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Baker's Yeast Production from Cactus *Opuntia* Cladodes Extract: Optimization of pH and Temperature

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Abstract

Aim: This study aims to evaluate the using of Cactus *opuntia* cladodes (*COC*) extract medium for growth of *Saccharomyces cerevisiae (*Baker's Yeast, BY), and to optimize pH and temperature values of the growth.

Methodology: The study design was a comparative study. The control media were both potatoes dextrose (PD) agar (PDA) and broth (PDB). The Opuntia plant aged 1-2 years was obtained from a farm located in the border of Jabalia Refugee Camp. The crude *COC* extract was diluted to 50% before using as experimental medium for BY. The preactivated BY sample was diluted to 10^{-7} before use. Experiments were carried out on both surface and submerged cultures. SPSS system was used to analyze the obtained data.

Results: The results showed that the BY can grow well and proliferate in *COC* extract (50% dilution) where the average specific growth rate (μ) was about 0.17 h^{-}. Similar to the growth of the BY on PDB medium, optimum pH and optimum temperature values for the yeast growth in the extract medium were 4.0 and 30 ^{0}C, respectively. It was also found that average yeast dry weight which was produced in *COC* extract was about 41.3 g/L, while an equal quantity of PDB produced about 54.1 g/L at the same conditions. Moreover, the results showed that 1000g fresh weight of COC produced 62.0 g of BY.

As a conclusion, *COC* extract could be used for production of BY at pH 4.0 and 30 ^{0}C.

Key words: Baker's Yeast, *Cactus opuntia* cladodes, pH, temperature, specific growth rate.

تنمية خميرة الخبز على عصير الصبار وتحديد الرقم الهيدروجيني ودرجة الحرارة المناسبين للنمو

الهدف: تهدف الدراسة لتقييم استخدام عصير الصبار في نمو فطر خميرة الخبز، وتحديد الرقم الهيدروجيني ودرجة الحرارة المناسبين لنموها.

المواد والطرق: أثناء الدراسة تم مقارنة استخدام عصير الصبار (كوسط غذائي تجريبي) مع وسط قياسي يسمى جلوكوز البطاطس (كوسط غذائي ضابط) في نمو فطرة خميرة الخبز. يبلغ عمر

الصبار المستخدم في التجارب من عام على عامين، وحصلنا عليه من مزرعة خاصة تقــع علــى حدود مخيم جباليا شمال قطاع غزة. وتم تخفيف عصير الصبار الخام إلي 50% قبل استخدامه في عملية نمو فطر الخميرة، وخفف فطر الخميرة المنشط إلي واحد من عشرة ملاين قبل زراعتها في الأوساط الغذائية المذكورة في الحالة الصلبة أو السائلة منها. واستخدم برنامج الحزمة الإحــصائية (SPSS) للتحليل بيانات الدراسة.

النتائج: تبين إثناء الدراسة أن خميرة الخبز تنمو و تتكاثر جيداً على عصير الصبار الخام، فكــان متوسط النمو النوعي لها هو 0.17 للساعة، وأن أنسب درجة حرارة ورقم هيدروجيني لنموها هما 30 درجة مئوية و 4.5 على التوالي ، وكل لتر من عصير الصبار أنتج حوالي 41.3 جراماً مــن فطر الخميرة، بينما كل لتر من الوسط القياسي الضابط أنتج 54.1 جراماً من الفطر تحت نفــس الظروف البيئية المذكورة، وأن كل كيلوجرام من الصبار أنتج 62.0 جراماً من الفطر.

Introduction

Yeasts are eukaryotic microorganisms classified in the kingdom Fungi, with approximately 1,500 species described [1]. Most reproduce asexually by budding, although few strains do that by binary fission. Yeasts are unicellular, although some species may become multicellular through formation of pseudohyphae, or true hyphae as seen in most molds [2]. Yeast size can vary greatly depending on the species, typically measuring 3– 4 µm in diameter [3]. Yeasts are chemoorganotrophs where the main source of carbon is obtained by hexose sugars such as glucose, or disaccharides such as sucrose and maltose. Yeast species are either obligate aerobes or facultative anaerobes. Yeasts are ubiquitous in the environment, but are most frequently isolated from sugar-rich samples.

The most common medium used for the cultivation of yeasts is PD. Most yeasts form small circular colonies when grown on agar media. Growth will at first be exponential until a mass of cells is produced. At this stage only the cells at the edge of the mass will have access of nutrients so the marginal growth zone will result. A linear increase in radius of the zone will result with time which will be slowed or even halted by nutrients depletion or by the accumulation of toxic metabolites such as ethanol [4].

When microorganisms are grown in a homogeneous batch culture, three phases of growth can be distinguished– the lag, exponential and stationary phases. The exponential phase is the period during which a constant specific growth rate (µ) is maintained and biomass increase is exponential. µ is maximal when all the nutrients required for growth are in excess and no inhibitor of growth is present. If any nutrient is present in sub optimal amounts then the value of µ relative to μ_{max} is determined by the affinity of the organism for the limiting nutrient and by the concentration of the nutrient. However, µ of BY growth on glucose is 0.47 h^{-1} and doubling

time (t_d) is 88 minutes. The growth rate equation used to calculate the specific growth rate is $\mu = 2.3 (\log x - \log x_0)/\Delta t$, where x and x_0 are biomass of BY that is directly proportional with optical density (OD) and Δt is the time difference. t_d is obtained from the relationship = $0.69/\mu$ [4].

BY is normally produced from molasses, grains or potatoes. Using of COC extract for BY production was not carried before. Prickly pear cactus (*Opuntia spp*.) is a fast growing xerophytic plant well adapted to arid conditions where it is widely used as fodder, forage, fruit and green vegetables [5]. Nowadays, *Opuntia* plants are grown in many countries. For cladodes, a mean hectare can yield 30–80 tons annually [6]. The plant is rich in energy, water and mucilage and poor in protein and has been used in livestock feeding since the nineteenth century [7]. Chemical compositions of COC sample from the Gaza Strip as measured in % wet basis are: Moisture 84.0, Crude protein 8.7, Crude fibers 2.9, Fat contents 0.02, Carbohydrates 7.0, Nitrogen free extract 10.9, Ash 1.2, Calcium (mg/ kg) 11.0, Iron (mg/ kg) 1.3. [8].

Mucilages are the major water soluble fibers in the COC. They are complex polymeric substances of carbohydrate nature, with a highly branched structure. Mucilages contains varying proportions of L-arabinose, D-galactose, L-rhamnose and D-xylose, as well as galacturonic acid in different proportions. The mucilage structure is proposed as two distinctive water-soluble fractions. One is a pectin with gelling properties with Ca^{2+}, and the other is a mucilage without gelling properties [9]. In another study it was found that the average pH of the young cladodes is 4.08 and for old cladodes 3.83, while the Ca and P contents of the young and old cladodes were 7.72 mg, 0 .60 mg and 6.56 mg, 0 .5 mg, respectively [10].

Significance and objectives of the study: In the light of the siege imposed on the Gaza Strip (GS) more than two years ago, suffering is spread among the whole people living in the GS. This led international commissions in GS to warn of the serious repercussions on the Palestinians and their future health and education, as a result of the siege.

In this regard, the siege affected availability of imported materials that the students should frequently use them in experimental courses such as some reagents and media required for growth of some microorganisms.

However, similar to PD, COC extract is very rich in fermentable sugars that some microorganisms such as BY can use to grow. Moreover, to date no scientific studies have been carried out using this local raw material for growing BY in a great economical reward for the local community.

The main objective of this study is, therefore, to describe the use of valuable and low cost COC extract for BY production. The other objective is to describe the optimal pH and temperature of BY growth on the extract.

Materials and Methods

The sample of *COC* aged 1-2 years harvested at 6:00 am from a local farm located in the West of Jabalia Refugee Camp. The BY (*Saccaromyces cerevisiae)* strain was from Maya Co., Turkey.

Study design: The present study compared between BY growth on *COC* extract and on the PDB.

Setting and duration of the study: The study experiments were carried out in the faculty of science laboratories at the Islamic university- Gaza. The study started on July, 2007 and ended on April, 2008.

Activation and dilution of BY: To activate BY, an autoclaved PDB medium (100 ml) at a pH 3.5 was inoculated by about 0.1g of dry BY. The sealed container used was left in shaker incubator (120 rpm) for 48 hours at 30 ^{0}C. A serial dilutions of (10^{-1}-10^{-8}) were obtained by adding 1.0 ml of thepre-activated BY in normal saline.

Preparation of PDA and PDB media

Thirty nine g of PDA powder were dissolved in distilled water to a final volume of one liter, then autoclaved for 15 minutes. Similarly, PDB media were prepared by dissolving 27g of the medium powder in distilled water to a final volume of 1.0 liter. Any ingredients added to these media were carried out after autoclaving under normal sterile conditions. The media pH was adjusted to 3.5 using tartaric acid. About 25 ml aliquots of PDA were poured into a set of Petri dishes, left to cool at 4^0C till used. Both media were used as control.

Preparation of the *COC* extract: The harvested *COC* were picked off from spines and washed well by tap water, then peeled by a sharp knife. The peeled cladodes were cut off into small cubes then blended. The resulted extract was filtrated by apiece of clothes then centrifuged by Sorvall centrifuge at 5000 rpm for 10 min at 30 ^{0}C. The clear extract was then transferred to a clean flask, and sealed by a Para- film then left at home refrigerator (4^0C) till used.

Preparation of COC extract dishes and broth: The filtrate of *COC* extract was diluted to 50 % by distilled water, and a suitable amount of agar was added (15 g/l). A pellet of NaOH was added to the extract while heated to prevent destruction of the agar. Required amounts of peptone (1 up to 6 g/100 ml) were added. After autoclaving, the pH of the medium was adjusted to the value 3.5 using 1M HCL and 1M NaOH, then it was poured into dishes. The dishes were left at 4^0C till used. Similarly, COC broth was prepared without addition of agar.

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Cultivation of BY on semi solid and liquid media: Inoculation of the semi solid media with 0.1 ml of the pre-activated BY was carried out in triplicates. Different dilutions of the pre-activated of BY (10^{-1}-10^{-7}) were used. All the dishes were incubated at 30 ^{0}C for two days. Similarly, 3 % of the preactivated BY with different dilutions were added to the liquid media (100 ml/ flask). The inoculated flasks were left in a shaker incubator (120 r.p.m) at 30 ^{0}C for a week.

Growth curve of BY on PDB: One tenth (0.1) gm of dry BY was added to 100 ml of autoclaved PDB flask (500 ml) with pH 4.0. The flask was incubated at 30 ^{0}C with a shaking rate of 120 r.p.m . A sample of 1 ml was aspirated at various times for four days and the optical density (O.D) at 660 nm was measured using single beam spectrophotometer (Spectronic 20, Germany). The experiment was done in duplicate

Calibration curve of BY

(1.0×10^{-5} up to 4.0×10^{-3} g) weights of dry BY were dissolved in normal saline solution and measured at 660 nm at room temperature. The optical density versus BY concentrations values were graphed.

Growth of BY in PDB and in COC extract with and without peptone: An enough volume of COC extract (50 %) at pH 5.0 was poured into 7 flasks (100 ml for each). The flasks were autoclaved for 15 min. The first six flasks were inoculated with different concentrations of peptone (0g/100ml up to 6g/100ml), the seventh one contained pure COC extract. The eighth flask contained PDB as control. Three percents (3% v/v) of the pre-activated yeast at the dilution 10^{-7} was added to every flask. The flasks were incubated in a shaker incubator (120 rpm) for a week at 30 ^{0}C. One ml from each flask was aspirated every 24 hours, and the O.D at 660 nm was measured. The experiment was done in triplicates.

Optimizing the pH of the yeast growth media: Several concentrations of the *COC* extract dishes have been prepared (100%, 75%, 50%, 25%,10%) in triplicates with different pHs (3.5, 4.0, 4.5, 5.0, 5.5) and inoculated with 0.1 ml of pre-activated yeast of the dilution 10^{-7}. The dishes were incubated at 30 ^{0}C for 48 hours. The produced colonies were counted visually. The same procedure was repeated in broth medium.

Optimization of temperature for BY growth: One tenth (0.1) gm of dry yeast was cultivated on 100 ml of PDB and *COC* extract (50% conc.) at pH 4.0, and at different temperatures range of (25 ^{0}C up to 40 ^{0}C) with an increment of 5 ^{0}C for each trial. One ml of the medium was taken at various times and measured at 660 nm. The experiment was also done in triplicates.

Data Analysis: Data obtained were analyzed using SPSS system (version11)and Microsoft excel. Student t-test and ANOVA test were carried out at

a significance level of 5%, for comparing two and more numerical means, respectively.Difference between variables was considered statistically significant if $p< 0.05$.

Results and Discussion

Surface cultures.

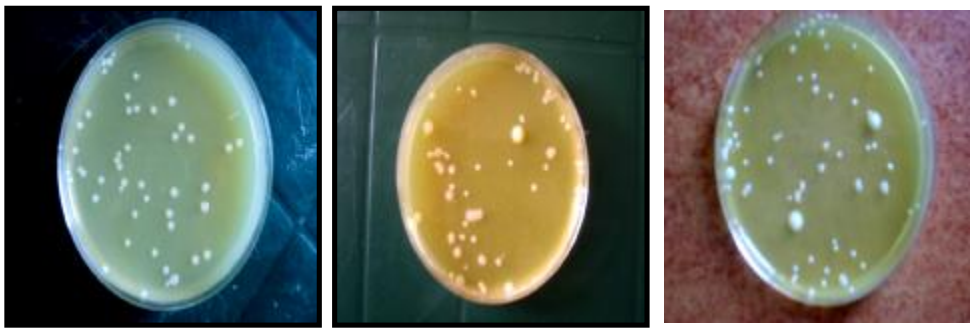
The first experiments showed that BY can grow in *COC* extract as good as on PDA. Number of BY colonies that were grown on both media with and without peptone at the same conditions (T=30 ^{0}C, pH 5) were too much to count up to dilution factor of BY to 10^{-6}. The best dilution of BY that gave good and measurable results was 10^{-7} where the size of the colonies was small and countable (data are not shown).

Table1 shows that at BY dilution of 10^{-7} there was no statistical significance differences between addition of peptone up to 4g % on COC extract and pure *COC* extract alone. Moreover, table 1 and figure 1 show that the growth of BY on PDA was significantly better than its growth on pure COC extract and COC extract plus peptone. In contrast, table 1 also shows that more addition of peptone to the COC extract (5g % and 6g %) has inhibitory effect on the BY growth. This inhibitory effect of peptone on BY growth was increased with increasing peptone amount which requires explanation.

Table 1: BY Growth on COC extract with and without peptone. PDA was a control.

Media	BY dilution	Peptone conc. g%	BY colonies No.			Average BY colonies No.* (SD)
			1	2	3	
PDA	10^{-7}	0	55	48	51	51 (3)^a
Pure *COC extract*	10^{-7}	0	45	40	38	41 (3)^b
COC extract + Peptone	10^{-7}	1	42	38	35	38 (3)^b
		2	40	36	36	37 (2)^b
		3	44	39	37	40 (3)^b
		4	39	35	36	37 (2)^b
		5	25	27	23	25 (2)^c
		6	19	16	17	17 (1)^d

***Means with different superscripts in the final column differ significantly($p<0.05$)**



a **b** **c**

Figure1: BY growth on COC extract plus peptone (a), COC extract alone (b) , and PDA (c).

Table 2 shows effects of different pHs and COC extract dilutions on growth of BY. The best pH of BY growth on the COC extract was about 4.0 and the best dilution was 75% (figure 2). Thus, there was no need to set up the COC extract pH values because its normal pH value upon harvesting at 6:00 am is already 4.1. Moreover, the slight difference between the number of BY colonies on 75% and 50% COC extract dilutions indicated that it was possible to use 50% COC extract dilution from economical point of view. The growth of BY on 100% and 50% COC dilutions were very similar at pH 4.0. The viscosity of 100% crude COC extract might be the cause of limiting the BY growth.

Table 2: Effect of pH values and different *COC* extract dilutions on BY growth.

***COC* extract dilution.**		**pH of the *COC* Medium**				
		3.5	**4.0**	**4.5**	**5.0**	**5.5**
100%	**Mean of colonies(SD)**	$31(2)^{c}$	$44(2)^{a}$	$35(1)^{b}$	$20(2)^{d}$	$8(2)^{e}$
75%		$35(1)^{c}$	$50(2)^{a}$	$38(1)^{b}$	$9(1)^{d}$	0
50%		$15(1)^{c}$	$45(2)^{a}$	$31(2)^{b}$	$3(1)^{d}$	0
25%		$10(1)^{b}$	$20(1)^{a}$	$10(1)^{b}$	$3(1)^{c}$	0
10%		$10(1)^{a}$	$7(1)^{b}$	$9(2)^{a}$	0	0

***** Means of different superscripts in the same raw show high significance difference (p=0.00)

***** The values given were an average of triplicate samples.

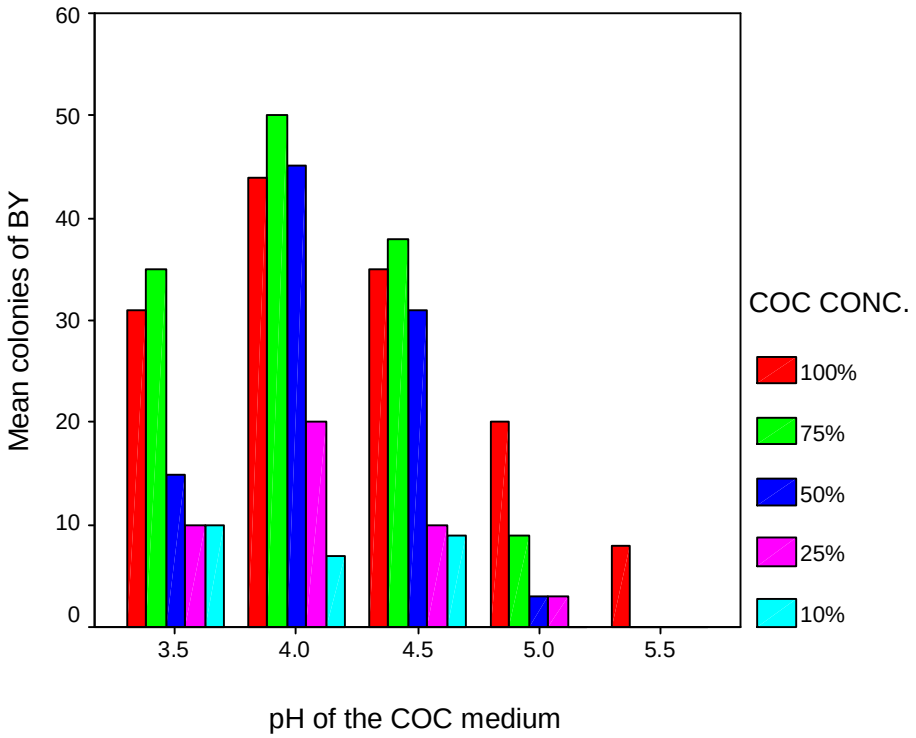


Figure 2: Multiple bar chart showing effect of pH and different COC extract dilutions on BY growth on the surface culture.

Submerged cultures.

Figure 3 shows all growth phases of BY in a standard PDB medium at 30 ^{0}C and pH 4.0. As shown, the lag, log and stationary phases were completed through the first three days and the death phase began beyond 72 hrs of growth. μ and t_d of BY in PDB were 0.25 h^{-1} and 83 minutes, respectively. The maximum O.D obtained was 22.91 which was equivalent to production of 54.1 g/L (D.W) of BY. These findings were very consistent with those results reported about growth of BY in glucose medium [11].

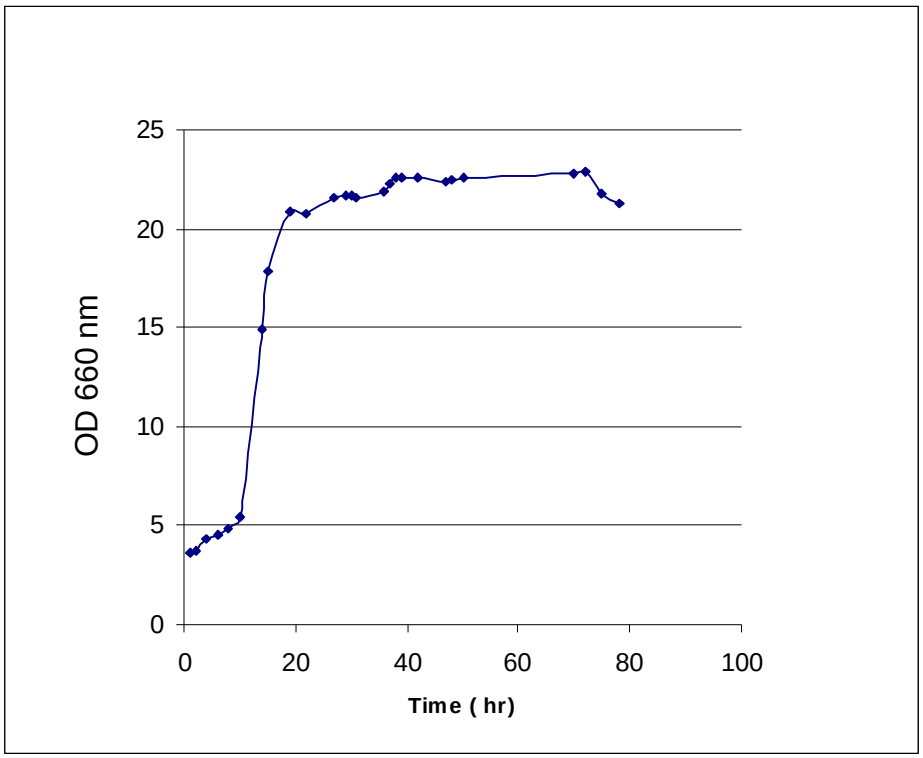


Figure 3: Growth curve of BY in PDB at 30 ^{0}C and pH 4.0.

Figure 4 illustrates the resultant calibration curve of BY growth at pH 4.0 and 30 ^{0}C. The extension coefficient (ϵ_{660}nm) of BY growth was 423.6 gm^{-1}cm^{-1}ml and each O.D unit corresponds to about 2.2 x10^{-3} g BY dry weight or about 3 x 10^{7} cells by considering that each one gram of BY counts about 15 billion live cells [12,13].

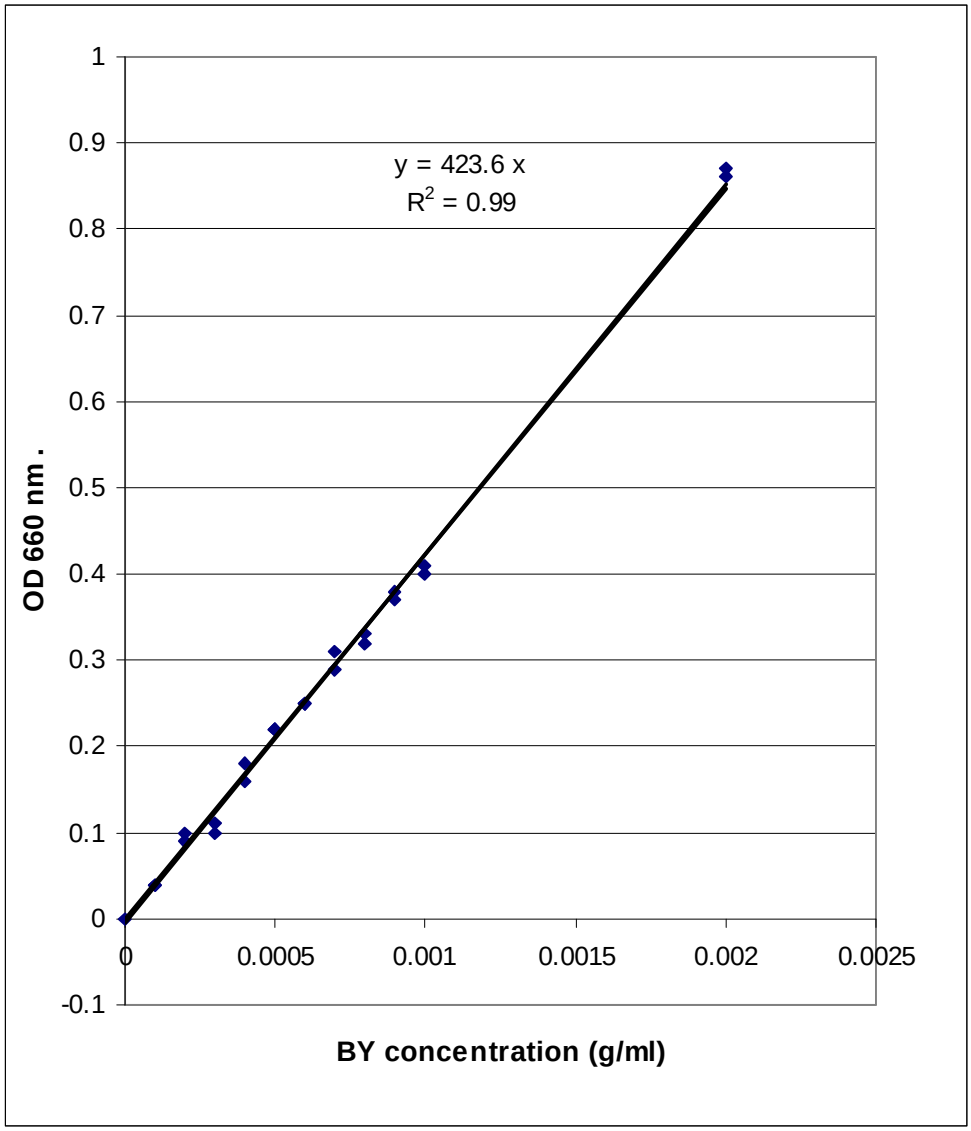


Figure 4: Calibration curve of BY cells at 660nm and 30 ^{0}C.(in g/ml dry weight).

Table 3 pointed out that BY growth in PDB was significantly better than the growth of BY in pure COC extract or in COC extract plus peptone. Growth of BY on pure COC extract medium is similar to addition of peptone to it (1 or 2 %) at the first 20 hrs of growth. At 28 hrs or more, growth of BY on COC extract and 1 or 2 g % peptone became better than its growth in the COC extract alone. In contrast, effect of addition of 3 or 4 g % peptone to the extract were very heterogeneous and not better than addition of 1 or 2 g % peptone to the extract. Moreover, the same experiment also showed more addition of peptone (5g % and 6 g %) has inhibitory

effect on the BY growth if compared with the lesser concentrations of peptone ($p>0.05$). Table 4 shows the resultant µ values of BY growth upon addition of different peptone concentrations. It was found that µ value of BY growth in COC extract increased significantly at (1 or 2g %) peptone concentration comparably with pure COC extract. In contrast, µ values decreased significantly as the peptone concentration became ≥3 % (table 4). However, these results indicated that, COC extract proteins is not enough for BY growth and proliferation, so adding 1 to 2 % nitrogen ingredients to the COC extract medium will improve the BY growth continuity.

Table 3: BY growth in COC extract at different peptone concentrations. PDB as control medium (BY dilution was 10^{-7})

Time (hrs)								
	PDB	**COC extract**	**COC+ pep. 1g%**	**COC+ pep. 2g%**	**COC+ pep. 3g%**	**COC+ pep. 4g%**	**COC+ pep. 5g%**	**COC+ pep. 6g%**
0	0.57^{a} (0.01)	0.56^{a} (0.01)	0.56^{a} (0.01)	0.54^{a} (0.03)	0.56 ^{a}(0.02)	0.55^{a} (0.02)	0.54^{a} (0.01)	0.52^{a} (0.01)
4	0.72^{a} (0.02)	0.64^{b} (0.01)	0.64^{b} (0.01)	0.59^{c} (0.01)	0.58^{c} (0.01)	0.57^{c} (0.01)	0.55^{d} (0.01)	0.54^{d} (0.01)
7	1.30^{a} (0.02)	1.10^{b} (0.02)	1.01^{c} (0.02)	0.97^{c} (0.02)	0.93^{d} (0.01)	0.93^{d} (0.02)	0.89^{e} (002)	0.88^{e} (0.01)
16	8.62^{a} (0.01)	5.00^{b} (0.01)	5.02^{b} (0.01)	4.30^{c} (0.01	4.29^{c} (0.02)	4.23^{d} (0.03)	3.91^{e} (0.02)	3.85^{e} (0.01)
20	9.85^{a} (0.06)	7.55^{a} (0.02)	7.33^{c} (0.03)	6.94^{d} (0.07)	6.41^{e} (0.05)	6.33^{f} (0.03)	4.44^{g} (0.02)	4.42^{g} (0.03)
28	12.40^{a} (0.04)	9.62^{c} (0.02)	10.23^{b} (0.01)	8.85^{d} (0.04)	8.45^{d} (0.04)	7.63^{e} (0.04)	4.84^{f} (0.03)	4.64^{f} (0.02)
48	13.73^{a} (0.03)	10.90^{c} (0.05)	11.21^{b} (0.07)	10.31^{d} (0.09)	9.09^{e} (0.03)	8.34^{f} (0.02)	4.12^{g} (0.03)	3.65^{h} (0.08)
70	14.08^{a} (0.03)	11.15^{c} (0.06)	11.72^{b} (0.03)	10.92^{d} (0.09)	9.36^{e} (0.04)	8.62^{f} (003)	3.34^{g} (0.09)	2.57^{h} (0.06)
96	13.86^{a} (0.09)	11.54^{c} (0.02)	12.01^{b} (0.04)	11.12^{d} (002)	9.70^{e} (0.04)	8.21^{f} (0.04)	3.15^{g} (0.04)	2.41^{h} (0.03)
120	9.96^{e} (0.02)	11.87^{b} (0.07)	12.24^{a} (0.03)	11.26^{c} (0.05)	9.88^{d} (0.04)	8.03^{f} (0.03)	2.84^{g} (0.02)	2.16^{h} (0.02)
144	9.32^{e} (0.04)	11.98^{b} (0.03)	12.68^{a} (0.03)	11.37^{c} (0.01)	9.93^{d} (0.03)	7.98^{f} (0.02)	2.41^{g} (0.02)	2.02^{h} (0.02)

*The values given were an average of triplicate samples.

*Means with different superscripts in the same raw differ significantly ($p< 0.05$)

Table 4: Specific growth rate of BY in COC extract at different peptone conc..

	(μ)* (SD) h^{-1}						
	COC extract + Peptone						
PDB	**0g%**	**1g%**	**2g%**	**3g%**	**4g%**	**5g%**	**6g%**
0.210^{a} (0.016)	0.168^{c} (0.012)	0.178^{b} (0.022)	0.176^{b} (0.015)	0.164^{d} (0.016)	0.163^{d} (0.024)	0.158^{e} (0.017)	0.156^{e} (0.016)

*The values given were an average of triplicate samples
*Means with different superscripts differ significantly ($p< 0.05$)

Table 5 illustrates that BY growth on the COC extract and PDB was not significantly different at first 8 hrs of cultivation. Clear significant difference of BY growth in both media was observed at 18 hrs or more. However, the best pH for optimum BY growth was 4.0 in both COC extract and PDB. It can be also noticed that BY production decreased at the beginning of the fifth day of cultivation because yeast cells began to die as a result of cell competition or wastes accumulation in the medium. However, 29.0 mg/ml of BY was produced in COC extract after 72 hours of cultivation at pH 4.0 compared with 37.0 mg/ml produced in PDB at the same conditions. However, μ values of BY in COC extract medium was 0.21 h^{-1} and 0.18 h^{-1} at the pHs of 4.0 and 4.5, respectively (Table 6). It also shows that μ of BY at different pH values in PDB medium was significantly more than in COC extract ($p<0.05$). These results and the previous ones on the surface culture showed that the growth of BY on PDB and the COC extract was optimal at pH 4.0.

Table 5: Growth of BY in *COC* extract and PDB at different pH values and 30 ^{0}C.

Time (hr)	pH	Average $O.D_{660nm}$ on COC (SD)	D.W of BY on COC gm/ml	Average $O.D_{660nm}$ on PDB (SD)	D.W of BY on PDB gm/ml	P-value
0	4.0	0.57(0.07)	0.001	0.59(0.01)	0.001	0.79
	4.5	0.58(0.01)	0.001	0.59(0.00)	0.001	0.83
	5.0	0.56(0.01)	0.001	0.57(0.02)	0.001	0.47
8	4.0	0.98(0.03)	0.002	1.02(0.01)	0.002	0.17
	4.5	1.09(0.08)	0.003	1.15(0.04)	0.003	0.39
	5.0	1.08 (0.03)	0.003	1.19(0.08)	0.003	0.15
18	4.0	8.15(0.08)	0.019	11.95(0.08)	0.028	0.00
	4.5	6.63(0.16)	0.016	11.54(0.11)	0.027	0.00
	5.0	5.54(0.18)	0.013	9.73(0.16)	0.023	0.00
24	4.0	9.59(0.196)	0.023	12.22(0.17)	0.029	0.00
	4.5	9.18(0.11)	0.022	11.90(0.16)	0.028	0.00
	5.0	8.83(0.12)	0.021	12.28(0.11)	0.029	0.00
48	4.0	9.98(0.07)	0.024	15.35(0.16)	0.036	0.00
	4.5	10.75(0.16)	0.025	15.35(0.11)	0.036	0.00
	5.0	10.36(0.07)	0.024	14.97(0.08)	0.035	0.00
72	4.0	12.11(0.09)	0.029	15.74(0.13)	0.037	0.00
	4.5	11.51(0.07)	0.027	15.35(0.11)	0.036	0.00
	5.0	11.13(0.06)	0.026	14.96(0.09)	0.035	0.00
96	4.0	12.05(0.11)	0.028	15.50(0.14)	0.037	0.00
	4.5	11.13(0.07)	0.026	14.97(0.09)	0.035	0.00
	5.0	10.36(0.09)	0.024	14.58(0.18)	0.034	0.00
120	4.0	11.51(0.16)	0.027	13.43(0.09)	0.032	0.00
	4.5	10.75(0.16)	0.025	12.28(0.15)	0.032	0.00
	5.0	8.83(0.07)	0.021	10.36(0.10)	0.029	0.00
144	4.0	9.98(0.02)	0.024	12.67(0.08)	0.030	0.00
	4.5	9.60(0.16)	0.023	11.51(0.17)	0.027	0.00
	5.0	8.06(0.02)	0.019	9.60(0.14)	0.023	0.00

Table 6: Specific growth rate of BY in COC extract at different pH values (PDB as control).

pH	μ On COC extract (SD) h^{-1}	μ On PDB (SD) h^{-1}	P- value
4.0	0. 21 (0.01)	0.25 (0.02)	0.04
4.5	0. 18 (0.01)	0.23 (0.01)	0.01
5.0	0. 16 (0.02)	0.21 (0.01)	0.03

Figure 5 shows that the growth curve of BY on the COC extract has more than one log phase because there was more than one carbon source that BY can use to grow and proliferate (diauxic growth behavior).The highest value of O.D. measured was 17.70 at the beginning of the seventh day of cultivation where 41.3 g/L of BY was produced in COC extract (50% dilution). The O.D was decreased to 13.2 in the last 3 hours of the BY growth. The second phase stationary phase was not completed and followed with fast death of the BY. It seems that the amount of the last fermented carbon source was very small in such away the second stationary phase was not started. According to figure 5, μ_1 and μ_2 of BY in COC extract were 0.21 h^{-1} and 0.01 h^{-1}, respectively. Thus, t_d of BY were 1.64 and 34.7 hours, respectively. Accordingly, it was estimated that each 1000g fresh weight of COC produced about 62.0 g (DW) of BY.

According to table 7, It was shown that the optimum temperature for BY growth in both *COC* extract and PDB media was at 30 ^{0}C. At this temperature, the growth of BY in COC extract continued for about 158 hours before exhaustion of the medium (data are not shown). However, table 7 shows μ of BY growth in PDB is significantly higher than its growth in COC extract at the mentioned temperatures ($p<0.05$). It was also noticed that the high temperatures (35 ^{0}C and 40 ^{0}C) and low temperature value (25 ^{0}C) values disturb the enzymes activity inside BY cells leading to decrease in the growth rate of the yeast.

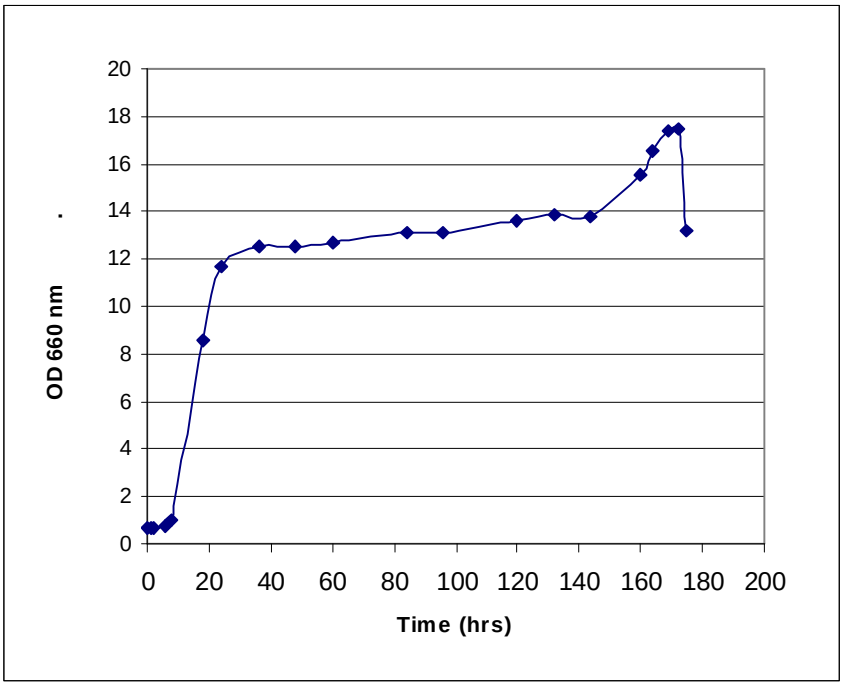


Figure 5 : Growth curve of BY on *COC* extract at 30 ^{0}C and pH 4.0 .

Table 7: Effect of temperature on specific growth rate of BY in *COC* extract and PDB

Temp. ^{0}C	**μ of BY on COC extract (h^{-1})**	**μ of BY on PDB (h^{-1})**	**P**
25	0.04 (0.05)	0.19 (0.01)	0.04
30	0.21 (0.01)	0.25 (0.02)	0.04
35	0.04 (0.01)	0.19 (0.01)	0.02
40	0.05 (0.01)	0.18 (0.01)	0.00

Conclusion

1- BY showed diauxic growth behavior in COC extract medium at pH 4.0 and 30 ^{0}C due to availability of more than one carbon source in the medium.

2- One kg of fresh COC produced about 62.0 g of BY (D.W) at pH 4.0 and 30 ^{0}C.

3- COC extract can be used as alternative to PD after enriching it e.g. adding some natural rich protein additives.

4- Production of a powdered media using dried and enriched COC as a cheap medium for BY growth may replace the currently used expensive PD media which are not available in the Gaza Strip.

5- Large scale studies are recommended on COC extract to produce BY in a commercial scale.

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