Utilization of date syrup as a substrate for carotenoid production by Rhodotorula glutinis

M. Moosavi-Nasab1E. Abedi1, S.S. Moosavi-Nasab1

1Department of Food Science and Technology, College of Agriculture, Shiraz University, Shiraz, I. R. Iran
2Seafood Processing Research Group, College of Agriculture, Shiraz University, Shiraz, I. R. Iran

*Corresponding Author: marzieh.moosavi-nasab@mail.mcgill.ca

ARTICLE INFO

Article history:
Received 15 August 2012
Accepted 28 April 2014
Available online 16 June 2015

Keywords:
By-Product
Date Syrup
Microbial Carotenoid
Rhodotorura glutinis

ABSTRACT-The potential use of date syrup, for the production of carotenoids by Rhodotorula glutinis in batch fermentation process, was investigated during 7 days. The results revealed that carbon (glucose or date syrup) and nitrogen sources [yeast extract, (NH₄)₂SO₄ and NH₄NO₃] had a significant influence on biomass and carotenoid production. Maximum yield of total carotenoid production (7.94 mg/L) with carotene content (2040 μg/g) and biomass (3.90 g/L) was obtained from R. glutinis after 7 days of fermentation in a substrate containing date syrup and yeast extract. The highest biomass (8.03 g/L) was obtained in the culture containing glucose and yeast extract, while the total carotenoid content of 6.72 mg/L with 836.86 μg/g carotene was produced in this medium. Significant differences were observed when comparing the average biomass and total carotenoid productions in different cultures and fermentation times. Our results demonstrated that date syrup (from low quality dates), as a by-product at a lower price, could be profitably used as a suitable carbon source for carotenoid production by R. glutinis.

INTRODUCTION

The agro-industrial by-products are economical sources of carbon and nitrogen for use in fermentation process. Date palm (Phoenix dactylifera) is one of the oldest cultivated plants in the Middle East and North Africa (Al-Farsiet al., 2005a). Date fruits contains, typically, total sugars (44-88%), fat (0.2-0.5%), protein (2.3-5.6%), dietary fiber (6.4-11.5%), minerals (0.1 to 0.916 mg/100 g date) and vitamins such as vitamin C, B₁, B₂, A and niacin (Al-Shahiband Marshall, 2003; Al-Farsiet et al., 2005b; Bigliarit al., 2008).

Date syrup is a product obtained from matured dates (tamar) and is used in the preparation of some traditional and industrial foods. The date fruits and date syrup are used for human consumption, in bakery and ice-cream products, and for the production of caramel color, ethanol, vinegar, and single cell protein (Mehaia and Cheryan, 1991). Because of the large amount of date production and also its high content of oligosaccharide and monosaccharide, this fruit, with its large amount of annual wastes, can be a good choice of microbial carotenoids production.

Carotenoids are natural bioactive compounds that play an important role in the function of all living organisms. They are used commercially as food colourants (yellow to red colour) and as a source in pigmentation of fish and shellfish in aquaculture, having crucial influence on the acceptability of many foods. Due to their anticancer and antioxidant properties and immune response stimulants, wider use of carotenoids as pharmaceuticals and nutraceuticals is expected (Edge et al., 1997; Hennekens, 1997). Recently, microbial production of carotenoids has attracted considerable interest. Yeasts in the genus Rhodotorula synthesize carotenoid pigments produce major pigments such as β-carotene, γ-carotene, torulene and torularhodin (Peterson et al., 1958; Simpson et al., 1971; Perrier et al., 1995).

Carotenoids production yield by R. glutinis affected by numerous environmental and fermentation parameters such as aeration, agitation, temperature, pH, and the concentration of the medium components. For effective carotenogenesis, the use of inexpensive alternative carbohydrate sources, which typically are the by-products of various industries and contribute to contamination of the environment and the use of yeast strains that produce high amount of carotenoid are important (Frengova et al., 1994). Recent researches have focused on the economical production of carotenoids (Edge et al., 1997; Bhosale and Gadre, 2001; Buzzini, 2001).

The aim of this study was to examine the potential of date syrup, an agro-industrial raw material, for the production of carotenoids by Rhodotorula glutinis at batch scale. In this study, date syrup was chosen as a substrate mainly because of its high sugar and other nutrient contents, low cost, ready availability and ease of storage. Date syrup is an important date fruit by-product which has not been investigated for β-carotene production. Furthermore, to the best of our knowledge, this is the first study investigating the carotenoids production by Rhodotorula glutinis using date syrup as an economical carbon source.
MATERIALS AND METHODS

Date syrup (DS) was obtained from the local shop (Kabkab). All media and chemicals were obtained from Sigma, Merck and Difco companies.

Microorganism and Growth Conditions

*Rhodotorula glutinis* Persian Type Culture Collection (PTCC) 5256 was purchased from Iranian Research Organization for Science and Technology (IROST). The yeast was maintained on a yeast and malt medium containing 50 ml of yeast and malt medium containing 15 g/L glucose, 2.5 g/L yeast extract, 2 g/L malt extract, 1 g/L (NH₄)₂SO₄, 1 g/L KH₂PO₄ and 0.25 g/L MgSO₄.7H₂O (1) and incubating the flask at 30°C for 72 h. Yeast cells were harvested by centrifugation (3500 rpm for 30 min), washed three times in sterile distilled water (A₀₅₈₀=0.5-0.75, corresponding to ca. 10⁶ cells/ml) in sterilized normal saline. Fermentation was carried out in 250 ml Erlenmeyer flasks containing 100 ml of sterilized medium. The basal medium used for carotenoid production had the following composition: 40 g/L glucose, 2 g/L yeast extract, 3 g/L KH₂PO₄, 5 g/L peptone, 10 g/L glucose and agar 20g/L at pH 6.2 ± 0.2 at 4°C until used.

A yeast starter was prepared by adding one loop of a 24h old slant culture into 250 ml Erlenmeyer flask containing 50 ml of yeast and malt medium containing 15 g/L glucose, 2.5 g/L yeast extract, 2 g/L malt extract, 1 g/L (NH₄)₂SO₄, 1 g/L KH₂PO₄ and 0.25 g/L MgSO₄.7H₂O (2) at 45ºC to a constant weight. To extract carotenoid, cells were resuspended in dimethyl sulfoxide (DMSO) and heated for 5 min at 55°C to break the cells and intercellular carotenoid extracted to DMSO phase. The suspension was then centrifuged and the supernatant was collected. Acetone was added and mixed with pellet and centrifuged until it became colorless. Supernatants were collected and the absorbance was measured at 455 and 470 nm for detection of carotene and total carotenoids, respectively. Commercial pure β-carotene (from Merck Company) was used as a standard.

<table>
<thead>
<tr>
<th>Table 1. Broth fermentation culture composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>1*</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>

* Presence in broth fermentation culture
* Basal medium

Analytical Methods

Total sugars and reducing sugars in date syrup were measured by the Munson and Walker method (Perrier et al., 1995). Total soluble solids were examined using refractometer by Brix measurement. Soluble proteins were measured by Biuret method (Córdova-Muruetal et al., 2013); moisture and ash content were determined (AOAC, 2002). Final pH of each broth culture media was measured.

10 ml sample was taken from each flask every day. After cultivation, the cells were harvested by centrifugation at 3500 rpm for 30 min, washed twice with distilled water and centrifuged again. The dry mass was gravimetrically determined by drying centrifuged and washed cells at 45°C to a constant weight. To extract carotenoids, cells were first harvested by centrifugation, washed twice with distilled water. Cells were resuspended in dimethyl sulfoxide (DMSO) and heated for 5 min at 55°C to break the cells and intercellular carotenoid extracted to DMSO phase. The suspension was then centrifuged and the supernatant was collected. Acetone was added and mixed with pellet and centrifuged until it became colorless. Supernatants were collected and the absorbance was measured at 455 and 470 nm for detection of carotene and total carotenoids, respectively. Commercial pure β-carotene (from Merck Company) was used as a standard.

Statistical Analysis

Data were subjected to the analyses of variance (ANOVA) and multiple comparison tests at 95% of confidence level. All the analyses were carried out using the statistical software, SPSS13. Means were separated using Duncan's multiple range tests.

RESULTS AND DISCUSSION

The date syrup obtained from the local shop contained a high concentration of sugar. It consisted of 68.80% total sugar, 68.08% reducing sugar, 1.63% ash, 76.80% solids, 24.75% moisture and 0.50% soluble protein. The effects of carbon and nitrogen source on the kinetics of growth and carotenoids synthesis of yeast *R. glutinis* in 7 days were evaluated (Figs. 1 and 2).

In this research, the same weight of glucose and date syrup was used. Significant differences were observed when the average biomass and total carotenoid productions were compared in different cultures and at different times. Whereas sugar content of date syrup was lower, it was observed that the total carotenoids production was higher when culture media contained date syrup. Figs. 1 and 2 reveal that carotenoids were produced in association with yeast growth.

As shown in Table 2, the culture medium 6 containing date syrup and yeast extract appeared to be
the most suitable medium for carotenogenesis (higher than basal medium) in which carotenoid content reached 7.94 mg/L/day and average carotenoid production rate was 2.11 (mg/L/day).

![Fig. 1. The growth kinetic of R. glutinis in various culture compositions: (◇): Glucose and yeast extract; (□): Glucose and (NH4)2SO4; (▲): Glucose and NH4NO3; (●): Date syrup and (NH4)2SO4; (■): Date syrup and NH4NO3; (●): Date syrup and yeast extract.](image1)

![Fig. 2. Carotenoids production kinetic by R. glutinis in various cultures (◇): Glucose and yeast extract; (□): Glucose and (NH4)2SO4; (▲): Glucose and NH4NO3; (●): Date syrup and (NH4)2SO4; (■): Date syrup and NH4NO3; (●): Date syrup and yeast extract.](image2)

**Table 2. Production of total carotenoids by R. glutinis grown in different fermentation culture, after 7 days**

<table>
<thead>
<tr>
<th>Culture type</th>
<th>Final pH</th>
<th>Total carotenoids (mg/L culture fluid)</th>
<th>Biomass (g/L culture fluid)</th>
<th>Carotene content (μg/g dry cells)</th>
<th>Average carotenoid production rate (mg/L/ day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.60±1.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.72±1.47&lt;sup&gt;f&lt;/sup&gt;</td>
<td>8.03±0.97&lt;sup&gt;d&lt;/sup&gt;</td>
<td>836.86±53.77&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.77±0.05&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>1.82±0.74&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.77±1.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.10±1.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>739.22±2.78&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.92±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>5.90±1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.89±2.41&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.00±0.88&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2295.00±16.78&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.47±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>4.05±1.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.40±0.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.46±0.65&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2136.94±11.31&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.30±0.82&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>4.05±0.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.59±0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.53±0.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1372.51±8.99&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.21±0.24&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>5.75±0.38&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.94±1.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.90±0.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2040.21±12.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.11±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Different superscripted letters indicate significant differences (P<0.05) among the observed values.

<sup>b</sup>Culture compositions have been reported in the text.

However, the basal medium stimulated a maximum biomass production of 8.03 g/L which contained glucose and yeast extract (culture medium1). Enhancement of the β-carotene production would be performed by the addition of other nutrients such as yeast extract, glucose and urea. Ammonium sulphate as a nitrogen source was added to the growth media 2 and 4 (in which carbon sources was glucose and date syrup, respectively) that enhanced the carotenoid production rate to 3.77 and 7.40 mg/L/day in culture 2 and 4, respectively. Thus, with the same nitrogen source, date syrup had a significant effect on carotenoid production. Minimum final pHs, total carotenoids, carotenoid content were obtained in culture 2 that contained ammonium sulphate instead of yeast extract and glucose as a carbon source. Furthermore, the addition of ammonium nitrate to culture 5 led to the enhancement of total carotenoid production and the organism growth was compared with ammonium sulphate when used as a nitrogen source in culture 4. Although the minimum biomass (3 g/L) was obtained in culture 3, the highest value of carotenoid content (2295 µg/g dry cells) was observed.

Carotenoid is a second metabolite of yeast produced at the stationary phase. With a decrease in biomass, carotenoid production was increased. Time course of carotenoid production in culture 6 is shown in Table 3. About half of total dry cell weight (maximum dry cell weight production rate after 24 h) and about one third of total carotenoids were produced within 48 h.

As seen in Fig. 3, the growth rate of yeast was maximum in date syrup with NH4NO3 medium (2.33 g/L/day) and decreased when other nitrogen sources were used in the media containing date syrup. On the other hand, in glucose media, maximum biomass production rate (1.28 g/L/day) was obtained in basal medium after 7 days. Also, the effect of carbon and nitrogen sources on carotenoid production rate was shown in Fig. 4. In media containing date syrup with (NH4)2SO4 and glucose with NH4NO3 the highest carotenoid production rates were 13.94 and 4.88 mg/g/day after 7 days, respectively. Fig 3 indicated that the nitrogen sources associated with glucose and date syrup had significant effects on the growth and carotenoid production rate but there was not a parallel relationship between them.
Table 3. Fermentation parameters of carotenoid production by *R. glutinis* in the culture 6 containing date syrup

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total carotenoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volumetric concentration (mg/L) *</td>
<td></td>
<td>0.69±0.01 f</td>
<td>2.69±0.86 f</td>
<td>3.08± 0.86 c</td>
<td>3.43±1.04 d</td>
<td>5.04±1.80 c</td>
<td>5.83±0.96 b</td>
<td>7.94±2.25 a</td>
</tr>
<tr>
<td>Produced carotenoids (%)</td>
<td></td>
<td>8.69±1.45 g</td>
<td>33.88±8.44 f</td>
<td>38.79±7.26 e</td>
<td>43.2±10.62 d</td>
<td>63.48±7.33 c</td>
<td>73.42±2.38 b</td>
<td>100±1.57 a</td>
</tr>
<tr>
<td>Volumetric rate (mg/L/day)</td>
<td></td>
<td>0.69±0.04 d</td>
<td>2.00±0.81 a</td>
<td>0.39±0.01 e</td>
<td>0.35±0.03 c</td>
<td>1.61±0.13 b</td>
<td>0.79±0.15 c</td>
<td>2.11±0.61 a</td>
</tr>
<tr>
<td>Cellular rate (μg/g dry cell weight day)</td>
<td></td>
<td>575±11.89 g</td>
<td>2222±5.98 d</td>
<td>1560±12.36 e</td>
<td>1400±1.2 f</td>
<td>3220±118.50 c</td>
<td>3950±1.80 a</td>
<td>3517±15.72 b</td>
</tr>
<tr>
<td><strong>Cell growth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellular biomass (g/L)</td>
<td></td>
<td>1.20±0.92 f</td>
<td>2.10±0.07 e</td>
<td>2.35±0.44 d</td>
<td>2.60±0.48 c</td>
<td>3.10±0.83 b</td>
<td>3.30±0.12 ab</td>
<td>3.90±2.25 a</td>
</tr>
<tr>
<td>Produced dry cell weight (%)</td>
<td></td>
<td>30.77±3.55 g</td>
<td>53.85±1.50 f</td>
<td>60.26±1.07 e</td>
<td>66.67±1.42 d</td>
<td>79.49±2.08 c</td>
<td>84.62±1.91 b</td>
<td>100±1.57 a</td>
</tr>
<tr>
<td>Dry cell weight production rate (g/L/day)</td>
<td></td>
<td>1.20±0.04 a</td>
<td>0.90±0.12 b</td>
<td>0.25±0.01 e</td>
<td>0.25±0.04 c</td>
<td>0.50±0.10 d</td>
<td>0.20±0.03 e</td>
<td>0.60±0.61 c</td>
</tr>
</tbody>
</table>

* Different superscripted letters indicate significant differences (*P*<0.05) among the observed values.

Fig. 3. Effect of carbohydrate and nitrogen sources on the growth rate of *R. glutinis* after 7 days in date syrup

The maximum total carotenoids (7.94 mg/L) and average carotenoid production rate (2.11 mg/L/day) were obtained from the culture containing date syrup and yeast extract as carbon and nitrogen sources that were higher than those at basal medium, respectively. *R. glutinis* is carotenoid producing yeast and synthesizes β-carotene as the major carotenoid (Perrier et al., 1995). The results (Table 3) suggest that date syrup had the potential to be employed as a sole substrate for *R. glutinis* for β-carotene production. Maximum carotenoids production, in line with some previous reports, occurred after the exponential phase (Buzzini and Martini, 1999; Buzzini, 2001). In addition, the maximum cell dry weight obtained from date syrup (8.03 g/L) was higher than the amount obtained from using glucose syrup (7.5 g/L), soybean flour extract (4.8 g/L) and maize flour extract (5.2 g/L) (Buzzini and Martini, 1999). Perhaps the difference is due to the differences of the yeast strain used, the nutrient compositions and chemical concentrations in the date syrup, the cultivation condition and the β-carotene extraction method.

The results revealed that the two factors, i.e., carbon and nitrogen sources, had a significant influence on biomass and carotenoid production. The inorganic nitrogen sources investigated produced only poor growth. In contrast to ammonium sulphate and ammonium chloride, ammonium nitrate resulted in distinctly higher β-carotene content, (Bhosale and Gadre, 2001). As seen in Table 2, the maximum total carotenoid was obtained from the date syrup medium containing yeast extract and the glucose medium containing NH$_4$NO$_3$. However, date syrup media containing NH$_4$NO$_3$ and (NH$_4$)$_2$SO$_4$ had the highest yeast growth and carotenoid production rate, respectively. During fermentation, the maximum carotenoid production seemed to occur after the exponential phase, in good agreement with some previous reports (Frengova et al., 1994) but in disagreement with some other findings (Shih and Hang, 1996).
CONCLUSIONS
The results demonstrated that date syrup as a by-product of low quality date can be used as a good and economical substrate for the production of yeast biomass and carotenoids by R. glutinis but its production was affected by nitrogen source. The best total carotenoids production rate could be obtained from culture contained date syrup and yeast extract as carbon and nitrogen sources, respectively.

REFERENCES
کاربرد شیره خرما به عنوان سوبسترا جهت تولید کاروتنوئید توسط رودوترولا گلوتينیس

پرورش موسمی نسبت به عنوان جهت تولید کاروتئین‌های میکروبی در کارهای مختلف مطالعه شده است. در این مطالعه بررسی کارکرد رودوترولا گلوتينیس به عنوان سوبسترا محیط‌کننده در فرآیند تخیر و تولید کاروتئین‌های میکروبی انجام شد.

چکیده- امکان استفاده از شیره خرما جهت تولید کاروتئین‌های میکروبی در فرآیند تخیر و تولید کاروتئینی در محیط‌های مختلف برای تولید کاروتنوئید توسط رودوترولا گلوتينیس در فرآیند تخیر غیر مداوم طی 7 روز بررسی شد. نتایج نشان داد که منابع کربن (گلکز یا شیره خرما) و منابع نیتروژن (عصاره مخمر، سولفات آمونیوم و نیترات آمونیوم) تأثیر قابل توجهی بر تولید کاروتئین داشت. حداکثر بارداری کاروتنوئید کل (mg/L 31/7) با محتوی کاروتن μg/g 2212 و زست توده g/L 32/9 توسط رودوترولا گلوتينیس در مرحله 7 روز در سوبسترایا حاوی خشخاش به عنوان منبع کربن و عصاره مخمر حاصل شد. در این مطالعه مایز تولید در محیط حاوی گلکز و عصاره مخمر ابتدایی بارداری کاروتنوئید کل (mg/L 72/6) هم گردد. بین زمان‌های مختلف تخیر و نیز میکروب‌های گلکز مثل ناپوز 4 روز نسبت به تولید زست توده و تولید کاروتئین تفاوت معناداری مشاهده شد. نتایج حاصل از این پژوهش نشان داد که شیره خرما از نظر محتوی کربن و همچنین به عنوان منبع مناسب برای تولید کاروتئین توسط رودوترولا گلوتينیس مورد استفاده قرار گرفت.

اطلاعات مقاله
تأریخ پذیرش: 8/2/1399
تأویج شد: 1399/12/26
واژه‌های کلیدی: محصول جانبی، کاروتنوئید، شیره خرما، رودوترولا گلوتينیس.