Oligosaccharides Production by *Leuconostoc holzaapfelii* Strain S7 Using Sugar Cane Molasses as Fermentation Medium

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Abstract

Oligosaccharides (OS) are considered as functional food and prebiotics. They employed in food, feed, pharmaceutical and agricultural applications as well as used as cosmetics and immune-stimulating agents. Oligosaccharides may be isolated from natural sources or chemically synthesized, but the prices are high. An alternative approach to oligosaccharide synthesis is to use microbial production. The high cost of OS production is the major restriction in the industrial production. So, this study was aimed to produce OS by *Leuconostoc holzaapfelii*strain S7 using sugar cane molasses as low cost fermentation medium. The yields of OS by this bacterial strain reached to 21.55, 28.82, 37.35, 44.23and 30.93$gl^{-1}$when used sugar cane molasses at concentrations of 130, 160, 200, 262 and 290 gl^{-1}, respectively, comparing with 64.80 gl^{-1} OS formed by the same strain grown on modified sodium gluconate medium at 7 pH fortified by 80 gl^{-1}sucrose and 5gl^{-1} of a mixture of equal weight of each of yeast extract, peptone & beef extract, incubated at 25°C for 72 hours. Chemical characterization of the obtained OS was achieved using Fourier Transform-Infra-Red spectroscopy (FT-IR*)* and Liquid Chromatography-Mass Spectrometric (LC-MS).Also, their melting point and monosaccharaides content in extracted product were determined. It could be concluded that sugar cane molasses used in

the current study was suitable as low cost medium for production of OS by the tested bacterial strain with considerable yield level.

Keywords: Oligosaccharides, polysaccharides, bacteria, molasses, fermentation.

Introduction

According to IUPAC & IUBMB nomenclature, oligosaccharides (OS) are low molecular weight carbohydrates, most of them are relatively short chains which contain between 3 and 10 sugar moieties[1]. Although the degree of polymerization could go down to 2 for some OS like lactulose (disaccharides) or up to 60 for some non-digestible oligosaccharides (NDOs), like chicory inulin[1-3].Short chain lengths of chicory inulin up to 20 fructose units are called fructo-oligosaccharides[4].Otherauthorities classify OS including anyone from 3 to 19 monosaccharides. However, there is no a rational physiological or chemical reason for setting these limits[5].

Some of OS consist of α-configuration which can be degraded by human α-glycolytic enzymes. Some other OS offer specific physicochemical properties (β-linkages) in which they are resistance to the digestive process in the upper part of the gastrointestinal tract and called non-digestible oligosaccharides (NDOs).NDOs are considered as functional food because they have beneficial effect on the host health. In addition, most of the NDOs are also classified as prebiotics. A number of OS have been used in foods and beverages such as candies, confectioneries, bakery products, fermented products, fruit juices, desserts, and spreads as taste improver, sweetener, fat replacer, emulsifier, increasing viscosity agent, stabilizer of proteins, flavors and colors. Most NDOs are produced enzymatically. Enzymes used to produce oligosaccharide compounds through degradation of polysaccharide or building up from mono- and disaccharide[4,6,7].Some non-food applications for oligosaccharides have also been proposed.NDOs can also be employed in feed, pharmaceutical, agricultural applications and in cosmetics as stabilizers, bulking agents and immune-stimulating agents[8].

The high cost of OS production is the major restriction in the industrial production and the cost of the sugar (carbon source)

consider one of the most important factors to determine the commercial success of OS production. Recent investigations were interested in production of lower cost OS and exo-polysaccharides (EPS) for biotechnological applications[9]**.**Therefore, an inexpensive substrate must be found to reduce the cost of the raw materials. Agro - industrial wastes are used as substrates for a cost-effective production[9].Since molasses was available as a cheaper substitute for sucrose, so cane molasses has been used as cheaper source of sucrose for industrial fermentations**.** Cane molasses is rich with high sucrose content, organic nitrogen, minerals and salts so it is good nutritional value for cultivated microbes[10]**.** Molasses is a by-product of sugarcane industry; it is obtained in the extraction process of sugar by repeated crystallization**.** Sugarcane molasses could be a better source of carbon due to higher content of total sugars nearly 48- 50 %**[11].**

Lactic acid bacteria (LAB)are common Gram positive and lactobacillus bacterium. LAB are a group of the richest sources of OS as well as EPS. They are microorganisms widely used for a long time particularly in fermented dairy industry and pharmaceutical applications. LAB are generally recognized as safe (GRAS) in which no reports of any illness or toxicity recorded or any harmful compounds produced by them[12]**.** Recently, LAB stirred up a sensation for their ability to produce many valuable products in particular OS and EPS[13]**.**OS and EPS from LAB have received increasing attention because of their potential applications in many fields[14-16].LAB able to produce a broad range of different OS and EPS with different composition and functionality and this led to widening their industrial applications. Most OS and EPS producing LAB belong to the genera *Lactobacillus, Streptococcus*, *Lactococcus*, *Pediococcus*, and *Leuconostoc*. Also, some strains of the genus *Bifidobacterium*are able to produce these biopolymers[17].

Thus, considering the importance of OS produced by bacterial strains and aiming to use a low cost substrate for it's production, the present work has the goal of evaluating the potential use of sugarcane molasses as alternative growth substrate to produce OS by *Leuconostoc holzaapfelii*strain S7(which recorded as a highly EPS-

producing strain in our previous study[18]under optima fermentation conditions.

Materials and Methods

Bacterial strain

Leuconostocholzaapfelii S7 was isolated from fermented apricot by the same authors in previous study[18]and itsgene sequences was deposited in the Gene bank database under accession number MN251672.Tryptic soy broth wasused for inoculum preparation. It consists of (gl^{-1}): Tryptone, 17; soy peptone, 3; glucose, 2.5; sodium chloride, 5 and di-potassium phosphate, 2.5. Medium pH was adjusted at 7.3.

Sugar cane molasses

Sugar cane molasses was kindly provided from Abo-Qurqas sugar factory, Al-Minia, Egypt. **Chemicals**

All chemicals used in this work were pure (Sigma and Merck) analytical grade.

Synthetic media

Sodium gluconate (NaG) medium modified from **Jakob*et al.*[19]**and it consists of the following (gl^{-1}): sucrose, 80; sodium gluconate, 20; yeast extract,3; peptone, 2; glycerol, 3 and mannitol, 10at 6pH.Other medium**(R)** described by **Rawal *et al.*[20]** and consist of (gl^{-1}): sucrose, 80; beefextraxt, 15; NaCl, 5; K_2HPO_4, 8; KH_2PO_4, 2 and $MgSO_4$, 0.5 at 7 pH. The third medium (IV) used was formed by **Iliev and Vasileva[21]** and consist of (gl^{-1}): sucrose, 80; yeast extract, 20; K_2HPO_4, 20;$MgSO_4.7H_2O$, 0.2; $MnSO_4.H_2O$, 0.01, NaCl, 0.01, $CaCl_2$, 0.02 and $FeSO_4.7H_2O$, 0.01 at 6.9 pH.

Molasses medium

The sugar cane molasses was clarified and diluted with distilled water containing solution of NaH_2PO_4(final concentration of 1.5 gl^{-1}) as method described by **Mattos *et al.*[22]** to obtain a series of sugarcane molasses media (W/V) with the following concentrations:13% (130 g/l), 16% (160 g/l), 20% (200 g/l),26.2% (262 g/l) and 29% (290 g/l).The diluted molasses was autoclaved at 120^{o}C for 30 min and allowed to settle for 24 h.[23].The sterilized clarified molasses was then separated from the solid material. Yeast extract (6gl^{-1}) was added to the clarified molasses and the pH was adjusted to 7.0.

Optimization of bacterial growth conditions for maximization of OS production.

Single-factor experiments were carried out in 500 ml flasks containing 100 ml medium. Four carbon source (glucose, sucrose, lactose and fructose) and six nitrogen sources (yeast extract, peptone, beef extract, gelatin,$(NH_4)_2SO_4$and NH_4Cl in addition to a mixture from yeast extract- peptone and beef extract at equal levels) were used. Four pH degrees (5, 6, 7 and 8) and five incubation temperatures (25, 30, 35, 37 and 40°C) were examined at different four incubation periods.

The effect of production media components.

To elucidate the effect of medium on OS production, three production broth media supplemented with 8% sucrose (NaG, IV and R)were assessed by inoculation each of the three media with 1% fresh inoculum of the bacterial strain, then incubation at 30^{o}C for 3 days. The OS was precipitated, purified, dried and weighted (gl^{-1}).

Effect of incubation period

Effect of incubation period on OS production by the bacterial strain was done using the NaG medium. Prepared medium was inoculated by 1% inoculum and incubated for 24, 48, 72 and 96 h at 30^{o}C after which the optical density (OD) and OS weight (gl^{-1}) were determined at the previous periods.

Effect of temperature

For studying the influence of temperature on OS production, the prepared NaG medium was inoculated with 1 % inoculum and incubated at different temperatures of 25, 30, 35,37 and 40 °C for 72 h. The OD and OS weight (gl^{-1}) were estimated.

Effect of initial pH

In order to determine the suitable pH improved OS production, bacterial strain was inoculated at1% into NaG medium with different pH (5.0, 6.0, 7.0 and 8.0, separately). Adjusting pH was done using 1M phosphoric acid and 1M NaOH before sterilization. Cultures were incubated at 25^{o}C for 72 h. The OD and OS weight (gl^{-1}) were determined.

Effect of nitrogen sources

Different nitrogen sources at 0.5% concentration (yeast extract, peptone, gelatin, beef extract, ammonium sulfate and ammonium chloride in addition to a mixture at equal weight of each of peptone, yeast extract& beef extract) were introduced, as sole nitrogen source, into the production medium (NaG broth with 8% sucrose as carbon source and without any nitrogen source), individually, to determine the effect of nitrogen source on OS production. The pH of the broth medium was adjusted to 7.0. Prepared medium was inoculated by 1% inoculum and incubated at 25^{o}C for 72 h. The OD and OS weight (gl^{-1}) were determined.

The effect of carbon sources

Different carbon sources at 4, 6 and 8% concentrations for each (sucrose, lactose fructose and glucose), individually, were introduced to NaG medium fortified without carbon source and with 0.5% mixture of equal weight of each of peptone, yeast extract& beef extract as nitrogen source at pH 7 to determine the effect of carbon source on OS production by the tested bacterial strain. Prepared medium was inoculated by 1% inoculum and incubated at 25^{o}C for 72 h. The OD and OS weight (gl^{-1}) were determined.

Production of OS using sugar cane molasses as fermentation medium

Five different concentrations of sugar cane molasses were used in this experiment to examine the possibility of OS production by the bacterial strain under study using molasses as low cost fermentation medium. These concentrations were 13, 16, 20,26.2 and 29%. The prepared molasses media at pH 7 were inoculated with 1 % inoculum and incubated at 25°C for 72 h. The OD and OS weight (gl^{-1}) were estimated.

Extraction and purification of OS

For extraction of OS, the culture broth was diluted with deionized water to reduce the viscosity[24]. The diluted culture broth was centrifuged (4000g for 30 min.) and the cell-free supernatant was mixed with two volumes of isopropanol slowly along the side wall of the conical flask and allowed to stand for 2 hours at room temperature for OS precipitation[25].

Purification and estimation of OS

The obtained OS was washed with cold ethanol three times for purification and then dried at 60^{o}C for two days and weighted[26].

Deproteinization of OS by Huang *et al.*[27] method (hydrochloric acid method).

Additional purification steps have become necessary to reduce the protein content and other components (production media components that could interfere with the OS) in the final OS preparation. The freeze-dried crude OS was re-dissolved overnight in 0.05 M phosphate buffer[28].The OS was shacked strongly until complete dissolving and then the crude OS solution was adjusted to pH 3 with 2 M hydrochloric acid overnight. The sample was centrifuged for 10 min at 5000g and the precipitate discarded to obtain the deproteinized solution. The OS was precipitated with 2 volumes of isopropanol from the aqueous phase and allowed to be stand at room temperature for 2 hours for OS precipitation. The precipitated OS was washed with ethanol three times and dried at 60^{o}C for two days[29].

Growth measurements[30]

The cell growth was evaluated by measuring the optical density (O.D) at 600 nm using a Jenway, 6850 UV/Vis (USA) spectrophotometer.

Chemical characterization

The extracted OS was partial purified, dried and kept for chemical characterization. Melting point of the extracted OS was recorded on a Gallen Kamp melting point apparatus. The sample was hydrolyzed using conc. HCl followed by dilution with water and titrated against Bendict´s solution for determination of their content of monosaccharides. The Fourier Transform Infrared (FTIR) spectra were recorded using potassium bromide disks on a FT- IR 8201 PC Shimadzu in the range of 4000-400 cm^{-1}at Chemistry Department, Faculty of Science, Assiut University, Egypt. The extracted OS was subjected to LC-MS analysis in Analytical Chemistry Lab. at Faculty of Science, Assiut University, Egypt.

Statistical analysis

Results obtained in this study were subjected to analysis of variance (ANOVA) and separation of means was carried out by Duncan's[31] Multiple Range Test at a 0.05 level of significance.

Results and Discussion

The total yield and the functional properties of OS and EPS produced by LAB depends on several factors such as medium composition[32,33], microbial strain, culture conditions[3]and environmental conditions[35,36].The using of different media lead to production of variable amounts of OS or EPS.In this study, the effect of production medium on OS yield by *Leuconostoc holzaapfelii* S7 has been studied using three production media (NaG, R and IV) supplemented with 8% sucrose. The results represented in **Table (1)** indicated that, the largest amount of OS formed by bacterial strain S7on the NaG medium (reached to 50.15 gl^{-1})followed by medium R and IV, respectively. Medium IV stated the drastically decrease in the OS amount. In this medium, the excess of nitrogen source (20 gl^{-1} yeast extract) and the presence of different salts in the medium led to sharp decline in OS compared to NaG and Rmedia. Also, in medium R increasing the amount of beef extract (15 gl^{-1}) resulted in the production of OS lower than the amount produced on NaG medium. These results elucidated that the high amounts of nitrogen sources in the production medium lead to decrease the yield of OS. According to these results, NaG medium was suitable for synthesis of large amount of OS. NaG medium was rich with sugars and poor in nitrogen source, indicating that the OS production by the tested strain was increased under limitation of nutritional value of the medium as mentioned in some studies that conclude the hard conditions for growth are thought to be optimal conditions for EPS production by mesophilic LAB[37,38].

Hermann[39]reported that addition of sodium gluconate to NaG medium stimulated the production of EPS. **Cerning**[40]mentioned that using of medium with limiting nutrients (deficiency of nitrogen, sulfur or phosphorous sources) in the presence of excess carbohydrate lead to increasing the polymer production and the addition of certain sugars can stimulate production of short chain OS.

According to**Sutherland**[41]**,**it is required sugar in excess to produce EPS.

Table 1 -: Effect of production media (NaG, IV and R) on EPS production.

Strain	OSlevel (gl^{-1}) on different media		
	NaG	R	IV
***Leuconostoc holzaapfelii* S7**	**50.15**	**43.52**	**16**

Fermentation period is one of the critical environmental parameters affecting OS composition, yield and molecular mass. The effect of the incubation periods (24, 36, 72 and 96 h) on the production of OS by the selected bacterial strain using NaG medium was studied. **Figure** 1,displayed the effect of the incubation period on OS production and optical density (OD) of fermentation medium. The OS production was significantly ($p \leq 0.05$) increased with increasing incubation period up to 72 h and also with increasing of the cell growth (OD). After 72 h., the rate of increasing was limited or stopped. OD and OS at 72 h were 0.70 and59.40gl^{-1}, respectively (Figure, 1). No significant difference of OS amount produced after fermentation period more than 72 h. The results indicated that no degradation for OS during fermentation and the bacterial strain S7 did not possess depolymerase enzymes to degrade and utilize the OS as carbon source.

Similar finding also mentioned by **Vaningelgem *et al.*[42].**They reported that, the EPS production at the growth phase was limited and no decrease of the EPS concentration was observed during the stationary phase. Also,**Wangpaiboon *et al.*[43]** noted that alternant produced from *Leuconostoccitreum* was stable since no degradation was observed. **De Vuyst *et al.*[44]; Jolly *et al.*[45] and van der Meulen *et al.*[46]** reported that, at the first three days of the fermentation period, the EPS-producer strains might produce EPS with gradual increase to avoid food shortage or to protect the cell from the around medium and produce adhesive medium round the cell.

Temperature is very important factor that affect the growth of bacterial strains and its ability to produce OS. Five degrees of

fermentation temperatures (25, 30,35, 37ºC and 40°C) have been used to determine the optimum temperature for growth and OS production by strain S7, whereas the other conditions were kept at the optima. Results were exhibited that the OS production was affected highly by different fermentation temperatures. **Figure 2** showed that the production of OS by selected strain at 25°C was significantly higher than at the fermentation temperatures of 35, 37 and 40°C, while no significant difference appeared at 30°C.On the other hand, the OD at 25°C was significantly higher than at the other fermentation temperatures (30, 35°C, 37 and 40 °C)**.**The OS yield of strain S7 was62.20 gl^{-1}at 25 °C, while at 35°Cthe yield was drastically decreased to 19.52 gl^{-1}(Figure, 2).

These results agreed with **Gamar-Nourani *et al*.**[47]**,**who noticed that low temperatures (20 or 25°C) were favored EPS formation by LAB. Also, **Looijesteijn and Hugenholtz**[34]; **Cerning *et al*.**[40,48]; **Racine *et al*.**[49]and**Sutherl and,**[50] reported that there was enhancement for the production of EPS at 25°C. EPS production by mesophilic LAB is almost 50% higher when the organisms are grown at 25°C instead of30°Cas reported by **Cerning *et al*.**[51],**Sutherland**[52] recorded that the most polysaccharide producing microorganisms are incubated near 30°C.On the other hand, the results of this study are contrast with **Benhadria *et al*.**[13]**,w**ho mentioned that the incubation temperature leading to the higher production of EPS by *Leuconostoc*sp was 37 °C. Also, **Elbanna *et al*.**[16] mentioned that the bacterial growth was decreased while the EPS production was increased at 40°C.According to these results, the fermentation temperature 25°C was applied in the next experiments.

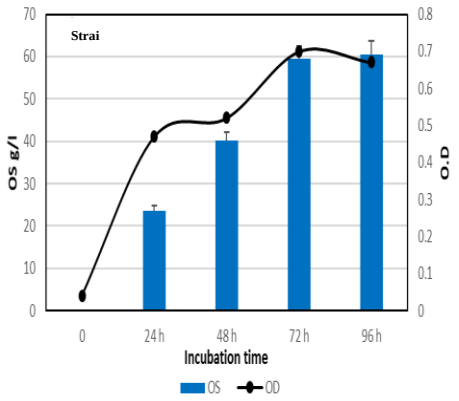

Figure1. Effect of incubation period on OS production by *Leuconostocholzaapfelii* strain S7

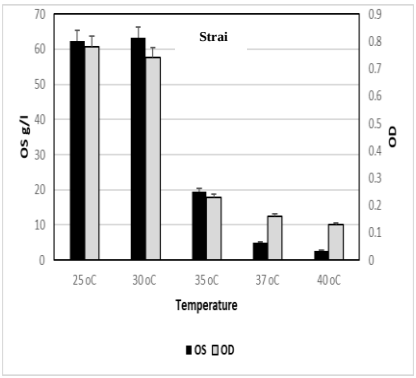

Figure2. Effect of different temperature on OS production by *L.holzaapfelii* strain S7

The pH is one of the most important factors which affect the growth and production of EPS by LAB[13]**.**To evaluate the influences of medium pH on the growth and production of OS by the selected strain, it was grown at different four pH (5, 6, 7 and 8) on NaG medium at 25°C for 3 days. F**igure 3** illustrated that a gradual increase in OS production with the increase in the initial pH from 5 to 7pH and reach the maximum at pH 7, then the production of OS was decreased when the pH of the medium reached to 8. The maximum OS production was 63.0 gl^{-1}at pH 7.0 (Figure, 3).

Nitrogen sources play an important role in the growth of the organism and the production of OS. It is the secondary energy sources for the organisms[53]**.** The influence of nitrogen sources on the tested bacterial strain was examined by using various organic and mineral nitrogen sources at the concentration of 0.5% added to the culture NaG medium. Organic nitrogen sources (yeast extract, beef extract, peptone, gelatin and mixture(at equal level)yeast extract, beef extract& peptone) in addition to ammonium sulphate and ammonium chloride as inorganic nitrogen sources have been used. **Figure 4**shown that all the organic nitrogen sources investigated in this study supported the growth and OS production by the tested bacterial strain. Yeast extract and the mixture of nitrogen sources used seems to be the preferred sources for cell growth and support the highest production of OS.

On the other side, when ammonium sulphate and ammonium chloride were separately used as sole nitrogen source in the medium, there was neither bacterial growth nor production for EPS (Figure, 4).This similar to results obtained by **Maalej *et al***.[54] who found that, when ammonium sulphate was used as inorganic nitrogen source no growth of bacteria was detected also no EPS production, but the optimized nitrogen source was organic yeast extract.

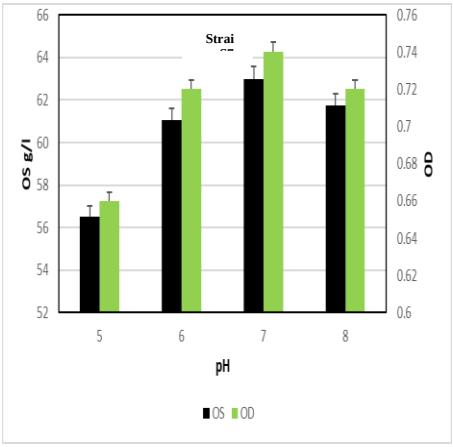

Figure3. Effect of pH on OS production by *Leuconostoc holzaapfelii* strain S7

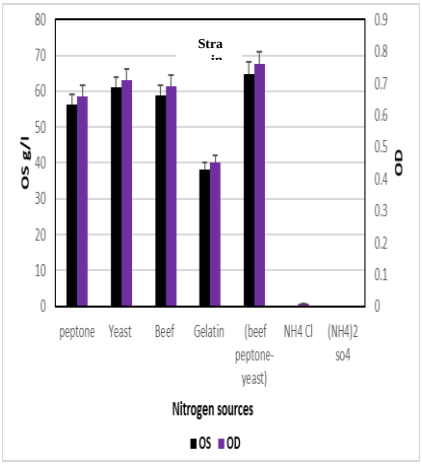

Figure4. Effect of nitrogen sources on OS production By Leuconostoc holzaapfelii strain S7

The type of available sugar in the medium strongly affect the monosaccharaides (glucose and fructose) and disaccharides (sucrose and lactose)at 4, 6 and 8% in Na G medium on the growth rate and OS production by strain S7 had been investigated. Except sucrose, we observed that none of those sugars support OS production by the tested strain S7 on NaG medium with the different concentrations under study (Figure,5). Similar finding was reported by Van Geel-Schutten et al.56.They observed that sucrose medium was the best sugar that was used for detecting of the EPS in medium. Also, sucrose being the most effective carbon source for the growth of Leuconostoc among different seven carbon sources tested(sucrose, lactose galactose, fructose, glucose, raffinose and maltose by de Man et al.57. Harutoshi58 reported that homo EPS are synthesized by LAB outside the cell by using extracellular fructosyl transferase

(FTF) "fructansucrases" or glycosyltransferase (GTF) "glucansucrases" and sucrose cause induction for the GTF enzymes of *Leuconostoc* species and the production of GTF by *Leu. mesenteroides* is low when using carbon sources other than sucrose. **Antunes *et al.*[59]**and **Holzapfel *et al.*[60]** mentioned that most of the *Leuconostocs* do not ferment lactose. **Frantzen *et al.*[61]**mentioned that most of the *Leuconostocs* unable to grow on medium supplemented with glucose.

Vijayendra&Sharath Babu,[62] reported that although glucose could support the bacterial growth, the amount of EPS produced was negligible when compared to that of sucrose. Significant amount of EPS production was observed only with sucrose, this observation could help in exploiting the sucrose-rich industrial by products for EPS production. **Hou *et al.*[63]** recorded that when glucose-based medium used for cultivation of *Leu.mesenteroides*, the production of dextran sucrase (which is responsible for catalyze the production of dextran) was inhibited and there is no EPS production but dextransucrase massively produced when the *Leu. mesenteroides* cultivated on sucrose-rich media. **Yan *et al.*[64]** explained that cultivation of *Leu.mesenteroides* CGMCC10064 in sucrose medium has an advantage in growth over that in glucose medium.

Bacterial strain S7 produced significant amount of OS under optima conditions examined in this study which was64.80 gl^{-1}. This yield of OS was higher than the EPS recorded by several other workers, they reported that the maximum EPS produced from different *Leuconostoc*species using sucrose medium were 30 gl^{-1}**(Vijayendra and Sharath Babu[62]),** 25.4 gl^{-1}**(Khue & Ngoc[65])**, 2.39 gl^{-1}**(Heperkan *et al.*[66])**, 15 gl^{-1}**(Yan et al.)[64]**

The high cost of OS production is the major restriction in the industrial production. Therefore, the search for an inexpensive substrate used as fermentation medium for the OS producer strains to reduce the cost of the fermentation raw materials is very important. Hence, sugarcane molasses (which has high concentration of sucrose) have been used as fermentation medium in this study. **Figure 6** represent the influence of using different concentrations of sugar cane molasses (13. 16, 20, 26.2 & 29%) as fermentation medium on the OS production by the tested bacterial strain. A gradual increase in

OS production was observed with the increase in the concentration of the sugarcane molasses from 13 to 26.2% and the yield reached to the maximum (44.23 gl^{-1}) at 26.2%molasses andthen was drastically decreased at 29% molasses medium (Figure, 6).Both cane and beet molasses have been used for the production of EPS by different bacterial strains[67,68].The amount of OS produced in this study using sugar cane molasses medium was higher than the EPS recorded in several previous studies, which reported that the maxima EPS produced by different *Leuconostoc* species using molasses medium or molasses as carbon source in the production media were 5.56 gl^{-1}(**Razack *et al.*[67]**), 7.6 gl^{-1}**(Moghannem *et al.*[69]),** 2,843gl^{-1} (**Sirajunnisa *et al.*[11]**).

It was noticed that the amount of OS produced using molasses medium (44.23 gl^{-1}, Figure, 6) was lower than that formed using NaG medium under optima conditions recorded in this study (64.80 gl^{-1}, Figure,5) by the same bacterial strain. This can be discussed in the light of molasses may have some undesirable constituents that may led to these results. Several studies have been confirmed the presence of some inhibitors in molasses such as heavy metals (iron, zinc), impurities, phenolic compounds and betaine'[10,70]. Hence sugar cane molasses retained undesirable constituents and the pretreatments used in this study (pH adjustment and clarification)may be insufficient and this led tothe lowering OS yields.

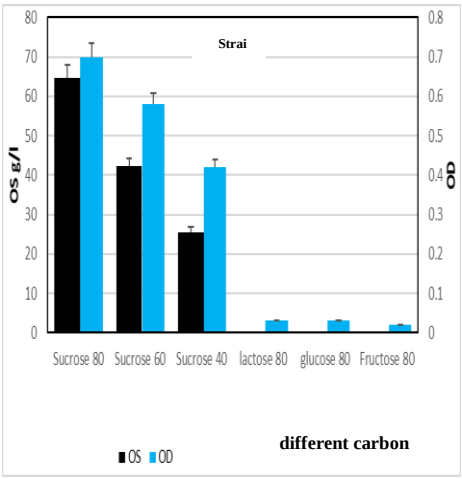

Figure 5. Effect of different carbon sources OS Production by *Leuconostocholzaapfelii* strain

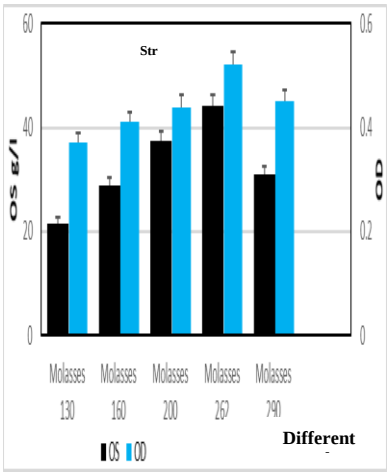

Figure6. Production of OS from different concentrations of sugar cane molasses by strains S7.

Chemical characterizations of the extracted OS were achieved. The OS obtained was insoluble in cold water but soluble in hot water and its melting point was more than 300ºC. The monosaccharide content of the extracted sample detected by Bendict´s reagent after hydrolysis was equal to 50% relative to the weight of starting sample. Sample IR: showed absorption broad band at 3418 cm^{-1}that characteristic for OH groups, at 2924 cm^{-1} for CH aliphatic and at 1652 cm^{-1} for CO group (Figure, 7). From the analytical data obtained by LC-MS (Figure, 8), we can conclude the OS sample containing eleven of glucose units. Whereas the gas mass spectrum fragmentation showed a molecular ion peak at m/z = 1980for 11 glucose units in addition to fragments at m/z = 360 for two glucose units, at 720 for four glucose units, at 1080 for six glucose units, at 1260 for seven glucose units, at m/z = 1440 for eight glucose units and at 1620 for nine glucose units.

The obtained results in this study indicated that sugar cane molasses was successfully utilized as economically fermentation medium for the production of OS by *Leuconostocholzaapfelii* strain S7 as an alternative to NaG medium on industrial large-scale production.

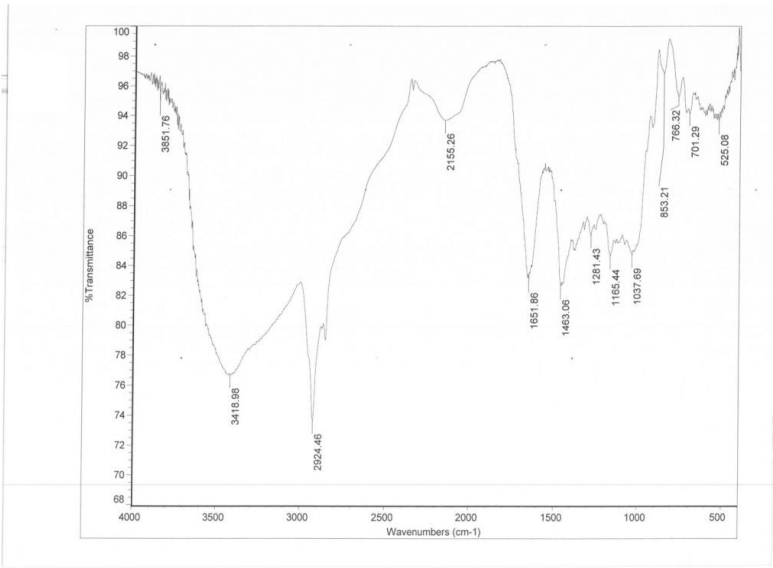

Figure 7: IR of OS sample

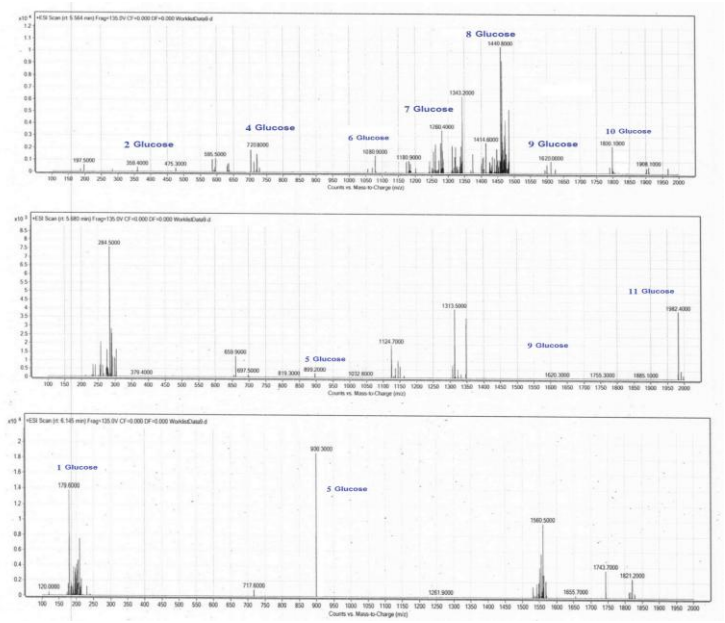

Figure 8: LC-MS analysis of OS sample

Reference

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الملخص العربي

إنتاج السكريات القليلة بالسلالة البكتيرية Leuconostoc holzaapfelii Strain S7باستخدام مولاس قصب السكر كوسيط تخمير

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تعد السكريات القليلة غذاء وظيفي و مساعدات حيوية. و تستخدم في انتاج الأغذية والأعلاف والمنتجات الدوائية والزراعية و مستحضرات التجميل بالإضافة إلى كونها عوامل محفزة للمناعة. قد تستخلص السكريات القليلة من المصادر الطبيعية أو قد تصنع كيميائيا إلا أنأسعارها مرتفعة جدا. الحل الأمثل للحصول عليهاهو إنتاجها بالميكروبات. ولكن نظرا لإرتفاع تكلفة الإنتاج يالميكروبات بسبب أرتفاع تكلفة مغذيات الميكروبات مما يجعل إنتاجها علي مستوي تجاري كبير محدود. كانت هذه الدراسة تهدف إلى إنتاج السكريات القليلة بواسطة سلالة *Leuconostoc holzaapfelii* S7 بإستخدام مولاس قصب السكر كوسط تخمير منخفض التكلفة. هذا وقد تمكنت السلالة البكتيرية المستخدمة من إنتاج 21.55 و 28.82 و 37.35 و 44.23 و 30.93 جرام من تلك السكريات لكل لتر من وسط التخمير المحتوي علي مولاس قصب السكر بتركيز 130 و 160 و 200 و 262 و 290 جرام، على التوالي بالمقارنة بـــكمية إنتاج وصلت إلي 64.80 جرام من تلك السكريات لكل لتر من وسط التخمير جلوكونات الصوديوم المدعم بـــ 80 جرام سكروز و 5 جرام من خليط متساوٍ لكل من مستخلص الخميرة والببتون والبيف عندأس هيدروجيني 7 والتحضين عند درجة حرارة 25 مئوية لمدة 72 ساعة بنفس السلالة البكتيرية. هذا وقد تم إجراء توصيف كيميائي للسكريات القليلة التي قد تم الحصول عليه بإستخدام التحليل الطيفي للأشعة تحت الحمراء (FT-IR) والطيف الكروماتوجرافي السائل (LC-MS) وتم كذلك تحديد نقطة إنصهارها ومحتواهامن السكريات الأحادية. ومن هذه الدراسه يمكن أن نخلص إلى أن مولاس قصب السكر مناسبًا كوسط تخمير منخفض التكلفة لإنتاج تلك السكريات بواسطة السلالة البكتيرية المختبرة.