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Fatty acid profiles of propolis and bee bread: effects on the shelf life of foods

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ABSTRACT: In recent years, increasing nutritional awareness has led to a growing demand for natural foods, with consumers preferring products containing natural ingredients over synthetic additives. This trend has accelerated the search for natural alternatives to synthetic preservatives in the food industry. Among natural additives, bee-derived products have attracted considerable attention due to their rich chemical composition and well-documented health-promoting properties. Although synthetic additives are widely used because of their cost-effectiveness, concerns regarding their potential adverse effects have intensified interest in natural preservation strategies. Propolis and bee bread (perga) are particularly promising in the food sector owing to their potential to extend shelf life. In this study, fatty acid methyl ester (FAME) analysis of the ethanol extracts of propolis and bee bread revealed the presence of 10 different fatty acids in propolis and 20 in bee bread. The dominant fatty acids were identified as oleic, linoleic, and linolenic acids. Furthermore, molecular docking analyses were performed to calculate the binding energies of these fatty acids to the tyrosinase enzyme (PDB ID: 5M8Q), which is associated with food spoilage reactions. The observed inhibitory interactions between fatty acids and tyrosinase suggest that propolis and bee bread may serve as effective natural alternatives to synthetic preservatives. Overall, the findings indicate that bee products hold strong potential for use in the natural preservation of food systems.

KEYWORDS: Propolis; Bee bread; Fatty acid; Docking; Tyrosinase.

INTRODUCTION

Although synthetic substances used to prevent food spoilage are extremely cheap, the fact that these substances have undesirable side effects has made the search for natural alternatives increasingly important [1]. Among natural substances, bee products obtained from bees (such as propolis, bee bread, pollen, etc.) are also found [2]. Edible coatings and films obtained from natural materials are among the commonly used methods to extend the shelf life of foods [3]. Propolis is a bee product with antiviral, antimicrobial, and antifungal properties, produced by *Apis mellifera* from the buds of plants, and from the resins and waxy substances collected from tree cones and bark [4]. The composition of propolis includes resins, beeswax, fatty acids, essential oils, pollen, and mineral substances [5]. Various studies have demonstrated the antibacterial and shelf life–extending effects of propolis in food technology [6]. The bee product that is fermented by lactic acid bacteria, enriched with secretions from the salivary glands of honeybees, and stored in honeycomb cells is called bee bread (*perga*) [7,8]. *Perga* is a bee product fermented by bee enzymes and is rich in proteins, amino acids, carbohydrates, phenolic acids, polyphenols, omega-3 and essential fatty acids, vitamins, and minerals [9]. Tyrosinase is an enzyme found in animals, fungi, and higher plants that uses molecular oxygen to oxidize monophenols to o-diphenols and o-diphenols to o-quinones [10]. Tyrosinase plays a significant role in food technology, as it contributes to plants' resistance against microbial and viral infections and enhances their tolerance to unfavorable climatic conditions [11]. In a study examining Argentine propolis, it was reported that propolis is a natural product with the potential to protect crops and extend the shelf life of foods. Therefore, it has a high potential for use in innovative products aimed at improving health, food preservation, and packaging [12]. Studies have shown that propolis delays spoilage in meat and meat products and prevents deterioration in milk and dairy products by reducing microbial activity. In particular, experiments conducted on hamburger patties made from beef showed no spoilage in

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frozen patties stored for three months. In addition, propolis has been found to have inhibitory effects against six yeast species that cause spoilage in pasteurized fruit juices [6]. In the food industry, interest in natural preservative components has been steadily increasing in parallel with consumers' tendency to avoid artificial additives. Enzymatic browning represents a major challenge in extending the shelf life of foods and preventing quality loss. This undesirable reaction, commonly observed in fruits and vegetables, occurs as a result of a series of oxidation processes catalyzed by the tyrosinase enzyme. Therefore, the inhibition of tyrosinase activity using natural compounds has become increasingly important in the food industry. Propolis and bee bread (*perga*), which contain biologically active compounds, are among the bee products that exhibit antimicrobial, antioxidant, and enzyme-inhibitory properties. In this study, the inhibition potential of oleic, linoleic, and linolenic acids found in propolis and *perga* against the tyrosinase enzyme was evaluated using the molecular docking method, and the potential use of these compounds as natural agents to prevent enzymatic browning in foods was investigated.

MATERIALS AND METHODS

Propolis and Perga Extraction

Propolis and *perga* samples obtained in 2022 from local beekeepers in the Karlıova district of Bingöl Province, collected from the same hives during the same season, were ground and stored at −18 °C. The ground samples were mixed with n-hexane at a ratio of 1:10 (W/V) and subjected to extraction at 40 °C for 48 hours using a magnetic stirrer operating at 150 rpm. At the end of the extraction period, the mixture was filtered through filter paper, and n-hexane was removed using a rotary evaporator, yielding the crude extracts [13].

GC-MS (Gas Chromatography-Mass Spectrometry) Fatty Acid Composition

From the hexane fraction obtained from propolis and perga samples, 0,1 g was taken and mixed with 10 mL of hexane by vortexing. Subsequently, 0,5 mL of 2N methanolic KOH was added, and the mixture was vortexed again, then left to stand for approximately 2 hours until the upper phase became clear. The clarified upper phase was transferred into amber-colored vials suitable for GC analysis, and the fatty acid profile of the samples was determined using this method. Fatty acid methyl ester (FAME) analysis was performed using a GC-MS system (7890A-Agilent 5975C). The MS and FID detectors were operated simultaneously. The injection volume was 1 µL, and the analysis was carried out in splitless mode. A BPx90 capillary column (100 m length, 0,25 mm internal diameter) was used. The column temperature was initially set at 120 °C and increased to 252 °C at a rate of 3 °C/min. The injector temperature started at 150 °C and was raised to 250 °C at a rate of 120 °C/min. The detector temperature was maintained at 260 °C. Helium was used as the carrier gas. The FAME analysis of the samples was performed by injecting the Supelco FAME Mix standard, and the retention times of each fatty acid were determined accordingly [14].

Molecular Docking

AutoDock Vina 1.5.4 was used as the primary software for docking calculations, while the RCSB Protein Data Bank and PubChem were utilized as auxiliary tools for protein and ligand selection, respectively. The structure of the target enzyme, tyrosinase (PDB ID: 5M8Q), was obtained from the Protein Data Bank along with its bound ligand information. To position the desired ligand in place of the existing one, the coordinates (x, y, z) of the binding site were determined based on the active site of the enzyme. For the three-dimensional structure of the enzyme alone, the codes corresponding to the bound ligands were removed, rendering the enzyme ready for binding with other ligands. The skeletal structures of the bioactive compounds selected as ligands were then introduced into the enzyme's binding pocket, and their binding energies were calculated [15]. Tyrosinase (5M8Q) was chosen as the target enzyme, while eicosatrienoic, oleic, linoleic, and linolenic acids were selected as ligands.

RESULTS AND DISCUSSION

Fatty Acid Determination by GC-MS

Fatty Acid Determination of Propolis by GC-MS

In the GC/MS analysis of propolis samples collected from two different regions of Bulgaria, 15 phenolic acids (7 of which were reported for the first time in propolis), 2 ketones, 8 phenolic acid esters (new for propolis), 6 flavanones, and 2 flavonols were identified [16]. In the GC/MS analysis of eucalyptus, poplar, and chestnut propolis samples, compounds such as butanedioic acid, 2-propenoic acid, and cinnamic acid from the organic acid group; 3,4-bis-cinnamate from the esters; and D-fructose and β-D-glucopyranose from the hydrocarbon and ketone group were detected in all three propolis types. However, the quantitative amounts of these compounds differed among the three propolis samples [17]. According to the FAME analysis of the ethanol extract of propolis, a total of 10 fatty acids were identified, including 4 saturated.

Table 1. Fatty acids identified in propolis

Saturated Fatty Acids (%)		Unsaturated Fatty Acids (%)	
C 16:0 (Palmitic acid)	8.0	C 18:1 (cis - 9) (omega 9) Oleic acid	0.27
C 12:0 (Lauric acid)	0.72	C 22:1 Erucic acid	0.02
C 14:0 (Myristic acid)	0.39	9-Octadecenoic acid (Z)-, methyl ester	0.80
		8-Octadecenoic acid, methyl ester, (E)-	0.73

Fatty Acid Determination of Perga by GC-MS

In the study, *perga* is shown to be a rich source of polyunsaturated fatty acids, which cannot be synthesized by the human body and must be obtained from dietary sources [18]. In a study conducted by Kaplan et al. [19], a total of 37 fatty acids 20 saturated and 17 unsaturated were identified in eight *perga* samples of different botanical origins. The unsaturated/saturated fatty acid ratio ranged from 1,38 to 2,39, indicating that *perga* could serve as a good source of unsaturated fatty acids [19]. In another study examining the chemical composition of *perga* samples obtained from different countries, it was found that the levels of unsaturated fatty acids were considerably high [20]. Additionally, another study reported that 37 different fatty acids were identified in the extracted oil from *perga*, with some fatty acids present in higher amounts than others [21]. According to the FAME analysis of the ethanol extract of *perga*, a total of 20 fatty acids were identified, including 9 saturated and 11 unsaturated fatty acids. The fatty acids and their quantities are presented in Table 2.

Table 2. Fatty acids identified in perga

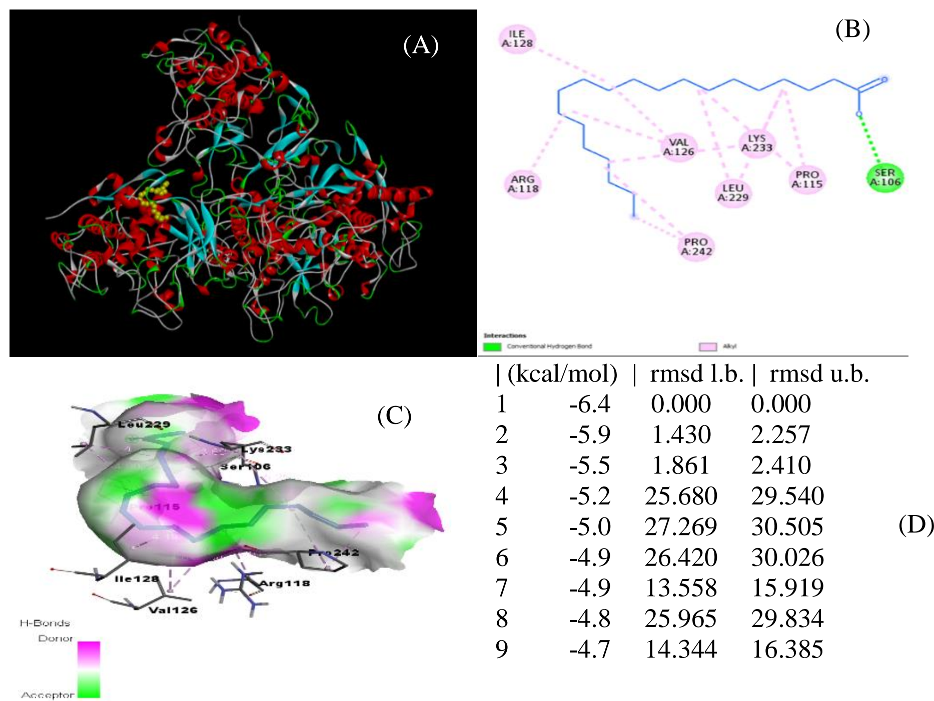
Saturated Fatty Acids (%)		Unsaturated Fatty Acids (%)	
C16:0 (Palmitic acid)	25.14	C18:1 (trans - 9) (omega 9) Oleic acid	9.74
C14:0 Methyl tetradecanoate	1.89	C18:3 (alpha) (all cis - 9,12,15) (omega 3)	3.21
C12:0 (Lauric acid)	0.71	C20:3 (cis-11,14,17; n-3) eikosatrienoik asit	3.00
C14:0 (Myristic acid)	0.56	C18:2 (trans-9, trans-12; n-6) linoleik asit izomeri	1.24
C18:0 (Stearic acid)	0.06	9-Hexadecenoic acid, methyl ester, (Z)-	1.04
Heptadecanoic acid, 16-methyl-, methyl ester	0.06	9-Octadecenoic acid (Z)-, methyl ester	0.12
Heptadecanoic acid, 15-methyl-, methyl ester	0.06	1-Octadecene	0.04
Heptadecanoic acid, 14-methyl-, methyl ester	0.06	Octadec-9-enoic acid	0.04
		9,12,15-Octadecatrienoic acid, methyl ester,	0.02
		8-Octadecenoic acid, methyl ester, (E)-	0.01

Molecular Docking

According to the docking results, the binding energy between oleic acid and the tyrosinase enzyme was determined as −6.0 kcal/mol; between linoleic acid and tyrosinase as −5.9 kcal/mol; and between linolenic acid and tyrosinase as −6.1 kcal/mol. In a study by Min et al. [22] investigating the anti-browning properties of paeonol on freshly cut apples, it was reported that the binding of paeonol to tyrosinase could alter the chromophore microenvironment and conformation of the enzyme. It was suggested that by binding to the active site of tyrosinase, paeonol could inhibit the enzyme and thus prevent browning in freshly cut apples. This finding indicates that natural tyrosinase inhibitors could be used in anti-browning applications to prevent food discoloration and extend shelf life [22]. Similarly, Shamsuri et al. [23] synthesized and characterized the molecule (1E)-1-(2-pyrazinyl)ethanone thiosemicarbazone (PT) to investigate its inhibitory effect against enzymatic browning and tyrosinase. The inhibitory effect of PT on tyrosinase was supported by molecular docking analyses. As a result, PT and structurally similar compounds were reported as promising inhibitors of tyrosinase and potentially effective in anti-browning applications [23].

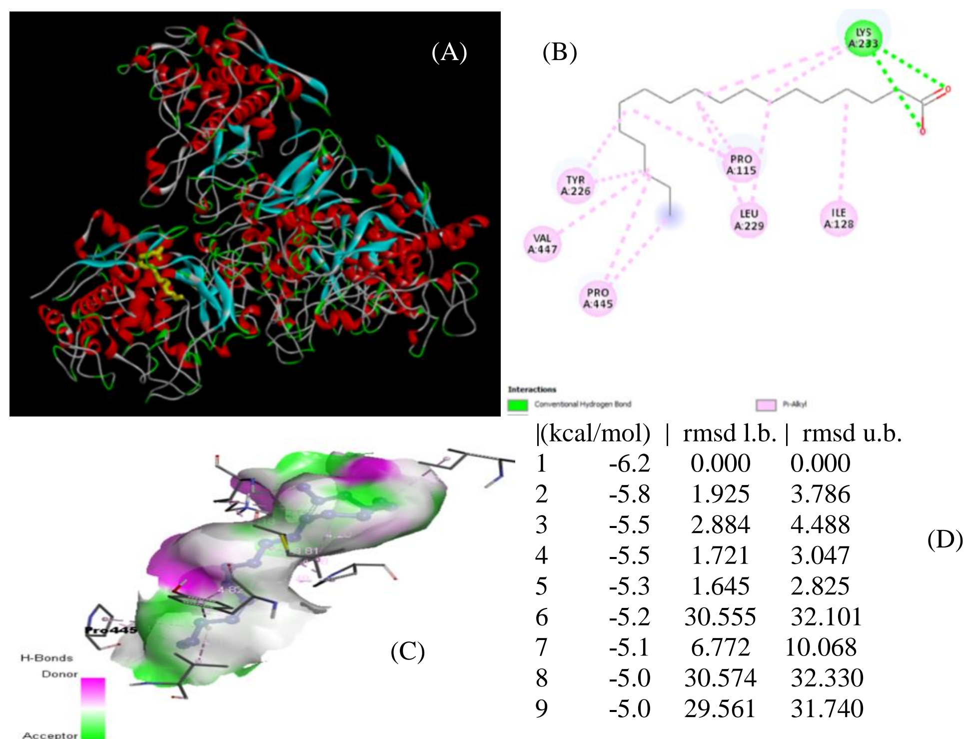
In the molecular docking analysis of eicosatrienoic acid with tyrosinase, the lowest binding energy was determined to be -6.4 kcal/mol. This value suggests that the ligand has a strong binding affinity to the enzyme's active site.

The figure shows the binding interactions of eicosatrienoic acid with the tyrosinase enzyme. The ligand forms a conventional hydrogen bond (green line) with the enzyme's Ser106 amino acid. This hydrogen bond contributes to the ligand's stable positioning in the active site. In addition, the hydrophobic portion of the ligand's chain exhibits alkyl/hydrophobic interactions (pink lines) with residues Pro115, Arg118, Val126, Ile128, Leu229, Lys233, and Pro242. These hydrophobic interactions may stabilize the ligand's intraenzymatic conformation, increasing binding affinity. This binding model demonstrates that eicosatrienoic acid binds to tyrosinase with a moderate-to-high affinity through a combination of hydrogen bonding and numerous alkyl interactions. This supports the potential inhibitory effect of eicosatrienoic acid on tyrosinase.



	(kcal/mol)	rmsd l.b.	rmsd u.b.
1	-6.4	0.000	0.000
2	-5.9	1.430	2.257
3	-5.5	1.861	2.410
4	-5.2	25.680	29.540
5	-5.0	27.269	30.505
6	-4.9	26.420	30.026
7	-4.9	13.558	15.919
8	-4.8	25.965	29.834
9	-4.7	14.344	16.385

Figure 1. Molecular docking of eicosatrienoic acid with tyrosinase enzyme and its binding energies. (A) Three-dimensional binding model showing the protein–ligand complex. (B) Two-dimensional interaction diagram indicating hydrogen and hydrophobic bonds.(C) Surface interaction map illustrating the binding pocket. (D) Binding energies (kcal/mol) and RMSD values of the docking poses.



	(kcal/mol)	rmsd l.b.	rmsd u.b.
1	-6.2	0.000	0.000
2	-5.8	1.925	3.786
3	-5.5	2.884	4.488
4	-5.5	1.721	3.047
5	-5.3	1.645	2.825
6	-5.2	30.555	32.101
7	-5.1	6.772	10.068
8	-5.0	30.574	32.330
9	-5.0	29.561	31.740

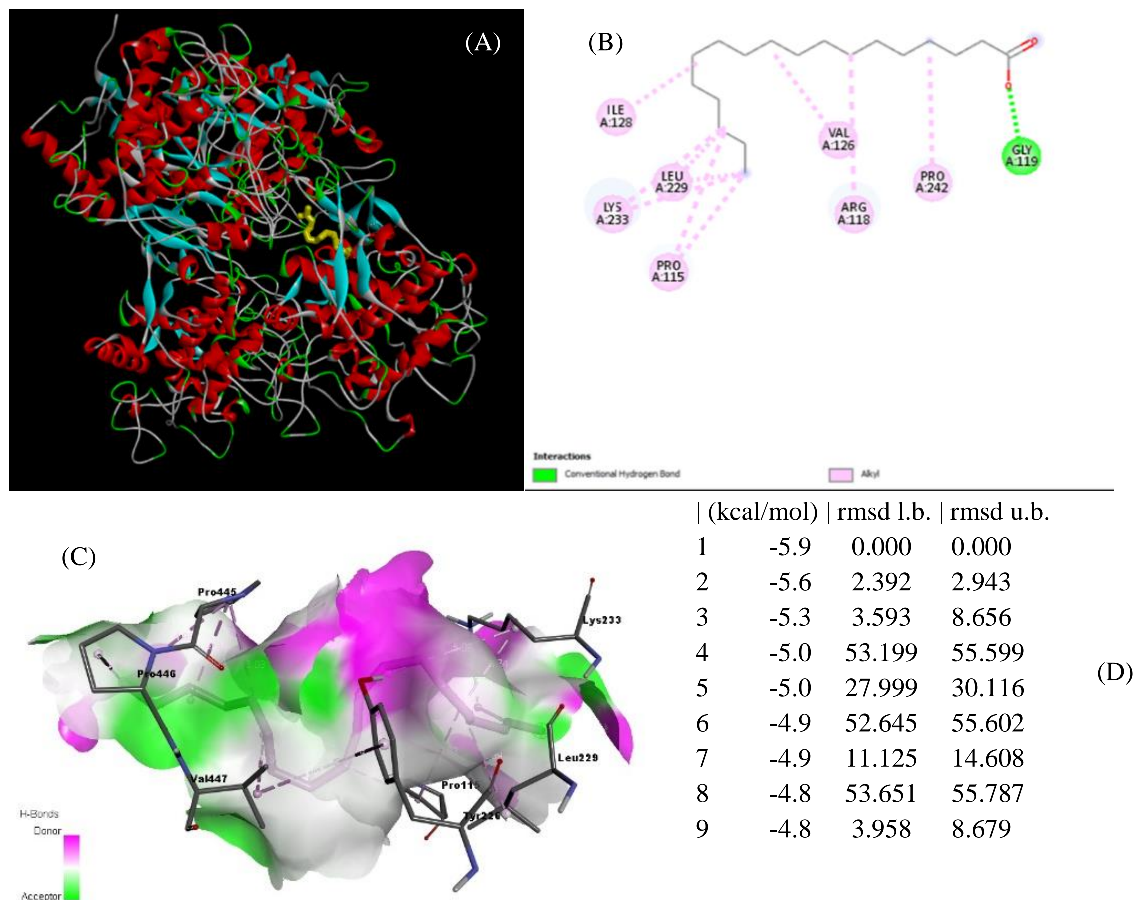
Figure 2. Molecular docking of linoleic acid with tyrosinase enzyme and its binding energies. (A) Three-dimensional binding model showing the protein–ligand complex. (B) Two-dimensional interaction diagram indicating hydrogen and hydrophobic bonds.(C) Surface interaction map illustrating the binding pocket. (D) Binding energies (kcal/mol) and RMSD values of the docking poses.

The lowest binding energy between linoleic acid and tyrosinase was determined to be -6.2 kcal/mol, indicating a strong interaction. The ligand formed two hydrogen bonds with the enzyme's Lys233 residue and exhibited hydrophobic interactions with residues Pro115, Ile128, Leu229, Tyr226, Pro445, and Val447. This binding pattern suggests that linoleic acid is firmly anchored in the active site of tyrosinase and could be a potential natural inhibitor

In the docking analysis of linolenic acid with tyrosinase, the lowest binding energy was determined to be -5.9 kcal/mol. The ligand formed a hydrogen bond with the enzyme's Gly119 residue and exhibited hydrophobic interactions with residues Pro115, Arg118, Val126, Ile128, Leu229, Lys233, and Pro242. These interactions suggest that the ligand binds stably to the active site and may have a potential inhibitory effect on tyrosinase.

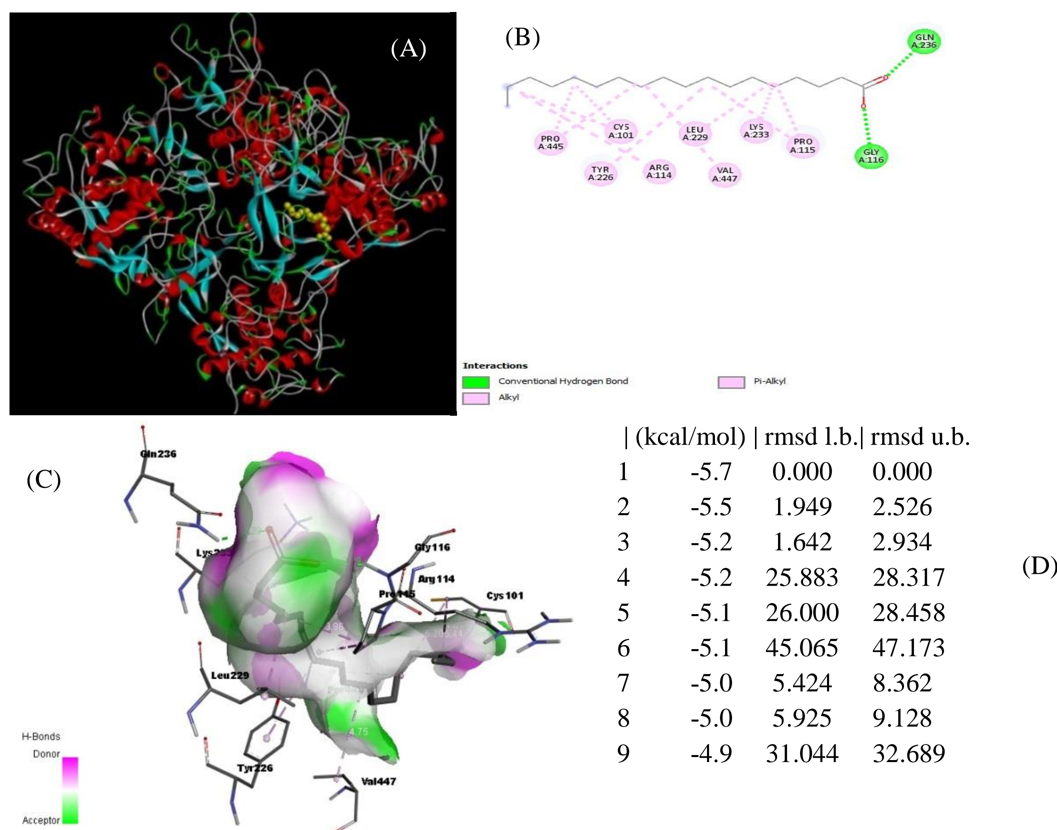
In molecular docking analysis of oleic acid with tyrosinase, the lowest binding energy was determined to be –5.7 kcal/mol. The ligand formed two hydrogen bonds with residues Gly116 and Gln236 of the enzyme and also exhibited hydrophobic (π-alkyl/alkyl) interactions with residues Pro115, Arg114, Tyr226, Val447, Leu229, Lys233, Cys101, and Pro445. This binding profile suggests that oleic acid is stable in the active site of the enzyme and may have potential as a tyrosinase inhibitör.

Molecular docking analyses performed with tyrosinase enzyme revealed that all fatty acids examined exhibited a specific binding affinity to the enzyme's active site. Binding energies ranged from -6.4 to -5.7 kcal/mol, with the highest binding affinity observed for eicosatrienoic acid (-6.4 kcal/mol) and the lowest for oleic acid (-5.7 kcal/mol). The results suggest that these fatty acids can exhibit a potential inhibitory effect by binding stably to the active site of tyrosinase. Therefore, oleic, linoleic, linolenic, and eicosatrienoic acids are biologically important compounds that can be considered natural tyrosinase inhibitors in foods or cosmetic products.



	(kcal/mol)	rmsd l.b.	rmsd u.b.
1	-5.9	0.000	0.000
2	-5.6	2.392	2.943
3	-5.3	3.593	8.656
4	-5.0	53.199	55.599
5	-5.0	27.999	30.116
6	-4.9	52.645	55.602
7	-4.9	11.125	14.608
8	-4.8	53.651	55.787
9	-4.8	3.958	8.679

Figure 3. Molecular docking of linolenic acid with tyrosinase enzyme and its binding energies. (A) Three-dimensional binding model showing the protein–ligand complex. (B) Two-dimensional interaction diagram indicating hydrogen and hydrophobic bonds.(C) Surface interaction map illustrating the binding pocket. (D) Binding energies (kcal/mol) and RMSD values of the docking poses.



	(kcal/mol)	rmsd l.b.	rmsd u.b.
1	-5.7	0.000	0.000
2	-5.5	1.949	2.526
3	-5.2	1.642	2.934
4	-5.2	25.883	28.317
5	-5.1	26.000	28.458
6	-5.1	45.065	47.173
7	-5.0	5.424	8.362
8	-5.0	5.925	9.128
9	-4.9	31.044	32.689

Figure 4. Molecular docking of oleic acid with tyrosinase enzyme and its binding energies. (A) Three-dimensional binding model showing the protein–ligand complex. (B) Two-dimensional interaction diagram indicating hydrogen and hydrophobic bonds.(C) Surface interaction map illustrating the binding pocket. (D) Binding energies (kcal/mol) and RMSD values of the docking poses.

CONCLUSION AND RECOMMENDATIONS

Oils are among the essential food ingredients, playing important roles not only as energy sources but also in nutrition and health due to the properties of the fatty acids they contain. The antimicrobial, antioxidant, and nutritionally enhancing properties of bee products provide significant advantages in preserving the quality and freshness of foods.

In this study, the potential inhibitory effects of oleic, linoleic, and linolenic acids found in propolis and perga on the tyrosinase enzyme were evaluated using molecular docking. The findings revealed that these fatty acids can bind to the active site of the tyrosinase enzyme and provide a certain level of inhibition. These results indicate that the fatty acids contained in propolis and perga have the potential to slow enzymatic browning in foods through tyrosinase inhibition, thereby extending the shelf life of fruits and vegetables. Therefore, these natural ingredients can be considered as alternatives to synthetic browning inhibitors in the food sector. The study results support the potential use of propolis and perga as functional food additives, and it is thought that future comprehensive in vitro and in vivo studies will reveal the biological activity and stability of these ingredients in food systems in more detail.

DECLARATIONS

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Authorship contributions

Concept and Writing: Z.D., E.B.; Data Collection and Interpretation: Z.D., E.B.,; Proofreading: B.K., F.C.

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Competing interests

The authors declared that there is no conflict of interest.

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