ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Production And Recognition Of Exopolysaccharide From Lactic Acid Bacteria Grown In Whey And Date Juice And Its Use In Processed Cheese Manufacturing.

Mustafa F. Jafar, and Ebtisam F Mousa*.

Department of Food Science, College of Agriculture, Baghdad University, Baghdad, Iraq

ABSTRACT

Ability of four pure isolations from lactic acid bacteria in production of exopolysaccharide was tested. The species of lactic acid bacteria were Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus plantarum, Streptococcus salivarius subsp. thermophilus and Lactococcus lactis subsp. lactis. Elementary biological tests were carried on these bacteria to select the most exopolysaccharide (EPS) produced bacteria. Lactococcus lactis subsp lactis was positive to Molish and negative to Fehling and Iodine tests with viscosity of 13.98 centipoises and had a production of total saccharide 196.2 $mg.L^{-1}$for expolysaccharide productions. The whey was used as natural culture medium for expolysaccharide production by Lactococcus lactis subsp. lactis. The total amount of the produced exopolysaccharide was 0.12 $mg.L^{-1,}$ The Whey medium was supplied 10% glucose, 1.5% casein, 0.1 mg L^{-1} $CaCl_2$ and 0.5 $mg.L^{-1}$ Vit.C and the produced exopolysaccharide amount was 1.545 $g.L^{-1}$.Recognition of expolysaccharide by using techniques of TLC, HPLC and quality tests showed that exopolysaccharide is a heteropolysaccharide type consists of monosaccharides (Galactose, Rhamnose and N-acetyle glucose amine). The produced expolysaccharides used as an emulsifier material in process cheese manufacturing instead of emulsion salts (sodium and potassium sorbet) at 2.5, 3.5 , 4.5% , and 4.5% conc. which showed values close to that of control treatment (3.5% commercial emulsion salts) in sensory properties.

Keywords: Lactic acid bacteria; Exopolysaccharide (EPS); Recognition; whey; emulsifier; process cheese.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Lactic Acid Bacteria (LAB) were used at wide range in dairy and therapy foods manufacturing and also in industrial biotechnology. It has large importance in medical side by increasing of microflora numbers in the intestinal tube besides its ability in expolysaccharide creation [1].LAB are"generally recognized as safe" (GRAS) microorganisms and many studies used exopolysaccharide produced by these bacteria as safe additives for improving consistency and viscosity and prevention exuding of the natural fermented milk, in addition to that , it was found that some of EPS produced by lactic acid bacteria can give medical advantages for consumers , The EPS is one of the material excreted by lactic acid bacteria through cell wall as capsules or is excreted in medium when they are used in dairy products manufacturing and these polysaccharides present in two types depending on their position (first as capsular polysaccharides at which the polymer attached to the cell surface forming capsules and the second type is slim polysaccharides that is excreted from cell wall in the medium and it is difficult to distinguished between them because that some of the bacterial strains release the capsular polysaccharides in the medium [2].

EPS are classified into two groups: homo-EPS, consisting of a single type of monosaccharides (α-D-glucans, β-D-glucans, fructans, and others represented by polygalactan) and hetero-EPS (HePSs) composed of different types of monosaccharides, mostly D-glucose, D-galactose, L-rhamnose, and their derivatives [3]. HePSs from LAB are produced in a greater variety with regard to monosaccharide composition, monosaccharide ratio, and molecular structure (monosaccharide components, ring forms, anomeric configurations, and stereo- and region-specific linkages) of the repeating unit, as well as the conformation and MM of the polymer [4].

Nevertheless, the quantity of EPS produced by LAB is adequate to be exploited for in situ applications, and LAB strain culture would be a useful method to produce EPS for food applications if the fermentation conditions using undefined media have been improved to maximize yields. LAB could be grown in edible and safe culture media such as whey, and if fermentation conditions are optimized a high yield can be obtained [5].

This study aimed to use numbers of pure strains of lactic acid bacteria and determine their ability in EPS production and use of cheap throughout the year available elementary material (whey) for its production and usage in process cheese manufacture due to a shortage in the available studies inside Iraq in this field.

MATERIALS AND METHODS

Lactic Acid Bacteria Isolates

Lactobacillus delbrueckii subsp.bulgaricus (produced by Danis sco-France company) and Lactobacillus plantarum bacteria were obtained from the research laboratories of college of Agriculture, University of Baghdad .These bacteria were grown on MRS liquid medium (prepared on instructions of Himedia company) and enriched by 0.5% lactose for inoculators preparation [6].

Streptococcus salivarius subsp. thermophilus bacteria (produced by Danis Sco- France company) and Lactococcus lactis subsp. lactis MWO 030 (From Sacco Sir- Italy company), these bacteria were grown on M17 liquid medium (prepared by Himedia company instructions) and enriched by 0.5% glucose for inoculator preparation [7, 6].

Inoculator size determination: it was done by using light absorption (standard curve method) for calculating number of bacteria living cells according to method described by Atlas et al. [8].

Physicochemical tests used for detection of polysaccharides in the fermentation medium

Molish test and Fehling's test were determined as described by A.O.A.C (1990) [9]. **Iodine test** was conducted according to Al- Dalale and Hakim [10]. **Total carbohydrate (sugars)** were determined by (phenol-H_2SO_4) method, that described by Dubois et al. [11]. Viscosity of the bacteria inoculated medium was determined by using Ostwald viscometer and was calculated as follows:

(Water viscosity / Liquid viscosity) = (time of water passage * its density) / (time of liquid passage * its density). Where, density= (weight of bottle with liquid – weight of empty bottle) / (weight of bottle with water – weight of empty bottle).

Use of whey as natural medium for EPS production

Whey is the by- product of soft cheese industry, The obtained whey was centrifuged to remove fat (<.05%) and then adjusting the pH to 5 by adding 0.1 N HCL and then heated for 30 min at 90° C and filtered with (Whatman No 0.4) filter papers to get rid of Whey proteins and then The resulting supernatant was adjusted to pH 6.8 with1M NaOH, Sterilization was done at 90 °C for 4-5 minutes.Whey was used either enriched with glucose 2.5, 5, 7.5, 10, 12.5% for EPS production by Lactococcus lactis subsp. lactis , or used after adding numbers of the enriched materials to its structure depending on result of previous study on EPS production by using the same bacteria in the fermentation medium M17 after fixation of the fermentation conditions [12]. The added materials were 10% glucose , 1.5 % casein , 1.5% K_2HPO_4 ,0.025% $MgCl_2$, 0.5% Vit.c and 0.01% $CaCl_2$.

Use of Date juice (hot, cold extraction) as natural medium for EPS production

The Date juice was prepared by mixing Date with distilled water as rate of 1:3 (weight / volume) and heated at 80 ° C for 2 hours with mixing in hot extraction method and at 40 ° C for 6 hours in cold extraction method, then the mixture was extracted by bdting cloth. Date juice was then used alone or enriched with glucose at rates (2.5, 5, 7.5, 10, 12.5 %) for EPS production. The enriched date juice by adding the same materials that were added to Whey were also studied.

Extraction and purification of EPS

The EPS was extracted from Lc. lactis subsp. lactis bacteria growing medium by using the methods that described by Van geel – Schutten, et al., and Prathima, et al., [13, 14].

EPS recognition using the thin layer chromatographic technique (TLC)

For EPS exploring that was prepared in this study , the standard monosaccharide and the monosaccharide resulted from EPS digestion [15] at 5 micro liter sample were put at 1 cm distance from silicate plates edge , and then mixture of solvents (propanol : acetic acid : water) was used in 3:1:1 volumetric ratios as moveable phase to separate the saccharides. The plates were sprayed by the indicators solution (85%- phosphoric acid: 0.2% α - naphthoresorcinol stain) as rate 1:9 (v /v), then R_f values were calculated for the standard saccharides and monosaccharide of EPS.

EPS recognition by using High Performance Chromatographic Technique (HPLC)

It was done by using HPLC instrument (provided by Shimadzu 10 AV-LC company) and the sample of acidified EPS was analyzed and the sample was injected under the following condition : column : (4.5x 50mm) , movable phase : 15ml – NaOH was mixed with 1 ml barium acetate, Runoff speed: 1.5ml / min. , Temperature: 40 ° C, Injection volume: 20 microliter , column : C18.

EPS concentration was calculated [16] as follows:

The unknown concentration = (sample area / area of standard) x standard concentration x dilution numbers.

Detection EPS by using qualitative tests

Rhaminose sugar and N-acetylglucose amine compound were detected by using qualitative tests which were conducted according to the method described by www.dreamjordan.com Internet [17].

Use of ESP in process cheese industry

The following materials were used in industry of the process cheese: One kilogram of manufactured soft cheese, 2.3% emulsion salts (type S4 JOHA), 3.25% Serum powder, 0.93% table salt, 0.23% lemon acid, 0.23% potassium sorbet and 18.6% water. The prepared EPS in this study was used instead of the emulsion salts in 2.3, 3.3, and 4.3% concentrations [18]. Soft cheese was chapped and minced and put in cooking pot and then the following materials : serum powder , emulsion material , table salt, lemon acid , potassium sorbet and water were added and cooked at 90c for 5 minutes with shaking and was filled while it was hot in glass cans and then it was cooled, storage.

Sensory evaluation

The product was evaluated by number of agriculture college staff- University of kofa (they were familiar with dairy products) by using a specified process cheese evaluation forms that based on evaluation of these characteristics: color, fat isolation, flavor, body, texture, taste and bitterness. Score was based on hedonic scale of 1-10 (1 = dislike extremely and 10 = like extremely) [19].

Statistical analysis

The data were analyzed using Randomized Complete Block Design (RCBD).The significant differences between means were separated by LSD and determined at $P \leq 0.05$ [20].

RESULTS AND DISCUSSION

Detection of sugars by physicochemical tests

Molich test

The results in Table (1) showed that all the studied lactic acid bacteria gave positive result in this test (formation of violet ring) indicating the presence of sugars generally in the inoculated media with this bacteria. These results were confirmed by Stingele et al., [21]they detected on sugars of Streptococcus thermophilus medium .Reaction of the added H_2SO_4 with the quintet hex sugars during this test work on removal of water molecules and every one of them may react with α- phenolephthol and formats violet-red compound as violet ring between the two separation surfaces [15].

Fehling test

Results of this test were negative in all the bacterial isolates (Table1) .This means no red precipitate formation was formed in the test tubes which reflected absence of reduced disaccharides in the these bacterial media , similar results were obtained by Seedevi et al., [22] when they detected on the exopolysaccharide produced in Lb. bulgaricus bacterial medium .

Iodine test

All studied lactic acid bacteria gave negative iodine test (the color of cursor has not changed) (Table1) and this indicates that the produced polysaccharide lactic acid bacteria grown in this media neither had the monosaccharide glucose nor the inulin , it may be concluded that the polysaccharide produced in the medium is a heterogeneous type (contains more than on type of monosaccharide) .This result agreed with those found by Nishimura [23] who mentioned that the polysaccharide produced by Lc. lactis subsp. cremoris was a heterogeneous polysaccharide formed from monosaccharide's glucose and galactose.

Determination of total carbohydrates (sugars)

Data in Table (1) showed the Lc. lactis subsp.lactis bacteria gave the highest carbohydrate amount (196.2 mg L^{-1}) ,While the less amount of total carbohydrate was in St. thermophilus medium (154.37 mg L^{-1}).

Viscosity test

The results showed that Lc. lactis subsp. lactis gave high viscosity medium (13.98 centipoises) followed by Lb. plantarum , then Lb. bulgaricus and lastly St. thermophilus bacteria as the viscosity values were: 12.3 , 11.31 and 8.37 centipoises respectively (Table 1), According to these results it may be said that Lc. lactis subsp. lactis were the highest producer to exopolysaccharide (EPS) in the fermentation medium .These results were in accordance with those reported by Looijesteijn et al., [24] in which increase of viscosity of medium that inoculated with Lc. lactis subsp. cremoris bacteria is an indicator of rise of the produced polysaccharide.

According to the last results (Table 1), it has been selected Lc. lactis subsp lactis bacteria for EPS production in this study.

Table (1): Physiochemical tests for the detection of sugar

Determination of total carbohydrates (sugars)	Viscosity	Iodine	Fehling	Molich	Tests / Bacteria
196.2	13.98	-	-	+	Lc. lactis*
193.57	12.30	-	-	+	Lb. Plantarum
189.72	11.31	-	-	+	Lb. bulgaricus
154.37	8.37	-	-	+	St. thermophilus

Use Whey as natural medium for EPS production

From fig (1), it may be noticed that enrichment of Whey with glucose in the following concentrations: 2.5 , 5 ,7.5 ,10 and 12.5% increased the production of EPS by the studied bacteria and the highest average of EPS production was 0.162 g L^{-1} in addition of 12.5% glucose to the fermentation media, compared with 0.115 mg L^{-1} in the control treatment (Whey + 0% glucose), the values were high significantly ($P<0.05$) different between these two treatment, this may be due to that the presence of glucose with whey increases the metabolic activity and then increases the mechanism of monosaccharide binding with nucleotides when EPS creation process in this bacteria[25, 26]. This result agreed with Zhang et al., [27] who reported that concentrated Whey was used in growing St. thermophilus STI strain medium, the EPS production increased often enrichment with glucose to 52.74% compared with EPS production in non- enriched whey by glucose. Kosikowski and Giec [28] mentioned that best EPS production was with using lactose sugar enriched Whey at rates ranged between 5 to 12% and clear decline in the biological mass was indicated in the higher of the last rate especially when 15% lactose sugar was used.

ISSN: 0975-8585

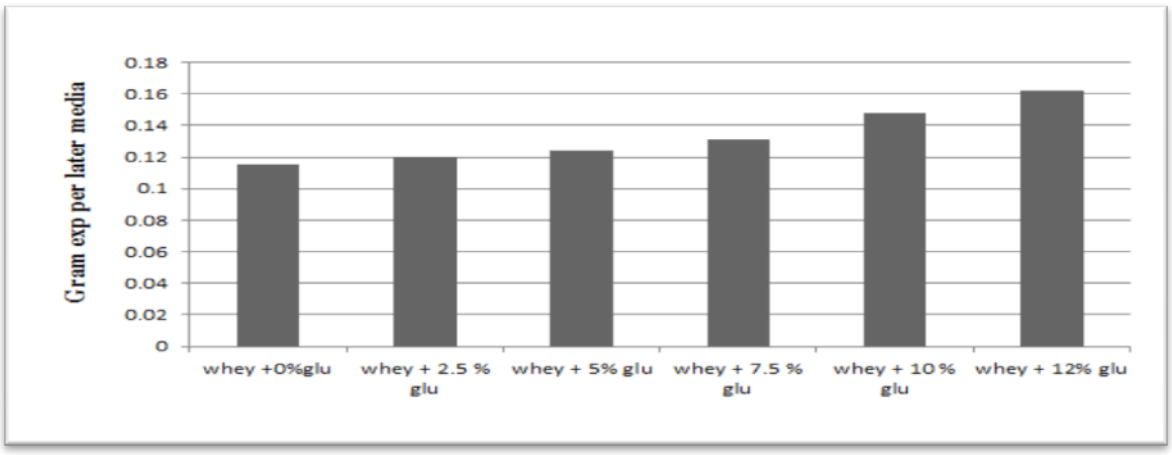

Fig (1): Effect of Whey use with different rates of glucose in EPS production.

It may be seen presence a clear increase in quantity of the produced EPS in the enriched Whey with (10% glucose, 1.5% casein , 0.1% $CaCl_2$ and 0.5% Vit.C),which reached 1.545 mgL^{-1} compared with 0.12mg L^{-1} EPS in the non-enriched Whey, significant differentes ($P<0.05$) were found between treatments (figure 2).This result exceeds on result of Prathimo et al., [14] when Whey enriched with 10% glucose , 1% casein, 0.1 mgL^{-1} $CaCl_2$ was used in EPS production by Lc. lactis bacteria in which EPS value was 153.39 mg L^{-1},While Schwartz and Bodie [29] found that use Whey that enriched with 10% sucrose , 0.05% yeast extract , 0.1% K_2HPO_4 resulted increase EPS(dextran) production by Leuconostoc mesenteroides compared with use non-enriched Whey.

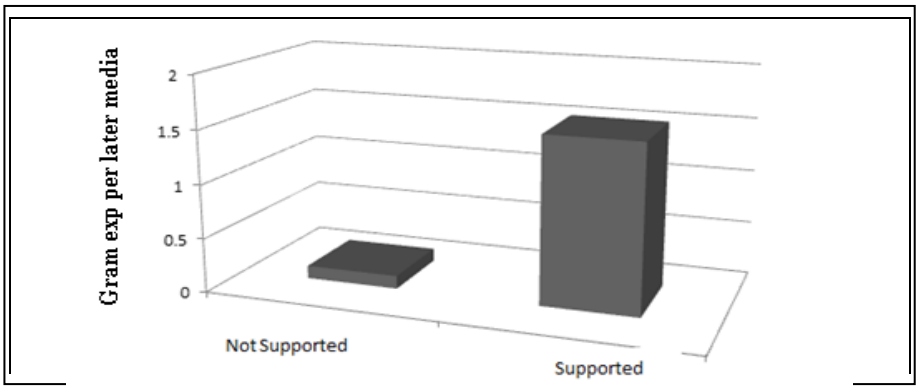

Fig (2): Use of Whey as natural medium for EPS production

Use of date juice (hot and cold extraction) as natural medium in EPS production

Date juice was used for EPS production it is a cheap and available source and the result in Fig (3) showed superiority of date juice (hot extraction) compared with date juice (cold extraction) in term of quantity of the produced EPS and they were 0.113 and 0.1 gm for each of them respectively.

Data juice enriched (hot extraction) with the carbonic source (glucose) had positive effect on EPS production from the studied bacteria (Fig 4). It can be shown from the result that the best of glucose concentration is 12.5% in which EPS concentration was 0.13 g L^{-1} often it was 0.113 gL^{-1} in control treatment, there were no significant ($P>0.05$) differences between treatments and the control, this increase may be due to the consideration of glucose in date juice and the added glucose as encouraging material in creation process of EPS [30].

ISSN: 0975-8585

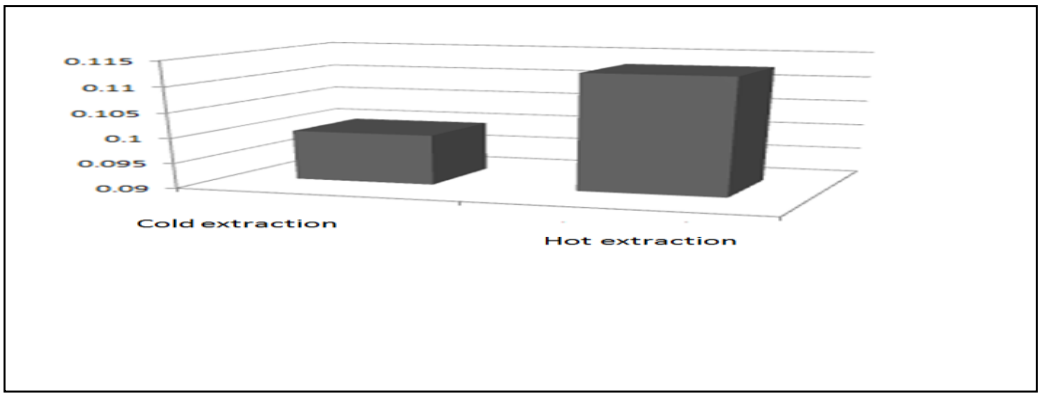

Fig (3): Effect of date juice use on EPS production

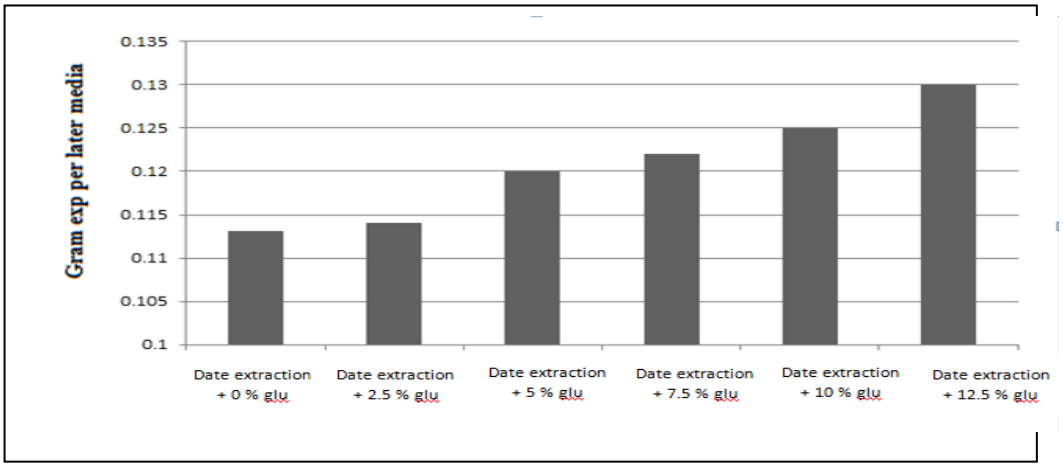

Fig (4) Effect of date juice and with different rate of glucose use on EPS production

The amount of EPS that produced in Date juice was not satisfied compared with that produced in Whey as natural media enriched with glucose (as it is shown in the last results),That may be due to containing Date juice of fructose sugar more than glucose sugar and the former effects negatively in creation of EPS bacteria process because it needs phosphorlation process to convert it to fructose -6- phosphate and the glucose -1- phosphate compound and this needs spending more energy by the cells to creation EPS process [31,32].

Recognition of EPS by using techniques of Thin Layer Chromatography (TLC)

It can be seen from Fig (5) that the produced EPS consists of the monosaccharides: Galactose , Rhaminose and N-acetylglucosamine through similarity of (R_F) value of EPS with (R_F) values of the standard monosaccharide, that shows that the produced EPS is a hetero-exopolysaccharides (HePSs). Suzuki et al., [33]found that EPS produced by strain of Lc. lactis subsp. lactis bv.diacetylactis was a hetero-exopolysaccharide but it differed in type of monosaccharides that was part in its structure according to the strain produced EPS. They observed that the monosaccharide composed ESP for the first strain belonging to the same species was formed from(glucose – N- acetylglucosamine ramons), while the EPS of the second strain formed from (galactose- glucose – fucose – rhaminose), that explains that the exopolysaccharides of different strain LAB vary greatly in monosacchamide composition . However,Sanalibaba and Cakmak [34] indicated that the EPS produced by Lc. lactis subsp . lactis was Homopolysaccharide (HoPs) and it is called polygalatan and consisted from monosaccharide galactose only.

ISSN: 0975-8585

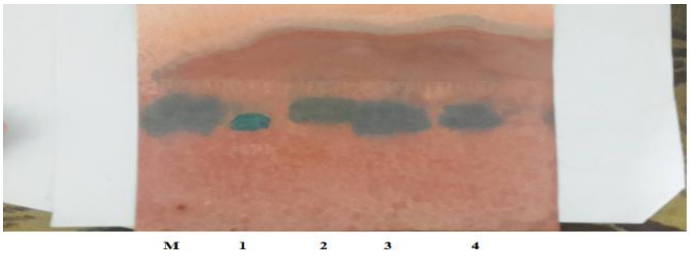

Fig (5): thin layer chromatography of the produced EPS.

M: represents model of the produced EPS, 1: glucose, 2: galactose , 3: Rhaminose and 4: N-acetyl glucosamine.

Recognition of EPS by using HPLC chromatography

Figure (6) and (7) shows that the EPS produced by Lc lactis consists from N-acetyl glucosamine and the two monosaccharide Galactose and Rhaminose with the following concentrations (0.122, 0.323 and 0.306) mg ml^{-1} respectively as it is shown in table (2).

Table (2): sugars and compounds in EPS structure

Saccharide	Focus mg /ml.	The proportion in EPS%
N- acetylglucoseamine	0.122	16.14
Galactose	0.323	43.1
Rhaminose	0.306	40.76

Presence of N-acetyl glucosamine and the monosaccharides with this ratios in the EPS structure produced by the used bacteria in this study may be due to the used strain of bacteria , there are other factors such as type of the growth media, incubation time and source of carbon in growth medium and its concentration may effect on the chemical structure of EPS and its molecular weight [35]. Suzuki et al., [33] indicated that one strain of Lc. lactis subsp lactis bv. diacetylactis produced polysaccharide consisted of glucose - N-acetylglucoseamine - Rhaminose with 54.4, 37.3 and while another strain of the same species of the bacteria produced polysaccharide consisted of 8.3% ratios respectively, galactose , glucose, fucose and Rhaminose with 39.2, 20.2 , 14.5 and 26.1 % ratios respectively. Kleerebezem et al., [36] mentioned that the produced EPS by Lc. lactis bacteria was consists of two type of monosaccharides galactose and rhaminose with 2:1 ratio series. Knoshaug et al., [37] found that Lactococcus lactis subsp. cremoris ropy 352 strain formed two type of polysaccharide, the first consisted of glucose and galactose with 1:0.72 ratios and the second type consisted of galactose, glucose and mannose with 1:0.5:0.22 ratios along the carbonic series of the produced EPS by these bacteria.

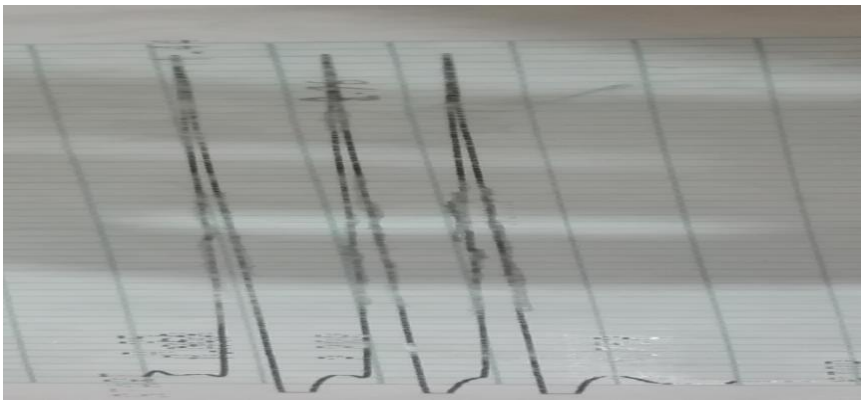

Fig (6): Spectral analyzing curve of the standard monosaccharides by HPLC.

ISSN: 0975-8585

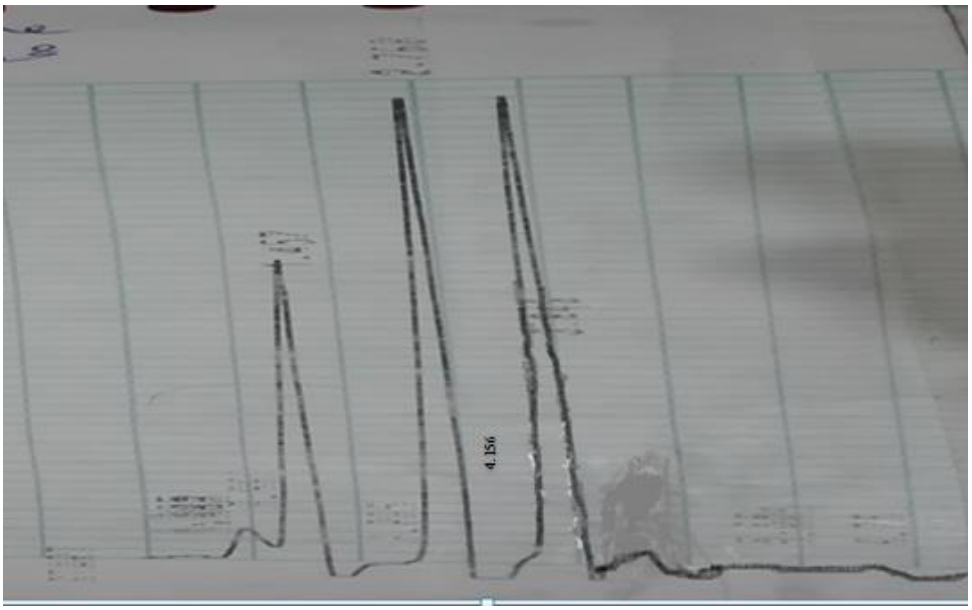

Fig (7): Spectral analysis curve of the monosaccharide consisted of the produced EPS by HPLC

Quality test results

Detection of Rhaminose sugar

Color of the mixture change from red to yellow color was observed and this indicates the presence of terminal – CH3 group and presence of Rhaminose sugar in the produced EPS by Lc. Lactis bacteria.

Detection of N- acetylglucoseamine compound

The result showed the change of sunflower paper from red to blue, and this is an indication of presence of N-acetyl glucose amine in the exopolysaccharide.

Results of using EPS in the process cheese manufacturing

Table (3) shows the marks that were given by the panelist of the process cheese produced by adding EPS in different concentrations 2.5, 3.5and 4.5% compared with the control treatment (2.3% emulsion salts), the results showed that the 4.5% ESP treatment in which the total score of grades was 60.833 did not significantly differ with the control treatment in which the total scores of grades was 62.667. And the grades that were given to the properties color, fat isolation, flavor, body, texture, taste and bitterness were 8.66, 8.83, 9.5, 8.66, 7.33, 8.83 respectively for 4.5% EPS treatment, that may be due to the fact that process cheese has a weak tendency to bind water and the EPS works on increasing cheese water content by water adsorption and increasing the size of casein micelle [38]. These results agreed with findings by Hassan et al., [39], the produced EPS by St. thermophilus bacteria used in improving AL-Koresh cheese texture. Caric [40] mentioned that the produced EPS by the thermophilic bacteria besides using heating and shaking may lead to the conversion of the cheese texture during cooking process to soft , homogenous texture like cream in which casein aggregation was dispensed and this is called creaming action to smaller and smaller aggregates and then to macro hydrophobic molecules. Meyen [41] found that the cooking process with help of the emulsion material, temperature ad shaking result to alteration the insoluble para casein that is being as gel to liquids form and then transformed to liquid mass during cooling to firm gel again, so we can get a new type of cheese differs in its properties from the original cheese used.

Table (3): the results of sensory evaluation of the process cheese that manufactured by using different concentrations of EPS and emulsion salts (control treatment)

ISSN: 0975-8585

Total degrees 70	bitterness	taste	Texture	Body	flavor	fat isolation	Color	Adjective / Focus
	10 degrees	10 degrees	10 degrees	10 degrees	10 degrees	10 degrees	10 degrees	
B 50.167	9.66	8.50	6.50	6.16	7.50	6.33	9.50	EPS 2.5%
51.333 B	8.00	7.83	5.83	6.00	7.83	6.33	8.33	EPS 3.5%
60.833 A	8.83	7.33	8.66	8.66	9.50	8.83	8.66	EPS 4.5%
62.667 A	9.33	8.16	8.50	8.50	9.16	8.66	9.50	Control
						5.4284 *		LSD

Each treatment represents seven replicates, the mean values that have the same letters mean presence of significant differences between them ($p < 0.05$).

CONCLUSIONS

This study proved that the bacteria Lactococcus lactis subsp. lactis was more efficient in production of Exopolysaccharide (EPS) compared with the bacterial species of lactic acid bacteria in this study. The highest productivity of EPS was obtained when the bacteria mentioned were grown in the whey as a natural economic medium with the addition some of the supports and in the optimal cultural conditions of production. The possibility of using EPS as an emulsifier in the manufacture of process cheese as an alternative to emulsifying salts of potassium and sodium sorbet.

REFERENCES

[1] Patel , A.K.; Michaud, P. ; Singhania , R . R.; Soccol,C.R.and Pandey ,A.(2010). Polysaccharides from Probiotics as Food Additives. Food Technol. Biotechnol. 48 (4) 451–463 .

[2] Tallon , R.; Bressollier , P. and Vrdaci , M . (2003). Isolation and characterization of two exopolys accharides produced by Lactobacillus plantarum EP56, Res . Microbiol. 154: 705 – 712.

[3] Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiology Letters, 87, 1990, 113–130.

[4] De Vuyst, L.; De Vin, F.; Vaningelgem, F.and Degeest, B. (2001). Recent developments in the biosynthesis of hetero-polysaccharides of lactic acid bacteria. International Dairy Journal. 11(9) 687–707.

[5] Garcia-Garibay, M and Marshall, V.M.E. Polymer production by Lactobacillus delbrueckii subsp. bulgaricus. Journal of Applied microbiology, 70, 1991, 325–328.

[6] Aslim , B. ; Yuksekday , Z . N.; Beyatli, y. and Marcan , N. (2005). Exopolysaccharide Production by actobacillus delbrukii subsp. bulgaricus and Streptococcus thermophillus strains under different growth condition . World Journal of Microbiology & Biotechnology. 21: 673 – 677.

[7] Akcelik , M . , Sanlibaba , P. (2002) . Characterization of an exopolysaccharide preventing phage adsorption in lactococcus lactis subsp. cremoris MA3a , TURK . J. Vetamin Sci . p.p 20: 1151 -1156.

[8] Atlas , R . M.; Parks, L. C. and Brown, A. E. (1995). Laboratory manual of experimental microbiology; Mosby-ear Book. Inc. Missouri. U.S.A.

[9] A.O.A.C (1990). Association of Analytical Chemists. Official method of Analysis. 15th edition. Washington, D.C.

[10] Al- Dalale, B. K. and Hakim, S. H. (1987). Food Analysis. Dar Al-Hikma Press Printing Press. University of Al Mosul

[11] Dubois , M. ; Gilles , K . A. ; Hamilton , J . K . ;Robes , P . A. and Smith, F. (1956) . Colorimetric method for determination of sugar and related sub stances. Anal. Chem . Vol. 28 pp: 350 – 356

[12] Mousa, I. F. and wit wit, M. F. (2017). Study the optimal conditions for the production of Exopolysaccharide byLactococcus lactis subsp lactis bacteria. Journal of Babylon University of Pure Sciences. Volume XXVI. Second issue.

[13] Van geel – Schutten , G . H.; Flesch , F. ; T en brink , B. ; Smith , M . R. and Dijkhuizen , L . (1998) Sareening and Charaterization of Lactobacillus srtrains producing large amounts of exopolysaccharides. Appl. Microbial biotechnol. 50: 697 – 703.

[14] Prathima , P. C. ; Lule , V. K . ; Tomar , S . K. and Singh , A . K. (2014) . Optimization of Exopolys accharide production by Lactococcus lactis NCDC 191 by Response Surface metodalong . Int. J . Microbiology and Appl .Sci 3(5): 835-854 .

[15] Harper, A. H. (1973) . Review of physiological chemistry 15 th Ed. Lange medical publication .

[16] Cataldi, T. R. I.; Margiotta, G.; Lasi, L.; Dichio, B.; Xiloyannis , C . and Bufo , S . A. (2000). Determination of sugar compounds in olive plant extracts by anion – exchange chromatography with pulsed amperaometric detection. Anal. Chem. 72: 3902 – 3907.

[17] http://www.dreamjordan.com Internet. (2012). Detection by using qualitative tests.

[18] Al-Khalayleh, N. I. and Tayfur, A. (2011). Study extensible process cheese manufacturing using local white cheese, Kashkaval, cottage cheese as raw materials. Damascus University of Agricultural Sciences Journal 27. (1) 391 - 403.

[19] Abdel Sada, F.H. (2013). Manufacturing an economic mix of process cheese, Kufa Journal of Agricultural Sciences. 5(1): 162 - 178.

[20] Al-Rawi, Kh. M. and Khalafallah, A. M. (1980).Design and analysis of agricultural experiments. Dar Al Kutub Printing & Publishing Est.University of Al Mosul.

[21] Stingele, F.; Neeser, J.R. and Mollet,B.(1996). Identification and characterization of the eps.(Exopolysaccharide) gene cluster from Streptococcus thermophilus Sfi6. J Bacteriol 178: 1680-1690.

[22] Seedevi, P.; Sudharsan, S.;Kumar, V. S.; Srinivasan, A. and Vairamani, S. (2013). Isolation and characterization of sulphated polysaccharides from Lactobacillus bulgaricus BN7 collected from yoghurt. Adv. Appl. Sci. Res5 (4):78–83.

[23] Nishimura,J.(2014). Exopolysaccharides produced from Lactobacillus delbrueckii subsp. bulgaricus. Laboratory of nutrition and life sciences. Int. technology. pp 1017-1023.

[24] Looijesteijn , P.J.; van Casteren , W.H.M. ; Tuinier , R. ; Doeswijk-Voragen , C.H.L. and Hugenholtz , J. (2000) . Influence of different substrate limitations on the yield composition and molecular mass of exopolysaccharides produced by Lactococcus lactis subsp. cremoris in continuous cultures. Journal of Applied Microbiology. 89: 116- 122.

[25] Pailin, T.; Kang, D.H.; Schmidt K. and Fung, D.Y.C. (2001). Detection of extracellular proteinase in EPS-producing lactic acid bacteria cultures on skim milk agar. Lett. Appl. Microbiol. 33: 45–49.

[26] Ludbrook , K.A. ; Russell, C.M. and Greig R.I. (2006) .Exopolysaccharide Production from Lactic Acid Bacteria Isolated from Fermented Foods . Journal of Food Science 62(3):597 – 600.

[27] Zhang, C.T.; Li, S. and Zhang, Y. (2011). Growth and exopolys accharide production by Streptococcus hermophilus ST1 in skim milk. *Braz J. Microbiol. 42(4): 1470–1478.*

[28] Kosikowski, F.V. and Giec, A. (2006). Activity of Lactose Fermenting yeasts in producing biomass from concentrated whey permeates. J. Food Sci. 47: 1892-1894.

[29] Schwartz, R. D. and Bodie, E. A. (1984). Production of viscous dextran – containing whey – sucrose broths by Leuconostoc mesenteroides ATCC 14935. Applied and Environ. Microbiol. 48(3): 678-679.

[30] Boels ,I.C. ;Van Kranenburg , R.; Hugenholtz, J. ; Kleerebezem ,M. and De Vos ,W. M.(2001).Sugar catabolism and its impact on the biosynthesis and engineering of exopolysaccharide production in lactic acid bacteria. International Dairy Journal . 9 (11): 723–732.

[31] Ryan, P.M.;Ross,R.P.; Fitzgerald, G.F. ; Caplice, N.M. and Stanson,C. (2015). Sugar coated: exopolysaccharides producing lactic acid bacteria for **food and human health applications. Food Funct 6: 679-693.**

[32] Juvonen, R.; Honkapää, K.;Maina, N.H.; Shi,Q. and Viljanen, K.(2015).The impact of fermentation with exopolysaccharide producing lactic acid bacteria on rheological, chemical and sensory properties of pureed carrots (Daucus carota L.). Int J Food Microbiol 207: 109-118.

[33] Suzuki C.; Kobayashi M. and Kimoto – Nira H. (2013). Novel exopolysaccharides produced by Lactococcus lactis subsp. lactis and the diversity of esp. E Gene in the exopolysaccharides biosynthesis Gene Clusters. Bio.Sci. Biotechnol. Biochem.77 (10):2013-2018.

[34] Sanalibaba, P. and Çakmak, G.A. (2016). Exopolysaccharides Production by Lactic Acid Bacteria. Appli Micro Open Access 2 (10): 2471-9315.

[35] Cerning, J. (1995). Production of exopolysaccharides by lactic acid bacteria and dairy propioni bacteria. Le lait: 463 –472.

[36] Kleerebezem, M.; van Kranenburg, R.; Tuinier, R.; Boels, I.C.; Zoon, P.; Looijesteijn, E.; Hugenholtz, J. and de Vos, W.M.(1999). Exopolysaccharides produced by Lactococcus lactis from genetic engineering to improved rheological properties. Ant Van Leeuwen. 76: 357-365.

[37] Knoshaug, E. P.; Ahlgreat, J. A. and Trempy, J. E. (2000). Growth Associated exopolysaccharide expression in Lactoccus lactis subsp. cremoris Ropy 352.National center for agricultural utilization research.Dairy Sci 83:633 –640.

[38] Kaya, S. (2002). Effect of salt on hardness and whiteness of Gaziantep cheese during short term brining; J. Food Eng 52: 155–159.

[39] Hassan, A.N.; Corredig, M.; FranK, J.F. and Elsoda, M. (2004). Microstructure and rheology of an acid-oagulated cheese (Karish) made with an exopolysaccharide-producing Streptococcus thermophilus strain and its exopoly saccharide non-producing genetic variant. J. Dairy Res.:71(1):116-120

[40] Caric, M. (1993). Processed cheese product, In cheese: Chemistry, physics and microbiology. P 476-505. volum. . P .f. Fox ed. chapmonaud hall; New york.

[41] Meyer, A. (1973). Processed Cheese Manufacture. Food Trade Press Ltd. London- UK.