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SPINY CACTUS PEEL WASTE AS SUITABLE SUBSTRATE FOR GROWING *Saccharomyces cerevisae* AND *Saccharomyces bulardii*

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ABSTRACT

Saccharomyces cerevisiae and *Saccharomyces bulardii* were grown on spiny cactus peel extract medium for 35h of growth. The optimum culture conditions necessary for the maximum growth was 30°C, 20h incubation period, and pH 5.0. A general increase in dry weight was observed for both yeast strains with a maximum in biomass yield at the 20h of growth (14.60g% and 12.10g% respectively) and of polymeric contents (13.40-19.2g/l respectively). Total sugar concentration of the culture medium was decreased during the fermentation period from 36.40 g/1 to 9.20 g/1 after 20h of growth. *S. cerevisiae* and *S. bulardii* contained crude protein (39.40 % and 30.60% respectively) at the end of the logarithmic phase (20h), while their true protein was 31.40% and 27.20%. The amino acids content in protein of both yeast cells were estimated and comparable to the FAO standard. Suitable amounts of essential amino acids (42.37%-47.54%) and reasonable amounts of sulfur-containing amino acids were also found in both yeast strains. lipid contents (6.80% and 5.60 %, respectively) were also found. Therefore, spiny cactus peel extract (mainly glucose, arabinose and mannose) was considered as suitable carbohydrate source for growing of *S.cerevisiae* and *S.bulardii* and production of maximal microbial protein and polysaccharides contents.

Keywords : Yeast, Waste, SCP, Polysaccharides.

INTRODUCTION

The need for more protein has elicited the study of variety of cheap sources aiming at production of protein biomass. Bioconversion of agricultural and industrial wastes would not only solve the pollution problem, but also yield useful products, especially single cell protein (Jwanny *et al.* 1996 and Gregorio *et al.*, 2002), polysaccharides (Leisola, 1998 and Shokri *et al.*, 2008) and enzymes (Diorio *et al.*, 2009). Beta-glucan, one of the major cell wall components of Saccharomyces cerevisiae has been found to enhance immune functions (Dallies *et al.*, 2008). Different agricultural wastes were used as microbial substrates for growing different yeast strains showed that agricultural wastes was considered as a good substrate for growth of different microorganisms (Rossi and Clementi, 1985; Rashad and Moharib, 2003; Gregorio *et al.*, 2002; Georgi *et al.*, 2007 and Ing-Lung *et al.*, 2008). Spiny cactus peel waste (Moharib, 2000) and other agricultural wastes were found to contain considerable carbohydrate which were used as suitable substrate for growing yeasts and production of high yield of protein and polysaccharides (Moharib, 1998 and Jwanny *et al.*,1995), they reported that these products have beneficial importance when they were incorporated into human foods and animal feeds. The aim of the present study was to investigate the use of spiny cactus peel extract as a substrate for cultivation of

S. cerevisiae and *S.bulardii* and production of cellular protein and polysaccharides.

MATERIALS AND METHODS

Organisms

Saccharomyces cerevisiae was purchased from Agricultural Research Center, Cairo, Egypt and *Saccharomyces bulardii* was transferred from capsule produced by an international pharmaceutical Co. on YPD/agar medium. Both yeast strains were routinely sub-culturing and stored at 4°C.

Substrate

Spiny cactus peel waste was obtained from the Egyptian local market, Cairo, Egypt. It was dried and ground to a very fine powder. A known weight of the dried powder (total carbohydrates, 46.6 %; nitrogen, 4.8 %; moisture, 16.2 % and ash, 9.8 %) was suspended in a known volume of 0.05 N HCl in a conical flask, shaken on a rotary shaker for 1h at 30°C and 200 rpm, then autoclaved at 121±1°C for 30 min. The peel suspension was filtered and the filtrate was designated as the extract (Jwanny *et al.*, 1989 and Moharib, 2003) and used as carbohydrate source for growing *S. cerevisiae and S.bulardii.* The chromatographic analysis (Wilson, 1959) of this extract indicated that the major components were glucose, galactose, mannose and arabinose and traces of xylose.

Fermentation procedure (s)

Fermentation was accomplished in 50ml of culture medium (g/l): KH_2PO_4, 2.0; $(NH_4)\ SO_4$, 3.0; $MgSO_4 .7\ H_2O$, 0.50; yeast extract, 0.30; $FeSO_4 .7\ H_2O$, 0.01; $MnSO_4 .\ 4\ H_2O$, 0.01; thiamine-HCl, 0.01; biotin, 0.01 and 50 ml of spiny cactus peel extract. The pH values, 5.0 and C/N ratio (10). A 24-h liquid culture was used as inoculum (3 % v/v) and added aseptically. Fermentation was carried out at 30°C on a rotary shaker at 150 rpm in 250 ml Erlenmeyer flasks. Samples were taken daily throughout the fermentation period (7 days) for analysis. The optical density of the yeast suspension was 0.3-0.4 at 610nm measured with a spectrophotometer LKB 4040. The yeast cells were harvested at different days of growth by cooling centrifugation at 4000 rpm for 10 min, washed and dried at 60°C to constant weight. The monosaccharide composition of both yeast polysaccharides were determined using paper chromatography (Wilson, 1959). Glucose, galactose, arabinose, mannose, xylose, and rhamnose, purchased from Sigma Chemical Co., were used as standards.

Analytical procedures

The dry weight was determined gravimetrically and crude protein in yeast cells by the standard Kjeldahl method as N x 6.25, while its true protein was estimated according to the method of Lowery *et al.* (1951), after extraction of the protein in dried residues using 0.5 N NaOH for 2 h at 45 °C (Cheng and Chang, 1984). The amino acid composition of yeast cells hydrolysates (acid hydrolysis with 6N HCl at 110 °C for 22 h) was determined with an amino acid analyzer (Eppendorf LC 3000) (Blook and Bolling, 1951). Nucleic acid of proteins was extracted by homogenization as described by Schneider (1945). DNA was determined according to Burton (1956) and RNA by orcinol method (Mejbaum, 1939). Total carbohydrates in the yeast

hydrolsates (2 MH_2SO_4) was estimated using the phenol-sulfuric acid test according to the method devised by Dubois *et al.* (1956). Crude fat was determined by the method of Folch *et al.* (1957). Harvested cells, washed, dried used for the determination of polysaccharides content according to the method described by Dallies *et al.* (2008) and Shokri *et al.* (2008).

RESULTS AND DISCUSSION

A great deal of interest has been focused on the potential of converting agricultural and industrial wastes into useful materials. In this study, an attempt is made to utilize the hydrolysed extract of the peel of spiny cactus fruit as the substrate for growing *S. cerevisiae* and *S. bulardii* and production of protein and polysaccharides at a low cost. The composition of some agricultural wastes was previously studied and nitrogen and carbohydrate contents were determined (Jwanny *et al.,* 1994 and Moharib, 2000). Therefore, it was considered to be a potential substrate for growing yeasts and edible fungi. Some investigators (Chanda *et al.,* 1990; Chanda, 1992 and Overchenko *et al.,* 1998) fortified agricultural wastes substrate with sucrose or molasses to give 10% - 15% total sugar in the medium and produced 30% of *Rodotorula* biomass protein, citric acid or other useful metabolites (Gregorio *et al.*, 2002; Georgi *et al.*, 2007 and Diorio *et al.*, 2009)

Effect of fermentation period

The growth of both yeast strains (*S.cerevisiae and S.bulardii*) on spiny cactus peel extract medium with in 20h is shown in Fig (1). The dry weight increased with time until the 20h of fermentation then a decrease was observed. The yeast biomass obtained was 12.10-14.6 g/l, respectively, while the total sugars consumed during growth phase was nearly at the same level for both yeast strains (74.72%). Maximum content of polysaccharides were achieved at the 20h of growth for *S.cerevisiae and S.bulardii* (19.20 and 13.40g/l respectively). These results are in accordance with those reported by other workers (Brillouet *et al.,* 1990; Wei *et al.*, 2008 and Diorio *et al.,* 2009) but higher than those reported by other investigators (Rashad *et al.*, 1993 and Leisola, 1998). Cultures of both yeast strains *(S.cerevisiae and S.bulardii)* were carried out until optimum of growth (20h) and the chemical composition of the collected dried biomass was determined. The results shown in Table1, illustrate the overall chemical composition of *S. cerevisiae and S. bulardii* dry biomass at the 20h of growth. The concentrations of components of *S. cerevisiae* biomass (90.40%) were higher than that of *S. bulardii* biomass (74.60%). The protein produced (39.40 % and 30.60% respectively) was within the same range reported by other workers (Rossi and Clementi, 1985; Rashad *et al.,* 1990; Gregorio *et al.,* 2002 and Paula *et al.,* 2009), but less than that mentioned by other investigators (Jwanny *et al.,* 1995). The lipid contents of *S. cerevisiae* and *S. bulardii* (6.80 and 5.60 respectively) were in the range of fat in yeast cells mentioned by other workers (Rashad *et al.,*1990 and Moharib, 1998). Therefore, we concluded that *S cerevisiae* and *S.bulardii* can utilize spiny cactus peel extract more efficiently as substrate for production of high yields of single cell protein (SCP). For

more consideration and evaluation of the nutritional quality of the two yeast strains, the amino acid profiles were determined. The present results (Table 2) show that the protein and total amino acid contents in *S. bulardii* (30.60 % and 66.84 %respectively) was less than that of. *S. cerevisiae* (39.40 % and 87.95 % respectively). The essential amino acids contents in protein of *S cerevisiae* and *S. bulardii* (47.54 and 42.37 % respectively) were comparable to those of other workers (Cheng and Chang, 1984 and Gregorio *et al.,* 2002) as well as the FAO standard proteins (Delaney *et al.,* 1975). Reasonable amounts of sulfur-containing amino acids were also found in the true protein of both yeast strains (Table 2). These results are consistent with those reported by other investigators used different yeast and fungi grown on different agricultural wastes (Cheng and Chang, 1984; Jwanny *et al.,* 1996 and Gregorio *et al.*, 2002).

Results in Table 2 show the nucleic acid content in yeast biomass varies. The maximum content of these nucleic acids were observed in both yeast strains at the end of logarithmic phase (20h). These contents are consistent with those reported by other investigators (Urakami *et al.*, 1983 and Rashad *et al.*, 1990), they suggested that RNA level of 10-14% may be of concern food grade SCP is produced. The proteins of both yeast strains having less amount of nucleic acids and reasonable amounts of essential amino acids that may be safely when they were incorporated into human food and animal feeds (Jwanny *et al.,* 1996). It could be concluded that spiny cactus peel extract can be considered as a potential substrate for the production of safely SCP and other useful metabolites. At the same time the disposal problem of agricultural wastes can be solved and may prove as an economical method to the overall control of industrial fermentation. Cultures of both *S. cerevisiae and S. bulardii* were grown on with in 20h of growth, using the extract of spiny cactus peel waste as carbon source. Chromatographic analysis of yeast cell polysaccharides revealed that glucose and mannose was the dominant and higher sugar in these polysaccharides, arabinose was the second most dominant sugars as well as small amounts of xylose. The monosaccharides librated were higher efficiency regarding the release of glucose from beta-glucan (Dallies *et al.*, 2008). These results agree with evidence reported by other investigators (Shokri *et al.,* 2008 and Morales-López *et al.,* 2009), they indicated that the yeast cell wall is composed entirely of beta-glucan and mannoprotein. Acid hydrolysis and extraction procedure methods used for polysaccharides determination in *S. cerevisiae* cell wall revealed that these results are similar to those obtained by other workers (Brillouet *et al.,* 1990; Dallies *et al.,* 2008; Shokri *et al.,* 2008; Ing-Lung *et al.*, 2008 and Avinash and Bhavanath, 2009), they reported that the major constituents of monosaccharides (glucose, mannose and arabinose) usually arising from some polysaccharides.

Effect of incubation temperature

The fermentation process was carried out for 20h at pH 5. The biomass, protein content and polysaccharide formation by these yeast strains were studied at different temperatures (20-45). Results in fig.2 show the biomass and polysaccharides of *S. cerevisiae and S. bulardii* under the influence of different incubation temperatures (20-45°C). Biomass, protein

content and polysaccharides were increased with increasing incubation temperature reaching a maximum at 30°C. At higher temperatures biomass, protein and polysaccharide contents of *S. cerevisiae and S. bolardii* were decreased. These results are consistent with those obtained by other workers (Rashad *et al.*, 1990; Gregorio *et al.*, 2002 and Wei *et al.*, 2008). They found that 30°C was the optimum temperature for increase of the above parameters of yeast strains which were grown on different substrates.

Table (1): Chemical analysis of *S. cerevisiae and S. bolardii* yeast cells (g%) grown on spiny cactus peel extract media at the 20h of growth.

Components	*Saccharomyces cerevisiae*	*Saccharomyces bulardii*
Total carbohydrate	44.20	38.40
Crude protein	39.40	30.60
True protein	31.40	27.20
Total lipid	6.8	5.60
RNA	9.80	9.20
DNA	0.86	0.80
polysaccharides		
Crude	19.20	13.40
Pure	13.86	9.40

Mean of three batches

Table (2): Amino acid profiles in hydrolsates of *S. cerevisiae* and *S. bulardii* dry cells grown on spiny cactus peel extract media at the 20h of growth.

Amino acids	*Saccharomyces cerevisiae* (g amino acids / 16 g N)	*Saccharomyces bolardii* (g amino acids / 16 g N)	Whole egg FAO standard*
Aspartic acid	8.86	8.30	
Serine	5.70	5.10	
Glutamic acid	9.90	9.20	
Proline	4.60	3.80	
Glycine	6.20	3.90	
Alanine	5.10	5.20	
Cystine	0.97	0.62	2.40
Tyrosine	4.80	2.40	4.20
Histidine	1.46	1.40	
Arginine	4.92	3.20	
Threonine	5.60	3.40	5.10
Valine	5.20	3.08	7.30
Methionine	0.92	0.64	3.20
Leucine	5.90	5.40	8.80
Isoleucine	6.40	3.80	6.60
Phenylalanine	5.80	3.20	5.80
Lysine	5.62	4.20	6.40

Mean of three batches FAO Standard* (Delaney *et al.* 1975)

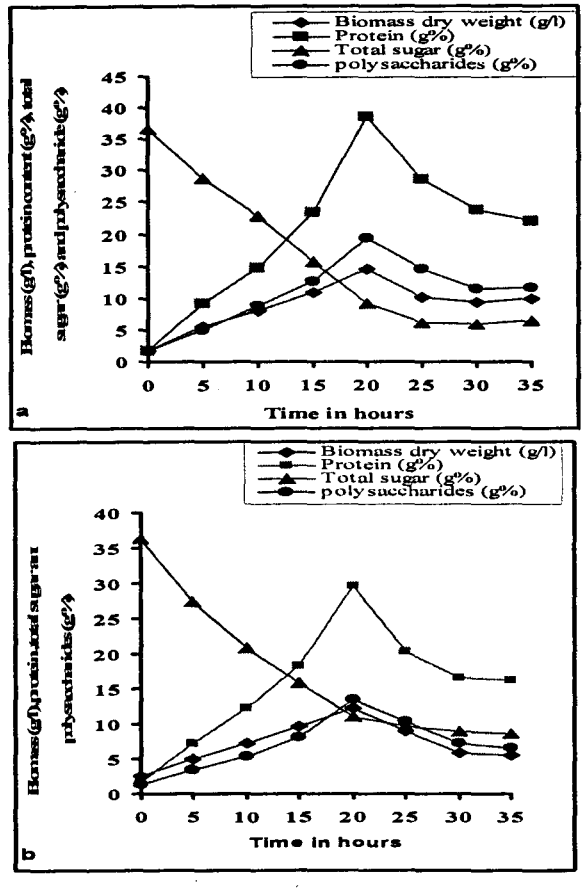

Figure (1): Effect of fermentation period on polysaccharides, biomass, protein content and total sugar of *S. cerevisae (a) and S.bolardii (b)* grown on spiny cactus peel extract.

Effect of pH values

The growth, protein content and polysaccharide formation by these yeast strains were studied at different pH values (fig.2). The fermentation process was carried out for 20h at 30 °C with different pH values (3-8). The effects of pH on yeast growth, protein and polysaccharides contents of *S. cerevisiae* and *S. bulardii* in the fermentation media are illustrated in fig.3. It is obvious that 5.0 was the optimum pH with maximum biomass production, protein and polysaccharide contents of both yeast strains. Lower or higher pH values, caused a decrease in protein and polysaccharide contents (fig.3).

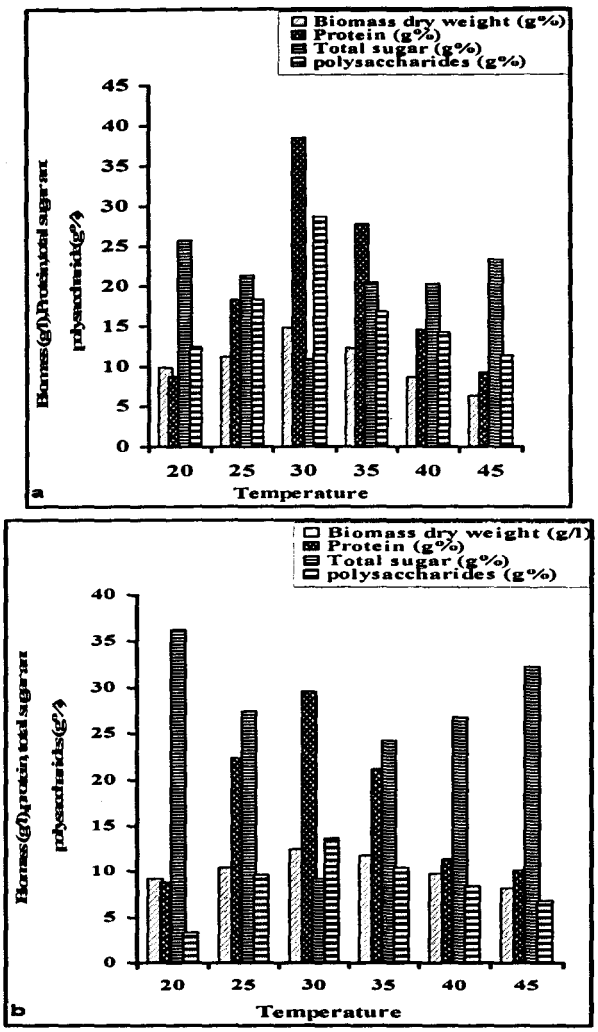

Figure (2): Effect of incubation temperature on polysaccharides, biomass, protein content and total sugar of *S. cerevisae (a) and S.bolardii (b)* grown on spiny cactus peel extract.

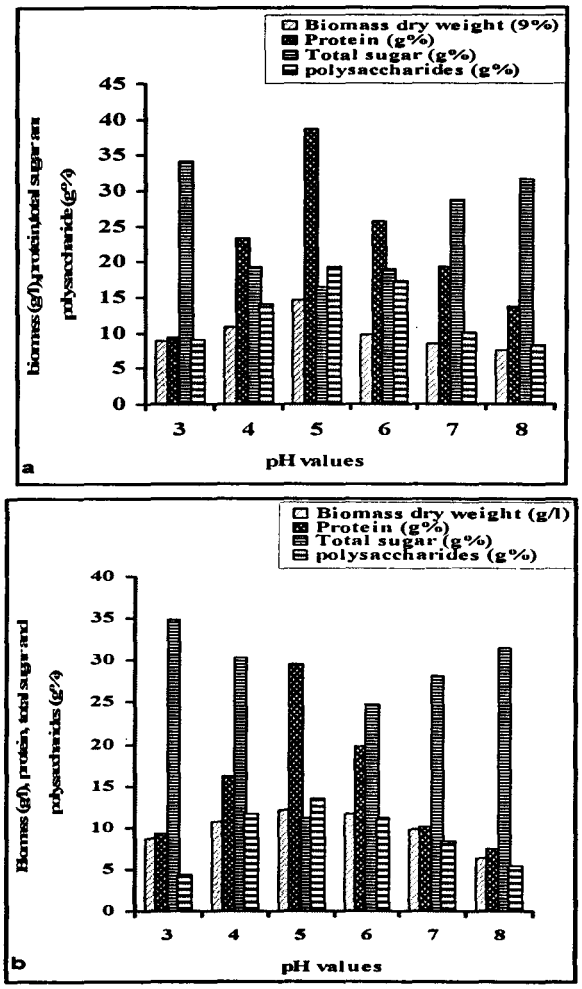

Figure (3): Effect of pH values on polysaccharides, biomass, protein content and total sugar of *S. cerevisae (a) and S.bolardii (b)* grown on spiny cactus peel extract.

These results are in accordance with those obtained by other investigators (Zhi-jie *et al.*, 2008; Avinash and Bhavanath, 2009 and Paula *et al.*, 2009), who indicated that the optimum pH values for growing different yeast strains ranged from pH 4.0 to 6.0. It can be finally concluded that 20h, 30 °C and pH 5.0, respectively, are the optimal values for incubation and growth of *S. cerevisiae* and *S. bulardii* on spiny cactus peel extract using liquid fermentation process. Under these conditions high yields of protein and polysaccharide were produced. These results agree with those obtained by

other investigators (Jwanny *et al.*, 1995; Zhi-jie *et al.*, 2008 and Avinash and Bhavanath, 2009). They have used different substrates for growing microorganisms. However, it can be concluded that spiny cactus peel extract is a suitable carbohydrate source for growing *S. cerevisiae* and *S. bulardii.* Simultaneously high yields of polysaccharide and microbial protein, which is rich in essential amino acids, particularly those of sulfur-containing amino acids, were produced. These products may be used as additives in the manufacturing of novel food and supplemented diets for human foods and animal feed.

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استخدام قشور مخلفات التين الشوكى كاوساط غذائية لتنمية الخمائر سكارواميسيس سيرفيزيا وسكارواميسيس بولارديای

سوريال عدلي محارب وايزيس منير عوض
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تم تنمية سكارواميسيس سيرفيزيا و سكارواميسيس بولارديای على اوساط غذائية تحتوى على مستخلص قشور مخلفات التين الشوكى كمصدر وحيد للكربون وذلك لمدة ٣٥ ساعة. وقد وجد ان درجة الحرارة المثلى لانتاج اعلى عائد من الكتلة الحيوية عند درجة حرارة ٣٠م وفترة التحضين لمدة ٢٠ ساعة والاس الهيدروجينى٥,٠ كما لوحظ زيادة مستمرة فى وزن الكتلة الحيوية الناتجة لنوعى الخميرة مع زيادة فترة النمو حتى٢٠ ساعة (١٤,٦٠ ج و١٢,١٠ج % على التوالى) وكذلك فى عديدى السكريات (١٣,٤٠ ج% و ١٩,٢٠ ج % على التوالى). وتبين النتائج انخفاض شديد فى تركيز السكريات فى الاوساط الغذائية خلال فترة التخمر(من ٠ الى ٣٥ ساعة) وخاصة بعد ٢٠ ساعة من نمو نوعى الخميرة (من ٣٦,٤٠ج الى ٩,٢٠ج %). وقد اوضحت النتائج احتواء خلايا السكارواميسيس سيرفيزيا و السكارواميسيس بولارديای على نسبة مرتفعة من البروتين الخام (٣٩,٤٠ % و ٣٠,٦٠% على التوالى) و النقى (٣١,٤٠ % و٢٧,٢٠ % على التوالى) خاصة عند نهاية منحنى النمو(٢٠ ساعة). وبتحليل الكتلة الحيوية وجد ان بروتينات خلايا نوعى الخميرة الناتجة تحتوى على كميات مناسبة من الاحماض الامينية الضرورية (٤٢,٣٧ % -٤٧,٥٤ %) مقارنة بمنظمة الاغذية الفاو كما تبين النتائج ان خلايا نوعى الخميرة سكارواميسيس سيرفيزيا و سكارواميسيس بولارديای تحتويان ايضاعلى كميات مناسبة من الاحماض الامينية الكبريتية بالاضافة الى نسبة لابأس بها من الدهون (٦,٨٠ % و ٥,٦٠ % على التوالى). لذلك يعتبر مستخلص قشور مخلفات التين الشوكى مصدرا جيدا للكربون لنمو سلالتى الخميرة سكارواميسيس سيرفيزيا و سكارواميسيس بولارديای للحصول على بروتينات تحتوى على نسب لاباس بها من البروتينات المحتوية على الاحماض الامينية (الضرورية والكبريتية) حيث يمكن استخدامها فى تغذية الحيوانات والدواجن وكذلك عديدى السكريات التى يمكن استخدامها فى علاج بعض الامراض وكذلك التخلص من تلك المخلفات.