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Attributes of Low-Fat Yogurt and Kareish Cheese Made Using Exopolysaccharides Producing Lactic Acid Bacteria

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ABSTRACT

To improve properties of low-fat yoghurt and kariech cheese, two mixed cultures of Exopolysaccharide (EPS)-producing Lactic Acid Bacteria (LAB) were used to manufacture them. To study the activity of cultures, pH was determined during fermenting of low-fat yoghurt samples. Some chemical, rheological, microbial and organoleptic examinations were done on low-fat yoghurt and kariech cheese during storage for 11 or 30 days, respectively. Culture that consists of [3% *S. thermophilus* MR-1C and *L. delbrueckii* sub sp., *bulgaricus* MR-1R (1:1) plus 1% *Bifidobacterium* spp. and *L. acidophilus* (1:1)] (SB-EPS) was the best in all characteristics of low-fat yoghurt or kariech cheese whether fresh or stored followed by culture that consists of [3% *S. thermophilus* TA061+*Lactobacillus helveticus* LH110 (1:1)+1% *Bifidobacterium* spp. and *L. acidophilus* (1:1)] then the control sample [4% *S. thermophilus*+*L. delbrueckii* sub sp., *bulgaricus* (traditional) (1:1)]. Generally, EPS-producing LAB improved the rheological properties of low-fat yoghurt and texture profile of kariech cheese.

Key words: EPS-producing LAB, low-fat yoghurt, kariech cheese, rheological properties, texture profile

INTRODUCTION

Yoghurt has a wide popularity around the world, because of its nutritional and healthy benefits that are discovered day after day for all ages of human. Although it has some technological defects which researchers have been trying to avoid them, as well as searching for the most advantage of it, using new bacterial strains like EPS-producing lactic acid bacteria or new production methods.

Kariech cheese is the most popular, cheaper, rich in nutrients and oldest cheese in Egypt. It is a soft acid cheese and low-fat because it is made from skimmed milk. Reduction of fat causes textural, functional and sensory defects such as rubbery texture, lack of flavour, bitterness, off-flavour, poor meltability and undesirable colour (Romeih *et al*., 2002) as well as high susceptibility to fragmentation and rough texture. Several visions have been proposed to improve kariech cheese include using therapeutic or probiotic cultures containing *Bifidobacterium* spp. (Abd-Elhamid, 2012) or using of EPS-producing *S. thermophilus* to enhance kariech cheese texture (Hassan *et al*., 2004).

Lactic Acid Bacteria (LAB) are the basic in dairy fermented products such as yoghurt and kariech cheese. In recent, LAB has drew attention for their efficiency to secrete extracellular-polysaccharides because of EPS-producing LAB have one of the largest technical

potentials for development of novel and improved products such as low-milk-solid and low-fat fermented dairy products which gave it great technological and commercial importance. Exopolysaccharides could be a potential attentive for thickening agents to increase moisture content and improve rheological properties of food such as yoghurt and cheese (Ghamari, 2009).

Worth mentioning, EPS include produced by LAB have various functional roles in human or animal health including immunomodulatory properties, antiviral activity, antioxidant activity and antihypertensive activity (cholesterol-lowering activity) (Maeda *et al*., 2004; Kodali and Sen, 2008; Nagai *et al*., 2011). In addition, its properties of anti-tumor, anti-ulcer interests, at the same time, it has benefits as prebiotics.

This investigation was carried out to study the impact of using two mixed culture of EPS-producing LAB on attributes of low-fat yoghurt and kariech cheese. Yoghurt and kareish cheese were made from mixed buffalo and cow's low-fat milk 1.5 and 0.5%, respectively. Some chemical, rheological, microbial and organoleptic examinations were done through the storage period.

MATERIALS AND METHODS

Fresh buffalo and cow's milk was obtained from the herds of Serw Station, Animal Production Research Institute, Ministry of Agriculture, Egypt. Sodium chloride was obtained from Al-Nasser Company for salt, Egypt. The two types of milk were standardized and mixed to obtain a mix suitable each product, yoghurt or kariech cheese (Table 1).

Bacterial cultures: The bacterial strains used in this study are listed in Table 2. All bacterial cultures were incubated at 37°C, stored at 4°C and maintained by biweekly transfer. *Streptococcus thermophilus* MR-1C and *Streptococcus thermophilus* TA061 were grown in M17 broth (Terzaghi and Sandine, 1975) containing 0.5% lactose while *Lactobacillus delbrueckii* sub sp., *bulgaricus* and *L. delbrue*ckii sub sp., *bulgaricus* MR-1R and *Lactobacillus helveticus* LH110 were cultured in MRS broth (De Man *et al*., 1960). These strains were obtained from Chr. Hansen-(Denmark). *Lactobacillus acidophilus* and *Bifidobacterium* spp., were obtained from

Table 1: Some chemical characteristics of milk used for manufacturing of low-fat yoghurt and kariech cheese

	Buffalo and cow's milk 1:1 (PSBCM)	
Chemical characteristics	Yogurt	Cheese-making
Acidity (%)	0.21	0.19
pH	6.52	6.68
Fat (%)	1.50	0.50
Protein (%)	3.70	4.00
Lactose (%)	4.70	4.70

PSBCM: Pasteurized Standardized Buffalo and Cow's Milk

Table 2: Used bacterial strains (EPS-producing, therapeutic and traditional LAB)

Inoculum rate (%)	Type of bacterial culture	Abbreviation
4	4% *S. thermophilus*+*L. delbrueckii* sub sp. *bulgaricus* (traditional) (1:1)	T
4	3% *S. thermophilus* MR-1C and *L. delbrueckii* sub sp. *bulgaricus* MR-1R (1:1) plus 1% *Bifidobacterium* spp. and *L. acidophilus* (1:1)	SB-EPS
4	3% *S. thermophilus* TA061+*Lactobacillus helveticus* LH110 (1:1) plus 1% *Bifidobacterium* spp. and *L. acidophilus* (1:1)	SH-EPS

Laboratorium wiesby, Niebull, Germany. *Bifidobacterium* spp. and *Lactobacillus acidophilus* were separately transferred into sterile skim milk containing 10 g dextrose and 1 g yeast extract/L, then incubation was carried out at 37°C until coagulation while *Bifidobacterium* spp., was incubated anaerobically at 37°C (in all experiments in this study) until coagulation. Further activation was achieved by three similar successive transfers in the same medium (Beena and Prasad, 1997).

Microbiological analysis: Microbiological analysis (total viable bacterial count, lactic acid bacteria, mould and yeast, spore-forming bacteria, psychrotrophic bacteria, *Staphylococcus aureus* and coliform group) were carried out (Vanderzant and Splittstoesser, 1992) using different selective media to enumerate different viable microorganism groups.

Rheological properties analysis: The curd tension (firmness) of yoghurt was determined as described by Chandrashekhara *et al.* (1957). Syneresis (whey separation) was determined using the drainage whey (mL/100 mL yoghurt) as described by Hassan *et al.* (1999). Viscosity was measured using Hoepler viscometer at 20°C according to Zbikowska and Zbikowski (1996).

Texture determination: Texture profile analysis of the kariech cheese samples was carried out by using the Texture Analyzer (CNS-Farnell, England). Cheese samples were cut into cubes 5 cm^3 and kept at 12°C for 1 h before analysis. The probe was TA 15 (45° and 30 mm diameter), at speed 1 mm sec^{-1} and 10 mm distance, using cycle or hold programs. Hardness, cohesiveness, springiness, gumminess and adhesiveness were calculated as described by Szczesniak and Kleyn (1963) and Bourne (1978).

Chemical analysis: All chemical analysis in this study was determined according to AOAC. (2003) except Total Volatile Fatty Acids (TVFAs) was estimated as mL 0.1 N NaOH/10 g cheese, according to Kosikowski (1978). The spectrophotometric method of Vakaleris and Price (1959) was used for measuring tyrosine and tryptophan.

RESULTS AND DISCUSSION

It was necessary to follow the develop acidity during manufacture of low-fat yogurt. Listed data in Table 3 indicated that control sample was the lowest pH. After 4 h, the control, SB-EPS and SH-EPS treatments recorded pH-values 4.68, 4.77 and 4.81, respectively. This may be due to dispersing the energy of EPS-producing LAB between production of EPS and metabolism of lactose and convert it into organic acids.

Rheological properties of low-fat yoghurt: As it is known, rheology of yoghurt associated with its gel structure and composition. Protein content, heat treatment and the presence of milk fat, thickening agents (stabilizers) and bacterial exopolysaccharides are factors with a direct effect on

Table 3: Development of pH-values during manufacture of yoghurt made with different cultures of LAB

	Incubation period (h)			
Samples of fermented milk with different cultures	After inoculation (zero time)	2	3	4
T	6.39	5.20	4.91	4.68
SB-EPS	6.42	5.71	4.95	4.77
SH-EPS	6.44	5.91	4.98	4.81

the structure of the protein matrix of yoghurt. Because of their contribution to enhancement of the consistency and rheology of fermented milk products, EPS-producing lactic acid bacteria are widely used these days. These bacteria impacted on yoghurt rheological attributes (Table 4).

At zero time, the highest mean viscosity was found in SB-EPS treatment (365 mPa.sec) followed by SH-EPS (332 mPa.sec) then control (281 mPa.sec). Viscosity gradually increased along cold storage, respectively. Although the positive relationship between pH and viscosity that appeared from results, viscosity increased with increasing storage, this may be due to the vitality of EPS-producing LAB and produced more of EPS that play a role in the colloidal case. These results are consistent with results of Narayana and Gupta (2013).

Syneresis (whey separation) in yoghurt is an important parameter, on which the quality of yoghurt is evaluated. Data listed in Table 4 indicated that at fresh and along storage, SB-EPS was the lowest syneresis followed by SH-EPS then control that was the greatest syneresis. These results confirmed the effect of EPS-producing cultures. Syneresis reached 17.6, 18.8 and 24.6 mL/100 mL for SB-EPS, SH-EPS treatments and control after 11 days, respectively. The important note was the inverse relationship between viscosity and syneresis, a positive relationship between the increase in viscosity and the high of pH-value thus clearly decreased the syneresis. Two factors may be responsible for those results such as the heighten in EPS concentration, capsule around bacterial cells or liberated in the medium which absorbs more water causing an increase viscosity as well as the reduction the phase separation of milk protein. Jaros *et al.* (2002) affirmed that the use of EPS-producing cultures helps decrease syneresis in set yogurt.

Regarding curd tension of low-fat yoghurt as a rheological parameter, it influenced by used EPS-culture; SB-EPS has achieved the greatest curd tension followed by SH-EPS then the control. From Table 4 curd tension was 34.4, 32.6 and 28.5 g/100 g at zero time and reached 42.2, 39.3 and 36.1 g/100 g after 11 day at 6±2°C, respectively.

Microbial analysis: The results in Table 5 appeared that the presence of EPS-producing LAB in SB-EPS and SH-EPS decreased total viable count against control (T), unlike lactic acid bacteria which slightly increased in SB-EPS and SH-EPS than T. Generally, *L. acidophilus* was more than *Bifidobacterium* spp. Mould and yeast could grow and detected on the seventh day of storage, control was suitable for growing mold and yeast more than SB-EPS and SH-EPS. These results may be due to increase the acidity which paved the conditions to grow these organisms. The antagonism effect of EPS-producing LAB against mold and yeast that can produce many types of mycotoxins, these results important to avoid chemical preservatives. Mihyar *et al.* (1997) needed 300-400 mg sodium benzoate/kg Labnehto limit growth of, mould and yeast. Mould and yeast were lower in SH-EPS than in SB-EPS. These results may be back to ability of *S. thermophilus* TA061

Table 4: Rheological properties of yoghurt samples made using EPS-producing LAB during storage period

	Rheological properties during storage period (days)											
	Curd tension (g/100 g)				Syneresis ratio (mL/100 mL)				Viscosity (mPa.sec)			
Treatments	0	3	7	11	0	3	7	11	0	3	7	11
T	28.5	30.7	33.2	36.1	26.8	25.9	23.8	24.6	281	290	295	299
SB-EPS	34.4	37.5	40.3	42.2	20.1	18.5	18.3	17.6	365	381	421	468
SH-EPS	32.6	34.3	36.2	39.3	21.2	20.5	19.3	18.8	332	351	376	410

Table 5: Some microbial attributes of yoghurt made using EPS-producing LAB

Treatments	S.P	T.V.C	LAB	*Bif*	*L.a*	M and Y
Control (T)	0.0	151	134	-	-	-
	3	157	133	-	-	-
	7	162	145	-	-	11
	11	166	157	-	-	15
SB-EPS	0.0	145	134	12	14	-
	3	149	135	13	15	-
	7	153	146	16	18	3
	11	160	158	21	22	6
SH-EPS	0.0	144	133	11	12	-
	3	146	136	13	15	-
	7	149	147	17	18	2
	11	151	159	20	23	3

S.P: Store period, T.V.C: Total viable count (CFU×10^6/g), LAB: Lactic acid bacteria (CFU×10^6/g), *Bif*: *Bifidobacterium* sp. (CFU×10^6/g), *L.a*: *L. acidophilus* (CFU×10^6/g), M and Y: Mold and Yeast (CFU×10^2/g), Psychrotrophic bacteria, Coliform group, *Staphylococcus aureus* and aerobic spore forming bacteria not detected

Table 6: Effect of using EPS-producing lactic acid bacteria on organoleptic properties of low-fat yoghurt during cold storage period

	Items of organoleptic analysis				
Treatments	Storage period (days)	Appearance (10)	Flavor (60)	Consistency (30)	Total (100)
T	0.0	9	55	26	90
SB-EPS		10	58	29	97
SH-EPS		10	57	27	96
T	3	8	53	25	85
SB-EPS		10	57	29	96
SH-EPS		9	56	26.5	91.5
T	7	7.5	50	24	81.5
SB-EPS		9	55.5	28	92.5
SH-EPS		8	54	25	87
T	11	7.5	47	22	76.5
SB-EPS		9	54	27	90
SH-EPS		8	52	24	84

or *Lactobacillus helveticus* LH110 screening anti-fungi in the medium higher than *S. thermophilus* MR-1C and *L. delbrueckii* sub sp., *bulgaricus* MR-1R. Undesirable bacteria such as *Staphylococcus aureus*, coliform group and psychrotrophic bacteria were not detected in the all treatments whether in fresh or stored yoghurt samples. This might be due to the efficient heat treatment of milk (95°C for 15 min) and high sanitation conditions during manufacture and storage, the obtained results agreed with Ammara (2000).

Sensory attributes: Organoleptically, employing of EPS- producing lactic acid bacteria impacted on acceptability of yoghurt, SB-EPS achieved the greatest points followed by SH-EPS then control at fresh or along storage period (Table 6). SB-EPS and SH-EPS treatments won the highest points may be due to the best rheological and chemical properties of low-fat yoghurt. Further improve the quality appeared in enhancing of appearance, consistency and flavour that recorded 58/60 at fresh and reached 54/60 after 11 days for SB-EPS.

Textural measurements: Sense in terms of the force required to compress the sample between the teeth grinding and the mechanical force necessary to achieve the vision a transformation is specified (Fox *et al*., 2000; Gunasekaran and Mehmet Ak, 2003). Generally, all parameters of textural profile (hardness, cohesiveness, adhesiveness, springiness) and secondary parameters (Gumminess) for control or treatments of Kareish cheese increased along the storage period (Table 7). Hardness affected by EPS-producing LAB, SB-EPS recorded the lowest hardness followed by SH-EPS then control that was more hardness, it's know that the low-fat cheese is more hardness than full-fat cheese. Usage of EPS-producing LAB decreased hardness kariech cheese. This is due to effect that water absorbed with EPS. In regard with other parameters, SB-EPS was the highest followed by SH-EPS then the control which was the lowest in all parameters except hardness. These findings in agreement with Rezazadeh *et al*. (2013) and also Ahmed *et al*. (2005) improved the textural properties of kariech cheese signed off by the acidic extracellular polysaccharides cultures. The water-holding capacity of fermented product increased when it was made with EPS-producing lactic acid strains (Narayana and Gupta, 2013). Observed in this experimental kariech cheese that fragmentation and rough texture disappeared during storage period, as well as the samples were more smoothness.

Chemical analysis of kariech cheese: Chemically, fresh or stored kariech cheese samples were analyzed at intervals 7, 14, 21 and 30 days (Table 8). Regarding yield, it strongly affected using EPS-producing LAB, SB-EPS and SH-EPS outputted the highest yield, 26.3 and 24.1% at fresh, respectively against 18.5% for control (traditional culture). These results confirm influence of EPS-producing culture, because EPS absorb more quantity of water that allows the interaction between EPS and milk proteins. By scanning electron microscopy, the EPS appear as strings attached to the bacteria and to the protein complexes, the bacteria were attached to the protein

Table 7: Rheological attributes of kariech cheese made using EPS-producing lactic acid bacteria during cold storage

Treatments store period (days)	Rheological parameters				
	Hardness (g)	Cohesiveness (ratio)	Adhesiveness (g sec^{-1})	Springiness (mm)	Gumminess (g sec^{-1})
T					
0.0	205	0.81	8.11	6.17	269
7	217	0.86	8.34	6.46	280
14	233	0.92	8.87	6.77	289
21	241	0.98	9.13	7.04	302
30	251	1.04	9.41	7.39	314
SB-EPS					
0.0	181	0.93	8.73	6.61	287
7	192	0.99	9.15	6.95	312
14	201	1.11	9.56	7.43	349
21	209	1.19	9.82	7.81	381
30	219	1.25	10.13	8.27	411
SH-EPS					
0.0	186	0.85	8.23	6.32	278
7	197	0.91	8.56	6.63	303
14	206	0.98	8.93	6.96	331
21	217	1.04	9.22	7.21	354
30	225	1.12	9.53	7.55	381

Table 8: Yield and some chemical analysis of fresh and stored kariech cheese made from PSBCM with different strains of LAB (traditional and EPS-producing strains)

	Storage period (days)				
Treatments and test	Fresh	7	14	21	30
Control (T)					
Yield (%)	18.50	18.20	17.90	17.70	17.20
Moisture (%)	71.11	70.91	70.43	70.06	69.02
Ash	1.31	1.43	1.56	1.73	1.81
Total solids	26.82	27.12	27.83	27.75	27.91
Titratableacidity (TA)	1.18	1.24	1.35	1.43	1.51
Acetaldehyde	270.00	298.00	316.00	230.00	216.00
Diacetyl	117.00	131.00	135.00	126.00	123.00
Lactose	2.60	2.30	2.00	1.80	1.40
Fat (%)	1.10	1.00	1.10	1.10	1.10
Tyrosine	15.80	25.50	33.30	35.60	44.80
Tryptophan	73.70	81.30	90.60	95.80	98.50
Total volatile	0.36	0.39	0.41	0.44	0.45
SB-EPS					
Yield (%)	26.30	26.10	25.90	25.70	25.50
Moisture (%)	73.96	73.81	73.03	72.75	72.41
Ash	1.47	1.85	2.11	2.25	2.32
Total solids	27.51	28.98	29.67	29.93	30.11
Titratable acidity	1.03	1.11	1.19	1.25	1.36
Acetaldehyde	286.00	313.00	332.00	247.00	231.00
Diacetyl	132.00	143.00	148.00	142.00	134.00
Lactose	2.90	2.50	2.30	2.01	1.80
Fat (%)	0.90	0.90	1.00	1.00	0.90
Tyrosine	11.20	18.80	22.80	29.20	34.30
Tryptophan	62.90	71.50	80.60	85.60	86.50
Total volatile	0.42	0.44	0.47	0.49	0.51
SH-EPS					
Yield (%)	24.10	23.80	23.50	23.20	22.90
Moisture (%)	72.85	72.34	72.02	71.69	71.13
Ash	1.37	1.69	2.05	2.23	2.27
Total solids	26.89	27.32	27.88	28.25	28.68
Titratable acidity (TA)	1.07	1.16	1.23	1.31	1.42
Acetaldehyde	279.00	307.00	326.00	242.00	227.00
Diacetyl	128.00	139.00	145.00	138.00	131.00
Lactose	2.80	2.40	2.10	1.90	1.70
Fat (%)	0.90	0.90	1.00	1.00	0.90
Tyrosine	13.20	20.80	24.80	31.20	36.30
Tryptophan	65.90	72.80	82.90	87.80	90.60
Total volatile	0.40	0.42	0.45	0.48	0.51

network through filamentous strands of the polysaccharide emerging from them. These strings or filaments were well attached to the protein aggregates. The EPS strings are intertwined in the protein matrix. The EPS molecules in their hydrated state may occupy much more volume. The background shows partial coverage by milk proteins attached to Self-Assembled Monolayers (SAM) (Ayala-Hernandez *et al*., 2008).

From previous information, naturally, the moisture increases in treatments SB-EPS and SH-EPS more than control but SB-EPS caused the highest moisture (73.96%) declined to 72.41% at the end of storage period against 69.02% for control.

From the same table, generally, TS increased with along storage period, it reached 30.11, 28.68 and 27.91% after thirty days for SB-EPS, SH-EPS and control, respectively. Ash analysis took the same trend, SB-EPS and SH-EPS treatments recorded 2.32 and 2.27 against 1.81 for control after 30 day and these results were expected due to EPS produced from SB-EPS and SH-EPS cultures. SB-EPS treatment was mild acidity (1.36%) but acidity of SH-EPS was middle (1.42%), these results may be due to the presence of *Bifidobacterium* sp. and *L. acidophilus* in that starter at ratio 1% or to slow the production of acidity by EPS-producing bacteria. At contrast, the control was the greatest acidity (1.51%) after storage month.

Production of flavour compounds (Acetaldehyde and Diacetyl) depends on the activity of type used starter and circumstances of fermentation. Table 8 indicates that the content samples of acetaldehyde and diacetyl influenced by the type of bacterial strains used. Control yielded the lowest content of these compounds contrary to SB-EPS treatment which yielded the greatest acetaldehyde and diacetyl either in fresh or stored samples followed by SH-EPS treatment. These findings due to a mixture of bacteria used, although these compounds decreased slightly with the low level of fat, perhaps the low level of fat is responsible for the low level of flavour compounds (Acetaldehyde and Diacetyl). These results in agreement with Amer *et al.* (1998) and Abou Ayana and Gamal El Deen (2011).

Results in the same Table 8 show clearly that the lactose content reflected the culture activity; the changes in acidity depend on the changes in lactose which plays a great role. Control had the lowest lactose content compared with the other two treatments; all fresh samples contained 2.6-2.9% lactose, this quantity gradually decreased to 1.4-1.8 after 11 days.

There is a positive relationship between fat and moisture and texture of cheese, the decrease of kariech cheese fat affects on its texture. Using EPS-producing LAB increased moisture content which offset the impact of fat lack. Thus a feeling of fatty taste appeared in the treatments. Fat ratio in all samples was nearly stable, fat ranged between 0.9-1%, this agree with Trancoso-Reyes *et al.* (2014) who reported that about 10% of milk fat has lost in whey.

Tyrosine and tryptophan content: Initially, estimation of amino acids, tyrosine and tryptophan is taken as an indicator on the efficiency of bacterial strains in proteolysis, thus the emergence of abnormal and unacceptable tastes. It is clear from the results in Table 8 that tyrosine and tryptophan content in all samples gradually increased as the age of the cheese produced. The tyrosine contents of control cheese ranged from 15.8-44.8 mg/100 g against 11.2-34.3 and 13.2-36.3 mg/100 g for SB-EPS and SH-EPS treatments, respectively. Regarding tryptophan, control sample recorded 98.5 mg/100 g and decreased to 86.5 and 90.6 mg/100 g for SB-EPS and SH-EPS treatments after a month store, respectively.

Noticeable, tyrosine and tryptophan clearly increased in the presence of traditional culture, control had the highest level of these amino acids which is considered proof of the proteolytic activity of the strains used. This may be due to increase the proteolytic activity traditional culture more than EPS-producing LAB these findings confirmed with Chervaux *et al.* (2000).

CONCLUSION

From the present study, it could be concluded that using EPS-producing lactic acid bacteria clearly improved the attributes of low-fat yoghurt and kariech cheese, particularly the rheological

properties as well as the yield of kariech cheese increased, increasing of moisture content offset the impact of fat lack. Thus a feeling of fatty taste appeared in the treatments. Thus providing functional cheese which is useful for diabetics and heart diseases, obesity and diet system with the benefit of good economic returns.

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