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FUNCTIONAL AND MICROSTRUCTURAL PROPERTIES OF LOW FAT MOZZARELLA CHEESE AS AFFECTED BY EXOPOLYSACCHARIDES-PRODUCING *S. THERMOPHILUS* AND STORAGE CONDITIONS

El –Sayed, Elham M.
Dairy Sci. Dept., Faculty of Agric., Cairo Univ.

ABSTRACT

This study was carried out to improve the functional properties of low fat mozzarella cheese and to extend its shelf-life via the use of exopolysaccharides (EPS) producing starter cultures and different freezing conditions. Conventional starter culture consisting of both non EPS producing *S. thermophilus* St CH-1 in association with *L. helveticus* Lh CH-5 with or without fat replacer (Simplesse.S-100), and two EPS-producing strains of *S. thermophilus* SFi-12 and SFi-39, each in combination with non-EPS *L. helveticus* Lh CH-5 were used in manufacture of low fat Mozzarella cheese. The resultant cheeses were ripened at 4ºC for 28 days (refrigerated cheese).

The results obtained indicated that cheeses made with EPS-producing *S. thermophilus* had significantly($p< 0.05$) higher level of moisture and moisture in non fat substances (MNFS) as compared with non-EPS cheeses with or without simpelesse when fresh or during the refrigerated storage period. In addition, the meltability and fat leakage values of EPS-cheeses were the highest among all experimented cheeses. However, the cheese made with EPS (SFi-39) exhibited the lowest apparent viscosity (AV). Statistical analysis showed that meltability, fat leakage and AV were significantly affected by type of EPS culture ($p < 0.001$), ripening period ($p < 0.001$) and their interactions ($p < 0.001$ for meltability, $p < 0.05$ for fat leakage and AV).

As Mozzarella cheese is considered short storage lives, a freezing scheme was designed to study its effect on the functional properties and shelf-life of the resultant cheese. The freezing schemes for the above experimental EPS and non EPS cheeses were as follows: a) Cheese ripening for 14d at 4ºC before freezing at -20ºC for 60 days, then again ripening at 4ºC for 14d., and b) Cheese freezing immediately after manufacturing at -20ºC for 60 days then ripening at 4ºC for 28d.

The results obtained indicated that frozen EPS cheeses retained the highest MNFS and TA at the end of frozen schemes (88d). Meltability and fat leakage of experimented frozen cheeses have increased upon ripening at two stages (before and after freezing) or one stage (after freezing). AV of frozen Mozzarella cheeses was fluctuated during freezing and ripening schemes.

Regarding the microstructural properties of non EPS and EPS experimented cheeses, Scanning electron micrographs (SEM) of the refrigerated cheeses (28d) showed that, the EPS-cheese was porous and had an open texture with numerous voids, whereas the non EPS cheeses had a closed and compact protein matrix. There were no marked differences between the microstructure of all refrigerated and frozen cheeses, which had a large voids in the cheese matrix.

The obtained results confirmed that EPS-producing *S. thermophilus* can be utilized to significantly increase cheese moisture content and to improve the texture and microstructure characteristics of resultant low fat Mozzarella cheese. In addition , freezing is a suitable alterative to prolong stability and shelf-life of low fat Mozzarella cheese without modifying its functional properties.

INTRODUCTION

Mozzarella cheese has with some justification, been called the glamour cheese of the 20th century. The pizza industry has played a major role in the dramatic rise in Mozzarella cheese production in the world. Nowadays, Mozzarella cheese become in great demand in Egypt, because of the wide spread of the fast food restaurants that deliver pizza. Additionally, the educational revolution brought a mass number of students to the school and universities who prefer pizza as a fast complete meal.

In recent years, consumer demand for low fat foods has created interest in the development and manufacture of low fat Mozzarella cheese (Merril *et al.*, 1994; Mistry *et al.*,, 1993; Oberg *et al.*,1996; Tunick *et al.*, 1991 and Broadbent *et al.*, 2001). But removal of fat from Mozzarella cheese affects several physical properties of the cheese especially melting capacity, stretchability, elasticity and free oil formation. Specifically resulted Mozzarella cheese becomes tough and rubbery, more heat is required for melting, and the cheese loses pliability rapidly during cooling (Mistry and Andrerson, 1993).

It was also established that these properties are heavily influenced by cheese moisture level (McMahon and Oberg, 1998). Examination of cheese microstructure shows that in full fat or part-skim Mozzarella cheese, much of the water is contained in channels formed in the protein matrix by entrapped fat globules (McMahon *et al.*,1993; Oberg *et al.*, 1993). Because there are very few fat globules to break up the protein matrix in low fat Mozzarella, these channels become much narrower with less space available for water retention. This results in cheese with a lower moisture level (Oberg *et al.*, 1993) and subsequently, a tough, rubbery texture and poor melt and stretch properties (McMahon and Oberg, 1998).

Since, exopolysaccharides (EPS) have excellent water-binding properties, and moisture retention are vital to functionality in low fat cheese, McMahon and Oberg (1998) and Perry *et al.*, (1997) investigated the influence of an EPS-producing starter pair *S. thermophilus* MR-1C and *L. bulgaricus* MR-1R on the moisture and melt properties of low fat (6%) Mozzarella cheese. As predicted, low-fat Mozzarella cheese manufactured with MR-1C and MR-1R contained significatntly ($P<0.05$) more moisture and had better melt properties than cheese made with an EPS^- commercial starter pair (*S. thermophilus* TAO61 and *Lactobacillus helveticus* LH100).

Moreover, the instability of some physical properties such as melting capacity and stretchability and the seasonality of milk production have led large-scale consumers and producers to freeze the cheese for storage (Alvarez, 1986; Pilcher and Kindstdt, 1990). The low temperatures suspend or reduce biochemical modifications which would occur during freezing storage.

Few studies have been carried out to investigate the effects of freezing and conditions of thawing on Mozzarella cheese. In studies conducted with Mozzarella, immediately after thawing, the cheese showed high fat leakage, low cohesiveness, free-surface moisture, bleached discoloration and poor melting (Dahlstrom, 1978). All these alterations were reverted after 3 wk of refrigerated storage at 4.4 ºC. Tunick *et al.*, (1991) froze Mozzarella cheese at –20 ºC for 8 wk. After thawing, they tempered the cheeses at 4ºC for 3 wk. and they observed that a greater melting capacity than that of the nonfrozen control. Bertola *et al.*, (1996) reported that frozen Mozzarella produced with a thermophilic starter, and subsequently tempered for 14 to 21 days at –20 ºC, showed the same quality as refrigerated cheese.

Evaluation of the extent of physical changes caused by freezing of Mozzarella is needed and this could contribute to a better understanding of changes occurring during refrigerated and frozen storage. Therefore, the objective of this study was to evaluate and compare effects of exopolysaccharides-producing strains of *S. thermophilus* and storage conditions (refrigerated and frozen stortage) on functional and microstructural properties of low fat Mozzarella cheese with and without adding fat replacer.

MATERIALS AND METHODS

Materials:

Milk: fresh cow's milk was obtained from the herd of the Faculty of Agriculture, Cairo University, Giza, Egypt.

Starter cultures: Four strains of thermophilic cheese cultures were used in this study. Two lyophilized non-EPS-producing cheese cultures (*S thermophilus* St CH-1 and *L. helveticus* CH-5) were obtained from Chr. Hansens, Denmark. Two lyophilized EPS-producing cheese cultures (*S thermophilus* SFi-12 and SFi-39) were obtained from Nestle Research Centre, Switerland.

Fat replacers: Simplesse® S100 based on whey proteins was obtained from Nutrasweet Co., Deerfield, II, USA.

Cheese Making: Fresh cow's milk standardized to a casein/fat ratio 3.5 was used for making low fat Mozzarella cheese according to the method of Merril et. al., (1994). Standarized milk was preacidified to pH 6.0 using diluted acetic acid (1:10 v/v) and devided into equal four portions (45 kg each), in the first portion regular starter culture consists of non EPS-producing *S thermophilus* (St CH-1) and *L. helveticus* (Lh CH-5) was added at the rate of 3% (v/v) (2% St CH-1 + 1% Lh CH-5,v/v). A starter culture of non-EPS producing culture of Lh CH-5 with each one of the EPS-producing cultures of SFi-12 and SFi-39 was separately added to the second and thired portions at the rate of 3% each. The remained fourth portion was mixed with 0.1% Simplesse S-100 (protein-based fat replacer) at 4ºC and vigorously agitated for 2 min and milk was then tempered to 34ºC and inoculated with regular starter culture (2% St CH-1 and 1% Lh Ch-2). After 30 min of ripening, 90 ml of standardized diluted calf rennet were added to each of the four portions. The cheese curds were cut 10 min after rennet addition using 1.9 cm knifes. Curd particles were left undisturbed for 15 min, followed by 30 s of gentle agitation every 15 min. The temperature of all curd particles from the four treatments was raised to 40ºC over 10 min with periodic gentle agitation. Then whey was drained at pH 6.0 and the curds were hand-cheddared (turning every 20 min) at 40 ºC until the curd pH reached 5.2. Curds were cut and salted with 1% (w/w) of dry salt. At pH 5.2, the curd was hand-stretched in 5% hot brine solution (80ºC) until elastic and smooth mass was attained. The curds were then immersed in ice bath for 1 h. Blocks of resulted cheese were vaccum-packaged. Three replicats were made for each control and experimental cheeses on 3 separate days. Each of the four cheeses produced was randomly divided into 3 parts, each one part being subjected to the following treatments: i- maintained under refrigeration at 4ºC for 28 days; ii-frozen at –20 ºC for 2 months, then tempered at 4ºC for 28 days; iii-tempered for 14 days at 4ºC, then frozen at –20 ºC for 2 mon., again tempered at 4 ºC for 14 days to complete their ripening period.

Analytical methods: For all analysis, except meltability, the cheese samples were ground in a blender to provide a particle size of about 2-3 mm. All analysis were done in triplicates.

Chemical analysis: Moisture, titratable acidity, total nitrogen (TN) and fat contents were determined according to the method mentioned by Ling (1963). pH-values were measured using an electric pH-meter (model Jenway 3305, England). Salt content was determined by the IDF method (1972). Calcium content was determined by the method of Natailianas and Whitney (1964) using calcein as an indicator.

Meltability tube test: The meltability of cheese samples was examined using tube test as described by Olson and Price (1958) which Modified by Rayan *et al.*, (1980).

A Pyrex glass tube 30 mm diameter and 250 mm length was used to hold the cheese during the test. One end of the tube was closed with a rubber stopper and the other end was covered with aluminum foil. A cylinder shape cheese sample (15 g ± 0.2) was placed in the tube and a reference line was marked on the tube aligned with the front edge of the cheese cylinder. Melting tubes were placed in a horizontal position in an oven at 110 ºC for 30 min. The distance between the reference line and the end line of flow (leading edge) of the melted cheese was recorded in mm as cheese meltability.

Free oil formation: The amount of free oil released from Mozzarella cheese during melting was measured using fat leakage test as described by Bertola *et al.*,, (1996).

Cheese disks (25-mm in diameter x 5-mm thick) were cut from the inside of cheese sample. the teat portions were placed in a Petri dishes. Filter paper disks (Whatman #1, 9-mm in diameter) were placed inside glass Petri dishes. Triplicate disks for each cheese sample were placed on the filter papers in an oven at 110 ºC for 30 min. The Petri dishes were cooled to room temperature. The diameter of each oil ring formed on filter paper was measured to the nearest 0.01 cm at four different angles, and means were calculated.

Apparent viscosity (AV): Apparent viscosity of mozzarella cheese was measured as described by Fife et. al. (1996). A Brookfield DV 11+ helical viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) fitted with a T-bar spindle (T-F with a 9.0 mm crossbar) was used to measure AV of melted cheese. An IBM-compatible computer, equipped with Brookfield DV Gather+ 1.0 software, was used to record AV every second for 2 min. The mean of the readings during period of 0.5 to 1.5 min was reported as AV and expressed as cP.

Cheese microstructure:

Cheese cubes (3 x 3 x 10 mm) were prepared from samples of stretched cheese and fixed in 4 % glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 2 hr to fix the protein. The cubes were washed several times in 0.1 M phosphate buffer (pH 7.2) for 15 min intervals, then post fixed in 1% osmium tetroxid (OsO4) in 0.1 M phosphate for 1-2 hr for fat fixation. The cheese samples were re-washed several times in 0.1 M phosphate buffer for 15 min intervals. Then specimens were dehydrated in series of aqueous ethanol solutions (25%, 50%, 75%, 95% and 100%) for 15 min each. The samples were dried to critical point using CO2 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.inc). Samples were examined at 5 KV through Scanning Electron Microscope JEOL – jsm 5200 equiped with an IBM-compatible computer to recording the images.

Statistical analysis:

The two-way statistical analysis of variance (ANOVA), mean separation, correlation and factor factorial was performed by running the MSTAT-C (ver.2.10, Michigan state university, USA.) package on a personal computer. The same program was used to analyze a factorial analysis of variance completely randomized design. The statistical significance of the data was determined using *P* value was equal to or less than 0.05.

RESULTS AND DISCUSSION

Initial composition of cheese:

The gross composition of the experimented low fat Mozzarella cheeses {non-EPS cheese (St CH-1/Lh CH-5) without simplesse, the St CH-1/Lh CH-5 cheese with simplesse and the EPS-cheeses (SFi-12/Lh CH-5 and SFi-39/Lh CH-5)} is summarized in Table 1. Cheese made with EPS-producing culture (SFi-12/Lh CH-5 and SFi-39/Lh CH-5) contained significantly ($P<0.05$) more moisture and moisture in non-fat substances (MNFS) than that of non-EPS-cheeses with or without simplesse (Table 1). This might be due to the water-binding effect of exopolysaccharides produced by EPS-strains (Hassan *et al.*, 1996 and Mistry, 2001). In comparison, SFi-39/Lh CH-5 cheese exhibited the highest content of moisture , MNFS and FDM followed by SFi-12/Lh CH-5 cheese. Using EPS- SFi-39 increased the contets of moisture and MNFS by 6.53 and 6.88%, over that of non-EPS cheese (St CH-1/Lh CH-5) respectively. The protein and S/M content of the experimented Mozzarella cheeses were found to be higher in non-EPS-cheese (control) than that of other cheeses. Contrary to this, calcium content and TA% were found to be higher in EPS$^+$ SFi-39 than that of other experimented cheeses.

The increase in the levels of moisture and MNFS in the cheeses made with EPS$^+$ *S. thermophilus* strains, is an agreement with earlier studies (Low *et al*, 1998; Perry *et al*, 1997 and Petersen *et al*, 2000).

Table 1 : Initial composition of low fat Mozzarella cheese as affected by EPS-producing *S. thermophilus*.

Composition	Strain cultures*			
	St CH-1/Lh CH-5	St CH-1/Lh CH-5 + simplesse	SFi-12/Lh CH-5	SFi-39/Lh CH-5
Moisture, %	56.05^d±0.069	58.03^c±0.023	58.75^b±0.001	59.71^a±0.028
MNFS†, %	59.74^d±0.017	61.86^c±0.011	62.84^b±0.025	63.86^a±0.037
Fat, %	6.2^b±0.010	6.2^b±0.020	6.5^a±0.020	6.5^a±0.040
FDM†, %	14.11^d±0.030	14.77^c±0.050	15.76^b±0.009	16.13^a±0.030
S/M†, %	2.32^a±0.010	2.29^b±0.030	2.02^d±0.010	2.26^c±0.020
Calcium, %	0.694^c±0.002	0.789^a±0.002	0.765^b±0.002	0.798^a±0.001
Protein, %	26.30^a±0.60	25.40^b±0.10	25.90^{ab}±0.40	25.70^{ab}±0.10
PH	5.21^a±0.010	5.20^a±0.010	5.17^b±0.010	5.17^b±0.010
TA, %	0.68^c±0.010	0.73^b±0.20	0.80^a±0.20	0.80^a±0.030

$^{a-d}$ Mean values (± SD n=3) with different superscripts in the same row are significantly different (P<0.005).
*** See text for details of strain cultures.**
† MNFS = Moisture-in-non-fat substances; FDM = Fat-in-dry matter; S/M = Salt-in-Moisture.

The statistical analysis showed that the moisture, MNSF, Ca and S/M contents of the low fat Mozzarella cheese were highly significant affected by the strain of *S. thermophilus* used (SFi-12 or SFi-39) in the starter blend. On contrary, the protein, TA and pH values were not highly significant (P<0.05) affected by them (Table 1).

Cheese properties as affected by EPS-culture and storage conditions:
Refrigerated cheese:

As shown in Table 2 MNFS contents of all cheeses were decreased by extending the refrigerated storage period. At day 28 of storage, the cheeses made with EPS-producing cultures (SFi-12/Lh CH-5 and SFi-39/Lh CH-5) resulted in the highest MNFS contents compared to non-EPS cheeses (St CH-1/Lb CH-5) with or without simplesse, the MNFS content of EPS-cheeses (SFi-12/Lh CH-5 and SFi-39/Lh CH-5) increased by 6.83 and 6.06% over than that of the non-EPS cheese (St CH-1/Lh CH-5), respectively, whereas MNFS content of non-EPS cheese with simplesse increased by 5.06%. The results are in agreement with that reported by Bhaskarachary & Shah (2001); Perry *et al.*, (1997) and Broadbent *et al.* (2003). Significant decrease (P<0.05) in S/M% of cheese made with EPS-cultures (SFi-39/Lh CH-5 and SFi-12/Lh CH-5) was noticed compared to that of non-EPS- chees

(St CH-1/Lh CH-5), at the end of refrigerated storage (Table 2). The results also indicated that, no significant changes ($P<0.05$) in the pH values of all cheeses throughout storage period. The pH values decreased from 5.17 – 5.21 when fresh to 4.90 – 5.01 at the end of storage. In contrast, the titratable acidity of all cheese increased significantly ($P<0.05$) over the storage period. At the end of storage, a significantly increase was observed in TA of cheeses made with EPS-cultures SFi-39/Lh CH-5 and SFi-12/Lh CH-5 compared to that of non-EPS cheese with or without fat replacer, but there was no significant differences between TA% of cheeses made either with SFi-12/LH CH-5 or SFi-39/Lh CH-5.

Table 2 :Changes in the chemical composition of low fat Mozzarella cheese as affected by EPS-producing *S. thermophilus* and refrigerated storage at 4ºC.

Strain culture[*]	Cheese component Refrigerated period, d				
	Fresh	**7**	**14**	**21**	**28**
			MNFS[†], %		
St CH-1/Lh CH-5	59.74^{i}±0.20	59.48^{j}±0.03	59.23^{k}±0.01	59.15^{k}±0.02	58.74^{l}±0.10
St CH-1/Lh CH-5 + simplesse	61.86^{g}±0.01	62.02^{g}±0.06	61.97^{g}±0.02	61.75^{h}±0.06	61.71^{h}±0.01
SFi-12/Lh CH-5	62.84^{c}±0.06	62.58^{d}±0.04	62.47^{de}±0.01	62.39^{ef}±0.03	62.30^{f}±0.03
SFi-39/Lh CH-5	63.86^{a}±0.01	63.63^{a}±0.04	63.27^{b}±0.03	63.18^{b}±0.02	62.75^{c}±0.04
			S/M[†], %		
St CH-1/Lh CH-5	2.319^{l}±0.03	2.506^{g}±0.02	2.575^{f}±0.05	2.758^{c}±0.04	2.905^{a}±0.02
St CH-1/Lh CH-5+ simplesse	2.294^{m}±0.02	2.353^{k}±0.02	2.412^{j}±0.03	2.653^{e}±0.02	2.775^{b}±0.05
SFi-12/Lh CH-5	2.016^{q}±0.02	2.069^{p}±0.01	2.193^{o}±0.02	2.305^{lm}±0.01	2.429^{i}±0.02
SFi-39/Lh CH-5	2.263^{n}±0.03	2.340^{k}±0.03	2.449^{h}±0.02	2.573^{f}±0.03	2.698^{d}±0.05
			pH		
St CH-1/Lh CH-5	5.17^{b}±0.01	5.15^{bc}±0.03	5.06^{bcde}±0.02	4.96^{de}±0.03	4.92^{de}±0.02
St CH-1/Lh CH-5 + simplesse	5.20^{b}±0.02	5.17^{bc}±0.04	5.40^{a}±0.43	5.07^{bcd}±0.02	5.01^{cde}±0.03
SFi-12/Lh CH-5	5.21^{b}±0.02	5.17^{bc}±0.01	5.07^{bcd}±0.02	4.92^{de}±0.02	4.90^{e}±0.02
SFi-39/Lh CH-5	5.17^{b}±0.10	5.13^{bc}±0.02	5.08^{bcd}±0.01	4.95^{de}±0.04	4.92^{de}±0.03
			TA, [†] %		
St CH-1/Lh CH-5	0.68^{i}±0.02	0.75^{g}±0.02	0.82^{de}±0.04	0.86^{c}±0.02	0.90^{b}±0.01
St CH-1/Lh CH-5 + simplesse	0.73^{h}±0.02	0.80^{f}±0.02	0.85^{c}±0.01	0.86^{c}±0.01	0.89^{b}±0.02
SFi-12/Lh CH-5	0.80^{f}±0.02	0.81^{ef}±0.01	0.86^{c}±0.02	0.90^{b}±0.03	0.96^{a}±0.02
SFi-39/Lh CH-5	0.80^{f}±0.02	0.83^{d}±0.02	0.85^{c}±0.01	0.90^{b}±0.02	0.95^{a}±0.02

[a – r] Mean values (± SD; n =3) with different superscripts in the same row are significantly different (P<0.005).
[*] See Table 1 and text for details of cheese component and strain cultures.
[†] MNFS = Moisture-in-non-fat substances; S/M = Salt-in-Moisture; TA = titratable acidity.

Functional properties:
Meltability:

Fig 1 illustrated the average meltability values in terms of cheese flow (mm) for low fat Mozzarella cheese made with or without EPS-producing cultures throughout 28 days of storage period at 4ºC. Meltability of fresh cheeses made with the EPS cultures (SFi-12/Lh CH-5 and SFi-39/Lh CH-5) was more than that of their non-EPS cheeses (St Ch-1/Lh CH-5) with or without added fat replacer. The meltability valued 40 mm for each EPS-cheeses and 32 mm for non-EPS cheeses (Fig 1).

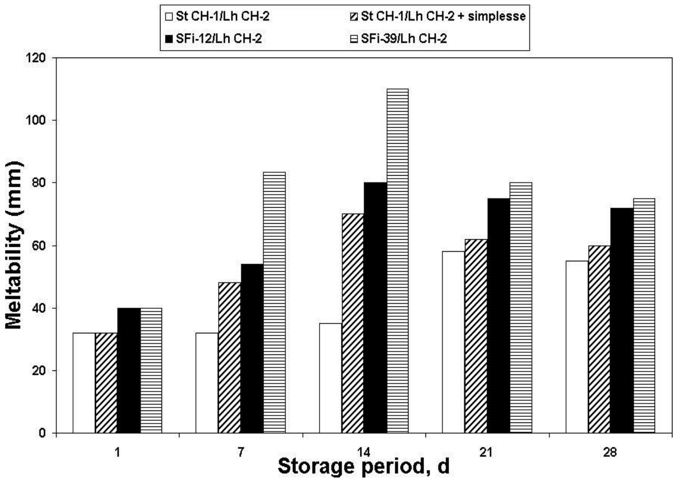

Fig. 1 : Mean values of meltability of low fat Mozzarella cheese as affected by EPS-producing *S. thermophilus* and refrigerated storage period at 4ºC.

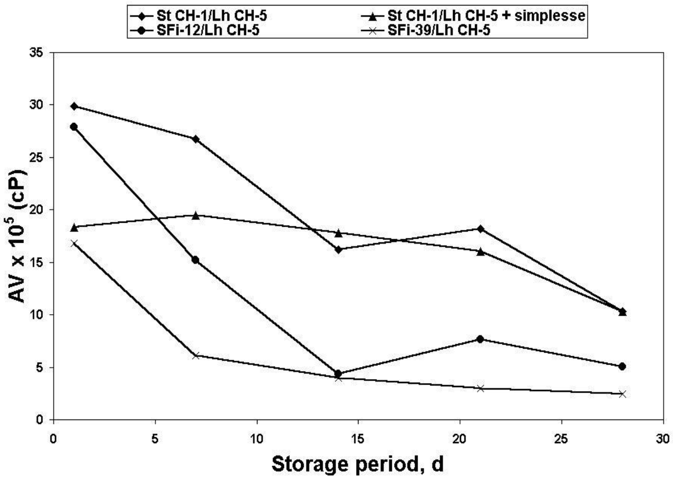

Fig 2 : Mean values of apparent viscosity (AV) of low fat Mozzarella cheese as affected by EPS-producing *S. thermophilus* and refrigerated storage period at 4ºC.

Meltability of all cheeses increased from d 1 to d 14 and then decreased in meltability from d 14 to d 28, except for the non-EPS cheese (control) (ST CH-1/LH CH-5 without added fat replacer), which decreased in meltability from d 21 to 28. The cheese made with EPS-cultures SFi-39/Lh CH-5 exhibeted the highest meltability at all days of storage, followed by cheese made with EPS-culture SFi-12/Lh CH-5 (Fig 1). The increase in meltability observed as affected by EPS-starter and refrigerated storage are in agreement with those reported by Fife *et al.* (1996); Perry *et al*. (1998) and Broadbent *et al*. (2003). Statistical analysis showed that the use of EPS-starter culture (SFi-39 or SFi-12), storage period and their interactions affected significantly ($P<0.001$) cheese melting (Table 4).

Fat leakage:

The free oil "fat leakage" test is important because an excess of free oil on the surface of a pizza after baking is a major quality defect for Mozzarella cheese manufacturers. The results in Table 3 showed that the oil ring diameter of fat leakage of EPS^{+}-cheeses (SFi-12/Lh CH-5 and SFi-39/Lh CH-5) were the largest values compared to those of non-EPS cheeses with or without simplesse at all days of refrigerated storage. These findings are in line with the results of Rudan *et al* (1999) who reported that the free oil results seemed to be related to melted cheese functionality.

Table 3 : Fat leakage values of low fat Mozzarella cheese as affected by EPS-producing *S. thermophilus* and refrigerated storage at 4ºC.

Strain culture*	Refrigerated storage, d				
	Fresh	7	14	21	28
	Fat leakage, mm				
St CH-1/Lh CH-5	$36.00^{jk}\pm1.0$	$39.00^{i}\pm2.0$	$41.00^{h}\pm1.0$	$35.00^{k}\pm1.5$	$33.00^{l}\pm1.0$
St CH-1/Lh CH-5 + simplesse	$41.00^{h}\pm1.5$	$43.00^{fg}\pm1.0$	$46.00^{cd}\pm2.0$	$43.00^{fg}\pm1.5$	$37.00^{j}\pm1.0$
SFi-12/Lh CH-5	$45.00^{de}\pm1.5$	$47.00^{c}\pm1.0$	$51.00^{a}\pm1.5$	$47.00^{c}\pm1.0$	$42.00^{gh}\pm1.0$
SFi-39/Lh CH-5	$46.00^{cd}\pm2.0$	$49.00^{b}\pm1.5$	$52.00^{a}\pm1.0$	$47.00^{c}\pm2.0$	$44.00^{ef}\pm1.5$

$^{a-l}$ Mean values (± SD; n =3) with different superscripts in the same row are significantly different ($P<0.005$).

*** See Table 1 and text for details of cheese component and strain cultures.**

The statistical analysis indicated that, significant ($P<0.05$) differences were found in fat leakage between EPS and non-EPS-cheeses. Fat leakage was significantly affected by EPS starter cultures ($P<0.001$), storage period ($P<0.001$) and their interaction (Table 4).

Apparent viscosity (AV) :

The data in Fig. 2 indicated that there were differences in AV-values between EPS and non-EPS-cheeses (St CH-1/Lh CH-5) with or without simplesse. During refrigerated storage, the AV of all cheeses (EPS and non-EPS) was decreased. Fig. 2 also showed that the lowest AV was attained when EPS SFi-39 or SFi-12 culture was used for making low fat Mozzarella cheese (2.45×10^5 and 5.04×10^5 cP, respectively) at the end of storage period. In contrast, low fat cheeses with higher AV were obtained by using non-EPS culture (St CH-1/Lh CH-5) without fat replacer (10.31 x 10^5 cP). These results are in line with that of Fife *et al* (1996) and Merill *et al* (1994) who found that AV of low fat Mozzarella cheese decreased with time of refrigerated storage. The analysis of variance indicated that the mean level of AV over the 28 d of storage period was significantly affected by EPS culture type, storage period and their interaction (Table 4).

Table 4 : Mean squares (MS) and probabilities (*P*) for meltability, fat leakage and apparent viscosity (AV) of low fat Mozzarella cheese made with EPS-producing cultures and stored at 4 ºC for 28 days.

Factor	**Meltability**		**Fat leakage**		**AV**	
	MS	***P***	**MS**	***P***	MS(x 10^{10})	***P***
EPS-culture type (A)	3352.13	***	360.00	***	526.30	***
Storage period (B)	2710.29	***	117.90	***	459.70	***
Interaction (A x B)	390.09	***	2.00	*	56.09	*
Error	4.23		1.00		22.87	

*** *P* <0.05   *** *P*<0.001**

Frozen cheese:

Chemical composition:

Tables 5 and 6 showed the effect of the use of both EPS-producing *S. thermophilus* and different frozen conditions on the chemical composition of low fat Mozzarella cheese. Regarded of frozen conditions, cheese made with EPS-producing culture (SFi-12/Lh CH-5 and SFi-39/Lh CH-5) contained significantly (P<0.05) more MNFS and TA than those of non-EPS cheeses with or without simplesse. While cheeses ripenied for 14 d before and 14 d after freezing for 60 d there was a slightly significant differences (P<0.005) in the chemical composition indices during the storage period (Table 5). On the other hand, low fat Mozzarella cheese, which immediately frozen -20 ºC) after manufacturing for 60 days and then ripened for 28 d at 4 ºC showed no significant differences in MNFS,S/M and TA during the freezing time (Table 6).

Meltability:

Fig 3 A, B illusterated the effect of EPS-producing culture and frozen conditions on the meltability of low fat Mozzarella cheese. Ripening of cheese at 4ºC for 14 d before freezing at -20 ºC for 2 mon, increased the meltability from d 1 to d 14 and reached the maximum value at the 14th d, then decreased during freezing time. Furthermore, the meltability re-increased during the second ripening process carried out (14 d/4ºC) after freezing, but the increment was not equal to that happened at first ripening stage prior freezing. For cheese frozen immediately after manufactureing, the melt capacity decreased slightly during the freezing period then significantly (P<0.05) increased when ripened and reached the maximum value at the end of ripening period (28 d after 2 months of freezing). In general, frozen storage decreased meltability of cheeses ripened for 7 d before and again ripened for 7d after freezing (Fig. 3 A, B). These results are in line with those reported by Bertola *et al.*,(1996); Oberg *et al.*, (1992) and Tunick *et al.*, (1993) who found that frozen storage decreased Meltability of stored cheeses.

However, it is of interest to note that, regardless of freezing conditions, meltability of cheeses made with EPS-producing strains of *S. thermophilus* retained the highest values during the frozen storage. It is also important to report that meltability of the experimented frozen cheeses did not differ than that of its refrigerated correspondences at the end of storage (Figs. 1 and 3 A&B).

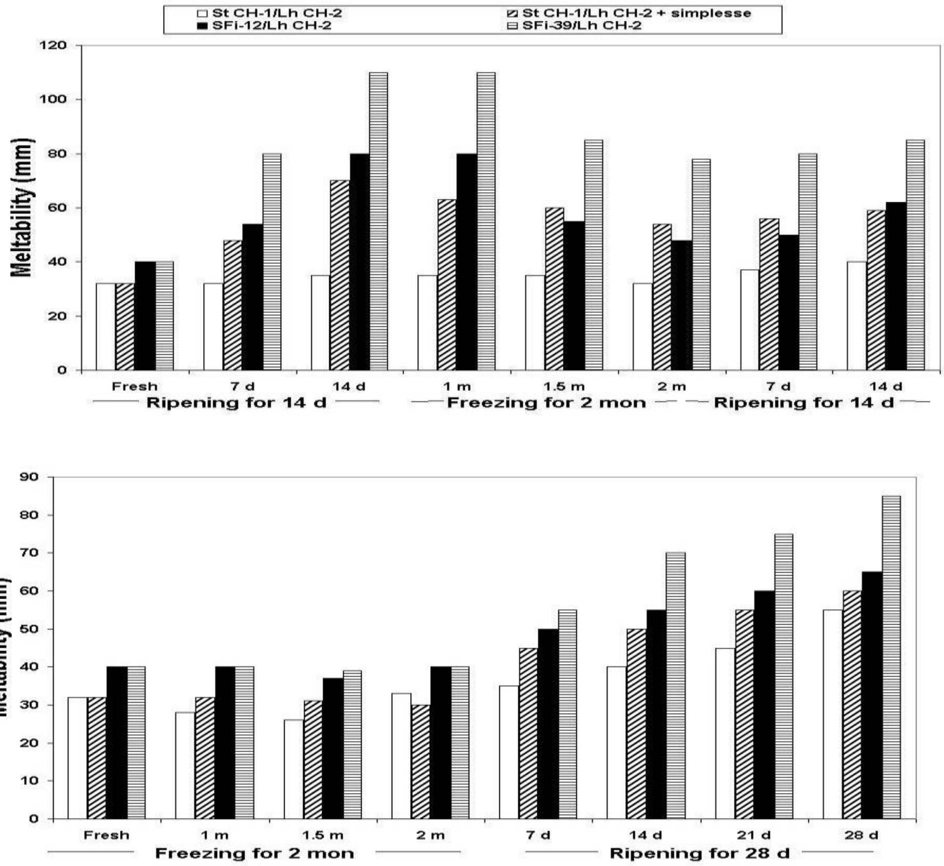

Fig. 3 : Mean values of meltability of low fat Mozzarella cheese as affected by EPS-producing *S. thermophilus* and frozen conditions; (A) 14 d ripening time at 4ºC before and after freezing time for 60 d at –20ºC; (B) freezing time for 60 d at -20ºC then 28 d ripening time at 4ºC.

T5

T6

Fat Leakage:

The results in Table 7 A& B showed that all cheeses exhibited the greatest diameter of oil ring during the ripening time either occurred before or after freezing as compared with that of the same cheeses during the freezing period. Moreover, the oil ring diameter of fat leakage of all cheeses was decreased upon freezing when employed after the first stage of ripening. This binding is in accordance with the results of Diefes *et al.*, (1993) who reported that Mozzarella cheese changed in rheological behaviour over 90-days of frozen storage.

Viscosity:

Apparent viscosity of cheeses was markedly affected by frozen storage and ripening time. Fluctuation changes over the storage period were observed (Table 8). Regardless of the ripening time (before or after freezing) the apparent viscosity values of all frozen cheeses were considerably decreased at the end of the storage period, except for AV of cheese made with the non-EPS culture (St CH-1/Lh CH-5) + simplesse which was increased (Table 8). The lowest AV was attained when EPS-producing culture of *S. thermophilus* (SFi-39) was used for making low fat Mozzarella cheese at the end of storage period.

The statistical analysis (Tables 9 a, b)showed that the rheological indices (meltability, fat leakage and AV) were significantly affected by the EPS-culture type and the frozen conditions as well as their interactions.

Microstructure :

Fig 4 A shows the microstructure of a 28 - day- old low fat Mozzarella cheese made with non EPS producing starter (St CH-1/Lh CH-5). The cheese showed a close and compact protein matrix, which was sparingly indented with the serum and fat voids located on the exposed surface. Using simplesse as a fat replacer in the manufacture of low fat Mozzarella cheese made with non EPS - starter culture (St CH-1/Lh CH-5) let to an increase in the openness of the cheese structure than that of the control cheese (Figs 4 A & B). As show in Fig. 4 C and d Low fat Mozzarella cheeses made with EPS- starter cultures (SFi-12/Lh CH-5 and SFi-39/Lh CH-5) were porous and had an open texture with numerous voids. Such voids were probably formed from dehydration of the EPS (secreted by the starter cultures) which might have formed globs . Such globs of EPS when extracted from the fractured surface of the cheese during SEM preparation , leave behind voids .

This effect of the EPS on the cheese matrix could lead to an increased porosity. These results are similar to those reported by Hassan *et al.* (2003) and Bhaskarachary and Shah (2000) Mistry and Anderson (1993) and Hassan *et al.* (1995) who observed that ropy EPS produced a spongy structure in the casein matrix.

For frozen cheeses which ripened in two stages (before and after freezing) , electron micrographs (Fig. 5 A,B,C,D) showed the EPS - cheeses (SFi-12/Lh CH-5 and SFi-39/Lh CH-5) exhibited also a porous and open texture, whereas the non EPS cheeses showed a closed and compact protein matrix and fewer voids.

T7

T8

The results of Bertola *et al* (1996) who reported that , as Mozzarella cheese aged, the para casein matrix porosity increased because of proteolysis, thus the para casein matrix presented less resistance to stretching and greater elasticity may confirm the obtained results.

Table 9 : Mean squares (MS) and probabilities (*P*) for meltability, fat leakage and apparent viscosity (AV) of low fat Mozzarella cheese made with EPS-producing cultures and stored as follows:
(a) : ripening for 14 days at 4ºC before and after freezing for 60 days at –20ºC.
(b): frozen at –20ºC for 60 days; then 28 days ripening time at 4ºC

(a)

Factor	Meltability		Fat leakage		AV	
	MS	*P*	MS	*P*	MS (x 10^{10})	*P*
EPS-culture type (A)	9590.09	***	286.79	***	379.85	*
Storage period (B)	1699.16	***	110.33	***	262.89	*
Interaction (A x B)	264.31	***	6.94	***	288.59	**
Error	2.47		1.79		131.00	

*** *P* <0.05 *** *P*<0.001**

(b)

Factor	Meltability		Fat leakage		AV	
	MS	*P*	MS	*P*	MS (x 10^{10})	*P*
EPS-culture type (A)	1583.25	***	376.84	***	421.29	***
Storage period (B)	1920.43	***	161.08	***	9.55.75	***
Interaction (A x B)	63.61	***	7.75	***	966.02	***
Error	3.03		3.14		11.53	

*** *P* <0.05 *** *P*<0.001**

In case of cheeses frozen immediately after manufacturing at -20ºC for 2 mon. and then ripened at 4ºC for 28 day (Fig. 6 A,B,C & D) , differences can be seen between the non EPS cheeses with and without simpelesse and the EPS cheeses. The non EPS- cheeses showed also a compact protein matrix , which was sparingly indented with serum and fat voids located on the exposed surface. Addition of simplesse (Fig. 6 B) increased the openness of the cheese structure than that of the control cheese (Fig. 6 A). As shown in Fig. 6 C&D , the EPS cheeses made with SFi-12/lh cH-5 and SFi-39/ lh cH-5 appeared more porous and open texture with a very large voids .

From the foregoing results it is recommended to use EPS-producing strains of *S. thermophilus* in order to increase the moisture of low fat Mozzarella cheese and to improve its melt properties. Moreover, it was found that the functional and microstructural properties of EPS frozen cheeses are comparable with those of their corresponding refrigerated cheeses. Therefore, freezing may be considered as a suitable alternative to prolong stability and shelf life of low fat Mozzarella cheese without modifying its functional properties.

Fig. 4: Scanning electron micrograph of a 28 days old low fat Mozzarella cheese made with different starter cultures: (A) St CH-1/Lh CH-5; (B) St CH-1/Lh CH-5 + simplesse; (C) SFi-12/Lh CH-5 and (D) SFi-39/Lh CH-5. All cheeses stored at 4 ºC for 28 d.

Fig. 5: Scanning electron micrograph of a 88 day-old low fat Mozzarella cheese made with different starter cultures: (A) St CH-1/Lh CH-5; (B) St CH-1/Lh CH-5 + simplesse; (C) SFi-12/Lh CH-5 and (D) SFi-39/Lh CH-5. All cheeses ripened at 4 ºC for 14 d and frozen at –20 ºC for 60 d, then ripened at 4 ºC for 14 d.

Fig. 6: Scanning electron micrograph of a 88 day-old low fat Mozzarella cheese made with different starter cultures: (A) St CH-1/Lh CH-5; (B) St CH-1/Lh CH-5 + simplesse; (C) SFi-12/Lh CH-5 and (D) SFi-39/Lh CH-5. All cheeses frozen at -20 ºC for 60 d and then ripened at 4 ºC for 28 d.

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الخواص الوظيفية والتركيبية للجبن الموزاريلا المنخفض الدهن بتأثير سلالات من *S.thermophilus* المنتجة للسكريات العديدة وظروف التخزين

الهام مصطفي السيد
قسم علوم الألبان – كلية الزراعة- جامعة القاهرة- الجيزة- مصر

الهدف من البحث هو تحسين الخواص الوظيفية والتركيبية للجبن الموزاريلا منخفض الدهن وإطالة مدة حفظه دون فقد ملموس في خواصه وذلك عن طريق استخدام المزارع البكتيرية المنتجة للسكريات العديدة والحفظ عن طريق التجميد. حيث تم تقسيم الدراسة إلي جزئيين:

في الجزء الأول: تم اختيار أربع سلالات بكتيرية منها سلالتين من بكتريا *S.thermophilus* المنتجة للسكريات العديدة هما SFi-12, SFi- 39 وسلالة بكتيرية من كل من *S thermophilus* St CH-1 , *L. helveticus* Lh CH-5 غير المنتجة للسكريات العديدة . حيث تم تصنيع أربع معاملات من الجبن الموزاريلا منخفض الدهن باستخدام ثلاث مزارع مختلطة من السلالات السابقة . واستخدم في المعاملة الأولي والثانية المزرعة التقليدية (St CH-1/Lh CH-5) غير المنتجة للسكريات العديدة كمقارنة بدون وبإضافة بديل دهني هو simplesse والمعاملة الثالثة والرابعة استخدامت المزارع المختلطة SFi-12/Lh CH-5 و SFi-39/Lh CH-5 المنتجة للسكريات العديدة وتم حفظ الجبن الناتج على درجة حرارة ٤ °م لمدة ٢٨ يوم .

أعطت جبن الموزاريلا المنخفض الدهن المصنع باستخدام السلالات البكتيرية المنتجة للسكريات العديدة SFi-39/Lh CH-5 و SFi-12/Lh CH-5 زيادة معنوية في محتواها من الرطوبة والرطوبة منسوبة إلى المكونات غير الدهنية (MNFS). كما تميزت بارتفاع خاصية القابلية للانصهار (Meltability) وقيم انفصال الدهن Fat leakage وذلك بالمقارنة بالجبن المصنع باستخدام المزرعة غير المنتجة للسكريات العديدة (St CH-1 /Lh CH-5) سواء بدون أو بإضافة البديل الدهني وهي طازجة أو أثناء مدة التخزين. وقد أعطى الجبن المصنع باستخدام SFi-39/Lh CH-5 أقل قيمة للزوجة عند نهاية مدة التخزين كما أظهرت نتائج التحليل الإحصائي تأثر الخواص الوظيفية (القابلية للانصهار- القابلية لانفصال الدهن - اللزوجة) معنوياًً بكل من نوع البادئ المستخدم ومدة التخزين و التفاعل بينهما.

وفي الجزء الثاني : تم دراسة تأثير الحفظ بالتجميد بطريقتين:

١ - خزنت الجبن بعد التصنيع مباشرة على ٤ °م لمدة ١٤ يوم ثم التجميد على –٢٠°م لمدة ٦٠ يوم ثم استكملت فترة التسوية على ٤°م لمدة ١٤ يوم أخرى.

٢- تم حفظ الجبن مباشرة بعد التصنيع بالتجميد على –٢٠ °م لمدة ٦٠ يوم ثم التخزين علي ٤° م لمدة ٢٨ يوم.

وقد أظهرت النتائج المتحصل عليها احتفاظ الجبن المصنع (SFi-39/Lh CH-5, /Lh CH-5 SFi-12) المنتجة للسكريات العديدة بأعلى نسبة رطوبة منسوبة للمكونات غير الدهنية وكذلك لقيم الحموضة عند نهاية مدة الحفظ في كلا الطريقتين. كما زادت درجة القابلية للانصهار وانفصال الدهن عند التخزين علي ٤°م قبل أو بعد التجميد بينما تذبذبت قيم اللزوجة للجبن المجمد بكلا الطريقتين .

وقد أظهرت دراسة التركيب البنائي الدقيق بالميكروسكوب الاليكتروني لمعاملات الجبن المصنع باستخدام المزارع البكتيرية (SFi-39/Lh CH-5, SFi-12/Lh CH-5) المنتجة للسكريات العديدة والتي تم حفظها على ٤°م لمدة ٢٨ يوم ببناء تركيبي مفتوح مع انتشار فجوات بينية صغيرة وكبيرة داخل شبكة البروتين مقارنة بتركيب بنائي مندمج خالي من الفجوات لجبن المقارنة غير المنتج للسلالات العديدة (St CH-1/LhCH-5). وفي حالة استخدام البديل الدهني لجبن المقارنة تميز الجبن الناتج بتركيب مفتوح نسبيا مع عدد فجوات أقل من الجبن المصنع باستخدام المزارع المنتجة للسكريات العديدة. كما لم يلاحظ اختلاف كبير لتأثير طريقتي التجميد على التركيب البنائي للجبن في المعاملات المختلفة بالمقارنو بالتركيب البنائي لنفس المعاملات والتي تم حفظها بالتبريد (٤°م /٢٨ يوم) حيث تميز بزيادة نسبة الفجوات الكبيرة خاصة بالنسبة للجبن المصنع باستخدام المزارع المنتجة للسكريات العديدة.

وتشير نتائج الدراسة إلى إمكانية استخدام سلالات *S.thermophilus* المنتجة للسكريات العديدة لتحسين الخواص التركيبية والوظيفية لجبن الموزاريلا منخفض الدهن كما يمكن استخدام التجميد كوسيلة مناسبة لإطالة مدة حفظ الجبن الموزاريلا منخفض الدهن بدون فقد ملموس لخواصه الوظيفية.

Table 5 : Changes in the chemical composition of low fat Mozzarella cheese as affected by EPS-producing *S. thermophilus* and frozen conditions (ripening for 14 days at 4ºC before and after freezing for 60 days at – 20ºC)

Strain culture*	Storage period							
	Ripening time at 4ºC, d			Freezing time at – 20ºC, mon.			Ripening time at 4ºC, d	
	Fresh	7	14	1	1.5	2	7	14
				MNFS†				
St CH-1/Lh CH-5	$59.74^{j}\pm0.02$	$59.48^{k}\pm0.02$	$59.23^{lm}\pm0.01$	$59.17^{m}\pm0.17$	$59.11^{m}\pm0.01$	$59.37^{kl}\pm0.49$	$58.45^{o}\pm0.42$	$58.79^{n}\pm0.08$
St CH-1/Lh CH-5 + simplesse	$61.86^{g}\pm0.04$	$62.04^{g}\pm0.02$	$61.97^{g}\pm0.02$	$61.94^{g}\pm0.03$	$61.68^{i}\pm0.41$	$61.91^{gh}\pm0.01$	$61.76^{hi}\pm0.03$	$61.74^{hi}\pm0.03$
Sfi-12/Lh CH-5	$62.84^{d}\pm0.06$	$62.58^{e}\pm0.05$	$62.47^{ef}\pm0.03$	$62.47^{ef}\pm0.03$	$62.49^{ef}\pm0.06$	$62.43^{ef}\pm0.01$	$62.33^{f}\pm0.04$	$62.36^{f}\pm0.04$
Sfi-39/Lh CH-5	$63.86^{a}\pm0.06$	$63.63^{a}\pm0.01$	$63.22^{b}\pm0.09$	$63.18^{b}\pm0.01$	$63.18^{b}\pm0.01$	$63.17^{b}\pm0.02$	$63.11^{bc}\pm0.02$	$62.96^{cd}\pm0.03$
				S/M†				
St CH-1/Lh CH-5	$2.30^{l}\pm0.3$	$2.51^{g}\pm0.02$	$2.58^{e}\pm0.03$	$2.58^{e}\pm0.03$	$2.58^{de}\pm0.01$	$2.58^{de}\pm0.02$	$2.70^{b}\pm0.03$	$2.817^{a}\pm0.02$
St CH-1/Lh CH-5 + simplesse	$2.29^{l}\pm0.02$	$2.34^{k}\pm0.10$	$2.41^{j}\pm0.10$	$2.41^{j}\pm0.02$	$2.42^{j}\pm0.01$	$2.42^{j}\pm0.01$	$2.47^{h}\pm0.03$	$2.64^{c}\pm0.03$
Sfi-12/Lh CH-5	$2.02^{p}\pm0.02$	$2.07^{o}\pm0.03$	$2.19^{n}\pm0.02$	$2.19^{n}\pm0.02$	$2.20^{n}\pm0.04$	$2.20^{n}\pm0.03$	$2.26^{m}\pm0.03$	$2.34^{k}\pm0.02$
Sfi-39/Lh CH-5	$2.26^{m}\pm0.05$	$2.34^{k}\pm0.05$	$2.45^{i}\pm0.04$	$2.45^{i}\pm0.03$	$2.45^{i}\pm0.03$	$2.45^{i}\pm0.02$	$2.53^{f}\pm0.20$	$2.60^{d}\pm0.04$
				PH				
St CH-1/Lh CH-5	$5.18^{b}\pm0.06$	$5.15^{c}\pm0.01$	$5.06^{gh}\pm0.20$	$5.06^{gh}\pm0.30$	$5.06^{gh}\pm0.01$	$5.05^{h}\pm0.06$	$5.03^{i}\pm0.80$	$4.93^{m}\pm0.30$
St CH-1/Lh CH-5 + simplesse	$5.20^{a}\pm0.10$	$5.17^{b}\pm0.10$	$5.13^{d}\pm0.05$	$5.13^{de}\pm0.01$	$5.12^{e}\pm0.06$	$5.12^{de}\pm0.10$	$5.05^{h}\pm0.01$	$4.97^{k}\pm0.05$
Sfi-12/Lh CH-5	$5.21^{a}\pm0.01$	$5.17^{b}\pm0.06$	$5.07^{fg}\pm0.01$	$5.07^{fg}\pm0.01$	$5.06^{gh}\pm0.01$	$5.06^{gh}\pm0.01$	$5.00^{j}\pm0.06$	$4.95^{l}\pm0.01$
Sfi-39/Lh CH-5	$5.18^{b}\pm0.01$	$5.13^{de}\pm0.01$	$5.08^{f}\pm0.01$	$5.08^{f}\pm0.01$	$5.07^{fg}\pm0.01$	$5.07^{fg}\pm0.01$	$5.00^{j}\pm0.06$	$4.92^{m}\pm0.05$
				TA, %†				
St CH-1/Lh CH-5	$0.68^{o}\pm0.01$	$0.74^{n}\pm0.01$	$0.81^{kl}\pm0.06$	$0.81^{kml}\pm0.01$	$0.82^{jk}\pm0.01$	$0.83^{ij}\pm0.10$	$0.89^{d}\pm0.01$	$0.94^{b}\pm0.01$
St CH-1/Lh CH-5 + simplesse	$0.73^{n}\pm0.01$	$0.80^{lm}\pm0.01$	$0.83^{ij}\pm0.01$	$0.83^{ij}\pm0.01$	$0.84^{hi}\pm0.01$	$0.85^{gh}\pm0.10$	$0.89^{d}\pm0.01$	$0.91^{c}\pm0.20$
SFi-12/Lh CH-5	$0.80^{m}\pm0.06$	$0.81^{klm}\pm0.10$	$0.84^{hi}\pm0.30$	$0.86^{fg}\pm0.01$	$0.87^{ef}\pm0.01$	$0.88^{de}\pm0.01$	$0.93^{b}\pm0.06$	$0.96^{a}\pm0.01$
SFi-39/Lh CH-5	$0.80^{lm}\pm0.01$	$0.83^{ij}\pm0.01$	$0.85^{gh}\pm0.01$	$0.85^{gh}\pm0.01$	$0.87^{ef}\pm0.01$	$0.88^{de}\pm0.06$	$0.94^{b}\pm0.01$	$0.97^{a}\pm0.01$

$^{a-o}$ **Mean values (± SD; n =3) with different superscripts in the same row are significantly different (P<0.005).**

* **See Table 1 and text for details of cheese component and strain cultures.**

† **MNFS = Moisture-in-non-fat substances; S/M = Salt-in-Moisture; TA = titratable acidity.**

Table 6 : Changes in the chemical composition of low fat Mozzarella cheese as affected by EPS-producing *S. thermophilus* and frozen conditions (frozen storage at –20ºC for 60 days; and then 28 days ripening time at 4ºC)

Strain culture[*]	Storage period							
	Freezing time at –20ºC, m				Ripening time at 4ºC, d			
	Fresh	1	1.5	2	7	14	21	28
	MNFS[†], %							
St CH-1/Lh CH-5	59.74^{l}±0.03	59.73^{l}±0.02	59.72^{l} ±0.01	59.72^{l} ±0.01	59.59^{m}±0.01	59.53^{m}±0.02	59.17^{n}±0.01	59.07^{n}±0.03
St CH-1/Lh CH-5 + simplesse	61.86^{i}±0.01	62.06^{i}±0.01	62.66^{f}±0.02	62.69^{ef}±0.01	61.90^{j}±0.01	61.84^{jk}±0.01	61.77^{k}±0.01	61.75^{k}±0.01
SFi-12/Lh CH-5	62.84^{d}±0.01	62.83^{d}±0.01	62.82^{d}±0.01	62.59^{fg}±0.39	62.80^{de}±0.01	62.59^{fg}±0.02	62.48^{gh}±0.01	62.37^{h}±0.01
SFi-39/Lh CH-5	63.86^{a}±0.01	63.65^{a}±0.01	63.64^{ab}±0.01	63.63^{ab}±0.02	63.60^{ab}±0.02	63.53^{b}±0.01	63.56^{ab}±0.01	63.32^{c}±0.03
	S/M[†], %							
St CH-1/Lh CH-5	2.3^{cdef}±0.01	2.3^{cdef}±0.01	2.3^{cdef}±0.01	2.3^{cdef}±0.01	2.5^{bcdef}±0.02	2.60^{bcd}±0.02	2.70bc±0.04	2.90^{b}±0.01
St CH-1/Lh CH-5 + simplesse	2.02^{f}±0.01	2.02^{f}±0.01	2.02^{f}±0.01	2.02^{f}±0.01	2.09^{ef}±0.01	2.12^{def}±0.01	2.25^{cdef}±0.01	2.5^{bcdef}±0.01
SFi-12/Lh CH-5	2.26^{cdef}±0.01	2.26^{cdef}±0.01	2.26^{cdef}±0.01	2.27^{cdef}±0.01	2.39^{cdef}±0.01	2.35^{cdef}±0.01	2.56^{bcde}±0.01	2.58^{a}±0.01
SFi-39/Lh CH-5	2.29^{cdef}±0.7	2.29^{cdef}±0.01	2.30^{cdef}±0.01	2.30^{cdef}±0.01	2.40^{bcdef}±0.09	2.44^{bcdef}±0.01	2.51^{bcdef}±0.01	2.53^{bcde}±0.01
	pH							
St CH-1/Lh CH-5	5.17^{b}±0.01	5.18^{b}±0.01	5.18^{b}±0.01	5.18^{b}±0.01	5.14^{c}±0.01	5.07^{f}±0.02	4.94^{j}±0.02	4.91^{k}±0.03
St CH-1/Lh CH-5 + simplesse	5.20^{a}±0.01	5.20^{a}±0.01	5.20^{a}±0.01	5.20^{a}±0.01	5.17^{b}±0.01	5.05^{g}±0.02	5.01^{h}±0.02	4.96^{i}±0.01
SFi-12/Lh CH-5	5.18^{b}±0.01	5.18^{b}±0.01	5.18^{b}±0.01	5.18^{b}±0.01	5.15^{c}±0.01	5.09^{e}±0.01	4.96^{i}±0.01	4.92^{k}±0.01
SFi-39/Lh CH-5	5.21^{a}±0.04	5.21^{a}±0.01	5.21^{a}±0.02	5.21^{a}±0.03	5.17^{b}±0.01	5.11^{d}±0.01	5.04^{g}±0.02	4.95^{ij}±0.02
	T.A[†], %							
St CH-1/Lh CH-5	0.68^{n}±0.01	0.68^{n}±0.01	0.69^{mn}±0.01	0.70^{m}±0.04	0.81^{hi}±0.01	0.87^{de}±0.01	0.90^{c}±0.01	0.93^{b}±0.01
St CH-1/Lh CH-5 + simplesse	0.73^{l}±0.01	0.73^{l}±0.01	0.74^{kl}±0.01	0.75^{k}±0.01	0.79^{j}±0.01	0.82^{gh}±0.01	0.86^{e}±0.01	0.91^{c}±0.01
SFi-12/Lh CH-5	0.80^{ij}±0.01	0.80^{ij}±0.01	0.82^{gh}±0.01	0.83^{fg}±0.01	0.88^{d}±0.01	0.90^{c}±0.01	0.94^{b}±0.01	0.97^{a}±0.01
SFi-39/Lh CH-5	0.80^{ij}±0.01	0.80^{ij}±0.01	0.83^{fg}±0.01	0.84^{f}±0.01	0.87^{de}±0.01	0.90^{c}±0.01	0.93^{b}±0.01	0.96^{a}±0.01

$^{a-n}$ Mean values (± SD; n =3) with different superscripts in the same row are significantly different (P<0.005).
[*] See Table 1 and text for details of cheese component and strain cultures.
[†] MNFS = Moisture-in-non-fat substances; S/M = Salt-in-Moisture; TA = titratable acidity.

Table 7 : Fat leakage of low fat Mozzarella cheese as affected by EPS-producing *S. thermophilus* and frozen conditions: (A): ripening for 14 days at 4ºC before and after freezing for 60 days at –20ºC; (B): frozen storage at –20ºC for 60 days; then 28 days ripening time at 4ºC

(A)

Strain culture*	Fat leakage, mm – Storage period: Ripening time at 4ºC, d			Freezing time at –20ºC, m			Ripening time at 4ºC, d	
	Fresh	7	14	1	1.5	2	7	14
St CH-1/Lh CH-5	$37.0^{ijk}\pm1.0$	$39.0^{ghi}\pm1.0$	$41.0^{fg}\pm1.0$	$39.0^{ghi}\pm2.0$	$36.0^{jk}\pm1.0$	$35.0^{k}\pm1.0$	$36.0^{jk}\pm2.0$	$37.0^{ijk}\pm2.0$
St CH-1/Lh CH-5 + simplesse	$45.0^{de}\pm1.0$	$47.0^{cd}\pm1.0$	$51.0^{ab}\pm2.0$	$43.0^{ef}\pm2.0$	$41.0^{fg}\pm1.0$	$37.0^{ijk}\pm2.0$	$38.0^{hij}\pm1.0$	$43.0^{ef}\pm1.0$
SFi-12/Lh CH-5	$46.0^{d}\pm2.0$	$49.0^{bc}\pm2.0$	$52.0^{a}\pm1.0$	$46.0^{d}\pm1.0$	$45.0^{de}\pm2.0$	$39.3^{gh}\pm1.0$	$43.0^{ef}\pm1.0$	$46.0^{d}\pm1.0$
SFi-39/Lh CH-5	$41.0^{fg}\pm1.0$	$43.0^{ef}\pm2.0$	$46.0^{d}\pm1.0$	$43.0^{ef}\pm2.0$	$41.0^{fg}\pm1.0$	$39.0^{ghi}\pm2.0$	$41.0^{fg}\pm1.0$	$42.0^{f}\pm1.0$

(B)

	Fat leakage, mm – Storage period: Freezing time at – 20ºC, m				Ripening time at 4ºC, d			
	Fresh	1	1.5	2	7	14	21	28
St CH-1/Lh CH-5	$37.0^{ijk}\pm2.0$	$35.0^{kl}\pm1.0$	$32.0^{mn}\pm1.0$	$31.0^{n}\pm1.0$	$31.7^{mn}\pm2.1$	$35.0^{kl}\pm2.0$	$37.0^{ijk}\pm1.0$	$38.0^{ij}\pm2.0$
St CH-1/Lh CH-5 + simplesse	$45.0^{de}\pm2.0$	$41.0^{gh}\pm1.0$	$36.0^{jkl}\pm1.0$	$34.0^{lm}\pm3.0$	$35.0^{kl}\pm1.0$	$41.0^{gh}\pm1.0$	$43.0^{efg}\pm2.0$	$44.0^{def}\pm2.0$
SFi-12/Lh CH-5	$46.0^{cd}\pm1.0$	$41.0^{gh}\pm1.0$	$39.0^{hi}\pm3.0$	$37.0^{ijk}\pm2.0$	$43.0^{efg}\pm2.0$	$48.0^{bc}\pm2.0$	$49.0^{ab}\pm1.0$	$51.0^{a}\pm1.0$
SFi-39/Lh CH-5	$41.0^{gh}\pm1.0$	$38.0^{ij}\pm1.0$	$37.0^{ijk}\pm1.0$	$34.0^{lm}\pm1.0$	$36.0^{jkl}\pm2.0$	$41.0^{gh}\pm2.0$	$42.0^{fg}\pm1.0$	$43.0^{efg}\pm3.0$

$^{a-n}$ Mean values (± SD; n =3) with different superscripts in the same row are significantly different (P<0.005).

*** See Table 1 and text for details of cheese component and strain cultures.**

Table 8 : Apparent viscosity (AV) of low fat Mozzarella cheese as affected by EPS-producing *S. thermophilus* and frozen condition: (A): ripening for 14 days at 4ºC before and after freezing for 60 days at –20ºC; (B): frozen storage at –20ºC for 60 days; and then 28 days ripening time at 4ºC

(A)

Strain culture*	AV†, cP Storage period							
	Ripening time at 4ºC, d			Freezing time at –20ºC, m			Ripening time at 4ºC, d	
	Fresh	7	14	1	1.5	2	7	14
St CH-1/Lh CH-5	29.87±0.26	56.73±0.16	16.18±0.17	9.97±0.07	6.07±6.46	9.25±6.88	18.81±0.39	10.38±0.15
St CH-1/Lh CH-5 + simplesse	18.31±13.1	19.46±14.5	17.84±0.24	20.25±0.05	16.81±0.19	13.42±0.38	15.37±0.15	21.79±28.2
SFi-12/Lh CH-5	27.90±1.98	15.21±0.10	4.34±0.01	28.72±0.14	4.65±0.04	8.30±0.06	36.86±0.021	2.72±35.7
SFi-39/Lh CH-5	16.78±0.07	6.09±0.08	4.00±0.10	4.20±0.04	5.51±0.05	36.73±40.5	5.81±0.29	2.16±0.04

(B)

	AV‡, cP Storage period							
	Freezing time at –20ºC, m				Ripening time at 4ºC, d			
	Fresh	1	1.5	2	7	14	21	28
St CH-1/Lh CH-5	29.87±0.26	28.40±0.18	23.02±0.07	17.74±0.20	29.63±0.26	21.31±0.24	2.98±0.05	2.33±0.11
St CH-1/Lh CH-5 + simplesse	26.05±0.30	28.76±17.55	23.33±0.12	5.04±0.49	19.62±0.17	9.96±7.41	3.33±0.04	5.97±0.08
SFi-12/Lh CH-5	27.90±1.98	32.87±0.52	14.94±0.04	13.27±0.09	32.90±0.26	17.62±0.39	2.66±0.04	5.24±1.00
SFi-39/Lh CH-5	16.78±.67	8.44±0.04	25.09±0.09	14.77±0.18	10.83±0.13	4.62±0.06	1.64±0.03	1.77±0.03

*** See Table 1 and text for details of cheese component and strain cultures.**
† Mean values (± SD; n =3), LSD = 5905 cP
‡ Mean values (± SD; n =3), LSD = 5540 cP