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Bee Pollen Extract- α -Cyclodextrin Inclusion Complexes for Improved Antioxidant Defense in Sperm Preservation

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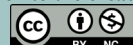
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Abstract. Bee pollen is a complex natural product rich in phenolic and flavonoid compounds with remarkable biological activities. However, its poor solubility limits its functional applications. In this study, bee pollen collected from Kherrata (Bejaia, Algeria) was extracted with ethanol and characterized using spectroscopic and chromatographic methods. High-performance liquid chromatography (HPLC) confirmed the presence of quercetin as one of the major bioactive compounds. To overcome its low solubility, inclusion complexes with α -cyclodextrin were prepared and analyzed by FTIR spectroscopy and phase solubility studies, revealing A_L -type diagrams consistent with 1:1 stoichiometric inclusion. Encapsulation with α -cyclodextrin resulted in superior complex stability and enhanced aqueous solubility. The encapsulated bee pollen extract and quercetin exhibited improved antibacterial activity, particularly against *E. coli* and *K. pneumoniae*, while retaining cytocompatibility.

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In sperm cryopreservation assays, the ethanolic extract and its α -cyclodextrin complexes significantly enhanced motility parameters (VCL, VSL, VAP) and preserved sperm integrity under oxidative stress. These effects are attributed to the sustained release and synergistic antioxidant action of phenolic constituents within the inclusion complex.

Finally, this study demonstrates that α -cyclodextrin encapsulation effectively enhances the physicochemical stability, solubility, and bioefficacy of bee pollen-derived bioactives. The combined antioxidant, antibacterial, and cryoprotective effects open new perspectives for the development of natural, functional, and biocompatible systems for biomedical and reproductive applications.

Resumen. El polen de abeja es un producto natural complejo, rico en compuestos fenólicos y flavonoides con notables actividades biológicas. Sin embargo, su baja solubilidad limita sus aplicaciones funcionales. En este estudio, el polen de abeja recolectado en Kherrata (Bejaia, Argelia) se extrajo con etanol y se caracterizó mediante métodos espectroscópicos y cromatográficos. La cromatografía líquida de alta resolución (HPLC) confirmó la presencia de quercetina como uno de los principales compuestos bioactivos. Para superar su baja solubilidad, se prepararon complejos de inclusión con α -ciclodextrina y se analizaron mediante espectroscopía FTIR y estudios de solubilidad de fases, que revelaron diagramas de tipo A_L compatibles con una inclusión estequiométrica 1:1. La encapsulación con α -ciclodextrina aumentó la estabilidad del complejo y mejoró la solubilidad en medio acuoso. El extracto encapsulado de polen de abeja y la quercetina mostraron una mayor actividad antibacteriana, particularmente frente a *E. coli* y *K. pneumoniae*, al tiempo que conservaron su citocompatibilidad. En los ensayos de criopreservación de espermatozoides, el extracto etanólico y sus complejos con α -ciclodextrina mejoraron significativamente los parámetros de motilidad (VCL, VSL y VAP) y preservaron la integridad de los espermatozoides bajo condiciones de estrés oxidativo. Estos efectos se atribuyen a la liberación sostenida y a la acción antioxidante sinérgica de los constituyentes fenólicos del complejo de inclusión.

Finalmente, este estudio demuestra que la encapsulación con α -ciclodextrina mejora eficazmente la estabilidad fisicoquímica, la solubilidad y la bioeficacia de los compuestos bioactivos derivados del polen de abeja. La combinación de efectos antioxidantes, antibacterianos y crioprotectores abre nuevas perspectivas para el desarrollo de sistemas naturales, funcionales y biocompatibles destinados a aplicaciones biomédicas y reproductivas.

Introduction

Bee pollen has been recognized for its nutritional and therapeutic benefits [1]. It is collected by worker bees from the stamens of flowering plants, mixed with nectar and salivary secretions, and serves as a primary food source for the colony [2,3]. Rich in flavonoids and polyphenols, bee pollen exhibits strong antioxidant activity that helps counteract oxidative stress [4]. Among its main constituents, quercetin stands out as one of the major flavonoids widely found in onions, apples, berries, tomatoes, and tea. Quercetin possesses significant antioxidant and antibacterial properties but suffers from poor water solubility, low stability, and limited bioavailability, which restrict its use in food and pharmaceutical formulations [5-7].

To address these limitations, cyclodextrin encapsulation has been proposed as an efficient strategy to improve solubility and stability of poorly soluble bioactives. Xu et al. (2023) encapsulated dihydroquercetin within β -cyclodextrin using co-precipitation [8], while Tang et al. (2023) and Wang et al. (2022) confirmed enhanced stability and biological performance of quercetin-cyclodextrin complexes [5,7]. Christaki et al. highlighted that α -cyclodextrin-based inclusion complexes improve the solubility, stability, and bioavailability of natural bioactive compounds, particularly polyphenols and flavonoids, enabling their effective use in pharmaceutical, food, and cosmetic applications, α -Cyclodextrin has no restrictions on its consumption and is recognized as safe. Its oral administration shows higher bioavailability and better solubility in ethanol compared to β -cyclodextrin [9]. α -CD contribute to molecular protection, Güneş Ak et al. investigated the enhancement of the bioactivity and bioavailability of *Limonium bellidifolium* through the formation of cyclodextrin-based inclusion complexes. Encapsulation of flavonoids, particularly quercetin, using α -cyclodextrin improved their

aqueous solubility, protected bioactive compounds from degradation, and enhanced their antioxidant activity, highlighting this approach as a promising strategy in natural product-based drug discovery [10].

Cyclodextrins (CDs) are cyclic oligosaccharides composed of α -(1,4)-linked glucose units, typically α -, β -, or γ -CD containing six, seven, or eight glucose monomers, respectively. Their hydrophobic cavity allows inclusion of guest molecules, increasing solubility and bioavailability [9-11]. β -CD is the most commonly used form due to its low cost and strong complexation ability [14], while derivatives such as hydroxypropyl- β -cyclodextrin (HP- β -CD) provide greater aqueous solubility and lower cytotoxicity [15].

In reproductive biotechnology, spermatozoa are highly sensitive to oxidative stress during storage, which compromises motility and fertilizing capacity [14]. The addition of antioxidants to semen extenders is known to improve sperm preservation [17]. Authaida et al. (2025) reported that *Moringa oleifera* leaf extract enhances post-thaw sperm quality [18], while Al-Amery and Banana (2019) found that bee pollen extracts improve the motility and viability of ram sperm stored at 5 °C [19].

However, no study has yet evaluated the antioxidant potential of ethanol extracts of bee pollen encapsulated in α -cyclodextrin on sperm preservation. The present work aims to extract and identify the principal bioactive compounds in bee pollen, form inclusion complexes with α -cyclodextrin, and evaluate their solubility, stability, and antioxidant efficacy to improve sperm storage outcomes.

Materials and methods

Materials

The raw bee pollen was collected from Kherrata region, 36° 29' 33,8" N, 5° 16' 39,32" E.

The following reagents were purchased from Sigma-Aldrich (BioChem): sodium nitrite (NaNO_2), aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), acetonitrile, acetic acid, methanol, ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Folin-Ciocalteu reagent. Quercetin and gallic acid standards were also obtained from Sigma-Aldrich.

Alpha-cyclodextrin (α -CD, 972 g/mol) and beta-cyclodextrin (β -CD, 1135 g/mol) were kindly provided by Roquette Frères (France). All reagents were of analytical grade.

Methods

Extraction procedure

Bee pollen samples were collected in 2024 from the Kherrata region (Bejaia, Algeria). The samples were commercially processed (The samples were obtained from a local beekeeper and had undergone standard apicultural processing: manual removal from hive traps, freezing to eliminate pests, coarse cleaning by sieving to remove large debris, and subsequent air-drying. Upon receipt in the laboratory), dried for 24 h at 60 °C to preserve bioactive compounds, and then finely ground using a laboratory mill and sieved to obtain particles < 250 μm to increase solvent contact surface area during extraction increase solvent contact during extraction.

Ethanolic extracts were obtained according to the protocol of Lawag et al., with minor modifications using magnetic stirring. Five grams of ground pollen were mixed with 150 mL of ethanol (99.9 %) and stirred for 4 h at room temperature. The mixture was filtered and concentrated using a rotary evaporator at 45 °C for 15 min [20]. While aqueous ethanol may maximize total phenolic yield, absolute ethanol was selected to favor the extraction of medium-polarity flavonoids and to minimize co-extraction of highly polar compounds, simplifying subsequent complexation and analysis.

The yield of extraction

The extraction yield was calculated as described by Ali-Rachedi et al. (2018) [20,21]:

$$\% R = (m_f/m_0) * 100 \quad (1)$$

where m_f is the mass of the dry extract and m_0 the mass of the initial sample. Yields were expressed as g of extract per 100 g of dry matter.

Determination of flavonoid content

The total flavonoid content (TFC) was determined following the method of Hadidi et al using the aluminum chloride colorimetric method, originally described for propolis samples and subsequently adapted for bee pollen analysis, as reported in the literature [22]. Briefly, 4 mL of ethanolic extract (0.05 mg/mL) was mixed with 1.2 mL of 5 % NaNO₂. After 5 min, 1 mL of 10 % AlCl₃ solution was added, followed by 8 mL of 1 M NaOH. Absorbance was recorded at 510 nm using a UV-Vis spectrophotometer. A calibration curve was established with quercetin as the standard.

Total phenolic content

The total phenolic content (TPC) was measured using the Folin-Ciocalteu method (Guendouzi et al., 2023) [22]. One milligram of ethanolic extract was dissolved in 20 mL of ethanol. Two milliliters of this solution were mixed with 1 mL of diluted Folin reagent (1:9, v/v) and 8 mL of 7.5 % Na₂CO₃. After 30 min at room temperature, absorbance was read at 765 nm. Gallic acid was used as a calibration standard.

High performance liquid chromatography HPLC

Quantitative analysis of quercetin was performed by HPLC equipped with a UV detector ($\lambda = 360$ nm). Separation was achieved on a C18 column (250 mm $\times$ 4.6 mm, 5 μ m) at 30 °C. The mobile phase consisted of solvent A (acetonitrile) and solvent B (acetic acid in water), delivered at a flow rate of 0.8 mL/min using a gradient system (Table 1). The injection volume was 10 μ L, and samples were filtered through 0.45 μ m membranes before analysis [1]. Quercetin calibration standards (0.002-0.33 mg/mL in methanol) were used to establish a standard curve. Bee pollen extract solutions were prepared at 2 mg/mL under identical conditions [4].

Table 1. Gradient system.

Time min	Gradient Elution
0-10	82 % A, 78 % B ;
10-27	78 % A, 78 % B
27-32	75 %A, 70 % B
32-40	70 % A, 65 % B
40-50	65 % A, 75 % B ;
50-55	75 % A, 82 % B

Fourier transform infrared (FTIR)

FTIR spectra were recorded using a Shimadzu spectrometer in the 400-4000 cm⁻¹ range to identify functional groups in raw bee pollen, pollen extract, and quercetin. Samples (0.02 g) were mixed with 0.8 g of KBr, pressed into pellets using a hydraulic press, and analyzed for characteristic absorption bands [23].

Thermogravimetric analysis TGA/DSC

Thermogravimetric analysis was conducted using a PerkinElmer STA 8000 analyzer to assess the thermal stability of bee pollen and its extract. Samples were heated from 50 °C to 700 °C under a nitrogen atmosphere, and weight loss was recorded as a function of temperature [24].

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were used to evaluate the thermal stability and decomposition behavior of quercetin and bee pollen extract, as well as their complexes with α -cyclodextrin prepared by physical mixing and kneading. The samples were heated from 50 C° to 600 °C

under a nitrogen atmosphere in order to prevent oxidative degradation. The weight loss was recorded as a function of temperature [5].

XRD analysis

X-ray powder diffraction (XRD) measurements were conducted using a Panalytical X'Pert PRO diffractometer equipped with a ceramic X-ray tube and a copper anode (Cu K α radiation, $\lambda = 1.5418 \text{ \AA}$). The instrument was operated at an accelerating voltage of 45 kV and a current of 40 mA. Diffraction data were collected over a 2θ range of 5° to 45° using a step-scan mode [5,8,25,26].

Solubility diagram

The solubility of quercetin and bee pollen extract in the presence of α - and β -cyclodextrins was studied according to the Higuchi-Conners method. Excess amounts of quercetin or extract were added to 5 mL aqueous CD solutions (α -CD: 0-150 mM; β -CD: 0-16 mM) and stirred for 7 days at 25°C . After filtration ($0.45 \mu\text{m}$), quercetin concentrations in the aqueous phase were determined spectrophotometrically at 360 nm using a calibration curve prepared with methanolic quercetin standards. The apparent stability constant (K) of the complex was calculated using:

$$K = \text{slope} / (S_0 * (1 - \text{slope})) \quad (2)$$

Where S_0 represents the intrinsic solubility of quercetin without CDs [4,5,13,27].

Preparation of α -CD inclusion complexes

Inclusion complexes of quercetin- α -CD and bee pollen extract- α -CD were prepared using the kneading method. Equimolar quantities (1:1) were mechanically mixed with minimal ethanol to form a homogeneous paste, which was dried and ground into a fine powder. The resulting complexes were characterized by FTIR to confirm molecular interactions and successful encapsulation, in comparison with physical mixtures. The encapsulation efficiency was determined using the method described by Liu et al, in which the α -CD quercetin complexes and α -CD extract were dissolved in methanol and centrifuged at 3000 rpm for 10 minutes. an aliquot of the complex suspension was centrifuged/filtered to separate free (non-complexed) quercetin/BPE. The free content was quantified by UV-Vis. The absorbances were read at 360 nm [5].

$$\text{EE}\% = [(\text{Total amount} - \text{Free amount}) / \text{Total amount}] * 100 \quad (3)$$

Antioxidant activity (DPPH assay)

The antioxidant activity was evaluated using the DPPH free radical scavenging assay. A 0.3 mM methanolic DPPH solution (5.126 mg/100 mL) was mixed with 0.3 mL of each sample (quercetin, bee pollen extract, and α -CD complexes) and 2.7 mL of DPPH solution. Absorbance was measured at 517 nm after 30 min. Ascorbic acid served as a positive control. The inhibition percentage (I %) was calculated as:

$$I \% = ((A_0 - A_1) / A_0) * 100 \quad (4)$$

Where A_0 and A_1 correspond to control and sample absorbances, respectively [20,24].

Antibacterial activity

The antibacterial activity of the ethanolic bee pollen extract was tested against Gram-positive (*Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC10876) and Gram-negative (*Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603) strains using the disk diffusion method.

Bacterial suspensions were prepared in nutrient broth ($\text{OD} = 0.08\text{-}0.1$ at 625 nm), and 20 mL of Mueller-Hinton agar was poured into Petri dishes. Disks impregnated with various concentrations of extracts were placed on inoculated plates and incubated at 37°C for 24 h. Inhibition zones were measured, using DMSO as a negative control and gentamicin as a positive control [29].

Effect on sperm motility and cytotoxicity assessment

Sperm motility was evaluated using a Makler chamber coupled with a Computer-Assisted Sperm Analysis (CASA) system (Sperm Class Analyzer, S.C.A. v3.2.0, Microptic S.L., Barcelona, Spain), allowing a detailed and objective assessment of sperm motility parameters. Epididymal bull spermatozoa were collected from testicular tissue using a sterile syringe and subsequently diluted in Tris buffer.

The experimental treatments included ethanolic bee pollen extract (BPE) at a concentration of 7.5 µg/mL and its corresponding inclusion complex at the same concentration. Quercetin (Q) and its α -cyclodextrin (α -CD) complex were also tested at 7.5 µg/mL. For the cryopreservation protocol, an aliquot of 0.1 mL of sperm suspension was mixed with 0.9 mL of BPE, Q, or their respective complexes. Control samples were prepared in Tris buffer without any treatment.

Sperm quality and motility were assessed immediately after preparation and following storage at 4 °C at different time points (30 min, 1 h, 2 h, 24 h, and 48 h). Cytotoxicity was evaluated over a short incubation period ranging from 0 to 1 h. Sperm motility parameters, including curvilinear velocity (VCL), straight-line velocity (VSL), and average path velocity (VAP), were analyzed using the CASA system [15,26].

Statistical analysis

All experiments were performed in triplicate (n=3), and results are expressed as mean $\pm$ standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p < 0.05$.

Results

Extraction yield

The extraction yield of bee pollen ethanolic extract was approximately 46.82 % (g/100 g dry matter) using the agitation method with ethanol as solvent. This value is consistent with that reported by Concetta Maria Messina et al., who obtained a yield of 48.61% using a similar procedure with 80 % ethanol (v/v) [31]. Such comparable yields highlight the influence of extraction method and solvent polarity on the recovery of bioactive compounds [20].

Total flavonoid content

The total flavonoid content (TFC) was determined using quercetin as the standard. The calibration curve ($Y = 2.212X$, $R^2 = 0.9956$) allowed the estimation of the flavonoid concentration at 324 mg QE/g of dry matter (Fig. S1). The high value suggests a strong presence of flavonoids forming stable complexes with aluminum chloride, which absorbs at 415 nm. This concentration was significantly higher than those reported by Nazli Mayda et al. for Turkish bee pollen [32], and by Lakhal Khadra et al. for Algerian bee pollen (8.1 mg QE/g) [33]. Similarly, Mladenova et al. reported lower values ranging between 1.74 and 3.01 mg QE/g for Bulgarian samples [34]. Our results are comparable to those reported by Mircea et al. regarding the flavonoid content of Romanian bee pollen, which is equal to 592.2 mg QE/g [35]. These variations can be attributed to geographical origin, botanical diversity, and extraction conditions.

Total phenolic content

The total phenolic content (TPC) was evaluated using gallic acid as the standard ($Y = 7.8932X + 0.2903$; $R^2 = 0.9957$). Based on the calibration curve (Fig. S2), the TPC of bee pollen extract was estimated at 189.2 mg GAE/g. This value greatly exceeds the range reported by Nazli Mayda et al. (8-43.42 mg GAE/g) [32] and is comparable to the values observed by Adriane Alexandre et al. (5.4-132 mg GAE/g) [36]. A five-year study on pollen from different regions and bee species, cited by Kacemi et al., reported that polyphenol contents ranged from 0.69 to 213.2 mg GAE/g, and our results fall within this range [37]. Such differences are likely due to pollen source, floral diversity, extraction methods and drying conditions.

High performance liquid chromatography (HPLC)

HPLC analysis was performed to quantify quercetin, as one of the major flavonoids in bee pollen. The chromatographic conditions were optimized based on previous studies. The quercetin standard (0.002-0.33 mg/mL)

exhibited a distinct peak at 22.5 min retention time [38]. The calibration curve ($Y = 1051X$; $R^2 = 0.9967$) indicated excellent linearity (Fig. S3), allowing estimation of 0.4776 mg quercetin/100 mg extract. The chromatograms (Fig. 1) confirmed the presence of multiple flavonoid peaks, notably quercetin, consistent with reports by Hong Duan et al. (0.476-0.717 mg/100 mg) [4]. Our findings also align with Soumeia Zeghoud et al., who reported quercetin concentrations ranging from 500.634 to 20749.05 $\mu\text{g}/\text{mg}$ depending on geographical origin [23].

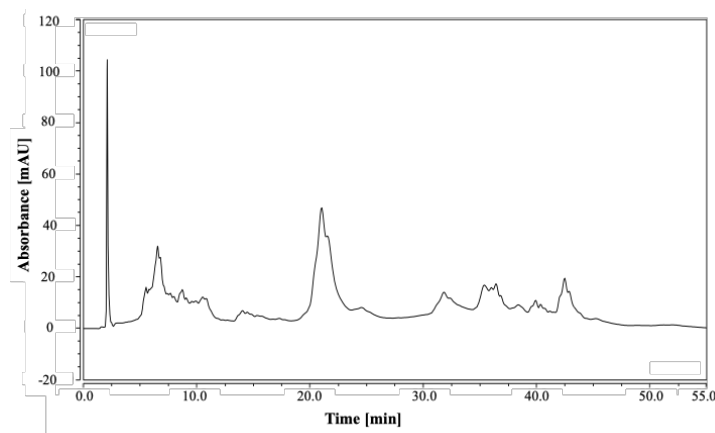


Fig. 1. Chromatogram of bee pollen extract sample.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of raw bee pollen, its ethanolic extract, and quercetin (Fig. 2) exhibited similar characteristic peaks, confirming the preservation of functional groups. Major absorption bands were observed between $3500\text{--}950\text{ cm}^{-1}$, corresponding to:

- O-H stretching ($3300\text{--}3500\text{ cm}^{-1}$) for phenols,
- C-H stretching ($2800\text{--}2750\text{ cm}^{-1}$) for carbohydrates,
- C=O (1600 cm^{-1}) for proteins, fatty acids, and flavonoids,
- C-O (1000 cm^{-1}) for saccharides,
- C-H ($900\text{--}750\text{ cm}^{-1}$) for aromatic alkenes [39].

These spectral similarities suggest that ethanol extraction did not alter the structural integrity of the pollen's main functional groups.

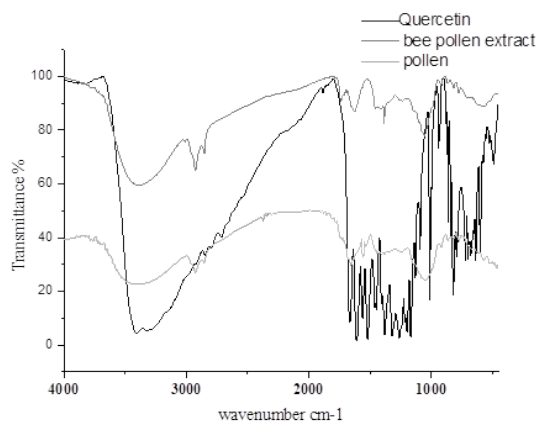


Fig. 2. FTIR spectra of raw bee pollen, ethanolic extract, and quercetin.

Thermogravimetric analysis (TGA/DSC)

Thermogravimetric analysis was used to assess the thermal stability of bee pollen and its ethanolic extract (Figures 3 and 4). Both samples exhibited comparable degradation profiles, indicating similar thermal behavior.

- 30-150°C: loss of residual water;
- 150-350°C: degradation of organic compounds (polyphenols, proteins, sugars);
- 350-450°C: decomposition of lipids and cellulose;
- >450°C: breakdown of sporopollenin.

The slightly greater stability of raw pollen may result from the presence of natural nectar and salivary secretions in the granules. These results are consistent with those of Volkan Aylanc et al. [24], who reported similar TGA patterns for natural sporopollenin microcapsules derived from bee pollen. The DSC thermograms show clear differences between raw bee pollen and bee pollen extract. Raw pollen exhibits a more complex thermal profile with multiple overlapping transitions, reflecting its heterogeneous composition and intact cell walls. A strong endothermic peak at low temperature is associated with moisture loss. In contrast, the bee pollen extract shows smoother and less intense thermal events, indicating lower water content and a more homogeneous composition after extraction. Both materials are thermally stable up to about 150 °C, while major degradation occurs above 200-250 °C. Overall, the extract displays more uniform and progressive thermal decomposition compared to raw pollen [24].

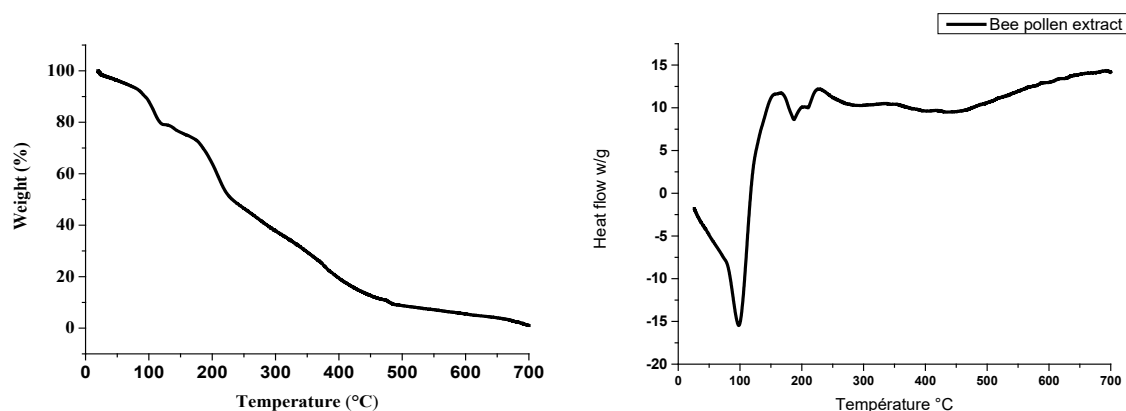


Fig. 3. TGA and DSC thermograms of bee pollen extract.

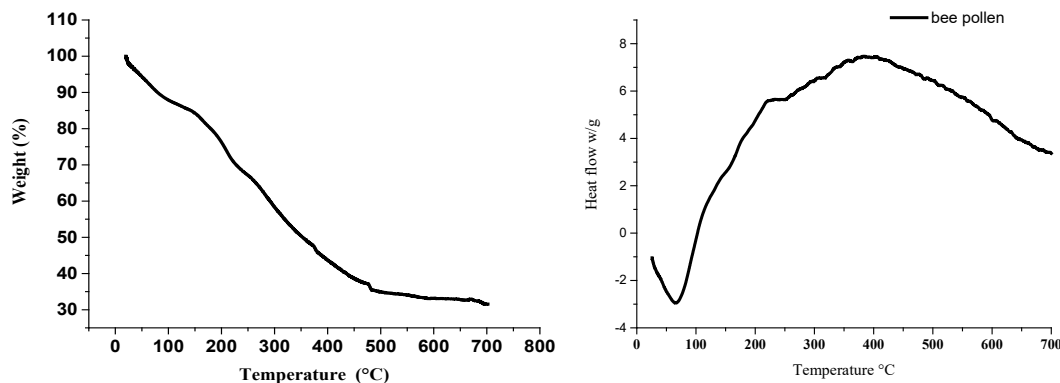


Fig. 4. TGA and DSC thermograms of raw bee pollen.

The physicochemical analyses confirmed the richness of bee pollen in polyphenolic and flavonoid compounds, particularly quercetin, which explains its strong antioxidant potential. The stability profiles (FTIR, TGA/DSC) demonstrated that ethanol extraction preserved the structural integrity of bioactive molecules, validating the suitability of the chosen extraction process.

Solubility studies

Calibration curve of quercetin

The quantification of quercetin in bee pollen ethanolic extract was determined using UV-Vis spectrophotometry. A calibration curve was established with methanolic quercetin solutions of different concentrations, showing a linear relationship described by the equation $y = 46.481x$ with a correlation coefficient of $R^2 = 0.9929$ (Fig. S4.), confirming excellent linearity from this calibration, the concentration of quercetin in 1 g of bee pollen extract was estimated to be approximately 18.9 mg, indicating that quercetin is one of the predominant compounds in the extract. This observation justifies the selection of quercetin as a representative bioactive molecule for subsequent encapsulation studies, since its major presence also reflects its contribution to the antioxidant properties of bee pollen.

Higuchi diagrams

Phase solubility studies were conducted to evaluate the interaction between quercetin or bee pollen extract and two types of cyclodextrins (α - and β -CD). The solubility of both the extract and pure quercetin increased linearly with increasing concentrations of cyclodextrins, as shown in Figures 5, 6, 7, and 8. The resulting phase solubility diagrams were classified as A_L type, characteristic of 1:1 stoichiometric inclusion complexes between the host (cyclodextrin) and guest (quercetin or extract) molecules [5,27].

The calculated stability constants (K_s) are summarized in Table 2. The results indicate that α -CD produced higher stability constants compared to β -CD for both the extract ($K_s = 34.41 M^{-1}$) and quercetin ($K_s = 101 M^{-1}$). This suggests that α -CD forms more stable inclusion complexes with both the crude extract and pure quercetin, possibly due to its smaller cavity size, which better accommodates the planar structure of flavonoids. These findings are consistent with those reported by Hongjie Tung et al [5] who observed similar A_L -type profiles in comparable systems. The solubility enhancement achieved through complexation with α -CD demonstrates its efficiency in improving the aqueous solubility of quercetin and bioactive components from bee pollen. Consequently, α -CD was selected as the preferred encapsulation material for the next stages of this work. The calculated K_s values, while indicative of stable complex formation, are apparent constants specific to our experimental conditions (aqueous medium, 25°C). Direct numerical comparison with literature values obtained via different methods should be made with caution. The key finding is the clear A_L -type behavior and significant solubility enhancement. According to Fenyvesi et al., the solubility constant of cyclodextrin inclusion complexes generally ranges from 10 to 2000 M^{-1} , depending on the nature of the guest molecule and the experimental conditions [38].

Table 2. Solubility constants (K_s) of quercetin and bee pollen extract calculated from solubility diagrams.

Host/Guest systems	$Y = \alpha X + S_0$	$K_s M^{-1}$
α -CD Extract	$Y = 0.02 X + 0.0594$	34.41
β -CD Extract	$Y = 0.02 X + 0.0648$	31
α -CD Quercetin	$Y = 0.0059 X + 0.0551$	101
β -CD Quercetin	$Y = 0.0041 X + 0.0047$	84.36

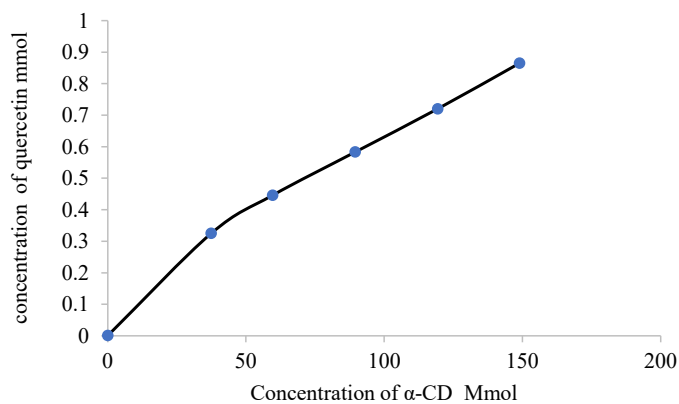


Fig. 5. α -CD-Quercetin solubility diagram.

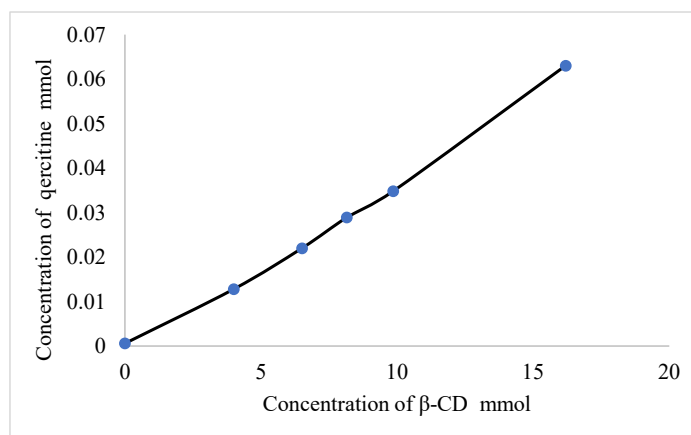


Fig. 6. β -CD-Quercetin solubility diagram.

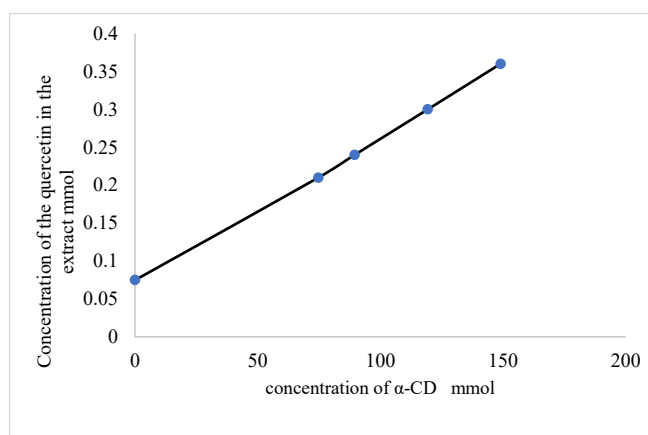


Fig. 7. α -CD-Extract solubility diagram.

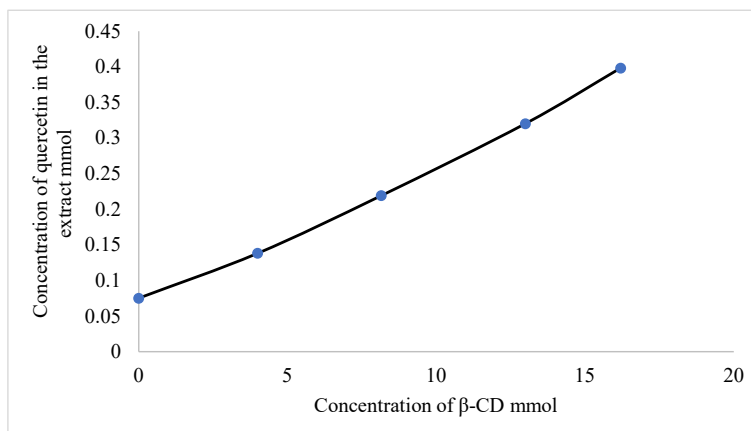


Fig. 8. β -CD-Extract solubility diagram.

Characterization of inclusion complexes

Encapsulation efficiency (EE%)

The encapsulation efficiency of the quercetin- α -CD complex was 55 %, and that of the extracted bee pollen- α -CD complex was 51 %. These values are consistent with those reported by Tang et al. (2024), who demonstrated efficient quercetin encapsulation using α -, β -, and γ -cyclodextrin/carbon quantum dot nanocomplexes, where encapsulation efficiencies were significantly enhanced compared to free quercetin, supporting the reliability and comparability of our results [5].

FTIR analysis

Fourier Transform Infrared (FTIR) spectroscopy was used to confirm the formation of inclusion complexes between α -cyclodextrins and the bee pollen extract or pure quercetin. The IR spectra of the extract and its complexes (Fig. 9) revealed similar global profiles, indicating that the main chemical structure of the bioactive compounds was preserved after encapsulation. However, noticeable differences in peak intensity and slight shifts in absorption bands were observed, suggesting interactions between the hydroxyl and carbonyl groups of the extract and the cyclodextrin molecules. Characteristic signals were observed at 3400-3000 cm^{-1} (O-H and N-H stretching, typical of polysaccharides and proteins), 3000-2800 cm^{-1} (C-H stretching of carbohydrates), and 1700-1600 cm^{-1} (C=O stretching of amides and fatty acids). The fingerprint region between 1540 and 900 cm^{-1} revealed C-O and C-C vibrations typical of saccharides. These observations are in agreement with the findings of Soumeia Zeghoud et al [23], confirming the presence of polyphenols, proteins, and carbohydrate components in bee pollen. Notably, the spectral changes in the O-H, C=O, and C-O/C-H regions reflect hydrogen-bond interactions between the bioactive components and the cyclodextrin cavity, confirming the successful formation of inclusion complexes.

Similarly, the FTIR spectra of pure quercetin and its complexes (Fig. 10) displayed characteristic absorption peaks corresponding to its functional groups. Pure quercetin showed strong bands at 3395-3268 cm^{-1} (O-H stretching), 1663-1518 cm^{-1} (C=O and aromatic ring stretching), and 1256-1164 cm^{-1} (C-O stretching in aryl ether and phenolic groups). After complexation with α -CD, the intensity of these peaks decreased and several characteristic quercetin bands, particularly those at 1663 and 1605 cm^{-1} disappeared or shifted to lower wavenumbers, indicating the inclusion of quercetin within the cyclodextrin cavity. The O-H stretching band shifted from 3282 cm^{-1} to 3142 cm^{-1} , confirming hydrogen bond formation between the hydroxyl groups of quercetin and the inner hydrophilic surface of α -CD. These spectral modifications support the occurrence of host-guest interactions and the establishment of stable inclusion complexes. These results are consistent with those of Hongjie Tang, Ying Zhu Liu et al. [5] who also observed similar band displacements confirming the successful encapsulation of flavonoids in cyclodextrin cavities.

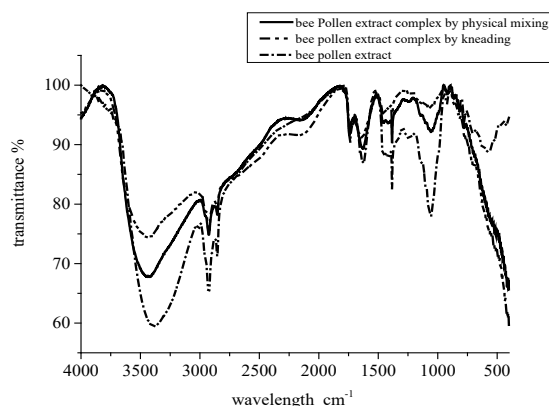


Fig. 9. FTIR spectrum of α -CD-extract complex.

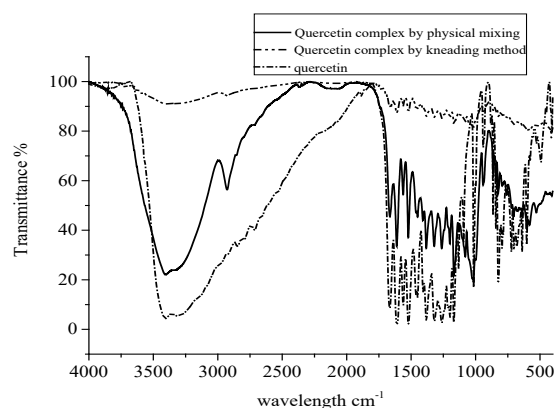


Fig. 10. FTIR spectrum of α -CD-quercetin complex.

TGA/DSC analysis of the inclusion complexes

The thermal behavior of quercetin, bee pollen extract, and their α -cyclodextrin (α -CD) complexes was investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) (Figs. 11 and 12).

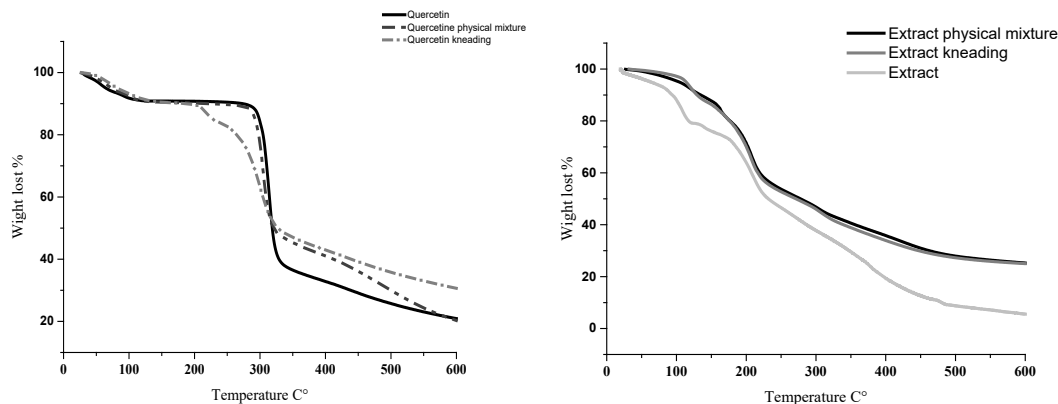


Fig. 11. Thermograms (ATG) of quercetin, quercetin physical mixture and quercetin kneading. And thermograms (ATG) of the bee pollen extract, bee pollen extract physical mixture and bee pollen extract complex.

For quercetin-based systems, all samples exhibited an initial weight loss below 120 °C, attributed to the removal of physically adsorbed water, which was more pronounced in α -CD-containing systems due to the hygroscopic nature of cyclodextrin. Pure quercetin showed a sharp mass loss between 250 and 350 °C, corresponding to its thermal degradation. A similar profile was observed for the physical mixture, indicating the absence of significant intermolecular interactions. In contrast, the kneaded complex displayed a broadened and shifted decomposition profile with a more gradual mass loss, reflecting enhanced thermal protection of quercetin within the α -CD cavity and confirming the formation of a true inclusion complex. At higher temperatures (>400 °C), the residual mass loss was associated with α -CD backbone degradation and carbonization processes, with the kneaded complex exhibiting improved overall thermal stability [5].

For the bee pollen extract systems, multistep degradation profiles typical of encapsulated organic materials were observed. The initial mass loss below 120 °C was attributed to surface and cavity-bound water, with lower values for the kneaded complex, suggesting stronger host–guest interactions. Compared to the physical mixture, the kneaded complex showed delayed and less pronounced degradation between 120 and 250 °C, indicating effective thermal protection of thermolabile pollen constituents. The main degradation step of α -CD (250–380 °C) was shifted toward higher temperatures in the kneaded complex, reflecting enhanced intermolecular interactions and structural stabilization. The higher carbonaceous residue above 400 °C further supports the formation of a compact and thermally stable supramolecular system, confirming successful pollen– α -CD inclusion complex formation [24].

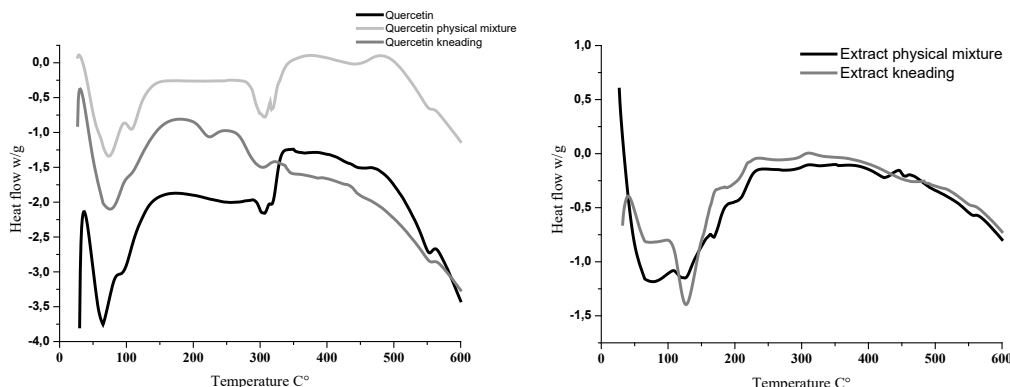


Fig. 12. The DSC curves of quercetin, quercetin physical mixture and quercetin kneading. And the DSC curves of the bee pollen extract physical mixture and bee pollen extract complex.

The DSC thermograms of the bee pollen extract, its physical mixture with α -cyclodextrin (α -CD), and the corresponding inclusion complex are presented in Figures 3 and 12. The raw bee pollen extract exhibited a broad endothermic event at low temperatures, mainly associated with the loss of bound water and the thermal transitions of its heterogeneous bioactive constituents. The physical mixture showed a simple superposition of the thermal events of the individual components, indicating the absence of significant intermolecular interactions.

In contrast, the kneaded pollen– α -CD complex displayed marked modifications in its DSC profile, characterized by attenuation, shifting, or partial disappearance of the characteristic endothermic peaks observed in both the raw extract and the physical mixture. At low temperatures (30–120 °C), the reduced intensity and broadening of endothermic peaks indicate stronger pollen– α -CD interactions and partial replacement of water molecules within the cyclodextrin cavity. In the intermediate temperature range (120–250 °C), the suppression or displacement of transitions related to pollen constituents (e.g., sugars and polyphenols) reflects their effective inclusion and thermal protection within the α -CD cavity. At higher temperatures (>250 °C), α -CD decomposition occurred more gradually and at slightly higher temperatures in the kneaded complex, indicating enhanced thermal stability and structural reorganization. Overall, these DSC features confirm the formation of a stable inclusion complex through the kneading method, in agreement with TGA results [24].

Similarly, DSC analysis of quercetin and its α -CD complexes revealed a broad endothermic event below 120 °C for all samples, attributed to moisture loss. Pure quercetin showed a sharp endothermic peak around 315–325 °C corresponding to its melting point, which remained visible in the physical mixture. In

contrast, this characteristic peak was significantly reduced or absent in the kneaded quercetin- α -CD complex, indicating loss of crystallinity and successful molecular inclusion within the cyclodextrin cavity [5].

These findings are consistent with previous reports on pollen extracts and flavonoid-cyclodextrin inclusion complexes, where the attenuation or disappearance of characteristic DSC transitions is commonly associated with effective encapsulation and improved thermal stability [8,24].

X-ray diffraction (XRD) analysis

The XRD patterns of bee pollen extract, quercetin, and their α -cyclodextrin (α -CD) systems (Figures 13 and 14) highlight marked differences between physical mixtures and kneaded complexes. The bee pollen extract exhibited a broad diffuse halo in the 2θ range of 15–25°, characteristic of an amorphous or poorly crystalline material. This amorphous profile was preserved in the physical mixture, indicating simple coexistence of components without structural modification or inclusion. In contrast, the kneaded pollen- α -CD complex showed further broadening and attenuation of the diffuse halo, accompanied by the disappearance or shift of residual diffraction features, reflecting increased amorphization and the formation of a new supramolecular solid phase driven by strong host-guest interactions [40].

Pure quercetin displayed numerous sharp diffraction peaks, confirming its highly crystalline nature, which remained largely preserved in the physical mixture. Conversely, the kneaded quercetin- α -CD complex exhibited a pronounced reduction or disappearance of quercetin crystalline peaks, together with a more diffuse diffraction profile, indicating loss of crystallinity and molecular dispersion of quercetin within the α -CD matrix. This structural reorganization confirms partial inclusion of quercetin into the cyclodextrin cavity and the formation of an amorphous inclusion complex, in agreement with previous reports on flavonoid-cyclodextrin systems [5,8,26,41]. Overall, the XRD results are fully consistent with TGA/DSC findings, collectively confirming that kneading promotes true inclusion complex formation, whereas physical mixing results only in component coexistence.

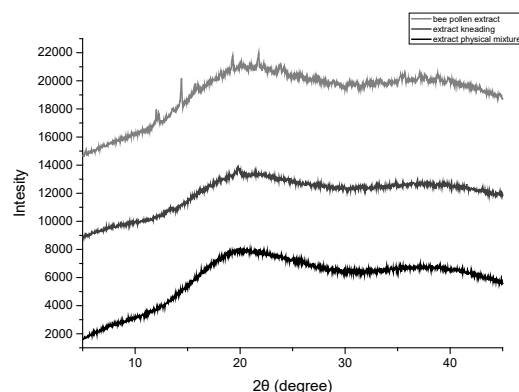


Fig. 13. The XRD patterns of bee pollen extract, extract complex and extract physical mixture.

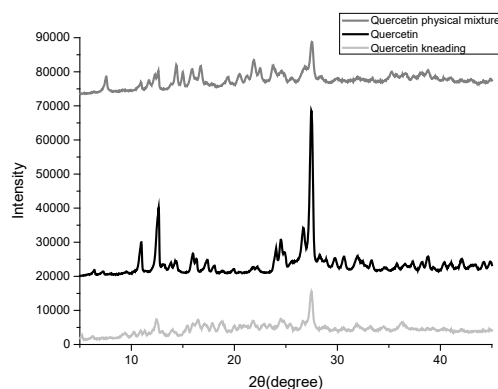


Fig. 14. The XRD patterns of Quercetin, its complex and its physical mixture.

Antioxidant activity

The antioxidant property of the samples was determined by the DPPH free radical scavenging assay which is based on electron transfer and hydrogen atom transfer reactions. This method offers advantages of being rapid, simple inexpensive and provides initial information on the antioxidant capacity of the test sample, measuring the ability of compounds to neutralize free radicals [42]. The DPPH radical (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical with a deep violet color, which is reduced to a non-radical form (DPPH-H) upon reaction with an antioxidant. This reaction involves the donation of an electron or a hydrogen atom from the antioxidant to the radical, resulting in a color change from violet to yellow, which can be quantitatively measured spectrophotometrically at 515-517 nm. The extent of decolorization reflects the radical scavenging potential of the sample, often expressed as IC_{50} value. In this study, pollen extracts exhibited significant DPPH radical scavenging activity, which correlates with their total phenolic and flavonoid content, confirming their potential as natural antioxidants, consistent with previous reports on bee pollen and other plant-derived extracts. The antioxidant activity of the bee pollen extract, pure quercetin, and their corresponding inclusion complexes with cyclodextrins was evaluated using the DPPH radical scavenging assay, with ascorbic acid serving as a reference antioxidant. The results (Fig. 15) revealed that ascorbic acid exhibited the highest radical scavenging capacity ($96.31 \pm 1.88 \%$), as expected. Quercetin and the bee pollen extract both demonstrated strong antioxidant potential, with inhibition percentages of $95.41 \pm 2.08 \%$ and $93.08 \pm 2.21\%$, respectively. Interestingly, the inclusion complexes, particularly those prepared via the kneading method showed a slight improvement in antioxidant efficiency compared to the physically mixed complexes, indicating that the encapsulation process influences the stability and reactivity of the encapsulated molecules.

For quercetin, the complex obtained by the kneading method exhibited a scavenging activity of $93.08 \pm 2.3 \%$, compared to $92.87 \pm 4.03 \%$ for the physical mixture. Similarly, the bee pollen extract complex prepared by kneading achieved $85.11 \pm 2.64 \%$ inhibition, surpassing the physical mixture ($82.86 \pm 0.76 \%$). These results confirm that the kneading method promotes stronger host-guest interactions, enhancing the stability and release of the active compounds.

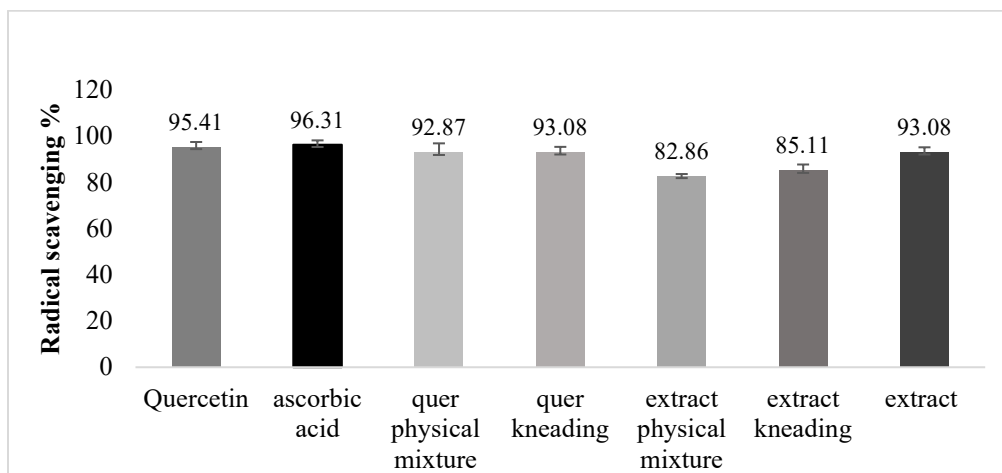


Fig. 15. Antioxidant activity of ascorbic acid, quercetin, bee pollen extract, and their inclusion complexes.

To further quantify the antioxidant potential, IC_{50} values (the concentration required to inhibit 50% of DPPH radicals) were calculated from the inhibition curves (Fig. 16). Lower IC_{50} values indicate stronger antioxidant activity. As shown below, the IC_{50} values were 0.341 ± 0.012 mg/mL for ascorbic acid, 0.668 ± 0.019 mg/mL for quercetin, and 0.694 ± 0.023 mg/mL for the bee pollen extract. For the inclusion complexes, the IC_{50} values were slightly higher: 0.92 ± 0.029 mg/mL for the quercetin- α -CD kneaded complex and 0.96 ± 0.027 mg/mL for the extract- α -CD complex.

These results confirm that both quercetin and bee pollen extract possess strong free radical scavenging capacities, and that complexation with α -cyclodextrin slightly modifies but maintains the overall antioxidant

profile. The modest variations in IC_{50} suggest that the inclusion process does not significantly alter the redox potential of the encapsulated compounds but instead contributes to their stabilization and controlled release. The antioxidant activity observed is closely linked to the presence of polyphenolic compounds and flavonoids in the bee pollen extract, which are known to donate hydrogen atoms or electrons to neutralize free radicals. This mechanism is consistent with literature reports highlighting that flavonoids are the major contributors to the antioxidant and pharmacological activities of bee pollen [22]. Furthermore, the ability of quercetin and phenolic compounds to inhibit lipid peroxidation and suppress the formation of reactive oxygen species (ROS) underscores their potential in preventing oxidative stress-related cellular damage. Our findings are consistent with the study by Hui Yang, Zhang Rui Liu et al. [2] who isolated and characterized bioactive compounds in pollen and demonstrated their antioxidant efficacy against oxidative stress. The high antioxidant capacity of the bee pollen extract supports its potential as a natural source of bioactive compounds with therapeutic applications, particularly when stabilized through cyclodextrin encapsulation.

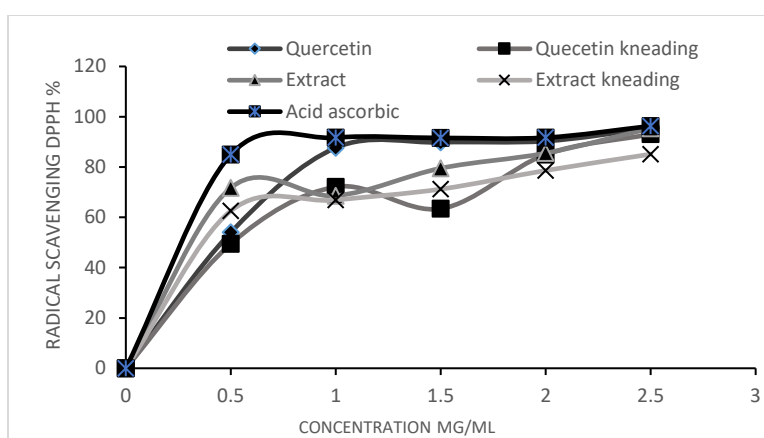


Fig. 16. IC_{50} values for ascorbic acid, quercetin, bee pollen extract, and their α -CD inclusion complexes.

Antibacterial activity of the ethanolic bee pollen extract

The antibacterial activity of the ethanolic bee pollen extract and raw pollen was assessed against four bacterial strains: *Escherichia coli* (E.C), *Klebsiella pneumoniae* (K.P), *Staphylococcus aureus* (S.A), and *Bacillus cereus* (B.C). The results, expressed as inhibition zone diameters (mm), are summarized in Table 3.

The ethanolic extract demonstrated a clear antibacterial effect, evidenced by the formation of inhibition zones around the discs, whereas the control (DMSO) showed no inhibition. The ethanolic extract exhibited higher antibacterial activity than crude pollen for most strains, confirming that ethanol enhances the extraction of active antimicrobial constituents such as flavonoids and phenolic compounds. At equivalent concentrations, the ethanolic extract showed notable inhibitory activity against *E. coli* (11 mm) and *K. pneumoniae* (13 mm), which increased proportionally with the extract concentration. The crude pollen dissolved in DMSO also exhibited antibacterial activity, though to a lesser extent, with inhibition zones of 9 mm for *E. coli* and 12 mm for *K. pneumoniae*.

In contrast, no significant inhibition was observed against *S. aureus* and *B. cereus* at low concentrations of both extract and crude pollen in DMSO. However, at higher concentrations, mild activity appeared for *S. aureus* (10 mm) and *B. cereus* (9 mm), indicating that Gram-positive bacteria are generally less sensitive to the bee pollen extract than Gram-negative strains. This difference may be attributed to structural variations in the bacterial cell wall; the outer membrane of Gram-negative bacteria facilitates the diffusion of small phenolic molecules, whereas the thick peptidoglycan layer of Gram-positive bacteria offers greater resistance to penetration. Interestingly, when comparing solvent systems, samples prepared in ethanol exhibited the most pronounced inhibition zones across all tested strains. For instance, at a concentration of 0.025 g/mL, the ethanolic extract produced inhibition zones of 11 mm (*E. coli*), 13 mm (*K. pneumoniae*), 15 mm (*S. aureus*), and 10 mm (*B. cereus*), surpassing those obtained with DMSO or water. This confirms the superior solubilizing

power of ethanol for bioactive compounds, particularly flavonoids and polyphenols, which contribute to antibacterial activity.

The results demonstrate that both raw bee pollen and its ethanolic extract possess antibacterial properties, with enhanced activity observed in the ethanolic extract, especially against Gram-negative bacteria. The stronger inhibitory effect on *K. pneumoniae* and *E. coli* highlights the efficiency of ethanol as an extraction solvent, improving the diffusion and bioavailability of active compounds. These findings are consistent with previous reports emphasizing the role of phenolic-rich bee products as natural antimicrobial agents [25,29].

For complexes, a slight improvement in inhibition diameters was observed, the bee pollen extract- α -cyclodextrin complex showed higher antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae* compared to free quercetin. Similarly, the quercetin- α -cyclodextrin complex exhibited improved inhibition zones relative to quercetin. This enhancement is likely due to the increased solubility and stability provided by α -cyclodextrin encapsulation, which facilitates better diffusion and interaction with bacterial cells. These results confirm that α -cyclodextrin complexation enhances the antibacterial efficacy of both pollen extracts and quercetin. Tian et al. demonstrated that cyclodextrin inclusion significantly enhances the solubility, stability, and antibacterial activity of natural bioactive compounds, resulting in improved efficacy against pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus*. These findings support the results of the present study, as the bee pollen extract- α -cyclodextrin inclusion complex may exhibit enhanced antibacterial activity through similar mechanisms, including improved protection of pollen-derived bioactive compounds and facilitated interaction with bacterial cell membranes [43].

Table 3. Inhibition zones (mm) observed for ethanolic bee pollen extract, crude pollen and extract- α -CD complex against bacterial strains.

Concentrations of ethanolic extracts of bee pollen	E.C	K.P	S.A	B.C
0.005 g / 10 mL DMSO (Extract)	8	10	-	7
0.05 g / 5 mL DMSO (Extract)	8	11	-	-
0.05 g / 5 mL DMSO (Bee pollen)	9	11	10	9
0.25 g / 2.5 mL DMSO (Extract)	11	13	-	-
0.25 g / 2.5 mL DMSO (Bee pollen)	9	12	-	-
0.01 g / mL (Water extract)	-	-	-	-
0.01 g / mL (Water bee pollen)	-	-	-	-
0.025 g / mL (Ethanol extract)	11	13	15	10
0.025 g / mL (Ethanol bee pollen)	12	15	10	9
0.25 g/2.5ml DMSO (complexe extract)	12	13.5	-	-
0.25g/2.5ml DMSO quercetin	10	11	-	-
0.25 g/2.5ml DMSO complex quercetin	11	12	-	-

Effect of ethanolic extracts of bee pollen and its complex on sperm cryopreservation and cytotoxicity assessment

Cryopreservation is a key method used to preserve sperm viability and functionality, especially in mammals, where spermatozoa are highly sensitive to cryodamage during the freezing-thawing process. This sensitivity is largely due to the high proportion of polyunsaturated fatty acids in sperm membranes, which are susceptible to peroxidation induced by reactive oxygen species (ROS) [18]. Excessive ROS generation can

compromise membrane integrity, motility, and DNA stability. Hence, supplementing cryopreservation media with antioxidant compounds is an effective approach to mitigate oxidative stress and enhance sperm survival.

Cryoprotective effect of bee pollen extract and its inclusion complex

To evaluate the cryoprotective potential of bee pollen ethanolic extract, quercetin, and their inclusion complexes, sperm samples were preserved at 4 °C and analyzed at 2, 4, 24, and 48 hours. The motility parameters studied included the curvilinear velocity (VCL), straight-line velocity (VSL), and average path velocity (VAP), as shown in Figures 17, 18 and 19.

At the initial time point (T₀), in the absence of oxidative stress, the control group exhibited the highest sperm motility values, reflecting the natural baseline activity. However, after 30 minutes, treatments containing quercetin and its cyclodextrin complexes showed a significant increase in motility parameters, indicating a rapid antioxidant effect. This can be attributed to the immediate release of quercetin molecules from the cyclodextrin cavity, neutralizing early ROS accumulation.

At 2 h and 4 h, all treated groups outperformed the control, confirming the ability of both the ethanolic bee pollen extract and quercetin-based systems to maintain sperm functionality. At 24 h, the ethanolic extract and its inclusion complexes continued to provide a notable protective effect, while free quercetin and the physical mixture of quercetin-cyclodextrin exhibited a decline in activity, likely due to faster degradation. This observation highlights the importance of encapsulation, which ensures gradual and sustained release of the antioxidant compound, maintaining its protective efficacy over time.

After 24 h, kneading complexes retained a significant advantage over both the control and physical mixtures. The latter likely represent simple mechanical associations without genuine molecular inclusion, leading to less stable interactions and faster compound loss. The inclusion process, in contrast, enhances the bioaccessibility, solubility, and stability of the bioactive molecule, thus prolonging its beneficial action during storage.

Bee pollen ethanolic extract and its inclusion complexes therefore contain a variety of synergistic antioxidants, notably flavonoids and phenolic acids that act collectively to protect sperm cells from oxidative stress. Their combined antioxidant and antibacterial effects contribute to maintaining sperm motility, membrane integrity, and overall viability during cryopreservation [12,35,25,44,45].

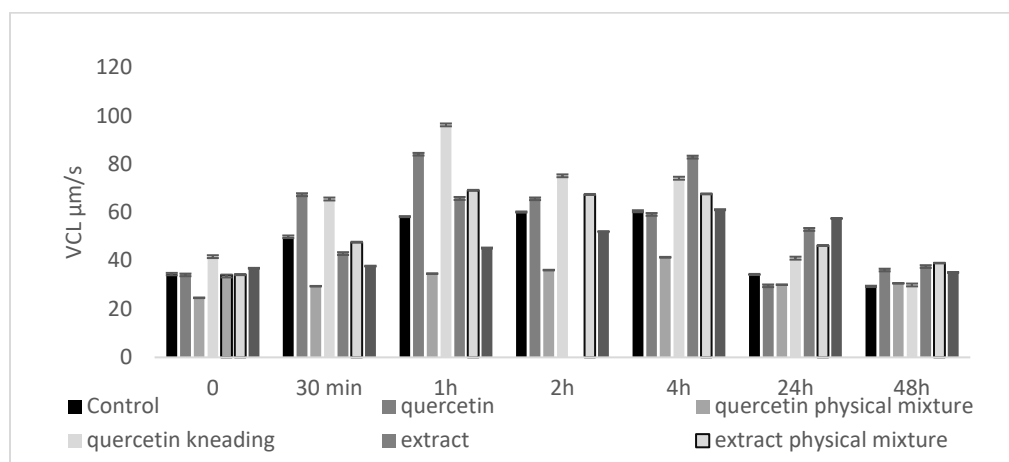


Fig. 17. Sperm motility parameter VCL after cryopreservation with bee pollen ethanolic extract, quercetin, and their complexes.

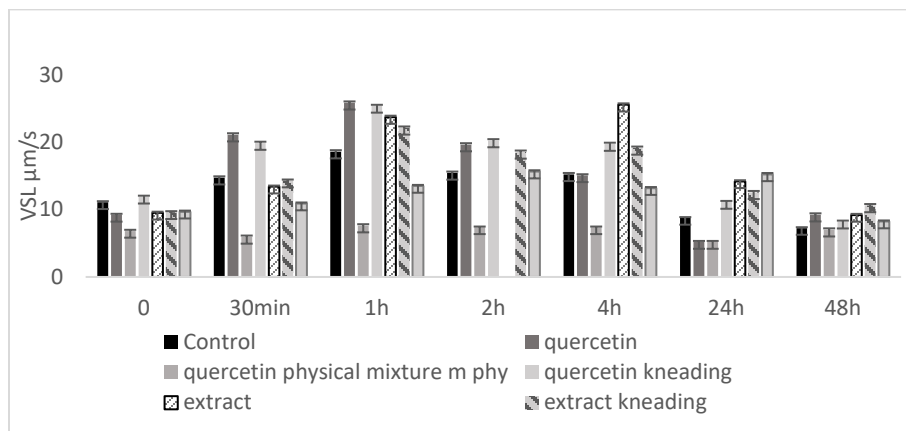


Fig. 18. Sperm motility parameter VSL after cryopreservation with bee pollen ethanolic extract, quercetin, and their complexes.

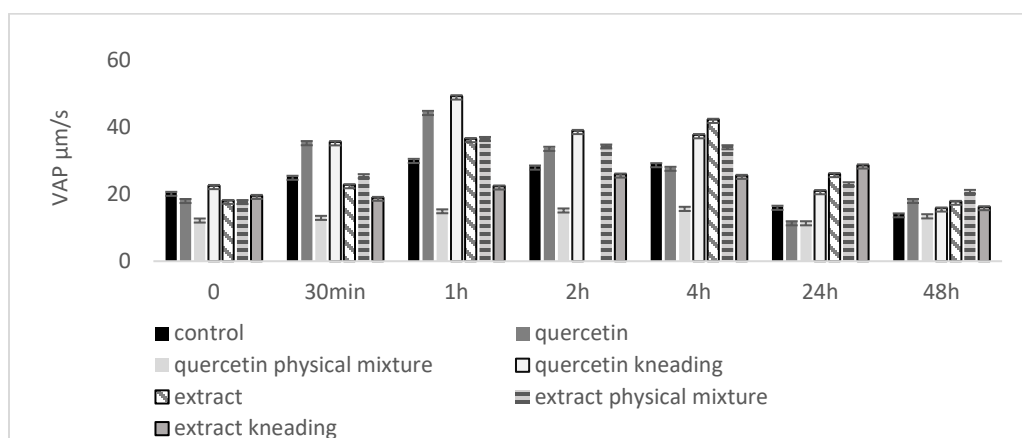


Fig. 19. Sperm motility parameter VAP after cryopreservation with bee pollen ethanolic extract, quercetin, and their complexes.

Cytotoxicity and cellular integrity assessment of bee pollen extract and its inclusion complex

In parallel, cytotoxicity analyses were performed to assess whether the tested concentrations of bee pollen ethanolic extract and its complexes exhibited any adverse effects on sperm cell viability. The results showed in (Figures 17,18,19.) at $t=0$ min, $t=30$ min and $t=1$ h; that at studied concentrations, no cytotoxic effect was observed sperm motility and morphology remained comparable or superior to the control. These findings confirm that the bee pollen ethanolic extract and quercetin inclusion complexes exert a dose-dependent protective effect rather than cytotoxicity. At studied concentrations, they enhance antioxidant defenses and sperm performance [25,37,44,45].

The combination of cryoprotective and cytocompatible properties demonstrates the potential of bee pollen-derived antioxidants, particularly when encapsulated in cyclodextrins as natural additives to improve sperm preservation media and mitigate oxidative stress-induced damage.

Conclusions

This study demonstrated that bee pollen from the Kherrata region (Bejaia, Algeria) is rich in bioactive phenolic and flavonoid compounds, with quercetin identified as the major constituent by HPLC. Encapsulation of bee pollen extract and quercetin with α -cyclodextrin significantly improved their solubility and stability, as confirmed by FTIR ATG/DSC and XRD. The phase solubility analyses indicating (1:1) inclusion complex. The encapsulated forms exhibited enhanced antioxidant and antibacterial activities, particularly against *E. coli* and *K. pneumoniae*, while maintaining low cytotoxicity. During sperm cryopreservation, these complexes markedly improved motility parameters (VCL, VSL, VAP) and reduced oxidative damage, confirming their sustained antioxidant protection.

Overall, α -cyclodextrin encapsulation effectively enhanced the physicochemical and biological properties of bee pollen bioactive, supporting their potential use as natural antibacterial, antioxidant and cryoprotective systems in biomedical and reproductive applications.

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References

1. Lu, P.; Takiguchi, S.; Honda, Y.; Lu, Y.; Mitsui, T.; Kato, S.; Kodera, R.; Furihata, K.; Zhang, M.; Okamoto, K.; Itoh, H.; Suzuki, M.; Kono, H.; Nagata, K. *Food Chemistry: Molecular Sciences*. **2022**, *5*, 100119. DOI: <https://doi.org/10.1016/j.fochms.2022.100119>
2. Zhang, H.; Liu, R.; Lu, Q. *Molecules*. **2020**, *25*, 1264. DOI: <https://doi.org/10.3390/molecules25061264>
3. Damulienė, V.; Kaškonienė, V.; Kaškonas, P.; Mickienė, R.; Maruška, A. *Pharmaceuticals*. **2024**, *18*(1), 15. DOI: <https://doi.org/10.3390/ph18010015>
4. Duan, H.; Dong, Z.; Li, H.; Li, W.; Shi, S.; Wang, Q.; Cao, W.; Fang, X.; Fang, A.; Zhai, K. *Food Chem. Toxicol.* **2019**, *134*, 110831. DOI: <https://doi.org/10.1016/j.fct.2019.110831>
5. Tang, H.; Liu, Y.; Xu, H.; Wang, F.; Xie, C.; Zhang, H.; Jiang, L. *Food Biophys.* **2024**, *19*, 269–283. DOI: <https://doi.org/10.1007/s11483-023-09821-7>
6. Yang, Y.; Zhang, J.; Zhou, Q.; Wang, L.; Huang, W.; Wang, R. *J. Sci. Food Agric.* **2019**, *99*, 5350–5357. DOI: <https://doi.org/10.1002/jsfa.9774>
7. Wangsawangrung, N.; Choipang, C.; Chairwut, S.; Ekabutr, P.; Suwantong, O.; Chuysinuan, P.; Techasakul, S.; Supaphol, P. *Gels*. **2022**, *8*, 573. DOI: <https://doi.org/10.3390/gels8090573>
8. Xu, Y.; Wang, Y.; Li, C.; Han, T.; Chen, H.; Chen, W.-X.; Zhong, Q.; Pei, J.; Haenen, G.; Li, Z.; Moalin, M.; Zhang, M.; Chen, W. *AAPS Open*. **2023**, *9*. DOI: <https://doi.org/10.1186/s41120-023-00083-8>
9. Christaki, S.; Spanidi, E.; Panagiotidou, E.; Athanasopoulou, S.; Kyriakoudi, A.; Mourtzinou, I.; Gardikis, K. *Pharmaceuticals*. **2023**, *16*, 1274. DOI: <https://doi.org/10.3390/ph16091274>
10. Ak, G.; Dall'Acqua, S.; Sut, S.; Selvi, S.; Eyupoglu, O. E.; Zengin, G. *Sci. Rep.* **2025**, *15*, 37049. DOI: <https://doi.org/10.1038/s41598-025-20905>

11. Poulson, G.; Alsulami, Q.; Sharfalddin, A.; Agammy, E. F.; Mouffouk, F.; Emwas, A.-H.; Jaremko, Ł.; Jaremko, M. *Polysaccharides*. **2021**, *3*, 1–32. DOI: <https://doi.org/10.3390/polysaccharides3010001>
12. Fatmi, S.; Bournine, L.; Iguer-ouada, M.; Malika, L.-S.; Bouchal, F.; Skiba, M. *Acta Pol. Pharm.-Drug Res.* **2015**, *72*, 179–192.
13. Fatmi, S.; Toutou, Z.; Chibani, N.; Lezrag, H. A.; Skiba, M.; Iguerouada, M. *biopolymer applications journal*. **2024**, *3*, 12–17.
14. Fatmi, S.; Taouzinet, L.; Skiba, M.; Iguer-ouada, M. *Cryo Letters*. **2023**, *44*, 89–99. DOI: <https://doi.org/10.54680/fr23210110712>
15. Santos, A. M.; Do Nascimento Júnior, J. A. C.; Cêzar, S. V. S.; Quintans Júnior, L. J.; Araújo, A. A. D. S.; Aragón, D. M.; Serafini, M. R. *Future Microbiology*. **2023**, *18*, 1363–1379. DOI: <https://doi.org/10.20944/preprints202304.0953.v1>
16. Taouzinet, L.; Fatmi, S.; Lahiani-Skiba, M.; Skiba, M.; Iguer-Ouada, M. *CryoLetters*. **2021**, *42*, 1–12. DOI: <https://doi.org/10.54680/fr21110110112>
17. Toutou, Z. Fatmi, S.; Chibani, N.; Bensidhoum, L.; Skiba, M.; Iguer-Ouada, M. *Acta Fytotech Zootech*. **2022**, *24*. DOI: <https://doi.org/10.15414/afz.2022.25.04.269-278>
18. Authaida, S.; Boonkum, W.; Chankitisakul, V. *Animals*. **2025**, *15*, 439. DOI: <https://doi.org/10.3390/ani15030439>
19. Al-Amery, K. S. M.; Banana, H. J. H. *Biochem Cell Arch*. **2020**, *20*, 723–729. DOI: <https://doi.org/10.35124/bca.2020.20.1.723>
20. Lawag, I.; Yoo, O.; Lim, L.; Hammer, K.; Locher, C. *Antioxidants*. **2021**, *10*, 1113. DOI: <https://doi.org/10.3390/antiox10071113>
21. Ali-Rachedi, F.; Meraghni, S.; Touaibia, N.; Mesbah, S. *Bull Société R. Sci. Liège*. **2018**, 13–21. DOI: <https://doi.org/10.25518/0037-9565.7398>
22. Boudou, F.; Guendouzi, A.; Belkredar, A.; Rasheed, M. *Notulae Scientia Biologicae*. **2024**, *16*, 13837. DOI: <https://doi.org/10.55779/nsb16211837>
23. Zeghoud, S.; Rebiai, A.; Hemmami, H.; Ben Seghir, B.; Elboughdiri, N.; Ghareba, S.; Ghernaout, D.; Abbas, N. *ACS Omega*. **2021**, *6*, 4878–4887. DOI: <https://doi.org/10.1021/acsomega.0c05816>
24. Aylanc, V.; Ertosun, S.; Peixoto, A. F.; Santamaria-Echart, A.; Russo-Almeida, P.; Vale, N.; Freire, C.; Vilas-Boas, M. *Emergent Mater*. **2025**, *8*, 3107–3122. DOI: <https://doi.org/10.1007/s42247-025-01002-1>
25. Toutou, Z.; Fatmi, S.; Chibani, N.; Taouzinet, L.; Harrate, I.; Inouri-Iskounen, A.; Skiba, M.; Iguerouada, M. *Starch*. **2023**, *75*, 2300017. DOI: <https://doi.org/10.1002/star.202300017>
26. Viswalingam, M.; Prabu, S.; Sivakumar, K.; Rajamohan, R. J. *Macromol. Sci., Part A*. **2016**, *53*, 781–790. DOI: <https://doi.org/10.1080/10601325.2016.1237817>
27. Benkő, B.-M.; Tóth, G.; Moldvai, D.; Kádár, S.; Szabó, E.; Szabó, Z.-I.; Kraszni, M.; Szente, L.; Fiser, B.; Sebestyén, A.; Zelkó, R.; Sebe, I. *Int. J. Pharm.* **2024**, *657*, 124187. DOI: <https://doi.org/10.1016/j.ijpharm.2024.124187>
28. Leila, K.; Djebli, N.; Pokajewicz, K.; Mostefa, N.; Wieczorek, P. *Molecules*. **2024**, *29*, 1997. DOI: <https://doi.org/10.3390/molecules29091997>
29. Çelik, S.; Kutlu, N.; Gerçek, Y.; Bayram, S.; Pandiselvam, R.; Bayram, N. *Foods*. **2022**, *11*, 3652. DOI: <https://doi.org/10.3390/foods11223652>
30. Guérin, Y.; Locatelli, Y.; Comizolli, P.; Mauget, R.; Mermillod, P.; Legendre, X.; Gatti, J.-L.; Dacheux, J.-L. *Les Actes du BRG*. **2003**, *4*, 173–183.
31. Messina, C.; Panettieri, V.; Arena, R.; Renda, G.; Espinosa Ruiz, C.; Morghese, M.; Piccolo, G.; Santulli, A.; Bovera, F. T. *Front. Vet. Sci*. **2020**, *7*. DOI: <https://doi.org/10.3389/fvets.2020.00095>
32. Mayda, N.; Özkök, A.; Ecem Bayram, N.; Gercek, Y.; Sorkun, K. *J. Food Meas. Charact*. **2020**, *14*. DOI: <https://doi.org/10.1007/s11694-020-00427-y>

33. Khadra, L.; Mokhnache, K.; Bouriche, H.; Ibrahim, D.; Harzallah, D. *Int. J. Res. Pharm. Chem.* **2019**, *9*. DOI: <https://doi.org/10.33289/IJRPC.9.4.2019.972>
34. Mladenova, R.; Aleksieva, K.; Solakov, N.; Loginovska, K. *Proc. Bulg. Acad. Sci.* **2022**, *75*, 672–679. DOI: <https://doi.org/10.7546/CRABS.2022.05.06>
35. Oroian, M.; Ursachi, F.; Dranca, F. *Antioxidants*. **2020**, *9*, 322. DOI: <https://doi.org/10.3390/antiox9040322>
36. De-Melo, A. A. M.; Estevinho, M. L. M. F.; Sattler, J. A. G.; Souza, B. R.; Freitas, A. D. S.; Barth, O. M.; Almeida-Muradian, L. B. *LWT - Food Sci. Technol.* **2016**, *65*, 808–815. DOI: <https://doi.org/10.1016/j.lwt.2015.09.014>
37. Kacemi, R.; Campos, M. G. *Molecules*. **2024**, *29*, 5893. DOI: <https://doi.org/10.3390/molecules29245893>
38. Aziza, N.; Khaydarov, K.; Zafar, M.; Alsakkaf, W.; Alkahtani, J.; Ahmad, M.; Makhkamov, T.; Djumayeva, Z.; Zengin, G.; Eshboyevich, T.; Beilerli, A.; Gareev, I.; Ochilov, U.; Islamov, B.; Iskandarovna, U.; Wibawa, I. *Biomed. Chromatogr.* **2024**, *38*. DOI: <https://doi.org/10.1002/bmc.5852>
39. Prđun, S.; Svečnjak, L.; Valentić, M.; Marijanović, Z.; Jerkovic, I. *Foods*. **2021**, *10*, 2103. DOI: <https://doi.org/10.3390/foods10092103>
40. Zheng, H.; Sun, Y.; Zeng, Y.; Zheng, T.; Jia, F.; Xu, P.; Xu, Y.; Cao, Y.; He, K.; Yang, Y. *Molecules*. **2023**, *28*, 4800. DOI: <https://doi.org/10.3390/molecules28124800>
41. Munot, N.; Kandekar, U.; Giram, P. S.; Khot, K.; Patil, A.; Cavalu, S. *Pharmaceutics*. **2022**, *14*, 1601. DOI: <https://doi.org/10.3390/pharmaceutics14081601>
42. Gulcin, İ.; Alwasel, S. H. *Processes*. **2023**, *11*, 2248. DOI: <https://doi.org/10.3390/pr11082248>
43. Tian, B. R.; Liu, Y. M. *Turk. J. Chem.* **2021**, *45*, 1707–1724. DOI: <https://doi.org/10.3906/kim-2106-51>
44. Benberkane, A.; Khellouf, A.; Karim, B.; Fatmi, S.; Iguer-ouada, M. *Cryo Letters*. **2019**, *40*, 219–225.
45. Lezrag, H. A.; Fatmi, S.; Taouzinet, L.; Toutou, Z.; Pokajewicz, K.; Merzeg, F. A.; Skiba, M.; Wiczorek, P. P.; Iguerouada, M. *J. Incl. Phenom. Macrocycl. Chem.* **2025**, *105*, 537–548. DOI: <https://doi.org/10.1007/s10847-025-01308-x>