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Exopolysaccharide from *Lactobacillus pentosus* Strain H2 and Its Impact on Rheological Properties and the Sensory Evaluation of Low Fat Yoghurt and UF-Soft Cheese

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Abstract: In this study, a total of 46 exopolysaccarides (EPS) producers were isolated from different Egyptian fermented milk products. Among these isolates, strain H2 was characterized and identified as *Lactobacillus pentosus* with similarity 98% based on 16S rRNA gene sequencing and phenotypic characterization. The maximum EPS secretion and cell biomass of strain H2 were 2.22 and 2.64 (g/L) at 30^{o}C after 72 and 96 h, respectively. Among eight sugars tested for EPS production, it was noticed that all industrial by-products tested were significantly increased the secretion of EPS from strain H2. The highest EPS amount was recorded for molasses and date debs permeate followed by lactose and glucose which were 4.54, 3.08, 2.86, 2.68 and 2.56 (g/l) respectively. Electrospray Ionization Mass Spectrometry (ESI MS) indicated that EPS of strain H2 was heteropolymer and consists of D-glucose, D-glucuronic acid and L-rhamnose. Different concentration of the purified EPS isolated from *Lactobacillus pentosus strain* H2 were applied to low fat UF-soft cheese and yoghurt. The results of these experiments indicated the purified EPS of strain H2 significantly improved the organoleptic properties including texture, flavors and mouth feel of both low fat cheese and yoghurt. Furthermore, all parameters of rheological properties of both products including firmness, cohesiveness, gumminess, chewiness, springiness were significantly improved as the purified EPS concentrations increased. The best sensory evaluation score was recorded for low fat cheese and yoghurt fortified with EPS 0.4 and 0.8% which were 94.90 and 95.60, respectively. Finally, the problems like bitterness, low viscosity, high syneresis formation and defects of consistency which are frequently encountered of fermented milk products can be solved by using purified EPS.

Keywords: Lactic Acid Bacteria, Exopolysaccharids, Low Fat Cheese and Yoghurt, Organoleptic, Rheological Properties

1. Introduction

Low fat yoghurt and cheese has gained popularity in recent years because of increasing demand for low-calorie products, super priority in health and economy aspects [1]. In spite of such popularity, but these products always suffers from weak body and poor texture. This is due to the removal of milk fat and low total solids of Low fat yoghurt and cheese. Consequently, Low fat yoghurt and soft cheese always exhibits some defects in the body and texture of the final product. In addition, milk fat helps to give smooth and rich mouth-feel, so it has a direct bearing on these products [2]. Some of the common methods adopted by manufacturers to avoid this problem have been to increase the level of non-fat milk solids or add animal and plant polymers, proteins, synthetic or other natural stabilizers. Exopolysaccharides (EPS) synthesized by lactic acid bacteria (LAB) play a major role in the manufacturing of fermented dairy products such as yoghurt,, cheese, fermented cream, milk based desserts. One of the major sensory attributes important for consumer preference of dairy products is firmness and creaminess. EPS's may act both as texturizers and stabilizers, firstly increasing the viscosity of a final product, and secondly by binding hydration water and interacting with other milk constituents, such as proteins and micelles, to strengthen the rigidity of the casein network [3, 4, 5, 6, 7]. In addition to, EPSs produced by LAB are thought to have beneficial effects on human health such as immunomodulators, and antitumor,

prebiotic effects and cholesterol-lowering ability. Furthermore it has been reported that EPS can positively affect gut health [8, 9, 10, 11]. The objectives of the present work are: (i) to screen for the most producing EPS producing lactic acid bacteria, (ii) identify the most promising strain based on phenotypic and 16S rRNA gene sequencing, (iii) optimize the culture conditions for growth and EPS production, (iv) studying the impact of the purified EPS produced from the most promising strain on the quality properties of low fat yoghurt and UF-soft cheese.

2. Material and Methods

2.1. Isolation and Screening of Polysaccharide Producing Strains

The polysaccharide lactic acid producing strains were isolated from different fermented butter milk and Kareish cheese. Serial dilutions of the enrichment cultures were incubated at 35 °C and spread on MRS agar plates. Exopolysaccharide producing isolates were detected by formation slimy colonies. The purity of the cultures was continuously controlled microscopically as well as by streaking on MRS agar to get single colonies. All strains were preserved at –70°C after mixing with sterile glycerol or DMSO at a final concentration of 20% and 7.5% (v/v), respectively. The most promising top three Gram-positive strains, H1, H2 and H4 were selected for detailed taxonomic studies.

2.2. Identification of EPS Producing Isolates

Morphological characterization for the top EPS isolates (H1, H2 and H4) was carried out according to Gerhardt *et al.* [12] and the biochemical characterization was determined by API 50 CHL (bioMe ´rieux, l'Etoile, France). The 16S rRNA gene was were sequenced using ABI 3730xl automated DNA sequencer (Applied biosystems USA) through Lab Biotechnology Company, Egypt. The 16S rDNA sequences were initially analyzed by using the program Blast (National Center Biotechnology Information, http//:www.ncbi.nml.nih.gov). The consensus sequence from the isolates and sequences of strains belonging to the same phylogenetic group and of other representatives of *Lactobacillus* strains (retrieved from the NCBI database) were aligned using the computer-program ClustalX. The phylogenetic reconstruction was done using the neighbour joining algorithm, with bootstrap values calculated from 1000 replicate runs, using the routines included in the MEGA software [13] and *Acinetobacter calcoaceticus* was used as outgroup.

2.3. Determination of Cell Biomass

Biomass concentration is obtained by means of the measurement of the optical density at 540 nm of the cultured broth after incubation. Biomass concentration was determined according to Bucke [14] by this formula:

$C_B = 0.2845 \times OD^{540\ nm}$, where: C_B is biomass concentration (g/l) and $OD^{540\ nm}$ is optical density at 540 nm.

2.4. Isolation, Purification and Identification of of EPSs

The EPS from selected strains was isolated and purified by ethanol according to the methods described by Cerning *et al.* [15] and the yields of EPSs were determined gravimetrically by measuring the polymer dry mass drying for 24h at 50°C. Chemical structure of EPS produced by H2 was analyzed by Electrospray Ionization Mass Spectrometry (ESI MS) according to Pesch *et al.* [16] by using Ion trap thermo Finnigan, PDA detector, LCQ advantage max.

2.5. Factors Affecting the Growth and Secretion of EPS from Strain H4

To detect the best fermentation conditions for growth H2 isolate and EPS production, a fermentation period (at 24, 36, 72 and 96 h), fermentation temperature (at 20, 30, and 40ºC) and the initial pH (at 6.0, 7.0 and 8.0) were conducted using MRS medium without any other additives. Furthermore, different sugars such glucose, fructose, galactose, lactose and sucrose at different concentrations (20, 40, 60 and 80 g/L) were evaluated. Also, food industrial by-products such as permeate, date debs and molasses were evaluated at different concentrations (20, 40, 60 and 80 g/L).

2.6. Source of Milk and Starters

Fresh skim milk and low fat ultra-filtrated milk were prepared from buffalo's milk obtained from the model dairy unit in the faculty of agriculture, Fayoum University. Lyophilized yoghurt starter culture YC-W11 (*Str. salivarius* subsp. *thermophilus* and *Lb. delbreukii* subsp. *bulgaricus*) and CHY-MAX rennet powder extra were obtained from CHR-Hansen laboratories, Denmark. Isolation and purification of EPSs from isolate H2 was performed according to Cerning *et al.* [15].

2.7. Manufacture of Low Fat Yoghurt

Fresh skim buffalo's milk (acidity 0.17%, fat 0.30%, protein 4.35% and moisture 90.34%) was heated at 95ºC for 10 min., cooled to 45ºC and divided into three treatments(C_1, Y_1 and Y_2), then different ratios of the EPS; 0.0, 0.4 and 0.8 % w/v were, respectively. Subsequently, all treatments were inoculated with 1% yoghurt starter culture YC-W11, poured into cups, incubated at 40±2ºC until coagulation and stored at 6±1ºC, for 10 days. Chemical and syneresis testes for fresh and stored (3, 6 and 10 days) of yoghurt samples were conducted. Furthermore, rheological and organoleptic properties at fresh and 10 days were determined.

2.8. Manufacture of Low Fat UF - Soft Cheese

Skim buffalo's milk was heated at 75ºC for 10 min., ultrafiltrated at about 50ºC (acidity 0.29%, fat 1.50%, protein 15.86% and moisture 72.10%). The ultrafiltrated milk was divided into four treatments (C, Ch_1, Ch_2, and Ch_3), then different concentrations of EPS (0.0, 0.2, 0.4 and 0.8 %) were

added, respectively. All treatments were inoculated with 1% yoghurt starter (YC-W11), incubated at 40±2ºC for one h, 1% salt. For coagulation, 1g/100 kg of rennet was added, subsequently stored in refrigerator at 6±1ºC, for 15 days. For chemical analysis, interval samples (fresh, 5, 10 and 15 days) of cheese treatments were taken. Also, rheological and organoleptic properties were determined for fresh and 15 days age.

2.9. Determination of Chemical, Physical, Rheological and Organoleptic Properties

Titratable acidity (TA), fat and moisture contents were carried out according to Ling [17]. The pH was determined by using pH meter (Kent EIL 7020). Total nitrogen (TN) and water soluble nitrogen (WSN) were determined using macro-kjeldahl method according to International Dairy Federation [18] and Kuchroo and Fox [19], respectively. Acetaldehyde and diacetyl content were estimated as described by Lees and Jago [20], respectively. The syneresis was determined as described by Folkenberg *et al.* [21], some rheological tests were performed by the textural profile analyzer (QC-tech- B type-Taiwan – universal testing machine). The organoleptic properties of fresh and stored low fat yoghurt and soft cheese from different treatments were assessed by panel taste of ten trained panelists from the staff members of Dairy Department and food Science Department, Faculty of Agriculture, Fayoum University, according to the scheme of Clark *et al.* [22].

2.10. Statistical Analysis

All data obtained were subjected to the statistical analysis that performed by SPSS version 19.0 [23] and Sigma plot 11.0 software programs.

3. Results and Discussion

3.1. Isolation and Identification of EPS Producing Strains

In this study, a total of 46 EPS producing isolates were isolated from different Egyptian dairy products. These isolates were Gram positive, non-spore former, non-motile and identified as lactic acid bacteria based on API 50CHL kit. Based on the EPS amount produced from these isolates, the most promising three isolates H1, H2 and H4 were chosen for further phenotypic and genotypic characterization. As shown in API 50 CHL profile (Table 1), isolates H1, H2 and H4 could ferment different sugars. It was noticed that isolates H1 and H2 were differed from isolate H4 by their ability to utilize melezitose, D-turanose, and D-arabinose and their inability to utilize glycerol. However, the characterization of some *Lactobacillus* to species level by biochemical methods alone is not reliable [[24,25], because of the considerable variations in biochemical attributes between strains currently considered to belong to the same species. so the 16s rRNA and DNA-DNA hybridization are considered as the best approaches to solve this problem.

Table 1. *API 50CHL carbohydrate profile for the selected EPS producing isolates.*

Carbon sources		Exopolysaccharide produducing isolates			
		H1	**H2**	**H4**	***Lb. pentosus****
1	Glycerol	-	-	+++	+++
2	Erythritol	-	-	-	-
3	D-Arabinose	+++	+++	-	-
4	L-Arabinose	+	+	+++	+++
5	Ribose	++	++	+++	+++
6	D-Xylose	-	-	+	+++
7	L-Xylose	-	-	-	-
8	Adonitol	-	-	-	-
9	β- Methyl-D-Xyloside	-	-	-	-
10	Galactose	+++	+++	+++	+++
11	Glucose	+++	+++	+++	+++
12	Fructose	+++	+++	+++	+++
13	Mannose	++	++	+++	+++
14	Sorbose	-	-	-	-
15	Rhamnose	++	+++	-	-
16	Dulcitol	-	-	-	-
17	Inositol	-	-	-	-
18	Mannitol	+++	+++	+++	+++
19	Sorbitol	+++	+++	+++	+++
20	α–Methyl-D-Mannoside	+	+	-	-
21	α –Methyl-D-Glucoside	-	-	-	-
22	N-acetyl-glucosamine	+++	+++	+++	+++
23	Amygdalin	+++	+++	+++	+++
24	Arbutin	+++	+++	+++	+++
25	Esculin Hydrolysis	++	++	+++	+++
26	Salicin	+++	+++	+++	+++
27	Cellobiose	+++	+++	+	+++
28	Maltose	+++	+++	+++	+++
29	Lactose	+++	+++	+++	+++
30	Melibiose	+++	+++	+++	+++
31	Sucrose	+++	+++	+++	+++
32	Trehalose	+++	+++	+++	+++
33	Inulin	-	-	-	-
34	Melezitose	+++	+++	-	-
35	Raffinose	+	+	++	+++
36	Starch hydrolysis	-	-	-	-
37	Glycogen	-	-	-	-
38	Xylitol	-	-	-	-
39	Gentiobiose	+++	+++	+	+++
40	D-Turanose	+++	+++	-	+++
41	D-Lyxose	-	-	-	-
42	D-Tagatose	-	-	-	-
43	D-Fucose	-	-	-	-
44	L-Fucose	-	-	-	-
45	D-Arabitol	+++	+++	-	-
46	L-Arabitol	-	-	-	-
47	Gluconate	+++	+++	+++	+++
48	2-Keto-gluconate	-	-	-	-
49	5-Keto-gluconate	-	-	-	-

Notes: Carbohydrate fermentation profiles were applied according to API 50 CHL strips (BioMériéux, Lyon /France). The Score of the result tests: - negative growth; + weak growth; ++good growth, +++ very good growth. (*) = Reference strain *Lb. pentosus* obtained from culture collection of department of Agricultural Microbiology and Biotechnology, Faculty of Agriculture, Fayoum University, Egypt.

Phylogenetic tree based on 16S rRNA gene sequences (Fig. 1) indicated that strains H1, H2 and H4 were similar to *Lactobacillus pentosus* with similarity 99%. Based on their superior EPS production from different carbon sources, isolate H2 was selected among the 47 isolates for studying the factors effect on EPS production.

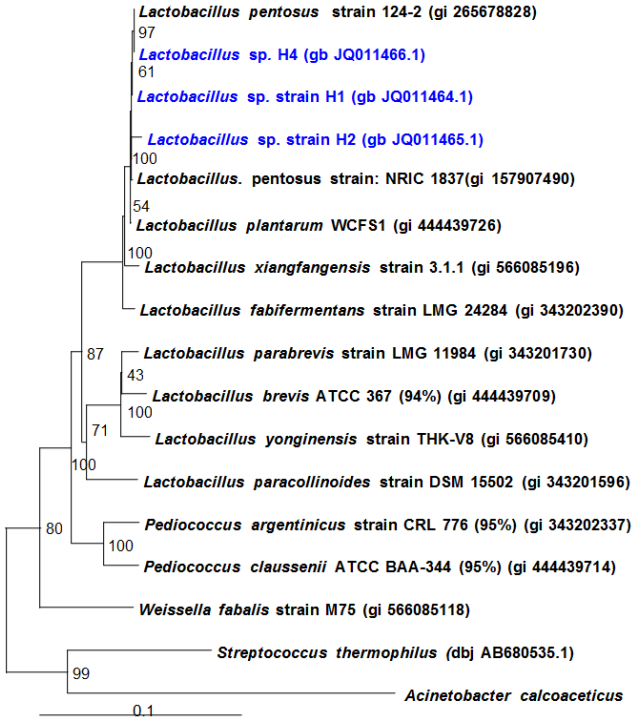

Fig. 1. *Neighbor joining tree showing the estimated phylogenetic relationships of H1, H2 and H4 (As presented with blue color) and nearest members of the Lactobacillus. Bootstrap values are shown as percentages of 1000 replicates. Bar: 1% sequence divergence. Acinetobacter calcoaceticus and Streptococcus thermophilus were used as out-group.*

3.2. Factors Affecting the Growth and Secretion of EPS from Strain H2

As shown in Fig. 2a, production of EPS from isolate H2 was significantly ($p \leq 0.01$) increased with incubation period up to 72 h, and then dramatically decreased up to 96 h. Isolate H4 exhibited the maximum EPS secretion (2.22 g/L) and cell biomass (2.64 g/L) after 72 and 96 h, respectively. Decreasing of EPSs yields with prolonged fermentation period is probably due to the presence of glycohydrolases, capable of hydrolyzing EPS and liberating monomer as a carbon source for lactic acid starter [26, 27]. In this context, it was reported that the EPS-producer strains might produce EPS with gradual increase at the first three days of the fermentation period to avoid food shortage or to protect the cells [28, 29]. Also, Pham *et al.* [30] reported that when acidification occurs due to lactate production during fermentation process, consequently, glycohydrolases are activated and polysaccharide yields decrease due to enzymatic digestion.

The results in Fig. 2b showed that the growth and EPS secretion of strain H2 was highly affected by different fermentation temperatures. It was noticed that the EPS secretion increased as fermentation temperature increased and the maximum EPS amount was recorded at 40°C (2.24 g/L), while the cell biomass was decreased (1.28 g/L) when the fermentation temperature increased. However, the unsuitable condition for growth is thought to be an optimal condition for EPS production by mesophilic LAB since sugar nucleotides, which are utilized by the cell wall, are needed for EPS production [31, 32]. Fig 2c indicated that, EPS secreted from strain H2 was 1.48, 1.44 and 1.40 (g/L) at pH 6, 7 and 8, respectively. While, the cell biomass of strain H4 was 2.21, 2.24 and 1.26 (g/L), respectively.

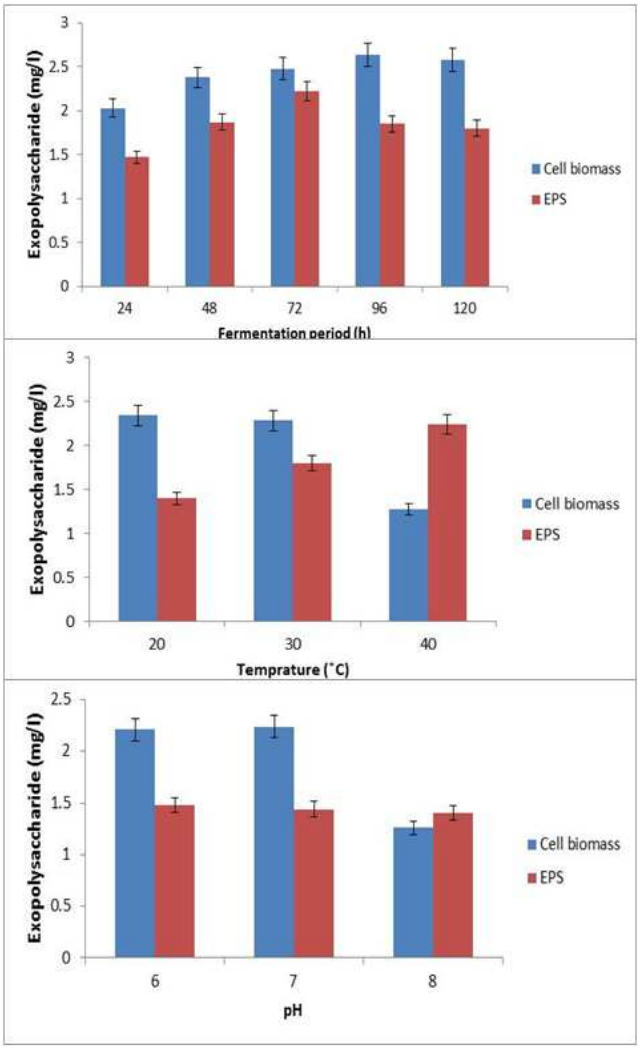

Fig. 2. *Effect of fermentation temperature (a), temperature (b) and pH on production of EPS and C_B from strain H2.*

3.3. EPS Production and the C_B from Different Sugars and Food Industrial by Products

In this study, different sugars including glucose, fructose, galactose, lactose and sucrose at different concentrations (20, 40, 60 and 80 g/L) were evaluated using MRS broth for EPS production by strain H2. Furthermore, food industrial by-products such as permeate, date debs and molasses were evaluated. The results presented in Table (2) showed that as sugar concentration increased, the EPS and C_B increased.

Among eight sugars tested, it was noticed that all industrial by-products tested were significantly increased the secretion of EPS from strain H2. The highest EPS amount was recorded for molasses and date debs, followed by permeate, lactose and glucose which were 4.54, 3.08, 2.86, 2.68 and 2.56 (g/l) respectively. While, the lowest EPS amount were recorded for galactose, fructose and sucrose, respectively. With respect to EPS production by lactic acid bacteria, the cost of fermentation medium represents an important aspect of their commercial production and the maximum yield of EPS should be obtained for economic reason [33, 34, 35, 36]. Therefore, an inexpensive substrate must be found to reduce the cost of the raw materials. Worth mentioning that all food industrial by-products tested, especially molasses and date debs were inducted approximately two times of EPS compared to the pure sugars. This might be attributed to the fact that these by-products contain some natural vitamins and minerals that can promote bacterial viability and EPS production.

Table 2. *Effect of different concentrations of different sugars and food by-products on cell biomass (CB) and Exopolysaccharide production (EPS) from strain H2.*

Sugar or Waste product	Cell biomass and Exopolysaccharide from different sugar concentration							
	20 g/L		40 g/L		60 g/L		80 g/L	
	CB	EPS	CB	EPS	CB	EPS	CB	EPS
Glucose	1.62^{d}	1.56^{e}	1.56^{e}	1.68^{d}	1.62^{e}	1.96^{d}	2.40^{a}	2.56^{cd}
Fructose	1.79^{c}	1.36^{f}	2.02^{b}	1.48^{e}	2.02^{b}	1.72^{e}	2.20^{b}	1.88^{e}
Galactose	1.56^{e}	1.36^{f}	1.61^{e}	$1.60d^{e}$	1.56^{f}	1.84^{de}	2.03^{c}	2.20^{de}
Lactose	1.34^{f}	2.48^{b}	1.49^{f}	2.68^{b}	1.45^{g}	2.56^{c}	1.96^{c}	2.68^{c}
Sucrose	1.96^{b}	0.96^{g}	1.96^{c}	0.92^{f}	1.76^{d}	1.32^{f}	1.80^{d}	1.42^{f}
Permeate	1.65^{d}	2.32^{d}	2.13^{a}	2.40^{c}	2.15^{a}	2.52^{c}	2.33^{a}	2.86^{bc}
Date debs	2.02^{a}	2.40^{c}	1.79^{d}	2.44^{c}	1.77^{d}	2.84^{b}	1.65^{e}	3.08^{b}
Molasses	1.91^{b}	3.34^{a}	1.82^{d}	3.74^{a}	1.96^{c}	4.52^{a}	2.22^{b}	4.54^{a}

3.4. Chemical Structure of EPS Produced by H2 Isolate

Ionization mass spectrometric (ESI/MS/MS) analysis of the purified EPS produced from strain H2 (Fig. 3) revealed the presence of oligomeric compounds, indicating that incomplete degradation of EPS was occurred. Partial degradation of EPS by acid hydrolysis is based on the fact that some glycosidic linkages are more labile than others. If polysaccharide contains only a limited number of acid-labile glycosidic linkages, a partial hydrolysis will afford a mixture of monosaccharaides and oligosaccharides [37]. The molecular weight of the oligomer (938.02) may be corresponding to glucose, glucoronic acid and rhamnose with ratio 1:2:2, respectively. Also, some monosaccharaides such D-glucose or D-galactose (MW: 174.33) and D-glucosamine 6-phosphate (MW 251.43) were detected (Fig 3). However, amount of the monomers depend on the ratio of EPS acid hydrolysis. Similar result was reported by Rodríguez-Carvajal *et al.* [38], reported that the EPS produced from *Lactobacillus pentosus* is a charged heteropolymer, with a composition of D-glucose, D-glucuronic acid and L-rhamnose in a molar ratio 1:2:2.

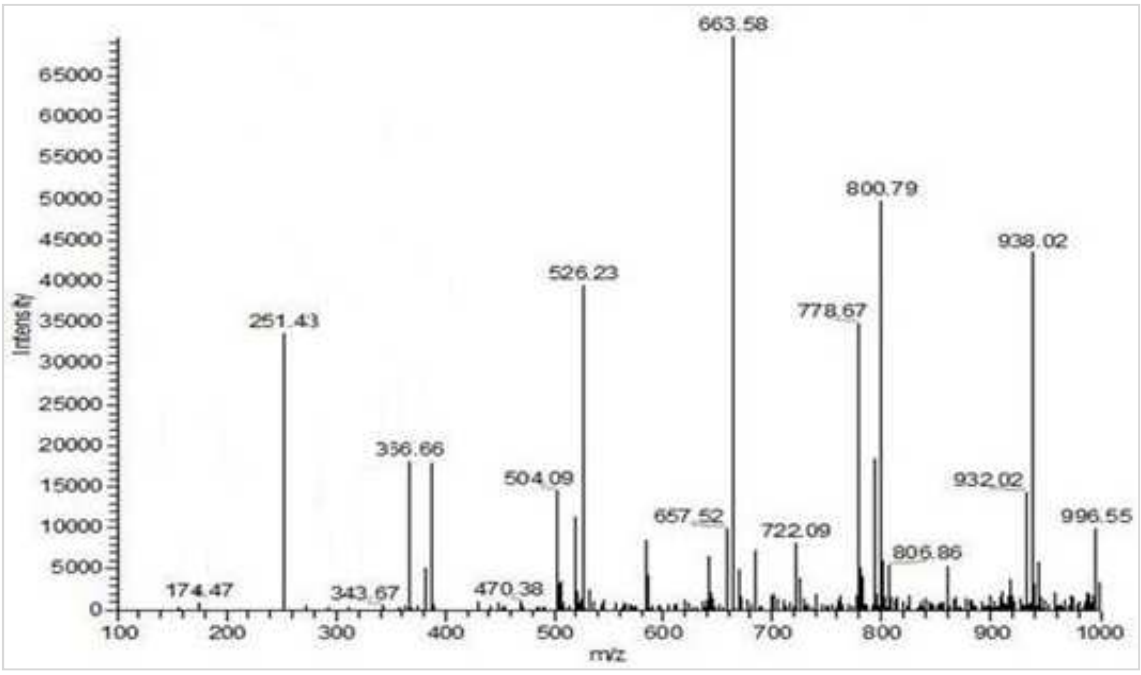

Fig. 3. *Chemical Structure of EPS produced by H2 analyzed by Electrospray Ionization Mass Spectrometry (ESI MS).*

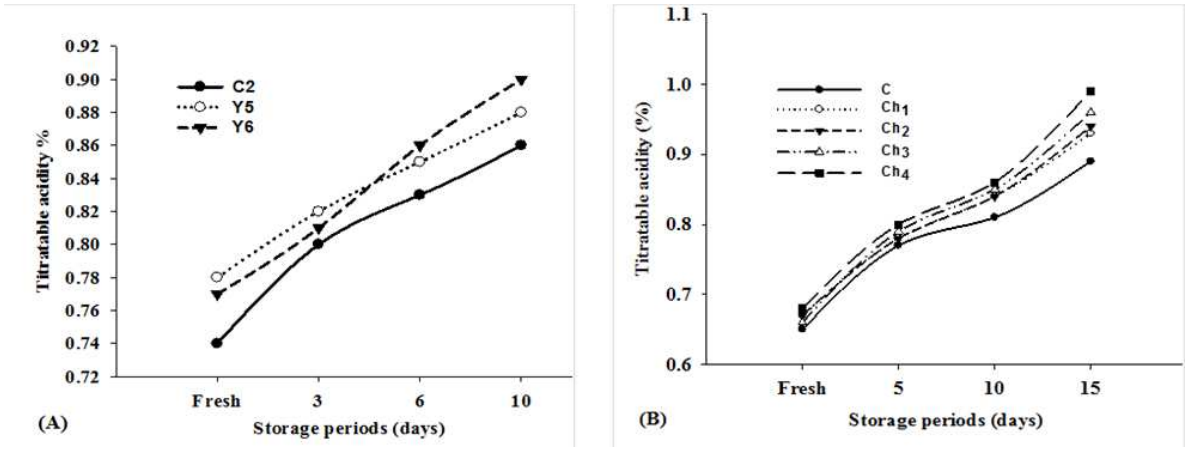

Fig. 4. *Changes in the titratable acidity (TA %) and pH values of low fat yoghurt (a) and low fat UF cheese (b) made with adding different concentrations of EPS produced by strain H2 during storage period. C2, Y5 and Y6: Control, Low fat yoghurt with 0.4 and 0.8 % EPS, respectively. C, Ch_1, Ch_2 Ch_3 and Ch4: Low fat UF – soft cheese with (control, 0.2, 0.4 and 0.8 %) of EPS, respectively.*

3.5. Chemical Analysis of Low Fat Yoghurt and UF-Soft Cheese

The composition of low fat yoghurt and UF-soft cheese which fortified with EPS as affected storage periods are given in Table (1). Moisture content of both products that contained EPS was increased with increasing the EPS concentration. These results are in agreement with Jimenez-Guzman *et al.* [39], Trancoso-Reyes *et al.* [39, 40] who mentioned that EPS have excellent water binding properties and moisture retention, which improve the quality of low fat dairy products. Generally, it was noticed that, fat, TN and WSN/TN contents in all EPS treatments were significantly *($P \leq 0.01$)* increased during storage periods. Also, as shown in Fig. (4), the titratable acidity (TA %) of low fat yoghurt and UF-soft cheese treatments gradually increased with progress the storage period. Increasing of WSN/TN content and the development of TA of the treatments containing the EPS were higher than the control at all the storage periods. This may attribute to the high activity of the starter culture strains in different treatments, since the EPSs can act as prebiotics for the LAB. Similar trend were reported by Barreteau *et al.* [41] and Salazar *et al.* [42], who mentioned that oligosaccharides as small parts from EPSs are consumed by *Lactobacillus* strains and consequently, their activities increased.

3.6. Rheological Properties of Low Fat Yoghurt and UF-Soft Cheese

The results of texture profile analysis (Table 4) showed that EPS of strain H2 improved rheology, texture, stability and mouth feel of all low fat yoghurt and UF- soft cheese treatments which fortified by EPS compared to control. The best rheological properties of low fat cheese were recorded for treatment (ch3) that contained 0.8% EPS. It noticed that, the texture profile values decreased as EPS ratio and storage periods increased. Vice versa, in case the low fat yoghurt, rheological properties including; firmness, cohesiveness, gumminess, chewiness, springiness and resilience were significantly *($p \leq 0.0001$)* improved as EPS concentrations increased, whereas, the best rheological values were recorded for yoghurt treatment (Y2) that contained 0.8% EPS, followed by yoghurt treatment (Y1) that contained 0.4% EPS. Furthermore, the fortification of the low fat yoghurt with EPS significantly decreased the amount of whey (syneresis) present on the surface of all yoghurt samples that contained EPS compared to control. This result suggests that the EPS has strengthened the protein network through the improvement of water binding properties. Furthermore, the ability of binding water for the EPS reflected the ability of the EPS to enhance the consistency and some other rheological properties of dairy products. Similar results were reported by Francois *et al.*, Trancoso-Reyes *et al.* [40,43] who mentioned that using EPS-producing cultures in cheese manufacture improved most of the rheological properties and proteolysis of low-fat cheese. Also, the data in Fig. 5 indicated that there was an inverse relationship between the EPS ratio and syneresis values during storage period at 6 ± 1°C. In this context, it was reported that LAB producing EPSs are often used to increase viscosity of set and stirred fermented milks, such as yoghurt and decrease susceptibility to syneresis [15, 44]. The increasing of syneresis during storage period progress is due to the development of acidity formed by the lactic acid starters. Since, the acidity leads to reinforcement of the strength of the protein network. So, it is important to say that the external addition of purified EPS instead of EPS producing lactic acid starters could be more suitable to improve the rheological properties of dairy products, especially that need heat treatments or freezing such ice cream.

Table 3. *Chemical changes in low fat yoghurt and UF - soft cheese containing different concentrations of EPS produced from strain H2 during storage period.*

Treatments	Storage periods (days)	Moisture (%)	Fat (%)	TN^{NS} (%)	WSN (%)	WSN/TN (%)
low fat yoghurt						
Control I	Fresh	89.65±0.01^{a}	0.30±0.01	0.68±0.01	0.18±0.003^{de}	26.47±0.28^{d}
	3	89.58±0.02^{cd}	0.32±0.02	0.69±0.00	0.19±0.007^{d}	27.54±1.03^{d}
	6	89.45±0.01^{e}	0.35±0.02	0.71±0.01	0.22±0.000^{c}	30.99±0.31^{c}
	10	89.27±0.03^{f}	0.38±0.00	0.72±0.01	0.27±0.000^{b}	37.50±0.37^{b}
Y_1	Fresh	89.66±0.01^{ab}	0.30±0.00	0.68±0.00	0.18±0.000^{de}	26.47±0.00^{d}
	3	89.60±0.01^{bc}	0.31±0.01	0.69±0.01	0.19±0.000^{d}	27.54±0.28^{d}
	6	89.56±0.01^{c}	0.33±0.02	0.70±0.01	0.23±0.007^{c}	32.86±1.34^{c}
	10	89.44±0.03^{e}	0.35±0.01	0.70±0.00	0.28±0.000^{ab}	40.00±0.00^{a}
Y_2	Fresh	89.66±0.01^{ab}	0.30±0.01	0.68±0.01	0.18±0.000^{de}	26.47±0.55^{d}
	3	89.62±0.02^{bc}	0.30±0.00	0.69±0.01	0.19±0.000^{d}	27.54±0.28^{d}
	6	89.59±0.01^{bc}	0.32±0.01	0.69±0.00	0.22±0.000^{c}	31.88±0.00^{c}
	10	89.50±0.01^{de}	0.34±0.00	0.70±0.01	0.29±0.007^{a}	41.43±0.60^{a}
low fat UF cheese						
Control II	Fresh	72.05±0.14^{a}	1.45±0.07^{fg}	2.48±0.01^{cde}	0.28±0.01^{ef}	11.29±0.51^{fg}
	5	71.85±0.04^{de}	1.52±0.14^{cde}	2.50±0.01^{bcde}	0.30±0.01^{ef}	12.00±0.32^{fg}
	10	71.65±0.09^{f}	1.59±0.21^{b}	2.51±0.02^{ab}	0.35±0.02^{cd}	13.94±0.72^{cde}
	15	71.26±0.21^{g}	1.66±0.14^{a}	2.53±0.01^{a}	0.40±0.01^{b}	15.81±0.06^{b}
Ch_1	Fresh	72.13±0.04^{a}	1.45±0.04^{fg}	2.48±0.02^{cde}	0.28±±0.00^{ef}	11.29±0.10^{fg}
	5	72.04±0.02^{ab}	1.48±0.04^{defg}	2.49±0.01^{cde}	0.30±0.00^{ef}	12.05±0.04^{fg}
	10	71.89±0.21^{bc}	1.54±0.07^{cdef}	2.50±0.01^{bcde}	0.35±0.01^{cd}	14.00±0.33^{cd}
	15	71.72±0.04^{e}	1.62±0.21^{bc}	2.51±0.01^{abcd}	0.45±0.01^{a}	17.93±0.00^{a}
Ch_2	Fresh	72.13±0.02^{a}	1.44±0.07^{fg}	2.48±0.01^{cde}	0.27±0.01^{f}	10.89±0.23^{f}
	5	72.05±0.14^{ab}	1.47±0.07^{efg}	2.49±0.01^{cde}	0.31±0.01^{e}	12.45±0.32^{ef}
	10	71.90±0.03^{ab}	1.55±0.05^{cdefg}	2.49±0.01^{cde}	0.36±0.01^{c}	14.46±0.49^{bc}
	15	71.75±0.14^{cd}	1.60±0.07^{cd}	2.51±0.02^{abcd}	0.47±0.01^{a}	18.73±0.13^{a}
Ch_3	Fresh	72.13±0.03^{a}	1.45±0.04^{efg}	2.47±0.01^{de}	0.29±0.01^{ef}	11.74±0.31^{fg}
	5	72.04±0.14^{ab}	1.47±0.04^{efg}	2.49±0.01^{cde}	0.31±0.02^{e}	12.45±0.78^{ef}
	10	71.92±0.07^{ab}	1.53±0.04^{efg}	2.51±0.01^{abcd}	0.37±0.01^{bc}	14.74±0.52^{bc}
	15	71.79±0.07^{cd}	1.58±0.07^{cdef}	2.52±0.01^{abc}	0.47±0.02^{a}	18.65±0.89^{a}

Notes: Means ± (St. Dev.) having different superscripts within each column are significantly different ($p \leq 0.01$). NS: Not significance, Control I and II : Low fat yoghurt and UF cheese without EPS, respectively. Y_1 and Y_2: Low fat yoghurt with 0.4 and 0.8 % EPS, respectively. Ch_1, Ch_2 and Ch_3: Low fat UF – soft cheese with (0.2, 0.4 and 0.8 %) of EPS, respectively.

Table 4. *Changes in rheological properties and sensory evaluation score of low fat yoghurt and UF – soft cheese made with adding different concentrations of EPS produced from strain H2 during storage period.*

Treatments*	Periods (days)	Rheological properties						scores of sensory evaluation				Notes
		Firmness (N)	Cohesiveness	Gumminess (N)	Chewiness (N)	Springiness	Resilience	Flavor (45)	Texture (40)	Appearance (15)	Total score (100)	
Low fat yoghurt												
Control I	Fresh	6.08^{d}	0.48^{b}	2.93^{c}	1.83^{b}	0.001^{d}	0.03^{d}	34.30^{a}	27.30^{b}	11.50^{c}	73.10^{b}	Almost no flavor
	10	4.71^{e}	0.15^{d}	1.66^{e}	0.98^{d}	0.592^{c}	0.22^{bc}	32.10^{b}	25.00^{b}	10.00^{d}	67.10^{b}	
Y_1	Fresh	6.47^{cd}	0.52^{b}	3.37^{b}	2.87^{a}	0.777^{b}	0.08^{cd}	42.10^{a}	38.40^{a}	14.00^{a}	94.50^{a}	creamy taste
	10	8.04^{b}	0.26^{cd}	2.12^{d}	1.38^{c}	1.253^{a}	0.34^{b}	42.00^{a}	36.60^{a}	13.30^{b}	91.90^{a}	
Y_2	Fresh	6.69^{c}	0.72^{a}	3.80^{a}	3.10^{a}	0.815^{b}	0.24^{bc}	42.20^{a}	38.90^{a}	14.50^{a}	95.60^{a}	
	10	8.89^{a}	0.37^{bc}	2.19^{d}	1.40^{c}	1.340^{a}	0.54^{a}	41.30^{a}	37.00^{a}	13.40^{b}	91.70^{a}	
Low fat UF - soft cheese												
Control II	Fresh	36.87^{a}	0.27^{bc}	15.52^{a}	15.03^{a}	1.08^{a}	0.75	35.90^{b}	36.50^{bc}	9.00^{ab}	82.40^{b}	rubbery and rigid slight flavor
	15	34.72^{b}	0.16^{c}	12.63^{c}	9.63^{d}	0.76^{bc}	0.18	39.40^{ab}	35.50^{c}	8.10^{b}	83.00^{b}	
Ch_1	Fresh	33.93^{b}	0.32^{bc}	13.93^{b}	12.88^{b}	1.00^{ab}	0.26	45.40^{a}	38.00^{ab}	9.20^{ab}	92.60^{a}	Creamy taste increased with increasing the concentration of EPS
	15	17.6^{e}	0.25^{bc}	3.72^{f}	1.93^{f}	0.34^{d}	0.05	43.40^{a}	38.50^{ab}	9.10^{ab}	91.00^{a}	
Ch_2	Fresh	32.95^{c}	0.47^{bc}	12.84^{c}	10.16^{c}	0.65^{cd}	0.16	46.40^{a}	38.90^{ab}	9.60^{a}	94.90^{a}	
	15	17.50^{e}	0.32^{bc}	3.22^{h}	1.26^{g}	0.36^{d}	0.04	45.50^{a}	39.10^{a}	9.60^{a}	94.20^{a}	
Ch_3	Fresh	25.69^{d}	0.50^{bc}	10.97^{d}	5.72^{e}	0.54^{cd}	0.15	40.40^{ab}	37.90^{ab}	8.80^{ab}	87.10^{ab}	
	15	16.67^{e}	0.39^{bc}	3.25^{fg}	1.16^{g}	0.39^{d}	0.04	42.00^{ab}	38.00^{ab}	9.40^{a}	89.40^{ab}	

Notes: Means ± (St. Dev.) having different superscripts within each column are significantly different ($p \leq 0.0001$) and ($p \leq 0.05$) for rheological and sensory evaluation, respectively. NS: Not significance, Control I and II : Low fat yoghurt and UF cheese without EPS, respectively. Y1 and Y2: Low fat yoghurt with 0.4 and 0.8 % EPS, respectively. Ch1, Ch2 and Ch3: Low fat UF – soft cheese with (0.2, 0.4 and 0.8 %) of EPS, respectively.

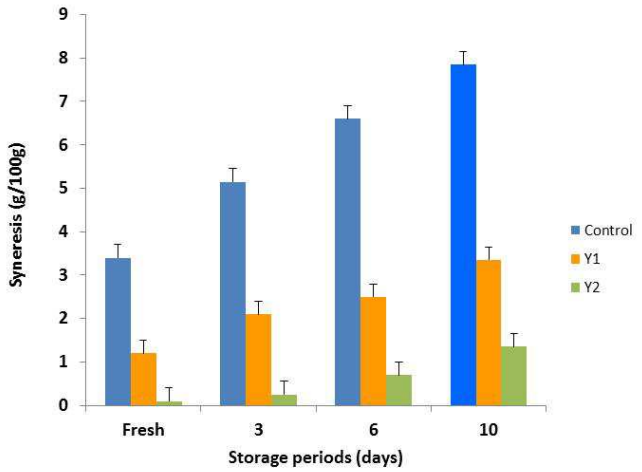

Fig. 5. *Changes in syneresis (g/100g sample) of low fat yoghurt made with adding different concentrations of EPS produced by strain H2 during storage period. Y1 and Y2: Low fat yoghurt with 0.4 and 0.8 % EPS, respectively.*

3.7. Sensory Evaluation of Low Fat Yoghurt and UF-Soft Cheese

Scores for organoleptic properties of low fat yoghurt and UF- soft cheese from different treatments are presented in Table (4). The results indicated that, all sensory properties profile including flavor, texture, appearance and mouth feel of both low fat yoghurt and cheese which fortified with EPS were significantly improved compared to control. The best sensory evaluation score was recorded for low fat yoghurt fortified with EPS 0.8% (Y1) followed by 0.4% (Y2) which were 95.60 and 94.50%, respectively. while, the best sensory evaluation score of low fat cheese was recorded for the treatment fortified with 0.4% (ch2) followed by 0.2% (ch1) and 0.8% (ch3) which were 94.90, 92.60, 87.10 and 82.40, respectively. During the storage period, the scores of appearance were also significantly increased in all low fat cheese treatments containing EPS compered to control. The results in accordance of Folkenberg *et al.* and Jimenez-Guzman *et al.* [21, 39] who mentioned that the sensory evaluation of ropy cheese that produced by EPS producing *Str. thermophilus* was softer and creamier. Worth mentioning that, in low fat cheese, panelists noticed that the cheese treatment fortified with 0.2 and 0.4% EPS gives creamy taste or mouth feel fat-richness. On the other hand the high ratio of EPS (0.8) caused unacceptable body, texture and flavor of cheese samples. Also, they recorded that Low fat yoghurt without EPS (control) were not firmed and the whey appeared on the surface of the yoghurt during storage.

4. Conclusion

From these results, it could be concluded that the purified EPS isolated from *Lactobacillus pentosus strain* H2 improved the organoleptic properties including texture, flavors and mouth feel of both low fat cheese and yoghurt. Furthermore, all parameters of rheological properties of both products including firmness, cohesiveness, gumminess, chewiness, springiness were significantly improved as the purified EPS concentrations increased. Finally, the problems like bitterness, low viscosity, high syneresis formation and defects of consistency which are frequently encountered of fermented milk products can be solved by using purified EPS.

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