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Replacing synthetic antioxidants in food emulsions with microparticles from green acerola (*Malpighia emarginata*)

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ABSTRACT

Among natural extracts with antioxidant potential, acerola fruit is an important source of bioactive compounds. The goal of this work was to evaluate the antioxidant capacity of microencapsulated and lyophilized extracts of unripe acerola fruits produced under environmentally friendly conditions. In vitro antioxidant activity was determined, and products were applied in oil-in-water emulsions. Lipid oxidation products were measured by the absorptivity at 232 nm and hydroperoxide content. Sensory characteristics resulting from the addition of acerola microparticles to emulsions were also investigated. The aqueous extracts of acerola fruits showed a high concentration of ascorbic acid (32.52 to 41.11 mg.100 mg^{-1}) and reducing power; the retention of ascorbic acid was 88% after spray drying. Oxidation inhibition was observed upon emulsion addition of acerola products: the hydroperoxide content after 9 days under accelerated conditions in the control samples was 14.03 mmol. L^{-1} and from 3.02 to 3.60 mmol. L^{-1} in samples with TBHQ or acerola microparticles (100-200 mg.kg^{-1}). In addition, the microparticles did not show sensory effects compared to synthetic antioxidants. Acerola microparticles obtained after simple water extraction from green fruits were effective and are a potential new ingredient for the oxidative stability of lipid emulsions.

1. Introduction

Lipid oxidation is considered one of the most important reactions in the food industry, occurring mainly in products with high lipid contents, such as emulsions and oils. This reaction disturbs the emulsion stability, such as mayonnaise and salad dressings, due to the formation of new compounds but also weakens the quality of the product and reduces the nutritional value of food (Frankel, 2005; Berton-Carabin et al., 2014). To improve oxidative stability, the use of antioxidants has become essential to maintain quality and extend the shelf life of food products (Shahidi and Zhong, 2010).

The main antioxidants currently used in the food for oils and fats are synthetic antioxidants, such as butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG) (Xu et al., 2021). However, despite being effective in controlling lipid oxidation, there is evidence of adverse effects on the body caused by the consumption of these antioxidants, increasing the preference of the consumer for antioxidant alternatives from natural sources present in compounds from different plant extracts (Shahidi and Ambigaipalan, 2015; Yashin et al.,2017; Cruz et al., 2018; Cavalaro et al., 2019).

Acerola (*Malpighia emarginata D.C.*) is a tropical fruit that originated in Central America and was introduced into Brazil in the 1950s due to its good adaptation to the soil and climate. Stemming from its undeniable potential as a natural source of vitamin C and other bioactive compounds and its great capacity for industrial use, the fruit has attracted industrial interest and has become economically important in several regions of Brazil (Cruz et al., 2018; Ramadan et al., 2019; Silva et al., 2019).

Among the bioactive compounds present in acerola fruit, there are vitamins such as thiamine (B1), riboflavin (B2), niacin (B3) and provitamin A; amino acids (AA) as asparagine, alanine, proline; β-carotenoids; phenolic compounds, including benzoic acid derivatives (gallic acid and syringic acid), phenylpropanoids (caffeic acid, ferulic acid, etc), flavonoids and anthocyanins and minerals as calcium, iron, potassium, magnesium and phosphorus. However, its greatest nutritional appeal is a very high vitamin C content, whereas some studies suggested that fresh Acerola juice contains 50 to 100 times more vitamin C than an equal portion of orange or lemon (Belwal et al., 2018; Prakash and Baskaran, 2018). This compound provides high antioxidant capacity *in vitro* systems, especially during the green maturation stage (Delva and Goodrich-Schneider, 2013; Xu et al. 2020).

Xu et al. (2020), studied the chemical variation at the two developmental stages (immature and mature) of acerola and found that ascor-

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bic acid (vitamin C) predominantly accumulated in the immature fruits, as well as the immature fruit possessed better scavenging ability of DPPH and ABTS than the mature one. On the other hand, amino acids, flavonoids, lipids, and terpenoids were predominantly in the mature fruits. Thus, this antioxidant capacity, present mainly in the unripe fruit, can be explored for application in oils and emulsions in the food industry as an alternative to synthetic antioxidants (Caetano et al., 2011). Therefore, there is a need for more studies involving the use of acerola in food products, which may contribute to the inhibition of lipid oxidation and the use of interesting technological properties.

Encapsulation and microencapsulation techniques have been applied to increase the stability of several unstable compounds under adverse environmental conditions, extending the storage time, and improving transportation conditions and marketing processes (Silva et al., 2014). Spray drying encapsulation is the most common technology used in the food industry and is considered a continuous and low-cost process that allows the production of small particles with interesting technological properties (Gharsallaoui et al., 2007). In the spray drying process, a liquid becomes a dry product in a very short time, with atomization by a spray nozzle, allowing for the protection of the compounds of interest (Borgogna et al., 2010).

Although the encapsulation process is widely used for microencapsulation of fruit extracts and other bioactive compounds from natural sources (Cabral et al., 2018; Rezende et al., 2018; Šeregelj et al., 2019), there is little information about using the encapsulated bioactive compounds of acerola in food emulsions as an ingredient. The addition of these compounds may imbue foods with interesting technological properties regarding the inhibition of lipid oxidation.

The objective of this work was to produce a new ingredient with high antioxidant activity by antioxidant recovery through the microencapsulation of bioactive compounds of unripe acerola fruits, aiming for its potential application as an antioxidant in oil-in-water emulsions model system.

2. Materials and methods

2.1. Chemicals, reagents, and encapsulating agents

Folin–Ciocalteu reagent was purchased from Dinâmica Química Contemporânea LTDA (Diadema, SP, Brazil). L-ascorbic acid, tert-butylhydroquinone (TBHQ), cumene hydroperoxide, ABTS (2.2′-azinobis-(3-ethylbenzothiazoline-6-acid)), DPPH (2,2-diphenyl-1-picrylhydrazyl), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Gum arabic (GA) was supplied by Nexira Brazil Comercial Ltd. (São Paulo, SP, Brazil). Tween 20 was purchased from Êxodo Cientifica (Hortolândia, SP, Brazil). The refined soybean oil was provided by Cargill Agricola SA. Other reagents were p.a grade.

2.2. Green acerola

Unripe acerola fruits were provided by the Junqueirópolis Agricultural Association, located in Junqueirópolis, (21° 30’ 52” S, 51° 26’ 01” O), São Paulo, Brazil. The fruits were harvested during early spring 2019, selected, and sanitized with sodium hypochlorite (Hidrosteril®, Brazil) according to package instructions and then rinsed and placed on a flat surface previously sanitized with 70% ethanol for natural drying at room temperature. The dried fruits were frozen at -80°C and subsequently lyophilized (Liobras, Liotop L-101, Brazil). The lyophilization process was carried out in the dark, during approximately 48h. The lyophilized acerolas were ground and stored in plastic bags covered by metalized bags at -28°C ± 1 until the analyses were performed.

2.3. Extracts preparation

Green acerola extracts were obtained by dispersing the lyophilized sample in distilled water, 1:40 (w/v), adjusted to 35°C using a water bath (Nova Instruments, Dubnoff, Brazil), for 10 minutes at 200 rpm. After the extraction period, the extracts were centrifuged at 5000 g in a 4°C refrigerated centrifuge (Hitachi Koki Co, Himac CF 16 RN, China) for 20 minutes and filtered through Qualitative Filter Paper (Whatman, USA) into amber vials for storage at -28 ± 1°C to preserve the antioxidant properties, the content of ascorbic acid and the reducing power.

2.4. Microencapsulated powders preparation

The unripe acerola fruit extract was atomized by a spray dryer. For this, a 6% (w/v) proportion of encapsulating material was added to the extract (Tonon et al., 2010). During processing, approximately 2 liters of the extract were used. The process was performed in duplicate. Gum arabic (GA) was used as an encapsulating agent (Nexira Brazil Comercial Ltda, Brazil). The mixtures were homogenized using a magnetic stirrer (IKA, Color squid, United States) until complete solubilization and then spray atomized (Eikonal, LM MSD 1.0, Brazil). A spray nozzle of 1.2 mm, an inlet temperature of 140°C, and an outlet temperature of 80°C, with a flow rate of 1 $L.h^{-1}$, was used for atomization. The samples were stored in glass containers at -28°C until further analysis and application.

2.5. Physicochemical properties determination from lyophilized and microencapsulated samples

2.5.1. Moisture content

The moisture content of the samples was calculated from the weight loss after heating the sample in a drying oven (Fanem, 315 SE, Brasil) at 105°C according to the method of AOAC (2005). The determinations were made in triplicate, and the values were analyzed as percentages.

2.5.2. Water activity (aw)

Water activity (aw) was measured by direct reading in bench water (TESTO, model 650, Germany) after samples were stabilized at 25°C for 15 min.

2.5.3. Solubility

Solubility was determined according to the methodology described by Eastman and Moore (1984), with some modifications by Cano-Chauca et al. (2005). One gram of sample was mixed with 100 mL of distilled water at room temperature. The mixtures were stirred in a magnetic stirrer for 5 minutes at 400 rpm (IKA RH, digital KT/C, Germany) at room temperature. The solution was centrifuged at 3000 x g for 5 minutes (Hettich, 320/320R, Germany) at 20°C. An aliquot of 25 mL of the supernatant was transferred into previously weighed crucibles and placed in an oven at 105°C for 5 hours. Solubility (%) was calculated by weight difference.

2.5.4. Hygroscopicity

Hygroscopicity was determined according to the method described by Cai and Corke (2000). Two grams of samples were placed in previously weighed Petri dishes and stored in a desiccator with saturated sodium sulfate solution (81RH), which remained for 7 days at 25°C, controlled by Biochemical Oxygen Demand refrigerator (BOD-Tecnal, TE -391, Brazil). After this time, the samples were weighed again, and the results were expressed in g of water absorbed per 100 g of dry solids.

2.5.4. pH determination

The pH of the samples was measured by a pH meter (Tecnal, Tec3-MP, Brazil) according to the AOAC method (2005).

2.6. Morphology of the microencapsulated powder

The structure and morphology of the microparticles were analyzed using a scanning electron microscope (SEM) (Hitachi, TM 3000, Japan) with vacuum and image capture. The samples were placed in small quantities on the surface of double-sided tape. The structures were observed with 1000 and 4000 times increases and 15 kV voltage.

2.7. Active compounds and antioxidant activity determination

For the determination of the bioactive compounds and antioxidant activity, the microparticles were solubilized in water (0.9 g of green acerola powder in 5 mL of water). The lyophilized extract samples were diluted in water at ratios of 1:100 (extract:solvent, v/v), 1:15, and 1:150 for reducing power, ABTS, and DPPH, respectively. The microencapsulated extract samples were diluted in water at ratios of 1:400, 1:20, and 1:300 for reducing power, ABTS, and DPPH, respectively. However, to determine the acid ascorbic (AA) content, a dilution of both extracts was made to obtain a concentration of 1 $mg.mL^{-1}$.

The losses caused by the microencapsulation process were analyzed by the retention of the compounds present in acerola, calculated as follows:

$$\text{Retention of compounds present in acerola (\%)} = \frac{(C_X)\,microencapsulated\ extract}{(C_X)\,\text{lyophilized extract}}\ X\ 100$$

where Cx is the concentration of compound x based on dried acerola extract (mg/100 mg microencapsulated or lyophilized extract).

2.7.1. Determination of reducing power (RP)

The reducing power of the extracts was determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965; Singleton et al., 1999). The absorbance was measured at 764 nm using a spectrophotometer (UV, Shimadzu, Japan). The results were calculated from a standard curve with known concentrations (5 to 60 $\mu g.mL^{-1}$) of gallic acid (linear equation $y = 0.0107x - 0.0211$; $R^2 = 0.9975$) and were expressed in mg of gallic acid equivalent (GAE). 100 mg^{-1} of dry extract.

2.7.2. ABTS radical scavenging capacity

The ABTS radical cation scavenging capacity was determined according to the methodology described by Re et al. (1999) and Camargo et al. (2012). The absorbance was measured at 734 nm using a spectrophotometer (UV, Shimadzu, Japan). The results were calculated using a standard curve of Trolox (from 500 to 2000 μM) (linear equation $y = -0.0003x + 0.7329$; $R^2 = 0.9936$), and the results were expressed in μM Trolox equivalent (TE).

2.7.3. DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical cation scavenging was performed according to the methodology described by Brand-Williams et al. (1995). The absorbance at 515 nm was measured using a spectrophotometer (UV, Shimadzu, Japan) at 515 nm. The results were calculated from a standard curve with known concentrations (from 20 to 160 μM) of Trolox (linear equation $y = -0.0021x + 0.408$; $R^2 = 0.9976$) and expressed μM TE.

2.7.4. Ascorbic acid (AA) content

The ascorbic acid contents of the extracts were analyzed by HPLC - High-Performance Liquid Chromatography with diode array detector (UV/VIS), according to the methodology proposed by Vendramini and Trugo (2004), with some modifications. High-performance chromatography was performed on an Agilent HP 1100 series quaternary pump chromatograph. Separation was performed on a C18 Phenomenex® octadecylsilane group derivatized silica column (dimensions 250 × 4.6 mm - 5 μm). In the mobile phase, two solvents were employed, A and B, a gradient of acidified trisodium citrate (0.01 M) (pH 3.1), and methanol, respectively. The following gradient was used for 20 minutes of running and 10 minutes of cleaning, totaling 30 minutes: 100% solvent A (up to 1.5 minutes), 30% solvent A and 70% solvent B (up to 13 minutes), and 10% solvent A and 90% solvent B (from 13.1 minutes). The flow rate was 1 $mL.min^{-1,}$ and the injection volume was 20 μL. The chromatographic run was monitored at 275 nm. The aqueous extracts were filtered through 0.45 μm membranes for sample injection, and quantification was performed by a standardization curve. The results were calculated from a standard curve with known concentrations (from 0.1 to 2 $mg.mL^{-1}$) of ascorbic acid (linear equation $y = 6412.5x - 845.21$; $R^2 = 0.9862$) and expressed in mg AA.100 g^{-1} of dry extract.

2.8. Oxidative stability of the emulsions

The ability of the acerola extracts to retard lipid oxidation in oil-in-water lipid emulsions was evaluated and compared with TBHQ, a synthetic antioxidant. Oil-in-water (o/w) emulsions were formulated according to the methodology of Huang et al. (1996), with modifications. Emulsions were prepared at 50%:45%:5% (oil:distilled water: Tween 20). The soybean oil was provided by CARGILL Agricola SA. Tween 20, the water, and the antioxidants (TBHQ and the acerola microparticles) were weighed and added to the container for homogenization, and only the oil was slowly added during the homogenization performed by Ultraturrax (Basic T18, IKA, Germany) at 18000 rpm for 5 minutes.

The added antioxidant concentrations (100 and 200 $mg.kg^{-1}$) were calculated based on the reducing power obtained for the lyophilized extract and microparticles. Seven treatments were performed in the emulsions: T1 (control - without antioxidants); T2 (lyophilized extract 100 $mg.kg^{-1}$); T3 (lyophilized extract 200 $mg.kg^{-1}$); T4 (microparticle 100 $mg.kg^{-1}$); T5 (microparticle 200 $mg.kg^{-1}$); T6 (TBHQ 100 $mg.kg^{-1}$) and T7 (TBHQ 200 $mg.kg^{-1}$).

For each treatment, triplicate aliquots of emulsions were made, distributed in 10 mL glass test tubes, and stored in a circulating oven (FANEM, 315 SE, Brazil) at a constant temperature of 40°C. The samples were collected for analysis after each storage period (0, 3, 6, and 9 days) (Kargar et al., 2011).

The evaluation of the oxidation degree and the stability of the emulsion was carried out by hydroperoxide content, according to the procedures of Shantha and Decker (1994) with some modifications, by conjugated diene assay according to standard method 2.501 AOCS (1998) and by the creaming index according to Keowmaneechai and McClements (2002).

For the hydroperoxide content, 300 μL of the upper layer of the emulsion were added with 1.5 mL of isooctane and isopropanol solution (3:1/v:v) in test tubes and shaken three consecutive times (10 seconds each) with vortexing at 20-second intervals (Labdancer, IKA, Germany). Two hundred microliter aliquots were then taken from the mixture and added to a second tube, followed by the addition of 2.8 mL of methanol and n-butanol solution (2:1/v:v) and vortexing. In this same tube, 30 μL of a solution of $NH_4SCN:Fe^{+2}$ (1:1/v:v), obtained from a mixture of NH_4SCN (3.94 M) and Fe^{+2}, was added. The Fe^{+2} (0.072 M) solution was obtained by mixing (1:1/v:v) $FeSO_4$ (0.144 M) and $BaCl_2$ (0.132 M). After the addition of NH4SCN:$Fe^{+\,2}$ (1:1/v:v), the tubes were shaken and stored at room temperature in low light for 20 minutes. The absorbance at 510 nm was measured using a spectrophotometer (UV, Shimadzu, Japan). A calibration curve was performed on cumene hydroperoxide, and the results were expressed as mmol hydroperoxide.kg^{-1} of oil.

To measure the content of conjugated dienes, 0.2 g of sample was added to volumetric flasks of 25 mL, completed with isooctane, and mixed manually for solubilization for approximately 30 seconds. The absorbance was measured using a spectrophotometer (UV, Shimadzu, Japan) at 232 nm. The results were calculated using Eq. (1):

$$E_{1\%\ (1cm)} = \frac{A}{C} \quad (1)$$

Table 1
Physicochemical characteristics of lyophilized unripe acerola and unripe acerola extract microparticles. Encapsulating material: Gum Arabic.

Analysis	Lyophilized Unripe Acerola	Unripe Acerola Extract Microparticles
Moisture (%)	21.20 ± 0.05[a]	4.42 ± 0.12[b]
Solubility (%)	47.83 ± 4.55[b]	96.56 ± 1.11[a]
Hygroscopicity (g absorbed water/100 g sample)	35.75 ± 3.81[a]	51.37 ± 3.87[a]
pH (Solution 1%)	3.67 ± 0.09[a]	4.48 ± 0.11[a]
Water activity (Aw)	0.64 ± 0.005[a]	0.39 ± 0.01[b]

Data are expressed as the mean ± standard deviation. Different letters within a row are significantly different ($p < 0.05$). T test was performed for two mean comparisons.

where $E_{1\%\ 1cm}$ = extinction of the 1% solution of the oil solution in the specific solvent, in a thickness of 1 cm; A = absorbance; and C = sample solution concentration (emulsion and isooctane) (g.100 mL^{-1}).

The cream index consists of measuring the total emulsion height and the phase separation height (serum). The analysis was evaluated according to the methodology described by Keowmaneechai and McClements (2002). The emulsions (10 mL) were stored in screw cap test tubes (9 × 1.5 cm) and subjected to the same accelerated oven test (40°C for 9 days).

2.9. Sensory analysis

A scale test was performed with emulsion samples prepared using five types of treatments, as shown below. Samples were properly named with three-digit numbers randomly served to sixteen volunteer judges in individual booths in a single session. The judges gave a record with four sensory attributes, two referring to the appearance of the product (yellow color and brightness) and two referring to the evaluation of aroma (oxidized aroma and acid aroma). Attributes were evaluated in grades from 1 to 5, with 5 being the highest grade of the attribute and 1 the lowest grade. The scorecard used in this test is summarized in Supplementary Fig. 1. The five treatments tested were as follows:

(i) Treatment 1 (oxidized control): the emulsion samples were subjected to an accelerated oxidation test at 40°C for 9 days.
(ii) Treatment 2 (control): the emulsion samples were prepared on the day of the sensory analysis without antioxidants added.
(iii) Treatment 3: The emulsion samples were prepared on the day of the sensory analysis with microencapsulated green acerola (100 ppm) added as an antioxidant.
(iv) Treatment 4: The emulsion samples were prepared on the day of the sensory analysis with microencapsulated green acerola (200 ppm) added as an antioxidant.
(v) Treatment 5: The emulsion samples were prepared on the day of the sensory analysis with synthetic antioxidant (TBHQ 200 ppm) added.

2.10. Statistical analysis

All results are expressed in the figures and tables as the mean ± standard deviation of triplicate measurements. A t test was performed for two mean comparisons and one-way analysis of variance (ANOVA) followed by Tukey's test for multiple means comparisons among the different treatment groups using GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA). Statistical significance was set at $p<0.05$.

3. Results and discussion

3.1. Physical properties of the samples

The basic characteristics of the powder, such as moisture, solubility, hygroscopicity, pH, and water activity, are important for determining the stability and storage of the extracts (Rezende et al., 2018). These properties of lyophilized unripe acerola and unripe acerola extract microparticles are shown in Table 1.

The moisture and water activity of the resulting product of the encapsulation process presented lower values, favoring conservation during storage. In addition, the pH, solubility, and hygroscopicity of the encapsulated extracts were higher than those of lyophilized acerola (Table 1). Microparticle water activity is low (0.39), which is within expectations for dehydrated products, reducing concerns about the development of pathogenic microorganisms (Beuchat et al., 2013). The water content (4.42%) is within the range evaluated by authors who worked with green acerola microencapsulated with gum arabic (6.39%) (Righetto and Netto, 2005).

Hygroscopicity is considered a very important property in powdered foods and is influenced by moisture. Lower hygroscopic encapsulating agents are more desirable because they tend to absorb less water from the environment, avoiding product degradation (Silva et al., 2013). The hygroscopicity of the microencapsulated acerola extract (Table 1) ranged from 48.75 to 55.81 g of absorbed water.100 g^{-1} of dry solids, which are comparable to the results obtained by Moreira et al. (2009) in the studies of the properties of microencapsulated acerola bagasse with maltodextrin and cashew gum, which showed results between 34.72 and 56.44 g of absorbed water.100 g^{-1} of powder. According to Moreira et al. (2009), these high hygroscopicity values are not absolute due to the exposure of the extracts to high relative humidity (90%) without proper packaging to separate the sample from the environment, which probably does not represent the actual hygroscopicity of the product under storage conditions because the powder would be packed with moisture control material.

3.2. Microparticle morphology

Observations made by electron microscopy showed the efficient formation of microparticles with the encapsulating agent used, Gum Arabic (Fig. 1) Fig. 1.A shows an image of 1000 × enlarged microparticles Fig. 1.B shows the microparticles at 4000x magnification. The microparticles showed regular intact walls with a rounded external surface and without cracks or apparent porosity. These characteristics are important for impermeability against undesired substances and greater protection of the properties of the core, that is, acerola (Santos et al., 2005).

It was also possible to observe the tendency of formation of aggregates of smaller microparticles around larger ones and the presence of concavities on the surface, which are characteristics of microparticles obtained from atomization with gum arabic as the encapsulating agent. These aspects were also reported by Trindade and Grosso (2000), Bertolini et al. (2001), Silva et al. (2013) and Subtil et al. (2014).

3.2. Antioxidant activity and bioactive compounds

The bioactive compound contents and antioxidant activity in lyophilized and microencapsulated green acerola extracts are presented in Fig. 2.

The antioxidant activity of lyophilized green acerola extracts, determined by the ABTS and DPPH assays, ranged from 154.24 to 165.15 μM TEAC.100 mg^{-1} and from 157.51 to 165.01 μM TEAC.100 mg^{-1} dry extract, respectively. For the microencapsulated extracts, the antioxi-

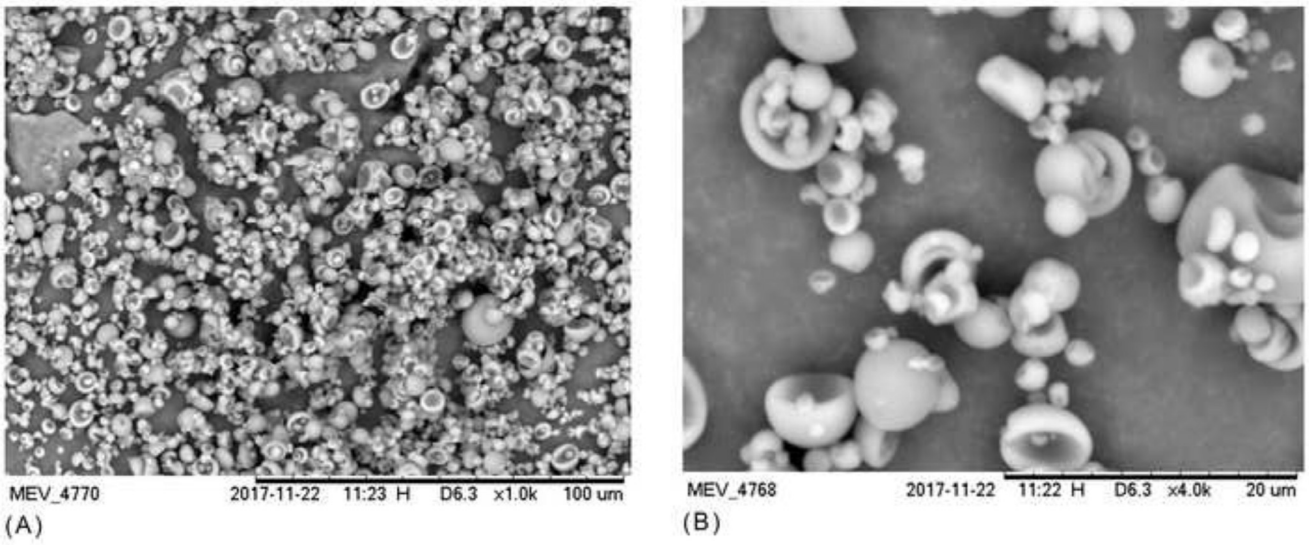

Fig. 1. Microparticles of lyophilized acerola extract: (A) 1000x magnification, (B) 4000x magnification Encapsulating agent: Gum Arabic.

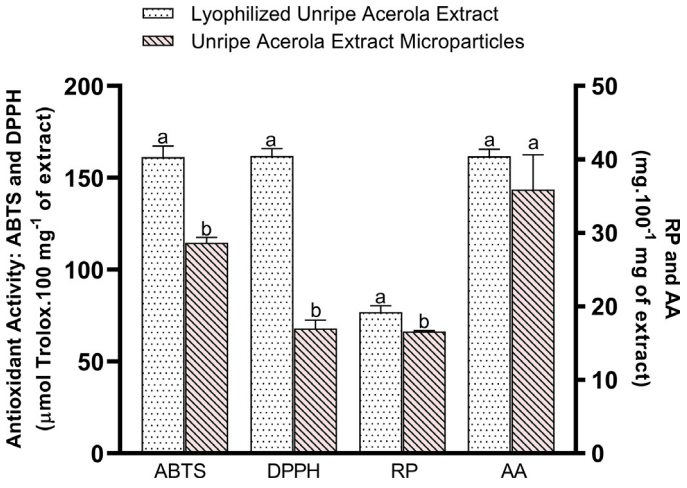

Fig. 2. Antioxidant activity (ABTS and DPPH), reducing power (RP) and ascorbic acid (AA) of lyophilized and microencapsulated green acerola extracts. Values are expressed as the mean ± standard deviation. Different letters within the extracts are significantly different ($p < 0.05$) using the T test.

dant capacity ranged from 111.22 to 116.55 μM TEAC.100 mg^{-1} and 64.73 at 73.30 μM TEAC.100 mg^{-1} of dry extract for ABTS and DPPH, respectively (Fig. 2).

In the assays for the evaluation of ascorbic acid contents and reducing power, the lyophilized extracts of green acerola presented concentrations ranging from 39.75 to 41.11 mg AA.100 mg^{-1} dry extract and from 18.50 to 20.16 mg EAG.100 mg^{-1} dry extract, respectively. In the microencapsulated extract, the concentrations of ascorbic acid ranged from 32.52 to 39.26 mg.100 mg^{-1} of dry extract, and the reducing power ranged from 16.48 to 16.64 mg of EAG.

The high bioactive activity found can be partially explained by the high amount of ascorbic acid present in acerola, since this compound influences the scavenging activity and the reducing power evaluated by the method used (Folin-Ciocalteu) (Cruz et al., 2018).

Stafussa et al. (2021) found that among 44 traditional and exotic Brazilian fruit pulps studied, acerola, açaí and jabuticaba had the highest values of reducing capacity and antioxidant activity. Similarly, Vieira et al. (2011) studied the reducing power and antioxidant capacity of aqueous and hydroalcoholic extracts of frozen tropical fruit pulps. The aqueous extracts of acerola presented a reducing power of 0.84 mg of gallic acid per 100 mg of pulp, which was lower than that found in this study, considering both the freeze-dried and microencapsulated extract of green acerola. However, this same work observed an antioxidant capacity of 160.5 μM TEAC.100 mg^{-1}, by the ABTS method, for the aqueous extracts of frozen acerola pulps, the results superior to those found in this work.

In addition, Silva et al. (2016) evaluated the effects of processing on bioactive compounds present in acerola residues. After dehydration processes, the authors found losses in citric acid contents due to the low sensitivity of this compound at high temperatures, while the reducing power found in the dry residues was higher than those obtained by fresh samples. The reducing power of acerola residues ranged from 0.727 to 0.914 mg gallic acid.100 mg^{-1}, results inferior to those found in the present work.

Nonetheless, Cruz et al. (2018) evaluated the comparison of the antioxidants present in acerola with synthetic antioxidants using an *in vivo* yeast method. The immature acerola fruit extracts in the *in vitro* studies presented higher levels of reducing power and antioxidant activities (DPPH and ABTS) than the ripe fruit extract. *In vivo* results showed that acerola extracts increased yeast resistance to menadione-induced oxidative stress treatments (prooxidant agents), showing no differences between mature and immature acerola extracts. However, neither extract protected the t-BOOH yeast membrane (oxidizing agent preferentially acting on cell membrane lipids). The results obtained with the synthetic antioxidant BHA in *in vivo* studies revealed pro-oxidant and harmful effects on cells. Thus, the study demonstrates the complexity of the antioxidant activity of acerola fruit and the need to study its application in each system to be analyzed.

Moreover, Rezende et al. (2018) studied the microencapsulation of bioactive compounds obtained from acerola pulp and residues by spray dryer and lyophilization. Among the various treatments performed, the content of ascorbic acid ranged from 0.22 to 0.46 mg AA.100 mg^{-1} extract, and the reducing power ranged from 1.02 to 1.05 mg EAG.100 mg^{-1} extract, values significantly lower than those found in this study. For the antioxidant capacity of the microencapsulated extracts, the authors observed a variation of 13.97 to 15.12 μM TEAC.100 mg^{-1} and of 12.92 to 15.52 μM TEAC.100 mg^{-1}, according to ABTS methods and DPPH, respectively, the results also lower than the values observed by this study.

In addition, Ferreira et al. (2009) obtained a range from 1.8 to 2.2 mg.100 mg^{-1} ascorbic acid in acerola depending on the fruit maturation stage Silva et al. (2019). found 0.13 ± 1.1 mg.100 mg^{-1} ascorbic acid on fresh acerola residues and 0.02 ± 0.1 mg.100 mg^{-1} ascorbic acid on

dry residues. Although these studies exhibited lower values than those found in the present study, some authors reported that the variations in the results may be related to the type of cultivation, handling, climate, soil, variety, geographical origin, and degree of fruit maturation, as well as the type of preparation, chemical composition, concentration and kinetic reactions of the sample, factors that make it difficult to compare the results obtained with those found in the literature (Vendramini and Trugo, 2000; Cruz et al., 2018).

Furthermore, it is important to emphasize that the extracts produced in this work were obtained under previously optimized conditions (variables: sample-to-solvent ratio (1:30 - 1:100) and extraction time (10 - 60 minutes)), resulting from the application of an experimental design and adjustment of mathematical models aiming for maximum antioxidant activity. The results presented in this study validate the optimized conditions determined for the extraction of active compounds.

The effect of the microencapsulation process was evaluated by retention of the active compounds (ascorbic acid (AA)), reducing power, and antioxidant capacity (ABTS and DPPH) in the microencapsulated extracts. The retention of the antioxidant capacity of the compounds present in acerola was 71.09% and 41.93% for ABTS and DPPH, respectively. In addition, for AA and reducing power, the retention of bioactive compounds was 88.76% and 86.25%, respectively.

Losses of these compounds during the drying process may have occurred due to different factors, such as surface cracking, rapid evaporation of water with the use of high temperatures in the spray drying process, pore formation in the microparticles and sublimation of water in the lyophilization process, which can cause loss of the encapsulated component and, consequently, its degradation. The use of a low encapsulating agent concentration (6%) may also have contributed to this microencapsulation efficiency range (Rezende et al., 2018).

Regardless of the losses, the acerola microencapsulation process allows us to control and delay the degradation reactions of its compounds during storage, protecting against undesired reactions caused by oxygen, heat, and humidity, since the moisture and water activity of the microparticles have been reduced.

3.3. Emulsion stability

Measurement of hydroperoxides and conjugated dienes are classical methods for quantifying lipid oxidation. The oxidation of polyunsaturated fatty acids occurs with the formation of hydroperoxides and displacement of double bonds, with consequent formation of conjugated dienes, which have absorptivity at 230-235 nm (Shahidi and Zhong, 2005) Fig. 3. shows the evolution of hydroperoxide contents during the 9 days of storage at 40°C. An increase in oxidation and a difference between the treatments performed were observed during the evaluated period. The highest values on the 9th day of analysis were for the control and treatment with lyophilized green acerola. The treatments with microencapsulated green acerola, at both concentrations, presented the lowest hydroperoxide contents until the end of the evaluated period. The performance of green acerola extract microparticles was similar to the effect of TBHQ.

Taghvaei et al. (2014) evaluated the oxidative stability of soybean oil at 110°C in different treatments with liquid extracts of olive leaves and microparticles of the same extract. They also observed that the best thermal stability was given to the microencapsulated extract with gum arabic and that the microencapsulation process conferred better protection of the bioactive compounds of the extract.

The results of the conjugated dienes are demonstrated in Fig. 4. The specific UV absorbance values ranged from 2.61 to 4.00, representing a variation in the content of conjugated dienes, and the results showed a small increase during the evaluation days. The highest specific absorbance values were observed for the control treatment, and at the end of the analysis period (9 days), the treatment with green acerola microparticles showed the lowest values.

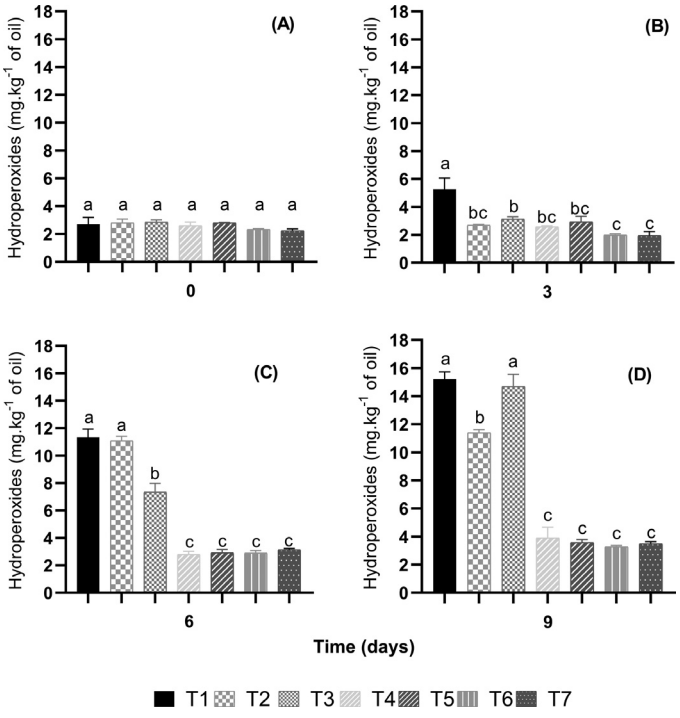

Fig. 3. Hydroperoxide content of soybean oil emulsions with added synthetic antioxidants (TBHQ) and green acerola extract during the accelerated test at 40°C performed each time and compared between treatments ($p < 0.05$). Legends: (A) Day 0; (B) Day 3; (C) Day 6; D: Day (9). T1 (control - without antioxidants); T2 (lyophilized extract 100 mg.kg-1); T3 (lyophilized extract 200 mg.kg-1); T4 (microparticle 100 mg.kg-1); T5 (microparticle 200 mg.kg-1); T6 (TBHQ 100 mg.kg -1) and T7 (TBHQ 200 mg.kg -1). Different letters indicate significant differences by Tukey's test ($P < 0.05$).

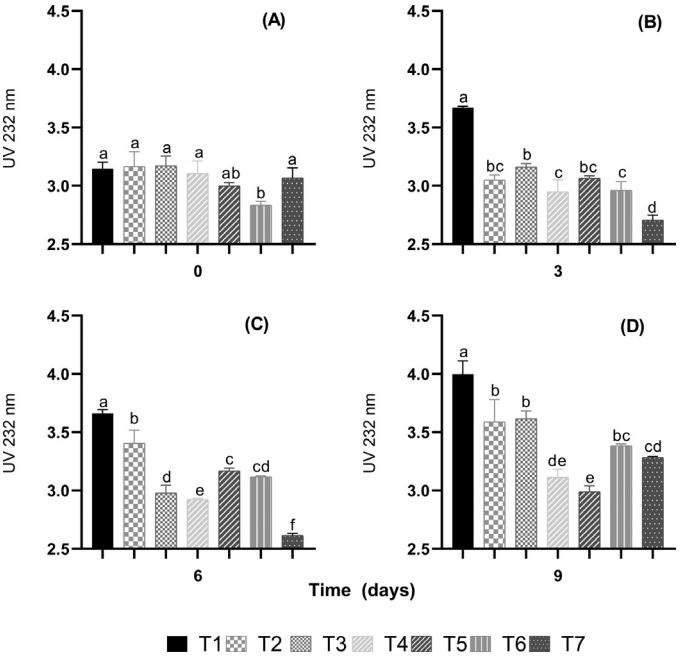

Fig. 4. Conjugated dienes of soybean oil emulsions supplemented with synthetic antioxidants (TBHQ) and green acerola extract during the accelerated test at 40°C were performed each time and compared between treatments ($p < 0.05$). Legends: (A) Day 0; (B) Day 3; (C) Day 6; D: Day (9). T1 (control - without antioxidants); T2 (lyophilized extract 100 mg.kg^{-1}); T3 (lyophilized extract 200 mg.kg^{-1}); T4 (microparticle 100 mg.kg^{-1}); T5 (microparticle 200 mg.kg^{-1}); T6 (TBHQ 100 mg.kg $^{-1}$) and T7 (TBHQ 200 mg.kg $^{-1}$). Different letters indicate significant differences by Tukey's test ($P < 0.05$).

Table 2
Average scores obtained by the tasters for the different treatments of emulsion samples according to the sensory parameters: oxidized odor, acid odor, yellowness, and brightness.

	PARAMETERS							
Treatment	Yellowness		Brightness		Oxidized Odor		Acid Odor	
1	3.31 ± 1.20	A	3.44 ± 1.09	a	1.50 ± 1.73	a	1.25 ± 0.58	a
2	3.19 ± 0.98	A	3.31 ± 1.30	a	1.81 ± 0.98	a	1.31 ± 0.60	a
3	1.69 ± 0.70	B	3.44 ± 0.96	a	1.31 ± 0.60	a	1.06 ± 0.25	a
4	1.50 ± 0.73	B	3.19 ± 1.11	a	1.19 ± 0.40	a	1.19 ± 0.40	a
5	1.00 ± 0.00	B	3.31 ± 1.14	a	1.31 ± 0.70	a	1.25 ± 0.58	a

Note:
[1]Different letters within a column are significantly different ($p < 0.05$). ANOVA followed by Tukey's test was performed for multiple means comparisons.

Caetano et al. (2011) studied the antioxidant capacity of acerola agroindustry residue through the application of a hydroethanolic extract of soybean oil, submitted to the accelerated test. The authors observed a progressive increase in conjugated dienes in the samples during the storage time, while also observing that extracts with acerola residues added low concentrations of conjugated dienes at seven days of the test, having statistically superior action than synthetic antioxidants.

The physical instability of emulsions can be observed through the creaming index, where emulsion droplet particles tend to separate from the emulsion body, settling or emerging depending on the density difference between the phases (Fredrick et al., 2010). The creaming index determined for the different treatments is summarized in Supplementary Fig. 2.

The emulsions studied, stored in an accelerated storage time at 40°C, showed an increase in the creaming index in all treatments. Low emulsion stability was observed and may be related to the high storage temperature (Supplementary Fig. 2). According to Chen and Tao (2005), heating can significantly affect emulsion stabilization since the use of high temperatures can change interfacial tension, emulsifier adsorption, and system viscosity. In addition, a sharp increase or decrease in the temperature rate tends to coagulate the particles, causing emulsion deterioration.

3.4. Sensory analysis

Sensory analysis was performed to indicate alterations caused by the addition of microencapsulated extracts in the emulsions. The results of the sensory scores given by each volunteer judge are presented in Supplementary Table 1. The results of the emulsion samples submitted to different treatments are shown in Table 2. There was no difference in the sensory attributes of appearance (brightness and acid odor), disregarding the judging factor (the interaction of each judge with the samples).

The oxidized odor scores also indicated no difference between the treatments performed, even for the sample that was submitted to the accelerated oxidation treatment, which may indicate consumers' insensitivity to the perception of this attribute.

The yellow color parameter was the only parameter that presented a difference in the sensory scores and the highest scores were obtained for the heated samples, not differing from the control samples prepared on the day of analysis. Samples with added synthetic antioxidant received the lowest scores for this attribute, not differing from the sample with the addition of green acerola microparticles at 100 and 200 $mg.kg^{-1}$ (Table 2).

Therefore, the results obtained revealed that the substitution of the synthetic antioxidant TBHQ by acerola extracts at the doses used did not differ in the sensory attributes studied. This observation is relevant because it demonstrates that the use of natural acerola-based antioxidants would not limit their use in food oil-in-water emulsions, such as mayonnaise and salad dressings, due to sensory changes, in addition to being potent antioxidants.

4. Conclusion

This study indicated that aqueous extracts of unripe acerola fruits, both lyophilized and microencapsulated, are a suitable option as an ingredient in food emulsions. The microencapsulated extracts, using gum arabic as an encapsulant agent, presented interesting characteristics for application as an active ingredient, presenting low humidity and water activity. The addition of microparticles to emulsions showed a significant protective effect on model systems, similar to the synthetic antioxidant TBH, at both 100 and 200 $mg.kg^{-1}$. The treatments with green acerola microparticles also showed no sensory difference when compared with synthetic antioxidant (TBHQ), not limiting their use in emulsions. Microparticles from green acerola can be produced by a clean process, using only water as a solvent for the recovery of aqueous antioxidant compounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Bianca Ferraz Teixeira: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft. **Jessica Bomtorin Aranha:** Methodology, Formal analysis. **Thais Maria Ferreira de Souza Vieira:** Conceptualization, Methodology, Writing – review & editing, Project administration, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2022.100130.

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