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Betacyanin Stability During Processing and Storage of a Microencapsulated Red Beetroot Extract

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Abstract: The objectives of this research were to evaluate betacyanin retention during processing and storage of a Microencapsulated Red Beetroot Extract (MRBE) and to evaluate if the product imparts off-flavors when added to plain yogurt. Blanched beetroot slices were ground in acidified medium, pressed and filtered to obtain red beetroot extract, which was microencapsulated by spray-drying with three maltodextrin:beetroot dry mass (M/B) ratios (3:1, 4:1 and 5:1). The resulting powders (MRBE3, -4 and -5, respectively) were packed in dark or translucent HDPE jars and stored under diffuse sunlight. Betacynin retention during microencapsulation was about 90%, independently on the M/B ratio. The addition of 1220 mg kg^{-1} of MRBE3, 1525 mg kg^{-1} of MRBE4, or 1830 mg kg^{-1} of MRBE5 to plain yogurt was not perceived in terms of flavor by panelists. Betacyanin degradation rates during storage were increased by light exposure and decreased by M/B ratio.

Key words: Betalains, natural pigments, microencapsulation, stability

INTRODUCTION

Color is one of the most important attributes of foods, being perceived as a quality indicator and determining frequently their acceptance. Many naturally colored foods, such as fruit products, are submitted to color losses during processing, requiring the use of colorants to restore their color. Natural colorants have many disadvantages when compared to synthetic ones, including higher costs, poorer tinctorial strength and lower stability. However, consumers tend to equate synthetic with unhealthy, when in contrast, natural additives are seen as harmless or even healthy. Moreover, some synthetic colorants are considered to be responsible for allergenic and intolerance reactions (Berzas Nevado *et al*., 1995). In this way, efforts have been made to replace synthetic food colorants by natural ones.

Betalains are natural water-soluble nitrogen-containing pigments, which comprise the red-violet betacyanins and the yellow-orange betaxanthins. The major commercially exploited betalain crop is red beetroot (*Beta vulgaris*), whose main pigment is betanidin-5-O-β-glycosidase, or betanin, which is the most common betacyanin (Strack *et al*., 2003; Stintzing and Carle, 2004).

Recent findings rank beetroots among the ten most potent antioxidant vegetables (Halvorsen *et al*., 2002; Ou *et al*., 2002). Betalains respond at least in part for these beneficial

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properties (Escribano *et al*., 1998; Kanner *et al*., 2001; Pedreño and Escribano, 2001; Wettasinghe *et al*., 2002). These reports have increased the interest in using red beetroot extracts to color food.

Although betacyanins cover about the same color range as anthocyanins, they have not been so much explored as the latter, probably because their relative scarceness in nature. However, in contrast with anthocyanins, betacyanins can be applied to low acidity foods, since they are not as susceptible as anthocyanins to hydrolytic cleavage, keeping their appearance over the broad pH range from 3 to 7 (Jackman and Smith, 1996), being the pH range 5-7 the ideal for their stability (Cai *et al*., 1998). Betalain degradation is also affected by temperature, light, oxygen and moisture (Constant *et al*., 2002). Microencapsulation techniques have been widely used to reduce interactions of food components with environment factors, such as oxygen, light and moisture (Depypere *et al*., 2003). Cai and Corke (2000) reported that microencapsulation enhanced chemical stability of betalains.

The objectives of this research are to evaluate the pigment retention during processing and storage of a microencapsulated red beetroot powder and to compare its tinctorial power with that of a synthetic food colorant.

MATERIALS AND METHODS

The red beetroots were purchased from the local market (Fortaleza, CE, Brazil). They were washed and sanitized (1% chloride solution), peeled, sliced and blanched in boiling water during 5 min. The blanched beetroot slices, added by an aqueous 1% citric acid solution (beetroot slices: solution weight proportion, 1:1), were ground by using a Robot Coupe R201 blender, model Ultra E, during 10 min. The acidification of the extraction medium was made in order to enhance betacyanin stability (Strack *et al*., 2003). The ground material was pressed and filtered in a 0.3 mesh sieve. The filtered red beetroot extract (RBE) was added with maltodextrin (DE 10), in three different maltodextrin: beetroot dry mass (M/B) ratios: 5, 4 and 3. The resulting dispersion was homogeneized and atomized in a Mini Spray Dryer Büchi B-290, with the following operation conditions: inlet temperature, 120°C; outlet temperature, 60°C; pump rate, 0.4 L/h. The obtained microencapsulated red beetroot extracts (MRBE) resulting from the different treatments (M/B ratios of 5, 4 and 3) will be further referred as to MRBE5, MRBE4 and MRBE3, respectively.

The following determinations were carried out on RBE and the resulting powders (MRBE): total betacyanin contents (Roy *et al*., 2004); total solid contents, water activity (AOAC, 1995) and color (Hunter). The powders were reconstituted before color analyses, in such a way as to equalize the beetroot solid contents of the MRBE with that of RBE.

The betacyanin retention during each microencapsulation treatment was defined as being:

$$BR(\%) = \frac{(BC/BS)_{powder}}{(BC/BS)_{extract}} \times 100 \quad (1)$$

$$(BC/BS)_{powder} = \frac{BC_{powder} \times (M/B + 1)}{TS_{powder}} \times 100 \quad (2)$$

BR: betacyanin retention during microencapsulation; BC: betacyanin contents; BC/BS: betacyanin contents based on beetroot solids; TS: total solid contents; M/B: maltodextrin: beetroot dry mass ratio.

The following microbiological determinations were carried out on powders previously to sensory analysis, in order to verify the safety of MRBE to panelists: coliform counts (total and faecal coliforms, *Escherichia coli*) and *Salmonella* research, according to American Public Health Association (Downes and Ito, 2001).

The relative tinctorial strength of the MRBE when compared to a synthetic colorant (Bordeaux Red, or Vermelho Bordeaux, M. Cassab, São Paulo, Brasil) was determined. A sample of plain yogurt was added with 231.25 mg kg^{-1} of the synthetic colorant (standard) and three other samples were added gradually with MRBE3, -4 or -5, until their color (Hunter) parameters (especially a* values) were equivalent to those of the standard.

A difference-from-control sensory test (Meilgaard *et al*., 1987) was conducted in Sensory Analysis Laboratory of Embrapa Tropical Agroindustry, using individual booths illuminated by red light in order to mask the color differences. The test was made to evaluate flavor differences between a control and four test samples. The control was plain yogurt added with 231.25 mg kg^{-1} of Bordeaux Red. The test samples were constituted by the same yogurt added with each MRBE in such a concentration as to obtain equivalent color parameters (as determined by the test for relative tinctorial strength). A fourth test sample was the hidden control. Concentrations of both the synthetic colorant and the MRBE were determined to obtain similar instrumental color values, avoiding the panelists to differentiate samples by appearance. The control (identified as C) and the four test samples coded with randomly selected three-digit numbers were presented to thirty untrained panelists. The order of presentation of the coded samples was randomized. Panelists were asked to taste the control and the coded samples and to rate the degree of flavor difference between each coded sample and the control, by using a scale ranging from 0 (no difference) to 8 (extreme difference). The mean rates for each sample were compared with the control by Dunnet test (at $p<0.05$).

The three powders were packed in sealed High Density Polyethylene (HDPE) white translucent or dark jars with HDPE lids and stored for six months at room temperature (27±2°C) under diffuse sunlight, in order to evaluate the betacyanin stability of the powders. The mean betacyanin losses throughout the storage time (i.e., the averages of the percent betacyanin losses from the 1st to the 6th month of storage) were compared among different M/B ratios by Tukey tests ($p<0.05$) and between the two kinds of HDPE jars by t-tests.

RESULTS AND DISCUSSION

Table 1 presents the results of the physicochemical analyses on RBE and the powders (MRBE). All final products presented a_w values lower than 0.6, the minimum a_w required for microbial growth (Troller, 1980). So, MRBE is expected to have high microbiological stability, provided that it is stored in high moisture barrier packages. The betalain retention for all powders was near 90%, with low variations among treatments, indicating that the M/B ratio had virtually no effect on pigment retention during microencapsulation. Similarly, the color attributes of the reconstituted MRBE different from those of RBE, but were not affected by M/B ratio.

The microbiological analyses indicated that all coliform counts were lower than 3 MPN g^{-1} and *Salmonella* was absent in 25 g of the samples, that is to say, MRBE was considered as safe for consumption and then adequate for being submitted to sensory analyses.

Table 1: Results from physicochemical determinations on red beetroot extract (RBE) and powders (MRBE)

Product	a_w	TS (%)	BC (mg/100 g*)	BC/BS (mg/100 g)	BR (%)	Color L*	a*	b*
RBE	0.99	4.65	28.82	619.78	-	16.48	+38.83	-3.78
MRBE5	0.46	96.63	90.71	563.42	90.91	10.92	+22.68	-8.24
MRBE4	0.53	95.69	106.09	554.28	89.43	10.64	+21.71	-8.39
MRBE3	0.49	96.28	135.98	564.94	91.15	10.51	+23.17	-8.12

TS: Total solid contents; BC: Betalain contents; BC/BS: Betalain contents, based on beetroot solids; BR: Betalain retention

The test for relative tinctorial strength indicated that the following MRBE concentrations in plain yogurt were required to obtain color parameters equivalent to those of the yogurt added with 231.25 mg kg^{-1} of Bordeaux Red: 1220 mg kg^{-1} of MRBE3, 1525 mg kg^{-1} of MRBE4, or 1830 mg kg^{-1} of MRBE5.

The results of difference-from-control test (Table 2) indicate that the flavor of yogurt added with any of the MRBE was not significantly different ($p<0.05$) from that added with a synthetic commercial colorant. Although some authors (Lu *et al*., 2003; Stintzing and Carle, 2004) mentioned a possible adverse earthy smell originated from geosmin and some pyrazines when adding beetroot extracts to dairy products, the MRBE did not impart off-flavors perceivable by panelists at the concentrations used.

Changes in betacyanin contents throughout storage of MRBE are indicated in Fig. 1 and 2, for translucent and dark HDPE jars, respectively. Light exposure greatly affected betacyanin degradation rates. While betacyanin storage in dark jars resulted in about 30% betacyanin degradation after 6 months, the material stored in translucent jars suffered at least 57% betacyanin degradation after the same time. These results are supported by those related by Von Elbe *et al*. (1974), Attoe and von Elbe (1981) and Cai *et al*. (1998), indicating an inverse relationship between light exposure and betalain stability. t-tests (Table 3) indicate that the MRBE packed in translucent jars suffered higher degradation than that packed in dark jars, independently on the M/B ratio, although the difference observed for MRBE5 has been less significant than those for MRBE3 and MRBE4. Results of Tukey tests (Table 3) indicate that, when the product was packed in translucent jars, higher M/B ratios resulted in better betacyanin stability. This indicates the positive effect of microencapsulation on chemical stability of these pigments, corroborating results reported by Cai and Corke (2000). On the

Table 2: p-values for differences-from-control (Dunnett's test)

Treatment	Mean differences-from-control	p-value
MRBE5	3.53	0.22
MRBE4	2.60	0.99
MRBE3	2.70	0.95
Control	2.60	

MRBE5, MRBE4 and MRBE3: red beetroot powders with maltodextrin: beetroot dry mass ratios of 5:1, 4:1 and 3:1, respectively

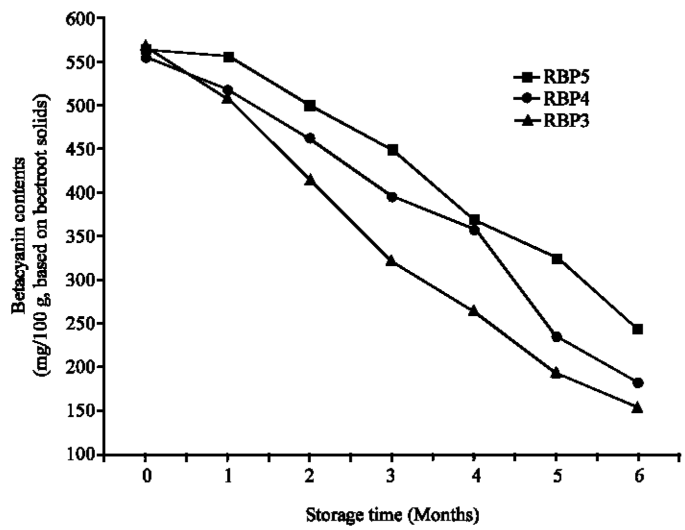

Fig. 1: Betacyanin contents throughout storage of MRBE in translucent HDPE jars

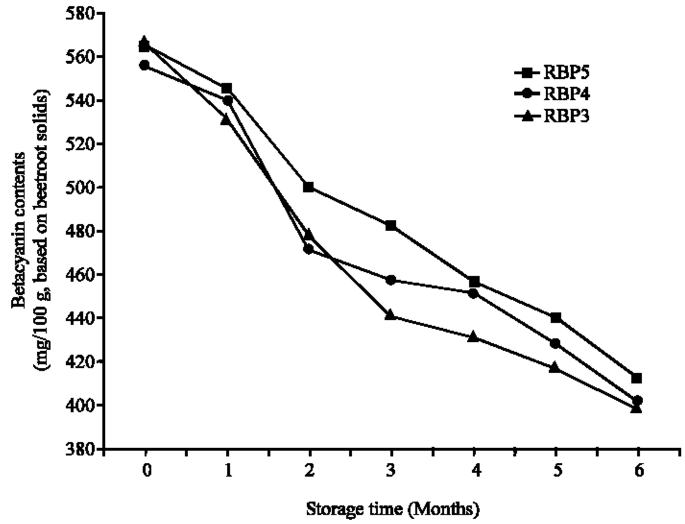

Fig. 2: Betacyanin contents throughout storage of MRBE in dark HDPE jars

Table 3: Comparisons of mean betacyanin losses throughout the storage time, among M/B ratios (Tukey tests, $p<0.05$) and between kinds of HDPE jars (t-tests)

	Mean betacyanin losses throughout the 6-month storage (%)		
Kind of jar	MRBE3	MRBE4	MRBE5
Translucent	45.55a	35.53ab	27.92b
Dark	21.72a	18.47a	17.57a
p-values (t-test)	<0.01	<0.01	0.04

Mean values in the same row followed by the same letter are not significantly different ($p<0.05$)

other hand, when the powder was packed in dark jars, the effect of M/B ratio was not significant ($p<0.05$), suggesting that the protective effect of the dark jars was sufficient for maintain a relatively good betacyanin stability on the powder, independently of the M/B ratio.

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