Microencapsulation by lyophilization of natural carotenoids using different wall materials

Microencapsulação por liofilização de carotenoides naturais utilizando diferentes materiais de parede

DOI:10.34117/bjdv7n7-263

Recebimento dos originais: 20/06/2021
Aceitação para publicação: 11/07/2021

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ABSTRACT

Due to the biological importance of carotenoids, several works have been developed aiming for the reduction of carotenoid degradation, and one notable proposed alternative has been the formation of microcapsules. Therefore, the aim of the current paper was the microencapsulation of carotenogenic extracts from *Rhodotorula mucilaginosa* and

Sporidiobolus pararoseus by a lyophilization method utilizing gum arabic, xanthan gum, sodium alginate and soy protein-like wall materials. The gum arabic showed the greatest efficiency of encapsulation for the *R. mucilaginosa* (66.3±0.8 %) and *S. pararoseus* (91.4±0.9 %) carotenogenic extracts, while the soy protein showed the lowest efficiency of encapsulation (40.7±1.1 % for *R. mucilaginosa* and 68.5±1.5 % for *S. pararoseus*). Scanning electron micrographs (SEM) showed irregular structure formation that was independent of the material utilized for the encapsulation. In this way, it was possible to observe that the wall materials directly affect the encapsulation efficiencies, morphology, and thermal behavior of the capsules of natural carotenoids.

Keywords: pigment, encapsulation, yeasts, bioactive, food, color, protein.

RESUMO

Devido à importância biológica dos carotenoides, diversos trabalhos têm sido desenvolvidos com o objetivo de reduzir a degradação destes compostos, sendo a microencapsulação uma notável alternativa. Assim o objetivo do presente trabalho foi a microencapsulação de extratos carotenogênicos das leveduras *Rhodotorula mucilaginosa* and *Sporidiobolus pararoseus* por liofilização utilizando como material de parede goma arábica, goma xantana, alginato de sódio e proteína de soja. A goma arábica apresentou a maior eficiência de encapsulamento para os extratos de *R. mucilaginosa* (66,3±0,8 %) e *S. pararoseus* (91,4±0,9 %). Já a proteína de soja apresentou a menos eficiência de encapsulamento para os dois extratos (40,7±1,1 % para *R. mucilaginosa* e 68,5±1,5 % para *S. pararoseus*). Análises de microscopia eletrônica de varredura (MEV) demonstraram a formação de estruturas irregulares independentemente do material utilizado para a encapsulação. Assim, observou-se que o material de parede afeta diretamente a eficiência de encapsulamento, morfologia e comportamento termal das cápsulas de carotenoides naturais.

Palavras-chave:pigmentos, encapsulação, leveduras, bioativos, alimentos, cor, proteína.

1 INTRODUTION

Carotenoids are synthesized by all photosynthetic organisms, filamentous fungi, and yeasts, some species of bacteria, microalgae and lichens, and they are chemically obtained for wide applications in industry (Cardoso et al., 2017). However, the biotechnological production of these pigments is increasing due to the demand of the consumer market for natural products. In addition, these processes allow better control of environmental and genetic manipulation (Yoo et al. 2016) and enable the production of isomers with biologically active configurations, compared to the chemical synthesis that produces mixtures of stereoisomers (Ausich, 1997).

During the manipulation and application of carotenoids, these bioactive compounds can be degraded by factors including temperature, light and oxygen. Therefore, protection techniques like microencapsulation can be used to improve the

stability of these pigments (Rutz et al., 2013; Tisch & Schmoll, 2010), assist in controlled release, and obtain products with homogeneous colour (Sauvant et al., 2012).

Bioactive compounds can be microencapsulated by physical-chemical methods (emulsification, liposomes, and encapsulation with polymers) and mechanical methods (spray-drying, extrusion, and lyophilization), which make it possible to obtain large quantities of particles with longer life (de Vos et al., 2010; Gonnet et al., 2010, Lopes et al., 2017; Andrade et al., 2020, Santos et al., 2020).

Several materials used as encapsulating agents are available for use in foods, including carbohydrates, proteins and lipids (Özkan & Bilek, 2014). However, the choice of the material used as cover for the microcapsules should be based on the characteristics of the compound to be encapsulated and the method of elaboration of the particles, with a view toward their subsequent application (Özkan & Bilek, 2014; Vaniski et al., 2017).

The microencapsulation of different compounds by lyophilization, including purple Brazilian cherry juice (Rutz et al., 2013), gallic acid (Da Rosa et al., 2014), palm oil and synthetic β-carotene (Rutz et al., 2016), have been previously studied, but the production of microcapsules with carotenoids obtained from yeasts is rare (Nogueira et al., 2017).

Thus, the aim of the current study was the microencapsulation of carotenogenic extract from *Rhodotorula mucilaginosa* and *Sporidiobolus pararoseus* by a lyophilization method using gum arabic, xanthan gum, sodium alginate and soy protein as wall materials.

2 MATERIAL AND METHODS

2.1 MICROORGANISMS AND INOCULUM PREPARATION

The *S. pararoseus* and *R. mucilaginosa* used in this study were previously isolated in Caçapava do Sul, a city located in the Escudo Sul-riograndense ecosystem (Rio Grande do Sul state, Brazil), from environmental samples (Otero et al., 2019) that were identified and deposited into the André Tosello Tropical Culture Collection (CCT 7689 and CCT 7688). The yeasts were kept at 4 °C, on sloping agar in malt and yeast (YM) medium with 3.0 g/L yeast extract (Kasvi, São José dos Pinhais, Brazil), 3.0 g/L malt extract (AcuMedia, San Bernadino, CA, USA), 5.0 g/L peptone (Kasvi) and 10.0 g/L glucose (Synth Diadema, Brazil) with the addition of 0.2 g/L KNO_3 (Synth) (Parajó et al., 1998). For reactivation, a stock culture was performed in test tubes with YM agar and incubated at 25 °C for 48 h.

After the reactivation, for the preparation of the pre-inoculum, cell resuspensions were performed in 1 mL of peptone water (0.1 %), added to 9 mL of YM broth and incubated under previously described conditions. This medium was added to 500 mL Erlenmeyer flasks with 90 mL of YM broth and incubated (Tecnal model TE 425, Piracicaba, Brazil) at 25 °C, 150 rpm, during the time needed to reach $1x10^8$ cel/mL, counted by Neubauer chamber (Optik Labor, Lancing, UK) (Rios et al., 2015).

2.2 CAROTENOID PRODUCTION IN SHAKE FLASKS

The carotenoid production for both yeasts was carried out in 500 mL Erlenmeyer flasks with 225 mL of YM medium, initial pH of 6.0 and 10 % of inoculum, at 25 °C, 180 rpm (Tecnal model TE 425) (Rios et al., 2015)for 192 h (*S. pararoseus*) and 216 h (*R. mucilaginosa*). The biomass was dried (Quimis Q314M242, Diadema, Brazil Eletrolab, São Paulo, Brazil) at 35 ºC for 48 h (da Fonseca et al., 2011) and macerated to a standardized degree by a 115-mesh sieve (Cipolatti, et al., 2015).

2.3 EXTRACTION OF CAROTENOIDS

Biomass lysis was performed by the ultrasonic wave method described by Lopes et al. (2017), with modifications. In the process of disruption, 0.05 g of biomass was added to 6 mL of acetone (Synth) and submitted to 3 cycles (10 min per cycle with 40 kHz) (Quimis, Diadema, Brazil). Between the cycles, the tubes were agitated by vortex (Biomixer model QL-901, Ningbo, China) mixing for 1 min.

After rupture, the biomass was centrifuged (CientecCT 5000 R, Belo Horizonte, Brazil) at 3439xg for 10 min. To the solvent phases obtained by centrifugation, 20 % NaCl solution (w/v) and petroleum ether (Neon, Suzano, Brazil) were added. After the formation of both phases, the apolar phase was collected, and excess water was removed by sodium sulfate (Synth) (Michelon et al., 2012), thus forming the carotenogenic extracts.

2.4 MICROENCAPSULATION OF CAROTENOIDS

Microencapsulation of carotenoids was performed by the lyophilization method, with xanthan gum (Sigma Aldrich, São Paulo, Brazil), gum arabic (Synth), sodium alginate (Sigma Aldrich) and soy protein (Sigma Aldrich) as the wall materials following the methods of Lain et al. (2010) and Pralhad & Rajenfrakumar (2004). Before the preparation of the microcapsules, the solvent was evaporated (Rotary evaporator 802, Fisatom, São Paulo, Brazil) (35 °C) from the carotenogenic extracts. The residue was

resuspended in distilled water at a ratio of 1:1 carotenoids:wall material, and this mixture was stirred (752A, Fisatom) for 1 h, then frozen at -80 °C (UFR30, Liotop, São Carlos, Brazil) and freeze-dried (L101, Liotop) for 48 h.

2.5 MORPHOLOGICAL CHARACTERIZATION OF THE PARTICLES AND DSC

Morphological analyses were made by scanning electron microscopy (SEM) (JSM - 6610LV, Jeol, Tokyo, Japan), and the measurements were performed by SigmaScan Pro 5.0.

Differential scanning calorimetry (DSC) analysis was performed by DSC-60 (Shimadzu, Japan), where approximated 3 mg of each sample was heated at the rate of 10 ºC/min, between 25 and 280 ºC, with a nitrogen flow of 40 mL/min.

2.6 MICROENCAPSULATION EFFICIENCY

For the quantification of the carotenoids in the surfaces of the microcapsules (SC), 0.1 g of capsules and 5 mL of hexane (Neon) were mixed in a vortex for 2 min (Biomixer model QL-901), followed by centrifugation (Cientec CT 5000 R) at 3420xg for 10 min, and then the supernatant was collected. For the quantification of total carotenoids (TC), 0.1 g of capsules and 2 mL of water were mixed in a vortex for 2 min, followed by the addition of 5 mL of hexane, centrifugation (Cientec CT 5000 R) at 3420xg for 10 min, and collection of the supernatant (Sutter et al. 2007).

The collected fractions were evaluated spectrophotometrically (Biospectro SP-220, Zhejiang, China) at 448 nm to obtain total carotenoid content. The results are expressed as a percentage of encapsulated carotenoids by Eq. 1 (Rutz et al., 2013):

$$EE = \frac{(TC-SC)}{TC} \times 100 \quad (1)$$

Where EE=encapsulation efficiency (%), TC=total carotenoids, and SC=carotenoids on the surface.

2.7 STATISTICAL ANALYSIS

Experiments were performed in triplicate, and the results were statistically evaluated by the t-test or analysis of variance. When differences were detected at 5 % of significance ($p<0.05$), the Tukey test was applied.

3 RESULTS AND DISCUSSION

3.1 MICROENCAPSULATION OF CAROTENOGENIC EXTRACTS BY LYOPHILIZATION

Due to its biological importance, several works have been developed aiming for the reduction of carotenoid degradation; among the alternatives proposed is the formation of microcapsules with wall materials that form physical barriers, reducing the effects of factors such as oxygen, light, and heat (Jafari et al., 2008).

Lyophilization was one of the proposed techniques to produce capsules. In this process, material drying occurs by the sublimation of ice into water vapor through the action of reduced pressures (Rajam & Anandharamakrishnan, 2015). This prevents the exposure of the sample to high temperatures, helping to protect thermolabile compounds (Chen et al., 2017) compared to other methods based on the removal of water by evaporation.

For the carotenogenic extract from *R. mucilaginosa*, capsules were developed with only the three materials proposed, since it was not possible to perform the solubilization of carotenoids with xanthan gum utilized in the present work. Rutz et al. (2013) reported that the xanthan gum is considered an anionic substance and that its interaction with the carotenoids occurs through ion-induced dipole interaction; thus, the nonsolubilization of this extract with xanthan gum is probably a result of the weak interactions between the materials. For the other wall materials tested (Table 1), significant differences ($p<0.05$) were observed between the EE (encapsulation efficiency) of each material. The gum arabic had the greatest efficiency of encapsulation at 66.3±0.8 %, followed by the sodium alginate (63.0±0.6 %) and, finally, the soy protein, with the lowest EE (%) of 44.7±1.1 %.

Table 1. Encapsulation efficiency (%) of the carotenogenic extracts from *R. mucilaginosa* and *S. pararoseus* by the lyophilization method.

	Wall materials			
Yeast	Sodium Alginate	Gum Arabic	Soy Protein	Xanthan Gum
R. mucilaginosa	$63.0\pm0.6^{b,B}$	$66.3\pm0.6^{a,B}$	$49.7\pm1.1^{c,B}$	ND
S. pararoseus	$86.8\pm0.8^{b,A}$	$91.4\pm0.9^{a,A}$	$68.5\pm1.5^{c,A}$	87.8 ± 1.8^{b}

Means±standard deviations (n=3). Means with different lowercase letters are significantly different ($p<0.05$) in the row using Tukey test. Means with different uppercase letters are significantly different ($p<0.05$) in the column using t-test. ND: not determined.

The carotenogenic extract from *S. pararoseus* (Table 1) was better encapsulated by the gum arabic, with an EE (%) of 91.4±0.9 %, followed by xanthan gum (87.8±1.8

%) and, with a similar ($p>0.05$) performance, the sodium alginate (86.8±0.8 %). The soy protein again showed the lowest EE (%) (68.5±1.5 %).

The arabic gum presented the highest encapsulation efficiency for both extracts. Similar results for the same wall material were described by (Barbosa et al., 2005) during the development of spray-dried bixin capsules using gum arabic, maltodextrin and sucrose. The authors described that the microcapsule with gum arabic was more stable to photodegradation than others, probably because the structure of this material, which is a highly branched heteropolymer of sugars, glucuronic acid and a small amount of protein that is covalently linked to the carbohydrate chain, which helps with the emulsifier effect for nonpolar substances such as gum arabic.

Proteins are good for wall material because of amphiphilic characteristics that make them excellent emulsifiers (Lam & Nickerson, 2013); however, in the present work, the soy protein was the wall material with the lowest EE (%) for both extracts. The protein structure is formed by polar and nonpolar amino acids and, when placed in aqueous solution, these groups tend to realign to reduce unfavorable thermodynamic interactions, and the time for these conformation modifications to occur is variable (Lam & Nickerson, 2013; McClements, 2004).

Gómez-Mascaraque et al. (2017) utilized whey protein for the microencapsulation of β-carotene through electrospraying. When the primary emulsion was subjected to only one cycle of high-speed homogenization, the formation of unstable emulsions occurred, with phase separation, but the emulsion was stabilized by adding an ultrasonication step, which the authors attribute to the higher exposure of the non-polar groups, allowing a faster adsorption at the interface. The wall materials in the current paper were homogenized with the aqueous phase and carotenogenic extracts through magnetic agitation, which probably was not enough energy for conformation modifications to occur, reducing the interaction between the hydrophobic extract and the soy protein chain.

The medium utilized can affect the profile of carotenoids produced by yeasts. In a medium composed of glycerol and corn steep liquor, *R. mucilaginosa* can produce β-carotene, astaxanthin and lutein, but the same yeast when cultivated in medium formulated with molasses and corn steep liquor is not capable of producing astaxanthin (Cipolatti, 2012). The strains of *R. mucilaginosa* and *S. pararoseus* utilized in the current research, when growing in YM medium, produce astaxanthin, lutein, and β-carotene (*R. mucilaginosa*), with lutein and β-carotene (*S. pararoseus*) as the main carotenoids (Cipolatti, 2012). Astaxanthin is a xanthophyll (contain oxygen in its structure), and the

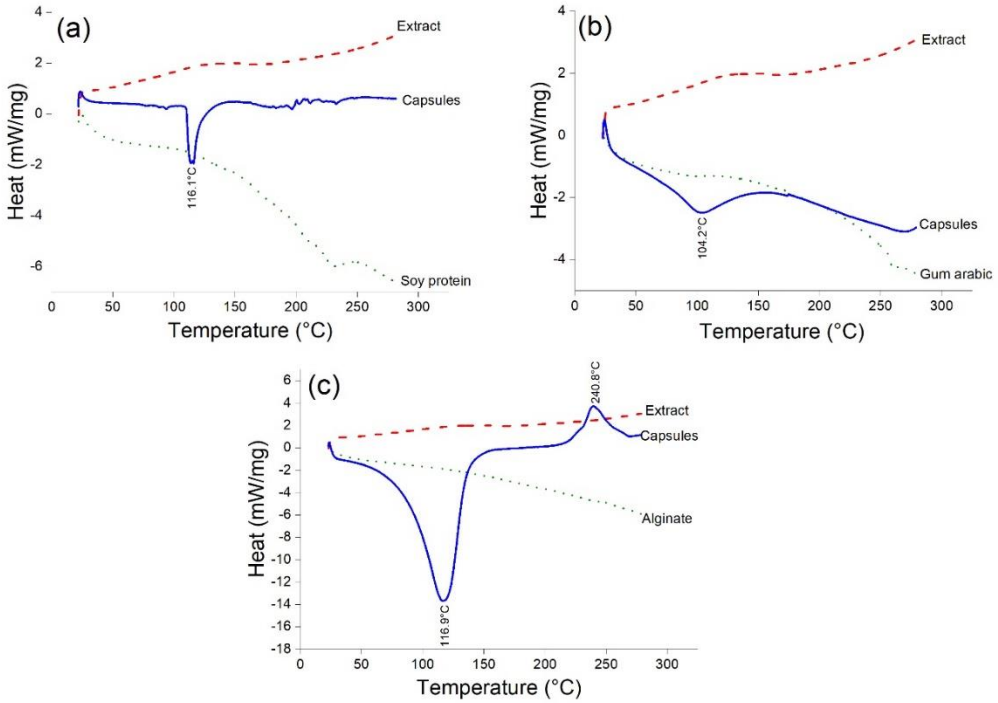
β-carotene is a carotene (contain only carbon and hydrogen in its structure); the presence of the oxygen causes the xanthophylls to be more polar than the carotenes (Cardoso et al., 2017). In the extracts utilized, no type of purification was performed; consequently, the other carotenoids produced by the yeasts were present in the extracts. The polar nature of the astaxanthin may have favored its affinity with the wall materials used, reducing the interaction of β-carotene (more apolar), which led to lower encapsulation efficiencies (Table 1) of the *R. mucilaginosa* extract compared to the *S. pararoseus* extract.

The presence of interferents in the rust carotenoid extract may also have caused smaller encapsulation efficiencies than those described elsewhere, such as by Rutz et al. (2016) describing encapsulation efficiencies greater than 95 % for the same technique with chitosan wall material and carboxymethylcellulose.

3.2 DSC THERMOGRAMS

DSC thermograms (Fig. 1 and 2) show endothermic and exothermic events by the representation of downward and upward curves, respectively. The capsules of carotenogenic extract from *R. mucilaginosa* with soy protein (Fig. 1a) and gum arabic (Fig. 1b) exhibited one endothermic event each, observed at 116.1 °C (soy protein) and 104.2 °C (gum arabic). The sodium alginate (Fig. 1c) showed one endothermic event at approximately 117 °C and one exothermic approximately 241 °C.

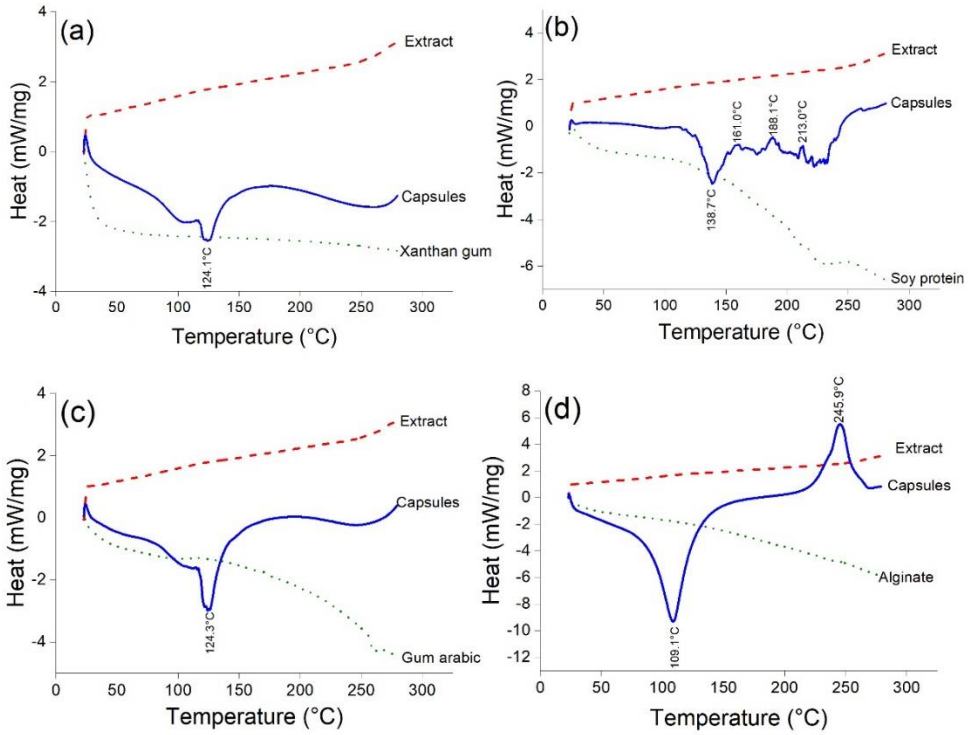
Figure 1. DSC thermograms of *Rhodotorula mucilaginosa* carotenoid capsules using (a) soy protein, (b) gum arabic and (c) sodium alginate as coating materials.



For the capsules of carotenogenic extracts from *S. pararoseus* with xanthan gum (Fig. 2a) and gum Arabic (Fig. 2c), only one endothermic event at approximately 124 °C was observed for both. In the DSC spectrum of the capsules from the same extract using soy protein, one endothermic event at approximately 139 °C and three other exothermic events at 161, 188 and 213 °C occurred.

For the sodium alginate capsules, two events were observed: one endothermic event at 109 °C and one exothermic event at 246 °C. Endothermic events observed from 100 to 140 °C were related to water loss (Daoub et al., 2018). The exothermic events approximately 240-245 °C in the capsules developed using alginate were related to the melting temperature of this wall material (Marques et al., 2018; Munavalli et al., 2018). The melting point of the gums was not observed because they are approximately 300 to 315 °C (Daoub et al., 2018), which are higher than the temperatures used in the present work.

Figure 2. DSC thermograms of *Sporidiobolus pararoseus* carotenoid capsules using (a) xanthan gum, (b) soy protein, (c) gum arabic and (d) sodium alginate as coating materials

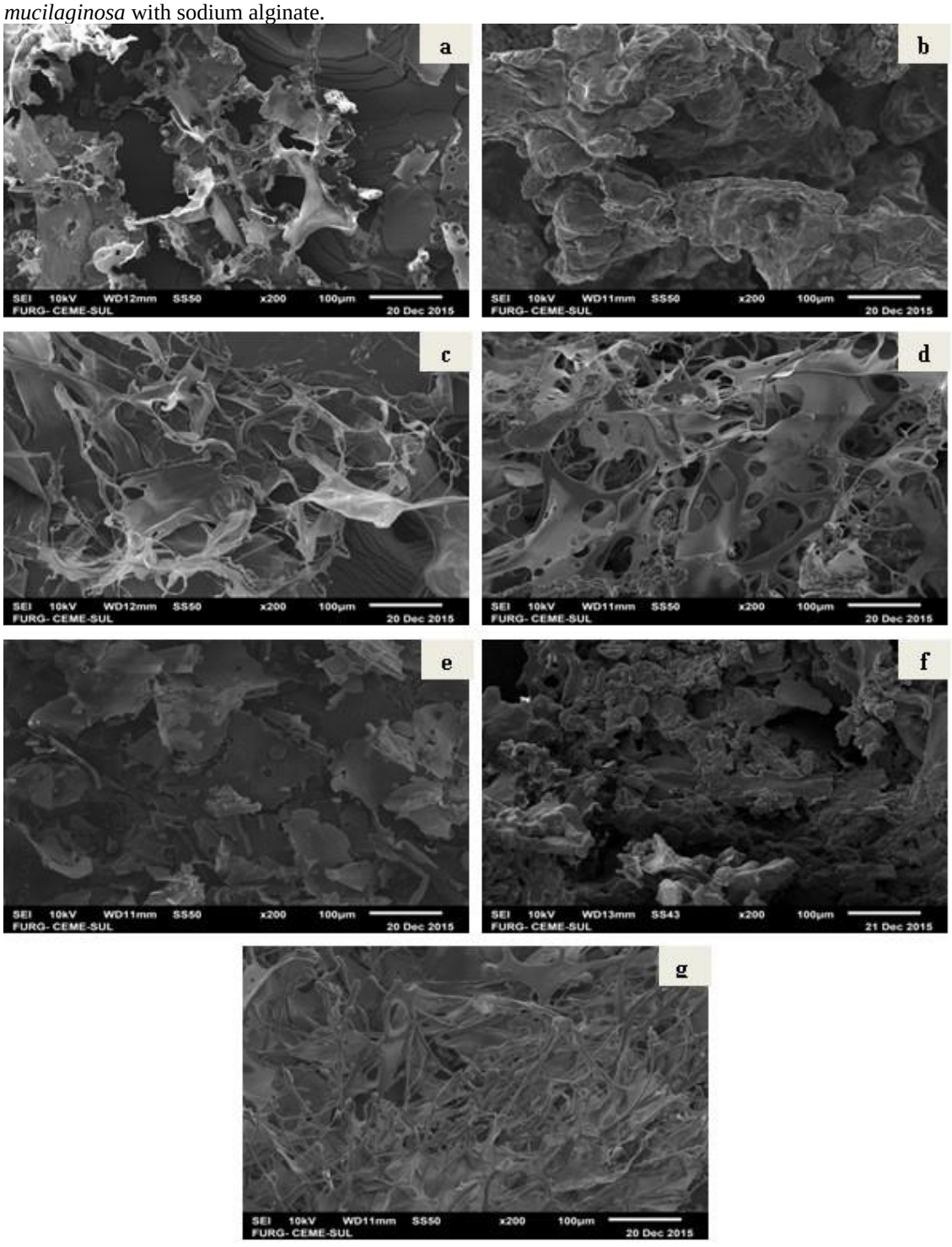


None of the endothermic or exothermic events observed in the DSC of the capsules were verified in the DSC of the extracts or wall materials alone, which seems to indicate interaction of the carotenoids with the wall materials.

3.3 SCANNING ELECTRON MICROGRAPHS (SEM)

Scanning electron micrographs (SEM) (Fig. 3) show irregular structure formation regardless of the material utilized for the encapsulation. Studies by Sousdaleff et al. (2013), Zuanon et al. (2013) and Rutz et al. (2016) using the freeze-drying method for the encapsulation of carotenoids obtained noncharacteristic particles and observed the nonformation of spherical microparticles, suggesting that, depending on the wall material, such conditions are not suitable for obtaining microcapsules, even though the method is commonly used.

Figure 3. SEM micrographs of microcapsules of carotenoids at 600x magnification: (a) *S. pararoseus* with gum arabic, (b) *S. pararoseus* with soy protein, (c) *S. pararoseus* with xanthan gum, (d) *S. pararoseus* with sodium alginate, (e) *R. mucilaginosa* with gum arabic, (f) *R. mucilaginosa* with soy protein, (g) *R. mucilaginosa* with sodium alginate.



When gum arabic and sodium alginate were used, the microstructures demonstrated a leaf-shape with heterogeneous pores. For the extract of *S. pararoseus* encapsulated with gum arabic (Fig. 3(A)), the diameters of the pores varied from 1.25 to 17.50 µm, while for the extract of *R. mucilaginosa* encapsulated with the same encapsulant (Fig. 3(E)), the diameters were between 1.00 to 36.00 µm. The pores in the

structures formed by the sodium alginate (Fig. 3(D) and 3(G)) varied from 1.25 to 39.25 µm (*S. pararoseus*) and between 1.08 to 13.92 µm (*R. mucilaginosa*). When xanthan gum and soy protein were used, no pores were observed in the microstructures formed. The structures for xanthan gum showed a leaf-shape between 0.75 and 6.00 µm in length.

4 CONCLUSIONS

The encapsulation efficiencies, the morphology and the thermal behavior of the capsules were affected by the wall materials used. Only the gum arabica was not able to be structured when the carotenogenic extract of *R. mucilaginosa* was used. The thermal events observed in the capsules were not present in the analyses of either the nonencapsulated extracts or the materials used for the encapsulation, which shows the interaction of the carotenoids with the wall materials.

Funding: This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) under Grant number [001].

REFERENCES

Andrade, S. F., Nascimento R. S., Carvalho, F. A., Santos, A. M., Pagani, A. A. C. (2020). Microencapsulação de cúrcuma longa (Açafrão) pelo método de gelificação iônica, agregando valor ao vinagre de álcool. *Braz. J. of Develop.*, *6*(3), 12387-12398. https://doi.org/ 0.34117/bjdv6n3-195

Ausich, R. L. (1997). Commercial opportunities for carotenoid production by biotechnology. *Pure and Applied Chemistry*, *69*(10), 2169–2173. https://doi.org/10.1351/pac199769102169

Barbosa, M. I. M. J., Borsarelli, C. D., & Mercadante, A. Z. (2005). Light stability of spray-dried bixin encapsulated with different edible polysaccharide preparations. *Food Research International*, *38*(8–9), 989–994. https://doi.org/10.1016/j.foodres.2005.02.018

Cardoso, L. A. A., Karp, S. G., Vendruscolo, F., Kanno, K. Y. F., Zoz, L. I. C., & Carvalho, J. C. (2017). Biotechnological Production of Carotenoids and Their Applications in Food and Pharmaceutical Products. In D. J. Cvetkovic & G. S. Nikolic (Eds.), *Carotenoids.* InTech. https://doi.org/10.5772/65523

Chen, J., Li, F., Li, Z., McClements, D. J., & Xiao, H. (2017). Encapsulation of carotenoids in emulsion-based delivery systems: Enhancement of β-carotene water-dispersibility and chemical stability. *Food Hydrocolloids*, *69*, 49–55. https://doi.org/10.1016/j.foodhyd.2017.01.024

Cipolatti, E P. (2012). *Obtenção de carotenoides microbianos com atividade antioxidante a partir de coprodutos agroindustriais* (Dissertação de mestrado). Universidade Federal do Rio Grande, Rio Grande.

Cipolatti, E. P., Bulsing, B. A., Sá, C. dos S., Burkert, C. A. V., Furlong, E. B., & Burkert, J. F. M. (2015). Carotenoids from *Phaffia rhodozyma*: Antioxidant activity and stability of extracts. *African Journal of Biotechnology*, *14*(23), 1982–1988. https://doi.org/10.5897/ajb2015.14682

Da Fonseca, R. A. dos S., Rafael, R. da S., Kalil, S. J., Burkert, C. A. V., & Burkert, J. F. de M. (2011). Different cell disruption methods for astaxanthin recovery by *Phaffia rhodozyma*. *African Journal of Biotechnology*, *10*(7), 1165–1171. https://doi.org/10.5897/AJB10.1034

Da Rosa, C. G., Borges, C. D., Zambiazi, R. C., Rutz, J. K., da Luz, S. R., Krumreich, F. D., Benvenutti, E. V., & Nunes, M. R. (2014). Encapsulation of the phenolic compounds of the blackberry (*Rubus fruticosus*). LWT - *Food Science and Technology*, *58*(2), 527–533. https://doi.org/10.1016/j.lwt.2014.03.042

Daoub, R. M. A., Elmubarak, A. H., Misran, M., Hassan, E. A., & Osman, M. E. (2018). Characterization and functional properties of some natural Acacia gums. *Journal of the Saudi Society of Agricultural Sciences*, *17*(3), 241–249. https://doi.org/10.1016/j.jssas.2016.05.002

De Vos, P., Faas, M. M., Spasojevic, M., & Sikkema, J. (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components.

International Dairy Journal, *20*(4), 292–302. https://doi.org/10.1016/j.idairyj.2009.11.008

Gómez-Mascaraque, L. G., Perez-Masiá, R., González-Barrio, R., Periago, M. J., & López-Rubio, A. (2017). Potential of microencapsulation through emulsion-electrospraying to improve the bioaccesibility of β-carotene. *Food Hydrocolloids*, *73*, 1–12. https://doi.org/10.1016/j.foodhyd.2017.06.019

Gonnet, M., Lethuaut, L., & Boury, F. (2010). New trends in encapsulation of liposoluble vitamins. *Journal of Controlled Release*, *146*(3), 276–290. https://doi.org/10.1016/j.jconrel.2010.01.037

Jafari, S. M., Assadpoor, E., He, Y., & Bhandari, B. (2008). Encapsulation efficiency of food flavours and oils during spray drying. *Drying Technology*, *26*(7), 816–835. https://doi.org/10.1080/07373930802135972

Laine, P., Kylli, P., Heinonen, M., & Jouppila, K. (2010). Does Microencapsulation Improve Storage Stability of Cloudberry (*Rubus chamaemorus*) Ellagitannins? *Water Properties in Food, Health, Pharmaceutical and Biological Systems: ISOPOW 10*, 563–569. https://doi.org/10.1002/9780470958193.ch50

Lam, R. S. H., & Nickerson, M. T. (2013). Food proteins: A review on their emulsifying properties using a structure-function approach. *Food Chemistry*, *141*(2), 975–984. https://doi.org/10.1016/j.foodchem.2013.04.038

Lopes, N. A., Remedi, R. D., dos Santos Sá, C., Burkert, C. A. V., & de Medeiros Burkert, J. F. (2017). Different cell disruption methods for obtaining carotenoids by *Sporodiobolus pararoseus* and *Rhodothorula mucilaginosa*. *Food Science and Biotechnology*, *26*(3), 759–766. https://doi.org/10.1007/s10068-017-0098-y

Marques, C. S. F., Rezende, P., Andrade, L. N., Mendes, T. M. F., Allegretti, S. M., Bani, C., Chaud, M. V., Batista de Almeida, M., Souto, E. B., Pereira da Costa, L., & Severino, P. (2018). Solid dispersion of praziquantel enhanced solubility and improve the efficacy of the schistosomiasis treatment. *Journal of Drug Delivery Science and Technology*, *45*, 124–134. https://doi.org/10.1016/j.jddst.2018.03.009

McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid and Interface Science*, *9*(5), 305–313. https://doi.org/10.1016/j.cocis.2004.09.003

Michelon, M., de Borba, T. de M., Rafael, R. da S., Burkert, C. A. V., & Burkert, J. F. de M. (2012). Extraction of carotenoids from *Phaffia rhodozyma*: A comparison between different techniques of cell disruption. *Food Science and Biotechnology*, *21*(1), 1–8. https://doi.org/10.1007/s10068-012-0001-9

Munavalli, B., Torvi, A., & Kariduraganavar, M. (2018). A facile route for the preparation of proton exchange membranes using sulfonated side chain graphite oxides and crosslinked sodium alginate for fuel cell. *Polymer*, *142*, 293–309. https://doi.org/10.1016/j.polymer.2018.03.044

Nogueira, M. B., Prestes, C. F., & Burkert, J. F. M. (2017). Microencapsulation by lyophilization of carotenoids produced by *Phaffia rhodozyma* with soy protein as the encapsulating agent. *Food Science and Technology*, *37*(spe), 1–4. https://doi.org/10.1590/1678-457x.05417

Otero, D. M., Bulsing, B. A., Da M Huerta, K., Rosa, C. A., Zambiazi, R. C., Burkert, C. A. V., & De M Burkert, J. F. (2019). Carotenoid-producing yeasts in the brazilian biodiversity: Isolation, identification and cultivation in agroindustrial waste. *Brazilian Journal of Chemical Engineering*, *36*(1), 117–129. https://doi.org/10.1590/0104-6632.20190361s20170433

Özkan, G., & Ersus Bilek, S. (2014). Microencapsulation of Natural Food Colourants. *International Journal of Nutrition and Food Sciences*, *3*(3), 145–156. https://doi.org/10.11648/j.ijnfs.20140303.13

Parajó, J. C., Santos, V., & Vázquez, M. (1998). Optimization of carotenoid production by *Phaffia rhodozyma* cells grown on xylose. *Process Biochemistry*, *33*(2), 181–187. https://doi.org/10.1016/S0032-9592(97)00045-9

Pralhad, T., & Rajendrakumar, K. (2004). Study of freeze-dried quercetin-cyclodextrin binary systems by DSC, FT-IR, X-ray diffraction and SEM analysis. *Journal of Pharmaceutical and Biomedical Analysis*, *34*(2), 333–339. https://doi.org/10.1016/S0731-7085(03)00529-6

Rajam, R., & Anandharamakrishnan, C. (2015). Spray freeze drying method for microencapsulation of Lactobacillus plantarum. *Journal of Food Engineering*, *166*, 95–103. https://doi.org/10.1016/j.jfoodeng.2015.05.029

Rios, D. A. da S., Borba, T. de M. de, Kalil, S. J., & Burkert, J. F. M. (2015). Rice parboiling wastewater in the maximization of carotenoids bioproduction by *Phaffia rhodozyma*. *Ciência e Agrotecnologia*, *39*(4), 401–410. https://doi.org/10.1590/s1413-70542015000400011

Rutz, J. K., Borges, C. D., Zambiazi, R. C., Cleonice, G., & Médelin, M. (2016). Elaboration of microparticles of carotenoids from natural and synthetic sources for applications in food. *Food Chemistry*, *202*, 324–333. https://doi.org/10.1016/j.foodchem.2016.01.140

Rutz, J. K., Zambiazi, R. C., Borges, C. D., Krumreich, F. D., da Luz, S. R., Hartwig, N., & da Rosa, C. G. (2013). Microencapsulation of purple Brazilian cherry juice in xanthan, tara gums and xanthan-tara hydrogel matrixes. *Carbohydrate Polymers*, *98*(2), 1256–1265. https://doi.org/10.1016/j.carbpol.2013.07.058

Santos, F. H. dos, Silveira, B. M. P. e, Souza, L. L. de, Duarte, A. K. C., Ribeiro, M. C., Pereira, K. C., & Costa, J. M. G. da. (2020). Influence of wall materials on the microencapsulation of pequi oil by spray drying. *Brazilian Journal of Food Technology*, *23*. https://doi.org/10.1590/1981-6723.13219

Sauvant, P., Cansell, M., Hadj Sassi, A., & Atgié, C. (2012). Vitamin A enrichment: Caution with encapsulation strategies used for food applications. *Food Research International*, *46*(2), 469–479. https://doi.org/10.1016/j.foodres.2011.09.025

Sousdaleff, M., Baesso, M. L., Neto, A. M., Nogueira, A. C., Marcolino, V. A., & Matioli, G. (2013). Microencapsulation by freeze-drying of potassium norbixinate and curcumin with maltodextrin: Stability, solubility, and food application. *Journal of Agricultural and Food Chemistry*, *61*(4), 955–965. https://doi.org/10.1021/jf304047g

Sutter, S. C., Buera, M. P., & Elizalde, B. E. (2007). β-Carotene encapsulation in a mannitol matrix as affected by divalent cations and phosphate anion. *International Journal of Pharmaceutics*, *332*(1–2), 45–54. https://doi.org/10.1016/j.ijpharm.2006.09.023

Tisch, D., & Schmoll, M. (2010). Light regulation of metabolic pathways in fungi. *Applied Microbiology and Biotechnology*, *85*(5), 1259–1277. https://doi.org/10.1007/s00253-009-2320-1

Vaniski, R., Corti, D., & Drunkler, D. A. (2017). Técnicas e Materiais Empregados na Microencapsulação de Probióticos. *Brazilian Journal of Food Research*, *8*(1), 156. https://doi.org/10.3895/rebrapa.v8n1.3651

Yoo, A. Y., Alnaeeli, M., & Park, J. K. (2016). Production control and characterization of antibacterial carotenoids from the yeast *Rhodotorula mucilaginosa* AY-01. *Process Biochemistry*, *51*(4), 463–473. https://doi.org/10.1016/j.procbio.2016.01.008

Zuanon, L. A. C., Malacrida, C. R., & Telis, V. R. N. (2013). Production of turmeric oleoresin microcapsules by complex coacervation with gelatin-gum arabic. *Journal of Food Process Engineerin*g, 36(3), 364–373. https://doi.org/10.1111/jfpe.12003