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Microencapsulation of non-polar extracts of Colombian propolis via spray drying

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Microencapsulación de extractos apolares de propóleos mediante secado por aspersión

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ABSTRACT

The oily extracts of propolis are matrices with a high antiradical, reducing, and antimicrobial properties. However, because of their susceptibility to oxidizing agents (oxygen and light) that react with the environment and negatively affect bioactivity and sensory properties, their use remains limited. Oily extracts of Colombian propolis (OECP) were microencapsulated (MOECP) by spray drying using maltodextrin and Gum arabic as the wall material and the conservation of active principles and the control of the release of bioactive components were evaluated. The formulations of MOECP with a higher concentration of propolis extract and lower drying temperature exhibited higher bioactivity because of less degradation of metabolites and less production of prooxidant substances. The samples exhibited a high antioxidant, antiradical, and antimicrobial potential. However, polyphenols and carotenoids were lost. Mixtures of maltodextrin and Gum arabic were suitable for microencapsulation and more than 50% of the phenols and carotenoids in MOECP were preserved.

Palabras clave:
Actividad antioxidante
Antimicrobianos
Bioactividad
Propóleos
Secado por aspersión

RESUMEN

Los extractos oleosos de propóleos se han caracterizado por ser matrices con un alto poder antirradical, reductor y antimicrobiano. Sin embargo, debido a su susceptibilidad a los agentes oxidantes (Oxigeno y luz) que reaccionan con el medio ambiente y afectan negativamente su bioactividad y propiedades sensoriales han limitado su uso. Para preservar la protección de los principios activos y permitir la liberación controlada de sus componentes bioactivos, se evaluó la microencapsulación de extracto oleoso de propóleos proveniente del bosque húmedo premontano colombiano (EOPC) por medio de secado por aspersión (Spray Drying). Para tal fin fueron utilizados como materiales de pared maltodextrina y goma arábiga. Los EOPC microencapsulados (EOPCM) fueron analizados mediante análisis fisicoquímicos y microbiológicos. Los resultados indican que los EOPCM presentaron un alto potencial antioxidante, antiradical y antimicrobiano y las formulaciones con mayor concentración de EOPC y menor temperatura de secado tuvieron mayor bioactividad debido a una menor degradación de metabolitos y a la menor producción de sustancias pro-oxidantes. Se concluye que es posible elaborar EOPCM usando mezclas de maltodextrina y goma arábiga y se conserva más del 50% de los fenoles y carotenoides en la muestra del EOPC.

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Propolis is an oily and resinous mixture naturally produced by bees (*Apis mellifera*) that isolates and protects hives from temperature changes, mechanical forces, and harmful biological vectors, while also providing collective social immunity at the colony level (Pasupuleti et al. 2017). This mixture is based on botanical materials collected by bees. Because the plant material has contact with the digestive system of bees before being incorporated into the hive compartments, propolis are considered an animal-based product (Seven et al. 2018). The profile of bioactive propolis molecules varies according to geographic and botanical origin, time of year, type of collection, bee genetics, and environmental factors (Gomes do Nascimento et al. 2019). For example, oily extracts are obtained from various bioactive compounds, such as polyphenols, carotenoids, terpenes, esters, amino acids, vitamins, minerals, and sugars (Bankova et al. 2014; Ahangari and Naseri 2018), that have antipathogenic properties both in vitro and *in vivo* (Šuran et al. 2021).

Recent studies have demonstrated that oily extracts of propolis (OEP) are rich in hydrophobic compounds like terpenoids, fatty acids, and waxes, which exhibit superior antimicrobial activity compared to hydrophilic fractions pointing their potential as a source of natural bioactives for diverse applications (Altabbal et al. 2023).

The aqueous and ethanolic extracts of propolis are more common; however, few studies have examined at the properties of oily propolis extracts in terms of their chemical composition and biological activity. EOPs contain nonpolar substances, such as essential oils, fatty acids, lipids conjugated with phenolic compounds, waxes, and resins, which are bioactive compounds of interest due to their antioxidant, antiradical, and antimicrobial activity (Ramanauskienė and Inkėnienė 2011). The antimicrobial potential of EOPs can exceed the bactericidal capacity of propolis extracts rich in polar substances (Almuhayawi 2020). However, the chemical constituents of the essential oil fraction in propolis are prone to degradation, mainly by oxidative agents such as light, pH, and oxygen (Pant et al. 2022). Consequently, encapsulation processes are needed to prevent chemical reactions, preserve bioactive compounds, and maintain biological activity for use as a food additive.

The microencapsulation process creates a microparticle of defined morphology with marked stability by wetting a mixture of bioactive compounds with a biopolymer solution (Kashif et al. 2022). Processes can be based on physical principles such as spray drying or chemical processes such as coacervation and the formation of β-cyclodextrin complexes, depending on many factors such as concentration, temperature, pH, chemical nature, and protective substances (Brahmi et al. 2021). Aspects such as the microparticle morphology and yield, which vary depending on the method and protocol, must be considered to reduce the loss of active ingredients and optimize the process to obtain a high content of biocompounds and enhance bioactivity for a desirable product (Đordevic et al. 2014).

In the microencapsulation process, the compounds used for the microcapsule wall must be determined to protect the substances, avoid unwanted reactions between the bioactive compounds and the medium, and avoid alterations to the organoleptic properties and degradation of organic compounds. The effects of temperature and pH changes also be studied. Microencapsulation improves the physical and rheological properties of material by increasing their solubility and facilitating their handling and incorporation, resulting in sensory defects, and increased bioactive compound activity (McClements et al. 2007).

The Spray drying method is an encapsulation process that forms a powder via atomization and continuous drying under hot air from micro drops, forming a high-quality powder with low water activity that protects the active material (Fernandes M et al. 2014).

The wall materials determine the effectiveness of the process and thus guarantee the stability of the final product. Two of the most widely used materials in microencapsulation processes are maltodextrin and Gum arabic. Maltodextrin is a low-cost polymer with a neutral flavor, low viscosity, and good protective capacity but poor emulsifying capacity. It must be supplemented with another biopolymer, such as Gum arabic, that can integrate with maltodextrin because of their similar properties, and has a greater emulsifying capacity. The use of biopolymers such as maltodextrin and Gum arabic in microencapsulation

processes, particularly through spray drying, has proven effective in protecting sensitive bioactive compounds and preserving their functional properties. The appropriate ratio of these biopolymers is crucial to minimizing the loss of non-polar compounds in oily propolis extracts (OPE), such as fatty acids, waxes, and resins, which significantly contribute to their antioxidant and antimicrobial activity (Brahmi et al. 2021). Studies have reported successful encapsulation of extracts and oils from various plants, including sunflower, soybean, and flaxseed (Carneiro et al. 2013). However, due to the novel of this matrix, an oily extract from Colombian beekeeping propolis has not been encapsulated. In this work, we considered the hypothesis that microencapsulation with Gum arabic and maltodextrin can preserve the bioactive properties of oily extracts of propolis.

The increasing demand for natural alternatives to synthetic antibiotics and preservatives highlights the importance of exploring bioactive compounds with antioxidant and antimicrobial properties. These compounds have potential applications in agri-food, cosmetic, and pharmaceutical industries, driving innovation in product development. Therefore, this study aimed to encapsulate oily extracts from propolis obtained in a Colombian premontane humid forest (OECP), using a combination of maltodextrin/gum arabic as wall materials, followed by physicochemical and microbiological characterizations.

MATERIALS AND METHODS

Propolis was supplied by Campo Colombia S.A.S, and obtained from a Colombian premontane humid forest (1,000–2,000 meters above sea level (masl), Latitude: 4.33323, Longitude: -75.8283, 4°19′60″ North, 75°49′42″ West, 18–24 °C) and the bee species *Apis mellifera*. Chemical reagents, such as dichloromethane: ethanol: water and β-carotene standard, were obtained from Sigma Aldrich®. Maltodextrin and Gum arabic were purchased from Cimpa® SAS. The reagents used for the microbiological analyses (Mueller Hinton® culture medium and microdilution plates) were obtained from Elementos Químicos Ltd.

OECP produced using organic solvents

To obtain OECPs, a mixture of non-polar and polar solvents was used, which allowed the effective extraction of total lipids, following the methodology of Kubiliene et al. (2015). 75 mL of dichloromethane: ethanol: water (1:1:1) were mixed with 25 g of bee propolis, which were shaken for 15 minutes at 4,000 rpm in an Eppendorf® 5427 R centrifuge. Three ultrasound cycles were applied at 60 Kw for 15 min at room temperature while protected from light exposure. Subsequently, the sample was filtered using Whatman® Grade 1:11 µm paper. The filtrate was subjected to funnel decantation to isolate the organic phase from the aqueous phase. The apolar region was subjected to another separation to remove waxes. Finally, the organic phase was rota evaporated at 40 °C in a Büchi® R-215 Rotavapor System to remove dichloromethane.

Microencapsulation of OECPs and characterization of microencapsulated (MOECP)

The OECPs were microencapsulated using the methodology proposed by Busch et al. (2017) with some modifications. Maltodextrin (30 g) and 0 Gum arabic (0.3 g) were used, dissolved in 150 mL of distilled water, maintaining a constant ratio between the solvent and the encapsulating agent following the formulations proposed in Table 1. Ten milliliters of oily propolis extracts were added, and the resulting mixtures were homogenized using an Ultra-Turrax T25: IKA®, Germany, at 15,000 rpm for 2 min. Then, the emulsion was dried, and the oily propolis extracts were encapsulated using a Büchi® B-191 Mini Spray Dryer. The spray drying operating conditions were suction (%) = 85; pump (%) = 10; air and feed flow: 0.60 m^3 min^{-1} and 1.3 mm diameter of the nozzle or injector. Yield was calculated according to Equation 1. Samples of microencapsulated OECPs under this methodology were called MOECP.

$$\text{Yield (\%)} = (Mm /(Me)+(Ms)) \times 100 \quad (1)$$

Where: “Mm” microencapsulated = Mass obtained in the encapsulation, “Me” encapsulant = Mass of the encapsulating agent “Ms” total solids = Mass of the total solids content of the propolis essential oil.

The water activity (A_w) of the MOECP was analyzed by placing the samples in a Rotronic® (HygroLab C1 model), and measurements were performed in triplicate at room temperature.

Table 1. Formulation of microencapsulation from two ratios of encapsulating agent and OECP, and parameters of microencapsulation.

Sample	Encapsulant/OECP ratio	Encapsulation temperature (°C)	Mass of OECP (g)
1	1:0.5	100	15.15
2	1:0.5	120	15.15
3	1:0.5	140	15.15
4	1:0.5	160	15.15
5	1:0.33	100	10.10
6	1:0.33	120	10.10
7	1:0.33	140	10.10
8	1:0.33	160	10.10

OECP: Oily extract of Colombian propolis.
Encapsulant: mixture of maltodextrin and Gum arabic.

Finally, color analysis of MOECP was performed using a Minolta® colorimeter (CM-3600), the CIE-Lab system (L*, a*, b*), illuminant D65 and an observation angle of 10°. The readings were performed in triplicate according to the methodology proposed by López-Patiño et al. (2021).

Table 1 lists the parameters used to describe the microencapsulation process using the Spray Dryer. Eight mixtures, two extract/encapsulant ratios, four temperatures, and two amounts of oily Colombian propolis extract (OECP) were used.

Total content of Phenols and β-carotene of OECP and MOECP

To evaluate the total content of phenols and β-carotene in the OECP and MOECP, the Folin-Ciocalteu method and the β-carotene test were used, respectively. First, pretreatment was carried out with 1:3 dilutions (extract/solvent) for both OECP and MOECP. For 0.5 mL of OECP and 0.5 mg of MOECP, the samples were diluted in 1.5 mL of dichloromethane.

Folin-Ciocalteu method was used to measure the phenol content. A standard curve was prepared from a stock solution of gallic acid in methanol at a concentration of 1 mg mL^{-1}. Once the standard curve was constructed, mixtures of 100 µL of control, 100 µL of OECP and 100 µL of diluted MOECP were made, with a 75 µL of Folin-Ciocalteu solution and 825 µL of 2% sodium carbonate solution. The mixtures were vortexed for 1 min, and 250 µL were poured into each well, repeating this process three times for each sample and control. The plate containing the microdilutions was then stored for 90 min in the dark, and, finally, the mixtures were read at 750 nm in a SpectraMax 190 Microplate Reader® plate reader according to the method proposed by Tiveron et al. (2016).

To measure β-carotene, a calibration curve was prepared from a stock solution made with a β-carotene standard (Sigma Aldrich®) whose concentration was 1 mg β-carotene mL^{-1} of solvent. Subsequently, 250 µL of the diluted OECP and MOECP samples were read at 470 nm using a SpectraMax 190 Microplate Reader® according to the method proposed by Nair and Meliani (2018).

Antiradical activity of OECP and MOECP determined using 1,1-diphenyl-2-picryl hydrazyl (DPPH) method

The DPPH method was used to obtain antiradical activity. A mixture of 0.0079 g of DPPH (Sigma Aldrich®) in 2.5 mL of ethanol was prepared and stirred in an amber bottle for 5 min at room temperature. Subsequently, 250 µL of this solution were diluted in 50 mL of 80% ethanol. Next, 300 µL of this mixture were used for a reading at 540 nm in a SpectraMax® 190 Microplate Reader to verify absorbance ranges between 0.5 and 0.6. Finally, 290 µL of the DPPH solution were mixed with 10 µL of diluted OECP or MOECP, respectively. The plate was left in the dark for 30 min, and the sample was read at 540 nm following the method proposed by Ramadan et al. (2012). The antiradical potential was obtained using Equation 2:

$$\text{DPPH (\%)} = (\text{Absorbance Blank} - \text{Absorbance Sample}) / (\text{Absorbance Blank}) \times 100 \quad (2)$$

Iron Reducing Antioxidant Power (FRAP) assay of OECPs and MOECPs

First, the calibration curve was prepared using a standard of ferrous sulfate diluted in 2 mM ferrous sulfate stock aqueous solution. The OECP and MOECP were diluted in dichloromethane at a 1:4 ratio. Subsequently, 90 µL of each dilution was diluted with 270 µL of distilled water in the dark, and then 2.7 mL of a 10 mM TPTZ Sigma Aldrich® solution was added. The mixtures were heated in a water bath at 37 °C for 30 min and then allowed to cool. Finally, absorbance was measured at 595 nm using a SpectraMax® 190 Microplate Reader. The tests were in triplicate, and the concentration was obtained using the slope and cut-off values, which were expressed in µM of ferrous sulfate, in 1 g of propolis following the method proposed by Thaipong et al. (2006).

Analysis of MOECP with Fourier Transform Infrared Spectroscopy (FT-IR)

To identify the functional groups, present in the MOECP compounds, infrared spectroscopy was used to obtain vibration bands characteristic of one or more specific functional groups. For the test, 0.1 g of each sample was weighed, and ground in an FT/IR-4700 FTIR Spectrometer, JASCO®. The readings were taken in the range of 400-4,000 cm^{-1}, in triplicate for each sample. Baseline corrections were made to eliminated outlier values and CO_2 and H_2O bands were removed. The spectra were plotted as a function of wavelength and transmittance percentage. To identify the functional groups of the spectra, the band values were compared with the bibliographic information using the method proposed by Fangio et al. (2018).

Microbiological analysis with minimum inhibitory concentration (MIC) of OECP and MOECP

The antimicrobial activity analysis was performed using the well microdilution technique. Plates with 24 wells were used, and 95 µL of Mueller Hinton® culture medium were added to each plate. Then, 0.05 mL of the OECP for the control treatment and dilutions of MOECP in dichloromethane (1:3) were added to achieve a final concentration in each well of 20 mg mL^{-1}. Once this concentration was reached, 95 µL of Mueller Hinton® medium was added, and serial dilutions were made. Subsequently, a solution of bacteria mix (*Escherichia, Staphylococcus aureus, Salmonella enteritidis, Enterobacter aerogenes, Enterobacter agglomerans, Klebsiella* sp.) was prepared with 100 µL of saline solution at a concentration of 100 µg mL^{-1} and a colony of the bacterial solution with absorbance values between 0.08 to 0.13. Twenty microliters of this solution were added to the wells. Finally, the solutions containing bacteria and OECP or MOECP were incubated for 24 h at 35 °C, and the minimum inhibitory concentration against the microorganisms was determined according to the method proposed by Bonou et al. (2016).

Scanning Electron Microscopy (SEM) of MOECP

The morphology of MOECPs where observed by using scanning electron microscopy (SEM). The tests were performed at the Laboratorio de Microscopía Electrónica de Barrido in the Universidad Nacional de Colombia. The samples were treated with a gold-palladium metallic coating applied with a Q150R ES metallizer (Quorum). Subsequently, they were observed using a Quanta 200 FEI® - USA scanning electron microscope. The operating conditions were at 2×10^{-2} torr and 25.0 kV, using secondary electrons. The aim was to determine the presence of agglomerations, the size of the particles, and the shape and presence of porous structures in MOECP according to the method suggested by Sanchez-Reinoso et al (2017).

Differential Scanning Calorimetry (DSC) of MOECP

DSC analysis was used to determine the enthalpy, glass transition temperatures, and endothermic and exothermic events of the MOECP during the useful life of the samples. A DSC 1-500/2722 Mettler Toledo® calorimetric analyzer was used with 2 mg of each sample placed in an aluminum capsule under N_2, which was supplied at a rate of 50 mL min^{-1}. The temperature range was 30-400 °C, and the heating rate was 10 K min^{-1}. Finally, thermograms were obtained and analyzed using Mettler Toledo® STARe Thermal Analysis System version 8, as proposed by Busch et al. (2017).

Statistical analysis

For each variable, the means and standard deviations were obtained. To analyze significant differences, ANOVA was performed using the Tukey test ($P \leq 0.05$). A principal component analysis (PCA) was also carried out to group the values of the analyses according to similarities and to determinate which variables had a higher incidence in the OECP or MOECP. Matlab® version 7.12.0.635 and Origin® 2018 64 Bit were used (Woźniak et al. 2019).

RESULTS AND DISCUSSION

OECPs extraction

The propolis used in this study were selected because it inhibits coliform microorganisms. Extraction was carried out on eight samples from premontane humid forests, the yield results are presented in Table 2.

Table 2. OECP yields obtained from the propolis collected during the study.

OECP Sample	Mass of propolis (g)	Mass of OECP (g)	Yield (%)
1	25.01	4.87	19.47±0.23[a]
2	25.14	4.95	19.69±0.60[a]
3	24.97	4.76	19.06±010[a]
4	25.03	4.94	19.74±0.57[a]
5	24.89	4.68	18.80±0.46[a]
6	24.96	4.88	19.55±0.52[a]
7	25.04	4.97	19.85±0.39[a]
8	25.01	4.83	19.31±0.28[a]

Different letters in the same column indicate significant differences (P<0.05). OECP: Oily extract of Colombian propolis. Samples 1 to 8 = OECP: Colombian propolis oily extract.

There were no significant differences between the propolis samples, and the yield percentage ranged between 19.06 and 19.85%. This consistency suggests that propolis collected from similar ecological zones under uniform conditions maintains a stable extraction efficiency with apolar organic solvents. Although the yield of the oily extracts was higher than that of essential oils, - had a maximum yield of 1.14%, it was lower than that of the ethanolic extracts, which was between 41 and 60%. Although propolis substances are lipophilic and have a greater affinity with apolar organic solvents, some factors explain the decrease in extraction -relative to that of ethanolic extracts (Sambou et al. 2020). First is that a large proportion of the hydrophobic compounds in propolis are resins (50-70%), whereas oils and waxes comprise 30 and 50% of the total composition (Abdelrazeg et al. 2020). Resins and waxes were removed during the filtration steps; therefore, only oils and substances contained within these matrices will remain (Ahangari and Naseri 2018). The literature reports 41 to 60%, the yields in this study were around 19%. According to Pobiega et al. (2019), water has little affinity to most propolis compounds. Yields with water ranged from 4 to 14%, which affected the results. Although water helped remove waxes and resins, facilitating precipitation, it resulted in a low yield. However, yields increased with ultrasound extraction techniques, which increased the concentration of secondary metabolites and the total mass of the extract. Although OECP yields are modest, the extracted components retain significant biological activity, emphasizing the potential of optimizing extraction techniques, particularly those incorporating ultrasound or alternative solvents, to maximize the recovery of bioactive compounds.

Microencapsulation of OECPs and their characterization (MOECP)

Table 3 presents the results of the yield and aqueous activity of MOECP.

The MOECP exhibited an aqueous activity that ranged from 0.2 to 0.3, which prevented the proliferation of pathogenic fungi and/or bacteria. A significant difference (P<0.05) was observed between the samples according to their concentration. The relationship between encapsulant concentration and the water activity was inversely proportional (the higher the concentration of the encapsulant, the lower the water activity). The yields ranged between 35 and 63%, with a higher value observed in samples that had a high concentration of OECP, such as formulations 1 and 2.

The observed inverse relationship between water activity and the concentration of encapsulating agents, such as maltodextrin and Gum arabic, aligns with findings from other studies. Recently, Iesa et al. (2023) indicated that a higher proportion of encapsulants reduces water activity by limiting free water within the microencapsulate matrix, enhancing both stability and microbial inhibition.

Table 3. Performance and aqueous activity of MOECP using different parameters.

Sample MOECP	Encapsulant/OECP ratio	Temperature (°C)	Encapsulant mass (g)	Yield (%)	Aqueous Activity (A_w)
1	1:0.5	100	18.92	62.44	0.31±0.06[a]
2	1:0.5	120	18.32	60.46	0.29±0.07[a]
3	1:0.5	140	15.71	45.25	0.28±0.07[a]
4	1:0.5	160	11.94	39.40	0.27±0.04[a]
5	1:0.33	100	12.90	42.58	0.28±0.05[a]
6	1:0.33	120	11.83	39.04	0.25±0.02[b]
7	1:0.33	140	11.06	36.50	0.23±0.03[b]
8	1:0.33	160	10.85	35.81	0.22±0.04[b]

MOECP: Microencapsulated oily extract of Colombian propolis.
Encapsulant: mixture of maltodextrin and Gum arabic.
OECP: Colombian propolis oily extract.

Moreover, the efficiency of microencapsulation can be influenced by the ratio of encapsulating agents and processing conditions, such as temperature, which affects the retention of bioactive compounds and the uniformity of the microcapsules.

The results of the colorimetry analysis for MOECP are presented in Table 4. The samples had a similar tone (h), without differences (P>0.05), but varied in terms of chroma (C) (P<0.05). This result was due to changes in luminosity and the blue-yellow ratio when the OECP concentration and temperature changed. As the temperature decreased and the concentration of the encapsulating material increased, the intensity of yellow in the samples increased, and the microencapsulation became less luminous. In the eight formulations, there was a significant difference between to the amount of OECP used and the temperature.

Table 4. OECP and MOECP color parameters.

Sample	L*	a*	b*	C	h
OECP	96.71±3.41[a]	0.17±0.02[a]	4.27±0.30[a]	4.27±0.43[a]	1.53±0.19[a]
1	91.65±2.59[a]	0.64±0.06[a]	5.37±0.41[b]	12.77±0.76[b]	1.54±0.21[a]
2	88.54±3.78[a]	0.66±0.09[a]	5.97±0,57[b]	12.01±0.80[b]	1.45±0.11[a]
3	90.19±4.12[a]	0.87±0.08[b]	6.13±0.39[c]	11.80±0.71[b]	1.43±0.15[a]
4	88.28±4.98[a]	0.86±0.10[b]	6.73±0.48[c]	11.40±0.63[b]	1.44±0.20[a]
5	95.98±4.21[b]	1.09±0.06[b]	11.39±0.76[d]	6.79±0.45[c]	1.48±0.12[a]
6	94.30±3.45[b]	1.67±0.06[c]	10.27±0.69[d]	6.19±0.37[c]	1.41±0.21[a]
7	95.05±7.3[b]	2.28±0.06[d]	11.58±0.81[d]	5.41±0.39[d]	1.38±0.14[a]
8	93.10±5.26[b]	3.44±0.06[c]	11.39±1.09[d]	5.09±0.28[c]	1.35±0.16[a]

Different letters in the same column indicate significant differences (P<0.05).
OECP: Oily extract of Colombian propolis.
Samples 1 - 8 = MOECP: Microencapsulated oily extract of Colombian propolis.

The concentrations of bioactive compounds, such as polyphenols and β-carotene, as well as antioxidant and antiradical activity, were reduced in samples 1 to 8 (MOECP), compared with OECP. This phenomenon can be attributed to increased temperature. The most affected metabolites were β-carotene, where the concentration was reduced by up to nine-fold. This can be attributed to the breaking of the double bonds between carotenoids and

xanthophylls with increasing temperature, which generated chemical changes in the carotenoids resulting in the appearance of low molecular weight substances, short-chain isoprene derivatives, and oxygenated substances.

Similarly, a decrease in polyphenols was observed with increasing temperature, which could change the original structure and functionality. Phenols react in the presence of substances such as organic acids, lipids, and nitrogenous compounds, giving rise to the appearance of substances such as benzocaine, ethers, glycolipids, aldehydes, and ketones, which have a lower antioxidant capacity than polyphenolic substances or compounds with - OH (Islam et al. 2014; Kalušević et al. 2017).

Total content of Phenols, β-carotene and Antiradical activity of OECP and MOECP

The antioxidant activity, and bioactive compounds content, such as polyphenols and carotenoids in OECP and MOECP are presented in Table 5. Antioxidant activity decreases with increase temperature, leading to the degradation of labile compounds.

Table 5. Antioxidant activity and polyphenol and carotenoid content of OECP and MOECP.

Sample	DPPH (% Inactivation)	mg gallic acid g^{-1} propolis	β-Carotene (µg g^{-1})	FRAP (µmol g^{-1} propolis)
OECP	75.94±3.47^{e}	280.99±4.18^{d}	9.93±0.19^{e}	571.88±21.67^{e}
1	59.09±2.40^{a}	200.12±8.51^{a}	3.55±0.06^{a}	300.25±5.01^{a}
2	57.94±3.47^{a}	180.99±4.18^{a}	2.93±0.19^{a}	318.90±21.67^{a}
3	46.12±3.73^{a}	158.10±15.09^{b}	2.21±0.53^{a}	145.05±4.35^{b}
4	43.80±4.60^{b}	154.52±5.43^{b}	1.95±0.23^{b}	117.72±19.89^{b}
5	29.09±2.40^{c}	100.12±8.51^{b}	1.55±0.06^{b}	200.25±5.01^{c}
6	27.94±3.47^{c}	70.99±4.18^{c}	0.93±0.19^{c}	118.90±21.67^{b}
7	16.12±3.73^{c}	58.10±15.09^{c}	0.21±0.53^{d}	95.05±4.35^{d}
8	13.80±4.60^{d}	54.52±5.43^{c}	0.15±0.23^{d}	67.72±19.89^{d}

Different letters in the same column indicate significant differences (P<0.05). OECP: Oily extract of Colombian propolis. Samples 1 - 8 = MOECP: Microencapsulated oily extract of Colombian propolis. DPPH= 1,1-diphenyl-2-picryl hydrazyl. FRAP= Ferric Reduction Antioxidant Power.

Formulations 1 and 2 maintained greater reducing capacity and antiradical activity, indicating less degradation of labile compounds, because they were prepared at lower temperatures. As the temperature increases, Maillard and Strecker degradation reactions generate substances with a lower antioxidant potential than bioactive compounds in OECP. The affected compounds, apart from polyphenols, are low molecular weight volatiles and nitrogenous substances, such as proteins, amino acids, and sugars that result in aldehydes, ketones, pyrazines, and furans. In this study, the values of antioxidant activity and bioactive compounds such as carotenoids were still present in the microencapsulated extracts (MOECP) although at a lower concentration than in the non-encapsulated extract (OECP). On the contrary, previous studies that evaluated the ethanolic extracts of propolis (Baysan et al. 2018) and encapsulated fruit extracts reported better antioxidant activity in encapsulated forms than in non-encapsulated extracts (Tamanna et al. 2015).

Iron Reduction Antioxidant Power Assay (FRAP) for OECP and MOECP

When analyzing the FRAP values for the OECP and MOECP (Table 5), the reducing capacity of the MOECP decrease with increasing temperature and decreasing OECP concentration. According to Rodrigues et al. (2021), microencapsulation reduces the levels of agents in propolis, such as polyphenols, via oxidative processes. Previous studies have shown that microencapsulating has a maximum reducing capacity at temperatures between 100 - 120 °C and that these encapsulates have a greater reducing capacity than encapsulates that only contain maltodextrin and Gum arabic (Pratami et al. 2020). Both polysaccharides and polyphenols have cyclic structures that reduce metal ions such as Fe^{+3}, which is transformed into Fe^{+2}. There are more phytochemical compounds in propolis extracts that contribute to the reducing activity, but they have not been correlated with the reducing power (Pratami et al. 2020).

Analysis of MOECP using FT-IR

Figure 1 shows the results of the infrared spectra reading with the MOECP treatments, which resulted from breaking the double bonds of the carotenoids and xanthophylls as the temperature increased, causing chemical changes in the carotenoids that resulted in the appearance of low molecular weight substances, short-chain isoprene derivatives, and oxygenated substances.

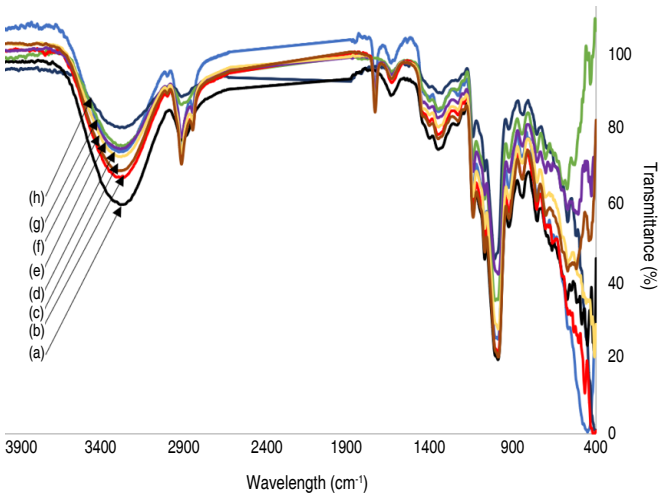

Figure 1. FT-IR spectra of MOECPs. (a) Sample 1 (100 °C), (b) Sample 2 (120 °C), (c) Sample 3 (140 °C), (d) Sample 4 (160 °C), (e) Sample 5 (100 °C), (f) Sample 6 (120 °C), (g) Sample 7 (140 °C), (h) Sample 8 (160 °C). MOECP: Microencapsulated oily extract of Colombian propolis.

The results of the FT-IR spectra suggest that as the temperature increased, there was a noticeable degradation of carotenoids, leading to the formation of low molecular weight substances, short-chain isoprene derivatives, and oxygenated compounds. This thermal degradation is characterized by the disappearance of characteristic absorption bands associated with carotenoid double bonds and the emergence of new peaks corresponding to these degradation products. Such findings are consistent with studies that have observed similar thermal-induced changes in carotenoid structures, which are known to contribute to the aroma profiles of various foods and natural products (Stutz et al. 2015). Additionally, the appearance of oxygenated compounds in the FT-IR spectra suggests oxidative processes occurring during encapsulation, which can influence the stability and bioactivity of the encapsulated propolis extract.

Microbiological analysis of OECP and MOECP

Table 6 presents the results of the microbiological analysis of the MOECP samples.

The MOECP activity against *Salmonella enteritidis* and *Klebsiella* sp. was null because the bioactive compounds that inhibit these bacterial strains were subjected to spray-drying and volatilization processes or other chemical reactions that altered their functional groups. In addition, the substances in the extract that do not undergo changes during encapsulation lack sufficient activity. This phenomenon was also evidenced -by MOECP activities against gram-positive and coliform organisms such as *Escherichia coli* and microorganisms of the *Enterobacter* genus, whose minimum inhibitory concentrations were higher than in OECP. In this study, the activity against gram-positive microorganisms such as *Staphylococcus aureus* was higher than that of other microparticles obtained from other propolis derivatives, such as ethanolic extracts (Da Cruz Almeida et al. 2017).

Previous studies have investigated microencapsulated with maltodextrin as the wall material, where the encapsulated bioactive compounds were plant extracts such as guava (*Psidium guajava* L) (Fernandes R et al. 2014), cinnamon (*Cinnamomum zeylanicum*) (Ostroschi et al. 2018), soybean (*Glycine max*) (Kalušević et al. 2017) and walnut (*Juglans regia*) (Cheraghali et al. 2018). The results of these studies indicated that none of the bioactive compounds exhibited activity against coliforms or gram-positive bacteria. Therefore, the results presented in Table 6 mean that MOECP had better *in vitro* antimicrobial

Table 6. Minimum inhibitory concentration (MIC) of propolis OECP- according to various parameters used in the Spray Dryer.

Microorganism (mg mL^{-1})	CMI (mg mL^{-1})								
	Sample (MOECP)								OECP
	1	2	3	4	5	6	7	8	
E. coli	5±0.2^{a}	5±0.2^{a}	5±0.2^{a}	5±0.2^{a}	5±0.2^{a}	10±0. 6^{b}	10±0. 6^{b}	10±0.6^{b}	5±0.2^{a}
Staphylococcus aureus	0.6±0.05^{a}	1.25±0.10^{a}	2.5±0.10^{b}	2.5±0.10^{b}	2.5±0.10^{b}	2.5±0.10^{b}	2.5±0.10^{b}	5±0.10^{c}	0.306±0.05^{a}
Salmonella enteriditis	-	-	-	-	-	-	-	-	5±0.2^{a}
Enterobacter aerogenes	5±0.10^{a}	5±0.10^{a}	5±0.10^{a}	5±0.10^{a}	10±0.30^{b}	10±0.30^{b}	-	-	5±0.5^{a}
Enterobacter agglomerans	5±0.20^{a}	5±0.30^{a}	10±0.30^{b}	10±0.30^{b}	10±0.30^{b}	10±0.30^{b}	-	-	10±0.5^{a}
Klebsiella sp.	-	-	-	-	-	-	-	-	10±0.5^{a}

Different letters in the same column indicate significant differences (P<0.05). MIC: Minimum Inhibitory Concentration. MOECP: Microencapsulated oily extract of Colombian propolis. OECP: Colombian propolis oily extract. -: No antimicrobial activity was evidenced.

activity than other plant extracts because of the high content of bioactives with a bactericidal capacity.

Figure 2 presents the results of the multivariate analysis performed on MOECP for antimicrobial activity.

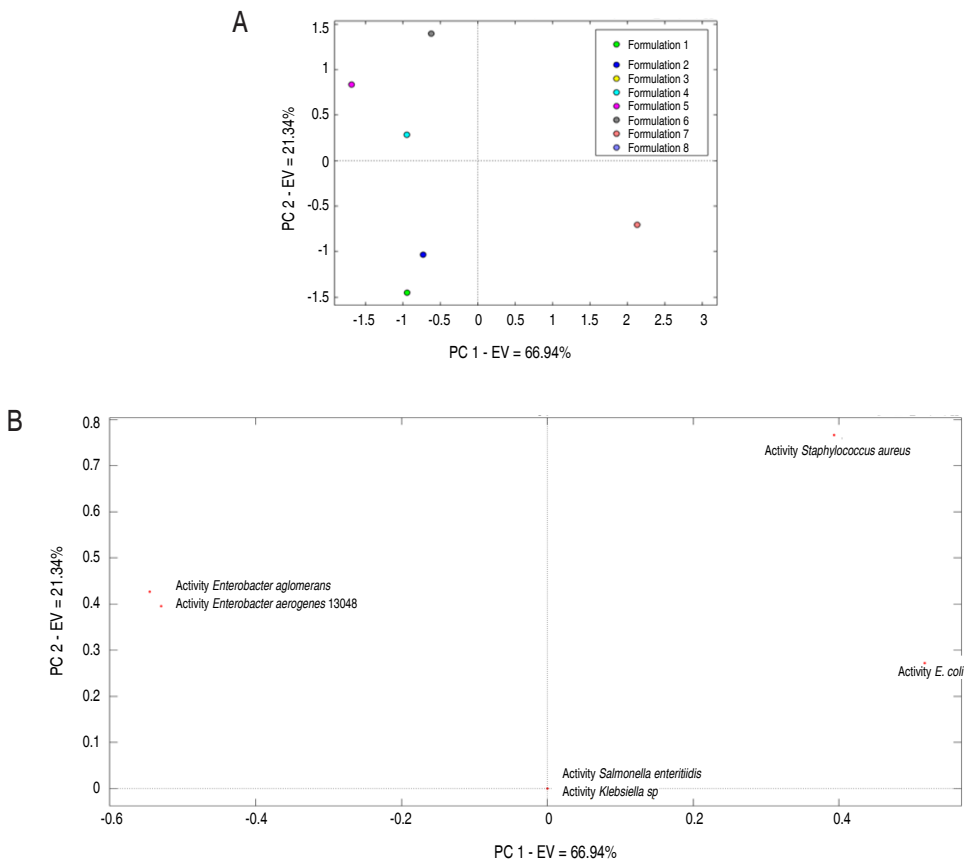

Figure 2. Principal Component Analysis. A) Score plot of microencapsulated propolis oily extracts and B) Loading plots of variables evaluated in the analysis of propolis oily extracts for antimicrobial activity.

One of the most important factors was a clear differentiation of samples according to their biological activity. Only formulations 1 and 2 shared similarities in terms of their bioactivity. Additionally, MOECP activity against *Staphylococcus aureus* and *Escherichia coli* differentiated the samples analyzed better. The activity against microorganisms of the genera *Enterobacter, Klebsiella* sp., and *Salmonella* was similar among the eight formulations, indicating that the drying conditions did not affect the activity against these pathogens. There were only two variables that effectively differentiated the samples, and the multivariate protocol allowed the samples to be differentiated.

Microscopy of the MOECP

Figure 3 shows the morphological characterization of MOECP samples using SEM. It is possible to identify that the microparticles from the oily propolis extract and the mixture of maltodextrin and Gum arabic were mostly spherical although there were amorphous particles with smaller particles were grouped. These clusters were larger in samples with higher concentrations of the oily propolis extract, such as formulations 1 and 2. Lower concentrations and higher temperatures resulted in larger particles with larger contact surfaces and lower densities of particles, possibly because of the higher evaporation rate in these treatments. The particles should be as smooth as possible to facilitates the retention of material. The results showed that temperature was the variable that most affected the micro-encapsulation process and that the samples subjected to higher temperature values had a rougher surface as determined by the microscopy analysis, meaning these particles presented higher bioactive losses.

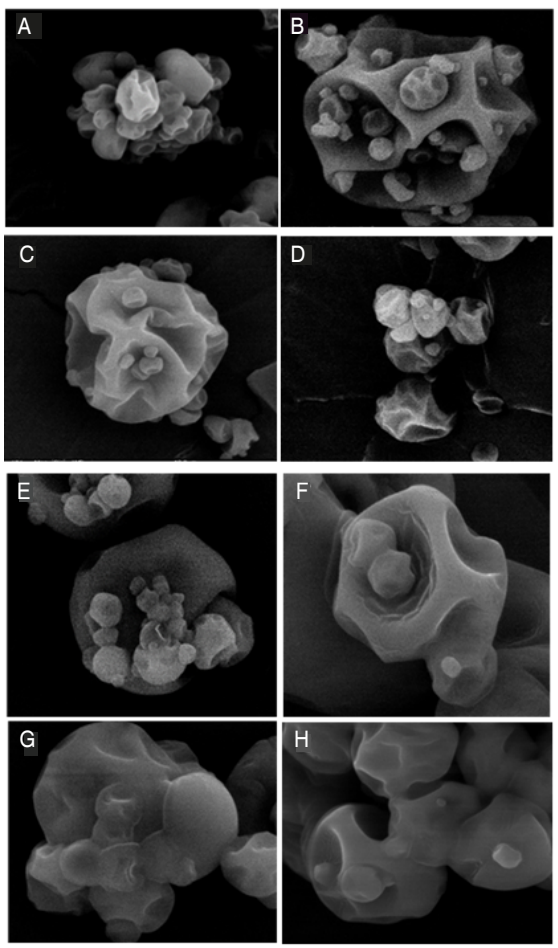

Figure 3. MOECP microparticles at 10 µm magnification. A) Sample 1 (100 °C), B) Sample 2 (120 °C), C) Sample 3 (140 °C), D) Sample 4 (160 °C), E) Sample 5 (100 °C), F) Sample 6 (120 °C), G) Sample 7 (140 °C), H) Sample 8 (160 °C). MOECP: Microencapsulated oily extract of Colombian propolis.

The results of three-dimensional microscopy of MOECP particles are related to their antioxidant activity. It has been reported that, as temperature increases, the presence of defects and cracks increases, which reduces the effectiveness of the wall material because they can degrade through oxidation and hydrolysis processes (Andrade et al. 2017). In terms of morphology, the macroparticles, regardless of their temperature and concentration, retained the same shape, as reported by Ramakrishnan et al. (2018), who used the same wall materials to encapsulate tamarind juice. Maltodextrin was responsible for circular morphology because the proportion of this polysaccharide with respect to Gum arabic was constant. Changes in the shape in the encapsulates were minimal. Microscopy revealed that in the particles with a higher concentration of the extract had more clusters because the spray drying process had a greater mass, which led to microparticles with greater thickness, higher molecular weight, and possibly more clusters (Insang et al. 2022). Samples with more clusters and greater thickness are expected to have a greater extract and, therefore, greater action against pro-oxidant substances and pathogenic bacteria.

DSC analysis of MOECP

Figure 4 and Table 7 show the results of the DSC analysis, which indicate the endothermic and exothermic changes in the MOECP.

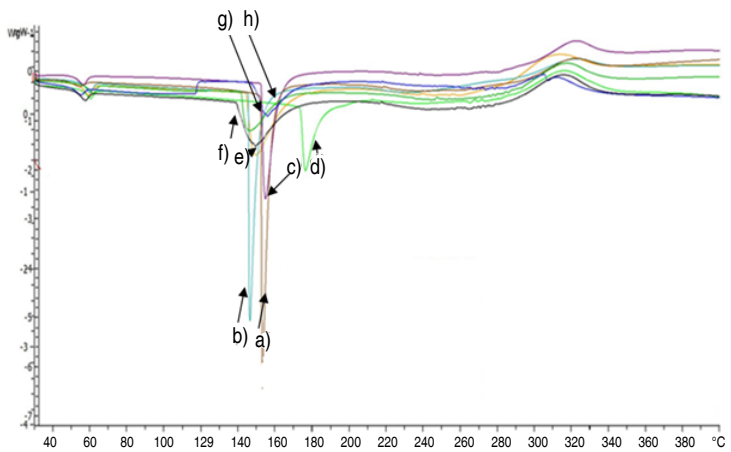

Figure 4. DSC spectra of the MOECPs. a) Light Blue: Sample 1 (100 °C), b) Brown: Sample 2 (120 °C), c) Purple: Sample 3 (140 °C), d) Light Green: Sample 4 (160 °C), e) Yellow Ochre: Sample 5 (100 °C), f) Black: Sample 6 (120 °C), g) Dark Green: Sample 7 (140 °C), h) Dark Blue: Sample 8 (160 °C). MOECP: Microencapsulated oily extract of Colombian propolis.

Table 7. Endothermic and exothermic changes in MOECP based on DSC thermal analysis.

Sample	Endothermic peak heat1 (J g^{-1})	Peak endothermic heat 2 (J g^{-1})	Peak exothermic peak heat (J g^{-1})
1	6.2±0.10^{a}	74.7±8.07^{a}	100.6±9,34^{a}
2	7.7±0.45^{a}	119.7±14.19^{b}	96±7.20^{a}
3	6.3±0.39^{a}	118.3±21.05^{b}	21.4±3.07^{b}
4	4.3±0.31^{b}	138.3±17.07^{b}	34±4.16^{b}
5	6.1±0.54^{a}	95.3±8.98^{b}	29.8±3.91^{b}
6	7±0.62^{a}	87.7±8.73^{a}	67.8±5.93^{c}
7	1.7±0.10^{c}	70.7±8.18^{a}	60.9±8.04^{c}
8	5.4±0.2^{b}	63±8.14^{b}	7.1±1.01^{d}

Samples 1 - 8 = MOECP: Microencapsulated oily extract of Colombian propolis. Different letters in the same column indicate significant differences (P<0.05).

The thermograms showed two endothermic events between 50 and 180 °C, while one exothermic event can be observed between 290 and 320 °C. The samples with a higher concentration of propolis (treatments four to eight) had endothermic peaks with increased height and thickness, while those with a lower concentration of propolis had narrower and weaker peaks. The two melting peaks correspond to Gum arabic and maltodextrin; they presented a difference in intensities, where the peak for Gum arabic (located between 50 and 65 °C) was fainter than that of maltodextrin (located between 150 and 180 °C). In turn, the samples with a higher concentration of the oily propolis extract (1, 2, 3, and 4) had less degradation than those with an extract: encapsulant ratio of (1:3) (5, 6, 7 and 8). It was concluded that, at a higher temperature and lower concentration, the intensity of the endothermic peaks increased, and, consequently, the amount of encapsulated extract decreased. It is recommended to maintain the encapsulation process conditions proposed in formulation two since it had the highest stability as represented by the higher endothermic peak.

The endothermic peak at 50 °C signaled volatilization of water, while the peaks at 150 °C corresponded to fusion processes where there were fusions between low molecular weight compounds, such as volatiles and polyphenols, and the wall material. The exothermic peak between 290 - 320 °C for all extracts indicated the loss of compound and rupture of some microencapsulated particles. This peak was more intense for samples with a higher oily extract concentration because more bioactive compound molecules underwent chemical changes, such as depolymerization and decomposition processes. The higher the intensity of the peaks, the greater the stability of the samples were, therefore, the formulations with higher OECP concentration were more stable. The heat values were higher for formulations 1 and 2, which had a higher concentration of OECP and a lower drying temperature, indicating that the samples were more resistant to high temperatures, pathogens, mechanical forces, degenerative reactions, and other decomposition processes (Ballesteros et al. 2017).

In the future, the addition of a protein system to the elaboration of microparticles, such as ovalbumin and pea protein, can be considered because encapsulates made with various bioactives from these biopolymers have presented more intense endothermic peaks, and exothermic events were not observed that signal the release of secondary metabolites from the particles and, therefore, the restoration of the structural polysaccharides to their original state (Jansen-Alves et al. 2018; Jansen-Alves et al. 2024).

CONCLUSION

OECP was encapsulated using a combination of maltodextrin and Gum arabic. The best microencapsulation seen with the 1:2 encapsulant/extract ratio and a temperature of 120 °C (formulation 2). Physicochemical characteristics and antimicrobial activity were affected by temperature and concentration. At a higher OECP concentration and a lower temperature, better antioxidant activity and greater potential against *Escherichia. coli, Staphylococcus aureus,* and microorganisms of the Enterobacter genera were observed. Formulations with higher concentration and lower drying temperature showed activity against coliforms, which was much higher than that of other encapsulates, where activity against *E. coli* provided a more effective differentiation. The Spray Drying process is effective for protecting the propolis extracts and it can maintain and enhance the activity of raw propolis samples, however, the spraying process volatilizes and degrades labile substances in the extract.

The microencapsulation of OECP enhances or preserves the activity of a sample against pro-oxidant substances and pathogens. Additionally, it allows the stable transport of active ingredients, such as gallic acid and beta-carotene, in a more reproducible manner. These results are important because MOECP could be preserved for much longer than in systems in which propolis was added without encapsulation. Furthermore, depending on the wall material and the optimal microencapsulation temperature/concentration ratio, the effective shelf-life of a bioactive can be extended. Microencapsulation of OECP using mixtures of maltodextrin and Gum arabic is possible, and more than 50% of the phenols and carotenoids in the raw sample are preserved.

A suggestion for future studies related to the encapsulation and preservation of propolis is to incorporate to the wall material formulation, new coatings that have a greater

synergism with the terpenoids and phenolic compounds contained in the raw propolis extract that do not suffer denaturation and other chemical reactions that have a negative impact on the bioactivity against pro-oxidant compounds and pathogenic bacteria. The use of coating materials different from polysaccharides such as ovalbumin is strongly recommended due to its synergistic actions with stable high molecular weight terpenoids, carotenoids and phenolic compounds. In addition, new works aimed at studying the encapsulation of propolis should employ other machines with higher speed and efficiency but with the same conditions as those advised in this study. The purpose of this study was to optimize the coating process and to obtain particles with similar conditions but with a higher yield.

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REFERENCES

Abdelrazeg S, Hussin H, Salih M and Shaharuddin B (2020) Propolis composition and applications in medicine and health. International Medical Journal 25(3): 1505-1542. https://doi.org/10.22037/iej.v13i3.20994

Almuhayawi M (2020) Propolis as a novel antibacterial agent. Saudi Journal of Biological Sciences 27(11): 3079-3086. https://doi.org/10.1016/j.sjbs.2020.09.016

Altabbal S, Athamnah K, Rahma A, Wali AF et al (2023) Propolis: A detailed insight of its anticancer molecular mechanisms. Pharmaceuticals 16(3). https://doi.org/10.3390/ph16030450

Ahangari Z and Naseri M (2018) Propolis: Chemical composition and its applications in endodontics. Iran Endodontic Journal 13(3): 285–292. https://doi.org/10.22037/iej.v13i3.20994

Andrade JKS, Denadai M, de Oliveira CS, Nunes ML and Narain N (2017) Evaluation of bioactive compounds potential and antioxidant activity of brown, green and red propolis from Brazilian northeast region. Food Research International 101: 129–138. https://doi.org/10.1016/j.foodres.2017.08.066.

Ballesteros L, Ramirez M, Orrego C, Teixeira J and Mussatto S (2017) Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. Food Chemistry 237(1): 623-631. https://doi.org/10.1016/j.foodchem.2017.05.142

Bankova V, Popova M and Trusheva B (2014) Propolis volatile compounds: Chemical diversity and biological activity: A review. Chemistry Central Journal 8(28): 1-8. https://doi.org/10.1186/1752-153X-8-28

Baysan U, Elmas F and Koç M (2018) The effect of spray drying conditions on physicochemical properties of encapsulated propolis powder. Journal of Food Process Engineering 42(4): 1-11. https://doi.org/10.1111/jfpe.13024

Bonou J, Baba-Moussa F, Adéoti Z, Ahouandjinou H, Dougnon V, Dossa D and Baba-Moussa L (2016) Antimicrobial activity of essential oils of Lippia multiflora, *Eugenia caryophyllata*, Mentha piperita and *Zingiber officinale* on five oral-dental microorganisms. Journal of Pharmacognosy and Phytochemistry 5(5): 271.

Brahmi R, Diaf K, Elbahri Z and Besbes N (2021) Microencapsulation of pharmaceutical drugs using Beeswax as a coated material: A review. Moroccan Journal of Heterocyclic Chemistry 20(1): 49-56. https://doi.org/10.48369/IMIST.PRSM/jmch-v20i1.24502

Busch V, Pereyra-Gonzalez A, Šegatin N, Santagapita P, Poklar Ulrih N and Buera M (2017) Propolis encapsulation by spray drying: Characterization and stability. LWT- Food Science and Technology, 75(1), 227-235. https://doi.org/10.1016/j.lwt.2016.08.055

Carneiro H, Tonon R, Grosso C and Hubinger M (2013) Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. Journal of Food Engineering 115(4): 443-451. https://doi.org/10.1016/j.jfoodeng.2012.03.033

Cheraghali F, Shojaee-Aliabadi S, Hosseini S, Mirmoghtadaie L et al (2018) Characterization of microcapsule containing walnut (*Juglans regia* L.) green husk extract as preventive antioxidant and antimicrobial agent. International Journal of Preventive Medicine 9(1): 1-6. https://doi.org/10.4103/ijpvm.IJPVM_308_18

Da Cruz Almeida E, Delgado da Silva M, Dos Santos Oliveira J, Umeko Kamiya R et al (2017) Chemical and microbiological characterization of tinctures and microcapsules loaded with Brazilian red propolis extract. Journal of Pharmaceutical Analysis 7(5): 280-287. https://doi.org/10.1016/j.jpha.2017.03.004

Đordevic V, Balanč B, Belščak-Cvitanović A, Lević S, Trifković K, Kalušević A, Nedović V (2014) Trends in encapsulation technologies for delivery of food bioactive compounds. Food Engineering Reviews 7(4): 452-490. https://doi.org/10.1007/s12393-014-9106-7

Fangio M, Orallo D, Gende L and Churio M (2018) Chemical characterization and antimicrobial activity against *Paenibacillus larvae* of propolis from Buenos Aires province, Argentina. Journal of Apicultural Research 58(4): 1-14. https://doi.org/10.1080/00218839.2019.1601318

Fernandes M, Dias A, Carvalho R, Souza C and Oliveira W (2014) Antioxidant and antimicrobial activities of *Psidium guajava* L. spray dried extracts. Industrial Crops and Products 60(1): 39-44. https://doi.org/10.1016/j.indcrop.2014.05.049

Fernandes R, Borges S and Botrel D (2014) Gum Arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. Carbohydrate Polymers 101(1): 524-532. https://doi.org/10.1016/j.carbpol.2013.09.083

Gomes do Nascimento T, Elleson dos Santos Arruda R, da Cruz Almeida ET et al (2019) Comprehensive multivariate correlations between climatic effect, metabolite-profile, antioxidant capacity and antibacterial activity of Brazilian red propolis metabolites during

seasonal study. Scientific Reports 9(18293). https://doi.org/10.1038/s41598-019-54591-3

Jansen-Alves C, Fernandes KF, Crizel-Cardozo MM and Krumreich FD (2018) Microencapsulation of propolis in protein matrix using spray drying for application in food systems. Food and Bioprocess Technology 11(4): 1422–1436. https://doi.org/10.1007/s11947-018-2115-4

Jansen-Alves C, Victoria FN, Borges CD and Zambiazi RC (2024) Encapsulation of propolis extract in ovalbumin protein particles: characterization and *in vitro* digestion. Natural Product Research 38(10): 1766–1770. https://doi.org/10.1080/14786419.2023.2214945

Iesa NB, Chaipoot S, Phongphisutthinant R, Wiriyacharee P Lim BG et al (2023) Effects of maltodextrin and gum arabic composition on the physical and antioxidant activities of dewaxed stingless bee cerumen. Foods 12(20). https://doi.org/10.3390/foods12203740

Insang S, Kijpatanasilp I, Jafari S and Assatarakul K (2022) Ultrasound-assisted extraction of functional compound from mulberry (*Morus alba* L.) leaf using response surface methodology and effect of microencapsulation by spray drying on quality of optimized extract. Ultrasonics Sonochemistry 82: 105806. https://doi.org/10.1016/j.ultsonch.2021.105806

Islam N, Khalil I, islam A and Hua Gan S (2014) Toxic compounds in honey. Journal of Applied Toxicology 34(7): 733–742. https://doi.org/10.1002/jat.2952

Kalušević ASL, Čalija B and Nedović V (2017) Microencapsulation of anthocyanin-rich black soybean coat extract by spray drying using maltodextrin, gum Arabic and skimmed milk powder. Journal of Microencapsulation 34(5): 475-487. https://doi.org/10.1080/02652048.2017.1354939

Kashif Maroof, Ronald FS Lee, Lee Fong Siow and Siew Hua Gan (2022) Microencapsulation of propolis by spray drying: A review, Drying Technology 40(6): 1083-1102. https://doi.org/10.1080/07373937.2020.1850470

Kubiliene L, Laugaliene V, Pavilonis A et al (2015) Alternative preparation of propolis extracts: Comparison of their composition and biological activities. BMC Complementary and Alternative Medicine 15: 156. https://doi.org/10.1186/s12906-015-0677-5

López-Patiño C, Arroqui C, Horvitz S and Vírseda P (2021) Strategies to enhance propolis ethanolic extract's flavor for its use as a natural preservative in beef. Current Research in Nutrition and Food Science 9(2). http://doi.org/10.12944/CRNFSJ.9.2.15

McClements D, Decker E and Weiss J (2007) Emulsion-based delivery systems for lipophilic bioactive components. Journal of Food Science 72(8): 109-124. https://doi.org/10.1111/j.1750-3841.2007.00507.x

Nair S and Meliani A (2018) Chemical composition and biological properties of propolis of Mascara (North-Western Algerian). World Journal of Life Sciences 1(1): 7-13. https://www.researchgate.net/publication/331074266

Ostroschi L, Brito de Souza V, Echalar-Barrientos M, Tulini F, Comunian T et al (2018) Production of spray-dried proanthocyanidin-rich cinnamon (*Cinnamomum zeylanicum*) extract as a potential functional ingredient: Improvement of stability, sensory aspects and technological properties. Food Hydrocolloids 79(1): 343-351. https://doi.org/10.1016/j.foodhyd.2018.01.007

Pant K, Thakur, M, Kumar Chopra H and Nanda V (2022) Encapsulated bee propolis powder: Drying process optimization and physicochemical characterization. LWT - Food Science and Technology 155(1): 1-10. https://doi.org/10.1016/j.lwt.2021.112956

Pasupuleti V, Sammugam L, Ramesh N and Hua Gan S (2017) Honey, propolis, and royal jelly: A comprehensive review of their biological actions and health benefits. Oxidative Medicine and Cellular Longevity 1-21. https://doi.org/10.1155/2017/1259510

Pobiega K, Krasniewska K, Derewiaka D and GniewoszM (2019) Comparison of the antimicrobial activity of propolis extracts obtained by means of various extraction methods. Journal of Food Science and Technology 56(12): 5386–5395. https://doi.org/10.1007/s13197-019-04009-9

Pratami DK, Mun'imA, Hermansyah H, GozanM and Sahlan M (2020) Microencapsulation optimization of propolis ethanolic extract from *Tetragonula* spp using response surface methodology. International Journal of Applied Pharmaceutics 12(4): 197-206. https://doi.org/10.22159/ijap.2020v12i4.37808

Ramadan A, Soliman G, Mahmoud S, Nofal S and RFAR (2012) Evaluation of the safety and antioxidant activities of *Crocus sativus* and propolis ethanolic extracts. Journal of Saudi Chemical Society 13-21. https://doi.org/10.1016/j.jscs.2010.10.012

Ramanauskienė K and Inkėnienė A (2011) Propolis oil extract: quality analysis and evaluation of its antimicrobial activity. Natural Product Research 25(15): 1463-1468. https://doi.org/10.1080/14786419.2010.529440

Ramakrishnan Y, NM Adzahan, YA Yusof, KJP Muhammad T (2018) Effect of wall materials on the spray drying efficiency, powder properties and stability of bioactive compounds in tamarillo juice microencapsulation, Powder Technology 328: 406–414. https://doi.org/10.1016/j.powtec.2017.12.018

Rodrigues R, Bilibio D, Plata-Oviedo MS, Pereira EA, Mitterer-Daltoé ML, Perin EC and Carpes ST (2021) Microencapsulated and lyophilized propolis co-product extract as antioxidant synthetic replacer on traditional Brazilian starch biscuit. Molecules 26(21): 6400. https://doi.org/10.3390/molecules26216400

Sambou M, Jean-François J, Moutombi FJN, Doiron JA et al (2020) Extraction, antioxidant capacity, 5-Lipoxygenase inhibition, and phytochemical composition of propolis from Eastern Canada. Molecules 25(2397):1-20. https://doi.org/10.3390/molecules25102397

Sanchez-Reinoso Z, Osorio C and Herrera A (2017) Effect of microencapsulation by spray drying on cocoa aroma compounds and physicochemical characterisation of microencapsulated. Powder Technology 318: 110–119. https://doi.org/10.1016/j.powtec.2017.05.040

Seven P, Seven I, Baykalir B, Mutlu S and Salem A (2018) Nanotechnology and nano-propolis in animal production and health: an overview. Italian Journal of Animal Science 17(4): 921-930. https://doi.org/10.1080/1828051X.2018.1448726

Stutz H, Bresgen N and Eckl PM (2015) Analytical tools for the analysis of β-carotene and its degradation products. Free Radical Research 49(5): 650–680. https://doi.org/10.3109/10715762.2015.1022539

Šuran J, Cepanec I, Mašek T, Radic B, Radic S, Gajger I and Vlainic J (2021) Propolis extract and its bioactive compounds—from traditional to modern extraction technologies. Molecules 26(10): 1-21. https://doi.org/10.3390/molecules26102930

Tamanna N and Mahmood N (2015) Food Processing and maillard reaction products: Effect on human health and nutrition. International Journal of Food Science 1(1): 1-6. https://doi.org/10.1155/2015/526762

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L and Byrne DH (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19(6-7): 669-675. https://doi.org/10.1016/j.jfca.2006.01.003

Tiveron A, Rosalen P, Franchin M, Coelho Lacerda R, Bueno-Silva B et al (2016) Chemical characterization and antioxidant, antimicrobial, and anti-inflammatory activities of South Brazilian organic propolis. PLOS One 11(11): 1-18. https://doi.org/10.1371/journal.pone.0165588

Woźniak M, Mrówczyńska L, Waśkiewicz A, Rogoziński T and Ratajczak I (2019) Phenolic profile and antioxidant activity of propolis extracts from Poland. Natural Product Communications 1(1): 1-7. https://doi.org/10.1177/1934578X1984977