Minor Bioactive Compounds of Cold-pressed Oils and Their Biomedical Functions: a Brief Review

Milan Vukic1, Vesna Vujasinovic2

1University of East Sarajevo, Faculty of Technology Zvornik, Zvornik, Bosnia and Herzegovina
2Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

Corresponding author: Milan Vukic, Assistant Professor, University of East Sarajevo, Faculty of Technology Zvornik, Karakaj 34A, 75400 Zvornik, Phone: +387 56 260 190, E-mail: milan.vukic@tfzv.ues.rs.ba, ORCID ID https://orcid.org/0000-0002-1699-2433.

Background: Consuming a diet rich in natural foods that include oilseed products containing minor bioactive compounds and a diverse array of fatty acids is not just a dietary choice; it is a critical element of maintaining human health. Objective: This paper aims to review the current state of knowledge on minor bioactive compounds in plant cold pressed oils, these are substances that are found in small amounts in plant cold pressed oils. Methods: This review provides a comprehensive analysis of the current state of the art, using data from peer-reviewed articles sourced from various databases. Results and Discussion: Cold-pressed oils extracted from various plant sources have emerged as vital allies in the battle against inflammation-related diseases, offering a versatile range of valuable compounds. These compounds contribute to the oils' multifaceted properties, which encompass potent anti-inflammatory, antioxidant, and anticancer effects, greatly enhancing their nutritional significance. This brief review delves deep into the intricate composition of cold-pressed oils, with a specific focus on the often overlooked but highly influential minor bioactive compounds, including phytosterols, phospholipids, tocols, phenols, squalene and pigments. Conclusion: This paper highlights the importance of cold-pressed oils as a source of various minor bioactive compounds that have the potential to promote human health and prevent or manage a range of diseases. The findings presented in this paper serve as a valuable resource for medical professionals in the field of integrative medicine, nutrition, and dietetics, as well as for consumers looking to make informed choices about their dietary and health needs.

Keywords: dietary choice, health, minor bioactive compounds, nutritional.

1. BACKGROUND

Consumers prefer food products that are natural, healthy, and beneficial (1). Plant oils are a major part of the human diet worldwide, making up more than 75% of the total lipids consumed every day (2). The food industry also gets oils from nuts, fruits, and beans. These plants are not grown as much as big oilseed crops, like sunflower or rapeseed, so the market is smaller and production cost for these non-traditional oils and higher. Plant oils can be extracted from oilseeds using different systems of pressing, solvent extraction, or a combination of both methods. Seeds with high oil content are pressed first then solvent extracted, or seeds with low oil content are directly solvent extracted. The extraction technology depends on the production cost, material traits, availability, use goal of the cake, and environmental factors (3, 4).

Cold pressing is the preferred way to get oil from oilseeds and fruits in recent years. Cold-pressed oils are not refined and only need centrifugation or filtration to get high-quality cold-pressed oils. Minor bioactive compounds that are usually lost during refining are kept in cold-pressed oils. Different vegetable cold pressed oils have potential biomedical uses (6). This way of oil production is also easy and cheap compared to other ways (1). Cold pressed oils are oils that are gotten by oil extraction without heat and chemical treatment, which may have more minor bioactive compounds, including natural antioxidants, than other refined oils (7, 8). These minor bioactive compounds that are naturally in cold pressed oils are phytosterols, phospholipids, toco-pherols, phenolic compounds, hydrocarbons (squalene), pigments (carotenoids and chlorophyll), (9,10,11). Minor fractions have an important role in the nutritional and health impact of edible oils (8).

2. OBJECTIVE

This paper aims to review the current state of knowledge on minor bioactive compounds in vegetable cold pressed oils, these are substances that are found in small amounts in vegetable cold pressed oils. They have various health benefits, such as antioxidant, anti-
inflammatory, and anticancer effects. They include phytosterols, tocopherols, phenols, carotenoids, and others. The paper also reviews the potential uses of cold pressed oils and their minor bioactive compounds for preventing and treating different diseases, such as cardiovascular diseases, diabetes, cancer, and skin disorders. The study also discusses the factors that affect the quality and stability of cold pressed oils, such as storage conditions, oxidation, and microbial contamination.

3. MATERIAL AND METHODS

This brief review is based on a comprehensive study of the current state of the art in the field. The materials used for this review include peer-reviewed articles collected through a systematic search of various databases including PubMed, Web of Science, and Google Scholar. Only those articles that provided a significant contribution to the understanding of the subject were included. The data from the selected articles were analyzed and synthesized to provide a comprehensive overview of the current state of the art. This review is subject to certain limitations. The findings are based on the available literature and may not include all relevant studies on the topic. Furthermore, the review is limited to articles published in English, which may exclude relevant studies published in other languages.

4. RESULTS AND DISCUSSION

The composition of minor bioactive compounds in plant cold pressed oils which can vary greatly depending on the source of the oil, dictates the therapeutic properties and potential uses of each oil. In the following section, we highlight the results of recent studies that have explored these benefits in depth, discussing the specific compounds found in various oils and how they contribute to health and wellness. We also discuss the implications of these findings for future research and potential applications in healthcare.

Specific minor bioactive compounds of cold-pressed oils

The health benefits of oils and its compounds are indeed determined by their composition. The profile of fatty acids, particularly omega-9, omega-6, and omega-3, along with high-value minor lipid compounds such as tococols, sterols, glycolipids, phospholipids, aroma compounds, and phenolics, have been shown to promote health and positively influence the biological functions of our body (12, 13). However, it’s important to note that the specific minor bioactive compounds found in cold-pressed oils are the ones that provide the most diverse positive effects on human health. These compounds, in their natural form and composition, can offer a range of health benefits that are not always found in other types of oils or food products.

Phytosterols

Phytosterols, also known as plant sterols, make up a significant portion of the unsaponifiable fraction of lipids (2). They can be found in vegetable oils either in their free form or esterified with fatty acids (14). Phytosterols are produced from the isoprenoid biosynthetic pathway via squalene from acetyl coenzyme-A. They are structural compounds of cell membranes and play a role in regulating membrane fluidity, permeability, and metabolism (15). Based on their chemical structure, phytosterols can be categorized into three groups: 4-desmethylsterols (cholestane series, i.e., normal phytosterols), 4-monomethylsterols (4-a-methylcholestane series), and 4,4-dimethylsterols (lanostane series, also known as triterpene alcohols) (14). Plant sterols, also known as 4-desmethylsterols, are unique in that they lack any methyl groups at the fourth position of the sterol ring structure (16). These phytosterols are part of the triterpenes family, which consists of a 27–30 carbon ring-based structure with hydroxyl groups. They closely resemble cholesterol in terms of both structure and biological function (17, 18). Phytosterols have a tetracyclic structure and a side chain in position C-17 (14, 16). Free phytosterols have a double bond in the B-ring between C-5 and C-6, or C-7 and C-8, also known as DS- and D7-sterols. The location of the double bond in the ring is specific to certain plant types (16).

Phytosterols that have a saturated ring structure, known as stanols, are found in nature but only in small amounts. The oxidation products of phytosterols in vegetable oils were identified and quantified by Dutta and Appelqvist (19). These include 7a and 7b hydroxy-sitosterol, 7-ketosito, and campesterol, 7-ketocampesterol, 5a, 6α-epoxy-sito- and campesterol, 5b, 6β-epoxy-sito and campesterol, and dihydroxysitosterol and dihydroxycampesterol. A study that looked at the contents of sterol oxidation products in cold pressed oils and refined oils from Polish markets found that the content of oxyphytosterols in refined oils was 2–2.5 times higher than in cold pressed oil. Epimers of 7-hydroxy-phytosterols and 7-keto-phytosterols were the main compounds in cold pressed oil, while epoxy derivatives were the main compounds in refined oil (20).

Vegetable oils, seeds, and nuts are the richest sources of phytosterols. The three most prevalent sterols are beta-sitosterol (29 carbons), campesterol (28 carbons), and stigmasterol (29 carbons) (14-16). The total phytosterol content and its composition can vary based on factors such as variety, farming and weather conditions, maturity, extraction and refining methods, and conditions before extraction and storage (21-23, 14, 24).

Numerous studies have shown that phytosterols can help prevent many chronic diseases such as cardiovascular diseases (15, 25), cancer (26, 27), ulcers (28), diabetes (29), and inflammation (30,31). Phytosterols have the ability to lower dietary cholesterol absorption in the intestine and serum low-density lipoprotein-cholesterol levels. Some studies suggest that consuming 2 g/day of sterol or stanols could reduce the risk of heart disease by 25% (15, 32, 33).

Phytosterols have been found to have a protective
effect against various types of cancer such as breast (30), prostate (34), lung (14), liver and stomach (35), and ovary and colon cancers (36). In vivo studies have demonstrated that diets enriched with phytoestrogens (2%, w/w) helped improve lipid profiles and reduced atherosclerotic lesions in apolipoprotein E-knockout (apo E-KO) mice (14, 37). Raicht et al. reported that the development of methyl nitrosourea induced tumors in mice was reduced when they were fed with 0.2% b-sitosterol in their diet for 28 weeks (38). The results showed a 39% reduction in overall tumor numbers and a 60% reduction in tumors per rat.

**Phospholipids**

Phospholipids or a cell membrane lipids are a minor but important group of lipids in seed oils, are highly abundant structural and functional lipids found in cell membranes (39,40). Major phospholipids are derivatives of glycerol, where the 1- and 2- positions are acylated by fatty acids while the 3-positions are esterified with phosphoric acid (41). The most common types of phospholipids are lecithin, cephalin, phosphatidylinositol, sphingomyelin, and phosphatidic acid. Lecithin, cephalin, and phosphatidylinositol are similar to triglycerides, except one fatty acid is replaced by phosphoric acid (42).

Phospholipids have the ability to act as surfactants and emulsifiers, which help to form and stabilize oil-water mixtures. PL are usually removed from the oil during the extraction process, because they can reduce the oil yield by forming emulsions (42). Phospholipids are found in different amounts in different seed oils, ranging from 1%–2% in soybean or corn oils to 0.3%–0.4% in peanut oil. Phospholipids can also enhance the oxidative stability of fats and oils by working together with other antioxidants (41). Phospholipids have been shown to have positive effects on human health (40). For example, phospholipids can provide arachidonic acid, which is involved in various metabolic processes, such as the synthesis of inflammatory mediators (39).

**Tocols (tocopherols and tocotrienols)**

Tocols, (Tocopherols and tocotrienols), are lipophilic antioxidants found naturally in vegetable oils that guard against oxidation (43). These compounds, which include a chromanol ring and a C16 phytol side chain, are divided into two categories: tocopherols, which have a saturated side chain, and tocotrienols, which have three double bonds at carbons 3, 7, and 11 (44). Both tocopherols and tocotrienols come in four isomers: alpha, beta, gamma, and delta, distinguished by the methylation pattern of the benzopyran ring with three methyl groups (at C-5, C-7, and C-8) (45). Among the tocopherols, alpha and gamma tocopherols are the most potent lipid-soluble antioxidants in vegetable oils. While alpha-tocopherol exhibits the highest vitamin E activity, gamma-tocopherol demonstrates the highest antioxidant activity (45).

In addition to alpha-tocopherol, vegetable oils also contain other tocopherols, particularly gamma and delta-tocopherol (46). Soybean and corn oils are typically rich in gamma-tocopherol, while olive oil predominantly contains alpha-tocopherol (47). Tocols are capable of neutralizing singlet oxygen and free radicals, especially lipid peroxyl radicals of polyunsaturated fatty acids (PUFA), thereby halting lipid peroxidation chain reactions (48). Tocols also play a crucial role in maintaining oil stability due to their strong antioxidant properties (49). Their biological effects at the molecular level have been linked to a reduced risk of numerous diseases such as cardiovascular diseases, anti-inflammatory and anti-diabetic effects, osteoporosis, hyperlipidemia, neurodegenerative diseases, and cancer (50,51). Vitamin E is also essential for immune defense. It has been proposed that tocopherols, acting as hormones or as secondary donors of genetic information, regulate the expression of certain genes (52).

A deficiency in Vitamin E can lead to the degradation of cell membranes due to the oxidation of unsaturated fatty acids in lipids. This deficiency can also manifest as muscle pain and progressive muscular disorders (52). The presence of tocols in oilseeds can be identified using various analytical techniques such as gas chromatography, liquid chromatography with diode array detection, thin-layer chromatography with evaporative light scattering detection, and liquid chromatography-mass spectrometry (51). The most abundant sources of tocopherols in our diet are vegetable oils and their derivatives. The amount of tocopherol in seed oils can vary, ranging from 2 to 8mg per 100g in coconut oil to 113 to 183mg per 100g in corn oil (52).

**Phenolic compounds**

Phenolic compounds, which are abundant secondary metabolites in plants, are substances that contain a phenol function (53). The most basic phenolic compound is benzene, which has one hydroxyl group (54). Phenolic compounds share a common chemical structure that includes an aromatic ring with one or more hydroxyl groups and can be categorized into several classes such as flavonoids, phenolic acids, tannins, stilbenes, and lignans (53). Phenolic compounds are significant due to their health benefits, effects on taste, odor, pigment formation, antioxidant and antimicrobial effect, enzyme inhibition, and control criteria in various foods (55; 53). Numerous studies have shown that phenolic compounds have a variety of effects such as antioxidant, antimicrobial, anticarcinogenic, antiinflammatory, and estrogen-related prevention of cardiovascular diseases, cancers, diabetes, and diseases associated with oxidative stress (53, 56-58).

Phenolic compounds have the ability to disrupt the radical chain reaction by donating a hydrogen atom to free radicals, thereby transforming themselves into a radical. In addition to this, phenolics can also function as metal chelators and oxygen scavengers. During the refining process, a certain amount of phenolics are removed, resulting in a higher concentration of phenolic compounds in cold-pressed oil compared to refined
oil (59). Natural olive oil is recognized for its stability, which is attributed to its high phenolic compounds content.

**Squalene**

Squalene, with a molecular formula of C30H50, is created by the fusion of six isoprene radicals, resulting in symmetry at the midpoint of the squalene molecule (60). This triterpene hydrocarbon, commonly found in nature, serves as a precursor to cholesterol and other steroids (61). It constitutes the majority of hydrocarbons in the unsaponifiable fraction of olive oil (62). While olive oil contains between 0.2% and 0.7% squalene, other oils contain between 0.02% and 0.03% (63). After ingestion, squalene is typically stored under the skin, making it useful as a moisturizer and emollient in cosmetics. It also has cell-protective properties against free radicals as a potential oxidation inhibitor (64). Squalene has been found to inhibit the activity of beta-hydroxy-beta-methylglutaryl-CoA (HMG-CoA) reductase, a key player in cholesterol synthesis. This displacement of the cell membrane reduces the risk of larynx, colon, and pancreatic cancers (65). One study suggested that daily consumption of a certain amount of olive oil reduces the risk of breast cancer in women by 25% (66).

In a study examining the effects of squalene on skin, colon, and lung cancer in mice, it was reported that daily consumption of squalene has antitumor, antibacterial, and anticarcinogenic effects. Researchers have also suggested that squalene plays a significant role in eye health, particularly for the rod photoreceptor cells of the retina (67).

**Pigments**

The concentration of pigments in cold-pressed oils surpasses that in refined oils (68). The primary pigments present in cold-pressed oils are carotenoids and chlorophyll (69).

**Carotenoids**

The distinctive yellow-red hue observed in the majority of vegetable oils can be attributed to the various carotenoid pigments present. Carotenoids, which are isoprenoid elements, are formed by the union of two C20 (geranylgeranyl diphosphate) molecules, typically consisting of 40 carbon (C40) atoms. These lipophilic compounds occur in acyclic, monocyclic, and bicyclic forms (70). The molecular skeleton of carotenoids is formed by carbon atoms linked by alternating single or conjugated dual bonds (71). Broadly, carotenoids can be categorized as terpenic alcohols, hydrocarbons, and organic acids. While a significant portion of carotenoids is found in the hydrocarbon structure, the remainder is present in the alcohol structure (69).

Hydrocarbon carotenoids are distinguished by the absence of oxygen in their structure. The most notable of these carotenoids is carotene (C40H56) (69, 72). Carotene has three isomers in nature: a, b, and g. a and b-carotenes usually coexist in nature. All three carotenes are physiological precursors of vitamin A (73). Another hydrocarbon carotenoid is lycopene, which gives tomatoes their red color (69, 70). The main carotenoid alcohols are lutein (xantho-phylls), cryptoxanthin, and rubixanthin. Lutein is a dioxi derivative of a-carotene that exhibits optical activity (69, 72).

The colors of carotenoids are due to the polienic conjugated bonds called chromophore structure (69). Carotenoids need to have at least seven conjugated double bonds to show a noticeable light yellow color. Therefore, poliens with fewer conjugated double bonds are not technically considered as carotenoids (71).

Carotenoids, as natural colorants, typically impart yellow, red, and orange hues to the foods they are found in. Specifically, a-carotene, b-carotene, lutein, and zeaxanthin contribute to a yellow color, lutein adds a greenish-yellow tint, and lycopene imparts a red color (74). Crude palm oil is known to have the highest carotenoid content, ranging from 500–700mg/kg oil. The carotenoid content of other crude vegetable oils is less than 100mg/kg oil (75).

**Chlorophyll**

Chlorophyll, a compound in the porphyrin group, is structured around four pyrrole units linked by methine bridges. Porphyrins are known for their ability to chelate with metal ions, and their color is determined by the compounds in the ring structure and the structure of the central atom. In chlorophyll, this central atom is magnesium (76). The chlorophyll pigment, which has a magnesium atom at its core, is made up of chlorophyll a and chlorophyll b (77). The structural difference between these two types of chlorophyll is that chlorophyll a has an additional oxygen atom and two fewer hydrogen atoms than chlorophyll b (69). The green color in all plant tissues is due to the presence of the chlorophyll pigment (76). Certain oils, such as early harvest olive oil, frost-exposed seed oils, rapeseed, and soybean oil, exhibit a green color due to the presence of chlorophyll and similar compounds (69).

However, chlorophyll is sensitive to temperature and can lose its magnesium during heat treatment, resulting in the formation of pheophytin and a change in color to brown-yellow (69). Factors such as oxygen, heat, light, and enzymes can easily break down the chlorophyll molecule (78). The degradation of chlorophylls occurs at a faster rate than that of carotenoids (79).

Chlorophyll a and b, present in concentrations of 1–10 ppm, give olive oil its greenish hue, while the brown color of the oil is attributed to the breakdown products of chlorophyll a and b, present in amounts of 0.2–24 ppm (69). Of all the types of chlorophyll, pheophytin, which constitutes 70%–80% of the total, is the most abundant and can be found in olive oil and other vegetable oils (62). Both chlorophyll and pheophytin can act as pro-oxidants in the photooxidation of oils (80). In the presence of light and O2, chlorophyll can transform into pheophytin. However, in the absence of light, it exhibits antioxidant activity in conjunction with phenolic antioxidants, playing a crucial role in maintaining the stability of olive oil (81, 82).
5. CONCLUSION

This paper highlights the importance of cold-pressed oils as a source of various minor bioactive compounds that have the potential to promote human health and prevent or manage a range of diseases. The composition of cold-pressed oils, including phytosterols, tocopherols, phenols, carotenoids, in their natural form and composition, offer diverse positive effects on human health, such as antioxidant, anti-inflammatory, and anticancer properties. It is evident that the retention of these minor bioactive lipids during the cold-pressing process distinguishes these oils from their refined counterparts, making them an attractive choice for individuals seeking natural and health-promoting food options.

The findings presented in this paper serve as a valuable resource for medical professionals in the field of integrative medicine, nutrition, and dietetics, as well as for consumers looking to make informed choices about their dietary and health needs. Further research in this area is warranted to uncover the full extent of the health benefits associated with the consumption of cold-pressed oils and their minor bioactive compounds.

- **Author’s contribution:** The authors were included in all processes of preparing this article. Final proofreading was made by the first author.
- **Conflict of interest:** None declared.
- **Financial support and sponsorship:** This study was supported by the Provincial Secretariat for Higher Education and Scientific Research, project name: "The potential and perspectives of cold-pressed oils in modern nutrition”, Grant No. 142-451-322/2023-01.

REFERENCES


45. Boschin G, Arnoldi A. Legumes are valuable sources of tocopherols, tocotrienols and tocomonoenols: Many similar molecules but only one vitamin E. Redox biology. 2019 Sep 1; 26:101259.


48. Aszi A. Tocopherols, tocotrienols and tococomonoenols: Many similar molecules but only one vitamin E. Redox biology. 2019 Sep 1; 26:101259.


