Food and Bioprocess Technology
https://doi.org/10.1007/s11947-025-03889-4

RESEARCH

Formulation and Evaluation of Propolis-Loaded Alginate Capsules by Ionic Gelation Technique

Seda Özgen[1] · **Müge Hendek Ertop**[2] · **Uğur Ertop**[3]

Received: 21 February 2025 / Accepted: 8 May 2025

Abstract

Propolis can be used as a stand-alone dietary supplement and has the potential to be used as an active ingredient in pharmaceuticals or functional foods because it has been shown to have various health benefits attributed to its phenolic compositions. However, its low solubility in water and strong taste and aroma limits the incorporation in food systems; it is thought that the different encapsulation techniques serve as an effective method to protect bioactive compounds while also helping to mask the taste of propolis and preserve its antioxidant properties. In this study, propolis extracts with final solvents of alcohol, honey, and glycerol were encapsulated using the ionic gelation method with sodium alginate (Na-Alg) at concentrations of 0.5%, 1.5%, and 2% and $CaCl_2$. The nine encapsulated propolis samples' physicochemical, microtextural, and bioactive properties were compared. Since the durability of the capsules until they reach the intestine is crucial, changes in color and bioactivity in digestive fluids after simulated gastric digestion were also assessed. The optical characterization of the capsules revealed that encapsulation with 1% Na-Alg resulted in mechanically weak, deformed, and heterogeneous capsules for all solvent bases. Increasing the Na-Alg concentration led to more uniform and homogeneous capsule structures. When 1.5% and 2% Na-Alg were used, the membranes effectively trapped the core material, producing successful capsules. Among the solvents, honey-based propolis extracts formed the best capsules. None of the samples with different bases and Na-Alg ratios lost their encapsulated form in the simulated gastric fluids. Considering the commercialization potential of the products, the combination of 1.5% honey-based propolis and the Na-Alg ionic gelation coating method is recommended as the most suitable approach.

Keywords Propolis · Encapsulation · Ionic gelation technique · Bioactivity · Stability

Abbreviations

Biological, Chemical and Microbiological

AA	Antioxidant activity
ANOVA	Analysis of variance
DPPH	1,1-Diphenyl-2-picrylhydrazyl
GAE	Gallic acid equivalent
MD	Mineral digestibility
Na-Alg	Sodium alginate
TCA	Trichloroacetic acid
TPC	Total phenolic content

Instrumental techniques

FTIR	Fourier transform infrared spectroscopy
SEM	Scanning electron microscope
UV	Ultraviolet

✉ Müge Hendek Ertop
mugeertop@kastamonu.edu.tr

[1] Faculty of Engineering, Department of Food Engineering, Çankırı Karatekin University, Çankırı, Turkey

[2] Faculty of Engineering and Architecture, Department of Food Engineering, Kastamonu University, Kastamonu, Turkey

[3] Institute of Science, Department of Genetics and Bioengineering, Kastamonu University, Kastamonu, Turkey

Introduction

Propolis, also known as "bee glue," is a sticky substance consisting of resinous, gummy, and balsamic components and waxes, essential oils, and pollen from specific plant species. Bees utilize it as a protective material to defend their hives (Alpat et al., 2023; Irigoiti et al., 2021). It is known that there are different types of propolis, such as poplar, red, green, Pacific, and Mediterranean, depending on the botanical origin and associated components

Published online: 28 May 2025

(El-Guendouz et al., 2019). The composition of propolis is strongly associated with its botanical and geographical origin; however, its overall percentage composition remains almost unchanged. The main components of propolis include resins and balsams (45–55%), waxes (8–35%), essential oils and aromatics (5–10%), pollen (5%), fatty acids (5%), mineral substances, and some other organic compounds (5%), such as polyphenols and terpenoids, in nature (Anjum et al., 2019; Rocha et al., 2023). Overall, more than 300 different bioactive compounds, such as phenolic aldehydes, ketones, and polyphenols (phenolic acids, flavonoids, and esters), have been identified in propolis. The beneficial effects of propolis are largely attributed to its high content of polyphenols (Meto et al., 2020).

Raw propolis cannot be consumed directly due to its wax, resin, herbal balsam content, and bioactive compounds; therefore, it must be extracted appropriately. There are different extraction methods, such as maceration, reflux, ultrasound, or microwave-assisted methods, for the preparation of propolis extracts. Furthermore, propolis extracts are often prepared using ethanol, but other solvents such as water, propylene glycol, chloroform, dimethyl sulfoxide, acetone, and ethyl acetate could also be used (Anjum et al., 2019; Irigoiti et al., 2021; Meto et al., 2020). The propolis extract has been demonstrated to possess an antimicrobial (Dantas Silva et al., 2017), antibiofilm (Mattigatti et al., 2012), anticancer (Kuo et al., 2015), antioxidant (da Silveira et al., 2016; Dantas Silva et al., 2017), and anti-inflammatory activities, as well as its ability to promote wound healing (Rosseto et al., 2017) in various studies. These properties have made propolis a compelling ingredient to meet the growing demand for functional foods. Therefore, producing propolis extracts using different solvents and processing them into various forms, such as powders, capsules, and vials, along with their inclusion in diverse foods like honey and fruit juices to develop functional food forms, has become a prominent topic of interest because the propolis-rich functional foods launched into the market are consumed by large populations to improve health and prevent diseases (Irigoiti et al., 2021). Incorporating propolis into food products poses a significant technological challenge due to its low water solubility and strong, unpleasant taste and odor, which often compromise food acceptability. Furthermore, the expected effects of propolis may be significantly altered during food processing. The antioxidant capacity of propolis depends not only on the individual antioxidant capacity of its components, but also on the microenvironment in which the compounds exist. These compounds interact with each other and may produce synergistic or inhibitory effects. Therefore, innovative technological solutions such as encapsulation are needed to overcome these challenges. Encapsulation technology is a viable method to suppress and reduce the strong odor and bitter taste of propolis, which are unpleasant for many consumers. This technique delays the release of the active ingredient into the oral cavity and prevents electrostatic interaction with chemoreceptors (Einhorn-Stoll et al., 2021), which has the potential to mask and neutralize the bitter effects. This technology could help to include such an active and pungent product in foodstuffs (Shakoury et al., 2022). In this process, bioactive substances such as propolis are encapsulated in coating materials to form nano- or microparticles, thereby improving their stability and bioavailability in food applications (Ray et al., 2016). Spray drying, spray coating, freeze-drying, emulsification, extrusion, and ionic gelation are just a few of the practical encapsulating techniques that are available; each has a unique cost–benefit analysis. Furthermore, the creation of encapsulated systems requires consideration of fundamental elements like the inherent characteristics and stability of the compound to be encapsulated, the characteristics of the encapsulating polymer, and the particular needs of the intended product, all of which promote ongoing innovation and technological advancement. The ionic gelation technique has potential advantages over other encapsulation methods, especially in terms of scalability and commercial potential. The production capacity can be relatively higher than methods that can be produced on a small scale (Jansen-Alveset al., 2019; Keskin et al., 2019; Ray et al., 2016). While the choice of coating method and material is critical in encapsulation, the type of solvent used for extracting the bioactive compound is equally important. However, studies have yet to be found in the literature evaluating together the combinations of solvent type and coating material.

Sodium alginate (NaAlg) has emerged as a viable coating material for encapsulation procedures with significant biodegradability, biocompatibility, and lack of toxicity. NaAlg is widely utilized in pharmaceutical formulations, food, and the chemical industry. It plays a key role in the creation of gels that carry essential biomolecules such as medications, additives, food ingredients, or bioactive compounds such as drugs, peptides, and phenolics, giving it a significant place in research and development (Barboza Duarte Rodrigues et al., 2024).

The aim of the study is to produce robust and stable capsules with high bioactive efficacy that are suitable for use as dietary supplements and functional food additives. To achieve this, combinations of propolis extracts—prepared with final solvents such as ethanol, honey, and glycerin—and coating material sodium alginate (Na-Alg) at varying concentrations were tested. The study evaluated the capsules' properties and their optical characterization, bioactivity, and bioavailability characteristics.

Material and Methods

Chestnut propolis samples (primary pollen content > 45% chestnut) were obtained from the apiaries of the Azdavay, Bozkurt, Cide, Doğanyurt, İnebolu, and Küre districts, which include the borders of the Kastamonu chestnut forest region, during the 2021 harvest period, through the Kastamonu Beekeepers Association. The raw propolis samples were divided into small pieces and kept in a deep freezer at – 18 °C. The samples were subsequently milled into powder and stored at − 18 °C until extraction.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and Folin–Ciocalteu's reagent were obtained from Sigma-Aldrich Co. (Munich, Germany). Ethanol and methanol were supplied by Merck KGaA (Darmstadt, Germany). Pepsin (P7000 ≥ 250 unit/mg) and pancreatin enzymes were purchased from Sigma-Aldrich Co. (Munich, Germany).

Preparation of Propolis Extract

The method used by Şenol Yazkan and Hendek Ertop (2025) was modified and applied according to our study. The mixture containing 20% raw propolis in absolute ethanol was extracted at 37 °C for 72 h with intermittent stirring. After extraction, the products were filtered, and the supernatant parts were separated by centrifugation (Nüve, Germany) at 1200 rpm for 10 min. The "Alcohol-Based Propolis" obtained by this method was stored at − 18 °C until use. The alcohol portion of the ABP was evaporated (Heidolph, Hei-Vap, Germany) at 40 °C under vacuum for approximately 25 min. The remaining portion was mixed with honey in the same amount as the removed alcohol to obtain "Honey-Based Propolis" and with glycerine to obtain "Glycerine-Based Propolis." The final products were homogenized by mixing with an ultraturrax (Ultra-Turrax IKA T25, Staufenim Breisgau, Germany) at 2500 rpm.

Encapsulation of Propolis Extract

Preparation of Sodium Alginate (Na-Alg) Solution

According to our previous experiments and several studies (Rodriguez et al., 2024), the concentration range (1%, 1.5%, and 2%) that we generally experimented with while encapsulating different core materials was also used in this study. To prepare sodium alginate (Na-Alg) solution at concentrations of 1% w/v, 1.5% w/v, and 2% w/v, an appropriate amount of powdered Na-Alg was dissolved in distilled water by stirring at 500 rpm for 2 h. Once fully dissolved, the solution was stirred at a low speed in a water bath for 1 h to remove gas bubbles. Before use, the solution was rested at + 4 °C for 24 h. A 2% calcium chloride solution was used as a crosslinker to prepare alginate capsules, and the calcium chloride concentration was kept constant throughout the experimental studies.

Production of Alginate Membrane Propolis Core Microcapsules

Glycerin, alcohol, and honey-based propolis extracts were used to prepare the emulsion media. The core material concentration was 5% by volume of the coating material. In order to form the stable emulsion, the solution was mixed with Na-Alg and the core material with an ultra turrax mixer at 12,000 rpm for 10 min. After the stable emulsion was formed, it was used in capsule production.

In this study, the inverse gelation method was applied to coat the droplets of the propolis and carrier emulsion with alginate gel, and a 6-channel syringe pump test setup was used to form a capsule. The experiment was performed under room conditions, and the propolis-alginate emulsion was dropped into the gelling solution using a programmable syringe pump. The dropping solution, $CaCl_2$ solution (2%), was continuously stirred with a magnetic stirrer at 500 rpm. A distance of 10 cm was left between the drip tip and the $CaCl_2$ surface so that the droplets could take a spherical shape.

After the encapsulation process was completed and the gels were formed, the mixing process was continued for 30 min in the hardener solution to stabilize the spheres. At the end of the period, the capsules formed were filtered with a laboratory-type strainer and stored in distilled water at + 4 °C until drying. In order to characterize the alginate capsules obtained in this way, different alginate gel concentrations (1, 1.5, and 2%) were studied, and the effects of these parameters on the sizes, shapes, and stabilities of the alginate microcapsules were evaluated. The flow rate of the emulsion from the syringe pump was kept constant at 5 mL/min. The produced capsules (Fig. 1) were dried in a constant temperature oven at 24 °C.

Encapsulation Efficiency

The method used by Jansen-Alves et al. (2018) and Shakoury et al. (2022) was modified and applied according to our study in order to compare all three bases. In order to determine, 5 mg of encapsulated propolis were mixed with 10 mL of phosphate buffer solution (pH 7.4 ve 0.05 M) via an Ultra-Turrax at room temperature for 1 min, and the encapsulated particles were blended. It was centrifuged at 4200 × *g* for 5 min at 25 °C. After phase separation, the absorbance of each supernatant was measured using a UV–VIS spectrophotometer at the wavelengths determined

Fig. 1 Appearance of propolis capsules with different solvent bases and Na-Alg rates (20 ×)

through spectrum scanning. Encapsulated propolis samples prepared with all three solvent bases (Fig. 1) exhibited different colored emulsions, leading to the determination of wavelengths through spectrum scanning. The amount of propolis transferred to the supernatant was compared with the theoretically added propolis amount, and the percentage encapsulation efficiency (EE%) was calculated from the absorbance difference.

Antioxidant Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which relies on the scavenging of free radicals, was used to determine antioxidant activity (Şenol Yazkan & Hendek Ertop, 2025). For the extraction, 2 g of the sample was stirred with 20 mL of 80% methanol solution by a magnetic stirrer at 37 °C for 2 h. The samples were then centrifuged at 10,000 rpm for 15 min, and the supernatant was filtered through filter paper and stored in glass tubes at – 18 °C until analysis. To determine the scavenging activity of phenolic compounds in propolis extracts on the DPPH radical, a mixture of 75 µL of 100-fold diluted extract and 1500 µL of DPPH solution was vortexed for 15 s and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a UV–VIS spectrophotometer, and the inhibition% values were calculated with the following equation;

$$\text{Inhibition\%} = \left[1 - \left(\frac{\text{AbsSample}}{\text{AbsControl}}\right)\right] * 100 \quad (1)$$

Total Phenolic Content (TPC)

The total phenolic content of the samples was determined according to the Folin–Ciocalteu. 0.1 mL of extract was mixed with 0.5 mL of Folin–Ciocalteu reagent (reagent:water mixture 1:10 v/v) and allowed to stand in the dark for 5 min. Subsequently, 0.4 mL of saturated sodium carbonate solution and 4 mL of pure water were added and mixed. The mixture was then left to stand at room temperature in the dark for approximately 1 h, and

the absorbance was measured at 760 nm using a UV–VIS spectrophotometer (Shahidi & Naczk, 1995). The results were calculated using the calibration curve obtained using gallic acid standard solutions and given as gallic acid equivalent (GAE).

Ash Content and In Vitro Mineral Digestibility (MD)

The ash content of the samples was determined using the AACC (2000) approved method (No: 08–01.01). For in vitro mineral digestibility, the sample (1 g) was incubated with 25 mL of pepsin solution (0.03 N 1 L HCl + 2 g pepsin) at 37 °C for 3 h. Each sample was filtered using ashless filter paper. The pellet and filter paper were burned together in the furnace, and the ash value was calculated. The digestible mineral content was obtained from their differences. The MD value (%) was obtained by using the following equation:

$$MD\% = \frac{Digestible\ Mineral\ Content}{Total\ Mineral\ Content} \times 100 \quad (2)$$

In Vitro Release

Under simulated gastrointestinal conditions, the in vitro release behavior of propolis from encapsulated samples based on three types of final solvents (glycerine, honey, and ethanol) was investigated using Mohammadian et al. (2019) and Hayta and Hendek Ertop's (2017) method with some modifications. One gram of encapsulated sample was taken into a borosilicate jar, which was subsequently charged with 25 mL of simulated gastric fluid (SGF) comprising 2 g of enzyme pepsin and 0.03 N 1 L of HCl. The mixture was incubated for three hours at 37 °C and 100 rpm continuous shaking. Each sample was filtered by ashless filter paper. The aliquots of the propolis-released gastric fluid medium were collected. Color intensity, antioxidant activity, and total phenolic content values in the simulated gastric fluid following gastric incubation were used to evaluate the concentration of propolis released from the capsules.

To confirm the release and disintegration behavior of the capsules in the small intestine, the encapsulated propolis samples were subjected to the intestinal digestion procedure following the gastric digestion protocol. A 1-g sample was subjected to gastric in vitro digestion with 15 mL of gastric fluid at 37 °C for 3 h. Neutralized with 7.5 mL of 2 N NaOH, 7.5 mL of phosphate buffer (pH = 8.0) containing 4 mg pancreatin was added and left for intestinal digestion at 37 °C for 24 h. The reaction was terminated with 10 mL of 20% trichloroacetic acid (TCA), and the capsule's deterioration and propolis release were evaluated.

Optical Characterization

Encapsulated propolis samples were analyzed using scanning electron microscopy (SEM) and light microscopy. For SEM analysis, samples stored under the same conditions post-encapsulation were adhered to a sample holder using double-sided tape. They were adhered to a sample holder with double-sided tape, and they were coated with about 135 Å Au/Pd (device coating speed 3 Å s^{-1}) with a SC 7620 mini Sputter Coater, and then, they were examined with the SEM (Aponte et al., 2014). The samples were also viewed under a stereo microscope at 20 × magnification (Fig. 2). Morphological characters were examined using a Zeiss Stemi SV-6 stereomicroscope. Photographs were taken with a Nikon SMZ25 stereomicroscope and a DS-Fi2 camera and were interpreted together with SEM images (Fig. 3).

Characterization by Fourier Transform Infrared Spectrometry (FTIR)

Encapsulated propolis was also characterized by Fourier transform infrared spectroscopy (FTIR-Bruker Tensor II). The spectrum of unloaded capsules which is not containing propolis coated with Na-Alg/$CaCl_2$ cross-linked capsulation was compared with the ATR-FTIR spectra of glycerin, alcohol, and honey-based capsules and was interpreted.

Color and Color Intensity

Color values (L*; brightness, a*; redness, b*; yellowness) were determined colorimetrically (3nh Colorimeter, NR145, China) by measuring from 5 different points on the encapsulated propolis samples and taking the average of the values (Rizzello et al., 2014). The method for determining the net absorbance value of the honey developed by Beratta et al. (2005) was used to measure color intensity with slight modification. According to the method, instead of the methanol/water extracts of samples prepared at 50% (w/v) were used SGF samples, after the gastric digestion procedure, were used and filtered through Whatman No. 1 filter paper, and their absorbance was measured at 450 and 720 nm using a UV–VIS spectrophotometer. The color intensity (mAU) was calculated using the following formula,

$$\text{Color intensity (mAU)} = (ABS_{450} - ABS_{720}) \times 1000 \quad (3)$$

Fig. 2 Light microscopy views of propolis capsules with different solvent bases and Na-Alg rates (20 ×)

Table 1 Some physicochemical and bioactive properties of encapsulated propolis samples

Solvent base	Na-Alg coating rate (%)	Antioxidant activity (% inhibition)	Total phenolic content (µg GAE/g)	Ash (%)	MD** (%)	Density (g/cm^3)	Color		
							*L**	*a**	*b**
Honey	1.0	35.66^{aA}	11650.00^{aA}	8.33^{a}	58.70^{aA}	0.455^{b}	34.92^{aA}	16.01^{bA}	25.21^{aA}
	1.5	33.58^{aA}	10910.67^{aA}	8.47^{a}	55.61^{aA}	0.828^{a}	34.31^{aA}	17.13^{aA}	27.62^{aA}
	2.0	30.35^{bA}	9100.00^{bA}	8.21^{a}	49.57^{bA}	0.862^{a}	34.30^{aA}	16.25^{abA}	28.01^{aA}
p value		0.007	0.005	0.614	0.024	0.002	0.422	0.030	0.470
Ethanol	1.0	23.58^{aB}	9850.00^{aB}	7.37^{a}	56.31^{aA}	0.476^{a}	17.25^{aC}	9.47^{aC}	4.87^{aC}
	1.5	19.22^{abB}	8960.67^{bB}	7.37^{a}	57.80^{aA}	0.608^{a}	17.41^{aC}	8.71^{aC}	4.14^{aC}
	2.0	17.29^{bB}	8850.00^{bB}	7.13^{a}	59.33^{aA}	0.751^{a}	17.97^{aC}	8.65^{aC}	3.76^{aC}
p value		0.027	0.007	0.598	0.250	0.294	0.562	0.089	0.063
Glycerine	1.0	25.21^{aB}	7710.67^{aC}	7.90^{a}	60.76^{aA}	0.441^{b}	31.90^{aB}	13.96^{aB}	17.47^{aB}
	1.5	14.86^{bB}	6730.33^{bC}	7.30^{a}	56.58^{aA}	0.813^{a}	31.27^{aB}	13.73^{aB}	17.26^{aB}
	2.0	11.50^{bB}	6610.67^{bC}	7.21^{a}	51.87^{aA}	0.842^{a}	29.68^{aB}	13.96^{aB}	15.18^{bB}
p value		0.013	0.007	0.602	0.418	0.003	0.259	0.340	0.043

*Lowercase letters within the same column indicate statistically significant differences based on the sodium alginate concentration ($p < 0.05$), while uppercase letters within the same column represent statistically significant differences between groups depending on the extraction base ($p < 0.05$)

***MD*, mineral digestibility

Table 2 Bioactive properties of honey-, alcohol- and glycerin-based propolis extracts

Solvent base	Antioxidan activity (% inhibition)	Total phenolic content (µg GAE/g)
Honey	90.12[a]	16475.00[a]
Ethanol	89.69[a]	11975.00[b]
Glycerine	73.34[b]	9100.00[c]
p value	0.003	0.000

Statistical Analysis

The obtained results were evaluated by Analysis of Variance (ANOVA) (IBM SPSS 17.0.1) for the comparison ($p < 0.05$) of them.

Results and Discussion

Encapsulation Efficiency

Encapsulation efficiency was calculated for capsules with a 1.5% Na-Alg ratio. The encapsulation efficiency (%EE) was an average of 78 ± 1.4 for alcohol-based capsules, 82 ± 2.3 for glycerin-based capsules, and 87 ± 1.8 for honey-based capsules. According to the obtained data, the type of the final solvent base affected the EE%, and the honey base was higher than the others. On the other hand, it was reported that in several researches, some capsulation factors, such as the acidity of the samples or the percentage of propolis loading, affected the EE% (Jansen-Alves et al., 2018; Shakoury et al., 2022).

Characterization of Encapsulated Propolis Samples

Optical Properties

The characterization results of nine encapsulated propolis samples obtained from combinations of three Na-Alg ratios (1%, 1.5%, and 2%) and three solvent bases (honey, alcohol, and glycerin) were presented in Tables 1, 2, and 3, and in Figs. 1, 2, 3 and 4. After the encapsulation process, the samples were dried under the same conditions on teflon plates and captured their surface views (Fig. 1). Although the encapsulation materials were the same, it was remarkable that the colors of the encapsulated granules in the images were visually different. They reflected their color of the final solvent base materials. The honey-based propolis appeared more yellowish, the alcohol-based propolis had a darker hue, and the glycerin-based propolis exhibited a more brownish appearance.

Additionally, it was evident that the Na-Alg ratios significantly influenced the shape of the encapsulated propolis granules. After the drying process, granules coated with 1% Na-Alg were found to have a more heterogeneous structure, appearing flattened and lenticular shaped. As the Na-Alg ratio increased, the granules assumed a more spherical appearance. Granules coated with 2% Na-Alg exhibited a more homogeneous shape, maintaining their spherical structure even after the drying process. Polymer concentration is seen to be the most crucial component because it can impact the spheres' size, entrapment efficiency, and morphology, which need to be accounted for when creating them. Numerous studies show that the viscosity of the solution, the substance's entrapment efficiency, and the average size and size of the spheres all rise as the concentration of

Table 3 Color and bioactivity changes in simulated gastric fluid after 3 hours of gastric digestion of encapsulated samples

Color					Bioactivity	
Extraction base	Na-Alg rate (%)	450 nm (abs)	720 nm (abs)	Color intensity	Inhibition (%)	Total phenolic content (µg GAE /ml)
Honey	1	0.2230	0.1525	70.50[a]	9.93[a]	64.00[a]
	1,5	0.1530	0.0975	55.50[b]	4.36[b]	52.75[a]
	2	0.1615	0.0995	62.00[ab]	2.67[b]	57.50[a]
P value				0.025	0.008	0.076
Ethanol	1	0.4180	0.2945	123.50[a]	15.58[a]	85.75[a]
	1,5	0.2335	0.1435	90.00[b]	11.05[b]	85.25[a]
	2	0.2215	0.1315	90.00[b]	11.15[b]	92.50[a]
P value				0.026	0.012	0.199
Glycerine	1	0.3250	0.2155	109.50[a]	16.17[a]	77.50[a]
	1,5	0.2235	0.1365	87.00[b]	10.56[ab]	75.25[a]
	2	0.2295	0.1330	96.50[ab]	7.40[b]	90.00[a]
P value				0.015	0.013	0.094

the polymer increases. The resulting microspheres will have a low density, a wide distribution area, and a quick release of the active ingredient when low amounts of polymer are used (Frent et al., 2022).

The general morphological structures of the dried encapsulated propolis were also imaged at 20 × magnification using a NIKON DS-Fi2 light microscope, as shown in Fig. 2.

According to images obtained via light microscopy, propolis capsules exhibited distinct morphological differences across the three solvent bases (honey, alcohol, and glycerin). The final solvent type significantly influenced the granule shape, as did the concentration of Na-Alg used in encapsulation. It was clearly observed that a 1% Na-Alg ratio was insufficient for stable encapsulation. Capsules based on glycerin displayed greater fragility, with glycerin-based propolis encapsulated with 1% Na-Alg showing a transparent, brittle, and non-homogeneous structure.

In contrast, honey-based propolis capsules achieved the most uniform and homogeneous morphology across all Na-Alg concentrations. These honey-based capsules demonstrated a quality comparable to commercially available gelatin-coated pharmaceutical capsules. Furthermore, as the concentration of Na-Alg increased, there was a notable improvement in capsule stability, sphericity, and encapsulation efficiency (Ghasemzadeh & Ghanaat, 2014; Mahou et al., 2015).

Images obtained through light microscopy were further analyzed with the support of scanning electron microscopy (SEM). SEM views confirmed that the morphological characteristics of the propolis capsules varied significantly across the three solvent bases. As shown in Fig. 3, when comparing the encapsulation images of the three solvent bases at the 1% Na-Alg usage, the capsules appeared amorphous and uncertain shapes and displayed aggregate-like structures. The SEM images further corroborated that a 1% Na-Alg concentration was insufficient for effective encapsulation.

In the case of 1.5% Na-Alg use, it was determined that the capsule shapes were more individually selectable, transformed from amorphous to oval form, and their surface smoothness became more pronounced in all three solvent bases. When the samples were compared in terms of final solvents (honey, glycerin, and alcohol) for this ratio (1.5%), it was seen clearly that the glycerin and honey-based samples were much better than the Alcohol-based samples in

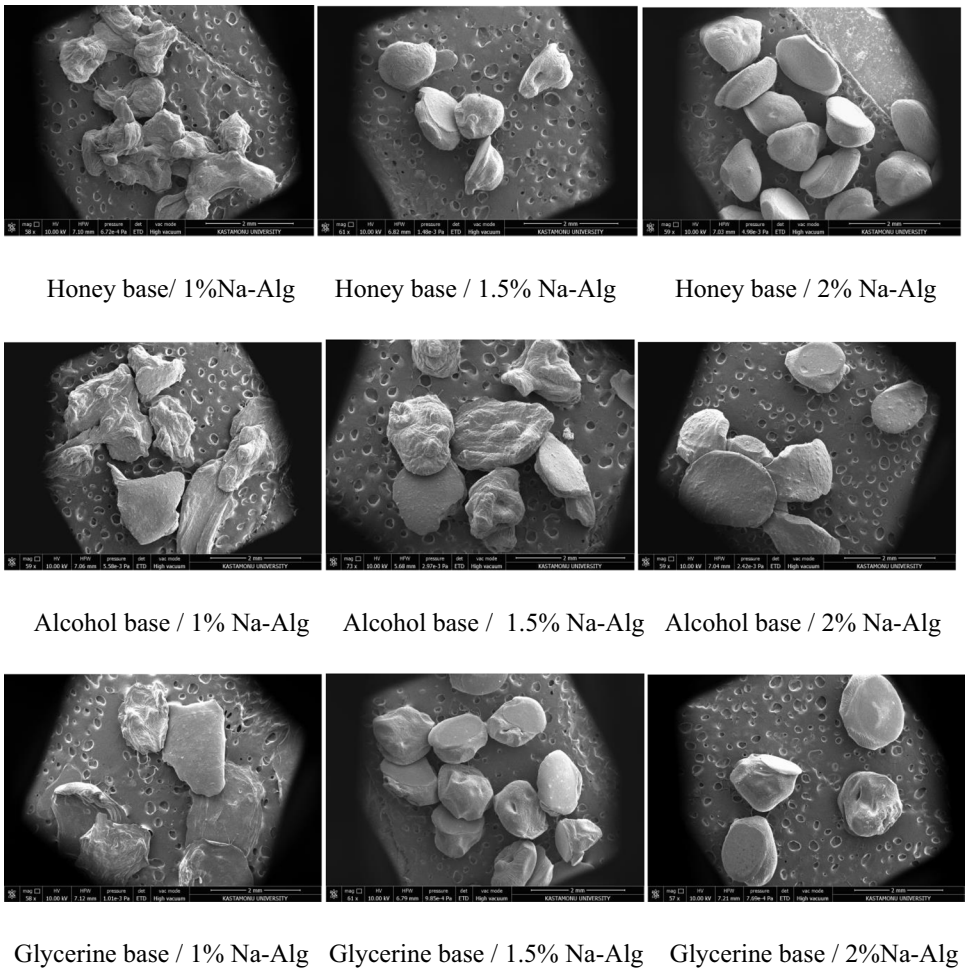

Fig. 3 Scanning electron microscopy (SEM) micrographs of propolis capsules with different bases and Na-Alg rates

terms of surface shape and smoothness. It was determined that the capsules using 2% Na-Alg (from top to bottom in the right column) were in the best form in terms of both individual shape morphology and surface smoothness, and the honey-based samples coated with 2% Na-Alg had the smoothest morphology. They had no gaps, which ensures better protection of the loaded material (Shakoury et al., 2022). When calcium salts are added to sodium alginate solution, they will form a gel very quickly due to the reaction between calcium ions and sodium alginate. Gelation occurs during casting due to the rapid gelation rate, thus resulting in various cross-linking densities and heterogeneity within the gel. The even distribution of calcium ions in the alginate solution ensures homogeneity.

It is responsible for the gel thickness increase as the concentration of alginate gel increases, ensuring homogeneity and achieving perfect smoothness (Sarıoğlu et al., 2024).

Physicochemical Properties

The antioxidant activities and total phenolic contents of encapsulated samples (Table 1) and the propolis extracts prepared different bases (Table 2) were determined. According to Table 2, in comparison with the final solvent types, the honey-based propolis extracts contained much more phenolic components and antioxidant activity than the other two types. Moreover, when the samples were compared in terms of the final solvent base for encapsulated samples, it was determined that the antioxidant activities of honey-based samples were significantly ($p < 0.05$) higher than those of alcohol and glycerin-based samples (Table 1). Raw propolis has high antioxidant activity with its rich polyphenol content, while antioxidant activity and phenolic substance content have changed depending on the final extraction agent used. Honey, being a food with antioxidant activity, has supported propolis bioactivity. This situation is seen in Table 2, where the antioxidant activities of honey-based extracts are higher than those of others. On the other hand, this situation may be due to the much better bonding of propolis and honey in the final extract, with honey capturing or covering the propolis droplets.

The increase in Na-Alg ratios in encapsulation also affected the bioactive properties of the samples. The increase in the amount of Na-Alg caused a decrease in both antioxidant activity and total phenolic content (Table 1). It was determined that samples containing 1% Na-Alg exhibited significantly higher bioactivity than other concentrations. This decrease in bioactivity is considered a relative decrease due to the increase in coating material in the total mass. The same situation is also seen between propolis extracts and encapsulated propolis samples. When comparing the antioxidant activity and total phenolic content of propolis extracts before and after encapsulation, a decrease in bioactive properties was observed due to the encapsulation process. This reduction could be attributed to including coating material in the encapsulated product on a per-unit basis (Table 1). However, the observed decrease in antioxidant activity and total phenolic content with increasing Na-Alg concentrations may not solely result from the encapsulation process itself. Higher alginate concentrations lead to the formation of denser gel matrices, which can restrict the diffusion and release of phenolic compounds during in vitro assays. Moreover, electrostatic and hydrogen bonding interactions between alginate and phenolic molecules may reduce their bioavailability. These interactions, combined with variations in encapsulation efficiency and loading capacity at higher polymer concentrations, likely contribute to the reduced measurable antioxidant activity. Similar effects of alginate concentration on phenolic release and antioxidant properties have been reported in the literature (Machado et al., 2022).

In the encapsulation technique used in this study, ionic gelation, Na-Alg cross-links can only be dissolved in basic media. The extraction method used for antioxidant activity is alcohol extraction, and there is no specific acidity regulation procedure. Therefore, as the amount of Na-Alg increases in encapsulation, cross-links will also increase, and stronger encapsulation will occur. Thus, the extraction of bioactive components from propolis, which forms the central phase in extraction, will become more difficult. Therefore, as the amount of Na-Alg increases, the decrease in extractable bioactives from propolis causes a decrease in antioxidant activity for in vitro media.

Although no statistically significant difference was found ($p > 0.05$), the higher ash content observed in honey-based samples is attributed to honey's higher total mineral content compared to other solvents.

While the Na-Alg ratio used in encapsulation did not cause significant changes in the color of the samples, the final solvents used—honey, alcohol, and glycerin—had a significant impact ($p < 0.05$) on the colors of the encapsulated final samples.

Characterization by Fourier Transform Infrared Spectrometry (FTIR)

For the characterization of the synthesized capsules using Fourier transform infrared (FTIR) spectroscopy, the spectrum of unloaded capsules cross-linked with Na-Alg and $CaCl_2$ without propolis was compared with the ATR-FTIR spectra of glycerin, alcohol, and honey-based propolis capsules, and the results were interpreted comparatively (Fig. 4).

As shown in Fig. 4, several peaks of propolis spectra indicated the diverse functional groups available in its structure. In the FTIR spectrum of $CaCl_2$ cross-linked Na-Alg, the strong absorption bands seen at 1589 and 1412 cm^{-1} are

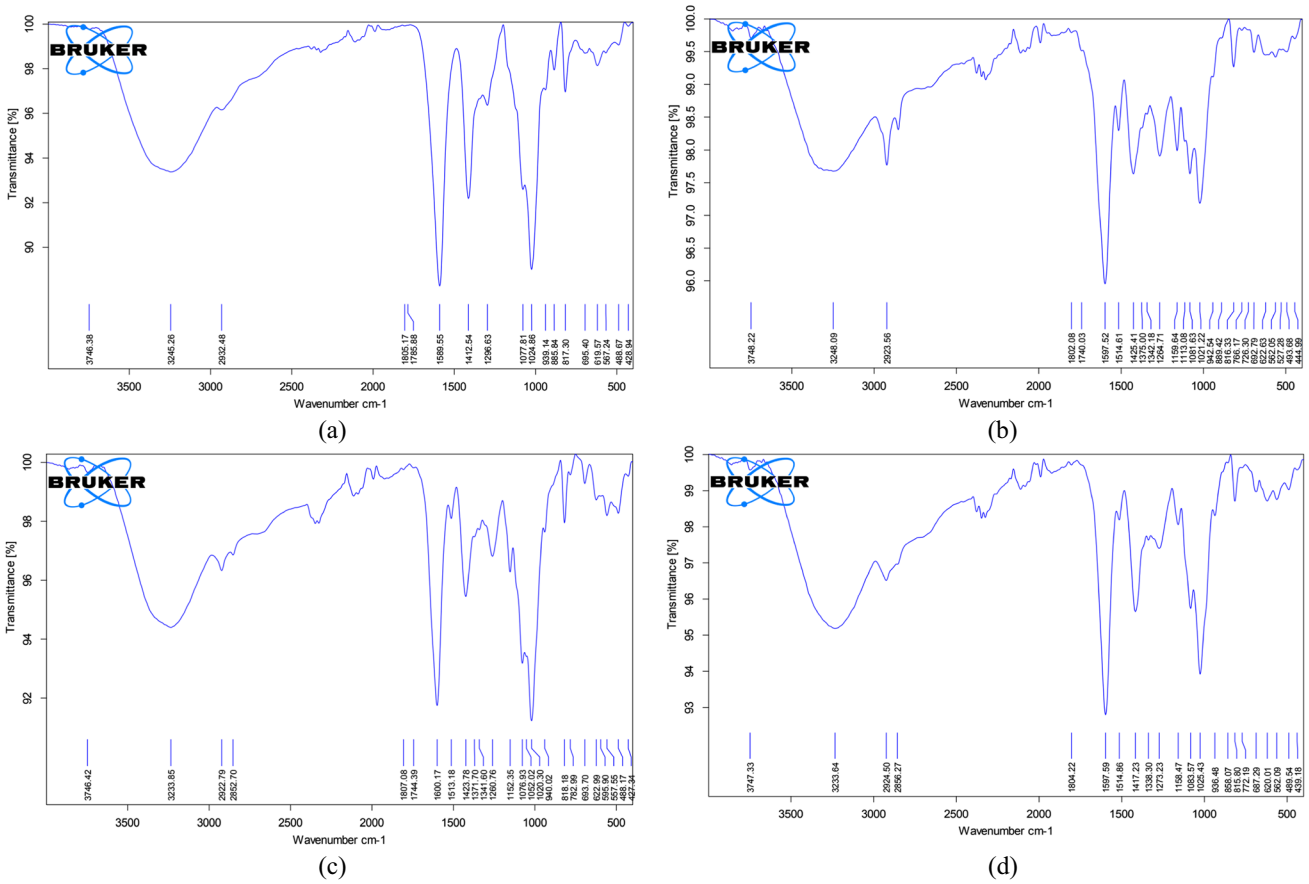

Fig. 4 FTIR spectra of cross-linked sodium alginate (**a**); alcohol-based propolis capsule with sodium alginate walls (**b**), honey-based propolis capsule with sodium alginate walls (**c**), and glycerin-based propolis capsule with sodium alginate walls (**d**)

attributed to the carboxyl groups' asymmetric and symmetric stretching vibrations. The band at 1024 cm^{-1} belongs to the C–O–C vibration. The polysaccharide structure's characteristic peaks are 939, 885, and 817 cm^{-1}. The results are similar to classical cross-linked Na-Alg structures (Bekin et al., 2014; Saarai et al., 2013).

The FTIR spectra of propolis-loaded capsules based on glycerin, alcohol, and honey exhibited peaks at similar wavenumbers, indicating structural similarities among them. However, all three spectra differed from the spectrum of empty capsules while showing similarities to each other.

The FTIR spectrum of sodium alginate capsules loaded with propolis showed partial similarity to the spectrum of unloaded capsules. However, distinctive new peaks observed at wavenumbers 2850–2856, 1513–1514, 1338, 1273, 1375, 1342, 1150, and 1050 cm^{-1} indicated the presence of propolis. The peak at 1296 cm^{-1} shifted to 1260 cm^{-1}, and its intensity decreased.

In the spectra, the band belonging to aliphatic C-H vibrations is seen in the 2850–2856 cm^{-1} region. The peaks around 1375–1380 cm^{-1} are associated with C-H bending, and those around 1050–1150 cm^{-1} are associated with C-O stretching (Keun et al., 2004). These results show that propolis enters the capsule structure where different bases are used in all three cases.

Release Studies and Digestive Behavior

One of the study's aims was to investigate the physical durability, capsule degradation properties and resistance to gastric conditions of encapsulated propolis samples, and the release of bioactive compounds until the point of intestinal discharge. It is an important feature for the capsules to reach the intestines with minimal deformation in the gastrointestinal system and release bioactive compounds by opening in the intestines.

Following a 3-h treatment of the encapsulated samples in acidic gastric fluid, absorbance values at 450 nm and 720 nm, representing the visible region, were measured using a UV–VIS spectrophotometer to determine color change values (Table 3). Due to the distinct and dominant color of the propolis extract, any degradation of encapsulation will result in the release of its color into the in vitro simulated gastric fluid. In this case, minimal measured color change

indicates limited propolis release into the medium, demonstrating the encapsulation's robustness. Thus, the color intensity value has been considered an indicator of color release of propolis (Table 3). Minimizing propolis release from the capsules until they reach the intestine is a desirable outcome.

According to the data presented in Table 3, the least color intensity was observed for the honey-based samples. The choice of final extraction solvent can influence bioactive compounds' solubility, stability, and release profile during digestion. Honey, as a natural deep eutectic solvent (NADES) (Dai et al., 2020), has unique properties that may affect bioactive compounds' encapsulation efficiency and release of bioactive compounds. Its composition, including sugars and phenolic compounds, can interact with the alginate matrix, potentially enhancing the stability and bioactivity of encapsulated propolis. Some studies have shown that honey can improve the dissolution of active components and their absorption after oral administration due to its NADES characteristics (Dai et al., 2020; Dimitriu et al., 2022). On the other hand, alcohol, as a polar solvent, can dissolve a wide range of bioactive compounds. However, its presence in the formulation may alter the gelation process of alginate, affecting the encapsulation efficiency and their releasing behavior. The interaction between alcohol and the alginate matrix could lead to changes in the structural integrity of the capsules, influencing their stability under gastric conditions. As a humectant, Glycerin can retain moisture and may impact the viscosity and gelation properties of alginate. Its interaction with the encapsulated compounds could affect the release kinetics and stability of the bioactive substances during digestion.

When comparing solvent bases individually, the highest color intensity—and consequently the highest release of propolis from the capsules—was observed in coatings with the lowest Na-Alg content (1%). In contrast, coatings with 1.5% and 2% Na-Alg demonstrated the lowest color intensities, with no statistically significant differences detected between them. The bioactivity values of the in vitro gastric fluid after three hours of digestion were analyzed and compared. A decrease in antioxidant activity was observed in the gastric fluid as the Na-Alg content used in encapsulation increased. However, no significant differences were found between using 1.5% and 2% Na-Alg ($p > 0.05$). The lowest antioxidant activity was identified in honey-based products, suggesting that capsules from honey-based products were more resistant to gastric conditions and exhibited minimal propolis release. The total phenolic content in the gastric fluid decreased for the honey-based product as the Na-Alg ratio increased. Although an increasing trend was observed in the other samples, these variations were not found to be statistically significant ($p > 0.05$). Polymer concentration is considered the most important factor to be taken into account when developing microspheres because it can affect the efficiency of the core material entrapping, the morphology, and the dimensions of the microspheres. In several studies, it was reported that with the increase in the concentration of the polymer, the efficiency of entrapping the core material increased (Uyen et al., 2020). In the case of using a low concentration of polymer, the resulting microspheres will have a large distribution area and a rapid release of the core substance.

To confirm the opening behavior of the capsules in the small intestine, encapsulated propolis samples underwent a gastric digestion procedure followed by the addition of TCA, pancreatin, and NaOH, and were subjected to 24 h of intestinal digestion. Photographs of the samples post-treatment are presented in the supplementary material (Supp. Mater.). The results showed that all encapsulated forms opened following the in vitro small intestine digestion (pH = 8.0) procedure. It also meant that the propolis capsules loaded to Na-Alg using the ionic gelation technique were opened, and bioactive compounds could be released in an alkaline intestinal medium. Studies on the stability of Na-Alg have reported that Na-Alg can withstand very short periods in alkaline media while gradually forming a gel of alginic acid at low-pH media. At acidic pH, the carboxyl groups in the alginate structure become protonated, forming an insoluble alginic acid layer on the surface of the microparticles, which plays a role in preventing the penetration of external liquid into the microparticles, and the stability of capsules (Frent et al., 2022; Guo et al., 2020).

Conclusion

We successfully demonstrated the fabrication of capsules containing chestnut propolis extract incorporated into an alginate matrix crosslinked by calcium ions. Capsules with a 1% sodium alginate ratio showed partial mechanical instability in their walls and irregular and non-uniform shapes. Conversely, when 1.5% and 2% Na-Alg were used, the membranes successfully encapsulated the core contents in all procedures, yielding effective and stable capsules. Considering the commercialization potential of the resulting products, a combination of 1.5% honey-based propolis and the Na-Alg ionic gelation coating method was identified as the most suitable approach. Regardless of the sodium alginate ratio used, it was observed that the encapsulated forms maintained their integrity after a 3-h gastric digestion simulation and reached the small intestine. Coating experiments with propolis extracts attached to different solvents were also performed, with honey-bound propolis extracts producing the most effective encapsulations.

In the continuation of the presented study, different wall materials can be tried in addition to the ionic gelation method, or another technique can be used for encapsulation. Product stability can be monitored throughout the shelf life, and testing can be carried out under realistic gastrointestinal conditions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11947-025-03889-4.

Acknowledgements The authors express their gratitude to Scientific and Technological Research Council of Turkey (TÜBİTAK) for its support.

Author Contribution The project was administrated by Müge Hendek Ertop (MHE). Seda Özgen and Uğur Ertop contributed to the supervision and project administration. All authors contributed to the methodology, software, formal analysis, and investigation of the project and the study. MHE wrote the original draft; other authors contributed to the writing, review, and editing. All authors have read and agreed to the published version of the manuscript.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This study is based on work supported by the Scientific and Technological Research Council of Turkey (TUBITAK), 1512- BIGG Entrepreneurship Support Program under Grant 2210436 entitled "Production of Encapsulated Forms of Chestnut Propolis Extract and Their Use in Functional Food Production."

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethical Approval Not applicable.

Competing interests The authors declare no competing interests.

References

AACC. (2000). Approved methods of the American Association of Cereal Chemists (Method 08-01.01, Determination of Ash), 10th ed. St. Paul MN, U.S.A.

Alpat, U., Nar, T., Karasu, S., & Sagdic, O. (2023). Optimization of propolis extraction with natural deep eutectic solvents using central composite design. *Phytochemistry Letters, 58*, 49–59. https://doi.org/10.1016/j.phytol.2023.09.013

Anjum, S. I., Ullah, A., Khan, K. A., Attaullah, M., Khan, H., Ali, H., ... & Dash, C. K. (2019). Composition and functional properties of propolis (bee glue): A review. *Saudi Journal of Biological Sciences*, *26*(7), 1695–1703. https://doi.org/10.1016/j.sjbs.2018.08.013

Aponte, M., Boscaino, F., Sorrentino, A., Coppola, R., Masi, P., & Romano, A. (2014). Effects of fermentation and rye flour on microstructure and volatile compounds of chestnut flour based sourdoughs. *LWT Food Science and Technology, 58*, 387–395. https://doi.org/10.1016/j.lwt.2014.03.022

Barboza Duarte Rodrigues IF, Pereira JM, Lima LMSD, Lins Silva KG, Silva MR, da Costa Silva V, ... Almeida Silva AC (2024) Development and evaluation of capsules loaded with red propolis extract and metallic nanoparticles using the ionic gelation method. J Apic Res: 1–12. https://doi.org/10.1080/00218839.2024.2391680

Bekin, S., Sarmad, S., Gürkan, K., Keçeli, G., & Gürdağ, G. (2014). Synthesis, characterization and bending behavior of electroresponsive sodium alginate/poly (acrylic acid) interpenetrating network films under an electric field stimulus. *Sensors and Actuators B: Chemical, 202*, 878–892. https://doi.org/10.1016/j.snb.2014.06.051

Beratta, G., Granata, P., Ferrero, M., Orıolı, M., & Facıno, R. M. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta, 533*, 185–191. https://doi.org/10.1016/j.aca.2004.11.010

da Silveira, C. C. S. D. M., Fernandes, L. M. P., Silva, M. L., Luz, D. A., Gomes, A. R. Q., Monteiro, M. C., ... & Maia, C. S. F. (2016). Neurobehavioral and antioxidant effects of ethanolic extract of yellow propolis. *Oxidative Medicine and Cellular Longevity, 2016*(1), 2906953.https://doi.org/10.1155/2016/2906953

Dai, Y., Jin, R., Verpoorte, R., Lam, W., Cheng, Y. C., Xiao, Y., ... & Chen, S. (2020). Natural deep eutectic characteristics of honey improve the bioactivity and safety of traditional medicines. *Journal of Ethnopharmacology, 250*, 112460. https://doi.org/10.1016/j.jep.2019.112460

Dantas Silva, R. P., Machado, B. A. S., Barreto, G. D. A., Costa, S. S., Andrade, L. N., Amaral, R. G., ... & Umsza-Guez, M. A. (2017). Antioxidant, antimicrobial, antiparasitic, and cytotoxic properties of various Brazilian propolis extracts. *Plos one*, *12*(3), e0172585. https://doi.org/10.1371/journal.pone.0172585

Dimitriu, L., Constantinescu-Aruxandei, D., Preda, D., Nichițean, A. L., Nicolae, C. A., Faraon, V. A., ... & Oancea, F. (2022). Honey and its biomimetic deep eutectic solvent modulate the antioxidant activity of polyphenols. *Antioxidants, 11*(11), 2194. https://doi.org/10.3390/antiox11112194

Einhorn-Stoll, U., Archut, A., Eichhorn, M., & Kastner, H. (2021). Pectin-plant protein systems and their application. *Food Hydrocolloids, 118*, 106783. https://doi.org/10.1016/j.foodhyd.2021.106783

El-Guendouz, S., Lyoussi, B., & Miguel, M. G. (2019). Insight on propolis from mediterranean countries: Chemical composition, biological activities and application fields. *Chemistry & Biodiversity, 16*(7), Article e1900094. https://doi.org/10.1002/cbdv.201900094

Frent, O. D., Vicas, L. G., Duteanu, N., Morgovan, C. M., Jurca, T., Pallag, A., ... & Marian, E. (2022). Sodium alginate—natural microencapsulation material of polymeric microparticles. *International Journal Of Molecular Sciences*, 23(20), 12108. https://doi.org/10.3390/ijms232012108

Ghasemzadeh, H., & Ghanaat, F. (2014). Antimicrobial alginate/PVA silver nanocomposite hydrogel, synthesis and characterization. *Journal of Polymer Research, 21*, 1–14. https://doi.org/10.1007/s10965-014-0355-1

Guo, X., Wang, Y., Qin, Y., Shen, P., & Peng, Q. (2020). Structures, properties and application of alginic acid: A review. *International*

Journal of Biological Macromolecules, 162, 618–628. https://doi.org/10.1016/j.ijbiomac.2020.06.180

Hayta, M., & Hendek Ertop, M. (2017). Optimisation of sourdough bread incorporation into wheat bread by response surface methodology bioactive and nutritional properties. *International Journal of Food Science Technology, 52*(8), 1828–1835. https://doi.org/10.1111/ijfs.13457

Irigoiti, Y., Navarro, A., Yamul, D., Libonatti, C., Tabera, A., & Basualdo, M. (2021). The use of propolis as a functional food ingredient: A review. *Trends in Food Science & Technology, 115*, 297–306. https://doi.org/10.1016/j.tifs.2021.06.041

Jansen-Alves, C., Fernandes, K. F., Crizel-Cardozo, M. M., Krumreich, F. D., Borges, C. D., & Zambiazi, R. C. (2018). Microencapsulation of propolis in protein matrix using spray drying for application in food systems. *Food and Bioprocess Technology, 11*(7), 1422–1436. https://doi.org/10.1007/s11947-018-2115-4

Jansen-Alves, C., Krumreich, F. D., Zandoná, G. P., Gularte, M. A., Borges, C. D., & Zambiazi, R. C. (2019). Production of propolis extract microparticles with concentrated pea protein for application in food. *Food and Bioprocess Technology, 12*, 729–740. https://doi.org/10.1007/s11947-019-2246-2

Keskin, M., Keskin, Ş, & Kolayli, S. (2019). Preparation of alcohol free propolis-alginate microcapsules, characterization and release property. *LWT, 108*, 89–96. https://doi.org/10.1016/j.lwt.2019.03.036

Keun, H. C., Ebbels, T. M., Bollard, M. E., Beckonert, O., Antti, H., Holmes, E., ... & Nicholson, J. K. (2004). Geometric trajectory analysis of metabolic responses to toxicity can define treatment specific profiles. *Chemical Research in Toxicology*, 17(5), 579–587. https://doi.org/10.1021/tx034212w

Kuo, Y. Y., Jim, W. T., Su, L. C., Chung, C. J., Lin, C. Y., Huo, C., ... & Chuu, C. P. (2015). Caffeic acid phenethyl ester is a potential therapeutic agent for oral cancer. *International Journal of Molecular Sciences*, *16*(5), 10748–10766. https://doi.org/10.3390/ijms160510748

Machado, A. R., Silva, P. M. P., Vicente, A. A., Souza-Soares, L. A., Pinheiro, A. C., & Cerqueira, M. A. (2022). Alginate particles for encapsulation of phenolic extract from Spirulina sp. LEB-18: Physicochemical characterization and assessment of in vitro gastrointestinal behavior. *Polymers, 14*(21), 4759. https://doi.org/10.3390/polym14214759

Mahou, R., Borcard, F., Crivelli, V., Montanari, E., Passemard, S., Noverraz, F., ... & Wandrey, C. (2015). Tuning the properties of hydrogel microspheres by adding chemical cross-linking functionality to sodium alginate. *Chemistry of Materials*, *27*(12), 4380–4389. https://doi.org/10.1021/acs.chemmater.5b01098

Mattigatti, S., Ratnakar, P., Moturi, S., Varma, S., & Rairam, S. (2012). Antimicrobial effect of conventional root canal medicaments vs propolis against Enterococcus faecalis, Staphylococcus aureus and Candida albicans. *The Journal of Contemporary Dental Practice, 13*(3), 305–309. https://doi.org/10.5005/jp-journals-10024-1142

Meto, A., Colombari, B., Meto, A., Boaretto, G., Pinetti, D., Marchetti, L., ... & Blasi, E. (2020). Propolis affects Pseudomonas aeruginosa growth, biofilm formation, eDNA release and phenazine production: Potential involvement of polyphenols. *Microorganisms*, *8*(2), 243. https://doi.org/10.3390/microorganisms8020243

Mohammadian, M., Salami, M., Momen, S., Alavi, F., Emam-Djomeh, Z., & MoosaviMovahedi, A. A. (2019). Enhancing the aqueous solubility of curcumin at acidic condition through the complexation with whey protein nanofibrils. *Food Hydrocolloids, 87*, 902–914. https://doi.org/10.1016/j.foodhyd.2018.09.001

Ray, S., Raychaudhuri, U., & Chakraborty, R. (2016). An overview of encapsulation of active compounds used in food products by drying technology. *Food Bioscience, 13*, 76–83. https://doi.org/10.1016/j.fbio.2015.12.009

Rizzello, C. G., Curiel, J. A., Nionelli, L., Vincentini, O., Cagno, R. D., Silano, M., Gobbetti, M., & Coda, R. (2014). Use of fungal proteases and selected sourdough lacticacid bacteria formaking whea tbread with an intermediate content of gluten. *Food Microbiology, 37*, 59–68. https://doi.org/10.1016/j.fm.2013.06.017

Rocha, V. M., Portela, R. D., Dos Anjos, J. P., De Souza, C. O., & Umsza-Guez, M. A. (2023). Stingless bee propolis: Composition, biological activities and its applications in the food industry. *Food Production, Processing and Nutrition, 5*(29), 1–13. https://doi.org/10.1186/s43014-023-00146-z

Rodriguez, Y. E., Laitano, M. V., Zanazzi, A. N., Fernández-Gimenez, A. V., de los Ángeles Pereira, N., & Rivero, G. (2024). Turning fishery waste into aquafeed additives: Enhancing shrimp enzymes immobilization in alginate-based particles using electrohydrodynamic atomization. *Aquaculture, 587*, 740846. https://doi.org/10.1016/j.aquaculture.2024.740846

Rosseto, H. C., de Toledo, L. D. A. S., de Francisco, L. M. B., Esposito, E., Lim, Y., Valacchi, G., ... & Bruschi, M. L. (2017). Nanostructured lipid systems modified with waste material of propolis for wound healing: Design, in vitro and in vivo evaluation. *Colloids and Surfaces B: Biointerfaces, 158*, 441–452. https://doi.org/10.1016/j.colsurfb.2017.07.029

Saarai, A., Kasparkova, V., Sedlacek, T., & Saha, P. (2013). On the development and characterisation of crosslinked sodium alginate/gelatine hydrogels. *The Journal of the Mechanical Behavior of Biomedical Materials, 18*, 152–166. https://doi.org/10.1016/j.jmbbm.2012.11.010

Sarıoğlu, K., Ülgen, A., & Oral, R. A. (2024). Encapsulation of anthocyanin with gelatin-alginate carrier by a specially designed ultrasonic encapsulator with adjustable frequency. *Journal of Applied Polymer Science, 141*(6), Article e54931. https://doi.org/10.1002/app.54931

Şenol Yazkan, S. N., & Hendek Ertop, M. (2025). Optimization of Bioactive Compound Extraction from Propolis by Reflux, Maceration and Ultrasound-assisted Methods and Characterization of the Extracts. *Journal of Agricultural Sciences, 31*(2), 359–372. https://doi.org/10.15832/ankutbd.1540221

Shahidi, F., & Naczk, M. (1995). Food phenolics: Sources, chemistry, effects and applications. *International Journal of Food Science and Technology, 44*(12), 287–293. https://doi.org/10.1111/j.1365-2621.2009.02077.x

Shakoury, N., Aliyari, M. A., Salami, M., Emam-Djomeh, Z., Vardhanabhuti, B., & Moosavi-Movahedi, A. A. (2022). Encapsulation of propolis extract in whey protein nanoparticles. *LWT, 158*, Article 113138. https://doi.org/10.1016/j.lwt.2022.113138

Uyen, N. T. T., Hamid, Z. A. A., Tram, N. X. T., & Ahmad, N. (2020). Fabrication of alginate microspheres for drug delivery: A review. *International Journal of Biological Macromolecules, 153*, 1035–1046. https://doi.org/10.1016/j.ijbiomac.2019.10.233