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A comparative study on date syrup (dips) as substrate for the production of baker's yeast (*Saccharomyces cerevisiae*)

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Abstract

The suitability of date syrup (dips) as a substrate for the production of baker's yeast (*Saccharomyces cerevisiae*) was examined and compared with molasses substrate as a reference. With regard to nutrient content, dips compared very well with molasses in this respect. Dips included more sugars, biotin and pantothenic acid than molasses, about similar contents of nitrogen, phosphorus and magnesium, about half the content of potassium (but still enough for baker's yeast production), and much less m-inositol. Like molasses, dips contained some compounds, including formic, acetic and propionic acid, toxic to *Saccharomyces cerevisiae* while other toxic compounds occasionally found in molasses such as nitrite, sulfite and butyric acid were not detected in dips. Addition of molasses to dips at 1:1 ratio will bring the end concentration of formic acid to values below toxicity level. Hence, dips can be used at 50% of the substrate for production of baker's yeast, which is a reasonable amount of utilization.

Key words: Date, dips, molasses, baker's yeast, biotin, pantothenic acid, substrates, formic acid, propionic acid.

Introduction

The annual production of date fruits in Saudi Arabia is estimated at about 712 thousand tons. It has been increasing steadily in the last ten years at a rate of about 1.9% every year [1]. All amounts of dates consumed locally as human food or exported to foreign markets make only about 50% of the annual production. A small part of the remaining produce is used as animal feed while the larger part is a surplus waiting for suitable means of economic utilization [2]. Baker's yeast can be produced from substrates that contain metabolizable sources of carbon, energy, nitrogen, minerals and essential vitamins. Potential substrates range from food materials like grains and dates, by-products of the food industry like molasses and whey, and wastes like agricultural residues and food rests. Substrates that contain carbon and energy sources readily metabolizable for *Saccharomyces cerevisiae* such as mono and disaccharides in dates and molasses can be directly used for production, while those containing complex carbohydrates such as starches in grains and cellulose in plant rests need costly hydrolytic treatments before use. The substrate of choice for baker's yeast production in the world today is molasses.

Commercial baker's yeasts produced from strains of *Saccharomyces cerevisiae* have the following average chemical composition: 47% C, 32% O_2, 6% H_2, 7.7% N_2, 2% K, 1.2% P, 1% S, 0.2% Mg, 0.1% Na and trace elements. In addition, the yeast cells contain small amounts of vitamin B complex, of which D-pantothenic acid, D-biotin and m-inositol are essential because the yeast cells cannot synthesize them [3]. These elements and compounds must be provided in the production medium in enough quantities and metabolizable forms.

Dates are supposed to make a good potential substrate for baker's yeast production serving mainly as carbon and energy source. According to Sawaya [4], dates contain 65-87% sugars and 1-3% proteins, in addition to many minerals important for yeast nutrition including potassium, magnesium, sulphur, phosphorus, iron, calcium and chlorine.

Few investigations about the production of baker's yeast from date extracts have been conducted [5-9]. Although most workers described dates as satisfactory for baker's yeast production, the yields they obtained were very low. A maximum of about 43% of the theoretical yield and only 10.7 g/l biomass concentration was reached in the fermentation medium compared to the optimum of about 40 g/l expected for an economical production [10]. Al Obaidi *et al.* [7] reported an average of 47% of the theoretical yield, and Nancib *et al.* [11] reached a maximum of 0.6 g/l biomass concentration in the fermentation medium.

The aim of the present study was to determine the chemical composition of date syrup (dips) and compare it with molasses as substrates for baker's yeast production.

Materials and Methods

Date syrup (dips) extraction: Extraction of dips used in this study was carried out using the technology adopted in a date processing factory in Al Hofuf City as follows: Dates (Ruzeiz variety) were de-pitted and the flesh heated with equal amount of water at 80°C for 30 minutes. The mixture was filter pressed to remove large impurities and insoluble matters and micro filtered to remove smaller impurities and obtain a clear extract using a NOVOX 200 sheet filter system (Filtrox AG, CH 9000 St. Gallen, Switzerland) with an effective clarifying filtration area of 1.02 m^2 and 2.0 µm pore size, operated at a pressure of 1 bar. Finally the extract was concentrated to 75ºBrix at low temperature (80ºC) by vacuum drying to give the syrup named dips.

Chemical analysis: Phosphorus, potassium, magnesium, ash, protein, nitrite and sulfite were determined according to A.O.A.C. standard methods of analysis [12].

Formic, acetic, propionic and butyric acids were determined using gas liquid chromatography: column LiChroCART 125-4, Purospher RP-18e, 5 µm; mobile phase A 20 mM sodium dihydrogen phosphate, pH 2.5; B acetonitrile; detection UV 220 nm.

Biotin and pantothenic acid were determined according to the VitaFast (R-Biopharm AG, Darmstadt, Germany) vitamin testing method, using ELISA Reader, Multiskan EX Model No. 355, ThermoLabsystem, Finland.

Sucrose, glucose, fructose and m-inositol were determined with High Performance Liquid Chromatography (HPLC) using a Shimadzu Model 2003 (Japan), equipped with a RID 10A refractive index detector, CLC NH_2 6 x150 column, LC 10ATP pump, and CTO 10AC VP oven. Mobile phase was 83% CH_3CN:17% water (v/v), flow rate 1 ml/min, column pressure 200 kgf/cm^2

Treatment of dips: To try to remove the volatile fatty acids contents in dips which represents the toxic substance for baker's yeast, a treatment similar to one performed for molasses clarification in baker's yeast factories (for the removal of similar compounds) was performed as follows: Dips was diluted 1:3 with water, pH brought to 4.5 with sulfuric acid, and then it was boiled in an open container under continuous aeration for about 15 minutes.

Results and Discussion

Chemical composition of dips as substrate for baker's yeast production: Dips was chemically analyzed to determine its contents of nutrients needed by baker's yeast (*Saccharomyces cerevisiae*) and also the presence of chemicals that could be toxic to the yeast. Results presented in Table 1 show that dips contained about 80% total sugars, i.e. 800 kg/ton, mainly in form of fructose (41%) and glucose (38%) and a small amount of sucrose (1%). It also contained 2% crude protein, 1.13 g/kg phosphorus, 14.88 g/kg potassium, 0.79 g/kg magnesium, 240 ppm pantothenic acid, 2.73 ppm biotin and no detectable amounts of m-inositol.

These results indicate that dips can be used as a main source of carbon and energy for baker's yeast production. Calculations based on the chemical composition of dips shown in Table 1 give the following: Since a ton of dips contains 800 kg sugar, then this ton, regarded as carbon and energy source, should produce about 400 kg dry yeast, because one kg sugar is known to yield about 0.5 kg yeast dry matter [13, 14], i.e. we need about 2.5 kg dips for every kg yeast dry matter produced. Protein content of dips is about 2%, i.e. 20 g/kg, whereas baker's yeast contains about 50% proteins, i.e. 500 g/kg yeast dry matter. This means that one kg yeast (i.e. 500 g protein) contains about 80 g nitrogen (16% nitrogen in proteins), and the content of 2.5 kg dips needed as carbon and energy source to produce one kg yeast (see above) is about 8 g nitrogen, hence a deficiency of about 72 g nitrogen for every kg yeast produced. This nitrogen deficiency in dips must be covered by adding to the production medium inorganic nitrogen sources such as ammonium salts. Phosphorus content in dips is 1.13 g/kg (about 2.8 g/2.5 kg dips), compared to 14.1 g/kg in the yeast, i.e. dips is deficient in phosphorus which has to be added to the production substrate in form of inorganic phosphorus at about 11.5 g phosphorus for every kg yeast produced. Magnesium content in dips is 0.79 g/kg (about 2 g/2.5 kg dips), and its content in baker's yeast is 2 g/kg, meaning that it is just enough. No m-inositol was detected in dips, thus about 2 g of this compound must be added to the production medium for every kg yeast produced. The content of dips from potassium is about 15 g/kg (37 g/ 2.5 kg dips), whereas the content of yeast from this nutrient is about 20 g/kg yeast dry matter. This means that the content of potassium in dips is enough for baker's yeast production. Similar calculations show that biotin and pantothenic acid are present in dips in quantities enough for baker's yeast production.

To compensate for the deficiencies in nutrients mentioned above, mineral media with compositions as shown in Table 2 were used in our laboratories for the production of up to 40 g/l yeast dry matter (e.g. 400 g yeast in a 10-litre fermentation volume). The substrates used were pure dips, pure molasses and 1:1 dips: molasses. The molasses and dips/molasses substrates gave satisfactory and comparable yields, while the yields from pure dips were unsatisfactory [15]. The reduced yields from pure dips substrate were attributed to the presence of compounds toxic to the yeast in dips.

Comparing dips and molasses as substrates for baker's yeast production: With regard to its content of nutrients, dips can compare very well with molasses which is the conventional substrate for baker's yeast production world wide (Table 3). Dips contain much more sugars, biotin and pantothenic acid than molasses, about similar contents from nitrogen, phosphorus and magnesium, about half the content of potassium (but still enough for baker's yeast production) and much less m-inositol.

Table 1. Dips contents of nutrients needed by baker's yeast.

	Concentration in dips	Contents in baker's yeast [3]
Phosphorus (g/kg)	1.13	14.10
Potassium (g/kg)	14.88	20
Magnesium (g/kg)	0.79	2
Total sugars (%)	80	30
Fructose	41	
Glucose	38	
Sucrose	1	
Crude protein (%)	2	50
Pantothenic acid (ppm)	240	150
Biotin (ppm)	2.73	0.30
m-Inositol (ppm)	0	2000

Table 2. Composition of mineral media required for 400 g yeast dry matter.

	Amount (g)		
Chemical	100% molasses	100% dips	1:1 dips/molasses
$(NH_4)_2SO_4$	61	60	60
$(NH_4)_2HPO_4$	54	53	52
Pantothenic acid	0.025	0	0
m-Inositol	0	0.8	0.4

Table 3. Comparing dips and molasses as substrates for baker's yeast production.

Nutrient	Dips	Beet molasses [3]	Cane molasses [3]
Sugars (%)	80	50	50
Nitrogen (%)	0.13	0.5	0.1
Phosphorus (%)	0.11	0.03	0.09
Potassium (%)	1.5	3.0	3.0
Magnesium (%)	0.08	0.01	0.3
Biotin (ppm)	2.73	0.05	2.0
Pantothenic acid (ppm)	240	80	25
m-Inositol (ppm)	0	6500	2000

Compounds toxic to baker's yeast detected in dips include formic acid at 3.06%, acetic acid at 2.38% and propionic acid at 0.68% (total acids 6.12%), and no detectable amounts of the toxicants nitrite, sulfite and butyric acid (Table 4). Formic acid becomes toxic to the yeast when its concentration in the medium exceeds 0.25% (Table 4), whereas the toxicity level of the other two acids is in excess of 3.0% for the sum of the two. In baker's yeast fermentation, a maximum of about 40 g/l yeast dry matter end concentration in the fermentor can be reached. In industrial fermentations this will mean adding dips to the mineral medium to an end dilution of 1:10 (dips:medium). Since dips is added in a fed-batch process, the concentration of toxic acids will increase gradually to reach about 0.3% for formic acid and about 0.7% for total acids at the end of fermentation (the amount present in dips diluted 1:10). This end concentration of formic acid is within levels toxic to baker's yeast (Table 4). Fermentation experiments done in our laboratories confirmed this, only 50-60% of the theoretical yields were obtained when pure dips was used as substrate [15].

To try to remove the toxic compounds (the volatile fatty acids) from dips, and hence increase yield, the treatment described in materials and methods was applied. This treatment did not bring about the desirable effect as can be seen from Table 4. This can be due to the fact that our treatment was performed in a simple pot, while the treatment of molasses is done in equipments specially designed for this purpose with heating up to 140°C under pressure followed by release of pressure and injection of air, which can result in a more effective evaporation of the volatile toxic compounds [3]. Such equipment is not available to us now, but can be regarded as a possible solution in plants for commercial production. Another solution for this toxicity problem can be reached by the addition of molasses to dips at 1:1 ratio. The dilution effect will bring the end concentration of formic acid to the half, i.e. 0.15%, which is below the toxicity level shown above. This solution was tried in fermentation experiments done in our laboratories and gave yields up to 90% of the theory, which were comparable to yields from pure molasses [15]. This means that dips can be used at up to 50% of the production substrate, which is a reasonable amount of utilization.

Table 4. Contents of dips from chemicals toxic to baker's yeast.

Chemical	Toxicity level (%) in yeast substrate [3]		Concentration (%) in dips	
	Tolerable	Toxic	Untreated	Treated
Nitrite	< 0.001	Up to 0.05	0	0
Sulfite	< 0.01	> 0.15	0	0
Formic acid	< 0.1	> 0.25	3.06	2.81
Acetic acid			2.38	2.38
Propionic acid	< 1.0	Up to 3.0	0.68	0.51
Butyric acid			0	0

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