Biocatalysis and Agricultural Biotechnology 7 (2016) 224–227

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Multiple optimization and statistical evaluation of astaxanthin production utilizing olive pomace

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ARTICLE INFO

Article history:
Received 24 May 2016
Accepted 13 June 2016
Available online 27 June 2016

Keywords:
Olive pomace
Solid state fermentation
Astaxanthin
Antioxidant capacity
Optimization
Experimental design

ABSTRACT

Solid State Fermentation (SSF) of olive pomace to astaxanthin pigment was accomplished within the frame of Box-Behnken and rotatable central composite experimental designs. Temperature, moisture content and pH for two astaxanthin producer yeasts, ATCC 24,202 (*Xanthophyllomyces dendrorhous*) and ATCC 24,259 (*Sporidiobolus salmonicolor*) were optimized at the first stage. 220.24 ± 17.47 μg/gdp as maximum astaxanthin yield was obtained by ATCC 24,202 at 15.0 °C, 4.5 pH and 90.0% moisture content. Inoculation rate and incubation time factors of the second stage were optimized for yield and antioxidant capacity of the produced astaxanthin. Confirmation of the experimental and statistical evaluations concluded that astaxanthin production from olive pomace could be performed with a high efficient accurately.

1. Introduction

Olive pomace as solid by-product of olive oil extraction is a valuable agricultural waste (Tekin and Dalgıç, 2000; Meziane, 2011). 35–45 kg olive pomace containing 2.1–3.6% oil is obtained after processing of 100 kg olive. The rest of the pomace consists of water, olive seed, and olive pulp (Güneşer et al., 2014). Organic ingredients of the pomace are phenolic compounds, lignin, cellulosic substances, fatty acids, lipids, sugars, proteins, ash, and fiber (Haddadin et al., 1999; Borja et al., 2005). To disposal the pomace as combustion materials (charcoal, fuel), animal feed, and other usages prevents conversion of it to substantial products commercially (Borja et al., 2005; Göğüş and Maskan, 2006).

The SSF technique appears as a biotechnological application for implementation of solid wastes to alternative products. It is emphasized that the SSF has many advantages in order to produce new, cheap, natural characterized products from natural sources (Nigam and Pandey, 2009). Processing the wastes to the products like microbial pigments which have notable usage areas (food, medical, feed, pharmaceutical, cosmetics etc.) (Joshi et al., 2003; Dufossé et al., 2005; Gupta et al., 2011) provides environmental pollution and incomes economic and industrially, besides obstructing the disposal of them by ineffective ways (Laufenberg et al., 2003; Krishna, 2005; Panesar et al., 2015). Astaxanthin pigment as valuable and important carotenoid in terms of commercial and health becomes a focal point for synthesizing by different paths like fermentation.

It is quite important to determine and optimize operational parameters of the SSF technique due to the fermentation takes time and is affected by many factors (such as homogenization of mass and heat distributions) in especial associated with pure culture implementation. Accordingly, performing an experimental design becomes first and outstanding step for the fermentation systems. Therefore, it was aimed to study the fermentation of olive pomace by two yeasts to produce astaxanthin pigment in the scope of experimental design.

2. Materials and methods

2.1. Yeast cultures

ATCC 24,202 (*Xanthophyllomyces dendrorhous*) and ATCC 24,259 (*Sporidiobolus salmonicolor*) were purchased from the American Type Culture Collection (Manassas, USA) and maintained in YM broth and YM agar at their optimum growth conditions; 20.0 °C, 4.5 pH and 18.0 °C, 6.0 pH respectively. YM medium has the composition: 3 g/L yeast extract (Merck, Germany), 3 g/L malt extract (Merck, Germany), 5 g/L peptone (Merck, Germany), 10 g/L dextrose (Sigma-Aldrich, Germany) and 20 g/L agar (Merck, Germany).

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http://dx.doi.org/10.1016/j.bcab.2016.06.012
1878-8181/

2.2. Fermentation process

Olive pomace supplied from local provinces in Turkey dried, milled and sieved to size 0.85 mm in order to obtain a uniform material for the fermentation.

The fermentation was carried out in 250 mL Erlenmeyer flasks containing a 100 g total amount of the prepared pomace and water during 12 days. Initial parameters of the fermentation were adjusted according to the experimental design. The flasks were sterilized by autoclave at 121 °C for 15 min and inoculated with 2 mL fresh culture (10^6 cells/mL).

2.3. Pigment analysis

5 g fermented content mixing with 20 mL pure methanol (Sigma-Aldrich, Germany) was waited during 2 h for extraction. Liquid phase of the mixture was taken and centrifugated. The pigment concentration of the supernatant was measured by double beam UV/VIS Spectrophotometer (Lambda 25 UV/VIS Spectrophotometer, USA) at 474 nm (Babitha et al., 2007).

2.4. Antioxidant capacity by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay

The assay was performed for both fermented and un-fermented content. Pure methanol was used as solvent for the extraction. Mixture of sample and methanol was shaken at 250 rpm and 30 °C for 2 h. Liquid part was filtered and centrifugated at 6000 rpm for 10 min. The mixture of 60 μM DPPH radical (Sigma-Aldrich, Germany) prepared with methanol and 250 μL extract (distilled water as control) were left at dark for 25 min. The antioxidant capacity (AC) was determined measuring remaining purple color at 517 nm (Kwon et al., 2006) and calculated by the following equation:

$$AC\% = \frac{(\text{Abs control} - \text{Abs extract})}{\text{Abs control}} \times 100.$$

2.5. Box-Behnken Design

Fermentation parameters (temperature, moisture content and pH) were optimized by 3 factors and 3 levels BBD. The parameters were selected based on the optimum growth conditions of the yeasts. The level intervals (Table 1) were determined after performing a preliminary study whose data not shown. The BBD comprises 17 runs with 5 center points (Table 2).

2.6. Rotatable central composite design

A second optimization by RCCD was employed with inoculation rate (IR) and incubation time (IT) factors. 13 experiments including 5 center points were conducted according to the design matrix (Table 3) to determine the maximum astaxanthin yield (AY) and antioxidant capacity.

Table 1
The factors and the level intervals of the BBD.

Factors	Units	Symbols	Level intervals
Temperature	°C	x_1	5
pH		x_2	1
Moisture content	%	x_3	10

Table 2
Design matrix for the optimization of the astaxanthin production by the yeasts.

Run	x_1	x_2	x_3	AY (μg/gdp)	
				ATCC 24,202	ATCC 24,259
1	0	0	0	125.19 ± 9.05	101.73 ± 3.40
2	–	0	+	220.24 ± 17.47	191.33 ± 2.81
3	+	–	0	125.41 ± 9.98	97.87 ± 5.66
4	+	0	–	76.23 ± 17.33	28.35 ± 5.83
5	0	–	+	199.87 ± 3.63	113.86 ± 65.10
6	+	+	0	113.84 ± 3.95	90.31 ± 1.70
7	0	–	–	92.51 ± 14.91	18.81 ± 2.06
8	0	0	0	125.56 ± 8.67	90.90 ± 6.25
9	0	0	0	132.22 ± 4.68	103.03 ± 7.63
10	+	0	+	199.46 ± 2.61	149.72 ± 1.50
11	0	+	+	202.01 ± 2.43	176.54 ± 6.82
12	0	0	0	125.06 ± 7.91	90.36 ± 3.03
13	–	–	0	140.31 ± 8.55	102.51 ± 1.91
14	–	+	0	141.46 ± 7.65	110.98 ± 3.60
15	–	0	–	74.89 ± 1.94	40.87 ± 9.13
16	0	+	–	83.01 ± 6.20	22.95 ± 5.65
17	0	0	0	151.72 ± 4.34	91.03 ± 2.25

μg/gdp: μg astaxanthin/g dried pomace.

Table 3
The design matrix of RCCD for ATCC 24,202.

Run	IR (%)	IT (h)	AY (μg/gdp)	AC %
1	10.00	355.88	195.22 ± 0.48	46.85
2	10.00	288.00	246.79 ± 3.42	81.92
3	10.00	288.00	223.17 ± 7.84	79.16
4	1.51	288.00	179.60 ± 4.75	73.62
5	10.00	288.00	225.61 ± 4.44	85.09
6	16.00	336.00	233.07 ± 4.19	43.53
7	4.00	336.00	193.42 ± 4.80	46.19
8	10.00	220.12	199.17 ± 5.48	56.99
9	10.00	288.00	217.58 ± 8.72	83.22
10	4.00	240.00	177.91 ± 7.79	62.30
11	10.00	288.00	239.09 ± 4.80	76.62
12	18.49	288.00	234.81 ± 0.44	84.01
13	16.00	240.00	203.30 ± 7.50	63.68

2.7. Statistical analysis

The experimental data were modeled and optimized in Response Surface Methodology (RSM) (Design Expert 7.1.6 Version) mathematically. Regression and ANOVA analyses were used for statistical evaluation. (1) and (2) equations of second order quadratic model which serves the best fittings (Aslan and Cebeci, 2007) generated for the BBD and for the RCCD designs respectively were stated below.

$$y_1 = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 \times_2 + \beta_{13} x_1 \times_3 + \beta_{23} x_2 x_3 + \beta_{11} x_{11} + \beta_{22} x_{22} + \beta_{33} x_{33} \quad (1)$$

$$y_2 = \beta_o' + \beta_1' IR + \beta_2' IT + \beta_{12}' IRIT + \beta_{11}' IR^2 + \beta_{22}' IT^2 \quad (2)$$

Where y_1 and y_2 are the responses or dependent variables; β_o and β_o' are model constants; β_1, β_2, β_3 and β_1', β_2' are linear coefficients; β_{12}, β_{13}, β_{23} and β_{12}' are cross product coefficients (present the interactions between the variables); β_{11}, β_{22}, β_{33} and β_{11}', β_{22}' are quadratic coefficients (Montgomery, 2001).

Modeling and optimizing procedures by RSM were confirmed by SigmaPlot 11 Version and MS Excel solver tool with steepest ascent method (SAM) respectively for both BBD and