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Antioxidant activities of spray-dried carotenoids using maltodextrin-Arabic gum as wall materials

Marco Antonio González-Peña¹, José Daniel Lozada-Ramírez² and Ana Eugenia Ortega-Regules^{3*}

Abstract

Background: Carotenoids are natural pigments that are highly sensitive to light, heat, acids, oxygen, metals, and free radicals, which degrade the antioxidant activities of carotenoids. Microencapsulation techniques have been used to prevent carotenoid degradation and preserve their antioxidant activities. In this work, we aimed to encapsulate mamey (*Pouteria sapota*) and carrot (*Daucus carota*) carotenoids in mixtures of maltodextrin (10% w/v) and Arabic gum (5 or 10% w/v) by spray-drying. The obtained powders were stored at different temperatures (4 and 25 °C) with or without access to daylight, and changes in color, carotenoid content, and antioxidant activity were analyzed monthly for three months. Moreover, the in vivo antioxidant activities of spray-dried carotenoids were evaluated in *Caenorhabditis elegans*.

Results: The carotenoid and antioxidant activity losses of stored carotenoids were found in the following order: 25 °C in daylight > 25 °C without access to daylight > 4 °C without access to daylight. Moreover, the combination of maltodextrin and Arabic gum (10%) was more effective to prevent carotenoid loss compared to maltodextrin and Arabic gum (5%). In vivo antioxidant activity results showed that spray-dried carotenoids reduced approximately 30% of reactive oxygen species (ROS) production in nematodes, even after three months of storage.

Conclusions: Mamey and carrot carotenoids were successfully encapsulated by the spray-drying technique. The spray-dried carotenoids effectively reduced the intracellular ROS levels and neutralized the oxidative stress damage in *C. elegans*, even after three months of storage. Moreover, the antioxidant activities of mamey carotenoids were equally effective as those of carrot carotenoids, meaning that mamey carotenoids could be seen as an alternative source of carotenoids.

Keywords: Spray-drying, Mamey, Carrot, Carotenoids, Antioxidant activity, *Caenorhabditis elegans*

Background

Carotenoids are a group of water-insoluble natural pigments, providing yellow, orange, and red colors to fruits and vegetables, such as carrot, tomato, mango, orange, guava, pepper, and sweet potato. They comprise repetitive units of isoprene with double conjugated bonds responsible for their colors and antioxidant activities

(Saini et al. 2015). However, carotenoids are highly sensitive to light, heat, acids, oxygen, metals, and free radicals, which are commonly found during food processing (Faria et al. 2010). This leads to structural changes, such as cycling, migration of double bonds, and addition of oxygen molecules, resulting in epoxy-carotenoids and apocarotenoids, which lost their biological activity (Ribeiro et al. 2018; Thakur et al. 2017).

One approach to prevent the degradation of carotenoids and preserve their antioxidant activities is to use encapsulation techniques, which not only improve their stability but also impact their bioavailability and

*Correspondence: ana.ortega@udlap.mx

³ Departamento de Ciencias de La Salud, Universidad de Las Américas Puebla, Santa Catarina Mártir S/N, Cholula, Puebla 72810, México
Full list of author information is available at the end of the article

solubility (Capelezzo et al. 2018). Encapsulation techniques consist of coating one or more sensitive substances (pigments, antioxidants, essential oils, and drugs) with another component that acts as a barrier and protects them from environmental conditions (Gul et al. 2015).

Microencapsulation involves covering a liquid, solid, or gaseous substance with a surrounding material (wall material) (Janiszewska-Turak 2017). Spray-drying is the most commonly used technique in the food industry for microencapsulation due to equipment availability and low production costs, especially when compared to other encapsulation techniques (Corrêa-Filho et al. 2019a, b; de Marco et al. 2013), such as freeze-drying (Capelezzo et al. 2018) coacervation (Soukoulis and Bohn 2018), drum-drying (Desobry et al. 1997) or extrusion (Rehman et al. 2020). Spray-drying consists of the formation of fine particles by passing a suspension through a sprayer in which compressed air heated to high temperatures flows. The flow of hot air causes particle dehydration and powder formation within which a substance is encapsulated (Gul et al. 2015). This allows obtaining stable powders with sizes ranging between 1 and 1000 μm at low cost and short production period using relatively low temperatures, enabling the encapsulation of thermolabile compounds and enhancing the stability of encapsulated substances (Mestry et al. 2011). Several researchers have employed the spray-drying technique for microencapsulation of various carotenoids, for example, astaxanthin (Pu et al. 2011; Shen and Quek 2014), β -carotene (Corrêa-Filho et al. 2019a, b; Desobry et al. 1997; Lim and Roos 2016; Przybysz et al. 2018, 2016; Shaaruddin et al. 2019), canthaxanthin (Hojjati et al. 2014), lutein (Lim and Roos 2016; Wang et al. 2012), and lycopene (Corrêa-Filho et al. 2019; Shu et al. 2006). They found that their stability improved under different storage conditions.

The stability and retention time of a pigment during microencapsulation and storage strongly depend on the wall material type employed to form the particles. Arabic gum (AG) is an effective carrier because of its high water solubility, low solution viscosity, and ability to form a protective film around the particles of the pigment (Przybysz et al. 2018). Maltodextrin (MD) also can be applied as a coating material at lower costs instead of AG, which constitutes an effective anti-oxygen barrier, have low viscosity, and is available in different average molecular weights (Desobry et al. 1997; Przybysz et al. 2018). Mesquite gum (MG), a natural exudate from the mesquite tree, is a neutral salt of a complex acidic branched polysaccharide, which has been reported as a good encapsulating agent (EA) (Beristain et al. 1999; Jiménez-Aguilar et al. 2011). However, the use of mixtures of AG and MD (de Marco et al. 2013; Przybysz et al. 2016) or MG and

MD (Beristain et al. 1999) are more effective than their individual use.

Mamey sapote (*Pouteria sapota*) is a tropical fruit of high nutritional value, native from Mexico and Central America, recognized as an alternative source of carotenoids and vitamin A (Moo-Huchin et al. 2014; Murillo et al. 2013; Yahia et al. 2011). The mamey fruit is characterized by its smooth texture, sweet flavor, and salmon-red pulp (Pinto et al. 2016). Therefore, the mamey is usually consumed fresh or as an ingredient in jam, ice cream, gelatin, yogurt, and desserts (Alia-Tejagal et al. 2007).

In this work, we aimed to evaluate the carotenoid retention and antioxidant activities of mamey (*Pouteria sapota*) and carrot (*Daucus carota*) carotenoids microencapsulated in mixtures of MD-AG and MD-MG stored at different temperature and light conditions. Moreover, the in vivo antioxidant activities of the carotenoids were evaluated in the nematode *Caenorhabditis elegans*. Microencapsulation of mamey and carrot carotenoid extracts by spray drying can result in stable powders of mamey and carrot carotenoids that preserve their antioxidant capacities, in vitro and in vivo, under different storage conditions.

Methods

Sample preparation

Mamey (*Pouteria sapota*) and carrot (*Daucus carota*) were purchased at local supermarket in the city of Puebla, Mexico. Mamey and carrot carotenoids were extracted based on the method described elsewhere (Moo-Huchin et al. 2014). Briefly, 10 g of mamey pulp or carrot were mixed with 50 mL of a hexane: acetone: ethanol solution (70: 15: 15 v/v/v) and stirred for 1 h. Afterwards, 5 mL of 40% KOH was added and incubated at room temperature in the dark for 2 h. Then, 30 mL of hexane was added, the mixture was shaken vigorously, the upper layer was collected, filtered through Na_2SO_4 powder to remove traces of water, and dissolved in ethanol. Mamey (10.70 ± 0.20 mg β -carotene/100 g fresh weight) or carrot (13.83 ± 0.23 mg β -carotene/100 g fresh weight) carotenoid solutions were mixed with a combination of MD DE 10 (CP Ingredients, Mexico) and AG (CP Ingredients, Mexico) or MG (Gomas Naturales, Mexico), as indicated in Table 1.

Spray-drying

Drying of all solutions was performed in a laboratory-scale spray-dryer (Büchi B-290, Switzerland) using an aspersion nozzle diameter of 150 μm , inlet temperature of 160 $^\circ\text{C}$, and feeding flow rate of 10 mL/min. Outlet temperatures and total soluble solids (TSS) are shown in Table 1. The collected powders were weighted, stored in

Table 1 Carotenoid solutions and conditions for spray-drying

Code	Maltodextrin (w/v) (%)	Arabic gum (w/v) (%)	Mesquite gum (w/v) (%)	TSS (°Brix)	Outlet temperature (°C)
M _{AG} 5	10	5	–	15.2	62
M _{AG} 10	10	10	–	19.1	63
C _{AG} 5	10	5	–	15.8	61
C _{AG} 10	10	10	–	18.6	60
M _{MG} 5	10	–	5	14.6	87
M _{MG} 10	10	–	10	20.2	87
C _{MG} 5	10	–	5	14.9	82
C _{MG} 10	10	–	10	20.1	81

M: mamey, C: carrot, AG: Arabic gum–maltodextrin, MG: Mesquite gum–maltodextrin

glass bottles, sealed in plastic bags, and kept at room temperature to prevent water intake until further analysis.

Characterization of microcapsules

Particle size

The particle diameter of each powder was measured with an automatic particle analyzer (Microtrac Blue-wave S3500, USA). Determinations were performed in duplicate.

Color

The color was determined from the CIELab scale parameters L^* (lightness), a^* (redness), and b^* (yellowness) using a Konica Minolta colorimeter (CR-400, Konica Minolta S.A., Germany) with a 1-cm path length of the optical glass cell (10 mL of the sample). Solutions of each powder were prepared by dissolving 2.70 g of each carotenoid powder in 10 mL water (Jiménez-Aguilar et al. 2011). Before the determinations, the colorimeter was calibrated with a white pattern. Additionally, the net color change (ΔE) was calculated from Eq. 1. Determinations were performed in triplicate.

$$\Delta E = \sqrt{(L^\circ - L^*)^2 + (a^\circ - a^*)^2 + (b^\circ - b^*)^2} \quad (1)$$

Moisture content and water activity

To determine the moisture contents of the powders, the gravimetric method AOAC 930.15 (2000) was used. Samples were dried at 105 °C for 24 h inside an oven. The water activities were measured at room temperature using a dew-point hygrometer (AQUA LAB, 4TEV,

Decagon Devices, Inc., USA). Determinations were performed in triplicate.

Density

Bulk density was calculated by weighing 2 g of each powder and measuring its volume in a 10-mL test tube. Compacted density was determined by measuring the volume occupied by the same mass of powder in the test tube after 425 hits. Additionally, Hausner's index (HR) was determined using Eq. 2. Determinations were performed in triplicate.

$$\text{Hausner's index} = \frac{\text{Compacted density}}{\text{Bulk density}} \quad (2)$$

Water solubility

200 mg of each powder was dissolved in 10 mL distilled water. The mixture was centrifuged at 760 × g for 10 min (Centrifuge Z 366 K, HERMLE Labortechnik, Germany), then 9 mL of the suspension was removed, and the residue was dried at 105 °C for 24 h (Montero et al. 2016). Water solubility was calculated using Eq. 3. Determinations were performed in triplicate.

$$\text{Solubility (\%)} = \frac{\text{Final weight} \times \left(\frac{10}{9}\right)}{\text{Initial weight}} \quad (3)$$

Carotenoid content and antioxidant activity

Carotenoid solutions were prepared by dissolving 1.35 g of each carotenoid powder in 5 mL distilled water (Jiménez-Aguilar et al. 2011). The absorbances of prepared solutions were analyzed at 450 nm (Genesys 10S UV–Vis spectrophotometer, Thermo Fisher Scientific, USA), using MD–AG or MD–MG solutions as blanks. A calibration curve was prepared using β -carotene (0–20 ppm) (purity > 97%, Sigma-Aldrich, Mexico) in ethanol using pure ethanol as the blank. Results were expressed as $\mu\text{g } \beta\text{-carotene/g powder}$. Determinations were performed in triplicate.

The antioxidant capacities of carotenoid extracts were determined by the method developed by Re et al. (1999) with some modifications. The ABTS^{•+} radical cations were produced by reacting 7 mM ABTS ([2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]; Sigma-Aldrich, Mexico) with 2.45 mM K₂S₂O₈ and allowing the mixture to stand at room temperature in the dark for 16 h. Then, the solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm (Genesys 10S UV–Vis spectrophotometer, Thermo Fisher Scientific, USA) and recorded as the initial absorbance. Then, 980 μL diluted ABTS^{•+} was mixed with 20 μL carotenoid microcapsule solutions, and the absorbance was measured after 6 min. The percentage of inhibition was calculated by Eq. 4:

$$\text{Inhibition(\%)} = \left(\frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \right) \times 100\% \quad (4)$$

A calibration curve was prepared by dissolving Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich, Mexico) in ethanol. Trolox solutions (0–1500 μM) were subjected to the same treatment with ABTS^{•+}, as previously described, using pure ethanol as the blank. Results were expressed as μmole of Trolox equivalents antioxidant capacity (TEAC)/g powder. Determinations were performed in triplicate.

In vivo antioxidant activity assay

The biological model used was a wild (Bristol N2) *C. elegans* strain fed with bacteria, uracil auxotroph, *Escherichia coli* OP50. Both organisms were obtained through the Department of Biological and Chemical Sciences of Universidad de las Américas Puebla.

To examine intracellular reactive oxygen species (ROS) production, adult nematodes were synchronized to obtain eggs (Surco-Laos et al. 2011), which were then placed in nematode growth medium (NGM) plates supplemented with carotenoid microcapsules (0 and 15 $\mu\text{g}/\text{mL}$), and they were incubated at 22 ± 2 °C until reaching the L3 stage. Upon reaching the L3 stage, 15 nematodes were transferred to new NGM plates with 100 μM 5-fluoro-2'-deoxyuridine (FUdR; Sigma-Aldrich, Mexico) to prevent progeny production and the pro-oxidant 150 μM 5-hydroxy-1,4-naphthoquinone (juglone; Sigma-Aldrich, Mexico) to induce oxidative stress for 12 h. Upon reaching the adult stage, nematodes were transferred into 200 μL M9 buffer containing 250 μM 2',7'-dichlorofluorescein diacetate (DCFH₂-DA; Sigma-Aldrich, Mexico) and were incubated in the dark for 1.5 h. Then, nematodes were placed in 50 μL M9 buffer containing 10 mM sodium azide for anesthesia, transferred into a thin dried agarose pad above a glass slide, and were mounted onto the fluorescence microscope (Zeiss Axio Imager 2, Jena, Germany) for capturing the fluorescence of the oxidized product DCF (Ren et al. 2017). To quantify DCF fluorescence, images were analyzed using ImageJ software.

Storage conditions

Carotenoid microcapsules were stored in Petri dishes (55 × 10 mm) inside polystyrene bags. They were kept at 25 °C in the presence of light and at 4 and 25 °C in the absence of light (Jiménez-Aguilar et al. 2011). Changes in color, carotenoid content, and antioxidant activities

of carotenoid microcapsules were analyzed in triplicate at the beginning, first, second, and third month of storage, whereas their in vivo antioxidant activities were determined at the beginning and end of storage.

Statistical analysis

Data were evaluated using ANOVA and means comparison routines (Fisher, $p < 0.05$), using Minitab Statistical Software (19th version, Minitab Inc., USA).

Results

Characterization of microcapsules

The yields obtained for mamey and carrot carotenoid solutions were 74% (M_{AG5}), 85% (M_{AG10}), 74% (C_{AG5}), 75% (C_{AG10}), 69% (M_{MG5}), 76% (M_{MG10}), 72% (C_{MG5}) and 77% (C_{MG10}). Slight differences in the yield of the eight systems were observed; however, higher yields were achieved with the MD–AG mixture. Regardless of the composition of the wall material, the encapsulation of carotenoids was achieved. Mamey microcapsules showed a salmon-pink color, while carrot microcapsules showed a yellow color. Carotenoid microcapsules (MD–AG) obtained by spray-drying are shown in Fig. 1. Table 2 shows the physical characteristics of mamey and carrot carotenoid microcapsules.

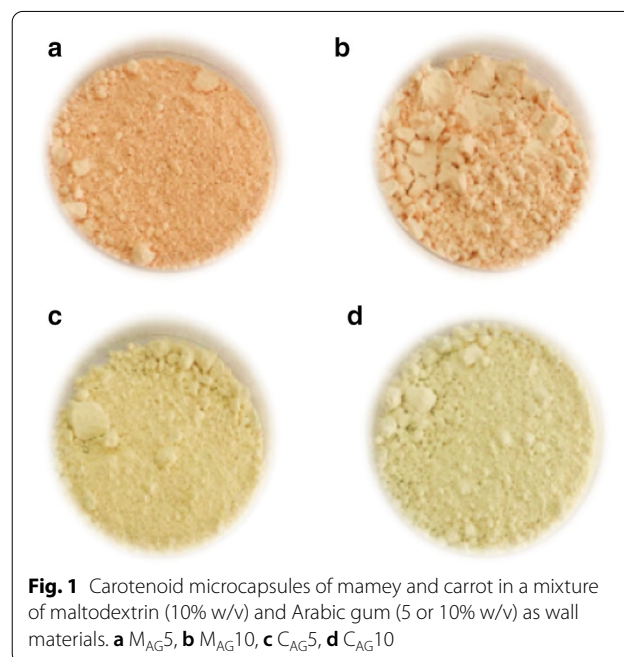


Fig. 1 Carotenoid microcapsules of mamey and carrot in a mixture of maltodextrin (10% w/v) and Arabic gum (5 or 10% w/v) as wall materials. **a** M_{AG5} , **b** M_{AG10} , **c** C_{AG5} , **d** C_{AG10}

Table 2 Characterization of mamey and carrot carotenoid microcapsules

Code	D_{50} (μm)	a_w	Moisture (%)	Solubility (%)	Bulk ρ (kg/m^3)	Compacted ρ (kg/m^3)	HR
M_{AG5}	2.86	0.207 ± 0.004^a	2.92 ± 0.13^e	87.82 ± 0.93^b	233 ± 8^a	365 ± 28^a	1.57
M_{AG10}	3.09	0.150 ± 0.006^b	2.58 ± 0.34^e	91.09 ± 2.96^a	215 ± 14^a	347 ± 23^a	1.61
C_{AG5}	3.09	0.201 ± 0.018^a	2.75 ± 0.10^e	86.64 ± 0.32^b	188 ± 10^b	286 ± 1^b	1.52
C_{AG10}	3.25	0.134 ± 0.004^b	2.74 ± 0.63^e	90.46 ± 3.15^a	187 ± 16^b	342 ± 6^a	1.83
M_{MG5}	2.94	0.312 ± 0.008^a	6.98 ± 0.08^a	85.85 ± 1.21^b	229 ± 18^a	370 ± 19^a	1.61
M_{MG10}	3.15	0.211 ± 0.018^c	5.85 ± 0.17^c	94.45 ± 3.39^a	210 ± 11^a	350 ± 24^a	1.67
C_{MG5}	3.12	0.292 ± 0.009^a	6.53 ± 0.28^b	83.63 ± 2.33^b	191 ± 10^b	290 ± 9^b	1.52
C_{MG10}	3.31	0.237 ± 0.015^b	4.88 ± 0.07^d	92.07 ± 4.60^a	175 ± 7^b	334 ± 7^a	1.91

M: mamey, C: carrot, AG: Arabic gum–maltodextrin, MG: Mesquite gum–maltodextrin. Different letters show significant differences ($p < 0.05$) between microcapsules within each parameter

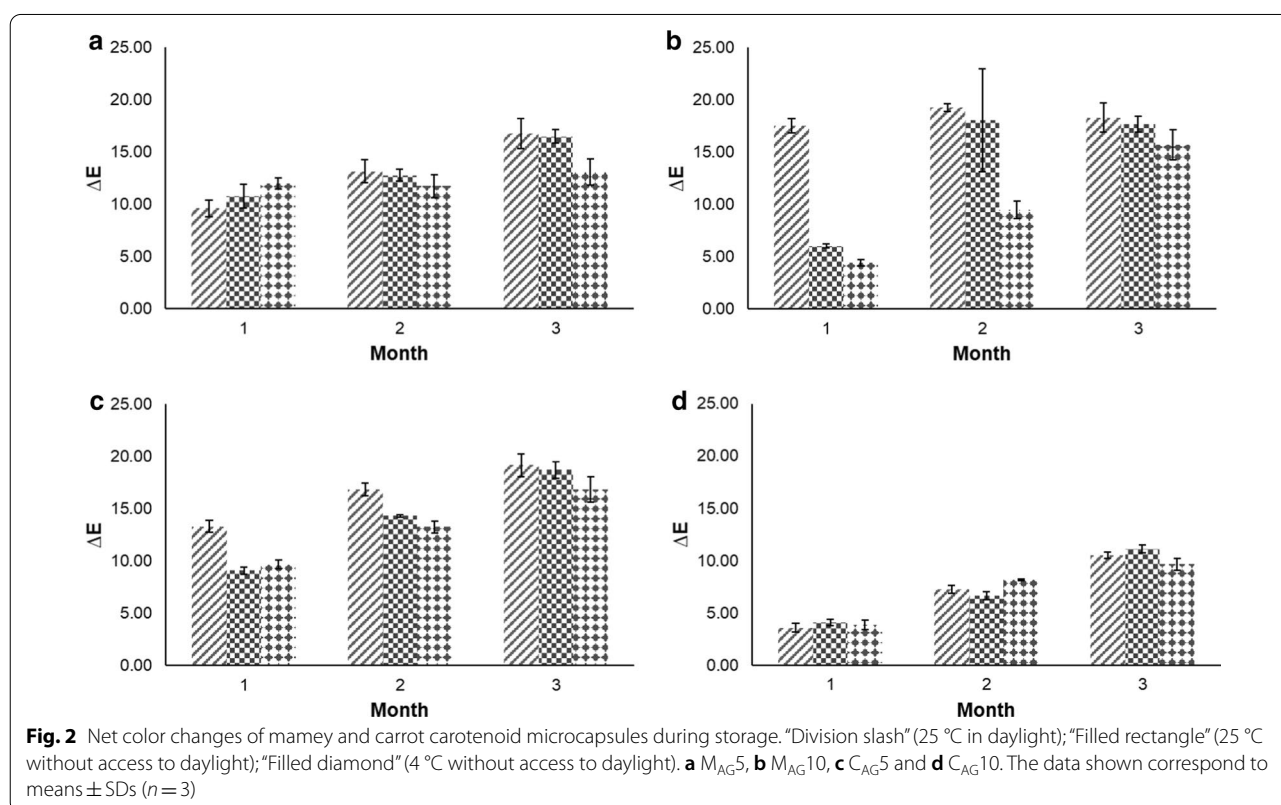


Figure 2 shows color changes of carotenoid powders during storage. A value of $\Delta E > 5$ is indicative of a significant and visually noticeable change from the initial color of powders. Thus, ΔE values close to or above 5 correspond to powders stored at 25 °C in daylight as these conditions accelerate the carotenoid (see Sect. 3.2.) and color losses ($p < 0.05$). Notably, powders stored in refrigeration (4 °C) showed minor color changes during storage. Moreover, L^* increased and a^* (mamey microcapsules)/ b^* (carrot microcapsules) decreased due to carotenoid losses as the storage temperature and period increased.

Carotenoid contents and antioxidant activities of microcapsules

The combination of MD–AG as EAs resulted in microcapsules with 23.06 ± 0.26 (M_{AG5}), 36.69 ± 0.38 (M_{AG10}), 62.68 ± 0.25 (C_{AG5}), and 73.50 ± 0.24 (C_{AG10}) μg β -carotene/g powder, whereas the combination of MD–MG led to minor carotenoid contents, 19.62 ± 0.52 (M_{MG5}), 16.06 ± 0.52 (M_{MG10}), 20.34 ± 0.48 (C_{MG5}) and 15.53 ± 0.40 (C_{MG10}) μg β -carotene/g powder, due to higher outlet temperatures (Table 1). The in vitro antioxidant activities of the MD–AG microcapsules

were 51.11 ± 4.30 (M_{AG5}), 49.08 ± 2.21 (M_{AG10}), 60.06 ± 1.52 (C_{AG5}), and 61.53 ± 2.74 (C_{AG10}) TEAC/g powder, whereas for the MD–MG microcapsules were 24.07 ± 3.42 (M_{MG5}), 22.08 ± 2.79 (M_{MG10}), 30.58 ± 2.48 (C_{MG5}) and 31.23 ± 2.96 (C_{MG10}) TEAC/g powder.

Cooler outlet temperatures such as the ones obtained for MD–AG microcapsules (Table 1) were more effective for the protection of carotenoids, leading to higher retention rates. For this reason, the storage studies and in vivo antioxidant activities were performed on only MD–AG microcapsules. Figure 3 shows the carotenoid contents of mamey and carrot microcapsules after three months of storage under different temperature and light conditions. The carotenoid contents in carrot powders were higher than those in mamey powders, and this is consistent with the data obtained from the extracts used for spray drying. Storage results showed that higher concentrations of EAs (10% MD and 10% AG) slowed down the carotenoid losses compared to the lower concentrations of EAs (10% MD and 5% AG) under the same storage conditions. In contrast, cooler storage temperatures reduce the rate of carotenoid degradation. At the end of the storage at 4 °C, almost 70% (M_{AG5} and C_{AG5}) and 80% (M_{AG10} and C_{AG10}) of carotenoids were preserved, showing that the

use of MD and AG as EAs was effective ($p < 0.05$) to protect the carotenoids from degradation.

Figure 4 shows the in vitro antioxidant activities of mamey and carrot microcapsules after three months of storage under different temperature and light conditions. The decrease in the antioxidant activities follows the same trend that the powders exhibit for the carotenoid losses. Cooler temperatures, lack of daylight, and higher concentrations of EAs resulted in minor antioxidant activity losses.

Figure 5 shows the ROS accumulation in the nematodes treated with MD–AG microparticles, expressed as the percentage of fluorescence relative to that of the control (nematodes not exposed to microparticles). At the beginning, nematodes treated with the carotenoid powders significantly decreased their intracellular ROS levels following exposure to juglone ($p < 0.05$). The ROS in the nematodes treated with M_{AG5} ($76.63 \pm 15.79\%$) and C_{AG5} ($80.78 \pm 8.12\%$) were significantly higher ($p < 0.05$) than those in the nematodes treated with M_{AG10} ($70.31 \pm 12.06\%$) and C_{AG10} ($70.71 \pm 8.06\%$); but in all cases, the ROS accumulation decreased ($p < 0.05$) due to the presence of antioxidants when compared to untreated nematodes. Moreover, these results were consistent with

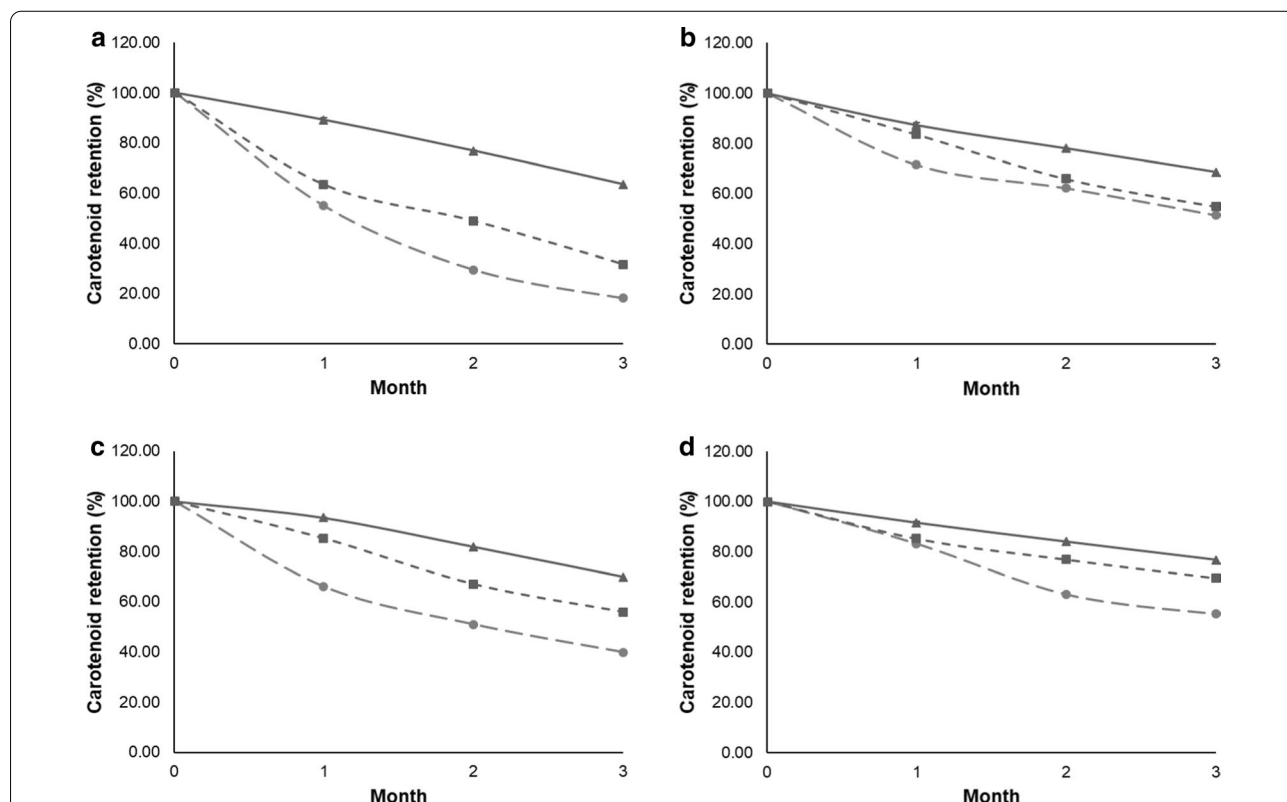
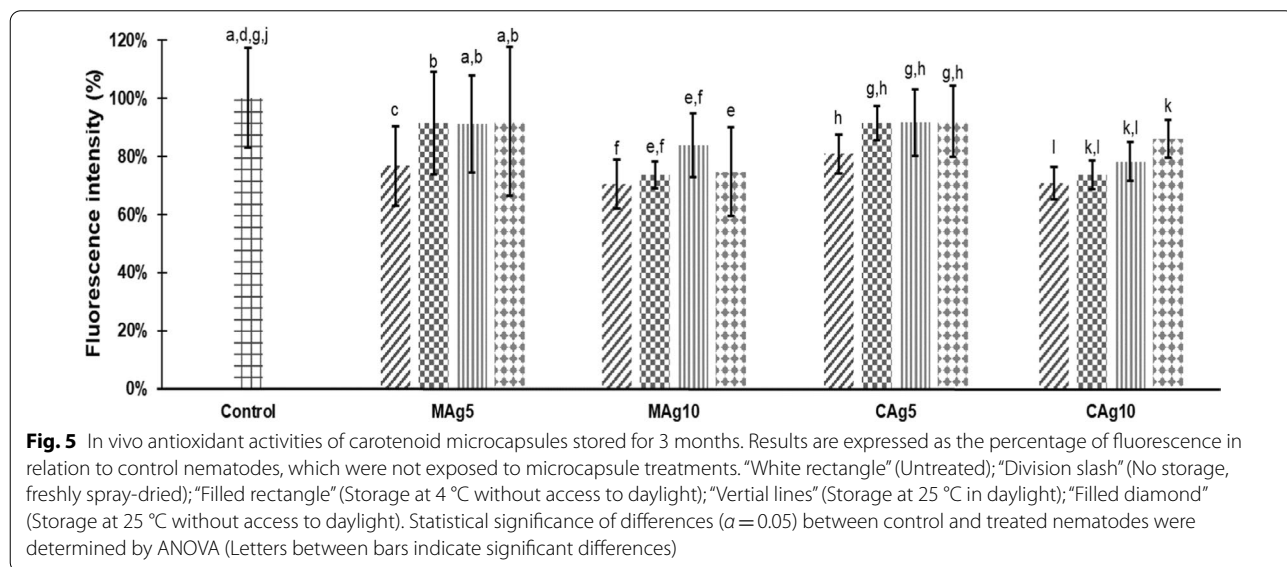
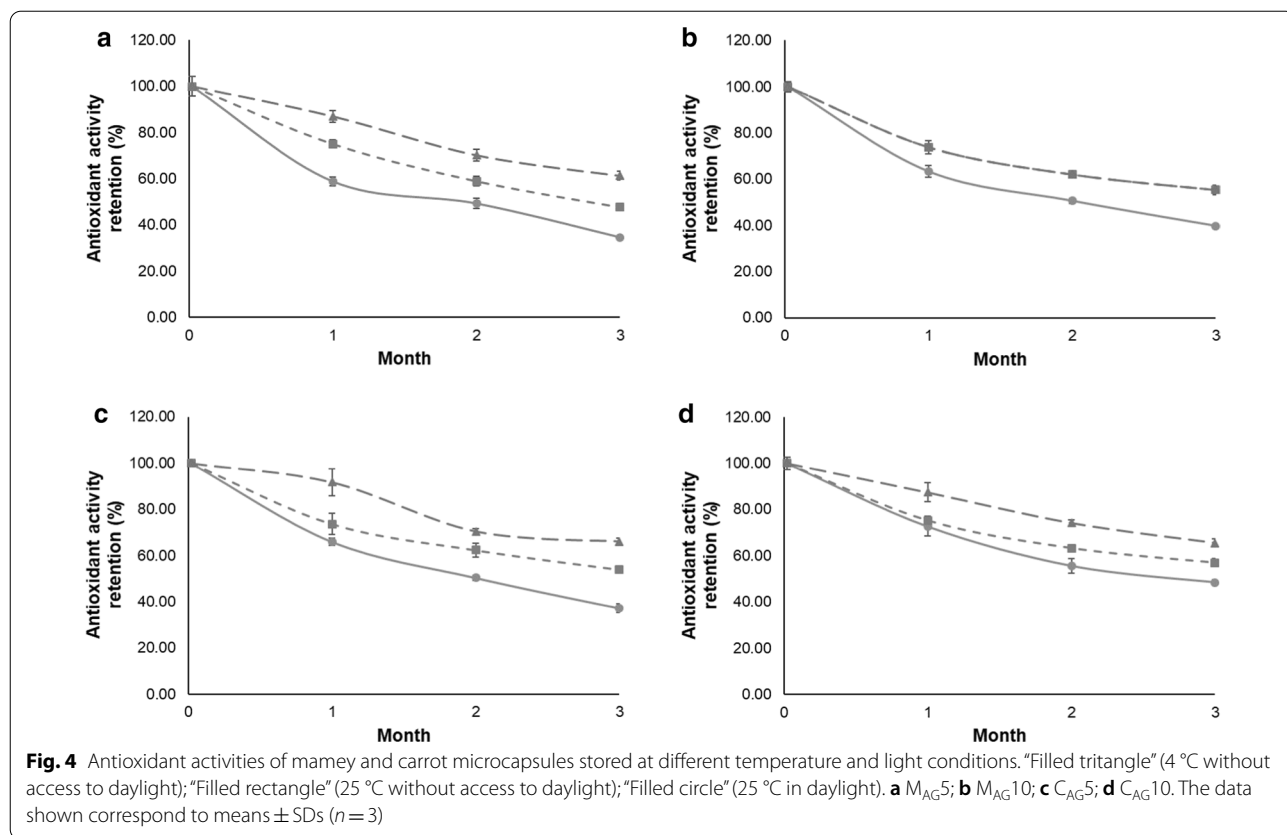


Fig. 3 Carotenoid contents of mamey and carrot microcapsules stored at different temperature and light conditions. “Filled triangle” (4 °C without access to daylight); “Filled rectangle” (25 °C without access to daylight); “Filled circle” (25 °C in daylight). **a** M_{AG5} ; **b** M_{AG10} ; **c** C_{AG5} ; **d** C_{AG10} . The data shown correspond to means \pm SDs ($n = 3$)



previous in vitro antioxidant activity results, showing that a higher concentration of EAs provided better protection to the carotenoids and their antioxidant activities.

After storage for three months, the antioxidant activities of M_{AG}5 and C_{AG}5 microcapsules decreased significantly ($p < 0.05$) compared to their initial activities,

and no significant differences ($p > 0.05$) were obtained between them and control nematodes due to major ROS accumulation in the nematodes, a phenomenon caused by the carotenoid loss upon storage (Fig. 3). On the other hand, no significant differences ($p > 0.05$) were found between M_{AG}10 and C_{AG}10 treatments, regardless

of the storage conditions (25 °C in daylight, 25 °C without access to daylight, and 4 °C without access to daylight) or their antioxidant activities at the beginning of the storage, despite the carotenoid (Fig. 3) and *in vitro* antioxidant activity (Fig. 4) losses. There were significant differences ($p < 0.05$) between control nematodes and the ones treated with M_{AG10} and C_{AG10} microcapsules, thus reducing the ROS accumulation. Moreover, these results suggest that mamey carotenoids possess an *in vivo* antioxidant activity similar to that of carrot carotenoids.

Discussion

Physical properties of microcapsules

Both the particle size (2.86–3.31 μm) and Hausner's index (1.52–1.91) indicate class "C" cohesive powders from Geldart's classification (1973). This powder type tends to form agglomerates of random size and shape due to interparticle forces between particles that affect their flow properties (Turki and Fatah 2008). The particle sizes of MD-AG (2.86–3.25 μm) and MD-MG (2.94–3.31 μm) microcapsules were smaller than the ones obtained by Faria et al. (2010) for β -carotene in MD (7.1 μm) or AG (10.7 μm), and by Hojjati et al. (2014) for canthaxanthin in soluble soybean polysaccharide (7.94 μm), AG (9.08 μm), or MD (10.42 μm). These differences are likely related to the temperatures used for spray-drying. Both authors used a temperature of 170 °C, whereas in this work 160 °C was used. In all cases, the microparticles tend to stick together through Van der Waals forces given that they have a larger contact surface, which increases particle size. These cohesion forces increase along with the temperature, leading to the formation of agglomerates (Esmailpour et al. 2015).

Moisture and water activity values of M_{AG5} , M_{MG5} , C_{AG5} , and C_{MG5} were higher ($p < 0.05$) compared to those of M_{AG10} , M_{MG10} , C_{AG10} , and C_{MG10} , as M_{AG5} , M_{MG5} , C_{AG5} , and C_{MG5} contain less amount of EAs and more water. Moreover, M_{AG10} , M_{MG10} , C_{AG10} , and C_{MG10} microcapsules were more soluble in water ($p < 0.05$) as they can attract more water due to the increased interaction between particles and water molecules (Juscamita Fabián et al. 2017). Moreover, de Marco et al. (2013) showed that bixin microencapsulation in MD-AG increased their water solubility by 72%. The increased solubility of carotenoids resulted in water-soluble powders, which can be used in aqueous systems, contrary to nonencapsulated carotenoids that are water-insoluble.

Carotenoid contents and antioxidant activities of microcapsules

The results of MD-AG microcapsules showed that the carotenoid contents in the carrot powders were significantly higher ($p < 0.05$) compared to the mamey powders.

These results are expected since the carotenoid contents in mamey are lower than in carrot (Alia-Tejacal et al. 2007). Moreover, it is evident from the carotenoid extracts that the content in carrot (13.83 ± 0.23 mg β -carotene/100 g fresh weight) is higher than in mamey (10.70 ± 0.20 mg β -carotene/100 g fresh weight). Nevertheless, Moo-Huchin et al. (2014) and Murillo et al. (2013) considered the mamey as an alternative source of carotenoids to the carrot, despite their differences in carotenoid contents.

The carotenoid contents in mamey (23.06–36.69 μg β -carotene/g powder) and carrot (62.68–73.50 μg β -carotene/g powder) powders were superior to the one obtained by Pu et al. (2011) for astaxanthin microcapsules (13.76 $\mu\text{g/g}$ powder) in sodium caseinate-lactose (1:1). The stability and retention of carotenoids during microencapsulation depend on the wall material type employed to form the particles (Przybysz et al. 2018). Desobry et al. (1997) found that the spray-drying and encapsulation process with MD degraded 11% of β -carotene due to the high temperature employed in the drying process. Moreover, Wang et al. (2012) reported that the blend of starch and gelatin helped to retain 80% of the pre-drying lutein content in the microcapsules after spray-drying. Although high temperatures are used in the spray-drying process, microcapsules help to prevent extensive losses of carotenoids and their antioxidant activities (Aissa et al. 2012; Thakur et al. 2017).

The differences between the outlet temperatures of AG and MG systems are consistent with several studies, which indicate that the temperatures of GM microcapsules are higher than those of AG microcapsules. The temperatures of MG microcapsules vary from 80 °C (Carrillo-Navas et al. 2011; Jiménez-Aguilar et al. 2011), 95 °C (Jiménez-Aguilar et al. 2011), and 104 °C (Ochoa-Velasco et al. 2017), while the temperatures of AG microcapsules go from 70 °C (Bastías-Montes et al. 2019) to 80 °C (Bednarska & Janiszewska-Turak 2020).

The carotenoid losses increased as the storage temperature increased. Hojjati et al. (2014) and Lim and Roos (2016) reported that the carotenoid levels (canthaxanthin and β -carotene/lutein, respectively) in microcapsules decreased as the storage temperature increased. Similarly, Pu et al. (2011) reported that the astaxanthin content in astaxanthin microcapsules decreased by 26.17% (5 °C) and 39.83% (25 °C) after 26 days of storage. Furthermore, Desobry et al. (1997) and Przybysz et al. (2018) reported that β -carotene contents decreased 70% (15 weeks at 25 °C) and 54% (12 weeks at 20 °C) after storage.

Carotenoids are sensible to heat, oxidation, and light because of their unsaturated structure; therefore, they gradually degrade during storage (Pu et al. 2011). The

results in Fig. 3 showed that daylight speeded up the degradation of carotenoids stored at 25 °C when compared to carotenoids stored at the same temperature without access to daylight. These results are consistent with color losses and major color changes ($\Delta E > 5$) shown in Fig. 2. Hojjati et al. (2014) reported that canthaxanthin retention values in soluble soybean polysaccharide, AG, and MD stored at 25 °C for 16 weeks with access to daylight were lower (58, 53, and 26%, respectively) when compared to powders without access to daylight (75, 72, and 57%, respectively).

The decreased antioxidant activities of the powders were closely related to the losses of carotenoids due to carotenoid oxidation and double bond losses (Jiménez-Aguilar et al. 2011), which are essential to maintain their antioxidant activities. However, the antioxidant activities of M_{AG}10 microcapsules stored at 4 and 25 °C without access to daylight did not show significant differences ($p > 0.05$). This is interesting because under the same storage conditions, significant differences ($p < 0.05$) were observed between the carotenoid contents of M_{AG}10 microcapsules. Although the carotenoid contents in the microcapsules decreased, it is possible that a synergic effect exists between its components allowing to maintain its antioxidant activities during these storage conditions. This tendency was not observed in the rest of the microcapsules (M_{AG}5, C_{AG}5 and C_{AG}10).

ROS measurement

The fluorescent probe DCFH₂-DA is commonly used to detect fluorescence linked to ROS production in in vitro and in vivo assays. DCFH₂-DA can easily penetrate the cellular membrane and is enzymatically deacetylated by esterases. This reaction converts DCFH₂-DA into the nonfluorescent compound DCFH₂, which is then rapidly oxidized to highly fluorescent DCF in the presence of ROS (Ren et al. 2017; Yoon et al. 2018). Since *C. elegans* can undergo experimental oxidative stress conditions upon exposure to certain pro-oxidant compounds (Zhou et al. 2011) leading to increased ROS levels, this characteristic allowed us to investigate the in vivo antioxidant capacity of carotenoid powders as the in vitro and in vivo efficacies of antioxidants could differ (You et al. 2015).

Previously, we investigated the antioxidant activities of mamey and carrot carotenoid extracts (20–40 µg/mL) and found that both increased the nematode survival by 20% to 40% (González-Peña et al. 2021). The effect of carotenoids on nematode survival has been well documented by Lashmanova et al. (2015), Pons et al. 2014, and You et al. (2015). Moreover, the protective effect of the carotenoids in *C. elegans* is related to their capacity to neutralize the ROS production and trigger the antioxidant defenses (Yazaki et al. 2011; You et al. 2015). In

this work, mamey and carrot carotenoid microcapsules (15 µg/mL) could neutralize the ROS production up to 30%, especially in the nematodes treated with M_{AG}10 and C_{AG}10 when compared to nontreated nematodes. These results reveal the antioxidant potential of mamey and carrot carotenoids on living organisms through their capacity to quench singlet oxygen and deactivate free radicals, thus decreasing the ROS levels, and possibly by activating the antioxidant defenses such as superoxide dismutase, catalase, and glutathione peroxidase, modulating transcriptional factors and signaling pathways, and reducing the mitochondrial ROS production (Liu et al. 2016; Pons et al. 2014; Saini et al. 2015; Yazaki et al. 2011; You et al. 2015).

The in vivo antioxidant activities of different antioxidants, which are responsible for decreased ROS production induced through oxidative stress, have been investigated. Chen et al. (2013) found that resveratrol (200 µM) decreased ROS production by 40%, indicating both the antioxidant activity of resveratrol and the nematode's capacity to incorporate the antioxidant into its metabolism to protect it against oxidative stress damage. Similarly, You et al. (2015) found decreased levels of fluorescence due to the use of carotenoids BTS (58%) and β-carotene (4%). Moreover, Liu et al. (2016) and Yazaki et al. (2011) reported that astaxanthin decreased the mitochondrial ROS levels (40%) after exposure to the carotenoid. Lee et al. (2015) showed that vixetin (100 µM) decreased ROS levels by 15%, leading to increased resistance against oxidative stress. On the other hand, Jara-Palacios et al. (2013) found that nematodes fed with pomace extract (300 µg/mL) reduced their ROS production by 30%. In contrast, water-soluble antioxidants such as betalains (phenylethylamine-betaxanthin and indicaxanthin) have been proved to be highly effective in the nematode's protection against oxidative stress, achieving up to 90% reduction in ROS production at the maximum concentration evaluated (100 µM) (Guerrero-Rubio et al. 2019). These results are consistent with our findings, showing that mamey and carrot carotenoids can effectively reduce the intracellular ROS levels and neutralize the oxidative stress damage of nematodes. Moreover, the in vivo antioxidant capacities of mamey and carrot carotenoids were equally effective to neutralize the oxidative stress damage, suggesting that mamey carotenoids are a good alternative to carrot carotenoids.

Conclusion

The combination of MD–AG provided successful encapsulation of mamey and carrot carotenoids by the spray-drying technique, whereas the combination of MD–MG was ineffective to properly encapsulate the carotenoids. In addition, the use of MD and AG at

equal concentrations (1:1) was adequate to the encapsulation and retention of carotenoids. The decreasing order of the carotenoid content and antioxidant activity losses of stored carotenoids was 25 °C in daylight > 25 °C without access to daylight > 4 °C without access to daylight. The encapsulation of carotenoids prevented some losses of the initial carotenoid contents and antioxidant capacities in MAG10 (40–70%) and CAG10 (50–80%) microcapsules, after three months of storage regardless of the light and temperature conditions. Mamey and carrot carotenoid microcapsules showed the capacity to reduce the oxidative stress damage in *C. elegans*. Regardless the overall loss of carotenoids and decreased antioxidant activities in all storage conditions, the spray-dried carotenoids could counteract ROS production in nematodes by 30%. Furthermore, these results revealed the potential of mamey as an alternative source of carotenoids, whose antioxidant capacity is on par with that of carrot.

Abbreviations

ΔE: Net color change; *a**: Redness; ABTS: 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); AG: Arabic gum; *b**: Yellowness; BTS: 13,13'-*p*-Tolyl β-carotene; C_{AG}10: Carrot microcapsules with maltodextrin (10%) and Arabic gum (5%); C_{AG}5: Carrot microcapsules with maltodextrin (10%) and Arabic gum (5%); C_{MG}10: Carrot microcapsules with maltodextrin (10%) and Mesquite gum (10%); C_{MG}5: Carrot microcapsules with maltodextrin (10%) and Mesquite gum (5%); CONACYT: Consejo Nacional de Ciencia y Tecnología; DCFH₂-DA: 2',7'-Dichlorofluorescein diacetate; EA: Encapsulating agent; FUDR: 5-Fluoro-2'-deoxyuridine; HR: Hausner's index; *L**: Lightness; M_{AG}10: Mamey microcapsules with maltodextrin (10%) and Arabic gum (10%); M_{AG}5: Mamey microcapsules with maltodextrin (10%) and Arabic gum (5%); MD: Maltodextrin; MG: Mesquite gum; M_{MG}10: Mamey microcapsules with maltodextrin (10%) and Mesquite gum (10%); M_{MG}5: Mamey microcapsules with maltodextrin (10%) and Mesquite gum (5%); NGM: Nematode growth medium; SD: Standard deviation; ROS: Reactive oxygen species; TEAC: Trolox equivalents antioxidant capacity; TSS: Total soluble solids; UDLAP: Universidad de las Américas Puebla.

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Authors' contributions

MAGP performed the experiments, the literature research, data analysis, and wrote the manuscript. JDLR and AEOR designed and supervised the experiments, and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Author details

¹ Departamento de Ingeniería Química, Alimentos Y Ambiental, Universidad de Las Américas Puebla, Santa Catarina Mártir S/N, Cholula, Puebla 72810, México. ² Departamento de Ciencias Químico-Biológicas, Universidad de Las Américas Puebla, Santa Catarina Mártir S/N, Cholula, Puebla 72810, México. ³ Departamento de Ciencias de La Salud, Universidad de Las Américas Puebla, Santa Catarina Mártir S/N, Cholula, Puebla 72810, México.

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