp *cremoris*.

Adjunct culture

Adjunct cultures of *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus curvatus*, *Lactobacillus rhamnosus*, *Lactobacillus helveticus* and 3 strains of *Enterococcus faecium* were obtained from the collection of Faculty of Agriculture, Alexandria University (El-Soda *et al*., 2003). Enterococci strains were examined for haemolytic activity before using in cheese making.

The adjunct cultures were grown and the cells were harvested and washed as described earlier (Awad *et al*., 2007). Two mixtures of adjunct culture isolated from Egyptian dairy products have been used in this study. The first mixture contains *Lactobacillus delbrueckii* subsp. lactis, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus helveticus* and 3 strains of Enterococcus faecium, while the second mixture contains *Lactobacillus delbrueckii* subsp. lactis, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus curvatus*, *Lactobacillus rhamnosus*, and 2 strains of *Enterococcus faecium*. The two mixtures, which adjusted to 1 OD in phosphate buffer pH 7.0 (Awad *et al*., 2007), were used at levels of 0.5, 1, 2 ml/ kg milk,

Commercial adjunct culture (DVS, CR401, Chr. Hansen's Laboratory, Denmark) contains *Lactobacillus delbrueckii* subsp. lactis, *Lactobacillus paracasei* subsp. *paracasei*, and *Lactobacillus helveticus*, was also used at level of 0.0275 g/ kg milk.

Milk

Raw whole cow and buffalo milk were obtained from the dairy farm at the Alexandria University. A mixture of 70:30 of raw cow and buffalo milk (fat: 4.5 %; acidity: 0.16 - 0.17 % as lactic acid) was used in this study. Raw milk was skimmed (<0.05 % fat) in mechanical separator (Alfa-Laval, Sweden).

Treatment of cheese milk

Heat pasteurization

The skim milk and cream were pasteurized using a high-temperature short-time technique in a tubular heat exchanger (Actini, France). The heat treatment employed was 74°C /15 s for skim milk and 78°C/15 s for cream, followed by quick cooling to 35°C. Pasteurized skim milk was mixed with pasteurized cream to obtain closest initial level of milk fat (4.4–4.5 %).

Microfiltration

Raw skim milk was microfiltered using microfiltration unit (Alfa-Laval, Sweden) with a ceramic membrane pore size of 1.4 μm, membrane area 0.2 m^2; flow rate 150 L/h^{-1} m^{-2}, and temperature 50°C. Cold pasteurized skim milk (micro-filtered) was mixed with pasteurized cream to obtain closest initial level of milk fat (4.4–4.5 %).

Cheese making procedure

Three replicates of experimental Ras cheeses for each treatment were processed using computer-controlled cheese equipment (INRA, Poligny, France) equipped with four 11 L vats. Seven treatments of Ras cheeses were made from pasteurized milk (Past) and other seven treatments from microfiltrated milk (MF). The four vats and these treatments were rotated for each replication to reduce systemic errors. Calcium chloride solution was added at a rate of 0.2 g/kg of pasteurized milk just before adding the starter culture. Starter cultures (DVS culture, R707, 0.15 g /Kg), different doses of freeze-shocked suspension of adjunct cultures (0.5, 1, 2 mL/ Kg) were added individually to milk at 35˚C, while DVS adjunct culture CR 401 was added at level of 0.0275 g/ Kg. The inoculated milk is held for 60-75 min at 35°C and then 15 ml of 2% rennet (Chymax-II 500: Chr. Hansen's Lab., Denmark) was added to each vat of pasteurized milk to coagulate the milk in 30 min, while 11 ml of 2% rennet was added to each vat of MF milk. The coagulum was cut into cubes (`~ 2 cm) and the curds were allowed to rest in the whey for 5 -10 min. The curds were cooked to 45°C over 50 and 40 min of pasteurized and microfiltrated milk respectively, and held at this temperature for 15 more minutes. A part of the whey was drained off when its acidity reached to 0.14 % (as lactic acid w/v), and then salt was added (3% salt) and mixed for 15 min as described by Hofi, *et al*., 1970. The obtained curd was hooped and pressed at 66 Psi for 4 h, and then the cheeses were turned and repressed at 120 Psi for 12 more h. During the first month of ripening, the second salting was performed as described earlier (Awad *et al*., 2007). When the salt in cheese reached to 3.5–4 %, the cheese surface was well washed and the Cheeses were waxed by quick immersion in the molten wax (Chr. Hansen's Lab., Denmark) and lifted up to cool for 2 h before removal to the ripening room (12±2°C and 80±5% relative humidity).

Cheese composition analysis

Total protein was measured by Kjeldahl (AOAC, 2000), Fat content by Gerber method (AOAC, 2000). A Corning flat surface combination electrode was used to measure the pH on the well-mixed ground cheese samples. The moisture content was determined using the moisture analyser (Mettler Toledo Model HR73). Salt content was determined using chloride meter (Jenway, England, UK).

Microbiological analysis

Cheese samples (10 g) were homogenized for 4 min with 90 mL of a sterile 2% sodium citrate solution and serially diluted using sterile 0.05% peptone. Appropriate dilutions of milk and sodium citrate solution of cheese were plated on Plate Count Agar (PCA) for enumeration the total microbial count at 32°C for 2 days, Violet Red Bile Agar (VRBA) for enumerating coliform bacteria at 37°C for 2 days, Potato Dextrose Agar (PDA) for the enumeration of yeasts and moulds at room temperature (20-25˚C) for 5 days and staphylococci 110 Medium for enumeration of staphylococci at 37°C for 2 days (Difco's Manual 1985).

Assessment of proteolysis and lipolysis

The water-soluble extract (WSE) was prepared by the method development by Kuchroo & Fox (1982) and the free amino acids (FAA) were determined in WSE by to the Cd-ninhydrin method of Folkertsma & Fox (1992) and expressed as mM leucine equivalent in water-soluble extracts, using a standard curve. Free fatty acids (FFA) were determined by the method of Deeth, *et al*. (1975) and expressed as mmol equivalent g-1 cheese fat.

Sensory evaluation

The earlier description (Awad *et al*., 2007) of sensory evaluation of Ras cheese was employed in this study.

Statistical analysis

Data reported are the average of three measurements. The SAS statistical analysis software package (SAS, 1999) was used for analysis of variance. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Removal of bacteria with microfiltration and pasteurization

Across 3 replicates, total bacterial counts of the raw skim milk were reduced from 150,000 cfu/mL to 210 and 0.19 cfu/mL by pasteurization and microfiltration respectively (Table 1). Microfiltration achieved an average 5.18 log reductions and pasteurization of whole milk achieved an average 2.85 log reduction (Table 1). The log reduction in bacterial count due to MF was comparable to that reported by Maubois (1997). Both coliforms and staphylococci were reduced by MF and pasteurization to an undetectable level (Table 1).

Cheese composition

Table (2) shows that the Ras cheeses made from pasteurized milk content more moisture than cheeses made from MF milk at day one of manufacture. The MF cheese milk had received a lower load of heat treatment than the pasteurized cheese milk. Minor protein denaturation resulting from pasteurization could explain the higher moisture in cheese from pasteurized milk. Similar results showing the effect of pasteurization and microfiltration of milk on the moisture content of cheese have also been reported by McSweeney *et al*. (1993), Skeie *et al*. (2001).

The moisture in all cheeses significantly decreased during ripening. Most of the moisture losses occurred during the first 30 days of ripening. This could be attributed to the second stage of salting that took place during this period (Awad, 2006; Awad *et al*., 2007). All cultures used in this study had little or no impact on the moisture content of Ras cheese. The average moisture content of Ras cheeses at one day of manufacture is comparable

Table 1: Microbiological analysis (CFU/ml) of skim milk before and after microfiltration and pasteurization

Milk	Total count bacteria	*Colifom* count	*Ştaphylococcus* spp	Yeast	Molds
Raw milk	1.5 × 105	3.3 × 103	8.1 × 102	2.2 × 103	ND
Pasteurized milk	2.1 x 102	ND	ND	ND	ND
Microfiltrated milk	0.19	ND	ND	ND	ND

CFU: colony forming unit ND: not detected

Table 2: Mean chemical composition (%) of Ras cheese made from pasteurized (Past) and microfiltered (MF) milk at different ripening times

		Moisture %			Fat %			Protein %			Salt %		
		Ripening time (days)											
Adjunct Culture	Milk	1	60	120	1	60	120	1	60	120	1	60	120
Mixture 1: 0.5 ml/ Kg	Past	44.5	33.1	31.8	31.0	37.5	39.5	16.5	22.0	23.5	3.0	3.7	4.0
	MF	43.8	32.6	31.2	32.0	38.0	38.0	16.4	21.7	23.2	3.1	3.7	3.9
Mixture 1: 1 ml/ Kg	Past	43.6	33.4	31.8	30.0	38.5	40.5	16.3	20.8	21.6	3.1	3.7	4.0
	MF	43.3	33.5	30.9	31.0	39.0	41.0	16.1	20.3	21.3	3.0	3.9	4.1
Mixture 1: 2 ml/ Kg	Past	43.9	33.7	32.2	30.5	38.0	41.0	16.5	20.0	21.7	3.0	3.5	4.1
	MF	43.7	33.9	31.4	31.0	39.5	42.0	16.0	20.5	21.1	3.2	4.0	4.2
Mixture 2: 0.5 ml/ Kg	Past	44.6	32.6	31.7	31.5	38.0	40.0	16.3	22.3	23.6	3.0	3.8	4.2
	MF	43.3	32.4	30.7	32.0	38.0	38.5	16.5	21.7	22.8	3.0	3.6	4.0
Mixture 2: 1 ml/ Kg	Past	44.5	33.8	32.4	30.5	38.5	40.5	16.1	20.5	21.1	3.0	3.8	4.2
	MF	43.2	33.5	31.1	31.0	38.0	40.5	15.9	20.4	21.2	3.1	3.9	4.1
Mixture 2:2 ml/ Kg	Past	44.8	33.5	32.6	31.0	39.0	41.0	16.3	20.7	21.8	3.0	3.7	4.1
	MF	43.6	33.1	32.1	31.5	39.0	41.0	16.2	20.7	21.4	3.1	3.8	4.1
DVS: 0.0275 g/Kg	Past	44.5	33.4	32.5	31.0	38.5	41.0	16.6	20.4	21.2	3.0	3.7	4.0
	MF	43.8	33.2	31.1	31.4	38.8	41.2	16.8	20.5	21.4	3.1	3.8	4.2

to that reported by Hofi, *et al*. (1970) and Awad *et al*. (2007).

The fat and protein content in cheeses were found to be related to the moisture content in cheeses during ripening. The protein and fat content on dry basis were not significantly different ($P<0.05$) in all cheeses. There is a gradual increase in salt content during the ripening period, similar results were also reported by Hofi, *et al*. (1970) and Awad (2006).

The gross chemical composition of aged Ras cheese are in agreement with the composition of Market Ras cheese (Awad *et al*., 2003), and were within the legal limit for Ras cheese in Egypt (Egyptian standards for Ras cheese, 2001).

Viability of total bacterial in cheese during ripening

The total bacterial count is higher in pasteurized milk cheeses than in MF milk cheeses throughout the ripening period (Table 3). The higher bacterial count in pasteurized milk cheese is related to the microbial level of used milk and moisture content in cheese (Tables 1, 2). On the other hand, McSweeney *et al*. (1993) found higher counts of lactobacilli in Cheddar cheese made from pasteurized milk than in cheese from MF milk. Counts of total bacteria are an indication of the normal course of ripening. Total bacterial count can have significant effects on extent of proteolysis and sensory attributes. Coliform bacteria, staphylococci, Yeasts and Molds were not detected in all cheese samples during ripening.

A gradual decline in numbers of total count bacteria was seen during ripening in all cheeses, resulting in about a 2.5-log reduction after 4 months. The results were in agreement with those reported by Awad *et al*. (2005), who found a decline in the viable counts of lactococci by 2 logs after 6 months of Cheddar cheese ripening. Midje *et al*. (2000) also reported that populations of starter cultures reached a maximum during Cheddar cheese manufacture and decreased thereafter. The reduction of total bacterial count during ripening was at higher rate in cheeses made from MF milk comparing to cheeses made from pasteurized milk (Table 3). The higher reduction rate of viable bacterial count in MF cheeses is may be related to low moisture content comparing to pasteurized milk cheeses.

Cheese pH during ripening

The pH values of experimental cheeses determined during ripening are presented in Table (4). The pH values on day 1 of manufacture of cheeses

Table 3: Bacterial populations and reduction of viability (log10 cfu/g) in Ras cheese made from pasteurized (Past) and microfiltered (MF) milk during ripening

Adjunct Culture	Milk	Viability of bacteria at different ripening time			Reduction of viability	
		Ripening time (days)				
		1	60	120	60	120
Mixture 1: 0.5 ml/ Kg	Past	7.32	7.00	4.88	0.32	2.45
	MF	7.02	6.65	3.70	0.37	3.32
Mixture 1: 1 ml/ Kg	Past	7.27	6.95	4.54	0.31	2.72
	MF	6.98	6.60	4.31	0.38	2.68
Mixture 1: 2 ml/ Kg	Past	7.37	7.18	5.00	0.19	2.37
	MF	7.18	6.92	4.85	0.26	2.33
Mixture 2: 0.5 ml/ Kg	Past	7.29	7.08	4.93	0.21	2.36
	MF	7.02	6.73	4.30	0.29	2.72
Mixture 2: 1 ml/ Kg	Past	7.18	7.01	5.04	0.16	2.13
	MF	7.31	6.98	4.95	0.33	2.36
Mixture 2: 2 ml/ Kg	Past	7.31	7.03	4.78	0.28	2.53
	MF	7.06	6.70	4.40	0.36	2.66
DVS: 0.0275 g/Kg	Past	7.28	6.85	4.48	0.43	2.80
	MF	7.04	6.52	4.08	0.51	2.96

made using MF milk are higher than that made using pasteurized milk. The lower pH values in pasteurized milk cheeses compared to MF cheeses are related to viable bacterial count and moisture content in cheeses (Tables 2, 3). The pH values of cheeses at first day of manufacture are dependent on adjunct culture doses, as the pH value reduced with increasing culture doses. There were no differences among mixtures of adjunct cultures used in this study at similar levels.

The lower pH found in cheeses from pasteurized milk than in cheeses from MF milk is in accordance with the findings made by Beuvier *et al*. (1997). Skeie and Ardo (2000) reported that the total amounts of organic acids, especially lactic acid, were higher in cheeses made from pasteurized milk than that made from MF milk.

During cheese ripening, starter and non starter lactic acid bacteria continue to produce acids and alkaline proteolytic products. pH can be a measure to observe the shifts in the balance between proteolysis and acid production. The pH of all cheeses decreased ($P<0.05$) gradually throughout the ripening period. During the first week or two, starter bacteria ferment the residual lactose and reduce pH (Choisy *et al*., 2000). At all ripening periods, cheeses made with 2ml/kg of cultures had significantly ($P \leq 0.05$) lower pH values than the rest of the treatments. Reducing the pH value in cheeses during the ripening, especially in cheeses made from pasteurized milk, could be attributed to the continued production of lactic acid by live cells of lactobacilli that could survive much longer in cheese than lactococci, and/or the liberation of certain amino acids, such as aspartic and glutamic acids that could influence cheese pH (Trepanier *et al*., 1992).

Proteolysis

Amino acid release, expressed as mM leucine equivalent, in water-soluble extracts of experimental cheeses at different ripening stages is shown in Table (4). The free amino acids values are higher in cheeses made using pasteurized milk than in cheeses made using MF milk throughout the ripening period. These is related to some factors such as; high moisture content, low pH values, high variable bacterial count and higher residual Chymosin activity, which is also related to high amount of rennet used for coagulation and high moisture content in pasteurized milk cheeses compared to UF milk cheeses. The free amino acids increased with increasing the amount

Table 4: Biochemical and organoleptic properties of experimental Ras cheese made from pasteurized (Past) and microfiltered (MF) milk.

		pH value			Free amino acids			Free fatty acids			Sensory evaluation at 120 days	
		Ripening time (days)										
Adjunct Culture	Milk	1	60	120	1	60	120	1	60	120	flavor	Body & texture
Mixture 1; 0.5 ml/ Kg	Past	5.22	5.05	5.01	1.20	2.50	4.80	1.90	3.50	6.20	83^{c}	78^{c}
	MF	5.33	5.12	4.94	0.80	1.70	3.20	1.50	3.00	5.20	72^{f}	84^{ab}
Mixture 1; 1 ml/ Kg	Past	5.12	5.02	4.94	1.50	2.90	5.20	2.50	4.10	6.80	88^{b}	80^{bc}
	MF	5.16	5.04	4.96	1.30	2.20	4.50	1.90	3.30	5.60	75^{e}	85^{a}
Mixture 1; 2 ml/ Kg	Past	5.07	5.01	4.92	1.80	3.10	5.90	2.80	5.40	7.40	92^{a}	81^{b}
	MF	5.13	5.03	4.96	1.70	2.70	5.50	2.40	3.90	6.50	85^{c}	87^{a}
Mixture 2; 0.5 ml/ Kg	Past	5.24	5.09	4.98	1.00	2.00	4.10	1.60	3.00	5.50	80^{d}	79^{bc}
	MF	5.34	5.17	5.03	0.70	1.30	3.80	1.40	2.80	5.00	71^{f}	82^{b}
Mixture 2; 1 ml/ Kg	Past	5.11	5.06	4.99	1.20	2.50	4.70	2.00	3.50	6.00	85^{c}	78^{c}
	MF	5.16	5.10	4.95	0.90	1.80	4.10	1.80	3.20	5.50	73^{f}	86^{a}
Mixture 2; 2 ml/ Kg	Past	5.05	5.00	4.95	1.80	2.80	5.10	2.60	4.30	7.20	90^{a}	82^{b}
	MF	5.12	5.05	4.92	1.20	2.30	4.80	2.10	3.70	6.20	83^{c}	88^{a}
DVS; 0.0275 g/Kg	Past	5.22	5.13	5.07	1.10	2.40	4.70	2.00	3.80	6.10	87^{b}	82^{b}
	MF	5.28	5.19	5.02	0.87	1.87	4.32	1.54	3.54	5.67	76^{e}	85^{a}

^{a,b,c,d,e,f}, Means within a column with no common subscript are significantly different ($P < 0.05$).

of added adjunct culture. The free amino acids concentration was higher in cheeses made with adjunct cultures of 2ml/kg than in cheeses made with 1ml/kg. Free Amino Acids concentration increased significantly ($P<0.05$) as ripening progressed in all cheeses. The major contributors to the production of small peptides and FAA are probably the starter and non-starter bacterial enzymes (El Soda *et al*., 2000). Differences were observed among the cheeses made using different strains and doses of adjunct culture, indicating that the adjunct culture seems to be responsible for the production of the free amino acids in Ras cheese during ripening. The first mixture of adjunct culture produced higher level of free amino acids than second mixture did. On the other hand, the both mixtures of adjunct cultures isolated from Egyptian dairy products produced more free amino acids during Ras cheese ripening than the DVS adjunct culture. These may be related to the DVS adjunct culture is not containing *Enterococcus* strains and the entercocci strains enhanced the free amino acid production in Ras cheese during ripening (Awad *et al*., 2007).

Lipolysis

The lipolysis in Ras cheese during ripening was measured in terms of total Free Fatty Acids (FFA) and expressed as mmol equivalent /g cheese fat. FFA increased gradually with increasing ripening time (Table 4). Cheeses made from pasteurized milk showed higher acid values during ripening than cheeses made from MF milk. However, cheeses containing Mixture 1 of adjunct culture exhibited a higher acid value than did those with Mixture 2 and DVS cultures. High acid values in cheeses containing entercocci strains has been previously reported by Awad *et al*. (2007), and attributed to the release of intracellular esterases and lipases.

Commonly, acid values follow the same trend as soluble nitrogen, suggesting that factors affecting proteolysis may have similar impact on lipolysis (Kebary *et al*., 1996). The higher values of free amino and free fatty acids were recorded in cheese made using pasteurized milk and 2ml/kg of adjunct culture *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus helveticus* and 3 strains of *Enterococcus faecium* (Mixture 1). These results indicate that adjunct cultures contribute to lipolysis in cheese and higher lipolysis is found in pasteurized milk cheese than in MF cheeses. Increasing the rate of lipolysis with added adjunct during cheese ripening has been observed by other authors (El-Soda *et al*., 1992; Awad *et al*., 2007).

Organoleptic quality of cheese

The mean grades for flavor intensity and body & texture acceptability of cheeses at 120 days of ripening are shown in Table 4. Generally, the pasteurized milk cheese received higher flavor intensity scores than MF milk cheeses, but both aged cheeses were considered acceptable. The typical Ras cheese flavor was noticed in aged cheeses made from pasteurized milk with added 2ml/kg of mixture1 adjunct culture. This might due to their relatively higher levels of free amino groups and free fatty acids. The concentrations of low molecular weight peptides and free amino acids have considerable influence on the cheese flavor (El Soda, 1993; Awad, 2006). In addition, the concentration of free fatty acids, especially the short chain ones, is responsible for the characteristic cheese flavor (Kanawjia *et al*., 1995).

At the 4 months evaluation, panelists detected differences ($P < 0.05$) in body and texture between pasteurized and MF milk cheeses (Table 4). The MF cheese received higher scores for overall body and texture than pasteurized milk cheeses. The main goal of this research was to produce high microbiological quality of Ras cheese with similar textural and flavor characteristics to its raw milk counterpart. Because the MF milk contains no nonstarter lactic acid bacteria that gave the best results, traditional Ras cheese flavor did not develop. On the other hand, pasteurization of milk reduced the body and texture of Ras cheese. These results indicated that the proteinase and peptidases of starter and non-starter bacteria is very important for the production of small peptides and the accumulation of amino acids in Ras cheese during ripening, similar results were reported by Awad *et al*., 2007. Therefore, to obtain a Ras cheese with typical flavor and texture using MF milk, it will been considerable interest in using defined strains of nonstarter lactic acid bacteria as adjunct cultures to improve flavor development in MF milk cheese.

ACKNOWLEDGEMENTS

The authors would like to thank the Egyptian Ministry of Agriculture and the Bureau de liaison Franco-Egyptien (BLAFE) for financial support.

REFERENCES

AOAC, **2000**. Official Methods of Analysis. Association of Official Analytical Chemists, International, Arlington, VA.

Awad, S. El Attar, A., Ayad, E. H. E. & El Soda, M. **2003**. Characteristic of Egyptian market Ras cheese: 1- Sensory evaluation, rheological, physico-chemical properties and microbiological analysis. Egyptian J. Dairy Sci. **31**: 289-303.

Awad, S. **2006**. Texture and Flavor development in Ras cheese made from raw and pasteurized milk. Food Chem., **97**: 394–400

Awad, S., Ahmed, N.H & El Soda, M. **2007**. Evaluation of isolated starter lactic acid bacteria in Ras cheese ripening and flavour development. Food Chem., **104**: 1192- 1199.

Awad, S., Hassan, A. N., & Muthukumarappan, K. **2005**. Applications of exopolysaccharides producing cultures in reduced-fat cheddar cheese: Texture and melting properties. J. Dairy Sci., **88**: 4204-4213.

Bech, A. **1993**. Characterizing ripening in UF-cheese. Int. Dairy J., **3**: 329–342

Beuvier, E., Berthaud K., Cegarra, S., Dasen, A., Pochet, S., Buchin S. & Duboz, G. **1997.** Ripening and quality of Swiss-type cheese made from raw, pasteurized or microfiltered milk. In. Dairy J., **7**: 311–323.

Bramley, A.J, & McKinnon, CH. **1990**. The Microbiology of Raw Milk. In: Robinson RK, ed. Dairy Microbiology. Vol 1. The Microbiology of Milk. 2nd ed. London: Elsevier, pp 163–208.

Cheryan, M. **1998**. Ultrafiltration and Microfiltration Handbook. Technomic Publication, Lancaster, PA.

Choisy, C., Desmazeaud, M., Gripon, J. C., Lambert, G. & Lenoir. J. **2000**. The Biochemistry of Ripening. pp 82–154 in Cheesemaking: From Science to Quality Assurance. A. Eck and J. C. Gillis, ed. Lavoisier Publishing, Paris, France.

Deeth, C. H., Fitz-Gerald, C.H. & Wood, A.F. **1975**. A convenient method for determining the extent of lipolysis in milk. Aus. J. Dairy Technol., **30**: 109-111.

Difco's manual **1985**. Dehydrated Culture Media and Reagents for Microbiology. 10th, USA. Difco laboratories Inc.

Egyptian Standards **2001**. Ras Cheese Part 5, ES: 1007/2001. Ministry of Industry and Technological Development, Egypt.

El Soda, M. **1993**. Control and Enhancement of Flavor in Cheeses. In Law, B. (Ed), Microbiology and Biochemistry of cheese and fermented milk. (pp. 219–246). Blackie Academic professional, London.

El Soda, M., Hantira, A. A., Ezzat, N., & El-Shafei, H. K. **1992**. Accelerated ripening of Ras cheese using freeze-shocked mutant strains of Lactobacillus casei. Food Chem., **44**: 197–184.

El Soda, M., Madkor, S.A, & Tong, P.S. **2000**. Adjuncts cultures: recent developments and potential significance to cheese industry. A review. J. Dairy Sci., **83**: 609 – 619.

El-Soda, M., El-Ziney, M., Awad, S., Osman, G., Omran, N., Gamal, G., Ezzat, N., and El-Shafei, H. **2003**. A culture collection of lactic acid bacteria isolated from raw milk and traditional Egyptian dairy products. Egyptian J. Dairy Sci., **31**: 23 – 41.

Folkertsma, B., & Fox, P.F. **1992**. Use of the cadmium ninhydrin reagent to assess proteolysis in cheese during ripening. J. Dairy Res., **59**: 217-224.

Giffel, M. C. & van der Horst, H.C. **2004**. Comparison between bactofugation and microfiltration regarding efficiency of somatic cell and bacteria removal. IDF 389, Brussels, Belgium.

Hoffmann, W., Klobes, H., Kiesner, Chr., Suhren, G., Krusch, U., Clawin-Radecker, I. & Larsen. P.H. **1996**. Use of microfiltration for the production of pasteurized milk with extended shelf life. IDF, 311, Brussels, Belgium.

Hofi, A.A., Youssef, E.H., Ghoneim, M.A., & Tawab, G.A. **1970**. Ripening changes in Cephalotyre "Ras" cheese manufactured from raw and pasteurised milk with special reference to flavour. J. of Dairy Sci., **53**: 1207-1212.

Jameson, G., & Lelievre.J. **1996**. Effects of whey proteins on cheese characteristics. IDF, **313**:3–8, Brussels, Belgium

Kanawjia, S.K., Rajesh, P., Sabikhi, L. & Singh, S. 1995. Flavor, chemical and texture profile changes in accelerated ripened Gouda cheese. LWT, **28**: 577–583.

Kebary, K.M.K., Khader, A.E., Zedan, A.N., & Mahmoud, S.F. **1996**. Accelerated ripening of low fat Ras cheese by attenuated lactobacilli cells. Food Res. Int., **29**: 705-713.

Kelly, P.M., & Tuohy, J.J. **1997**. The effectiveness of microfiltration for the removal of microorganisms. IDF, **320**: 26–31.

Kuchroo, C.N., & Fox, P.F. **1982**. Soluble nitrogen in Cheddar cheese. Comparison of extraction proce¬dures. Milchw., **37**: 331-335

Maubois, J. **1997**. Current uses and future perspectives of MF technology in the dairy industry. IDF, **320**: 37–39.,

McSweeney, P.L.H., Fox, P.F., Lucey, J.A., Jordan, K.N., and Cogan, T.M. **1993**. Contribution of the indigenous microflora to the maturation of Cheddar cheese. Int. Dairy J., **3**: 613–634.

Midje, L.D., Bastian, E.D., Morris, H.A., Martin, F.B., Bridgeman, T. & Vickers, Z.M. **2000**. Flavor enhancement of reduced fat Cheddar cheese using an integrated culturing system. J. Agri. Food Chem., **48**:1630–1636.

Olesen, N. & Jensen. F. **1989**. Microfiltration. The influence of operation parameters on the process. Milchw., **44**:476–479.

Papadatos, A., Neocleous, M., Berger, A. M. & Barbano, D. M. **2003**. Economic Feasibility Evaluation of Microfiltration of Milk Prior to Cheesemaking. J. of Dairy Sci., **86**:1564–1577

SAS® **1999**. User's Guide: Statistics, Version 8 Edition. SAS Inst., Inc., Cary. NC.

Skeie, S., & Ardo,Y. **2000**. Ripening study of model cheeses made from raw, pasteurized and microfiltered milk. Food Sci. Technol., **33**: 499–505.

Skeie, S., Lindbergb, C. & Narvhusa, J. **2001**. Development of amino acids and organic acids in Norvegia, influence of milk treatment and adjunct *Lactobacillus*. Int. Dairy J., **11**: 399–411

Trepanier, G., El Aboudoi, M, Lee, B. H. & Simard, R.E. **1992**. Accelerated maturation of cheddar cheese: Microbiology of cheese supplemented with *Lactobacillus casei* subsp. *casei*. J. Food Sci., **57**: 345-347.

تأثير معاملة اللبن و البادئات المساعدة علي خواص الجبن الراس

سامح علي محمد عوض، نورالهدي أحمد محمد، مرسي أبوالسعود السوده

قسم علوم و تكنولوجيا الألبان، كلية الزراعة، جامعة الاسكندرية

تعتبر المعاملات الحرارية للبن مثل البسترة و التعقيم من الطرق التقليدية للتخلص من ميكروبات اللبن و زيادة فترة الحفظ، ولكن تلك المعاملات يكون لها تأثير غير مرغوب فيه علي الخواص الطبيعية و الكيميائية للبن و علي سبيل المثال عملية التجبن بالمنفحة. وقد بدأ حديثا استخدام الترشيح الدقيق كبديل للمعاملات الحرارية في التخلص من الميكروبات بدون تأثير علي الخواص الطبيعية و الكيماوية للبن. و في هذه الدراسة تم دراسة مقارنة لتأثير عملية البسترة و عملية الترشيح الدقيق علي التحلل البروتيني و التحلل الدهني و إظهار نكهة الجبن خلال فترة التسوية لمدة أربعة أشهر. تم كذلك تقييم خلطات من البادئات المساعدة لبكتريا حمض اللاكتيك المعزولة من منتجات الألبان التقليدية في صناعة الجبن الراس و إظهار نكهة الجبن خلال فترة التسوية. تم إجراء عملية الترشيح الدقيق علي اللبن الفرز الخام ثم تم تعديل نسبة البروتين الي الدهن باستخدام قشدة مبسترة. و المعاملة الثانية كانت استخدام لبن مبستر معدل نسبة البروتين الي الدهن. أظهرت النتائج أن المعاملة الأولية علي اللبن "البسترة أو الترشيح الدقيق" كان لها تأثير علي عملية الصناعة مثل كمية المنفحة المطلوب إضافتها للتجبن و زمن عملية السمط. وقد تأثر التركيب الكيماوي للجبن بالمعاملة الأولية للبن ولكنه لم يتأثر بنوع البادئ المستخدم في الصناعة. وكانت نسبة الرطوبة مرتفعة و درجة الـ pH كانت منخفضة في الجبن الطازج المصنع من لبن مبستر بالمقارنة بالجبن المصنع من لبن معامل بالترشيح الدقيق. أدت عملية الترشيح الدقيق الي خفض التحلل البروتيني و التحلل الدهني في الجبن بالمقارنة بعملية البسترة. كان هناك تفاوت كبير في نسب الأحماض الأمينية الحرة و الأحماض الدهنية الحرة و الخواص الحسية بين الأجبان المستخدم في صناعتها بادئات مساعدة مختلفة و تركيزات مختلفة منها. كان للجبن المصنع من لبن معامل بالترشيح الدقيق درجات تحكيم حسي عالية للقوام ودرجات تحكيم حسي منخفضة للنكهة مقارنة بالجبن المصنع من اللبن المبستر.