THERMAL DEGRADATİON KİNETİCS AND IN VİTRO GASTROİNTESTİNAL STABİLİTY OF 10-HYDROXY-2-DECENOİC ACİD İN ROYAL JELLY-BASED FUNCTİONAL FORMULATİONS

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ABSTRACT

Royal jelly (RJ) is a bee-derived secretion rich in 10-hydroxy-2-decenoic acid (10-HDA), a fatty acid responsible for its antimicrobial, antioxidant, and neuroprotective effects. However, 10-HDA is sensitive to heat and digestive conditions, which limits its stability in functional formulations. This study investigated the thermal degradation kinetics and in vitro gastrointestinal stability of 10-HDA in pure RJ and its mixtures with honey, pollen, and propolis. Samples were thermally treated at 30, 50, 70, and 90 °C for 0–20 minutes. LC-MS/MS analysis showed that in pure RJ, 10-HDA content decreased from 1.905 g/100 g to 0.149 g/100 g at 90 °C, following first-order kinetics with an activation energy of 29,27. In contrast, all mixtures followed zero-order kinetics, suggesting that matrix components provided thermal protection. Calculated activation energies and Q_{10} values confirmed the compound's thermal sensitivity. Gastrointestinal stability was assessed using simulated salivary, gastric, and intestinal fluids. The highest degradation occurred in the oral and intestinal phases. The soft candy formulation showed the highest intestinal loss (57.14%), while the RJ- honey-propolis mixture retained the most 10-HDA (31.19 g/100 g). Propolis-containing formulations consistently performed better. In conclusion, 10-HDA degradation is strongly affected by temperature, digestion time, and formulation composition. Propolis and honey enhanced 10-HDA retention, especially under digestive conditions. These findings offer practical insights for developing heat-stable and bioaccessible RJ-based functional products for food, supplement, and apitherapy applications.

Keywords: Royal jelly, bee products, 10-HDA degradation, LC-MSMS, in vitro Bioaccessibility, gastrointestinal simulation.

1. INTRODUCTION

Royal jelly is a valuable bee product characterized by a thick and dense consistency, ranging from a milky white to a yellowish hue [1,2]. It has a distinctive sweet and acidic taste and is secreted by nurse bees from the hypopharyngeal glands. RJ exhibits notable antioxidant and anti-inflammatory activities, along with a variety of other biological effects [3]. RJ is widely used as a nutritional supplement and is notable for its unique fatty acid, 10-hydroxy-2-decenoic acid (10-HDA), which is considered a marker for both freshness and authenticity [4]. The concentration of this acid is considered a parameter for assessing the freshness and authenticity of royal jelly [5]. Traditional processing techniques used to convert royal jelly into therapeutic or functional food products often lead to significant changes in its chemical profile [6,7]. Despite its widespread application**,** its potential in functional food applications remains underexploited due to limited research on formulation strategies that preserve 10-HDA.

In addition to 10-HDA**,** royal jelly (RJ) contains a variety of bioactive substances, including proteins (9–18%), total sugars (10–16%), lipids (3–8%), free amino acids, peptides, minerals, and vitamins. These components synergistically interact with each other to perform numerous biological functions within royal jelly [7]. 10-HDA is a major fatty acid component found in royal jelly [8,9]. 10-HDA is a unique component specific to royal jelly and is not found in other bee products [10]. 10-HDA is known for its various biological activities [1] such as immuno-regulation [11–16], anti-inflammatory [17–25], antioxidants [26–29], anti-tumor [30,31] and antimicrobial properties [23,32–34].

Given its biological significance**,** 10-HDA is not only used as a quality marker in commercial RJ products [35] but also as a bioactivity indicator. However, its stability is highly sensitive to thermal degradation, primarily due to oxidative degradation, isomerization, and hydrolysis reactions. These degradative changes can occur during storage, processing, and transportation. In particular, temperature fluctuations significantly affect degradation kinetics, reducing its concentration and compromising bioactivity. For instance, Kamakura et al. (2014) [36] demonstrated that RJ stored above 40 °C shows a marked reduction in 10-HDA content compared to samples kept below 4 °C.

Furthermore, recent evidence suggests that royal jelly presents a potential therapeutic value and a beneficial action for treating Alzheimer's in neurodegenerative processes[37], it also, has the potential to prevent fatty liver disease by cholesterol accumulation [38]. Nevertheless, these functional benefits may be lost if RJ is preserved or processed under suboptimal conditions. Structural and functional degradation may reduce or eliminate its biological activity. While RJ continues to be widely used in the health, medical, and cosmetic sectors [7,39], existing studies have primarily focused on isolated properties such as biological activity, purity (10-HDA), and freshness (e.g., glucose oxidase, furacin). However, no research has comprehensively evaluated the thermal degradation of 10-HDA as a function of its formulation into functional food products. There are many studies on the direct use and processing of bee products (royal jelly, propolis, pollen, honey, bee bread, beeswax, apilarnil, and bee venom). Among these products, royal jelly has a therapeutic effect against some diseases due to its biochemical properties. Studies have been carried out on the biological and pharmaceutical properties [25,40], purity (10-HDA), and freshness criteria (glucose oxidase, furacin) of royal jelly [41–46] These studies have typically evaluated RJ in its raw or minimally processed forms. However, no study has been found that includes a holistic approach to determine the thermal degradation of 10-HDA in royal jelly as a function of the processes used to produce functional foods from royal jelly. This remains a critical research gap, especially given the increasing interest in RJ-enriched formulations such as lozenges, soft candies, and nutraceutical supplements.

To fully understand the stability of 10-HDA in the gastrointestinal system as well, it is important to consider the digestion process. The digestive system extends approximately 8–10 meters and includes organs such as the stomach and intestines, where digestion is mediated by enzymes and pH variations [47]. Fat digestion predominantly occurs in the small intestine, and the digestibility of fatty acids is affected by chain length and saturation [48]. Short- and medium-chain saturated fatty acids are absorbed more efficiently than long-chain ones, while unsaturated fatty acids are more digestible than their saturated counterparts [49]. These conditions could also influence the structural integrity and bioavailability of 10-HDA during digestion.

This study aims to (i) investigate the thermal degradation kinetics of 10-HDA in pure RJ and its combinations with honey, pollen, and propolis at 30–90 °C using kinetic modeling (reaction order, rate constants, activation energy, Q_{10}), and (ii) assess its gastrointestinal stability in functional RJ-based products (soft candies and lozenges) using a standardized in vitro digestion model covering oral, gastric, and intestinal phases. The findings will contribute to the development of thermally and physiologically stable royal jelly formulations for use in apitherapy, nutraceuticals, and functional foods.

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2. MATERIALS AND METHODS

2.1 Materials

Raw materials, including pure royal jelly, honey (pine), propolis, and pollen, were obtained from local beekeepers.

2.2 Experimental design

1. Preparation of functional mixtures containing royal jelly
2. Preparation of soft candies containing royal jelly and its mixtures
3. Descriptive analysis
4. Thermal treatment
5. 10- HDA analysis
6. Determination of thermal degredation kinetics of 10-HDA in royal jelly and royal jelly based products
7. In vitro bioaccesibility evaluation

2.3 Sample preparation

2.3.1 Preparation of functional mixtures containing royal jelly

Functional apitherapy mixtures were prepared according to table 1 using pure royal jelly, honey, pollen, and propolis.

Table 1. Composition (g/100 g) of Royal Jelly-Based Functional Confectionery and Honey Mixtures.

Ingredient	Mixture 1	Mixture 2	Mixture 3	Mixture 4	Royal Jelly Soft Candy (g)	Royal Jelly + Propolis Soft Candy (g)	Royal Jelly + Propolis + Pollen Soft Candy (g)	Royal Jelly Lozenge (g)
Royal Jelly	4.34	4.34	4.34	4.34	5	5	5	5
Honey	95.66	89.14	93.49	86.97	-	-	-	30
Pollen	-	6.52	-	6.52	-	-	3	-
Propolis	-	-	2.17	2.17	-	2	2	3
Water	-	-	-	-	61.3	59.3	56.3	100
Sugar	-	-	-	-	15	15	15	25
Gelatin	-	-	-	-	10	10	10	-
Carob Gum	-	-	-	-	0.2	0.2	0.2	-
Glycerol	-	-	-	-	3.5	3.5	3.5	-
Polydextrose	-	-	-	-	5	5	5	-
Lemon Juice	-	-	-	-	-	-	-	3
Cinnamon + Clove + Green Tea	-	-	-	-	-	-	-	2
Freeze-dried Royal Jelly	-	-	-	-	-	-	-	5

2.3.2 Preparation of Soft Candies Containing Royal Jelly and Its Mixtures

Three different soft candies containing royal jelly, royal jelly, propolis, and royal jelly, propolis, and pollen were produced following the method method of Hacıoğlu (2017) [50]. For soft candy production, the following ingredients were used: water (ultrapure), granulated sugar, gelatin (Alfasol, bovine gelatin, 250 bloom), carob gum (Incom, Turkey), polydextrose, glycerol (Uparc, extrapure, min. 98–100%, USA) for plasticizing and preservation properties, and royal jelly-based mixtures (royal jelly + propolis, royal jelly + propolis + pollen).

The ingredients were weighed in the specified amounts and mixed at 50°C using a magnetic stirrer at 250 rpm. The mixing process was terminated when the soft candy mixture reached 70°Bx, after which the mixtures were poured into molds. The molds were stored at +4°C for 24 hours, and the soft candies were removed from the molds after the storage period.

2.3.3 Production of Lozenges from Royal Jelly

Lozenges were produced using commercial freeze-dried royal jelly. The production was carried out with modifications based on the methods of Edwards (2018) [51] and Witzler et al. (2017) [52].

For this purpose, the following ingredients were heated and mixed to 90°Bx: ultrapure water, sugar, honey, lemon juice, a blend of cinnamon, clove, and green tea, and powdered propolis. After reaching the desired consistency, freeze-dried royal jelly was added, and the mixture was poured into molds. The lozenges were removed from the molds after one hour.

2.4 Thermal treatment of samples

Heat treatment experiments were carried out by slightly modifying the earlier methods applied [53]. All samples were weighed 2-3 g into Pyrex tubes (75x10 mm ID) and closed tightly to prevent evaporation. Heat treatments were carried out in a water bath at different temperatures (30, 50, 70, 90 °C) and time (0, 5, 10, 15, 20 min) combinations. These temperatures were chosen based on industrial processes applied during the stabilization and packaging of beekeeping products. The experiment time was started after the temperature measured by the thermocouple reached the set temperature. Then, the samples taken at 5-minute intervals were immediately cooled in a cold-water bath. Considering the 10-HDA change in heat treatment samples, the reaction degree, linear regression equations, reaction rate constants, activation energy, and Q_{10} values were calculated [54].

2.5 Descriptive analysis

The samples' moisture content, viscosity, and pH values were calculated. The color values were analyzed using Hunter UltraScan-VIS in the appropriate mode (RSEX-Reflectance Specular Excluded) and image area (0.375 in.). The products' color values were shown in terms of L, a, and b. In addition, the chroma values of the samples were calculated. The sugar content of the samples was determined spectrophotometrically with the 3,5-dinitrosalicylic acid method [55]. The calibration curve (Equation 1**)** for the spectrophotometric measurements (Thermo Scientific™ Evolution 201 UV-Vis, Waltham, MA, USA) at 575 nm was obtained using glucose standard solutions at different concentrations. Viscosity analysis was performed using a Brookfield R/S Plus (Brookfield TC-502) viscometer. The data obtained from the pure royal jelly and the mixtures with T-F spindle at 50 rpm mixing speed for 2 minutes were recorded and their averages were calculated.

$$FSC\ (g/L) = 60.401 \times Abs_{575} + 0.5751 \qquad \text{(Equation 1)}$$

2.6 10-HDA analysis

Royal jelly sample (200 mg) was transferred (250-fold dilution) into a 50 ml flask, and 25 ml of ultrapure water was added to dissolve the royal jelly. Then, 0,5 ml of 2 molar NaOH solution was added, waiting 10 minutes, and the flask was filled to 50 ml with ultrapure water. Then, 4 ml of this solution was taken and put into a 50 ml falcon tube, and 27 ml of saturated NaCl solution was added. The pH value was adjusted to the range of 2-2,5 by adding 1 ml of 0,1 molar hydrochloric acid to this solution. Then 8 ml of diethyl ether was added and shaken (4 ml of sample solution + 27 ml of NaCl solution + 1 ml of HCl solution + 8 ml of diethyl ether = 40 ml in total, thus 10 times dilution). The solution obtained was centrifuged at 3000 rpm, and the supernatant was separated. Under nitrogen gas media, 4 ml of the supernatant phase was dried. The dried sample was then dissolved in 1,6 ml of acetonitrile. The concentrated solution was taken from the vial, completed to 1 ml, diluted 150 times with acetonitrile, and 1 ml was taken into a 2 ml vial and injected into the UHPLC-MS/MS device [41,56]. Analysis of 10-hydroxy 2-decenoic acid (10-HDA) in all samples was performed in an ultra-high-performance liquid chromatography-tandem mass spectrometer (Thermo brand UHPLC-MS/MS). The 10-HDA standard with a concentration of 1 mg/kg was injected into the UHPLC with a C18 column and determined by the method developed for MS/MS. The retention time for the 10-HDA standard on the C18 column was defined as 1,30 minutes. After this process, a quantification method for 10-HDA analysis was created using the UHPLC-MS/MS device software. The resulting method uses a gradient flow program with methanol-water as the UHPLC mobile phase. The flow rate was 200 µL /min, and the total analysis time was 5 min.

The LOD value of 10-HDA was calculated as 0.0030 mg/kg, and the LOQ value was 0.0101 mg/kg. The regression constant of the drawn calibration line was obtained as R^2= 0.9996.

2.6.1 Determining the thermal degradation kinetics of 10- HDA

The 10-HDA changes in royal jelly and its mixtures were mathematically modeled [56]. The heat-treated royal jelly and its mixtures were taken at specific time intervals to determine the reaction degree, and the 10-HDA amounts were plotted on arithmetic and logarithmic scale graphics against time. The degree of reaction is determined according to the graph type in which the linear curve is obtained. Accordingly, if the linear curve is acquired when the arithmetic is machined into the graph, it is considered zero. If plotted, the logarithmic scale is processed on the graph; it is considered first-degree. In addition, the 1/(10-HDA concentration) versus time was plotted, and its conformity to the second-order reaction kinetics was checked.

If a straight line is obtained when the change in a reaction is measured and processed on a logarithmic scale graph against time, it is understood that this reaction is a first-order reaction, and the equation of the straight line is;

$$y = a + kx$$

where,
y: Concentration
a: The point where the line intersects the y-axis (the value of y when x=0)
k: the slope of the line
x: Time

Linear regression equations were obtained for each temperature. The slope of the line for the reaction (k) is taken as the first-order reaction rate constant (k).Creation of Arrhenius Curve, Calculation of Activation Energy, and Q_{10} Value.An Arrhenius curve was created to determine the change of 10-HDA in royal jelly and its mixtures depending on temperature. Here, the natural logarithms of the reaction rate constants (k) (ln k) were plotted against 1/T values, and a line and its linear regression equation were obtained. The reaction's activation energy was calculated using the slope of the line ($—E_a/R$).

$$k = k_0 e^{-Ea/RT}$$

where,
k: Reaction rate constant, k_0: Frequency factor, E_a: Activation energy, R: Ideal gas constant, and
T: Absolute temperature (K)
In following equation was used in the calculation.

$$Q_{10}=(k_2/k_1)^{10/(T2-T1)}$$

where,
k: Reaction rate constant, and T: Temperature.

2.7 In Vitro bioaccesibility evaluation

The in vitro Bioaccessibility of 10-HDA in different product formulations produced under various conditions was evaluated under oral, gastric, and intestinal environments. The Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF), and Simulated Intestinal Fluid (SIF) were prepared using electrolyte stock solutions, enzymes, $CaCl_2$, and water. The electrolyte stock solutions consisted of four parts electrolyte solution and one part water, ensuring the appropriate ionic composition in simulated conditions. The gastrointestinal digestion model was adapted from Samtlebe et al. (2016) [57] for assessing the in vitro bioaccessibility of 10-HDA.

For the simulated gastric environment, a solution containing 0.2% NaCl and 80 mM HCl was prepared, and pepsin enzyme (3.2 mg/mL) was added. The pH was adjusted to 2.0 using 0.5 M NaOH. Then, 1 g of royal jelly or royal jelly-based apitherapy formulations was added to 9 mL of preheated (37°C) simulated gastric fluid. Samples were collected at 0, 60, and 120 minutes, and the 10-HDA content was analyzed.

For the simulated intestinal environment, a 0.1 mM sodium phosphate buffer (pH 6.8) containing 10 mg/mL pancreatin enzyme was prepared and preheated to 37°C. 1 g of royal jelly or royal jelly-based formulations was added to 9 mL of this solution, and samples were collected at 0, 30, 60, 90, and 120 minutes for 10-HDA analysis.

2.8 Statistical analysis

The SPSS (IBM SPSS Statistics, Version 25.0) statistical program was used to evaluate the obtained data. First, normal distribution and homogeneity of variance tests were applied to the data, and ANOVA and Kruskal-Wallis H tests were used according to the results obtained from these tests. Tukey HSD and Tamhane's T2 tests were performed for multiple comparisons.

3. RESULTS

3.1 Descriptive properties of samples

The chemical and physicochemical properties of royal jelly and its mixtures were analyzed, with findings presented in Table 2. The 10-HDA content varied between 0.09% and 1.94%, with pure royal jelly meeting the minimum standard of 1.4%. The moisture content of pure royal jelly was determined to be 62.92%, whereas the lowest moisture content was observed in Mixture 2 (12.45%).

Table 2: Descriptive Analysis

Sample	Dry Matter (%)	pH	Acidity (%)	Total Sugar (g/100 g)	Viscosity (mPa.s)	10-HDA (g/100 g)	Chroma
Royal jelly	37,08^{a}	3,9^{a}	42,13^{a}	22,56^{a}	1,94^{a}	1,94^{a}	12,47^{a}
Mixture 1	87,54^{b}	5,8^{b}	3,49^{b}	85,04^{b}	13,91^{b}	0,09^{b}	1,01^{b}
Mixture 2	86,96^{b}	5,7^{c}	5,86^{c}	82,79^{b}	20,71^{c}	0,08^{c}	11,17^{c}
Mixture 3	87,03^{b}	5,9^{b}	4,12^{b}	77,69^{b}	14,64^{b}	0,09^{b}	6,25^{d}
Mixture 4	87,14^{b}	5,6^{d}	6,97^{c}	78,01^{b}	21,47^{c}	0,09^{b}	10,06^{ac}

The moisture content of royal jelly is influenced by bee genotype and the time of collection after larval grafting, as extended collection periods tend to increase moisture loss. It is reported that the moisture content of royal jelly ranges from 60.1% to 67.1%, with an average value of 65.2% [58]. In this study, the pH value of pure royal jelly was determined to be 3.9, which falls within the typical range reported for royal jelly (3.55–4.02) [59] , and was lower than that of the mixtures containing honey, pollen, and propolis.

The viscosity and chroma values of the mixtures showed significant differences ($p<0.05$). Pollen content was found to increase the viscosity of the product, which can be attributed to the physicochemical composition of bee pollen. Bee pollen possesses a high content of carbohydrates, proteins, lipids, and fibers [60]. Its rich composition - particularly the elevated levels of proteins and carbohydrates - can enhance water absorption and promote gel formation. This, in turn, increases the viscosity of the mixture. The 10-HDA content ranged from 0.08 to 1.94 g/100 g across the samples.

3.2 Change of 10-HDA content in mixtures depending on temperature and time

Thermal processes were conducted at different temperatures (30, 50, 70, 90°C) and durations (0, 5, 10, 15, 20 min) on royal jelly, Mixture 1, Mixture 2, Mixture 3, and Mixture 4. The changes in 10-HDA levels following heat treatment were evaluated, with results presented in Table 3. Figure 1 illustrated change of 10-HDA content of samples with time during thermal treatment. The findings indicated a reduction in 10-HDA content with increasing temperatures, following first-order kinetics in pure royal jelly. The natural logarithm of the 10-HDA content exhibited a linear decline at varying temperatures, confirming a first-order degradation reaction. In contrast, 10-HDA degradation in mixtures followed zero-order reaction kinetics.

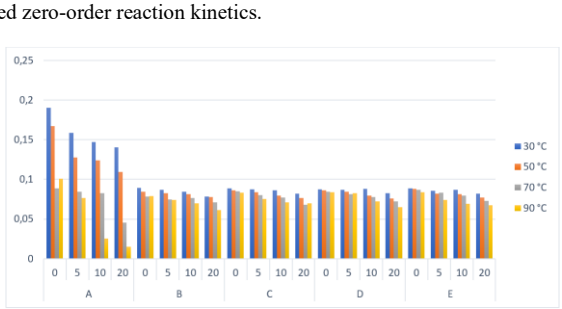

Figure 1. Change of 10-HDA content of samples with time during thermal treatment.
A-Pure Royal Jelly, **B-** Mixture 1, **C-** Mixture 2, **D-** Mixture 3, **E-** Mixture 4
*10-HDA values for sample A were divided by 10 for visual comparison.

Table 3. 10-HDA content (g/100 g) of samples, and rate equations depending on heat treatment (N=4)

Heating Temperature					
Sample	Heating Time (min)	30 °C	50 °C	70 °C	90 °C
Pure Royal Jelly	0	1,906	1,670	0,884	1,008
	5	1,588	1,277	0,842	0,764
	10	1,473	1,238	0,827	0,252
	15	1,436	1,214	0,458	0,276
	20	1,401	1,093	0,456	0,149
	Rate Equation	y = -0,311lnx+1,86 R^2 = 0,95	y = -0,33lnx+1,61 R^2 = 0,90	y = -0,28lnx+0,97 R^2 = 0,70	y = -0,57lnx+1,03 R^2 = 0,92
Mixture 1	0	0,089	0,084	0,078	0,078
	5	0,087	0,082	0,074	0,073
	10	0,084	0,081	0,076	0,069
	15	0,081	0,080	0,072	0,069
	20	0,078	0,07	0,070	0,061
	Rate Equation	y = -0,0028x+0,09 (R^2 = 0,99)	y = -0,0015x+0,09 (R^2 = 0,96)	y = -0,0017x+0,08 (R^2 = 0,83)	y = -0,004x+0,08 (R^2 = 0,94)
Mixture 2	0	0,088	0,086	0,084	0,082
	5	0,087	0,084	0,080	0,075
	10	0,086	0,079	0,076	0,071
	15	0,085	0,079	0,069	0,066
	20	0,081	0,076	0,067	0,069
	Rate Equation	y = -0,0013x+0,09 R^2 = 0,99	y = -0,0027x+0,09 R^2 = 0,98	y = -0,0033x+0,09 R^2 = 0,98	y = -0,0048x+0,09 R^2 = 0,90
Mixture 3	0	0,087	0,086	0,084	0,083
	5	0,086	0,084	0,081	0,082
	10	0,087	0,079	0,077	0,072
	15	0,083	0,078	0,073	0,072
	20	0,082	0,075	0,072	0,065
	Rate Equation	y = -0,0013x+0,09 R^2 = 0,99	y = -0,0027x+0,09 R^2 = 0,98	y = -0,0033x+0,09 R^2 = 0,98	y = -0,0048x+0,09 R^2 = 0,90
Mixture 4	0	0,088	0,088	0,087	0,084
	5	0,085	0,081	0,083	0,074
	10	0,086	0,081	0,079	0,069
	15	0,085	0,080	0,079	0,070
	20	0,082	0,077	0,073	0,067
	Rate Equation	y = -0,0014x+0,09 R^2 = 0,87	y = -0,0024x+0,09 R^2 = 0,86	y = -0,0032x+0,09 R^2 = 0,93	y = -0,0037x+0,08 R^2 = 0,77

Activation energy and Q_{10} values were calculated to determine the degradation rate of 10-HDA at different temperatures (Figure 1). The initial 10-HDA content was 1.905 g/100 g at 30°C and decreased to 0.149 g/100 g after 20 minutes at 90°C. The degradation rate followed a logarithmic trend, as described by the rate equation $y = -a.ln(x) + b$, where y represents the natural logarithm of 10-HDA content, x denotes heating time, and b is a constant. The R^2 values for these equations ranged from 0.70 to 0.95, indicating a satisfactory fit. In contrast, the degradation in mixtures followed a zero-order reaction model, with the rate equation $y = -a.x + b$.

The reaction rate constants for royal jelly and its mixtures increased with rising temperatures. Similarly, Q10 values in pure royal jelly increased with temperature (Table 4). However, Q10 and Ea values were not calculated for mixtures, as their thermal degradation followed zero-order kinetics. The activation energy of 10-HDA degradation in pure royal jelly was found to be higher than in mixtures, likely due to the influence of added sugars from honey.

Table 4. Reaction rate constants, Q_{10} values, and activation energies of royal jelly and the mixtures (N=4).

Sample	$Kx10^3$ (1/min)				Q_{10}	E_a (kJ/mol)
	30°C	50°C	70°C	90°C		
Royal jelly	14,3	18,0	38,6	96,6	1,39	29,27
Mixture 1*	6,6	3,6	4,7	11,3		
Mixture 2*	3,8	6,0	11,9	9,4		
Mixture 3*	3,1	6,6	8,5	12,8		
Mixture 4*	3,2	5,8	8,0	9,7		

The findings indicate that the physicochemical properties of royal jelly are significantly altered when combined with honey, pollen, and propolis. The pH values obtained align with previous reports, where the pH of pure royal jelly ranged between 3.4 and 4.5 [61]. The 10-HDA content in commercial royal jelly samples has been reported between 0.75% and 3.11% [62], while the European Standard mandates a minimum of 1.4% [63]. Additionally, Bloodworth et al. 1995 [64] identified a 10-HDA content ranging from 1.98% to 6.37% in 39 commercial royal jelly samples. Similarly, fresh royal jelly samples from Eastern Croatia exhibited values between 1.56% and 3.78% [5]. These findings suggest that the composition of royal jelly mixtures varies depending on formulation factors, with components such as pollen influencing viscosity and honey altering sugar composition.

The results confirm that 10-HDA is highly susceptible to thermal degradation, with degradation kinetics differing between pure royal jelly and its mixtures. The logarithmic trend in pure royal jelly degradation aligns with first-order reaction kinetics, whereas mixtures follow zero-order kinetics. This difference may be attributed to interactions with other components such as sugars and proteins, which can affect the stability of 10-HDA.

The literature on the kinetic parameters of 10-HDA thermal degradation remains limited, preventing direct comparisons. However, previous studies indicate that heat treatment significantly impacts royal jelly's bioactive components. Saricaoglu et al. 2019 [44] demonstrated that elevated temperatures induce substantial structural changes in royal jelly, suggesting potential degradation of bioactive constituents, including 10-HDA. Another study reported a marked decline in 10-HDA levels when royal jelly was stored at 30°C for 90 days, highlighting the impact of prolonged heat exposure. Similarly, research on lyophilized royal jelly showed that lower temperatures preserve 10-HDA content, whereas even moderate temperatures (25°C) can lead to degradation [65].

Although specific kinetic studies on 10-HDA are scarce, the degradation trend observed - particularly the increase in degradation rate with rising temperature - was comparable to those reported for other bioactives such as ascorbic acid, vitamin B12, and phenolic lipids in cashew nut shell liquid. Karhan et al. (2004) [66]reported first-order degradation kinetics for ascorbic acid in rosehip puree, with an activation energy of 66.87 kJ/mol, and rapid thermal loss observed above 100 °C. Similarly, Ceribeli et al. (2023) [67] demonstrated that vitamin B12 in fortified milk degraded via first-order kinetics with a significantly higher activation energy (Ea = 130 ± 5 kJ/mol), showing substantial instability above 120 °C. In contrast, Sangaré et al. (2025) [68]reported that cashew nut shell liquid constituents such as anacardic acid and cardanol degraded sequentially, with Ea values ranging from 95.3 to 124.2 kJ/mol under pyrolytic conditions.

Compared to these compounds, 10-HDA displayed moderate thermal sensitivity, particularly in the 70–90 °C range, while its degradation was significantly attenuated in formulations containing honey and pollen. This matrix effect resembles the protective roles of milk proteins in B12 stability and polyphenols in rosehip puree, indicating that formulation design may be key to enhancing the thermal stability of 10-HDA in functional food matrices. The thermal degradation of 10-HDA in royal jelly and its mixtures appears to be influenced by both temperature and the presence of additional components. The lower Q_{10} value in royal jelly, compared to typical first-order reactions in food systems, suggests a relatively slower degradation rate. However, no previous studies have reported comparable Q_{10} values for royal jelly. Given that 10-HDA degradation in mixtures follows zero-order kinetics, Q_{10} values were not calculated. These findings highlight the need for controlled storage and processing conditions to maintain 10-HDA stability in royal jelly and its derived products.

3.3 In vitro bioaccesibility evaluation

3.3.1 Stability in the Oral Phase

The initial content of 10-HDA and its degradation during simulated oral, gastric, and intestinal digestion phases in various royal jelly-based formulations are presented in Table 5. Additionally, Figure 2 presented the stability of 10-HDA in royal jelly-based formulations and their mixtures following the simulated oral digestion phase. The oral phase was characterized by the most pronounced degradation, with losses ranging from 51.43% to 53.62%. The greatest reduction occurred in Royal Jelly Soft Candy (53.62%), while the Royal Jelly + Propolis Lozenge showed the least degradation (51.43%). Despite these differences being relatively small, they emphasize the vulnerability of 10-HDA to salivary enzymes and initial matrix dissolution, suggesting that this stage poses a critical barrier to its preservation. In the gastric phase, further losses were recorded, varying between 12.07% and 26.58%. The highest degradation occurred in pure RJ, which lacks protective excipients, whereas the lowest was observed in the soft candy formulation, indicating potential protective effects of its structural and compositional features under acidic conditions.

Table 5. Stability of 10-HDA in Royal Jelly Formulations During Simulated Gastrointestinal Digestion.

Sample	Initial 10-HDA (%, g/100 g)	Mouth Loss (%)	Stomach Loss (%)	Intestine Loss (%)
Royal jelly		53.21	26.58	45.6
Royal jelly + Honey		53.59	21.95	35.42
Royal jelly + Honey + Pollen		53.0	15.38	45.45
Royal jelly + Honey + Propolis		51.94	21.43	17.39
Royal jelly + Honey + Pollen + Propolis		52.38	14.63	35.56
Royal jelly Soft Candy	0,1272±0,0038	53.62	12.07	57.14
Royal jelly + Propolis Soft Candy	0,1007±0,0035	53.33	20.0	20.41
Royal jelly + Propolis + Pollen Soft Candy	0,1143±0,0034	51.88	18.18	22.03
Royal jelly + Propolis Lozenge	0,035±0,0013	51.43	17.86	22.58

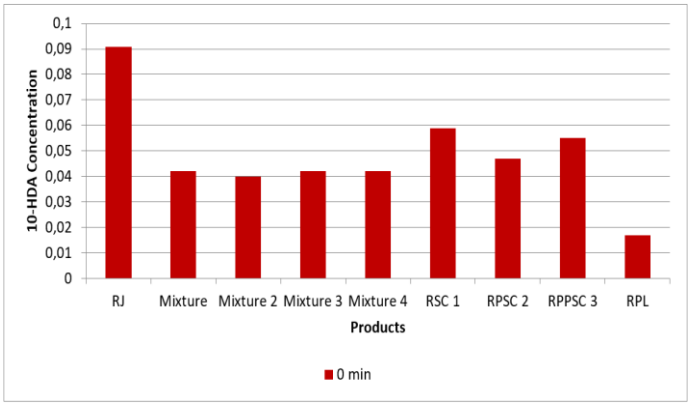

Figure 2: 10-HDA Stability in Royal Jelly Mixtures and Products After Simulated Oral Phase.

*For visualization purposes, the 10-HDA values for pure royal jelly were divided by 10.

Samples were incubated for 120 minutes in simulated gastric fluid, and 10-HDA levels were assessed at 0, 60, and 120 minutes. A control experiment containing only gastric fluid confirmed that no external degradation occurred in the absence of sample matrices. The absolute 10-HDA content in the gastric environment varied substantially among the samples. Pure RJ retained the highest concentration (0.888%), whereas the RJ + Propolis Lozenge retained the lowest (0.028%). +The consistent decline in 10-HDA concentration over time underscores its sensitivity to acidic and enzymatic environments. The inclusion of additional bioactive components such as propolis and pollen led to a modest improvement in 10-HDA retention; however, these enhancements were insufficient to prevent the substantial losses observed, particularly during the oral phase.

These findings highlight the critical role of matrix design in functional food formulations aimed at improving the stability and potential bioavailability of 10-HDA throughout gastrointestinal transit. Phenolic compounds (polyphenols) neutralize free radicals and terminate the lipid oxidation chain through their hydroxyl (-OH) groups attached to aromatic rings. The primary mechanism involves either the transfer of a hydrogen atom from the phenolic antioxidant to the free radical or the donation of an electron to stabilize the radical [69]. Phenolic antioxidants serve as natural alternatives to synthetic additives such as BHT/BHA and are widely utilized to extend the shelf life of foods [70]. Bee pollen and propolis exhibit strong antioxidant activity due to their high phenolic and flavonoid content [71].

3.3.2 Stability in the Gastric Phase

The stability of 10-HDA during simulated gastric digestion at various time intervals in royal jelly mixtures and derived products was shown in Figure 3. The stability of 10-HDA during gastric digestion was significantly influenced by both formulation composition and incubation duration. The effect of different incubation times on the 10-HDA content of royal jelly in the gastric environment was found to be statistically significant, with multiple comparison tests indicating significant differences between all time points (0-60 min, 0-120 min, and 60-120 min).

Similarly, the 10-HDA content of the royal jelly and honey mixture showed significant degradation over time, with a statistically significant reduction at each measured time point. The combination of royal jelly with honey and pollen also resulted in a notable decrease in 10-HDA, though in this case, significant differences were only observed between 0-60 min and 0-120 min, suggesting a slower degradation rate after the first hour.

For the royal jelly and honey with propolis mixture, the impact of different incubation times was statistically significant, with significant differences observed across all time points. The addition of propolis appears to provide some protective effect, although degradation was still evident. A similar trend was observed in the royal jelly with honey, pollen, and propolis mixture, where incubation time had a statistically significant impact, and significant differences were detected between all measured time points. These results indicate that while honey, pollen, and propolis may contribute to 10-HDA stability, degradation remains unavoidable under gastric conditions.

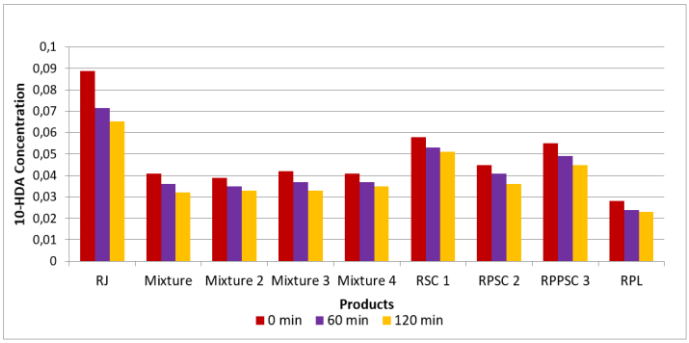

Figure 3. Stability of 10-HDA During Simulated Gastric Digestion at Different Time Points in Royal Jelly Mixtures and Derived Products.

*For visualization purposes, the 10-HDA values for pure royal jelly were divided by 10.

The effect of incubation time was also significant for royal jelly-containing soft candy, where multiple comparisons revealed differences between 0-60 min and 0-120 min. However, the soft candy matrix appeared to offer some protection, as the gastric loss was lower compared to other formulations. The royal jelly with propolis-containing soft candy exhibited a more pronounced protective effect, with statistically significant differences observed at all time points, suggesting that the presence of propolis may contribute to increased stability. A similar pattern was seen in the royal jelly with propolis and pollen-containing soft candy, where significant differences were detected between all incubation times, reinforcing the idea that the combined effects of propolis and pollen may help delay degradation.

The 10-HDA content of the royal jelly and propolis lozenge was also significantly affected by incubation time, with differences observed between 0-60 min and 0-120 min. This suggests that while the lozenge matrix may slow down degradation, it does not completely prevent it. Among all formulations, propolis-containing products exhibited the highest retention rates, likely due to the protective effects of its phenolic and flavonoid compounds. Honey-based formulations showed moderate stability, which may be attributed to its prebiotic properties and its ability to form hydrogen bonds that stabilize 10-HDA.

Studies have demonstrated that gelatin/pectin-based soft candy matrices effectively encapsulate bitter or astringent bioactive compounds, thereby enhancing the sensory quality of the product while maintaining the stability of active ingredients to a large extent [72]. The incorporation of 10-HDA in soft candy formulations (e.g., gelatin or pectin-based gummies) and lozenges offers advantages in terms of stability preservation and taste masking.

3.3.3 Stability in the Intestinal Phase

The stability of 10-HDA during simulated intestinal digestion at various time intervals in royal jelly mixtures and derived products was shown in Figure 4. All samples were incubated in simulated intestinal fluid for 120 minutes, and 10-HDA levels were monitored at 0, 30, 60, 90, and 120 minutes. A control sample containing only intestinal fluid confirmed that no degradation occurred in the absence of test materials.

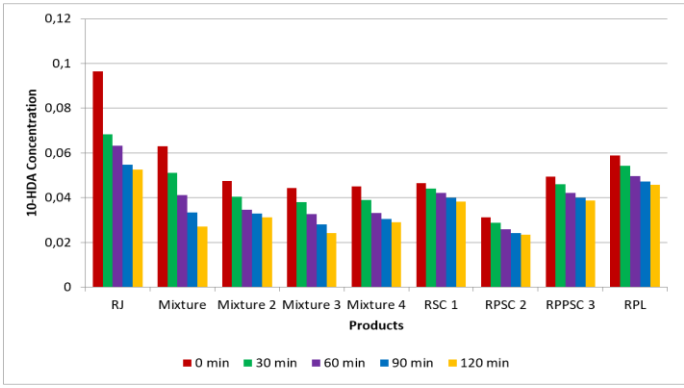

Figure 4. Stability of 10-HDA During Simulated Intestine Digestion at Different Time Points in Royal Jelly Mixtures and Derived Products.

Among all formulations, pure royal jelly retained the highest 10-HDA concentration (0.965%) at the start of the intestinal phase, while the lowest was recorded in the Royal Jelly + Propolis Lozenge (0.031%). A progressive, time-dependent decrease was observed in all samples, indicating that 10-HDA undergoes enzymatic degradation throughout the intestinal process.

Statistical analysis revealed significant differences in 10-HDA content over time ($p < 0.05$) for all formulations. In pure royal jelly and the RJ + Honey + Pollen formulation, significant degradation occurred between all time points, with no plateau observed. For the RJ + Honey + Propolis formulation, degradation was more pronounced in the early stages (0–60 min), while the rate slowed thereafter. A similar trend was seen in the RJ + Propolis Soft Candy and RJ + Propolis Lozenge, where multiple comparisons showed that degradation plateaued after 60 minutes, suggesting a stabilization effect in the later stages of digestion.

The intestinal phase resulted in the widest variability in 10-HDA loss across samples, ranging from 17.39% to 57.14%. RJ + Honey + Propolis exhibited the lowest loss (17.39%), indicating that this combination provided the most protection against intestinal degradation, likely due to the synergistic effects of phenolic and prebiotic components. In contrast, RJ Soft Candy exhibited the highest degradation (57.14%), despite performing better in the gastric phase, indicating high susceptibility to intestinal enzymatic activity.

Final retention values of 10-HDA after complete digestion ranged from 17.49 g to 31.19 g per 100 g of the original sample. The highest stability was observed in formulations containing propolis—especially RJ + Honey + Propolis (31.19 g), RJ + Propolis Lozenge (30.89 g), and RJ + Propolis Soft Candy (30.71 g)—supporting the hypothesis that propolis exerts a protective effect, possibly due to its rich phenolic content and antioxidant capacity. Honey-containing samples showed moderate stability, likely due to hydrogen bonding and prebiotic effects, while RJ Soft Candy showed the lowest residual content, suggesting formulation limitations under intestinal conditions.

Overall, these results highlight that formulation plays a decisive role in determining 10-HDA stability during digestion. While all formulations suffered losses during the intestinal phase, those containing propolis exhibited the most consistent protective effects across all stages. This underlines the importance of optimizing RJ-based functional products with stabilizing components to enhance 10-HDA bioaccessibility and efficacy. Given that medium-chain fatty acids like 10-HDA are absorbed in the small intestine, maintaining stability through oral and gastric phases is critical for ensuring absorption and bioactivity in the lower gastrointestinal tract.

4. CONCLUSION

This study evaluated the thermal degradation of 10-HDA in pure royal jelly and its mixtures with honey, pollen, and propolis under controlled heating conditions. The results demonstrated that temperature and heating time had a pronounced effect on 10-HDA stability, with the highest degradation occurring at 90 °C after 20 minutes. In pure royal jelly, degradation followed a first-order kinetic model, while mixtures showed zero-order kinetics, indicating matrix-dependent protection.

The calculated activation energies (Ea) and Q_{10} values confirmed the compound's high sensitivity to heat. Kinetic modeling highlighted the role of formulation in slowing thermal degradation, and provided useful predictions for shelf-life and process optimization in heat-processed functional foods. In the simulated gastrointestinal digestion model, the oral and intestinal phases caused the most significant 10-HDA degradation. Propolis-containing formulations consistently showed higher retention, suggesting that phenolic compounds contributed to stabilization through antioxidant mechanisms or molecular interactions. Although the soft candy formulation performed well in the gastric phase, it exhibited the highest loss in the intestinal phase, indicating limited resistance under enzymatic conditions. Time-dependent degradation was observed in all samples, emphasizing the importance of matrix design in preserving 10-HDA during digestion. In conclusion, thermal stability and gastrointestinal behavior of 10-HDA are strongly influenced by both processing conditions and formulation composition. Optimizing heat treatment and incorporating stabilizing ingredients like propolis and honey are key strategies for enhancing the functionality and bioaccessibility of royal jelly-based products in food, health, and apitherapy applications.

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Conflict of interest

The authors declare no competing interests.

Author Contributions

I.Y.: Investigation, Analyses, Conceptualization, writing. **H.G.H.Y.:** Investigation, Writing. **A.H.:** Investigation. **M.K.:** Conceptualization, Development, or design of methodology, Resources, Supervision, Project administration, and Funding acquisition.

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