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Physicochemical, Antioxidant, Antimicrobial, and Sensory Characteristics of Selected Kinds of Edible Oils

Eva Ivanišová [1,2], Veronika Juricová [1], Július Árvay [1], Miroslava Kačániová [3], Matej Čech [1], Zbigniew Kobus [4], Monika Krzywicka [4,*], Wojciech Cichocki [5] and Katarzyna Kozłowicz [6]

[1] Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia; eva.ivanisova@uniag.sk (E.I.); veronika.juricova@gmail.com (V.J.); julius.arvay@uniag.sk (J.Á.); matej.cech@uniag.sk (M.Č.)
[2] Food Incubator SPU v Nitre s.r.o, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia
[3] Institute of Horticulture, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture in Nitra, 949 76 Nitra, Slovakia; miroslava.kacaniova@gmail.com
[4] Department of Technology Fundamentals, University of Life Sciences in Lublin, 28 Głęboka, 20-612 Lublin, Poland; zbigniew.kobus@up.lublin.pl
[5] InnPlantFood Research Group, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland; wojciech.cichocki@up.poznan.pl
[6] Department of Biological Bases of Food and Feed Technology, University of Life Sciences in Lublin, Głęboka 28, 20-612 Lublin, Poland; katarzyna.kozlowicz@up.lublin.pl
* Correspondence: monika.krzywicka@up.lublin.pl

Abstract: The aim of this study was to determine the peroxide values, acid numbers, oxidative stability (Rancimat method), antioxidant activity (DPPH method), antimicrobial activity (disc diffusion method), sensory properties (9-point hedonic scale), and fatty acid profiles (FAME) of five edible oils purchased from local Slovakian producers—grape seed oil, flax seed oil, walnut oil, poppy seed oil, and milk thistle seed oil. The peroxide value ranged from 2.27 (milk thistle oil) to 8.51 (flax seed oil) mmol O_2/kg. All these values were in accordance with regulations (upper limit of 20 mmol O_2/kg). The values of the acid number ranged from 0.11 (walnut oil) to 2.49 (milk thistle oil) mg KOH/g, and were in accordance with regulations as they did not exceed the value of 4 mg KOH/g. The oxidation stability was the lowest in flax seed oil (0.18 h) and the highest in grape seed oil (2.05 h). In milk thistle oil, the highest amounts of oleic and behenic acids, in flax seed oil, the highest amount of α-linolenic acid, and in grape seed oil, the highest amount of linolic acid were determined. Antioxidant activity was the strongest in the sample of grape seed oil—65.53 mg TEAC/L (Trolox equivalent antioxidant capacity). Samples of flax seed oil showed the strongest inhibition of *Candida albicans* CCM 8186 (4.58 mm) and *Bacillus subtilis* CCM 2010 (0.31 mm). Poppy seed oil was determined to be the most inhibiting towards *Klebsiella pneumoniae* CCM 2318 (3.68 mm). Milk thistle oil showed the strongest inhibition of *Clostridium perfringens* CCM 4435 (6.31 mm). Grape seed oil was the most inhibitory towards *Staphylococcus aureus* subs. *aureus* CCM 2461 (5.32 mm). Walnut oil showed the strongest activity towards *Yersinia enterocolitica* CCM 5671 (6.33 mm). The sensory analysis resulted in the samples of walnut and grape seed oil being awarded the highest scores for smell, taste, and overall acceptability. The tested edible oils are rich in biologically active compounds with antioxidant and antimicrobial activities. Their consumption can have a positive effect on the functioning of the human body and its health. Proper storage conditions are, however, necessary because of the susceptibility of these oils to oxidation.

Keywords: acid number; peroxide value; Rancimat; antioxidant activity; fatty acids

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1. Introduction

Fats are one of the main sources of nutrients essential to human nutrition and proper functioning of the body. They supply energy, constituents of cell membranes, fat-soluble vitamins, antioxidants, and other biologically active substances [1].

Nonetheless, they are often associated with the risk of civilization diseases such as obesity, *diabetes mellitus*, and cardiovascular diseases. These problems, however, are not the result of the properties of fats themselves, but are rather caused by their excessive consumption, unbalanced intake of fatty acids, or inappropriate culinary processing (especially usage of cold-pressed oils for hot kitchen processes [2]. Complete exclusion of fat from diets can lead to deficits of fat-soluble vitamins, fluctuations in the level of hormones, or weakening of overall immunity.

Edible fats and oils are the carriers of essential fatty acids, and vegetable oils are the primary source of these acids [3]. Fatty acids present in these oils are known to absorb sunlight. With absorption, their ability to react with oxygen improves so they become unstable and very active chemically. Therefore, the greatest enemy of these acids is light, which causes the destruction of their vital biological properties, which manifests in altered organoleptic properties, including bitter taste [4]. Flax seed oil is known as the richest source of the essential omega-3 acid α-linolenic, which amounts to 50 to 62% of the total fatty acid content [3]. Positive effects of this fatty acid in the prevention and improvement of cardiovascular diseases, selected cancer types, rheumatoid arthritis, and autoimmune diseases have been reported [5]. Poppy seed oil is used in human nutrition and cosmetics, but it is also a raw material for the technical industry. The nutritional value of oil is based on the quantitative ratio of mono- and polyunsaturated fatty acids to saturated fatty acids. The dominant fatty acids in poppy seed oil are linoleic, oleic, and palmitic acids [4]. Stearic, palmitoleic, and α-linolenic acids are less abundant. Grape seed oil has a high content of essential omega-6 acids, important for the synthesis of prostaglandin, which has an impact on the aggregation of blood platelets and inflammatory processes [6]. It has a strong antioxidant effect, due to the high content of vitamin E, which helps reduce the risk of arteriosclerosis. It has a positive effect on the reduction of low-density cholesterol and, on the contrary, increases the level of high-density cholesterol, thereby reducing the risk of developing cardiovascular diseases. It is also rich in β-sitosterol and γ-tocopherol [7]. Grape seed oil has proven to have great potential for use in the pharmaceutical and food industries [8]. The predominant fatty acids in walnut oil are linoleic, oleic, and linolenic acids. The proportions of these acids determine the nutritional and economic value of the oil, e.g., a lower amount of linoleic and linolenic acid in the oil will ensure a longer shelf life, while a higher amount of polyunsaturated fatty acids is desirable for their potential health benefits [9]. The optimal ratio between n-6 and n-3 polyunsaturated fatty acids is 4:1. Together with antioxidant substances such as tocopherols and phytosterols, unsaturated fatty acids help prevent diseases and maintain health [10]. The content of oil in milk thistle seeds is approximately 22% [9]. This crop is also used to produce edible oil that has a positive impact on human health. It was found that the oil is rich in unsaturated fatty acids, which make up 75.1% of the total amount of fatty acids [11]. Among the tocopherols contained in milk thistle seed oil, α-tocopherol is the most abundant at 84.5%, while β-, γ- and δ-tocopherols constitute 9.9%, 5.4%, and 0.2%, respectively [12]. The analysis of fats and oils includes their identification, the determination of their compositions, and the detection of antioxidants, colour pigments, foreign substances, and impurities (residues of solvents, pesticides, trace elements) [13]. Additionally, other quality parameters, such as the extent of lipolysis, autoxidation, or heat treatment, are determined [14]. The main goal of this study was to determine the physicochemical, antioxidant, antimicrobial, and sensory characteristics of oils from grape seed, flax seed, walnut, poppy seed, and milk thistle seed, originated from Slovakia. These kinds of oils are currently very popular in the Slovak Republic, especially as part of a healthy lifestyle.

2. Materials and Methods

2.1. Chemicals

All the chemicals used were of analytical grade and purchased from either Sigma-Aldrich (St. Louis, MO, USA) or CentralChem (Bratislava, Slovak Republic).

2.2. Oil Preparation

The samples of oils (grape seed, flax seed, walnut, poppy seed, and milk thistle seed oil) were purchased from a local Slovakian producer who uses cold-pressing technology with a process temperature below 50 °C. Immediately after their production, the oils were hermetically sealed in dark glass bottles and stored in the dark at a low temperature.

2.3. Acid Value Determination

A sample of 5 g of each vegetable oil was added to 100 mL of mixed solvent (ethanol and chloroform, 1:1). The prepared solution was gently heated to boiling in a hot bath (GFL 1013, Zevenhuizen, The Netherlands). Then, 2–3 drops of phenolphthalein were added to the mixed solution and stirred. The mixture was then titrated with 0.1 M of KOH until the colour changed (from white to pink). The result was expressed as the number of milligrams of KOH required to neutralize the free fatty acids in 1 g of the sample. The acid value was calculated using the following formula [15]:

$$\text{Acid value} = \frac{V \times C \times 56.1}{m} \quad (1)$$

where V is the consumption of the titration KOH solution volume, mL; C is the concentration of the KOH solution, mol/L; m is the amount of the oil, g; 56.1 is the potassium hydroxide molar mass, g/mol.

2.4. Peroxide Value Determination

The concentration of peroxides in the oil was determined by measuring the amount of iodine released from potassium iodide. The determination of the peroxide concentration was based on the AOCS official method [16] (AOCS, 2005). A sample of 5 g of oil was dissolved in a 100 mL mixture of acetic acid and chloroform (3:2). The flask was shaken vigorously to release iodine from the chloroform layer after the saturated potassium iodide solution and distilled water were added. A starch solution was used as an indicator and the mixture was titrated with 0.01 N sodium thiosulphate. The result was expressed as mmol peroxide/kg. The peroxide value was calculated using the following formula:

$$\text{Peroxide value} = \frac{1000 \times M \times (a - b) \times f}{m} \quad (2)$$

where 1000 is the conversion factor; M is the molecular weight of sodium thiosulphate, mol/L; a is the consumption of the titrant (sodium thiosulphate solution) for titrating the sample, mL; b is the consumption of the titrant for the blank run, mL; m is the amount of the oil, g; f is the concentration of sodium thiosulphate, g/mol.

2.5. Oxidative Stability Determination

A sample of 3.0 g of oil was used to determine the oxidative stability in the 892 Rancimat apparatus from Metrohm (Zofingen, Switzerland) according to AOCS, (2005) [16]. The temperature used for all experiments was 120 °C, with a steady air flow of 20 L/h. The apparatus software was used to determine the induction times with a precision of 0.005.

2.6. Antioxidant Activity Determination—DPPH Method

According to the procedure outlined by Sánchez-Moreno et al. [17], the 2,2-difenyl-1-picrylhydrazyl (DPPH) test was used to measure the sample's capacity to scavenge free radicals. The DPPH solution was prepared by dissolving 0.025 g in 100 mL of ethanol;

next, 3.6 mL of this solution was added to the oil (0.4 mL). At 515 nm, the absorbance of the oil was measured using a Jenway spectrophotometer (6405 UV/Vis, London, UK). The sample's ability to neutralize free radicals was calculated as mg of Trolox equivalent antioxidant capacity (TEAC) per litre of sample.

2.7. Determination of Fatty Acid Methyl Esters (FAME) Content by GC-FID Method

The oil—0.1 g was taken into a 40 mL glass vial, then mixed with 5 mL of 0.50 N methanolic NaOH, and the mixture was heated for 3 min at 60 °C. The mixture was allowed to cool at room temperature, and then 6 mL of 14% BF3 solution (boron trifluoride methanol complex solution) was added to the mixture and again heated for 3 min at 60 °C. The mixture was once more cooled to room temperature before we added 10 mL of isooctane and shook it thoroughly, followed by allowing it to settle. Once settled, the upper layer of the mixture was transferred to a tube containing sodium sulphate to eliminate any excess moisture. The esterified oil samples were then diluted at a ratio of 1:19 (50 mL FAME + 950 mL n-hexane) before analysis. The qualitative and quantitative determination of FAME was conducted using gas chromatography analysis performed with an Agilent 7890B gas chromatograph equipped with a flame ionization detector. A CombiPAL autosampler was used to inject 1 µL of the diluted sample into the instrument. An HP-88 GC capillary column (Agilent Technologies, Santa Clara, CA, USA, 60 m × 0.25 mm × 0.20 µm) was used to separate fatty acid methyl esters. The purity of all the analytical gases employed (He, N2, H2, and synthetic air) was 5.0. The information was processed online with Agilent OpenLab ChemStation software (OpenLab CDS 2.X and OpenLab CDS ChemStation Edition C.01.08–C.01.10). A 37-component standard Supelco 37 was used for calibration. The component FAME Mix includes certified reference material (CRM), TraceCERT, and Supelco USA, used as a quantitative indicator. The Agilent 5977A MSD mass spectrometer was used to determine chromatographic analyses using the GC-Fit method.

2.8. Antimicrobial Activity Determination

The effectiveness of the antimicrobial activity was assessed using the disc diffusion method. The experiment involved testing eight different strains of microorganisms, which included one yeast (*Candida albicans* CCM 8186), five Gram-negative bacteria (*Haemophilus influenzae* CCM 4454, *Escherichia coli* CCM 3954, *Klebsiella pneumoniae* CCM 2318, *Yersinia enterocolitica* CCM 5671, *Salmonella enterica* subs. *enterica* CCM 3807), and four Gram-positive bacteria (*Staphylococcus aureus* CCM 2461, *Clostridium perfringens* CCM 4991, *Bacillus cereus* CCM 2010, *Streptococcus pneumoniae* CCM 4501). All strains were obtained from the Czech Collection of Microorganisms. Prior to testing, the bacterial and yeast suspensions were cultured in nutrient broth (Imuna, Bratislava, Slovakia) at 37 °C for 24 h. A suspension of 0.1 mL of the tested microorganism with a density of 10^5 cfu/mL was spread on Mueller Hinton Agar (MHA, Oxoid, Basingstoke, UK). Filter paper discs (diam = 6 mm) impregnated with the oil (15 µL) were placed on the agar. Agars were left at 4 °C for 2 h and then after incubating at 37 °C aerobically for 24 h, the inhibition zones were measured in millimetres. The analysis was conducted in triplicate.

2.9. Sensory Characteristic Determination

A sensory panel of 30 evaluators—15 women and 15 men, aged 22 to 65—determined the organoleptic properties of the edible oils. The panellists were asked to assess the overall acceptance, taste, smell, and general appearance. A 9-point hedonic scale was used to rate the samples, with values ranging from 9 (like very much) to 1 (strongly dislike).

2.10. Statistical Analysis

All experiments were carried out in triplicate and the mean of replicates was reported together with standard deviation. The experimental data were subjected to analysis of variance (Duncan's test) at the significance level of 0.05. Statistic calculations were performed using SAS (2009) software.

3. Results and Discussion

3.1. Acid Value, Peroxide Value, and Oxidative Stability

The results of acid value testing ranged from 0.11 (walnut oil) to 2.49 (milk thistle seed oil) mg KOH/g (Table 1). All these values were in accordance with the limit provided by AOCS (2004) for oils produced with cold-pressing technology (4 mg/g). Milk thistle seed oil showed the highest value, primarily because this kind of oil is rich in unsaturated fatty acids, which are extremely susceptible to hydrolysis in the presence of light and moisture. This results in higher concentrations of free fatty acids and a greater acid value [18]. The acid value is an important indicator of the quality of oils, especially from the point of view of storage. Higher values indicate oxidation, which decreases the quality of oil. This parameter reflects the abundance of acidic substances that are produced through the oxidation of hydrocarbons. Thus, the acid value directly represents the degree of oil degradation [19]. Eating rancid edible oil is unlikely to have an immediate negative effect on human health [20]. Such fats, however, have drastically decreased nutritional value because of the degradation of nutrients and vital fatty acids [20]. Similar results were reported by Wazed et al. [21], who analysed soybean, palm, mustard, and bran oil. The acid value was the lowest in soybean oil—0.61 mg KOH/g and the highest in mustard oil—6.29 mg KOH/g. The flax seed oil samples in our study showed an acid value of 0.15 mg KOH/g, which was lower than that reported in a study of Mohanan et al. [22], where a value of 0.89 mg KOH/g was given for oil originating from Canada. Negash et al. [20] confirmed that a high moisture content in oil negatively impacts the acid value and causes rancidity, which makes storage conditions very important in shaping the quality of oil. Preferably, the storage should be done in a cold, dry, and dark place.

Table 1. Results of acid value, peroxide value, and oxidative stability of analysed oils.

Sample	Acid Value [mg KOH/g]	Peroxide Value [mmol O_2/kg]	Oxidative Stability [h]
FO	0.15 ± 0.01^{d}	8.51 ± 0.12^{a}	0.18 ± 0.01^{e}
PO	1.95 ± 0.05^{b}	5.43 ± 0.02^{c}	0.58 ± 0.05^{d}
MTO	2.49 ± 0.08^{a}	2.27 ± 0.04^{e}	1.94 ± 0.05^{b}
GO	0.32 ± 0.03^{c}	5.94 ± 0.04^{b}	2.05 ± 0.06^{a}
WO	0.11 ± 0.01^{d}	2.64 ± 0.23^{d}	1.09 ± 0.02^{c}

Mean ± standard deviation; different letters in a column denote mean values that statistically differ from one another; h—hours; FO—flax seed oil; PO—poppy seed oil; MTO—milk thistle seed oil; GO—grape seed oil; WO—walnut oil.

The degree of oxidation of lipids, fats, and oils is determined by analysing the concentration of peroxides. The amount of total peroxides in a sample is represented by the peroxide value, which is associated with rancidity in lipid-rich food products. Oxidation is one of the primary causes behind the development of off-flavours, quality deterioration, and the formation of toxins in oils [23]. In our study, peroxide values ranged from 2.27 (milk thistle oil) to 8.51 (flax seed oil) mmol O_2/kg. A high susceptibility to oxidation limits the use of flax seed oil in the food industry. During storage, lipids undergo hydrolysis and oxidation, which leads to the formation of polymers and volatile substances rich in oxygen. Oxidation of flax seed oil, rich in α-linolenic acid, is accompanied by significant colour changes. Flax seeds contain many cyclic peptides and cyclolinopeptides. Some of these peptides are coextracted during cold pressing, which usually gives the product a mild nutty flavour when fresh [21]. However, a persistent bitter aftertaste develops during storage of the oil at room temperature. It is caused by methionine sulfoxide originating from the conjugated linoleic acid present in flax seed oil in amounts of 485 to 925 mg/kg [23]. Higher peroxide values were also determined in grape seed and poppy seed oil, but they were still in accordance with AOCS [16]. Generally, the peroxide value should not be higher than 20 mmolO_2/kg in cold-pressed oil [16]. This requirement was fulfilled in all the samples tested in our study. Aydoğan et al. [24] determined the peroxide value of

11.39 ± 2.26 mmol O_2/kg in cold-pressed milk thistle oil from Turkey; this was higher compared to the value determined in our sample. In a study by Melo et al. [25], walnut kernel cold-pressed oil showed a peroxide value of 1.95 mmol O_2/kg. This was lower compared to our observations for walnut oil—2.64 mmol O_2/kg. According to Gilbraith et al. [23], peroxide values in fresh oils are below 10 mmol O_2/kg, whereas oils that have spoiled and gone rancid show results that exceed 30 mmol O_2/kg. Notably, peroxide values that reach 100 mmol O_2/kg have been associated with cases of food poisoning.

Oxidation resistance is one of the most crucial quality parameters for edible oils. It determines their usefulness in technological processes and shelf life. Storage tests are the most reliable, but they are time-consuming. The possibility of determining oil stability in a short time is therefore valued [26]. In our study, the oxidative stability values ranged from 0.18 h (flax seed oil) to 2.05 h (grape seed oil) (Table 1). The stability of oils was generally shorter, which is not surprising because the tested cold-pressed oils contained high levels of polyunsaturated fatty acids. The poppy seed oil and flax seed oil were the most susceptible to oxidation. A similar tendency was observed by Dedebas [27], who tested the oxidative stability of cold-pressed poppy seed oil using the Rancimat instrument at temperatures of 110, 120, 130, and 140 °C. The induction time was the lowest at 140 °C—0.61 h, while at 120 °C it was 2.73 h. This value was higher compared to the one determined for poppy seed oil in our study in the same conditions (120 °C). Due to the dominancy of α-linolenic acid, poppy seed and flax seed oils are prone to oxidation.

The shelf life of oils can be prolonged by antioxidants. In a study by Mohanan et al. [22], flax seed oil containing 80 mg/kg tocopherol, 40 mg/kg ascorbyl palmitate, 40 mg/kg phytic acid, and 240 mg/kg tea polyphenol palmitate was determined to be very resistant to oxidation. The shelf life of this oil was 3.22-fold longer than that of the control flax seed oil. The addition of the right mix of antioxidants can, thus, result in obtaining oil products with enhanced oxidative stability that are suitable for commercialization. In our study, grape seed oil was found to be the most resistant to oxidation (2.05 h). This result is comparable with the findings of Maszewska et al. [26], who reported the value of 2.4 h for this type of oil.

3.2. Antioxidant Activity

The capacity of cold-pressed oils to scavenge free radicals in oils has been linked to *alpha*-tocopherol and polyphenols [28]. The antioxidant activity (Figure 1) was the best in grape seed oil (65.53 mg TEAC/l), followed by milk thistle (61.49 mg TEAC/l), walnut (60.82 mg TEAC/l), poppy seed (53.56 mg TEAC/l), and flax seed oil (28.88 mg TEAC/l). The lowest value was detected in flax seed oil. The antioxidant potential of grapes and the products of their processing, such as leaves, skin, wine, and seeds, were compared in a study by Xia et al. [29]. By using the oxygen radical absorbance capacity assay, grape seeds were found to have the highest antioxidant capacity (42.18 mmol of Trolox equivalent/g). This high antioxidant potential may be the consequence of the synergistic interaction of various phenolic compounds and is linked to the high concentration of procyanidins, epicatechin, gallic acid, and proanthocyanidins in grape seeds and the related oil. Saeed et al. [30] used poppy seed oil in concentrations ranging from 20 to 100 µL and determined the antioxidant activity to be between 22.28 ± 1.40 and 58.32 ± 3.40%. These authors concluded that poppy seeds are a useful source of natural antioxidants that can be utilized to substitute for synthetic antioxidants. Li et al. [31] determined the antioxidant activity of walnut oil and walnut extract using the DPPH method. The walnut oil samples were found to have lower antioxidant activity than the walnut extract (0.47 and 1026.19 µmol TEAC/g, respectively). This might have been the result of the high abundance of polyphenols in walnut kernels. Emir et al. [32] analysed the antioxidant activity in cold-pressed poppy seed oil from blue, white, and yellow poppy varieties using the DPPH method. The highest value was found in the blue variety—31.23 mmol TEAC/g, followed by white (30.37 mmol TEAC/g) and yellow (22.61 mmol TEAC/g) varieties. Antioxidants, especially polyphenols, contained in oil can improve its oxidative stability [33]. In our study, grape seed and milk thistle

oils were found to have the highest activity, which has probably influenced the oxidative stability results (Table 1)—these oils also showed the longest times in the stability test.

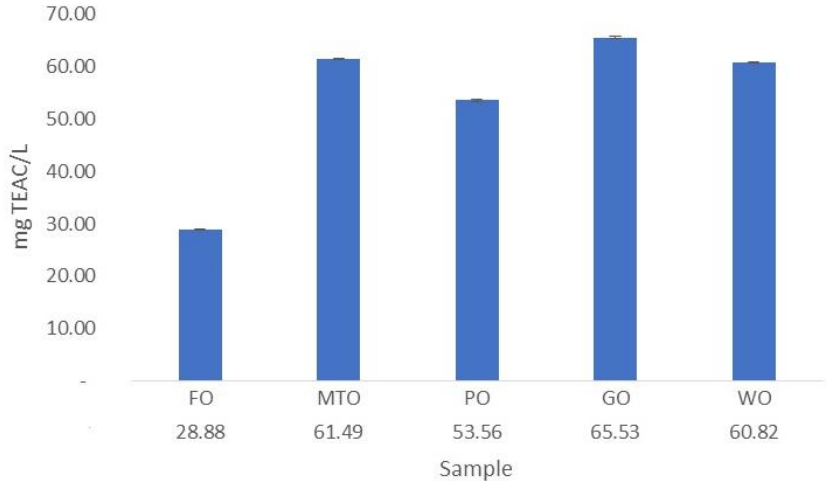

Figure 1. Results of antioxidant activity of analysed oils. (FO—flax seed oil; PO—poppy seed oil; MTO—milk thistle seed oil; GO—grape seed oil; WO—walnut oil; TEAC—Trolox equivalent antioxidant activity).

3.3. Fatty Acid Methyl Esters (FAME) Content

The fatty acid composition of the analysed oils is presented in Table 2. The major fatty acids in flax seed oil were α-linolenic (C18:3n3c), oleic (C18:1n9c), and linoleic (C18:2n6c) acids. Minor ones were stearic (C 18:0), palmitic (C 16:0), and elaidic (C18:1n9t) acids. Similar results were reported by Silska and Walkowiak [34] in a study on 84 varieties (α-linolenic acid 48.4–58.9%, linoleic acid 10.3–17.3%, palmitic acid 4.2–6.6%, stearic acid 2.6–5.1%). Supplementing the diet with flax seeds and the related oil, which contains significant levels of α-linolenic acid and lower levels of linoleic acid, enables the body to receive the required ratio of diunsaturated (n-6) to triunsaturated fatty acids (n-3). Only a few food products contain α-linolenic acid, which must be supplemented in the diet to safeguard health. The ingestion of 2.2 g of *alpha*-linolenic acid per day from flax seed oil enhanced verbal fluency in healthy people without cognitive impairments, despite an age-related reduction in cognitive function [35]. Major fatty acids in poppy seed oil (Table 2) were linoleic (C18:2n6c), oleic (C18:1n9c), and palmitic (C 16:0) acids. Minor fatty acids were stearic (C 18:0), α-linolenic (C18:3n3c), and elaidic (C18:1n9t) acids. In a study by Xia et al. [29] linoleic acid (71%), followed by oleic (15%) and palmitic (9%) acids, were found to be dominant in four Turkish poppy seed varieties. These findings are comparable with our results. Due to its high linoleic and low linolenic acid content, poppy seeds are a suitable crop for the food industry. Due to its instability and changes brought on by auto-oxidation, high levels of linolenic acid are problematic in food production. Özbek and Ergönül [36] analysed expeller-pressed poppy seed oils from Turkey and reported a high level of linoleic acid (70%), followed by oleic acid (15%) and palmitic acid (8%). These authors also confirmed that poppy seed oil is very rich in antioxidants, especially apigenin, syringic acid, luteolin, *p*-coumaric acid, quercetin, and ferulic and sinapic acids. Linoleic (C18:2n6c), oleic (C18:1n9c), and palmitic (C 16:0) acids were dominant in milk thistle oil (Table 2). Stearic (C 18:0), α-linolenic (C18:3n3c), and elaidic (C18:1n9t) acids were also present. This oil was also found to contain arachidic (C 20:0), eicosenoic (C20:1n9c), and behenic (C22:0) acids, which were absent in the other samples. Our results are comparable with the results of Fathi-Achachlouei and Azadmard-Damirchi [11], who analysed the fatty acid profiles of oil obtained in Iran from different milk thistle varieties. The average amounts of acids in their study were 51% linoleic, 23% oleic, 8% palmitic, 6% stearic, 1% arachic, 2.3% behenic, and 0.9% eicosenoic acid. Linoleic acid (52%), β-sitosterol (68 mg/100 g oil), and γ-tocopherol (54 mg/kg oil) were determined as the dominant

components in cold-pressed milk thistle oil in a study by Aydoğan et al. [24]. In grape seed oil (Table 2), linoleic acid (C18:2n6c) was the main fatty acid. It was followed by oleic (C18:1n9c) and palmitic (C 16:0) acids. Minor fatty acids were stearic (C 18:0), α-linolenic (C18:3n3c), and elaidic (C18:1n9t). Only in this oil was linoelaidic acid detected. A similar tendency was observed in a study by Yu et al. [37], who reported linoleic (72%) and oleic (13%) acids dominating the composition of grape seed oil processed by chloroform. In a study by Nash (2004), individuals who consumed up to 45 g of grape seed oil daily showed decreased LDL cholesterol and increased HDL cholesterol, respectively. In walnuts, the dominant fatty acid was linoleic acid (C18:2n6c), followed by oleic (C18:1n9c) and α-linolenic (C18:3n3c) acids. Flax seed oil as well as walnut oil are some of the best plant sources of α-linolenic acid. Compared to other analysed oils, these two oils showed the highest content of this fatty acid. However, this feature makes these oils very sensitive to oxidation, which was reflected by the results of oxidative stability tests (Table 1). Elaidic acid was found to be the least abundant among the fatty acids identified in walnuts (C18:1n9t). Zwarts et al. [38] reported that walnut oil contained 7% palmitic, 2% stearic, 15% oleic, 60% linoleic, and 10% linolenic acids. The oleic acid content of walnut oil determined by this author was lower compared to our study. The content of individual fatty acids is strongly influenced by variety and agro-ecological conditions. The high levels of linoleic and linolenic acids found in walnuts are particularly advantageous to human health, especially the condition of the cardiovascular system [39].

Table 2. Fatty acid methyl ester contents in analysed oil.

FAME [%]	FO	PO	MTO	GO	WO
C16:0	5.56 ± 0.03 ^{e}	8.99 ± 0.03 ^{a}	7.93 ± 0.02 ^{b}	7.46 ± 0.02 ^{c}	6.64 ± 0.29 ^{d}
C18:0	3.85 ± 0.03 ^{b}	2.16 ± 0.08 ^{d}	5.13 ± 0.02 ^{a}	3.85 ± 0.02 ^{b}	2.56 ± 0.02 c
C18:1n9c	19.69 ± 0.02 ^{b}	15.46 ± 0.13 ^{e}	30.34 ± 0.25 ^{e}	17.39 ± 0.75 ^{d}	18.26 ± 0.19 ^{e}
C18:1n9t	0.73 ± 0.04 ^{c}	1.11 ± 0.02 ^{a}	0.62 ± 0.01 ^{d}	0.73 ± 0.02 ^{c}	0.84 ± 0.02 ^{b}
C18:2n6t	-	-	-	0.62 ± 0.03 ^{a}	-
C18:2n6c	17.92 ± 0.67 ^{e}	68.31 ± 0.26 ^{b}	47.41 ± 0.25 ^{d}	69.25 ± 0.16 ^{a}	60.06 ± 0.42 ^{c}
C18:3n3c	51.64 ± 0.49 ^{a}	3.19 ± 0.08 ^{c}	1.47 ± 0.05 ^{d}	-	10.50 ± 0.17 ^{b}
C20:0	-	-	0.54 ± 0.01 ^{a}	-	-
C20:1n9c	-	-	0.62 ± 0.04 ^{a}	-	-
C22:0	-	-	2.97 ± 0.02 ^{a}	-	-

FO—flax seed oil; PO—poppy seed oil; MTO—milk thistle seed oil; GO—grape seed oil; WO—walnut oil; mean ± standard deviation; different letters in a column denote mean values that statistically differ one from another; FAME—fatty acid methyl esters.

3.4. Antimicrobial Activity

The shelf life of edible oils and their applicability in industry are greatly dependent on their oxidative stability and antioxidant and antibacterial activity [40]. In our study, the best activity of flax seed oil (Table 3) was determined against *Bacillus cereus* CCM 2010 (4.58 mm), followed by *Salmonella enterica* subs. *enterica* CCM 3807 (3.07 mm). Increased flax seed oil proportions in the human diet can reduce immune function markers. The improvement of acute pneumonia was reported to be mediated by enhancing the intake of flax seed oil-based polyunsaturated fatty acids [4]. The effect of flax seed oil supplementation on the course of pneumonia caused by *Streptococcus pneumoniae* was studied in a mouse model [41]. Long-term supplementation safeguarded animals against lung colonization with *S. pneumoniae*, with a decrease in histopathological involvement of lung tissue. Supplemented infected mice showed moderate pneumonia compared to severe pneumonia in unsupplemented mice [41]. Jabbar et al. [42] showed that methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA), *Klebsiella pneuminae*, and *Staphylococcus epidermidis* were all affected in various ways. The application of flax seed oil also aided in the healing of experimental wounds. In their research, the most potent antibacterial activity of poppy seed oil was observed against Haemophilus influenzae CCM 4454, Escherichia coli CCM 3954, *Klebsiella pneumoniae* CCM 2318, and *Streptococcus pneumoniae* CCM 4501.

Flax seeds demonstrated different levels of inhibitory and antibiofilm effects on a range of bacterial strains, such as MRSA, *S. epidermidis*, *K. pneumoniae*, and MSSA. The application of flax seed oil showed positive effects on wound healing in experiments [42]. The most potent antibacterial activity observed in our study was seen in poppy seed oil, particularly against *Bacillus cereus* CCM 2010 (4.32 mm), *Escherichia coli* CCM 3954 (0.31 mm), *Klebsiella pneumoniae* CCM 2318 (3.68 mm), and *Streptococcus pneumoniae* CCM 4501 (0.14 mm). This oil had the best overall antimicrobial activity. Our results are comparable with the results of Kumaravel and Alagusundaram [43], who determined an inhibition zone for *Staphylococcus aureus* of 2.00 mm, but the activity of this oil against *Escherichia coli* and *Bacillus cereus* was stronger in our study (inhibition zone diameters of 0.31 mm and 4.32 mm, respectively). In the case of the referenced study, these zones were smaller (0.10 for both). Moreover, these authors determined antifungal activity of poppy seed oil towards *Aspergillus niger* (MTCC 281) and *Aspergillus oryzae* (MTCC 624)—3 mm—and for *Peniciliium chrusogenum* (MTCC 6795)—2 mm. The studied milk thistle seed oil exhibited the strongest activity against *Clostridium perfringens* CCM 4991 (6.31 mm) (Table 3). In a study by Al-Madhagy et al. (2023) [41], a strong antibacterial activity with a maximum zone of inhibition of 15.50 mm was determined for an oil extract of milk thistle leaf against *Staphylococcus aureus.* Moreover, antifungal activity was also reported, with a maximum zone of inhibition (13.83 mm) in tests against *Aspergillus versicolor*. Gaber [44] determined a stronger activity of milk thistle oil compared to our results. This was especially noticeable for *Escherichia coli*—6 mm, *Staphylococcus aureus*—7 mm, and *Candida albicans*—9 mm. Grape seed oil (Table 3) showed the strongest activity against *Staphylococcus aureus* CCM 2461 (5.32 mm) and *Streptococcus pneumoniae* CCM 4501 (0.11 mm). Khan et al. [45] determined antibacterial properties of different active emulsified films containing grape seed oil (0.15 g/g). These active films showed antibacterial effects against *Salmonella typhimurium* (12.10 mm), *Staphylococcus aureus* (10 mm), *Pseudomonas fluorescens* (11.06 mm), and *Escherichia coli* 157:H7 (11.03 mm). Walnut oil showed the highest activity against *Yersinia enterocolitica* CCM 5671 (6.33 mm) (Table 3). The extracts from walnut kernels were reported to inhibit strains of *Escherichia coli, Bacillus subtilis,* and *Klebsiella pneumoniae* in a study by Elouafy et al. [46]. Generally, it can be stated that the antimicrobial activity of all the tested oils can be effectively utilised in salad dressings because this kind of oil is suitable for cold culinary applications, especially for salads. The addition of selected oils can not only improve the nutritional value but also introduce an antimicrobial effect, facilitating a prolonged shelf life when packing a seasoned, ready-to-eat salad.

Table 3. Antimicrobial activity of analysed oils.

Microorganism	FO [mm]	PO [mm]	MTO [mm]	GO [mm]	WO [mm]
Yeast					
***Candida albicans* CCM 8186**	0.31 ± 0.01^{a}	0.09 ± 0.01^{c}	0.08 ± 0.01^{c}	0.12 ± 0.01^{b}	0.12 ± 0.01^{b}
Gram-negative bacteria					
***Haemophilus influenzae* CCM 4454**	0.09 ± 0.01^{e}	0.22 ± 0.01^{a}	0.03 ± 0.01^{e}	-	0.09 ± 0.01^{b}
***Escherichia coli* CCM 3954**	0.09 ± 0.01^{d}	0.31 ± 0.01^{a}	0.03 ± 0.01^{e}	0.13 ± 0.01^{c}	0.17 ± 0.01^{b}
***Klebsiella pneumoniae* CCM 2318**	1.31 ± 0.02^{d}	3.68 ± 0.14^{a}	3.11 ± 0.11^{b}	2.24 ± 0.12^{c}	2.11 ± 0.09^{c}
***Yersinia enterocolitica* CCM 5671**	2.09 ± 0.05^{d}	2.11 ± 0.01^{d}	3.13 ± 0.02^{b}	2.64 ± 0.03^{c}	6.33 ± 0.02^{a}
***Salmonella enterica* subs. *enterica* CCM 3807**	3.07 ± 0.04^{e}	3.63 ± 0.03^{d}	4.31 ± 0.03^{b}	4.11 ± 0.02^{c}	4.65 ± 0.03^{a}
Gram-positive bacteria					
***Staphylococcus aureus* CCM 2461**	2.61 ± 0.07^{c}	2.32 ± 0.01^{d}	4.14 ± 0.03^{b}	5.32 ± 0.03^{a}	2.34 ± 0.02^{d}
***Clostridium perfringens* CCM 4991**	2.11 ± 0.01^{e}	2.02 ± 0.01^{d}	6.31 ± 0.02^{a}	3.08 ± 0.01^{c}	6.11 ± 0.02^{b}
***Bacillus cereus* CCM 2010**	4.58 ± 0.08^{a}	4.32 ± 0.03^{b}	4.05 ± 0.06^{c}	4.31 ± 0.02^{b}	2.65 ± 0.03^{d}
***Streptococcus pneumoniae* CCM 4501**	-	0.14 ± 0.01^{a}	-	0.11 ± 0.02^{a}	-

FO—flax seed oil; PO—poppy seed oil; MTO—milk thistle seed oil; GO—grape seed oil; WO—walnut oil; mean ± standard deviation; different letters in a column denote mean values that statistically differ one from another; mm—millimetre.

3.5. Sensory Characteristics

Walnut oil was rated as the best within all the assessed categories (Table 4). The probands positively evaluated its overall appearance (8.12 p)—a pleasant yellow colour, odour—very pleasant nutty smell (8.52 p), taste—a very pleasant nutty flavour (7.92 p), as well as its overall acceptability (8.43 p). Similar results were also published by Martínez et al. [47], who evaluated the sensory attributes of walnut oils. Higher intensity ratings were associated with nutty and oily, and lower with pungent and astringent qualities, and were indicative of fresh walnut oil. Low and comparable levels of sweetness and bitterness were also found. Negative qualities and oxidized odour/flavour showed ratings close to zero, indicating their near absence. Grape seed oil was the second-best rated. The evaluators positively rated its overall appearance (7.11 p)—nice colour and good consistency, taste (7.11 p)—pleasant vinous and fruity taste, smell (5.09 p) and overall acceptability (7.15 p). The third place was occupied by poppy seed oil, characterised by a pleasant poppy smell (7.38 p) and overall acceptability (7.13 p). According to Veličkovska et al. [48], virgin grape seed oil has a delightful, vinous, fruity taste and smell that is reminiscent of raisins, while refined grape seed oil is neutral in both aroma and taste. This is a desirable combination for a variety of foods, including salads. Some evaluators also felt bitter tones. The flax seed oil was ranked fourth. The evaluators rated its taste lower (3.15 p)—spicy and bitter, as well as its overall acceptability (2.37 p). Some probands reported that products of oxidation were detectable in this oil. This is probable, considering the high abundance of α-linolenic acid. Milk thistle oil was rated as the worst of all the tested oils, with the majority of the evaluators reporting medicine-like tones. The high content of polyphenols in this oil might have been the reason behind these observations. According to Kalinowska et al. [49], the polyphenols amount to 1.46 mg GAE (gallic acid equivalent) per g of milk thistle oil. A similar score was also determined for milk thistle oil by Aydoğan et al. [24]. The authors reported moderate consumer satisfaction and intermediate acceptance levels for cold-pressed milk thistle seed oil.

Table 4. Sensory characteristics of analysed oils.

Properties	FO [p]	PO [p]	MTO [p]	GO [p]	WO [p]
Overall appearance	6.11 ± 0.01^{d}	7.11 ± 0.02^{c}	6.02 ± 0.01^{e}	7.11 ± 0.02^{b}	8.12 ± 0.01^{a}
Smell	6.13 ± 0.02^{c}	7.38 ± 0.23^{b}	3.91 ± 0.01^{e}	5.09 ± 0.23^{d}	8.52 ± 0.02^{a}
Taste	3.15 ± 0.07^{e}	6.96 ± 0.04^{c}	5.12 ± 0.01^{d}	7.11 ± 0.02^{b}	7.92 ± 0.01^{a}
Overall acceptability	2.37 ± 0.29^{c}	7.13 ± 0.04^{b}	6.92 ± 0.01^{b}	7.15 ± 0.07^{b}	8.43 ± 0.03^{a}

FO—flax seed oil; PO—poppy seed oil; MTO—milk thistle seed oil; GO—grape seed oil; WO—walnut oil; mean ± standard deviation; different letters in a column denote mean values that statistically differ one from another; p—points; sum of all evaluators.

4. Conclusions

In the present study, Slovakian edible flax seed, poppy seed, milk thistle, grape seed, and walnut oils were analysed in terms of various physicochemical parameters, and antioxidant and antimicrobial activities. Furthermore, a sensory evaluation of the oil samples was done. Regarding physicochemical characteristics, all the samples were in accordance with the requirements. Cold-pressed oils are a very good source of healthy fatty acids, which we confirmed in our study. Another positive element is the antioxidant activity of the oils. The most important finding, which we consider to be innovative, is the antimicrobial activity of the oils, as nowadays attention is drawn to natural substances with antimicrobial activity, not only for the pharmaceutical (as a part of food supplements), but also for the food industry. Strong antioxidant properties, desirable fatty acid profiles, as well as antimicrobial activity make the studied oils suitable for gastronomy applications, especially for cold dishes (prolonged shelf life when packing a seasoned, ready-to-eat salad), and food fortification, because the use of natural substances with biological activity is very attractive for consumers nowadays. For the future, it is necessary to continue in this study, especially with a focus on the determination not only of benefits, but also of some

risks connected with cold-pressed oil, especially oxidative products, which can negatively influence sensory attributes as well as health benefits. The major limitation is related to the long-term storage of these oils, which are sensitive to oxidation, so in the future, it will be necessary to study also the effect of the addition of natural antioxidants on prolonging their oxidative stability.

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