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Enhancing gelatin matrices with propolis and royal jelly: antioxidant, physico-chemical, techno-functional, and physico-mechanical properties

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ABSTRACT

This study evaluated the effects of incorporating propolis and royal jelly into gelatin matrices (solution, powder, and film) to enhance their stability and functionality. Both bee products significantly modified the physico-chemical and techno-functional properties of gelatin. Propolis reduced conductivity (1205 to 890 μS/cm), osmolarity (195 to 130 mOsm/kg), and zeta potential (−51 to −42 mV), while royal jelly increased them (conductivity to 1390 μS/cm, osmolarity to 256 mOsm/kg, zeta potential to −60 mV). Viscosity decreased with propolis but increased with royal jelly. Antioxidant activity improved significantly with both additives ($p < 0.05$), especially propolis. Techno-functional properties such as emulsification activity (55.6 % to 66.8 %) and oil-holding capacity (2.14 to 3.25 g/g) were enhanced. Mechanical testing showed decreased tensile strength (from 48 to 35 MPa) and increased flexibility in films. Water-binding capacity was reduced by propolis and increased by royal jelly. These findings suggest that gelatin matrices enriched with bee products can serve as multifunctional delivery systems for food and pharmaceutical applications.

1. Introduction

The scientific community has paid much attention to beekeeping products due to their potential health benefits. Honeybees produce honey as their main product [1], along with several other valuable substances such as beeswax [2], bee propolis [3], bee pollen [4], bee bread [5], royal jelly [6], apilarnil [7], and bee venom [8]. Bee-derived products are rich in bioactive compounds, including vitamins, proteins, peptides, amino acids, lipids, functional polysaccharides, and polyunsaturated fatty acids, and are increasingly recognized for their nutritional density and health-promoting properties [9]. **Bee products are exceptional sources of both macronutrients and micronutrients, exhibiting a wide range of biological activities. These include antioxidant [10], antimicrobial [11], anti-inflammatory [12], immunomodulatory [13], anticancer [14], and anti-allergic [15] properties. Their unique composition and health benefits make bee products valuable for both nutritional and therapeutic applications.**

Although the medicinal properties of beekeeping byproducts were recognized by ancient civilizations, their modern use remains largely limited to nutritional supplements and health-related products. The broader application of bee products, particularly propolis, is constrained by factors such as unpleasant taste, low water solubility, strong hydrophilicity, and a distinctive odor. Despite not being officially classified as a food supplement, propolis is a key active component in many antimicrobial and pharmaceutical formulations for external use. Among bee-derived substances, propolis and royal jelly are among the most widely used due to their notable health benefits, underscoring their growing importance in the health and wellness industry [9,16]. Moreover, exposure to oxygen, heat, light, or interactions with other substances can typically deplete the bioactive chemicals and biological activity of bee products. Microencapsulation offers a promising solution to protect these compounds, enabling them to conceal their disagreeable sensory qualities and enhance them [17].

Since gelatin is a versatile natural polymer with numerous advantageous properties, it is widely used in healthcare-related fields. Gelatin

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is also used as a biomaterial to create microparticles in the biomedical field since it may be employed in various synthesis techniques, such as electrospray, desolvation, coacervation, nanoprecipitation, emulsion, and spray drying [18]. Gelatin-based microparticles have been designed to carry a variety of substances, including growth hormones, medications, proteins, cells, and genes. Gelatin is the preferred biopolymer for creating microparticles due to its numerous advantages, such as its ease of production, low cost, accessibility, biocompatibility, minimal immunogenicity, suitable biodegradability and availability of exposed chemical groups. Readily accessible functional groups found in gelatin enable a variety of coupling alterations with targeting ligands. These modifications are beneficial in developing targeted food encapsulation vehicles [19].

Several studies have explored gelatin-bee product systems, highlighting their potential to enhance the functional and sensory properties of food products. For instance, research has shown that incorporating honey and propolis extract into gelatin-based gummy jellies can significantly improve their physicochemical, textural, and antimicrobial properties, with the addition of propolis notably boosting phytochemical content and bioactivity. Other studies have also demonstrated that varying concentrations of gelatin and bee products can influence the mechanical strength and sensory acceptance of such formulations [20]. Rivero et al. [21] developed honey and propolis gummy jellies with high antioxidant capacity and demonstrated that propolis addition effectively delayed fungal growth during storage, highlighting the potential of these systems for functional food applications. Furthermore, in vivo and in vitro studies of propolis-enriched silk fibroin-gelatin scaffolds showed enhanced antibacterial activity against common pathogens, supporting the broader application of gelatin-bee product composites for bioactive and therapeutic uses [22].

To the best of our knowledge, this study is the first to comparatively evaluate the effects of both propolis and royal jelly on gelatin matrices in solution, powder, and film forms. By examining their influence on physicochemical, techno-functional, and mechanical properties, this work provides novel insights into the multifunctional enhancement of gelatin-based delivery systems. Microencapsulation of royal jelly and propolis in gelatin or functionalization of gelatin with royal jelly and propolis are nice candidates for safeguarding bee product chemicals, enabling them to contribute to a better understanding of the health-promoting properties of bee products for human health and food.

2. Materials and methods

2.1. Preparation of gelatin solutions

Gelatin (5 % w/v) was dissolved in distilled water at 40 °C. To ensure complete solubilization of the gelatin powder, ethanolamine was added to the solution at a final concentration of 0.2 %. Ethanolamine served as both a cross-linker and an emulsifier [23]. Before incorporation, the bee products (propolis and royal jelly) were dissolved in a 0.2 % ethanolamine solution at a concentration of 50 mg/mL. Different volumes of the bee product solutions (1.0, 2.0, 3.0, and 4.0 mL) were added to the gelatin solution, resulting in final concentrations of 0.2, 0.4, 0.6, and 0.8 mg/mL, respectively. The gelatin solution was thoroughly mixed and incubated at 50 °C for 12 h. Glycerol (20 % w/w) was added as a plasticizer, and the solution was homogenized to create a stable emulsion. The final volume of each solution was adjusted to 100 mL. A control gelatin solution, without bee products, was prepared using the same procedure. Based on the amount of propolis added, the resulting solutions were labeled as gelatin, gelatin/PP1, gelatin/PP2, gelatin/PP3, and gelatin/PP4. Similarly, based on the amount of royal jelly added, the solutions were labeled as gelatin, gelatin/RJ1, gelatin/RJ2, gelatin/RJ3, and gelatin/RJ4 [24].

2.2. Physico-chemical properties of gelatin solutions

The physico-chemical and rheological properties of gelatin solution, including conductivity, osmolarity, zeta-potential, dynamic particle size, viscosity, and surface tension determined according to the practical approach at ambient temperature. The conductivity was measured using a Mettler-Toledo instrument (Cleantech, Schaffhausen, Switzerland). The osmolarity of the gelatin solution was measured using an OSMOMAT 3000 osmometer (Gonotec, Germany) by comparing the freezing point of water with that of the gelatin solution. Particle size, polydispersity electrophoretic mobility and zeta-potential of gelatin particles were determined using a Horiba SZ-100 particle size analyzer (Japan) based on Dynamic Light Scattering (DLS) principles. The surface tension was measured with a Du Nouy tensiometer (Kruss, Germany). The MCR302 rheometer (Anton Paar) measured the quantitative viscosity at different shear rates [24]. The UV-Vis absorbance and fluorescence intensity of gelatin dispersions containing propolis or royal jelly were analyzed. The UV absorption spectra of the gelatin dispersions were recorded using a UV-Vis absorption spectrophotometer (UV1280, Shimadzu, Japan). Additionally, a fluorescence spectrophotometer (Varian Cary Eclipse, Agilent, USA) was used to examine the intrinsic fluorescence of the enzyme-inhibitor solutions, with excitation set at 280 nm and emission recorded across a wavelength range of 290–500 nm [16].

2.3. Total antioxidant capacity of gelatin solutions

The gelatin-propolis and gelatin-royal jelly solution was prepared as mentioned in section 2.1. The total antioxidant capacity of the mentioned gelatin solution was assessed by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical decoloration. ABTS radical solution was prepared prepared using mixing 2.54 mM potassium persulfate and 7 mM non-radocal ABTS in dark. The ABTS radical solution has maximum light absorbance at 734 nm. For total antioxidant capacity assay different concentration pof the gelatin solution (20–200 μg/mL) was mixed with 1.0 mL of ABTS radical solution and incubated for 5.0 min. After which a the light absorbance was measured at 734 nm. Distilled water was used as blasnk. ABTS radical solution was used as control was used as control. Trolox was used as standard reference. A calibration curve was generated using Trolox (0.10 mg/mL). The total antioxidant capacity was expressed as milligrams of Trolox equivalents (TE) per gram The percentage of radical inhibition and the 50 % inhibitory concentration (IC50) were calculated based on the change in absorbance at 734 nm using following equation [25].

Inhibition present (%) = [(Absorbance of pure ABTS solution-Absorbance of ABTS solution in presence of antioxidant)/Absorbance of pure ABTS solution] × 100.

2.4. Techno-functional properties of gelatin powders

Before experimentation, gelatin dispersions were freeze-dried to obtain powders. The following techno-functional properties of these powders were assessed: water content, water solubility, hygroscopicity, surface hydrophobicity, emulsification activity/stability, foam activity/stability, oil-holding capacity, and water-holding capacity (All techniques are provided in the supplementary file). These properties were determined using established methods. The interaction between gelatin and royal jelly or propolis was evaluated by Fourier transform infrared spectroscopy (FTIR) using a Bruker (Germany) FTIR spectrophotometer [26].

2.5. Preparation of gelatin films by casting method

The casting method was employed to produce the films. Centrifugation at 300×*g* for 10 min was used to eliminate the air bubbles in the film solution. The gelatin homogeneous solutions (15 mL) were poured into a disposable culture dish with a 90 mm diameter and dried at 40 °C for film

casting. The plates were placed in flat trays to obtain films with uniform thickness. Finally, the gelatin-bee product films were obtained and named as mentioned above according to the bee product addition [27].

2.6. Mechanical behavior of gelatin films

The Gotech testing machine (Germany) was used to determine tensile strength (TS), elongation at break (EAB), and elastic modulus (EM) of gelatin film. Before testing, balancing the films in a desiccator at 50 ± 5 % relative humidity for two days was done. Each film was cut into 60 mm × 10 mm strips. However, only 20 mm of each strip was clamped within the jaws, resulting in an effective initial length of 40 mm. Film thickness was measured at various points with a micrometer, and the mean was taken. The initial cross-sectional area of the film cut was 10 mm × average thickness in mm. The film samples were mounted between two grips with an initial grip separation of 4 cm and then pulled apart at a speed of 50 mm/min. TS, EM, and EAB were determined in three samples from each type of film [28].

Tensile strength (MPa) = maximum load (N)/cross-sectional area (mm^2)

Elongation at break (%) = [(length at break - initial length)/initial length] × 100

Elastic modulus (MPa) = Tensile strength/strain.

2.7. Water vapor permeability of gelatin films

According to previous studies, water vapor permeability (WVP) was evaluated gravimetrically using a bottle sealed with a film sample [29]. The penicillin bottle was filled with water and sealed with circular film samples. It was then placed inside a silica-filled box for regulated humidity and temperature. A digital balance was used for three days to monitor the sealed bottle weight reduction. The WVP value was calculated using the following equation.

$$WVP\ (g/m.Pa.s) = (W \times X)/(A \times T \times \Delta P)$$

Where W is the weight loss of the bottle (g), X is the thickness of the film (m), A is the measuring area of exposed film (m^2), t is the time (s), and ΔP is partial vapor pressure difference of the atmosphere with silica gel and pure water (3.169 × 103 Pa, 25 °C).

2.8. Water content of gelatin films

The water content measurement followed earlier studies [30]. Film samples were weighed initially, dried for 2 h at 105 °C, and weighed once again. The water content was computed using the following formula as the weight reduction percentage.

Water content% = 100 × [(initial weight - dried weight)/initial weight]

2.9. Water solubility of gelatin films

The water solubility was determined following earlier studies [31]. The film samples were dried at 105 °C for 2.0 h and weighed. After drying, the film was placed in sealed falcon tubes with 25 mL of distilled water and incubated for 1 h at 30 °C. The undissolved film residue was then recovered by filtering the mixture through the Whatman filter paper. After film drying, the remaining film segments, the final dry mass, were weighed. The following formula was used to determine the water solubility.

Water solubility% = 100 × [(initial weight - remaining weight)/initial weight]

Table 1
Physico-chemical properties of gelatin-propolis (PP) solutions, techno-functional properties of freeze-dried gelatin-propolis powder, and physico-mechanical properties of gelatin-propolis film.

Physico-chemical properties of gelatin solutions	Gelatin	Gelatin-PP1	Gelatin-PP2	Gelatin-PP3	Gelatin-PP4
Conductivity (mS/cm)	1.20 ± 0.01^{a}	1.14 ± 0.01^{b}	1.05 ± 0.01^{b}	1.04 ± 0.01^{c}	0.98 ± 0.01^{d}
Surface tension (mN/m)	47.00 ± 2.50^{a}	43.00 ± 2.00^{ab}	40.00 ± 2.40^{bc}	38.00 ± 1.70^{c}	36.00 ± 1.70^{c}
Osmolarity (mOsmol/kg)	195.00 ± 8.00^{a}	173.00 ± 9.00^{b}	155.00 ± 7.00^{c}	140.00 ± 5.00^{d}	136.00 ± 5.00^{d}
Electrophoretic mobility (mm^2/Vs)	0.04 ± 0.002^{a}	0.03 ± 00.001^{b}	0.025 ± 0.001^{c}	0.024 ± 0.001^{cd}	0.0235 ± 0.001^{d}
Zeta-potential (-mV)	51.00 ± 3.00^{a}	43.00 ± 2.80^{b}	35.00 ± 2.50^{c}	31.00 ± 2.00^{cd}	30.00 ± 2.20^{d}
Particle size (nm)	181.00 ± 8.00^{c}	184.00 ± 8.70^{c}	240.00 ± 12.00^{b}	263.00 ± 13.00^{a}	280.00 ± 15.00^{a}
Polydispersity	0.52 ± 0.02^{b}	0.75 ± 0.03^{a}	0.70 ± 0.03^{a}	0.59 ± 0.02^{b}	0.77 ± 0.04^{a}
Viscosity (mPa.s)	1.80 ± 0.27^{a}	1.66 ± 0.25^{a}	1.42 ± 0.20^{b}	1.18 ± 0.22^{c}	1.05 ± 0.24^{d}
Antioxidant capacity (mg Trolox equivalent per gram)	256.00 ± 0.12^{e}	534.00 ± 13.00^{d}	713.00 ± 18.00^{c}	944.00 ± 17.00^{b}	1018.00 ± 22.00^{a}
Techno-functional properties of freeze-dried gelatin powders	**Gelatin**	**Gelatin-PP1**	**Gelatin-PP2**	**Gelatin-PP3**	**Gelatin-PP4**
Water content (%)	10.22 ± 1.50^{a}	10.35 ± 1.20^{a}	9.89 ± 1.00^{ab}	8.55 ± 0.70^{b}	7.70 ± 0.80^{b}
Water solubility (%)	74.39 ± 3.40^{a}	73.35 ± 4.00^{a}	73.53 ± 3.70^{a}	73.07 ± 4.20^{a}	70.11 ± 3.00^{a}
Water swelling (%)	123.90 ± 7.70^{a}	120.60 ± 8.00^{a}	120.50 ± 7.00^{ab}	110.80 ± 8.50^{ab}	101.20 ± 6.50^{b}
Water hygroscopicity (%)	31.64 ± 1.70^{a}	31.50 ± 1.80^{a}	30.78 ± 1.50^{a}	28.22 ± 1.40^{ab}	25.65 ± 2.00^{b}
Hydrophobicity (μ/g)	6.16 ± 0.50^{a}	6.30 ± 0.44^{a}	6.41 ± 0.40^{a}	7.09 ± 0.54^{a}	7.50 ± 0.50^{a}
Water holding capacity (g/g)	4.02 ± 0.25^{a}	3.87 ± 0.28^{a}	3.68 ± 0.20^{a}	3.42 ± 0.23^{ab}	3.16 ± 0.20^{b}
Oil holding capacity (g/g)	2.14 ± 0.10^{c}	2.25 ± 0.13^{bc}	2.31 ± 0.15^{b}	2.57 ± 0.17^{ab}	2.82 ± 0.20^{a}
Emulsifying activity (%)	55.58 ± 4.00^{a}	55.80 ± 4.40^{a}	57.29 ± 4.70^{a}	58.14 ± 5.00^{a}	58.86 ± 4.60^{a}
Emulsion stability (%)	47.03 ± 3.60^{a}	47.70 ± 3.50^{a}	49.47 ± 5.00^{a}	52.16 ± 4.80^{a}	54.72 ± 5.00^{a}
Foaming capacity (%)	44.46 ± 3.00^{a}	45.00 ± 3.30^{a}	47.03 ± 4.00^{a}	48.74 ± 3.80^{a}	49.50 ± 4.50^{a}
Foam stability (%)	37.62 ± 2.80^{a}	36.90 ± 2.40^{a}	35.91 ± 2.80^{a}	35.06 ± 3.00^{a}	34.20 ± 3.20^{a}
Physico-mechanical properties of gelatin films	**Gelatin**	**Gelatin-PP1**	**Gelatin-PP2**	**Gelatin-PP3**	**Gelatin-PP4**
Tensile strength (MPa)	48.00 ± 2.50^{a}	45.00 ± 2.60^{ab}	40.00 ± 2.30^{bc}	35.00 ± 2.00^{d}	32.00 ± 2.20^{d}
Elastic modulus (MPa)	43.30 ± 3.00^{a}	40.20 ± 3.40^{ab}	35.10 ± 2.80^{b}	29.30 ± 2.50^{c}	27.00 ± 2.30^{c}
Elongation at break (%)	112.00 ± 5.00^{a}	112.00 ± 5.30^{a}	114.00 ± 6.00^{a}	120.00 ± 5.60^{a}	118.00 ± 5.00^{a}
WVP (ng/m.Pa.s)	11.10 ± 1.60^{a}	10.30 ± 1.20^{b}	9.76 ± 1.30^{c}	9.47 ± 1.00^{d}	9.30 ± 1.10^{d}
Water content (%)	16.50 ± 1.70^{a}	16.00 ± 1.50^{a}	15.30 ± 2.00^{ab}	13.00 ± 1.40^{bc}	11.00 ± 1.00^{c}
Water solubility (%)	22.00 ± 2.50^{d}	25.00 ± 2.80^{cd}	28.00 ± 2.50^{bc}	32.00 ± 3.00^{ab}	37.00 ± 2.70^{a}
Water swelling (%)	188.00 ± 7.40^{a}	183.00 ± 8.00^{ab}	175.00 ± 6.50^{abc}	170.00 ± 6.00^{bc}	168.00 ± 5.70^{c}

The values are expressed as means ± standard deviation for three independent experiments. Mean values with different letters within a row are significantly different.

2.10. Swelling of gelatin films

The water swelling capacity was measured according to previous works [27]. The dried film samples were immersed in 25 mL of distilled water in a falcon tube for 1 h. At this time, solubility is negligible. Each sample was picked, surface water was removed with filter paper, and the final weight of the swelling film was determined. The following formula was used to determine the swelling.

Swelling% = 100 × [(swelled weight - initial weight)/initial weight]

2.11. Morphology of gelatin films

The morphology of films were visualized using a Tescan-vega3 scanning electron microscope (Tescan, Czech). The film was submerged in liquid nitrogen and then fractured to prepare the sample to limit deformation while fracturing. Then, the sample was mounted onto a bronze stub with conducting resin and sputtered with gold in an ion sputter coater (R-ES150Q, Quorum Technologies, England) for 2 min. All samples were photographed at a voltage of 15 kV with 10000× magnification [28].

2.12. Statistical analysis

The data are presented as mean values plus standard deviations based on three studies. Significant differences between treatments were analyzed using one-way analysis of variance (ANOVA) and Tukey posthoc testing in a statistical package for the social sciences (SPSS, Abacus Concepts, Berkeley, CA). The relationships between the active observation (gelatin solution, powder, film) and the association between different active variables (conductivity, osmolarity, surface taction, zeta potential, particle size, viscosity, water-holding capacity, oil-holding capacity, emulsion capacity, foaming capacity, mechanical properties and water binding capacity) of the gelatin materials was evaluated by principal component analysis (PCA) using using Minitab software (version 20.1.2).

3. Results and discussions

3.1. Physico-chemical properties of gelatin-bee product solutions

The physico-chemical behavior of gelatin solution was electrical conductivity (1205 μS/cm), osmolarity (195 milliosmol/kg), surface tension (47 mN/m), zeta potential (−51 mV), particle size (182 nm), particle size distribution (0.52). The addition of bee propolis significantly altered the physicochemical properties of the gelatin solution, leading to a decrease in electrical conductivity, osmolarity, surface tension, zeta potential, and an increase in particle size (Table 1 and Figs. S1 and S2 in supplementary file). The addition of royal jelly significantly altered the physicochemical properties of the gelatin solution, leading to an increase in electrical conductivity, osmolarity, surface tension, zeta potential, and particle size (Table 2 and Figs. S3 and S4 in supplementary file). Gelatin primarily consists of glycine, proline, and hydroxyproline. The incorporation or loading of propolis (naturally hydrophobic due to its high content of wax and balsam) and royal jelly (naturally hydrophilic due to its sugar and protein content) onto the gelatin chain network leads to differential modifications in the physicochemical properties of gelatin. Our previous work (Table S1 in supplementary file) suggested that propolis is mainly composed of balsam > wax > sugar > fatty acid > protein > phenol > flavonoid with

Table 2
Physico-chemical properties of gelatin-royal jelly (RJ) solutions, techno-functional properties of freeze-dried gelatin-royal jelly powder, and physico-mechanical properties of gelatin-royal jelly film.

Physico-chemical properties of gelatin solutions	Gelatin	Gelatin-RJ1	Gelatin-RJ2	Gelatin-RJ3	Gelatin-RJ4
Conductivity (μS/cm)	1.20 ± 0.01^{b}	1.20 ± 0.01^{b}	1.23 ± 0.01^{a}	1.26 ± 0.02^{a}	1.25 ± 0.02^{a}
Surface tension (mN/m)	47.00 ± 2.50^{a}	43.00 ± 2.50^{a}	44.00 ± 2.80^{a}	47.00 ± 2.60^{a}	48.00 ± 3.00^{a}
Osmolarity (mOsmol/kg)	195.00 ± 8.00^{b}	198.00 ± 6.00^{b}	215.00 ± 7.00^{a}	220.00 ± 7.00^{a}	230.00 ± 9.00^{a}
Electrophoretic mobility (mm^2/Vs)	0.04 ± 0.002^{b}	0.04 ± 0.002^{b}	0.044 ± 0.002^{a}	0.046 ± 0.002^{a}	0.0315 ± 0.001^{a}
Zeta-potential (-mV)	51.00 ± 3.00^{b}	53.00 ± 3.30^{b}	56.80 ± 3.20^{a}	60.40 ± 3.50^{a}	58.00 ± 3.50^{a}
Particle size (nm)	181.00 ± 8.00^{b}	196.00 ± 10.00^{b}	237.00 ± 13.00^{a}	270.00 ± 12.00^{a}	265.00 ± 15.00^{a}
Polydispersity	0.52 ± 0.02^{b}	0.82 ± 0.04^{a}	0.94 ± 0.04^{a}	0.99 ± 0.05^{a}	0.44 ± 0.01^{c}
Viscosity (mPa.s)	1.80 ± 0.27^{d}	2.05 ± 0.22^{cd}	2.27 ± 0.25^{bc}	2.30 ± 0.30^{ab}	2.70 ± 0.35^{a}
Antioxidant capacity (mg Trolox equivalent per gram)	256.00 ± 12.00^{e}	466.00 ± 14.00^{d}	560.00 ± 15.00^{c}	617.00 ± 14.00^{b}	856.00 ± 17.00^{a}
Techno-functional properties of freeze-dried gelatin powder	**Gelatin**	**Gelatin-RJ1**	**Gelatin-RJ2**	**Gelatin-RJ3**	**Gelatin-RJ4**
Water content (%)	10.22 ± 1.50^{a}	9.61 ± 1.00^{a}	10.42 ± 1.30^{a}	11.22 ± 1.10^{a}	12.26 ± 1.40^{a}
Water solubility (%)	74.39 ± 3.40^{a}	76.11 ± 4.60^{a}	76.91 ± 3.80^{a}	76.91 ± 4.30^{a}	79.41 ± 4.60^{a}
water swelling (%)	123.90 ± 7.70^{ab}	130.80 ± 7.70^{b}	138.60 ± 8.30^{ab}	147.21 ± 8.50^{a}	151.41 ± 9.00^{a}
Water hygroscopicity (%)	31.64 ± 1.70^{c}	34.45 ± 1.60^{bc}	35.25 ± 2.00^{ab}	36.05 ± 2.20^{a}	40.36 ± 2.50^{a}
Hydrophobicity (μ/g)	6.16 ± 0.50^{a}	5.85 ± 0.47^{a}	5.51 ± 0.40^{ab}	5.01 ± 0.43^{b}	4.51 ± 0.37^{b}
Water holding capacity (g/g)	4.02 ± 0.25^{c}	4.01 ± 0.18^{c}	4.09 ± 0.25^{c}	4.77 ± 0.28^{b}	5.13 ± 0.35^{a}
Oil holding capacity (g/g)	2.14 ± 0.10^{a}	1.76 ± 0.10^{b}	1.84 ± 0.13^{b}	2.00 ± 0.14^{a}	2.16 ± 0.13^{a}
Emulsifying activity (%)	55.58 ± 4.00^{a}	57.19 ± 4.50^{a}	61.59 ± 5.30^{a}	62.49 ± 5.70^{a}	64.09 ± 5.50^{a}
Emulsion stability (%)	47.03 ± 3.60^{b}	52.08 ± 4.40^{ab}	53.88 ± 4.70^{ab}	58.57 ± 4.50^{a}	59.47 ± 5.00^{a}
Foaming capacity (%)	44.46 ± 3.00^{b}	50.47 ± 4.00^{ab}	53.88 ± 3.80^{a}	57.60 ± 4.70^{a}	61.20 ± 5.20^{a}
Foam stability (%)	37.62 ± 2.80^{a}	37.66 ± 2.40^{a}	39.55 ± 2.70^{a}	40.16 ± 3.00^{a}	43.56 ± 3.50^{a}
Physico-mechanical properties of gelatin film	**Gelatin**	**Gelatin-RJ1**	**Gelatin-RJ2**	**Gelatin-RJ3**	**Gelatin-RJ4**
Tensile strength (MPa)	48.00 ± 2.50^{a}	45.00 ± 2.60^{ab}	44.00 ± 2.30^{ab}	43.00 ± 2.00^{ab}	42.00 ± 2.20^{b}
Elastic modulus (MPa)	43.30 ± 3.00^{a}	40.30 ± 3.50^{ab}	38.40 ± 3.40^{ab}	36.30 ± 2.50^{b}	34.20 ± 2.80^{b}
Elongation at break (%)	112.00 ± 5.00^{a}	112.00 ± 5.50^{a}	114.00 ± 5.00^{a}	118.00 ± 6.30^{a}	123.00 ± 6.00^{a}
WVP (ng/m.Pa.s)	11.10 ± 1.60^{b}	11.30 ± 1.40^{b}	11.45 ± 1.20^{ab}	11.70 ± 1.50^{a}	11.67 ± 1.20^{a}
Water content (%)	16.50 ± 1.70^{b}	17.00 ± 2.00^{ab}	18.00 ± 1.50^{ab}	20.00 ± 1.70^{a}	21.00 ± 2.00^{a}
Water solubility (%)	22.00 ± 2.50^{b}	23.00 ± 1.60^{b}	28.00 ± 1.80^{a}	33.00 ± 2.30^{a}	36.00 ± 2.60^{a}
Water swelling (%)	188.00 ± 7.40^{a}	184.00 ± 6.00^{a}	186.00 ± 7.70^{a}	192.00 ± 8.00^{a}	197.00 ± 7.00^{a}

The values are expressed as means ± standard deviation for three independent experiments. Mean values with different letters within a row are significantly different.

strong antioxidant activity. Royal jelly is mainly composed of sugar > protein > fatty acid > balsam > wax > phenol > flavonoid with strong antioxidant activity [16].

3.2. Viscosity of gelatin-bee product solutions

The gelatin solution exhibited Newtonian behavior under the shear rates applied. The addition of propolis significantly decreased the viscosity of the gelatin solution (Fig. 1A). In contrast, adding royal jelly significantly increases the viscosity of the gelatin solution (Fig. 2A). Our experimental results suggested the differential effect of propolis and royal jelly on the viscosity of gelatin solution that could be attributed to different chemical compositions and physico-chemical properties. Viscoelasticity of a biopolymer solution depends on the shear rate (flow rate), particle concentration, particle shape (spherical, non-spherical, ellipsoid, regular, irregular, star-shape), particle size distribution (polydisperse or monodisperse), particle surface charge (zeta-potential), electrical conductivity, surface tension and osmolarity [32].

The viscosity of a Newtonian liquid stays constant in response to an increase in shear rate. No substantial particle-particle interactions take place in Newtonian fluids with an increase in shear rate. The viscosity of Newtonian liquids rose with the rise in particle concentration. Particles collide with one another as the concentration increases, and these interactions significantly raise the shear tension needed to shear the solvent. The viscosity increased by increasing the osmolality of a given particle solution. Increased polar molecular mobility results in increased electrical conductivity and decreased viscosity [33].

In the case of smaller particles, the increased number of particles per unit volume, larger surface area, and greater Brownian motion result in higher viscosity at a given shear rate and particle concentration. The maximal packing density of polydisperse suspensions is more significant than that of monodisperse suspensions, and for a given particle concentration, a higher packing density results in a lower viscosity [34]. Irregular particle surfaces led to a higher viscosity compared to regular

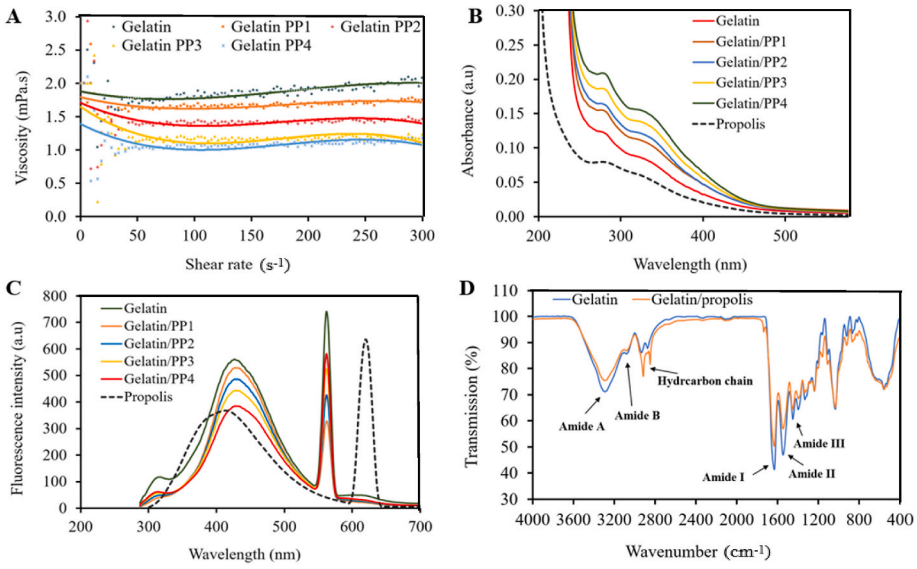

Fig. 1. Viscosity-shear rate profile (A), (UV-Vis) light absorbance (B), fluorescence intensity (C), and Fourier transform infrared spectroscopy of (D) gelatin incorporated with different concentrations of propolis (PP).

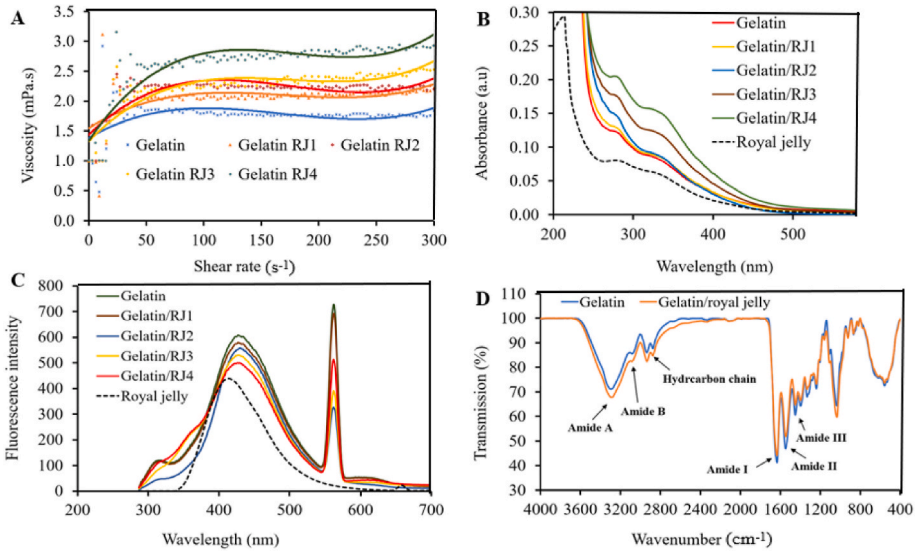

Fig. 2. Viscosity-shear rate profile (A), (UV-Vis) light absorbance (B), fluorescence intensity (C), and fourier transform infrared spectroscopy of (D) gelatin incorporated with different concentrations of royal jelly (RJ).

particles. An increase in specific particle surface in unstable particles raises the likelihood of particle-particle interactions; hence, a more significant impact of inter-particle friction is anticipated, increasing viscosity. Because of the more significant aspect ratio and surface area, suspensions containing ellipsoid particles have higher viscosities at low shear rates than those containing typical spherical particles. Because elongated particles are randomly oriented but align to the flow direction at a greater shear rate, suspensions containing ellipsoid particles exhibit lower viscosities at higher shear rates than ordinary spheral particles [35].

Sedimentation, flocculation, or particle accumulation are preferred for a sample with a low zeta potential. Particles may effectively reject each other in samples with a high zeta potential, preventing agglomeration. With a given particle size, particle shape, and low shear rate, the viscosity increases with increasing zeta potential due to particle repulsion, increased hydrodynamic volume, and increased Brownian motion. Particles clump together with low zeta potential, and as the agglomeration grows more significant over time, gravity will eventually lead to sedimentation and a rise in viscosity [34]. Surface tension and viscosity depend on molecular interactions, and with the increase in surface tension, the particle interaction increases; finally, the viscosity also increases [36].

Thus, it is essential to consider all relevant factors, including shear rate, particle concentration, size distribution, surface charge, electrical conductivity, surface tension, and osmolarity, to comprehend the rheological behavior of the gelatin-bee product suspensions.

3.3. UV-vis light absorption and fluorescence intensity analysis

UV-Vis light absorption and fluorescence intensity analysis are efficient tools for detecting protein-protein and protein-ligand interactions. The UV absorption of gelatin in the 270–320 nm range increased from 0.12 to 0.21 with increasing concentrations of propolis (Fig. 1B) and showed a similar trend with royal jelly (Fig. 2B). As a result, propolis and royal jelly can form non-covalent complexes with gelatin strains, modify gelatin strand conformation, and depict the aromatic group to UV radiation, enhancing UV absorption. Furthermore, propolis and royal jelly at 270–330 nm had strong UV absorption capacity, and this increase in UV may be related to these ingredients. Gelatin, gelatin/propolis, and gelatin/royal jelly emit fluorescence at 400–500 nm, while maximum emission was absorbed at 440 nm. The fluorescence emission intensity of gelatin decreased from approximately 580 to 380 upon the addition of propolis (Fig. 1C), while a reduction to 500 was observed with royal jelly (Fig. 2C). Both propolis and royal jelly at 400–500 nm had strong fluorescence emission. The fluorescence quenching establishes the interaction between gelatin and propolis or royal jelly, reflecting environment and polarity changes. The non-covalent interactions between propolis or royal jelly and gelatin form a non-fluorescent compound, altering the microenvironment of gelatin. The increase in the collision of fluorescent groups in the gelatin, propolis, and royal jelly lowers the intrinsic fluorescence intensity [16].

3.4. Antioxidant activity of propolis and royal jelly

The total antioxidant activity of gelatin is low but the addition of propolis (Table 1, Fig. S5 in supplementary file) and royal jelly (Table 2, Fig. S6 in supplementary file) significantly increases the antioxidant activity of gelatin. Bee products are considered a potential source of natural antioxidants that can counteract the effects of oxidative stress underlying the pathogenesis of many diseases. The antioxidant capacity of several extractions (water, ethanol, methanol, butanol, hexane, cyclohexane, dichloromethane, petroleum ether, ethyl acetate) of bee products such as honey, pollen, propolis, beeswax, royal jelly, and bee venom, and the analytical methods used were reviewed by Martinelli & Mutinelli [10]. The high content of phenolic compounds, flavonoids, monoterpenoids, and monoterpenes in these bee products and their synergistic effects are considered the primary contributors to anti-radical activities. Nonetheless, bee products are complex natural substances and therefore also contain other substances presenting antioxidant activity, including minerals, amino acids, peptides, proteins, organic acids, polyunsaturated fatty acids, fibers, and polysaccharides. Apart from phenolic compounds, other components of bee products, such as protein, lipid, and carbohydrate, also exhibit antioxidant activity. Proteins containing sulfur/hydrophobic/aromatic/acidic/basic amino acids can react with free radicals and donate protons or electrons to convert them into stable forms. Lipids containing long-chain polyunsaturated omega-3 and omega-6 fatty acids can scavenge superoxide and display antioxidant activity dependent on their degree of unsaturation. Water-soluble non-starch polysaccharides in bee products have significant bioactivities such as anti-oxidation, hypoglycemic, anti-cancer, and anti-bacterial activity [10].

3.5. Techno-functional properties of gelatin-protein hydrolysate powders

The techno-functional properties of the gelatin powders were evaluated, including water content (10.2 %), water solubility (74.4 %), water swelling (124 %), hygroscopicity (31.6 %), hydrophobicity (6.2 μg/g), emulsification activity (55.6 %), emulsification stability (47 %), foam activity (44.5 %), foam stability (37.6 %), water-holding capacity (4.02 g/g), and oil-holding capacity (2.14 g/g). The addition of bee propolis resulted in minor changes to these properties, including a decrease in water content, water solubility, water swelling, and hygroscopicity. Conversely, it led to an increase in hydrophobicity, emulsification activity, emulsification stability, foam expansion activity, and oil-holding capacity (Table 1). Similarly, the addition of royal jelly resulted in minor changes to these properties, including an increase in water content, water solubility, water swelling, hygroscopicity, emulsification activity, emulsification stability, foam expansion activity, foam stability, water-holding capacity, and oil-holding capacity (Table 2). Our experimental results suggested the differential effect of propolis and royal jelly on the physico-chemical properties of gelatin freeze-dried powder that could be attributed to different chemical compositions.

The techno-functional activities of the protein solution depend on various internal protein characteristics like; primary structure (i.e., the amino acid sequence, amino acid composition, distribution of hydrophilic and hydrophobic amino acids, the position of cysteine amino acid), secondary structure (the length, sequence, and position of α-helix, β-sheet, β-turn, loop), tertiary structure (globular or fibrous protein), quaternary structure (heteromer or homomer), protein size, protein mass (molecular weight), protein net charge (isoelectric point), protein conformation, folding/unfolding status, surface hydrophobicity, amphipathic character, molecular flexibility, segmental mobility, and existence of non-covalent interactions (electrostatic, hydrophobic, hydrogen bond). Furthermore, the techno-functional activity of protein depends on various external factors such as protein concentration, solvent, pH, temperature, ionic strength, salts (salting-in and salting-out), ingredient, protein treatment (germination, fermentation, soaking, toasting, autoclaving), protein etching, protein oxidation, reducing agents, protein cross-linking, nonprotein components and the mechanical stress [37]. Gelatin is a fibrous protein, and the gelatin structure is very cooperative. The structural changes of gelatin under intrinsic and external factors will be less relevant. The biochemical nature of royal jelly and bee propolis may be the reason for a variation in the techno-functional activity of royal jelly and bee propolis. Generally, royal jelly is more hydrophilic due to high amounts of protein and carbohydrates, but bee propolis is more lipophilic due to high amounts of wax and balsam [38].

The water retention capacity of protein (water-holding capacity, water content, hygroscopicity, wettability, swelling, water retention, solubility) is the capacity of the protein to retain water (physically entrapped water, capillary water, hydrodynamic water, bound water) in the polymer matrix. The volume of water associated with a protein is

linked to its amino acids (in gelatin proline and hydroxyproline), charged residues (acidic and basic amino acid), conformation (globular or fibrous), and hydrophobicity (hydrophobic amino acid). Royal jelly with high amounts of proteins and polysaccharides could improve the water-binding capacity of gelatin. Propolis with high amounts of wax and balsam reduced water binding capacity [39].

The oil retention capacity of proteins depends on the existence of non-covalent bonds (van der Waals, hydrogen bonds, hydrophobic, electrostatic) contributed to lipid-to-protein interactions. Oil retention capacity depends on the surface availability of nonpolar hydrophobic amino acid chains. As a result, protein denaturation or partial hydrolysis exposes hydrophobic regions and thus increases the oil holding capacity value. Royal jelly with high amounts of hydrophobic amino acid must improve the oil-binding capacity of gelatin, but this change is non-significant. Propolis with a high amount of wax and balsam improves oil-holding capacity [40].

Proteins produce more stable emulsions and foams than low molecular-weight surfactants. Protein surface properties strongly influence emulsion and foam formation. Emulsifiers or foaming agents generate stable oil-water and air-water borders by reducing surface tension. The functional surface activity of a protein depends on its conformational factors (distribution of hydrophilic and hydrophobic residues, flexibility, conformational stability, segmental mobility) and external factors (temperature, pH, ionic strength). Royal jelly with high amounts of protein improved emulsion and foam capacity. Besides wax and balsam, propolis has proteins and polysaccharides and could improve emulsion and foam capacity but reduce foam stability [41].

3.6. FTIR

The FTIR patterns of gelatin/propolis, and gelatin/royal jelly are shown in Figs. 1D and 2D, respectively. The pattern of the FTIR profile of gelatin is similar to the gelatin/propolis and gelatin/royal jelly. The intensity of transmission changes at some wave numbers is due to the propolis and royal jelly. The main bands in the gelatin including amide A (3308 cm^{-1}), amide B (3100 cm^{-1}), amide I (1650 cm^{-1}), amide II (1550 cm^{-1}), and amide III (1450 cm^{-1}) remained unchanged after propolis and royal jelly addition (Table S2 in supplementary file). Based on FTIR analyses, the interactions between gelatin and propolis or royal jelly are likely non-covalent [42].

3.7. Mechanical properties of gelatin films

The control gelatin film exhibited a tensile strength of 48 MPa, an elastic modulus of 43.3 MPa, and an elongation at break of 112 %. The addition of bee propolis to the gelatin films resulted in significant changes to their mechanical properties, including a decrease in tensile strength and elastic modulus, and an increase in elongation at break (Table 1, Fig. S7 in supplementary file). Similarly, the addition of royal jelly to the gelatin films led to a decrease in tensile strength and elastic modulus and an increase in elongation at break (Table 2, Fig. S8 in supplementary file). Our experimental results indicate a differential impact of propolis and royal jelly on the physico-mechanical properties of gelatin films, likely attributable to their distinct chemical compositions. Royal jelly was more compatible with the gelatin matrix due to its hydrophilic nature [43].

The tensile strength, rigidity, and flexibility of protein polymer depend on various internal protein polymer characteristics like primary structure, secondary structure elements (α-helix, β-sheet), tertiary structure, protein size, protein mass, protein net charge, protein conformation, folding/unfolding status, surface hydrophobicity, amphipathic character, molecular flexibility, segmental mobility, existence of non-covalent interactions, protein treatment, protein etching, protein oxidation, reducing agents, protein cross-linking, and nonprotein ingredients [44]. The intermolecular force between polymer chains and the film network microstructure is typically linked to the tensile strength of biodegradable films. The discontinuous film microstructure is caused by incompatible chemicals within the film. As a result, the external force is distributed unevenly across each matrix bond, which reduces the mechanical strength of the system. Adding propolis or royal jelly in protein-based films may partially replace stronger polymer-polymer interactions with weaker polymer-propolis or

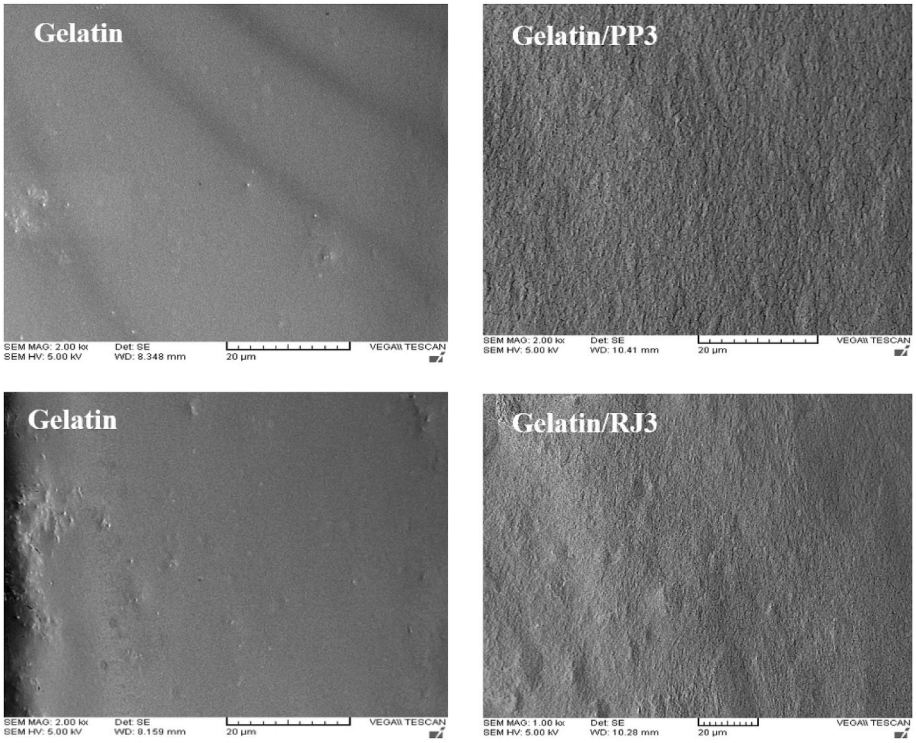

Fig. 3. Cross-sectional morphology of gelatin film and gelatin film incorporated with propolis (PP) and royal jelly (RJ).

polymer-royal jelly interactions in the film network, decreasing tensile stress [45]. The decrease in tensile strength in the presence of propolis and royal jelly is probably due to intermolecular interactions between the functional groups of polymers and these substances. It is possible to substitute new contacts between the polymer, propolis, and royal jelly components for the initial interactions between the polymer chains. The film tensile strength may be lowered, and the integrity of the polymer matrix may be altered by these changes [46]. By creating new bonds between the propolis and royal jelly and the polymer chains, replacing the old ones, and interfering with non-covalent interactions between polymer chains, the addition of propolis and royal jelly to the gelatin film increased its flexibility and decreased its rigidity. Consequently, the polymer chains' segmental mobility rose, causing the chains to slide against one another more frequently and become more flexible [47]. The increase of propolis and royal jelly led to the reduction of the polymer chain cohesion forces, creating a heterogeneous matrix and subsequently lowering the tensile strength and increasing the flexibility of the films.

3.8. Water binding capacity of gelatin films

The water binding capacity of gelatin film was water vapor permeability (1110×10^{-10} mg/m Pa s), water content (16.5 %), water solubility (22 %), and water swelling (188 %). The addition of bee propolis to the gelatin films significantly reduced water vapor permeability, water content, water solubility, and water swelling (Table 1). Conversely, the addition of royal jelly increased these properties (Table 2). Because propolis and royal jelly have diverse chemical compositions, our experimental results revealed that they have different effects on the water-binding capacity of gelatin films [48]. The difference in water binding capacity may be related to the microstructure of gelatin films added with propolis or royal jelly and the hydrophilic/lipophilic nature of propolis and royal jelly. Royal jelly with high amounts of proteins and polysaccharides could increase the water-binding capacity of gelatin. Propolis with high amounts of wax and balsam reduced water binding capacity [39]. The water retention capacity of protein-based polymeric films (water content, hygroscopicity, wettability, swelling, water retention, water solubility, water vapor permeability) is the capacity of the protein polymer to retain water (physically entrapped water, hydrodynamic water, bound water, capillary water) in the polymer matrix. The volume of water associated with a

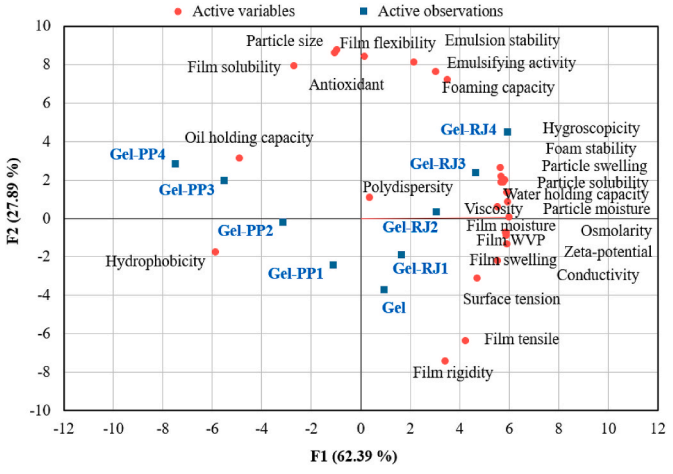

Fig. 4. Relationships between the gelatin materials (solution, powder, film) and the association between different variables including onductivity, osmolarity, surface taction, zeta potential, particle size, viscosity, water-holding capacity, oil-holding capacity, emulsion capacity, foaming capacity, mechanical properties, and water binding capacity of gelatin materials as analyzed by principal component analysis.

protein is linked to its amino acid composition, charged residues, conformation (globular or fibrous), hydrophobicity, hygroscopy, existence of non-covalent interactions, protein treatment, protein etching, protein oxidation, protein cross-linking, and nonprotein ingredients [47].

Bee propolis could reduce water vapor permeability. At the same time, royal jelly increased the water vapor permeability of the gelatin film, which could be correlated to the different lipophilic/hydrophilic natures of these materials. The rate at which water vapor can permeate a substance is measured as its water vapor permeability. Since less water vapor can permeate the material and alter the food moisture content, food packaged in biopolymers with low water vapor permeability will remain fresher for longer. By doing this, spoiling, discoloration, and flavor loss may be avoided. Another factor that preserves the food texture is low water vapor permeability. Food quality, texture, and appearance may be impacted if food packaging material with a large water vapor permeability allows excessive moisture to escape or enter the package. This could lead to the food drying out or becoming overly moist [47]. Accordingly, gelatin films incorporated with bee propolis are more suitable for packaging films with lower water permeability. Furthermore, propolis had a higher content of polyphenol and flavonoid compounds with higher antioxidant and antimicrobial activities, which helped to increase the shelf life of food [49].

The water content and swelling capacity of gelatin film could differentially change by propolis (reduce) and royal jelly (increase), and both could increase the solubility of the gelatin film. Gelatin is a fibrous and hydrophilic protein that can interact with high amounts of water molecules, mainly due to its proline and hydroxyproline content. Due to high hydrophilicity and fibrous structure, the gelatin matrix absorbs large quantities of water molecules, leading to high water content and swelling. Water binding effectiveness depends on how the addition of lipophilic/hydrophilic compounds affects the microstructure of the gelatin film. Through non-covalent binding, the functional groups of gelatin polymer can interact with components of bee propolis, enhancing the polymer-propolis interaction. This phenomenon causes the propolis to oversaturate the polymer network, blocking the interaction of the water molecules with the polymer chain and reducing the amount of water and swelling [48]. Protein and carbohydrates, which comprise most of the royal jelly, absorb enormous amounts of water molecules, increasing the water content and causing gelatin films to inflate [50].

The contacts between the polymer and the component, or the roughness of the films, which reduces the interactions stabilizing the polymer network, could cause an increase in solubility. The functional groups in the polymer chain may interact with the additive. It can reduce the integrity of the polymer matrix network and, as a result, enhance the solubility of the film by competitively breaking the hydrophobic interaction or chain-to-chain hydrogen bond. It can also increase film roughness. However, the new non-covalent connections between the polymers and functional groups of the additives replaced the original hydrogen bonds between the polymer chains. Water solubility was enhanced by the polymer chain distance and the addition of twisting pores to the polymer matrix. The water binding capacity of gelatin powder obtained by freeze-drying differs from that of gelatin film obtained by gelatin solution casting [51].

3.9. Morphology of gelatin films

SEM images were used to examine the cross-sectional morphology of the gelatin films (Fig. 3). In pure gelatin films, the cross-section was compact, homogeneous, continuous, and glassy. The cross-section morphology of films strongly depends on the polymer chain interactions and cross-linking. The homogeneous structure represents the extensive interactions between polymer chains with each other or with the cross-linker. Ethanolamine, to some extent, can cross-link gelatin chains. Thus, to some extent, pure gelatin compact, homogeneous,

continuous, and glassy structure could be attributed to the comprehensive solubilization of gelatin in an alkaline solution by adding ethanolamine. Royal jelly, mainly composed of protein and polysaccharides, did not introduce a significant change in the morphology and topology of the gelatin film. This indicates that royal jelly did not reduce gelatin polymer chain connection during the film dispersion, drying, and casting processes. Accordingly, royal jelly did not significantly impact the gelatin film morphology and structure, which may be attributed to the excellent distribution of royal jelly in the gelatin matrix [52]. Propolis, mostly made of balsam and wax, slightly altered the topology and morphology of the gelatin film by introducing microscopic pores and fissures into the matrix of the film. When propolis was added the gelatin film matrix continuity was somewhat disrupted by the wax and balsam droplets, which resulted in a small hole in the film cross-section. Therefore, the appropriate addition of propolis could improve film structure and morphology. Furthermore, propolis had a high content of polyphenols and flavonoids with vigorous antioxidant activity that improved the functional activity of gelatin films [51].

3.10. Principal component analysis (PCA)

Relationships between the gelatin materials (solution, powder, film) and the association between different variables (conductivity, osmolarity, surface taction, zeta potential, particle size, viscosity, water-holding capacity, oil-holding capacity, emulsion capacity, foaming capacity, mechanical properties, and water binding capacity) were analyzed by PCA. The PC1 accounted for 62.39 % and, PC2 for 27.89 % of the total variance. Gelatin materials and gelatin/royal jelly materials are strongly related to conductivity, osmolarity, surface tension, zeta potential, polydispersity, viscosity, water-holding capacity, water-binding capacity, hygroscopicity, foam stability, film tensile strength, and film rigidity. Gelatin/propolis materials are strongly related to hydrophobicity, oil-holding capacity, and antioxidant capacity. As a result although royal jelly has a lower antioxidant capacity than propolis but higher compatibility with gelatin materials probably due to high amounts of carbohydrates and protein (Fig. 4).

4. Conclusion

This study demonstrated that the incorporation of propolis and royal jelly into gelatin matrices, across solution, powder, and film forms, can significantly enhance their physicochemical, techno-functional, and mechanical properties. The distinct effects observed are attributable to the unique chemical compositions of each bee product: propolis (rich in waxes and balsams) and royal jelly (rich in proteins and polysaccharides). Notably, both additives improved antioxidant activity and functional performance, with propolis showing stronger effects on stability-related parameters, and royal jelly enhancing hydration and flexibility. These modifications, while causing only minor structural changes to the gelatin matrix, substantially broaden its functionality. This is the first study to compare the impact of both propolis and royal jelly on gelatin in multiple physical forms. The results highlight their promise as multifunctional components for bioactive food and pharmaceutical delivery systems.

CRediT authorship contribution statement

Maryam Behfar: Writing – original draft, Data curation. **Fatemeh-Sadat Hashemirad:** Investigation, Formal analysis, Conceptualization. **Gholamreza Kavoosi:** Supervision, Funding acquisition. **Seyed Mohammad Mahdi Dadfar:** Writing – review & editing, Methodology.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used OpenAI's tool ChatGPT to enhance the readability, clarity, and fluency of the text. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2025.102219.

Data availability

No data was used for the research described in the article.

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