Bee Propolis, Bee Bread, and Royal Jelly: Proximate Analysis, Fatty Acid Composition, Nutritional Quality, and Anti-Amylase Activity

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Abstract

This work explores the proximate composition, fatty acid profile, nutritional quality, and anti-amylase activity of propolis, royal jelly, and bee bread. The differential FTIR patterns of propolis, royal jelly, and bee bread reflect these products have different proximate compositions and nutritional properties. The values for carbohydrate, fat, and protein values of be products are similar to egg and soya. The primary fatty acids bee bread and bee propolis are palmitic, linolenic, oleic, linoleic, myristic, and docosanoic acids. The primary fatty acids in royal jelly are 2-dodecenedioic, 10-hydroxy-2-decenoic, decanedioic, linoleic, 10-hydroxydecanoic acid 3-hydroxy-decanoic acids, respectively. The propolis, bee bread, and royal jelly have well-balanced saturated, unsaturated, monounsaturated, polyunsaturated, omega-3, and omega-6 fatty acids. Their nutritional quality, including omega-6/omega-3, thrombogenicity, atherogenicity, hypocholesterolemic, nutritional value, and peroxidizability indexes, are similar to egg and soya. Fatty acids inhibit amylase by increasing K_m/V_{max} and decreasing V_{max} and K_m through an un-competition or non-competition strategy. Molecular docking, ultraviolet absorption, and fluorescence quenching analysis reveal that fatty acids interact with amino acid residues of amylase through Van der Waals and hydrogen bonds interactions. Functional fatty acids from bee products can be used in a number of food supplements, food ingredients, and medications to provide carbohydrate-degrading enzymes.

1. Introduction

The increasing shortage of world food and pharmaceutical sources and the growing world population compels us to search for alternative resources such as bee products. Bee propolis, royal jelly, pollen, and bee bread possess nutritional value and health-protecting properties and recommend a superfood for human food and feed. Propolis, bee bread, and royal jelly are applied in the management of different illnesses since they have several biological properties including, antioxidant, anti-fungal, anti-bacterial, antiseptic, anti-cancer, and anti-inflammatory, depending on chemical composition and region of collection. Current research demonstrated the advantageous properties of propolis in the management of obesity, diabetes mellitus, and dyslipidemia¹. Bee propolis is a sticky mixture of wax, resin, protein, fatty acid, polysaccharides, and exudate from the plant's flower produced by mixing with the bee salvia. Commonly, propolis contains resins (50–60%), waxes (30–40%), essential oil (5.0–10%), and other constituents, including proteins, amino acids, free fatty acids, vitamins, and micronutrients, depending on plant sources². Bee bread is a mixture of plant flower pollen, wax, honey, and salivary gland secretion of bees compacted into honeycombs. Bee bread mixture is a perfectly complete food, has high energy, and is a nutrient resource for bee larvae and adult bees³. The components of bee bread encompass proteins and amino acid (8.0–28%), carbohydrates (15–45%), lipids and fatty acids (7.0–15%), vitamins, minerals, and antioxidant compounds such as flavonoids, and phenolic acids, depending on the plant's sources and collationed region⁴.

Royal jelly is a yellowish and creamy emulsion of protein (8.0–22%), amino acid (1.5–3.5%), sugars (7.0–52%, primarily consists of fructose and glucose), lipids (7–33%, short hydroxyl fatty acids, and dicarboxylic acids), and polyphenols, flavonoids, and vitamins⁵. This high-energy emulsion is a valuable food for the

queen in adult and larvae stages and young bee larvae. One of the main characteristics of royal jelly emulsion is hydroxylated fatty acids and dicarboxylic acids. The primary hydroxylated fatty acids in royal jelly are 10-hydroxydecanoic and 10-hydroxy-2-decanoic acids with various biological activities⁶. Proximate and nutrient analysis of bee products plays a crucial role in assessing their nutritional significance. As various bee products are also used as food and medicinal ingredients, evaluating their nutritional effectiveness can help understand the worth of these products. As far as herbal drug standardization is concerned, WHO also emphasizes the need and importance of determining proximate and macronutrients analysis. Soybean⁷ and egg⁸ are significant sources of protein and fatty acid for the human diet, recommended by WHO/FDA.

The fundamental goal of current work is to determine the nutritional properties of bee bread, bee propolis, and royal jelly through examining their proximate composition and comparing them with egg (as an animal resource) and soybean (as a plant resource). The lipid nutritional quality and fatty acid composition of bee bread, bee propolis, and royal jelly were determined and compared with egg and soybean fatty acids. The data matrix of the fatty acid composition and nutritional value in the samples were subjected to principal component analysis to illustrate the apparent discrepancy of distribution of these components among the samples. The researcher examines the anti-amylase activity of fatty acids from bee bread, bee propolis, and royal jelly. Double reciprocal Lineweaver-Burk plots will investigate the mechanism of inhibition of fatty acid on the amylase activity. The interactions between amylase and fatty acids were analyzed using ultraviolet absorption spectroscopy and fluorescence quenching spectroscopy. Furthermore, molecular modeling does use to determine the interaction between fatty acids and amylase.

2. Materials And Methods

2.1. Proximate composition of bee product

Bee products including bee propolis, bee bread, and royal jelly are prepared from beekeepers in Marvdasht region of Fars province (Fars, Iran). The predominant vegetation in this area is wild almond. To evaluate the differential chemical components of bee propolis, bee bread, and royal jelly, Fourier transforms infrared spectroscopy (FT-IR), in the range between 4000 – 400 cm^{-1} , performed utilizing Bruker FTIR spectrophotometer (Germany). The moisture content of bee products determines by drying the samples at 105°C for two h through a vacuum oven (model: BM55E, Fan Azma Gostar, Iran). The bee samples were heated at 550°C for five h in electric ovens (model: FM2P, Fan Azma Gostar, Iran) for ash determination. The phenol-sulfuric acid method applies to assessing total carbohydrate content⁹. According to the Bradford method, total protein content in the bee products evaluates through Coomassie brilliant blue G-250¹⁰. The sulfuric acid-phosphoric acid-vanillin reagent uses to quantify total lipid and fatty acids¹¹. The following formula measured the total energy content of the bee product: Total energy = (4.0 kcal/g $\times$ g protein) + (9.0 kcal/g $\times$ g fat) + (4.0 kcal/g $\times$ g carbohydrate).

Bee products (1.0 g) mix with pepsin (2 mg/100 ml) in 7.5 mM HCl (100 ml). These mixtures incubate at 40°C for 15 h with continuous shaking. The undigested protein content in the samples before and after

pepsin treatment was determined as mentioned above. The ratio of digested protein to the original protein reflects the digestibility level. The protein digestibility level expresses as the digested protein (g) per 100 g of total protein in the raw materials.

2.2. Fatty acid preparation

The bee products biomass wash digested for 48 hours using hydrochloric acid: normal saline: methanol (2:1:1) solution at 60°C. Hexane was applied to the digestion solution and vortexed for 10 minutes to separate fatty acids. The fatty acids in the hexane phase separate from the bottom phase. The fatty acid chemical composition was examined by gas chromatography/mass spectrometry (GC-MS). A rotary evaporator uses to evaporate the hexane phase at ambient temperature. The residual fatty acids dissolve in ethanol for further experiments¹².

2.3. Fatty acid profiling by GC-MS

Fatty acid profile attains by means the gas chromatograph (Agilent 7890B GC 7955A MSD) equipped with a single quadrupole mass spectrometer. The standard column for fatty acid determination was the silica capillary HP-5MS column (30 m × 0.25 mm, 0.25 µm). Helium at a flow rate of 1.0 ml/min applies as carrier gas. Interface and ion source temperatures were 300°C and 250°C, respectively. The oven temperature program was 80–250°C as follow: 80°C for four min, rise at 20°C/min to 140°C, grow at ten °C/min to 250°C, then hold at 250°C for 10 min. The types of fatty acids are determined by comparison the fragmentation patterns of the associated peaks with these suggested in the libraries of Wily and NIST¹³.

2.4. Indices of lipid nutritional quality

The nutritional quality of the bee products measures by calculating the total saturated fatty acids (SFA), total unsaturated fatty acids (UFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), total omega-3, and total omega-6¹⁴. Based on total SFA, total UFA, total MUFA, and total PUFA, we calculate hypocholesterolemic / hypercholesterolemic index (H/H), thrombogenicity index (TI), atherogenicity index (AI), PUFAs/SFAs (P/S), omega-6 PUFAs/omega-3 PUFAs index, nutritive value index (NVI), and peroxidizability index (PI)¹⁵.

2.5. Amylase activity inhibition

To the porcine pancreatic α-amylase (EC: 3.2.1.1, 5.0 U/mg) solution (1.0 Unit/ml), different concentrations (0.3, 0.6, 0.9 mg/ml) of fatty acid and acarbose solutions was added, then incubated at 37°C for 20 minutes. To the amylase-inhibitor, starch solution (5.0 mg/ml in 100 mM phosphate buffer, pH 7) add. The amylase-inhibitor-starch mixture incubates for 20 min at 37°C. By adding 0.02 ml of 1.0 M HCl to the mixture, the amylase reaction stops. To assess undigested starch, iodine solution pours on the amylase reaction. The amylase reaction dilutes with distilled water, and the absorbance of samples is recorded at 580 nm. The activity (velocity) of amylase in the absence and presence of acarbose or fatty acid was calculated via monitoring decomposition of starch per min by measuring light absorbance at 580 nm for 30 min at an interval of 3.0 min. The slope of starch decomposition versus time plot represented the amylase activity and expressed as µg/min. The kinetic parameters of amylase activity were obtained from the Lineweaver-Burk curve that plotted based on 1/V versus 1/[starch] in the absence and presence of fatty acid

or acarbose (0.3, 0.6, 0.9 mg/ml). The kinetic parameters like K_m/V_{max} , K_m , and V_{max} and the type of inhibition attained from the Lineweaver-Burk plot¹².

2.6. Fluorescence and ultraviolet spectroscopy analysis

The interactions between amylase and fatty acid investigated using ultraviolet absorption spectroscopy (UV1280, Shimadzu, Japan) and fluorescence quenching spectroscopy (Varian Cary Eclipse, Agilent, USA)¹⁶. Amylase solution (5.0 ml of 500 $\mu\text{g/ml}$) was prepared in phosphate buffer (100 mM, pH = 7). To the enzymatic solutions, 5.0 ml of different concentrations (0.3, 0.6, 0.9 mg/ml) of acarbose or fatty acid were added. The reaction mixtures incubate at ambient temperature for 10 min. The UV absorption spectrum of the amylase-inhibitor mixture was recorded from 200 to 400 nm using quartz cuvettes at room temperature. The intrinsic fluorescence absorption spectrum of the amylase-inhibitor solutions records at 280 nm for excitation wavelength and 290–500 nm for emission wavelengths.

2.7. Molecular docking

Three-dimensional structure of the amylase downloads from the protein data bank (5U3A, PDB). Three-dimensional structures of fatty acid and acarbose download from the chemspider website. AutoDock vina platform was used to examine interactions between amylase and ligands. The evaluation of the docked positions and amylase-ligand interactions and docking scores (binding free energy) was attained employing PYMOL software¹².

2.8. Statistical analysis

All results show mean values $\pm$ standard deviations. The data were analyzed with a one-way analysis of variance (ANOVA) using SPSS software (SPSS Inc., Chicago, IL, USA). The significant differences between samples were examined by the Tukey test at $P < 0.05$. Minitab software was used to perform the principal component analysis (PCA).

3. Results And Discussions

3.1. Proximate composition

The differential FTIR patterns from bee bread, royal jelly, and bee propolis reflect the different components in these products (Fig. 1). The bands in the $3700 - 3100 \text{ cm}^{-1}$ might connect to stretching vibration of OH groups of water or aromatic compounds. The peaks in the $2900 - 2700 \text{ cm}^{-1}$ may link to the stretching vibration of CH, CH₂, and CH₃ groups of lipid and fatty acids. The bands in $1300 - 900 \text{ cm}^{-1}$ related to the stretching of C = O of amides, C = C of aromatic, N-H of amines, or carboxyl groups in proteins. The bands at $1100 - 500 \text{ cm}^{-1}$ are due to vibration of polysaccharides, including symmetric stretching of C-O-C and OH groups¹⁷.

The chemical composition (moisture content, ash, carbohydrate, fat, and protein and energy values) of bee bread, bee propolis, and royal jelly product were compared with egg and soya and summarized in Table 1. Principal component analysis showed that the sum of the first and second major components accounted

for 94.4% total variance of the changes, with the first major component (PC1) accounting for 65.7% (eigenvalue = 4.60) and the second major component (PC2) for 28.7% (eigenvalue = 2.00) (Fig. 2). The first principal component (PC1) is positively correlated with energy (0.458), ash (0.444), fatty acid (0.351), and protein (0.318). Therefore, increasing energy, ash, fatty acid, and protein values increase the value of the first principal component. PC1 is negatively correlated with digestibility (-0.421) and moisture (-0.414). The second principal component (PC2) is positively correlated with sugar (0.670). Therefore, increasing the values of sugar increase the value of the PC2. PC2 is negatively correlated with digestibility (-0.215), moisture (-0.279), protein (-0.462) and fatty acid (-0.456). Royal jelly in the first quarter is not closely related to the measured parameters. The bee bread and propolis in the second quarter are the most related to sugar and energy. Egg in the 3rd quarter is most associated with digestibility and moisture. In the 4th quarter, soya is most associated with a fatty acid, protein, and ash¹⁸.

Table 1
Proximate analysis of bee propolis, bee bread and royal jelly in comparison with egg and soya.

Chemicals	Bee propolis	Bee bread	Royal jelly	Egg	Soya
Moisture content (g/100g)	15.45 ± 1.14b	11.81 ± 0.90a	25.50 ± 1.90c	72.00 ± 3.0b	9.80 ± 0.75a
Ash (g/100g)	2.76 ± 0.22b	2.11 ± 0.15b	1.46 ± 0.12a	1.10 ± 0.05a	3.37 ± 0.27c
Protein (g/100g)	11.00 ± 0.85a	18.50 ± 1.40b	21.70 ± 1.55b	13.70 ± 1.11a	39.75 ± 1.50c
Fatty acid (g/100g)	13.34 ± 1.16a	11.40 ± 0.84a	12.50 ± 1.00a	11.50 ± 0.87a	33.86 ± 2.22b
Sugar (g/100g)	57.15 ± 3.25d	55.60 ± 3.30d	38.35 ± 2.45c	1.40 ± 0.20a	12.66 ± 1.12b
Energy (kcal/100g)	392.66 ± 14c	399.00 ± 12c	352.70 ± 11b	163.90 ± 6.7a	505.38 ± 15d
Digestibility (g/100g raw material)	5.69 ± 0.45a	11.56 ± 0.85b	15.85 ± 1.20c	11.25 ± 0.75b	21.35 ± 1.55d
Digestibility (g/100g protein)	51.68 ± 2.84a	62.49 ± 3.70b	73.04 ± 4.30c	82.12 ± 4.76d	53.71 ± 3.60a
The values are expressed as means ± SD for three replicate experiments. Mean values with different letters within a row are significantly different by Tukey test at ($p < 0.05$).					

3.2. Fatty acid composition

The fatty acid composition of bee bread, bee propolis, and royal jelly product were stated in Table 2. Linoleic, palmitic, oleic, α -linolenic, behenic, and myristic acids are the primary fatty acids in propolis. Linolenic, palmitic, α -linolenic, myristic oleic, and behenic acids are the primary fatty acids in bee bread. α -linolenic, 2-dodecenedioic, 10-hydroxy-2-decenoic, decanedioic, linoleic, 10-hydroxydecanoic, 3-hydroxy-decanoic, palmitic, 11-hydroxy-dodecanoic, palmitoleic, and oleic acids are primary fatty acids in the royal

jelly (Table 2). The fatty acid composition of fatty acids from bee bread, bee propolis, royal jelly from other geographical regions differs. Fatty acids such as; capric, linoleic, palmitic, stearic, linolenic, oleic, behenic, arachidic, lauric, decanoic, dodecanoic, tetradecanoic, octadecenoic, tetracosanoic, eicosanoic, and hexacosanoic acids, are reported in bee products¹⁹.

Table 2
Fatty acid composition (percent of area) of fatty acid from bee bread, bee propolis, and royal jelly in comparison with egg and soya.

Common Name	Systematic Name	Lipid Numbers	Bee propolis	Bee bread	Royal jelly	Egg	Soya
Levulinic acid	Levulinic acid	C5H8O3	0.00 ± 0.00a	0.75 ± 0.03a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Benzoic acid	Benzoic acid	C7H6O2	0.43 ± 0.02b	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Hyrocinnamic acid	Hyrocinnamic acid	C9H10O2	0.70 ± 0.03b	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Cinnamic acid	Cinnamic acid	C9H8O2	0.43 ± 0.02b	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Anethole	Anethole	C10H12O	2.80 ± 0.15c	0.00 ± 0.00a	0.40 ± 0.01b	0.00 ± 0.00a	0.00 ± 0.00a
Octadecane	Octadecane	C18H38	3.65 ± 0.17c	0.00 ± 0.00a	1.30 ± 0.05b	0.00 ± 0.00a	0.00 ± 0.00a
Pentacosane	Pentacosane	C25H52	3.46 ± 0.18b	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Heptadecane	Heptadecane	C27H56	3.10 ± 0.14b	3.19 ± 0.17b	0.50 ± 0.02a	0.00 ± 0.00a	0.00 ± 0.00a
Queen bee acid	10-hydroxy-2-decenoic acid	C10:1	0.00 ± 0.00a	0.00 ± 0.00a	19.50 ± 1.70b	0.00 ± 0.00a	0.00 ± 0.00a
Sebacic acid	decanedioic acid	C10:0	0.00 ± 0.00a	0.00 ± 0.00a	16.60 ± 1.45b	0.00 ± 0.00a	0.00 ± 0.00a
10-Hydroxydecanoic acid	10-hydroxydecanoic acid	C10:0	0.00 ± 0.00a	0.00 ± 0.00a	8.80 ± 0.73b	0.00 ± 0.00a	0.00 ± 0.00a
3-hydroxy-decanoic acid	3-hydroxy-decanoic acid	C10:0	0.00 ± 0.00a	0.00 ± 0.00a	7.50 ± 0.65b	0.00 ± 0.00a	0.00 ± 0.00a
2- dodecenedioic acid	2- dodecenedioic acid	C12:1	0.00 ± 0.00a	0.00 ± 0.00a	19.90 ± 1.65b	0.00 ± 0.00a	0.00 ± 0.00a

Common Name	Systematic Name	Lipid Numbers	Bee propolis	Bee bread	Royal jelly	Egg	Soya
11-hydroxy-dodecanoic acid	11-hydroxy-dodecanoic acid	C12:0	0.00 ± 0.00a	0.00 ± 0.00a	2.40 ± 0.13b	0.00 ± 0.00a	0.00 ± 0.00a
Lauric acid	Dodecanoic acid	C12:0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Myristic acid	Tetradecanoic acid	C14:0	1.89 ± 0.008c	13.43 ± 0d	0.00 ± 0.00a	0.47 ± 0.03b	0.30 ± 0.01b
Myristoleic acid	9-tetradecenoic acid	C14:1n-5	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.15 ± 0.02b	0.12 ± 0.01b
Pentadecanoic acid	Pentadecanoic acid	C15:0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.15 ± 0.02b	0.00 ± 0.00a
Palmitic acid	Hexadecanoic acid	C16:0	33.50 ± 2.00d	20.31 ± 1.30c	5.00 ± 0.23a	31.37 ± 2.35d	11.36 ± 1.00b
Palmitoleic acid	9-hexadecenoic acid	C16:1n-7	0.00 ± 0.00a	0.00 ± 0.00a	1.60 ± 0.07c	3.55 ± 0.25d	0.23 ± 0.02b
Hexadecadienoic acid	7,10-hexadecadienoic acid	C16:2n-6	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Hexadecatrienoic acid	7,10,13-hexadecatrienoic acid	C16:3n-3	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Margaric acid	heptadecanoic acid	C17:0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.14 ± 0.03b	0.37 ± 0.06c
Heptadecanoic acid	10-heptadecenoic acid	C17:1n-7	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.16 ± 0.02b	0.08 ± 0.001a
Stearic acid	octadecanoic acid	C18:0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	11.00 ± 1.13c	3.77 ± 0.77b
Oleic acid	9-Octadecenoic acid	C18:1n-9	27.27 ± 1.450d	10.89 ± 0.94b	1.55 ± 0.03a	38.10 ± 1.75e	20.88 ± 1.56c
Linoleic acid	9,12-octadecadienoic acid	C18:2n-6	11.04 ± 0.97b	24.04 ± 1.40c	4.45 ± 0.56a	9.53 ± 0.65b	52.70 ± 2.35d

Common Name	Systematic Name	Lipid Numbers	Bee propolis	Bee bread	Royal jelly	Egg	Soya
α -Linolenic acid	9,12,15-octadecatrienoic acid	C18:3n-3	8.03 $\pm$ 0.54b	24.40 $\pm$ 1.30c	8.55 $\pm$ 0.55b	0.03 $\pm$ 0.001a	8.22 $\pm$ 0.37b
γ -Linolenic acid	6,9,12-octadecatrienoic acid	C18:3n-6	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	1.23 $\pm$ 0.05b	0.03 $\pm$ 0.01a	0.04 $\pm$ 0.01a
Arachidic acid	Eicosanoic acid	C20:0	0.00 $\pm$ 0.00a	0.02 $\pm$ 0.01a	0.00 $\pm$ 0.00a	0.07 $\pm$ 0.02a	0.04 $\pm$ 0.02a
Paullinic acid	13-Eicosenoic acid	C20:1n-7	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.47 $\pm$ 0.03a	0.00 $\pm$ 0.00a
Gondoic acid	11-eicosenoic acid	C20:1n-9	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.03 $\pm$ 0.01a	0.00 $\pm$ 0.00a
Eicosatrienoic acid	11,14,17-eicosatrienoic acid	C20:3n-3	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.07 $\pm$ 0.01a	0.02 $\pm$ 0.01a
Dihomo- γ -linolenic acid	8,11,14-Eicosatrienoic acid	C20:3n-6	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.07 $\pm$ 0.01a	0.05 $\pm$ 0.01a
Arachidonic acid	5,8,11,14-eicosatetraenoic acid	C20:4n-6	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	1.10 $\pm$ 0.04c	0.26 $\pm$ 0.02b
Eicosapentaenoic acid	5,8,11,14,17-Eicosapentaenoic acid	C20:5n-3	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.28 $\pm$ 0.02b	0.32 $\pm$ 0.02b
Behenic acid	Docosanoic acid	C22:0	3.09 $\pm$ 0.17b	2.18 $\pm$ 0.11b	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
Dosapentaenoic acid	4,7,10,13,16-docosapentaenoic acid	C22:5n-6	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.27 $\pm$ 0.012b	0.39 $\pm$ 0.02b
Cervonic acid	4,7,10,13,16,19-docosahexaenoic acid	C22:6n-3	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	2.13 $\pm$ 0.13b	0.00 $\pm$ 0.00a
Total FA	Total FA	Total FA	99.39 $\pm$ 2.80a	99.21 $\pm$ 3.0a	99.28 $\pm$ 2.65a	99.20 $\pm$ 2.0a	99.25 $\pm$ 2.70a
The values are expressed as means (standard deviation) of three replicates. Mean values with different letters within a row are significantly different by Tukey test at (p < 0.05).							

Principal component analysis showed that the sum of the first and second major components accounted for 73.3% total variance of the changes, with the first major component (PC1) accounting for 45.7% (eigenvalue = 16.43) and the second major component (PC2) for 27.6% (eigenvalue = 9.93) (Fig. 3). The first principal component (PC1) is positively correlated with C14:1n5 (0.239), C15:0 (0.210), C16:1n7 (0.174), C17:0 (0.153), C17:1n7 (0.245), C18:0 (0.241), C18:1n9 (0.179), C20:0 (0.242), C20:1n7 (0.210), C20:1n9 (0.210), C20:3n3 (0.238), C20:3n6 (0.242), C20:4n6 (0.234), C20:5n3 (0.220), C22:5n6 (0.203), and C22:6n3 (0.210). PC1 is negatively correlated with benzoic acid (-0.105), hydrocinnamic acid (-0.105), cinnamic acid (-0.105), anethole (-0.123), octadecane (-0.146), pentacosane (0.105), heptadecane (-0.165), 10-hydroxy-2-decenoic acid (-0.103), decanedioic acid (-0.103), 10-hydroxydecanoic acid (-0.103), 3-hydroxy-decanoic acid (-0.103), 2-dodecenedioic acid (-0.103), 11-hydroxy-dodecanoic acid (-0.103), α -Linolenic acid (-0.146), and docosanoic acid (-0.146). The second principal component (PC2) is positively correlated with 10-hydroxy-2-decenoic acid (0.269), decanedioic acid (0.269), 10-hydroxydecanoic acid (0.269), 3-hydroxy-decanoic acid (0.269), 2-dodecenedioic acid (0.269), and 2-dodecenedioic acid (0.269). PC2 is negatively correlated with benzoic acid (-0.223), hydrocinnamic acid (-0.223), cinnamic acid (-0.223), anethole (-0.190), octadecane (-0.131), pentacosane (-0.223), heptadecane (-0.201), palmitic acid (-0.262), oleic acid (-0.194), and docosanoic acid (-0.246). Royal jelly in the first quarter is closely related to 2-dodecenedioic acid, 10-hydroxy-2-decenoic acid, decanedioic acid, 10-hydroxydecanoic acid, 3-hydroxy-decanoic acid, and 6,9,12-octadecatrienoic acid. The soya in the second quarter is the most related to linoleic acid, oleic acid and palmitoleic acid. Bee bread and bee propolis in the 3rd quarter are extremely associated with 9,12,15-octadecatrienoic acid, tetradecanoic acid, heptadecane, levulinic acid, hexadecanoic acid, octadecane, pentacosane, heptadecane, and docosanoic acid. In the 4th quarter, the whole egg is most associated with 9-octadecenoic acid, hexadecanoic acid, octadecanoic acid, 9,12-octadecadienoic acid, 9-hexadecenoic acid, 4,7,10,13,16,19-docosahexaenoic acid, and 5,8,11,14-eicosatetraenoic acid. Hydroxylated acids (like 10-hydroxy-2-decenoic, 10-hydroxydecanoic, and 3-hydroxydecanoic acids) and dicarboxylic fatty acids (like decanedioic and 2-dodecenedioic acids) are only found in royal jelly¹⁷. Myristic acid is only found in bee bread. Stearic acid finds in eggs and soya but not in bee products. Linoleic, palmitic, linolenic, and oleic acids are found in bee bread, bee propolis, royal jelly, egg, and soya but in different quantities¹⁸.

3.3. Lipid nutritional quality

The lipid nutritional quality of bee products including UFA, PUFA, omega-3 PUFA, SFA, omega-6 PUFA, MUFA, omega-7, omega-9, H/H, PUFA/SFA, AI, TI, NVI, and PI are stated in Table 3. Principal component analysis showed that the sum of the first and second major components accounted for 80.9% total variance of the changes, with the first major component (PC1) accounting for 50.0% (eigenvalue = 8.00) and the second major component (PC2) for 30.9% (eigenvalue = 4.94) (Fig. 4). The first principal component (PC1) is positively correlated with UFA (0.340), PUFA (0.325), Omega-6 (0.349), Omega-5 (0.135), Omega-6/Omega-3 (0.244), PUFA/SFA (0.352), H/H (0.325), PI (0.293), and NVI (0.241). PC1 is negatively correlated with SFA (-0.326), MUFA (-0.188), Omega-7 (-0.140), AI (-0.109), and TI (-0.168). The second principal component (PC2) is positively correlated with PUFA (0.138), Omega-3 (0.398), AI (0.216), PI (0.127). PC2 is negatively correlated with MUFA (-0.292), Omega-5 (-0.392), Omega-7 (-0.350), Omega-9 (-0.334), Omega-6/Omega-3 (-0.318), TI (-0.283), NVI (-0.309). Propolis and royal jelly in the first quarter is closely related to the AI. The bee bread in the second quarter is the most related to omega-3, PI, omega-6 and PUFA/SFA. Egg in the 3rd

quarter is most associated with TI, MUFA, omega-9 omega-7. In the 4th quarter, soya is most associated with omega-5, NVI, omega-6/omega-3, UFA, H/H and PUFA/SFA¹⁷.

Table 3

Lipid nutritional quality of fatty acid from bee bread, bee propolis, and royal jelly in comparison with egg and soya.

Common Name	Bee propolis	Bee bread	Royal jelly	Egg	Soya
Saturated fatty acid (SFA)	38.48 ± 2.25d	35.94 ± 1.10cd	32.80 ± 1.85b	43.20 ± 2.50e	15.921 ± 0.90a
Unsaturated fatty acid (UFA)	46.34 ± 2.70a	59.33 ± 3.40b	56.78 ± 3.17b	55.99 ± 3.05b	83.33 ± 4.25c
Monounsaturated fatty acid (MUFA)	27.27 ± 1.70c	10.89 ± 0.75a	42.55 ± 2.57d	42.47 ± 2.40d	21.32 ± 1.24b
Polyunsaturated fatty acid (PUFA)	19.07 ± 1.20	48.44 ± 2.70	14.23 ± 0.85	13.52 ± 0.75	62.00 ± 3.50
Omrg-3 PUFA	8.03 ± 0.55b	24.40 ± 1.35c	8.55 ± 0.54b	2.51 ± 0.16a	8.56 ± 0.53b
Omega-6 PUFA	11.04 ± 0.77b	24.04 ± 1.40c	5.68 ± 0.45a	11.00 ± 0.70b	53.44 ± 3.00d
Omega-5	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.15 ± 0.01b	0.12 ± 0.01b
Omega-7	0.00 ± 0.00a	0.00 ± 0.00a	1.60 ± 0.07c	4.19 ± 0.27d	0.32 ± 0.02b
Omega-9	27.27 ± 1.75d	10.89 ± 0.76b	1.55 ± 0.08a	38.13 ± 2.25e	20.88 ± 1.35c
Omega-6/Omega-3	1.37 ± 0.08b	0.95 ± 0.06a	0.66 ± 0.05a	4.37 ± 0.26c	6.24 ± 0.40d
PUFA/SFA	0.49 ± 0.03a	1.35 ± 0.07b	0.43 ± 0.03a	0.31 ± 0.02a	3.89 ± 0.20c
Atherogenicity index (AI)	0.88 ± 0.04a	1.25 ± 0.05b	0.09 ± 0.01a	0.59 ± 0.03a	0.15 ± 0.01a
Thrombogenicity index (TI)	0.80 ± 0.05b	0.37 ± 0.02a	0.10 ± 0.01a	1.24 ± 0.07b	0.24 ± 0.01a
Hypocholesterolemic/hypercholesterolemic fatty acids (H/H)	1.30 ± 0.08a	1.76 ± 0.10a	2.91 ± 0.16a	1.49 ± 0.08a	7.02 ± 0.35b
Peroxidizability index (PI)	27.80 ± 1.70a	73.12 ± 4.0c	25.10 ± 1.50a	35.80 ± 2.00b	75.20 ± 4.40c
Nutritive value index (NVI)	0.81 ± 0.05b	0.54 ± 0.03a	0.31 ± 0.02a	1.56 ± 0.09b	2.17 ± 0.14c
The values are expressed as means (standard deviation) of three replicates. Mean values with different letters within a row are significantly different by Tukey test at (p < 0.05).					

The high omega-3 diet causes a decrease in triglycerides, increases mitochondrial biogenesis, prevents inflammation, and restores insulin sensitivity. Besides, an omega-3 rich diet reduces chronic diseases such

as obesity, diabetes, heart disease, increases blood vessels' elasticity, and prevents lipid plaques in the arteries²⁰. Prostaglandin E3, thromboxane A3, prostacyclin I3, and leukotriene B5 generated from the omega-3 fatty acids can suppress thrombosis and inflammation. On the other hand, prostaglandin E2, prostacyclin I2, thromboxane A2, and leukotriene B4 are produced from omega-6 fatty acids, leading to pro-inflammatory and prothrombotic with the enhancement in blood viscosity²¹. The proportion of omega-6 / omega-3 is vital for lowering the risk of chronic diseases and preventing and managing obesity disorders. The ratio of omega-6/omega-3 lower than 3.0 is significant for healthy food. Bee products are important as sources of functional fatty acids with a well-proportioned omega-6 to omega-3, which is a priority for dietary supplements.

The H/H values describe the impacts of specific fatty acids on cholesterol level and metabolism. The higher proportion of H/H directly relates to the higher content of PUFA, which is beneficial for human healthiness. The P/S ratio is an index that expresses the lipid's nutritional quality in a specific diet. The high ratios of P/S consider undesirable for foodstuffs due to their potentials in inducing high blood cholesterol levels. The favorable ratio of PUFA/SFA that reflects the lipid nutritional quality of a specific diet is the range of 1–2 that reduce blood cholesterol and the risk of cardiovascular diseases¹⁵.

Low AI values indicate the higher protective of foodstuffs potentials in preventing heart and coronary diseases and general and abdominal obesities and AI values. Moreover, there is a direct relationship between gestational diabetes mellitus and AI in pregnant women¹⁴. Myristic acid and palmitic acid consider as the most atherogenic agents. Stearic acid is a thrombogenic fatty acid²². Foodstuffs with low TI values have a high potential to protect against coronary and heart diseases, reduce general and abdominal obesities, and prevent gestational diabetes mellitus in pregnant women. The AI and TI values of bee products are somewhat lower or similar to the AI values of egg and soya⁸.

The highest NVI values belong to soya, followed by egg and then bee propolis, bee bread, and royal jelly (Table 3). The NVI values of bee products range between 0.310 to 0.814, which is lower than an egg (1.565) and soya (2.172) (Table 3). The higher NVI values in the soya and egg attribute to the higher proportion of stearic and oleic acids and the lower levels of palmitic acid⁸. The lowest PI value belongs to royal jelly, followed by bee propolis, egg, and bee bread, then soya oil. Royal jelly and bee propolis had a PI value similar to egg, while the PI value of bee bread was identical to soya (Table 3). The higher PI value of soya and bee bread can be related to higher PUFA and MUFA in the soya and bee bread oil (Table 3). The PI represents the susceptibility of fatty acid to oxidation, and this index signifies the stability of PUFA against oxidation processes. The favorable ratio of PI that reflects the lipid nutritional quality of a specific diet is the range of 70–90 that reduces blood cholesterol and the risk of cardiovascular diseases. All bee products in this work have PI values lower than 90. Big PI values lead to a higher level of fatty acids oxidation. However, great PI values owing to the high content of omega-3 and omega-6 lead to more protective effects against oxidation and inflammation²³.

3.4. Inhibition of the α -amylase activity

Bee propolis, bee bread, and royal jelly inhibit amylase with a lower level than acarbose. Kinetic parameters of amylase in the presence of acarbose and bee products measure through kinetic analysis. Figure 5a demonstrates plots of amylase activity at varying concentrations of acarbose. According to the Lineweaver-Burk plot, acarbose inhibits with a competitive inhibition trend. Since V_{max} of amylase remains constant, in contrast, the K_m/V_{max} and K_m increase (Table 4). Acarbose is a competitive inhibitor of amylase activity, and as the concentration of acarbose increases, the V_{max} values do not change, but the K_m values increase (Fig. 5A). Fatty acid from bee bread (Fig. 5B), royal jelly (Fig. 5C) and, bee propolis (Fig. 5D), on the other hand, are mixed un-competitive or non-competitive inhibitors. The K_m/V_{max} increases while K_m and V_{max} value decrease. By binding fatty acid to the allosteric site of the enzyme, this rises in the K_m/V_{max} ratio links to the reduction in the V_{max} and functional enzyme²⁴. The active compounds in fatty acid attach to allosteric sites, avoiding starch degradation. The enzyme conformation varies when inhibitors bind to the enzyme, and the affinity of the enzyme active site for starch reduces²⁵.

Table 4

The values of kinetic parameters (K_m/V_{max} , K_m , V_{max}) of α -amylase in response to acarbose bee bread, bee propolis and royal jelly fatty acid extract.

α					
Samples	Parameters	0.00 mg/ml	0.30 mg/ml	0.60 mg/ml	0.90 mg/ml
Acarbose	K_m/V_{max}	$4.78 \pm 0.25a$	$9.07 \pm 0.52b$	$10.04 \pm 0.57b$	$13.12 \pm 0.70c$
Acarbose	V_{max}	$0.73 \pm 0.04a$	$0.74 \pm 0.05a$	$0.70 \pm 0.04a$	$0.68 \pm 0.03a$
Acarbose	K_m	$3.47 \pm 0.20a$	$6.74 \pm 0.35b$	$7.06 \pm 0.40b$	$8.96 \pm 0.45c$
Bee bread	K_m/V_{max}	$5.05 \pm 0.25a$	$7.52 \pm 0.40b$	$8.39 \pm 0.44c$	$9.40 \pm 0.50d$
Bee bread	V_{max}	$0.73 \pm 0.04c$	$0.47 \pm 0.03b$	$0.39 \pm 0.02a$	$0.31 \pm 0.02a$
Bee bread	K_m	$3.84 \pm 0.21c$	$3.60 \pm 0.20bc$	$3.29 \pm 0.18ab$	$2.96 \pm 0.15a$
Bee propolis	K_m/V_{max}	$4.69 \pm 0.25a$	$5.79 \pm 0.32b$	$6.78 \pm 0.35c$	$7.68 \pm 0.42d$
Bee propolis	V_{max}	$0.93 \pm 0.05d$	$0.70 \pm 0.04c$	$0.48 \pm 0.03b$	$0.36 \pm 0.02a$
Bee propolis	K_m	$4.38 \pm 0.25b$	$4.05 \pm 0.22b$	$3.28 \pm 0.17a$	$2.78 \pm 0.15a$
Royal jelly	K_m/V_{max}	$4.21 \pm 0.23a$	$6.71 \pm 0.35b$	$7.97 \pm 0.45c$	$9.36 \pm 0.50d$
Royal jelly	V_{max}	$0.81 \pm 0.04c$	$0.48 \pm 0.03b$	$0.36 \pm 0.02ab$	$0.28 \pm 0.02a$
Royal jelly	K_m	$3.43 \pm 0.20c$	$3.23 \pm 0.17bc$	$2.91 \pm 0.15ab$	$2.63 \pm 0.14a$
The values are expressed as means (standard deviation) of three replicates. Mean values with different letters within a row are significantly different by Tukey test at ($p < 0.05$).					

3.5. Ultraviolet and fluorescence spectroscopic analysis

Ultraviolet-visible absorption is one of the most effective methods for monitoring enzyme conformation changes during inhibitor binding²⁶. This study examines the absorption spectra of amylase in the presence

of acarbose and fatty acids from bee products (Fig. 6). The ultraviolet absorption peak around 210–220 nm relates to the carbonyl group $\pi\pi$ transition of the peptide bond. The ultraviolet absorption peak around 255–280 nm attributes to the $\pi\pi$ shift of aromatic groups in the protein sequence like tryptophan, tyrosine, and phenylalanine¹⁶. Fatty acid and acarbose at these wavelengths did not have ultraviolet absorption. By increasing the concentration of acarbose and fatty acid, the ultraviolet absorption of amylase regularly increases. Accordingly, ligands such as fatty acid and acarbose can establish complexes with amylase, alter their conformation and expose the aromatic group to ultraviolet light in this manner, increasing the $\pi\pi$ transition of aromatic groups²⁷.

Another effective technique to monitoring the interactions between amylase and inhibitors such as acarbose and fatty acid is fluorescence quenching analysis (Fig. 7). The fluorescence emission spectra of the enzyme contribute to aromatic amino acids such as tyrosine, tryptophan, and phenylalanine²⁸. Acarbose and fatty acid did not have a fluorescence emission at this condition. The amylase exhibits a fluorescence emission peak at 360 nm after excitation at 280 nm. By increasing the concentrations of acarbose and fatty acid, the fluorescence emission of amylase declines.

Furthermore, the fluorescence intensity slightly shifts toward the blue region (Fig. 7). The fluorescence quenching and shifting of amylase directly confirm the interaction between the enzyme and its inhibitors that reflect the changes in the structural architecture, conformation, surrounding environment, and polarity of the enzyme²⁶. The molecular interactions between fatty acid or acarbose with amylase generate a non-fluorescent amylase-inhibitor complex and change the enzyme architecture and microenvironment. These effects increase the collision between fluorescent groups in the enzymes (aromatic amino acid) and quenching agents (inhibitors), leading to a decrease in the intrinsic fluorescence intensity²⁸.

3.6. Molecular docking simulation of amylase

Molecular docking is a technique for predicting the binding direction of small molecules (ligands) to their protein targets, as well as their affinity. Acarbose is the competitive inhibitor of amylase, and the results of docking reveal that the interaction of acarbose with amylase occur mainly through Van der Waals interactions (with Ala106, Ala198, Arg195, Asn105, Asp197, Gly104, His101, His299, Ile235, Ile51, Leu162, Leu165, Trp58, Trp59, Tyr62, and Val107) and then hydrogen binding (with Gln63, Glu233, and Thr163 of amylase) (Table S1, Figure S1-S4 in supplemental file). The related affinity of amylase for acarbose was – 8.2 kcal/mol (Table S1, Figure S1-S4 in supplemental file). Similarly, molecular docking results revealed that the interactions of fatty with amylase occurred mainly through Van der Waals interactions (Ala198, Arg10, Arg195, Arg252, Arg303, Arg398, Asn298, Asp197, Asp300, Asp356, Gln63, Gln7, Gln8, Glu233, Gly403, Gly9, His101, His201, His299, His305, Ile235, Leu162, Leu163, Leu165, Lys200, Phe335, Pro332, Pro4, Thr11, Thr163, Thr6, Trp357, Trp58, Trp59, Tyr151, Tyr62) and then hydrogen binding (with Arg421, Asp197, Asp300, Asp356, Asp402, Gln63, Glu233, Gly334, His201, Thr163, Trp59) (Table S1, Figure S1-S4 in supplemental file). But the binding pocket for fatty acid differs from the binding pocket of acarbose. The related affinity of fatty acids for acarbose ranges from – 5.8 to 4.8 kcal/mol, lower than the affinity of amylase for acarbose (Table S1, Figure S1-S4 in supplemental file). The highest binding affinity related to the γ -linolenic acid and then 3-Hydroxy-decanoic acid. The fatty acid interacts with a binding pocket other

than the active site or binding pocket near the active site. It inhibits amylase activity via an un-competitive or non-competitive inhibition strategy.

4. Conclusions

In summary, the differential FTIR patterns of bee bread, royal jelly, and bee propolis reflect the variations in proximate compositions and discrepancy in nutritional properties of bee bread, bee propolis, and royal jelly. The fat of bee propolis, bee bread, and royal jelly, to some extent, is identical and equal to egg fat and lower than soya. The protein of royal jelly and bee bread was higher than egg, while the propolis had lower protein than an egg. Bee propolis had the most elevated sugar, followed by bee bread and royal jelly and then soy and egg. The highest energy was found in the soya, followed by bee bread, bee propolis, royal jelly, and then egg. The higher energy of soya may be related to the higher level of lipid and protein contents. The primary fatty acid in bee propolis and bee bread were palmitic, oleic, linolenic, linoleic, behenic, and myristic acids. The primary fatty acid in royal jelly was 2-dodecenedioic, 10-hydroxy-2-decenoic, decanedioic, 10-hydroxydecanoic, linolenic, 3-hydroxy-decanoic, palmitic, linoleic, 11-hydroxy-dodecanoic, oleic, palmitoleic, and γ -linolenic acids. The bee products have a well-balanced omega-6 to omega-3 ratio, thrombogenicity index, atherogenicity index, PUSA/SFA, and hypocholesterolemic/hypercholesterolemic index. Besides, kinetic analyses and molecular docking reveals that fatty acids inhibit amylase activity through non-competition or non-competition strategy. Fatty acid similar to acarbose increases ultraviolet absorption and quenches the intrinsic fluorescence intensity of amylase by forming a complex with the enzymes. In conclusion, bee products can consider as purposeful sustenance with nutritive values like egg and soya, and valuable fatty acids are essential as the non-competitive and un-competitive inhibitor for amylase intended for diabetes management.

Declarations

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Author contributions

Gholamreza Kavooosi, Masoomah Fallah, and Fatemeh Najafi conceived and designed research, conducted experiments, contributed reagents and analytical tools, and wrote the manuscript.

Conflicts of interest

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

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Figures

Figure 1

Fourier transform infrared spectrum of bee bread, royal jelly and bee propolis. The differential FTIR patterns of bee bread, royal jelly and bee propolis reflect the different components in these products.

Figure 2

Principal Component Analysis (PCA) biplot illustrating the relationships among the biochemical composition of bee bread, bee propolis, royal jelly, egg and soyabean. For the abbreviation of the analyzed samples and the compounds see Table 1.

Figure 3

Principal Component Analysis (PCA) biplot illustrating the relationships among the fatty acid composition of bee bread (BB), bee propolis (BP), royal jelly (RJ), egg and soyabean. For the abbreviation of the analyzed samples and the compounds see Table 2.

Figure 4

Principal Component Analysis (PCA) biplot illustrating the relationships among the fatty acid nutritional quality of bee bread, bee propolis, royal jelly, egg and soyabean. For the abbreviation of the analyzed samples and the compounds see Table 3.

Figure 5

Double reciprocal Lineweaver-Burk plot of α -amylase activity in the absence and presence of varying concentrations of acarbose (A) and fatty acid from bee bread (B), royal jelly (C) and propolis (D).

Figure 6

Ultraviolet spectra of amylase in the absence and presence of varying concentrations of acarbose (A) and fatty acid from bee bread (B), royal jelly (C) and propolis (D).

Figure 7

Fluorescence intensity of amylase in the absence and presence of varying concentrations of acarbose (A) and fatty acid from bee bread (B), royal jelly (C) and propolis (D).

Supplementary Files

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