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Original article

Study on efficiency of betacyanin extraction from red beetroots

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Summary Red beetroots are rich in betalain pigments, which are divided into betacyanins (predominating in red beetroots) and betaxanthins. The objective of this work was to study efficiency of aqueous extraction of betacyanins from red beets. The experiment was conducted according to a central composite design, with the following variables: solvent pH (pH, 3.0–5.0), solvent-to-beetroot ratio (S/B, 1:1–5:1), solvent initial temperature (ST, 30–70 °C) and grinding time (GT, 2–10 min). Beetroots were ground with the solvent, filtered and evaluated for betacyanin contents. A highly significant model was obtained for betacyanin extraction efficiency (BEE), which was positively affected by ST and S/B, and inversely affected by pH. The most adequate extraction conditions were pH, 3.0; S/B, 5:1; ST, 70 °C and GT, 2 min. At such conditions, BEE was near 70%. The method is simple and produces a bright red–purple extract to be later dried or concentrated for use as a food colourant.

Keywords *Beta vulgaris*, extraction/separation, natural products, pigments.

Introduction

Colour is one of the most determining attributes to sell a food product, being perceived as a quality indicator and determining frequently their acceptance. Many naturally coloured foods, such as fruit products, suffer colour changes during processing, requiring the use of colourants to restore a good appearance. At times, when consumers are increasingly aware of the importance of a healthy diet, they have been demanding for naturally coloured foods, as they tend to associate the terms 'synthetic' and 'artificial' with 'unhealthy'. Thus, although natural pigments have many technological disadvantages when compared to synthetic ones, including higher costs and lower colour stability, consumers have preferred them over the synthetic counterparts.

Betalains are natural water-soluble nitrogen-containing pigments, with a colouring power competitive to those of synthetic colourants (Cassano *et al.*, 2007). They are synthesised from tyrosine into the red–violet betacyanins and the yellow-orange betaxanthins. Betalamic acid (Fig. 1a) is the chromophore common to all betalain pigments. The nature of the betalamic acid addition residue determines the pigment classification as betacyanin (Fig. 1b) or betaxanthin (Fig. 1c) (Gandía-Herrero *et al.*, 2005).

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The major commercially exploited betalain crop is red beetroot (*Beta vulgaris*), which contains two major pigments, betanin (a red betacyanin) and vulgaxanthine I (a yellow betaxanthin). Interest in red beets has increased in last years, as findings ranked them among the ten most potent antioxidant vegetables (Halvorsen *et al.*, 2002; Ou *et al.*, 2002), and betalains respond at least in part for these health-related beneficial properties (Pedreño & Escribano, 2001; Wettasinghe *et al.*, 2002). Red beetroot concentrate is universally permitted as a food ingredient, termed beetroot red (Kujala *et al.*, 2000).

Red beetroot pigment is usually obtained from sliced roots by pressing or centrifuging blanched ground beets or by aqueous extraction of shredded beetroots, a process which results in a yield of 45–70% betanines (private communication by Y. Lee, 1976, according to López *et al.*, 2009). The extracting medium is water at pH 5 added with ascorbic acid (López *et al.*, 2009). The betanine extract is then concentrated up to 60–65% total solids or dehydrated to a powder (Henry, 1992). Some pretreatments to improve betalain extraction have been proposed, such as low direct-current electric fields (Zvitov *et al.*, 2003), gamma irradiation (Nayak *et al.*, 2006) and pulsed electric fields (López *et al.*, 2009). However, such techniques are costly when compared with the most usual solvent extraction methods. According to Delgado-Vargas *et al.* (2000), although betalains are water soluble, the use of methanol or ethanol with

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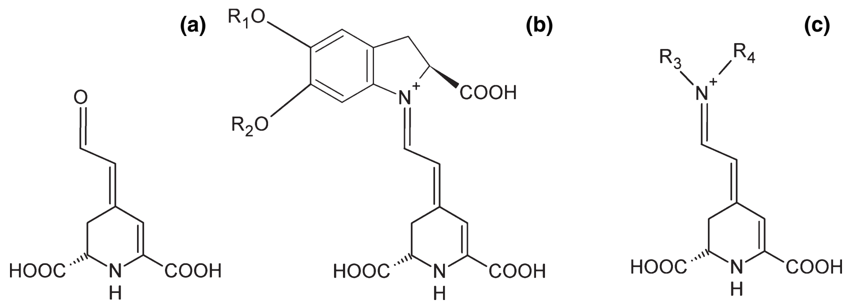

Figure 1 General structures of betalamic acid (a), betacyanins (b) and betaxanthins (c). R_1 and R_2: hydrogen, acyl or sugar moieties; R_3: amine or amino acid group; R_4: usually hydrogen (Gandía-Herrero *et al.*, 2005).

water is generally required to completely extract pigments. Nevertheless, Castellar *et al.* (2006) reported that pure water was more efficient than ethanol; water to extract pigments from *Opuntia* fruits. Moreover, it is the most inexpensive, abundant and environmentally friendly solvent.

Acidification of the solvent has been reported to enhance betacyanin extraction (López *et al.*, 2009) and pigment stability (Schliemann *et al.*, 1999; Strack *et al.*, 2003). Temperature was also reported to affect extraction (Roy *et al.*, 2004). Some other factors, such as solvent, tissue ratio and grinding time, may affect the extraction step. However, no studies were found in quantification of effects of all those factors on betalain extraction efficiency.

The objective of this study was to evaluate the effects of four variables – solvent pH and initial temperature, grinding time and solvent-to-beetroot ratio (w/w%) – on betalain extraction from red beetroots, to obtain an extract, which can be later processed to be used as a natural food colourant with antioxidant properties. Some further processing methods for beetroot extracts can be used, such as freeze drying (Roy *et al.*, 2004; Prudencio *et al.*, 2008), spray drying (Azeredo *et al.*, 2007), ultrafiltration (Bayindirli *et al.*, 1988; Roy *et al.*, 2004), as well as conventional (evaporation) or nonconventional (reverse osmosis) concentration methods (Thakur & Das Gupta, 2006). The colour properties and stability of red beetroot colourants have been already reported to be adequate for several products, such as soft drinks (Havlíková *et al.*, 1985), a typical Indian dessert called sandesh (Roy *et al.*, 2004), yoghurts (Azeredo *et al.*, 2007) and petit suisse cheese (Prudencio *et al.*, 2008). According to Stintzing & Carle (2007), the most interesting applicative feature of betalains is their pH stability over a wide range (3–7), which make them suitable for application in a broad variety of foods, in contrast with the less hydrophilic anthocyanins, which lose colour under low-acid conditions.

Materials and methods

The red beetroots were purchased in the local market, and the extraction treatments were conducted at the Agroindustrial Process Laboratory of Embrapa Tropical Agroindustry (Fortaleza, CE, Brazil). The roots were washed in running water, sanitised with a 50 mg kg^{-1} sodium hypochlorite solution for 10 min, manually peeled and cut in slices (5 mm in thickness, 50 mm in diameter) from the middle third of the roots, perpendicularly to the root axis. Each slice was cut in two halves, one for betacyanin determination in raw beetroots and the other for processing. Half-slices were blanched in water (beetroots:water weight ratio, 1:10) at (90 ± 1) °C for 2 min, without stirring; this step is desirable to inactivate enzymes, although it may destroy some pigments (Delgado-Vargas & Paredes-Lopez, 2003). Moreover, a heat treatment previously to the extraction may reduce microbial load, and increase permeability of the tissues, facilitating betacyanin extraction. The betacyanin contents of raw and heat treated beet slices were determined according to the method proposed by Roy *et al.* (2004), consisting basically on juice extraction, addition of a citric-phosphate buffer and measurements of absorbances at different wavelengths against the buffer as blank.

Twenty-nine treatments (runs) were conducted, according to a central composite design (Table 1), with the following independent variables: solvent pH (ranging from 3 to 5), solvent: beets weight ratio (1:1:5:1), solvent initial temperature (30–70 °C) and grinding time (2–10 min). More extreme values of such variables were not adopted by economic and/or technical/sensory reasons. A further decreasing in solvent pH could impair the application of the final extract as a food ingredient because of an excessive acidity. Higher solvent:beet ratio would increase the volume and weight of the extract, making its concentration more expensive. Temperatures above 70 °C could result in thermal degradation of betalains (Fernández-López & Almela, 2001), and higher grinding times would implicate in increasing processing cost.

Triplicates of extraction treatments were conducted, each run with 30 g samples of heat treated beet half-slices. For each run, the water pH was adjusted to the determined value by addition of citric acid, and the acidified water was then heated until the specified temperature. Previously heat-treated beet half-slices were then ground at $277 \times g$ with the solvent in a

Table 1 Coded (C) and uncoded (UC) levels of the independent variables for each extraction treatment

Treatment	Solvent pH C	Solvent pH UC	Solvent/beet ratio (w/w) C	Solvent/beet ratio (w/w) UC	Solvent initial temperature (°C) C	Solvent initial temperature (°C) UC	Grinding time (min) C	Grinding time (min) UC
1	−1	3.5	−1	2:1	−1	40	−1	4
2	1	4.5	−1	2:1	−1	40	−1	4
3	−1	3.5	1	4:1	−1	40	−1	4
4	1	4.5	1	4:1	−1	40	−1	4
5	−1	3.5	−1	2:1	1	60	−1	4
6	1	4.5	−1	2:1	1	60	−1	4
7	−1	3.5	1	4:1	1	60	−1	4
8	1	4.5	1	4:1	1	60	−1	4
9	−1	3.5	−1	2:1	−1	40	1	8
10	1	4.5	−1	2:1	−1	40	1	8
11	−1	3.5	1	4:1	−1	40	1	8
12	1	4.5	1	4:1	−1	40	1	8
13	−1	3.5	−1	2:1	1	60	1	8
14	1	4.5	−1	2:1	1	60	1	8
15	−1	3.5	1	4:1	1	60	1	8
16	1	4.5	1	4:1	1	60	1	8
17	−2	3.0	0	3:1	0	50	0	6
18	2	5.0	0	3:1	0	50	0	6
19	0	4.0	−2	1:1	0	50	0	6
20	0	4.0	2	5:1	0	50	0	6
21	0	4.0	0	3:1	−2	30	0	6
22	0	4.0	0	3:1	2	70	0	6
23	0	4.0	0	3:1	0	50	−2	2
24	0	4.0	0	3:1	0	50	2	10
25	0	4.0	0	3:1	0	50	0	6
26	0	4.0	0	3:1	0	50	0	6
27	0	4.0	0	3:1	0	50	0	6
28	0	4.0	0	3:1	0	50	0	6
29	0	4.0	0	3:1	0	50	0	6

Robot Coupe R201 blender, model Ultra E (BBS Limited, Farnham, Surrey, UK). After grinding, the material was filtered through a 0.3-mm mesh sieve, and the filtered extract was submitted to analyses of total betalains – i.e. total betacyanins and betaxanthins (Roy *et al.*, 2004). The betacyanin extraction efficiency (BEE) was defined as being:

$$\text{BEE}\ (\%) = \frac{\text{BC}_\text{e} \times w_\text{e}}{\text{BC}_\text{bb} \times w_\text{bb}} \times 100, \qquad (1)$$

where BC_e is the betacyanin content of the final extract (mg per 100 g); w_e is the weight of extract (g); BC_bb is the betacyanin content of the heat treated beet half-slices (mg per 100 g); and w_bb is the weight of heat treated beet half-slices used for obtaining the extract (g).

The software MINITAB 15 (Minitab Inc., State College, PA, USA) was used as two kinds of models (a full quadratic and a simplified one with only the linear terms plus the significant nonlinear ones) and to chose the most adequate one to represent the experimental responses, that is to say, those with the most significant *F*-values for each response. Contour plots were generated from the models and were helpful in evaluating the influences of each independent variable on the responses.

The definition of the most adequate conditions to obtain a highest possible BEE value (within the experimental ranges) was based on the 'response optimizer' function of MINITAB, along with a careful evaluation of the model and the contour plots. A verifying test was then carried out under such experimental conditions (in five replicates), to compare the experimental BEE with the value predicted by the model and to evaluate the predictive power of the model for those conditions.

Results and discussion

Raw peeled beetroot half-slices presented the following betalain contents: betacyanins, 84.26 mg per 100 g and betaxanthins, 27.54 mg per 100 g. The blanching step resulted in decreased pigment contents (betacyanins, 67.58 mg per 100 g, and betaxanthins, 21.36 mg per 100 g), indicating that part of the betalains were leached out by water.

Table 2 presents the extraction efficiency values resulted from all treatments, which varied between less than 40% and more than 60%. Table 3 presents the regression coefficients for the two proposed models for BEE. The full quadratic model was highly significant ($P < 0.001$), but only one of its nonlinear terms (the quadratic term for solvent-to-beetroot ratio) was significant ($P < 0.05$); thus, a simplified model was proposed, by considering linear terms plus the only significant nonlinear term. Although the full quadratic model presented a higher determination coefficient (R^2), the simplified model was preferred because it was simpler, but still similar in terms of other statistical indexes. The regression terms for the simplified model (Table 3) and the contour plots (Fig. 2) indicate that, except for pH, all independent variables had positive effects on BEE. That is to say, the pigment extraction efficiency was favoured by higher temperatures, higher solvent-to-beetroot ratio and lower pH. The effect of grinding time was nonsignificant, but there was a tendency for it to enhance pigment extraction. The significance of the quadratic term for solvent-to-beetroot ratio indicates that the impact of this variable on extraction efficiency decreases as the solvent-to-beetroot ratio increases.

The enhancement of betalain extraction with acidification corroborates claims by other authors (Delgado-Vargas & Paredes-Lopez, 2003; López *et al.*, 2009). The positive effect of temperature on extraction efficiency is consistent with reports by Roy *et al.* (2004), although López *et al.* (2009) have reported that extraction at 30 °C was more efficient than at 60 °C, which can be

Table 2 Betacyanin extraction efficiency (BEE) from the experimental runs

Run	BEE (%)
1	45.13 ± 2.13
2	39.89 ± 1.74
3	51.47 ± 2.79
4	44.82 ± 2.54
5	53.13 ± 3.01
6	47.36 ± 2.46
7	60.90 ± 3.58
8	51.38 ± 2.80
9	47.97 ± 2.51
10	38.45 ± 1.90
11	56.48 ± 3.34
12	47.03 ± 2.67
13	50.73 ± 2.96
14	42.97 ± 2.39
15	61.28 ± 3.68
16	54.34 ± 3.08
17	52.98 ± 2.57
18	43.38 ± 2.19
19	45.47 ± 2.92
20	57.10 ± 3.24
21	43.42 ± 2.35
22	52.67 ± 2.78
23	45.62 ± 2.72
24	52.35 ± 2.98
25	48.84 ± 2.11
26	45.58 ± 2.67
27	46.93 ± 2.18
28	49.47 ± 2.99
29	49.23 ± 2.65

BEE values were expressed as means ± standard deviations from three runs.

Table 3 Regression coefficients of the full quadratic and simplified models for betacyanin extraction efficiency (in coded form)

	Full quadratic		Simplified	
Term	Coefficients	*P*	Coefficients	*P*
Constant	48.01	<0.01	48.51	<0.01
X_1	−3.34	<0.01	−3.34	<0.01
X_2	3.56	<0.01	3.56	<0.01
X_3	2.89	<0.01	2.89	<0.01
X_4	0.78	0.06	0.78	0.06
X_1^2	0.12	0.75	–	–
X_2^2	0.90	0.03	0.82	0.03
X_3^2	0.09	0.82	–	–
X_4^2	0.32	0.40	–	–
$X_1 \times X_2$	−0.27	0.58	–	–
$X_1 \times X_3$	0.05	0.91	–	–
$X_1 \times X_4$	−0.41	0.40	–	–
$X_2 \times X_3$	0.33	0.49	–	–
$X_2 \times X_4$	1.00	0.05	–	–
$X_3 \times X_4$	−0.75	0.13	–	–
R^2	94.39%	90.63%		
R^2 (adjusted)	88.78%	88.59%		
F-value (regression)	16.83 ($P < 0.001$)	44.50 ($P < 0.001$)		
F-value (lack of fit)	1.35 ($P = 0.41$)	1.33 ($P = 0.43$)		
S	1.88	1.90		

X_1: solvent pH; X_2: solvent/beet ratio; X_3: solvent initial temperature; X_4: grinding time.

explained by temperature-dependent pigment degradation reactions (Herbach *et al.*, 2006; Stintzing & Carle, 2007). As the solvent in this study was previously heated, but the heating was not maintained during grinding, the temperature may have soon dropped to values, which did not promote significant pigment losses. The effect of solvent-to-beetroots ratio in increasing BEE is explained by the concentration gradient of betacyanins between the solvent and the beetroots being an important driving force during mass transfer. Anthocyanin extraction has also been reported to be enhanced by increasing solvent-to-solid ratios (Gao & Mazza, 1996; Cacace & Mazza, 2003).

The response optimizer function of MINITAB indicated, as evident from the graphs (Fig. 2), that the conditions, which provided the highest pigment extraction efficiency (within the ranges studied) were: pH, 3; solvent-to-beetroot ratio, 5:1; temperature, 70 °C; and grinding time, 10 min. Under such conditions, the predicted extraction efficiency was 72.91%. However, grinding time was nonsignificant ($P < 0.05$), its decreasing could be proposed to reduce processing time and energy-related costs. The decrease of grinding time to only 2 min instead of 10 min resulted in only a little decrease in the predicted BEE (that is to say, from 72.91% to 69.81%). Thus, instead of unnecessary high grinding time, a much lower one (2 min) was considered to define the most adequate extraction conditions.

The verifying treatment, carried out under the specified conditions (pH, 3; solvent-to-beetroot ratio, 5:1; temperature, 70 °C; and grinding time, 2 min), resulted in a BEE of 67.12%, with a standard deviation of 3.28%. So, the experimental (observed) BEE was about 4% lower than the predicted one, indicating that the predictive power of the model was satisfactory for those conditions.

The process resulted in a bright purple-red extract (betacyanin content, 10.14 mg per 100 g; soluble solid content, 1.69%; pH = 3.6), which could be later processed to be used as food colourant for a wide variety of foodstuffs.

Conclusions

- A model consisting in all linear terms plus the quadratic term for solvent-to-beetroot ratio was proposed to represent BEE. The model was highly significant and adequate to represent variations among treatments, within the investigated ranges.

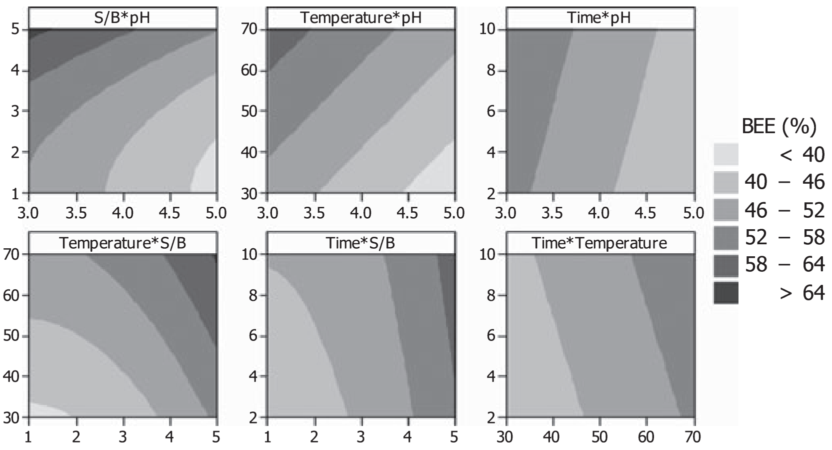

Figure 2 Contour plots for betacyanin extraction efficiency. S/B: solvent-to-beetroot weight ratio. The header of each graph indicates (*Y*-axis × *X*-axis).

- The BEE was enhanced by increasing solvent-to-beetroot ratio and initial temperature of the solvent, and by decreasing solvent pH. The effect of grinding time was nonsignificant.
- The maximum betalain extraction efficiency values, predicted by the model (within the experimental ranges studied) and corroborated experimentally, were around 70%. The most adequate extraction conditions were defined as being: pH, 3; solvent-to-beetroot ratio, 5:1; temperature, 70 °C; and grinding time, 2 min.
- The study suggests a simple, inexpensive and reasonably efficient method to produce a bright red–purple extract, which can be later processed to be used in several applications as a food colourant.

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