



Modulation of Hot Pepper Pungency by Melt Dispersion Encapsulation: Role of Wax Type on Microcapsule Structure, Bioactive Compound Content, and Antioxidant Activity

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Abstract

In this study, it was aimed to suppress pungency and obtain a new product by using carnauba, beeswax, and their mixture in the encapsulation of hot pepper oleoresin. Melt dispersion technique was used in production and physical, chemical, and structural analyzes of the obtained capsules were examined. The suppression of the pungency sensation was determined by sensory analysis, and it was determined that the beeswax-based capsule (BW50) was the most effective formulation in suppressing pungency perception (0.83). When looking at the sensory and Scoville Heat Units (SHU) results, there is a parallelism, and it can be stated that the pungency sensation is suppressed. The highest encapsulation efficiency values were observed in CW20 (91.92%), BW20 (90.69%), BC20 (86.92%), and CW30 (86.49%) capsules. When capsule bioactivities were examined, beeswax capsules showed the highest values, while carnauba wax capsules generally showed the lowest values. As expected, as the amount of oleoresin in the capsules increased, the capsules exhibited higher phenolic, flavonoid, carotenoid, and antioxidant activity scores. SEM, FT-IR, and TGA determined that the encapsulation process did not cause any adverse structural reactions in oleoresin. This study broadened the potential application of encapsulated hot pepper oleoresin in food formulations, particularly in functional foods and products intended for consumers sensitive to pungency, by converting it into a more tolerable form.

Keywords Encapsulation · Melt dispersion · Pungency · Hot pepper · Beeswax · Carnauba wax

Introduction

Pepper is a fruit belonging to the Solanaceae family, which has both hot and sweet forms grown in many parts of the world. Pepper fruits can be thin and pungent, while large, thick, and sweet can be produced and can be used raw, in the form of spices or as a coloring agent. Different maturity levels, chlorophyll, and carotenoid production capabilities

allow peppers to be obtained in various colors such as green, red, yellow, and orange (Wang et al., 2025).

Hot pepper oleoresin is a hydrophobic pepper extract containing capsaicinoids such as capsaicin, dihydrocapsaicin, and carotenoids such as capsanthin and capsorubin. Hot pepper oleoresin, which has a dark red or black-red color, is a commercial viscous product used in the food, pharmaceutical and cosmetic sectors (Baenas et al., 2019). Capsaicinoids (capsaicin is the dominant capsaicinoid), vitamin C, vitamin E, and xanthophylls constitute the active ingredient group in hot pepper oleoresin (Sricharoen et al., 2017). Studies have shown that secondary metabolite products extracted from plants provide therapeutic benefits such as antibacterial, antiviral, anticancer, analgesic, antiinflammatory, and antitumor properties (Khanam et al., 2025). Likewise, anti-inflammatory (Eya et al., 2025), antiarthritic (Akter et al., 2026), anticancer (EL-Haggag, 2025), and antifungal (Abraham et al., 2025) properties of pepper have been reported by researchers. These health benefits have made pepper a basic ingredient in pharmaceutical and medical formulations as

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well as its commercial use in the food industry. However, in order to benefit from the health benefits of a product, it is necessary to consume that product in a healthy and regular manner. The sharp and pungent taste of peppers is undesirable for many consumers, limiting their ability to benefit from their health advantages. Therefore, encapsulation technology is widely used in the food industry to suppress or reduce undesirable taste and aroma characteristics. Different wall materials are available for encapsulation, including protein, lipid, and carbohydrate-based materials. Natural waxes are one of these materials and stand out for their hydrophobic properties, enabling the protection and controlled release of bioactive compounds (Klojdová et al., 2023). Carnauba wax and beeswax were selected in this study because they are food-grade, natural, and widely approved for use in food applications. Carnauba wax, obtained from *Copernicia prunifera* leaves, has a high melting point and low viscosity, which facilitates encapsulation processes and enhances capsule stability (Bezerra et al., 2025). Beeswax was chosen as the wall material due to its good barrier properties, compatibility with lipid-based systems, and authorization as a food additive (E901) (Gundev et al., 2022). The increasing use of both waxes in capsule production in the food, pharmaceutical, and cosmetic sectors in recent years supports the rationale for choosing these materials (Medeiros et al., 2019).

Encapsulation is the process of confining the target component (core) within another material (wall). Many encapsulation techniques are applied, such as spray cooling, spray drying, co-encapsulation, melt dispersion, and encapsulation in polymer microspheres (Lalarukh et al., 2024). In the pharmaceutical industry, mostly non-hydrophilic wall materials (such as lipids and waxes) are used for encapsulating hydrophobic compounds. The melt dispersion technique is one of these techniques. Here, the target component and wax mixture are brought to the melting point with water and the entrapment process is carried out by mixing, then with the cooling process, the melted wax turns back into solid form, entrapping the target component (Lalarukh et al., 2024). This method has several important advantages, including: the absence of organic solvents, suitability for lipid-based coating materials, and a relatively simple and economical process (Lazăr et al., 2024). Melt-dispersion technology yields effective results in preserving bioactive compounds and masking undesirable flavors, particularly in hydrophobic wax-based systems. Numerous studies in the literature demonstrate the successful application of this technique in food production (Assadpour & Jafari, 2019; Nandy et al., 2020; Q. Huang & Zhang, 2025; Lopes et al., 2025). In recent studies, the main objectives are to increase the physicochemical stability and improve the solubility of red pepper oleoresin capsules (Costa et al., 2023; Ferraz et al., 2022; Figueiredo

et al., 2022; Zhang et al., 2023). However, a review of the literature reveals that there is still a significant gap regarding the suppression or controlled release of capsaicinoids that cause a sudden burning sensation (pungency) in the mouth. In current studies, reducing oral secretion with the spray-chilling method (Günel et al., 2021) or using beta-cyclodextrin to mask the flavor (Khoshtinat et al., 2023) has been preferred (Khoshtinat et al., 2023). To our knowledge, the potential of lipid-based wall materials, particularly waxes, to mitigate the intense pungency effect of hot peppers has not yet been explored. This study aims to address this gap in the literature by developing wax-based microcapsules specifically designed to mask pungency, and to expand the application of hot pepper oleoresin in the food industry.

In our study, the aim was to encapsulate hot pepper oleoresin with carnauba, beeswax, and a mixture of these two waxes using the melt dispersion technique. In this way, the pungent taste will be suppressed/mitigated, and the usage possibilities of hot pepper and hot pepper extract will be increased and it will be provided to benefit consumers and the sector. The oleoresin converted from liquid to solid form will be more easily incorporated into formulations. Other purposes are to examine the bioactive, structural, and thermogravimetric properties of the obtained capsules and to test their sensory pungency.

Materials and Methods

Materials

Hot pepper (Sena cultivar), a local product of Kahramanmaraş (Turkey), was provided in dry form from the regional institute and stored in the dark at 4 °C until analyzes were performed.

Oleoresin Extraction from Hot Pepper

The Soxhlet extraction method, which has a high yield, was preferred to obtain oleoresin from dry hot pepper. First, refuse substances such as seeds and leaves contained in the hot pepper were removed and the hot pepper was ground (Waring, Staufen, Germany). After the particle size was standardized between 300 and 500 µm, 250 mL acetone was added to 15 g of sample and extracted for 6 h. Acetone was removed and oleoresin was obtained in a rotary evaporator (Heidolph Hei-Vap, Germany) under 150 mbar vacuum at 40 ± 2 °C conditions. Oleoresin was stored in a dark environment at − 18 °C until the encapsulation process and analysis were performed.

Encapsulation of Hot Pepper Oleoresin in Carnauba and Beeswax

Encapsulation of hot pepper oleoresin in carnauba wax, beeswax, and blend wax (carnauba:bees, 1:1) was realized by the melt dispersion technique (Milanovic et al., 2010). Carnauba, beeswax and blend wax (carnauba:beeswax, 1:1) were melted in a water bath at 95 °C, and hot pepper oleoresin was added and mixed with high-speed homogenizer (Ultra-Turrax T18, IKA, Staufen, Germany) at 10,000 rpm for 2 min to ensure the confinement process. After the homogenization process, 150 mL of about 5 °C cold water was added to the mixture to solidify the oleoresin wax structure. Then, the obtained solid material was filtered and washed with pure water (the same volume as that added for solidification) to remove the unencapsulated part on its surface and the capsules were obtained by drying in an oven at 35 °C for 18 h. Capsules containing different percentages of oleoresin (20, 30, and 50%) were produced with three different wall materials. All encapsulation processes were conducted in two replicates to ensure the reproducibility of the production method. For each sample, analyses were performed in triplicate parallel without waiting, resulting in a total of six measurements ($n=6$) for each data point. The results are expressed as mean $\pm$ standard deviation. Capsule production diagram is given in Fig. 1.

Encapsulation Efficiency

The extracts were obtained by modifying the method proposed by Zhang et al. (2022) to conduct efficacy and compositional analyses of the capsules prepared using

carnauba wax, beeswax, and blend wax via the melt dispersion technique. The total capsaicin content was determined by adding 5 mL of acetone to 100 mg of capsules, followed by ultrasonic treatment for 5 min. The mixture was then centrifuged at 4 °C and 4000 rpm for 10 min. The supernatant was collected, and an additional 5 mL of acetone was added to the pellet, repeating the same process. The combined supernatants were subjected to rotary evaporation to remove acetone, and the residue was dissolved in 10 mL of ethanol.

For the surface capsaicin content, a modified version of the method proposed by Zhang et al. (2022) was employed. Specifically, 2 mL of ethanol was added to 50 mg of capsules and vortexed for 1 min. The mixture was then centrifuged at 4000 rpm for 5 min, after which the supernatant was collected. The absorbance of the supernatant was measured at 280 nm using a spectrophotometer, with ethanol serving as the blank (B. Zhang et al., 2022).

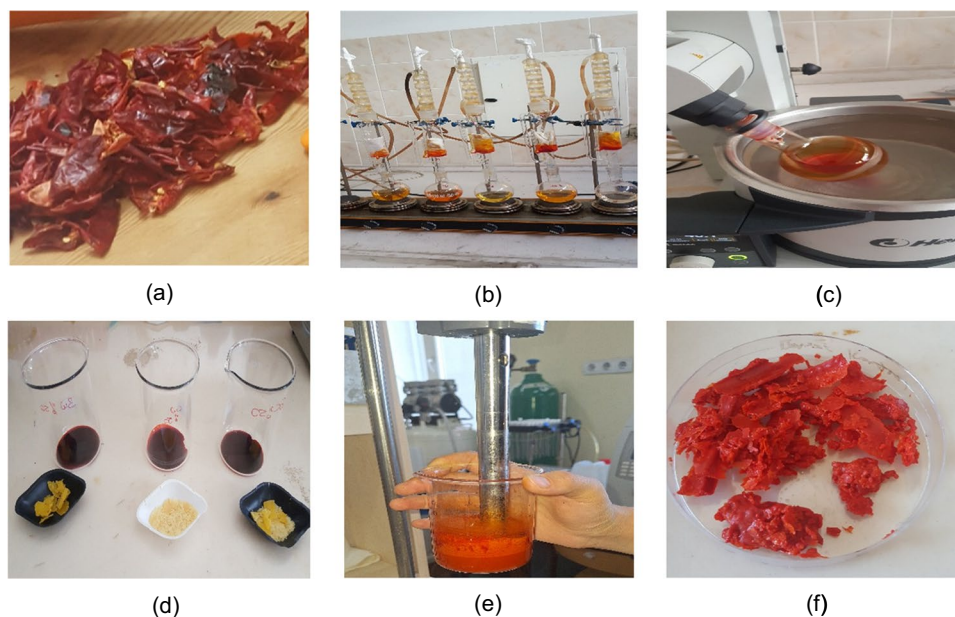
$$EE (\%) = 1 - \frac{\text{Surface capsaicin content of the capsule}}{\text{Total capsaicin content of the capsule}} \times 100$$

Physical, Bioactivity, and Structural Properties of the Samples

Color Measurement

The color data of the produced capsules (3 g) were determined by color analyzer (Konica Minolta, CR400, Chiyoda City, Japan). Lightness (L^*), redness (a^*), and yellowness (b^*) color parameters were recorded.

Fig. 1 Preparation steps of wax-encapsulated hot pepper oleoresin. **a** Dried hot pepper used as the raw material, **b** Soxhlet extraction process for obtaining hot pepper oleoresin, **c** removal of the solvent by rotary evaporation to obtain concentrated oleoresin, **d** Mixing of hot pepper oleoresin with the wax matrix at different weight ratios, **e** Homogenization process to ensure uniform dispersion of oleoresin within the wax matrix, **f** final wet wax capsules containing hot pepper oleoresin



Water Solubility

To determine the water solubility of the samples, 10 mL of distilled water was added to 0.2 g of sample, vortex (VWR International, Germany) was applied for 5 min and centrifuged at 8500 rpm for 5 min. Then, 4 mL of supernatant were transferred to aluminum petri dishes and kept at 70 °C for 24 h. The solubility (%) was calculated according to the formula below (Do, Hoa T & Nguyen V, 2018).

$$\text{Water solubility (\%)} = \frac{\text{Dried supernatant weight}}{\text{Initial sample weight}} \times 100$$

Water Activity (aw)

The water activity data of the capsules obtained with the melt dispersion technique were determined by taking double measurements with a water activity device (Aqualab T3, USA).

Moisture Content

The moisture determination of capsules was carried out based on the AOAC-942.05 method.

Total Extractable Color (ASTA)

ASTA values of the capsules were specified by extraction with acetone based on the Fikiru et al. (2024) method. In this technique, 100 mL of acetone was added to 100 mg of sample and kept in the dark for 16 h, and at the end of the period, it was gently mixed once and blanked with acetone at 460 nm, and spectrophotometric measurements were taken with a dilution appropriate for the samples (Fikiru et al., 2024). Calculations were made according to the formula below.

$$\text{Total extractable color (in ASTA units)} = \frac{A * 16.4}{w} \times 100$$

where A is the absorbance of the acetone extract and w is the dry mass of the sample (g).

Determination of Total Carotenoids

Curves were obtained with solutions of the β -carotene standard prepared at different concentrations (5–40 mg/L) in ethanol, and the absorbance of the sample extracts at 460 nm was read (Sricharoen et al., 2017). As a result of the calculations, the total carotenoid amounts contained in the samples are given as mg β -carotene per gram capsule (mg β CE/g).

Antioxidant Activity (AA)

Antioxidant activity values of the capsules were investigated by spectrophotometry using the ABTS method as reference. A 2.45 mM ABTS solution was prepared using a 12.25 mM $\text{K}_2\text{S}_2\text{O}_8$ solution, and it was left to stand in the dark for 12 h. The absorbance of the ABTS was then adjusted to a range of 0.68–0.72 by diluting it with saline phosphate buffer (pH 7.4). Initially, 2 mL of the radical solution was transferred to a cuvette, followed by the addition of 10–40 μL of the sample extract. The mixture was incubated in darkness for 6 min. Phosphate buffer was used as the blank, and ethanol as the solvent for the control. After incubation, absorbance readings were taken at 734 nm (Re & Pellegrini, 1999). A linear calibration curve was generated using the absorbances of Trolox solutions against standard concentrations. The results were expressed as mg Trolox equivalents antioxidant activity (TEAC) per gram capsule.

Total Phenolic Content (TPC)

The total phenolic content of the samples was assessed using the Singleton and Rossi method. Two milliliters of Folin reagent, diluted 10 times with distilled water, were added to a test tube containing 0.4 mL of the sample extract. Subsequently, 1.6 mL of Na_2CO_3 solution (7.5%, w/v) was added, and the mixture was vortexed. The samples were then allowed to rest in the dark at room temperature for 1 h. Once the incubation period concluded, readings were obtained at 765 nm with a UV spectrophotometer, using ethanol for the blank solution. A linear calibration curve was generated from a series of gallic acid concentrations, and the results were calculated as gallic acid equivalents (GAE) per gram of capsule (Singleton & Rossi, 1965).

Total Flavonoid Content (TFC)

To a test tube, 1 mL of the sample solution, 4 mL of distilled water, and 0.3 mL of 5% NaNO_2 were added and allowed to stand for 5 min. After this period, 0.3 mL of 10% AlCl_3 was introduced into the tube. At the 6 min mark of the incubation, 2 mL of 1 M NaOH was added to the mixture. In the final step, the volume was adjusted to 10 mL using distilled water, and absorbance was measured at 510 nm. The final results were calculated as catechin equivalents (CE) per gram of capsule, based on a calibration curve derived from catechin solutions (Zhishen et al., 1999).

Capsaicinoid Analysis

To generate a calibration curve, solutions with concentrations ranging from 10 to 100 mg/L in ethanol were prepared using a capsaicin standard (55% capsaicin and 45% dihydrocapsaicin, Sigma-Aldrich, USA). The capsaicinoid content in the capsule extracts was measured spectrophotometrically at 280 nm according to a method described in the literature (González-Zamora et al., 2015). Total capsaicinoids were expressed as milligrams of total capsaicinoid per kilogram capsules.

Pungency

The capsules' pungency values were assessed in Scoville heat units (SHU). For this evaluation, two primary capsaicinoids (capsaicin and dihydrocapsaicin) served as references. After conducting spectrophotometric analysis of these two capsaicinoids, the final results were calculated using the following formula (Scoville, 1912).

$$\text{Total SHU} = [\text{Capsaicin}(\text{ppm}) + \text{Dihydrocapsaicin}(\text{ppm})] \times 16.1$$

Sensory Analysis

A sensory evaluation of the capsules was conducted with 15 trained panelists to determine whether the pungency of the hot pepper oleoresin was effectively masked or reduced and to assess the perceived intensity of pungency. The panelists were selected from among the volunteer students and instructors of the Food Engineering Department at Erciyes University who had completed a 28-h sensory analysis course. Panelists received training using three reference standards prepared in tomato puree (Tat, Turkey). Because hot pepper oleoresin is highly pungent and difficult to distribute uniformly across the tongue, direct oral testing is impractical. Tomato puree was therefore selected as a carrier matrix, as it allowed an increased sample volume to be placed in the mouth while maintaining the target oleoresin concentration. This approach also facilitated better dispersion of the oleoresin in the oral cavity and helped obscure its color. For instance, 0.1 g of oleoresin was incorporated into 100 g of tomato puree by mixing a pre-blended oleoresin–puree mixture for 1 min at 50 rpm.

Panelists were introduced to three reference preparations: S0 (pure tomato puree), S1 (0.1% oleoresin), and S2 (0.2% oleoresin). The amount of capsule added to the puree was adjusted so that the final concentration of oleoresin in the mixture was 0.1%, ensuring that pungency perception could be compared at equivalent levels.

A unipolar intensity scale was employed to evaluate pungency. Panelists used a 0–10 scale, where 0, 5, and 10 corresponded to S0, S1, and S2, respectively. At the beginning of each session, panelists first tasted the reference standards before proceeding to evaluate the test samples. They were instructed to hold each sample in their mouth for 20 s, cleanse their palate with a yogurt-based beverage (Pinar Ayrın) and water between samples, and wait at least 5 min before proceeding to the next evaluation.

Scanning Electronic Microscopy (SEM)

The morphological characteristics and microstructure of the capsules were analyzed using a scanning electron microscope (Zeiss, Germany) at an accelerating voltage of 5 kV and a magnification of 2000×, with applying a gold coating. For imaging, a random point was selected from samples cut into 1 cm × 1 cm sections, and the photography process was initiated.

Fourier Transform Infrared Spectroscopy (FT-IR)

The structural characteristics of the capsules were evaluated using Fourier-transform infrared spectroscopy (FT-IR). The spectra were recorded using an FT-IR spectrophotometer (Spectrum 400, Perkin Elmer Instruments, Waltham, USA) in the wavelength range of 4000–400 cm^{−1}.

Thermal Analysis of the Samples (TGA)

The mass changes of the capsules with increasing temperature and time were carried out using a thermogravimetric thermal analyzer (Perkin Elmer, USA). Approximately 2–3 mg of capsule samples were placed in a platinum dish and exposed to the following conditions:

- Nitrogen flow rate: 20 mL/min,
- Temperature increase procedure: from 25 to 500 °C with a heating rate of 10 °C/min

The results are presented as TGA curves showing the mass loss and mass change of the sample with the effect of temperature.

Statistical Analysis

Multiple comparisons and a post hoc test (Duncan test) of the samples were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) at a significance level of $p < 0.05$. All analyses were performed in two replicates, with three parallel measurements for each replicate. All results are presented as mean ± standard deviation.

Results and Discussion

Physicochemical Properties

ASTA value, color values as L^* , a^* and b^* , water activity and water solubility of the samples were listed in Table 1. The redness value of red pepper and its products is closely related to oleoresin quality and carotenoid content. Huang et al. reported that ASTA values of *Capsicum frutescens* oleoresin obtained using microwave, ultrasound-assisted, and ASE techniques were found to range from 607 to 850. It has been stated that the best solvent for the ASTA value of oleoresin is acetone, and in this study, oleoresin was obtained with acetone and accordingly the ASTA data were higher (Huang et al., 2022). A review of studies in the literature reveals a study using a spray-drying technique to determine the extractable color value in capsules. In this study, ASTA values were found to range from 1395.18 to 1649.68 (Zhang et al., 2023), which is similar to our BC50, CW30, and BW30 capsule values. Differences in values may vary depending on many factors, such as the encapsulation technique used, the wall material, the amount of oleoresin they contain, the type of pepper, and the drying conditions. Most researchers have focused on dried peppers rather than capsule studies (Fikiru et al., 2024).

When the L^* , a^* , and b^* values of the capsules are examined, it is seen that there is a general decreasing trend with the increase in oleoresin they contain. Adding wall materials to capsules resulted in a decrease in dark color. Light scattering, and therefore color clarity, can vary depending on capsule geometry, content, and microstructure (McClements, 2002). When Table 1 is examined, the a_w values of the capsules, except for BW30 (a_w : 0.64), are lower than 0.6. When the wax capsules were subjected to multiple comparisons among themselves, it was determined that there was no

difference between BC and CW capsules, while three different percentages of BW capsules (containing 20–30–50% oleoresin) exhibited statistically significant differences. In a study encapsulating green tea extract with beeswax and carnauba wax, the capsules' a_w values were found to range from 0.46 to 0.93. Carnauba wax had a water activity of 0.46 to 0.92, while beeswax had a_w values of 0.76 to 0.93. The current study, as in our study, reported that beeswax capsules had higher a_w values (Tay, 2022). In the moisture results of the capsules, it is seen that most capsules give results in close range and the difference is statistically insignificant. The water solubility values of wax capsules range from 0.98% to 2.21%. These values indicate that the capsules can be easily added to a water-based product to obtain a final product suitable for the intended purpose. In our previous study, water solubility values were found to be 12–21% in capsules obtained by ionic gelation and 100% in co-crystal capsules (İncegöl et al., 2025). In studies where *Capsicum* oleoresin was encapsulated with different wall materials, water solubility values were found to be in the range of 91.97–97.90 (Gabriela et al., 2021) and 65.94–92.17. *Capsicum* oleoresin was encapsulated with gum arabic, corn starch and modified malt wall materials in spray dryer and the water solubility was found to be 74.55–94.50% in the related study (da Silva Anthero et al., 2022).

Total Contents of Phenolics, Flavonoids, Carotenoids, and Antioxidant Activity

An examination of current studies on the bioactive compounds of hot pepper and hot pepper oleoresin shows that the results depend on multiple factors. The species of the pepper, its maturity, cultivation conditions, harvest time, post-harvest treatments, drying parameters, extraction technique, operational conditions, and the solvent composition

Table 1 Physicochemical properties of samples

Sample	Total extractable color (ASTA)	L^*	a^*	b^*	Moisture (%)	Water activity	Water solubility (%)
CW20	610.71 ± 0.91 ^b	40.40 ± 0.11 ⁱ	44.98 ± 0.18 ^h	34.56 ± 0.25 ^h	0.92 ± 0.03 ^e	0.36 ± 0.04 ^a	1.09 ± 0.07 ^a
CW30	1041.19 ± 2.23 ^e	36.67 ± 0.02 ^f	41.90 ± 0.02 ^f	28.93 ± 0.06 ^f	0.63 ± 0.04 ^{bcd}	0.36 ± 0.05 ^a	0.99 ± 0.17 ^a
CW50	2307.67 ± 9.17 ⁱ	33.08 ± 0.02 ^d	35.71 ± 0.02 ^d	19.46 ± 0.03 ^d	0.54 ± 0.15 ^{abc}	0.35 ± 0.04 ^a	1.23 ± 0.11 ^{ab}
BW20	753.35 ± 7.68 ^c	29.54 ± 0.03 ^b	28.96 ± 0.05 ^c	13.60 ± 0.04 ^c	0.81 ± 0.02 ^{de}	0.46 ± 0.07 ^b	0.98 ± 0.01 ^a
BW30	1082.72 ± 15.01 ^f	29.96 ± 0.02 ^c	28.32 ± 0.07 ^b	12.36 ± 0.13 ^b	0.68 ± 0.03 ^{cd}	0.64 ± 0.02 ^c	1.79 ± 0.36 ^c
BW50	2264.82 ± 8.27 ^h	25.99 ± 0.06 ^a	16.17 ± 0.05 ^a	7.15 ± 0.08 ^a	0.68 ± 0.02 ^{cd}	0.50 ± 0.00 ^b	2.21 ± 0.17 ^d
BC20	502.78 ± 7.85 ^a	37.21 ± 0.02 ^g	45.01 ± 0.01 ^h	32.37 ± 0.08 ^g	0.50 ± 0.05 ^{abc}	0.38 ± 0.06 ^a	1.20 ± 0.11 ^{ab}
BC30	972.86 ± 6.16 ^d	35.30 ± 0.10 ^e	40.32 ± 0.05 ^e	24.62 ± 0.09 ^e	0.49 ± 0.15 ^{ab}	0.32 ± 0.00 ^a	1.43 ± 0.03 ^b
BC50	1638.7 ± 1.84 ^g	38.31 ± 0.02 ^h	42.65 ± 0.04 ^g	35.93 ± 0.12 ⁱ	0.38 ± 0.03 ^a	0.35 ± 0.01 ^a	1.17 ± 0.05 ^{ab}

All data are represented as mean ± SD for triplicate determinations. Different letters in the same column indicate significant differences between samples according to the Duncan test ($p < 0.05$). CW20-30-50: carnauba wax capsule containing 20–30–50% oleoresin, BW20-30-50: beeswax capsule containing 20–30–50% oleoresin, BC20-30-50: carnauba: beeswax capsule containing 20–30–50% oleoresin

used are the primary factors influencing the outcomes. In a study investigating the production of spice oleoresins using different solvents, Soxhlet extraction of chili pepper was performed with ethanol, acetone, and their mixtures. In this study, conducted by the researchers, the total phenolic content of the oleoresin was reported to range from 30.83 to 54.53 mg GAE/g oleoresin, while the total flavonoid content ranged from 35.63 to 54.14 mg QE/g oleoresin (Devage et al., 2023). In our previous study, oleoresin bioactivity properties showed a similar range to the literature (İncegöl et al., 2025).

An examination of Table 2 reveals that the highest values among wax capsules were found to be those containing 50% oleoresin. In terms of wall material, beeswax capsules had the highest values, while carnauba wax generally had the lowest. The BW20 and CW50 capsules were in the same group in terms of total phenolic content. In other words, the 50% oleoresin in carnauba wax may not have been adequately trapped by the wax. As the amount of oleoresin contained in the capsules increased, the capsules exhibited higher phenolics, flavonoids, carotenoids and antioxidant activity scores. When the capsules were evaluated for phenolic, flavonoid, carotenoid, and antioxidant substances, the highest values were observed in the BW50, CW50, and BC50 capsules. Antioxidant activity remained low in BW20, suggesting that the matrix properties of beeswax may hinder the measurement of antioxidant activity at low oleoresin loads or limit the release of compounds.

When examining the bioactive substance content of oleoresin-containing capsules, very few studies have been conducted with existing wall materials. Studies on waxes and hot peppers are not yet available, while a few studies exist for sucrose and sodium alginate. The antioxidant activity of propolis-loaded carnauba wax capsules was determined using the DPPH radical and reported as 5.4 µg TE/µg capsule

(Shalaby et al., 2024). Anthocyanins were encapsulated by combining them with sodium alginate and oil/carnauba wax/beeswax at different percentages (1%, 2%, and 3%). However, the relevant study did not focus on phenolic, flavonoid, or antioxidant contents; instead, it examined encapsulation efficiency, structural properties, and the percentage release of phenolics and anthocyanins (Soiklom et al., 2024). A study in which thyme essential oil was encapsulated using sugarcane wax also exists, and in this study, the total phenolic content of the capsules was reported to range between 31.34 and 39.27 mg GAE/g capsule (Hashim et al., 2024). For stability studies of pure capsaicin, direct encapsulation was performed. The color change of capsicum oleoresin encapsulated by co-crystallization with sucrose was investigated (Federzoni et al., 2019), but no data were available on the phenolics, flavonoids, antioxidants, or carotenoids of the capsules. In encapsulation processes, the characteristics of the final product vary according to the properties of both the core material and the wall material. A wide range of factors such as the encapsulation technique used, the type of wall material, the nature of the active compound, processing time and temperature, the presence of other components in the system, the wall-to-core ratio, and the interactions between the core and wall collectively influence capsule quality. When the current literature is evaluated as a whole, the phenolic, flavonoid, carotenoid, and antioxidant contents obtained in our study appear to be consistent and within the expected ranges. According to the results obtained, there is a general upward trend in total phenolic, flavonoid, and carotenoid contents, as well as antioxidant activity, depending on the increase in core material; in this context, it was determined that BW50 and BC50 samples, in particular, reached the highest values in terms of these biological properties. This suggests that the oleoresin load is a direct determinant of the bioactive profile of the capsules. In our study, the

Table 2 Total phenolic substance, total flavonoid, total carotenoid, and antioxidant activity contents in capsules

Sample	Total phenolics (mg GAE/g capsule)	Total flavonoids (mg CE/g capsule)	Total carotenoids (mg (βCE/g capsule)	Antioxidant activity (mg TEAC/g capsule)
CW20	3.24 ± 0.19 ^a	20.23 ± 0.77 ^a	59.23 ± 2.12 ^{ab}	6.07 ± 0.35 ^d
CW30	4.70 ± 0.39 ^b	33.72 ± 3.66 ^b	93.33 ± 1.44 ^c	8.32 ± 0.08 ^e
CW50	9.11 ± 0.55 ^d	48.78 ± 1.93 ^c	170.24 ± 1.86 ^e	9.19 ± 0.10 ^f
BW20	8.93 ± 0.40 ^d	23.61 ± 2.16 ^a	63.98 ± 6.75 ^b	1.14 ± 0.21 ^a
BW30	13.24 ± 1.42 ^{fg}	31.32 ± 4.68 ^b	103.98 ± 3.40 ^d	2.61 ± 0.69 ^b
BW50	23.27 ± 1.05 ⁱ	59.90 ± 2.82 ^e	210.03 ± 13.58 ^f	9.25 ± 0.69 ^f
BC20	7.60 ± 0.09 ^c	19.34 ± 1.33 ^a	52.51 ± 0.68 ^a	4.36 ± 0.31 ^c
BC30	11.39 ± 0.14 ^e	29.69 ± 0.94 ^b	91.09 ± 1.75 ^c	6.51 ± 0.30 ^d
BC50	20.14 ± 1.53 ^h	53.38 ± 2.93 ^d	170.83 ± 3.37 ^e	10.13 ± 0.34 ^g

All data are represented as mean ± SD for triplicate determinations. Different letters in the same column indicate significant differences between samples according to the Duncan test ($p < 0.05$). CW20-30-50: carnauba wax capsule containing 20-30-50% oleoresin, BW20-30-50: beeswax capsule containing 20-30-50% oleoresin, BC20-30-50: carnauba: beeswax capsule containing 20-30-50% oleoresin. GAE, gallic acid equivalent; CE, catechin equivalent; TEAC, Trolox equivalent; βCE, beta carotene equivalent

biological activity of the capsules was evaluated based on their total phenolic content, flavonoid, carotenoid, and capsaicinoid content, as well as their antioxidant capacity. Bioavailability analyses were excluded from the scope of this study, which is considered a limitation. Future studies are recommended to investigate the bioavailability of the capsules using in vitro digestion models and in vivo analyses.

Encapsulation Efficiency

Encapsulation efficiency of CW20 (91.91%) and BW20 (90.69%) were similar and the highest but CW30 and BC20 were not in the same group with CW20 and BW20 pair. It can be interpreted that the BW30 and BW50 samples do not present a statistically significant difference, meaning that the increase in oleoresin percentage does not affect the activity value, and that the beeswax cannot effectively encapsulate 50% oleoresin. When examining the overall efficiency values, it was determined that the efficiency value decreased as the core (oleoresin) ratio increased. This may mean that as the amount of core material (oleoresin) increases, there may not be enough wall material left to completely surround the core or maintain the capsule structure intact. While beeswax and carnauba wax alone yielded high encapsulation efficiency, using a mixture of these two waxes resulted in lower EE values (Table 3). The main reason for this difference may be that the incompatibility in the crystallization and melting properties of the different waxes caused a more heterogeneous structure within the mixture matrix (Galvão et al., 2020). Heterogeneous crystal structures can increase the micropermeability of the capsule wall, facilitating diffusion of the core component to the surface and

thus reducing encapsulation efficiency. Galvão et al. (2020) reports that disruption of the crystalline jarring in such lipid matrix mixtures negatively affects microstructural integrity and therefore provides relatively lower protection. Other researchers have similarly reported that increased core material negatively impacts efficacy (Zahran et al., 2022; Song et al., 2025; Twumasi et al., 2025).

When existing studies are examined, the efficiencies of capsules obtained by ionic gelation technique were reported as 87–89% for pure capsaicin (Kulig et al., 2023), 80% for fish oil (Bannikova et al., 2018), and 96–98% for peppermint essential oil (Yilmaztekin, et al., 2019). In the study where green tea extract was encapsulated using sodium alginate, maltodextrin, canauba wax and beeswax, the efficacy data were found to be in the range of 47.3–52.75% for carnauba wax and 30.38–31.99% for beeswax, while the efficiency of sodium alginate capsules was determined to be in the range of 38.93–78.32% (Tay, 2022). The lower efficiency values found here than in our study can be explained by the hydrophilic properties of the core material. Studies conducted with waxes in the literature report efficacy data ranging from 47 to 90% (Rocha et al., 2020). Encapsulation of capsicum oleoresin was carried out with gum arabic, modified malt, and corn starch and the efficiency was found in the range of 68.5–91.6% (da Silva Anthero et al., 2022). Trapani et al. (2019) encapsulated grape seed extract using solid lipid particles, and in the relevant study, encapsulation efficiency values were reported to be in the range of 40.1–82.7% (Trapani et al., 2019). Thyme and sage extracts were encapsulated using carnauba wax and witepsol lipids, and the efficiency values in the study were determined as 79–87.4% (Campos et al., 2015). In a study where flurbiprofen, the active pharmaceutical ingredient, was encapsulated using a mixture of carnauba and beeswax, the efficiency value was determined in the range of 96.4–96.9%, while increasing the percentage of beeswax in the mixture did not positively affect the efficiency (Baviskar et al., 2012). Another study using carnauba wax was the encapsulation of propolis, the efficiency value was found to be 99.28–99.81% (Shalaby et al., 2024). In a study encapsulating clove oil using carnauba and beeswax, the encapsulation efficiency was found to be 58–60% for carnauba and 63–66% for beeswax (Cristina et al., 2019). Researchers encapsulating omega-3 and vitamin D3 with beeswax reported encapsulation efficiency of 92.3% and 86.3% for vitamin D3 and omega-3, respectively (Ghobadi & Sohrabvandi, 2024).

Various factors such as the type of wall material, wall-to-core ratio, encapsulation technique, mixing ratios and properties of the active ingredient play an important role in determining encapsulation efficiency. As the amount of wall material/wall-to-core ratio increases, the effectiveness of the capsules can be expected to improve up to a certain point. In general, it has been observed that the encapsulation

Table 3 Encapsulation efficiency of capsules

Sample	Wall-to-core (g/g)	Encapsulation efficiency (%)
CW20	4	91.92 ± 0.06 ^e
CW30	2.3	86.49 ± 0.06 ^d
CW50	1	81.40 ± 0.26 ^b
BW20	4	90.69 ± 0.12 ^e
BW30	2.3	78.43 ± 0.76 ^a
BW50	1	77.15 ± 0.03 ^a
BC20	4	86.92 ± 1.68 ^d
BC30	2.3	83.47 ± 0.45 ^c
BC50	1	80.67 ± 0.27 ^b

All data are represented as mean ± SD for triplicate determinations. Different letters in the same column indicate significant differences between samples according to the Duncan test ($p < 0.05$). CW20–30–50: carnauba wax capsule containing 20–30–50% oleoresin, BW20–30–50: beeswax capsule containing 20–30–50% oleoresin, BC20–30–50: carnauba: beeswax capsule containing 20–30–50% oleoresin

efficiency obtained with single wax coating systems (beeswax or carnauba wax) is higher compared to mixed beeswax–carnauba wax systems. This can be attributed to the encapsulation of oleoresins and active compounds, which exhibit hydrophobic properties, within solid lipids that also possess hydrophobic structures. Although the wall-to-core ratio in solid lipids is relatively low, the higher efficiency compared to the other two techniques can be associated with the compatibility between the wall and core materials. In the encapsulation process with the melt dispersion technique, it is predicted that the solubility of the hydrophilic or hydrophobic core component in the molten lipid, the physico-chemical structure of the lipid as the wall material, and the polymorphic structure of the lipid during the solidification process after melting affect the encapsulation efficiency.

Total Capsaicinoids and Scoville Heat Unit

As the pungency values of hot pepper are calculated based on the amounts of capsaicinoids they contain, there is a linear relationship between pungency and capsaicinoid content (Lozada et al., 2021). The capsaicinoid content, SHUs and pungency levels of the capsules and the samples were listed in Table 4. It was determined that BC20 and BW20 shared the same group in terms of SHU and capsaicinoid content, with the lowest values among the capsules. These two capsules were followed by BW30 and BC30, sharing the same group.

Kaiser et al. produced chitosan-containing nanocapsules of capsaicin for sensory testing, and it was determined that the encapsulation process changed the threshold value and affected the release (Kaiser et al., 2015). In a study in which pure capsaicin capsules were obtained by encapsulating standard capsaicin using the spray cooling technique, it was reported that the powder capsules contained 13.25–66.23 mg/kg total capsaicin and SHU values were

in the range of 490–15800 (Günel et al., 2021). The health effects of gum arabic-modified malt wall materials containing 2.5% and 5% Capsicum oleoresin and the oleoresin particles obtained by spray drying on mice were investigated (Hulagannavar et al., 2024). When existing studies were examined, very few studies aimed at suppressing the pungency of hot peppers, and these studies did not address capsule properties, capsaicinoids, or SHU values. When comparing the capsaicinoid, SHU, and bioactive properties of hot pepper oleoresin with existing studies, parallel results and average values were obtained. An examination of the capsules' pungency category reveals that all are super-hot. A significant increase in total capsaicinoid content and SHU values is observed with increasing oleoresin content. For example, in the CW group samples, CW50 (50% oleoresin) has the highest values. These findings indicate that increasing the oleoresin content increases the encapsulation efficiency of capsaicins and facilitates the preservation of bioactive compounds through hydrophobic interactions within the lipid matrix. Even samples with low oleoresin content maintained the “super hot” pungency level, demonstrating effective encapsulation in all formulations. Differences observed depending on the system and lipid type used may be due to changes in wall-core compatibility and lipid matrix structure.

Morphological Analysis

The surface morphology of the capsules was analyzed using scanning electron microscopy (SEM) to evaluate how the encapsulation process influenced their structural and surface characteristics. SEM micrographs of the carnauba (CW), beeswax (BW), and blend (BC) capsules are presented in Figs. 2, 3, and 4, respectively.

When the surface morphology of carnauba wax capsules is examined, it is observed that an increase in

Table 4 Total capsaicinoids, SHU and pungency levels of capsules

Sample	Total capsaicinoids (mg/kg)	SHU	Pungency level
CW20	116,707.46 ± 5301.21 ^c	1,878,990.06 ± 85,349.43 ^c	Super hot
CW30	132,826.47 ± 2108.88 ^d	2,139,126.36 ± 34,627.97 ^d	Super hot
CW50	184,854.75 ± 1734.21 ^g	2,976,161.43 ± 27,920.81 ^g	Super hot
BW20	91,924.87 ± 1072.64 ^a	1,482,424.29 ± 14,009.71 ^a	Super hot
BW30	101,130.37 ± 2811.17 ^b	1,645,800.86 ± 20,366.91 ^b	Super hot
BW50	177,573.07 ± 1233.64 ^f	2,851,202.09 ± 8937.74 ^f	Super hot
BC20	90,358.88 ± 1437.82 ^a	1,454,778.04 ± 23,148.83 ^a	Super hot
BC30	105,305.18 ± 2767.00 ^b	1,683,343.73 ± 42,457.98 ^b	Super hot
BC50	161,473.73 ± 9945.27 ^e	2,603,528.53 ± 164,186.10 ^e	Super hot

All data are represented as mean ± SD for triplicate determinations. Different letters in the same column indicate significant differences between samples according to the Duncan test ($p < 0.05$). CW20–30–50: carnauba wax capsule containing 20–30–50% oleoresin, BW20–30–50: beeswax capsule containing 20–30–50% oleoresin, BC20–30–50: carnauba: beeswax capsule containing 20–30–50% oleoresin. SHU, Scoville Heat Unit

Fig. 2 SEM image of carnauba wax capsules. CW20–30–50: carnauba wax capsules containing 20–30–50% oleoresin (F: ×2000)

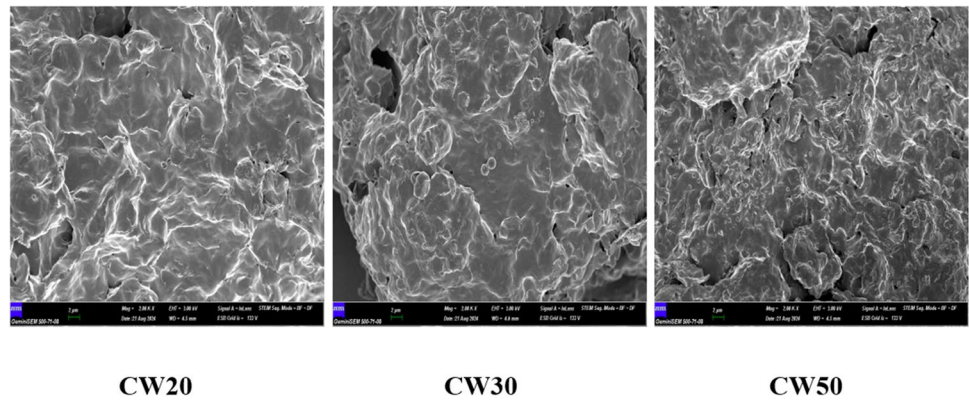


Fig. 3 SEM image of beeswax capsules. BW20–30–50: beeswax capsules containing 20–30–50% oleoresin (F: ×2000)

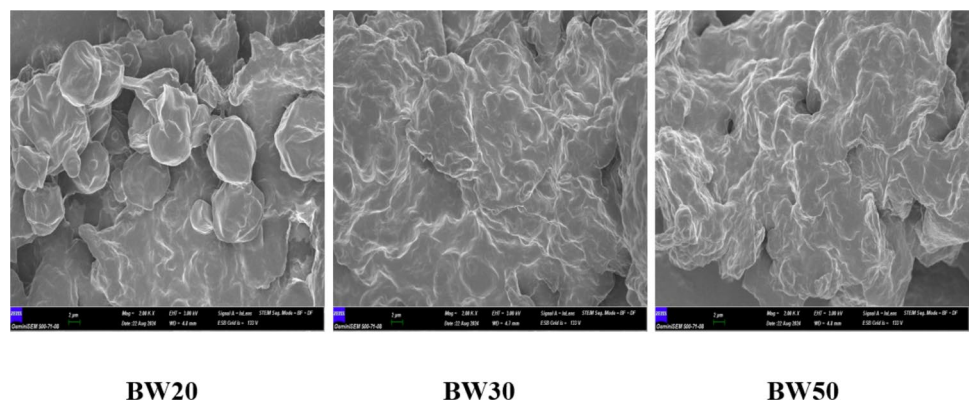
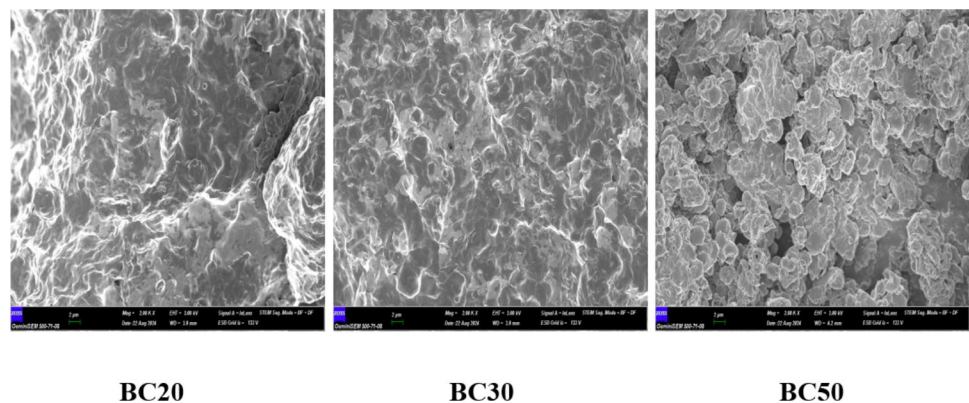


Fig. 4 SEM image of blend wax capsules. BC20–30–50: beeswax: carnauba capsules containing 20–30–50% oleoresin (F: ×2000)



oleoresin content causes an increase in surface porosity, which can be said to deteriorate homogeneity. Studies on solid lipids have shown that emulsion, mixing speed, and time affect capsule surface morphology (Costa et al., 2020). A review of existing studies indicates that surface morphology largely depends on the initial wall-to-core ratio and emulsification time; however, carnauba

wax microcapsules typically exhibit porosity and localized agglomeration (Rocha et al., 2020). In contrast, SEM analyses of pure carnauba wax microcapsules have shown that they possess irregular, non-porous surfaces and generally maintain a spherical morphology (Khosro-nia et al., 2023). In a study encapsulating baking powder with carnauba and beeswax, it was stated that the empty

forms of both wall materials were spherical and that the shape changed with the addition of the target component, revealing roughness and pores on the surface (Khosronia et al., 2023) (Milanovic et al., 2010). The SEM images obtained from the encapsulation of salt with carnauba and the capsule images in our study were not spherical and showed similar results (Henrique et al., 2021). In our study, the capsules contained a significant amount of core material, so the lack of a spherical structure can be interpreted as the morphological properties of the oleoresin are also effective.

Figure 3 shows the morphological characteristics of beeswax oleoresin capsules. The BW20 capsule appears to be more spherical than the other two capsules. As the oleoresin content increases, the observed spheres disappear, revealing agglomerated structures. This can be attributed to the irregular structure of the oleoresin. Generally, the capsule images reveal an irregular appearance, which has been attributed to the slow solidification tendency of beeswax (Milanović et al., 2017).

Surface images of the BC (blend capsule) are given in Fig. 4. It is observed that the blend capsules have a more porous structure than the 50% capsules of carnauba and beeswax.

Milanović et al. (2017) achieved flavor encapsulation using carnauba wax, beeswax, and a mixture of the two. In the study, they also stated that increasing beeswax content caused irregularities and roughness in the structure (Milanović et al., 2017). Differences in SEM images of solid lipids vary depending on various factors, such as seed percentage, mixing speed and duration, melting point of the wax, and solidification rate. Particle size and appearance vary depending on the physicochemical properties of the target material to be immobilized, the amount of wall material included, and the encapsulation method. Considering the SEM image results, the fact that the mixed capsules have a more porous structure than the individual wax capsules may be related to their sensorially perceived pungency. Carnauba and beeswax are the most effective wall materials in suppressing capsaicin pungency, and considering the surface morphologies of the capsules, the capsule with the highest oleoresin content in beeswax exhibits the smoothest morphology, which may be directly proportional to the greatest suppression of pungency.

FT-IR Analysis

FT-IR spectra of oleoresin and its capsules are shown in the Figs. 5, 6, and 7 below. FT-IR spectra were scanned to identify any adverse reactions to oleoresin and oleoresin-containing capsules using the chemical groups examined in the infrared region. According to the CW capsule

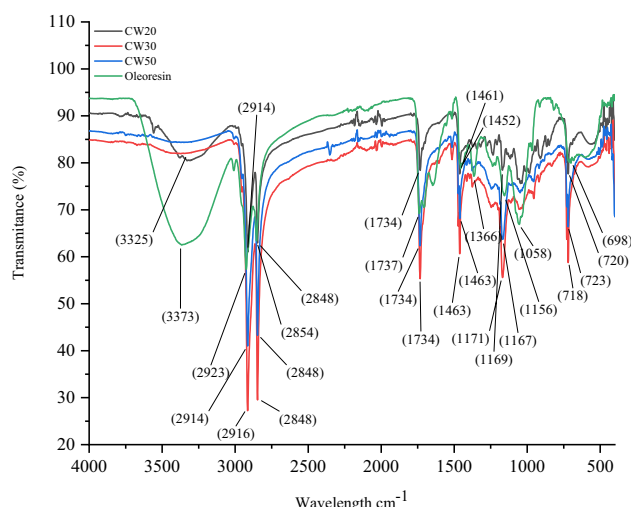


Fig. 5 FT-IR analysis of carnauba wax capsules. CW20–30–50: carnauba wax capsules containing 20–30–50% oleoresin

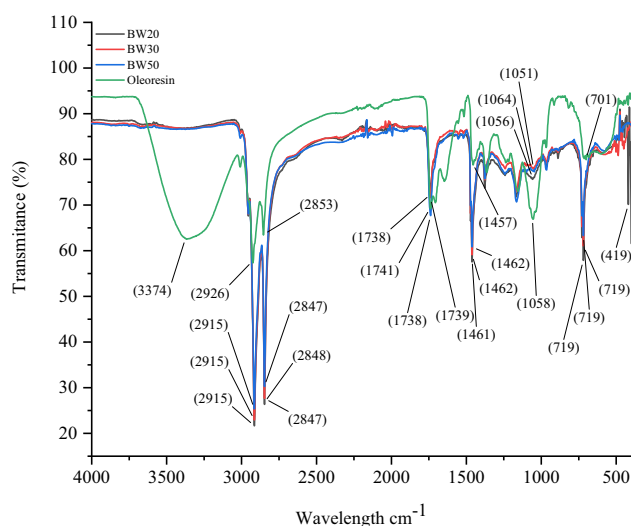


Fig. 6 FT-IR analysis of beeswax capsules. BW20–30–50: beeswax capsules containing 20–30–50% oleoresin

spectrum (Fig. 5), the sharpest peaks in carnauba were observed at around 2900 cm^{-1} . Other prominent peaks were observed at wavenumbers 1700, 1100, and 700. An examination of the 3300 band revealed that these peaks in capsules were significantly less pronounced than in oleoresin, suggesting that a decrease in the number of OH groups may have occurred due to core-wall interactions.

The sharpest peaks were produced by the CW30 sample. C–H at $2916\text{--}2848$, C=O at 1734, and C–O–C stretching at $1100\text{--}1400$ represent the C–O–C group. The C–O–C group of the skeletal stretching vibration of aromatic rings was observed around 1100 cm^{-1} . There are studies in which similar peaks were obtained at similar wavenumbers (Khosronia

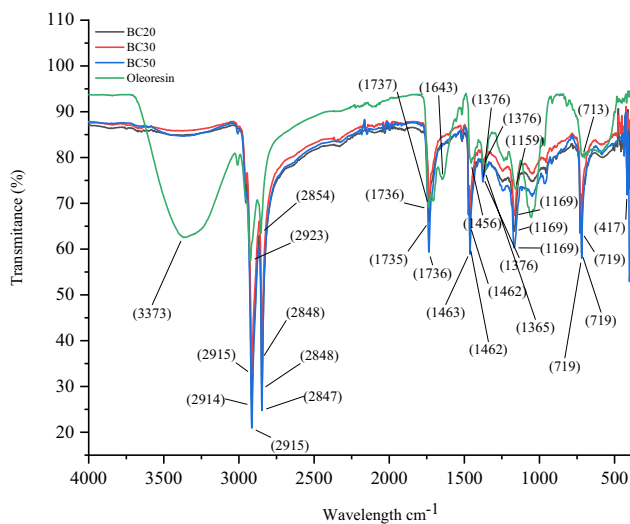


Fig. 7 FT-IR analysis of blend capsules. BC20–30–50: beeswax: carnauba wax capsules containing 20–30–50% oleoresin

et al., 2023; Shalaby et al., 2024; Soiklom et al., 2024). When the spectrum of pure carnauba wax was examined, peaks were obtained at wave numbers of 2964–2901, 1842, 1715, 1632, 1585, 1387, 1297, 980, 878, and 680 cm^{-1} (Khosronia et al., 2023; Soiklom et al., 2024). Peaks obtained at similar locations to our study showed minor changes, which may be due to the interaction with oleoresin. Generally speaking, encapsulation by carnauba wax can be achieved without altering the structure of the core material.

The FT-IR spectrum of beeswax capsules is shown in Fig. 6. When the spectrum is examined, it is seen that the BW20, BW30, and BW50 capsules exhibit very similar peaks, but these peaks differ from the oleoresin at some points. The characteristic oleoresin peak at 3374 cm^{-1} was not observed in the beeswax capsules. The peaks around 2900 were sharper and more distinct in the beeswax capsules. Similarly, the capsules produced sharper and more pronounced peaks at wavenumbers 1400 and 700. When the pure beeswax spectrum is examined, it can be said that the results obtained in our study are consistent and similar to those in the literature (Khosronia et al., 2023; Soiklom et al., 2024).

It has been reported that small peaks at 2000 cm^{-1} , not present in pure beeswax encapsulation of baking powder, are produced by wall-core interactions. In a study yielding very similar data to ours, the core material, baking powder, produced a peak in the 3200 cm^{-1} band. However, this peak was not observed in beeswax capsules, and it was reported that the wall-core interactions and changes in the number of H_2 and O_2 atoms within the capsule affected the results (Khosronia et al., 2023). The decrease in the wave number of the capsules can be explained by the decrease in free

charged groups and the increase in bond interaction due to the presence of two substances.

Spectral analyses of the blended capsules are shown in Fig. 7. When the spectrum is examined, distinct peaks are observed at 2900, 1700, 1400, 1100, and 700. Similar peaks were observed with the pure oleoresin peaks. However, the capsule peaks are sharper and clearer. The blended capsules also yielded results similar to both oleoresin and wax spectra. However, the peak in the 3300 band observed in the carnauba wax capsules did not occur in the beeswax and blended capsules. This may be due to the presence of two different wax blends, which may have interacted with the oleoresin differently than in the other capsules, resulting in a decrease in the number of OH groups. Similarly, the beeswax capsules did not exhibit peaks in the 3300 band, with the wax group being the dominant group in the blended capsules.

The sharpest peaks were found in the BC50 group. A literature review revealed that aroma compounds are encapsulated by preparing a wax blend. In this study, a blend of carnauba and beeswax (10–50%) was prepared to encapsulate the aroma compound ethyl vanillin. The capsule dimensions, shapes, and thermal properties were investigated in the study (Milanović et al., 2017). The data obtained indicate that the original functional structure remains unchanged after encapsulation. These findings are consistent with other studies reporting that no chemical interactions occur in the capsules during encapsulation (Gundev et al., 2022; Soiklom et al., 2024; Techawinyutham et al., 2019).

Thermogravimetric Analysis

The water present in the food structure is removed from the structure in 3 different stages: free water at 40–60 °C, bound water at 80–120 °C, and water more tightly bound to carboxylate groups at 160 °C (Laurienzo et al., 2005).

TGA was applied to determine the thermal stability of the samples, and the resulting data are given in Fig. 8. According to the graph, the capsule residue amounts were 4.84% for CW20, 6.53% for CW30, and 1.73% for CW50, while in our previous study, the residue amount of oleoresin was found to be 3.28% (Incegül et al., 2025). Based on these data, it can be concluded that carnauba wax has better thermal stability than oleoresin. Increasing the CW ratio (especially CW50) increased the stability of the system. A similar situation has been observed in the encapsulation of urea with carnauba (Medeiros et al., 2019). For CW20, approximately half of the mass was lost at 375 °C, for CW30 at 367 °C, and for CW50 at 377 °C.

According to Fig. 8, the temperatures at which 50% mass loss occurred for BW20, BW30, and BW50 wax capsules were 305, 294, and 318 °C, respectively. Similarly, the residue amounts were determined to be 2.80%, 4%, and 0.5%.

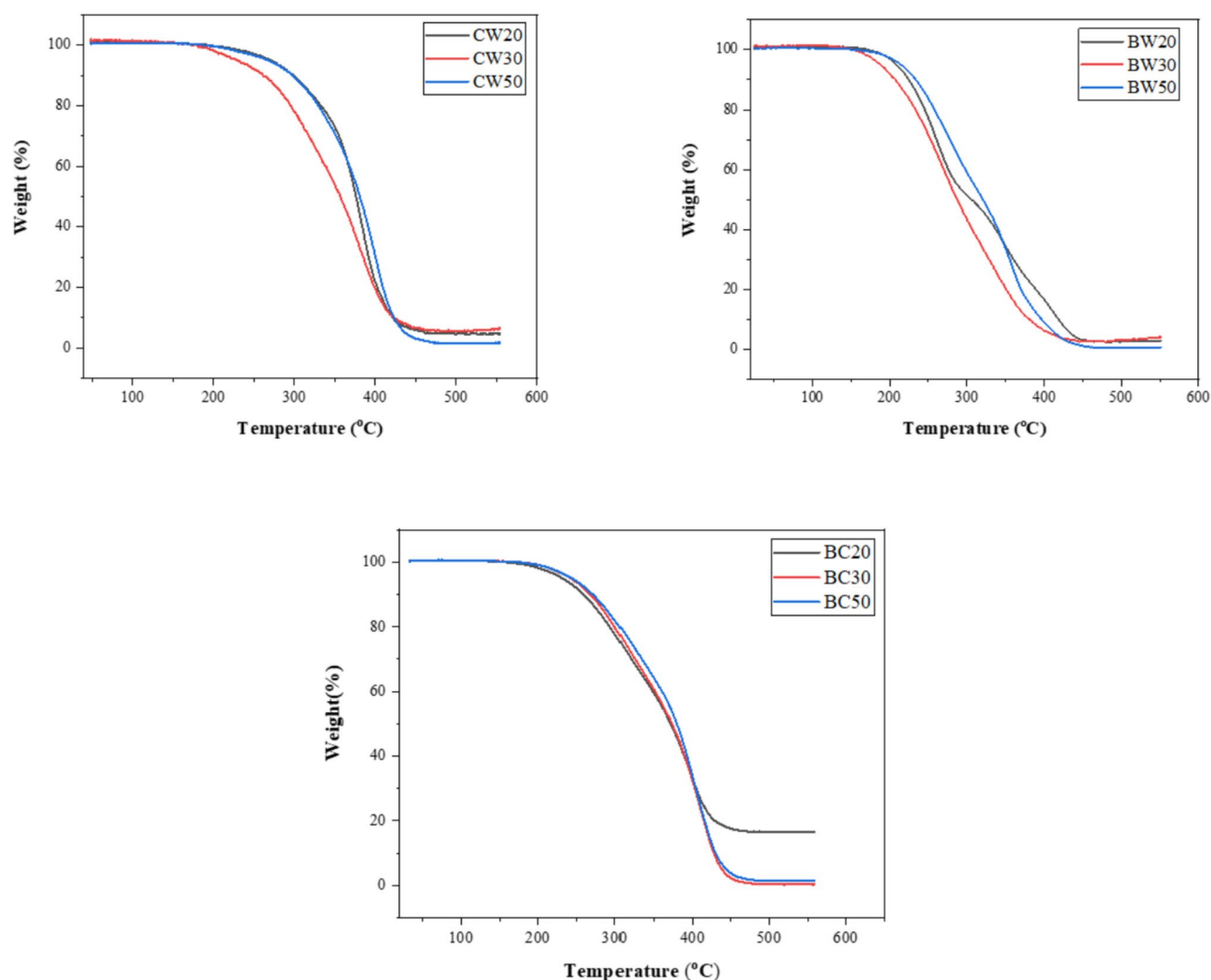
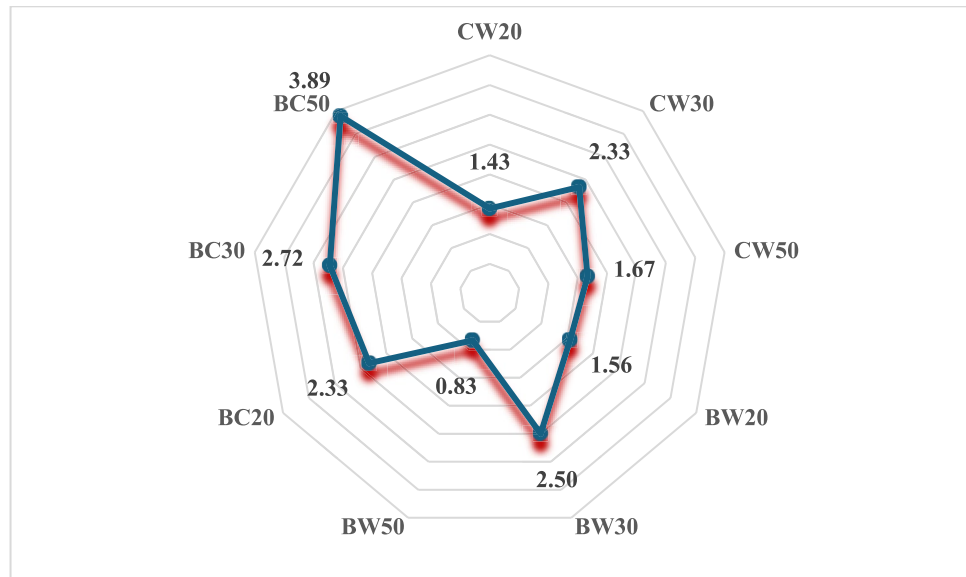


Fig. 8 TGA graph of capsules. CW20–30–50: carnauba wax capsules containing 20–30–50% oleoresin, BW20–30–50: beeswax capsules containing 20–30–50% oleoresin, BC20–30–50: beeswax: carnauba wax capsules containing 20–30–50% oleoresin

In the study where retinyl palmitate was encapsulated with beeswax, paraffin, and carnauba wax, it was stated that the beeswax samples showed initial degradation around 312 °C and completely degraded after 465 °C (Nandy et al., 2020). Similarly, in our study, complete degradation occurred at approximately 450 °C. Nandy et al. also reported residue levels close to zero for carnauba and beeswax capsules. The sample leaving the highest residue in the mixture capsules was the BC20 capsule, followed by the oleoresin, BC50, and BC30 capsules. Looking at the general curves of the waxes, a uniform deformation without complexities is observed. Capsule losses are 50% at 371 °C for BC20, 362 °C for BC30, and 366 °C for BC50. Examination of the graphs shows that thermal decomposition and mass losses occurred in a single stage. Parallel graphs were obtained in green tea and carvacrol encapsulation, similar to our study (Galvão et al., 2020; Tay, 2022). It has been reported

that the temperature range for beeswax decomposition is 180–480 °C with a mass loss of 99.5%, while for carnauba wax, this temperature range is 250–500 °C with a mass loss of 98.5% (Galvão vd., 2020). The relevant study determined that the loss was greater in beeswax, and the same situation is observed in our study. The greater loss can be attributed to the lower melting point of beeswax. Among the three capsule types, the initial temperature at which the waxes decomposed was found to be higher than that of oleoresin; this can be attributed to the wall-core material relationship. It has been stated that encapsulation of retinyl palmitate with carnauba and beeswax resulted in better thermal stability of carnauba wax compared to beeswax (Nandy et al., 2020). The same situation has been observed in green tea capsules (Tay, 2022). The lower amount of beeswax residue in this study was found to be consistent with the literature. The incorporation of the produced capsules into food formulations and

Fig. 9 Radar plot of capsules displaying the level of perceived pungency at equal level of 0.1. CW20-30-50: carnauba wax capsules containing 20-30-50% oleoresin, BW20-30-50: beeswax capsules containing 20-30-50% oleoresin, BC20-30-50: beeswax: carnauba wax capsules containing 20-30-50% oleoresin



their stability within these matrices are of critical importance. The assessment of thermal stability indicates that the majority of the capsules are resistant to the processing conditions commonly applied in food systems. Moreover, thermal decomposition does not occur at typical processing temperatures, suggesting that these capsules can be safely and effectively incorporated into food formulations without compromising their structural integrity or functionality.

Sensory Evaluation

Daily consumption of hot peppers varies greatly depending on geographical region and dietary habits. While hot peppers are an integral part of daily meals in Asian, Latin American, and Mediterranean cuisines, their consumption is more restricted in some societies (Siebert & Lee, 2022). The pungency (burning sensation) characteristic of hot peppers is primarily due to the presence of capsaicinoid compounds, particularly capsaicin and dihydrocapsaicin. These compounds activate TRPV1 (Transient Receptor Potential Vanilloid 1) receptors in the oral cavity, creating a sensory perception similar to burning, heat, and pain. Individual tolerance, habits, cultural background, and genetic factors significantly influence the perception of pungency in chili pepper consumption (Siebert & Lee, 2022). In the development and formulation of foods containing chili peppers and capsaicin, it is not enough to simply determine the chemical components; evaluating the intensity of pungency, persistence in the mouth, and overall acceptability through sensory analysis is of great importance. In this study, sensory analysis was conducted to quantitatively measure consumers' perception of spiciness for the developed capsules. Especially for products intended for daily consumption, ensuring that the pungency level is consistent with consumer expectations

is considered a critical factor for sustainable consumption and product success.

Overall, Fig. 9 shows that the pungency scores of the capsules are low. The CW20 sample exhibited the lowest pungency value (1.43), while the CW30 and CW50 samples had values of 2.33 and 1.67, respectively. This suggests that the harder and hydrophobic structure of carnauba wax can suppress the perceived bitterness by limiting the release of capsaicin. The lowest perception was observed in the BW50 sample (0.83), which has the lowest wall-to-core ratio among the BW capsules. Beeswax-Carnauba wax mixture (BC) capsules generally exhibited the highest perceived pungency values. In particular, the BC50 sample had the highest perceived bitterness among all samples with a value of 3.89. A relatively high pungency value (2.72) was also obtained in the BC30 sample. This suggests that the combination of the two types of wax creates a synergistic effect by facilitating capsaicin release, resulting in a stronger chemesthesia response in the mouth. Overall, the results indicate that perceived pungency is strongly dependent not only on the wall material but also on the proportion of capsule wall material, its physicochemical properties, and the oleoresin content. Although numerous studies have investigated the encapsulation of red pepper oleoresin using various techniques and wall materials to improve physicochemical stability, research specifically aimed at reducing or controlling pungency perception remains limited (Costa et al., 2023; Ferraz et al., 2022; Figueiredo et al., 2022; Zhang et al., 2023). Among the limited studies addressing pungency suppression, Günel et al. (2021) reported that spray-chilled microencapsulation of capsaicin significantly reduced its oral release after *in vitro* digestion, resulting in an approximately 4500-fold decrease in perceived pungency as determined by sensory evaluation (Günel et al., 2021).

In another study, Zhang et al. (2024) microencapsulated Sichuan red pepper oleoresin to enhance stability, ease of use, solubility, and rapid oral release of pungency, which is in direct contrast to the aim of the present study focused on pungency suppression (Zhang et al., 2024). For a similar purpose, to facilitate and expand the use of garlic oil, sensory evaluation was carried out by adding β -cyclodextrin capsules to sausage. In the relevant study, it was determined that the capsules were indistinguishable from the control group, meaning that β -cyclodextrin suppressed the pungent taste and aroma of garlic (Khoshtinat et al., 2023). No studies have been found that use waxes to suppress the burning sensation of hot peppers. In our study, the most successful capsule type in this regard was the BW50 capsule. Beeswax (E901) and carnauba wax (E903), used as encapsulation materials, are commonly used in the food industry and are considered food-grade. While largely indigestible, these substances are considered safe within permitted usage levels. Although rare, a possible risk of allergic reactions in sensitive individuals has been reported in the literature. Therefore, further studies are needed regarding long-term consumption and bioavailability. Beeswax and carnauba wax were used in this study not as direct food additives, but as microcapsule carrier matrices. Due to their low added levels, their amounts in the final product are considered compliant with regulations under the ‘‘ADI not specific’’ and ‘‘quantum satis’’ approaches defined by Authority (EFSA, 2012).

Conclusions

In our study, we selected hot pepper oleoresin as a material that cannot be consumed alone by most individuals due to personal preferences, health conditions, and various side effects, and investigated its potential for use by transforming it into different forms. In this context, beeswax, carnauba wax, and 50% mixtures of these two were selected as wall materials, and hot pepper oleoresin capsules were obtained using the melt dispersion technique. The liquid form of the oleoresin was converted to a solid form, and its pungency sensation was sensorially tested. Regarding the suppression of pungency sensation, which was the main starting point of the study, beeswax was found to be the most successful wall material with a BW50, followed by CW20 and BW20 capsules. By transforming the hot pepper oleoresin into a more tolerable form for sensitive individuals, its usage area has been increased. A parallel is observed between the sensory and SHU results, and it can be stated that the pungency sensation is suppressed. The highest encapsulation efficiency values were observed in CW20. When the biological activities of the capsules were examined, beeswax capsules showed the highest values, while carnauba wax capsules generally showed the lowest values. As the

amount of oleoresin in the capsules increased, the capsules exhibited higher bioactivity. ASTA color values increased with increasing oleoresin content in the capsule, with the highest values observed in CW50. The capsule with the highest oleoresin content in beeswax exhibits the smoothest morphology, which may be directly proportional to the greatest suppression of pungency. FT-IR results revealed that encapsulation with carnauba wax did not alter the structure of the core material. Water activity values indicate that the capsules are microbiologically safe, thus allowing for safe inclusion in various food formulations for individuals seeking the health benefits of capsaicinoids but with low sensory tolerance levels. Given the non-polar nature of waxes, they can be appropriately incorporated into aqueous formulations in various categories such as lemonade, sauces, soups, and the beverage industry. Further studies could involve conducting model food trials to investigate changes in the human body and to test the changes and stability occurring in food processing. Furthermore, the physical properties of microcapsules, such as their chemical structures, crystallization, melting point, and barrier properties, can be investigated for their use in model foods for applications involving products with different characteristics.

Author Contributions Yasemin İncegöl : Conceptualization, writing-original draft, methodology, formal analysis, writing-review and editing, investigation, validation, resources, data curation. Serap Berktaş : Formal analysis, investigation, writing-review and editing. Mustafa Çam: Conceptualization, writing-original draft, writing-review and editing, supervision, investigation, validation.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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