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Beetroot Extract Encapsulated in Inulin: Storage Stability and Incorporation in Sorbet

Jéssica Mayuri Omae, Priscila Akie Goto, Letícia Misturini Rodrigues, Suelen Siqueira dos Santos, Carolina Moser Paraiso, Grasiele Scaramal Madrona, Rita de Cássia Bergamasco*

Department of Food Engineering, State University of Maringa, Colombo Avenue, 5790, Bl 13, Maringa, Parana, Brazil
ritabergamasco@bol.com.br

Betacyanin is a natural colorant extracted, principally, by beetroot, being a good alternative to substitute synthetic colorant, because it not shows toxic effect and established daily intake rate. However, betacyanin is instable to conditions of heat, light and oxygen. The objective of this study was to encapsulate beet root extract, by spray drying, using inulin as encapsulating agent. The study proposed also, the incorporation of colorant microcapsules in sorbet. Solutions of microcapsules (10%) and beet root extract (control) were prepared and storage at 4° and 25°C, and sob influence of light and absence of light. The stability of colorant was determined by betacyanin quantification, by spectrophotometry. Sorbet was prepared with incorporation of microcapsules and beet root extract (control), and color stability of sorbet was evaluated during 6 months of storage at -18°C. Encapsulation efficiency was 58.4%. A linear relationship was observed from the plots of the betacyanin content versus time, implying first-order reaction kinetics for beetroot extract and microcapsules degradation. For all conditions evaluates, it was observed that the highest rate constant was obtained by microcapsules solutions, i.e., microencapsulation was not effective to protect the beet root extract. However, color stability of sorbet prepared with beet root microcapsules was higher than compared to sorbet prepared with beet root extract. All the samples of sorbet had good acceptance in the sensory analysis, with acceptance index higher than 80%.

1. Introduction

Color is an important feature in food, and of great influence on its acceptability. Generally, in the food industry artificial colorants are used in the products, however there is great concern as to their applicability, as these colorant can cause harmful effects to human health (Azeredo, 2009). Thus, the search for natural colorant to replace artificial in food has increased every year.

Beet is a natural source of red dye, and its main pigment is betacyanin. Beet extract is widely used in the food industry in products such as yogurts, jellies, sweets. However, its applicability is limited because of its low stability when exposed to light and heat (Serris and Biliaderis, 2001).

One way to improve the stability of natural colorant 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). Encapsulation is a technique in which the bioactive compound, pigments or other component are trapped in an encapsulating matrix, which can protect it from adverse ambient conditions such as light, heat and oxygen, thus increasing its stability (Saénz et al., 2009).

Inulin is a fructooligosaccharide (FOS) obtained from vegetable sources, being extracted mainly from chicory. It is an interesting encapsulating agent, as it shows prebiotic effect, is a dietary fiber, and improves the bioavailability of calcium. In addition, products supplemented with inulin have low glycemic indexes (Robert et al., 2012).

Saénz et al. (2009) used inulin and maltodextrin as encapsulating agents of betalain extracted from cactus pear (*Opuntia fícus-indica*), and observed that inulin-encapsulated dye presented better performance when compared to maltodextrin when analyzing the stability of the dye during the storage.
Thus, considering the growing concern of consumers with regard to health and processed foods, the objective of this study was to encapsulate beet root extract, by spray drying, using inulin as encapsulating agent. The study proposed also, the incorporation of colorant microcapsules in a food matrix (sorbet).

2. Materials and Methods

Beetroot vegetable and the ingredients used in the formulation of sorbet were purchased from a local market. Inulin was obtained from Clariant (São Paulo, Brazil). All other reagents were of analytical grade.

2.1. Extraction of beetroot colorant and betacyanin quantification

Red beetroot were washed and sanitized with chlorinated water (100 ppm, 8 minutes). Then they were crushed and mixed with water, at 60 °C, in a ratio of 1:10 (beetroot mass: volume of water). This solution was stirred for 10 s on a shaker, then centrifuged at 6000 rpm for 10min.
The supernatant was collected and the same procedure was repeated two more times to ensure maximum extraction. Quantification of betacyanin was performed by the spectrophotometric method according Stintzing et al. (2005).

2.2. Extract encapsulation

An amount of 15 g of inulin was dissolved in 500 ml of distilled water. Then, 75 mL of 1:10 beet extract was added to the solution, this solution was dried in a spray dryer at 120 ºC, air flow of 600 L/h, feed rate of 10 mL min and pressure of 20 psi.
The collected powder was stored in a glass bottle packed in aluminum foil, under refrigeration (4 °C) for further analysis (Saenz et al., 2009).

2.3. Microcapsules: extraction and quantification of the betacyanin

The extraction and quantification of the betacyanin from the microcapsules was performed according to the methodology described by Saenz et al. (2009).

2.4. Process yield and encapsulation efficiency

The yield of the process of encapsulation of the betacyanin in inulin by spray drying was calculated as a function of the mass of total solids in solution before drying in spray dryer (g) and the mass of powder obtained after drying in spray dryer (g).The encapsulation efficiency was determined according to the methodology described by Shu et al. (2006).

2.5. Stability of encapsulated betacyanin

A solution of the microcapsules in distilled water (10 %), with addition of 5 % of potassium sorbate, was prepared to avoid microbial contamination. The solution was divided into 10 mL containers, with screw caps. An aqueous solution was also prepared with the beet extract, called control solution. Solutions were stored under different conditions: wrapped in aluminum foil under refrigeration (4 °C); Wrapped in aluminum foil at room temperature (25 °C), in a box at room temperature, and in a box in the presence of two 20 W fluorescent lamps at room temperature. Samples were collected during 24 days for the quantification of the betacyanin according to item 2.3.

2.6. Food application (sorbet)

Sorbet formulation was composed by 70.7% of coconut water (Vita coco®), 17.7% of sugar, 4.3% cream, 3.5% of flavour, 0.7% of stabilizer, 0.7% neutral league, 0.7 % of Hx cream (Hexus®) and 1.7 % of dye. After preliminary tests, two sorbet formulations were developed, one containing the encapsulated dye and one containing the extract.

After, the ingredients were mixed and homogenized, and transferred to an ice cream discontinuous producer for incorporation of air and freezing at -18 ° C for 30 minutes by agitation according Corradini et al. (2014).

2.7. Sorbet: color stability during storage

Sorbet samples were stored under refrigeration at -18 °C during six months (180 days). In pre-established periods of time, instrumental color analysis of sorbet was performed in order to observe the color change (ΔE) between samples during storage according Óbon et al. (2009). For that, a Konica Minolta® CR400 portable colorimeter was used, with an integration sphere and a 3 ° viewing angle, that is, d/3 illumination and D65 illuminant. The system used was CIEL * a * b *. Color difference values (ΔE *) were calculated to study color changes in the samples, data were measured in triplicate.

2.8. Sorbet consumer test

Sensorial analysis of the sorbet samples was performed according to the methodology described by Meilgaard (2006), with 100 untrained consumers of both sexes. The samples were coded from a table of random numbers, arranged in disposable cups and delivered to the tasters.
Each formulation was evaluated, after the production for color, flavor and overall appearance parameters using a nine-point hedonic scale, the extremes being 1-very disagreeable and 9-liked very much, and the acceptance index was also calculated. This work was approved in the ethics committee of the State University of Maringá under number 33456114.5.0000.0104.

2.9. Statistical

Statistical analysis was applied to the consumer test by using analysis of variance (ANOVA) and Tukey averages at the 5% probability level, in the statistical program STATISTICA version 7.0.

3. Results and Discussion

3.1 Results of betalain Stability

In the production of microcapsules, the process yield was 75.3%. Powders obtained by spray drying generally have yield in this value range, due to the adhesiveness and cohesiveness of the particles while interacting with the drying chamber (Behboudi-Jobbehdar et al., 2013).
The encapsulation efficiency was 99.6 %, a result very closed of Saénz et al. (2009), which used cactus pear (*Opuntia ficus-indica*) as a source of betacyanin. The authors obtained an encapsulation efficiency of 99.16-99.56% for betacyanin encapsulated in maltodextrin, and 98.34-99.54% for betacyanin encapsulated in inulin.
The stability of the beetroot microcapsules and extract was evaluated in terms of betacyanin content, during 24 days of storage at 4°C and 25°C, and 17 days of storage under influence of light and absence of light. A linear relationship was observed from the plots of the betacyanin content versus time, implying first-order reaction kinetics for beetroot extract and microcapsules degradation. The rate constant (k) and half-life period ($t_{1/2}$) of microcapsules and extract were determined and are presented in Table 1.

Table 1. Betacyanins degradation rate constant at 4 and 25°C, and under influence of light and absence of light (of 25 ºC) for beetroot (microcapsules and extract).

Sample	At 4°C		At 25°C		Presence of light		Absence of light	
	k x 10^3 (days^{-1})	$t_{1/2}$ (days)	k x 10^3 (days^{-1})	$t_{1/2}$ (days)	k x 10^3 (days^{-1})	$t_{1/2}$ (days)	k x 10^3 (days^{-1})	$t_{1/2}$ (days)
Extract	28.6	24.2	35.1	19.7	53.9	12.8	41.8	16.5
Microcapsules	41.0	16.9	99.9	6.9	159.4	4.3	123.3	5.6

For all conditions evaluated, it is observed that the microcapsules sample had the highest rate constant (around 2 times superior) and, consequently, shorter half-life period.
Literature relates several researches about the use of encapsulating agents to prevent betacyanin degradation (Saénz et al., 2009, Robert et al., 2015, Otálora et al., 2015), however the most of stability studies is realized with powders samples. In the present study the experiment was conducted with microcapsules suspended in aqueous solution, and, consequently, the protection of beetroot extract was reduced.
Cai et al. (1998) studied stability of betacyanin extracted from Amaranthus, in the liquid and solid form, during 10 month of storage at 4 and 25 °C, and they observed that dry extract presented greater storage stability when compared to the liquid extract.
Comparing degradation rate constants of samples exposed to light and in the absence of light, it is observed that there was a reduction of this parameter, when the samples are stored in the absence of light. This fact was also observed when comparing the degradation rate constants obtained from the samples stored at 4 and 25 °C. Factors such as oxygen, temperature and light contribute to the degradation of betacyanin (Azeredo, 2009). In this study it was observed that the lower degradation rate constants were obtained for samples stored at 4 °C and in the darkness, being less expressive for the samples stored at 4 °C. This corroborate with data from literature (Azeredo, 2009), where the degradation of betacyanin is more influenced by the temperature factor. For samples contained microcapsules it was observed that half-life period of the samples

stored at 4 °C is more than twice the half-life period of the samples stored at 25 °C, which is not observed for the control sample.

3.2 Sorbet stability and consumer´s acceptance

Sorbets with beet root extract and microcapsules kept under -18 ºC were evaluated for color stability, and Figure 1 presented results in terms of total color difference.

It can be observed (Figure 1) that there was no significant change in sorbet color in the first 15 days of storage. However, after that period, the sorbet made with beet extract had its color changed, while the sorbet made with microcapsules kept its color until 150 days of storage. With 180 days of storage, there was a significant variation in the color of the sorbet containing the microcapsules, but even so, this variation was lower when compared to the sorbet made with extract.

Is important highlight that although the microcapsule stability study (Table 1) has shown that inulin is not so effective in the microencapsulation of beet extract, when the microcapsule was added to the sorbet, certain color stability is observed during 150 days of storage. This fact complements what has already been explained previously, that the microcapsules in powder form (sorbet) are more stable than in liquid form.

Óbon et al. (2009) describe that the color difference can be described by the total distance between two colors in the three-dimensional CIELab color space (ΔE), and that ΔE values between 1.5 and 5 are not very visually different, while values above 5 indicate that the color difference is evidently high, the author evaluated the addition of powdered dye obtained from Opuntiastricta fruits in yogurt and soft drink during 30 days.

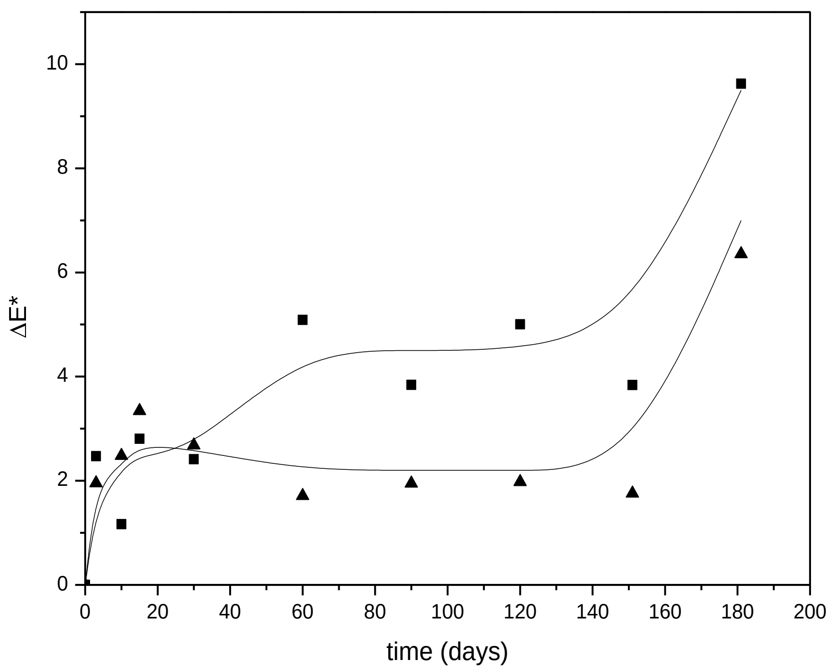

Figure 1. Total color difference of sorbet elaborated with beetroot extract (filled square) and beetroot microcapsules (filled triangle), during 180 days of storage at -18 °C.

Results of consumer’s test are presented in Table 2 for sorbet with beetroot extract and microcapsules, for all samples the acceptance index was higher than 80%. No difference was observed in flavor and overall appearance for sorbets, and both obtained scored above 7, which corresponds to “I enjoyed regularly”.

In the color attribute there was a significant difference ($p<0.05$) between the formulations, and sorbet with beetroot extract had the highest score. This difference can be justified by the fact that the extract was pure and added in greater proportion in the sorbet, when compared to the microcapsules (that was composed by inulin and extract), presenting a more intense coloration and, consequently, pleasing the consumer`s.

Table 2.Results of consumer's test for sorbet with beetroot extract and microcapsules

Sorbet Sample	Color	Flavor	Overall appearance
Extract	7.8ª	7.3ª	7.5ª
Microcapsules	7.1ᵇ	7.2ª	7.3ª

*Equal letters in the same column represent statistically equal results by the Tukey test ($p < 0.05$).

Literature presents the application of microcapsules in many different foods, as dairy products, fruit juices, cereals, and bakery products (Tolve et al., 2016), however the use in sorbet has not been reported yet, and may this product can represent an interesting product.

Comparing our results with data closer to the literature, is important to highlight a study which applying the microencapsulated phenolic for enrichment of the functional properties of regular ice cream, they observed that the addition of pomegranate peel at 0.5 and 1.0% showed significant improvement of the antioxidant activity, and also presented scores between 7.1 and 7.3 in the consumers test (Çam et al., 2014), being this product widely accepted, as well our studied sorbet (scores 7.1 to 7.8, Table 2).

4. Conclusion

The spray drying microencapsulation technique proved to be efficient in the encapsulation of the natural dye obtained from the beet extract, presenting good applicability in the evaluated food product and greater durability than the extract.

Inulin used as a betacyanin encapsulating agent showed high efficiency of microencapsulation, although it did not present satisfactory storage stability results under different ambient conditions (light and temperature). However, the application of the microcapsules in sorbet contributed to a higher color stability of the product during storage (at -18 ºC) when compared to the formulation using the dye as an extract.

Finally, in the sorbet consumer test, both samples had a good acceptance rate, with values higher than 80%. These results show that the encapsulation of beet extract in inulin is a good alternative for use in formulations of sorbet.

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