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Effect of ph on the stability of red beet extract (*Beta vulgaris* l.) microcapsules produced by spray drying or freeze drying

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Abstract

Red beets is rich in phenolic acids and has high antioxidant capacity, and can be used to produce a natural dye. This study evaluated the effect of pH (3 to 6) on the stability of red beet extract microcapsules, dried by freeze drying and spray drying and stored at room temperature. The microcapsules were produced using a combination of maltodextrin and xanthan gum as encapsulating agents and stored for 7 days. For all evaluated microcapsules, a degradation of betanin was observed, however, that degradation was independent of pH, with the exception of the sample with maltodextrin and dried by spray drying. The freeze dried products showed lower degradation constants and higher half-life ($t_{1/2}$) when comparing with the spray dried samples. The microcapsules containing maltodextrin and xanthan gum, dried by spray drying, showed the highest change in the content of phenolic compounds after storage for 7 days. The color parameters showed a reduction for a*, and increase in b* and L*, for all samples during the storage time. In general, the microcapsules produced using maltodextrin and xanthan gum, and dried by freeze dryer, showed higher stability in terms of betanin content, phenolic compounds and color parameters during storage at different pHs.

Keywords: encapsulation; natural dye; betanin; phenolic compounds; maltodextrin; xanthan gum.

Practical Application: The best drying parameters and encapsulating agent for the natural beet dye were defined for foods with different pHs.

1 Introduction

The red beet root (*Beta vulgaris* L.) is a traditional vegetable that is widely consumed in several countries. The caloric value is moderate, but it is a rich source of fiber and sugars (Straus et al., 2012). Consumption of red beet, which is rich in phenolic acids and has high antioxidant capacity, can help protect against age–related diseases (Ravichandran et al., 2013).

The natural dye present in red beet is betalain, which contains two groups of pigments: betacyanins (red-violet) and betaxanthin (yellow), and this pigments together result in a number of varieties of red color. The betacyanin present mainly in the roots of red beets is known as betanin (Nemzer et al., 2011).

The stability of betanin depends directly on its pH, which ranges from 3 to 7, with the optimum pH being between 4 and 5. Its spectrum ranges from pink to red. It is unstable in the presence of light and oxygen, and is degraded when subjected to high temperatures (Huang & Von Elbe, 1987). According to Serris & Biliaderis (2001), the possibility of the betanin regeneration at 30, 40 and 50 °C is minimal. This is an important factor, once the regeneration of betanin can interfere the kinetics degradation.

Natural dyes generally have higher costs and exhibit a lower stability under storage and processing conditions when compared with artificial dyes (Cardoso-Ugarte et al., 2014). One way to improve the stability of natural dyes is the encapsulation process, which creates a barrier between the core material and the environment. This barrier is formed by the supporting material (encapsulating agent) which protects the encapsulated material, making the final product more stable (Janiszewska, 2014).

There are various encapsulating agents, such as polysaccharides, lipids and proteins, and the most widely used is maltodextrin, due to the low cost (Saénz et al., 2009). Furthermore, some gums, such as xanthan gum, can also be used in combination with maltodextrin during the encapsulation process, for increase the encapsulation yield. Ravichandran et al. (2014) found that the microencapsulation of betalain with maltodextrin associated with xanthan gum (0.5%) showed better stability and an increase of up to 65% in betanin content when compared to maltodextrin encapsulation alone.

One of the most widely used techniques for microencapsulation is spray drying, which is the atomizing of an emulsion or suspension containing the encapsulated material and the encapsulant agent in a drying chamber with hot air circulation. The contact of the solution with hot air causes the water evaporation, resulting in the production of the microcapsules (Laohasongkram et al., 2011). Another technique that can be used is freeze drying, which consists in the frozen of the solution containing the encapsulating agent and the encapsulated material, followed by drying sublimation under vacuum, and the change in ice occurs directly from the solid in vapor without becoming liquid (Kumar et al., 2011).

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The objective of this study was to encapsulate the natural dye of red beets in a combination of maltodextrin and xanthan gum, using freeze drying and spray drying techniques, and to evaluate the stability of the resulting microcapsules at different pHs.

2 Materials and methods

The red beet used in this research was acquired in the local market of the city of Maringa-Brazil, which has latitude 23° 25 '31 "S and longitude 51° 56' 19" W, during the period from July to November.

The maltodextrin (DE 10) was provided by Cargill (São Paulo-Brazil) and xanthan gum was purchased from Doce Aroma (São Paulo-SP). All reagents used in the study were of analytical grade.

2.1 Extraction of betanin

The beets were washed, sanitized (using 200 ppm of active chlorine during 15 min) and sliced. The juice was extracted with a centrifugal Turbo Juicer CF-06 (Mondial) and filtered through filter paper.

2.2 Processing of the microcapsules

The encapsulating agents were added to the red beet juice in a quantity of 30 g dry material/100 g total matter. Two solutions were prepared: one containing only maltodextrin and another containing maltodextrin (99.5%) and xanthan gum (0.5%). The solutions were dried by spray drying or freeze drying.

For microencapsulation by spray drying, the solutions were dried on a mini spray drier (LM brand; model MSD 1.0). The drying conditions were: nozzle diameter of 0.7 mm; drying air inlet temperature of 150 °C and outlet 90 °C; atomization pressure: 0.08 to0.14 bar; average flow drying air of 3.8 m^3/h; average feed flow rate 0.6 L/h. The powders collected were stored in plastic bags with nylon polyethylene thermosoldable for later use.

For microencapsulation by freeze drying, the samples were frozen for 48 hours at –10 °C. The samples were subsequently dried by freeze drying for 2 days (–36 °C, 1.09 Pa) to ensure complete drying of the product (L108 freeze dryer, Liobras). The final product was stored in plastic bags with nylon polyethylene thermosoldable for later use.

The dried powders were named: powder containing maltodextrin as the encapsulating agent and dried by spray drying (MAS), powder containing maltodextrin and xanthan gum and dried by spray drying (MXS), powder containing maltodextrin as the encapsulating agent and dried by freeze drying (MAL), and powder containing maltodextrin and xanthan gum and dried by freeze drying (MXL).

2.3 Betanin stability at different pHs

Solutions containing the microcapsules (MAL MXL, MAS and MXS) (10% w/v) were prepared in buffers at pH 3, 4, 5 and 6. Potassium sorbate (5%) was added to each of these solutions to prevent contamination by microorganisms. Sodium acetate was used as the buffer for pH 3 to 5 solutions, and sodium phosphate was used for pH 6 (Gandía-Herrero et al., 2009). The buffer solutions of microcapsules were storage in BOD (Biochemical oxygen demand) chamber for 7 days, at a constant temperature of 30 °C. Periodic measurements of betanin content, phenolic compounds and color parameters of buffered solutions were determined.

2.4 Quantification of betanin

The betanin content in the microcapsules was determined according to the methodology of Stintzing et al. (2005). The color degradation was monitored on time zero (control) and 1, 3, 4 and 7 days. The betanin degradation kinetics were verified by periodic absorption measurements of buffer solutions of microcapsules at 538nm, at 30 °C.

To evaluate the reaction kinetics during the dye storage in buffer solutions, we tested first order kinetic models. Previous research demonstrated that the degradation of natural dyes such as betanin follows first order kinetics during storage (Serris & Biliaderis, 2001; Bustos-Garza et al., 2013). The color degradation can be calculated using Equation 1.

$$\frac{dC}{dt} = -kC_0 \quad (1)$$

Where: C is the remaining concentration; C_0 is the initial concentration of dye; t is the time interval between C_0 and C; k is the degradation constant of first order (1/time). In plotting concentration (-ln C) versus time, the conversion constant (k) is simply the slope of the line.

The half-life ($t_{1/2}$) for the reaction is the time required for the quantity of betanin to be reduced by half its initial value. The half-life, which, according to Kirca & Cemeroglu (2003), is directly related to the speed constant for a first order reaction, is described by Equation 2.

$$t_{1/2} = -\frac{ln(0.5)}{k} \quad (2)$$

2.5 Analysis of total phenolic compounds

The total phenolic content was determined by spectrophotometry, following the Folin-Ciocalteu method, with the same modifications of Ruiz-Gutiérrez et al. (2014). Measurements were performed in triplicate using a gallic acid curve as a standard. It was used a spectrophotometer model 700S of the brand FEM and wavelength 765 nm. Results were expressed in mg of gallic acid equivalents (GAE)/100 g powder.

2.6 Color analysis

Color was evaluated using a portable colorimeter CR400 Konica Minolta® with integrating sphere and 3° of viewing. The color parameters were measured using a CIEL*a*b* system. Hue angle (*h*) was calculated according to the Equation 3.

$$h = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (3)$$

2.7 Statistical analysis

Data were expressed as mean ± standard deviation, after tests of normality and homogeneity, data were underwent to analysis of variance (ANOVA) and correlation analysis, with Statistica software version 8.0 (StatSoft, Inc., Tulsa, OK, USA). We adopted the significance level of 5% for rejection of the null hypothesis ($p < 0.05$) for the Tukey test. The Pearson correlation coefficient is a measure of the degree of linear relationship between two variables and this quantitative test was used to determine the correlation between the betanin content, color parameters and phenolic compounds content of beet extract microcapsules.

3 Results and discussion

3.1 Quantification of betanin

The degradation of betanin, evaluated at different pHs, followed first order kinetics, as previously demonstrated in the literature (Serris & Biliaderis, 2001; Bustos-Garza et al., 2013; Cano-Higuita et al., 2015). The degradation constant and half-life of the microcapsules, stored in different buffer solutions, are presented in Table 1.

Samples MXS, MAL and MXL present no significant differences in the degradation constant at the evaluated pHs (Table 1). As the stability of betanin is pH dependent, being more stable between pH 4 and 5 (Huang & Von Elbe, 1987), this result shows the betanin encapsulation efficiency in maltodextrin and xanthan gum, where a dye degradation was observed, but this degradation is independent of pH of the solution.

The degradation constants for microcapsules containing maltodextrin dried by spray drying (MAS), differed significantly depending on pH, wherein the pH 3 and 4 did not differ, but differed in relation to pH 5 and 6, with the lowest values observed at pH 3 and 4. It can be explained because maltodextrin is an encapsulating agent widely used in encapsulation of active materials by spray drying; however, it has low emulsifying capacity, and the incorporation of other encapsulating agents, such as xanthan gum, can improve this capability (Poshadri & Kuna, 2010). The use of xanthan gum in the formation of the microcapsules increased the stability of betanin, the sample dried in spray drying presented lower constant degradation at pH 3 and 4; and no difference was observed for the freeze drying method.

When the methods of drying were compared, freeze drying produced lower degradation constants for all evaluated pHs, as compared to spray drying. The microcapsules obtained by spray drying and freeze drying have different structures, due to the drying mechanisms. While the spray drying the homogenization of solution before drying has an important role in encapsulation efficiency, ensuring that the material is completely entrapped in the matrix, and not permitting a significant amount of this component in the particle surface, in the freeze drying homogenization has little influence on microcapsule structure (Cano-Higuita et al., 2015). In our study, spray drying produced higher degradation constants compared with freeze drying, probably because there were large quantities of betanin on the microcapsules surface produced by this technique.

In a study by Ravichandran et al. (2014), the authors observed a 21% increase in the stability of microcapsules of red beet extract dried by spray drying and a 65% increase for the microcapsules dried by freeze drying, when the combination of maltodextrin and xanthan gum (0.5%) was used as an encapsulating, as compared to the use of maltodextrin alone.

It was observed (after 7 storage days in pH 6) that the sample MXS decreased 44.9% in constant degradation when compared to the MAS sample. The freeze dried sample MXL this reduction was around 19.6% compared to MAL, but statistically ($p < 0.05$) the freeze dried samples did not differ.

The half-life of the microcapsules was also calculated and ranged from 3.2 to 6.3 days for the spray drying and 5.5 to 7.6 days for freeze drying, being higher in this last drying methodology. Bustos-Garza et al. (2013) evaluated the microencapsulation of astaxanthin from *Haematococcus pluvialis* at 25 °C, with maltodextrin and arabic gum (50:75), and dried by a spray dryer

Table 1. Degradation constant (k) and half-life ($t_{1/2}$) of microcapsules stored in different buffer solutions.

Sample	$k \times 10^3$ (day^{-1}) Time of half-life (days)			
	pH 3	*pH 4*	*pH 5*	*pH 6*
Spray drying				
MAS	$158.9^{cA} \pm 9.7$ $t_{1/2} = 4.3$	$163.2^{cA} \pm 20.0$ $t_{1/2} = 4.2$	$189.4^{bB} \pm 6.9$ $t_{1/2} = 3.6$	$215.6^{bC} \pm 6.3$ $t_{1/2} = 3.2$
MXS	$127.3^{bA} \pm 11.9$ $t_{1/2} = 5.4$	$119.1^{bA} \pm 5.6$ $t_{1/2} = 5.8$	$108.4^{aA} \pm 1.6$ $t_{1/2} = 6.3$	$118.8^{aA} \pm 5.7$ $t_{1/2} = 5.8$
Freeze drying				
MAL	$118.4^{bA} \pm 13.0$ $t_{1/2} = 5.8$	$121.6^{bA} \pm 1.4$ $t_{1/2} = 5.7$	$110.6^{aA} \pm 2.9$ $t_{1/2} = 6.2$	$126.0^{aA} \pm 4.0$ $t_{1/2} = 5.5$
MXL	$91.3^{aA} \pm 4.9$ $t_{1/2} = 7.6$	$91.8^{aA} \pm 1.9$ $t_{1/2} = 7.5$	$105.7^{aA} \pm 4.0$ $t_{1/2} = 6.5$	$101.4^{aA} \pm 4.9$ $t_{1/2} = 6.8$

Different lowercase letters in the same column (considering the samples) and different capital letters in the same line (considering the evaluated pHs) are significantly different statistically ($p < 0.05$).

obtained half-lives ranging from 1.6 to 2.5 days at different pHs, lower values by comparing with our study.

3.2 Color parameters and phenolic compounds of the microcapsules in buffer solutions

Phenolic compounds content and color parameters (L*, a*, b*) in pH 3, 4, 5 and 6 were determined in day zero (t_0) and after 7 days of storage at 30 °C (t_7). Results (Table 2) demonstrate that, at pH 4, no significant reduction was observed in phenolic content of any microcapsules after 7 storage days at 30 °C. The microcapsules containing maltodextrin and xanthan gum, dried by spray drying and freeze drying, showed greater variation in phenolic content at pH 3, 5 and 6, being more significant for the microcapsules dried by spray drying.

An interesting trend was observed by comparing the results of betanin content with the results of phenolic content of red beet extract microcapsules stored at 30 °C, in different buffer solutions: the degradation of betanin was more significant than the degradation of phenolic compounds. It is important to highlight that betanins are the major phenolic compounds present in red beet, therefore it was expected that the rate of betanin degradation would be consistent with the degradation rate measured for all phenolic compounds. However, this result was also observed by Kujala et al. (2000) that evaluated the betacyanin content and the phenolic content of red beet, stored at low temperatures, they reported that most variation in betanin content of red beet extract is due to poor stability of the compound, even though it was encapsulated and not all betanin degradation products are phenolic.

After 7 storage days the a* parameter was reduced in all samples, the smallest reduction was observed at pH 3, where a* decreased 78.17% in the MAS sample, and 65.58% for sample MAL, followed by MXL sample with 54.99% and 54.52% for MXS. The addition of xanthan gum in the formation of microcapsules increased the stability of betanin during storage and buffering

Table 2. Content of phenolic compounds and color parameters of the microcapsule solutions at varying pHs.

	pH 3							
	Phenolic compounds (mg GAE/100g)		L*		a*		b*	
	t_0	t_7	t_0	t_7	t_0	t_7	t_0	t_7
Spray drying								
MAS	523.11^{bA}	471.60^{bA}	13.49^{aA}	16.78^{aA}	18.60^{bA}	4.06^{cB}	7.65^{aA}	11.72^{aB}
MXS	583.42^{aA}	481.30^{abB}	13.15^{aA}	17.48^{aA}	19.24^{aA}	8.75^{aB}	9.35^{bA}	13.54^{bB}
Freeze drying								
MAL	562.51^{abA}	514.94^{abA}	12.38^{aA}	17.50^{aB}	19.67^{aA}	6.77^{bB}	7.60^{aA}	12.59^{abB}
MXL	593.72^{aA}	530.69^{aB}	14.62^{aA}	17.11^{aA}	18.35^{bA}	8.26^{aB}	8.45^{abA}	13.38^{bB}
	pH 4							
	Phenolic compounds		L*		a*		b*	
	t_0	t_7	t_0	t_7	t_0	t_7	t_0	t_7
Spray drying								
MAS	447.66^{aA}	371.30^{aA}	9.13^{aA}	13.12^{aA}	9.18^{aA}	1.54^{aB}	4.80^{aA}	9.06^{aB}
MXS	565.54^{aA}	447.96^{aA}	8.84^{aA}	14.26^{bcA}	9.11^{aA}	3.55^{bB}	5.08^{bA}	10.47^{bB}
Freeze drying								
MAL	488.87^{aA}	405.24^{aA}	8.73^{aA}	13.88^{bB}	8.39^{bA}	1.71^{aB}	4.69^{aA}	9.73^{cB}
MXL	583.11^{aA}	501.60^{aA}	8.90^{aA}	14.59^{cA}	9.16^{aA}	3.09^{cB}	5.15^{bA}	10.57^{bB}
	pH 5							
	Phenolic compounds		L*		a*		b*	
	t_0	t_7	t_0	t_7	t_0	t_7	t_0	t_7
Spray drying								
MAS	505.24^{aA}	417.97^{aA}	8.82^{aA}	11.61^{aB}	7.28^{aA}	0.86^{abB}	4.43^{aA}	7.96^{aB}
MXS	603.72^{bA}	424.33^{aB}	9.05^{bA}	10.86^{bB}	4.07^{bA}	0.95^{bB}	3.67^{bA}	6.97^{bB}
Freeze drying								
MAL	521.60^{abA}	411.30^{aB}	8.28^{cA}	11.77^{aB}	6.19^{cA}	0.63^{aB}	4.11^{cA}	7.55^{cB}
MXL	579.48^{abA}	463.11^{aB}	8.86^{abA}	12.11^{bB}	9.18^{dA}	1.72^{cB}	4.92^{dA}	8.56^{dB}
	pH 6							
	Phenolic compounds		L*		a*		b*	
	t_0	t_7	t_0	t_7	t_0	t_7	t_0	t_7
Spray drying								
MAS	571.91^{aA}	357.06^{aB}	6.93^{bA}	9.15^{aB}	2.04^{aA}	-0.23^{aB}	3.09^{aA}	5.63^{aB}
MXS	585.24^{aA}	423.12^{bB}	6.83^{aA}	8.90^{bB}	1.87^{bA}	-0.05^{bB}	3.17^{abA}	5.09^{bB}
Freeze drying								
MAL	403.11^{bA}	369.94^{abA}	7.02^{cA}	9.95^{cB}	2.22^{cA}	-0.13^{abB}	3.25^{bA}	5.72^{aB}
MXL	576.75^{aA}	463.11^{cB}	6.85^{aA}	8.98^{dB}	1.89^{dA}	-0.12^{abB}	3.20^{abA}	5.05^{bB}

Different lowercase letters in the same column, and different capital letters on the same line differ statistically ($p < 0.05$).

Table 3. Pearson coefficients for microcapsules produced with maltodextrin and xanthan gum by spray drying and immersed in a pH 3 solution.

	Betanin	Phenolic compounds	L*	a*	b*	h
Betanin	-	0.907	–0.926	0.993	–0.841	–0.918
Phenolic compounds	0.907	-	–0.991	0.946	–0.988	–0.996
L*	–0.926	–0.991	-	–0.960	0.977	0.992
a*	0.993	0.946	–0.960	-	–0.896	–0.957
b*	–0.841	–0.988	0.977	–0.896	-	0.985
h	–0.918	–0.996	0.992	–0.957	0.985	-

with a lower reduction of a* parameter. This result is in agreement with the results of betanin content in the microcapsules where a shorter half life (Table 1) was observed in the sample containing the maltodextrin microcapsules dried by spray drying.

Analyzing the parameter b* after 7 days, an increase was noticed in all samples, with the greatest increases occurring at pH 4.The value of b*increased by 88.75% for the MAS sample, 105.24% for the MXL sample, 106.10% for the MXS sample and 107.46% for the MAL sample. The degradation of betanin leads to the formation of compounds with a yellow color, as reflected by the significant increase in the b* parameter. The degradation of betanin is usually accompanied by a marked change in color as a result of formation of degradation products with yellow coloring such as betalamic acid, neobetacianins and betaxantins (Herbach et al., 2006).

3.3 Correlation between betanin content, color parameters and phenolic compounds contents

Pearson correlation (PC) test was used to evaluate the correlation between betanin contents, color parameters and phenolic compounds content of buffer solutions of microcapsules. All samples of microcapsules showed the same behavior independent of pH, and in general, there was a good correlation Pearson for all parameters (CP > 0.8), a 5% significance level, and as example Table 3 shows the Pearson correlations for the microcapsules produced with maltodextrin and xanthan gum, dried by spray drying and immersed in a pH 3 solution.

The betanin content was significantly correlated with the phenolic compounds in microcapsules, this correlation was positive, since betanin is the main phenolic compound found in red beet. Kujala et al. (2000) also observed a positive Pearson correlation of 0.98 between betanin and total phenolic compounds in the root of red beet (*Beta vulgaris*), a value close to that found in this study.

The highest Pearson correlation was observed between betanin content and the color parameter a* (0.993). This finding can be explained by the fact that betanins produce red coloring, but when they degrade a discoloration of the product occurs resulting in a reduction in the parameter a*.

Although the betanin sample initially has an opaque red color, during the process of degradation the color changes to a clearer yellow, which leads to an increase in the parameters b* and L*. In view of this, the correlation between the betanin content and these parameters is significantly negative. Accordingly, the hue angle (h) also increases, and a negative correlation is also observed between betanin content and the hue angle (PC = –0.918).

As phenolic compounds have a positive correlation with the betanin content, the same finding is observed when correlates phenolic compounds with color parameters, having a positive coefficient with the parameter a* (0.946) and negative with the parameters b*, L* and h (PC = –0.991, PC = –0.988, PC = –0.996, respectively).

4 Conclusion

In general, all microcapsules evaluated, except the MAS, showed good encapsulation efficiency because, although degradation of the dye occurred, this degradation was independent of pH. Microcapsules produced using the combination of maltodextrin and xanthan gum and drying by freeze drying, showed the lowest degradation constant (k) and the longest half-life ($t_{1/2}$).Thus, the best samples suggested for use in food products at pH 3 to 6 is sample MXL, after MAL and MXS.

The greatest variation in phenolic content during storage was observed in the microcapsules containing maltodextrin and xanthan gum, dried by spray drying. The parameter a* decreased with storage time and the parameters b* and L* increased during storage for all samples, due to degradation of the red color of betanins and the resulting formation of new compounds that were a lighter yellow color. Finally, we conclude that microencapsulation by freeze and spray drying using maltodextrin and xanthan gum as encapsulating agents, may be suitable for use as a color stabilizer for red beet extracts.

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