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EFFECT OF HOMOGENIZATION ON THE PROPERTIES AND MICROSTRUCTURE OF MOZZARELLA CHEESE FROM BUFFALO MILK

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ABSTRACT

Background. The name pasta filata refers to a unique plasticizing and texturing treatments of the fresh curd in hot water that imparts to the finished cheese its characteristic fibrous structure and melting properties. Mozzarella cheese made from standardized homogenized and non-homogenized buffalo milk with 3 and 1.5%fat. The effect of homogenization on rheological, microstructure and sensory evaluation was carried out.
Material and methods. Fresh raw buffalo milk and starter cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were used. The coagulants were calf rennet powder (HA-LA). Standardized buffalo milk was homogenized at 25 kg/cm^2 pressure after heating to 60°C using homogenizer. Milk and cheese were analysed. Microstructure of the cheese samples was investigated either with an application of transmission or scanning electron microscope. Statistical analyses were applied on the obtained data.
Results. Soluble nitrogen total volatile free fatty acids, soluble tyrosine and tryptophan increased with using homogenized milk and also, increased with relatively decrease in case of homogenized Mozzarella cheese. Meltability of Mozzarella cheese increased with increasing the fat content and storage period and decrease with homogenization. Mozzarella cheese firmness increased with homogenization and also, increased with progressing of storage period. Flavour score, appearance and total score of Mozzarella cheese increased with homogenization and storage period progress, while body and texture score decreased with homogenization and increased with storage period progress. Microstructure of Mozzarella cheese showed the low fat cheese tends to be harder, more crumbly and less smooth than normal. Curd granule junctions were prominent in non-homogenized milk cheese.
Conclusion. Homogenization of milk cheese caused changes in the microstructure of the Mozzarella cheese. Microstructure studies of cheese revealed that cheese made from homogenized milk is smoother and has a finer texture than non-homogenized but is also, firmer and more elastic.

Key words: Mozzarella cheese, homogenization, microstructure, buffalo milk, sensory evaluation

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INTRODUCTION

Mozzarella cheese, like other pasta filata group of cheese has a unique characteristic manufacturing method in which the curd is kneaded by immersion into hot water before being moulded [Abd El-Rafee 1998, El-Batawy et al. 2004, Abou-Donia 2005]. Mozzarella cheese is classified by standards of identity into four separate categories based on moisture content and fat in dry matter. Mozzarella and part skim milk Mozzarella are high in moisture (> 52%). Low--moisture (LM) and low-moisture part skim (LMPS) Mozzarella have lower water content and are used primarily as ingredients for pizza. Homogenization of cheese milk was originally proposed as a possible means of improving cheese quality. Milk homogenization reduces the size of fat globules and disrupts the fat globules membrane, which is replaced by membrane fragments complexes with casein submicelles [Peters 1964, Henstra and Schmidt 1970, Schmidt and Buchheim 1970, Tunick et al. 2000]. The onset of coagulation is faster when milk is homogenized [Robson and Dalgleish 1984], but syneresies and draining are slower due to curd shattering [Green et al. 1983, Tunick et al. 2000]. Homogenization has been employed in the manufacture of Mozzarella from cow milk to reduce milk solids losses in whey and molding water and reduce excessive fat leakage when used on pizza. Also, low pressure homogenization decreased stretchability and meltability of Mozzarella, which was attributed to the effect to the presence of casein at the water fat droplet interface, which causes the fat droplets to be cross-linked in the protein matrix. The effect of homogenization pressures and temperatures on rheology, meltability and other characteristics of buffalo milk Mozzarella has also been investigated [Jana and Upadhyay 1991, Lelievre et al. 1990, Tunick et al. 1993 a, 1995, 2000, El-Batawy et al. 2004, Ahmed et al. 2011].

This research was focused on the effect on standardizing milk fat for different levels and milk homogenization on the compositional, rheological, microstructure and sensory evaluation of Mozzarella cheese made from buffalo milk.

MATERIAL AND METHODS

Materials

Fresh raw buffalo milk was obtained from the herd of the Faculty of Agriculture, Cairo University. Starter cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* were obtained from Microbiological Resources Centre (MIRCEN), Faculty of Agriculture, Ain Shams University. Calf rennet powder (HA-LA) from CHR-Hansen's Denmark.

Cheese manufacture

Fresh buffalo milk obtained from Faculty of Agriculture, Cairo University was separated to cream and skim milk. The obtained skim milk was divided to 4 equal portions. Two portions were standardized to 3% fat, while the 2 others were standardized to 1.5% fat using the separated cream. One portion of each fat standardized milk was homogenized at 25 kg/cm^2 pressure after heating to 60°C using (Rannie Copenhagen) homogenizer. The other 2 portions were left as control without homogenization. Non-homogenized milk (control) was manufactured to Mozzarella cheese according to Kosikowski [1982 a]. In case of homogenized milk some modifications were carried out according to Jana and Upadhyay [1992] who described a method for manufacturing Mozzarella cheese from buffalo homogenized milk. Three replicates of all treatments were analysed for chemical, physical and rheological properties when (fresh, 1, 2, 3 and 4) weeks of cold storage (4°C).

METHODS OF ANALYSIS

Milk and fresh cheese

Dry matter (DM), fat, total protein (TP) contents and titratable acidity were determined according to the methods AOAC... [2007], soluble nitrogen (SN) content in cheese was determined by the micro-kjeldahl method [Kosikowski and Mistry 1997], sodium chloride content according to Richardson [1985] and pH values was measured using a digital pH meter (M41150 USA) equipped with glass electrode. The cheese was also analysed for total volatile fatty acids (TVFA) as described by Kosikowski [1982 b] soluble tyrosine and tryptophan contents of cheese samples were determined according to Vakaleris and Price

[1959], total phosphorus (P) content as described by IDF... [1966] and calcium (Ca) content as described by Raadsveld and Klomp [1971]. The cheese recovery was calculated according to Vandeweghe and Maubois [1987].

The cheese samples were also analysed for meltability (tube) test as described by Muthukumarappan et al. [1999] meltability (disc) and fat leakage test were determined according to Guinee et al. [1999]. The method described by Kindstedt and Fox [1991] was adapted for estimation of free oil in method Mozzarella cheese using Gerber fat testing equipment Bytrometer.

Cheese firmness

The firmness of cheese was measured at 15°C using Koehlerik K19500penetrometer (Sycamore, AVE USA). The penetrometer was equipped with 2 different weights, the standardized rod weight of 47.5 gm and an additional 35 gm. The weights (total 82.5 gm) used a type of cone and an additional weight was selected to allow a better differentiation of firmness values. The whole scale was calibrated into (35) units; each unit was further divided into 10 parts, 0.1 mm each. In making the measurement, the cheese sample was placed on the base and the rod moved down until the tip of cone release button was depressed 5 secs. for recording the distance in units of 0.1 mm. The measurement was repeated three times using three different locations on the surface of a cheese sample and the average of three values was taken.

Microstructure of cheese

The fine structure of the cheese samples was investigated using either transmission or scanning electron microscope: a. Transmission using the thin-sectioning technique for preparation according to Taneya et al. [1980]. b. Scanning electron microscope (SEM) was carried out according to Tunick et al. [1993 b].

Sensory evaluation of cheese

Cheese samples were sensory evaluated according to the score card suggested by Land and Shepherd [1988]. Samples were judged by 20 persons of the staff at the Dairy Department, NRC.

Statistical analysis

Statistical analysis was preformed using the GLM procedure with SAS [2004] software. Duncan's multiple comparison procedure was used to compare the means. A probability to $P < 0.05$ was used to establish the statistical significance.

RESULTS AND DISCUSSION

Chemical composition of milk

Data in Table 1 show the chemical composition of the standardized buffalo milk 3 and 1.5% fat. The obtained results indicate that fat/DM increased with increasing the fat content of the original milk.

Chemical composition of Mozzarella cheese

The chemical composition of fresh Mozzarella cheese made using non-homogenized and homogenized buffalo milk standardized with 3 and 1.5% fat are presented in Table 2. The same table shows that, the fat/DM of Mozzarella cheese slightly increased when homogenized buffalo milk was used. On the contrast TP/DM decreased when pre-cheese milk was homogenized. Furthermore, Table 2 shows that, the Ca and P/DM also, decreased when homogenized buffalo milk was used.

Table 1. Chemical composition of standardized fresh buffalo milk

Fat %	DM	Fat/DM	TP	TP/DM	Ca	Ca/DM	P	P/DM	Acidity	pH
					%					
3	12.58[a]	23. 86[a]	3.98	31.65[b]	0.170	1.34[b]	0.162	1.30[b]	0.17	6.7
1.5	10.65[a]	14.08[b]	3.99	37.47[a]	0.172	1.62[a]	0.163	1.52[a]	0.18	6.6

DM – dry matter, TP – total protein, Ca – calcium, P – phosphorus.
Dissimilar superscripts at the same row for treatments are significantly differed ($P < 0.05$). Each value is a mean of 3 replicates.

Table 2. Effect of homogenization on the chemical composition of fresh Mozzarella cheese manufactured from milk with 3 and 1.5% fat

Components %	Non-homogenized milk cheese		Homogenized milk cheese	
	3%	1.5%	3%	1.5%
DM	45.22^{a}	42.72^{b}	38.48^{a}	36.17^{b}
Fat/DM	37.88^{a}	24.81^{b}	38.20^{a}	24.89^{b}
TP/DM	51.44^{b}	65.26^{b}	49.45^{b}	64.00^{a}
Ca/DM	1.63^{b}	1.98^{a}	1.54^{b}	1.88^{a}
P/DM	1.62^{b}	1.96^{a}	1.42^{b}	1.84^{a}
Salt	1.51	1.58	1.68	1.78
Yield	15.57^{a}	13.18^{b}	19.10^{a}	15.90^{b}

Dissimilar superscripts at the same row for treatments are significantly differed ($P < 0.05$). Each value is a mean of 3 replicates.

These results are in agreement with those reported by Emmons et al. [1980] and Tunick et al. [2000] who mentioned that, the moisture was slightly higher in homogenized milk reduced fat cheese compared with the treatments receiving little or non-homogenized. Also Jana and Upadhyay [1991] and El-Batawy et al. [2004] indicated that, homogenization of milk had a significant effect on composition of cheese through a higher moisture and fat retention. Higher acidity of whey at draining yielded cheese with low pH and lower ash content compared to control cheese.

Recovery of dry matter (DM), fat and protein of Mozzarella cheese

Recovery of milk DM, fat and TP of homogenized and non-homogenized Mozzarella curd and cheese are present in Table 3. Data reveal that, the recovery of DM and fat in the cheese increased when homogenized milk was used either with 3 or 1.5% fat. The recovery of DM, fat and TP were higher in case of homogenized cheese than non-homogenized one. Homogenized cheese showed less loss in DM and fat. Their loss was higher in low fat cheese with 1.5% than cheese with 3% fat.

These results are in agreement with those reported by Emmons et al. [1980] who mentioned that, the moisture content was slightly higher in reduced fat cheese made from homogenized milk compared with non-homogenized milk. Also Jana and Updahyay [1991], Tunick et al. [2000] and El-Batawy et al. [2004] indicated that, homogenization of milk had a significant effect on composition of Mozzarella cheese through a higher moisture and fat retention.

Soluble nitrogen (SN) content

The soluble nitrogen content of Mozzarella cheese made from buffalo milk homogenized or non-homogenized with 3 and 1.5% fat during storage period at 4°C is shown in Table 4. The data show that, cheese soluble nitrogen content was higher in case of fresh homogenized buffalo cheese than non-homogenized one. Throughout storage period cheese soluble nitrogen content of all treatments gradually increased till 4 weeks storage. Ahmed et al. [2011] clearly that, the soluble nitrogen of Mozzarella cheese made from

Table 3. Effect of homogenization on recovery percentage of some components during manufacture of Mozzarella cheese

Components %	Non-homogenized milk				Homogenized milk			
	3%		1.5%		3%		1.5%	
	curd	cheese	curd	cheese	curd	cheese	curd	cheese
DM	58.24^{a}	55.98^{ab}	53.51^{b}	52.86^{b}	59.76^{a}	58.91^{a}	54.04^{b}	53.94^{b}
Fat	91.97^{ab}	88.75^{b}	94.63^{a}	93.14^{a}	94.60^{ab}	93.59^{b}	96.66^{a}	95.40^{a}
TP	91.42^{b}	90.99^{b}	92.42^{a}	92.09^{a}	92.21^{a}	91.56^{b}	92.51^{a}	92.25^{a}

Dissimilar superscripts at the same row for treatments are significantly differed ($P < 0.05$). Each value is a mean of 3 replicates.

standardized milk containing 3 or 1.5% fat increased with increasing the storage period. However, the values of homogenized milk cheese were always higher than non-homogenized cheese. When homogenized milk was used cheese soluble nitrogen contents were higher either when fresh or after 4 weeks storage. El--Batawy et al. [2004] were in agreement of the present data. They revealed that cheese made from homogenized milk had higher SN/TN ratio compared with that of cheeses made from non-homogenized milk showed a marked increase during storage period.

The statistical analysis showed that, the soluble nitrogen contents were highly significant affected by the storage period (F = 948.5). Furthermore, a highly significant influence on the soluble nitrogen of Mozzarella cheese was found by interaction between the storage period and the effect of homogenization (F = 278.28).

Total volatile free fatty acids (TVFA) content

Data in Table 4 show the TVFA content of Mozzarella cheese made from buffalo milk homogenized or non-homogenized with 3 and 1.5% fat in pre-cheese milk. The content of TVFA in case of non-homogenized Mozzarella cheese were lower compared with that made using homogenized buffalo milk. Furthermore, the fat content of the original milk directly affected TVFA content during cold storage. It could be seen from the same data that, the TVFA content increased with increasing of the storage period. In accordance Ahmed et al. [2011] showed that, the total volatile fatty acids (TVFA) of Mozzarella cheese made from standardized milk containing 3 or 1.5% fat increased with increasing the storage period. It can be seen that Mozzarella cheese made from homogenized milk contained slightly higher total volatile fatty acids (TVFA) either fresh or during storage, compared with non-homogenized ones all though the storage period. The (TVFA) increased during storage in cheese from all treatments. These results are in agreement with that recorded by El-Batawy et al. [2004].

The statistical analysis of the results revealed that, TVFA content of Mozzarella cheese were high significantly affected by the storage period (F = 3312.66). Moreover, the TVFA values were found to be high significantly affected by the effect of homogenization (F = 21 105.75). Furthermore, a highly significant influence on the TVFA of Mozzarella cheese was observed by interaction between the storage period and the effect of homogenization (F = 4880.88).

Table 4. Effect of homogenization of pre-cheese milk on soluble nitrogen and total volatile free fatty acids contents of Mozzarella cheese during storage at 4°C

Properties	Storage period weeks	Non-homogenized milk		Homogenized milk	
		3%	1.5%	3%	1.5%
Soluble nitrogen %	fresh	0.162^{Aa}	0.210^{Ba}	0.166^{Aa}	0.218^{Ba}
	1	0.214^{b}	0.221^{a}	0.224^{b}	0.227^{a}
	2	0.232^{b}	0.242^{b}	0.235^{b}	0.248^{b}
	3	0.254^{c}	0.268^{b}	0.256^{c}	0.278^{c}
	4	0.274^{c}	0.295^{c}	0.325^{d}	0.335^{d}
TVFA ml 0.1 N NaOH/ 100 g cheese	fresh	2.00^{Aa}	2.00^{Ba}	2.40^{Aa}	2.20^{Ba}
	1	3.50^{b}	2.90^{b}	3.80^{b}	3.19^{b}
	2	7.42^{c}	3.61^{c}	7.73^{c}	4.24^{c}
	3	9.01^{d}	6.24^{d}	9.81^{d}	7.15^{d}
	4	12.20^{d}	9.89^{d}	15.17^{d}	12.11^{d}

Dissimilar superscripts at the same row (for treatments) and the same column (for storage periods) are significantly differed ($p < 0.05$). Each value is a mean of 3 replicates.

Soluble tyrosine and tryptophan contents

Soluble tyrosine and tryptophan of Mozzarella cheese during cold storage are shown in Table 5. Soluble tyrosine and tryptophan considered a good indicator for cheese ripening during storage period. From the obtained data it could be noticed that, the amount of soluble tyrosine and tryptophan increased with higher values in case of Mozzarella cheese made using homogenized buffalo milk either with 3 or 1.5% fat in pre-cheese milk. Also, it could be seen that, soluble tyrosine and tryptophan had the same trend during the storage period. Their values increased with increasing the cheese period and decrease with increasing the fat percent of cheese milk. In agreement Ahmed et al. [2011] indicated that, the soluble tyrosine and tryptophan of Mozzarella cheese made from standardized milk containing 3 or 1.5% fat increased with increasing the storage period.

Table 5. Effect of homogenization of pre-cheese milk on soluble tyrosine and soluble tryptophan contents of Mozzarella cheese during storage at 4°C

Properties	Storage period weeks	Non-homogenized milk		Homogenized milk	
		3%	1.5%	3%	1.5%
Soluble tyrosine mg/100 g cheese	fresh	3.54^{Aa}	3.80^{Ba}	4.22^{Aa}	5.95^{Ba}
	1	10.41^{d}	11.74^{d}	16.56^{b}	11.89^{b}
	2	22.14^{c}	25.95^{c}	30.47^{c}	23.21^{c}
	3	43.64^{b}	49.85^{b}	51.52^{d}	56.45^{d}
	4	53.81^{b}	56.42^{b}	60.82^{d}	68.74^{d}
Soluble tryptophan mg/100 g cheese	fresh	1.99^{Aa}	2.00^{Aa}	2.14^{Aa}	2.25^{Aa}
	1	6.94^{b}	7.41^{b}	8.48^{b}	9.81^{b}
	2	12.90^{c}	14.86^{c}	18.67^{c}	20.11^{c}
	3	28.46^{d}	31.10^{d}	32.76^{d}	37.12^{d}
	4	38.04^{d}	42.05^{d}	42.83^{d}	44.20^{d}

Dissimilar superscripts at the same row (for treatments) and the same column (for storage periods) are significantly differed ($p < 0.05$). Each value is a mean of 3 replicates.

Table 6. Effect of homogenization of pre-cheese milk on the acidity and pH value of Mozzarella cheese during storage at 4°C

Properties	Storage period weeks	Non-homogenized milk		Homogenized milk	
		3%	1.5%	3%	1.5%
Acidity %	fresh	0.64^{b}	0.70^{b}	0.67^{c}	0.72^{c}
	1	0.66^{b}	0.72^{b}	0.69^{b}	0.74^{b}
	2	0.68^{ab}	0.76^{ab}	0.72^{b}	0.78^{b}
	3	0.70^{a}	0.78^{a}	0.74^{a}	0.79^{a}
	4	0.72^{a}	0.80^{a}	0.76^{a}	0.82^{a}
pH	fresh	5.3^{a}	5.3^{a}	5.3^{a}	5.3^{a}
	1	5.2^{a}	5.2^{a}	5.1^{a}	5.1^{b}
	2	4.9^{ab}	5.0^{b}	4.75^{b}	4.8^{b}
	3	4.8^{ab}	4.8^{b}	4.6^{bc}	4.65^{bc}
	4	4.6^{b}	4.65^{c}	4.5^{c}	4.50^{c}

Dissimilar superscripts at the same row (for treatments) and the same column (for storage periods) are significantly differed ($p < 0.05$). Each value is a mean of 3 replicates.

The statistical analysis of soluble tyrosine and tryptophan contents illustrated that soluble tyrosine highly significant affected by the storage period ($F = 6.573$) and by the pre-cheese milk homogenization ($F = 376.54$). Furthermore, the interaction between storage period and homogenization had highly significant influence ($F = 38.14$). Statistical analysis also indicated that, storage period had significant effect on soluble tryptophan content ($F = 3.03$). While, the effect of pre-cheese milk homogenization had highly significant effect on soluble tryptophan ($F = 238.40$). Furthermore, a significant influences on tryptophan of Mozzarella cheese were found by the interaction between storage period and homogenization ($F = 4.19$).

Acidity and pH values

Data presented in Table 6 show the acidity and pH values of Mozzarella cheese made from non-homogenized and homogenized buffalo milk with 3 or 1.5% fat when fresh and during storage period for 4 weeks at 4°C. It was observed that, the acidity increased and pH decreased with the progressing of the storage period in all cheese treatments. Moreover, the interaction of acidity was higher in case of Mozzarella cheese made using homogenized buffalo milk compared with those made using non-homogenized buffalo milk either with 3 or 1.5% fat in pre-cheese milk. These results are in accordance with that reported by El-Batawy et al. [2004].

Fat leakage and oiling off

The fat leakage and oiling off of homogenized and non-homogenized Mozzarella cheese prepared from standardized buffalo milk to 3 or 1.5% fat are presented in Table 7. Fat leakage and oiling off were clearly decreased in case of homogenized Mozzarella cheese compared with the one made using non-homogenized milk. Furthermore, the fat leakage and oiling off increased with increasing the storage period at 4°C. These results are in agreement with the findings of Breen et al. [1964] and Emstrom and Anis [1985], who reported that, the cheese made from homogenized

Table 7. Effect of homogenization of pre-cheese milk on fat leakage and oiling off of Mozzarella cheese during storage at 4°C

Properties	Storage period weeks	Non-homogenized milk		Homogenized milk	
		3%	1.5%	3%	1.5%
Fat leakage cm^2	fresh	64.87^{Aa}	46.36^{Ba}	58.26^{Aa}	38.29^{Ba}
	1	88.21^{b}	52.01^{b}	79.18^{b}	45.22^{b}
	2	92.47^{c}	56.48^{c}	81.34^{c}	51.41^{c}
	3	96.71^{d}	60.21^{d}	87.60^{d}	55.67^{d}
	4	104.16^{d}	68.65^{d}	90.45^{d}	60.32^{d}
Oiling off %	fresh	3.5^{Aa}	1.0^{Ba}	1.5^{Aa}	0.0^{Ba}
	1	4.5^{b}	1.5^{b}	1.9^{b}	0.5^{b}
	2	6.0^{c}	2.0^{c}	2.5^{c}	1.0^{c}
	3	7.4^{d}	2.5^{d}	3.0^{d}	1.5^{d}
	4	8.5^{d}	3.0^{d}	3.8^{d}	2.0^{d}

Dissimilar superscripts at the same row (for treatments) and the same column (for storage periods) are significantly differed ($p < 0.05$). Each value is a mean of 3 replicates.

milk had lower fat leakage than its non-homogenized counterparts. Furthermore, Breen et al. [1964], Rudan and Barabano [1998], El-Batawy et al. [2004], who confirmed that the fat leakage can be reduced by decreasing the fat content of the cheese milk or by low pressure homogenization of the whole milk standardization. Tunick et al. [1993 a] and Tunick [1994] found that homogenization decreased significantly of oiling off in Mozzarella cheese and suggested that the reduction in fat particle size and the subsequent changes in the cheese structure were responsible for the change in oiling off.

The statistical analysis of the obtained results showed that the fat leakage of Mozzarella cheese was highly significant affected by the homogenization of the initial milk used ($F = 392.89$). Also, the storage period was found to be highly significant effected the fat leakage ($F = 253.11$). Fat leakage was found to be highly significant affected as results of the interaction between the homogenization of the original milk used and the storage period ($F = 7.22$). Results, also indicated that oiling off was highly significant affected by the homogenization of the original milk used ($F = 152.21$). Furthermore, the storage period was found to be highly significant effected oiling off of the cheese ($F = 36.58$). The interaction between homogenization and storage period had highly significant effect ($F = 10.85$) on Mozzarella cheese oiling off.

Meltability

It was evident from the data presented in Table 8 that the meltability of Mozzarella cheese either by disc or by tube methods increased with increasing the storage period in case of both homogenized and non-homogenized cheese. It was clear that all cheeses had no meltability when fresh, after which it gradually increased during cold storage. On the other hand homogenization of pre-cheese milk seemed to have adverse effect on meltability. The increased moisture entrapped in curd from homogenized milk may be responsible for the low meltability in homogenized young cheese. These results are in agreement with

Table 8. Effect of homogenization of pre-cheese milk on the meltability of Mozzarella cheese during storage at 4°C

Properties	Storage period weeks	Non-homogenized milk		Homogenized milk	
		3%	1.5%	3%	1.5%
Meltability mm (tube method)	fresh	0.0^{Aa}	0.0^{Aa}	0.0^{Aa}	0.0^{Aa}
	1	6.8^{b}	56^{b}	55^{b}	45^{b}
	2	87^{c}	75^{c}	68^{c}	62^{c}
	3	94^{d}	80^{d}	86^{d}	73^{d}
	4	116^{d}	88^{d}	92^{d}	82^{d}
Meltability cm^2 (disc method)	fresh	10.24^{Aa}	7.5^{Ba}	6.82^{Aa}	4.81^{Ba}
	1	19.36^{b}	9.87^{b}	12.41^{b}	7. 64^{b}
	2	25.42^{c}	12.36^{c}	18.98^{c}	9.37^{c}
	3	28.10^{c}	19.24^{d}	22.64^{d}	14.22^{d}
	4	38.25^{d}	23.38^{d}	26.83^{d}	18.82^{d}

Dissimilar superscripts at the same row (for treatments) and the same column (for storage periods) are significantly differed ($p < 0.05$). Each value is a mean of 3 replicates.

those reported by Lelievre et al. [1990], Harvey et al. [1982] and El-Batawy et al. [2004], who mentioned that Mozzarella spread, increased with either fat content increase or proteolysis during storage because of breakdown of α_{s-1} casein, solublization of the resulting fragments and release of fat. Also Keenan et al. [1988] and Lelievre et al. [1990] observed that, the fate of fat globule membranes in homogenized milk is not certain but most of the membrane is generally assumed to be removed from the globules and replaced at least in part by casein micelles sub micelles. Crosslinking of this casein with the protein matrix would make the cheese harder. Also Fox and Guinee [1987], Lelievre et al. [1990] and El-Batawy et al. [2004] confirmed that, homogenization decreased the meltability of Mozzarella cheese due to adsorption of casein on to the lipid droplets apparently prevents the melted fat from spreading out.

The statistical analysis of the obtained results showed that, meltability measured by either disc or tube methods was affected highly significantly by the homogenization of the original milk used ($F = 321.24$ and 7701.40). Also, the storage period was found to be highly significantly affecting the meltability of Mozzarella cheese ($F = 135.21$ and 486.10). Moreover, the interaction of the fat percentage of the original milk used with storage period, was highly significant and affected meltability of cheese ($F = 7.42$ and 49.32).

Firmness

Mozzarella cheese firmness values made from standardized buffalo milk to 3 or 1.5% fat, either homogenized or not, are presented in Table 9. It was clear that, firmness of the cheese increased with using homogenized buffalo milk compared with those made using non-homogenized buffalo milk (the higher record the lower firmness). It was noticed, from the same table that, the firmness of the cheese increased with increasing the storage period. These results are in full agreement with the findings of Emmons et al. [1980] who found that, homogenization of cow milk tented to increase the moisture content and decreased firmness and elasticity of Mozzarella cheese, but not markedly. Also Metzger and Mistry [1993], Tunick et al. [2000] and El-Batawy et al. [2004] showed that the hardness of the cheese increased with increasing the storage period. The cheese made from the homogenized milk exhibited lower hardness, gumminess, and chewiness, but higher cohesiveness values when compared with those of control cheese. Homogenization of milk adversely affects protein structure and causes casein micelles and their subunits to be incorporated into the fat globule membranes. These interactions between fat and casein lead to a weaker rennet curd, curd shattering, and improper curd matting [Metzger and Mistry 1993, El-Batawy et al. 2004].

Table 9. Effect of homogenization of pre-cheese milk on the firmness* of Mozzarella cheese during storage at 4°C (as penetration distance in mm)

Storage period weeks	Non-homogenized milk		Homogenized milk	
	3%	1.5%	3%	1.5%
Fresh	70^{Aa}	66^{Ba}	65^{Aa}	62^{Ba}
1	64^{b}	62^{b}	60^{b}	59^{b}
2	60^{c}	57^{c}	56^{c}	54^{c}
3	57^{d}	54^{c}	52^{d}	51^{d}
4	55^{d}	52^{d}	50^{d}	49^{d}

*The higher record the less firmness.
Dissimilar superscripts at the same row (for treatments) and the same column (for storage periods) are significantly differed ($p < 0.05$). Each value is a mean of 3 replicates.

The statistical analysis of the results showed that the firmness of Mozzarella cheese was highly significant affected by homogenization of the original milk used ($F = 389.1$). Furthermore, the storage period of Mozzarella cheese had also a highly significant influence on the firmness ($F = 87.89$). Moreover, their interaction had a highly significant influence on the firmness of Mozzarella cheese ($F = 19.17$).

Sensory evaluation

The sensory evaluation records of Mozzarella cheese made using standardized buffalo milk 3 or 1.5% fat, either homogenized or not, are illustrated in Table 10. Flavour scores of the cheese increased during the storage period; this increase could be attributed to the proteolysis of cheese. However, the rate of increasing in case of Mozzarella cheese was rapid and higher, compared with that made using non-homogenized buffalo milk.

Table 10. Effect of homogenization of pre-cheese milk on sensory evaluation of Mozzarella cheese during storage at 4°C

Sensory evaluation	Storage period weeks	Non-homogenized milk		Homogenized milk	
		3%	1.5%	3%	1.5%
Flavour (50)	fresh	35	30	37	33
Body and texture (35)		24	21	22	20
Appearance (15)		12	13	14	15
Total (100)		71^{Aa}	64^{Ba}	73^{Aa}	68^{Ba}
Flavour (50)	1	36	31	38	34
Body and texture (35)		25	22	23	21
Appearance (15)		11	12	13	14
Total (100)		72^{a}	65^{a}	74^{a}	69^{a}
Flavour (50)	2	37	32	39	35
Body and texture (35)		26	23	24	22
Appearance (15)		10	11	12	13
Total (100)		73^{b}	66^{ab}	75^{b}	70^{b}
Flavour (50)	3	38	33	40	36
Body and texture (35)		28	24	25	23
Appearance (15)		9	10	11	12
Total (100)		75^{c}	67^{ab}	76^{c}	71^{c}
Flavour (50)	4	40	35	42	38
Body and texture (35)		29	25	27	24
Appearance (15)		8	9	10	11
Total (100)		77^{d}	69^{cd}	79^{d}	73^{d}

Samples were judged by 20 persons of the colleague staff members.

The statistical analysis of sensory evaluation scores showed that, the effect of cheese milk homogenization on flavour scores was highly significant (F = 185.18). Moreover, the storage period of the cheese had highly significant effect on flavour scores (F = 41.43). Furthermore, a highly significant effect could be seen on flavour scores by the interaction between storage period and homogenization (F = 3.15).

Body and texture scores of Mozzarella cheese decrease in case of Mozzarella cheese made using homogenized buffalo milk compared with those made using non-homogenized buffalo milk. Moreover, body and texture scores of Mozzarella cheese increased with increasing the cheese storage period. Also, it could be seen from the same table that, body and texture scores of cheese increased with rising fat content of the initial milk used homogenized or non-homogenized.

The statistical analysis foregoing results showed that, the body and texture scores of Mozzarella cheese was highly significant affected by the homogenization of the original milk used (F = 22.77). Furthermore, the storage period of Mozzarella cheese had also, a highly significant influence on the body and texture scores (F = 16.0). Moreover, the interaction of homogenization and storage period had highly significant influence on the body and texture of Mozzarella cheese (F = 9.43).

The data presented in Table 10 also, show that the cheese appearance scores increased in case of Mozzarella cheese made using homogenized buffalo milk compared with those made using non-homogenized buffalo milk. However, the appearance scores of all cheese decreased with progressing the storage period at 4°C.

The statistical analysis of the appearance scores showed that, the appearance was highly significant affected by the homogenization of the original milk used (F = 34.93). Moreover, the storage period was found to be highly significant effected the appearance of the cheese (F = 11.99). Furthermore, the appearance was found to be highly significant affected as a results of the interaction between the homogenization of the original milk used and the storage period (F = 41.18). From the previous results it is evident that the total scores of Mozzarella cheese increased in case of using homogenized buffalo milk compared with those made using non-homogenized buffalo milk. Also, the total scores of Mozzarella cheese increased in case of cheese made from with 3% fat. The obtained results are in agreement with those reported by Kosikowski [1960], Jana [1988] and El-Batawy et al. [2004] who mentioned that, homogenization of milk in Mozzarella cheese manufacture leads to whiter appearance and improved flavour. Also Emmons et al. [1980] and Emstrom and Anis [1985] found that the full fat cheese was different from the reduced fat cheese in all attributes, it was less open, less elastic, less crumbly,

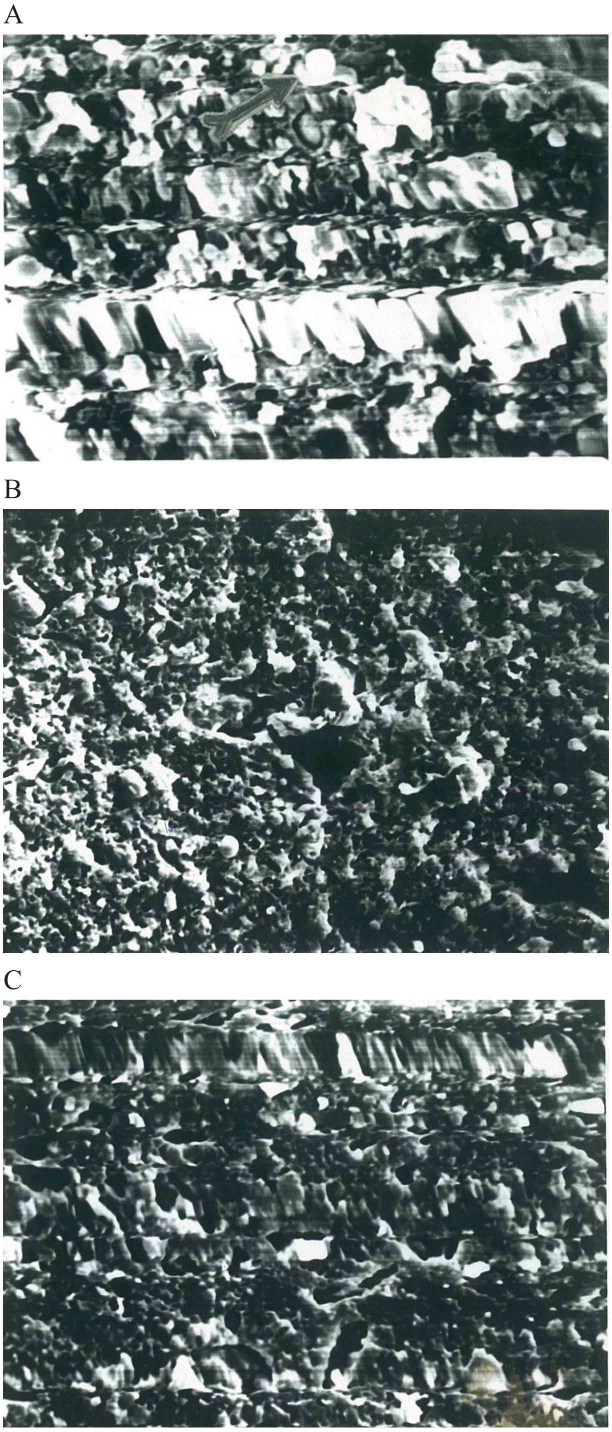

Fig. 1. Scanning electron microscopy of Mozzarella cheese made from non-homogenized milk 1.5% fat. More protein can be deformed per unit volume (A, B and C). Curd granule junctions were prominent (A). Calcium phosphate (arrow, A)

softer and smoother. Also, homogenization had some effect on the sensory properties of reduced fat cheese. It should be noted that the moisture content tended to increase the differences in texture related to homogenization maybe simply a reflection of the latter on the moisture content. Jana and Updahyay [1991] mentioned that, the homogenized milk cheese had superior flavour with acidic taste. Tunick et al. [1992] found that, homogenized cheeses were white in appearance whereas the non-homogenized cheeses were slightly yellow.

Microstructure

Microstructure of Mozzarella cheese was studied by both scanning and transmission electron microscopy. In the production of most cheese, the basic step is coagulation of milk characterized by aggregation of casein micelles into chains and clusters. Because of small dimensions of casein micelles and fat globules which depend on whether milk has been homogenized or not. Electron microscopy was used to study the microstructure of individual components in milk products such as casein micelles and fat globules, and changes with these components which undergo either alone or by interaction with each other and the effect of decreasing the fat levels and homogenization of the milk used. The microstructure of Mozzarella cheese prepared from standardized buffalo milk of 3 or 1.5% fat with or without homogenization is studied.

When the milk fat is reduced by half, the part-skim product tends to be harder, more crumbly and less smooth than normal. Probably due to increasing cross-linking with curd, introduced into cheese, together with reduced plasticizer (the fat present in curd acts as a plasticizer and inhibits the formation of cross-links between the casein chains). These are in agreement with those reported by Masi and Addeo [1986] who, presented an inverse relationship between the ratios of fat/solid non fat and the modulus of elasticity in experimentally manufactured Mozzarella cheese. Their data showed a linear increase in cheese firmness as fat content decrease. Badawi et al. [2004] found that Mozzarella cheese made from low fat milk (1.5%) had elongated protein matrix and contained few channels between protein strands and had firm and rubbery texture. As seen from Figure 3 B and 5 A, B fat globules are aggregated in large fat particles which acquired

Fig. 2. Scanning electron microscopy of Mozzarella cheese made from homogenized milk 1.5% fat. Rate of aggregation of the casein particles is reduced. Fat globules became part of the paracasein network. Junctions are more difficult to see

irregular shapes. Yun [1985] and Emmons et al. [1980] mentioned that fat globules in both stretched and stirred curd cheeses occurred in clusters. Also Merrill et al. [1996] mentioned that, reduced fat cheese had greatly denser protein matrix with fewer small vacuoles. The cross sections of the cheese showed more protein (Fig. 1 B, C; 5 A, B) to be cut or deformed per unit volume in the reduced fat cheese than in full fat cheese (Fig. 3 B and 6 A, B). Round-shaped (Fig. 1 A and 3 C) crystalline structures, tentatively identified as calcium phosphate, were also, observed in the granule junctions. The round or oval dark objects in light area indicate lactic bacteria (Fig. 3 A and B). Light area surrounding bacteria are believed to have been caused by digestion of protein which did not stain [Emmons et al. 1980, Tunick et al. 1993 a, b]. Milk may be homogenized for some period for cheese making. The fat globules become smaller and the total surface area large, to an extent depending on the pressure. When milk was homogenized, the volume of the casein particles reduced (Figs 1 B, 2, 5 A, B, 3 B, 4 and 6 A, B). Many micelles were linked to fat particles in homogenized milk without altering the micelles size distribution. This may reduce the total surface area of the casein, reducing the amount available for interaction and slowing it in this way. Homogenization of milk significantly decreases syneresis rate. This is related to the incorporation of micelles casein in the surface coat of the fat globules which causes the fat globules

A

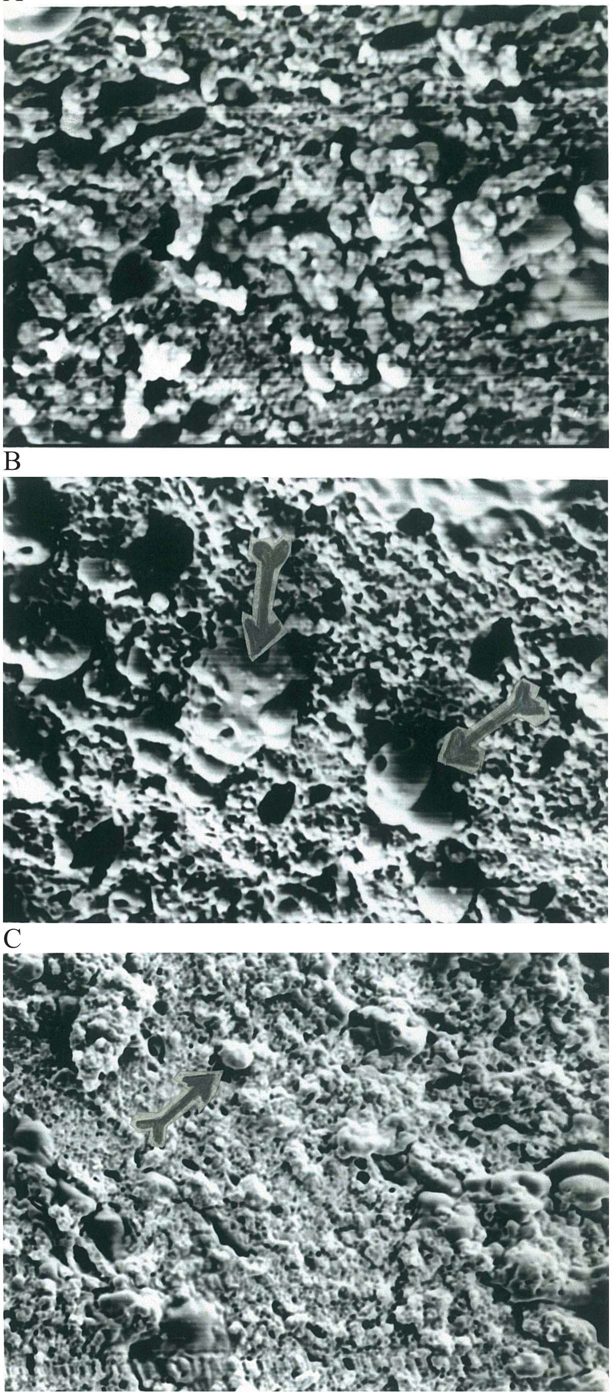

Fig. 3. Scanning electron microscopy of Mozzarella cheese made from non-homogenized milk 3% fat. Curd granule junctions were prominent (A). Fat globules are aggregated in large fat particles (arrow) which acquired irregular shapes (B). Calcium phosphate (C)

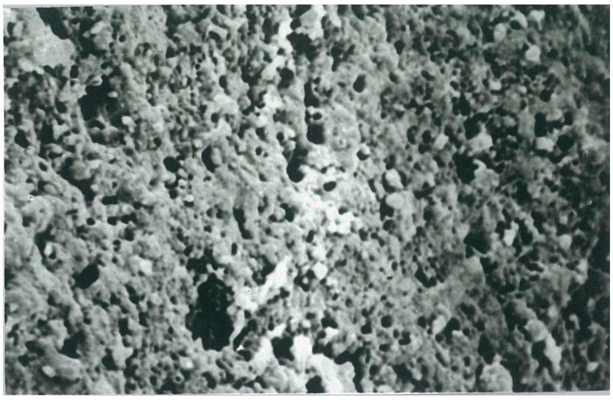

Fig. 4. Scanning electron microscopy of Mozzarella cheese made from homogenized milk 3% fat. Rate of aggregation of casein particles is reduced. Fat globules became part of the paracasein network Junction is more difficult to see

to become part of the paracasein network. Fox [1993] mentioned that the fat in cheese is normally held there by entrapment within the casein network and between the curd particles. (The only interaction between it and the casein being by friction). In cheese made from homogenized milk the fat globules are smaller and of more uniform size. During homogenization the natural fat globule membranes are partly replaced by casein micelles and this casein will probably bind to the casein network formed by the rennet action. Thus, the fat can interact strongly with the matrix. Cheese made from homogenized milk is smoother and has a finer texture than non-homogenized milk but is also, firmer and more elastic. Walstra and Jenness [1984] and El-Batawy et al. [2004], reported that homogenization results in cheese with appreciably firmer mesh structure, which explains the present findings. Curd granule junctions were observable in non-homogenized milk cheese, because large fat globules were lost at the granule surfaces leaving protein-dense junctions. In homogenized milk cheese they were less apparent because of the protein-dense areas, which results from the smaller fat globules, were narrower (Fig. 1 A, 2 A and 3 A, 4 A). These results are in agreement with those reported by Emmons et al. [1980] and Tunick et al. [2000], who found that, where fat is distributed

A B

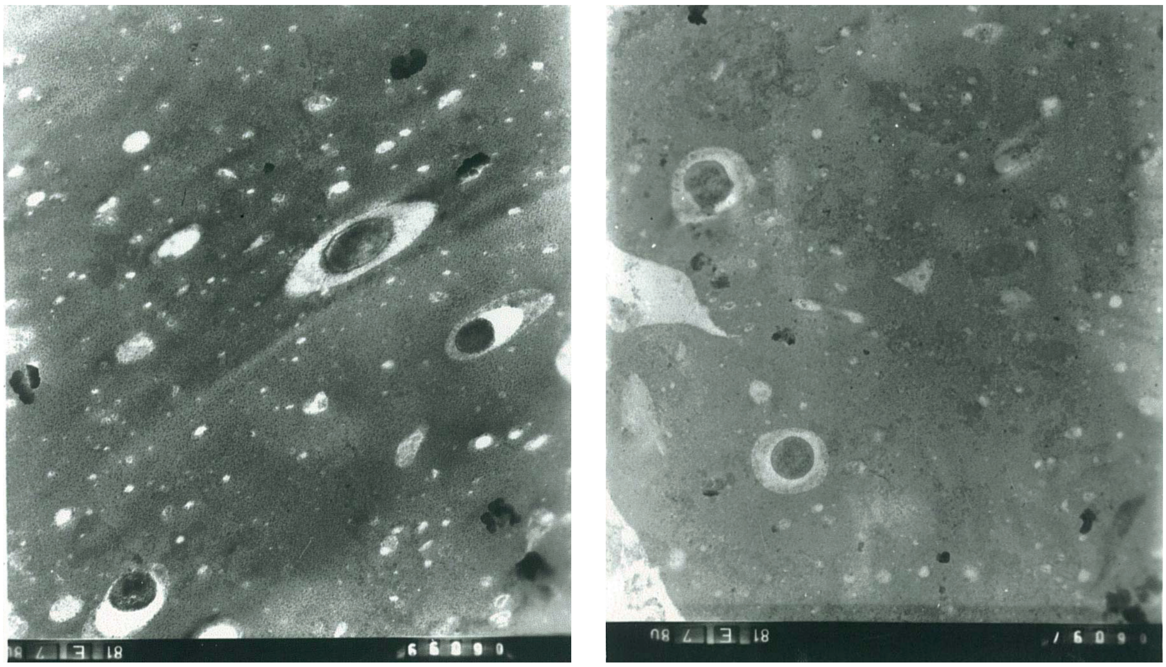

Fig. 5. Transmission electron microscopy. Mozzarella cheese made from 1.5% fat (A) non-homogenized and (B) homogenized milk. Light areas indicate fat, dark areas indicate protein. Round or oval dark objects in light areas indicate lactic bacteria; light areas surrounding bacteria are believed to have been caused by digestion of protein

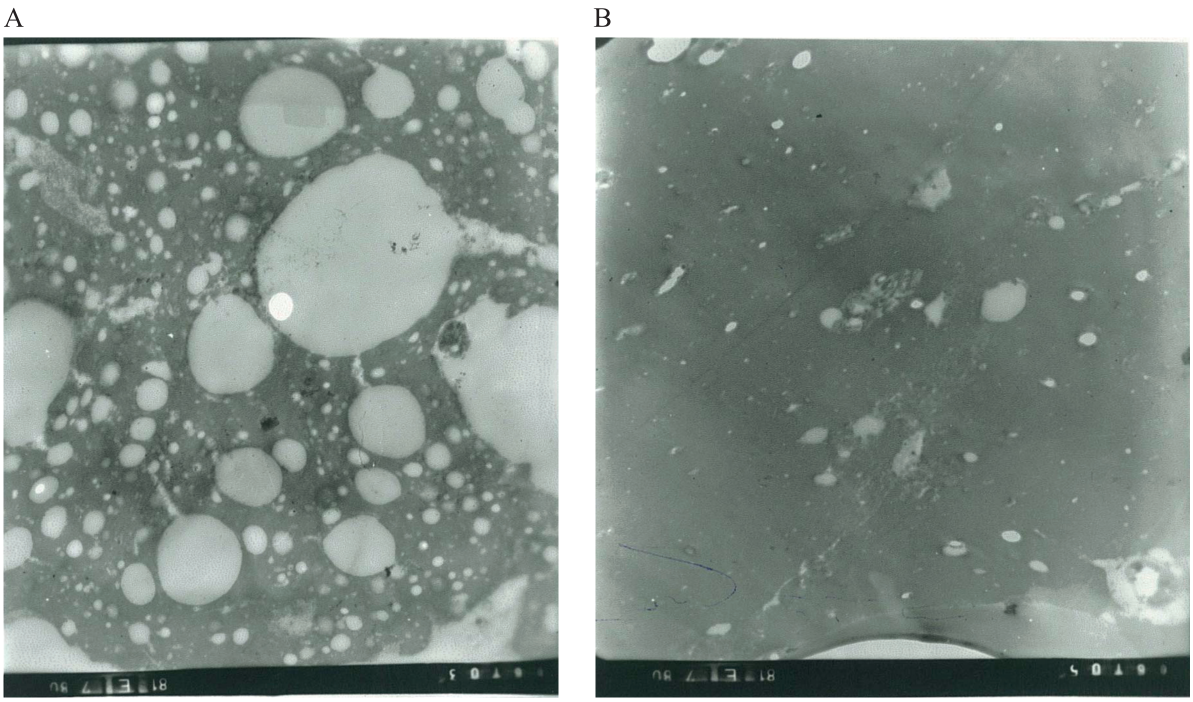

Fig. 6. Transmission electron microscopy. Mozzarella cheese made from 3% fat (A) non-homogenized and (B) homogenized milk. Light areas indicate fat, dark areas indicate protein (8000×)

uniformly in the form of small fat globule fragments produced by homogenization, less of the small globules leave a thinner super facial layer depleted of fats and thus, enriched in protein. The junctions, formed by the fusion of two such narrow areas, are more difficult to see than the wide protein-dense junction in the non-homogenized milk cheese. In addition, scattering of light on the empty cavities in the curd granule interior following fat removal for the visualization of the junction patterns is decreased in the reduced fat cheese, thus lowering the contrast between the junction and granule interior.

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WPŁYW HOMOGENIZACJI NA WŁAŚCIWOŚCI I MIKROSTRUKTURĘ SERA MOZZARELLA Z MLEKA BAWOLEGO

STRESZCZENIE

Wstęp. Charakterystyczna włóknista struktura sera Mozzarella oraz jego rozpływalność wynikają z procesu plastyfikowania masy sera w gorącej wodzie. Tekstura sera Mozzarella zależy także od procesu homogenizacji przerabianego mleka bawolego o wystandaryzowanej zawartości tłuszczu na poziomie 3 i 1,5%. W pracy oceniono wpływ procesu homogenizacji mleka przerobowego na cechy reologiczne, mikrostrukturę i cechy sensoryczne sera Mozzarella.
Materiał i metody. Do świeżego surowego mleka bawolego wprowadzono kultury startowe *Streptococcus thermophilus* i *Lactobacillus delbrueckii* ssp. *bulgaricus*. Koagulantem był roztwór podpuszczki cielęcej w proszku (HA-LA). Mleko bawole znormalizowano pod względem zawartości tłuszczu i zhomogenizowano przy ciśnieniu 25 kG/cm^2 po uprzednim ogrzaniu do 60°C. Analizie poddano mleko i otrzymany ser. Mikrostrukturę próbki sera badano z użyciem transmisyjnego lub skaningowego mikroskopu elektronowego. Uzyskane dane poddano analizie statystycznej.
Wyniki. Proces homogenizacji mleka przerobowego zwiększa w nim zawartość azotu rozpuszczalnego, wolnych kwasów tłuszczowych, tyrozyny i tryptofanu, a tym samym w otrzymanym z tego mleka serze Mozzarella. Rozpływalność sera Mozzarella zwiększała się wraz ze wzrostem zawartości tłuszczu i czasu przechowywania, a zmniejszała wskutek homogenizacji. W wyniku homogenizacji i wydłużenia czasu przechowywania zwiększyła się jędrność sera. Zwiększyły się także wyniki sensorycznej oceny smaku i wyglądu, natomiast punkty przyznane za strukturę były mniejsze po homogenizacji, a większe po przechowywaniu. Mikrostruktura sera o mniejszej zawartości tłuszczu jest bardziej twarda, krucha i mniej płynna niż przy normalnej zawartości tłuszczu. Nitkowata struktura skrzepu była bardziej widoczna w serze z mleka niehomogenizowanego.
Wnioski. Homogenizacja mleka przerobowego wpłynęła na zmiany w mikrostrukturze sera Mozzarella. Analizując mikrostrukturę, stwierdzono, że ser z mleka homogenizowanego ma delikatniejszą teksturę, jest gładszy, bardziej jędrny i elastyczny niż ser z mleka niehomogenizowanego.

Słowa kluczowe: ser Mozzarella, homogenizacja, mikrostruktura, mleko bawole, ocena sensoryczna

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