

Single cell protein production using low quality fruits of some dates

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Abstract

Single cell protein (SCP) is a good source of essential amino acids. This study aimed to utilize some date by-products such as date fruit flesh to produce single cell protein. Fruit flesh of three unknown dates and three date varieties were used to produce a single cell protein by *Saccharomyces cerevisiae* ATCC64712 under different condition (sugar concentrations in the extract media, temperatures and pH values). The results showed that the optimum production of the biomass (42.85 g/l) was produced from date extracts media at 18% sugar, 30°C and pH 4.5. To reduce nucleic acids content in the biomass, heat shock was used. The effect of heat shock on protein content and nucleic acids percentage for the studied yeast strain was 75.83%, and the maximum reduction of nucleic acids was observed at pH4 and 60°C for 30 sec with 20.88% loss of its protein content. The present study provides evidence that date flesh is a great source for single cell protein that can be used in variable fermentations. Additionally, single cell protein has a high level of essential amino acids such as lysine, methionine and threonine. This source of protein has been proved a good replacement of other expensive protein sources like fish and soybean meals. Therefore, conclusion can be made that single cell protein can easily replace traditional (plant and animal) protein sources in human, animal as well as fish diets without any detrimental effect.

Keywords: single cell protein, date fruit, *Saccharomyces cerevisiae*, amino acids.

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1. Introduction

The date palm (*Phoenix dactylifera* L.) is one of the most economically important fruit tree grown in Egypt. Production of Egypt alone represent about 20% of the total world production at 2012 (Bekheet, 2013; FAO, 2018). There were a gradually increase in the worldwide production; it was about 8.5 million tons in 2016. About half of date production is wasted and thus unutilized. Dates are rich in certain nutrients and provide a good source of rapid energy due to their high carbohydrates content (70 – 80%). Most of the carbohydrates in date fruits are in the form of fructose and glucose, which are easily absorbed by the human body (Al-Farsi *et al.*, 2005; 2008; Mrabet *et al.*, 2008). Date seed oil has been used to replace the portions of other vegetable oils in body creams, shampoos, and shaving soap formulations, and, in general, the quality of these cosmetic formulations is encouraging (Devshony *et al.*, 1992). Besides, date seed has also been introduced recently to the market as a coffee substitute (Rahman *et al.*, 2007). Almost half of the sugar in dates is fructose; and over 75% of dates are reduced sugars on dry basis. Thus, date is a prospective raw material for the production of fructose and single cell protein (Lattieff, 2016). To reduce the production cost of single cell proteins, the most important thing is the selection of cheap and suitable substrates or biodegradable agro industrial byproducts as a nutrient source for the microorganisms to grow and produce tons of protein (Anupama and Ravindra, 2000). For this purpose, different substrates were used and

compared in the past. Some of the commonly used substrates are apple pomace, yam peels, citrus pulp, potato peels, pineapple waste, papaya waste etc. (Nasseri *et al.*, 2011). It is also very important to choose suitable waste product for the proliferation of single cell protein producing microorganism. Nowadays, for both research and industrial purpose, availability of microorganisms is not an issue as many strains of bacteria, algae, fungi and yeast can be cultured in the laboratory by different ways. Mainly, microorganisms do not depend on substrate properties with exception of few. However, substrate availability is very limited regarding the consumer and concerned economy (Ferreira *et al.*, 2010). The aim of this study was to optimization condition of single cell protein production.

2. Materials and methods

2.1 Materials

2.1.1 Date samples

This study was carried out on low quality date fruits of three of unclassified dates (Manthour or seedling) called Mt.1, Mt.2 and Mt.3, and three classified date include Hayany and Zaghloul (soft varieties) and Saily (semi dry date variety). The unclassified (Manthour, Mt.) date fruits called Mt.1, Mt.2 and Mt.3. Date fruits of Hayany, Zaghloul, Mt.1 and Mt.2 were obtained from Qena governorate, Egypt while, Saily and Mt.3 fruits obtained from El-Kharja Date Packing Factory, El-Kharja oasis, The New Valley governorate, Egypt during the 2013

seasons (Figure 1).

2.1.2 Yeast strain

Saccharomyces cerevisiae EMCC ATCC64712 obtained from Microbiological Resources Center (MIRCEN), Ain Shams University, Egypt.

2.1.3 Media for yeast

Glucose peptone yeast extract medium was used for yeast maintenance composition is follows DSM Medium 186 (Yeast extracts 3 g, glucose 10 g, peptone from soybeans 5 g, agar 15 g, malt extracts 3 g, distilled water 1000 ml).



Figure (1): Morphological picture of the studied date samples: A (Hayany), B (Zaghloul), C (Saidy), Mt1, Mt2 and M3 (Manthour).

2.2 Single cell protein production

2.2.1 Preparation of date juice

The mixture of six date fruits was cleaned from extraneous materials then washed and the pits were removed. The date flesh was cut into small pieces to decrease their size in order to improve the extraction efficiency. The small pieces of flesh were mixed with water (1 flesh: 4 water, w/v) and soaked for 12 hr under cooling, boiled gently for 30 min, then blended in an electric mixer (Blinder) for one minute

and the date fruit juice filtered through double fold cheese to exclude all large undigested particles. Date juice was packaged in double polyethylene bags and kept at -15°C till further experiments. The date fruit juice was prepared from date mixture (Shubbar, 1981) as illustrated in Figure (2).

2.2.2 Effect of environmental factors

The most environmental conditions were studied to produce the highest biomass and protein content from the tested yeast

strain as follows:

- The pH value: Date juice medium was adjusted at different pH values ranged from 3.5 to 6.0 pH. Flasks of different pH levels were incubated at 30°C for 96 hr on rotary shaker at 150 rpm.
- Temperature: Flasks containing date juice media with 18% sugar content were adjusted to pH 4.5 and incubated at different temperatures; 25, 30, 35 and 40°C for 96 hr using rotary shaker at 150 rpm.
- Inoculum size: Suspension of cell obtained from yeast strain was used as inoculum size in a range between 1-6 % (0.5 to 3.0 ml) inoculum. The inoculated flasks contained 50ml media were incubated at 30°C on rotator 150 rpm for 48 hr. Biomass production using fermenter incubation period at optimal condition, however yeast strain incubated in date juice medium

three liter in fermenter at aeration rate 2.5 l/min, pH 4.5 and 30°C for 96 hr. Biomass, crude protein (CP) and total crude protein (TCP) were determined.

2.2.3 The dried yeast cells

The produced yeast cells were harvested by centrifugation at 3000 rpm for 15 min. The supernatant was then decanted and the residual cell suspended in distilled water and re- centrifugation. This was repeated twice. Finally, the cells were transferred to measuring flask (100) using distilled water. Aliquots of 5 ml were taken and dried at 105°C overnight and results were given as g dried yeast per litre of the used medium (Kishan and Neelakantan, 1989; White, 1954).

2.2.4 Yeast biomass

The biomass of yeast strain was centrifuged, washed twice with distilled water and dried at 70°C till constant weight was obtained.

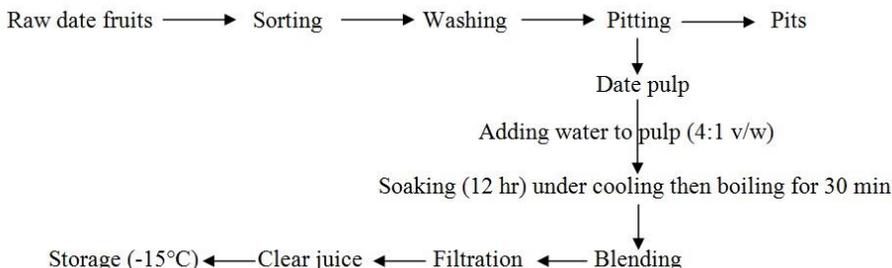


Figure (2): Extraction of date juice flow diagram.

2.2.5 Total soluble solids (TSS)

Total soluble-solids values as brix were determined with Digital Refract meter (Model no. RX 5000, ATAGO, Japan) at 25°C.

2.2.6 The pH value

The pH values were measured using digital pH meter model no. APX 175, Control Dynamics Ltd., Bangalore, India.

2.2.7 Crude protein and amino acids

The nitrogen content was analyzed using the standard Kjeldahl procedure (AOAC, 2016). Protein content was calculated by multiplying the nitrogen content by 6.25 according to Merrill and Watt (1973). Amino acids were determined using Automatic Amino Acid Analyzer (AAA 400INGOS Ltd). Total amino acids hydrolysis was carried out according to the method of Csomós and Simon-Sarkadi (2002). Free amino acids extraction was carried out according to the method of Shalabia (2011).

3. Results and Discussion

3.1 Production of single cell protein

3.1.1 Environmental conditions

Saccharomyces cerevisiae EMCC ATCC64712 utilize the available wastes and use them as growth medium to increase their cell masses which are made up of the SCP. Fermentation is the main

process responsible for SCP production. After the completion of the fermentation process, the available biomass is harvested which can further be utilized as a protein source. Then this source undergoes further processing techniques like purification, cell disruption, washing followed by protein extraction to give generally high production rates along with better yield and makes the production control relatively easier. The most important variables affecting the cultivation and production of SCP are the environmental conditions under which the microorganisms were cultivated (incubation temperature, pH value, incubation period, inoculums size and composition of the growth medium) (Kishan and Neelakantan, 1989).

3.1.2 Effect of pH value

Extracts of date fruits containing 18 % sugar, adjusted to different pH values such as 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 were used to produce single cell protein by the selected yeast. Results in Table (1) showed that the slightly acidic pH (3.5–6.0) was appropriate for biomass production by the *S. cerevisiae* ATCC 64712. The maxima of biomass, crude protein (CP) and total crude protein (TCP) were obtained at pH 4.5 followed by pH 5.0. The maxima production of biomass (42.85 g/l), crude protein CP (67.8%) and total crude protein TCP (29.05g/l) were obtained at pH 4.5 followed by 32.45 g/l, 60.18% and 19.52g/l, respectively at pH 5.0. This finding was supported by the

suggestion that a weak acidic medium is more appropriate for the overall growth of yeasts (Pramanik, 2003). The results agree with those obtained by Abou-Aly (1996) and Hassan (2012). They found that the biomass and total crude protein were gradually increased by increasing pH up to 4.5. However, other observations suggest that the pH range of any yeast strain could vary depending on the medium composition. In this context, Onishi (1963), and Kishan and Neelakantan, (1989) showed that the pH range for the growth of *Z. Rouxii* strain

isolated from the soy sauce process without NaCl was very broad (pH 3.0–7.0), while in a medium containing 18 % NaCl, the pH range for growth was narrow (pH 4.0–5.0). Data of statistical analysis of biomass (Table 1) showed that there were high significant differences among biomass, CP and TCP produced by yeast strain at pH 4.5 and at the other pH values with the same conditions. On the other hand, there were no significant differences among biomass and CP production at pH 4.0, 5.0 and 5.5, and among TCP at pH 5.0 and 5.5.

Table (1): Effect of initial medium pH on biomass production and protein content of *Saccharomyces cerevisiae* ATCC 64712.

Initial pH	Biomass g/l	CP %	TCP g/l
3.5	28.07 ^c ±0.043	42.00 ^d ± 0.15	11.79 ^e ±0.30
4.0	31.94 ^b ±0.54	58.0 ^b ±0.154	18.51 ^c ±0.06
4.5	42.85 ^a ±0.164	67.8 ^a ±0.06	29.05 ^a ±0.100
5.0	32.45 ^b ±0.259	60.18 ^b ±0.748	19.52 ^b ±0.089
5.5	32.00 ^b ±0.66	59.46 ^b ±0.308	19.02 ^b ±0.355
6.0	27.60 ^c ±0.398	45.60 ^c ±0.396	12.57 ^d ±0.087
LSD	1.249	2.347	0.627

3.1.2 Effect of incubation temperature

Samples of date extracts were adjusted to 20 brix (18 % sugar concentration) and pH 4.5, then inoculated with yeast and maintained for produced single cell protein at 25, 30, 35 and 40°C. The optimum temperature for single cell protein production by *S. cerevisiae* ATCC 64712 after 60 hr with standard inoculum grown in sugar date juice (18%) are shown in Table (2). Results showed that *S. cerevisiae* ATCC 64712 strains was able

to grow up to 40°C. The maximum yield was 42.85 g/L at 30°C and decreased to 16.51g/l at 40°C. These results demonstrated the 30°C was the optimum degree for biomass production, crude protein and total crude protein. Based on the information available in the literature, the optimum temperature varies widely among the yeast strains, that 28°C is the most favorable temperature for biomass production by *Kluyveromyces lactis* grown on whey permeate. Lee et al. (1993) reported that the optimum

temperature for thermo-tolerant *Candida tropicalis* used for SCP production was 38 °C. Rajoka, (2004)) and Rajoka et al. (2006) studied the production of SCP by *Candida utilis* at different temperatures (20-45°C) in a stirred fermenter and reported that the maximum production of CP was realized when the fermentation temperature was maintained at 35°C. They also found that the production of CP decreased above 35°C. High temperature can cause inactivation of enzymes of the metabolic pathway, while low temperature may not permit the flow of

nutrients across the cell membrane, resulting in a high demand for maintenance energy. However, at low temperature, the enzyme activities are expectedly low (Converti and Dominguez, 2001; Roels, 1983). From statistical analysis, data provided that there were high significant difference among biomass, CP and TCP produced by yeast strain at 30°C and other produced at different temperatures used except in between 25 and 35°C, there was no significant difference in biomass produced by yeast.

Table (2): Effect of incubation temperature on biomass production and protein content of *Saccharomyces cerevisiae* ATCC 64712.

Incubation temperature °C	Biomass g/l	CP %	TCP g/l
25	41.36 ^b ±0.29	51.71 ^c ±0.053	21.29 ^c ±0.203
30	42.85 ^a ±0.16	67.8 ^a ±0.05	29.05 ^a ±0.10
35	42.4 ^{ab} ±0.14	62.57 ^b ±0.057	26.52 ^b ±0.083
40	16.51 ^d ±.811	49.6 ^d ±0.58	8.17 ^d ±0.31
LSD	1.456	0.955	0.636

3.1.3 Effect of inoculum size

Samples of date extracts were adjusted to 20 Brix (18% sugar concentration), 4.5 pH, 30°C and inoculated with suspension of yeast cells obtained from active slants prepared to study the effect of inoculum size, which were used in range between 1-6% (0.5-3.0 ml inoculum medium) on biomass production. Table (3) showed that the biomass, CP and TCP gradually increased with the inoculum size increasing to the maximum (42.85 g/l, 67.8% and 29.05 g/l, respectively) at the

inoculum 5%. These results are agreement with Alian et al. (1990), Francisco et al. (2010) and Hassan (2012). They reported that the best ratio of inoculum size for single cell protein production of *Saccharomyces cerevisiae* was 5% v/v. Statistically, it was clear that there were high significant differences among biomass and TCP produced by yeast strain at all inoculum except in between 1.5 and 3.0 inoculum size. On the other hand, data also revealed that there was no significant difference in between 1.0 and 3.0 inoculum size in CP production.

Table (3): Effect of inoculum size on biomass and protein content of *Saccharomyces cerevisiae* ATCC 64712.

Inoculum size		Biomass g/l	CP %	TCP g/l
ml	%			
0.5	1	32.09 ^e ±0.57	45.46 ^e ±0.57	14.57 ^e ±0.07
1.0	2	35.2 ^d ±0.57	54.54 ^d ±0.56	19.18 ^d ±0.11
1.5	3	37.60 ^c ±0.58	56.54 ^c ±0.58	21.24 ^c ±0.10
2.0	4	40.50 ^b ±0.57	65.16 ^b ±0.58	26.38 ^b ±0.32
2.5	5	42.85 ^a ±0.16	67.8 ^a ±0.05	29.05 ^a ±0.10
3.0	6	38.70 ^c ±0.58	53.73 ^d ±0.44	20.78 ^c ±0.15
LSD		1.63	1.55	0.52

3.1.4 Effect of sugar concentration

The suitability of date extracts containing different sugar concentrations to produced single cell protein by *S. cerevisiae* ATCC 64712 was studied (Table 4). The tested sugar concentrations in date extract were 10, 12, 14, 20, 22 and 24 %. Results in Table (4) illustrated that concentration of the date juice significantly affected the productivity of biomass and crude protein of the yeast. The biomass and CP increased with the increase in concentration of the substrate up to 18%. The maximum production of biomass and CP were recorded when the yeast grown in 18% sugar of date juice after 60 hr at 30°C and 4.5 pH. However, when the sugar concentration of substrate increased up to 20%, the biomass and CP of the yeast strains decreased by 20.91 and 16.62%, respectively. These results proved that 18% sugar in date juice is the most appropriate concentration to encourage the growth and production of the biomass by the yeast strain. They seemed to carry out all normal physiological processes in a moderate

concentration of sugars, while the increase in date juice concentration slowed down their growth. The decrease in growth rate in high concentrations of date juice could be attributed to the viscosity of the medium and plasmolysis of yeast cells that retard or stop their growth (Pramanik, 2003). In similar studies, Hashem *et al.* (2014) optimized the cultural conditions for production of single-cell protein by yeast strains (*Zygosaccharomyces rouxii* KKUY-0157 and *Hanseniaspora uvarum* KKUY-0084) spoilage date juice (SDJ). They showed the best growth and production of biomass at 25 °C in a 20 % date juice concentration, they could resist an increase in temperature to 30 °C, and they could grow in higher concentrations of date juice. They noticed that the growth and biomass productivity of the two strains were greatly enhanced by adding metals such as Mn or Mg as well as a nitrogen source (tryptone). Data given in Table (4) indicated that there were high significant differences in the biomass production in all used sugar concentration except inbetween 16, 22 and 24% sugar.

Table (4): Effect of sugar concentration on biomass production and protein content of *Saccharomyces cerevisiae* ATCC 64712.

Sugar concentration (%)	Biomass g/l	CP %	TCP g/l
10	22.93 ^f ±0.672	44.3 ^b ±0.057	10.15 ^e ±0.29
12	24.96 ^e ±0.49	44.8 ^g ±0.058	11.18 ^f ±0.20
14	27.49 ^d ±0.017	56.32 ^d ±0.005	15.47 ^d ±0.008
16	31.3 ^c ±0.09	64.61 ^b ±0.005	20.21 ^b ±0.060
18	42.85 ^a ±0.16	67.8 ^a ±0.05	29.05 ^a ±0.10
20	33.62 ^b ±0.18	56.53 ^c ±0.005	19.00 ^c ±0.11
22	31.35 ^c ±0.12	46.83 ^e ±0.05	14.64 ^e ±0.03
24	31.28 ^c ±0.16	45.6 ^f ±0.008	14.26 ^e ±0.07
LSD	0.954	0.107	0.423

Also, there were high significant differences in CP between all used sugar concentrations. Besides, there was high significant difference in TCP except among 22 and 24% sugar concentration. Furthermore, Table (5) and Figure (3) were illustrated the amino acid composition of the protein hydrolysate of *Saccharomyces cerevisiae* ATCC 64712. Data revealed that fifteen amino acids were detected and identified. Aspartic acid (Asp) was the predominant amino acid (16.37 g /100 g), followed by glutamic acid (12.54 g/100 g), histidine (9.72 g/100 g), alanine (8.97 g/100 g) and isoleucine (8.76 g /100 g). While,

methionine had the lowest value (0.62 g /100 g). Regarding the determined concentrations of the *S. cerevisiae* (ATCC 64712) amino acids it was contained over levels of all amino acid except methionine and leucine than those reported by Hassan (2012) in the *S. cerevisiae*. He found that asparatic acid 6.55, threonine 3.06, alanine 4.56, methionine 0.67, isoleucine 3.09, leucine 5.01, tyrosine 2.17, phenylalanine 2.57, histidain1.76, lysine 4.59, glutamic acid 10.10, valine 3.72, cystine 2.00 and arginine 2.74g/100g protein. The differences in amino acids concentration may be due to the yeast used and the mediums of growth.

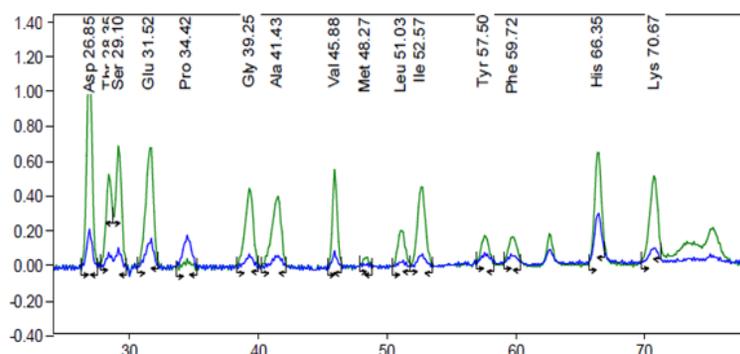


Figure (3): Amino acid content of *Saccharomyces cerevisiae* ATCC 64712).

Table (5): Amino acid content of *Saccharomyces cerevisiae* (ATCC 64712).

Peak No.	Area	Amino acid	Total acids (%)
1	35.62	Aspartic acid	16.37
2	8.16	Threonine	3.75
3	12.98	Serine	5.97
4	27.28	Glutamic acid	12.54
5	1.82	Proline	0.84
6	18.43	Glycine	8.47
7	19.52	Alanine	8.97
8	14.08	Valin	6.47
9	1.35	Methionine	0.62
10	7.99	Leucine	3.67
11	19.07	Iso Leucine	8.76
12	5.80	Tyrosine	2.67
13	5.77	Phenylalanine	2.65
14	21.14	Histidin	9.72
15	18.58	Lysine	8.54

4. Conclusion

From the obtained results, it could be concluded that date flesh was suitable material for single cell protein production by yeast strain; *Saccharomyces cerevisiae* ATCC64712. In addition, the maximum production of biomass and CP were recorded when the yeast grown in 18% sugar of date juice after 60 hr at 30°C and 4.5 pH.

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