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Propolis microencapsulation by complex coacervation using whey protein and gum arabic: An approach to the assessment of the stability and controlled release of phenolic compounds

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Abstract

The aim of this study was to microencapsulate the propolis extract via complex coacervation using whey proteins and gum arabic, followed by oven drying. It assessed encapsulation efficiency, yield, morphology, and physicochemical properties of the microparticles, including moisture content, water activity, bulk density, hygroscopicity, and dispersibility. Antioxidant activity was determined in both free and encapsulated extracts, and the stability of bioactive compounds was evaluated over 90 days at −75 and 25 °C. The release profile of phenolic compounds was assessed in acidic and neutral media. The encapsulation process showed high efficiency (80.3%) and yield (74.3%), producing microcapsules with an average diameter of 3.14 µm, 6.7% moisture content, 0.30 water activity, 0.56 mg/mL bulk density, 3.2% hygroscopicity, and 25% water dispersibility. Microencapsulation effectively maintained the antioxidant activity of propolis extract. The stability of the bioactive compounds was influenced by temperature, with a more pronounced reduction in flavonoid content (86%) at room temperature and better retention of phenolic compounds (15%) at −75 °C. The release profile of phenolic compounds was similar in acidic and neutral media, with higher retention (62%) in the neutral medium. These findings highlight the potential of encapsulated propolis extract as a food additive.

Keywords: bioactive compounds; antioxidant activity; complex coacervate; encapsulation; hydrocolloid; functional ingredient.

Practical Application: Oven-dried propolis microcapsules preserve bioactive compounds and functionality.

1 INTRODUCTION

Propolis is a natural resinous product produced by honey bees from plant sources and mixed with bees' salivary enzymes, wax, and pollen (Balasubramaniam et al., 2025; El-Sakhawy et al., 2024; Zheng et al., 2017) and is available worldwide. Its chemical composition is complex and variable, depending mainly on the proximity of specific plant sources available for bee feeding, geographic location, and the season of collection (Jansen-Alves et al., 2023). Propolis is recognized as a source of bioactive compounds and a potential substance for the development of natural and functional additives. Regular consumption of propolis may contribute to a reduced risk of developing different pathologies due to its antimicrobial, anti-inflammatory, wound-healing, anesthetic, anticarcinogenic, antiviral, antioxidant, and phytotoxic properties (Martinotti & Ranzato, 2015; Nani et al., 2020). Despite the potential use of propolis in the food industry, its intense characteristic odor and bitter taste pose major challenges, as they adversely affect the sensory qualities of the final product. Additionally, raw propolis is not easily soluble in water due to the presence of hydrophobic compounds (Mendez-Pfeiffer et al., 2021), making it difficult to incorporate into food products directly (El-Sakhawy et al., 2024).

An alternative method that may be used for masking undesirable flavors and aromas is encapsulation, which involves creating an external membrane or coating around an active material (Saifullah et al., 2019). Encapsulation also helps protect bioactive compounds, such as phenolic compounds and flavonoids found in propolis, from degradation by creating a physical barrier against external factors, such as light and oxygen (Mendez-Pfeiffer et al., 2021). This process can promote greater stability of bioactive compounds, maintaining their functional and biological properties, enhancing solubility, and minimizing undesirable sensory characteristics of propolis (Jansen-Alves et al., 2023).

Nowadays, there are numerous techniques and approaches available for the production of microcapsules, such as spray drying (a physical method) (Wang et al., 2024), interfacial polymerization (a chemical method) (Wang et al., 2023), and complex coacervation (a physicochemical method) (Djihad et al., 2024). This last technique has proven to be an interesting option in preventing and reducing the degradation of target molecules, as well as masking unpleasant tastes and smells, thereby enhancing the sensory appeal of the product to which the compound is added (Souza et al., 2020; Yavuz-Düzgün et al., 2020).

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As well as the choice of the encapsulation method used, selecting the appropriate wall material to cover or create an external membrane over the target material is an important factor in microencapsulation and in the protection of food industry products. This is because it must provide adequate properties and be compatible with the matrix to which it will be applied (Nascimento Filho et al., 2022). In the literature, various coating materials have been reported for the microencapsulation of different components (Choudhury et al., 2021; Li et al., 2023). Among them, polymeric pairs of proteins and gums have shown promising results in encapsulating various compounds through complex coacervation (Carpentier et al., 2022; Meiguni et al., 2023; Zhao et al., 2022).

Two-phase systems formed from the interactions between proteins and polysaccharides in aqueous media can create coacervates with functional properties suitable for food systems. The present study aimed to assess the potential of complex coacervation using whey protein (WP) and gum arabic (GA) as wall materials, combined with oven drying, for the encapsulation of the propolis extract. Additionally, the study evaluated the properties of the resulting microcapsules, including their antioxidant activity, controlled release behavior, and the stability of the bioactive compounds.

1.1 Relevance of the work

This study investigated the encapsulation of a propolis extract by complex coacervation using whey protein and gum arabic, followed by oven drying. The obtained capsules were characterized in terms of physical properties, antioxidant activity, compound stability, and release behavior in different media, aiming for their application as a natural food additive. Results showed an efficient encapsulation with preserved antioxidant activity, highlighting oven drying as a simpler and more economical alternative to lyophilization or spray drying. The process yielded capsules rich in bioactive compounds with promising characteristics for food applications, supporting the incorporation of natural and functional additives in the food industry.

2 MATERIAL AND METHODS

2.1 Material

Raw propolis was collected in the city of Bocaiuva (17°6'55" S and 43°49'16" W geographic coordinates), Brazil (SisGEn registration number: A8A85D9). This region is surrounded by a beekeeping pasture containing *Baccharis dracunculifolia*, commonly known as "field rosemary," which is considered the primary botanical source of green propolis, enabling its presence in the sample of propolis used in the present study. The crude propolis was stored in polyethylene bags and kept at −18 °C until the production of the concentrated propolis extract (CPE), which was used as the core (or active material). WP concentrate (Gemacon Tech, Brazil) and GA (Exodus Científica, Brazil) were used as the wall material. All chemicals and reagents used in this study were of analytical grade.

2.2 Propolis extract production

Propolis ethanolic extract (PEE) was obtained according to the methodology described by Nori et al. (2011), with modifications. A 10 g aliquot of previously crushed propolis was mixed with 200 mL of ethanol at 80% (v/v) in bottles protected from light. The extraction was carried out at 50 °C with mechanical stirring at 200 rpm for 30 min using a shaker incubator (6430B, Thoth Equipamentos, Brazil), followed by centrifugation at 3,000 rpm for 20 min (ST16-R, Thermo Scientífica, Brazil). The supernatant was collected and concentrated at 50 °C under reduced pressure using a rotary evaporator (LGI-52CS-1, LGI Scientific, Brazil) coupled to a vacuum pump and thermostatic bath. Afterward, the extract was placed in a BOD (biochemical oxygen demand) incubator (SL-102, SOLAB, Brazil) at 50 °C for complete ethanol evaporation. The resulting CPE was stored in closed, light-protected bottles at −18 °C until use.

2.3 Preparation of coacervates

WP and GA were used in the encapsulation process at a proportion of 3:1 (in mass) protein-polysaccharide, with a total concentration of biopolymers (wall materials) of 2% (w/v). Solutions of 3% (w/v) WP and 1% (w/v) GA were separately prepared by dispersing each biopolymer powder into 50 mL of distilled water. The WP solution's pH was adjusted to 7.5, and 0.63 g of CPE was added. Afterward, the solutions were gently stirred at room temperature (25 ± 1 °C) for 1 h to allow complete hydration of macromolecules. Then, 50 mL of GA solution was slowly added to 50 mL of WP solution, under stirring at 6,000 rpm for 2 min (IKA T18 digital, Ultra Turrax, Germany). HCl (1 mol/L) was used to acidify the solution to the desired pH of 4.0, and the mixture was held at room temperature under a stirring rate of ~ 400 rpm for 30 min. Finally, the material was cooled in the refrigerator and kept at rest overnight to promote sedimentation. The supernatant was carefully removed, and the phase containing the coacervate was dried in an oven (SL-102, SOLAB, Brazil) at 50 °C for 24 h. The dried material was macerated using a mortar and pestle, and the resulting WP-GA coacervate powder, was collected for further analysis.

2.4 Disruption of the microcapsules

For the evaluation of antioxidant activity, total flavonoid content (TFC), and total phenolic compounds, the disruption of the microcapsules was carried out following the methodology described by Nori et al. (2011), with slight modifications. To 0.2 g of microcapsules, 2.0 mL of 10% (w/v) sodium citrate was added. The pH was adjusted to 7.5 using a 0.1 mol/L NaOH solution, and the mixture was homogenized by vortexing (Warmnest, Brazil) for about 2 min. Subsequently, 5 mL of 99.5% (v/v) ethanol was added, and the mixture was homogenized for an additional 2 min. The resulting mixture was then centrifuged at 4,000 × *g* for 20 min. The supernatant obtained from this process was used for the analyses mentioned above.

2.5 Microencapsulation yield and efficiency

The encapsulation yield *(EY)* was expressed as the ratio between the amount of capsules obtained after drying and the amount of polymers and core material used in the process (Šturm et al., 2019). According to Equation 1, to measure the encapsulation efficiency (*EE*), 2.0 mL of 99.5% ethanol was added to 0.2 g of propolis extract capsules to dissolve the phenolic compounds on the surface of the microcapsules without

disrupting them. The mixture was homogenized by vortexing and centrifuged at 4,000 × *g* for 2 min. The *EE* was determined based on the total phenolic compounds' content (section 2.5.4) and calculated using the following Equation 2.

$$EY\,(\%) = \frac{weight\ of\ microcapsules\ obtained\ after\ drying}{propolis\ weight\ +\ weight\ of\ the\ encapsulating\ material} \times 100 \quad (1)$$

$$EE\,(\%) = \frac{W_2 - W_1}{W_2} \times 100 \quad (2)$$

where W_1 is the amount of total phenolics present in the supernatant of a known quantity of microcapsules and W_2 is the amount of total phenolics used to prepare the same quantity of microcapsules (Nori et al., 2011).

2.6 Evaluation of bioactive compounds

2.6.1 Total phenolic content determination

The propolis extract and the propolis extract microcapsules (PEM) (after disruption of the capsules, section 2.4) were submitted to total phenolic content (TPC) determination by the Folin-Ciocalteau colorimetric method (Waterhouse, 2002). The previously diluted sample (0.5 mL) was homogenized by vortexing with 2.5 mL of Folin-Ciocalteau reagent (1:10 v/v ratio of reagent to distilled water) and 2 mL of 4% (w/v) sodium carbonate for 1 min. The reaction mixture was incubated in the dark at room temperature for 2 h, and its absorbance was measured spectrophotometrically (Shimadzu UV-1280, Japan) at 720 nm. A calibration curve was prepared using gallic acid solutions (20–160 μg/mL). Ethanol (99.5%, v/v) was used instead of the sample to determine the blank. The results were expressed as mg of gallic acid equivalent per g of sample (mg GAE/g).

2.6.2 Total flavonoid content determination

A colorimetric assay was used to quantify the TFC in the PEM (after disruption of the capsules, as described in section 2.4). Aliquots of 0.5 mL of previously diluted sample were mixed with 1.5 mL of 99.5% (v/v) ethanol. Then, 0.1 mL of 10% (w/v) aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water were added to the tubes. The mixture was homogenized by vortexing, and its absorbance was taken at 415 nm after 30 min of incubation at room temperature in the dark to complete the reaction (Bonvehí & Gutiérrez, 2011). A calibration curve was prepared using quercetin solutions (0–200 μg/mL). The aluminum chloride aliquot was replaced by the same amount of distilled water for blank determination. The TFC was expressed as mg of quercetin equivalent per g of sample (mg QE/g).

2.7 Physicochemical characterization of propolis extract microcapsules

2.7.1 Physical properties

The morphology of microcapsules before the drying step was evaluated using light microscopy (Axio Lab. A1, Carl Zeiss, Germany) at 400 × magnification. A 10 μL aliquot of the sample was placed on a slide and examined. Photographs of microcapsules were taken using ZEN Blue software. Particle size was measured with ImageJ software (version 1.53 m, National Institutes of Health, USA). The average diameter was determined from at least 200 representative measurements of the particle diameter in the image.

The moisture content of the samples was determined according to the gravimetric method (Association of Official Analytical Chemists [AOAC], 1995). Briefly, 1.00 g of the sample ($n = 5$) was dried at 105 °C until a constant weight was achieved. The moisture content was expressed as a percentage. The water activity value of the sample ($n = 5$) was determined using a water activity analyzer (Aqualab 4TE, USA) at room temperature.

The bulk density (ρ) of the propolis-encapsulated powders was determined by measuring the samples' weight and the corresponding volume. Aliquots of approximately 2 g of the sample ($n = 5$) were transferred into a 10-mL graduated cylinder (Fernandes et al., 2014), and ρ was calculated by dividing the mass of the powder by the volume it occupied in the cylinder.

Hygroscopicity was determined according to the method described by Saikia et al. (2015). One gram of sample ($n = 5$) was placed in Petri dishes and then positioned inside a desiccator containing a saturated solution of sodium chloride (75% relative humidity, 25 °C). After 6 days, hygroscopicity was evaluated by weighing the final mass of the sample. It was expressed as a percentage, calculated as the ratio between the mass of water absorbed and the initial mass of the dried sample.

The determination of water dispersibility (WD) followed the procedure described by Busch et al. (2017) with slight modifications. Approximately 0.5 g of sample ($n = 5$) was taken and dissolved in 50 mL of distilled water. The suspension was vortexed for 5 min, and centrifuged at 1,200 × *g* for 10 min. Then, an aliquot of 20 mL of supernatant was withdrawn and dried at 105 °C for 3 h. The WD was determined using the Equation 3:

$$WD(\%) = \frac{mass\ of\ dry\ matter\ (powder\ after\ supernatant\ drying) \times 2.5}{initial\ mass\ of\ sample\ (g)} \times 100 \quad (3)$$

2.7.2 Antioxidant activity by 1,2-diphenyl-2-picrylhydrazyl assay

The antioxidant activity of the propolis extract and the PEM (after disruption of the capsules, section 2.4) was measured using the 1,2-diphenyl-2-picrylhydrazyl (DPPH) method as described by Zainal et al. (2022), with slight modifications. Aliquots of 4 mL of sample (propolis extract or supernatant obtained from the rupture of microparticles (section 2.4), diluted in methanol to final concentrations of 0.6, 1.5, and 3.0 mg/mL) were transferred to test tubes containing 1 mL of 0.5 mmol/L DPPH solution and homogenized using a vortex for 2 min at room temperature and protected from light. The mixture was incubated in the dark at room temperature for 20 min. The absorbance was measured at 517 nm using a spectrophotometer (Shimadzu UV-1280, Japan). Replacement of the sample with 50% (v/v) ethanol was used as the control. All analyses were performed in triplicate. The percentage of inhibition (% inhibition) of the samples was calculated using the Equation 4:

$$\% \, inhibition = \frac{blank \, absorbance - sample \, absorbance}{blank \, absorbance} \times 100 \quad (4)$$

2.7.3 Stability of propolis extract capsules during storage

Dried PEM were stored in closed, Eppendorf-type tubes, protected from light, and kept at −75 and 25 °C for 90 days. Samples (approximately 0.2 g) were analyzed for TPC and TFC after 1, 8, 15, 30, 45, 60, and 90 days using the methods described in sections 2.6.1 and 2.6.2, respectively.

2.7.4 Release of bioactive compounds from capsules

The release of phenolic compounds from propolis extract capsules was evaluated in different media. For this, suspensions containing 200 mg of propolis extract capsules and 25 mL of medium were prepared and maintained under magnetic stirring. Aliquots of 1.0 mL were collected over time (0, 15, 30, 60, 120, 180, 240, 300, and 360 min) and immediately centrifuged at 1,200 × *g* for 10 min. The supernatant was collected for analysis of TPC (section 2.6.1), while the sediment was resuspended in 1.0 mL of fresh medium and returned to the system to maintain the volume and quantity of capsules constant. The release of phenolic compounds from capsules was evaluated separately in acidic (1% (v/v) acetic acid solution, pH 2.7 at 37 °C) and neutral media (0.1 mol/L phosphate buffer, pH 7.1 at 25 °C).

2.8 Statistical analysis

All treatments and assays were carried out at least in triplicate, and results were presented as mean ± standard error (SE). The analysis of variance (ANOVA) was performed, and the Tukey test was used to evaluate significant differences at $p < .05$. The analyses were performed using Statistica software version 10.0 (StatSoft, USA).

3 RESULTS AND DISCUSSION

3.1 Encapsulation efficiency and yield

Efficiency and yield are important parameters in the development of the encapsulation process, regardless of the method or the encapsulated substance. Relatively high values were obtained for the microencapsulation of propolis extract by complex coacervation between WP and GA: *EE* = 80.3 (± 0.2)% and *EY* = 74.3 (± 1.1)%. The present study achieved better *EE* compared to Nori et al. (2011), who reported 66–72%, but lower than Sukri et al. (2023), who achieved 88.27–91.86% efficiency when encapsulating propolis extract using different coating materials. In contrast, the observed propolis microcapsule yield was higher than that reported by these latter authors. It is worth noting that *EE* depends on the temperature, ionic strength, and pH of the reaction medium; the polymers' mixing ratio; their molecular weight; total concentration; rate of stirring; and charge densities (Prata & Grosso, 2015; Sukri et al., 2023; Timilsena et al., 2019). Additionally, variations in the properties of bioactives, such as their initial amounts, potential interactions, methods of entrapment, and localization within the carrier, may generate greater or lesser release of the entrapped compounds (Boostani & Jafari, 2021), due to differences in the stimuli responsible for the release.

3.2 Characterization of microcapsules

The PEM obtained exhibited a relatively spherical form with varied sizes before the drying step (Figure 1), similar to those reported by other researchers who also studied propolis encapsulation (Andrade et al., 2018; Silva et al., 2013). The mean particle size was 3.14 ± 0.42 μm. This value was slightly higher than those observed by Šturm et al. (2019), who reported mean particle sizes between 0.6 and 3.0 μm, with the majority around 1 μm, using different measurement methods (light microscopy, scanning electron microscope, and dynamic light scattering). These authors also reported the difficulty in making comparisons regarding particle sizes with other studies due to the different types of core substances, coating materials, mesh sizes, encapsulation settings, machines, and techniques used. The recommended particle size for application in food products should be less than 100 μm to avoid sensory perception in the mouth (Kaushik et al., 2015). According to Jansen-Alves et al. (2024), large microcapsules are likely to trap a greater amount of propolis extract, which may affect the sensory perception of taste.

The moisture content of the PEM was 6.7 ± 0.3%. This result is consistent with findings from other studies. Sukri et al. (2023) reported moisture content of 4.55–5.94% for propolis microcapsules prepared with gelatin and sodium alginate via the complex coacervation method. Similarly, values between 5.28 and 7.33% were reported in the microencapsulation of propolis and honey using mixtures of maltodextrin/tara gum and modified native potato starch/tara gum (Ligarda-Samanez et al., 2023). It is important to note that low moisture content is essential for ensuring the stability of dry products. The conditions under which the product is obtained, as well as its composition, can influence the final moisture content. Typically, foods with moisture levels ranging from 3 to 10% can be stored effectively without undergoing substantial alterations (Silva et al., 2013).

The water activity of the microcapsules was 0.30 ± 0.02, consistent with values reported in the literature for microcapsules of various types of propolis obtained through different encapsulation methods and using diverse wall materials (Andrade et al., 2018; Baysan et al., 2021; Jansen-Alves et al., 2018). Water activity is related to the availability of free water in a food product, which drives ongoing biochemical reactions. Values below 0.3 are desirable for food materials, as they indicate a

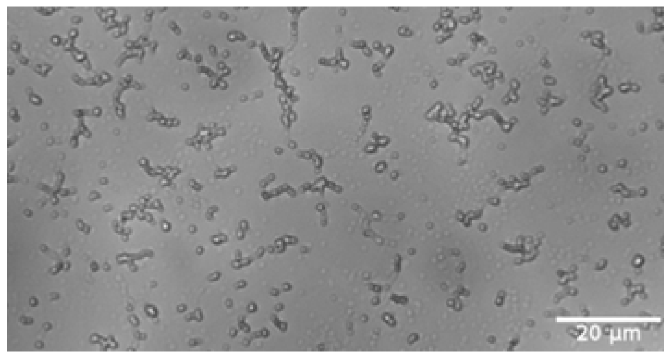


Figure 1. Image from light microscopy (magnification of × 400) of propolis extract microcapsules produced by complex coacervation of whey protein and gum arabic before the drying step.

lower amount of free water available for microbial growth and biochemical reactions (Damodaran & Parkin, 2018).

The bulk density of the PEM was 0.56 ± 0.01 g/mL. This result falls within the typical range of 0.3–0.8 g/mL for food powders (Barbosa-Cánovas & Juliano, 2005) and aligns with the values reported by Pant et al. (2022), which range from 0.414 to 0.612 g/mL for developed encapsulated propolis powders. It is important to note that bulk density is a critical parameter for handling and storing powdered products. Lower-density materials require more storage space, which can lead to increased storage costs as well as higher expenses related to packaging, handling, and transport. According to Koç et al. (2017), lower bulk density in food products is associated with greater susceptibility to oxidation processes and, consequently, reduced storage stability due to the presence of more air between the voids.

The hygroscopicity of a material refers to its capacity to absorb humidity present in the environment, making it a crucial parameter in determining the shelf life and appropriate packaging (Vardanega et al., 2019). Additionally, the presence of humidity in powdered products can impact their flowability. High hygroscopicity values can lead to powder agglomeration or increased stickiness, making it more challenging to disperse and handle the material. The microparticles obtained in the present study can be classified as non-hygroscopic (< 10%) (Pisecký, 2012). The hygroscopicity of the microcapsules produced (3.2 ± 0.1%) was lower than the values reported by Andrade et al. (2018) (6.69 ± 0.42%), Pant et al. (2022) (9.12–15.41%), Sukri et al. (2023) (7.96–10.23%), and Laureanti et al. (2023) (10.93–16.43%) for other microencapsulated propolis.

Water dispersibility refers to the ability of a powder to distribute evenly in water, determining its capacity to separate into distinct particles upon dissolution. The propolis microcapsules produced showed low WD (25.04 ± 0.02%) as compared to the results reported by Pant et al. (2022) and Šturm et al. (2019). The dispersibility of the microcapsules was expected to be higher, given that the wall materials have good water solubility. However, it is important to note that the propolis used in this study did not undergo a de-waxing process to remove waxes, which may have contributed to the product's low dispersibility.

3.3 Antioxidant activity

The antioxidant activity of the CPE and the PEM was assessed using the DPPH assay, and the results are shown in Table 1. All samples demonstrated some level of antioxidant activity, which can be attributed to the phenolic compounds present in propolis. The results revealed that the antioxidant activity value showed an increasing trend as the propolis concentration increased for all samples, with the CPE at 3.0 mg/mL exhibiting the highest antioxidant activity (88.40%). However, the complex coacervation process seemed to slightly diminish the antioxidant compounds, as evidenced by the reduction in inhibition percentage (< 22%) compared to the CPE used in the encapsulation process ($p < .05$) (Table 1). Nonetheless, the encapsulation process, including drying the capsules at a low temperature (50 °C), had a minimal detrimental effect on the DPPH radical scavenging activity, as high antioxidant activity was maintained in the produced capsules.

3.4 Stability

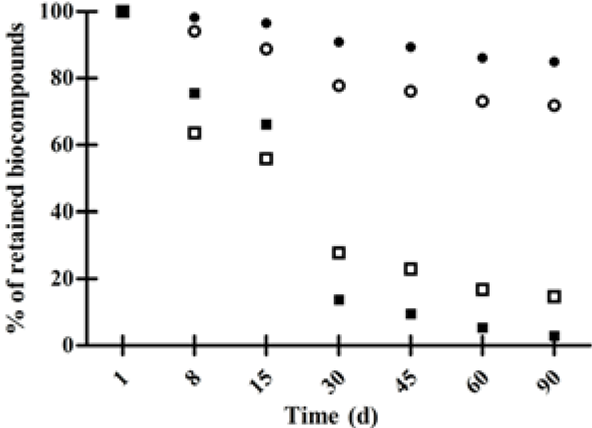
ANOVA results for the bioactive compounds (TPC and TFC) in microencapsulated propolis showed that the individual effects of storage variables (temperature and time) as well as their interaction on the phytochemical characteristics were highly significant ($p < .001$). The storage time had the greatest impact on the compounds, accounting for 54.31 and 92.64% of the total variance for TPC and TFC, respectively (data not shown).

For encapsulated bioactive compounds to exert their functionality in specific applications, they must exhibit stability throughout storage. The stability evaluation of the bioactive compounds (TPC and TFC) in the propolis extract capsules was carried out over 90 days (Figure 2). The results revealed that the TPC decreased by around 28% in capsules stored at 25 °C (21.8–15.7 mg GAE/g); however, the reduction was only 15% when stored at −75 °C (22.9–19.5 mg GAE/g). The amount of TFC in the microcapsules stored showed quick initial degradation. With 30 days of storage at −75 °C and 25 °C, TFC degradation reached 72 and 86%, respectively. Subsequently, there was a constant loss of content or degradation of the flavonoid compounds. Finally, after 90 days of storage, TFC losses were greater than 85%. In the current research, Sá et al. (2023) reported a similar reduction in

Table 1. Evaluation of the antioxidant activity of concentrated propolis extract and propolis extract microcapsules using the 1,2-diphenyl-2-picrylhydrazyl method.

Concentration (mg/mL)	Sample	Antioxidant activity* (% inhibition)
0.6	CPE	17.97 ± 0.55^{aB}
	PEM	14.00 ± 0.55^{bB}
3.0	CPE	88.40 ± 0.36^{aA}
	PEM	77.01 ± 0.58^{cA}

*Means followed by different lowercase letters for the same concentration differ statistically from each other according to the Tukey test ($p \leq .05$). Means followed by different uppercase letters for the same sample differ statistically from each other according to the Tukey test ($p \leq .05$). CPE: concentrated propolis extract; PEM: propolis extract microcapsules.



The standard errors ranged from 0.11 to 0.44 and from 0.09 to 0.78, respectively, for total phenolic content and total flavonoid content.

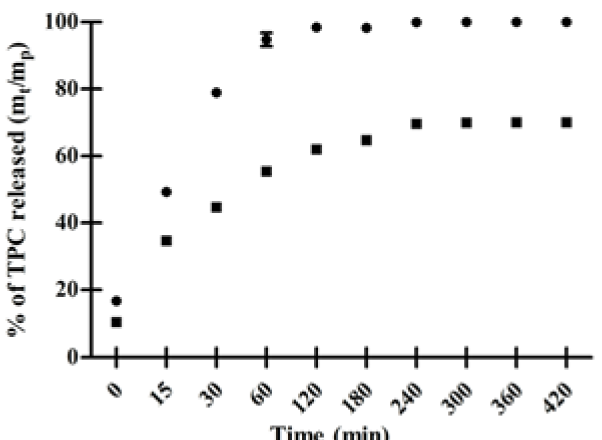
Figure 2. Percentage of total phenolic content (circle) and total flavonoid content (square) retained in propolis extract microcapsules stored at −75 °C (black) and 25 °C (white) during 90 days.

the content of flavonoids (96%) after 60 days of storage at 25 °C, but a higher degradation of the phenolic compounds (92%) than that observed in the present study. The stability of encapsulated compounds may be linked to higher *EE* (Wang et al., 2021). The initial low retention values obtained, mainly in TFC, may be attributed to the degradation of molecules present on the surface, which are more susceptible to interaction with degradative agents such as oxygen and humidity (Souza et al., 2020). For both bioactive compounds, degradation was more pronounced at the higher temperature, consistent with the findings of Nori et al. (2011), whose propolis microcapsules were stored in glass vials, sealed under vacuum. This result was expected, as higher temperatures typically accelerate certain reactions. In general, the results suggest that the encapsulation provided some protection for the phenolic compounds during storage at room temperature and under freezing conditions over a period of 3 months.

3.5 Release of phenolic compounds from capsules

Propolis extract microcapsules exhibited a similar release profile of phenolic compounds in the tested media (Figure 3). The release behavior of TPC from capsules is consistent with the typical release profile of active compounds from polymeric capsules, which usually occurs in two phases: first, an initial burst release, likely due to the rapid diffusion of compounds attached to or near the surface of the particles; and subsequently, a much slower but constant release until equilibrium is reached.

A higher release of TPC was observed in acidic media (~ 55%) compared to neutral media (~ 38%). Additionally, the release rate of active compounds from capsules in the acidic medium was faster than in the neutral medium. The microcapsules tended to reach a maximum release within 120 min, with minimal variation in the compounds released thereafter. In a neutral medium, it took twice as long to reach a plateau in TPC release (Figure 3).



m_t refers to the mass of total phenolics released at time *t* and m_p represents the mass of total phenolics present in the microcapsules in the medium. The error bar represents the standard error, which was less than 1.1%.

Figure 3. Release profiles of phenolic compounds from propolis extract microparticles in (●) acid (1% v/v acetic acid, pH 2.7) and (■) neutral (0.2 mol/L phosphate buffer, pH 7.1) media.

Changes in medium pH may cause microcapsule degradation in complex coacervation due to the altered solubility of wall polymers (Choudhury et al., 2021). Zhang et al. (2018) reported a greater release of polyphenols under gastric conditions (simulated gastric fluid containing salts and enzymes) compared to intestinal conditions (simulated intestinal fluid with salts and enzymes) when evaluating the in vitro controlled release from free propolis and propolis encapsulated using zein and zein-carboxymethyl chitosan as wall materials.

Our results were in agreement with a previous study reported by Cruz et al. (2019), who emphasized that understanding the release profile of an active ingredient is crucial for elucidating its behavior and release mechanism, particularly in the context of the food matrices in which it may be applied. The results of the present study suggest that the microencapsulation provided better protection for the bioactive compounds in a neutral environment. Given this information, along with the low WD and the good stability of phenolics during storage, the microcapsules produced could serve as a source of bioactive compounds in solid food products with a near-neutral pH or in the elaboration of active food packages with antioxidant activity.

4 CONCLUSIONS

The findings demonstrate that propolis extract can be effectively microencapsulated through complex coacervation followed by oven drying. This process produced propolis extract capsules with desirable characteristics, including low water activity, low moisture content, typical bulk density for food powders, and a non-hygroscopic nature. Encapsulation effectively preserved the antioxidant activity and minimized the degradation of phenolic compounds during capsule storage at both studied temperatures, unlike the TFC. Regarding the release of bioactive compounds, phenolic compounds exhibited greater retention in a neutral medium compared to acidic conditions. Combined with the relatively low WD and the other observed results, these findings suggest that encapsulated propolis extract has potential as a functional ingredient in solid food products with near-neutral pH. Further studies are recommended to elucidate the bioavailability and release kinetics of the bioactive compounds in various food matrices. In addition, evaluating the sensory attributes and consumer acceptance of food products fortified with encapsulated propolis extract powder will be essential to assess its commercial viability.

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