## 15

# Encapsulated pequi oil with cashew gum/gelatin by complex coacervation for use in food: characterization and quality assessment

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### ABSTRACT

**Purpose:** Pequi fruit (*Caryocar coriaceum* Wittm.) has a great economic and sensorial importance for the State of Ceará, the largest producer in Brazil. This fruit is an excellent nutritional source rich in vitamins, carotenoids and phenolic compounds, in addition to having good antioxidant capacity, fatty acids and minerals. **Method:** Pequi oil was microencapsulated by complex coacervation for protect compounds of interest. The physicochemical characterization of the oil was before and after encapsulation to evaluate its characteristics and quality employing this process. **Results:** The saponification index, color analysis and iodine index presented lower molecular weight, carotenoids content and a lower degree of unsaturation after microencapsulation, respectively. The peroxide index presented low presence of degradation compounds. The acidity index and fatty acid composition did not change significantly, with a predominance of oleic and palmitic acids. **Conclusion:** Complex coacervation is a viable technique for the preservation of Pequi oil, enabling its use for various purposes such as food and nutraceutical.

Keywords: Caryocar Coriaceum, Microencapsulation, Peroxide, Total Carotenoids, Fatty Acids.

#### **INTRODUCTION**

Pequi (*Caryocaraceae Caryocar* coriaceum. (Wittm.) is a drupaceous fruit present in the state of Ceará, Brazil, consisting of an exocarp and greenish pericarp, an external mesocarp, a yellowish internal mesocarp (edible pulp), and a spiny endocarp that protects the edible seed, called an almond (CARDOSO *et al.*, 2013; DE OLIVEIRA SOUSA *et al.*, 2011). The pulp is an excellent nutritional source rich in vitamins A, E, C, carotenoids, phenolic compounds, presenting good antioxidant capacity, fatty acids, phosphorus, potassium, and magnesium (MACHADO; MELLO; HUBINGER, 2012). The abundance of essential fatty acids in this species and the therapeutic potential suggests them to be a promising species for the food and pharmaceutical industries. The chemical composition and antibacterial properties of the Pequi pulp oil show it a valuable source of unsaturated fatty acids with high energy content, and potential application against some bacterial infections (COSTA *et al.*, 2011; De Figueiredo *et al.*, 2016).

The quality of the vegetable oil depends on factors such as the raw material quality, the extraction method, the storage type, as well as exposure to light, oxygen, high temperatures and humidity. Hydrolysis and lipid oxidation reactions affect its quality, indicating how important these factors are for evaluating its conservation. Lipid oxidation produces primary toxic substances such as hydroperoxides, precursors of the final degradation compounds such as aldehydes, ketones and alcohols (MARIUTTI; BRAGAGNOLO, 2017; SADOUDI; AMMOUCHE; ALI, 2014).

The complex coacervation process is a microencapsulation method under mild conditions, by which labile or easily damaged substances can be protected into microparticles. Nevertheless, in the health and food area, biocompatibility, biodegradability and nontoxicity are required, which reduces the number of potentially available polyelectrolytes to be used. Polysaccharides and proteins have been employed extensively to encapsulate biologically active substances by complex coacervation (DA SILVA *et al.*, 2018; SARAVANAN; RAO, 2010; SILVA; ANDRADE, 2009).

Gelatin is a polyampholyte obtained from denatured collagen. In acid pH, the amino groups of gelatin are protonated, and the polymer behaves as a polycation. On the other hand, cashew gum in water is a polyanionic polymer. The interaction between the biopolymers forms insoluble complexes and produces phase separation (DE KRUIF; WEINBRECK; DE VRIES, 2004). The deposition of such complexes around a hydrophobic core creates a barrier, thereby allowing its encapsulation.

The choice for the microencapsulation method as well as the compatibility of the nucleus with the wall materials are determining factors for the process effectiveness. Naturally, derived polymers represent a self-renewing potentially economical bio-source when compared to synthetic and environmentally safe products (LEITE *et al.*, 2017; SHELKE; JAMES; LAURENCIN, 2014; KIM *et al.*, 2009).

Cashew gum is a polysaccharide extracted from the exudates of *Anacardium occidentale* L. tree, a low-cost and easily available source, and is widely distributed in northeast Brazil. It is a branched acidic heteropolysaccharide composed of a main chain of  $\beta$ -d-galactose 1  $\rightarrow$  3 linked with side chains of galactose and glucose (PITOMBEIRA *et al.*, 2015). Technological interest in cashew gum is related to its rheological characteristics, and biodegradability (HOGAN *et al.*, 2001; LEITE *et al.*, 2017).

Therefore, the objective of this study was to evaluate Pequi oil encapsulated with cashew gum as a wall material, aiming at preserving the oil's characteristics of interest, in addition to the possibility of using it as a bioactive for alimentary, nutraceutical and cosmetic purposes.

#### MATERIAL AND METHODS

The Pequi oil was purchased from a local market in the city of Crato, Ceará, Brazil (coordinates: 07°18'19" S and 39°18'08" W) and kept in refrigerated temperature (10 °C) under light protection until its use in microparticle formation. Gelatine (GE) 225H type B was provided from Rousselout ®. Cashew gum (CG) was collected from *Anacardium occidenta-le L*. plants from Embrapa Tropical Agroindustry Experimental Field in Ceará - Brazil (coordinates: 4°11'26.62" S and 38°29'50.78" W).

#### Cashew gum (CG) isolation

The isolation of polysaccharides from the cashew tree exudate was carried out according to (TORQUATO *et al.*, 2004) with modifications. The exudate was ground in a knife mill and solubilized in water in the proportion of 300:1 (g/L). After solubilization, the sample was vacuum filtered, centrifuged at 15.303 *x g* for 10 min at 25 °C and precipitated in 1:3 ethanol (v/v) for 24 hours at 10 °C. The precipitate was dried in an air circulating oven at 60 °C and in the sequence was ground, resulting in isolated CG. The isolated CG had contents of 8.1% of moisture, 0.6% of ashes, 0,5% of proteins and 87,8% of carbohydrates.

#### **Microparticle formation**

The microparticles were produced according to Da Silva *et al.* (2018). 100 mL of each suspension of CG 2% (w/v) and gelatin 1% (w/v) were prepared and homogenized by Ultra-Turrax at 10,000 rpm for 5 min at room temperature. 3g of pequi oil was introduced into gelatin suspension at 10,000 rpm for 5 min at room temperature. The CG solution was then slowly added to the gelatin-stabilized emulsion to a final aqueous volume of 200 mL. Then, 400

mL of distilled water was added and homogenized by Ultra-Turrax at 10,000 rpm for 3 min at room temperature. The pH was adjusted with hydrochloric acid (2M) to 4.5 the emulsion was refrigerated ( $8 \pm 2 \,^{\circ}$ C) overnight for precipitation of the particles. Subsequently, excess water was eliminated and coacervate suspensions were obtained for the analyses. The microparticles were stored in screw-capped Falcon tubes and protected from light covered with aluminum foil. The material was kept refrigerated at 5  $^{\circ}$ C until use.

#### Morphology of the particles

The morphology of the microparticles freeze-dried was examined in a scanning electron microscope (Zeiss, DSM940A). The samples were coated by a gold thin film in a sputter coater (Emitech, k550).

#### Oil extraction from the microparticles

Oil extraction was performed using an adaptation of the method developed by Bahrami *et al.* (2014), using mixture of chloroform, methanol and water in the ratio of 1:2:0.8 (v/v), resulting in a single phase system. Chloroform and distilled water were subsequently added in the ratio of 1:1 (v/v) to achieve a biphasic system. Pequi oil dissolved in the chloroform phase was extracted with a rotary evaporator stored under refrigeration (10 °C) and kept protected from light.

#### Physicochemical analysis

Pequi oil before and after microencapsulation was evaluated for peroxide, acidity, iodine, saponification, total carotenoid content, composition of fatty acids, color analysis and the Kreis test. All analyses were performed in triplicate for each batch of microparticle formation totalizing nine replications and the results were expressed as mean and standard deviation.

#### Peroxide index

Determination of peroxides was carried out using an adaptation of the American Oil Chemists' Society (AOCS, 1995) methodology, by solubilizing 5 g of Pequi oil in 30 mL of acetic acid-isooctane solution (3:2 v/v) and 0.5 mL of saturated potassium iodide solution, then allowing it to rest for 1 minute. Next, the solution was titrated with 0.01 N sodium thio-sulphate solution using 1% (w/v) starch solution as indicator.

#### Acidity index

The acid index was obtained according to the methodology described by Official Methods of Analysis of the Association of Office Analytical Chemists (AOAC, 2005), solubilizing 2 g of Pequi oil in 25 mL of neutral ether solution (2:1), using 0.01 M sodium hydroxide solution as the titrant.

#### lodine index

The iodine index determination was performed according to Wijs method as described by (AOCS, 1995), solubilizing 0.25 g of the sample in 10 mL of cyclohexane and 25 mL of the Wijs solution, and keeping in the dark for 30 minutes. Next, 10 ml of 15% potassium iodide solution and 100 ml of distilled water were added. Titration was performed with 0.1 M sodium thiosulphate solution using 1% starch solution as indicator.

#### Saponification index

The saponification index was obtained according to the methodology described by (AOCS, 1995), solubilizing 2 g of Pequi oil in 20 ml of alcoholic 4% potassium hydroxide solution. The solution was heated by reflux for 30 minutes and titrated with 0.5 N hydrochloric acid.

#### Total carotenoid content

The carotenoid content was determined by spectrophotometric analysis, according to the methodology described by (HIGBY, 1962). An amount of 2.5 g of Pequi oil was homogenized in 30 mL of isopropyl alcohol and 10 mL of hexane. The mixture was transferred to a separate funnel and after 30 minutes, the lower phase was discarded. The residue was then washed 3 times and the oily part was collected and filtered. The filtrate was transferred to a 50 mL volumetric flask containing 5 mL of acetone, and the volume was completed with hexane. The samples were read in a spectrophotometer at a wavelength of 450 nm.

#### Fatty acid profile

To determine the fatty acids, 0.05 g of Pequi oil in 1 mL of hexane were solubilized in a test tube. Then 1.33 mL of 0.5 M sodium hydroxide in methanol was added and the tube was heated in a water bath at 65-70 °C until the solution became clear. After cooling, 1.67 mL of esterification solution was added, stirred for 30 seconds and again heated in a water bath at 65-70 °C for 5 minutes. Fatty acid methyl esters were formed at this stage. After cooling, 1.33 mL of saturated sodium chloride solution was added with subsequent stirring, then 1 mL of

n-hexane was added and stirred for 30 seconds. After phase separation the upper phase was collected and dried in the exhaust hood for 24 hours. An aliquot of 10  $\mu$ l was removed and diluted into 990  $\mu$ L and the samples were analyzed by GC–MS 7890B/MSD-5977 A (Agilent, California, USA). Chromatographic separations were performed using a 5% phenyl-methyl column (HP-5MS 30 m x 0.25 mm x 1.0  $\mu$ m; Agilent Technologies).

#### Color

The color evaluation was performed by colorimetry using the Chroma Meter CR-400 equipment (Konica Minolta). Pequi oil was placed in sufficient quantity on a petri dish to cover it, and the reading was performed based on the CIELAB scale using the L, a\*, b\* coordinates which represent brightness, variation between green (negative values) and red (positive values), and variation between blue (negative values) and yellow (positive values), respectively.

#### Kreis Test

The Kreis test was performed according to the methodology described by the (AOAC, 2005). An amount of 5 ml of Pequi oil and 5 ml of hydrochloric acid were solubilized in a test tube and homogenized by vortex for 30 seconds. 5 ml of 0.1% solution of floroglucin in ether were added and again stirred for 30 seconds. After 10 minutes there was a phase separation and the appearance of red coloration, in case the solution presented rancid substances.

#### Statistical analysis of the data

The samples averages were compared by Tukey test (p < 0.05) using Statistic 10.0 software.

#### RESULTS AND DISCUSSION

Undoubtly, complex coacervation is an important method of encapsulation because it is simple, fast, and relatively inexpensive and does not involve high temperatures, reducing the risk of degradation of the encapsulated interest substance. Coacervate microparticles are impervious to oxygen as the core is completely surrounded by the continuous layer of wall material, conferring high protection to the core (SIOW, 2012). In order to increase shelf life and also facilitate transport, the coacervates can be dehydrated. In this work, the dry particles by freeze-drying presented structures in bridges (Fig. 1), being a characteristic of structures submitted to this kind of process (COMUNIAN *et al.*, 2018). These bridges are important to avoid the formation of an agglomerated mass of the material.

**Figure 1.** Micrograph of Pequi oil particles using cashew gum and gelatin matrices (2:1 w/w) at pH 4.5. The microparticles were obtained by complex coacervation and dehydrated by freeze-drying.



Source: Research data.

It can be considered that the adjustment of pH to acidic condition is the main factor that may cause changes in the characteristics of the oil during the process of formation of the particles by complex coacervation. Associated with this, post-encapsulation events, during the oil extraction from microparticles may also occur changes resulting from the use of solvents and the extraction method used.

In this work we established a comparative of the oil before and after the encapsulation method in order to identify and discuss possible reasons for the observed changes in oil characteristics. The results of the physicochemical analyzes performed in Pequi oil before and after microencapsulation by complex coacervation can be seen in Table 1.

Evaluated parameters	Before encapsulation	After encapsulation
Acidity index (mg KOH/g)	$0.69 \pm 0.063^{\circ}$	$0.74 \pm 0.03^{a}$
Peroxide index (meq/1000g)	$0.76 \pm 0.12^{a}$	$4.03 \pm 0.12^{b}$
Saponification index (mg KOH/g)	194.3 ± 4.27°	326.5 ± 2.39 <sup>b</sup>
lodine index (gl <sub>2</sub> /100g)	55.84 ± 2.22 <sup>a</sup>	39.53 ± 0.5 <sup>b</sup>
Total carotenoids (mg/100g)	2.76 ± 0.19 <sup>a</sup>	$0.61 \pm 0.04^{b}$

**Table 1.** Physico-chemical characteristics of Pequi oil before and after microencapsulation. The results are expressed asmean ± standard deviation.

Similar letters on the same line indicate no significant difference among the samples by the Tukey test (p <0.05). **Source:** Research data.

The Pequi oil acidity index analyzed after microencapsulation did not present a significant increase in relation to the non-encapsulated sample. The values obtained for the two samples were lower than those found by Dos Santos *et al.* (2010), equals to 3.23 mg KOH/g. The acidity content provides important information about the oil conservation state, since it is directly related to the hydrolysis of triacylglycerols; the process responsible for increasing the concentration of free fatty acids in the oil. According to Resolution RDC No. 270 (ANVS, 2005), the acidity index may not exceed 4 mg KOH/g for cold-pressed and unrefined oils, allowing to infer that the analyzed sample presented good conservation status after the microencapsulation process.

The peroxide content obtained in the oil evaluated after microencapsulation remained below the limit established by the same RDC of up to 15 meq/1000 g. Therefore, even if there was an increase in the value of this index when compared to unencapsulated oil, there was no pronounced lipid oxidation process, which according to Choe and Min (2006) is influenced by the fatty acid composition of the oil, oil processing, heat or light energy, concentration and type of oxygen, free fatty acids, mono and diacylglycerols, transition metals, peroxides, thermally oxidized compounds, pigments and antioxidants. These factors interactively affect the oil's oxidation and it is not easy to differentiate the individual effect of the factors. Low molecular weight compounds are produced during oxidation resulting in abnormal flavor that is less acceptable or unacceptable to consumers or for use as a food ingredient. Oil oxidation also destroys essential fatty acids producing toxic compounds and oxidized polymers, thus this parameter is an important indicator for determining oil quality and shelf-life.

The Kreis test indicated the low presence of degradation compounds that characterize a beginning rancification process, since the analyzed oil samples presented a slightly pink coloration. The Kreis test is considered a qualitative analysis and it indicates the occurrence of lipid oxidation of oils and fats at an early stage of rancidity development (JORGE, 2009). The intensity of the red coloration is directly proportional to the lipid oxidation degree.

The iodine index of the microencapsulated sample presented a significant reduction (p < 0.05) in relation to the unencapsulated oil. This parameter is related to the amount of double bonds present in the sample, so the higher the degree of unsaturation in the sample, the greater the absorption capacity of iodine, and consequently the higher the index. The obtained results showed that some unsaturations were degraded, probably during the oil extraction from microparticles, however the values were lower than those found by (dos Santos *et al.*, 2010) of 60.39 gl<sub>2</sub>/100g. According to Garcia *et al.* (2007), oils with a high degree of unsaturation are more prone to oxidative rancidity.

The Pequi oil saponification index analyzed before microencapsulation was similar to that found by Facioli and Gonçalves (1998) of 200 mg KOH/g, and lower than the sample evaluated after microencapsulation ( $326.5 \pm 2.39$  mg KOH/g), indicating a composition with higher molecular weight fatty acids. The saponification index corresponds to the amount of potassium hydroxide in milligrams needed to saponify one gram of oil sample. According to Dos Santos *et al.* (2010) this parameter is inversely proportional to the average molecular weight of the triglyceride fatty acids present.

Studies evaluating the quality of the encapsulated material are much less frequent than those related to the encapsulation method (efficiency, operational parameters and etc.) and release particles, although it is even more important to evaluate the influence of the encapsulation method conditions on the substance of interest, above all, especially, when the final objective is a practical application. Some studies, for example, have indicated that the complex coacervation method maintained the antibacterial activity of vegetable oil (DIMA *et al.*, 2013) and conserved characteristic of fish oil (PATRICK *et al.*, 2013).

Microencapsulated samples showed a significant reduction in total carotenoid content and natural liposoluble pigments attributed to the yellow, orange or red coloration of food. The reduced carotenoid content in the encapsulated oil can also be evidenced in the results of the chromaticity analysis presented in Table 2. The conjugated polyene chain that is characteristic of carotenoids also makes these compounds susceptible to degradation from a number of agents. Depending on the carotenoid, the terminal end groups may suffer degradation, for example by light, temperature and presence of oxidizing substances (BOON *et al.*, 2010; JANISZEWSKA-TURAK, 2017). Rutz *et al.* (2017) demonstrated that palm oil particles formed by complex coacervation had losses in the carotenoid content. It was also observed by them that the freeze-drying method is more suitable than the spray drying method for dehydration of the particles and avoid carotenoid losses.

Evaluated parameters	Before microencapsulation	After microencapsulation
L* (Luminosity)	$80.47 \pm 0.37^{\circ}$	84.07 ± 0.006 <sup>d</sup>
a* (red/green coordinate)	- 4.79 ± 1.79°	- 10.61 ± 0 <sup>d</sup>
b* (yellow/blue coordinate)	86.52 ± 1.12°	34.07 ± 0.038 <sup>d</sup>

 Table 2. Color analysis of Pequi oil before and after microencapsulation. The results are expressed as mean ± standard deviation.

Different letters on the same line indicate a significant difference between the samples by the Tukey test (p<0.05). **Source:** Research data.

The values obtained for the b\* coordinate indicate that the Pequi oil presented a significant reduction (p < 0.05) in the yellow coloration because of pH changes during the encapsulation process. The L\* coordinate indicate that the encapsulated oil showed a higher luminosity, making it lighter in color.

According to Table 3 the samples presented composition of other very similar fatty acids (mainly saturated and monounsaturated acids), mainly consisting of oleic and palmitic acid, as was also found by Facioli e Gonçalves (1998) being 53.9% and 40.2%, respectively. According to De Souza (2020), fatty acids with a lower degree of unsaturation make the oil more stable to the oxidative process. Linolenic acid was not identified in the oil analyzed after microencapsulation. Probably, the oil extraction from microparticles caused the degradation this unsaturated acid that it was present in minimum quantity before encapsulation. Many

studies reported differences in the concentrations of unsaturated and saturated fatty acids from oils submitted to different methods and use of solvents (ABDOLSHAHI *et al.*, 2015; BAHRAMI *et al.*, 2014; MEZZOMO *et al.*, 2010).

**Table 3.** Percent composition of Pequi oil fatty acids before and after microencapsulation. The results are expressed asmean ± standard deviation.

Fatty acids	Before microencapsulation (%)	After microencapsulation (%)
Palmitoleic acid (C 16:1)	0.511 ± 0.08 <sup>a</sup>	0.453 ± 0.02 ª
Palmitic acid (C 16:0)	34.19 ± 0.93 °	34.52 ± 0.79 °
Linolenic acid (C 18:2)	1.55 ± 0.06	nd
Oleic acid (C 18:1)	60.43 ± 1.97 °	62.65 ± 0.80 °
Stearic acid (C 18:0)	1.56 ± 0.04 °	1.61 ± 0.01 °

nd = not detected. Similar letters on the same line indicate no significant difference between the samples by the Tukey's test (p<0.05).

Source: Research data.

#### CONCLUSION

Pequi oil microencapsulated by complex coacervation presented alterations in some properties; however, regarding the acidity and peroxide parameters, it remained within the limits established by legislation for cold pressed oils. In this sense, the technique of microencapsulation is viable as a way of preserving Pequi oil for food, pharmaceutical and nutraceutical purposes. However, further studies are recommended which consider the temperature of oil extraction and the storage time of the microencapsulated oil, as well as its exposure to light and oxygen as factors that may alter its quality.

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