POTENTIAL USE OF COLD PRESSED BLACK SEED OIL IN PRODUCING NOVEL FOOD AND FUNCTIONAL FOOD

ПОТЕНЦИЈАЛНА УПОТРЕБА НА ЛАДНО ЦЕДЕНО МАСЛО ОД ЦРНО СЕМЕ ВО ПРОИЗВОДСТВОТО НА НОВА И ФУНКЦИОНАЛНА ХРАНА

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Abstract: *Black seed oil (Nigella sativa oil) is obtained through the extraction of seeds from the Nigella sativa plant, also known as "black seed" or "black cumin," and grows in countries around the Mediterranean Sea, as well as in Pakistan, India, and Iran. Due to its richness in bioactive substances, NSO is used in traditional medicine to treat various ailments and shows significant medical and pharmacological properties. Nigella sativa oil is considered a promising trend for producing products with greater functional benefits. In recent decades, the biological properties and medicinal aspects of NSO have been studied, particularly the active compounds such as thymoquinone, thymohydroquinone, ρ-cymene, carvacrol, and t-anethole, which exhibit antioxidant, antimicrobial, antifungal, anti-inflammatory, anticancer, antidiabetic, antihypertensive, and hypolipidemic activities with low toxicity. These properties make NSO useful for food applications, such as improving sensory characteristics, stability, and shelf life of various products like bread, baked goods, and other functional food items.*
However, its use in the food industry is limited due to its low water solubility, high volatility, and specific organoleptic properties, which may make the product unacceptable if used in higher concentrations. To successfully incorporate NSO into food products, innovations in stabilization processes and improving its bioavailability and organoleptic properties are needed.
The aim of this paper is to provide an overview of NSO and its application in the food industry, as well as to explore its biological activities and potential industrial uses of NSO as an ingredient in food products.

Key words: *Nigella sativa, cold-pressed oil, bioactive compounds, food industry, antioxidant.*

Апстракт: *Маслото од црно семе (Nigella sativa oil) се добива со екстракција од семките на растението Nigella sativa, познато и како "црно семе" или "црн ким", и расте во земјите околу Средоземното Море, во Пакистан, Индија и Иран. Поради богатството со биоактивни супстанции, NSO се користи во традиционалната медицина за лекување на различни заболувања и покажува значајни медицински и фармаколошки својства. Маслото од Nigella sativa се смета за ветувачки тренд за добивање на производи со поголеми функционални придобивки. Во последните децении, истражени се биолошките својства и медицински аспекти на NSO, посебно на активните соединенија во неговиот состав, како што е тимокинон, тимохидрокинон, ρ-цимен, карвакрол и t-анетол кои покажуваат антиоксидативни, антимикробни, антифунгални, антиинфламаторни, антиканцерогени, антидијабетични, антихипертензивни и хиполипидемични активности со ниска токсичност, што го прави корисно и за прехранбени апликации. Во прехранбената индустрија, NSO ги подобрува сензорните карактеристики, стабилноста и рокот на траење на различни производи*

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како што се леб, пекарски производи и други функционални намирници. Сепак, неговата примена е ограничена поради ниската растворливост во вода, високата испарливост и специфичните органолептички својства, кои може да го направат производот неприфатлив доколку се користи во поголеми концентрации.
За успешно вклучување во прехранбените производи, потребни се иновации во процесите за стабилизација и подобрување на неговата биорасположивост и органолептички својства. Целта на овој труд е да даде преглед на NSO и неговата примена во прехранбената индустрија, како и да ги разгледа биолошките активности и потенцијалните индустриски примени на NSO како состојка во прехранбени производи.

Клучни зборови: *Nigella sativa, ладноцедено масло, биоактивни соединенија, прехранбена индустрија, антиоксиданси.*

1. INTRODUCTION

Nigella sativa is an annual flowering plant from the Ranunculaceae family, which originates and is cultivated in South and Southwestern Asia, but it is also grown in Mediterranean countries, Central Europe, and Western Asia (Hassanien et al., 2015; Mazaheri et al., 2019; Fidan et al., 2019; Alu'datt et al., 2024). The fruit of the plant is a spherical capsule containing numerous black seeds with a bitter and aromatic taste (Rahim et al., 2022).

In traditional medicine, black seed is considered a miraculous herb and is used to treat various disorders and diseases (Ahmad et al., 2013), including headaches, respiratory diseases, diabetes, hypertension, male infertility, paralysis, infections, and digestive system diseases. Additionally, it is used to strengthen the immune system and as a remedy for coughs (Hassanien et al., 2015; Mazaheri et al., 2019; Mukhtar et al., 2019; Jufri et al., 2022). Furthermore, Nigella sativa shows analgesic, antipyretic, contraceptive, anti-oxidative, antitussive, and anti-inflammatory properties (Jufri et al., 2022). The mechanism of its anti-inflammatory action involves the inhibition of pro-inflammatory cytokines, interleukin 1-beta (IL-1β) and interleukin 6 (IL-6) (Rahim et al., 2022). The oil of *Nigella sativa* is considered a potential therapeutic supplement for autoimmune disorders, offering a natural and promising approach for the treatment of psoriasis, arthritis, systemic lupus, ankylosing spondylitis and type 2 diabetes (Saifullah, 2024). Avicenna referred to black seed as "the canon of medicine" due to its ability to stimulate the body's energy and contribute to recovery from fatigue and discomfort (Ahmad et al., 2013). Besides its use in medicine, *Nigella sativa* seeds have traditionally been used as a spice and natural preservative in various food products, including yogurt, pickles, sauces, and salads, especially in the bakery industry and cheese production (Hassanien et al., 2015; Kiralan et al., 2015). Its nutritional value is significant, as it contains an appropriate amount of proteins and fats, as well as essential fatty acids, vitamins and minerals (Hannan et al., 2021).

Cold-pressed black seed oil (BSO) is known for its antioxidant properties and health benefits. This oil contains a wide range of bioactive compounds with significant health improving effects and is used in various food applications (Kiralan et al., 2020). Lipid oxidation poses a significant challenge in the food industry, especially in unsaturated and polyunsaturated fatty acids (PUFAs), which are prone to degradation (Ramadan et al., 2011). Oxidation of these lipids can occur through photooxidation, autoxidation, or enzyme-catalyzed oxidation (Sun et al., 2011).

2. CHARACTERISTICS AND COMPOSITION OF NIGELLA SATIVA

Cold-pressed black seed oil contains fats in the range of 26–34%, of which 0.4% – 2.5% are essential oils, proteins (26%), carbohydrates (25%), dietary fibers (8.4%), alkaloids, and saponins (Hassanien et al., 2015; Zeyada et al., 2023). *Nigella sativa* seeds mainly consist of essential (volatile) and fixed (stable) oils, which are responsible for the health benefits of this plant. The

fixed oil contains significant amounts of unsaturated fatty acids such as linoleic and oleic acids, while saturated fatty acids such as arachidonic and eicosanoic acids are present in smaller quantities, along with thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone) (Mukhtar et al., 2019). The oil from black seed exhibits pharmacological potential, including antioxidant, antifungal, and antimicrobial activities (Kiralan et al., 2013; Hassanien et al., 2015). Antimicrobial activity of essential oils obtained from spices and culinary herbs originates from their phenolic compounds (Hassanien et al., 2015; Liao et al., 2020). Thymoquinone and melanin are among the most important active ingredients of *Nigella sativa* and are responsible for its antioxidant and antibacterial effects against a wide range of pathogens and spoilage bacteria, such as *Staphylococcus aureus, Micrococcus luteus, Listeria monocytogenes, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Enterococcus faecalis, Escherichia coli* and *Klebsiella pneumoniae* (Mahros et al., 2020).

Medicinal plants are rich with phytochemicals, such as phenols, proteins, and peptides are recognized for their potential application as functional food products (Alu'datt et al., 2024). The correlation between total phenolic compounds and the oxidative stability of oils is an important factor for their quality, as it is related to shelf life, sensory quality, and resistance to autoxidation (Mazaheri et al., 2019). The phenolic compounds in *Nigella sativa* seeds contribute to its stabilizing, antioxidant, and antimicrobial properties, which help extend the shelf life of food and beverages (Mohammed et al., 2021). Natural antioxidants are essential in human nutrition because they reduce the presence of free lipid radicals in food and the body after ingestion (Mohammed et al., 2021). Although phenolic compounds play a key role in the antioxidant capacity of oils, research by Kiralan et al. (2015) didn't found correlation between total phenols, tocopherols, and the oxidative stability of oils (Kiralan et al., 2015).

Black seed oil contains significant amounts of carbohydrates, amino acids and proteins. Its nutritional value includes 84 g of fiber, 216 g of protein, 45 g of ash, 38 g of moisture, 406 g of fats, 249 g of free nitrogen extract, as well as minerals such as 60 mg zinc, 105 mg iron, 527 mg phosphorus, 15.4 mg thiamine, 18 mg copper, 57 mg niacin, 0.16 mg folic acid, and 1860 mg calcium per kg (Rahim et al., 2022). Among vitamins, the most prevalent are vitamin C (ascorbic acid), vitamin E (tocopherol), vitamin B1 (thiamine), B6 (pyridoxine), niacin, folic acid, and B2 (riboflavin) (Rahim et al., 2022).

Triacylglycerols are the main components of oils and fats, making up 96–98% of their composition. The dominant triacylglycerols (TAGs) in black seed oil are tri-linoleoyl, oleoyl-di-linoleoyl, palmitoyl-di-linoleoyl, palmitoyl-oleoyl-linoleoyl, and dioleoyl-linoleoyl (Mazaheri et al., 2019). Fatty acid profiling shows the presence of saturated (22.7% – 25.5%) and unsaturated fatty acids (74.8% – 77.5%) (Mazaheri et al., 2019). *Nigella sativa* oil contains high levels of linoleic acid (61.6%), oleic acid (22.8%), palmitic acid (12.4%), stearic acid (3.5%) and α-linolenic acid (0.4%). The fatty acid composition depends on the quality of the seed, ecological conditions and extraction method (Zeyada et al., 2023). Oil obtained by the Soxhlet method contains high concentrations of unsaturated fatty acids (85.16%) and saturated fatty acids (15.02%), with linoleic acid (57.71%) and oleic acid (24.46%) being dominant (Albakry et al., 2022). Cold-pressed black seed oil is categorized as a functional oil due to its high content of omega-9 (oleic, 15–24%) and omega-6 (linoleic, 54–70%) fatty acids (Rahim et al., 2022). Based on its fatty acid composition, it falls into the category of oils with a high content of linoleic acid, similar to sunflower oil, corn, grape seed, and sesame oil (Mazaheri et al., 2019). The essential oils of black seed contain bioactive components such as p-cymene, thymoquinone, α-thujene, longifolene, β-pinene, α-pinene, and carvacrol, which are considered responsible for its antimicrobial potential (Hassanien et al., 2015; Mohammed et al., 2021). These compounds have antioxidant, antibacterial, and anti-inflammatory properties with low toxicity and are useful for food applications (Liao et al., 2020; Mohammed et al., 2021).

2.1. Extraction Methods

Extraction methods for essential oil that can be applied to *Nigella sativa* seeds include hydrodistillation (steam distillation), solvent extraction, supercritical fluid extraction (CO2) and cold pressing (Mohammed et al., 2021). The cold-pressing method is becoming a popular alternative to conventional practices due to consumer demand for natural and safe food products. Cold pressing is a technology that don't involves heat or chemical treatments during the oil extraction process. This method does not include refining and can preserve high levels of lipophilic phytochemicals, including antioxidants and thymoquinone derivatives (Lutterodt et al., 2010; Kiralan et al., 2015). Cold-pressed oils are important sources of essential fatty acids, phenols, sterols, tocopherols and carotenoids, possessing health-promoting properties. Additionally, they retain their characteristic taste, aroma and color and can be classified as natural products. Cold pressing avoids the degradation of thermosensitive compounds and oils obtained by this method contain more bioactive compounds, including pro-oxidants (free fatty acids, hydroperoxides, chlorophylls and carotenoids) and antioxidants (tocopherols, phenols, and polar lipids) (Kiralan et al., 2017; Kiralan et al., 2019). The stability of cold-pressed oils is typically from 6 to 12 months and depends on the level of polyunsaturated fatty acids (PUFAs) and present antioxidants (Kiralan et al., 2017).

2.2. Stability of BSO

Cold-pressed black seed oil (BSO) is unrefined and rich in lipophilic phytochemicals such as natural antioxidants and thymoquinone derivatives (Lutterodt et al., 2010). Due to its minimal processing, cold-pressed oils may contain components with prooxidant effects, including metal ions, chlorophylls, and lipid peroxides (Grajzer et al., 2020). Unlike refined oils, cold-pressed oils often exhibit a higher potential for auto-oxidation and less predictable oxidative stability (Grajzer et al., 2020). The quality and stability of vegetable oils are influenced by factors such as growing conditions, raw material quality, pressing method and storage conditions (Cichocki et al., 2023). Lipid oxidation is one of the main changes that can occur during the processing, marketing, and storage of food (Hassanien et al., 2015). Autoxidation is a non-enzymatic process between unsaturated fatty acids and oxygen, influenced by factors such as fatty acid composition, degree of unsaturation, presence of antioxidants, and storage conditions (Colakoglu et al., 2006). Cold-pressed oils are considered more favorable than refined oils due to the presence of bioactive substances like phenols, tocopherols, polyphenols and squalene, which can help delay lipid oxidation (Grajzer et al., 2020). The antioxidant activity of phenolic compounds arises from their high reduction potential (Viuda-Martos et al., 2011). According to the analysis of Symoniuk et al. (2022) and Kiralan (2013), black seed oil demonstrated resistance to oxidation under accelerated conditions (60°C and 100°C). The high resistance of NSO at 60°C and 100°C is attributed to stable compounds like thymoquinone, α-longipinene, carvacrol and 4-terpineol (Mazaheri et al., 2019).

Lipid oxidation results in the formation of lipid hydroperoxides through the replacement of a hydrogen atom in the lipid hydrocarbon chain with a hydroperoxide group. Once formed, hydroperoxides are susceptible to further oxidation, leading to the formation of secondary reaction products such as aldehydes, ketones, acids and alcohols. Many of these secondary products negatively impact the taste, aroma, flavor, nutritional value, and overall quality of food (Turner, 2013). During auto-oxidation, oxygen interacts with unsaturated lipids, limiting the use of unsaturated lipids in functional foods (Sun et al., 2011). Factors influencing oxidation include the presence of oxygen, light, heat, metals, and the degree of unsaturation of lipids, with PUFAs being especially sensitive (Ramadan et al., 2011).

Unlike refined oils, cold-pressed oils often exhibit higher initial auto-oxidation rates and less predictable oxidative stability. Pro- and antioxidant interactions can influence the antioxidant activity of the oil, which in turn affects its shelf life. Numerous methods have been developed to assess the antioxidant capacity of oils. Among them, the ability of antioxidants in oil to reduce the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (DPPH analysis) is commonly used to determine the radical-scavenging activity of hydrophilic and lipophilic antioxidants in oils (Grajzer et al., 2020). The radical-scavenging capacity of oils strongly depends on the content of tocopherols and other polyphenols (Grajzer et al., 2020). In Grajzer's study, it was noted that the tocopherol content in cold-pressed oils negatively correlates with their antioxidant capacity, while the content of phytosterols showed a positive correlation with the radical-scavenging capacity in the lipophilic fraction of cold-pressed oils rich in n-3 polyunsaturated fatty acids (PUFAs) (Grajzer et al., 2020). Antioxidants can slow down this reaction by neutralizing free radicals or binding metals. Oxidation control is achieved by adding antioxidants, optimizing processing and storage and using techniques such as vacuum packaging and inert gases (Ramadan et al., 2011). The most commonly used primary antioxidants in food include synthetic compounds such as phenolic antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ) (Sun et al., 2011).
The selection of natural antioxidants that could be used to extend the shelf life of oils is limited and depends on several factors, such as the fatty acid profile of the oil, the minor components and the structure of the antioxidants. Food producers are increasingly interested in finding natural alternatives to synthetic antioxidants that not only slow down oxidation processes but also have a positive impact on consumer health (Odeh et al., 2021).

Although the auto-oxidation process cannot be completely prevented, it can be delayed and minimized by adding antioxidants that donate hydrogen atoms to free radicals, thus leading to stable products (Köckritz & Martin, 2008; Moigradean et al., 2014). Today, natural antioxidants from herbs and spices are increasingly used for toxicological reasons, including rosemary, garlic, parsley, black seed, thyme and others (Temelkovska & Pavlovska, 2021). The use of various synthetic antioxidants in food products is limited due to potential health risks and toxicity. The most commonly used antioxidants, BHT and BHA, have been shown to induce DNA damage (Wangensteen et al., 2004).

Phenolic compounds, ascorbic acid, carotenoids, phospholipids, sterols, and others are natural antioxidants found in food (Embuscado, 2015). Herbs and spices, whether whole or ground, along with their extracts and essential oils, are also used in inhibiting lipid oxidation and microbial growth in food, and are crucial in reducing the formation of harmful substances such as heterocyclic amines (HCAs). Their use results in clean-label products, entirely natural and GMO-free, which are desirable qualities for consumers (Embuscado, 2015).

3. USE OF BSO IN THE FOOD INDUSTRY

Cold-pressed black seed oil (BSO) has been used as an additive in meat due to its antibacterial and antioxidant properties, which improve the safety, quality, and shelf life of meat. This was investigated in a study by Mahros et al. (2020), which aimed to examine the effects of black seed oil and black seed powder during meat storage. The best antibacterial effect was achieved with the addition of 3% BSO, which significantly reduced the colony count and psychotropic bacteria during the first week of storage and BSO caused a reduction of *Escherichia coli* (Mahros et al., 2020). The study by Wojtasik-Kalinowska et al. (2017) demonstrated a positive effect of adding BSO on the fatty acid profile of meatballs made with pork. In samples with added oil, there was a 60% slower lipid oxidation compared to control samples, without changes in the sensory properties of the meatballs (Wojtasik-Kalinowska et al., 2017).

In order to produce a low-fat, dairy-free cream, BSO extracted by supercritical fluid technique was used. The sensory evaluation of the obtained coffee cream showed high sensory acceptability and the high potential of microencapsulated oil for mass production and commercialization of dairy-free functional cream (Mohammed et al., 2019). The addition of encapsulated oils from *Moringa oleifera* and *Nigella sativa* in yogurt affected its viscosity, pH, acidity and microbiological properties. There was absence of harmful bacteria and the sensory evaluation showed that both oils, used at a concentration of 4%, improved the taste and texture (Elshiekh & Omar, 2024). The addition of essential black seed oil in Feta cheese reduced the number of bacteria and fungi compared to the control sample and affected the sensory characteristics of the cheese during a 90-day storage period. The results also showed that the addition of 0.5% essential *Nigella sativa* oil prevented microbial growth and oxidative degradation, but also improved the taste and overall acceptability of the cheese (Al-Kaabi et al., 2024). Incorporating black seed oil into Edam cheese reduced the number of yeasts and inhibited the growth of coliform groups, while proteolytic bacteria were found in larger numbers than lipolytic bacteria. Panelists accepted the taste, appearance and aroma of the Edam cheese with the addition of 0.6% black seed oil (Elmontaleb et al., 2020).The goal of the study by Mohamed et al. (2020) was to develop an ice cream product enriched with nanoemulsion of Nigella sativa oil in four ratios (0% control, 3%, 5%). Improvements in rheological and sensory properties were observed and the ice cream enriched with 5% nanoemulsion of BSO was the most preferred form of the four formulations (Mohamed, 2020).

The impact of natural oils from black seed, cumin, thyme and wheat on bread was investigated in relation to dough and bread quality. The use of oils resulted in a reduction of dough consistency and the greatest influence on the extension of dough development time was observed in bread enriched with thyme, cumin and black seed oils (Debonne et al., 2018). Results from a study aimed at developing enriched biscuits by incorporating BSO as a nutrient showed that the product with 6% added BSO had the most acceptable sensory attributes, physical and nutritional quality (Bornare et al., 2015). A chickpea paste (hummus), enriched with BSO at a concentration of 6%, showed increased sensory acceptability compared to the control sample and other samples with the addition of cold-pressed black seed oil at concentrations of 4%, 8%, and 12% (Delinikolova & Jankuloska, 2025).

BSO can also be used to improve the oxidative stability of other vegetable oils. For example, the addition of black seed oil to sunflower oil improved its oxidative stability (Mazaheri et al., 2019). To increase the oxidative stability and phytochemical content of rapeseed oil, blends with 5%, 10%, and 20% rice bran oil and black seed oil were prepared. The blends of rapeseed oil with BSO showed higher levels of α- and γ-tocopherols and tocotrienols, a reduction in the PUFA/SFA ratio, an increase in the omega-6/omega-3 ratio from 2.1 to 3.7, and improved stability (Rudziń ska et al., 2015).

Improved oxidative stability and taste were observed in mayonnaise enriched with BSO. Four different formulations of mayonnaise were used (control, and samples with 5%, 10%, and 20% BSO). After four weeks of storage at 20°C, the peroxide values were lower and sensory analysis showed better acceptability for the mayonnaise with 5% BSO compared to the control sample (Ozdemir et al., 2018). The results of the effect of essential black seed oil on butter stability showed that it could be considered a source of natural antioxidant. In the samples with 0.05%, 0.1%, and 0.2% BSO and the sample with BHT (100 ppm), the addition of 0.2% BSO showed strong antioxidant activity, nearly equal to that of BHT, and a reduction in the total number of aerobic mesophilic bacteria, lactic acid bacteria, and coliform bacteria during storage. The samples with added essential oil were preferred by panelists compared to the control sample (Çakmakçi et al., 2014).

4. CONCLUSION

Cold-pressed black seed oil is a rich source of bioactive compounds, including p-cymene, thymoquinone, α-thujene, longifolene, β-pinene, α-pinene, and carvacrol. The oil has antioxidant potential and demonstrates various biological activities, including antimicrobial, antifungal, and antibacterial properties. The benefits of cold-pressed oil are of particular health interest when used in stable production formulations.

Black seed has recently become an important research topic worldwide. Cold- pressed black seed oil has been registered as a novel food in the register of food produced with innovative technologies, and given that, its potential for implementation in food products is even greater. However, its application in food products remains modest and therefore further research is needed to explore new possibilities for its incorporation into novel and functional foods

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