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Carob Fruit as Source of Carbon and Energy for Production of *Saccharomyces cerevisiae*

Manal H. Ahmad and Ghaleb M. Abuerreish*

Department of Biological Sciences, University of Jordan, Amman 11942, Jordan

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Abstract

Carob (*Ceratonia siliqua L.*) aqueous extracts were prepared from carob pods and kibble (pods minus seeds) particles of 0.5 to 1.0 cm diameter, at 45 °C for 2hr. *Saccharomyces cerevisiae* cells were grown on extracts by fed-batch method at 32 ºC, an optimal pH 5.5, and shaking at 300 rpm for 10 h. The cell yield and yield coefficient were 12.6 % and 0.27 g /g sugar utilized, respectively. Aeration effect and continuous feeding on the yield of *Saccharomyces cerevisiae* were studied on extracts prepared from carob pod powder or from chopped kibble. The biomass yield coefficient was about 0.27 g- yeast/g sugar utilized for both the pod and the kibble extracts. The cells maintained the availability of reducing sugars on the expense of non-reducing sugar present in carob extract; therefore, reducing sugars were maintained at their initial level. However, aeration and continuous feeding increased the yield to 113 %. Instantaneous growth rate constant after 10 h-period was 0.18 h^{-1} which decreased with time. Supplementation of Ca, Mg, N, and P salts to extract in a continuous fed-batch culture did not significantly increase the cell mass above the yield in the control. These nutrients were important in fixed batch culture. This indicates that, in cell division, the carbon source in the extract is not limiting factor, while the above additives become the limiting factor. Comparing the yield of the yeast grown on carob kibble extract with other substrates, previously and currently employed in the industry, reveals that the carob kibble extract is more economical substrate for industrial production of baker's yeast.

Keywords: Baker's Yeast, Carob, Carob Pod, *Ceratonia siliqua.* Yeast, *Saccharomyces cerevisiae*

1. Introduction

Carob tree (*Ceratonia siliqua L.* Leguminosae family) is widely cultivated in the Mediterranean regions, and in areas of North America (Blendford, 1979; Manso *et al.*, 2010). The tree is considered an important flora for economic and environmental reasons (Batlle and Tous, 1997). The world annual production carob fruit is > 315000 tons, which is distributed among Spain (42 %), Italy (16 %), Portugal (10 %), Morocco (8 %), Greece (6.5 %), Cyprus (5.5 %), and Turkey (4.8 %) (Santos *et al.*, 2005). Carob fruit, or pod, consists of kibble (pulp or locust bean) and the seeds, or locust kernel gum. The seeds which contain the polysaccharides, galactomannans, are used in gum production (Davies *et al.*, 1971). The chemical compositions of carob kibble are compiled from various published reports (Yousif and Alghzawi, 2000; Calixto, 1987; Petit and Pinilla, 1995; Avallone *et al.*, 1997) and represented in Table 1. The sugar contents in carob pods, 48 % - 56 %, (Santos *et al.*, 2005; Ahmed, 2001) were reported to be higher than those in sugar cane, 14 % -18 %, (Sugarcane, Wikipedia). However, the profiles of carbohydrates which were determined in g /100 g carob pod were: Fructose at 10.2 – 11.5, glucose at 3.3 – 3.68, and sucrose at 29.9 – 38.4 (Biner *et al.*, 2007).

Table 1. Chemical composition of carob kibble.

Composition	Concentrations g /100g	composition	Concentration mg /100g
Total sugars[a]	45.0±0.30	Potassium[c]	802.00
Tannins[b]	18.5 ±0.20	Sodium[c]	10.10
Ash[a]	2.79±0.22	Magnesium[c]	66.89
Proteins[a]	5.54±0.33	Calcium[c]	440.00
Fats[a]	0.3±0.04	Iron[c]	2.34
Crude fiber[a]	10.99±0.51	Zinc[c]	0.70
		Copper[c]	0.62
		Manganese[c]	0.56
		Manganese[c]	0.56
		Phosphorus[c]	31.58

[a]Tannins were reported at 3.15±0.03 (Yousef and Alghzawi, 2000), [b](Calixto, 1987), [c](Petit and Pinilla, 1995).

These three main sugar components were also reported as 11.6 %, 12.8 %, and 37.2 %, respectively by other investigators (Santos *et al.*, 2005). Tannins in carob pod

* Corresponding author. e-mail: ghalebmusa@hotmail.com.

were identified and quantified (Papagianonopoulos *et al.*, 2004), and the chemical structures of major individual polyphenols in carob fiber were studied (Owen *et al.*, 2003). In order to increase the availability of free sugars for the production of protein from carob pod extracts using certain microorganisms, the media were supplemented with ammonium salt. The organisms of interest to produce protein through that process were *Aspergillus niger* (Imrie & Vitos, 1975), *Fusarium moniliforme* (Drouliscos *et al.*, 1976; Macris and Kokke, 1977, 1978), *Rhizopus oligosporus* and *Monascus ruber* (Righelato *et al.*, 1976). Carob pod is used in various processes of food technology, medicine, and other industrial processes. At the industrial level, the pod was employed in the production of ethanol by *Saccharomyces cerevisiae* (Turhan *et al.*, 2010; Roukas, 1995; Roukas, 1994a, b, Roukas, 1993), by *Zymomonaras mobilis* (Vaheed *et al.*, 2011), the production of citric acid by *Aspergillus niger* (Roukas, 1998), the production of mannitol by lactic acid bacteria (Carvalheiro *et al.*, 2011), and the production of biocontrol agent *Pantoea agglomerans* PBC-1 (Manso, 2010). The economical and industrial aspects of carob products were reviewed by (Davies *et al.*, 1971; Ayaz *et al.*, 2009). The nutritional and health beneficial aspects of the carob fruit products have been reviewed (Ayaz *et al.*, 2009; Zunft *et al.*, 2003, 2001; Makris and Kefalas, 2004). Many countries of the third world are carob-cultivating, and rely heavily on bread and pastry products that utilize great amounts of baker's yeast, *S. cerevisiae*. Because of the cheaper price of carob fruit compared to other sources of sugars utilized in the production of yeast, and the higher content of the sugars that are consumable by the yeast than that of the sugarcane, it is highly feasible to utilize carob fruit as source of carbon and energy to produce the yeast, *Saccharomyces,* which is the scope of this study.

2. Materials and Methods

2.1. Materials

Carob fruit and sucrose were purchased from local grocery markets. Anthrone was obtained from Merck, Germany. Bovine serum albumin and sodium potassium tartrate were obtained from British Drug House, UK. Coomassie Blue-G250, ethanol, Folin-Denis reagent, glucose monohydrate, phosphoric acid, and tannic acid were obtained from Fluka, USA. Malt broth and nutrient agar were obtained from HiMedia Laboratories, Ptv. Limited, Bombay, India. Yeast extracts were obtained from Oxoid, England. Bacteriological peptone was obtained from Mast Laboratories, UK. 3,5-Dinitrosalycylic acid was obtained from Sigma, USA. Baker's yeast was obtained from Astrico, Yeast Industries Co. LTD, Jordan. All other reagents were analytical grade, and double distilled water was used when required.

2.2. . Methods

2.2.1. Inoculum Preparation

Pure colony of *S. cerevisiae* was prepared as follows: Dry commercial yeast (one to two pellets) was added to 2 ml of sterile malt broth and shaken in a water-shaker bath for two h at 32 ºC. Then, 0.1 ml of the yeast malt broth was diluted to 10 ml with sterile physiological saline solution. Finally, one drop of diluted yeast suspension was placed on culture medium composed of: malt extract (0.5 %), yeast extract (0.5 %), sucrose (2 %), peptone (0.5 %), agar (2 %) (MYSP), and incubated at 32 °C for 48 h (Campbell, 1988; Ahmed, 2001). Thirty ml of YPS-medium (yeast extract, 1 %; peptone, 2 %; sucrose, 2 %) were inoculated with this colony to produce inoculum for larger cultivation culture.

2.2.2. Preparation Of Carob Extract

Three carob pod extracts (CE) were prepared at different conditions and were used to grow *S. cerevisiae.* The extracts were prepared as follows:

Carob Extract-1 preparation (CE-1). Carob pods were cut into small pieces with particle size of 0.5 to 1.0-cm. The pieces were packed into a column (30-cm x 2-cm). The column was eluted with water at a rate of 2 ml /min using peristaltic pump. The pod to water (p:w) ratio was 1:4 by weight. The eluant, which was centrifuged at 3000 x g and 0 °C for 30 min, was designated carob extract-1 (CE-1).

Carob Extract-2 preparation (*CE-2*). Carob pods were milled to fine powder using Moulinex miller. The powder was mixed with water at a ratio of 1:4 by weight at 45±3 °C for 2 h. The extract, which was centrifuged at 3000 x g and 0 °C for 30 min, was designated carob extract-2 (CE-2).

Carob Extract-3 preparation (*CE-3*). The preparation of CE-3 was similar to CE-2 but kibble (pods minus seeds) with particle size around 0.5 to 1.0-cm was used.

2.2.3. The Effect of Ph On the Growth of S. Cerevisiae

CE aliquots at various pH values (30 mL in 100-mL arm-flask) that contain 2 % sugar (expressed as glucose) were inoculated with the cells. The flasks were shaken at 250 rpm in a water bath at 32 °C for 24 h.

2.2.4. Determination of Water-Extractable Materials

Total protein was determined according to Bradford (1976) using bovine serum albumin as the standard. Tannins were determined by Folin-Denis reagent as described (AOAC, 1990) using tannic acid as the standard. Total sugars were determined by the Anthrone method using glucose as the standard (Graf *et al.*, 1951). The reducing sugars were measured using 3,5-dinitrosalicylic acid (DNS) reagent (Miller, 1959). Non-reducing sugars were calculated as the difference between total sugars and the reducing sugars.

2.2.5. Effect of Aeration and Continuous Feeding

Diluted CE-2 (at 28 mmol /L sugar, pH 5.5) was added continuously to 2-L arm-flask using peristaltic pump. The rate of feeding was calculated according to the following equation:

$$F_s = [\mu/y_{xs}+m_s][(C_{xo}.V_o)/C_{si}].e^{\mu t},$$

where F_s, L /h, is the flow rate of the feed medium; μ, μh^{-1}, is the specific growth rate constant; y_{xs}, g cells /g substrate, is biomass yield on the substrate; m_s, g substrate/g cells /h, is maintenance coefficient; V_o, L, is initial culture volume; C_{xo}, g /L, is initial biomass concentration; C_{si}, g /L, is substrate concentration in the

feed; and t, h, is time after starting the feed (Van Hoek *et al.*, 2000). The working volume was 1L. The air was provided by air pump that is connected to the arm of the flask. The rate of air pumping was 2 m^3 /min. Olive oil, 0.13 ml /L, was used as antifoam (Drouliscos *et al.*, 1976).

2.2.6. Cultivation of Cells

Two doses (200 mL each) of diluted CE were added to 2L arm-flask containing 200 ml diluted CE to give 28 mmol /L sugar as glucose equivalent and inoculated with the yeast at 3 %, (3.31±0.16) x10^7 cells /mL of the initial volume. Sugar concentration was maintained at 28 mmol /L. The first dose was added after 3.5 h, and the second dose was added after 7.0 h. The final volume was 600 ml, pH 5.5. The inoculated media were shaken at 32 °C and 300 rpm. Aerobic condition was provided by passing the air through Millipore filter.

2.2.7. Determination of Cell Number and Weight of Yeast

The growth of yeast was monitored by the change in absorbance at 550 nm using Bausch and Lomb spectrophotometer, and by counting the number of viable cells on MYSG-medium. The dry weight of the yeast was determined as follows: Cultivation medium was centrifuged at 2500 x g for 20 min, and 0 °C, and the pellet was washed with saline solution. The yeast was filtered on filter paper and dried to constant weight in an oven at 105 °C for 12 h.

2.2.8. Purity of the Culture

The yeast culture was examined microscopically using methylene blue as stain and bacterial contamination was examined by Gram stain (Benson, 1985).

2.2.9. Bread Preparation

Bread was prepared in two ways: one is using *Saccharomyces cerevisiae* that was produced in this study and the other one was using commercial yeast that was from the local bakeries, which is sold in local grocery markets. The method of preparation was based on that applied in the local bakeries. A flour quantity, 100 g, was mixed with 55 mL water and 1g yeast. The mix was let stand at room temperature for 45 min, cut into small pieces and let stand fort 15 min followed by baking the dough in the oven at temperature used by the bakeries.

2.2.10. Statistical analysis

Data averaging was performed on samples of three or more runs. The standard deviation (±) was calculated from a computer-run program.

3. Results

3.1. Chemical Composition of CE-

The concentrations of reducing and non-reducing sugars, protein, and tannins as g /100g, and % of total sugars extracted are shown in Table 2. The CE-1, CE-2, and CE-3 contained reducing sugars as 2.1, 2.6, and 1.8 g/100 ml, respectively. CE-2 and CE-3 have essentially equal concentration of non-reducing sugars, 12.5 and 14.6 g /100ml, respectively. However, the total sugar contents, as g /100 ml, were 23.2, 17.7, and 16.4, in CE-1, CE-2, and CE-3, respectively. Protein and tannin contents were low and almost equal in these extracts, except CE-2 showed 0.11g / 100mL, which is high if compared to the other values.

Table 2. Protein, sugar, and tannin contents in carob extracts (CE) prepared under variable conditions. The values are g /100ml extract.

	CE1	CE2	CE3
Total sugar	23.2±4.5	17.7±1.9	16.4±0.4
Reducing sugar	2.1±0.2	2.5±0.1	1.8±0.0
Non-reducing sugar	21.1±4.3	12.5±1.8	4.6±0.4
Protein	0.0±0.0	0.06±0.0	.02±0.0
Tannin	0.06±0.0	0.11±0.14	.07±0.0

3.2. Growth Kinetics of S. cerevisiae on CE.

The growth kinetics was studied by following the increase in turbidity at light wavelength of 550 nm which is related to cell growth. At the same times, changes in the levels of reducing and non-reducing sugar were measured. The pattern of growth and the consumption of reducing and non-reducing sugar using CE-1, CE-2, and CE-3 media are presented in Figure 1. The non-reducing sugar values were deduced from the total and the reducing sugars values were determined experimentally (see Methods). In this study, the cells were grown in the fed-batch culture. CE was added to maintain initial sugar concentration at ~ 28 mmol /L as glucose at time intervals, 3.5 h each.

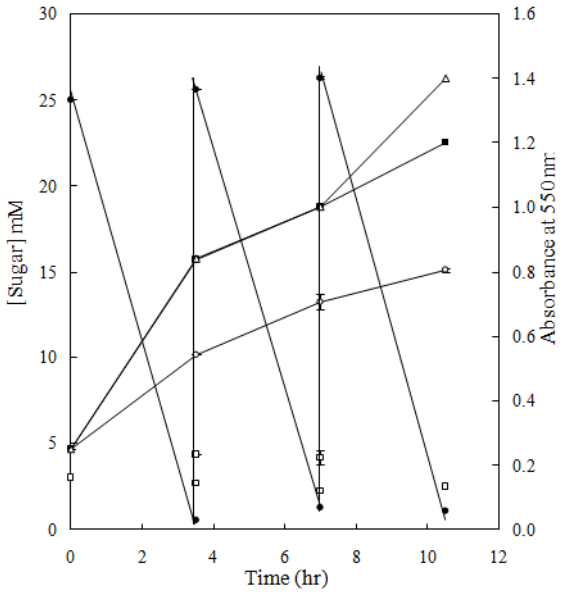

Figure 1. Yeast growth kinetics on CE. S. cerevisiae cells were grown in CE fed-batch medium, at pH 5.5, 32 °C, and shaking at 300 rpm for 10 h. At 3.5 h intervals (i.e. after 3.5 h and 7 h) two CE doses of each extract were added to maintain sugar concentration at ~ 28 mM . -□- Reducing, -●- non-reducing sugar, A550nm cell growth -○- : on CE-1, -■- on CE-2, -Δ- on CE-3.

Instantaneous growth rate constant (μ_i, h^{-1}), biomass yield (Y_{Ns} CFC /g sugar utilized), cells biomass yield on substrate ($Y_{x/s}$, g yeast /g sugar utilized), and specific biomass production rate (q_x, CFC /g sugar utilized /h) are presented in Table 3. The level of reducing sugar was

maintained at ≅ 3 mM, while non-reducing sugar level decreased to almost zero at the end of the interval period. The yield of cellular mass values were 0.04, 0.15, and 0.27 as g of dry cells per one g sugar utilized using CE-1, CE-2, and CE-3, respectively (Table 3).

Table 3. Kinetic parameters of yeast growth on carob extracts (CE) prepared under variable conditions (for abbreviation in the table, see footnotes below).

	CE1			CE2			CE3		
Time, h	3.5	7	10	3.5	7	10	3.5	7	10
μ_i, h^{-1}	0.22	0.22	0.21	0.53	0.28	0.19	0.4	0.24	0.06
Y_{Ns}[a]	-	-	0.53	-	-	1.46	-	-	3.7
q_x[b]	-	-	0.52	-	-	1.46	-	-	3.7
$Y_{x/s}$	-	-	0.04	-	-	0.15	-	-	0.27

Abbreviations: CFC is colony forming cells, instantaneous growth rate constant (μi, hr^{-1}), biomass yield (YNs CFC/g sugar utilized), cells biomass yield on substrate (Yx/s, g yeast/g sugar utilized), and specific biomass production rate (qx, CFC/g sugar utilized/hr). [a]The values are multiplied by 10^{-9} i.e. number of colony-forming cells is $x10^9$).[b]The values are multiplied by 10-8 (i.e. colony forming cells is $x10^8$)

Apparently, the parameter μ_i was constant at 0.22 h^{-1} for CE-1 at times of growth, 3.5, 7, and 10 h. For CE-2 and CE-3, the values were 0.53 h^{-1}, 0.28 h^{-1}, and 0.19 h^{-1} and 0.4 h^{-1}, 0.24 h^{-1}, and 0.06 h^{-1}, respectively.

3.3. Effect of Ph on S. Cerevisiae Growth on CE

The pattern of yeast growth on CE-2 at pH range of 3 to 7 was studied. The sugar level was kept at 2 %. The profiles show an optimal pH for yeast growth at 5.0 to 6.0 (figure is not shown).

3.4. Effect of Aeration and Continuous Feeding on Yeast Growth

The effect of aeration and continuous feeding of CE on cell growth is presented in Table 4. The biomass yield was, somehow, improved by 113.2 %. Typical results of such experiments are presented using CE-2. Table 2 shows the reducing sugar concentration increased and non-reducing sugar decreased with increasing feeding rate. Also, it is observed that reducing sugar concentration was maintained around 3 mM. The values of μ_i at 2, 4, 6, 8, and 10 h were 1.41 h^{-1}, 0.502 h^{-1}, 0.369 h^{-1} , 0.308 h^{-1}, and 0.184 h^{-1}, respectively. $Y_{x/s}$, q_x, and Y_{Ns} were $6.37x10^8$, $6.37x10^7$, and 0.27, respectively, at 10 h growth period.

Table 4. Aeration effect on parameters of yeast growth on continuous feeding of CE-2. Aeration rate was $2m^3$ / min, and feeding flow rate was 26 mL /h. The cellular growth was at pH 5.5, 32 °C, and shaking at 150 rpm (abbreviations of parameters are the same of those in table 3).

Time, h	2	4	6	8	10
μ_i, h^{-1}	1.41	0.502	0.369	0.308	0.184
Y_{Ns}[a]	-	-	-	-	0.637
q_x[b]	-	-	-	-	0.637
$Y_{x/s}$	-	-	-	-	0.27

[a]The values are multiplied by 10^{-9} (i.e. number of colony-forming cells is $x0^9$).

[b]The values are multiplied by 10^{-8} (i.e. number of colony-forming cells is 10^8).

The effect of growth limiting nutrients (i.e. Ca, Mg, N, P, and S as the salt forms) that may be depleted before the depletion of the carbon source is presented in Table 5 which summarizes the yield (g dry cells /100g carob kibble) of yeast grown on the carob extracts prepared under various conditions. The yield from CE-1, CE-2, and CE-3 were 2.0±0.2, 7.2±0.6, and 12.6±1.8, respectively. These values were obtained from cultures without aeration and continuous feeding. Up on aeration and continuous feeding, the yield increased by 20 % (15.4±2.2) for CE-3. The additives increased the yield to the level obtained from the aeration and continuous feeding (Table 5).

Table 5. The yield of yeast (g of dry cells /100g carob kibble) using CE prepared by different procedures, and the effects of certain exogenous nutrients added to the extract.

Extract	Yield
CE-1	2.0±0.2
CE-1[a]	11.9±0.6
CE-2	7.2±0.6
CE-2[a]	12.6±0.1
CE-3	12.6±0.8
CE-2, aeration, continuous feeding	15.4±2.1
CE-3[a]	12.4±.1.2

[a] plus 22.7 mM $(NH4)_2SO_4$ + 13.2 mM KH_2PO_4 + 0.9 mM $MgSO_4$ + 0.1 mM $CaCl_2$

3.5. The bread which was prepared using the produced yeast in this study and that prepared using commercial yeast for leavening the dough were tasted by seven persons. Five persons said that there is no difference in the two types of bread, and two persons said that the bread prepared using CE yeast had a better taste and a distinct flavor.

4. Discussion

4.1. Procedure to Prepare CE

In this study, the best procedure for preparation of CE from carob kibble was to mix kibble particles of 0.5-1.0 cm diameter in size with water in 1:4 p:w ratio for 2 h at 45 °C. This was based on information in the literature and this work. The CE-1 and CE-3 extraction methods were mainly developed to reduce the extraction of tannins. It was suggested that tannins have an inhibitory effect on yeast growth; although tannins have antioxidant property by their antiradical-scavenging effect (Yoshida *et al.*, 1989). Since the smaller the size of the particle the larger the extractable material, CE-1 lacks a number of nutrients such as nitrogenous substances. The protein in carob is associated with tannins (Calixto, 1987). Thus, CE-1 lacks the protein that is important as nitrogen source for yeast growth. Therefore, for this reason preparation procedure of CE-2 was developed. Applying temperature at 45°C improved the extractability of nutrients. Hence, a combination between CE-1 and CE-2 was designed to give CE-3 that has lower tannins level than CE-2 and more nutrients than CE-1.

CE-1 contained the highest sugar level among the carob extracts obtained in this study, and this may be explained in two ways. In one way, heating during preparation of CE-2 and CE-3 causes degradation of some sugars. In another way, tannins, proteins, and other nutrients are present in higher concentrations in CE-2 and CE-3 than in CE-1. These nutrients may compete with sugars for water in the extraction process. Because degradation of sugars starts at temperature above 50 °C (Mulet *et al.*, 1988) and CE-2 and CE3 have higher concentration of protein and tannin (Table 2), it was suggested that the nutrients - water competition assumption is more reasonable. Tannins concentration was lower in CE-1 and CE-3 than in CE-2. This is because tannins in carob are in granules (Mulet *et al.*, 1988). When the particle size is small the granules are degraded, and this event frees tannins.

4.2. Growth Kinetics of S. Cerevisiae on CE

The growth of yeast gave the highest yield on CE-3. This might be due to higher content of nitrogen source and lower tannin concentration compared to CE-1 and CE-2, respectively. Furthermore, *S. cerevisiae* consumed sugars from CE-1, CE-2 and CE-3 in the same pattern. Apparently, nonreducing sugars decreased to zero level, while reducing sugars remained around 3 mM. It means that 10% of the sugars remained not consumed. This observation is in agreement with (Roukas, 1993). Also, this may suggest that consumption of reducing sugars becomes concentration-dependent >3 mM. This was seen in the growth abrupt upon the addition of CE. Alternatively, accumulation of reducing sugars could be explained in another way. Sucrose, the main sugar present in carob, is converted by the yeast to fructose and glucose. So in order to consume all reducing sugars, it may require longer time than 3.5 h. Since carob has small concentrations of free glucose, fructose, xylose, and others (Calixto, 1987), the accumulation of the reducing sugars could be due to a combination of reasons. Furthermore, previous studies showed that *S. cerevisiae* cannot utilize xylose because it lacks the enzyme xylose isomerase (Gong *et al*., 1981). Xylitol and penititol are poor carbon source for yeast due to its limited permeability into cells (Singh and Mishra, 1995).

The parameter μ_i decreased within 3.5 h-interval, and this was in agreement with reported values. The yield coefficient was highest with CE-3 because it might be of low tannins and high protein contents.

S. cerevisiae growth parameters obtained from batch versus continuous feeding media of CE were lower in batch. Y_{xs} and q_x were 1.8 x 10^9 CFC /g sugar utilized and 5 x 10^7 CFC /g sugar utilized /h, respectively, in batch-culture (Roukas, 1993). The yield coefficient for molasses and sorghum hydrolysates was found 0.28 (Reed and Nagodawithana, 1991; Konlana *et al.*, 1996), which is comparable to 0.27 for CE-3 in this study. Molasses yield coefficient could not be compared with that for CE-3 because molasses yield coefficient was under optimal condition of nutrient supplements, that requires further investigated on carob fruits.

4.3. Effect of pH

The optimal pH for growth of baker's yeast was examined in batch-culture for 24 h at 32 °C. It appears that at pH values, 5.0 to 6.0, the yeast has the same growth rate. This indicated that the optimal pH for yeast growth under the conditions of this study is between 5.0 and 6.0. This range of pH value is consistent with that for molasses (Reed and Nagodawithana, 1991) and for whey (Champagne *et al.*, 1990).

4.4. Effect of Aeration, Continuous Feeding, And Ph

Aeration and continuous feeding increased the biomass yield. Under strict aerobic conditions the best yield was 54 g yeast solid per 100 g of glucose (Reed and Nagodawithana, 1991). So if carob kibble has 50 % sugar, it suggests that the maximum yield would be 27 %. The obtained result, 26 %, is essentially the theoretical value. The parameter μ_i (0.18) after 10 h is in agreement with van Hoek *et al.*, 2000, equation. This high value indicates that ethanol essentially was not produced. Its production would lower the availability of carbon source for cell growth.

Therefore, the optimal method to prepare CE was to use carob kibble with particle diameter between 0.5-1.0 cm, and 1:4 p:w ratio for 2h. Accordingly, continuous (column extraction) and discontinuous methods both gave the same results. But, to automate the carob extraction for large-scale production, continuous extraction may be the method of choice (Petit and Pinilla, 1995).

Comparing carob with other substrates used for baker's yeast production, carob gave equal biomass yields in some cases, and higher values in others.

The seeds of carob pods contribute more than 60 % of the pod market price (Makris and Kefalas, 2004) and carob kibble, that is left, mostly, is thrown away as waste. Thus, carob kibble is a cheap substrate for production of baker's yeast. The present results point out that carob kibble is an excellent economical substrate for industrial production of baker's yeast. The production of yeast from carob fruit may be another motive to enhance the cultivation of vast areas of land that are suitable for forestry.

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