ISSN: 2347-467X, Vol. 12, No. (1) 2024, Pg. 462-470

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Improvement of Storage Stability of Lutein Contained in Arazá Pulp Through Microencapsulation Process

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Abstract

Arazá is an Amazonian fruit rich in carotenoids such as lutein, which has several biological properties. This paper evaluates the storage stability of lutein in microencapsulated arazá pulp by spray drying with maltodextrin as wall material. The physicochemical properties of the pulp and microencapsulation, encapsulation efficiency, percentage yield, thermal stability (DTG and TGA), morphology, particle size, storage stability and lutein degradation kinetic were evaluated. The results were low Aw (0.33), good yield (64.22%), spherical and smooth particles, the lutein degradation kinetic with a degradation constant of 1.49 × 10-4 days-1, which is thirty-five times lower than that obtained for the freeze-dried arazá pulp used as control. In conclusion, it was possible to obtain stable microencapsulates, being a promising alternative for the preservation of this perishable fruit.

Article History

Received: 20 March 2024

Accepted: 17 April 2024

Keywords

Bioactive Compound;
Carotenoids;
Eugenia Stipitata;
Spray Drying;
Microencapsulate.

Introduction

Arazá (*Eugenia stipitata*) is an evergreen tree of the Myrtaceae family which grows in the Amazon region of Brazil, Peru, Ecuador and Colombia.[1] It has a high-perishable, oval shape and thin skin yellow fruit. Its pulp is aromatic and acidic,[2] it is rich in ascorbic acid, malic acid, terpenes, phenolic compounds, flavonoids,[3,4] and carotenoids,[5] these bioactive compounds provide it antioxidant and other biological properties.[4] Among its carotenoids, zeaxanthin, β-carotene, α-carotene and lutein have been found, being lutein with the highest concentration, with 154 µg/100 g.[5]

Lutein, a yellow pigment classified as a xanthophyll, has been shown to have antioxidant and anti-inflammatory activities,[6–8] neuroprotective effects[9] and anti-tumor properties.[10] Furthermore, therapeutic properties have been attributed to lutein such as: treatment of diseases related to the optic nerve, macular degeneration, retinal diseases.[6,11] However, this metabolite, as the same as other carotenoids,

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Doi: https://dx.doi.org/10.12944/CRNFSJ.12.1.37

is sensitive to heat, light, pH and oxidation conditions,[12] the high number of C=C double bonds in its structure confers it little stability and easy degradation, weakening or even losing its properties.[13] In order to preserve the bioactivity of this fruit, different conservation methods, such as microencapsulation, are being sought.

In spray drying microencapsulation, a mixture of an interest matrix and a wall material is atomized and hot air is used to remove water from the mixture, leaving behind dry powder at the dryer's bottom.[14] Microencapsulates obtained by spray drying have several advantages, including minimum water activity, good reconstitution properties, and suitability for storage and transportation,[15] allowing it to be handled and incorporated into food systems. The composition of the wall material greatly influences the efficacy and stability of microencapsulation, the biopolymers such as maltodextrin with different dextrose equivalents are often used as encapsulating agents.[16] Some authors report the use of maltodextrin as a wall material to microencapsulate lutein, with a high yield and storage stability.[17–20] Arazá fruit pulp have been previously microencapsulated by spray drying,[21] resulting in powders with thermal and storage stability at temperatures between 20 and 40 °C, without becoming sticky. Others autors microencapsulated arazá by frezee drying with maltodextrin and gum Arabic, being maltodextrin the best for keeping the storage stability at 20 °C.[22] Likewise, it was reported[17] lutein microcapsules prepared with maltodextrin and sucrose by spray drying, obtaining storage stability at 23°C and high yields.

Considering the benefits of lutein, its low stability and its important concentration in arazá, a fruit with a great potential for obtaining functional foods, but with high perishability, this work aims to obtain microencapsulates from arazá pulp to assess storage stability of lutein into the powders, its physicochemical characterization, morphology and thermal stability.

Materials and Methods

Plant Materials

Ripe fruits of arazá (*Eugenia stipitata*) were acquired in local markets of Florencia, Caquetá located in southern Colombia. The peel and seeds were removed from fruits and homogenized with a blender. They were freeze-dried and ground to obtain a powder. The powder was sieved through 30 mesh and stored at -20 °C before use.

Microencapsulation

Microencapsulation process was carried out using the spray drying technique on laboratory scale. Labplant dryer (SD-6, London, United Kingdom), at an air flow rate of 4.3 mL/s with a 4-speed air compressor bar and a pump speed of 485 mL/h. The dimensions of the drying chamber were 1110 × 825 × 600 mm and a nozzle with an internal diameter of 2.0 mm was used. For this study, 120 °C as drying temperature was applied and composition of the equipment's feeding solution, composed of arazá pulp and maltodextrin, in proportion 1:1 were used according with the bibliography.[21,23] Finally, the microparticles were then sealed in airtight bags and kept in a desiccator at room temperature, regulated humidity, and dark conditions until they were subjected to additional examination.

Reagents

Lutein standard were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents such as methanol, acetonitrile, ethanol, dichloromethane, ethyl acetate, and high-performance liquid chromatography (HPLC) grade n-hexane were purchased from Merck KGaA, (Darmstadt, Germany). Maltodextrin (MD) [dextrose equivalent (DE) 20] and butylated hydroxyanisole (BHA), was procured from (Sigma-Aldrich, St. Louis, MO, USA).

Characterization of Microencapsulates

Moisture

Moisture was determined with 2 g of the arazá microencapsulate using an Halogen Moisture Analyzer (HC103 METTLER TOLEDO, Canada) at 110 °C. The determinations were made at 0 and 45 days; the analysis were carried out at temperatures between 18 and 25 °C, with a relative humidity of 70%.[24]

Water Activity, pH, and Total Soluble Solid Content

At 20 °C, the powders water activity (Aw) was measured using 1.0 g of powder by an Aqualab 3TE (Decagon Devices Pullman, WA, USA) apparatus. The solid soluble content (expressed in °Brix) and

pH, was measured TEC-5, Tecnal (Piracicaba, SP, Brazil) and potenciometer Atago HSR-500 (Tokyo, Japan), respectly.

Yield (Y)

The amount of maltodextrin and soluble solids in the pulp expressed as degrees Brix, 4.3 °Brix for arazá pulp, was used to determine the spray drying yield as a percentage. This was done by dividing the total solids content in the collected powders by the total solids in the feed solution. The equation (1) was used to express the yield of encapsulation (Y).

Y (%)=(Total solids content in the collected powders (g))/(Total solids in the feed solution (g))×100% ...(1)

Microencapsulates Morphology and Size Particle

The structure of the particles was examined using scanning electron microscopy (SEM). The samples were coated with graphite and observed using a Vega 3 SB microscope (Tesca, Czech Republic) under conditions of 20 kV and a vacuum of 0.009 Pa. The particle size determination was carried out using ImageJ® software (Version 1.54), at a scale of 200 µm.

Lutein Encapsulation Efficiency

The equation (2) was used to calculate the encapsulation efficiency (E), which was tested using the methodology documented in the literature.[20] Lutein concentration was assessed by HPLC as mentioned below.

E (%) = (Lutein content into the microencapsulate)/(Lutein content in the pulp before drying)×100% ...(2)

Determination of Lutein Analyses by HPLC-PDA

An UHPLC system equipped with SIL-30AC autosampler was used to analyze the lutein content. The column used for chromatographic separation was a YMC 30-CarotenoidTM (5 mm, 150 mm, 4.6 mm). The oven temperature was 20 °C. The mobile phase was composed of methanol (A) and acetone (B) at a flow rate of 0.8 mL/min. The gradient program was used to perform the elution: 20% B for 15 minutes, 20%-50% B for 15-20 minutes, 50%-20% B for 20-25 minutes, and 20% B for 25 to 40 minutes. The volume of injection was 15 µL and the wavelength of detection was 445 nm. The retention time and UV spectrum of the microencapsulated and lyophilized arazá were compared with the standard to identify lutein. The analysis was performed in triplicate. The data analysis was carried out using Labsolutions (LC/PDA) software (Shimadzu, Japan). The lutein content is expressed in milligrams per 100 g of sample.

Lutein Extraction

The lutein extraction procedure was performed according to previous studies with some modifications.[22,25] 100 mg of powder (freeze dried or microencapsulate) were weighed and 1000 µL of an organic mixture solution of hexane, ethanol and ethyl acetate (3:2:1) and 0.1% Butylated hydroxyanisole (w/v) were added. The mixture was then centrifuged at 10000 rpm for 5 min at 20 °C in a Sovall LYNX 6000 centrifuge from Thermo Fisher Scientific, Waltham, MA, USA. Then, 150 mL of 10% (w/v) NaCl was added to the organic phase. The aqueous phase of the extraction solution became colorless following this procedure. Each phase was dried using amber vials at a sample concentrator (Savant SpeedVac AES2010 Thermo Scientific, Waltham, MA). After, the dried extracts were obtained, they were stored at -20 °C for subsequent analysis. Extractions for each sample were carried out in triplicate for each sample. To measure the lutein content by HPLC, lutein extracts were reconstituted in 250 µL of ethanol.

Lutein storage stability

The microencapsulated and freeze-dried pulp samples (2.5 g of each) were placed in Petri dishes (5 cm in diameter) and stored in dark at controlled relative humidity and constant temperature. A constant humidity value was 75% was obtained by using 200 mL of saturated solutions of KNO_3, NaCl and NaBr in each desiccator. A thermohygrometer (SH109 - Kex Germany) was used to measure the relative humidity. The storage time measurements for the two samples (lyophilized and microencapsulated) were 5, 10, 15, 20, 25, 30, 35, 40, 40, and 45 days. Measurements were performed in triplicate. The total lutein content was determined as aforementioned.

Lutein Degradation Kinetic Analysis

Data on the kinetics of lutein degradation were analyzed and adjusted using a Weibull model, based on previous studies[22] with software OriginPro 2021.

Rate (*k*) and shape (γ) constants as well as the half-life were estimated according to equation (3).

$$y_t = \frac{C_t}{C_0}\, exp\,(-(kt)^{\gamma}) \quad ...(3)$$

Were, y_t is the retention of lutein, C_0 is the initial lutein content, and C_t is the lutein content at a specific time (days).

Statistical Analysis

For statistical analysis was used InfoStat - Statistical Software. The results were expressed as means ± standard deviations (SD). Fischer's LSD test was used to determine differences between means. Values with p values less than 0.05 were considered significant.

Results and Discussion

Physicochemical Characterisation of *Eugenia stipitata* Pulp and Microencapsulates

Edible fraction of arazá had a titratable acidity of 3042.94 ± 2.11 mg malic acid/100 g FW, pH 2.5 ± 0.3, total soluble solids 4.3 ± 0.5 °Brix, and a moisture content of 91.57 ± 1.04 %. These data are similar to those previously reported,[5,21] except for titratable acidity which was higher than that reported by Garzón *et al*.[5]

Microencapsulation process had a yield of 64.22%, being a higher value than the one reported for the microencapsulation of pitanga juice by spray drying using maltodextrin as single wall material.[25] Microencapsulates had a titratable acidity of 3038.72 ± 0.5 mg malic acid/100 g FW, total soluble solids 14.00±1.03 ºBrix, pH 3.12 ± 0.19, being higher than that reported for freeze-dried microencapsulated arazá.[22] Moisture for day zero and day forty five of 1.01±0.02 % and 2.14±0.11 %, respectively; and Aw 0.33 ± 1.14, this value being lower than that reported previously[26] for microencapsulation of seafood flavor enhancer from Indonesian brown seaweed with maltodextrin, gum arabic and β-cyclodextrin and for microencapsulation of umami flavor enhancer from Indonesian water brown seaweed with maltodextrin by spray drying.[27] The values obtained for moisture and water activity are optimal values, because the lower the water activity, the lower the availability of microorganism growth, which causes high stability for the microencapsulation.[28] The encapsulation efficiency was 79.83% in the preparation of lutein microcapsules with maltodextrin, it was reported similar values of 90.7-74.1% encapsulation efficiency of microencapsulated lutein by spray drying prepared with mixtures of maltodextrin and sucrose.[17]

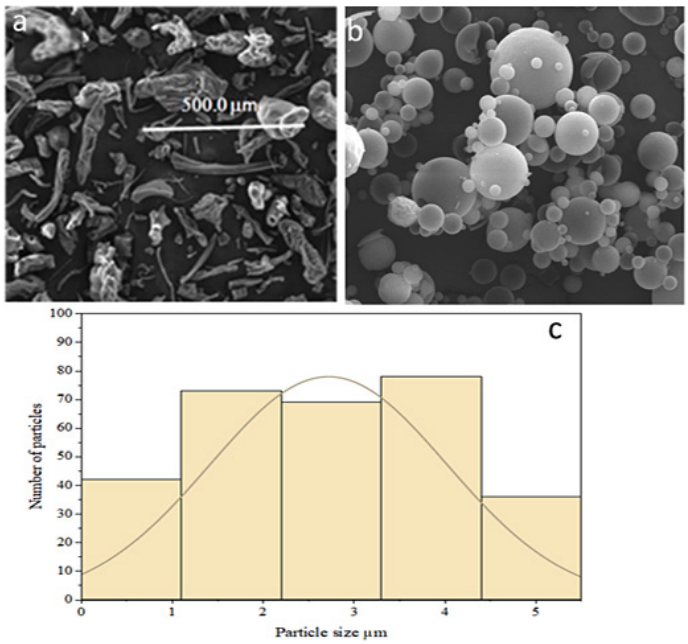

Fig. 1: SEM images of maltodextrin (a), spray-dried arazá pulp (b), and particle size distribution (c).

Morphology and Size Particle of Microencapsulates

Micrographs for wall material and microencapsulates are shown in figures 1a and 1b, respectively. Particle size distribution for microencapsulates is shown in Figure 1c. The micrographs of arazá microencapsulates compared to maltodextrin (fig.1a. and 1b), show the success of the microencapsulation process, obtaining particles with different morphology. Figure 1b shows the formation of spherical microcapsules, without evidence of fractures, as compared to some spray-dried microencapsulates that have a characteristic wrinkled and flattened appearance.[29]

The spray-dried microencapsulates have consistent spherical shape, with smooth surface, in comparison to the freeze-dried microencapsulates previously obtained,[22] where wrinkled surfaces, pores and visible dents were found. They also are more smooth and spherical, compared to microencapsulates of crude lutein powder, with inulin and modified starch particles,[20] where micrographs with characteristic of partially collapsed spherical shape with surface dents, where obtained. Likewise, microencapsulation of umami flavor enhancer from Indonesian waters brown seaweed with different percentages of maltodextrin, showed a morphology that forms irregular particles and stick together, being better those obtained in this work.[27]

The presence of pores is one of the characteristics of microencapsulates prepared by spray drying using carbohydrates as wall materials, they are created by increasing the particle temperature and vapor pressure.[30] The microencapsulates had a size distribution between 0.528 and 4.98 µm (Fig. 1c).

DTG and TGA

Thermal stability of the microencapsulates (fig.2) was evaluated by thermogravimetric analysis (TGA) and the first derivative of the weight variation with respect to temperature (DTG). Different inflection points were observed, the first inflection point with a temperature range between 65-106°C and 4.6% weight loss, the surface evaporation of water and the thermal desorption of volatile compounds account for this,[31] the presence of this surface water is the result of rehydration of the capsule after the spray drying process.[32] The second inflection point with a temperature range between 216-263°C and 14.6% weight loss, according to the literature[33] is linked to the internal water loss that occurs when capsules break. The third turning point with a temperature range between 333-377 °C and 66.6% weight loss, is linked to decomposition of maltodextrin as a wall material, corresponding to most of the capsule composition[34,35] and the decomposition and volatilization of molten materials.[33]

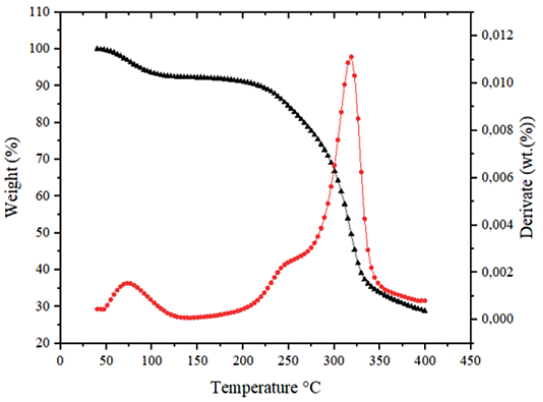

Fig.2: Thermogravimetric (black) and Derivative Thermogravimetric (red) curves (TGA/DTG) of spray-dried arazá pulp.

Lutein Storage Stability

Figure 3 shows the behavior of lutein retention from day 0 to day 45, where it can be clearly observed the great difference in the degradation kinetics, being faster (about 35 times higher) for the lyophilized one ($k = 5.24 \times 10^{-3}$ days^{-1}) with respect to the microencapsulated powder ($k = 1.49 \times 10^{-4}$ days^{-1}), making the lutein more stable in the microencapsulated powder, with a non-significant drop on day thirty, compared to the lyophilized lutein powder. A similar result was found in the literature[22] where the studies of storge stability of carotenoids in freeze-dried arazá powders showed a degradation rate of $k = 7.10 \times 10^{-3}$ days^{-1}, being this a similar value to that obtained for the freeze-dried lutein powder. Arazá microencapsulates showed better lutein storage stability, than the ones obtained from lutein crude powder, which showed a drastic and sustained reduction in lutein content, which was below 10% on day 24 at two temperatures (25 and 40°C) for 72 days.[20]

Table 1: Kinetic and Weibull distribution values for lutein degradation in freeze-dried and spray-dried arazá pulp.

Sample	k (days^{-1})	γ	$t_{1/2}$(days)	R^2 Adj
Freeze-dried	$5.24\times10^{-3} \pm 7.91\times10^{-5}$	1.049±0.010	134	0.985
Spray-dried	$1.49\times10^{-4} \pm 1.63\times10^{-5}$	0.791±0.016	>200	0.975

k: Rate constant, γ: shape constant, $t_{1/2}$: half-life, R^2_{Adj}: Adjusted R-squared. Values of k and γ are reported as the mean value ± standard deviation (n =3).

The stability of lutein into the microencapsulates could be related to its morphology. The microparticles need to be flawless and devoid of pinholes in order for the coating material to be more stable and to protect its bioactive components.[36] Micrographs at figure 1b for the microencapsulates, showed no holes or dents, which could improve the storage stability of lutein.

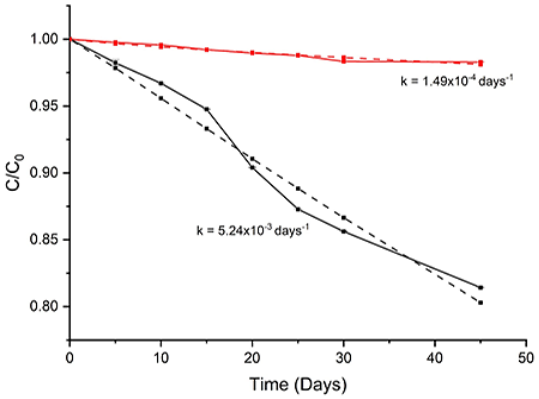

Fig. 3: Stability of lutein in spray-dried (red) and freeze-dried (black) arazá pulp. Dashed lines represent fitted data.

Conclusions

Microencapsulation by spray drying proved to be an effective technique for preserving the lutein contained in arazá during the storage. The product obtained showed low moisture content, water activity, being these values optimal, because there is a lower possibility of growth of organisms, which provides stability to the microencapsulates. The lutein degradation kinetic presented a constant of 1.49×10^{-4} days^{-1}, which is thirty-five times lower than that obtained for the freeze-dried arazá pulp used as a control. The microencapsulation stability is also influenced by the smooth and spherical morphology obtained, the flawless and pore-free microparticles allow the coating material to be more stable and thus protect the bioactive compounds, such as lutein. These results confirm that it is possible to obtain stable arazá microencapsulates from spray drying process, by using an economical wall material as maltodextrin, being a promising alternative for the preservation of bioactive compounds in Amazonian fruits, to be implemented in the food industry.

Acknowledgments

The authors thank Universidad de la Amazonia, for providing necessary requirements in completing this research work. Liceth N. Cuéllar Álvarez thanks to MinCiencias and SGR of Colombia for “Beca de Excelencia Doctoral del Bicentenario – Corte I”

Funding

The research was funded by MinCiencias and SGR of Colombia trough BPIN 2020000100031 project.

Conflict of interests

The authors declare no potential conflicts of interest.

Data Availability Statement

The manuscript incorporates all datasets produced throughout this research study.

Ethics Statement

The document accurately and thoroughly presents the authors' original research and analysis.

Author contributions

Conceptualization and Methodology: Liceth N.Cuéllar Álvarez, formal analysis: Francis S. Sánchez-Garzón, writing - original draft: Dayana Trujillo-Candela, writing - review & editing: Luz Stella Nerio.

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