Research, Society and Development, v. 9, n. 9, e159996691, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i9.6691

Potencial bioativo de nanopartícula de subproduto de acerola (*Malpighia* sp. L): Bioacessibilidade em néctar

Bioactive potential of nanoparticles of acerola byproduct (*Malpighia* sp. L): Bioaccessibility in nectar

Potencial bioactivo de nanopartículas de subproducto de acerola (*Malpighia* sp. L): Bioaccesibilidad en néctar

Recebido: 13/07/2020 | Revisado: 05/08/2020 | Aceito: 08/08/2020 | Publicado: 14/08/2020

Alessandra Pinheiro de Góes Carneiro
ORCID: https://orcid.org/0000-0001-5784-3808
Universidade Federal do Ceará, Brasil
E-mail: alessandrapgc@hotmail.com
Antonia Livânia Linhares de Aguiar
ORCID: https://orcid.org/0000-0003-1193-9163
Universidade Federal do Ceará, Brasil
E-mail: livania_linhares@hotmail.com
Ana Cristina Silva de Lima
ORCID: https://orcid.org/0000-0003-0249-5281
Universidade Federal do Ceará, Brasil
E-mail: anacristinalima@ufc.br
Larissa Moraes Ribeiro da Silva
ORCID: https://orcid.org/0000-0001-7302-401X
Universidade Federal do Ceará, Brasil
E-mail: larissamrs@yahoo.com.br
Paulo Henrique Machado de Sousa
ORCID: https://orcid.org/0000-0001-7005-6227
Universidade Federal do Ceará, Brasil
E-mail: phmachado@ufc.br
Raimundo Wilane de Figueiredo
ORCID: https://orcid.org/0000-0002-1520-2233
Universidade Federal do Ceará, Brasil
E-mail: figueira@ufc.br

Resumo

O processamento industrial da acerola gera uma grande quantidade de resíduos que geralmente são descartados, causando perdas econômicas e ambientais. Estudos mostram que os resíduos de frutas são uma fonte rica de compostos bioativos, o que leva a atenção para novos estudos para viabilizar a aplicação em produtos alimentícios. Nesse estudo, foram analisados β-caroteno, antocianinas, flavonoides amarelos, vitamina C, polifenóis extraíveis totais e atividade antioxidante pelo método ABTS$^{\bullet+}$ na polpa e no subproduto da acerola liofilizado. Logo, o extrato do subproduto da acerola foi submetido ao processo de encapsulação por pulverização, utilizando goma arábica e maltodextrina como material de parede. Foi acompanhando a estabilidade da vitamina C, compostos fenólicos e atividade antioxidante pelo método ABTS$^{\bullet+}$ presentes na nanopartícula e atividade antimicrobiana contra os microorganismos *E. coli* e *L. monocytogenes*. Por fim, a nanopartícula foi aplicada no néctar e verificada a biacessibilidade para compostos fenólicos e capacidade antioxidante. Os resultados mostraram que a polpa e o subproduto da acerola apresentaram resultados relevantes para polifenóis (1,214.54 mg GAE/100g e 9,802.97 mg GAE/100g, respectivamente) e vitamina C (1,113.10 mg/100g e 6,039 mg/100g, nessa ordem). Os compostos bioativos e atividade antioxidante foram mantidos no extrato encapsulado, assim como a nanopartícula apresentou atividade bactericida para *E. coli*. Os resultados demonstram a qualidade do resíduo de acerola agroindustrial na forma de nanopartículas como fonte de compostos bioativos.

Palavras-chave: Aplicação; Compostos bioativos; Encapsulação; Frutas.

Abstract

The industrial processing of acerola generates a large amount of waste that is usually discarded, causing economic and environmental losses. Studies show that fruit residues are a rich source of bioactive compounds, which calls attention to new studies to enable the application in food products. In this study, β-carotene, anthocyanins, yellow flavonoids, vitamin C, total extractable polyphenols, and antioxidant activity by the ABTS$^{\bullet+}$ method were analyzed in the pulp and lyophilized acerola by-product. Therefore, the extract of the acerola by-product was subjected to the spray encapsulation process, using gum arabic and maltodextrin as wall material. The stability of vitamin C, phenolic compounds, and antioxidant activity by ABTS$^{\bullet+}$ method present in the nanoparticle and antimicrobial activity against the microorganisms *E. coli* and *L. monocytogenes*. Finally, the nanoparticle was applied to the nectar and the bioaccessibility for phenolic compounds and antioxidant capacity

was verified. The results showed that the pulp and by-product of acerola showed relevant results for polyphenols (1,214.54 mg GAE/100g and 9,802.97 mg GAE/100g, respectively) and vitamin C (1,113.10 mg/100g and 6.039 mg/100 g, in that order). The bioactive compounds and antioxidant activity were maintained in the encapsulated extract, just as the nanoparticle showed bactericidal activity for *E. coli*. Overall, the results demonstrate the quality of the agroindustrial acerola residue in the form of nanoparticles as a source of bioactive compounds.

Keywords: Application; Bioactive compounds; Encapsulation; Fruits.

Resumen

El procesamiento industrial de la acerola genera una gran cantidad de residuos que generalmente se descartan, causando pérdidas económicas y ambientales. Los estudios muestran que los residuos de frutas son una rica fuente de compuestos bioactivos, lo que llama la atención sobre nuevos estudios para permitir la aplicación en productos alimenticios. En este estudio, se analizaron β-caroteno, antocianinas, flavonoides amarillos, vitamina C, polifenoles extraíbles totales y actividad antioxidante mediante el método $ABTS^{\bullet+}$ en la pulpa y subproducto de acerola liofilizado. Por lo tanto, el extracto del subproducto de acerola se sometió al proceso de encapsulación por pulverización, usando goma arábiga y maltodextrina como material de pared. La estabilidad de la vitamina C, los compuestos fenólicos y la actividad antioxidante se monitoreó mediante el método $ABTS^{\bullet+}$ presente en la nanopartícula y la actividad antimicrobiana contra los microorganismos E. coli y L. monocytogenes. Finalmente, la nanopartícula se aplicó al néctar y se verificó la biacesibilidad de los compuestos fenólicos y la capacidad antioxidante. Los resultados mostraron que la pulpa y el subproducto de la acerola mostraron resultados relevantes para los polifenoles (1,214.54 mg GAE/100g y 9,802.97 mg GAE/100g, respectivamente) y vitamina C (1,113.10 mg / 100g y 6.039 mg/100g, en ese orden). Los compuestos bioactivos y la actividad antioxidante se mantuvieron en el extracto encapsulado, así como la nanopartícula mostró actividad bactericida para E. coli. Los resultados demuestran la calidad del residuo de acerola agroindustrial en forma de nanopartículas como fuente de compuestos bioactivos.

Palabras clave: Aplicación; Compuestos bioactivos; Encapsulamiento; Frutas.

1. Introduction

The acerola (*Malphigia* sp. L) is a tropical fruit originating in South and Central America, genus *Malphigia*, belonging to the family Malpighiaceae, and called the Antilles cherry. A pleasant-tasting fruit and an excellent source of ascorbic acid and other photo-chemical compounds, such as carotenoids and polyphenols, is commercially exploited in the form of juice, nectar, jam, candy, among other products (Filho et al., 2018; Mazza et al., 2020).

The industrial production of acerola generates an enormous amount of residues, such as seeds, bark and bagasse, which have a high potential for use, such as food supplements or natural additives, due to the high amount of nutrients, antioxidant potential and antimicrobial properties. On the other hand, more efficient waste management, by means of recovery, contributes to adding economic value and minimizing environmental impacts, due to the reduction of production costs and generated waste (Leao et al., 2017; Milani et al.; 2018). The use of acerola residues, whether for the application of existing foods or in the development of new products, leads to an increase in the commercial value and profitability obtained from the raw material (Çam et al., 2014).

However, there are several limitations to the application of bioactive fruit compounds in food products due to the low stability resulting from pH, humidity, temperature, enzymes, oxygen, light, among other factors. In this context, the nanoencapsulation process appears to reduce the limitation of the technological application of sensitive substances to the processing steps and shelf life. The nanoencapsulation technique is a physical process that consists of coating a compound within a matrix, which has the ability to protect and isolate the contents from the external environment (Rezende et al., 2018; Labuschagne, 2018; Mar et al., 2020).

Many authors have shown studies that report the presence of a high amount of bioactive compounds and antioxidant properties in acerola, characterized in different stages of maturation (Mezadri et al., 2008; Belwal et al., 2018), with variations in the extraction methodologies, simulating processes that could be applied at an industrial level to obtain better performance (Silva et al., 2020; Mariano-Nasser et al., 2017; Xu et al., 2020). However, it is limited as publications on extraction, characterization and application of bioactive compounds from the acerola residue (Rezende et al., 2017; Silva et al., 2014). In order to have a potential view as a functional ingredient, the objective of this study was to characterize the bioactive compounds and antioxidant capacity of the pulp and by-product of acerola. Also, encapsulate the by-product of acerola and monitor the stability of vitamin C, phenolic

compounds, and antioxidant activity by the ABTS$^{\bullet+}$ method. Then, verify the bioaccessibility of phenolics and the antioxidant potential of the nanoparticle applied to nectar.

2. Materials and Methods

The present research is characterized as a quantitative laboratory study. The pulp and by-products obtained from the processing of acerola (*Malpighia* sp. L), both lyophilized, were supplied by an organic production company located in Ubajara, Ceara, Brazil.

2.1 Bioactive compounds and antioxidant capacity present in the pulp and by-product of acerola (*Malpighia* sp. L)

2.1.1 β-carotene

The levels of β-carotene were obtained according to the methodology described by Nagata & Yamashita (1992). The results were expressed in mg of β-carotene/100g of sample.

2.1.2 Yellow flavonoids and total anthocyanins

They were determined according to the methodology described by Francis (1982). Initially, 1 g of the sample was homogenized with an extraction solution (1.5M HCl and 85% ethanol) for its extraction. The samples were homogenized, and the contents transferred to a 50 mL volumetric flask protected from the light, which was checked with the extraction solution, homogenized, and transferred to an amber flask. The system was subjected to 13 hours of rest under refrigeration and in the absence of light. After this period, the extract was filtered and subjected to readings, in a Shimadzu spectrophotometer model UV-1800, at 374 nm for yellow flavonoids and at 535 nm for total anthocyanins. The results were expressed in mg.100 g^{-1} calculated using the formula: absorbance x dilution/76.6 or 98.2.

2.1.3 Vitamin C

The ascorbic acid content was determined using the titration method with Potassium Iodate and the results were expressed in mg/100g of ascorbic acid (IAL, 2008).

2.1.4 Total extractable polyphenols

The total extractable polyphenols were determined using a methodology that uses the Folin-Ciocalteu reagent and gallic acid as standard, according to the methodology described by Larrauri et al. (1997). The reading was performed on a Shimadzu spectrophotometer, model UV-1800 at 700 nm, using the standard curve of gallic acid as reference. The results were expressed in mg of gallic acid (GAE)/100 g of sample.

2.1.5 Total antioxidant activity by ABTS$^{\bullet+}$ method (TAA)

Total antioxidant activity was determined by using the methodology described by Re et al. (1999) adapted by Rufino et al. (2007), whose method is based on the ability of the antioxidants, present in the fruit extract, to capture the free radical ABTS$^{\bullet+}$ [2,2'-azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid)]. The results were expressed as an antioxidant capacity equivalent to the Trolox in μM Trolox/g of sample (μM TE/100 g).

2.2 Nanoencapsulation of the by-product of acerola (*Malpighia* sp. L)

To obtain the nanoparticle, initially, 250 mg of gum arabic was solubilized in distilled water at 50 °C, under stirring. Separately, the 250 mg maltodextrin was solubilized under stirring in distilled water and added to the gum arabic solution. The solution was added Tween 40, 10 mg, previously solubilized in 10 mL of distilled water together with 1 g of the extract of the lyophilized acerola by-product. The final volume was adjusted to 100 mL. Then, the solution was homogenized in Ultra Turrax for 2 minutes at 11,000 rpm and subjected to the spray dryer drying process. The parameters used in the equipment for atomizing the material were: inlet air temperature of 165 ºC, a suction rate of 90%, a pump rate of 10% (Büchi Mini Spray Dryer B-290).

2.3 Antimicrobial activity and accelerated stability of the acerola by-product nanoparticle (*Malpighia* sp. L)

The antimicrobial activity of the nanoparticle was evaluated through the minimum inhibitory concentration (MIC) and minimum bacterial concentration (CBM) for *Listeria*

monocytogenes and *Escherichia coli* and analysis of shelf life through an accelerated stability test.

2.3.1 Antimicrobial activity of the acerola (Malpighia sp. L) by-product nanoparticle

To check the antimicrobial activity of intraocular glasses used using *E. coli* strains ATCC 25922 and *L. monocytogenes* ATCC 19115, grown in Triptcase Soya Ágar medium (Difco, Sparks, USA) at 35 °C for 24 hours. The antimicrobial potential of the samples was determined through the limits of minimum inhibitory concentration (MIC) and minimum bacterial concentration (CBM), according to the methodologies described by Branen & Davidson (2004) and Brandt et al. (2010), with adaptations. The tested samples were 40, 50, 55, 60, 75, 80 and 100 mg/mL on the pathogenic microorganisms: *E. coli* and *L. monocytogenes.* A MIC was used in the microdilution method in plates (96 wells, 300 µL of capacity/well (MicrotestTM, Becton Dickinson and Co.). After the distribution of the plate, the optical density (OD) was read at 630 nm using the ELx 808 absorbance reader (BioTek instruments), Inc. Winooski, VT, USA, with no time after distribution (T_0) and after incubation at 35 °C for a period of 24 hours (T_{24}). Results were moderated by variation of readings (T_{24} - T_0), being considered as MIC with values ≤ 0.05 nm.

From the test wells that indicated inhibitory activity (≤ 0.05 nm), 100 µL spread plate was plated in selective and differential culture media specific to each microorganism, using MacConkey agar (Oxoid) for *E. coli* and Listeria Oxford agar (HiMedia) for *L. monocytogenes*. Then, the plates were incubated at 35 °C for 48 hours. CBM was the lowest concentration tested whose plaque showed no microbial growth.

2.3.2 Accelerated stability of the acerola by-product nanoparticle (*Malpighia* sp. L)

The nanoparticle was stored in a refrigerated incubator, biological oxygen demand - BOD, under controlled temperature (50 ºC) and in the absence of light for 44 days. The nanoparticle, 400 mg, was stored in a 250 mm amber glass flask, in duplicate. Five times (0, 11, 22, 33, 44 days) were established to monitor the stability of the bioactive compounds present: vitamin C, total extractable polyphenols, and antioxidant activity by $ABTS^{\bullet+}$ method, according to the methodology described by Saénz et al. (2009).

2.4 Addition of the nanoparticle of the acerola by-product in acerola nectar (*Malpighia* sp. L)

To obtain the acerola flavor nectar, 0.5 mg/mL of nanoparticles was added with extract of the acerola by-product obtained by spray-drying (Ruiz-Rico et al., 2017). The nectar was developed according to Normative Instruction 12/2003, which describes the Identity and Quality Standards for tropical juice and nectar (Brasil, 2003).

2.4.1 *In vitro* simulated gastrointestinal digestion of acerola nectar (*Malpighia* sp. L)

Digestions with simulated gastric fluid and simulated intestinal fluid, both prepared according to Miller et al. (1981). The bioaccessibility assessment of the bioactive compounds present was carried out after the end of the 2-hour digestion step, where the membrane content called dialysate was removed, and the samples were stored under refrigeration pending analysis. The determination of total extractable polyphenols and antioxidant activity by the $ABTS^{\bullet+}$ method.

The bioaccessible percentage was calculated according to Briones-Labarca et al. (2011), using Equation 1.

$$\% \text{ Bioaccessible} = 100 \times (D \mid E) \quad (1)$$

Where D is the data of the dialysable content and E corresponds to the total data of the sample (data corresponding to each determination).

2.5 Statistical analysis

Data were expressed as mean ± standard deviation, in triplicate. The results of the analysis of the nanoparticle storage time were performed using linear regression and Tukey analysis between the sampling times. The data were submitted to analysis of variance (ANOVA) and Tukey ($p < 0.05$) using the software Statistica 7.0.

3. Results and Discussion

3.1 Bioactive compounds and antioxidant capacity present in the pulp and by-product of acerola (*Malpighia* sp. L)

The results presented in Table 1 represent the contents of different compounds with bioactive properties, in addition to the antioxidant capacity present in the pulp and by-product of the acerola obtained after industrial processing and submitted to the lyophilization process. Statistically, all parameters showed a significant difference between them for the two products, and higher data are present in the by-product. This demonstrates the quality of the material that is industrially rejected.

The values obtained were lower than the values obtained by Silva et al. (2014) for β-carotene, 1.56 mg/100 g and 2.62 mg/100 g and anthocyanins, 144.27 mg/100 g^{-1} and 245.90 mg/100 g^{-1}, in that order, for pulp and by-product of acerola. These same authors did not identify significant levels of flavonoids in the pulp and for by-product obtained data (98.05 ± 0.19 mg/100 g^{-1}) higher than those reported in this study (20.63 ± 4.34 mg/100 g^{-1}).

Rodriguez-Amaya et al. (2006) point out that among the precursors of vitamin A, β-carotene is the one with the greatest activity. This highlights the importance of incorporating this vitamin in the diet.

Table 1 - Bioactive compounds and antioxidant capacity present in the pulp and by-product of acerola (*Malpighia* sp. L)

Analysis	Pulp	By-product
β-carotene (mg/100 g)	0.11 ± 0.06[b]	0.56 ± 0.16[a]
Yellow flavonoids (mg.100 g^{-1})	7.98 ± 1.66[b]	20.63 ± 4.34[a]
Anthocyanins (mg.100 g^{-1})	6.74 ± 1.12[b]	63.97 ± 1.70[a]
Vitamin C (mg/100 g)	1,113.10 ± 52.60[b]	6,039 ± 530.79[a]
Total extractable polyphenols (mg GAE/100 g)	1,214.54 ± 60.24[b]	9,802.97 ± 906.13[a]
Antioxidant activity $ABTS^{\bullet+}$ (μM TE/ 100 g)	106.89 ± 44.99[b]	452.02 ± 120.74[a]

Mean values ± standard deviation. Averages followed by the same letter on the same line do not differ statistically from each other at the 5% level of significance. Dry basis.
Source: Research data (2020).

Anthocyanins are the pigments responsible for the red coloring of acerola. This demonstrates the importance of measuring them, since the commercial interest also considers

the sensory aspects of appearance and color, since yellow-colored pulp has less acceptance than red by the consumer.

In a study by Guevara et al. (2019) in order to quantify different sources of vitamin C, found in avocado (1.63 ± 0.43 mg/100 g), cherimoya (78.96 ± 6.13 mg/100 g), star fruit (199.44 ± 11.28 mg/100 g), guava (496.73 ± 14.32 mg/100 g) and papaya (341.98 ± 20.22 mg/ 00 g) lower than those obtained by Mariano-Nasser et al. (2017) for different acerola cultivars (824.6 to 2,331.6 mg/100g of vitamin C). Araújo et al. (2016) obtained in pasteurized acerola juice, on average 1,016.35 mg/100g of vitamin C.

The high content of vitamin C found in the by-product of acerola, 6,039.0 mg/100 g, suggests an excellent source for enriching food products poor in this vitamin. At the industrial level, the by-product is discarded, and the data presented demonstrate the nutritional and technological potential for its use.

In different acerola cultivars Sousa et al. (2014) found levels from 862.86 to 2.534.70 mg/100 g for vitamin C. Also, levels from 5.99 to 9.82 mg/100 g^{-1}, mg / 100 g-1, 2.29 to 12.37 mg/ 100 g^{-1} and 1,561.67 to 4,338.89 mg GAE/100 g, in that order, for flavonoids, anthocyanins and total extractable polyphenols. Silva et al. (2020) observed variations in total extractable polyphenols for acerola by-product submitted to ultrasound processing (100.7 to 910.5 mg GAE/100 g) lower than the data present in this study for pulp (1,214.54 mg GAE/100 g) and by-product (9,802.97 mg GAE/100 g) of acerola.

In a study with fruits of different acerola cultivars, Mariano-Nasser et al. (2017) obtained for total extractable polyphenols variations from 914.2 to 2,428.3 mg GAE/100 g. The high content of the phenolic compounds in ripe acerola is due to the degradation of compounds such as chlorophyll into substances such as carotenoids, pigments, and phenols during the maturation process (Cruz et al. 2019).

In a comparative study between different methods of extracting bioactive compounds and antioxidant activity from acerola residue, Rezende et al. (2017) reported that the ultrasound extraction method showed better results for anthocyanins (20.3 ± 0.1 mg / 100 g), ascorbic acid (489 ± 26 mg/100 g), total flavonoids (405 ± 14 mg/100 g), compounds phenolic (1034 ± 12 mg GAE/100 g) and antioxidant activity by the $ABTS^{\bullet+}$ method (179.8 ± 0.6 μM TE/100 g).

The concentration of ascorbic acid, polyphenol content and antioxidant activity can vary in fruits between different regions of the country. In addition, factors such as temperature, light intensity, moisture content, maturation stage and fruit processing can influence the result (Rufino et al., 2010). Finally, the extraction method and reagent used to

release these compounds are examples of other factors that can interfere in the quantification of the final content.

Due to the high presence of bioactive compounds, especially ascorbic acid and polyphenols, acerola shows beneficial health effects, being, therefore, an excellent option for application as food supplements and the development of new products with functional properties (Xi et al., 2020).

3.2 Antimicrobial activity and accelerated stability of the acerola by-product nanoparticle (*Malpighia* sp. L)

The antimicrobial potential of the nanoparticle by-product of acerola in a gum arabic and maltodextrin matrix against the microorganisms *Escherichia coli* and *Listeria monocytogenes* can be seen in Table 2.

No bacterial growth was observed for *L. monocytogenes* and *E. coli* at all concentrations tested. With this, the minimum inhibitory concentration (MIC) for the nanoparticle against the two microorganisms was determined, with minimum inhibition in the concentration of 40 mg/mL. Regarding the minimum bactericidal concentration (CBM), the nanoparticle was effective for Gram-negative bacteria *E. coli*. The antimicrobial potential of the nanoparticle is due to the synergistic effect of the bioactive compounds present.

Table 2 - Antimicrobial activity of acerola by-product nanoparticles (*Malpighia* sp. L).

Parameters (mg/mL)	**Microorganisms**	
	Listeria monocytogenes	*Escherichia coli*
[1]CIM	40	40
[2]CBM	------	100

Legend: [1]Minimal inhibitory concentration; [2]Minimal bactericidal concentration.
Source: Research data (2020).

In a study looking at the antimicrobial activity of thyme oil emulsions stabilized with ovalbumin and gum arabic, Niu et al. (2016) described that the emulsions showed a long-term inhibition for the growth of *E. coli*, and emulsions with a final pH of 4.0 showed longer antibacterial properties. The results showed that simple emulsions or complex emulsions of

ovalbumin and gum arabic can be used in the design and use of antimicrobial distribution systems, being useful at the industrial level.

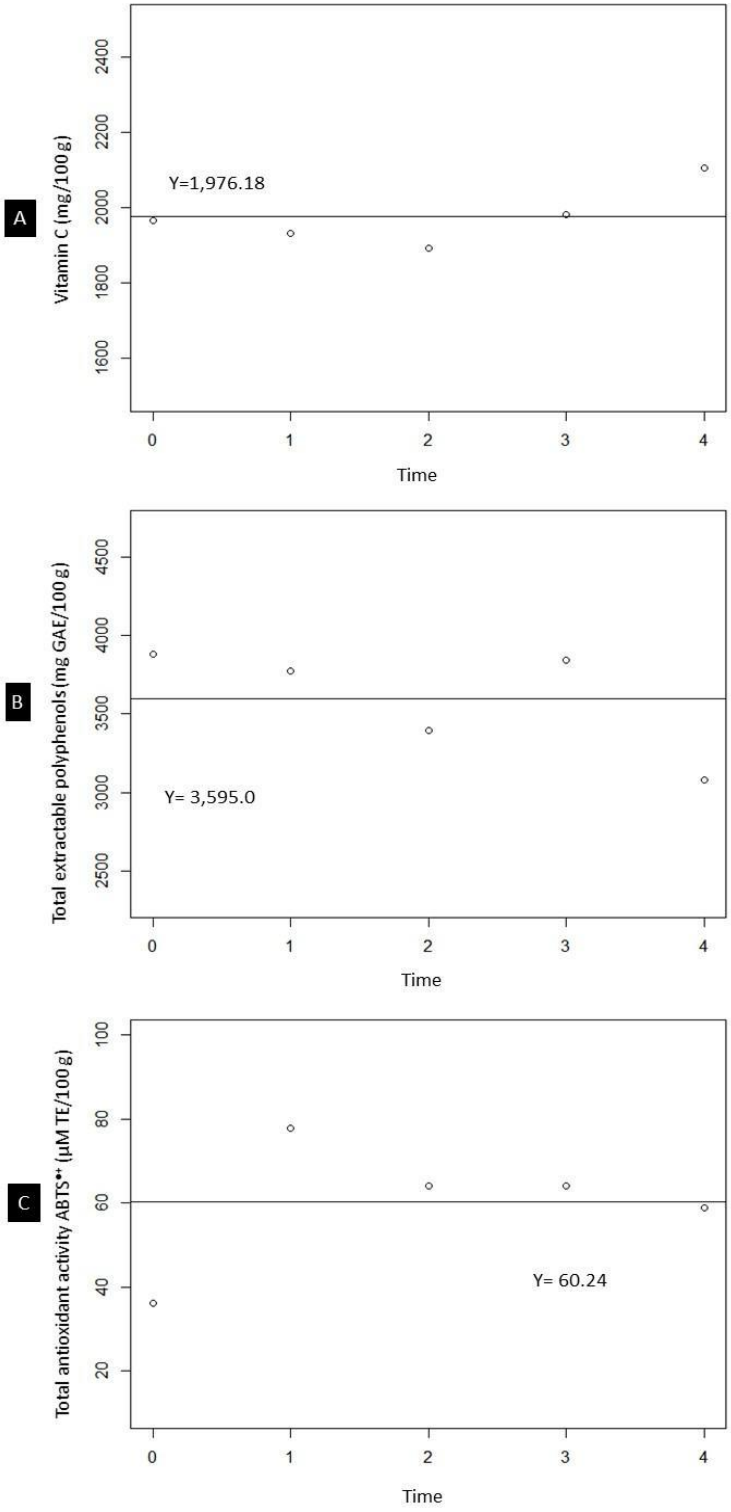
Herculano et al. (2015) found for CBM in a eucalyptus oil nanoparticle in cashew gum matrix the values of 4 g/L for *S. Enteritidis* (Gram-negative) and 3 g/L for *L. monocytogenes* (Gram-positive), indicating more effective bactericidal action for Gram-positive than Gram-negative microorganisms.

Figure 1 shows the accelerated stability data for vitamin C, total extractable polyphenols, and antioxidant capacity by the $ABTS^{\bullet+}$ method as a function of the time (44 days) of the nanoparticle during storage at 50 °C. This analysis indicates a storage time of approximately 6 months (Cheong et al., 2017), which would favor the storage of nanoparticles during the period, guaranteeing acceptable conditions for their use as an enricher in food processing.

The nanoparticle did not show significant degradation, at the level of 5% during stability, with averages of 1,976.18 mg/100 g, 3,595.0 mg GAE/100 g and 60.24 μM TE/100 g, for vitamin C, total extractable polyphenols, and antioxidant capacity by $ABTS^{\bullet+}$ method, in that order. These results demonstrate the ability of the wall material to preserve the properties of the acerola by-product extract during the storage time.

In a study with the purpose of verifying the physicochemical, sensory and antioxidant characteristics of mango juice, Londoño et al. (2017) pointed out that the antioxidant capacity and polyphenol content were maintained after pasteurization, 85 °C for 10 minutes, and storage for 44 days.

Figure 1 - Record of the content of bioactive compounds and antioxidant capacity of the nanoparticle by-product of acerola (*Malpighia* sp. L) maintained for 44 days at a controlled temperature at 50 ºC.



Legend: (A) Vitamin C; (B) Total extractable polyphenols; (C) Total antioxidant activity ABTS$^{\bullet+}$.
Source: Research data (2020).

Tolun et al. (2016) describe in their study of microencapsulation of grape extracts in arabic gum and maltodextrin matrix the favorable effects present in grape seed extracts provide the possibility of being used as a functional ingredient in many food products.

The deterioration at the microbiological and oxidative level of food imposes several negative effects on consumer health, such as food poisoning, and on the industrial sector mainly limiting the useful life. The bioactive compounds present in acerola fruits such as ascorbic acid and polyphenols have numerous secondary metabolic properties that develop several biological properties including antioxidant potential and antimicrobial activity (Souza et al., 2020; Prakash et al., 2020).

3.3 Nanoparticle of the by-product of acerola in acerola nectar (*Malpighia* sp. L): Gastrointestinal digestion simulated *in vitro*

The data on total extractable polyphenols and antioxidant capacity in acerola nectar with addition nanoparticles containing extract of the acerola processing byproduct in gum arabic and maltodextrin matrix before and after simulated gastrointestinal digestion *in vitro* can be verified in Table 3.

Table 3 - Average values for total extractable polyphenols and total antioxidant activity present in acerola nectar (*Malpighia* sp. L) with the addition of nanoparticles before and after simulated *in vitro* gastrointestinal digestion.

Parameters	Nanoparticle
Total extractable polyphenols mg GAE/100 g (before the DGS *in vitro*)	6,585.31 ± 219.66
Total extractable polyphenols mg GAE/100 g (after DGS *in vitro*)	631.19 ± 25.13
Bioaccessibility (%)	9.59
Antioxidant activity $ABTS^{\bullet+}$ µM TE/100 g (before the DGS *in vitro*)	10.86 ± 1.46
Antioxidant activity $ABTS^{\bullet+}$ µM TE/100 g (after DGS *in vitro*)	8.47 ± 0.65
Bioaccessibility (%)	77.99

Mean values ± standard deviation. GAE: Gallic acid. DGS: Gastrointestinal digestion simulated *in vitro*.
Source: Research data (2020).

The low bioaccessible percentage of total extractable polyphenols (9.59%) can be justified by the fact that some polyphenols are linked to macromolecular compounds that are non-dialysable, or that they can form mineral complexes, further reducing their solubility,

explains Bouayed et al. (2011). The polyphenol content present in the nectar before gastrointestinal digestion (6,585.31 mg GAE/100 g) was higher than that detected only in the nanoparticle (3,595 mg GAE/100 g). Although the bioactive compounds present in the pulp used to obtain nectar contributed to the phenolic content, the high content demonstrates that nanoparticles have the potential for applications in functional and nutraceutical foods.

The application of simulated gastrointestinal digestion *in vitro* demonstrates, according to Lima et al. (2014) that, in some cases, only a fraction of the total amount of nutrients in food is potentially bioaccessible. The results obtained in relation to the total extractable polyphenols and antioxidant activity, show that the percentage of absorption of these compounds varies a lot depending on the components of the food and the elements of the matrix.

González et al. (2019) point out that the spray dryer encapsulation process does not affect the phenolic profile of olive leaves. In addition, the process prevented the degradation of these compounds during gastric digestion. Thus, it allows gradual degradation at the end of intestinal digestion, increasing the potential bioaccessibility of the substances present.

In a study with the objective of verifying the effects of prebiotics (inulin and galactooligosaccharide) during the microencapsulation process in alginate and chitosan matrix, Krasaekoopt & Watcharapoka (2014) verify that the microencapsulation process contributed to improve protection against probiotics and growth of these microorganisms in the simulated digestive system.

There are reports in the literature demonstrating the potential of phenolic compounds and their biological properties. However, there are no studies on the *in vitro* bioaccessibility of phenolic compounds in a ready-made product, such as nectar, which is added with nanoparticles to enhance their polyphenol content, increasing its functionality.

The industrial applicability of nanoparticles dispersed in an aqueous medium, for example, drinks such as yogurt, juice or néctar, of a complex food matrix (polysaccharides such as gum arabic, chitosan, maltodextrin or galactomannan, proteins or lipids) can be limited due to problems of low stability physico-chemical in prolonged storage periods. The main limitations are the aggregation of the particles, the chemical stability of the polymer and the premature release of the active substance. This calls for new research to address this issue.

4. Conclusion

The lyophilized pulp and by-product of acerola showed a high source for the bioactive compounds analyzed and antioxidant potential. The process of encapsulating the extract of the byproduct of acerola by spraying contributed to the maintenance of bioactive compounds during the stability of the nanoparticle and protection during gastrointestinal digestion *in vitro* simulation of nectar. These promising studies make possible the potential application of bioactive compounds extracted from acerola residues in the food industry.

Acknowledgments

The authors would like to thank CAPES (Coordination for the Improvement of Higher Education Personnel), CNPq (National Council for Scientific and Technological Development) and the University Federal of Ceara (UFC) through the Tropical Fruit Laboratory (DEAL) and the Chemistry Laboratory Biopolymers (LABIO POL) for their support provided for this study.

References

Araújo, A. P. O., Santos, E. C. C., Damasceno, F. S., Deboni, T. M., Cuevas, M. S., & Mota, R. V. (2016). Utilização de planejamento experimental no estudo da pasteurização do suco de acerola. *Scientia Plena*. 12, 1-8.

Belwal, T., Devkota, H. P., Hassan, H. A., Ahluwalia, S., Ramadan, M. F., Mocan, A., & Atanasov, A. G. (2018). Phytopharmacology of acerola (*Malpighia* spp.) and Its potencial as functional food. *Trends in Food Science & Technology*. 74, 99-106.

Brandt, A. L., Castillo, A., Harris, K. B., Keeton, J. T., Hardin, M. D., & Taylor, T. M. (2010). Inhibition of Listeria mmonocytogenes by food antimicrobials applied singly and in combination. *Journal of Food Science*. 75, 557-563.

Branen, J. K., & Davidson, P. M. (2004). Enhancementofnisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. *International Journal of Food Microbiology*. 90, 63-74.

Brasil, Instrução Normativa nº 12, de 4 de setembro de 2003. (2003). Regulamento técnico geral para fixação dos Padrões de Identidade e Qualidade para néctar. Diário Oficial da União, Brasília, DF.

Briones-Labarca, V., Venegas-Cubillos, G., Ortiz-Portilla, S., Chacana-Ojeda, M., Maureira, H. (2011). Effects of high hydrostatic pressure (HHP) on bioaccessibility, as well as antioxidant activity, mineral and starch contents in Granny Smith apple. *Food Chemistry*. 128, 520-529.

Çam, M., Içyer, N. C., & Erdogan, F. (2014). Pomegranate peel phenolics: Microencapsulation, storage stability and potential ingredient for functional foods development. *LWT - Food -Science and Technology*. 55, 117-123.

Cheong, A. A. M., Tan, B. C. P., & Nyam, A. K. L. (2017). Physicochemical, oxidative and antioxidante stabilities of kenaf seedoil-in-water nanoemulsions under different storage temperatures. *Industrial Crops and Products*. 95, 374-382.

Cruz, R. G., Beney, L., Gervais, P., Lira, S. P., Vieira, T. M. F. S., & Dupont, S. (2019). Comparison of the antioxidante property of acerola extracts with synthetic antioxidants using na in vivo method with yeasts. *Food Chemistry*. 277, 698-705.

Francis, F. J. (1982). Analysis of anthocyanins in foods. In: Markakis, P. *Anthocyanins as Food Colors*. New York, Academic Press, 181-207.

Filho, E. G. A., Silva, L. M. A., Brito, E. S., Wurlitzer, N. J., Fernandes, F. A. N., Rabelo, M. C., Fonteles, T. V., & Rodrigues, S. (2018). Evalution of termal and non-thermal processing effect on non-prebiotic and prebiotic acerola juices using ^{1}H *q*NMR and GC-MS coupled to chemometrics. *Food Chemistry*. 265, 23-31.

González, E., Gómez-Caravaca, A. M., Giménez, B., Cebrián, R., Maqueda, M., Martínez-Férez, A., Segura-Carretero, A., & Robert, P. (2019). Evolution of the phenolic compounds profile of olive leaf extract encapsulated by spray-drying during in vivo gastrointestinal digestion. *Food Chemistry*. 279, 40-48.

Guevara, M., Tejera, E., Granda-Albuja, M., Iturralde, G., Chisaguano-Tonato, M., Granda-Albuja, S., Jaramillo-Vivanco, J., Giampieri, F., Battino, M., & Alvarez-Suarez, J. M. (2019). Chemical Composition and Antioxidant Activity of the Main Fruits Consumed in the Western Coastal Region of Ecuador as a Source of Health-Promoting Compounds. *Antioxidants*. 14, 1-8.

Herculano, E. D., Paula, H. C. B., Figueiredo, E. A. T., Dias, F. G. B., & Pereira, V. A. (2015). Physicochemical and antimicrobial properties of nanoencapsulated Eucalyptus staigeriana essential oil. *LWT - Food Science and Technology*. 61: 484-491.

IAL. (2008). Métodos Físico-químicos Para Análise de Alimentos. (4th ed.), Instituto Adolfo Lutz, São Paulo, Brasil.

Krasaekoopt, W., & Watcharapoka, S. (2014). Effect of addition of inulin and galactooligosaccharide on the survinal of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice. *LWT - Food Science and Technology*. 57, 761-766.

Labuschagne, P. (2018). Impact of wall material physicochemical characteristics on the stability of encapsulated pytochemicals: A review. *Food Research International*. 107, 227-247.

Larrauri, J. A., Rupérez, P., & Saura-Calixto, F. (1997). Effect of drying temperature on the stabilitity of polyphenols and antioxidant activity of red grape pomace peels. *Journal Agriculture and Food Chemistry*. 45, 1390-1393.

Leão, D. P., Franca, A. S., Oliveira, L. S., Bastos, R., & Coimbra, M. A. (2017). Physicochemical characterization, antioxidant capacity, total phenolic and proanthocyanidin content of flours prepared from pequi (*Caryocar brasilense* Camb.) fruit by-product. *Food Chemistry*. 225, 146-153.

Lima, A. C. S., Soares, D. J., Silva, L. M. R., Figueiredo, R. W., Sousa, P. H. M., & Meneses, E. A. (2014). *In vitro* bioaccessibility of copper, iron, zinc and antioxidant compounds of

whole cashew apple juice and cashew apple fibre (Anacardium occidentale L.) following simulated gastro-intestinal digestion. *Food Chemistry*. 161, 142–147.

Londoño, M. B. Z., Chaparro, D., Rojano, B. A., Arbelaez, A. F. A., Betancur, L. F. R., & Celis, M. E.M. (2017). Effect of storage time on physicochemical, sensorial, and antioxidante characteristics, and composition of mango (cv. Azúcar) juice. *Emirates Journal of Food and Agriculture*. 29, 367-377.

Mar, J. M., Silva, L. S., Lira, A. C., Kinupp, V. F., Yoshida, M. I., Moreira, W. P., Bruginski, E., Campos, F. R., Machado, B. M., Souza, T. P., Campelo, P. H., Bezerra, J. A., & Sanches, E. A. (2020). Bioactive compounds-rich powders: Influence of different carriers and drying techniques on the chemical stability of the *Hibiscus acetosella* extract. *Powder tecnnology*. 3607 383-391.

Mariano-Nasser, F. A.7 Nasser, M. D.7 Furlaneto, K. A.7 Ramos, J. A.7 Vieites, M. K. P. (2017). Bioactive compounds in different acerola fruit cultivares. *Semina: Ciências Agrárias*. 387 2505-2514.

Mazza, P. H. S.7 Jaeger, S. M. P. L.7 Silva, F. L.7 Barbosa, A. M.7 Nascimento, T. V. C.7 Hora, D. I. C.7 Da Silva Júnior, J. M.7 Bezerra, L. R., & Oliveira, R. L. (2020). Effect of dehydrated residue from acerola (Malpighia emarginata DC.) fruit polp in lamb diet on intake, ingestive behavior, digestibility ruminal parameters and N balance. *Livestock Science*. 233, 103938.
7

Mezadri, T., Villaño, D., Fernández-Pachón, M. S., García-Parrilla, M. C., & Troncoso, A. M. (2008). Antioxidantes compounds and antioxidante activity in acerola (*Malpighia emarginata* DC.) fruits and derivates. *Journal of Food Composition and Analysis*. 21, 282-290.

Milani, L. P. G., Garcia, N. O. S., Morais, M. C., Dias, A. L. S., Oliveira, N. L., & Conceição, E. C. (2018). Extract from byproduct *Psidium quajava* standardized in ellagic acid: additivation of the *in vitro*photoprotective efficacy pf a comestic formulation. *Revista Brasileira de Farmacognosia*. 28, 692-696.

Miller, D. D., Schricker, B. R., Rasmussen, R. R., & Van Campen, D. (1981). *In vitro*method for estimation of iron availability from meals. *The American Journal of Clinical Nutrition*. 34, 2248-2256.

Nagata, M., & Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon. *Shokuhin Kogyo Gakkaisk*. 39, 925-928.

Niu, F., Pan, W., Su, Y., & Yang, Y. 2016. Physical and antimicrobial properties of thyme oil emulsions stabilized by ovalbumin and gum arabic. *Food Chemistry*. 212, 138-145.

Prakash, B., Kumar, A., Singh, P. P., & Songachan, L. S. (2020). Antimicrobial and antioxidant properties of phycochemicals: current status and future perspective. *Functional and Preservative Properties of Phytochemicals*. 1-45.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved $ABTS^{\bullet+}$ radical cation decolorization assay. *Free Radical Biology and Medicine*. 26, 1231-1237.

Rezende, Y. R. R. S., Nogueira, J. P., & Narain, N. (2017). Comparison and optimization of conventional and ultrasound assisted extraction for bioactive compounds and antioxidant activity from agro-industrial acerola (*Malpighia emarginata* DC) residue. *LWT - Food Science and Technology*. 85, 158-169.

Rezende, Y. R. R. S., Nogueira, J. P., & Narain, N. (2018). Microencapsulation of extracts of biactive compounds obtained from acerola (*Malpighia emarginata* DC) pulp and residue by spray and freeze drying: Chemical, morphological and chemometric characterization. *Food Chemistry*. 254, 281-291.

Rodriguez-Amaya, D. B., Rodriguez, E. B., & Amaya-Farfan, J. (2006). Advances in food carotenoid research: Chemical and technological aspects, implications in humna healh. *Malaysian Journal of Nutrition*. 12, 101-121.

Rufino, M. S. M., Alves, R. E., Brito, E. S., Morais, S. M., Sampaio, C. G., Pérez-Jimenez, J., & Saura-Calixto, F. D. (2007). Metodologia Científica: Determinação da atividade

antioxidante total em frutas pela captura do radical livre ABTS$^{\bullet+}$. Comunicado Técnico, 128. Fortaleza: Embrapa Agroindústria Tropical.

Ruiz-Rico, M., Pérez-Esteve, E., Lerma-García, M. J., Marcos, M. D., Martínez-Máñez, R., & Barat, J. M. (2017). Protection of folic acid through encapsulation in mesoporous sílica particles included in fruit juices. *Food Chemistry*. 218, 471-478.

Saènz, C., Tapia, S., Chávez, J., & Robert, P. (2009). Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus-indica*). *Food Chemistry*. 114, 616-622.

Silva, L. M. R. da, Figueiredo, E. A. T. de, Ricardo, N. M. P. S., Vieira, I. G. P., Figueiredo, R. W. de, Brasil, I. M., & Gomes, C. L. (2014). Quantification of bioactive compounds in pulps and by-products of tropical fruits from Brazil. *Food Chemistry*. 143, 398-404.

Silva, P. B., Mendes, L. G., Rehder, A. P. B., Duarte, C. R., & Barrozo, M. A. S. (2020). Optimization of ultrasound-assisted extraction of bioactive compounds from acerola waste. *Journal of Food Science and Technology*. 1-10.

Souza, K.O., Moura, C. F. H., Brito, E. S., & Miranda, M. R. A. (2014). Antioxidant compounds and total antioxidant activity in fruits of acerola from cv. Flor Branca, Florida Sweet and BRS 366. *Revista Brasileira de Fruticultura*. 36, 294-304.

Souza, N. C., Nascimento, E. N. O., Oliveira, I. B., Oliveira, H. M. L., Santos, E. G. P., Mata, M. E. R. M., Gelain, D. P., Moreira, J. C.F., Dalmolin, R. J. S., & Pasquali, M. A. B. (2020). Anti-inflammatory and antioxidant properties of blend formulated with compounds of *Malpighia emarginata* D.C (acerola) and *Camellia sinensis* L. (green tea) in lipopolysccharide-stimulated RAW 264.7 macrophages. *Biomedicine & Pharmacotherapy*. 128, 110277.

Tolun, A., Altintas, Z., Artik, N. (2016). Microencapsulation of grape polyphenols using maltodextrin andgum arabic as two alternative coating materials: Development and characterization. *Journal of Biotechnology*. 239, 23-33.

Xu, M., Shen, C., Zheng, H., Xu, Y., Xue, C., Zhu, B., & Hu, J. (2020). Metabolomic analysis of acerola cherry (*Malpighia emarginata*) fruit during ripening development via UPLC-Q-TOF and contribution to the antioxidante activity. *Food Research International*. 130, 108915.

Percentage of contribution of each author in the manuscript

Alessandra Pinheiro de Góes Carneiro – 25%

Antonia Livânia Linhares de Aguiar – 25%

Ana Cristina Silva de Lima – 10%

Larissa Moraes Ribeiro da Silva – 10%

Paulo Henrique Machado de Sousa – 20%

Raimundo Wilane de Figueiredo – 10%