

**Microencapsulation of beet dye (Beta vulgaris L.) using maltodextrin and xanthan gum
as encapsulant agents and application in yogurt**

**Microencapsulação do corante de beterraba (Beta vulgaris L.) usando maltodextrina e
goma xantana como agentes encapsulantes e aplicação em iogurte**

**Microencapsulación de colorante de remolacha (Beta vulgaris L.) utilizando
maltodextrina y goma xantana como agentes encapsulantes y aplicación en yogur**

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Abstract

Beet is a vegetable rich in antioxidant activity and phenolic compounds, besides being used as a natural dye, which has as a disadvantage the instability in relation to several factors, such as temperature, pH, oxygen and light. Therefore, this work aimed to microencapsulate the beet dye, evaluate the stability of powders and their application in yogurt. Beet extract was encapsulated in a combination of maltodextrin and xanthan gum, with subsequent drying in spray dryer or lyophilizer. In the powders obtained, color parameters, betanin content,

phenolic compounds content, yield, microcapsule morphology and powder storage stability were evaluated for 40 days, with light, temperature and oxygen variation. The samples with the best results were applied in yogurt and the products were evaluated sensorially. The performance of powders dried by spray dryer was approximately 43% lower than lyophilized, with no variation in moisture content and soluble solids. Morphologically the samples dried by spray dryer were different from those dried by lyophilization. The content of phenolic compounds in the powders showed an increase after 40 days of storage in all samples, while the content of betanin showed a considerable drop in the first 7 days, followed by stability up to 40 days. With respect to color the parameter a^* , was stable in storage, and the samples dried by spray dryer showed a higher value than the samples dried by lyophilization. The samples lyophilized with maltodextrin and with xanthan gum were added to the yoghurt, both in concentrations 0.5 and 1%. There was no significant difference between the yogurt samples prepared with the powders both in the sensory analysis and in the physical-chemical analysis, except for the ashes. Therefore, all the microcapsules evaluated presented good encapsulation efficiency and good stability during the 40 days evaluated.

Keywords: Betanin; Spray-dryer; Lyophilization, *Beta vulgaris* L.; Phenolic compounds; Colorimetry.

Resumo

A beterraba é um vegetal rico em atividade antioxidante e compostos fenólicos, além de ser utilizada como corante natural, o qual tem como desvantagem a instabilidade frente a fatores diversos, como temperatura, pH, oxigênio e luz. Sendo assim, este trabalho teve como objetivo microencapsular o corante de beterraba, avaliar a estabilidade dos pós e sua aplicação em iogurte. O extrato de beterraba foi encapsulado em uma combinação de maltodextrina e goma xantana, com posterior secagem em spray dryer ou em liofilizador. Nos pós obtidos, avaliou-se parâmetros de cor, teor de betanina, teor de compostos fenólicos, rendimento, morfologia das microcápsulas e estabilidade ao armazenamento dos pós por 40 dias, com variação de luz, temperatura e oxigênio. As amostras com melhores resultados foram aplicadas em iogurte e os produtos avaliados sensorialmente. O rendimento dos pós secos por spray dryer foi aproximadamente 43% menor que o liofilizado, sem variação do teor de umidade e de sólidos solúveis. Morfologicamente as amostras secas em spray dryer se apresentaram diferentes das secas por liofilização. O teor de compostos fenólicos dos pós apresentou aumento após 40 dias de armazenamento em todas as amostras, enquanto o teor de betanina apresentou uma queda considerável nos primeiros 7 dias, seguida de estabilidade até

40 dias. Com relação a cor o parâmetro a^* , foi estável na estocagem, sendo que as amostras secas por spray dryer apresentaram um valor maior que as amostras secas por liofilização. As amostras liofilizadas com maltodextrina e com goma xantana foram adicionadas nos iogurtes, ambas nas concentrações 0,5 e 1%. Não houve diferença significativa entre as amostras de iogurte preparadas com os pós tanto na análise sensorial quanto nas análises físico-químicas, com exceção das cinzas. Portanto, todas as microcápsulas avaliadas apresentaram boa eficiência de encapsulação e boa estabilidade durante os 40 dias avaliados.

Palavras-chave: Betanina; Spray-dryer; Liofilização, Beta vulgaris L.; Compostos fenólicos; Colorimetria.

Resumen

La remolacha es una verdura rica en actividad antioxidante y compuestos fenólicos, además de ser utilizada como colorante natural, lo que tiene el inconveniente de la inestabilidad ante diversos factores, como la temperatura, el pH, el oxígeno y la luz. Por ello, este trabajo tuvo como objetivo microencapsular el tinte de remolacha, evaluar la estabilidad de los polvos y su aplicación en yogur. El extracto de remolacha se encapsuló en una combinación de maltodextrina y goma de xantano, con posterior secado en un secador por atomización o liofilizador. En los polvos obtenidos se evaluaron parámetros de color, contenido de betanina, contenido de compuestos fenólicos, rendimiento, morfología de microcápsulas y estabilidad al almacenamiento de polvos durante 40 días, con variación de luz, temperatura y oxígeno. Las muestras con mejores resultados se aplicaron en yogur y los productos se evaluaron sensorialmente. El rendimiento del secador por pulverización de polvo seco fue aproximadamente un 43% menor que el del liofilizado sin variación del contenido de humedad y sólidos solubles. Muestras Morfológicamente secos en un pulverizador secador realizan de diferente seca por liofilización. El contenido de compuestos fenólicos en los polvos aumentó después de 40 días de almacenamiento en todas las muestras, mientras que el contenido de betaninamostró una caída considerable en los primeros 7 días, seguida de estabilidad hasta los 40 días. Con respecto al color parámetro a^* era estable en el almacenamiento, y las muestras secadas por pulverización secador mostró un valor más alto que las muestras secadas por liofilización. Las muestras liofilizadas con maltodextrina y goma xantana se agregaron a los yogures, tanto en concentraciones de 0.5 como de 1%. No hubo diferencia significativa entre las muestras de yogur preparadas con los polvos tanto en el análisis sensorial como en los análisis físico-químicos, a excepción de las cenizas. Por tanto,

todas las microcápsulas evaluadas mostraron buena eficiencia de encapsulación y buena estabilidad durante los 40 días evaluados.

Palabras clave: Betanina; Spray-secado; Liofilización, *Beta vulgaris* L.; Compuestos fenólicos; Colorimetría.

1. Introduction

Stable colors are value attributes in food, especially when used in processed foods (Kumar et al., 2015). In this sense, the food industries make use of a large amount of synthetic dyes in order to retain the appearance of the original material and make the products more attractive, however, the effluents discarded by the factories are considered an important source of pollution of water bodies (Sá & Nunes, 2017). In addition, consumers are increasingly concerned about potential health effects due to food allergies and intolerances (Kumar et al., 2015). Thus, the need for studies to evaluate the stability and application of natural colorants in processed foods has increased.

Beet is a vegetable known for its antioxidant properties, is rich in phenolic acids and vitamins. In addition, beet can be used as a natural colorant due to its water-soluble pigments, known as betalains (Ozaki et al., 2021). These pigments, also classified as betacyanins, give the product a red-violet color, and betaxanthins, responsible for the orange-yellow color. Betaxanthins are present in beets in a lower proportion than betacyanins, with betanin being considered the main one in this class (Nemzer et al. 2011; Pitalua et al. 2010).

The stability of betanin depends directly on the pH (which presents an optimal value between 4 and 5), presence of light, oxygen and temperature, as these factors, when not controlled, can lead to oxidation and degradation of the compound (Huang & Von Elbe, 1987).

Therefore, an alternative to improve the stability of natural food colorants would be the use of microencapsulation, a physical process that traps food particles through a film formed by an encapsulating agent, so that it creates a physical barrier between the material and the core protecting from oxygen, water or other conditions (Ravichandran et al., 2014).

Different encapsulating agents have been used in food microencapsulation to improve encapsulation efficiency, stability and controlled release (Mar et al., 2020). Thus, polysaccharides, lipids and proteins can be used as encapsulating agents in the process of microencapsulation, being maltodextrins in different degrees of dextrose equivalent or when combined with gums, the most used (Antigo et al., 2020). Ravichandran et al. (2014)

observed that the association of maltodextrin and xanthan gum in the microencapsulation of beet betalain improved the stability and a 65% increase in the betalain content of lyophilized microcapsules and 21% in the betalain content of spray-dried microcapsules than when compared to encapsulation with maltodextrin alone.

The microencapsulation technology can be performed by different methods, atomization and lyophilization being the most used. Atomization, also known as spraying or spray drying, consists of a technique that, in addition to removing water, microencapsulates substances susceptible to rapid changes in environmental conditions, for this reason, the products obtained can retain beneficial properties for the human body (Tomczyk et al., 2020).

On the other hand, freeze-drying is a drying technique in which an aqueous solution is frozen and then dried by sublimation under vacuum, with the ice change taking place directly from the solid in steam, without passing through a liquid phase (Kumar et al., 2011).

In view of this, the objective of this work was to encapsulate the natural beet dye in a combination of maltodextrin and xanthan gum, from the freeze-drying or atomization techniques; besides evaluating the stability of the powders obtained in terms of temperature, presence of light and oxygen and applying them in yogurt.

2. Methodology

2.1 *Materials*

The beets were always selected from the same lot and purchased in local commerce in the city of Maringá, Brazil (23° 25' 31" S/ 51° 56' 19" W) in 2015. The maltodextrin (DE10) and xanthan gum were ceded, respectively, by the companies Cargill (Campinas, Brazil) and Doce Aroma (São Paulo, Brazil). All the reagents used were of analytical grade.

2.2 *Extraction of betanin and processing of microcapsules*

The beets were selected, washed, sanitized (200 ppm of active chlorine) and sliced. Beet extract was extracted in centrifuge (Mondial, Turbo Juicer CF-06, Brazil) and filtered on filter paper.

The encapsulating agents were added so that the total soluble solids content in the solution was 30% (w/v). Two solutions with encapsulating agents were evaluated:

maltodextrin and maltodextrin with xanthan gum (0.5%). Both were submitted to atomization processes (spray drying) and lyophilization.

Spray drying microencapsulation followed the methodology described by Valduga et al. (2008). The solutions were dried in a mini spray dryer (LM, model MSD 1.0, Brazil) being the operational conditions of drying: temperature of the drying air inlet 150°C and outlet 90°C; atomization pressure: 0.08 to 0.14 bar; average drying air flow rate of 3.8 m³/h; average feed rate of 0.6L/h.

In freeze-drying microencapsulation the samples were frozen for 48 hours at -10°C. Subsequently, the lyophilization process took place for 2 days at -36°C to ensure complete drying of the product (Liobras, lyophilizer L108, Brazil).

The final products of the lyophilizer and spray dryer, in dry powder form, were stored in nylon plastic packaging with thermo-weldable polyethylene for later use.

The powders obtained were named as follows: spray drying powder (MS), powder containing maltodextrin as an encapsulating agent and spray drying powder (MAS), powder containing maltodextrin and xanthan gum, and spray drying powder (MXS), freeze-drying powder (ML), powder containing maltodextrin as an encapsulating agent and freeze-drying powder (MAL), powder containing maltodextrin and xanthan gum, and freeze-drying powder (MXL).

2.3 Total soluble solids content, water activity (aw) and process yield

The content of soluble solids in liquid extract (initial) and yogurt was measured using a refractometer Model RX-5000. The determination of the water activity of the powders was performed in triplicate, using the apparatus Aqua Lab Model Cx2T, operating at a temperature of 25.0 ± 0.3°C. The yield of the microencapsulation process was calculated using equation 01.

$$\text{Process yield (\%)} = (\text{actual quantity of samples} / \text{theoretical quantity of samples}) \times 100$$

Eq. (1)

2.4 Morphology of powders

The morphology of beet extract microcapsules was determined by scanning electron microscopy (SEM) (Shimadzu, Super Scanning Electronic Microscope SS-550, Thailand).

The samples were coated in gold and placed on a support, the images of which were obtained at a 200-fold magnification for freeze-dried samples and 2000-fold for spray-dried samples.

2.5 Stability of powders

To evaluate the stability of the powders, the four samples were stored under different conditions: under refrigeration at 4°C (MASa, MXSa, MALa and MXLa); at 30°C packaged in aluminum foil (MASb, MXSb, MALb and MXLb), at 30°C inside a cardboard box, exposed to 20W fluorescent lamps (MASc, MXSc, MALc and MXLc) and at 30°C packaged in aluminum foil and vacuum (MASd, MXSd, MALd, MXLd). All samples were temperature controlled in a BOD (Biochemical oxygen demand) chamber. The quantification of betanin, total phenolic and color compounds were evaluated in triplicate on days 0, 1, 4, 8, 14, 21, 29 and 40.

2.6 Extraction and quantification of betanin from microcapsules

The methodology for extraction of betanins from microcapsules was according to Saenz et al. (2009). One hundred milligrams of the microcapsules were dispersed in 1 mL of ethanol solution: acetic acid: water (50:8:42). This dispersion was shaken in Vortex, for 1 minute. The supernatant was centrifuged for 5 min, and then filtered. This solution was used for analysis of phenolic compounds and quantification of betanine.

The betanin content in the microcapsules was determined according to the methodology of Stintzing et al. (2005).

To evaluate the kinetics of the reaction during dye storage in the parameters light temperature and oxygen, it was chosen to test kinetic models of 1st order. Previous researches showed that the degradation of natural dyes such as betanine follows a first order kinetics during storage (Serriset al., 2001; Bustos-Garza et al., 2013; Cano-Higueta et al, 2015). For this purpose, color degradation can be calculated using equation 2.

$$\frac{dC}{dt} = -k.C \quad (2)$$

In what: C is the remaining concentration; C₀ is the initial concentration; t is the time interval between C₀ and C; k is the 1st order (1/time) transformation constant.

In a plot of concentration (-ln C) versus time, the transformation constant (k) is simply the inclination of the line.

The half-life ($t_{1/2}$) for the reaction is the time required for the amount of betanin to fall by half its initial value. It is directly related with the constant of the speed for a reaction of first order that, according to Kirca et al. (2003), is described by equation 3.

$$t_{1/2} = \frac{\ln(2)}{k} t_{1/2} = \frac{\ln(2)}{k} \quad (3)$$

2.7 Analysis of total phenolic compounds

The total phenolic content was determined following the Folin-Ciocalteu method, described by Ruiz-Gutiérrez et al. (2014). The measurements were performed in triplicate using a calibration curve with gallic acid as standard. The readings were performed in spectrophotometer (Femto, model 700S, Brazil) with a wavelength of 765nm.

2.8 Color analysis

The color of powders and yogurt was evaluated by means of a portable colorimeter (Konica Minolta®, CR400, Japan) with integration sphere and 30° viewing angle. The system used was CIEL*a*b*. The color variation (ΔE) was calculated for yogurt according to equation 4:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

2.9 Application of the dye in yogurt

In the processing of yoghurts, Parmalat brand UHT whole milk was heated to approximately 45°C, added sugar (10% w/v) and inoculated with 0.04% commercial yeast (Christian Hansen®), containing cultures of *Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp. *Lactise S. thermophilus*. Fermentation was performed for approximately 4.5 hours in a BOD chamber, until the milk reached pH 4.6.

After fermentation, the dough was cooled to a temperature of approximately 4°C and then the dough was broken together with the addition of 5% strawberry pulp (North Pulp) and 0.01% strawberry flavoring identical to natural (Two Wheels).

In preliminary tests, it was observed that lyophilized dyes showed greater stability in different buffers, so in the application in yogurt only MAL and MXL powders were used. A

batch of yogurt was produced and separated into 4 samples, one containing the MAL dye and another containing the MXL dye, both at a concentration of 0.5% (w/v) and 1.0%.

The sensory evaluation of the yogurt samples was carried out soon after production, by 80 (eighty) untrained tasters who were university students and public employees of both sexes and aged between 17 and 65 years, 62.5% were women and the average age of the tasters was 21 years. The tests were conducted in the Sensory Laboratory of the Food Engineering course of the State University of Maringá (UEM). The taster was provided with yogurt samples (randomly coded) in a sequential manner, in a portion of 25 to 30g of sample. For tasters' evaluation, a 9-point structured hedonic scale was used (1 = I liked it very much, 9 = I liked it very much) for the attributes appearance, aroma, flavor, texture and overall acceptance, and on a 3-point scale the intention to buy (3= I would certainly buy, 1= I would certainly not buy). The data were evaluated by ANOVA and Tukey's test ($p < 0.05$), with the help of the Sisvar program.

Yogurt samples were also evaluated for physical-chemical analysis and storage stability, under refrigeration at 4°C. For the physicochemical analyses, a sample formulated with the beet liquid extract (2% v/v) was also prepared as dye.

2.10 Physical-chemical analysis of yogurt

The method described by the Adolf Lutz Institute (IAL, 1985) was used for the analysis of the pH and titratable acidity of yogurt.

The humidity of the yogurt was done by gravimetry, drying the sample in an oven at 105°C, until constant weight (IAL, 1985). The ash content of the yogurt was determined by gravimetry, according to the methods described by the Adolf Lutz Institute (IAL, 1985).

3. Results and Discussion

3.1 Characterization of the powders obtained

The spray drying powders showed lower yield when compared to the freeze-drying powders (Table 1). This fact may be associated with the chemical composition of both extract and maltodextrin which, due to their high sugar content (fructose and glucose), caused greater adhesion by caramelization of sugars and agglomeration of encapsulated material on the walls of the spray dryer chamber (Valduga et al., 2008).

Table 1. Yield, encapsulation efficiency, water activity and moisture of spray dryer and lyophilizer powders, using maltodextrin and xanthan gum as encapsulating agents.

Parameters**	Sample*			
	MS	MXS	ML	MXL
Process yield (%)	62,58	48,04	98,87	99,06
Encapsulation efficiency (%)	64,70	71,79	42,19	43,45
Water activity	0,252 ^a ± 0,01	0,315 ^a ± 0,00	0,219 ^a ± 0,01	0,226 ^a ± 0,00
Humidity (%)	1,87 ^a ± 0,28	4,84 ^b ± 0,10	4,78 ^b ± 0,24	4,95 ^b ± 0,17

*Sample: spray drying (MS) microcapsules, powder containing maltodextrin and xanthan gum, and spray drying (MXS) microcapsules, freeze-drying (ML) microcapsules, powder containing maltodextrin and xanthan gum, and freeze-drying (MXL) microcapsules.

**Averages ± standard deviation on the same line accompanied by different letters are significantly different ($p < 0.05$).

Source: Authors, 2020.

The encapsulation efficiency was calculated taking into account the amount of betacyanine present in the solution before drying, and in the powder after drying. According to Table 1, the samples dried in spray dryer showed better results of encapsulated dye, when compared to the samples dried by lyophilization. An unexpected result, since freeze-drying is a recommended drying technique for high temperature sensitive compounds, such as dyes. A possible explanation for this fact is that the homogenization of the encapsulating agents in beet extract was not enough for a complete interaction between the components, and part of the dye may have sublimated together with the water at the time of lyophilization, resulting in a lower encapsulation efficiency.

The combination of maltodextrin with xanthan gum resulted in a higher encapsulation efficiency of betacyanin, in the case of microencapsulation by spray drying. The use of gums associated with maltodextrin has given good results of microencapsulation in spray drying because the gum has a film forming capacity, a characteristic that maltodextrin does not have, resulting in a good combination of encapsulating agents (Ravichandran et al., 2014; Janiszewska, 2014).

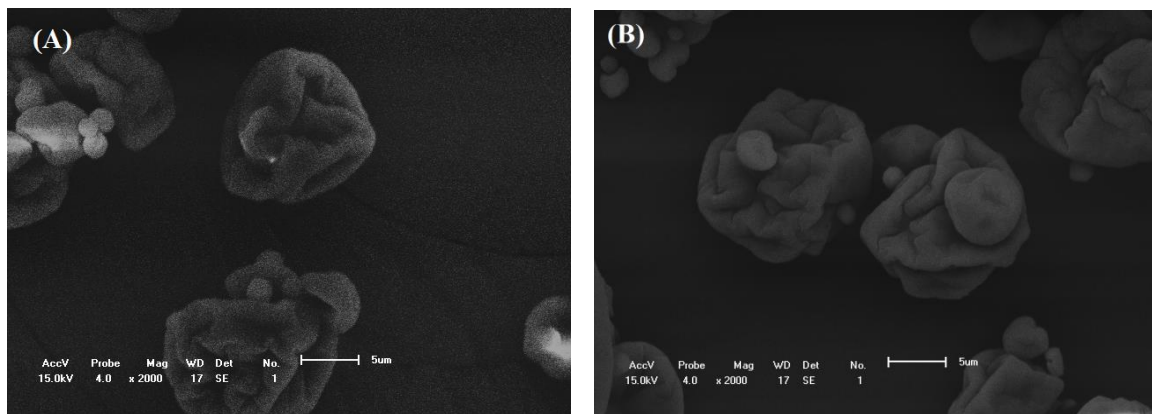
The samples showed no significant difference in relation to water activity (Table 1), and the values found are below those reported in the literature, for example, according to Pitalua et al. (2010), water activity below 0.521 does not contribute to significant color changes in spray dryer microcapsules.

Regarding the moisture content, only the MS sample showed a significant difference compared to the other samples. This is probably due to the fact that maltodextrin has lower hygroscopicity than xanthan gum, resulting in a lower moisture content (Cai & Corke, 2000; García-Ochoa et al., 1999).

3.2 Scanning electron microscopy

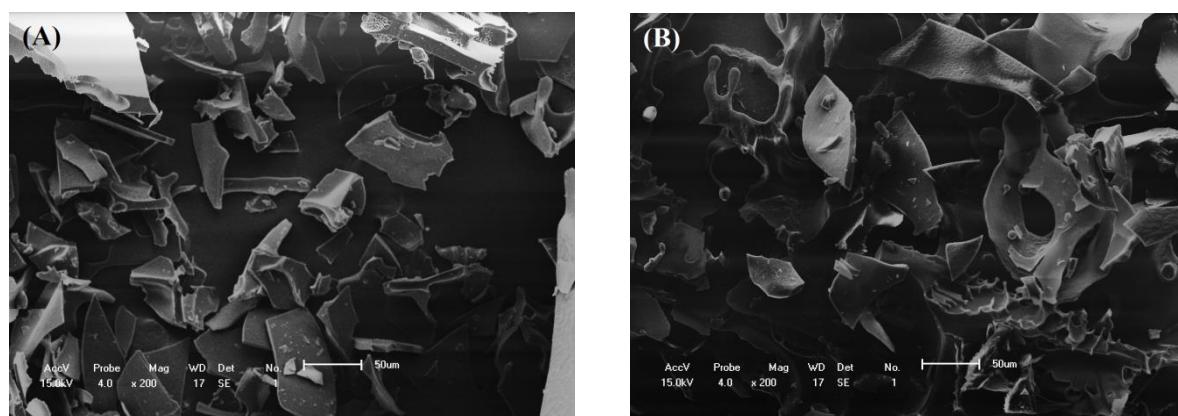
The morphological characterization of the samples was performed by scanning electron microscopy (SEM), and the results are shown in Figures 1 and 2, for spray drying and freeze-drying microcapsules, respectively.

Figure 1. Scanning electron microscopy of microencapsulated beet extract by spray dryer with maltodextrin (A), and microencapsulated beet extract with maltodextrin and xanthan gum (B).



Source: Authors.

Figure 2. Scanning electron microscopy of microencapsulated beet extract in maltodextrin (A) microencapsulated beet extract in maltodextrin and xantham gum, dried by lyophilization.



Source: Authors.

The spray drying microcapsules (Figure 1), regardless of the encapsulating agents, have a spherical shape with many wrinkles, which are formed on the surface of the particles, according to the literature due to shrinkage during the drying process (Janiszewska, 2014). No microcapsules with ruptures have been observed, which is a positive point, since the rupture favors the release of the microencapsulated dye.

The freeze-drying method used had an influence on the structure formed after drying. It is observed in Figure 2 that the formed product presents irregular shape and of different sizes, result of the maceration of the mass obtained after drying, becoming a powder. Similar results were observed by Sánchez et al (2006), who microencapsulated betalain from *Opuntia lasiacantha*, using maltodextrin as an encapsulating agent.

3.3 Betanin content

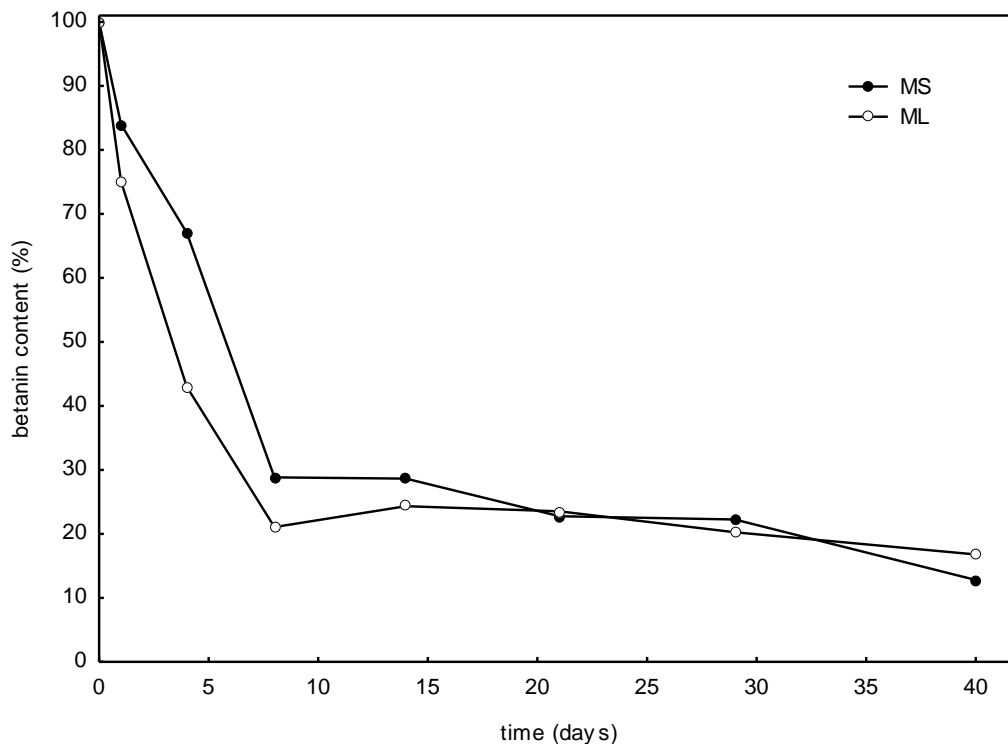
The dye microcapsules were tested for stability under different storage conditions. Figure 3 shows the degradation curve of the maltodextrin microcapsules, and spray dried and lyophilized, for samples stored at 30°C, without the presence of light. In general, all microcapsules showed similar degradation behavior, with a steeper curve in the first week of storage followed by a reduction in the degradation rate. Freeze dried samples showed a greater reduction in betanin content in the first week of storage, followed by a stabilization in dye content in the microcapsule after this period. The spray dryer samples showed a lower

loss in the betanin content, when compared to the lyophilized samples, however, the dye degradation remained until the 40 days of analysis, but at a lower rate.

This difference in the degradation behaviour of the betanin of the microcapsules in relation to the drying method is related to the shape of the final product formed. The freeze-drying technique produces a material of irregular shape, as can be observed in Figure 2, leaving the dye more exposed to the external environment, accelerating its degradation. The spray drying technique produces matrix type microcapsules (Figure 1), offering a greater protection to the dye. The accelerated degradation observed in the first week of storage is due to the dye stored on the surface of the microcapsule and, after this period, a slower degradation of betanin is observed, which occurs as the dye is released from the microcapsule. It is interesting to note that although the lyophilized sample does not present a microcapsule characteristic, a betanin content of approximately 20% still remains in the product until the end of the analysis period (40 days).

Based on the data of the betanin degradation curve of the microcapsules, a first order kinetic model was adjusted, for a period of 15 days of storage, and the adjusted parameters (degradation constant (k) and half-life ($t_{1/2}$)) are presented in Table 3.

Figure 2. Degradation curve of betanin from spray drying (MS) and lyophilization (ML) microcapsules.



Source: Authors.

Table 2. Parameters of the degradation kinetics (first order) of encapsulated betanine, and exposed under different storage conditions.

	Storage condition	$k \times 10^3$ (days ⁻¹)	$t_{1/2}$ (days)	R^2
Temperature**				
Samples*				
MS	4°C	108.30 ^a ± 0.54	6.40	0.9403
	30°C	117.42 ^b ± 2.87	5.90	0.9430
MXS	4°C	111.70 ^a ± 3.51	6.20	0.9539
	30°C	118.64 ^b ± 1.50	5.84	0.9503
MAL	4°C	154.89 ^d ± 0.08	4.47	0.9099
	30°C	186.60 ^c ± 0.07	3.71	0.9196
MXL	4°C	171.13 ^e ± 0.44	4.05	0.8796
	30°C	183.44 ^c ± 0.21	3.78	0.9146
LIGHT				
MS	Light	136.67 ^b ± 2.48	5.07	0.9165
	darkness	117.42 ^a ± 2.87	5.90	0.9430
MXS	Light	139.56 ^b ± 3.55	4.96	0.9005
	darkness	118.64 ^a ± 1.50	5.84	0.9503
MAL	Light	151.89 ^d ± 1.55	4.56	0.9349
	darkness	186.60 ^e ± 0.07	3.71	0.9196
MXL	Light	165.01 ^c ± 2.55	4.20	0.9267
	darkness	171.13 ^c ± 0.44	4.05	0.8796
VACUUM				
MS	Air	117.43 ^a ± 2.87	5.90	0.9430
	vacuum	133.29 ^d ± 1.18	5.20	0.9339
MXS	Air	118.64 ^a ± 1.50	5.84	0.9503
	vacuum	138.78 ^e ± 2.41	4.99	0.9367
MAL	Air	186.60 ^c ± 0.07	3.71	0.9196
	vacuum	171.98 ^b ± 1.98	4.03	0.9285
MXL	Air	171.13 ^b ± 0.44	4.05	0.8796
	vacuum	190.20 ^c ± 0.09	3.64	0.9432

*Sample: spray drying (MS) microcapsules, powder containing maltodextrin and xanthan gum, and spray drying (MXS) microcapsules, freeze-drying (MAL) microcapsules, powder containing maltodextrin and xanthan gum, and freeze-drying (MXL) microcapsules.

**Averages ± standard deviation on the same line accompanied by different letters are significantly different ($p < 0.05$).

Source: Authors, (2020).

The data obtained from the kinetics of microencapsulated dye degradation were well adjusted to a first order kinetic model, as observed in the R² values of the curves (> 0.87) (Table 2).

In general, it is observed that the samples dried by spray drying had the lowest degradation constants, and consequently, the longest half-life. Freeze dried products generally do not present the shape of a microcapsule, as can be seen in figure 2, exposing the dye more easily to the external environment. The opposite can be seen in spray drying products (Figure 1), which present a matrix type microcapsule, contributing to a lower degradation of the dye.

Regarding the encapsulating agents, no significant difference was observed in the degradation constants with the addition of xanthan gum in the formulation, for spray drying products. For lyophilized products, the presence of xanthan gum in the formulation of microcapsules did not contribute to the protection of the dye, being the samples with higher degradation constants. A surprising fact, because the xantana gum generally can act as a film on the microcapsule, thus increasing the shelf life of the microcapsule. This fact has been observed when using the combination of maltodextrin and acacia gum in the encapsulation of dyes by spray drying (Ancient et al., 2020). Probably xantham gum, in the concentration used, may have been the cause for the low half-life.

With respect to storage conditions, an increase in storage temperature resulted in an increase in the degradation constant. Temperature is a relevant parameter in the stability of natural dyes, and betanin has low stability at high temperature, causing its degradation. The longer half-life was observed for the microencapsulated dye in maltodextrin, by spray drying, and stored at 4°C, of 6.40 days, demonstrating that the temperature has a greater influence on the stability of the dye, when compared to the parameters light and vacuum.

The presence of light also accelerates the degradation of the dye, the degradation constant being greater for samples stored in the presence of light.

For vacuum storage, an interesting result was found, the greatest degradation constants were found in the samples stored under vacuum. An unusual result, since the presence of oxygen can also accelerate the degradation of the dye. The shortest half-life was observed for the dye microencapsulated in xanthan gum and maltodextrin, by lyophilization, and stored under vacuum.

3.4 Phenolic compounds

Table 3 shows the content of phenolic compounds present in the microcapsules, stored for 30 days, under controlled conditions of temperature, light and oxygen. In general, the samples showed increases and reductions in the content of phenolic compounds, during the 30 days of storage, in all conditions evaluated. One explanation for this fact is that betacyanine, when degraded, forms some phenolic compounds, changing their content in microcapsules (Castro, 2017).

The spray drying samples showed a higher content of phenolic compounds, after 30 days of storage, when compared to freeze-dried samples, which may be related to the higher degradation of betacyanine. In both types of drying it was observed that the content of phenolic compounds (table 3) increased for all samples during 40 days, and this shows that the samples exposed to temperature, incidence of light and presence of oxygen were stable and the degradation process was not initiated. The freeze-dried samples obtained the highest percentages of increase of phenolic compounds.

Kujala et al. (2000) observed that it was possible that decreases and increases in phenolic compounds could cancel each other out and thus alter the total phenolic content.

Table 3. Phenolic compounds content** of microcapsules stored under controlled temperature, light and oxygen conditions.

Storage conditions	Microcapsules*	Remaining (days) (%)			
		0	8	15	30
4°C, darkness	MS	220,69	201,30	207,66	259,48
	MXS	328,87	278,57	196,15	317,06
30°C, darkness	MS	220,69	260,09	164,63	230,69
	MXS	328,87	254,63	202,51	223,72
30°C, Under light	MS	220,69	227,66	156,45	230,39
	MXS	328,87	226,75	181,00	218,57
30°C, darkness, under vacuum	MS	220,69	210,39	156,15	232,21
	MXS	328,87	232,51	164,33	215,24

Storage conditions	Microcapsules*	Remaining (days) (%)			
		0	8	15	30
4°C	ML	249,18	316,15	301,00	184,33
	MXL	233,12	295,84	256,45	214,93

30°C, darkness	ML	249,18	292,51	217,97	158,57
	MXL	233,12	267,97	267,06	116,15
30°C, Under light	ML	249,18	280,09	242,51	135,84
	MXL	233,12	308,57	247,67	143,12
30°C, Under vacuum	ML	249,18	256,15	254,63	151,00
	MXL	233,12	253,72	277,67	167,06

*Microcapsules: spray drying (MS) microcapsules, powder containing maltodextrin and xanthan gum, and spray drying (MXS) microcapsules, freeze-drying (ML) microcapsules, powder containing maltodextrin and xanthan gum, and freeze-drying (MXL) microcapsules.
Source: Authors, (2020).

3.4.1 Effect of temperature

Both samples had higher phenolic preservation, after 30 days of storage, when stored at 30°C (86°F). At a higher temperature, betacyanine degradation is observed, which can form phenolic compounds, therefore the highest preservation. The xanthan sample had the lowest amount of phenolic at 4°C (highest betacyanine retention), and at 30°C the lowest amount was for the maltodextrin sample. Overall, the amount of phenolic in the samples stored at 4°C and 30°C was good, with retention greater than 70% after 30 days of storage. But it is worth remembering that this method of folin is widespread and the final amount may be related to the amount of phenolic compounds formed during storage from betacyanine degradation.

A reduction in phenolic content is observed with time and storage conditions. At 4°C, phenolic loss occurred after 8 days of storage for MS and MXS samples, but this loss was gradual for MXS, while for MS there was stabilization for 15 days.

3.4.2 Effect of luminosity

Regarding the light factor, it can be observed that the variation of the phenolic remaining samples after 30 days of storage was small, both for the light factor and the type of encapsulating agent.

At 30°C in the dark, there was loss only after 15 days of storage for MXS and MS samples. At 30°C (86°F) under light, there was a loss in the first week, stabilizing after that period for MS, while for MXS this loss was gradual.

3.4.3 Effect of vacuum

Under vacuum, there was loss in the first week, stabilizing MS and MXS after this period.

In general, phenolic compounds can be produced during the degradation of betanin, as well as they can be degraded depending on the storage conditions. In general, the encapsulation acted in the preservation of the phenolic compounds, after 30 days of storage in drastic conditions, the retention was above 70%.

For freeze-dried samples a lower phenolic retention is observed after 30 days of storage. And this is in accordance with betacyanine, where lyophilization has improved dye retention, avoiding its degradation and, consequently, reducing the production of phenolic compounds.

3.5 Color

Comparing all the analyzed samples (data not presented because they do not present great variations), in the parameter a^* (red coloration) the samples had similar behavior, and the microencapsulation was able to reach stability during 40 days of analysis, even with exposure to light, temperature and oxygen, and the values in general were between 20 and 40. The spray dryer showed a a^* value higher than the samples dried by lyophilization during all the time.

3.6 Physical-chemical and sensory analysis of yogurt

Although the spray drying microcapsules present the highest stability parameters at storage, the results were calculated based on the first 15 days of storage. Taking into consideration all the evaluated storage period, the microcapsules obtained by freeze-drying showed a higher dye content after 40 days of storage. Therefore, yogurt was prepared with the microcapsules obtained by lyophilization. The results of the physical-chemical analysis of the yogurt are presented in Table 4.

Table 4. Results of physical-chemical analysis of yogurt.

Parameters**	Microcapsules*				
	MAL 0,5%	MAL 1%	MXL 0,5%	MXL 1%	Extract 2%
Humidity (%)	81,43 ^a ± 0,05	81,07 ^a ± 0,11	81,69 ^a ± 0,04	81,08 ^a ± 0,15 ^a	81,16 ^a ± 0,00
Ash (%)	0,61 ^a ± 0,01	0,65 ^{ab} ± 0,01	0,61 ^a ± 0,03	0,73 ^b ± 0,00	0,73 ^b ± 0,04
pH	4,63 ^a ± 0,01	4,61 ^a ± 0,01	4,61 ^a ± 0,01	4,64 ^a ± 0,01	4,62 ^a ± 0,02
Acidity (% lactic acid)	0,79 ^a ± 0,01	0,81 ^a ± 0,01	0,79 ^a ± 0,04	0,79 ^a ± 0,04	0,76 ^a ± 0,03

*Microcapsules: powder containing maltodextrin as an encapsulating agent and dried by lyophilization (MAL), powder containing maltodextrin and xanthan gum and dried by lyophilization (MXL).

**Equal lines in the same line do not differ at 5% significance level.

Source: Authors, (2020).

According to Table 4, it can be observed that the use of betanin in microencapsulated form did not significantly alter the moisture, pH and acidity of yogurt. As for the ash content, a reduction in the content for samples added of 0.5% of microcapsules is noted. This difference may have been evidenced by the lower concentration of added microencapsulated dye, when compared to the product containing only beet extract.

What is interesting to note is that the presence of encapsulating agents has not significantly affected the physical-chemical characteristics of yogurt, with the exception of the ash content, however this change may be related to the amount of dye present in the formulation, and not to the encapsulating agent, since beet extract has a high mineral content.

Table 5 presents the results obtained in sensory analysis. It is noted that there was no significant difference between the samples in any of the evaluated attributes, at a 5% significance level. It is worth mentioning that the sensory analysis was carried out with untrained tasters, who liked beet or not.

For a product to be well accepted the acceptance rate must be higher than 70% (Dutcosky, 2007), so it can be noted that all concentrations were well accepted, thus enabling the application of natural beet dye microencapsulated in yogurt. In general, the notes on a 9-point hedonic scale varied between 6 (I liked it slightly) and 7 (I liked it moderately), and the highest notes obtained (above 7) were for color, which shows that the tasters approved of the color of yogurt with natural dye. It is also verified that the sample that obtained the highest acceptance rate was ML 0.5% > ML 1% > MXL 1% > MXL 0.5%.

Table 5. Results of the Sensory Analysis.

Attributes	ML 0,5%	ML 1%	MXL 0,5%	MXL 1%
Global Appearance	7,05 ^a	6,98 ^a	6,81 ^a	6,88 ^a
Aroma	6,76 ^a	6,89 ^a	7,08 ^a	7,14 ^a
Consistency	6,64 ^a	6,84 ^a	6,60 ^a	6,44 ^a
Color	7,23 ^a	7,46 ^a	7,04 ^a	7,3 ^a
Taste	6,58 ^a	6,23 ^a	6,48 ^a	6,28 ^a
Intention to buy	2,24 ^a	2,13 ^a	2,23 ^a	2,14 ^a
Index of acceptance	78,33(%)	77,5(%)	75,69(%)	76,39(%)

*Microcapsules: lyophilization-dry powder (ML), powder containing maltodextrin and xanthan gum and lyophilization-dry (MXL).

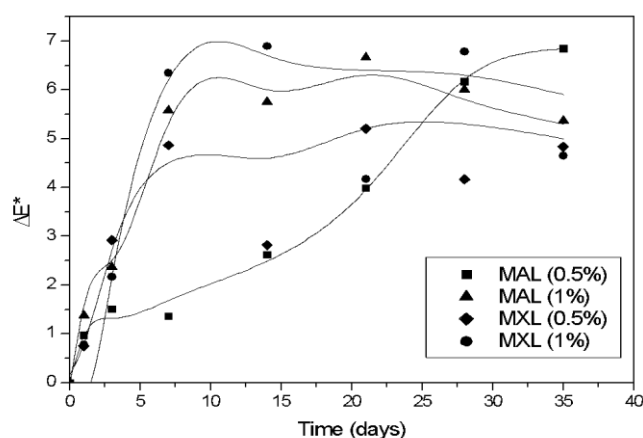
**Equal lines in the same line do not differ at 5% significance level.

Source: Authors, (2020).

Color Variation (ΔE)

To investigate the effect of the global variation in color in yogurt, the calculation of ΔE was performed with the parameters a^* , b^* and L^* , which were expressed by Figure 3.

Figure 3. Global variation in color in yogurt (ΔE).



Source: Authors.

The ML sample color variation of 0.5% was linear, while in the other samples there was a large variation in the first eight days and then it stabilized. From the figure it is possible

to observe that the smallest color variations were from the MXL samples (0.5%) < ML (1%) < ML (0.5%).

Samples containing larger amounts of microcapsules show greater color variation during storage.

4. Final Considerations

In general, all the microcapsules evaluated presented good encapsulation efficiency and good stability during the 40 days evaluated, because, despite the initial degradation of betanin, this degradation was the same for all the encapsulating agents.

As for the encapsulation technique, lyophilization presented lower values of a^* than spray dryer. Morphologically, the spray dryer samples were spherical and wrinkled, differently from the freeze-drying samples if they were smooth and of irregular sizes. The content of phenolic compounds, during storage, increased after 30 days of storage, which is due to the fact that 40 days of storage maintains a good stability of the product, even when exposed to temperature, light and oxygen.

Regarding the yogurt samples, there was good acceptance (>75%) and no significant difference between the treatments both in sensory analysis and physical-chemical, with the exception of ashes, where the sample MXL 1% and extract 2% differed from the samples MAL 0.5% and MXL 0.5%.

Finally, it is concluded that microencapsulation by lyophilization and spray drying, using maltodextrin and xanthan gum as encapsulating agents, described in this work and applied in yogurt, can be suggested as a color stabilizer suitable for beet extracts.

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