

Green-solvent extraction of guabijú (*Myrcianthes pungens*) fruit: a sustainable antioxidant source for oil-in- water emulsions

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Research Article

Keywords: Antioxidant capacity, catechin, lipid oxidation, microencapsulation, phenolic compounds, ultrasound extraction

Posted Date: December 22nd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8148354/v1>

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Additional Declarations: No competing interests reported.

Abstract

This study investigated the antioxidant activity of guabijú (*Myrcianthes pungens*) peel extracts and their encapsulated forms in oil-in-water emulsions. Ultrasound-assisted extraction with 50% ethanol produced phenolic-rich peel extracts that exhibited superior bioactivity compared to the pulp, including high catechin (797.28 mg·100 g⁻¹) and cyanidin-3-glucoside (163.75 mg·100 g⁻¹) contents quantified by HPLC-DAD. The peel extract was encapsulated with maltodextrin and gum Arabic by freeze-drying, achieving 87.39% encapsulation efficiency, with significant reductions in moisture, water activity, and hygroscopicity. FTIR analysis confirmed the preservation of phenolic structures. The encapsulated extract was then incorporated into oil-in-water emulsions. At concentrations $\geq 0.08\%$, it exhibited antioxidant performance comparable to synthetic BHT, effectively inhibiting both primary (peroxide value) and secondary (TBARs) lipid oxidation during 60 days of storage. Optical microscopy revealed improved droplet stability and delayed coalescence and Ostwald ripening in emulsions containing the encapsulated extracts. These results demonstrate the potential of guabijú peel as a natural source of phenolic antioxidants and show that freeze-drying microencapsulation effectively preserves their activity, enabling their incorporation as natural substitutes for synthetic antioxidants in emulsified systems.

1. Introduction

Guabijú (*Myrcianthes pungens* (O.Berg) D. Legrand) is a fruit native to Brazil, characterized by its velvety dark violet peel when ripe and its yellow, sweet, slightly astringent pulp (Spinelli et al., 2023). This fruit stands out for its high levels of phenolic compounds, which are highly valued for their health benefits, particularly due to their antioxidant properties (Machado et al., 2024). The growing interest in phenolic compounds and their biological activities has driven research and innovation in the development of phenolic-enriched products (González-Ortega et al., 2020; Laureanti et al., 2023). However, a major limitation to the application of phenolic compounds is their high sensitivity to environmental and processing conditions (Laureanti et al., 2023; Lu et al., 2021).

Despite their antioxidant potential attributed to their unsaturated bonds and hydroxyl groups, phenolic compounds are chemically unstable and prone to degradation, which can be triggered by several factors, including heat, light, pH variations, oxygen, metal ions, and the presence of enzymes (Ferreyra, Bottini, & Fontana, 2023). In this regard, microencapsulation emerges as a promising technique to protect these compounds from degradation (Yeasmen; Orsat, 2024), enhancing their stability, shelf life, and bioavailability (Ali et al., 2024; Nguyen et al., 2024).

A wide range of processed foods, pharmaceuticals, and cosmetics are formulated as oil-in-water emulsions, in which the lipid phase is dispersed in an aqueous medium (El-Guendouz et al., 2018). These emulsions are particularly vulnerable to lipid oxidation owing to the high surface area of the dispersed phase, which facilitates contact with dissolved oxygen and leads to the formation of rancid compounds, thereby compromising product quality (Skowrya et al., 2014; Springer, Ziegler, & Bach, 2023). To prevent such degradation, synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are often used (El-Guendouz et al., 2018; Gallego et al., 2013). However, studies have linked these compounds to adverse health effects, including allergy, liver toxicity, inflammatory responses, and tumor promotion in animal models, raising concerns about their long-term safety (Bauer et al., 2001; Yamaki et al., 2007).

As a safer and eco-friendly alternative, plant-derived antioxidants, particularly phenolic compounds, have attracted considerable attention for their ability to scavenge free radicals and prevent oxidative damage to cells and tissues (Gallego et al., 2013; Yeasmen & Orsat, 2024).

In this context, the present study aimed to investigate the phenolic composition and evaluate the *in vitro* antioxidant and anti-inflammatory activities of ethanolic extracts from guabijú peel and pulp. The extract exhibiting the highest antioxidant potential was subsequently freeze-dried, encapsulated, and incorporated into oil-in-water emulsions to assess its efficacy in preventing lipid oxidation.

2. Materials and methods

2.1 Reagents

The reagents used in the experiments - [Folin-Ciocalteu reagent (1.9–2.1 N), ($\pm$)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) ($\geq 97\%$), sulfanilamide ($\geq 98\%$), sodium nitroprusside ($\geq 98\%$), N-(1-naphthyl)ethylenediamine dihydrochloride ($\geq 98\%$), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) ($\geq 98\%$), ferric chloride ($\geq 97\%$), fluorescein ($\geq 95\%$), 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) ($\geq 97\%$), trichloroacetic acid ($\geq 99.0\%$), thiobarbituric acid ($\geq 98.0\%$), 1,1,3,3-tetraethoxypropane (TEP) ($\geq 96\%$), cyanidin-3-glucoside ($\geq 98.0\%$), catechin ($\geq 99.0\%$), gallic acid ($\geq 97.5\%$), caffeic acid ($\geq 98\%$), quercetin ($\geq 95\%$), trans-cinnamic acid ($\geq 97\%$), ellagic acid ($\geq 95\%$), rutin ($\geq 94\%$), luteolin ($\geq 97.0\%$), trans-resveratrol ($\geq 95\%$), hesperidin ($\geq 80\%$), naringin ($\geq 95\%$), naringenin ($\geq 95\%$), ascorbic acid ($\geq 99.0\%$), citric acid ($\geq 99.5\%$), and HPLC-grade acetonitrile] - were purchased from Sigma-Aldrich. Cold-pressed, unrefined sunflower oil and BHT were obtained in their commercial forms. All other reagents used were of analytical grade.

2.2 Plant material and extraction

Guabijú fruits were collected in the municipality of Chapecó, Santa Catarina, Brazil (27°05'49.8"S, 52°41'07.7"W) (Fig. S1 – Supplementary Material). The fruits were manually cleaned and deseeded. The peel and pulp fractions were frozen at -86°C for 24 h, followed by freeze-drying at -55.0°C under a vacuum pressure of 0.006 Pa for 72 h. The dried material was milled, sieved through a 32-mesh, and stored in metallized packages at -86°C until analysis (Fig. S2 – Supplementary Material).

The phenolic compounds were extracted from the peel and pulp of guabijú using a solid-to-solvent ratio of 1:15 (plant material:solvent) as described by Machado et al. (2024). For that, 1 g of freeze-dried powder was mixed with 15 mL of 50% (v/v) ethanol solution, and the extraction was carried out using an ultrasonic processor (Hielscher, UP200Ht, Teltow, GER) equipped with a 13 mm titanium probe, operating at 100 W for 5 min, under temperature control ($25.0 \pm 1.0^{\circ}\text{C}$). After extraction, the mixtures were centrifuged at $366,5 \text{ rad}\cdot\text{s}^{-1}$ for 15 min, and the supernatants were concentrated by rotary evaporator under reduced pressure to obtain the concentrated extracts.

2.3 Identification and quantification of phenolic compounds by HPLC-DAD

The concentrated peel and pulp extracts were analyzed by high-performance liquid chromatography with diode array detection (HPLC-DAD) using a Vanquish system (ThermoFisher Scientific, Germering, GER), as proposed by Machado et al. (2024), with modifications. A reverse-phase C18 column (Acclaim 120, $4.6 \text{ mm} \times 150 \text{ mm} \times 5 \mu\text{m}$) was used, with a flow rate of $0.2 \text{ mL}\cdot\text{min}^{-1}$, column oven temperature set at 35°C , and injection volume of $10 \mu\text{L}$. The mobile phase consisted of solvent A: water acidified with 0.5% acetic acid (v/v), and solvent B: acetonitrile. The chromatographic run time lasted 70 minutes with the following solvent B gradient: 0 min, 0%; 0.01–15 min, 20%; 15–20 min, 30%; 20–40 min, 40%; 40–45 min, 50%; 45–53 min, 75%; 53–60 min, 100%; 60–63 min, 0%; 63–70 min, 0%.

The quantification of cyanidin-3-glucoside was carried out using a flow rate of $1 \text{ mL}\cdot\text{min}^{-1}$, a column oven temperature of 35°C , and an injection volume of $10 \mu\text{L}$. The mobile phase consisted of solvent A: water acidified with 0.1% formic acid (v/v), and solvent B: acetonitrile. The chromatographic gradient for mobile phase B started at 0 min, 8%; 0.01–2 min, 12%; 2–5 min, 18%; 5–10 min, 20%; 10–12 min, 30%; 12–15 min, 50%; 15–16 min, 75%; 16–17 min, 100%; 17–18 min, 100%; 18–18.5 min, 8%; 18.5–20 min, 8%.

The quantification method was validated according to the guidelines of ANVISA (2017) and INMETRO (2020), which recommend a minimum of five concentration levels for analytical calibration curves, with three replicates per level. Linearity was evaluated using calibration curves comprising at least six concentration points, which were randomly injected in triplicate. Intraday precision was determined at three concentration levels (low, medium, and high). The limits of detection (LOD) and quantification (LOQ) were calculated based on the slope of the calibration curves and the standard deviation of the response at the lowest concentration level (Table S1 – Supplementary Material).

2.4 Antioxidant capacity

The ferric reducing antioxidant power (FRAP) was determined according to the method described by Rufino et al. (2006). Three dilutions of each extract were prepared, and $90 \mu\text{L}$ of each dilution was mixed with $270 \mu\text{L}$ of distilled water and 2.7 mL of the FRAP reagent. The FRAP reagent was freshly prepared by combining 25 mL of 0.3 M acetate buffer, 2.5 mL of 10 mM TPTZ solution, and 2.5 mL of 20 mM ferric chloride solution. After vortexing, the samples were incubated at 37.0°C for 30 minutes.

Quantification was performed using a ferrous sulfate standard curve at 595 nm, and the results were expressed as $\mu\text{M FeSO}_4\cdot\text{g}^{-1}$ of sample.

The oxygen radical absorbance capacity (ORAC) was determined according to the method described by Dávalos, Gómez-Cordovés, and Bartolomé (2004), with modifications. A volume of 25 μL of diluted extract was added to 150 μL of fluorescein solution (1 μM in phosphate buffer, pH 7.4) and incubated in a microplate reader (ThermoFisher Scientific, Varioskan LUX, Singapore) at 37.0°C for 10 min. Subsequently, 25 μL of AAPH solution (50 $\text{mg}\cdot\text{mL}^{-1}$ in phosphate buffer, pH 7.4) was added. Fluorescence readings were performed using water as a blank. The fluorescence intensity was monitored for 2 h, until the end of the decay kinetics, with excitation at 485 nm and emission at 520 nm. The area under the fluorescence decay curve (AUC) was calculated using Eq. 1. Quantification was performed using a Trolox standard curve, and the results were expressed as $\mu\text{M Trolox}\cdot\text{g}^{-1}$ of sample.

$$AUC = 1 + \sum_{i=1}^{i=120} \frac{f_i}{f_0} \quad (1)$$

Where:

f_0 : Initial fluorescence reading;

f_i : Fluorescence reading at time i ;

2.5 Anti-inflammatory capacity *in vitro*

The *in vitro* anti-inflammatory activity was assessed based on the nitric oxide (NO) radical scavenging capacity, as described by Hazra, Biswas, and Mandal (2008), with modifications. For that, 1 mL of extract was mixed with 3 mL of sodium nitroprusside solution (in 10 mM phosphate buffer, pH 7.4) and incubated at $25.0 \pm 1.0^\circ\text{C}$ for 150 min. After, 1 mL of 0.33% (w/v) sulfanilamide (in 20% v/v acetic acid) was added to 0.5 mL of the reaction mixture. After 5 min, 1 mL of 0.1% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride was added, and the mixture was incubated for 30 min at 25°C. Absorbance was measured at 540 nm. Quantification was performed using a Trolox standard curve, and the results were expressed as $\mu\text{mol Trolox}\cdot\text{g}^{-1}$ of sample.

2.6 Antimicrobial activity

The antimicrobial activity was evaluated against three Gram-negative pathogenic bacteria: *Escherichia coli* (ATCC 25922), *Salmonella enterica* serovar Typhimurium (ATCC 14028), and *Listeria monocytogenes* (ATCC 19115), using the disk diffusion method, as described by Ostrosky et al. (2008). Pathogenic cultures were inoculated at a concentration of 10^8 CFU $\cdot\text{mL}^{-1}$ onto Mueller-Hinton agar plates. Paper disks (6 mm in diameter) were placed on the surface of each plate, and 15 μL of extract was applied to each disk. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured using a caliper. The results were expressed in millimeters (mm).

2.7 Encapsulation of guabijú peel extract

The extract and coating materials were dissolved in water ($0.4\text{ g}\cdot\text{mL}^{-1}$) and stirred at $26.18\text{ rad}\cdot\text{s}^{-1}$ at $25.0 \pm 1.0^\circ\text{C}$ for 2 hours. The resulting mixture was frozen at -86°C for 24 h and freeze-dried at -55°C and 0.006 Pa for 72 h. The encapsulated extract was then ground, sieved through a 32-mesh screen ($< 500\text{ }\mu\text{m}$), and stored at -86°C , protected from light. For comparative purposes, three different samples were studied: (i) the non-encapsulated lyophilized extract, (ii) the encapsulated extract, and (iii) the wall material (MD and GA in a 3:2 ratio).

The encapsulation efficiency was determined as described by Laureanti et al. (2023), using Eq. 2:

$$\% \text{ Efficiency} = \left[1 - \left(\frac{\text{surface TPC}}{\text{total TPC}} \right) \right] \times 100 \quad (2)$$

The surface phenolic content (surface TPC) was determined by mixing 0.4 g of the encapsulated extract with 4 mL of 80% ethanol (v/v), followed by vortexing for 1 min and centrifugation at $628.32\text{ rad}\cdot\text{s}^{-1}$ for 5 minutes. The total phenolic content (total

TPC) was obtained by disrupting the encapsulated extract particles, as described by Buratto et al. (2019), with modifications. For that, 0.5 g of encapsulated extract was homogenized with 15 mL of 80% methanol (v/v) acidified with 0.1% HCl, followed by incubation at 25°C for 15 min. The mixture was then centrifuged at 628.32 rad·s⁻¹ for 5 min. The resulting supernatants were used for the quantification of phenolic compounds using the Folin–Ciocalteu method, adapted from Singleton, Orthofer, and Lamuela-Raventós (1999). Gallic acid was used as the standard for quantification at 760 nm, and the results were expressed as mg of gallic acid equivalents per 100 g of sample (mg GAE·100 g⁻¹).

2.8 Physicochemical properties of the lyophilized extract, encapsulated extract, and wall material

The moisture content of the samples was determined according to method 925.45b of the AOAC (2016). Water activity was measured by direct reading using a digital analyzer (Aqualab, AquaLab PRE, Pullman, USA). Hygroscopicity was determined as described by Cai and Corke (2000).

2.9 Surface morphology and particle size distribution

The external structures of the lyophilized extract, encapsulated extract, and wall material were evaluated by scanning electron microscopy (SEM) using a field emission scanning electron microscope (Jeol, JSM-6701F, Peabody, USA). For the analysis of average particle size and size distribution, a laser diffraction particle size analyzer was used (Shimadzu, SALD-2201, Tokyo, JPN).

2.10 Fourier-Transform Infrared (FTIR) Spectroscopy

The lyophilized extract, encapsulated extract, and wall material were analyzed by Fourier-transform infrared (FTIR) spectroscopy using an FTIR spectrophotometer (Bruker, INVENIO-S model, Billerica, USA). Sample spectra were acquired in transmission mode over the range of 4000 to 400 cm⁻¹, with a resolution of 4 cm⁻¹.

2.11 Stability tests of phenolic compounds in the encapsulated extract

Stability was determined as described by Abdin et al. (2021). The encapsulated particles were stored in Falcon tubes, protected from light, and kept at 25.0 ± 1.0°C. The determination of total phenolic content was performed on days 0, 1, 7, 15, 21, 30, 45, and 60 of storage. On days 0 and 60, the samples were also analyzed by HPLC-DAD, as described in Section 2.3.

2.12 Preparation of the emulsions

Oil-in-water (O/W) emulsions were prepared as described by Springer, Ziegler, and Bach (2023) and Skowrya et al. (2014), with modifications. Initially, 1% Tween-20 was dissolved in ultrapure water, followed by the addition of 20% sunflower oil and 7% glyceryl monostearate under mechanical stirring at 324.63 rad·s⁻¹ for 10 min.

A total of 11 formulations were prepared: a negative control without encapsulated extract (NC); a positive control (PC) containing 0.04% of the synthetic antioxidant BHT (butylated hydroxytoluene), as recommended by the manufacturer; emulsions containing lyophilized extract at concentrations of 0.04%, 0.08%, 0.16%, and 0.32% (EX0.04, EX0.08, EX0.16, and EX0.32, respectively); emulsions containing encapsulated extract at the same concentrations (ENC0.04, ENC0.08, ENC0.16, and ENC0.32, respectively); and one emulsion containing only the wall material (MD:GA at 3:2 ratio). The pH was adjusted to 7.0 ± 0.1 using citric acid (1 M), and the emulsions were incubated at 33.0 ± 1.0°C in the absence of light and under constant orbital motion (10.47 rad·s⁻¹) for 60 days.

2.13 Lipid Oxidation in O/W Emulsions

The primary oxidation products were evaluated by determining the peroxide value on days 1, 7, 30, and 60 of emulsion storage, according to the methodology 8–53 of AOCS (2017). The secondary oxidation products were measured by quantifying the thiobarbituric acid reactive substances (TBARs) in the emulsions on days 1, 7, 15, 30, 45, and 60 of storage, as described by Gallego et al. (2013). For the analysis, 0.5 mL of the emulsion (diluted in water) was mixed with 2.5 mL of TBARs reagent and vortexed for 30 s. The samples were incubated for 5 min at 25°C, then placed in a boiling water bath for 10 min. After cooling, the samples were centrifuged at 628.32 rad·s⁻¹ for 10 min, and the absorbance of the supernatant was measured at 532 nm. The quantification was carried out using a standard curve prepared with malondialdehyde (MDA) standard solution (10 mM), obtained

by the acid hydrolysis of 239 μL of 1,1,3,3-tetraethoxypropane in 100 mL of 1% (v/v) sulfuric acid for 2 hours at room temperature (Sim et al., 2003). The results were expressed as mg MDA $\cdot\text{kg}^{-1}$ of emulsion.

2.14 Emulsion Microscopy

The negative control emulsion (NC), as well as the emulsions with the highest concentrations of lyophilized extract and encapsulated extract (EX0.32 and ENC0.32, respectively), were analyzed by optical microscopy at 50 $\times$ magnification using a metallographic microscope (Kontrol, IM100, São Paulo, BRA). The analyses were performed in transmission mode on days 1, 15, and 60 of storage to assess emulsion stability.

2.15 Statistical analysis

All analyses were performed in triplicate. Data was analyzed through analysis of variance (ANOVA) using Origin 2024 software, followed by Tukey's multiple comparison test at a 5% significance level.

3. Results and discussion

3.1 Characterization of Guabijú peel and pulp extracts

The peel extract exhibited significantly higher levels of phenolic compounds ($p < 0.05$) compared to the pulp (Table 1). This higher concentration may be associated with the protective role of the peel against abiotic and biotic stresses, such as UV radiation and pathogen attack, which trigger the synthesis of specialized metabolites including phenolic compounds (Ahlawat et al., 2023).

Table 1. Phytochemical, antioxidant, and anti-inflammatory characterization of guabijú peel and pulp extracts

Parameter	Peel	Pulp
*Cyanidin-3-glucoside (mg. 100 g ⁻¹ sample)	163.75 $\pm$ 6.67	-
*Gallic acid (mg. 100 g ⁻¹ sample)	27.65 $\pm$ 2.46 ^a	11.20 $\pm$ 3.62 ^b
*Catechin (mg. 100 g ⁻¹ sample)	797.28 $\pm$ 16.61 ^a	7.77 $\pm$ 0.64 ^b
*Caffeic acid (mg. 100 g ⁻¹ sample)	33.16 $\pm$ 1.00 ^a	10.12 $\pm$ 0.07 ^b
*Ellagic acid (mg. 100 g ⁻¹ sample)	30.90 $\pm$ 0.30 ^a	8.18 $\pm$ 0.11 ^b
*Quercetin (mg. 100 g ⁻¹ sample)	32.16 $\pm$ 1.03 ^a	23.34 $\pm$ 0.12 ^b
*Trans-cinnamic acid (mg. 100 g ⁻¹ sample)	13.23 $\pm$ 0.02	-
Antioxidant capacity- FRAP (μmol ferrous sulfate. g ⁻¹ sample)	748.88 $\pm$ 1.62 ^a	117.44 $\pm$ 4.52 ^b
Antioxidant capacity - ORAC (μmol Trolox. g ⁻¹ sample)	558.87 $\pm$ 58.02 ^a	169.72 $\pm$ 17.55 ^b
Anti-inflammatory activity (μmol Trolox. g ⁻¹ sample)	22.03 $\pm$ 0.14 ^a	6.26 $\pm$ 0.03 ^b

Values are expressed as mean $\pm$ standard deviation. Means followed by different letters in the same row differ significantly according to Tukey's test at a 5% significance level. Compounds marked with * were identified and quantified by HPLC-DAD.

The validation parameters applied to the HPLC-DAD analytical methods for phenolic compounds showed correlation coefficients (r^2) higher than 0.99 for all calibration curves, intraday precision values below 10%, and low limits of detection and quantification, which were below 0.8 $\mu\text{g}\cdot\text{mL}^{-1}$ and 2.5 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively (Table S1, Supplementary Material).

Seven phenolic compounds were identified and quantified, with uneven distribution between the fruit parts and significantly higher concentrations in the peel ($p<0.05$). Among them, catechin was the major constituent found in guabijú peel. This flavanol is well known for its high antioxidant capacity and its role in preventing lipid oxidation (Ruengdech, Mishra, & Siripatrawan, 2025). Cyanidin-3-glucoside, an anthocyanin, was also identified as one of the predominant compounds in the peel. It is a water-soluble pigment responsible for the characteristic purple color of the fruit and is closely associated with antioxidant activity (Zhao et al., 2019; Machado et al., 2024).

In addition, significant amounts of other phenolic compounds were detected in the peel, including gallic acid, caffeic acid, ellagic acid, quercetin, and trans-cinnamic acid, all with significantly higher concentrations than those found in the pulp ($p<0.05$). On the other hand, compounds commonly reported in plant matrices, such as rutin, luteolin, hesperidin, naringin, naringenin, ascorbic acid, and citric acid, were not detected in the samples, which may reflect the unique phytochemical profile of guabijú fruit.

The antioxidant capacity of the extracts was evaluated using the FRAP and ORAC assays. The FRAP assay demonstrated greater effectiveness of the peel extract in donating electrons, facilitating the reduction of ferric (Fe^{3+}) to ferrous ions (Fe^{2+}), confirming its role as a reducing agent. In turn, the ORAC assay indicated a higher capacity of the peel extract to neutralize peroxy radicals, reflecting its effectiveness in hydrogen atom transfer-based antioxidant mechanisms.

Regarding *in vitro* anti-inflammatory activity, the peel extract showed greater efficacy in neutralizing nitric oxide (NO) radicals when compared to the pulp extract. This result can be due to the higher concentration of phenolic compounds in the peel, which are known to inhibit inflammatory mediators (Bouhlali et al., 2020). NO inhibition is considered a key parameter in assessing the anti-inflammatory potential of plant extracts, given its role in inflammatory processes and cellular oxidative stress (Gómez-Maqueo et al., 2019; Bouhlali et al., 2020).

Both extracts exhibited antimicrobial activity against *Escherichia coli* (inhibition zones of 14.3 ± 0.2 mm for peel and 12.2 ± 0.4 mm for pulp), *Salmonella* sp. (inhibition zones of 9.4 ± 0.4 mm for peel and 8.3 ± 0.2 mm for pulp), and *Listeria* sp. (inhibition zones of 14.4 ± 0.3 mm for peel and 9.5 ± 0.1 mm for pulp), indicating the potential of guabijú fruit as a source of natural antimicrobial agents. This activity may be associated with the presence of flavonoids such as quercetin and catechin, which are widely recognized for their ability to inhibit the growth of pathogenic microorganisms (Dias, Pinto, & Silva, 2021).

The results highlight the functional potential of guabijú peel as a promising source of natural antioxidants. Utilizing this often-discarded part of the fruit represents a sustainable approach for developing bioactive ingredients with promising applications in the food, pharmaceutical, and cosmetic industries.

3.2 Physicochemical properties of the lyophilized and encapsulated extracts

The guabijú peel extract, due to its higher bioactivity (Table 1), was encapsulated with maltodextrin and gum Arabic, achieving an encapsulation efficiency of $87.39 \pm 0.03\%$.

The encapsulation led to a significant reduction in moisture content, water activity, and hygroscopicity when compared to the non-encapsulated extract (Table 2). The decrease in moisture content from 26.37 to 3.45% after the encapsulation process is noteworthy, as moisture levels between 1% and 6% are considered ideal for maintaining product stability during storage (da Silva Júnior et al., 2023). The reduction in water activity further contributes to the microbial stability of the encapsulated extract, remaining within the recommended limit (< 0.30) for adequate preservation (Nunes et al., 2015; Rezende, Nogueira, & Narain, 2018).

Table 2 Physicochemical properties of the lyophilized extract, encapsulated extract, and wall material

Parameter	Lyophilized extract	Encapsulated extract	Wall material
Moisture (%)	26.37 ± 0.27 ^a	3.45 ± 0.45 ^b	3.19 ± 0.15 ^b
Water activity	0.125 ± 0.002 ^a	0.118 ± 0.002 ^b	0.037 ± 0.003 ^c
Hygroscopicity (g.100 g ⁻¹)	75.25 ± 1.56 ^a	45.19 ± 0.10 ^b	38.99 ± 0.33 ^c

Values are expressed as mean ± standard deviation. Means followed by different letters in the same row differ significantly according to Tukey's test at a 5% significance level.

The decrease in hygroscopicity further contributes to enhanced protection, promoting product stability during storage. This behavior is consistent with literature reports on extracts encapsulated with gum Arabic and maltodextrin by freeze-drying (Khazaei et al., 2014).

3.3 Surface morphology and particle size distribution

Microscopy of the lyophilized extract (Fig. 1a, 1b, and 1c) revealed a homogeneous surface with slight roughness. In contrast, the encapsulated extract and the wall material mixture exhibited a heterogeneous morphology, with sharp, straight edges, as indicated by the arrows in the images (Fig. 1d to 1i). This sawdust-like appearance is typical of the freeze-drying process and has been previously reported in the literature (Ballesteros et al., 2017; da Silva Júnior et al., 2023; Pashazadeh et al., 2021).

The analysis of particle size distribution revealed a broad distribution, with the highest concentration of particles ranging from 1.858 µm (D_{10}) to 56.096 µm (D_{90}) (Fig. 1j). The median particle size (D_{50}) was 13.476 µm. Although some particles presented diameters smaller than 1 µm, most particles were in the micrometer range, indicating that the material can be classified as a microparticle (Joudeh & Linke, 2022).

3.4 Fourier-Transform Infrared Spectroscopy

FTIR analysis revealed a broad band between 3600 and 3000 cm⁻¹ in all materials, associated with O–H and N–H stretching vibrations, typical of phenolic compounds, carbohydrates, and polypeptides (Fig. 2)(Akbarmehr et al., 2023; Ligarda-Samanez et al., 2022). This region also coincides with non-free O–H stretching, related to residual water, and C–H stretching vibrations (Nguyen et al., 2024).

The band at 2930 cm⁻¹, which is more intense in the wall material, is associated with CH₂ and CH₃ groups from the polymeric matrix (Akbarmehr et al., 2023; Silverstein, Webster, & Kiemle, 2005), while in the encapsulated extract it is related to aromatic compounds with phenyl bonds, such as flavonoids (Ballesteros et al., 2017). The region around 2000 cm⁻¹ corresponds to overtones or combination bands of aromatic rings from phenolic compounds (Silverstein, Webster, & Kiemle, 2005), which were previously identified and quantified in the peel extract through TPC and HPLC analysis.

In the region near 1640 cm⁻¹, signals were attributed to carbonyl groups of flavonoids (Ligarda-Samanez et al., 2022) and to primary amide stretching derived from gum Arabic peptides (Ali et al., 2024; Nguyen et al., 2024). At 1420 cm⁻¹, CH₃ vibrations adjacent to carbonyl groups were observed, while C–O stretching vibrations of alcohols/phenols appeared at 1160 cm⁻¹, which typically produce strong bands in the 1260–1000 cm⁻¹ range (Silverstein, Webster, & Kiemle, 2005). Bands between 1040 and 1020 cm⁻¹ are indicative of carbohydrate structures, which are commonly detected in this spectral region (Ali et al., 2024). In the 900 to 675 cm⁻¹ region, out-of-plane C–H bending vibrations from aromatic rings of polyphenols were also identified (Ligarda-Samanez et al., 2022; Silverstein, Webster, & Kiemle, 2005).

The variation in spectral intensity among the samples is associated with the extract-to-wall material ratio in the encapsulated formulations, which directly influences the observed signals (Nguyen et al., 2024). Overall, the FTIR spectrum of the microparticles showed only characteristic bands of the wall material and the extract, with no appearance of new peaks, indicating that no structural changes occurred during encapsulation (Meng et al., 2017).

3.5 Stability of the encapsulated extract during 60 days of storage

A sharp decline of total phenolic compounds was observed in the microparticles during the first 7 days of storage (Fig. 3a), followed by a slower degradation rate. After 60 days, the total reduction was 17% compared to the initial value. No significant changes were observed for the concentrations of gallic acid, ellagic acid, catechin, and quercetin, indicating good stability over time and reinforcing the potential of the encapsulated extract for applications requiring longer shelf life and prolonged antioxidant efficacy (Fig. 3b). In contrast, a decrease of 23% was observed for cyanidin-3-glucoside, probably due to the high instability of its phenolic hydroxyl groups, known to be sensitive to light, oxygen, and temperature (Xue et al., 2024).

3.6 Antioxidant effect of the encapsulated extract in O/W emulsions

The evolution of the peroxide value in O/W emulsions over 60 days indicates the formation and decomposition of primary oxidation products (Table 3). No hydroperoxides were detected on the first day; however, their presence was confirmed from day seven onward. Since hydroperoxides are primary products of lipid oxidation, their breakdown into secondary oxidation products can explain the lower peroxide values observed in some samples over time (Ganjeh et al., 2024).

Table 3 Peroxide value of the oil-in-water emulsions stored at 33.0 °C

Sample	Peroxide value (meq.kg ⁻¹ emulsion)		
	day 7	day 30	day 60
NC	3.48 ±0.18 ^{aBC}	3.65±0.23 ^{aBC}	4.86±0.33 ^{cA}
PC	2.55±0.02 ^{bcdA}	2.83±0.16 ^{bA}	1.96±0.22 ^{fB}
EX0.04	2.17±0.20 ^{ceB}	1.91±0.30 ^{deB}	5.93±0.90 ^{bA}
EX0.08	1.59±0.12 ^{eB}	1.64±0.22 ^{defB}	2.12±0.06 ^{fA}
EX0.16	1.73±0.18 ^{eAB}	1.19±0.20 ^{fgB}	2.49±0.32 ^{efA}
EX0.32	1.62±0.10 ^{eC}	1.45±0.12 ^{efC}	3.97±0.17 ^{cdB}
ENC0.04	1.95±0.17 ^{deB}	0.86±0.12 ^{ghC}	2.03±0.11 ^{fB}
ENC0.08	1.66±0.24 ^{eA}	1.85±0.12 ^{deA}	1.84±0.04 ^{fA}
ENC0.16	0.25±0.12 ^{fC}	2.20±0.15 ^{cdB}	4.07±0.33 ^{cdA}
ENC0.32	2.96±0.43 ^{abA}	0.41±0.07 ^{hC}	3.17±0.30 ^{deA}
WM	2.73±0.61 ^{abcC}	2.58±0.33 ^{bC}	8.41±0.27 ^{aA}

Means followed by different letters differ significantly (Tukey’s test, p<0.05); lowercase letters indicate differences within columns and uppercase letters within rows. NC: Negative control; PC: Positive control (BHT); EX0.04: 0.04% lyophilized extract; EX0.08: 0.08% lyophilized extract; EX0.16: 0.16% lyophilized extract; EX0.32: 0.32% lyophilized extract; ENC0.04: 0.04% encapsulated extract; ENC0.08: 0.08% encapsulated extract; ENC0.16: 0.16% encapsulated extract; ENC0.32: 0.32% encapsulated extract; WM: Wall material (MD:GA 3:2).

The samples EX0.08, ENC0.04, and ENC0.08 demonstrated statistically similar performance to BHT (p > 0.05) in inhibiting primary oxidation over 60 days, suggesting equivalent effectiveness in delaying the formation of primary oxidation products. In contrast, the sample containing only wall material (WM), with no antioxidant active compounds, showed peroxide values comparable to the negative control (NC) on day 30 (p < 0.05) and even higher on day 60. This result suggests that maltodextrin and gum Arabic may act as pro-oxidants in the absence of antioxidants, accelerating oxidation.

Despite these variations, none of the samples exceeded the limit of 15 meq·kg⁻¹ established by international standards for cold-pressed, unrefined oils (CODEX, 2024), indicating that all formulations maintained acceptable quality throughout the evaluation period.

The hydroperoxides formed during primary oxidation are unstable and decompose into secondary products such as short-chain aldehydes (e.g., malondialdehyde), ketones, alcohols, hydrocarbons, and volatile organic acids, which are generated in the later oxidation stages (Ganjeh et al., 2024). These TBARS-reactive compounds were not detected on days 1 and 7 and were only identified from day 15 onward (Table 4).

Table 4 Concentration of thiobarbituric acid reactive substances (TBARs) of the oil-in-water emulsions stored at 33.0 °C

Sample	TBARS concentration (mg MDA.kg ⁻¹ de emulsion)			
	day 15	day 30	day 45	day 60
NC	2.36±0.02 ^{cA}	2.61±0.11 ^{aA}	2.90±0.34 ^{abA}	2.95±0.29 ^{bA}
BHT	5.25±0.17 ^{aA}	0.47±0.17 ^{deC}	1.43±0.79 ^{cdBC}	1.86±0.04 ^{cB}
EX0.04	2.37±0.34 ^{cBC}	1.92±0.26 ^{bC}	3.09±0.06 ^{aA}	2.77±0.08 ^{bAB}
EX0.08	2.63±0.53 ^{cA}	1.11±0.30 ^{cdB}	1.52±0.36 ^{cdB}	1.37±0.04 ^{dB}
EX0.16	0.73±0.21 ^{eBC}	0.99±0.10 ^{cdB}	1.36±0.05 ^{cdA}	0.47±0.04 ^{eC}
EX0.32	1.05±0.31 ^{deBC}	1.59±0.32 ^{bcAB}	2.08±0.09 ^{bcA}	0.63±0.06 ^{eC}
ENC0.04	1.98±0.08 ^{cdB}	1.38±0.07 ^{bcC}	2.07±0.29 ^{bcB}	5.36±0.07 ^{aA}
ENC0.08	1.95±0.06 ^{cdA}	1.34±0.18 ^{bcB}	0.63±0.27 ^{dC}	1.60±0.10 ^{cdB}
ENC0.16	0.62±0.14 ^{eBC}	0.96±0.34 ^{cdB}	1.93±0.06 ^{cA}	0.27±0.03 ^{eC}
ENC0.32	1.03±0.76 ^{deAB}	0.27±0.06 ^{eB}	1.84±0.06 ^{cA}	0.24±0.03 ^{eB}
WM	3.70±0.23 ^{bB}	1.41±0.31 ^{bcC}	2.09±0.16 ^{bcC}	4.97±0.32 ^{aA}

Means followed by different letters differ significantly (Tukey's test, p<0.05); lowercase letters indicate differences within columns and uppercase letters within rows. NC: Negative control; PC: Positive control (BHT); EX0.04: 0.04% lyophilized extract; EX0.08: 0.08% lyophilized extract; EX0.16: 0.16% lyophilized extract; EX0.32: 0.32% lyophilized extract; ENC0.04: 0.04% encapsulated extract; ENC0.08: 0.08% encapsulated extract; ENC0.16: 0.16% encapsulated extract; ENC0.32: 0.32% encapsulated extract; WM: Wall material (MD:GA 3:2).

After 60 days of storage, the samples containing lyophilized and encapsulated extracts at concentrations above 0.08% showed similar efficacy to the synthetic antioxidant BHT in inhibiting secondary oxidation. The samples EX0.04 and ENC0.04 behaved similarly to the negative control. The sample containing only the wall material showed an increase in TBARs levels, reinforcing its pro-oxidant effect.

The present results indicate the high antioxidant potential of guabijú extract, with performance comparable to BHT, representing a promising natural alternative to this synthetic additive. In addition to inhibiting lipid oxidation, its phenolics and anthocyanins contents may have presented health benefits, including anti-inflammatory, antioxidant, antibacterial, antiviral, and antitumor activities (Dwibedi et al., 2022; Lu et al., 2021).

3.7 Emulsion microscopy

At the beginning of storage (day 1), all samples presented a heterogeneous distribution of oil droplets in the aqueous phase, characterized by a broad range of droplet sizes (Fig. 4a, 4d, and 4g). After 15 days, the sample NC exhibited coalescence, indicating thermodynamic instability of the emulsion (Fig. 4b). Coalescence is an irreversible process in which smaller droplets fuse with larger ones, resulting in an increase in droplet size (Rocha et al., 2023). In contrast, the samples EX0.32 and ENC0.32 did not exhibit this phenomenon (Fig. 4e and 4h).

On day 60 of storage, Ostwald ripening was observed in the samples NC and EX0.32 (Fig. 4c and 4f), accompanied by advanced coalescence. This phenomenon results from the diffusion of smaller droplets into larger ones as a result of pressure differences between them (Rocha et al., 2023). In contrast, the sample ENC0.32 (Fig. 4i) exhibited Ostwald ripening at an early stage, indicating that the use of the encapsulated extract contributed to greater thermodynamic stability of the emulsions.

4. Conclusion

Guabijú peel exhibited higher concentrations of flavonoids and anthocyanins compared to the pulp, resulting in significantly greater in vitro antioxidant and anti-inflammatory activities ($p < 0.05$). This effect is mainly attributed to the high catechin concentration, identified as the main bioactive compound in the peel.

Microencapsulation of the peel extract using maltodextrin and gum Arabic via freeze-drying proved to be effective in protecting phenolic compounds from degradation. After 60 days of storage, the total phenolics content decreased by only 17%, with notable stability observed for catechin, gallic acid, ellagic acid, and quercetin.

Moreover, the incorporation of the encapsulated extract into O/W emulsions efficiently inhibited lipid oxidation, showing comparable performance to the synthetic antioxidant BHT. These results highlight the potential of guabijú peel extract as a promising natural alternative to synthetic antioxidants in food systems, adding functional value due to its antioxidant and anti-inflammatory properties.

Declarations

CRedit authorship contribution statement

Liciani Inaê Putti: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Validation, Visualization, Writing – original draft. **Luana Bettanin:** Investigation, Validation, Writing – review and editing. **Thais Fernanda de Marco:** Methodology, Investigation. **Marcia Bär Schuster:** Methodology, Investigation. **Jaqueline Scapinello:** Methodology, Writing – review and editing. **Alexandre Tadeu Paulino:** Resources. **Ilizandra Aparecida Fernandes:** Methodology, Investigation. **Elisandra Rigo:** Funding acquisition. Resources. **Darlene Cavalleiro:** Conceptualization, Project administration, Writing – review and editing. **Georgia Ane Raquel Sehn:** Conceptualization, Project administration, Supervision, Methodology, Writing – review and editing.

Acknowledgements

The authors are thankful for the Multi-User Facility infrastructure from Universidade do Estado de Santa Catarina (UDESC) Technological Sciences Center, *campus* Joinville, as well as the laboratories of the Department of Food Engineering and Chemical Engineering (UDESC) for their support in carrying out this study.

Funding

This study was supported by Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) (Grant 2023TR565).

Declaration of Competing Interest

The authors declare no competing financial interests or personal relationships that could have appeared to influence the study reported in this manuscript.

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Figures

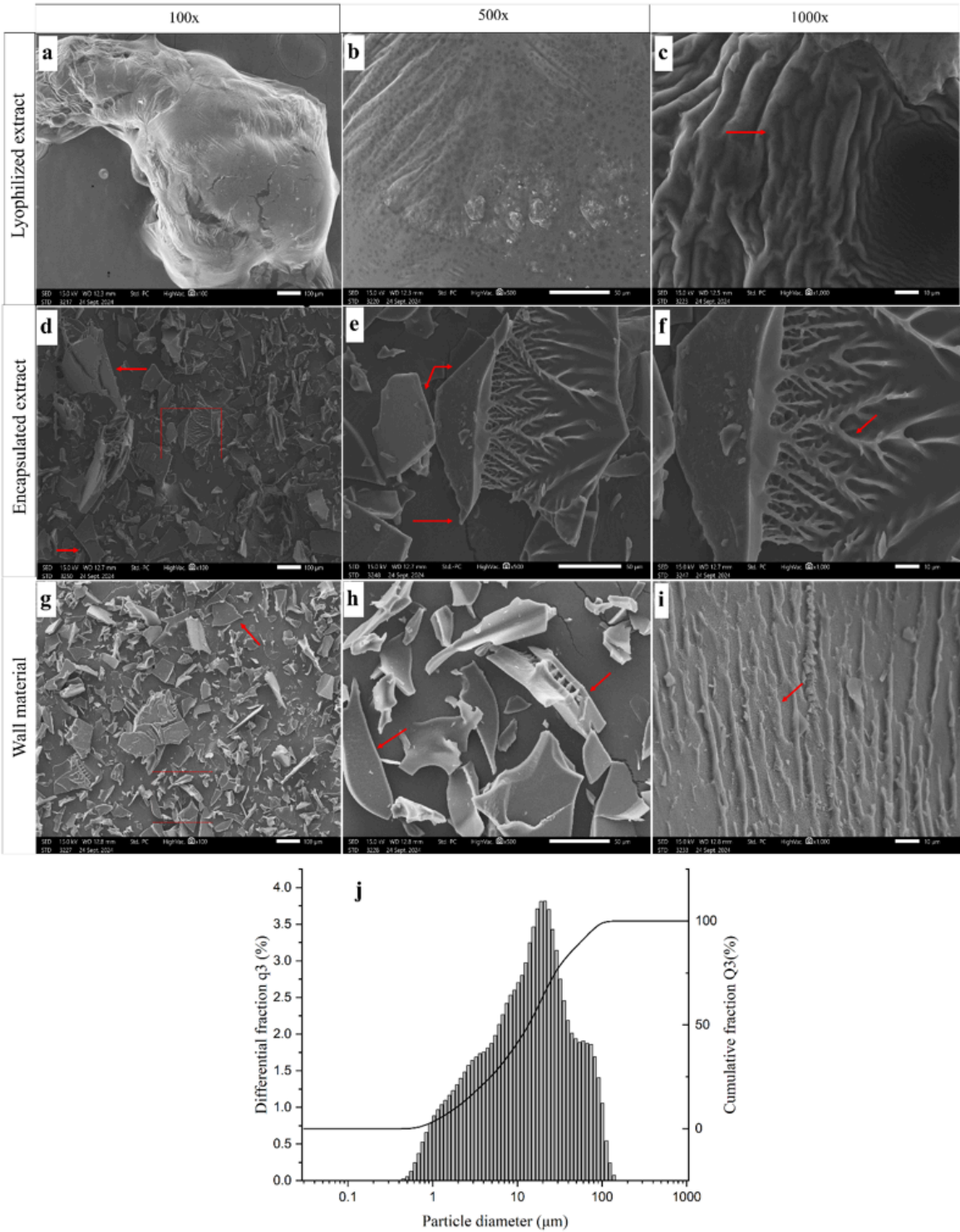


Figure 1

Scanning microscopy of the lyophilized extract (a-c), encapsulated extract (d-f), wall material (maltodextrin/gum Arabic 3:2) (g-i), and particle size distribution of the encapsulated extract (j)

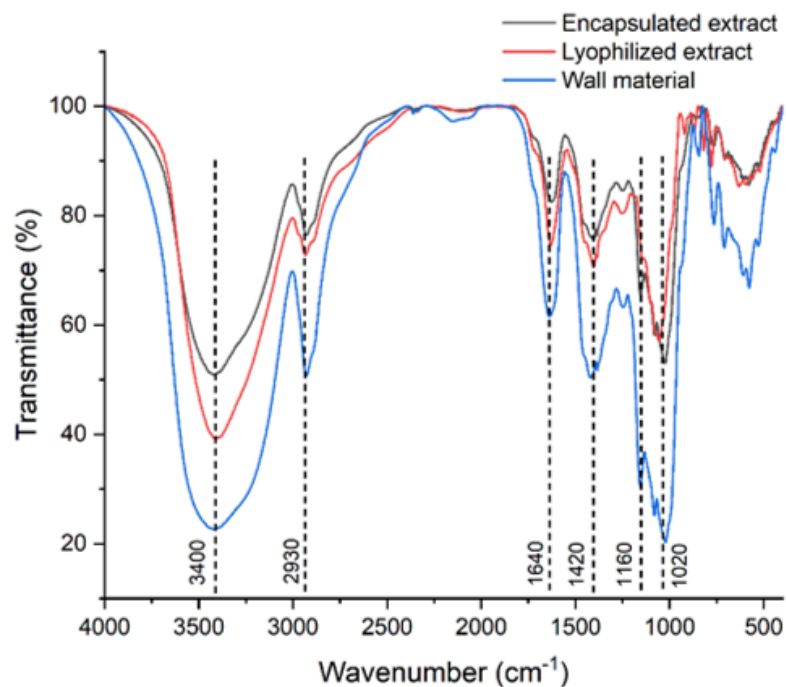


Figure 2

FTIR spectra of the lyophilized extract, encapsulated extract, and wall material (maltodextrin/ gum Arabic 3:2)

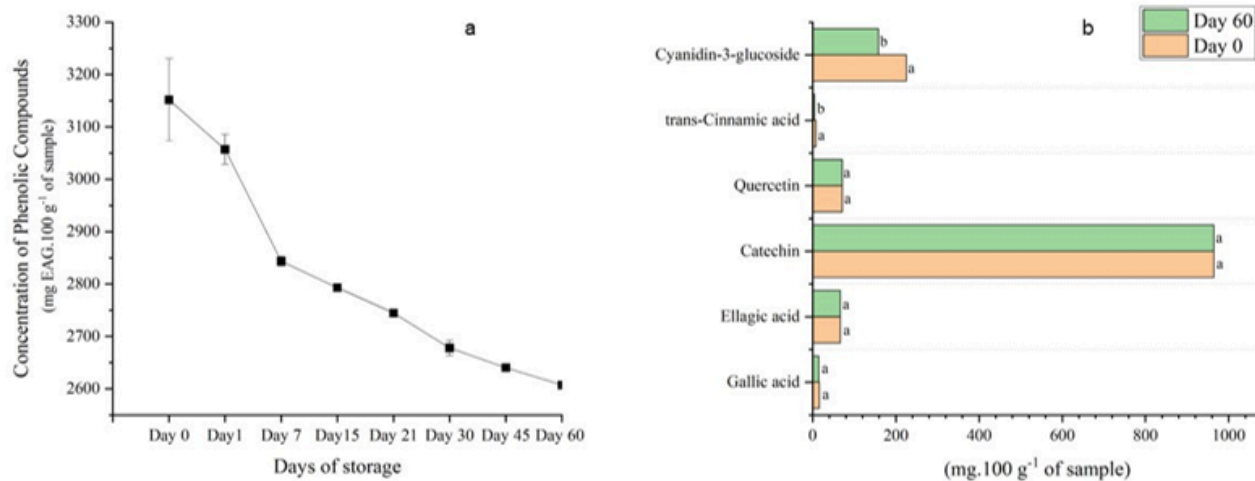


Figure 3

Concentration of total phenolic compounds in the encapsulated extract particles, determined by the Folin–Ciocalteu method on days 0, 1, 7, 15, 21, 30, 45, and 60 of storage (a), and by HPLC–DAD on days 0 and 60 of storage (b). Similar letters indicate no significant difference at a 5% level using Tukey's test.

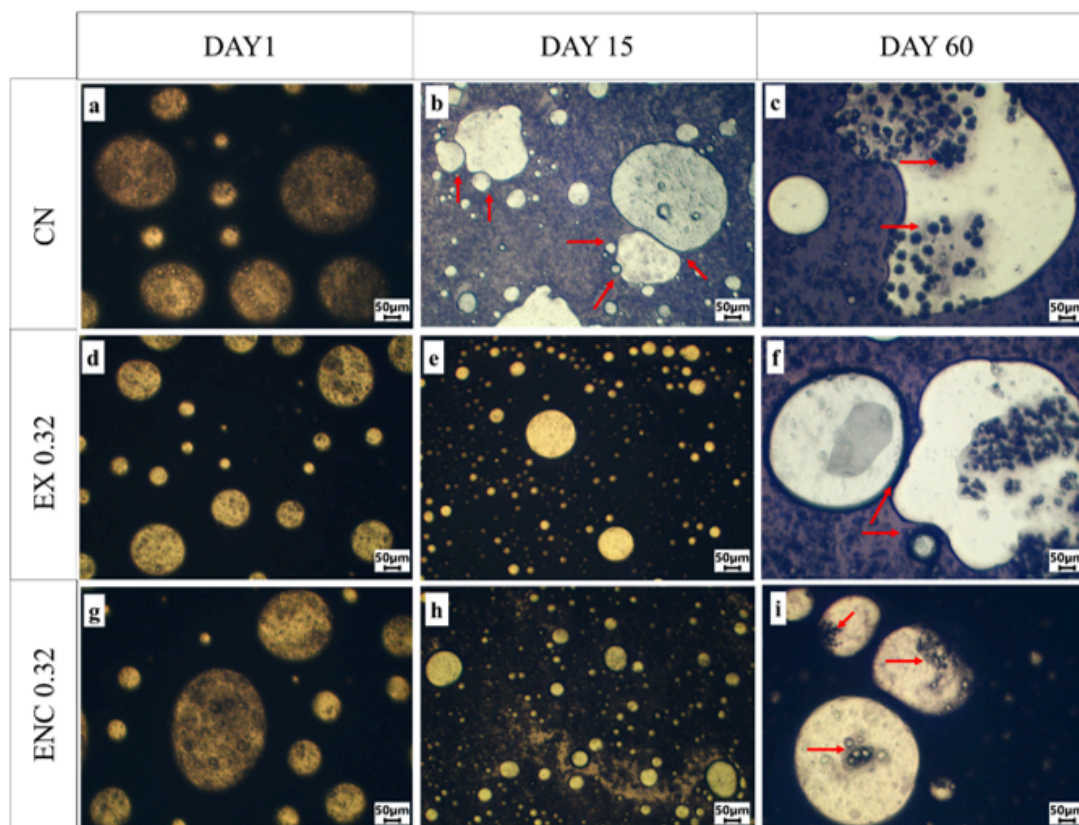


Figure 4

Optical transmission microscopy of oil-in-water emulsions ENC0.32 (with encapsulated extract), EX0.32 (with lyophilized extract), and CN (negative control), on days 1, 15, and 60 of storage at 33.0 °C.

Supplementary Files

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