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RESEARCH ARTICLE

# Microencapsulated propolis in chewing gum production

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## ABSTRACT

Propolis is a resin with high antibacterial and antioxidant properties that honey bees (*Apis mellifera L.*) gather and then harmonize through their metabolic secretions. Propolis is beneficial to dental health because it contains antibacterial activity against the *Streptococcus mutans*, which causes tooth decay. The aim of this study is to use microencapculated propolis extract (MPE) in chewing gum formulation for the first time, in order to protect propolis from environmental effects during production and to ensure controlled release in mouth. Spray drying method was applied where maltodextrin acted as coating material. The particle structure of MPE was investigated by scanning electron microscope. The encapsulation efficiency was determined as 62.19%. The hygroscopicity and moisture content of the MPE particles was found as 31.50% and 7.98% respectively. The DPPH scavenging ability, total flavonoid and total phenolic content of the MPE appeared lower than propolis extract. The total flavonoid content of MPE was calculated as 29.06 mg/kg and total phenolic content as 89.17 mg/kg. *In vitro* antimicrobial activity against *Streptococcus mutans* of 9% MPE containing gum samples was measured highest with 4.20 mm zone diameter. Hunter b* value increased with increasing MPE concentration. The final MPE incorporated chewing gum appear to be highly functional.

**Keywords:** Propolis; Spray drying; Chewing gum; Functional food

## INTRODUCTION

Propolis is one of the most extensively utilized natural bee products. Propolis' antibacterial capabilities have long been known. Honey bees (*Apis mellifera* L.) blended with their own metabolic secretions to produce propolis from the young shoots of trees, bark, leaves, cones and plant buds of different types of oils, waxy substances, special resins and pollen. Propolis is a resinous substance, which has very effective anti-inflammatory, anticancer, antioxidant, anti-fungal, antimicrobial and antiviral effects (Koo et al., 2000; Popova et al., 2005; Ahn et al., 2007; Laskar et al., 2010; Jafari et al., 2022).

Propolis is normally composed of phenolic compounds, wax, resin, water, inorganic substances, and essential oils, although the exact composition varies depending on the plant source. The color of propolis may vary from yellow to green to dark brown depending on storage time and source. It is brittle and hard in cold, sticky and soft in hot temperatures. Calcium, potassium, iodine, magnesium, zinc, manganese, iron, copper, cobalt, and salt are the primary minerals contained in propolis. Vitamin A, B1, B2, B3, B6, C, and E are the primary vitamins (Marcucci, 1995).

Encapsulation in foods, in particular, is used to promote nutritional content, extend shelf life, improve digestibility, and shorten ripening time. The main microencapsulation methods of food components appear as freeze-drying, spray-drying, fluidized bed coating, cocrystallization, molecular residue and aggregation. Spray drying is preferred as an encapsulating technology in the food sector because to its low cost, better particle structure, speed, practicability, and high efficiency (Gökmen et al., 2012; Tavares et al., 2022).

In general, chewing gum is known to enhance plaque cleaning on the teeth, stimulate saliva flow Propolis has been proven to prevent tooth decay due to its significant *in vivo* and *in vitro* antibacterial activity against *Streptococcus mutans*, which is effective in dental caries (Duarte et al., 2006; Zulhendri et al., 2021). Tulsani et al. (2014) stated that chewing gum incorporating raw propolis is superior to xylitol one in reducing bacterial counts in mouth.

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In literature some novel study on microencapsulation of propolis by different methods i.e spray drying, freeze drying etc. are present (Busch et al., 2017; Sturm et al., 2019; Baysan et al., 2021; Shakoury et al., 2022; Sa et al., 2023). Tavares et al. (2022) recently listed propolis encapsulation studies and novel use of encapsulated propolis in food and pharmeceutical industries in their review study. Some attempts to enrich chewing gum or candies with microencapsulated functional ingredients were reported (Potineni and Peterson, 2008; Altınok et al., 2020; Thivya et al. 2021; Vergara et al., 2023). Propolis, either raw or in extract form, was employed in chewing gum formulations in certain studies (Soekanto et al., 2018; Bölük et al., 2021), however no studies found that microencapsulated propolis was used in chewing gum.

In this study, propolis extract was microencapsulated by spray drying method to be incorporated in chewing gum formulations in varying concentrations for the first time, in order to protect propolis' functionality from environmental effects during production as well as to achieve controlled release of propolis through chewing. For this purpose raw propolis was extracted first, then microencapsulated by spray drying method. MPE particle structure was examined by SEM. Additionally, encapsulation efficiency, moisture, hygroscopicity, free radical scavenging ability, amount of total flavonoid and total phenolic compound of MPE were determined. Then, MPE was incorporated into chewing gum formulations in varying concentrations. *In vitro* antimicrobial activity against *Streptococcus mutans* and color of the chewing gum samples were observed. The ultimate goal was to create a highly functional product.

## MATERIALS AND METHODS

### Material

Propolis samples used in the experiments were freshly scraped from the beehives in İmranlı district of Sivas province. Samples were stored at +4°C until extraction.

### Preparation of propolis extract

Approximately 14 g of propolis were dissolved in 100 ml of ethanol (Sigma-Aldrich, Germany) while being stirred for 24 hours. It was then filtered under vacuum with a Buechner funnel using filter paper. To eliminate any residual wax, the ethanol extract was frozen (-20°C) for 10 hours and centrifuged twice (Sigma, 2-16PK, Germany) for 10 minutes at 4500 rpm at 5°C. The clear liquid remaining in the supernatant was removed and stored at + 4°C (Busch et al., 2017).

### Analysis of propolis extract

Free radical scavenging ability of the propolis extract was determined by spectrophotometric DPPH method at 517 nm (Chen et al., 2003). Calculation of the total amount of flavonoid of the propolis extract by spectrophotometric aluminum nitrate method at 415 nm where quercetin (Sigma-Aldrich, India) was used as standard (Park et al., 1995). The total amount of phenolic compounds was determined by Folin-Ciocateau method using gallic acid (Sigma-Aldrich, China) as standard (Woisky and Salatino, 1998).

### Emulsion preparation

30 g maltodextrin (Alfasol, Turkey) (DE 14-17) was added to 100 ml distilled water and thoroughly mixed at 25 °C (Wisestir, MSH 20-A, Korea). The mixture was then homogenized by Ultra-Turrax (Yellow Line IKA, DI 25 basic, Germany) at 12000 rpm, while 10 ml propolis extract was being added dropwise for 10 minutes to form an emulsion.

### Microencapsulation by spray drying

Laboratory scale The Buchi B-290 (Flawil, Switzerland) mini-spray dryer in the Pilot Plant of TÜBİTAK Marmara Research Center (Istanbul) was employed in the study. The feed rate was 4 ml/min and the air inlet temperature was set to 120 °C. The aspirator setting was 85%, the pump setting was 13% and the compressed air setting was 0.30 bar. The outlet temperature varied between 70-65 °C. Encapsulation was performed in the spray dryer for 8 hours. The final product was stored in plastic beakers in the refrigerator at +4 °C.

### Analyzes applied to microencapsulated propolis extract (MPE)

Mira3 XMU A Tescan (Tescan Inc., Czech Republic) brand Scanning Electron Microscope (SEM) located in the Sivas Cumhuriyet University Advanced Technology Application and Research Center Laboratory was used to examine the particle structure and pores of the products obtained through microencapsulation of propolis extracts. Gold-palladium-plated Au-Pd physical vapor deposition model Quorum Q150 (Quorum Tech, UK) was used in the SEM. The samples were acquired and imaged at a working range of 15 mm using a Tescan Mira3 XMU (Czech Republic) at 10 kV field emission.

Encapsulation efficiency was determined by the spectrophotometric method used by Nori et al. (2011). To assess the moisture of the MPE powder, the air oven method 945.14 (AOAC, 2000) and the hygroscopicity Na2SO4 method proposed by Cai and Corke (2000) were utilized. The free radical scavenging ability, total flavonoid content, and total phenolic compound analyses of MPE were performed using the same procedures described above in the section on propolis extract analysis.

### Production of the chewing gum samples

Initially, gum base (Maykar Maya Chemical Industrial and Trading Co., Turkey) was melted (at 60-65°C) in a gum mixer. The MPE were then diluted with lecithin solution (Alfasol, Turkey). This solution was then combined for 2 minutes with molten gum base and allowed to cool. The solution was agitated for 2 minutes during cooling by adding saturated sorbitol solution (Alfasol, Turkey). At around 55°C, sorbitol crystals (Takita, Turkey) were added and agitated for 4 minutes. Lastly, glycerin (Tekkim, Turkey) was added and mixed for 1 minute. The resultant chewing gum dough was flattened and then conditioned at room temperature for 12 hours before being cut into little cubes (Fig. 1). The chewing gum samples were wrapped in aluminum foil and stored at 22±2 °C. Table 1 shows the components of the various gum formulations obtained by adding MPE in various quantities.

### Analyzes applied to produced chewing gum samples

The disc diffusion technique was used to assess the biological reaction of MPE-incorporated chewing gum samples in ethanol. Tryptic soy agar (TSA) (Merck, Germany) was employed as medium. Autoclaved medium (core, OT 012, Turkey) was poured into sterilized petri dishes. After gelation of the agar, *Streptococcus mutans* (ATCC 25175) (Kwik Stik, France) were inoculated to obtain 105 cfu/petri dish by spread plate method. The disks (BLK, Turkey) soaked in ethanolic solutions of the control group and samples containing MPE were placed in the middle of each petri plate. Petri dishes were incubated for 24 h in an oven at 37°C (Thermal, H130S, Turkey). The inhibition zones were observed (Duran et al., 2007). CIE L*, a* and b* values were determined using Hunter colorimeter (CR-400 Konica, Minolta, Japan). Each gum sample was measured at 3 different points. The equipment was calibrated with the white calibration disk before the measurements.

### Statistical analysis of data

SPSS 22 (Statistical Package for Social Sciences) was used for statistical analysis of the data. Descriptive statistics, One-way ANOVA Test to determine the mean scores of groups with more than two, Tukey Post Hoc Test to determine differences between homogeneously distributed groups, Tamhane Post Hoc Test to determine differences between unhomogeneously distributed groups were applied ($p < 0.05$).

## RESULTS AND DISCUSSION

### Analysis of particle structure

Microphotographs of powder propolis microcapsules obtained by spray drier at a given temperature and feed rate are shown in Figs. 2 and 3. According to the figures, there is a combination of MPE and non-MPE particles. The spray dryer's nozzle effect is assumed to be responsible for the almost spherical formations. Maltodextrin was observed as brittle, angular and having different surface fractures

**Fig 1.** Produced gum samples

**Table 1: Gum formulations**

| Sample name | Gum base (g) | Liquid Sorbitol (g) | Powder Sorbitol (g) | Lecitin (g) | Glycerin (g) | MPE (g) |
|---|---|---|---|---|---|---|
| Control | 52.5 | 28.5 | 11.5 | 0.5 | 7.0 | 0.0 |
| 1% MPE | 52.0 | 28.5 | 11.0 | 0.5 | 7.0 | 1.0 |
| 3% MPE | 51.0 | 28.0 | 10.5 | 0.5 | 7.0 | 3.0 |
| 5% MPE | 49.5 | 28.0 | 10.0 | 0.5 | 7.0 | 5.0 |
| 9% MPE | 48.0 | 26.0 | 9.5 | 0.5 | 7.0 | 9.0 |

(Fig. 1). The average size of powder varied between 40μm and 200μm. Maltodextrin swells with water and it formed spherical partial hydrogels. In Fig. 2(c), the structure of the maltodextrin on the spherical propolis powder is formed by the formation of microcapsules in the form of smaller particles were observed. Water evaporation during the spray-drying process may cause morphological irregularities. Following spray drying, all samples had a distorted shape with huge creases and a hollow surface. According to Rosenberg, Kopelman, and Talmon (1985), the production of bruised surfaces on spray-dried particles is caused by particle shrinkage during the drying process. According to Re (1998), wrinkles or cracked surface defects emerge when sluggish film formation occurs during the drying of atomized droplets. A similar shape was seen in microcapsules made using maltodextrin as an encapsulating agent for Orthosiphon Stamineus extract (Pang et al., 2014; Negrao-Murakami et al., 2017). Microcapsules had a rough surface with voids and structural flaws. These morphological irregularities are probably due to the evaporation of water during spray drying. According to Alamilla-Beltran et al. (2005), when the drying temperature rises, the evaporation rate rises, resulting in smoother surfaces. Higher air inlet temperatures result in harder microporous surfaces, while lower air inlet temperatures have observed that irregularly shaped microcapsules form with curved surfaces. It was reported that a drying process at 160°C and a low feed rate produced more microcapsules with hemispherical morphology, whereas large cracks and sticky procedures produced no lumps (Medina-Torres et al., 2016). Tonon et al. (2009) found similar findings for acai juice encapsulated with maltodextrin with irregular, smooth, spherical, and hemispherical forms.

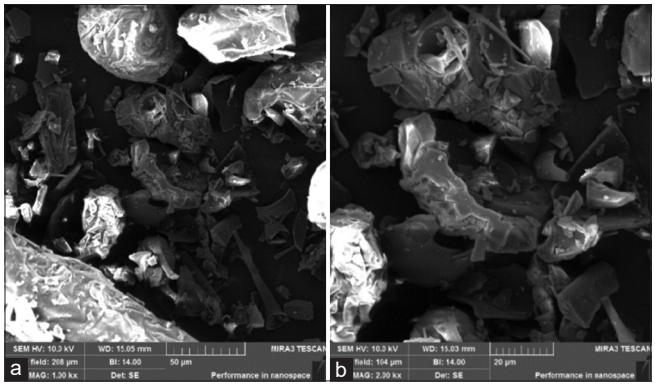

Fig 2. SEM images of hollow malthodextrin capsules (a) 1000 and (b) 2000 times magnified

## Encapsulation efficiency

The spray dryer encapsulation efficiency of the MPE samples was determined to be 62.19%. Similar results were reported in the literature under comparable drying circumstances, namely 69-80% in blackberry (Ferrari et al., 2012), 57.3-89.4% in purple maize (Lao and Giusti, 2017), and 48.59-62.32% in bay leaves (Medina-Torres et al., 2016). Bruschi et al. (2003) reported that the combination of liquid feed rate, desiccant air inlet temperature and spray pressure is effective. The operational parameters such as coating material type, atomization airflow, drying air temperature and emulsion feed flow conditions might affect encapsulation efficiency (Ferreira et al., 2019).

## Hygroscopicity

The moisture adsorption of MPE particles at 25 °C (81% relative humidity) after 7 days was determined to be 31.50±0.22%. Spray-dried particles can easily absorb moisture from the air and cake formation can occur unless necessary precautions are taken (Goula and Adamapoulos, 2010). Karaaslan et al. (2010) stated that higher hygroscopic properties of powders produced at low temperatures were associated with higher moisture content. An important factor affecting dust stability is the moisture content, because small amounts of water can cause the temperature to fall sufficiently to increase the mobility of the matrix during storage. Negrao-Murakami et al. (2017) encapsulated concentrated mate water (*Ilex paraguariensis A. St. Hil.*) using maltodextrin as coating material in spray dryer. They reported the hygroscopicity values ranged between 17.05-26.30% (75.3% relative humidity). Nori et al. (2011)

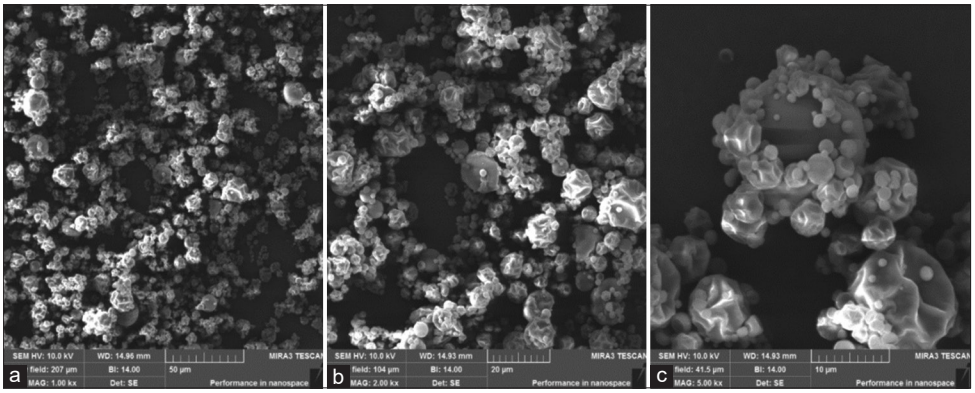

Fig 3. SEM images of microencapsulated propolis extracts (a) 1000, (b) 2000 and (c) 5000 times magnified

calculated the hygroscopicity value (at 81% relative humidity) (33.46±0.66 and 34.10±1.20g absorbed water/100g powder) in their study by encapsulating the propolis by coacervation with soy protein and pectin. As a result, they indicated that the concentration of the substance used in their formulations has no effect on the absorption capacity of water from the environment and that the material should be packed immediately in a high relative humidity environment in water vapor-impermeable containers.

### Moisture determination

It is not ideal to have a particularly high moisture content in the spray drying process since the durability of the dried samples is related to moisture. The amount of moisture affects the water activity and the agglomeration of the product. The calculated moisture content of propolis extracts (91.89±0.07%) was higher than the MPE (7.98±0.07%). The moisture content of the microencapsulated substances of various food substances i.e. cinnamon, watermelon and pitaya were reported to change between 1.34% to 5.46% (Shaaruddin et al., 2017; Negrao-Murakami et al., 2017; Medina-Torres et al., 2016). According to Bruschi et al. (2003), the moisture content was influenced by the feed rate, the dryer's air inlet temperature, and the spray pressure factors. In their study they encapsulated propolis coated with mannitol and gelatin and the moisture content of the formed microencapsules ranged from 4.12% to 9.40%. Righetto and Netto (2005) discovered that maltodextrin (25 DE) was more successful than other coating materials in reducing the moisture content of spray-dried acerola powder, most likely owing to changes in chemical composition.

### Free radical scavenging ability

The DPPH radical procedure was employed to determine the free radical scavenging activity of propolis extract and MPE. As the DPPH solution interacts with a dark violet antioxidant molecule, the structure changes to yellow diphenylpyrylhydrazine. The difference measured by monitoring this color shift in the spectrophotometer is exactly proportional to the concentration of the antioxidant ingredient. Table 2 shows the percentage inhibition data at various levels of DPPH free radical scavenging capabilities of propolis extract and MPE; the difference between the group averages was statistically significant ($p<0.05$). At 500 mg/l concentration the propolis extract had 88.49±0.11% and MPE had 59.52±2.35% DPPH removal capacity. Alencar et al. (2007) found DPPH activity as 57.0±3.2% in Brazilian red propolis extract. In another study performed in Taiwanese propolis % DPPH activity of propolis extracts taken at different concentrations ranged from 18.3 to 99.8% (Chen et al., 2003). Nori et al. (2011) calculated % DPPH activities of propolis microcapsules at different concentrations as 24.65±1.48% at 600 ppm, 56.40±0.81% at 1500 ppm and 84.94±0.13% at 3000 ppm. Busch et al. (2017) applied encapsulation process to propolis with three different coating materials (maltodextrin, vinal gum, arabic gum) and calculated the free radical capture ability as 0.58±0.11, 0.55±0.09 and 0.79±0.06 mg gallic acid/g, respectively.

**Table 2: Evaluation of the antioxidative capacity of the propolis extract (PE) and microencapculated propolis extract (MPE) at different concentrations**

| PE Conc. (mg/L) | % DPPH | MPE Conc. (mg/L) | % DPPH |
|---|---|---|---|
| 100 | 37.03±0.80[a] | 100 | 14.30±0.86[a] |
| 200 | 85.37±0.74[b,c,d] | 200 | 28.00±2.92[b] |
| 300 | 86.84±0.17[b,c] | 300 | 39.79±3.25[c] |
| 500 | 88.49±0.11[b,d] | 500 | 59.52±2.35[d] |

The difference between the a, b, c, d groups are statistically significant ($p<0,05$)

### Calculation of total flavonoid amount

Terpenes and flavonoids present in propolis are extremely powerful antioxidants. Flavonoids are the most common type of chemical found in organic solvents. Flavonoids are pigments that contain pigments present in nearly all sections of plants. Certain flavonoids are distinguished by enzymes involved in bee secretions. Flavonoids are efficient against a wide range of microorganisms. Flavonoids protect the stomach mucosa by reducing capillary fractures, have a beneficial impact on the cardiovascular system, control blood circulation, and prevent ulcers. They also regulate the internal secretion system and have a positive effect on exhaustion (Kumova et al., 2002). In our study the total flavonoid content of the MPE was calculated as 29.06±1.20 mg/kg and propolis extract as 6412±331mg/kg. Marquele et al. (2006) found the total amount of flavonoid between 10.58-14.03 mg/kg. In the study carried out in Brazilian red propolis extract, the total amount of flavonoid was calculated as 4300 mg/kg (Alencar et al., 2007).

### Calculation of total phenolic compounds

The majority of the compounds responsible for antioxidant activity in the propolis are determined by the amount and type of phenolic substances. The total phenolic content of the MPE was calculated as 89.17±0.96 mg/kg and propolis extract as 14280±634 mg/kg in the study. Because of the encapsulation process circumstances, the phenolic content of the core material may drop. The amount of phenolics was lowered due to the high temperature and oxygen exposure during spray drying. Maltodextrin forms amorphous glassy matrices, which serve as an oxidation barrier. High DE maltodextrins may form more solid and oxygen-impermeable wall structures, extending the life of pigment storage (Cai and Corke, 2000). Alencar et al. (2007) reported the total amount of phenolic substance to be 23200 mg/kg in Brazilian red propolis extract. Marquele et al. (2006) found the total amount of phenolic material as 37.83-45.57 mg/kg.

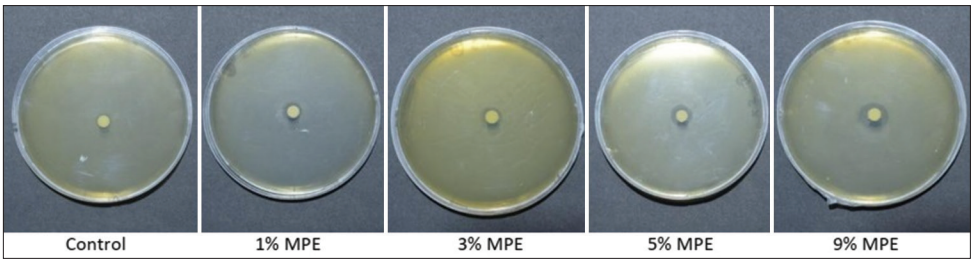


Fig 4. The zones observed in the gum samples

**Table 3: Evaluation of the antimicrobial activity of the gum samples on *Streptococcus mutans* (ATCC 25175)**

| Sample name | Zone diameter (mm) |
|---|---|
| Control | 0.39±0.09[a] |
| %1 MPE | 1.84±0.23[a] |
| %3 MPE | 2.50±0.24[a] |
| %5 MPE | 3.50±0.24[a] |
| %9 MPE | 4.20±0.14[b] |

The difference between the a and b groups are statistically significant (p<0,05)

**Table 4: CIE L * a * b * values of the chewing gum samples**

| Sample name | L* | a* | b* |
|---|---|---|---|
| Control | 81.87±0.23[a] | 0.40±0.06[a] | 18.26±0.25[a] |
| %1 MPE | 81.56±0.77[a] | 0.64±0.03[a] | 19.12±1.10[a] |
| %3 MPE | 78.07±0.49[b] | 0.46±0.17[a] | 18.10±0.57[a] |
| %5 MPE | 81.93±0.47[a] | 0.01±0.00[a] | 20.14±0.05[b] |
| %9 MPE | 76.61±0.29[c] | 0.38±0.06[a] | 23.50±0.70[c] |

The difference between the a, b, c groups are statistically significant (p<0,05)

### *In vitro* determination of antimicrobial activity

The Disk Diffusion Technique was used to test the antimicrobial activity of gum formulations against *Streptococcus mutans* (ATCC 25175). Fig. 4 depicts the zones found in the gum samples. The zone size increased with concentration (Table 3), indicating a statistically significant difference between the group averages ($p<0.05$). As predicted, the gum samples containing 9% MP had the greatest antibacterial activity against *Streptococcus mutans*. According to Duran et al. (2007), microcapsulatedd raw propolis has excellent antibacterial activity against *Streptococcus mutans*. Furthermore, they proposed that it may be widely employed as an antibacterial agent in medicine. *Streptococcus mutans* and *Streptococcus sobrinus* species have been linked to the development of dental caries. According to the literature, propolis has strong antibacterial action against *S. mutans* and *S. sobrinus* (Park et al., 1998; Uzel et al., 2005; Duarte et al., 2006; Alencar et al., 2007; Tulsani et al., 2014; Zulhendri et al., 2021).

### Color analysis of chewing gum samples

CIE L* color parameter level was determined to be highest in 5% MPE and lowest in 9% MPE in the current investigation (Table 4). The CIE +a* color parameter representing red color was found to be highest in gum containing 1% MP and lowest in gum containing 5% MPE. In terms of red color, no statistically significant difference was discovered between gum samples having different concentrations of MPE ($p<0.05$). The +b* color parameter level values were found to be the highest in gum containing 9% MPE and the lowest in control gum samples, expressing the yellow color in the gum samples. The yellow color in chewing gum samples rose as MPE content increased ($p<0.05$).

## CONCLUSION

Raw propolis and its extract form have a significant unpleasant taste and odor for customers. The encapsulation approach has permitted the creation of alcohol-free propolis powders with large levels of encapsulated phenolic compounds, outstanding antioxidant properties, superior cold water solubility, and low water activity. Yet, the undesirable features were hidden, and the bioactive components were stable throughout the gum manufacturing process and storage. In this study, MPE was used for the first time in chewing gum formulation to preserve propolis extract from environmental impacts during manufacture and storage, as well as to ensure regulated release in the mouth. Propolis extract was microencapsulated using spray drying and maltodextrin as the coating material. Maltodextrin was chosen because it is less expensive and can build a better antioxidative barrier than other coating materials. As compared to similar functional components in the literature, formed MPE exhibited equivalent or greater DPPH scavenging capacity, total flavonoid and total phenolic content. MPE is regarded to have a significant potential for usage in culinary applications. Due to the antibacterial qualities of propolis, chewing gums containing 9% MPE demonstrated strong inhibitory impact in vitro against *Streptococcus mutans*, which is the major cause of tooth decay. This is a positive development for the chewing gum sector. The yellow color (+b*) of the chewing gums likewise became more pronounced as the MPE level rose. Yet, it is apparent that by changing coating type, microencapsulation method, operation parameters and etc. different types of MPE having differing propoerties

could be produced to be used in the confectionery and chocolate sectors. Finally, the MPE containing chewing gum appears to be quite innovative in the market with functional properties.

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### Conflict of interest

Authors declare that there is no conflict of interest.

### Authors' contributions

Emre Bostancı: Data collection, literature review, writing of the manuscript.

Evren Gölge: Concept, planning methodology, writing of the manuscript, final manuscript review and corrections, submission.

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