

Enhancing Quality Characteristics of Cold-Pressed Sesame Oil with Ethanolic Extract of Quince (*Cydonia oblonga*) and Exploring the Interaction of Trans Fatty Acids with Key Pro-inflammatory Cytokines via Molecular Docking

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Abstract

Currently, there is a growing trend of replacing synthetic antioxidants with natural alternatives to delay or prevent the oxidation of edible oils. In this paper, we assessed the phenolic compounds and antioxidant properties of ethanolic extracts obtained from *Cydonia oblonga* (SQ). Furthermore, we incorporated SQ at two different concentrations (1% and 2%) into cold-pressed sesame oil, storing it for 30 days under ambient conditions. We then assessed the peroxide value (PV), acid value (AV), oxidative stability using the Rancimat apparatus, and the fatty acids (FAs) composition. Additionally, we conducted molecular docking analyses to explore the interaction between trans fatty acids (TFAs; C18:1 and C18:2) and key pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and C-reactive protein (CRP). The total phenol, flavonoid content, and antioxidant activity of SQ were found to be 95.33 ± 5.03 mg GAE/g, 343.67 ± 16.44 mg QE/g, and 465.67 ± 5.51 mmol Fe²⁺/g, respectively. The presence of SQ exhibited a significant impact on reducing PV and AV when compared to the control group. Furthermore, the addition of SQ resulted in a significant increase in the induction period (IP) compared to the control. The predominant FAs in the samples were 18:2n-6, 18:1n-9, 16:0, and 18:0, respectively. The levels of TFAs in all samples at 30 days were higher than those at 0 days. TNF- α and IL-6 showed a higher binding affinity for the trans-C18:1 ligand, with a docking score of -6.81 and -5.82, respectively, compared to the trans-C18:2 ligand. In this context, SQ can be proposed as a natural antioxidant to enhance the oxidative stability of sesame oil. Additionally, the binding preferences and specific interactions of TFAs with these pro-inflammatory cytokines indicate their potential role in modulating inflammation.

Introduction

Sesame oil is a consumable vegetable oil derived from the seeds of the *Sesamum Indicum* Linn plant. Due to its great resistance to oxidation during storage and frying, which distinguishes it from other vegetable oils, it is particularly used in cooking. This exceptional stability can be attributed to its rich content of antioxidants, including tocopherols and lignans (1, 2). Sesame oil is usually produced using the cold-pressing technique, which requires less processing and preserves the seed's natural deliciousness. Cold-pressed sesame oil is a preferred option in culinary uses and food preparations because of its distinctive flavor and distinctive nutty scent (3, 4). Beyond its culinary appeal, sesame oil is also recognized for its potential health-enhancing properties. It contains a high concentration of unsaturated FAs, which are useful in decreasing the risk of cardiovascular diseases (5). The composition of Sesame oil consists of approximately 14% saturated FAs, 39% monounsaturated FAs, and 46% polyunsaturated FAs (6). The robust antioxidant activity of sesame oil arises from its primary components, notably γ -tocopherol and three essential lignan compounds: sesamin, sesamol, and sesamol. These compounds collectively contribute to the potent antioxidant properties exhibited by sesame oil (7). However, oil is susceptible to oxidation upon exposure to various elements, including light, heat, trace metals, and oxygen. This vulnerability stems from the presence of unsaturated bonds within its chemical structure. The process of oxidation has detrimental effects on both the nutritional integrity

and shelf life of the oil (8). Lipid peroxidation is a multifaceted process that unfolds in distinct stages, swiftly giving rise to a multitude of compounds. These compounds play a pivotal role in the development of lipid rancidity. Among them, hydroperoxides emerge as prominent primary oxidation products, which subsequently undergo additional oxidation steps, culminating in the formation of secondary oxidation products. Examples of these secondary oxidation products encompass various organic compounds such as alkanes, alkenes, aldehydes, and ketones (9). To curtail the incidence of lipid oxidation and extend shelf life, the application of antioxidants has proven effective. These compounds act as safeguards against the autoxidation of oils and fats by bestowing their hydrogen atoms upon free radicals, thereby diminishing the likelihood of oil oxidation and the subsequent development of rancidity (8, 10).

Research has highlighted that the consumption of oils and oily foods containing oxidative metabolites comprising reactive oxygen species can inflict damage on various organs within the body. This damage ensues through the generation of cytotoxic and genotoxic compounds, fostering the development of chronic ailments. These repercussions include perturbation of the body's redox equilibrium, compromising the robustness of the antioxidant network. As a consequence, the risk escalates for afflictions such as atherosclerosis, diabetes, obesity, and cancer. This oxidative stress further exacerbates the pathogenesis of myeloma and lymphoma by inflicting DNA damage, influencing chemotherapy outcomes in non-Hodgkin's lymphoma, accelerating the aging process, augmenting plasma total cholesterol levels, fostering larger atherosclerotic lesions, and promoting atherogenesis that impacts arterial walls and precipitates heart diseases, ultimately culminating in cell death (9, 11–13).

TFAs are a type of unsaturated FAs characterized by the presence of one or more unconjugated double bonds in the trans configuration. The majority of TFAs are produced during industrial processing, particularly through the partial hydrogenation of vegetable oils that are abundant in polyunsaturated fatty acids (PUFAs) (14). Partially hydrogenated vegetable oils can contain as much as 60% TFAs, primarily composed of various isoforms of trans-octadecenoic acid (trans-C18:1), which typically constitute 80–90% of the total TFA content. Several studies have furnished evidence suggesting that specific TFAs exert an influence on the regulation of crucial physiological processes, including lipid metabolism, inflammation, oxidative stress, endoplasmic reticulum stress, autophagy, and apoptosis. Disruption of these biological pathways by TFAs serves as a potential underlying mechanism contributing to the detrimental impacts of TFAs on cardiometabolic health (15, 16). Furthermore, recent evidence highlighted a potential link between TFAs and the regulation of pro-inflammatory cytokines, such as TNF- α , IL-6, and CRP (17, 18). Studies have indicated that elevated levels of TFAs in the diet have been associated with increased circulating levels of pro-inflammatory cytokines, leading to the activation of inflammatory pathways (19, 20). Pro-inflammatory cytokines play key roles in immune responses and are involved in the development and progression of various inflammatory diseases, ranging from cardiovascular diseases and cancer to diabetes and obesity (21–23). Therefore, understanding the molecular interactions between TFAs and pro-inflammatory cytokines may shed light on the mechanisms underlying the harmful effects of TFAs on cardiometabolic health and further emphasize the importance of discovering potential interventions to alleviate their impact.

C. oblonga Mill., commonly known as Quince, belongs to the Rosaceae family. This plant is utilized for both nutritional and medicinal purposes due to its substantial polyphenol content. The polyphenols found in quince predominantly comprise hydroxycinnamic acids (such as caffeoyl and coumaroyl derivatives), flavonols (including quercetin and kaempferol derivatives), and, to a lesser extent, flavanols (such as catechin and epicatechin). Quince serves as a dietary source of health-enhancing compounds, contributing to its antioxidant capacity. Moreover, quince exhibits various beneficial properties, encompassing anti-ulcerative, anti-carcinogenic, anti-inflammatory, anti-allergic, and antimicrobial activities (24–26).

Hence, the aim of this research was to investigate the impact of an ethanolic extract derived from *C. oblonga* on the quality attributes of sesame oil obtained through cold pressing over a span of 30 days under ambient storage conditions (ranging from 25–30°C). Moreover, we conducted molecular docking analyses to explore the interaction between TFAs (specifically trans-C18:1 and trans-C18:2) and key pro-inflammatory cytokines, aiming to elucidate the potential molecular mechanisms by which TFAs may contribute to inflammation-related processes.

Material and methods

Preparation of oil sample and quince extract

Fresh sesame oil and quince were procured from Rosalita company and a local fruit shop, respectively, located in Kashan, Iran. The quince fruit samples were carefully air-dried in shaded conditions for a period of 2 weeks. Subsequently, the dried quince samples were finely powdered utilizing a mixer grinder. Each batch of powdered quince was subjected to extraction using 80% ethanol employing the maceration technique. Specifically, 100 grams of the powdered quince material were mixed with 400 milliliters of 80% ethanol and left to undergo extraction at room temperature for a duration of 8 hours (27). The sesame oil was divided into 3 different categories: sesame oil without extract (control; C), sesame oil treated with 1% quince extract (SQ1) and sesame oil treated with 2% quince extract (SQ2).

Estimation of total phenolics, flavonoids and total antioxidant activity

The quantification of total phenolic and flavonoid constituents was conducted on ethanolic extracts using colorimetric methods, as described in previous studies (28). The determination of the overall antioxidant activity of the plant extract was carried out through iron reduction, employing the Ferric Reducing Antioxidant Power assay (FRAP), as detailed in a prior study (29).

Peroxide value

The PV serves as a measure to assess the concentration of peroxides and hydroperoxides that emerge during the initial phases of the lipid oxidation process. As the oil initiates the oxidation process, peroxides begin to develop, subsequently liberating iodine from potassium iodide. The resulting iodine can then be

quantified through titration using standard sodium thiosulfate, employing the methodology outlined in the AOCS Cd 8–53 standard methods. This analysis offers valuable insights into the oxidative status of the oil (30). In summary, the procedure involved adding 30 ml of acetic acid-chloroform solution to a 250 ml flask containing 5 g of oil sample and shaking to ensure complete dissolution. Once the oil had completely dissolved, 0.5 ml of saturated potassium iodide solution was augmented and quickly swirled for one minute, after which 30 ml of deionized water was added. An indicator volume of one ml of starch was added to the mixture. Subsequently, the sample was titrated against 0.1 N sodium thiosulfate until the blue-gray color ceased to exist. The PV was calculated utilizing the volume of titrant as input, employing the subsequent equation: The outcomes were denoted in milliequivalents of peroxide per kilogram of oil:

$$\text{Peroxide value} = \frac{(S - B) \times N \times 1000}{W}$$

Where,

S = Volume consumed during titration of the sample (ml)

B = Volume consumed during titration of the blank (ml)

N = Normality of sodium thiosulfate solution

W = weight of oil (Kg)

Acid value

The AV experiment was employed in this research to determine the proportion of unbound FAs released through the hydrolysis of triglycerides. The procedure followed the methodology outlined in AOCS Cd 3d-63 (30). In summary, in a 250 ml flask, 10 ml of ethanol and 1 g of oil were combined to dissolve the oil. Then, as an indication, add 2 drops of phenolphthalein. Then, 0.1 N potassium hydroxide (KOH) was added to oil samples in titrations until a faint pink tint emerged. The free fatty AV was calculated using the volume of the titrant using the formula below:

$$\text{Acid value} = \frac{(V - B) \times N \times 56.1}{W}$$

Where,

V = titrant volume consumed during titration of the sample

B = titrant volume consumed during the titration of the blank

N = normality of KOH

W = weight of oil (g)

Oxidative stability

The oxidative stability of the oils was evaluated following the ISO 6886 standard method, utilizing a Rancimat 982 apparatus manufactured by Metrohm CH in Zofingen, Switzerland. The experimental conditions entailed setting the temperature to 110°C, while maintaining an air stream of 20 liters per hour (31).

Fatty Acids Composition

The FAs composition of both the control and treated sesame oil samples was assessed subsequent to alkaline trans-esterification. This analysis was conducted using gas-liquid chromatography with flame ionization detection (GC-FID), following the methodology outlined in Regulation EEC 2568/91 (6, 32). Fatty acid methyl esters (FAME) along with standard compounds were subjected to analysis via gas chromatography (Agilent 6890), which was outfitted with a flame ionization detector and an Hp-88 capillary column (100m length, 250µm inner diameter, 0.2 µm film thickness). The identification of FAME was established through a comparative assessment of their retention times against those of a standard FAME mixture. The quantification process was based on peak areas represented in electronic units, with the resulting fatty acid content being expressed as the relative percentage of each individual fatty acid.

Molecular docking analysis

The molecular docking study is useful to predict the binding interaction of the protein and small molecules (33). We chose Autodock Vina for molecular docking and investigation the binding interactions between TNF-α, IL-6, and CRP proteins and ligands, TFAs (trans-C18:1 and trans-C18:2). Blind docking was performed using AutoDock Vina software, which allowed the calculation of binding scores for each protein-ligand complex.

Three-dimensional crystal structure of the selected molecular targets was downloaded from a protein data bank (PDB). The PDB ID of the downloaded proteins were 2AZ5, 1ALU, and 3PVN for TNF-α, IL-6, and CRP, respectively. Ligand molecules, trans-C18:1 (Elaidic acid; PubChem CID: 637517) and trans-C18:2 (Linoelaidic acid; PubChem CID: 5282457) were retrieved from the PubChem database. AutoDockTools were used to prepare structure of proteins and ligands by adding hydrogens, assigning Gasteiger charges and to convert the ligand and protein into the pdbqt format required by AutoDock Vina (34).

A docking grid box of dimensions 40 × 40 × 40 with a grid spacing of 1 Å was centered on each protein structure to cover the entire surface, with the exhaustiveness parameter of 8.

The top binding pose ranked by binding affinity were retained to investigate interaction each protein-ligand pair. The interaction plot analyses were carried out by using BIOVIA Discovery Studio 2020 software.

The interactions between the protein residues and ligands were analyzed and categorized based on their type. The distances between the interacting residues and ligands were calculated. The specific residues involved in each interaction were noted.

Statistical analysis

All experiments in this study were performed in triplicate, and the data are presented as the mean $\pm$ standard deviation (SD). Statistical analysis was performed using SPSS software (version 21, Chicago, IL, USA). One-way ANOVA and the least significant differences test were employed to determine the statistically significant differences between the samples. A p-value less than 0.05 was considered indicative of significant differences.

Results

Table 1 presents the results acquired from tests assessing total phenol and flavonoid content, as well as antioxidant activity, for the hydro-ethanolic extract derived from the whole fruit of *C. oblonga*.

Table 1
Total phenols, flavonoids content and antioxidant activity of hydro-ethanolic extract of whole fruit of *C. oblonga*

	Results
Total phenols content (mg GAE/g)	95.33 $\pm$ 5.03
Flavonoids content (mg QE/g)	343.67 $\pm$ 16.44
Antioxidant activity (mmol Fe ²⁺ /g)	465.67 $\pm$ 5.51

Figure 1 depicts the PV trends for sesame oil samples, including both oil without extract and oil treated with the hydro-ethanolic extract from the whole fruit of *C. oblonga* (at concentrations of 1% and 2%), during a 30-day storage period. The findings reveal a notable elevation in PVs across all samples as the storage duration extends. At the initial point (zero time), no significant distinctions were observed between the control, SQ1, and SQ2 samples. However, over the course of the 10, 20, and 30-day storage intervals, the presence of quince extract exhibited a significant impact in reducing PVs when contrasted with the control group ($p < 0.05$).

The AVs for sesame oil without extract and sesame oil with the hydro-ethanolic extract of the whole fruit of *C. oblonga* (at concentrations of 1% and 2%) during a 30-day storage period are presented in Fig. 2. At zero and 10 time points, the results indicated no significant differences among all samples. After 20 days, the AV of all samples increased compared to the initial measurement, but only SQ2 exhibited a notable difference from the other samples ($p < 0.05$). As the storage period extended to 30 days, the presence of quince extract demonstrated significance in reducing the AV when compared to the control ($p < 0.05$).

The IP results obtained using the Rancimat method for sesame oil without extract and sesame oil with the hydro-ethanolic extract of the whole fruit of *C. oblonga* (at concentrations of 1% and 2%) are illustrated in Fig. 3. The addition of quince extract showed a significant increase in IP when compared to the control ($p < 0.05$).

Table 2 provides the results of fatty acid quantification for sesame oil without extract and sesame oil with the hydro-ethanolic extract of the whole fruit of *C. oblonga* (at concentrations of 1% and 2%) at both 0 and 30 days. The predominant FAs were 18:2n-6, 18:1n-9, 16:0, and 18:0, respectively. The levels of TFAs in all samples at 30 days were higher than those at 0 days ($p < 0.05$). Additionally, the quantities of 18:2n-6 and 18:1n-9 at 30 days were lower than those at 0 days ($p < 0.05$).

Table 2

Fatty acids composition expressed in mg/g of sesame oil without extract and sesame oil with hydro-ethanolic extract of whole fruit of *C. oblonga* (1%; SQ1 and 2%; SQ2) at 0 and 30 days.

Fatty acids	0 day			30 day		
	C	SQ1	SQ2	C	SQ1	SQ2
C14:0	0.12 ± 0.00 ^A	0.12 ± 0.00 ^A	0.12 ± 0.00 ^A	0.12 ± 0.00 ^A	0.12 ± 0.00 ^A	0.12 ± 0.00 ^A
C16:0	92.4 ± 0.04 ^A	92.4 ± 0.04 ^A	92.4 ± 0.04 ^A	92.6 ± 0.04 ^A	92.5 ± 0.04 ^A	92.4 ± 0.04 ^A
C16:1n-7	1.1 ± 0.02 ^A	1.1 ± 0.02 ^A	1.1 ± 0.02 ^A	1.08 ± 0.02 ^A	1.1 ± 0.02 ^A	1.1 ± 0.02 ^A
C17:0	0.33 ± 0.00 ^A	0.33 ± 0.00 ^A	0.33 ± 0.00 ^A	0.31 ± 0.03 ^A	0.30 ± 0.02 ^A	0.34 ± 0.04 ^A
C18:0	42.57 ± 0.02 ^A	42.57 ± 0.02 ^A	42.57 ± 0.02 ^A	42.54 ± 0.04 ^A	42.55 ± 0.04 ^A	42.53 ± 0.03 ^A
C18:1n-9	361.4 ± 0.08 ^A	361.4 ± 0.08 ^A	361.4 ± 0.08 ^A	354.4 ± 0.09 ^B	356.4 ± 0.07 ^C	360.5 ± 0.08 ^A
C18:1n-7	5.66 ± 0.04 ^A	5.66 ± 0.04 ^A	5.66 ± 0.04 ^A	5.57 ± 0.09 ^A	5.59 ± 0.06 ^A	5.63 ± 0.04 ^A
C18:2n-6	415.25 ± 0.05 ^A	415.25 ± 0.05 ^A	415.25 ± 0.05 ^A	409.1 ± 0.05 ^B	412.25 ± 0.03 ^C	413.1 ± 0.06 ^C
C18:3n-3	3.14 ± 0.04 ^A	3.14 ± 0.04 ^A	3.14 ± 0.04 ^A	3.12 ± 0.06 ^A	3.11 ± 0.04 ^A	3.15 ± 0.06 ^A
C20:0	4.8 ± 0.02 ^A	4.8 ± 0.02 ^A	4.8 ± 0.02 ^A	4.7 ± 0.09 ^A	4.8 ± 0.05 ^A	4.7 ± 0.08 ^A
C20:1n-9	1.6 ± 0.04 ^A	1.6 ± 0.04 ^A	1.6 ± 0.04 ^A	1.52 ± 0.03 ^B	1.58 ± 0.04 ^A	1.59 ± 0.04 ^A
C20:4n-6	0.62 ± 0.02 ^A	0.62 ± 0.02 ^A	0.62 ± 0.02 ^A	0.59 ± 0.03 ^A	0.61 ± 0.04 ^A	0.62 ± 0.06 ^A
C24:0	0.51 ± 0.04 ^A	0.51 ± 0.04 ^A	0.51 ± 0.04 ^A	0.50 ± 0.02 ^A	0.51 ± 0.06 ^A	0.50 ± 0.07 ^A
SFA	140.73 ± 0.12 ^A	140.73 ± .012 ^A	140.73 ± .012 ^A	140.77 ± .022 ^A	140.78 ± .021 ^A	140.59 ± .026 ^A

Mean ± standard deviation of twice analyses. Different letters in the same line indicate significant difference at 95% by the ANOVA test ($p < 0.05$).

Fatty acids	0 day			30 day		
	C	SQ1	SQ2	C	SQ1	SQ2
MUFA	369.76 ± 0.18 ^A	369.76 ± 0.18 ^A	369.76 ± 0.18 ^A	362.57 ± 0.23 ^A	364.67 ± 0.19 ^A	368.82 ± 0.18 ^A
PUFA	419.01 ± 0.11 ^A	419.01 ± 0.11 ^A	419.01 ± 0.11 ^A	412.81 ± 0.14 ^A	415.97 ± 0.11 ^A	416.87 ± 0.18 ^A
Trans fatty acid	0.4 ± 0.01 _A	0.4 ± 0.01 _A	0.4 ± 0.01 _A	0.5 ± 0.02 ^B	0.49 ± 0.03 _B	0.47 ± 0.02 _B
Mean ± standard deviation of twice analyses. Different letters in the same line indicate significant difference at 95% by the ANOVA test (p < 0.05).						

The docking simulations provided insights into the binding affinities of TNF- α , IL-6, and CRP with trans-C18:1 and trans-C18:2 ligands, as well as the specific interactions formed between the protein residues and ligands. The docking scores obtained for each protein-ligand complex are presented in Table 3, while Table 4 provides information on the interactions, distances, and categorization of interactions for each protein-ligand complex.

Table 3
The binding affinities of the top docking poses

Protein	Ligand (TFA)	Score
TNF- α	trans-C18:1	-6.81
	trans-C18:2	-6.24
IL-6	trans-C18:1	-5.82
	trans-C18:2	-5.84
CRP	trans-C18:1	-5.69
	trans-C18:2	-5.77

Table 4

Intermolecular interactions between the ligands and key amino acid residues in the binding site of each docked protein complex.

Protein	Interactions	Distance	Category	Type
TNF- α	A: GLY122-C18:1	2.49227	Hydrogen Bond	Carbon Hydrogen Bond
	A: TYR59-C18:1	4.69657	Hydrophobic	Pi-Alkyl
	A: TYR59-C18:1	4.55788	Hydrophobic	Pi-Alkyl
	B: TYR119-C18:1	5.20912	Hydrophobic	Pi-Alkyl
	B:TYR151-C18:1	5.1787	Hydrophobic	Pi-Alkyl
	A: TYR151-C18:2	2.10425	Hydrogen Bond	Conventional Hydrogen Bond
	A: SER60-C18:2	2.60046	Hydrogen Bond	Conventional Hydrogen Bond
	B: TYR119-C18:2	5.37121	Hydrophobic	Pi-Alkyl
IL-6	ARG30- C18:1	2.75109	Hydrogen Bond	Conventional Hydrogen Bond
	ASP34-C18:1	2.00213	Hydrogen Bond	Conventional Hydrogen Bond
	ARG30-C18:1	4.8115	Hydrophobic	Alkyl
	ARG179-C18:1	5.18369	Hydrophobic	Alkyl
	LEU33-C18:1	4.82652	Hydrophobic	Alkyl
	LEU178-C18:1	4.31064	Hydrophobic	Alkyl
	ARG182-C18:1	5.02992	Hydrophobic	Alkyl
	SER176-C18:2	2.33641	Hydrogen Bond	Conventional Hydrogen Bond
	LYS66-C18:2	2.73064	Hydrogen Bond	Carbon Hydrogen Bond
	SER176-C18:2	3.07451	Hydrogen Bond	Carbon Hydrogen Bond
	ARG179-C18:2	4.58308	Hydrophobic	Alkyl
	SER176-C18:2	2.33641	Hydrogen Bond	Conventional Hydrogen Bond
CRP	LYS69- C18:1	2.81528	Hydrogen Bond	Conventional Hydrogen Bond
	ASP70- C18:1	2.08868	Hydrogen Bond	Conventional Hydrogen Bond
	LYS69- C18:1	2.59002	Hydrogen Bond	Carbon Hydrogen Bond
	PRO87- C18:1	4.86508	Hydrophobic	Alkyl
	LYS69- C18:2	5.38624	Hydrophobic	Alkyl
	VAL89- C18:2	4.59074	Hydrophobic	Alkyl
	PRO87- C18:2	3.86619	Hydrophobic	Alkyl

Representative protein-ligand complexes with the most favorable binding affinities are illustrated in Figs. 4–6. TNF- α and IL-6 exhibited a higher binding affinity for the trans-C18:1 ligand with a docking score of -6.81 and - 5.82, respectively compared to the trans-C18:2 ligand (with a docking score of -6.24 and - 5.84, respectively). The analysis of interactions revealed that TNF- α formed hydrogen bond interactions between residue GLY122 and trans-C18:1, as well as hydrophobic interactions involving residues TYR59, TYR119, and TYR151 with both trans-C18:1 and trans-C18:2 ligands. In the sense of IL-6, results indicated hydrogen bond interactions involving residues ARG30, ASP34, and ARG182 with trans-C18:1 ligand, while hydrophobic interactions were observed with residues ARG179, LEU33, LEU178, and SER176. For trans-C18:2 ligand, hydrogen bond interactions were formed with residues SER176, LYS66, and ARG179. CRP demonstrated slightly lower binding affinities for both ligands, with a docking score of -5.69 for trans-C18:1 and - 5.77 for trans-C18:2. The interaction analysis demonstrated hydrogen bond interactions involving residues LYS69, ASP70, and PRO87 with trans-C18:1 ligand, while hydrophobic interactions were observed with residue PRO87. For trans-C18:2 ligand, hydrophobic interactions were observed with residues LYS69, VAL89, and PRO87. The distances and interaction types are detailed in Table 4.

Discussion

This study investigated the impact of an ethanolic extract derived from *C. oblonga* as a potential natural antioxidant on the oxidative stability of cold-pressed sesame oil over a 30-day period of environmental storage. Furthermore, a molecular docking assay was employed to explore the potential interactions between TFAs (trans-C18:1 and trans-C18:2) and key pro-inflammatory cytokines, including TNF- α , IL-6, and CRP.

The findings of this study revealed that the PVs of both the control and treated sesame oil samples remained below the CODEX standard threshold (15 meq/kg oil) over the 30-day testing period (35). PV served as a metric to quantify the primary oxidation products generated within fats as a consequence of oxidation. Our study's outcomes demonstrated noteworthy variations between the control samples and those treated with SQ1 and SQ2 during the storage period, as depicted in Fig. 1. Notably, prior research has indicated that phenolic compounds present in various extracts could potentially enhance the stability of oils (36, 37). Soybean oil samples enriched with olive leaves extract (OLE) displayed reduced PVs compared to the control group. Furthermore, the oil samples supplemented with varying concentrations of OLE (1000 and 1500 ppm) demonstrated a noteworthy and statistically significant decrease ($p < 0.05$) in the presence of secondary products when compared to the control (37). Yazdanpanah et al. demonstrated that the PV of sesame oil infused with an aqueous extract of white tea was notably lower than that observed in the control group (38). Omid et al. (2018) similarly reported comparable findings. They discovered that varying concentrations of methanolic extract derived from *spirulina microalgae* (ranging from 500 to 2000 ppm) exhibited a significant difference in terms of PV compared to the control group (39).

Throughout this study, the AV of all sesame oil samples displayed a gradual increase as the storage time advanced. Nevertheless, these values remained below the permissible level set for cold-pressed virgin oils, which stands at 4 mg KOH per gram of oil. (3). The AV experiment quantifies the quantity of free FAs released through the hydrolysis of fat within the oil sample. This measurement serves as an indicator of the quality of sesame oil. A higher AV indicates a greater degree of oil oxidation, which corresponds to lower quality (3). The upward trend in AV over time was previously documented by Zanardi et al. (40) in sesame oil, also in other oils like palm, peanut, and sunflower. In our work, there was no noteworthy disparity between the control samples and SQ1 and SQ2 at 0 and 10 days. However, a significant divergence emerged between the control samples and SQ1, SQ2 at the 30-day mark. This result aligns with findings from another study in which sunflower oil treated with either natural or synthetic antioxidants exhibited a similar pattern (41).

The IP in hours, determined through the Rancimat method, serves as a widely accepted measure to assess the capacity of oils and fats to withstand accelerated oxidation under stressful conditions like air and heat. Notably, the IP values for SQ1 and SQ2 significantly surpassed those of the control sample ($p < 0.05$), as depicted in Fig. 2. This enhancement could be attributed to the presence of phenolic compounds within the quince extract. A prior study demonstrated a synergistic effect between phenolic compounds such as sesamol and γ -tocopherol, observed in a model system involving linoleic acid. Arab et al. demonstrated a robust correlation ($R^2 = 0.84$) between the IP determined via the Rancimat method and the total phenolic compound content. This finding highlights the significant involvement of these compounds in effectively curbing oil oxidation (6).

The FAs composition observed in sesame oil, SQ1, and SQ2 (Table 2) closely resembled or fell within the ranges previously reported in literature. (2, 6, 42). The predominant FAs identified both before and after the addition of quince extract over the 30-day period included linoleic acid (C18:2n-6; ranging from 409.1 to 415.25 ± 0.05 mg/g), oleic acid (C18:1n-9; ranging from 354.4 to 361.4 mg/g), palmitic acid (C16:0; ranging from 92.4 to 92.6%), and stearic acid (C18:0; ranging from 42.53 to 42.57 mg/g). All three samples - sesame oil, SQ1, and SQ2 - contained TFAs within the range of 0.4 to 0.5 mg/g. Importantly, a significant difference ($p < 0.05$) was observed between the amounts of TFAs at 0 and 30 days, a result consistent with previous findings (43).

The experimental results support the findings from molecular docking simulations, which revealed the potential interaction of TFAs with pro-inflammatory cytokines, such as TNF- α , IL-6, and CRP. The higher binding affinities observed for TNF- α and IL-6 towards trans-C18:1 suggest that these proteins may have a stronger preference for this ligand.

The hydrogen bond interactions observed between TNF- α and IL-6 with their respective ligands indicate the involvement of specific residues, such as GLY122, TYR59, TYR119, TYR151, ARG30, ASP34, ARG182, and SER176, in stabilizing the protein-ligand complexes. In addition, the hydrophobic interactions involving TYR59, TYR119, TYR151, ARG179, LEU33, LEU178, and SER176 further contribute to the binding interactions. CRP exhibited lower binding affinities for both ligands compared to TNF- α and IL-6.

However, the hydrogen bond and hydrophobic interactions observed between CRP and its ligands suggest potential binding modes and stabilization of the complexes.

The binding preferences and specific interactions of TFAs with these pro-inflammatory cytokines indicate their potential role in modulating inflammation.

Conclusion

The results of our work showed that the total phenol, flavonoid content, and antioxidant activity of SQ were 95.33 ± 5.03 mg GAE/g, 343.67 ± 16.44 mg QE/g, and 465.67 ± 5.51 mmol Fe²⁺/g, respectively. The presence of SQ had a significant impact on reducing PV and AV when compared to the control group. Additionally, the addition of SQ resulted in a significant increase in IP compared to the control group. The predominant FAs in the samples were 18:2n-6, 18:1n-9, 16:0, and 18:0, respectively. The levels of TFAs in all samples at 30 days were higher than those at 0 days. Moreover, TNF- α and IL-6 exhibited a higher binding affinity for the trans-C18:1 ligand with a docking score of -6.81 and -5.82, respectively, compared to the trans-C18:2 ligand. In this context, SQ can be proposed as a natural antioxidant to improve the oxidative stability of sesame oil. Additionally, the binding preferences and specific interactions of TFAs with these pro-inflammatory cytokines indicate their potential role in modulating inflammation.

Declarations

Acknowledgments

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Authors' Contributions

H.M. and R.S.C. were responsible for investigation, methodology, formal analysis, and writing of the original draft. K. S., A.J. and R.S.C. were responsible for visualization, data curation, and supervision (equal). All of the authors were responsible for writing, reviewing, and editing.

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Data Availability

The datasets of the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval and Consent to Participate

IR.KAUMS.MEDNT.REC.1400.081

Consent for Publication

All authors agreed to publish the results in Plant Foods for Human Nutrition.

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Figures

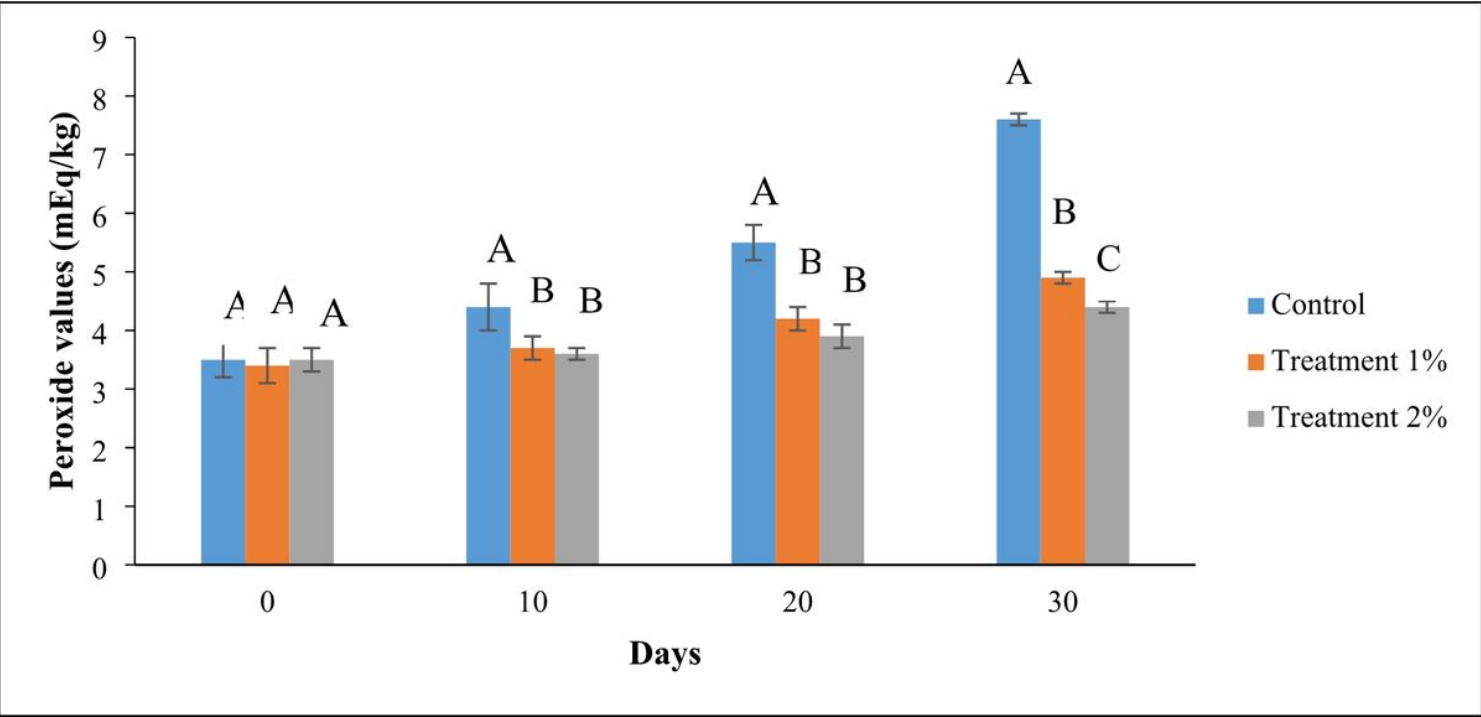


Figure 1

Peroxide value (mEq/kg) for sesame oil without extract (control) and sesame oil with hydro-ethanolic extract of whole fruit of *C. oblonga* (1% and 2%) during storage period 30 days. Different letters A, B and C in the groups indicate significant differences ($p < 0.05$).

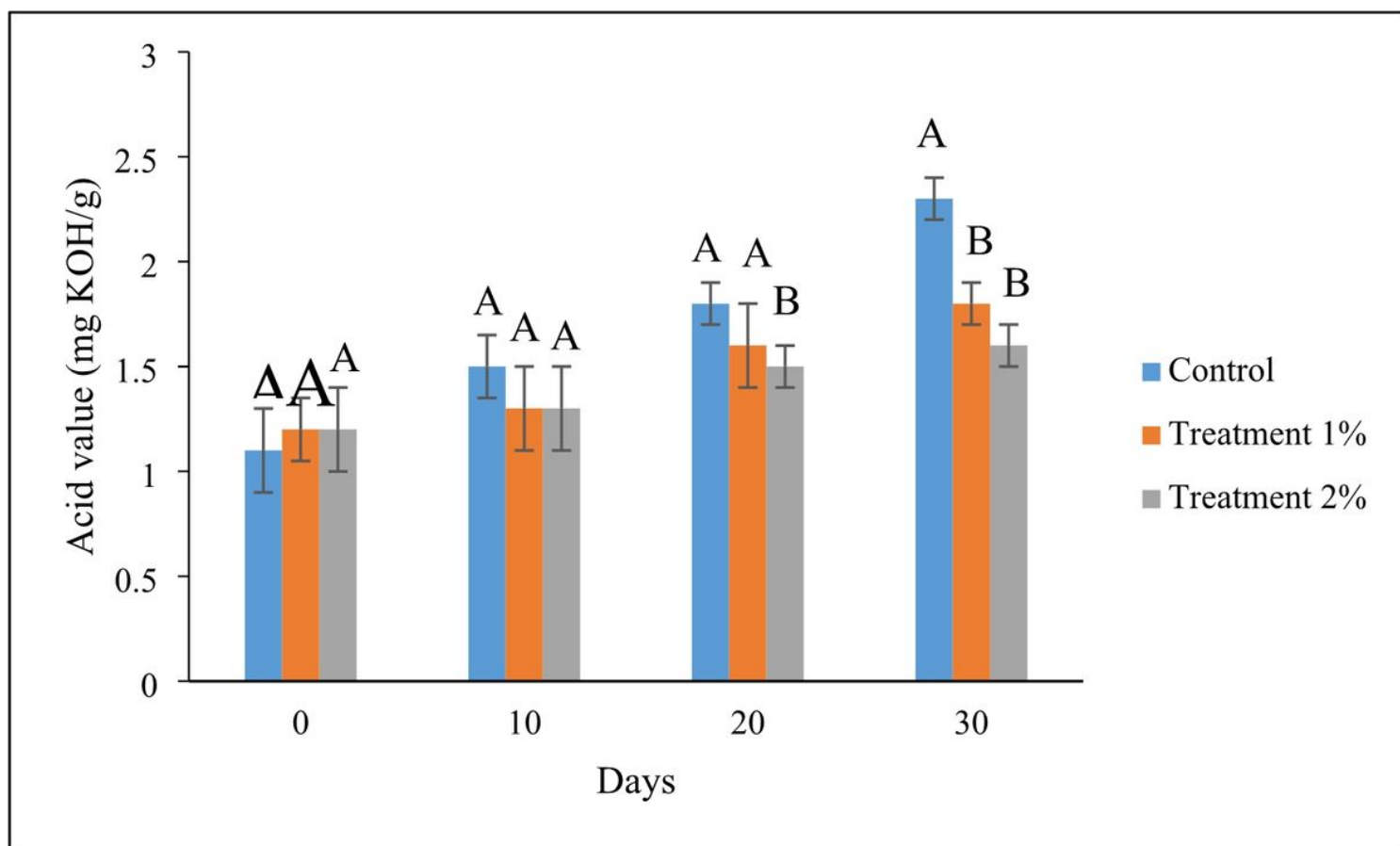


Figure 2

Acid value (mg KOH/g) for sesame oil without extract (control) and sesame oil with hydro-ethanolic extract of whole fruit of *C. oblonga* (1% and 2%) during storage period 30 days. Different letters A, B and C in the groups indicate significant differences ($p < 0.05$).

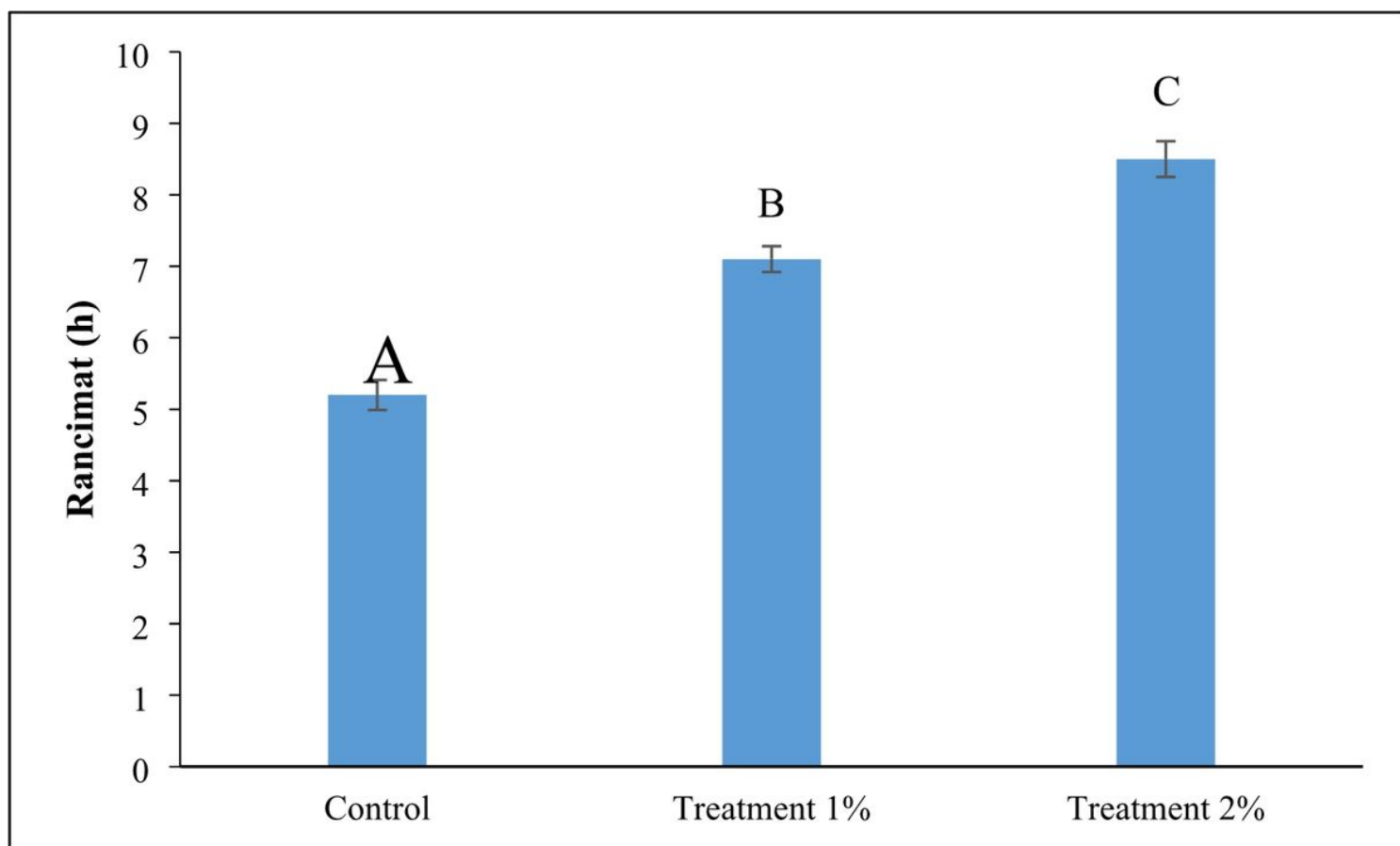


Figure 3

Evaluation of the oxidative stability of sesame oil without extract (control) and sesame oil with hydro-ethanolic extract of whole fruit of *C. oblonga* (1% and 2%) at 110 °C and the air stream was 20 Lit/h. Different letters A, B and C in the groups indicate significant differences ($p < 0.05$).

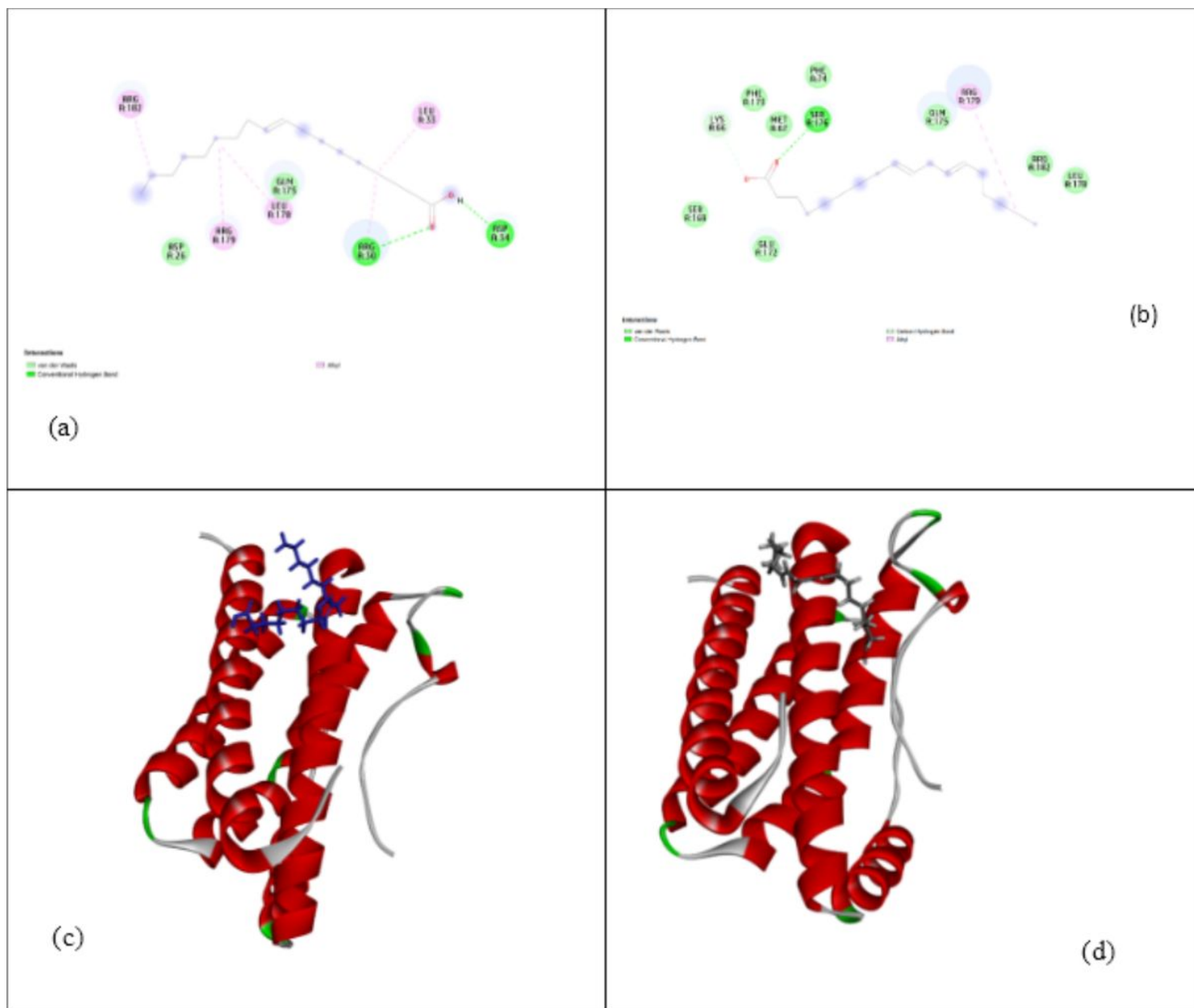


Figure 5

Representation of 2D and 3D protein-ligand interactions. a) 2D interaction of IL 6- trans-C18:1, b) 2D interaction of IL 6- trans-C18:2, c) 3D representation of the protein-ligand complex between IL 6- trans-C18:1 (gray), d) 3D representation of the protein-ligand complex between IL 6- trans-C18:2 (gray).

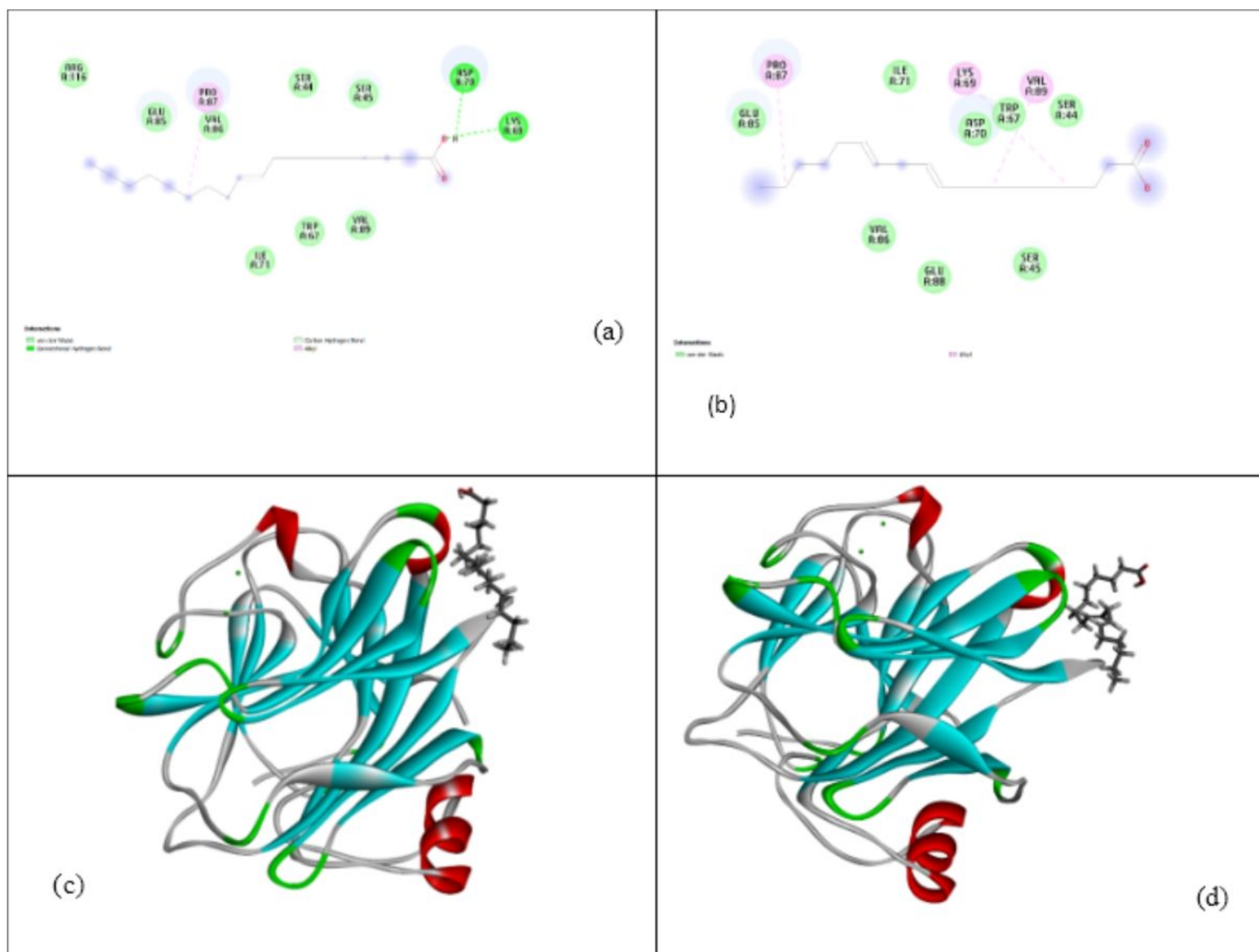


Figure 6

Representation of 2D and 3D protein-ligand interactions. a) 2D interaction of CRP- trans-C18:1, b) 2D interaction of CRP - trans-C18:2, c) 3D representation of the protein-ligand complex between CRP- trans-C18:1 (gray), d) 3D representation of the protein-ligand complex between CRP- trans-C18:2 (gray).