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PHYSICO-CHEMICAL AND ORGANOLEPTIC PROPERTIES OF LOW FAT YOGHURT MADE WITH MICROBIAL EXOPOLYSACCHARIDE PRODUCED FROM SALTED WHEY

Ali, A. A.; M. A. A. Azzam; A. M. Metwally and A. A. Awad
Dairy Dept., Fac. Of Agric. Cairo Univ. Giza, Egypt.

ABSTRACT

Yoghurt samples of high fat (3.1%), low fat (1.5%) and low fat mixed with 35, 70 and 100 mg of exopolysaccharide /liter of milk were prepared and stored for 10 days at 5ºC. EPS were prepared in the lab by growing *Halomonas eurihalina* F_{2-7} & *Xanthomonas campestris pv. campestris* in salted whey and was compared with commercial xanthan gum.

Supplementation with EPS significantly increased rate of acid development thus shortened the period of coagulation, enhanced growth of lactic acid bacteria, lowered curd tension and whey separation and increased apparent viscosity and greatly helped the sensory attributes of low fat yoghurt. The degree of improvement was dependent on type of the EPS and the concentration. Therefore, it is important to select the proper EPS and the concentration for each product and the property requested to affect.

Keywords: low fat yoghurt, EPS, Syneresis, curd tension, viscosity.

INTRODUCTION

Low fat yoghurt (LFY) suffers low organoleptic properties and exhibits whey separation (Harwalker and Kalab, 1986). Stabilizers and fat replacers are usually used to improve low fat yoghurt (Hess *et al.,* 1997). However, stabilizers can adversely affect the true yoghurt taste, aroma and mouthfeel. So, an alternative way to improve yoghurt texture and stability is the use of bacteria that produce exopolysaccharides (EPS) (Hassan *et al.*1996) or addition of exopolysaccharides powder as bio-ingredients in yoghurt making (Doleyres *et al.* 2005).

Extracelluler polysaccharides are produced by sevral number of bacteria such as *Xanthomonas campestris* (Evans *et al.,*1979), *Pseudomonas* (Jarman, 1979), Alcaligenes (Sutherlaland, 1990), *Halomonas eurihalina* (Bejar *et al.,*1996), etc. These polymers may be assembled as capsular polysaccharides that are tightly associated with cell surface, or they may be liberated in the growth medium (i.e. ropy polysaccharide).

Xanthan gum, is an exopolysaccharides synthesized by Xanthomonas campestris pv. Campestris which is one of the major commercial biopolymers produced with an annual worldwide production of 30 000 tons (Sutherland 1998 and Demain, 2000). Because of its superior rheological properties, xanthan gum has been available as bio-ingredient for application in the food industry, where, it is used as a rheological control agent in aqueous systems and as stabilizer for emulsions and suspension (Yoshida and Tanner, 1993).

Exopolysaccharide produced by a marine bacterium Halomonas eurihalina, increases the viscosity of solution at low pH values and acts as emulsifying hydrocarbons (Calvo *et al*., 1995). This property would make it

valuable for use in the food industry as an additive in salad sauces or citric desserts, where the pH is usually acidic.

Because EPS can reduce syneresis and enhance product texture and viscosity, these substances are used as a substitute for commercial stabilizers of plant or animal origin, in fermented dairy products manufacture (Cerning, 1992). Therefore, yoghurt is the most important commercial application for EPS in dairy foods (De vuyst and Degest, 1999).

In our previous work (Ali *et al.*, 2007), EPS was produced by Halomonas eurihalina F2-7 and Xanthomonas campestris pv. campestris which were grown in salted Domiati cheese whey since these microorganisms can tolerate high salt.

Therefore, the objective of the present work was to use the prepared EPS in the manufacture of low-fat yoghurt hoping to improve the low quality of low fat yoghurt. Different levels of EPS were added and physical, chemical and organoleptic properties were followed. The prepared EPS was compared with the commercial xanthan.

MATERIALS AND METHODS

Fresh cows' milk (4.2% fat, 3.6% protein) was obtained from the faculty of agriculture herd, Cairo University, was standardized, using a cream separator (Kamdhenu, Sinhal Metal Industrial, PVI. LTD. India), into 3.1% fat (control full fat yoghurt) (FFY) and 1.55 % fat (low fat yoghurts) (Table, 1), milk was fortified at 40°C with commercial low-heat nonfat dry milk (T.S 96% & fat 1.25%) (Volio Co. Helsinki- Finland) to increase milk to total solids to 15 %. Low fat milk (1.55% fat) was fortified with two type of EPS prepared from Halomonas eurihalina F2-7 and Xanthomonas campestris pv. campestris (Ali et al., 2007) as well as commercial xanthan gum® (Ketrol-F, CP, Kelco, Danish). Each substance was added to milk at 3 levels; 35, 70 and 100 mg/L. Milk was heated at 90°C /15min., cooled to 42°C and inoculated with 2.5% yoghurt culture, streptococcus thermophilus and Lctobacillus delprueckii ssp. bulgaricus YC, X-11, YO-Flex® (1:1 by volumes)(CHR-Hansen, Danish). Then transferred into 125 g plastic cups, incubated at 42°C. When yoghurt coagulum pH attained ~4.7, cups were removed from incubator and placed in cold storage at 5°C for10 days.

Table (1): Yoghurt milk composition, (%)

Milk	TS	T. protein	Fat	Ash	Carbohydrate
Full fat	15.02	5.75	3.10	0.95	5.22
Low fat	15.32	6.60	1.55	0.98	6.17

Yoghurt samples were analyzed for total solids, fat, titratable acidity and pH (ling, 1963), total carbohydrates (Barrnet & Abd El-Twab, 1957), total protein and ash (AOAC, 1990) and acetaldehyde (Lees & Jago, 1969). Physical properties of yoghurt samples were also determined by measuring of viscosity (Trachoo & Mistry, 1998), curd tension (Abd El-Fatah, 1994) whey separation (Hassan *et al.*, 1996) and water holding capacity (Parnell Clunies *et al*., 1986). For viscosity, Brookfield viscometer LTV with spindle RV4 was used. Yoghurt samples were also microbiologically analyzed for lactic acid

bacteria (LAB) Coliform and molds & yeasts counts according to Atlas (2004), Harrigan and McConkey (1996) and APHA, (1994), respectively. The organoleptic properties of yoghurt samples were assessed for flavor, body & texture and appearance according to the scheme described by El-Shibiny et al. (1979). Analysis of yoghurt samples was performed when fresh and at 5°Cand 10 days of cold storage (5°C).

The two-way analysis of variance (ANOVA) was performed by running the MSTAT-C (ver.2.10, MSU, USA) Package on a personal computer. The same program was used to analyze two and three Factor factorial Randomized Complete Block Design. The statistical significance of the data was determined by using P value at $\alpha = 0.001$. Linear regressions were used to correlate the measured parameters.

RESULTS AND DISCUSSION

1 -Titratable acidity (TA) and pH values:

Table (2) presents the development of TA & pH during coagula formation. Rate of acidity development was almost similar in both full fat and low fat controls. Low fat yoghurt fortified with prepared EPS or xanthan gum developed acidity at a faster rate particularly at the 100 mg/L concentration. Acidity content on milk coagulation was higher in the fortified yoghurt and the difference was significant ($p< 0.01$) at the 100 mg/L level. pH development followed the acidity development trend. The fast rate of acidity development was manifested in faster rate of coagulation, thus the 100 mg/L level, coagulated in shorter period than the control.

Table (3) shows the acidity and pH development of yoghurt during cold storage. All yoghurts continued to develop acidity at a lower rate with no significant effect of the EPS fortification. These results are consisted with those of Salah (2000), Abd El-Salam et al. (1996) and Tamime and Deeth (1980).

3-Curd tension

As shown in Table (4), fresh coagula strength markedly influenced by reducing fat content. Curd tension was increased from 40.8 to 48.1 gram, by reducing the fat percentage from 3.1% to 1.55%. The lower curd tension in the high fat yoghurt may be attributed to presence of more fat globules between the micelles network thus reducing the number of bounds. White, (1995) noticed that increasing milk protein content resulted in increase of the level of bound water (water of hydrated proteins) in yoghurt coagulum leading to firm and viscous yoghurts. The presence of EPS reduced the curd tension of low fat yoghurt and the reduction was concentration dependent. The curd tension of low fat control was reduced from 48.1gm. to 33.9 with the lowest EPS concentration and to reach 21.2 g with 100 mg/L EPS concentration. Amataykul *et al.*(2006) and Puvanenthiran *et al.* (2002) and Hassan *et al.* (1996) reported the same effect of EPS on curd tension. The incompatibility between EPS and milk protein maybe also an explanation (Dekruif & Tuinier, 2001; Abd El-Salam *et al.*, 1996, Taggatz and Morris, 1990). By storage, curd tension values increased gradually to reach 12 & 9% in full & low fat control, but the increase was slight (2-4%) in EPS fortified yoghurt. This decrease

may be due to the ability of the EPS to binned the water and prevent synersis of the coagulum (Amataykul *et al.,*2006 and Puvanenthiran *et al.,* 2002).

Table (2): pH and T.A of full and low fat yoghurt made with EPS during coagulum formation.

Treatment	EPS (mg/L)	Coagulation period (42°C/ hrs.)								C.T.*
		T.A				pH				
		Onset	1	2	Co.**	Onset	1	2	Co.**	(hr.)
Full fat yoghurt (FFY)										
Control	**0**	0.17	0.33	0.62	0.79	6.68	6.3	5.1	4.74	3.14
Low fat yoghurt (LFY)										
Control	**0**	0.19	0.37	0.66	0.80	6.67	6.1	5.7	4.88	3.10
	35	0.18	0.54	0.68	0.82	6.65	5.82	5.1	4.67	2.45
Com.[a]-X	**70**	0.18	0.52	0.77	0.86	6.65	5.81	4.81	4.65	2.42
	100	0.19	0.52	0.69	0.91	6.66	5.81	4.83	4.63	2.40
	35	0.19	0.55	0.65	0.80	6.63	5.80	5.2	4.68	2.47
X[b]–EPS	**70**	0.19	0.50	0.66	0.85	6.67	5.81	5.2	4.58	2.45
	100	0.19	0.51	0.74	0.88	6.67	5.80	4.93	4.58	2.43
	35	0.19	0.56	0.63	0.83	6.65	5.78	4.9	4.65	2.44
H[C]–EPS	**70**	0.18	0.57	0.70	0.88	6.63	5.80	4.87	4.65	2.42
	100	0.19	0.53	0.71	0.90	6.63	5.72	4.89	4.63	2.38
	LSD= 0.1534					**LSD= 0.2937**				

***C.T.= Coagulation time** **Co.** = Coagulation**
[b]X- EPS= Lab. Xanthomonas EPS **[c]H- EPS= Lab. Halomonas EPS**
[a]Com.-X=Commercial Xanthan gum

Table (3): pH and T.A development of full and low fat yoghurt made with EPS during cold storage (5ºC)

Treatment	EPS (mg/L)	TA			pH		
		Storage period, days at 5ºC					
		Fresh	5	10	Fresh	5	10
Full fat yoghurt (FFY)							
Control	**0**	0.82	0.92	1.00	4.70	4.56	4.48
Low fat yoghurt (LFY)							
Control	**0**	0.88	0.96	1.04	4.87	4.62	4.52
	35	0.97	1.0	1.18	4.47	4.52	4.37
Com.[a]-X	**70**	1.04	1.04	1.2	4.52	4.52	4.37
	100	1.08	1.1	1.21	4.62	4.47	4.35
	35	0.91	1.0	1.12	4.66	4.50	4.41
X [b]–EPS	**70**	1.02	1.14	1.18	4.52	4.45	4.39
	100	1.02	1.14	1.2	4.51	4.43	4.35
	35	0.98	0.99	1.08	4.57	4.53	4.43
H[C] –EPS	**70**	1.0	1.1	1.17	4.56	4.42	4.38
	100	1.02	1.12	1.2	4.64	4.41	4.36
	LSD= 0.2295				**LSD= 0.7275**		

[a]Com.-X= Commercial Xanthan gum **[b]X- EPS= Lab. Xanthomonas EPS**
[c] H- EPS= Lab. Halomonas EPS

Table (4): Influence of EPS and refrigerated storage on curd tension (g) of low fat yoghurt.

Treatment	EPS (mg/L)	Storage period, days at 5°C		
		Fresh	5	10
		Full fat yoghurt (FFY)		
Control	0	40.83	45.64	45.97
		Low fat yoghurt (LFY)		
Control	0	48.11	51.01	51.88
Com.[a]-X	35	33.97	34.11	34.71
	70	32.66	32.90	33.00
	100	26.19	26.81	26.93
X[b]–EPS	35	26.30	26.61	26.97
	70	25.28	25.88	26.18
	100	21.24	22.00	22.13
H[C]–EPS	35	25.01	25.59	25.61
	70	24.82	25.11	25.77
	100	23.97	24.25	24.98

LSD = 9.353

[a]Comm.-X= Commercial Xanthan gum [b]H- EPS= Lab. Halomonas EPS
[b]X- EPS= Lab. Xanthomonas EPS

4- Susceptibility to syneresis:

The levels of syneresis reported in Table (5) show that reducing fat content significantly, reduced yoghurt syneresis, either fresh or during 10 days of cold storage.

Table (5): Synersis of low-fat yoghurt fortified with EPS during cold storage.

Treatments	EPS (mg/L)	Storage period, days at 5°C		
		Fresh	5	10
		Synersis, ml whey/ 100 ml yoghurt		
Full-fat control (3.1% fat)	0	16.82	17.22	17.49
Low-fat control (1.6 % fat)	0	14.33	14.87	14.91
Low fat yoghurt mixed with EPS:				
Com.[a]-X	35	13.17	13.36	13.37
	70	11.23	11.38	11.44
	100	10.42	10.70	11.01
X [b]–EPS	35	13.90	13.94	13.96
	70	13.26	13.38	13.45
	100	13.21	13.30	13.35
H[C] –EPS	35	10.84	11.37	11.40
	70	10.30	10.64	10.67
	100	10.08	10.16	10.24

LSD = 2.728

[a]Com.-X= Commercial Xanthan gum [b]X- EPS= Lab-Xanthomonas EPS
[b]H- EPS= Lab-Halomonas EPS

This may be attributed to the increase in protein matrix strength (higher compactness) of yoghurt which conserves more water, consequently reducing the syneresis of yoghurt coagulum (Puvanenthiran, *et al.* 2002; Harwalker and Kalab, 1986). The presence of the EPS significantly reduced low fat yoghurt synersis particularly the commercial Xanthan which showed significant reduction effect than the other EPS.

This reduction effect may be du to the high water binding capacity of EPS (De vest and Degeest, 1999, Cerning, 1990) which reached its maximum in acidic products, such as yoghurt (Bejar, *et al.* 1998). In addition, EPS present in yoghurt coagulum is associated with large pores of protein network resulting in density aggregated protein and adsorption increase of water and viscosity of yoghurt (Amatayakul *et al.,* 2006).

At the end of storage, the levels of synersis slightly increased for all yoghurts, regardless of type and concentration of EPS. These results agreed with those reported by EL-Sayed *et al.* (2002). Guinee *et al.* (1994) and Foley & Mulcahy (1989).

5-Water-Holding Capacity (WHC)

Table (6) presents data of the water-holding capacity (WHC) of all yoghurts studied. LFY coagulum exhibited higher ability to bind the water compared to that of FFY coagulum when fresh or throughout 10 days cold storage. This may be due to the higher ability of the protein bind water (Trachoo & Mistry, 1998; White, 1995). The EPS addition to LFY milk increased significantly the WHC of fresh and stored product coagulum, reach the maximum for LFY coagulum made with H-EPS (100 mg/L) (48.35 & 49.88% for fresh and stored yoghurt). On the other hand, the WHC of yoghurts increased during storage at 5°C. This increase agree with those reported that EPS producing cultures and/or the presence of EPS channels in the coagulum serum improved the WHC of yoghurt (Amataayakul et al.,2006, Doleyres *et al*.,2005 and Hassan *et al.*,1996).

6- Apparent viscosity (AV):

Values of AV were significantly affected ($P< 0.001$) by the EPS type & concentration and the period of storage while no significant interaction between those factors was found. As shown in Fig (1), Low fat yoghurt exhibited lower AV values compared to that of full fat yoghurt when fresh or throughout 10 days storage. This may be due to the effect of fat. Because of EPS interacts with yoghurt free water forming a gel-like structure, the LFY made with EPS had the highest viscosity which in agreement with others (Hassan *et al.,* 2003 , Dave and Degeest, 1999). The viscosity of LFY coagulum increased with EPS concentration and xanthan was the most effective than the other EPS.

Yoghurt viscosity slightly decreased after 5 days of cold storage then followed by a significant increase on the 10 days of storage. However, cold storage did not change the relative effect of fat & EPS on viscosity. The increase in the AV values may be du to the hydration of casein complexing with EPS (Dave and Shah, 1998), and as a result of rearrangement of protein under the acidic conditions (Hassan *et al.*, 1995) or by storage (Ozer *et al.*, 1998). These results agree with those of Doleyres *et al.* (2005), El-Sayed *et al.* (2002), Foly and Mulcahy (1989), and Guven (1998).

Table (6) : Influence of EPS and refrigerated storage on water-holding capacity (WHC) (% w/w) of low-fat yoghurt.

Treatments	EPS (mg/L)	Storage period, days at 5°C		
		Fresh	5	10
		Full fat yoghurt (FFY)		
Control	0	59.22	58.70	58.1
		Low fat yoghurts (LFY)		
Control	0	55.35	54.97	54.55
Com.[a]-X	35	53.30	53.18	53.15
	70	53.70	53.40	53.11
	100	52.95	52.07	51.81
X [b]–EPS	35	54.44	53.07	53.10
	70	54.18	53.15	53.15
	100	53.55	52.87	52.65
H[C] –EPS	35	52.77	52.02	51.85
	70	52.32	51.55	51.37
	100	51.65	50.62	50.12

LSD = 7.640

[a]Com.-X= Commercial Xanthan gum
[b]X- EPS= Lab-Xanthomonas EPS
[b]H- EPS= Lab-Halomonas EPS

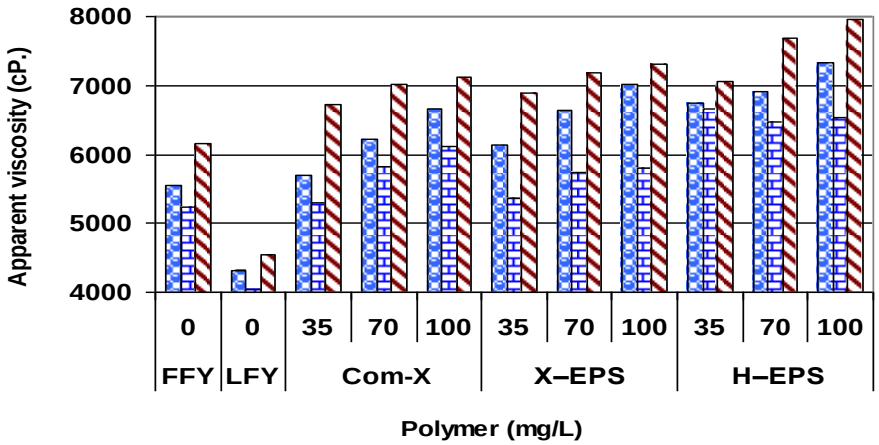

Fig (1) : Apparent viscosity of fresh , 5 days/ 5°C and 10 days / 5°C stored yoghurt made with EPS.

7- Lactic acid bacteria (LAB) viable counts:

As illustrated in Fig (2), the presence of the EPS in yoghurt enhanced LAB growth during coagulum formation, consequently fresh yoghurt contained higher counts than non-fortified yoghurts. Type of EPS and the concentration affected rate of growth and final counts. As expected, the development of high acidity caused a detrimental effect on LAB viability, thus their counts decreased on storage but still after the 10 days of storage, had

higher viable cells than the count requested for functional yoghurt. The higher counts accompanied with EPS may attributed to the activating effect of EPS on *Lb. delberuekii ssp. bulgaricus* (Amataykul *et al.,*2006) and available nutrients associated with EPS such as fine whey proteins which may partially influence the growth of yoghurt starter organism (Dave and Shah, 1998). These results agreed with those of El-Sayed *et al.* (2002) and Gould (1991).

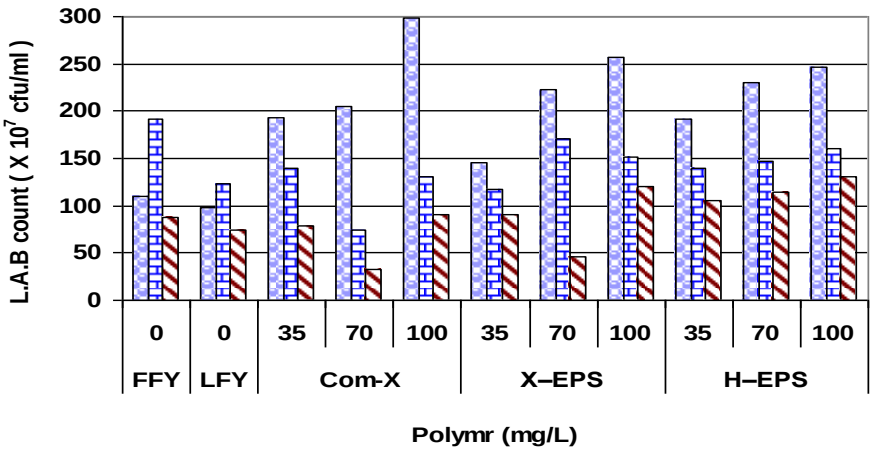

Fig (2): Lactic acid bacteria count of fresh and stored yoghurt for, 5 days and 10 days of cold storage at 5ºC made with EPS.

8- Acetaldehyde:

Fig (3) shows acetaldehyde contents of yoghurts when fresh and throughout storage period. The effect of EPS on acetaldehyde production was variable according to the type and concentration.

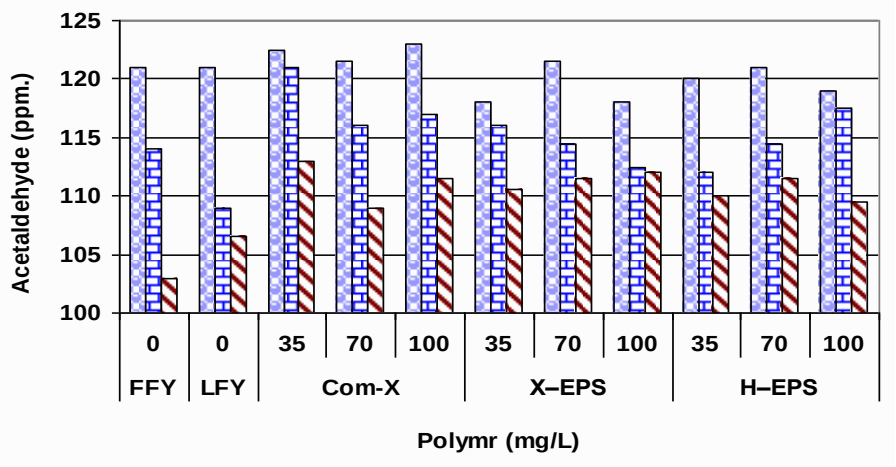

Fig (3): Acetaldehyde of fresh , 5 days and 10 days of cold storage at 5ºC of yoghurts made with EPS.

The high EPS concentration (70 & 100 mg/L) showed enhancing effect on acetaldehyde production particularly Comm-Xanthan type on fresh yoghurt. During storage, acetaldehyde gradually decreased in all yoghurts. This decrease may be due to the ability of LAB to produce alcohol dehydrogenase, that convert acetaldehyde to ethanol (Bills and Day, 1966). These results are in accordance with those reported by Estevez *et al.*, (1998); Abou-Dawood *et al.*, (1993) and Vasvada and White, (1979).

9- Sensory evaluation:

According to Table (7), low-fat yoghurt with or without different EPS matched the sensory evaluation of high fat yoghurt.

Table (7): Sensory attributes scores of fresh and stored (5°C/ 10 days) yoghurts made with EPS

Treatments	EPS (mg/L)	Flavor (60)	Body & Texture (30)	Appearance (10)	Total (100)
			Fresh		
[a]**FFY**	**0**	57.5	29	9.5	96
[b]**LFY**	**0**	52.5	18.5	8	79
	35	55	21	8	84
[c]**Com. -X**	**70**	54.5	21	8.5	84
	100	54.5	19	8	81.5
	35	54	22.5	9	85.5
[d] **X–EPS**	**70**	55	22	9.5	86.5
	100	55.5	21.5	9	86
	35	57	27	9.5	93.5
[e]**H–EPS**	**70**	57	26.5	9.5	93
	100	57.5	27	9.5	94
			5 days		
[a]**FFY**	**0**	58	29	9	96
[b]**LFY**	**0**	52	17.5	8	77.5
	35	55.5	19.5	8	83
[c]**Com. -X**	**70**	54.5	21	8	83.5
	100	52.5	20.5	8	81
	35	54.5	21.5	9	85
[d] **X–EPS**	**70**	55.5	20.5	9.5	85.5
	100	54	21.5	9.5	85
	35	57	27	9	93
[e]**H–EPS**	**70**	56.5	27	9.5	93
	100	57.5	27	8.5	93
			10 days		
[a]**FFY**	**0**	57.5	28	9	94.5
[b]**LFY**	**0**	51	18	8	77
	35	55	20	8	83
[c]**Com. -X**	**70**	54	20	8	82
	100	52	21	8	81
	35	54	21	9	84
[d] **X–EPS**	**70**	56	20	9	85
	100	53	22	9	84
	35	57	27	9	93
[e]**H–EPS**	**70**	56	27	9	92
	100	56.5	27	9	92.5
LSD		**3.429**	**2.345**	**0.4383**	**0.6529**

[a] FFY= Full-fat yoghurt **[b] LFY= Low-fat yoghurt**
[c] Comm-X= Commercial Xanthan gum. **[d] X-EPS= Lab- Xanthomonas EPS**
[e] H-EPS= Lab-Halomonas EPS

However, EPS addition improved low-fat yoghurt organoleptically with varying degrees according to type. Prepared H-EPS was the most effective in improving the organoleptic low-fat yoghurt giving scores (94) somewhat close to high fat yoghurt (96). This effect of H-EPS presented during storage ending with 94.5 and 92.5 total for full fat and H-EPS-low fat yoghurt after 10 days of storage.

Conclusion

The use of EPS in low-fat yoghurt manufacture resulted in change and in some improvement in physical, viable lactic acid bacteria and organoleptic properties of the product. However, the improvement depends on type and concentration of the EPS. Therefore, it is important to select the proper EPS and the concentration for each product. EPS prepared from *H. eurihalina* F_{2-7} and *X. campestris pv. campestris* which grow in salted whey proved effective in improving low fat product.

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الخـواص الفيزيوكيمائيـة و الحسـية للزبـادي مـنخفض الـدهن المصـنع باسـتخدام السكريات العديدة المنتجه ميكروبيا من بيئة الشرش المملح

عبد الرحمن عبد العاطي علي، محمد أحمد عبد الخالق عزام، أحمد محمد السيد متولي و عوض عبد الرحمن عوض

قسم الألبان – كلية الزراعة – جامعة القاهرة – الجيزة - مصر

جهـزت عينـات الزبـادي كاملـة الدسـم (٣,١% دهـن) ومنخفضـة الدسـم (١,٥% دهـن) وأخـري منخفضـة الدسـم مدعمـة بـسكريات عديـدة (٣٥, ٧٠, ١٠٠ ملجـم / لتـر) منتجـه معمليـا بواسطة بكتريـا *Halomonas eurihalina* F_{2-7} & *Xanthomonas campestris pv. Campestris* منماة على الشرش المملح. وعينات زبـادي منخفض الدسم مدعمـه بصـمغ الزانثـان التجاري بنفس التركيزات السابقة للمقارنة .

وقد لوحظ أن التدعيم بالسكريات العديدة Halomonas EPS و Xanthomonas EPS أدي إلى زيادة تقدم الحموضة وأعداد بكتريا البادئ زيادة معنوية مع قصر مدة التخثر. كما أدى إلى زيادة لزوجة الزبـادي وانخفاض جذبـه الخثـري ومعدل انفصـال الشرش منـه علاوة علـى تحسن خواصه الحسيه كثيرا وذلك عند مقارنته بالزبادي كامل الدسم.إلا أن درجه التحسن هذه حددها نوع وتركيز السكر العديد المستخدم وبالتـالي يجب تحديد نـوع وتركيز السكر المناسب لكل منتج للحصول على الخواص المرجوة.