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# Encapsulation of Japanese grape (*Hovenia dulcis*) pseudofruits by freeze-drying: characterization and antioxidant potential

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**ABSTRACT:** The pseudofruit of *Hovenia dulcis* is recognized as a source of bioactive compounds; however, like most fruits, it is highly susceptible to deterioration. Encapsulation techniques are used to protect and stabilize compounds while also minimizing changes in the properties of the supplemented product. This study analyzed freeze-dried pulp of *H. dulcis* pseudofruit (HD) and pseudofruit pulp microencapsulated by freeze-drying using whey protein concentrate and gum arabic as coating materials (En-HD). The samples (HD and En-HD) were characterized for their physicochemical properties, total phenolic compounds (TPC), antioxidant activity (ABTS and DPPH), $\alpha$-amylase inhibition, particle size distribution, and scanning electron microscopy. Microencapsulation retained 95.8% of TPC after 75 days of storage at −80 ºC. En-HD showed higher antioxidant activity by the ABTS assay and greater inhibition of the $\alpha$-amylase enzyme, demonstrating the protective effect of microencapsulation. This technique, with further testing, particularly *in vivo*, could be promising for managing blood sugar levels. En-HD exhibited a smaller particle size and 76% greater solubility than HD, likely due to the coating materials used in the microencapsulation process and the freeze-drying of the encapsulated sample made with previously freeze-dried HD. En-HD shows potential as a functional additive for use in food industry.

**Keywords:** ABTS; DPPH; encapsulation techniques; particle size; phenolic compounds; $\alpha$-amylase inhibition.

# *Encapsulamento de pseudofrutos de uva japonesa (Hovenia dulcis) por liofilização: caracterização e potencial antioxidante*

**RESUMO:** *O pseudofruto de Hovenia dulcis é reconhecido como uma fonte de compostos bioativos; entretanto, como a maioria das frutas, é altamente susceptível à deterioração. Técnicas de encapsulamento são utilizadas para proteger e estabilizar esses compostos, ao mesmo tempo que minimizam as alterações nas propriedades do produto suplementado. Este estudo analisou*

*a polpa liofilizada do pseudofruto de H. dulcis (HD) e a polpa de pseudofrutos microencapsulados por liofilização usando concentrado de proteína de soro de leite e goma arábica como materiais de revestimento (En-HD). As amostras (HD e En-HD) foram caracterizadas por suas propriedades físico-químicas, compostos fenólicos totais (TPC), atividade antioxidante (ensaios ABTS e DPPH), inibição da α-amilase, distribuição do tamanho das partículas e microscopia eletrônica de varredura. A microencapsulação reteve 95,8% do TPC após 75 dias de armazenamento a −80 ºC. O En-HD apresentou maior atividade antioxidante pelo ensaio ABTS e maior inibição da enzima α-amilase, demonstrando o efeito protetor da microencapsulação. Essa técnica, com mais testes – particularmente in vivo –, pode ser promissora para o gerenciamento dos níveis de açúcar no sangue. O En-HD exibiu um tamanho de partícula menor e 76% maior solubilidade do que o HD, provavelmente devido aos materiais de revestimento utilizados no processo de microencapsulação e à liofilização da amostra encapsulada feita com HD previamente liofilizado. O En-HD mostra potencial como um aditivo funcional para uso na indústria de alimentos.*

**Palavras-chave:** *compostos fenólicos; ensaio ABTS; ensaio DPPH; inibição da α-amilase; tamanho de partícula; técnicas de encapsulamento.*

## 1 Introduction

*Hovenia dulcis*, commonly known as the Japanese grape, belongs to the Rhamnaceae family and is an edible fruit with medicinal properties, notably in managing alcoholism and liver diseases (Cai *et al*., 2021). It contains bioactive compounds with antioxidant, antimicrobial, and antidiabetic effects, making in an potential additive for the food industry (Cavalheiro *et al*., 2025; Schaefer *et al*., 2022).

Incorporating bioactive compounds into food products poses challenges, as these substances are often unstable or poorly soluble under processing and storage conditions, such as heat, oxidation, pH, and water activity. These factors can lead to degradation, reduced absorption, and diminished bioavailability in the gastrointestinal tract (Grgić *et al*., 2020).

Encapsulation has emerged as a promising technique for incorporating bioactive compounds into foods by enclosing them in tiny capsules or particles (El-Messery *et al*., 2019). Various materials, including proteins, lipids, and polysaccharides, are used for microencapsulation (Simões *et al*., 2017). Proteins are widely applied for their advantages, including biodegradability, biocompatibility, and water solubility (Grgić *et al*., 2020). Polysaccharides, conversely, can interact with bioactive compounds, serving as versatile carriers to binding and trapping compounds with hydrophilic and hydrophobic properties (Simões *et al*., 2017; Santos *et al*., 2020).

Gum Arabic, a complex heteropolysaccharide with a highly branched structure, has proven to be an excellent film-forming agent, providing enhanced protection for encapsulated particle. Whey protein concentrate is recommended as a coating material due to its emulsifying capacity and its role in stabilizing microparticles (El-Messery *et al*., 2019; Lourenço; Moldão-Martins; Alves, 2020).

Utilizing *H. dulcis* pseudofruit as a food ingredient offers a potential method to increase its commercial value and add functional properties to final products through

encapsulated supplementation. This study, therefore, evaluated the feasibility of microencapsulating *H. dulcis* pseudofruit in whey protein concentrate and gum Arabic using freeze-drying focusing on the resultingphysicochemical and bioactive characteristics.

The remainder of this article is organized as follows.. Section 2 describes the experimental procedures used in this study, while Section 3 discusses the results obtained and compares them with findings from the existing literature. Finally, Section 4 presents the conclusion of this study.

## 2 Materials and methods

This section outlines the primary materials and methods used to prepare and assess microencapsulation.

### 2.1 Reagents and chemical standards

Analytical-grade reagents, including sodium nitroprusside, sulfonamide, N-(1-naphthyl) ethylenediamine (NED), Trolox, alpha-amylase, acarbose, Folin-Ciocalteu, ABTS (2,2-azinobis-3-ethyl-benzothiazoline-6 sulfonic acid), and DPPH (2,2-diphenyl-1-picryl-hydrazil), were obtained from Sigma Aldrich (San Luis, EUA). Whey protein concentrate (WPC) and gum Arabic (GA) were sourced from Êxodo Científica (São Paulo, Brazil). All other reagents were of analytical grade.

### 2.2 Preparation of *Hovenia dulcis* pseudofruit powder (HD)

The *H. dulcis* pseudofruits were collected in April 2022 in Faxinal dos Guedes, Santa Catarina, Brazil (latitude: 26° 51' 21" S, longitude: 52° 15' 23" W), at the mature stage, with a soluble solid-to-titratable acidity ratio of 16.2. The pseudofruit were separated from seeds and stems, washed in running water (Figure 1), and excess water was removed. The sample was ground using a domestic processor (Clinck, Brazil) to particle sizes of approximately of 2-5 mm (measured with a caliper), then packaged in 1 cm layer in 250 mL high-density polyethylene plastic bags and frozen at –80 ºC in a ultra-freezer (IULT 335D, Indrel, Brazil). Freeze-drying was conducted in a tray dryer at –50 ºC and 0.05 mTorr for 24 hours (TDF 5503, IIShin, Korea).

Figure 1 ▶
Japanese grape at ripening stage.
*Source: authors' archive*

The freeze-dried sample was ground in a blender (Diamante 800, Britânia, Brazil), and particle size was standardized using a 32 Tyler mesh size (< 500 mm). The resulting *H. dulcis* powder (HD) was stored in metallized pouch bag at –80 ºC in an ultra-freezer (IULT 335D, Indrel, Brazil) until use.

## 2.3 Preparation of microencapsulated *Hovenia dulcis*

The microencapsulated *H. dulcis* (En-HD) was produced following the methodology of Yadav *et al*. (2020), with modifications. WPC and GA were used as coating materials in a 3:2 (w:w) ratio. HD and the coating materials in a 1:5 (w:w) ratio were dissolved in water (0.6 $g.mL^{-1}$) and stirred on a magnetic stirrer (CE-1540, Cienlab, Brazil) at 1800 rpm for 2 hours. The mixture was then subjected to an ultrasound bath (SSBuc, Solid Steel, Brazil) at 160 W and 40 kHz for 30 minutes at 25 ºC, then arranged in 2- and 3-mm layers in Zip Lock bags, frozen at –80 ºC in an ultra-freezer (IULT 335D, Indrel, Brazil), and subsequently freeze-dried at –50 ºC and 0.05 mTorr for 24 hours (TDF 5503, IIShin, Korea).

The resulting *H. dulcis* microparticles, with particle sizes smaller than 500 mm, were stored in an ultra-freezer at –80 ºC (IULT 335D, Indrel, Brazil) in a light-protected environment until use.

## 2.4 Microencapsulation efficiency of the encapsulated *Hovenia dulcis* powder

The microencapsulation efficiency of En-HD was evaluated by estimating the total phenolic compounds (TPC) on the particle surface and within the microencapsulated particles, following the methodology of Robert *et al*. (2010), with modifications.

To determine the TPC, 1 g of En-HD was vortexed (Vortex Mixer K45-2820, Kasvi, Brazil) with 10 mL of methanol:acetic acid:distilled water (50:8:42 v/v/v) for 1 minute. The solution was placed in an ultrasound bath (SSBuc, SolidSteel, Brazil) at 160 W and 40 kHz for 20 minutes, then centrifuged at 4856 g (SL-700, Solab, Brazil) for 5 minutes at 25 °C. For the TPC content on the particle surface, 1 g of En-HD was vortexed (Vortex Mixer K45-2820, Kasvi, Brazil) with 10 mL of ethanol:methanol (1:1 v/v) for 1 minute, and subsequently centrifuged at 4856 g (SL-700, Solab, Brazil) for 5 minutes at 25 °C.

The extracts were used to determine the TPC, and the microencapsulation efficiency was calculated using Equation 1.

$$\%\ Efficiency = \frac{\left(TPC_{total\ particle} - TPC_{surface}\right)}{TPC_{total\ particle}} x\,100 \quad (1)$$

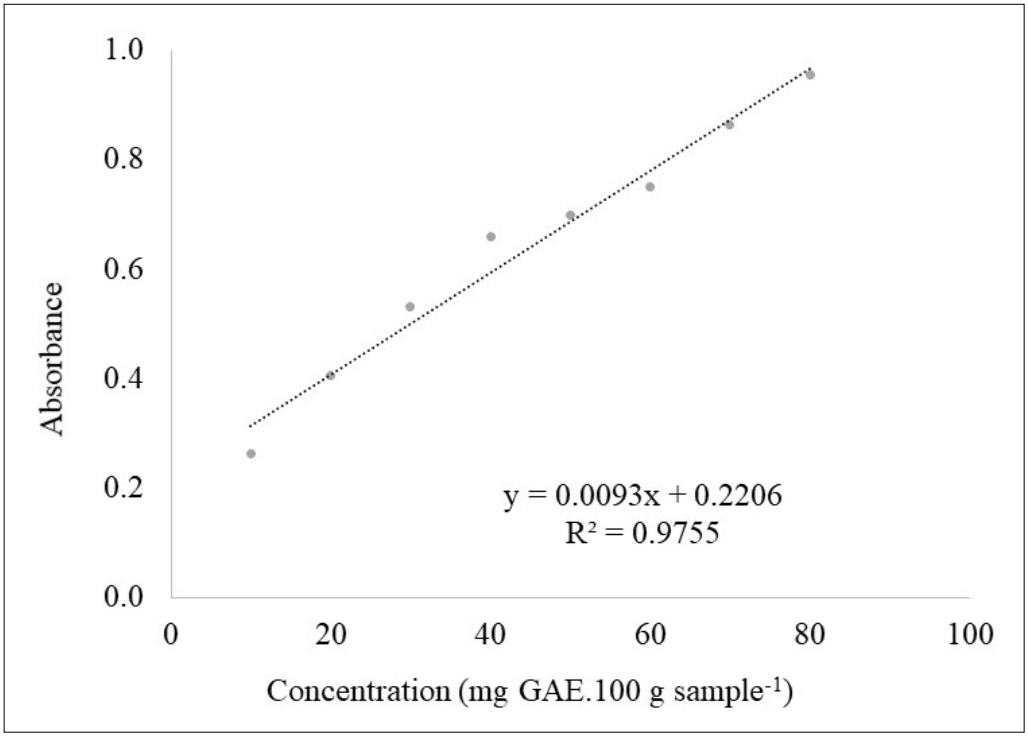
## 2.5 Determination of total phenolic compounds (TPC) and antioxidant activity

To assess TPC and antioxidant activity, HD and En-HD extracts were prepared according to the method of Larrauri, Rupérez and Saura-Calixto (1997), with

modifications. Specifically, 4 grams of sample were mixed with 40 mL of 50% methanol and held at 25 ºC for 60 minutes. The mixture was then centrifuged at 4856 g (SL-700, Solab, Brazil) for 15 minutes at 25 ºC, and the supernatant was transferred to a 100 mL volumetric flask. Subsequently, 40 mL of 70% acetone was added to the residue, homogenized, and left at 25 ºC for 60 minutes, followed by centrifugation at 4856 g (SL-700, Solab, Brazil) for 15 minutes at 25 ºC. The supernatant was added to the methanolic solution in the volumetric flask, and the volume was completed with distilled water.

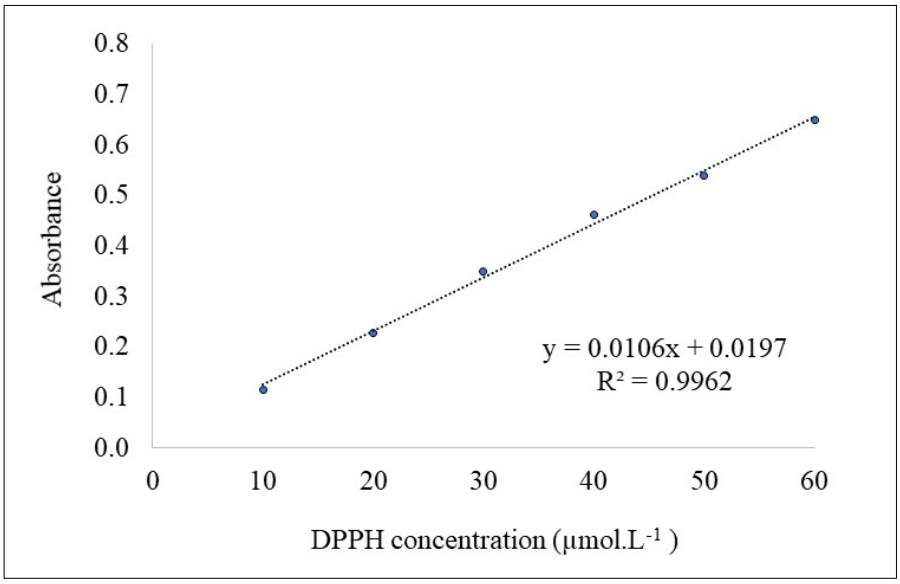
TPC determination followed the method described by Roesler *et al*. (2007), with adaptations. For this, 0.5 mL of the extract was mixed with 2.5 mL of 0.1 N Folin-Ciocalteu reagent and left to react for 5 minutes. Then, 2 mL of 7.5% sodium carbonate was added, and the mixture was incubated at 25 °C for 2 hours, protected from light. Absorbance was measured at 760 nm (80 AS, Cirrus, Brazil), with ultrapure water used as a blank. TPC was calculated using a standard curve of gallic acid (GAE) with concentrations ranging from 10 to 80 mg GAE.L$^{-1}$ ($R^2$ = 0.9755) (Figure 2). Results were expressed as mg GAE per 100 g.sample$^{-1}$.

Figure 2 ▶
Standard curve of total phenolic compounds.
*Source: research data*



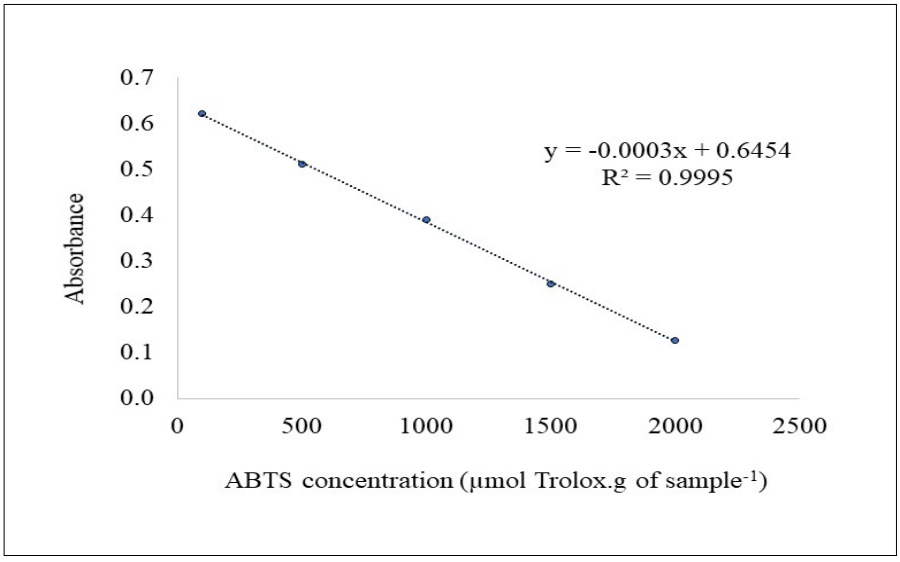

Antioxidant activity was determined by the DPPH assay according to Rufino *et al*. (2007a). The extract was diluted to 4 concentrations (0.5:100; 1:100; 1.5:100; 2:100, extract:water), and 0.1 mL of each dilution was combined with 3.9 mL of 0.06 mM DPPH solution in a light-protected environment. A control solution containing 50% methyl alcohol, 70% acetone, and distilled water (40:40:20, v:v:v) was used. Absorbance was measured at 515 nm (80 AS, Cirrus, Brazil). The DPPH concentration in µmol.L$^{-1}$ was derived from a DPPH standard curve ($R^2$ = 0.9962) (Figure 3), with results expressed in EC50 mg.mL$^{-1}$.

Figure 3 ▶
Standard curve for DPPH analysis.
*Source: research data*




The ABTS assay for antioxidant was conducted as described by Rufino *et al*. (2007b), using the same dilutions prepared for the DPPH assay. A 30 µL aliquot of each dilution was mixed with 3 mL of ABTS solution, and after a 6-minute reaction, absorbance was measured at 734 nm (80 AS, Cirrus, Brazil). A standard Trolox curve ($R^2$ = 0.9995) (Figure 4) was used to determine concentrations equivalent to 1000 µmol.$L^{-1}$ of Trolox, with results expressed as µmol Trolox.g of sample$^{-1}$.

Figure 4 ▶
Standard curve for ABTS analysis.
*Source: research data*




## 2.6 Determination of α-amylase inhibition

To analyze α-amylase inhibition, an aqueous extract was prepared following the method of Wang *et al*. (2019) with modifications. HD and En-HD samples were diluted in distilled water at a concentration of 2% (w/w) and stored for 12 hours at 25 ºC, protected from light. The solutions were centrifuged at 25 ºC for 15 minutes at 4856 g (SL-700, Solab, Brazil), and the supernatant was collected and stored at –80 °C (IULT 335D, Indrel, Brazil).

The α-amylase inhibition assay was conducted following the method of González-Muñoz *et al*. (2013). A 500 µL aliquot of the extract was homogenized with 500 µL of 0.02 M sodium phosphate buffer (pH = 6.9) containing α-amylase enzyme (0.5 $mg.mL^{-1}$) and incubated at 25 °C for 10 minutes. Then, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH = 6.9) was added, and the mixture was incubated at 25 ºC for 10 minutes. Following, 1 mL of dinitrosalicylic acid was added, and the mixture was placed in a boiling water bath for 10 minutes. After cooling to room temperature, the mixture was diluted with 15 mL of distilled water, and absorbance was measured at 540 nm (80 AS, Cirrus, Brazil). Sodium phosphate buffer (0.02 M, pH = 6.9) was used as a control, and each sample's blank was prepared by omitting the α-amylase enzyme. The percentage of inhibition was calculated using Equation 2. The experiment also included acarbose as a positive control.

$$Alpha\ amylase\ inhibition(\%) = \frac{\left(A_{control} - \left(A_{extract} - A_{blank}\right)\right)}{A_{control}} \times 100 \qquad (2)$$

where: $A_{control}$ is the absorbance with sodium phosphate buffer in place of the sample; $A_{blank}$ is the absorbance without enzyme addition; and $A_{extract}$ represents the absorbance of the evaluated sample. TPC, antioxidant activity, and the α-amylase inhibition determinations of En-HD were conducted on days 1 and 75 of storage at –80 ºC to estimate potential compound degradation.

## 2.7 Physicochemical characterization of HD and En-HD

Physicochemical and morphological characterization of HD and En-HD samples were conducted on day 1 post-storage. Moisture content was determined at 105 ºC until a constant weight achieved, according to AOAC method 925.45-b (AOAC, 2016).

Water solubility was measured as described by Sánchez-Madrigal *et al*. (2019) by calculating the ratio of the dried supernatant mass (evaporation residue) to the sample's initial mass. A 10 mL aliquot of distilled water and 0.2 g of HD or En-HD were vortexed in 15 mL centrifuge tubes and vortexed for 1 minute (Vortex Mixer K45-2820, Kasvi, Brazil). The mixture was centrifuged at 4856 g (SL-700, Solab, Brazil) for 20 minutes at 25 °C, and the supernatant was oven-dried at 105 ºC to constant weight. Solubility was calculated from the mass ratio between of the dried supernatant (evaporation residue) to the initial sample mass.

Particle size distribution was measured using a laser diffraction particle analyzer (SALD-2201, Shimadzu, Japan) with samples dispersed in a 2% sodium hexametaphosphate solution to prevent particle agglomeration.

## 2.8 Scanning Electron Microscopy (SEM) of HD and En-HD

SEM analysis was determined with a field emission scanning electron microscope (FEG) (JSM6701F, JEOL, Japan) and microanalysis by energy-dispersive X-ray spectrometry (EDS). Samples were mounted on aluminum supports and sputter-coated with gold. The analysis was performed at an acceleration voltage of 15 kV and a magnification of 1000×.

### 2.9 Statistical analysis

Results were evaluated using analysis of variance (ANOVA) and Tukey's test at a 95% significance level in Statistica 14 – Trial (Staftsoft, Tulsa, USA). All analyses were performed in triplicate, with results presented as mean ± standard deviation (Montgomery; Runger, 2021).

## 3 Results and discussion

This section presents, analyzes, discusses, and compares the results obtained from microencapsulation efficiency, bioactive compounds, α-amylase inhibition, physicochemical properties, and morphological analyses with findings previously reported in the literature.

### 3.1 Microencapsulation efficiency, total phenolic compounds (TPC), antioxidant activity, and α-amylase inhibition

Encapsulation efficiency is a key parameter influencing the retention of active compounds in the encapsulate, thereby determining the stability of the encapsulated active compound (Indrawati *et al*., 2015). The microencapsulation efficiency, measured in terms of TPC, was 79.91%, suggesting a high potential for preserving bioactive compounds. In this study, encapsulation efficiency is likely associated with the compatibility of the coating materials, which demonstrated good interaction. This result aligns with studies by Dag, Kilercioglu and Oztop (2017), who achieved efficiency between 77.03% and 84.44% for encapsulated golden berries using maltodextrin, gum arabic, alginate, and pectin as coating materials.

TPC levels (Table 1) were significantly higher ($p < 0.05$) for HD compared to En-HD over various storage periods. This may be due to the coating materials used for encapsulation and other processing factors, such as double freeze-drying and environmental exposure during milling of the final product (Hussain *et al*., 2018). Therefore, nutrients levels might have been impacted due to chemical degradation during storage and physical losses during processing. Dag, Kilercioglu and Oztop (2017) reported similar findings in freeze-dried golden berry juice (242.02 mg GAE.100 g dry.sample$^{-1}$) compared to encapsulated powder with various coating materials (59.61 to 95.89 mg GAE.100 g dry.sample$^{-1}$).

Table 1 ▶
Total phenolic compounds (TPC), antioxidant activity (ABTS and DPPH), and α-amylase inhibition of *Hovenia dulcis* powder (HD) and encapsulated *Hovenia dulcis* powder (En-HD) stored at −80 °C for 75 days.
*Source: research data*

| Analyses | HD | En-HD | |
|---|---|---|---|
| | Storage time (days) | | |
| | 1 | 1 | 75 |
| TPC (mg GAE.100 g.sample$^{-1}$) | 159.65 ± 0.88$^{a}$ | 134.17 ± 2.02$^{b}$ | 128.56 ± 2.99$^{b}$ |
| DPPH ($EC_{50}$ mg.mL$^{-1}$) | 308.75 ± 6.64$^{c}$ | 7043.24 ± 11.31$^{a}$ | 1182.49 ± 7.05$^{b}$ |
| ABTS (µmol Trolox.g.sample$^{-1}$) | 159.87 ± 1.91$^{c}$ | 230.48 ± 0.14$^{a}$ | 170.65 ± 0.12$^{b}$ |
| α-amylase inhibition (%) | 54.03 ± 5.59$^{a}$ | 96.88 ± 6.82$^{b}$ | 92.80 ± 3.90$^{b}$ |

Mean ± standard deviation. Means followed by the same lowercase letters in the same row do not differ significantly by Tukey's test ($p < 0.05$)

The encapsulated powder retained 95.8% of its initial TPC content after 75 days of storage, indicating compound preservation under the study conditions, likely due to the chosen coating materials. The En-HD sample showed higher antioxidant activity than HD, likely due to encapsulation creating a physical barrier, allowing antioxidants to maintain their activity longer and preventing chemical degradation from unwanted chemical reactions.

Antioxidant activity measured by the DPPH assay was higher than that by the ABTS assay. The coating material, composed of proteins and polysaccharides with opposite electrical charges, likely contributed to interactions between two biopolymers, influenced by environmental conditions (Simões *et al*., 2017). Thus, electrical charges may affect oxidation rates between the core material and the external environment, explaining the differing results from ABTS and DPPH assays based on oxidation reactions with free radicals.

Gum Arabic may interact with bioactive compounds through functional groups, making it a versatile carriers for binding and trapping both hydrophilic and hydrophobic compounds (Simões *et al*., 2017), possibly enhancing the antioxidant activity of En-HD relative to HD as measured by the ABTS assay. These interactions may also have influenced the results for En-HD during the storage, showing reduced antioxidant activity in both ABTS and DPPH assays.

It is suggested that some *Hovenia dulcis* was not fully encapsulated, as observed in the particle size analysis, resulting in greater exposure of the bioactive compound and less stability over 75 days, possibly contributing to the decrease in DPPH values. Various factors can impact antioxidant compound degradation, including chemical structure, water availability, and the presence of other food components (Ramírez; Giraldo; Orrego, 2015).

En-HD demonstrated greater α-amylase inhibition than acarbose (100% inhibition) on day 1 post-encapsulation (Table 1). This result suggests that microencapsulation successfully protected the active-inhibitory compound from environmental exposure.

Regulating blood sugar level is an effective approach to managing *Diabetes mellitus* and associated complications. Although synthetic α-amylase inhibitors like acarbose and miglitol are widely used, they come with high costs and side effects (Hussain *et al*., 2018). Consequently, there is growing interest in natural inhibitors, with En-HD emerging as promising alternative, though further research is needed to validate *in vivo* effectiveness. Hussain *et al*. (2018) reported similar results, with 93.33% α-amylase inhibition in encapsulated freeze-dried powder composed of various roots and seeds using gum Arabic as a coating material.

To date, no studies have examined the microencapsulation of HD via freeze-drying. The findings of this study suggest that En-HD has the potential to provide bioactive compounds, as it exhibits higher TPC, antioxidant activity, and α-amylase inhibition. The results were satisfactory under the methods employed, suggesting HD is a promising candidate for food industry applications. However, further studies are needed to assess food supplementation potential and gastrointestinal behavior.

## 3.2 Physicochemical and morphological properties of HD and En-HD

Moisture content is a critical factor in the food industry, as it is directly reflects the efficiency of the drying process (Dag; Kilercioglu; Oztop, 2017). HD and En-HD samples displayed low moisture contents ($0.95 \pm <0.01\%$ and $4.91 \pm 0.07\%$, respectively), indicating microbiological stability under the study conditions.

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The En-HD sample exhibited significantly higher ($p < 0.05$) water solubility (76.42 ± 2.58%) compared to HD (17.85 ± 0.79%), likely due to the high solubility of the gum Arabic and whey proteins (Rezende; Nogueira; Narain, 2018; Lourenço; Moldão-Martins; Alves, 2020; Simões *et al*., 2017). The solubility of food powders influences their behavior upon contact with aqueous solutions (Hinestroza-Córdoba *et al*., 2020), which is affected by factors such as composition and particle size (Hussain *et al*., 2018).

Increased solubility may also result from reduced porosity due to freeze-drying (Hinestroza-Córdoba *et al*., 2020) and the use of WPC and gum Arabic. Hussain *et al*. (2018) reported water solubility ranging from 84.06% and 92.31% for encapsulated powder composed of various roots and seeds with different concentrations of coating materials, corroborating the values observed in this study for En-HD.

The coating materials possess important properties for bioaccessibility, and parameters such as flexibility, resistance, stability, and impermeability are crucial for delivering and releasing bioactive compounds (Grgić *et al*., 2020). Therefore, a suitable combination of coating and core materials can yield encapsulated material with favorable physicochemical properties and stability.

Figure 5 ▼
Particle size distribution of *Hovenia dulcis* powder (HD).
*Source: research data*

Figures 5 and 6 display the particle size distribution differences between HD and En-HD samples. Both samples exhibited bimodal curves, indicating the presence of particles of varying sizes, likely due to the freeze-drying process, which generates a wide range of particle sizes (Hinestroza-Córdoba *et al*., 2020).

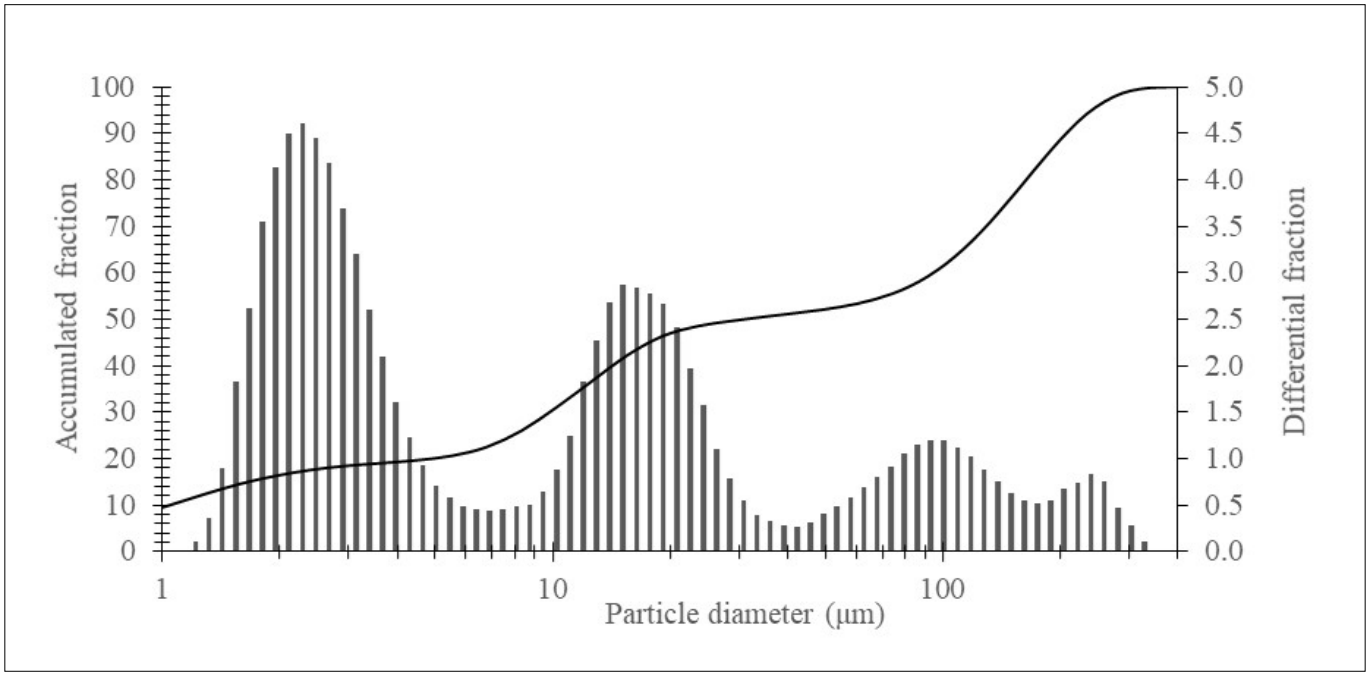

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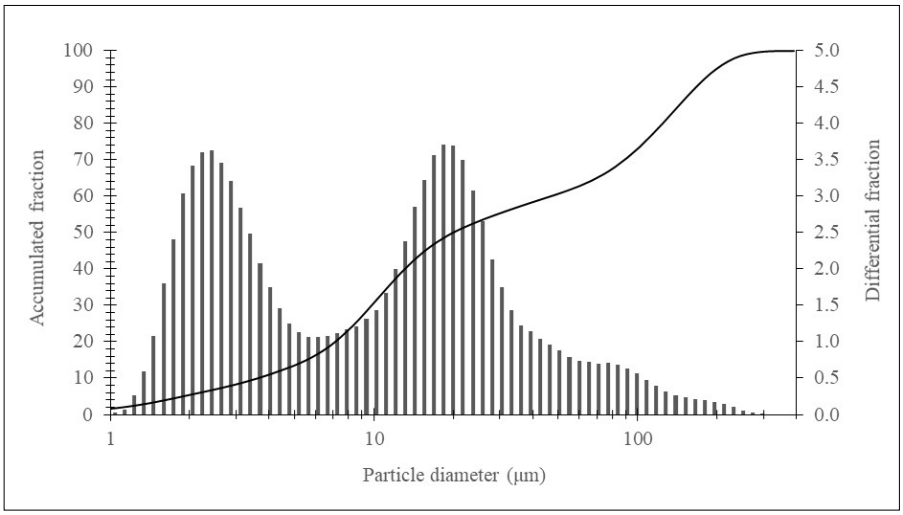
Figure 6 ▶
Particle size distribution of encapsulated *Hovenia dulcis* powder (En-HD).
*Source: research data*



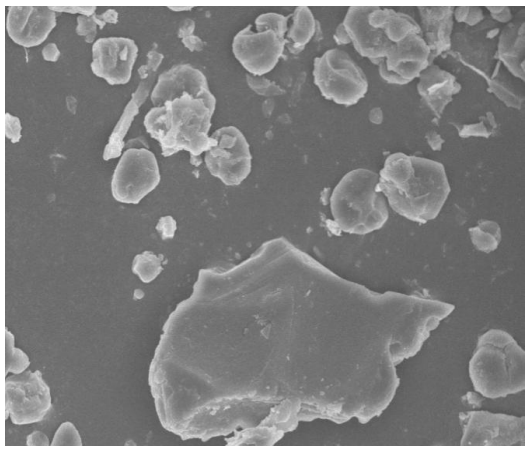

The HD sample exhibited a higher average particle size diameter (50% of the sample) at 30.74 µm compared to En-HD at 19.99 µm. Overall, particle sizes up to 207.71 µm and 167.26 µm were observed for 90% of the particles in HD and En-HD, respectively.

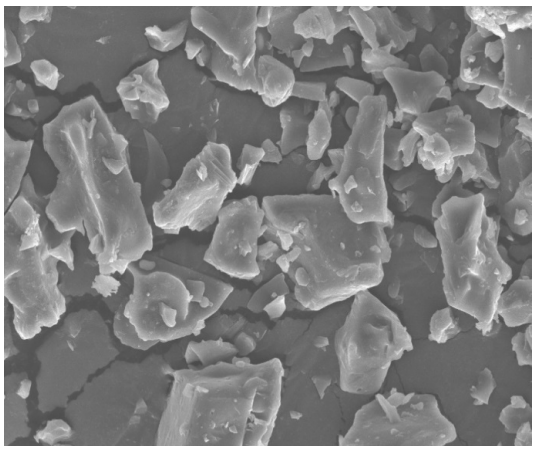
The smaller particle sizes observed in En-HD may be attributed to the coating materials utilized in the microencapsulation process. Ultrasound baths are less efficient in reducing particle size compared to ultrasonic probes (Jambrak *et al*., 2014). Thus, the percentage of small particles found in En-HD may also be attributed to the wall material (whey protein and gum Arabic), as some of these materials may have remained dispersed, without forming a complete capsule.

Figure 7 ▼
Scanning Electron Microscopy images at a magnification of 1000× of (a) *Hovenia dulcis* powder (HD), and (b) encapsulated *Hovenia dulcis* powder (En-HD).
*Source: research data*

The presence of smaller particles also explains the increased water solubility, as smaller particles possess enhanced properties and greater interactions with water molecules (Hinestroza-Córdoba *et al*., 2020). The smaller the particle size, the greater the surface area available for hydration (Kuck; Noreña, 2016).

Figures 7a and 7b display the SEM images of HD and En-HD, respectively. Undefined shapes of varying sizes were observed, with a greater number of smaller particles found in the En-HD sample, which aligns with the particle size distribution results. The freeze-drying process significantly affects the structure of the product. According to Fang and Bhandari (2010), encapsulation by freeze-drying occurs as the core materials are homogenized within matrix solutions and subsequently co-lyophilize, resulting in irregular shapes.


(a)


(b)

As reported by Ramírez, Giraldo and Orrego (2015), the freezing step preceding freeze-drying plays a crucial in shaping the final structure, as rapid freezing generates small ice crystals that directly impact pore size. This, following the sublimation process, enhances the quality of freeze-dried products (Kuck; Noreña, 2016).

Similar results were obtained by Dag, Kilercioglu and Oztop (2017), as well as Hussain *et al*. (2018), who studied various coating materials for the freeze-dried encapsulation of golden berries and polyherbal formulations, respectively, reporting irregular shapes resembling fractured glass flakes.

## 4 Conclusion

The microencapsulated *Hovenia dulcis* powder (En-HD) showed a 95.8% retention of TPC after 75 days of storage at –80 ºC, high antioxidant activity by the ABTS assay, and substantial α-amylase inhibition compared to the pseudofruit powder (HD), suggesting potential as a promising candidate for further testing, particularly *in vivo*, to control blood sugar levels. Freeze-drying encapsulation increased sample solubility by 76%, likely due to whey protein, gum Arabic, and the smaller particle size of the En-HD sample. The freeze-drying microencapsulation of *H. dulcis* effectively preserved bioactive compounds, indicating as an additive for the food industry.

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## Declaration of competing interest

The authors declare no conflict of interest.

## Article contributions

**RIGO, E.; SEHN, G. A. R.; CAVALHEIRO, D.:** conception and design of the study/research; data analysis and interpretation; critical intellectual review of the manuscript. **HANAUER, D. C.:** conception and design of the study/research; data analysis and interpretation. **KNAKIEWICZ, L.:** data analysis and interpretation. All authors participated in writing, discussing, reading, and approving the final version of the paper.

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