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Impact of Different Types of Exopolysaccharide-Producing Adjunct Starter Cultures on Enhancement of Karish Cheese Properties

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Abstract

Karish cheese is acid coagulated traditional Egyptian cheese and it exhibited undesirable textural characteristics.Therefore, Exopolysaccharide (EPS) producing*Lactobacillus*(*Lb.*) *paracasei* S12, *Lb. paracasei* S8, *Enterococcus* (*Ent.*) *durans* S27 and *Ent. hirae* S9 were used as adjunct cultures in manufacturing of Karish cheese. The effect of these adjunct cultures on the moisture content, fat, pH, yield, proteolytic activity, texture profile and over all sensory acceptance were studied. The moisture content in Karish cheese was significantly increased when EPS starter cultures were added particularly with two strains of enterococci.No significant changes were found in fat content and pH values between different Karish cheese treatments.The range of Fat/dry matter was between 3.57 to 5.67%.Cheese yield increased by 1.5 to 4% as a result of adding EPS producing cultures. Also, values of texture profile of different Karish cheese treatments were enhanced. Karish cheese manufactured with *Ent. durans*S27 had the highest proteolytic activity and had the lowest overall sensory acceptance score. The results of this study indicate that *Lb. paracasei* S12 and S8 and *Ent. hirae* S9 are recommended as adjunct cultures for manufacturing of Karish cheese.

Key words: Enterococcus hirae; EPS producing cultures; Karish cheese; Texture profile; Chemical properties; Proteolytic activity

1. Introduction

Over consumption of dietary fat is associated with obesity and other metabolic syndrome [1-3] resulting in an increase in the consumer demand in the low-fat dairy products. On the other hand, low fat cheeses have lower overall acceptability than full fat cheeses whereas low fat cheeses were characterized as hard, rubbery, dry, and grainy texture[4, 5]. In order to enhance the flavor and texture of low-fat cheese, exopolysaccharides (EPS) producing adjunct culture were used in the manufacture of different types of low-fat cheeses [6]. Application of EPS producing adjunct cultures could enhance the softness and spread-ability of cheese [7] with high water holding capacity [8]. Furthermore, the texture profile was appeared more closely to full fat cheese [9].Karish cheese is acid coagulated traditional Egyptian cheese and it is manufactured from skim milk [10]. Therefore, it exhibited undesirable textural characteristics. Hassan et al. [9] reported that manufacture of Karish cheese with EPS producing *Streptococcus* (*St.*) *thermophilus* culture exhibited a porous structure with more deformable curd than that made using EPS-nonproducing cultures. Also, addition of EPS producing *St. thermophilus* could enhance moisture content, yield, texture profile and

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overall acceptability[11]. However, there is still gab in application of wide range of EPS producing cultures. Therefore, the main objective of this study is to evaluate the influence of different EPS producing lactic cultures (*Lb. paracasei* S12, *Lb. paracasei* S8, *Ent.durans* S27 and *Ent. hirae* S9) on enhancement of moisture content, yield, texture profile and flavor through proteolytic activity of different Karish cheese treatments.

2. Materials and Methods

2.1 Materials

Skim milk was obtained from Dairy plant, Faculty of Agriculture, Cairo University. O-phthaldialdehyde (OPA) reagent was purchasedfrom Sgima-Aldrich Co., St Louis, MO, United States. Mesophilic starter culture *Lactococcus*(*Lc.*)*Lactis* CHOOZIT DIA-A was gratefully gifted from Danisco Co. (Paris, France). *Lb.paracasei* S12, *Lb.paracasei* S8, *Ent.durans* S27 and *Ent.hirae* S9 were isolated from traditional dairy products.

2.2 Experimental procedure

Different Karish cheese treatments were made in the Dairy Science Department, Faculty of Agriculture, Cairo University, Egypt. Skim milk (0.5% fat) was heat treated at 72 ºC for 2 min. and then cooled to 37 ºC. Milk was divided into five equal portions. The first portion served as control whereas milk was inoculated with 1% non-producing EPS mesophilic starter culture *Lc. Lactis*CHOOZIT DIA-A. The second(T1), third(T2), fouth(T3)and fifth (T4)portions were inoculated with 0.5% non-producing EPS mesophilic starter culture *Lc. Lactis* CHOOZIT DIA-A and 0.5% of *Lb.paracasei* S12, *Lb.paracasei* S8, *Ent.durans* S27 or*Ent.hirae* S9 respectively. At pH 4.5-4.6 after overnight incubation at 32 ºC, the curd of different treatments was scooped into polystyrene moulds, dry salt (1g /100g milk) was sprinkled on the surface of curds and drained overnight at 4 ºC. The resultant cheesewas then cut into blocks, enveloped in parchmentpaper, and stored at 4 ºC for fifteen days. Data were reported as the average of three independenttrials.

2.3 Methods of analysis

The moisture and fat contents of each sample were determined according to AOAC [12].The proteolytic activity of crude extracts was determined by reacting free amino acids with o-phthaldialdehyde (OPA), according to Luo et al. [13]. The absorbance of the mixture was measured at 340 nm using spectrophotometer (Jenway®Genova Life Science Spectrophotometer UV/Visible).At the end of cold storage period, the degree of protein hydrolysis (%DH) was calculated by the following equation:

$$DH\% = A_{sample} - A_{blank} / A_{total} - A_{blank} \times 100$$

The Textural profile of different Karish cheese treatments were evaluated using texture analyzer (CNS-Farnell, Borehamwood, Hertfordshimre, England). Cheese samples presented to the instrument were 30 mm in diameter and 20 mm in height. A TA15-451Perspex cone was used as the probe with a penetration of 10 mm at 1 mm/s. The textural parameters were determined according to the method of Szczesniaket al [14].

The sensory evaluation of different Karish cheese treatments was carried out according IDF Standard 99A [15]. Samples presented to the panelists (n = 10) in a random order. Scores were obtained for the three sensory attributes. Panelists were also instructed to report any defects they notice.

2.4 Statistical analysis

All data are expressed as means of three separate replicates and were presented as mean ± standard deviation. Results were analyzed using analysis of variance (ANOVA) by MSTAT-C software (Michigan State University, East Lansing, Michigan, USA). Differences between means were deemed significant at 95% ($P < 0.05$) confidence level.

3. Results and Discussion

3.1 Chemical composition

Generally, levels of moisture content were significantly decreased during cold storagepresented in Table 1. Among different Karish cheese treatments, the moisture content was significantly increased when EPS producing adjunct cultures were added. Karish cheese manufactured with thetwo strains of enterococci had higher content of moisture than Karish cheese manufactured with two strains of *Lb. paracasei* and control during fifteen days of cold storage period. This is due to the ability of EPS producing cultures to increase the water holding capacity [9, 11]. Moreover, the differences between moisture content in Karish cheese treatments indicate that starter cultures that used were differencing in their production of EPS. The average of Fat/dry matter was between 3.57 to 5.67% for different Karish cheese treatments.No significant differences were observed in pH values between different Karish cheese treatments.Addition of EPS producing *S. thermophilus* strain did not significantly effect on the fat content, fat: moisture ratio and pH values of Karish cheese [11].

3.2 Cheese yield

Data in Fig. 1 show the changes in yield of Karish cheese as a result of adding different EPS producing cultures. Karish cheese manufactured with two enterococci strainshad significantly higher yield than Karish cheese manufactured with two lactobacilli strains and control. The yield of different Karish cheese treatments was parallel to moisture content. The cheese yield of Karish cheese treatments increased in different ratios ranged from 1.5 to 4%. Results obtained by Ahmed et al. [11] showed that the yield of Karish cheese was increased by 2% when *S. thermophilus* (EPS producing strain) was applied.

3.3 Proteolytic activity

During cold storage of different Karish cheese treatments, levels of proteolytic activity increased (Table 2 and Fig. 2). Crude extract of Karish cheese manufactured using*Ent. durans* S27 strain had the highest proteolytic activity during fifteen days of storage. Crude extract from fermented milk by *Lb. casei* NRRL-B-1922 strain had the highest proteolytic activity [16]. *Lb. paracasei* M11 had a proteolytic activity in fermented goat milk [17]. Enterococci play an important role in ripening of wide range of chesses as nonstarter lactic acid bacteria through its proteolytic activity [18-20].

3.4 Texture profile.

Texture possesses a crucial role in assessment the quality of different dairy products oftentimes relies on protein structure and matrix. The texture attributes of different Karish cheese treatments are listed in Table 3. Variations in starter culture of Karish cheese resulted in the formation of curds with distinctly different texture properties. At day one of cold storage, the hardness, adhesiveness, gumminess, and chewiness of control treatment was found to be significantly higher than those of other treatments. The cohesiveness and springiness had the highest values for T2, T3 and T4 treatments. The cold storage for 15 days led to increase hardness, adhesiveness, chewiness, cohesiveness, and gumminess and decrease springiness of all Karish cheese treatments.The protein-polysaccharide interactions and coagulation as well as aggregation behavior have a significant effect on rheological properties and physical stability of multi-component food products including Karish cheese [11, 21].

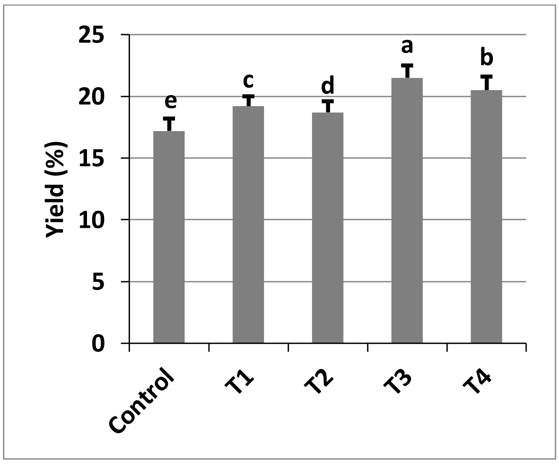

Figure 1. Yield (%) of different Karish cheese treatments

Control: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain), T1: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain)+ *Lactobacillus paracasei* S12(EPS positive producing strain), T2: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain) + *Lactobacillus paracasei* S8 (EPS positive producing strain), T3: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain) + *Enterococcus durans* S27 (EPS positive producing strain) and T4: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain) + *Enterococcus hirae* S9 (EPS positive producing strain).

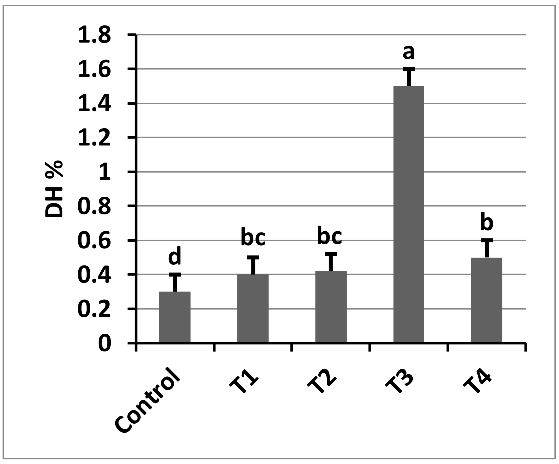

Figure 2. Degree of protein hydrolysis (DH %) of different Karish cheese treatments

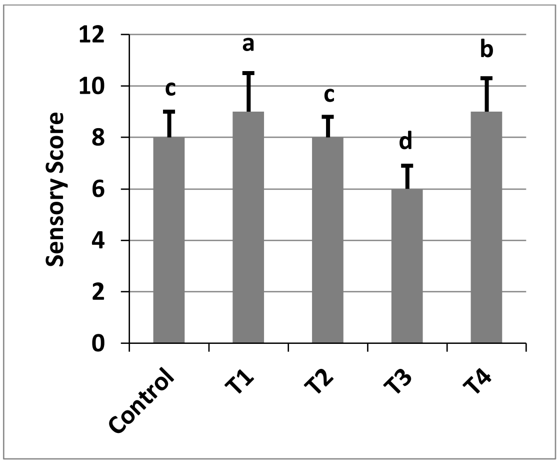

Figure 3.Overall acceptance score (1 to 9) of different Karish Cheese treatments

Control: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain), T1: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain)+ *Lactobacillus paracasei* S12(EPS positive producing strain), T2: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain) + *Lactobacillus paracasei* S8 (EPS positive producing strain), T3: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain) + *Enterococcus durans* S27 (EPS positive producing strain) and T4: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain) + *Enterococcus hirae* S9 (EPS positive producing strain).

Control: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain), T1: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain)+ *Lactobacillus paracasei* S12(EPS positive producing strain), T2: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Lactobacillus paracasei* S8 (EPS positive producing strain), T3: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Enterococcus durans* S27 (EPS positive producing strain) and T4: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Enterococcus hirae*S9 (EPS positive producing strain).

3.5 Sensory evaluation

Data in Fig. 3 show the overall acceptance of sensory evaluation of different Karish cheese treatments. Karish cheeses manufactured with EPS producing culture were described by panelists as smooth and creamy while control cheese was described as dry, granular and lowest creaminess. Results obtained by Ahmed et al [11] showed that Karish cheese manufactured with EPS producing strains had highest softness and creaminess. Also, Perry et al.[8, 22] reported that using EPS producing lactic culture could enhance the sensory attributes of low-fat Mozzarella cheese. Karish cheese manufactured with *Ent. durans*S27 (T3) had the lowest overall acceptability because its bitterness which was in a good correlation with the proteolytic activity as shown in Table 2 and Fig. 2.

4. Conclussion

This work declared the positive effect of EPS producing adjunct cultures on enhancement of moisture content, yield, texture profile and overall sensory acceptance of Karish cheese. Karish cheese made with two strains of *Lb. paracasei* or *Ent. hiare*was smother, softer, moisture content and overall sensory acceptance higher than control. Therefore, these strains could be recommended as adjunct cultures for enhancing the characteristics of Karish cheese.

Table 1.Chemical composition and pH of different Karish cheese treatments during storage at 5ºC for 15 days

Treatments (T)	Storage Period (day)	Moisture %	Fat %	Fat / Dry Matter %	pH
Control	0	70.60 ± 1.05^{e}	1.05 ± 0.10	3.57	4.45 ± 0.10
	15	69.52 ± 1.10^{d}	1.25 ± 0.0	4.10	4.30 ± 0.20
T1	0	78.60 ± 1.00^{b}	1.00 ± 0.0	4.67	4.43 ± 0.10
	15	77.50 ± 0.80^{c}	1.10 ± 0.0	4.88	4.35 ± 0.10
T2	0	78.70 ± 0.50^{b}	1.20 ± 0.0	5.63	4.47 ± 0.15
	15	77.01 ± 0.40^{c}	1.30 ± 0.0	5.65	4.31 ± 0.11
T3	0	79.80 ± 0.80^{a}	1.05 ± 0.0	5.19	4.45 ± 0.13
	15	78.50 ± 0.80^{b}	1.25 ± 0.0	5.81	4.30 ± 0.15
T4	0	79.10 ± 1.00^{a}	1.15 ± 0.0	5.50	4.48 ± 0.17
	15	77.10 ± 0.90^{c}	1.30 ± 0.0	5.67	4.30 ± 0.15

Control: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain), T1: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain) + *Lactobacillus paracasei* S12(EPS positive producing strain), T2: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain) + *Lactobacillus paracasei* S8 (EPS positive producing strain), T3: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain) + *Enterococcus durans* S27 (EPS positive producing strain) and T4: Karish cheese manufactured using *Lactococcus lactis* (EPS negative producing strain) + *Enterococcus hirae* S9 (EPS positive producing strain).

Table 2.Proteolytic activity of crude extract of Karish cheese during storage at 5ºC for 15 days

Treatments (T)	Proteolytic activity		
	Zero time	**7th days**	**15th days**
	Optical Density (340 nm)		
Control	0.14	0.15	0.20
T1	0.09	0.11	0.16
T2	0.13	0.15	0.17
T3	0.26	0.30	0.51
T4	0.12	0.14	0.16

Control: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain), T1: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Lactobacillus paracasei* S12(EPS positive producing strain), T2: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Lactobacillus paracasei* S8 (EPS positive producing strain), T3: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Enterococcus durans* S27 (EPS positive producing strain) and T4: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Enterococcus hirae*S9 (EPS positive producing strain).

Table 3. Texture profile of different Karish cheese treatments during storage at 5ºC for 15 days

Treatments	Storage, day	Hardness (g)	Adhesiveness (g)	Cohesiveness	Springiness (%)	Gumminess	Chewiness
Control	0	2.75	0.31	0.43	3.37	2.70	7.92
	15	3.15	0.36	0.53	3.07	3.00	8.22
T1	0	2.40	0.24	0.59	4.05	1.70	5.25
	15	2.70	0.28	0.75	3.85	1.90	5.45
T2	0	2.45	0.26	0.61	3.71	1.90	6.41
	15	2.75	0.30	0.80	3.56	2.10	6.61
T3	0	1.80	0.17	0.51	4.21	1.00	4.13
	15	1.95	0.19	0.60	4.09	1.10	4.23
T4	0	2.00	0.21	0.55	4.22	1.20	4.72
	15	2.20	0.25	0.67	4.04	1.35	4.87

Control: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain), T1: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Lactobacillus paracasei* S12(EPS positive producing strain), T2: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Lactobacillus paracasei* S8 (EPS positive producing strain), T3: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Enterococcus durans* S27 (EPS positive producing strain) and T4: Karish cheese manufactured using *Lactococcuslactis* (EPS negative producing strain) + *Enterococcus hirae*S9 (EPS positive producing strain).

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