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Research Article Effect of Exopolysaccharides-producing Starter Culture on the Flavor Profile and Characteristics of Low Fat Ras Cheese

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Abstract

Background and Objective: The application of the exopolysaccharide-producing starter culture for improving the texture and technical properties and evaluating flavor profile of low-fat Ras cheese was studied. The experimental design was performed to compare flavour compounds of traditional and exopolysaccharide producing starters (EPS) for different levels of fat milk cheese. **Materials and Methods:** Control (4% fat) with traditional starter, T_1 (0% fat) with EPS, T_2 (1% fat) with EPS), T_3 (2% fat) with EPS and T_4 (3% fat) with EPS were used. The physicochemical, textural profile analysis and organoleptic properties of fresh and stored cheeses (4 months) were determined. Also, the microscopic structural changes in fresh low-fat Ras cheese with EPS were evaluated. **Results:** The results indicated that addition of EPS producing cultures with decreasing fat of cheese milk lead to an increase in the moisture of treatments as well as hardness, cohesiveness, springiness, chewiness and gumminess of the resultant cheese. The data indicated that control cheese (full-fat and without EPS-producing cultures) had the lowest values of acidity. The changes in pH values among all cheese treatments and during storage period followed opposite trend to that of titratable acidity. There were negative correlation between the rate of fat reduction and the values of SN (soluble nitrogen). **Conclusion:** Addition of EPS-producing cultures in Ras cheese milk improved sensory evaluation of resultant cheese, whereas cheese with 3% fat and EPS-producing cultures (T_4) selected as best Ras cheese sample.

Key words: Exopolysaccharides starter culture, low-fat Ras cheese, textural profile analysis, flavor profile, organoleptic properties

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nowadays, consumption of low-fat foods, including cheeses, has grown steadily because of consumer awareness about health concerns related to decreasing the risks associated with obesity, atherosclerosis and coronary heart disease and elevated blood pressure¹. Consumers also want to consume cheese with desirable flavor and texture characteristics, while reducing dietary fat allowance, because mouth feel still remains a basic factor when making a preference to food^{2,3}. Fat has an important role in the development of texture, flavour and appearance of cheese⁴. Textural attributes are believed to be important criteria in determining the identity and guality of a cheese and its consumer acceptability¹. Manufacturing low-fat or reduced-fat cheese with an acceptable flavour and texture demonstrates some difficulties as reported previously by Banks et al.^{5,6}, Jameson⁷, Anderson *et al.*⁸ and Rogers *et al.*⁹.

Starter culture selection is one of the alternative ways to overcome problems associated with fat reduction. Using strains of lactic acid bacteria (LAB) to produce an extracellular material called exopolysaccharide (EPS) in the growth medium could be an alternative for increasing the moisture content and improving sensory attributes of reduced and/or low-fat cheeses¹⁰⁻¹⁴.

Ras cheese, the main traditional hard cheese in Egypt is manufactured in a high proportion under artisan conditions from raw cow's or mixture of cow's and buffalo's milk without using starter cultures and marketed when it has a queried sharp flavour closed to kefalotyic cheese after 3-6 months¹⁵⁻¹⁸.

Cheese flavour is a result of a complex balance among volatile and non-volatile chemical compounds, coming from milk fat, milk proteins and carbohydrates during the ripening of cheese^{19,20}. Although, there are many studies concerning Ras cheese, but only Ayad *et al.*²¹ studied the aroma profile of Egyptian Ras cheese ripened at different stages. Based on the chromatographic separation and identification using mass spectrometry in addition to the sensory evaluation of the prepared samples, various classes of volatile constituents have been identified as being responsible for the final aroma and taste of cheese. These compounds comprise fatty acids, esters, alcohols and ketones²²⁻²⁵.

Studies aimed at producing low-fat cheeses generally show that low-fat products made by conventional methods have sensory defects, such as poor aroma, undesired flavours (a meaty or bitter taste) and a texture which is over firm, elastic (often described as 'rubbery'), or affected by crystallization of calcium lactate³. The effect of EPS-producing LAB has been extensively evaluated in the production of fermented dairy products, especially in yoghurt and in low-fat Mozzarella in the cheese industry^{13,26-28}, but not with reduced and/or low-fat cheeses especially low fat Ras cheese. Therefore, the objective of this study was to improve the volatile and sensory quality of low-fat Ras cheese using EPS-producing cultures.

MATERIALS AND METHODS

The study was conducted during the month of July, 2018 to December, 2018 at Food Technology Research Institute, Agriculture Research Center, Giza, Egypt and Chemistry of Flavour and Aroma Dept. National Research Center, Dokki, Giza, Egypt

Materials: Fresh cow milk was obtained from the Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. Starter culture; which consists *of Streptococcus salivarius* subsp. *thermophillus* and *Lactobacillus delbruckii* subsp. *bulgaricus* (1:1) (Chr. Hansen's Lab A/s Copenhagen, Denmark) were used. *Streptococcus salivarius* subsp. *Thermophillus* and *Lactobacillus delbruckii* subsp. *Bulgaricus* EPS-producing cultures were obtained from Microbiology Lab. at Dairy Department, National Research Center, Dokki, Egypt. Rennet powder (Hanelase) was obtained from Chr. Hansen's Lab., Denmark. Fine cooking salt produced by EL-Naser Saline's Company was obtained from the local market.

Cheese manufacture: The experimental design was performed to compare flavour compounds of traditional and exopolysaccharide producing starters for different levels of fat milk cheese. Five different types Ras cheeses consisted of the as following:

- Control: 4% fat with traditional starter
- T₁: 0% fat (free fat) with exopolysaccharide starter
- T₂: 1% fat with exopolysaccharide starter
- T₃: 2% fat with exopolysaccharide starter
- T₄: 3% fat with exopolysaccharide starter

Ras cheese treatments were manufactured according to the method adopted²⁹. Resultant cheese was stored at 15° C ± 2 for 4 month. All cheese treatments were sampled and analyzed when fresh and 2 and 4 month for chemical, rheological, sensory properties, microstructure and flavour compounds. The whole experiment was duplicated. **Method of analysis:** Cheese samples were analyzed for moisture, protein, fat, salt, SN contents and pH value and titratable acidity were determined according to AOAC³⁰. Scanning electron microscopy was performed using modified method of Tamime *et al.*³¹. Texture profile analysis (TPA) of Ras cheese was measured at 23 °C as described by Bourne³². Sensory evaluation of cheese was carried out for fresh and after 2 and 4 months of storage according to the method of El-Shafei *et al.*³³. All data were analyzed by the General Linear Models procedure of SAS³⁴. Least significant difference test was performed to determine differences in means at p<0.05.

Isolation of headspace volatiles: The volatiles in the headspace of each sample under investigation were isolated by using a dynamic headspace system. The samples were purged for 1 h with nitrogen gas (grade of N2<99.99) at a flow rate 100 mL min⁻¹ the headspace volatiles were swept into cold traps containing diethyl ether and pentane (1:1, v/v) and held at -10°C. The solvents containing the volatiles were dried over anhydrous sodium sulfate for 1 h. the volatiles were obtained by evaporation of the solvents under reduced pressure.

Gas chromatographic (GC) analysis: GC analysis was performed by using Hewlett-Packard model 5890 equipped with a flame ionization detector (FID). A fused silica capillary column DB-5 (60 m×0.32 mm id,) was used. The oven temperature was maintained initially at 50°C for 5 min, then programmed from 50-250°C at a rate of 4°C min⁻¹. Helium was used as the carrier gas, at flow rate of 1.1 mL min⁻¹. The injector and detector temperatures were 220 and 250°C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated using hydrocarbons (C8-C22, Aldrich Co.) as references.

Gas chromatographic-mass spectrometric (GC-MS) analysis:

The analysis was carried out by using a coupled gas chromatography Hewlett-Packard model (5890)/mass

spectrometry Hewlett-Packard MS (5970). The ionization voltage was 70 eV, mass range m/z 39-400 a.m.u. The GC condition was carried out as mentioned above. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology) and compared with those of authentic compounds and published data Adams³⁵. The quantitative determination was carried out based on peak area integration.

Chemical analysis: RCFC samples were analyzed for their total acidity (%), pH, moisture (%), fat/DM (%), WSN/TN (%) according to Amatayakul *et al.*¹⁴. The total volatile fatty acids (TVFA) as described by Costa *et al.*¹⁵. Analysis of the total free amino acids method was conducted according to Hofi *et al.*¹⁶. All analysis were performed in triplicate and results reported as mean \pm standard deviations.

Sensory analysis: Prepared RCFC samples were evaluated for flavor (100 points) according to El-Hofi *et al.*³⁶ by ten panelists of the staff members at Dairy Technology Research Department, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

Statistical analysis: Statistical analyses were performed using SPSS software version 16. The varying degree of the result is expressed as mean \pm standard deviation (Mean \pm SD). The differences between the samples were determined using t-tests ($\alpha = 0.05$).

RESULTS

Physiochemical characterization of low-fat Ras Cheese with EPS cultures: Moisture content of low-fat Ras cheese as affected by using producing cultures (EPS) during storage up to 4 months are shown in Table 1. The results indicate that cheese with EPS and free fat (T_1) had the lowest moisture content while Ras-cheese 3% fat and using EPS (T_4) had the highest among treatments. Low-fat Ras cheese treatments

Table 1: Chemical characterization of fresh Ras cheese by using different levels fat and EPS cultures

Parameters	Treatments								
	Control	T ₁	T ₂	Т ₃	 T ₄				
Moisture	36.91 ^c	33.82 ^D	38.47 ^B	40.19 ^{AB}	43.51^				
Fat	37.20 ^A	6.30 ^D	10.80 ^c	18.50 [⊂]	25.30 ^B				
Protein	21.50 ^D	37.70 ^A	32.90 ^B	28.20 ^c	26.70 ^{CD}				
Salt	1.30 ^A	1.30 ^A	1.26 ^{AB}	1.20 ^B	1.20 ^B				

Control: 4% fat with traditional starter, $T_1:0\%$ (free fat) with exopolysaccharide starter, $T_2:1\%$ fat with exopolysaccharide starter, $T_3:2\%$ fat with exopolysaccharide starter, $T_4:3\%$ fat with exopolysaccharide starter, $T_3:2\%$ fat with exopolysaccharide starter, $T_4:3\%$ fat with exopolysaccharide starter, $T_3:2\%$ fat with exopolysaccharide starter, $T_4:3\%$ fat with exopolysaccharide starter, $T_4:3\%$ fat with exopolysaccharide starter, $T_4:2\%$ fat with exopolysaccharide starter, $T_4:3\%$ fat with exopolysaccharide starter, $T_4:2\%$ fat wit

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	Storage periods	Treatments						
Parameters		Control	T ₁	T ₂	T ₃	T_4		
Hardness (N)	Fresh	21.50	64.00	29.10	20.40	18.10		
	4 month	11.00	34.80	15.08	10.40	9.07		
Cohesiveness (-)	Fresh	0.68	0.65	0.77	0.78	0.72		
	4 month	0.61	0.62	0.60	0.71	0.50		
Springiness (mm)	Fresh	4.65	4.30	4.58	4.86	5.06		
	4 month	4.41	3.43	4.10	4.34	4.47		
Gumminess (N)	Fresh	14.80	41.70	22.50	15.80	13.00		
	4 month	6.30	21.40	11.30	7.80	7.00		
Chewiness (N m ⁻¹)	Fresh	70.95	211.09	103.01	68.64	63.03		
	4 month	63.88	95.67	75.00	67.56	57.54		

Table 2: Textural profile analysis (TPA) of Ras cheese by using different levels fat and EPS cultures during the storage periods



Fig. 1(a-c): (a) Soluble nitrogen (%), (b) Titratable acidity and (c) pH value of Ras cheese by using different levels fat and EPS cultures during the storage periods

with EPS and different levels of milk fat exhibited significantly higher moisture content compared to full-fat Ras cheese with traditional culture (control).

The data showed that the increase of fat content in cheese milk increased the fat content of the resultant cheese. Replacement of normal starter with EPS-producing cultures in low fat cheese milk led to increase protein content compared to full-fat Ras cheese (control). On the other hand, protein content of low-fat Ras cheese was decreased with increasing fat content of cheese milk.

Changes in soluble nitrogen (SN) of low-fat Ras cheese as affected by using exopolysaccharide-producing cultures (EPS) during storage up to 4 months are shown in Fig. 1a. SN content was increased by decreasing fat content in all cheeses.

The changes in titratable acidity of low-fat Ras cheese with EPS-producing cultures during storage period are illustrated in Fig. 1b-c. The data indicate that control cheese (full-fat and without EPS-producing cultures) had the lowest values of acidity. Free-fat Ras cheese made with adding EPS-producing cultures (T_1) was lower acidity value among treatments.

Textural profile analysis: From the obtained results Table 2, it could be seen that, replacing traditional with EPS starter with higher level of fat in cheese milk caused decrease in hardness in fresh cheeses. Springiness is described to the panelists as bouncing properties of the sample through several consecutive bites. The obtained values of this property (Table 2) for Ras cheese with different treatments ranged from 4.30-5.06 mm which meaning that the hardness decreased. Treatment (4) with EPS starter showed the lowest in hardness, gumminess and chewiness compared to other treatments.

Flavor compounds: The volatile compounds produced in five samples (control + 4 treatments) were identified using GC-MS. The volatile compounds identified are listed in Table 3 and the GC-MS aroma profiles of samples were seventeen volatile compounds were included 7 esters, 5 alcohols, 4 ketones and 1 fatty acid compounds. Samples had almost the same volatile constituents. Esters e.g., ethyl hexanoate, ethyl octanoate and ethyl butanoate were the dominants among the identified aroma compounds in all samples, followed by alcohols especially 3-methyl butanol and 2-butanol. Ketones as

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	, , ,		Treatments	Treatments					
Flavor									
compounds	Compounds	KI	Control	T ₁	T ₂	T ₃	T_4		
Esters	Ethyl propionate	709	0.62	0.04	0.70	0.28	0.33		
	Ethyl butanoate	803	13.09	9.55	12.56	15.33	17.85		
	Ethyl pentanoate	898	1.65	0.75	1.35	1.95	2.50		
	Ethyl hexanoate	1001	38.83	22.35	34.85	36.90	35.85		
	Ethyl heptanoate	1095	0.65	0.10	0.55	0.72	0.75		
	Ethyl octanoate	1195	18.35	11.44	17.65	19.45	20.55		
	Ethyl decanoate	1394	1.44	1.88	1.56	1.36	0.97		
Alcohols	2-Butanol	605	2.85	1.85	2.99	3.08	3.88		
	2-Pentanol	730	0.11	0.05	0.10	0.15	0.14		
	3-Methyl butanol	735	6.85	7.35	6.95	6.12	2.29		
	2-Methyl butanol	739	0.21	0.13	0.19	0.28	0.25		
	1-pentanol	760	0.95	0.43	0.85	1.29	1.35		
Ketones	Diacetyl	590	1.40	1.95	1.68	1.78	1.54		
	2-Pentanone	700	3.75	3.00	4.20	4.50	6.35		
	2-Heptanone	890	0.45	0.18	0.33	0.55	0.62		
	2-Nonanone	1092	0.89	0.25	0.75	0.95	1.00		
Acids	Butyric acid	820	1.75	1.09	1.55	2.22	2.77		

Table 3: GC-MS analysis of volatile compounds in Ras cheese by using different levels fat and EPS cultures during the storage periods

Table 4: Sensory evaluation of Ras cheese by using different levels fat and EPS cultures during the storage periods

		Treatments						
	Storage periods							
Parameters		Control	T ₁	T ₂	T ₃	T_4		
Flavor (50)	Fresh	46 ^{Aab}	40 ^{cd}	42 ^{Bc}	43 ^{ABc}	44 ^{ABb}		
Body and texture (40)		36 ^{Aab}	30 ^{Bcd}	32 ^{ABc}	33 ^{ABc}	35 ^{Aab}		
Appearance (10)		8 ^{Aa}	6 ^{Bb}	7 ^{ABab}	7 ^{ABab}	7 ^{ABab}		
Total (100)		90 ^{Aab}	76 ^{Ce}	81 ^{Bd}	83 ^{Bcd}	86 ^{ABc}		
Flavor (50)	2 month	47 ^{Aab}	40 ^{BCd}	43 ^{Bc}	45 ^{ABb}	46 ^{Aab}		
Body and texture (40)		37 ^{Aa}	30 ^{Ccd}	33 ^{Bc}	35 ^{ABab}	36 ^{Aab}		
Appearance (10)		9 ^{Aa}	7 ^{ABab}	7 ^{ABab}	8 ^{Aa}	9 ^{Aa}		
Total (100)		93 ^{Aa}	77 ^{Dde}	83 ^{Ccd}	88 ^{Bb}	91 ^{ABab}		
Flavor (50)	4 month	48 ^{Aa}	41 ^{Ccd}	44 ^{Bb}	46 ^{ABab}	47 ^{Aab}		
Body and texture (40)		38 ^{Aa}	31 ^{Bcd}	35 ^{ABab}	36 ^{ABab}	37 ^{Aa}		
Appearance (10)		9 ^{Aa}	7 ^{Bab}	8 ^{ABa}	8 ^{ABa}	9 ^{Aa}		
Total (100)		95 ^{Aa}	79 ^{Dde}	87 ^{Cbc}	90 ^{Bab}	93 ^{ABa}		

A-CMeans with same letter among treatments in the same storage period are not significantly different, a-CMeans with same letter for same treatment during storage periods are not significantly different

2-pentanone, diacetyl and 2-nonanone were also detected in a higher concentrations (Table 3). Butyric acid was the only short-chain fatty acid detected in all samples with concentrations ranged from 1.09-2.77%.

Microscopic structural changes: The microscopic structural changes in fresh low-fat Ras cheese with EPS-producing starter are shown in Fig. 2. The protein matrix (gray area) formed a continuous phase permeated by an amorphous system of voids filled with serum (black area), which in turn revealed the spatial dimensions of these images. An extremely porous, open, was obtained in full-fat Ras cheese (con.), whereas a continuous phase of protein aggregate network characterized by a more compacted and dense structure accompanied by less voids was revealed in the fat-free Ras cheese (T_1) reflecting the hardness texture that was revealed

by sensory evaluation. As it might be seen in Fig. 2, the using 1 and 2% fat and replacing of normal starter with EPS-producing cultures in low fat cheese milk of Ras cheese (Fig. 2, T_2 , T_3) promoted regularly aggregated protein matrices characterized by a fine-meshed network accompanied by small pores that was much smaller in size compared to that of fat-free Ras cheese (Fig. 2, T_1). Treatment cheese, T_4 showed many big pores maybe cause be the higher moisture in this cheese. It had an open structure that resembled the control cheese.

Sensory evaluation: Results from sensory evaluation of Ras cheeses during ripening are given in Table 4. The flavor points for Ras cheeses were significantly (p<0.05) affected using EPS-producing cultures. Full and reduce-fat with EPS-producing cultures cheeses received significantly lower



Fig. 2(a-e): Scanning electron micrographs (SEM) of fresh Ras cheese by using different levels fat and EPS cultures, (a) Control, 4% fat (full-fat) with traditional starter, (b) T_1 , 0% (free fat) with EPS-producing starter, (c) T_2 , 1% fat with EPS-producing starter, (d) T_3 , 2% fat with EPS-producing starter and (e) T_4 , 3% fat with EPS-producing starter Black arrows indicate fibrous-like protein aggregates and white arrows indicate clusters of protein folds, scale bar is 10 μ m flavor scores than the control during ripening. Flavor scores in Ras cheeses were rapidly increased by the age of 120 days of ripening.

Cheeses made using the EPS-producing cultures were described as smooth and soft however Ras cheese without fat (T_1) was described as dry texture: The body and texture scores in full and reduce-fat cheeses including EPS-producing cultures $(T_2, T_3 \text{ and } T_4)$ had higher points than those of the cheeses without fat (T_1) .

There were significant differences noted in appearance scores between cheeses at the beginning and during of ripening period. At the end of ripening, full and reduce-fat with EPS-producing cultures cheeses (T_2 , T_3 and T_4) received the highest appearance scores in the expert panel compared to free fat with EPS-producing cultures cheese (T_1). Generally, the appearance, body and texture and flavour scores significantly increased all cheeses during ripening.

DISCUSSION

Generally, addition of EPS producing cultures with decreasing fat of cheese milk lead to an increase in the moisture of treatments which could be related to the changes that occurred in low-fat Ras cheese compared to control (full-fat and traditional culture). These changes due to retention of water in cheese curd depending up on EPS-producing capacity of the cultures³⁷. A gradual decrease in moisture content in all treatments including control (full-fat and without EPS-producing culture) was observed as the storage period advanced. The results are in agreement with those reported by El Soda³⁸.

Dabour *et al.*¹ reported that protein content of EPS-containing cheeses were significantly low than that of control cheese (without EPS cultures) and appeared to be proportionally affected by the amount of water retained in cheese. The salt contents followed the same trend to that of protein content.

There were negative correlation between the rate of fat reduction and the values of SN. These results are in agreement with those reported by Guinee *et al.*³⁹ who found that reducing the fat level of Cheddar-cheese resulted in a decrease in SN/TN. Meanwhile, SN content of all cheese treatments including control increased throughout the ripening period which are in a agreement with those reported by Romeih *et al.*⁴⁰. Higher fat content of cheese milk from 1-3% with adding EPS-producing cultures led to increase values of acidity in the resultant cheese. This could be attributed to

higher moisture contents for these treatments (T_1 , T_3 and T_4). In agreement with Tohamy *et al.*⁴¹, the acidity of low-fat Ras cheese samples formulated in the current study was decreased compared with full-fat Ras cheese (control). All values of acidity increased with prolonging the storage period. The increase in acidity value at the end of storage was different to that of fresh samples.

The general trend of these results is in agreement with those reported by Tohamy *et al.*⁴¹. The changes in pH values among all cheese treatments and during storage period (Fig. 1b-c) followed opposite trend to that of titratable acidity. This means that the pH values of treatments with EPS-producing cultures were higher than control when fresh and along the storage period.

The replacement of traditional starter culture with EPS, led to increase in cheese moisture content, as a result of water adsorption or binding by EPS starter. Because the increase in moisture content weakness the casein micelles. Hardness is described as the force required to penetrate the sample with the molar teeth (from soft to firm)⁴².

Chewiness is described to be the number of chews required to swallow a certain amount of sample. This property expressed mathematically as the product of gumminess and springiness, therefore, it took the same trend of these property. Fat content in the cheese is responsible for its many desirable functional and texture. In addition, decreasing moisture content might result in decrease in the level of free moisture in cheese; this increased the hardness⁴³. Values of hardness, cohesiveness, springiness, gumminess and chewiness decrease during storage period for 4 months.

The aroma profile was found to be in agreement with Ayad *et al.*⁴⁴ whereas aroma release from full-fat cheese (except for diacetyl) is lower than low-fat cheese (Table 3). This difference could be related to the solubility of hydrophobic aromatic compounds in fat of full fat cheese⁴⁵. When an EPS-producing culture was added to low-fat Ras cheese, aroma release was decreased compared to T₁ (zero fat) thin it increases with increasing level of fat in milk of cheese. This decrease could be attributed to entrapment of aromatic compounds in gel network as a result of water binding capacity of polysaccharide. Results of the present study are in agreement with Aminifar and Attar⁴⁵ who stated that release of aromatic compounds from Iranian white brined cheese was affected by the amount of fat and polysaccharide compounds.

According to Olson⁴⁶ and Manning⁴⁷ formation of aroma depend on the enzymes present, so different flavors can be obtained. Therefore, the flavour of samples under investigation in the present work results from the interaction of enzymes from the microorganisms. Alcohols detected in

samples result from catabolism of amino acids⁴⁸. However, the concentrations of the different ketones varied between the different cheese samples and are formed by enzymic oxidative decarboxylation of fatty acids, through the action of mould⁴⁹. From Table 3 it is clear that low-fat Ras cheese are rich in ketones. Diacetyl, found in high level in all treatments (1.54-1.95%), is known to come from citrate conversion and is responsible for a creamy flavor⁵⁰. Some differences in the levels of ethyl-esters were also encountered among treatments (Table 3). These compounds are formed by chemical or enzymatic reactions of fatty acids with primary alcohols, as reported by Barbieri et al.51. Generally, the results showed that use of EPS-producing cultures in the manufacture of low-fat Ras cheese caused an increase in esters, ketones and alcohols compounds. However, in all cheeses, esters, ketones and alcohols are principal chemical groups of flavour cheese. The resultant low-fat Ras cheese with EPS-producing cultures was similar with full-fat Ras cheese based on volatile profiles and sensory scores. It was concluded that the low-fat Ras cheeses could be manufactured using EPS-producing starter culture to improve volatile composition and sensory properties.

In general, using EPS-producing cultures in low-fat Ras cheese was more pronounced on microscopic structural changes of resultant cheese. Casein micelles aggregate to form a protein network in which the fat globules are entrapped⁵². The microstructure of milk proteins matrix changes during processing of cheese according to the type of starter and content of milk fat (Fig. 2).

Reduction of fat in cheese may cause a lower amount of fatty acids due to the cheese may perceive as lacking flavor¹⁰. The sensory results of T₁ sample which is fat-free and described as dry-texture one, found to be similar to the studies of Ahmed *et al.*⁵³ for Karish cheese made using EPS-producing cultures. Low-fat cheeses are typically characterized as weak and coarse texture as described by Kosikowski and Mistry⁵⁴ and the greater fat reduction give the more defects⁵. A small degree of casein breakdown is required for body and texture development and an acceptable functionality. When the fat content was reduced to below 4% a slow proteolysis has been observed during ripening⁵⁵. The higher water and fat content of the EPS-producing cultures cheeses changed its sensory characteristics, giving a softer texture for product⁵⁶.

The obtained results, exopolysaccharide enhance viscosity, texture and mouth feel and to avoid syneresis in yoghurt. The results of this study suggest that the use of EPS-producing cultures could provide better textures for camel milk yoghurt than those imparted by additives.

CONCLUSION

Using EPS-producing cultures in Ras cheese milk were improved sensory evaluation of resultant cheese and cheese with 3% fat and EPS-producing culture (T_4) selected as best cheeses by sensory panel to produce high quality Ras cheese.

SIGNIFICANCE STATEMENT

This study reveals the possible effect of EPS-producing cultures that can be beneficial for producing low-fat Ras cheese with better volatile and sensory properties. This study will help the researcher to uncover the critical area of low-fat cheese products that many researchers were not able to explore. Thus, a new application on EPS-producing cultures, may be arrived at.

REFERENCES

- Dabour, N., E. Kheadr, N. Benhamou, I. Fliss and G. LaPointe, 2006. Improvement of texture and structure of reduced-fat cheddar cheese by exopolysaccharide-producing lactococci. J. Dairy Sci., 89: 95-110.
- 2. Buttriss, J., 1987. Diet, health and the dairy industry. Int. J. Dairy Technol., 40: 61-64.
- Bryant, A., Z. Ustunol and J. Steffe, 1995. Texture of cheddar cheese as influenced by fat reduction. J. Food Sci., 60: 1216-1219.
- 4. Koca, N. and M. Metin, 2004. Textural, melting and sensory properties of low-fat fresh kashar cheeses produced by using fat replacers. Int. Dairy J., 14: 365-373.
- 5. Banks, J.M., E.Y. Brechany and W.W. Christie, 1989. The production of low fat Cheddar-type cheese. Int. J. Dairy Technol., 42: 6-9.
- 6. Banks, J.M., E.A. Hunter and D.D. Muir, 1993. Sensory properties of low fat cheddar cheese: effect of salt content and adjunct culture. J. Soc. Dairy Technol., 46: 119-123.
- 7. Jameson, G.W., 1990. Cheese with less fat. Aust. J. Dairy Technol., 45: 93-98.
- Anderson, D.L., V.V. Mistry, R.L. Brandsma and K.A. Baldwin, 1993. Reduced fat cheddar cheese from condensed milk. 1. Manufacture, composition and ripening. J. Dairy Sci., 76: 2832-2844.
- Rogers, N.R., D.J. McMahon, C.R. Daubert, T.K. Berry and E.A. Foegeding, 2010. Rheological properties and microstructure of Cheddar cheese made with different fat contents. J. Dairy Sci., 93: 4565-4576.
- 10. Mistry, V., 2001. Low fat cheese technology. Int. Dairy J., 11: 413-422.

- Chen, C.M. and M.E. Johnson, 1996. Process for manufacturing reduced-fat Cheddar cheese. United States Patent No. 5554398, September 10, 1996. https://patents.google.com/patent/US5554398A/en
- Broadbent, J.R., D.J. McMahon, C.J. Oberg and D.L. Welker, 2001. Use of exopolysaccharide-producing cultures to improve the functionality of low fat cheese. Int. Dairy J., 11: 433-439.
- 13. Ruas-Madiedo, P., J. Hugenholtz and P. Zoon, 2002. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. Int. Diary J., 12: 163-171.
- 14. Amatayakul, T., F. Sherkat and N.P. Shah, 2006. Physical characteristics of set yoghurt made with altered casein to whey protein ratios and EPS-producing starter cultures at 9 and 14% total solids. Food Hydrocolloids, 20: 314-324.
- Costa, N.E., J.A. Hannon, T.P. Guinee, M.A.E. Auty, P.L.H. McSweeney and T.P. Beresford, 2010. Effect of exopolysaccharide produced by isogenic strains of *Lactococcus lactis* on half-fat Cheddar cheese. J. Dairy Sci., 93: 3469-3486.
- Hofi, A.A., E.H. Youssef, M.A. Ghoneim and G.A. Tawab, 1970. Ripening changes in cephalotyre "RAS" cheese manufactured from raw and pasteurized milk with special reference to flavor. J. Dairy Sci., 53: 1207-1211.
- 17. Scott, R., 1981. Cheesemaking Practice. Applied Science Publisher Ltd., London, UK., ISBN-13: 978-0853349273, Pages: 475.
- Phelan, J.A., J. Renaud and P.F. Fox, 1993. Some Non-European Cheese Varieties. In: Cheese: Chemistry, Physics and Microbiology, Volume 2: Major Chees Groups, Fox, P.F. (Ed.). 2nd Edn., Chapman and Hall, London, UK., ISBN-13: 9780412535109, pp: 421-465.
- 19. Fox, P.F. and J.M. Wallace, 1997. Formation of flavor compounds in cheese. Adv. Applied Microbiol., 45: 17-85.
- 20. McSweeney, P.L.H. and M.J. Sousa, 2000. Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Lait, 80: 293-324.
- Ayad, E.H.E., S. Nashat, N. El-Sedek, H. Metwaly and M. El-Soda, 2004. Selection of wild lactic acid bacteria isolated from traditional Egyptian dairy products according to production and technological criteria. Food Microbiol., 21: 715-725.
- 22. Bosset, J.O. and R. Gauch, 1993. Comparison of the volatile flavour compounds of six European 'AOC' cheeses by using a new dynamic headspace GC-MS method. Int. Dairy J., 3: 359-377.
- 23. Engels, W.J.M., R. Dekker, C. de Jong, R. Neeter and S. Visser, 1997. A comparative study of volatile compounds in the water-soluble fraction of various types of ripened cheese. Int. Dairy J., 7: 255-263.
- 24. Urbach, G., 1995. Contribution of lactic acid bacteria to flavour compound formation in dairy products. Int. Dairy J., 5: 877-903.

- Maarse, H. and C.A. Visscher, 1989. Volatile Compounds in Food: Qualitative and Quantitative Data. 6th Edn., TNO-CIVO Food Analysis Institute, Zeist, The Netherlands, ISBN-13: 9789067431682, pp: 49.
- Hamdy, S., H. Shaaban, H.S. Mahmoud, K. Abbas and A.Farouk, 2017. Preparation of ras cheese flavour concentrate using lipolyzed cream and skim milk Curd. Int. J. Dairy Sci., 12: 275-281.
- 27. Hassan, A.N., M. Corredig and J.F. Frank, 2002. Capsule formation by nonropy starter cultures affects the viscoelastic properties of yogurt during structure formation. J. Dairy Sci., 85: 716-720.
- 28. Perry, D.B., D.J. McMahon and C.J. Oberg, 1997. Effect of exopolysaccharide-producing cultures on moisture retention in low fat Mozzarella cheese. J. Dairy Sci., 80: 799-805.
- 29. Abdel-Tawab, G., 1963. Manufacturing of Ras cheese from pasteurized milk. M.Sc. Thesis, Ain Shams University, Cairo, Egypt.
- 30. AOAC., 2000. AOAC official method 920.124: Acidity of cheese. Titrimetric method. Association of Official Analytical Chemists, Washington, DC., USA.
- 31. Tamime, A., M.F. Younis, G. Davies and I. Bradbury, 1990. The quality of processed cheese made from reconstituted skim milk powder cheese base. Egypt. J. Dairy Sci., 18: 115-131.
- 32. Bourne, M.C., 1978. Texture profile analysis. Food Technol., 32: 62-66.
- 33. El-Shafei, H., A. Wahba, F. El-Abbasy and A. Sameh, 1995. Manufacture of Ras cheese with different milk clotting enzymes. Egypt. J. Dairy Sci., 23: 271-283.
- 34. SAS., 1990. SAS User's Guide/STAT Version 6.04. 4th Edn., SAS Institute Inc., Cary, NC., USA.
- Adams, R.P., 2001. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. 3rd Edn., Allured Publication, Carol Steam, IL., USA., ISBN-13: 9780931710858, Pages: 456.
- El-Hofi, M., A. Ismail, F. Abd Rabo, S. El-Dieb and O. Ibrahim, 2010. Studies on acceleration of Ras cheese ripening by aminopeptidase enzyme from buffaloes' pancreas. Il-Utilization of buffaloes' pancreas aminopeptidase in acceleration of Ras cheese ripening. N. Y. Sci. J., 6: 91-96.
- 37. Sanli, T., A. Gursel, E. Sanli, E. Acar and M. Benli, 2013. The effect of using an exopolysaccharide-producing culture on the physicochemical properties of low-fat and reduced-fat Kasar cheeses. Int. J. Dairy Technol., 66: 535-542.
- El Soda, M., 2014. Production of low fat cheddar cheese made using exopolysaccharide-producing cultures and selected ripening cultures. Adv. Microbiol., 4: 986-995.
- Guinee, T.P., M.A.E. Auty, C. Mullins, M.O. Corcoran and E.O. Mulholland, 2000. Preliminary observations on effects of fat content and degree of fat emulsification on the structurefunctional relationship of Cheddar-type cheese. J. Texture Stud., 31: 645-663.

- Romeih, E.A., M. Abdel-Hamid and A.A. Awad, 2014. The addition of buttermilk powder and transglutaminase improves textural and organoleptic properties of Fat-Free buffalo yogurt. Dairy Sci. Technol., 94: 297-309.
- 41. Tohamy, M.M., M.A. El-Nawawy, A.A. Kandeel and M.A. Moustafa, 2011. Modification of the traditional method of making Ras cheese and its effect on the properties and quality of the resultant low-fat Ras cheese. J. Food Dairy Sci. Mansoura Univ., 2: 101-114.
- 42. Lee, C.H., E.M. Imoto and C.K. Rha, 1978. Evaluation of cheese texture. J. Food Sci., 43: 1600-1605.
- 43. Awad, S., 2011. Texture and Microstructure. In: Practical Food and Research, Cruz, R.M.S. (Ed.). Chapter 14, Nova Science Publishers Inc., Hauppauge, NY., USA., ISBN: 978-1-61728-506-6, pp: 361-391.
- 44. Ayad, E.H.E., S. Awad, A. El Attar, C. de Jong and M. El-Soda, 2004. Characterisation of Egyptian Ras cheese. 2. flavour formation. Food Chem., 86: 553-561.
- 45. Aminifar, M. and F. Attar, 2014. The relation between proteinprotein and polysaccharide-protein interactions on aroma release from brined cheese model. Proceedings of the 3rd International Conference on Nutrition and Food Science, June 18-20, 2014, Copenhagen, Denmark, pp: 19-22.
- 46. Olson, N.F., 1990. The impact of lactic acid bacteria on cheese flavor. FEMS Microbiol. Rev., 7: 131-147.
- 47. Manning, D.J., 1979. Chemical production of essential Cheddar flavour compounds. J. Dairy Res., 46: 531-537.
- 48. Hemme, D., C. Bouillanne, F. Metro and M.J. Desmazeaud, 1982. Microbial catabolism of amino acids during cheese ripening. Sciences des Aliments, 2: 113-123.

- 49. Kinsella, J.E., D.H. Hwang and B. Dwivedi, 1976. Enzymes of *Penicillium roqueforti* involved in the biosynthesis of cheese flavor. Crit. Rev. Food Sci. Nutr., 8: 191-228.
- 50. Welsh, F.W., W.D. Murray, R.E. Williams and I. Katz, 1989. Microbiological and enzymatic production of flavor and fragrance chemicals. Crit. Rev. Biotechnol., 9: 105-169.
- Barbieri, G., L. Bolzoni, M. Careri, A. Mangia, G. Parolari, S. Spagnoli and R. Virgili, 1994. Study of the volatile fraction of Parmesan cheese. J. Agric. Food Chem., 42: 1170-1176.
- Ong, L., R.R. Dagastine, S.E. Kentish and S.L. Gras, 2013. Microstructure and composition of full fat Cheddar cheese made with ultrafiltered milk retentate. Foods, 2: 310-331.
- 53. Ahmed, N.H., M. El Soda, A.N. Hassan and J. Frank, 2005. Improving the textural properties of an acid-coagulated (Karish) cheese using exopolysaccharide producing cultures. LWT-Food Sci. Technol., 38: 843-847.
- 54. Kosikowski, F.V. and V.V. Mistry, 1997. Cheese and Fermented Milk Foods, Volume 1: Origins and Principles. 3rd Edn., F.V. Kosikowski, Madison, WI., USA., ISBN-13: 9780965645614, Pages: 728.
- Rudan, M.A., D.M. Barbano, J.J. Yun and P.S. Kindstedt, 1999. Effect of fat reduction on chemical composition, proteolysis, functionality and yield of Mozzarella cheese. J. Dairy Sci., 82: 661-672.
- Jimenez-Guzman, J., A. Flores-Najera, A.E. Cruz-Guerrero and M. Garcia-Garibay, 2009. Use of an exopolysaccharideproducing strain of *Streptococcus thermophilus* in the manufacture of Mexican Panela cheese. LWT-Food Sci. Technol., 42: 1508-1512.