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 known as a source of bioactive compounds; however, like most fruits, it is easily susceptible to deterioration. Encapsulation techniques have been used to protect and stabilize compounds, in addition to minimizing changes in the characteristics of the supplemented product. In the present study, the freeze-drying pulp of *H. dulcis* pseudofruit (HD) and pulp of pseudofruits microencapsulated by freeze-drying using whey protein concentrate and gum arabic as coating materials (En-HD) were investigated. The samples (HD and EN-HD) were characterized for their physicochemical properties, total phenolic compounds (TPC), antioxidant activity (ABTS and DPPH), α-amylase inhibition, particle size distribution, and scanning electron microscopy. The microencapsulation retained 95.8% TPC after 75 days of storage at –80 ºC. En-HD showed higher antioxidant activity by the ABTS assay, and inhibition of the α-amylase enzyme, demonstrating the protective effect of the microencapsulation technique, which can be promising, after further testing, especially *in vivo*, for controlling blood sugar levels. The En-HD presented a smaller particle size and higher solubility when compared to HD (76% higher), probably due to the coating materials used in the microencapsulation process, as well as the freeze-drying of the encapsulated sample made with HD previously subjected to freeze-drying. En-HD proved to be a promising candidate to provide functional properties for use as an additive in food industries.
Keywords: ABTS; DPPH; particle size; phenolic compounds; α-amylase inhibition.

Encapsulamento do pseudofruto da Uva do Japão (Hovenia dulcis) por liofilização: caracterização e potencial antioxidante

Resumo
O pseudofruto da Hovenia dulcis é conhecido como fonte de compostos bioativos, entretanto, como a grande maioria dos frutos, é facilmente susceptível à deterioração. Técnicas de encapsulamento têm sido utilizadas para proteger e estabilizar compostos, além de minimizar alterações nas características do produto suplementado. No presente estudo, a polpa do pseudofruto H. dulcis liofilizada (HD) e a polpa dos pseudofrutos microencapsulados por liofilização usando concentrado de proteína de soro de leite e goma arábica como materiais de revestimento (En-HD) foram investigados. As amostras (HD e En-HD) foram caracterizadas quanto às propriedades físico-químicas, compostos fenólicos totais (TPC), atividade antioxidante (ABTS e DPPH), inibição da α-amilase, distribuição granulométrica e microscopia eletrônica de varredura. A microencapsulação reteve 95,8% de TPC após 75 dias de armazenamento a -80 ºC. En-HD apresentou maior atividade antioxidante pelo ensaio ABTS e inibição da enzima α-amilase, demonstrando o efeito protetor da técnica de microencapsulação, que pode ser promissora, após maiores testes, especialmente in vivo, para o controle da glicemia. O En-HD apresentou menor tamanho de partícula e maior solubilidade quando comparado ao HD (76% maior), provavelmente devido aos materiais de revestimento utilizados no processo de microencapsulação, bem como à liofilização da amostra encapsulada feita com HD previamente liofilizado. En-HD mostrou ser uma matéria-prima promissora para fornecer propriedades funcionais para uso como aditivo na indústria de alimentos.
***Palavras-chave**: ABTS; compostos fenólicos; DPPH; inibição da α-amilase; tamanho de partícula.*

1 Introduction

Hovenia dulcis, popular as the Japanese grape, belongs to the Rhamnaceae family and is an edible fruit with medicinal properties for controlling alcoholism and liver diseases (Cai *et al*., 2021). It has bioactive compounds with antioxidant, antimicrobial, and antidiabetic effects, and can be considered an excellent additive for the food industry (Cavalheiro *et al*., 2024; Schaefer *et al*., 2022).

Manufacturers have faced several challenges related to the incorporation of bioactive compounds in foods, as these substances tend to be unstable or poorly soluble during processing and storage conditions, such as heat, oxidation, pH, and water activity, which can lead to degradation, poor absorption, and reduced bioavailability in the gastrointestinal tract (Grgić *et al*., 2020).

Encapsulation has stood out as a technique for incorporating bioactive compounds into foods, in tiny capsules/particles (El-Messery *et al*., 2019). Numerous materials can be used for microencapsulation, including proteins, lipids, and polysaccharides (Souza *et al.,* 2017). Proteins have a wide application due to some advantages including biodegradability, biocompatibility, and solubility in water (Grgić *et al.,* 2020). Polysaccharides, in turn, can interact with bioactive compounds, making them versatile carriers to bind and trap a variety of compounds with hydrophilic and hydrophobic characteristics (Souza *et al.,* 2017; Santos *et al*., 2020).

Gum Arabic is a complex heteropolysaccharide with a highly branched structure, which has proven to be an excellent film-forming agent, with better protection of the encapsulated particle. The whey protein concentrate is indicated for use as a coating material due to its emulsion capacity and stabilization of the microparticles (El-Messery *et al*., 2019; Lourenço; Moldão-Martins; Alves, 2020).

The use of *H. dulcis* pseudofruit as a food ingredient can be an alternative approach to increase its commercial value and add functionality to end products through supplementation in encapsulated form. Thus, the present study evaluated the feasibility of microencapsulating *H. dulcis* pseudofruit in whey protein concentrate and gum Arabic by the freeze-drying technique regarding their physicochemical and bioactive characteristics.

In this way, the rest of this article is divided into three sections. In Section 2, the experimental procedures used in the development of this project will be presented, and Section 3 contains a discussion of the results obtained and correlations with other works already published in the literature. Finally, Section 4 presents the conclusion of this article.

2 Materials and methods

The following section outlines the primary materials and methods utilized to prepare and assess microencapsulation.

2.1 Reagents and chemical standards

Analytical grade reagent, 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) e DPPH (2,2-diphenyl-1-picryl – hydrazil), were obtained, from Sigma Aldrich (San Luis, EUA). Whey protein concentrate (WPC) and gum Arabic (GA) were from Êxodo Científica (São Paulo, Brazil). All other reagents were of analytical grade.

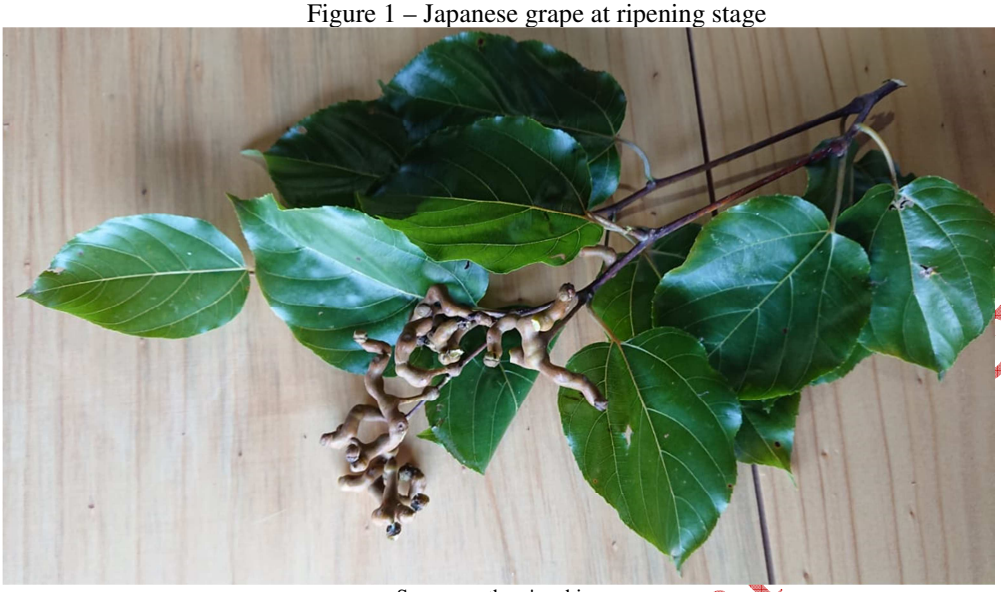
2.2 Obtaining of *Hovenia dulcis* pseudofruit powder (HD)

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

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

use.

Figure 1 – Japanese grape at ripening stage



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2.3 Obtaining the microencapsulated of *Hovenia dulcis*

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

Then, *H. dulcis* microparticles with particle sizes smaller than 500 mm were stored protected from light, in an ultra-freezer at –80 ºC (IULT 335D, Indrel, Brazil) 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 the microencapsulated particles, according to the methodology described by 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, and then centrifuged at 4856 g (SL-700, Solab, Brazil) for 5 minutes at 25 °C. To obtain the TPC content of 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 then 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.

$$\% \text{ Efficiency } = \frac{(TPC_{total\ particle} - TPC_{surface})}{TPC_{total\ particle}} x100 \quad (1)$$

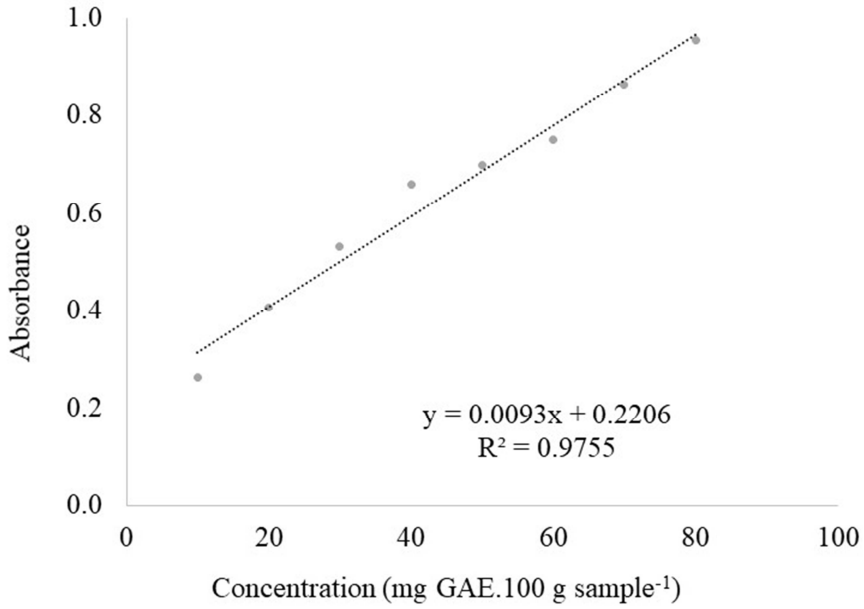
2.5 Determination of total phenolic compounds (TPC) and antioxidant activity

To assess TPC and antioxidant activity, HD and En-HD extracts were prepared according to

Larrauri, Rupérez and Saura-Calixto (1997), with modifications. For that, 4 grams of sample was mixed with 40 mL of 50% methanol and kept at 25 ºC for 60 minutes. Subsequently, the mixture was 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. Then, 40 mL of 70% acetone was added to the residue, homogenized, and kept at rest 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 transferred to the same volumetric flask containing the methanolic solution, and the volume was completed with distilled water.

The TPC were determined as described by Roesler *et al*. (2007), with adaptations. For that, 0.5 mL of the extract was mixed with 2.5 mL of 0.1 N Folin-Ciocalteu reagent and kept in reaction for 5 minutes. Then, 2 mL of 7.5% sodium carbonate was added and kept at rest protected from light at 25 °C for 2 hours. The absorbance of the mixture was measured at 760 nm (80 AS, Cirrus, Brazil). The blank was prepared by replacing the extract with ultrapure water. The 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). The results were expressed in mg GAE.100 g.sample^{-1}.

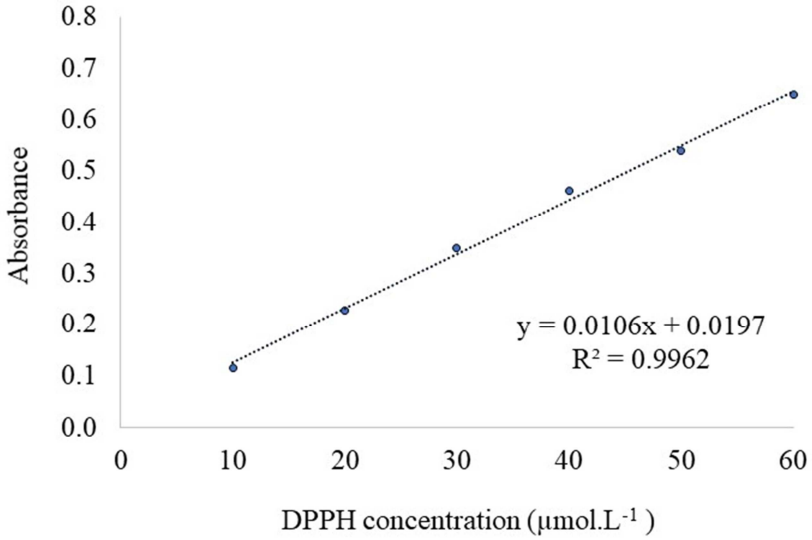
Figure 2 – Standard curve of Total phenolic compounds



Source: research data

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

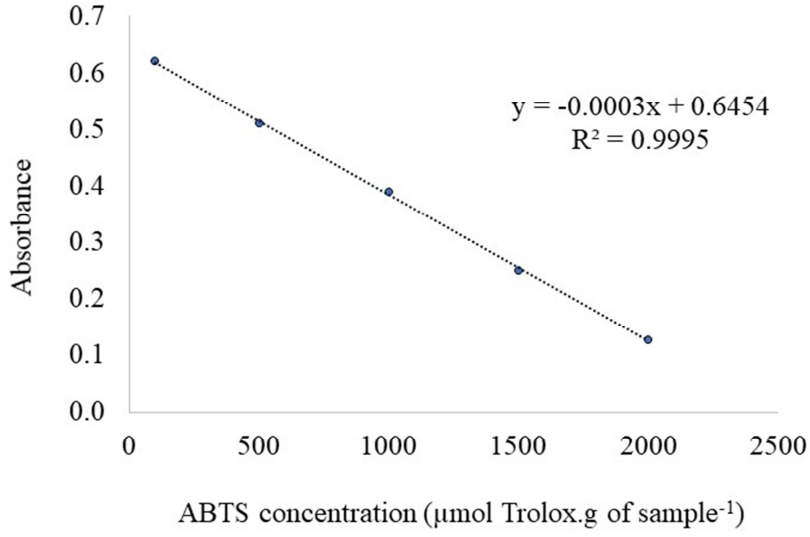
Figure 3 – Standard curve for DPPH analysis



Source: research data

The antioxidant activity by the ABTS assay was determined as described by Rufino *et al.* (2007b), using the same DPPH dilutions previously described. In an environment protected from light, a 30 µL aliquot of each dilution was mixed with 3 mL of the ABTS solution, and after 6 minutes of reaction, absorbance readings were measured at 734 nm (80 AS, Cirrus, Brazil). A standard Trolox curve (R^2 = 0.9995) (Figure 4) was used to define the concentration equivalent to 1000 µmol.L^{-1} of Trolox. The results were expressed in µmol Trolox.g of sample^{-1}.

Figure 4 – Standard curve for ABTS analysis



Source: research data

2.6 Determination of α-amylase inhibition

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

The α-amylase inhibition was evaluated as described by González-Muñoz et al. (2013), using 500 µL of extract, which was homogenized with 500 µL of 0.02 M sodium phosphate buffer (pH = 6.9) containing the α-amylase enzyme (0.5 mg.mL^{-1}) and incubated at 25 °C for 10 minutes. Then, 500

µL of 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. Subsequently, 1 mL of dinitrosalicylic acid was added, and the mixture was placed in a water bath at boiling temperature for 10 minutes. The mixture was cooled to room temperature and diluted with 15 mL of distilled water. The absorbance was measured at 540 nm (80 AS, Cirrus, Brazil). A 0.02 M sodium phosphate buffer (pH = 6.9) was used as a control. The blank of each sample was defined by mixing the extract and 0.02 M sodium phosphate buffer (pH = 6.9) without the addition of the α-amylase enzyme. The percentage of inhibition was calculated according to Equation 2. Simultaneously, the experiment was conducted substituting the sample with acarbose as a positive control.

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

where: $A_{control}$ represents absorbance using sodium phosphate buffer instead of the sample; A_{blank} represents absorbance using a buffer without enzyme addition; and $A_{extract}$ represents the absorbance of the evaluated sample. The determinations of TPC, antioxidant activity, and the α-amylase inhibition of En-HD were performed on days 1 and 75 of storage in an ultra-freezer at –80 ºC, estimating the possible losses of compounds during this period.

2.7 Physicochemical characterization of HD and En-HD

The physicochemical and morphological characterization of HD and En-HD samples was performed on day 1 after storage. The moisture content was determined at 105 ºC until constant weight according to the method 925.45-b (AOAC, 2016).

The water solubility of the samples was determined as described by Sánchez-Madrigal *et al.* (2019), calculating the ratio between the mass of dried supernatant (evaporation residue) and the initial mass of the sample. For that, 10 mL of distilled water and 0.2 g of HD or En-HD were placed in 15 mL centrifuge tubes and vortexed for 1 minute (Vortex Mixer K45-2820, Kasvi, Brazil). Then, the mixture was centrifuged at 4856 g (SL-700, Solab, Brazil) for 20 minutes at 25 °C. The supernatant was transferred to a Petri dish and oven-dried at 105 ºC until constant weight. The water solubility was calculated by the ratio between the mass of the dried supernatant (evaporation residue) and the initial mass of the sample.

The average particle size distribution was determined in a laser diffraction particle analyzer (SALD-2201, Shimadzu, Japan). The samples were dispersed in a 2% sodium hexametaphosphate solution to prevent agglomeration of particles and then dispersed in deionized water directly in the equipment.

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

The SEM was determined in a field emission scanning electron microscope (FEG) (JSM6701F, JEOL, Japan), and microanalysis by energy-dispersive X-ray spectrometry (EDS). The samples were placed on aluminum support and sputter-coated with gold. The analyses were conducted at 15 kV of acceleration voltage with a magnification of 1000×.

2.9 Statistical analysis

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

3 Results and discussion

In this section, the results obtained from microencapsulation efficiency, bioactive compounds, α-amylase inhibition, physicochemical properties, and morphological analyses will be presented, analyzed, discussed, and compared with findings already published in the literature.

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

The encapsulation efficiency is an important parameter that influences the amount of active compound remaining in the encapsulate, thereby determining the stability of the encapsulated active compound (Indrawati *et al.*, 2015). The microencapsulation efficiency, calculated as the amount of TPC, was 79.91%, indicating a high potential for the preservation of bioactive compounds. In the present study, the encapsulation efficiency may be associated with the compatibility of the coating materials, as they demonstrated good interaction between them. This result agrees with the studies of Dag, Kilercioglu and Oztop (2017), who obtained efficiency between 77.03% and 84.44% for encapsulated golden berries using maltodextrin, gum arabic, alginate, and pectin as coating materials.

The TPC levels (Table 1) were significantly higher ($p < 0.05$) for HD when compared to En-HD in the different storage periods, which may be due to the coating materials used to obtain the encapsulated material, in addition to other processing conditions such as double freeze-drying and exposure to the environment during milling of the final product (Hussain *et al.*, 2018). Therefore, the amount of nutrients may have been affected due to chemical degradation caused by the storage and physical losses during processing. Dag, Kilercioglu and Oztop (2017) reported a similar result in freeze-dried golden berry juice (242.02 mg GAE.100 g dry.sample^{-1}) when compared to the encapsulated powder with different 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

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 lower-case letters on the same line do not differ significantly by the Tukey's test ($p < 0.05$)
Source: research data

The encapsulated powder exhibited a residual TPC content of 95.8% after 75 days of storage, indicating the preservation of the compounds for the conditions studied, possibly due to the coating materials used. The En-HD sample exhibited higher antioxidant activity than the HD sample, considering that encapsulation creates a physical barrier, allowing antioxidants to maintain their activity for longer. Additionally, it helps prevent chemical degradation, protecting them from unwanted chemical reactions.

The antioxidant activity determined by the DPPH assay was higher than that observed with the ABTS assay. The coating material formed by the combination of proteins and polysaccharides has opposite electrical charges that contribute to the interactions between the two biopolymers, which are affected by environmental condition (Souza *et al.,* 2017). Therefore, electrical charges can affect the oxidation rates between the core material and the external environment. This can elucidate the opposite results obtained for the ABTS and DPPH assays based on oxidation reactions with free radicals.

Gum Arabic can interact with bioactive compounds through functional groups, making them versatile carriers to bind and trap hydrophilic and hydrophobic compounds (Souza *et al.,* 2017), which may have contributed to the greater antioxidant activity of En-HD when compared with HD, determined by the ABTS assay. These interactions may also have influenced the results of En-HD during the storage, which showed a decrease in the antioxidant activity by the ABTS e DPPH assay.

We also believe that part of the *Hovenia dulcis* was not fully encapsulated (as observed in the particle size analysis), and this fact resulted in greater exposure of the bioactive compound,

consequently not providing stability during the 75 days of storage, possibly leading to the decrease in DPPH value.

It is noteworthy that several factors can affect the degradation of antioxidant compounds, including the chemical structure, the availability of water, and the presence of other food components (Ramírez; Giraldo; Orrego, 2015).

A greater α-amylase inhibition was observed for the sample En-HD when compared to acarbose (100% inhibition) on day 1 after the microencapsulation (Table 1). The result indicates that microencapsulation was able to protect the active-inhibitory compound from the external environment.

It is known that regulating the blood sugar level is an effective approach to overcome *Diabetes mellitus* and related complications. Although synthetic α-amylase inhibitors such as acarbose and miglitol have been successfully used in various processes, they have high costs and clinical side effects (Hussain *et al*., 2018). In this context, there is an increasing demand for studies on natural inhibitors, and En-HD is a promising alternative, even though further investigation is necessary to prove its effectiveness *in vivo*. Hussain *et al*. (2018) found similar results, 93.33% α-amylase inhibition in encapsulated freeze-dried powder composed of different roots and seeds, using gum Arabic as a coating material.

To date, there are no studies on the microencapsulation of HD by freeze-drying. The results of the present study indicate that En-HD has the potential to provide bioactive compounds, once it presents higher TPC, antioxidant activity, and α-amylase inhibition. The results were satisfactory by the methods studied, making HD a promising alternative for application in the food industry. However, further studies are needed to assess food supplementation and gastrointestinal behavior.

3.2 Physico-chemical and morphological properties of HD and En-HD

The moisture content of samples is of great importance in the food industry, as it is directly related to the efficiency of the drying process (Dag; Kilercioglu; Oztop, 2017). The samples HD and En-HD showed low moisture contents (0.95 ± <0.01 and 4.91 ± 0.07%, respectively), thus both presented microbiological stability under the conditions of the study.

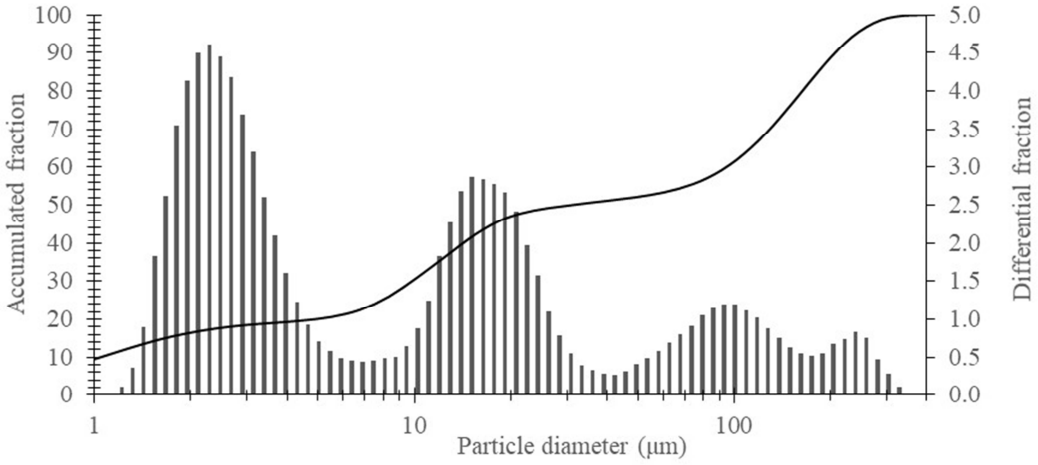
The sample En-HD showed higher ($p < 0.05$) water solubility (76.42 ± 2.58%) when compared to HD (17.85 ± 0.79%), probably due to the high solubility of the gum Arabic and whey proteins (Rezende; Nogueira; Narain, 2018; Lourenço; Moldão-Martins; Alves, 2020; Souza *et al.,* 2017). The solubility of food powders is responsible for the behavior of the powder when in contact with an aqueous solution (Hinestroza-Córdoba *et al*., 2020), which is affected by several factors including the composition and particle size of the powders (Hussain *et al*., 2018).

In addition, the high solubility can also be due to the lower porosity caused by freeze-drying (Hinestroza-Córdoba *et al*., 2020), as well as the use of WPC and gum Arabic. Hussain *et al*. (2018) reported water solubility between 84.06 and 92.31% for encapsulated powder composed of different roots and seeds, using different concentrations of coating materials, corroborating the values observed in the present study for En-HD.

The coating materials have significant properties in the bioaccessibility of the compounds, and the parameters flexibility, resistance, stability, and impermeability are of great importance for the delivery and release of the bioactive compounds (Grgić *et al*., 2020). Therefore, an effective combination of coating materials and core materials can result in an encapsulated material with good physicochemical properties and stability.

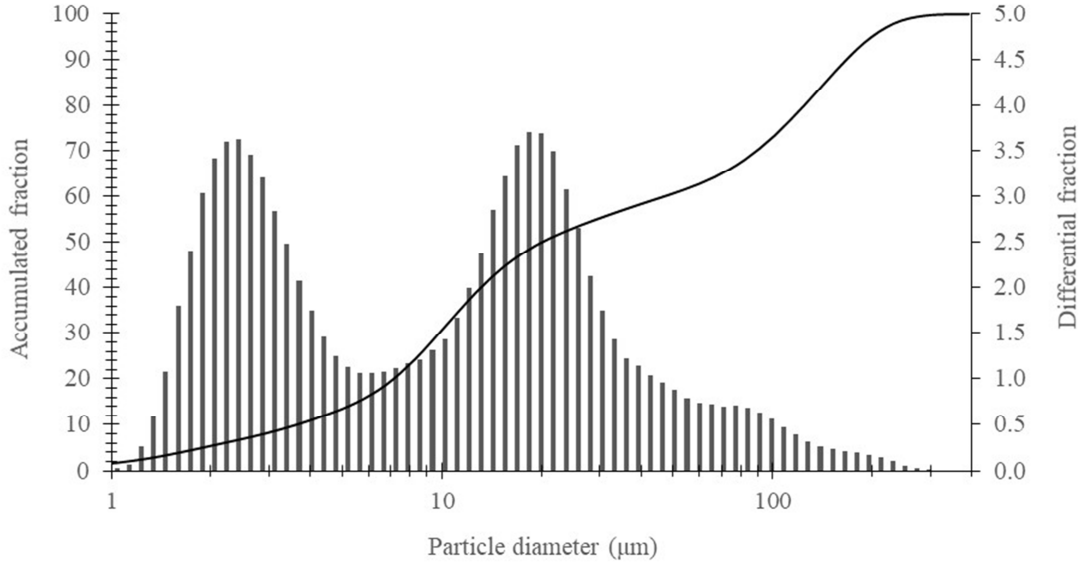
Figures 5 and 6 shows the difference in the particle size distribution of the samples HD and En-HD. Both samples presented bimodal curves, indicating the existence of particles of different sizes, probably due to the freeze-drying process, which generates many particles of varying sizes (Hinestroza-Córdoba *et al*., 2020).

Figure 5 – Particle size distribution of *Hovenia dulcis* powder (HD)



Source: research data

Figure 6 – Particle size distribution of encapsulated *Hovenia dulcis* powder (En-HD)



Source: research data

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

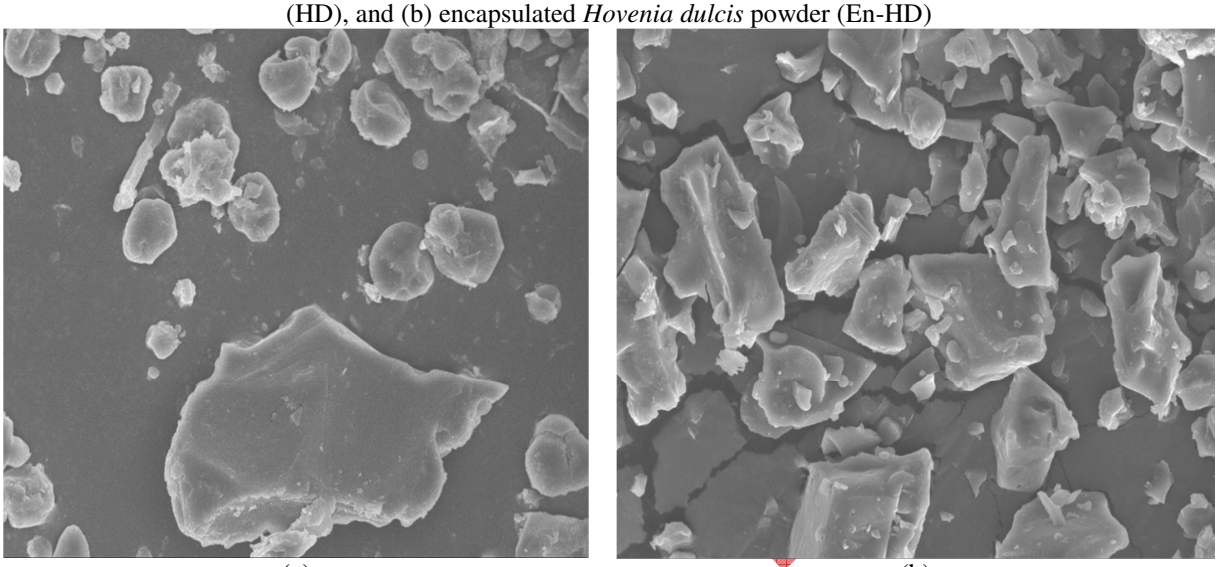
The smaller particle sizes observed for En-HD may be due to the coating materials used in the microencapsulation process. Ultrasound baths are less efficient in reducing particle size when compared to ultrasonic probes (Jambrak *et al*., 2014). Therefore, the percentage of small particles found in En-HD can also be attributed to the wall material (whey protein and gum Arabic) as part of it may have remained dispersed, meaning it did not form the capsule.

The presence of smaller particles also explains the results of water solubility, once smaller particles have better 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 shows the SEM images of HD and En-HD, respectively. Undefined forms with different sizes were observed, with a great number of smaller particles observed for the sample

En-HD, which was also observed in the particle size distribution. The freeze-drying process has a major impact on the product structure. According to Fang and Bhandari (2010), encapsulation by freeze-drying is achieved as the core materials homogenize in matrix solutions and then co-lyophilize, resulting in uncertain forms.

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)



(a) (b)

Source: research data

As reported by Ramírez, Giraldo and Orrego (2015), the freezing process before freeze-drying is an important step in forming the product structure, once fast freezing generates small ice crystals that directly affect the pore size, which after a sublimation process improves the quality of frozen products (Kuck; Noreña, 2016).

Similar results were obtained by Dag, Kilercioglu and Oztop (2017), and Hussain *et al*. (2018), who studied different coating materials for the encapsulation by freeze-drying of golden berry and polyherbal formulation, respectively, and reported irregular forms that resemble broken flakes of glass.

4 Conclusion

The microencapsulated *Hovenia dulcis* powder (En-HD) showed 95.8% retention of TPC after 75 days of storage at –80 °C, high antioxidant activity by the ABTS assay, and a marked α-amylase inhibition when compared to the pseudofruit powder (HD), thus being a promising candidate after further testing, especially *in vivo,* for controlling blood sugar levels. The encapsulation by freeze-drying increased the solubility of the samples by 76%, probably due to the presence of whey protein and gum Arabic, as well as the smaller particle size of the sample En-HD. The microencapsulation of *H. dulcis* by freeze-drying proved to be an effective alternative for the preservation of bioactive compounds, demonstrating the potential for use as an additive in food industries.

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Declaration of competing interest

The authors declare no conflict of interest.

References

AOAC – ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTRY. Official methods of analysis, 20. ed. Arlington: AOAC International, 2016.

CAI, W.; XU, S.; MA, T.; ZHANG, X.; LIU, B.; XU, F. Five novel triterpenoid saponins from *Hovenia dulcis* and their Nrf2 inhibitory activities. **Arabian Journal of Chemistry**, v. 14, n. 8, 103292, 2021. DOI: https://doi.org/10.1016/j.arabjc.2021.103292.

CAVALHEIRO, D.; MORANDIM, G. C.; SCHAEFER, S. V.; AMARAL, A. M. P.; BETTANIN, L.; SEHN, G. A. R. *Hovenia dulcis* T. extract and *Glycyrrhiza glabra* as natural antioxidants in Bologna-type mortadella. **Revista Principia - Divulgação Científica e Tecnológica do IFPB**, Early View, 2024. DOI: http://dx.doi.org/10.18265/2447-9187a2024id8195.

DAG, D.; KILERCIOGLU, M.; OZTOP, M. H. Physical and chemical characteristics of encapsulated goldenberry (*Physalis peruviana* L.) juice powder. **LWT – Food Science and Technology**, v. 83, p. 86-94, 2017. DOI: https://doi.org/10.1016/j.lwt.2017.05.007.

EL-MESSERY, T. M.; EL-SAID, M. M.; SHAHEIN, N. M.; EL-DIN, H. M. F.; FARRAG, A. Functional yoghurt supplemented with extract orange peel encapsulated using coacervation technique. **Pakistan Journal of Biological Sciences,** v. 22, n. 5, p. 231-238, 2019. DOI: https://doi.org/10.3923/pjbs.2019.231.238.

FANG, Z.; BHANDARI, B. Encapsulation of polyphenols: a review. **Trends in Food Science & Technology,** v. 21, n. 10, p. 510-523, 2010. DOI: https://doi.org/10.1016/j.tifs.2010.08.003.

GONZÁLEZ-MUÑOZ, A.; QUESILLE-VILLALOBOS, A. M.; FUENTEALBA, C.; SHETTY, K.; RANILLA, L. G. Potential of Chilean native corn (*Zea mays* L.) accessions as natural sources of phenolic antioxidants and in vitro bioactivity for hyperglycemia and hypertension management. **Journal of Agricultural and Food Chemistry,** v. 61, n. 46, p. 10995-11007, 2013. DOI: https://doi.org/10.1021/jf403237p.

GRGIĆ, J.; ŠELO, G.; PLANINIĆ, M.; TIŠMA, M.; BUCIĆ-KOJIĆ, A. Role of the encapsulation in bioavailability of phenolic compounds. **Antioxidants**, v. 9, n. 10, 923, 2020. DOI: https://doi.org/10.3390/antiox9100923.

HINESTROZA-CÓRDOBA, L. I.; SERNA, S. D.; SEGUÍ, L.; BARRERA, C.; BETORET, L. Characterization of powdered lulo (*Solanum quitoense)* bagasse as a functional food ingredient. **Foods,** v. 9, n, 6, 723, 2020. DOI: https://doi.org/10.3390/foods9060723.

HUSSAIN, S. A.; HAMEED, A.; NAZIR, Y.; NAZ, T.; WU, Y.; SULERIA, H. A. R.; SONG, Y. Microencapsulation and the characterization of polyherbal formulation (PHF) rich in natural polyphenolic compounds. **Nutrients,** v. 10, n. 7, 843, 2018. DOI: https://doi.org/10.3390/nu10070843.

INDRAWATI, R.; SUKOWIJOYO, H.; INDRIATMOKO; WIJAYANTI, R. D. E.; LIMANTARA, L. Encapsulation of brown seaweed pigment by freeze drying: characterization and its stability during storage. **Procedia Chemistry**, v. 14, p. 353-360, 2015. DOI: https://doi.org/10.1016/j.proche.2015.03.048.

JAMBRAK, A. R.; MASON, T. J.; LELAS, V.; PANIWNYK, L.; HERCEG, Z. Effect of ultrasound treatment on particle size and molecular weight of whey proteins. **Journal of Food Engineering**, v. 121, p. 15-23, 2014. DOI: https://doi.org/10.1016/j.jfoodeng.2013.08.012.

KUCK, L. S.; NOREÑA, C. P. Z. Microencapsulation of grape (*Vitis labrusca* var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating

agents. **Food Chemistry,** v. 194, p. 569-576, 2016. DOI: https://doi.org/10.1016/j.foodchem.2015.08.066.

LARRAURI, J. A.; RUPÉREZ, P.; SAURA-CALIXTO, F. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. **Journal of Agricultural and Food Chemistry**, v. 45, n. 4, p. 1390-1393, 1997. DOI: https://doi.org/10.1021/jf960282f.

LOURENÇO, S. C.; MOLDÃO-MARTINS, M.; ALVES, V. D. Microencapsulation of pineapple peel extract by spray drying using maltodextrin, inulin, and Arabic gum as wall matrices. **Foods,** v. 9, n. 6, 718, 2020. DOI: https://doi.org/10.3390/FOODS9060718.

MONTGOMERY, D. C.; RUNGER, G. C. **Estatística aplicada e probabilidade para engenheiros**. 7. ed. Barueri: LTC, 2021, 416p.

RAMÍREZ, M. J.; GIRALDO, G. I.; ORREGO, C. E. Modeling and stability of polyphenol in spray-dried and freeze-dried fruit encapsulates. **Powder Technology**, v. 277, p. 89-96, 2015. DOI: https://doi.org/10.1016/j.powtec.2015.02.060.

REZENDE, Y. R. R. S.; NOGUEIRA, J. P.; NARAIN, N. Microencapsulation of extracts of bioactive compounds obtained from acerola (*Malpighia emarginata* DC) pulp and residue by spray and freeze drying: chemical, morphological and chemometric characterization. **Food Chemistry**, v. 254, p. 281-291, 2018. DOI: https://doi.org/10.1016/j.foodchem.2018.02.026.

ROBERT, P.; GORENA, T.; ROMERO, N.; SEPULVEDA, E.; CHAVEZ, J.; SAENZ, C. Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying. **International Journal of Food Science and Technology**, v. 45, n. 7, p. 1386-1394, 2010. DOI: https://doi.org/10.1111/j.1365-2621.2010.02270.x.

ROESLER, R.; MALTA, L. G.; CARRASCO, L. C.; HOLANDA, R. B.; SOUSA, C. A. S.; PASTORE, G. M. Atividade antioxidante de frutas do cerrado. **Food Science and Technology,** v. 27, n. 1, p. 53-60, 2007. DOI: https://doi.org/10.1590/S0101-20612007000100010. In Portuguese.

RUFINO, M. S. M.; ALVES, R. E.; BRITO, E. S.; MORAIS, S. M.; SAMPAIO, C. G.; PÉREZ-JIMÉNEZ, J.; SAURA-CALIXTO, F. D. Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. **Comunicado Técnico 127, Embrapa Agroindústria Tropical**, Fortaleza, p. 1-4, julho, 2007a. Available at: https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPAT/10224/1/Cot_127.pdf. Acessed on: 9 dec 2023.

RUFINO, M. S. M.; ALVES, R. E.; BRITO, E. S.; MORAIS, S. M.; SAMPAIO, C. G.; PÉREZ-JIMÉNEZ, J.; SAURA-CALIXTO, F. D. Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre ABTS^{+}. **Comunicado técnico 128, Embrapa Agroindústria Tropical.** Fortaleza, p. 1-4, julho, 2007b Available at: https://ainfo.cnptia.embrapa.br/digital/bitstream/CNPAT/10225/1/Cot_128.pdf. Acessed on: 9 dec 2023.

SÁNCHEZ-MADRIGAL, M. Á.; QUINTERO-RAMOS, A.; AMAYA-GUERRA, C. A.; MELÉNDEZ-PIZARRO, C. O.; CASTILLO-HERNÁNDEZ, S. L.; AGUILERA-GONZÁLEZ, C. J. Effect of agave fructans as carrier on the encapsulation of blue corn anthocyanins by spray drying. **Foods,** v. 8, n. 7, 268, 2019. DOI: https://doi.org/10.3390/foods8070268.

SANTOS, F. H.; SILVEIRA, B. M. P.; SOUZA, L. L.; DUARTE, A. K. C.; RIBEIRO, M. C.; PEREIRA, K. C.; COSTA, J. M. G. Influence of wall materials on the microencapsulation of pequi oil

by spray drying. **Brazilian Journal of Food Technology,** v. 23, 2020. DOI: https://doi.org/10.1590/1981-6723.13219.

SCHAEFER, S. V.; AMARAL, A. M. P.; CHEROBIN, A. K.; MONTEIRO, L. K.; MORANDIN, G. C.; FISCHER, C.; CAVALHEIRO, D.; SEHN, G. A. R. Japanese grape (*Hovenia dulcis*) powder as an antioxidant agent in Bologna sausages. **Journal of the Science of Food and Agriculture,** v. 102, n. 14, p. 6255-6262, 2022. DOI: https://doi.org/10.1002/jsfa.11974.

SOUZA, L. S.; MADALENA, D. A.; PINHEIRO, A. C.; TEIXEIRA, J. A.; VICENTE, A. A.; RAMOS, O. L. Micro- and nano bio-based delivery systems for food applications: *in vitro* behavior. **Advances in Colloid and Interface Science**, v. 243, p. 23-45, 2017. DOI: https://doi.org/10.1016/j.cis.2017.02.010.

WANG, L.; CLARDY, A.; HUI, D.; GAO, A.; WU. Y. Antioxidant and antidiabetic properties of Chinese and Indian bitter melons (*Momordica charantia* L.). **Food Bioscience**, v. 29, p. 73-80, 2019. DOI: https://doi.org/10.1016/j.fbio.2019.03.010.

YADAV, K.; BAJAJ, R. K.; MANDAL, S.; MANN, B. Encapsulation of grape seed extract phenolics using whey protein concentrate, maltodextrin and gum arabica blends. **Journal of Food Science and Technology**, v. 57, n. 2, p. 426-434, 2020. DOI: https://doi.org/10.1007/s13197-019-04070-4.