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Research Article

The Antioxidant Effect of Rosemary on the Oxidation Stability of Refined Sunflower Oil

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Abstract

In this study, the essential oil chemical compounds of rosemary (*Rosmarinus officinalis* L.) and its antioxidant activity on the oxidation stability of refined sunflower oil (RSO) were investigated by the Rancimat method. The plant material was obtained from Afyonkarahisar Medicinal and Aromatic Plants Center/Turkey. Based on the GC/FID-MS analysis result, 1,8-Cineole (15.18%), Camphor (11.39%), Borneol (11.39%), Germacrene D (11.12%), Carvacrol (11.05%), α-Pinene (6.01%) and p-Cimene (3.07%) were identified as the major constituents of rosemary essential oil. The total antioxidant activity of rosemary essential oil was determined using the DPPH method. The EC50 value was measured as 3.35 mg mL-1. While the induction time of RSO is 1.57 hours on average,the induction time of RSO with 1 g of 100 g-1 rosemary added was 1.68 hours on average, and the induction time of RSO with 5 g of 100 g-1 rosemary added was 1.79 hours on average. According to the results, rosemary, as an economic medicinal and aromatic plant, significantly increased the oxidation stability of RSO. Therefore, rosemary can be recommended as a natural antioxidant to extend the shelf life of edible fixed oils.

Keywords: Antioxidant, Essential Oil, Rancimat, Rosmarinus Offinalis, Sunflower Oil

Rafine Ayçiçek Yağının Oksidasyon Kararlılığı Üzerine Biberiye'nin Antioksidan Etkisi

Öz

Bu çalışmada, biberiye (*Rosmarinus officinalis* L.) uçucu yağının bileşenleri ve rafine ayçiçek yağının oksidasyon kararlılığı üzerine antioksidan aktivitesi Ransimat metodu ile incelenmiştir. Bitki materyali Afyonkarahisar Tıbbi ve Aromatik Bitkiler Merkezinden temin edilmiştir. GC/FID-MS analizi sonucu, 1,8-Cineole (15.18%), Camphor (11.39%), Borneol (11.39%), Germacrene D (11.12%), Carvacrol (11.05%), α-Pinene (6.01%) ve p-Cimene (3.07%) biberiye uçucu yağının major bileşenleri olarak tespit edilmiştir. DPPH metodu kullanılarak toplam antioksidan tayini yaılmış olup, EC50 değeri 3.35 mg ml^{-1} olarak belirlenmiştir. Rafine ayçiçek yağının indüksiyon periyodu ortalama 1.57 saat iken, 1 g 100 g^{-1} oranında biberiye eklendiğinde 1.68 saat, 5 g 100 g^{-1} oranında biberiye eklendiğinde ise 1.79 saat olarak tespit edilmiştir. Elde edilen sonuçlara göre ekonomik değere sahip, tıbbi ve aromatik bitkisi olan biberiye, ayçiçek yağının oksidasyon kararlılığını önemli ölçüde artırmıştır. Yenilebilir sabit yağların raf ömrünü uzatmak amacıyla doğal bir antioksidan olarak biberiye önerilebilmektedir.

Anahtar Kelimeler: Antioksidan, Esansiyel Yağ, Ransimat, Rosmarinus Officinalis, Ayçiçek Yağı

1. Introduction

The formation of hydroperoxides by reacting unsaturated fats with oxygen is called the free radical chain, and it takes place in three steps. In the first step, free radical formation (R.) occurs. In the growth step, R. reacts with oxygen to form a peroxy radical (ROO.) which forms unsaturated fats and peroxides (ROOH.). At the termination step R. and ROO. create non-radical products. Antioxidants (AH.) intervene at the beginning or the development stage of the reaction and cause the chain reaction to be interrupted (Frankel, 1985). According to Codex Alimentarius Commission (CAC.), antioxidants are substances that prevent the decomposition of food oils (rancidity and discoloration) by intercepting oxidation reactions and increasing their shelf life (Targan et al., 2008). Recently, numerous studies have been conducted to enhance the effect of essential oils and other secondary metabolites of plants as natural antioxidants that raise oxidation stability of edible oils through different methods (Topuz, 2014; Tomsone and Krūma, 2015).

The essential oils have antioxidant, anti-inflammatory, cancer chemoprotective, cytotoxicity, allelopathic, antimicrobial, repellent, and insecticidal activities (Dhifi et al., 2016).

Rosmarinus officinalis L. (rosemary) is a medicinal, aromatic, and spice plant belonging to Lamiaceae family used widely in fragrance, cosmetics, food, and pharmaceutical industries. This plant grows all over the world, especially in the Mediterranean region (Omidbeigi, 2011). Today, the demand for rosemary and its products has increased in traditional medicine, pharmaceutical industries, cosmetic sector (Kassahun et al., 2019). Rosemary has antioxidant, antimicrobial, cytotoxic, antimutagenic, antiphlogistic, and chemo-preventive properties (Hussain Abdullah et al., 2013; Özguven et al., 2013). 1,8-cineole (38.5%), camphor (17.1%), α-pinene (12.3%), limonene (6.2%), camphene (6%), linalool (5.7%), borneol (3.2%), and α-terpineol (2.3%) were found as the main compounds of rosemary essential oil (Hussain Abdullah et al., 2013). In another study, 1,8-cineole and camphor were identified 49 and 12% (Risaliti et al., 2019). The anti-inflammatory and activities of 1,8-cineol have been proven (Sampath et al., 2017; Juergens et al., 2017). Camphor is also an organic compound with antifungal, antibacterial, antiseptic, and antipruritic effects (Pragadheesh et al., 2013; Singh and Jawaid, 2012; Zhou et al., 2017). Camphor has a strong smell and taste and is easily absorbed by the skin. This constituent can be used topically to relieve pain, irritation, and itching (Garg and Jain, 2017). Sunflower oil is one of the most widely used edible oils used in frying food and salads worldwide. Like other vegetable source oils, Sunflower oil is part of a healthy diet containing unsaturated fatty acids and fat-soluble vitamins. Sunflower oil is rich in linoleic acid, an essential n-6 polyunsaturated fatty acid (Salas et al., 2015).

The current study aimed to investigate the antioxidant activity of rosemary on edible sunflower oil by the Rancimat method. In this way, rosemary was used as an alternative natural antioxidant source.

2. Material and Method

2.1. Plant Material

The plant material was *Rosmarinus officinalis* L. from the scientific project carried out in Afyonkarahisar Medicinal and Aromatic Plants Centre/Turkey (38° 46' N, 30° 30' E). The flowering branches were harvested at the beginning of the flowering stage. After the leaves were separated from other parts, they were dried by a cabin dryer at 37 °C for 72 h. The sunflower oil used in the experiments was refined and obtained from a local market in Afyonkarahisar/Turkey.

2.2. Radical Scavenging Activity

The DPPH method is one of the methods used in the determination of total antioxidants. The solution of the DPPH radical shows maximum absorption between 515-520 nm and is purple in color. In the presence of any antioxidant, the DPPH solution becomes stable and the % radical scavenging activity is calculated with the help of the decrease in absorption accompanied by a decrease in color intensity. Radical scavenging activity (%) was determined by DPPH method with some modifications (Brand-Williams et al., 1995; Locatelli et al., 2009; Türk Baydır, 2019). Accordingly, radical scavenging activities (%) of 0.001 g ml^{-1} rosemary extract were prepared using different solvents as follows: ethyl alcohol, methyl alcohol, acetone, ethyl ether, and 2-propanol. However, the experiments were continued with methyl alcohol because of the obtained best results. For calculating the EC50 value, 500 µL of 0.5, 1, 1.5, 2, and 2.5 mg ml^{-1} of methyl alcohol extract of powdered rosemary leaves were completed to 1 ml with methyl alcohol. Finally, 0.5 ml of 0.002 g 100 ml^{-1} DPPH in methyl alcohol was added to the samples. The prepared samples were placed in a dark cabin at laboratory temperature (24±2 °C) for 30 minutes. The absorption values were read at 517 nm by UV/Vis Microplate Spectrophotometer (Thermo Scientific Multiskan Go). Thus, 0.5 ml of the DPPH solution with 1 ml of methyl alcohol was mixed and read at 517 nm as the control value. Methyl alcohol solution was evaluated as the blank sample. Radical scavenging activity (%) was calculated from the equation below:

Radical scavenging activity (%) = $(A_{control} - A_{sample}) / A_{sample} \times 100$

Experiments were performed in triplicate. The average and standard deviation values of the test results were calculated and given.

2.3. Rancimat method

One of the methods to evaluate the oxidation stability of oils is Rancimat device. This technique is based on the automatic determination of time before the maximum change of oxidation rate. Rancimat also provides some other helpful information on the

oxidative stability of edible fats and oils. By drawing the oxidative stability index (OSI.) logarithm against accelerated temperature and extrapolating to room temperature, the shelf life of the sample can be estimated under environmental conditions (Farhoosh, 2007). The time required to produce a sudden increase of the conductivity due to volatile acid formation, mainly formic acid, determines the OSI, which can be defined as a measure of the resistance to oxidation of a fat or oil (García-Moreno et al., 2013). According to the rancimat method, it can be said that the higher induction period under the same conditions, the more stable the oil is (Li et al., 2019). Oxidation stability is characterized by induction time. Stability analyses were performed according to the standard Rancimat method by the Rancimat (Metrohm/743). During the analysis, vessels containing 3 g of RSO samples were placed in heating blocks. After, 3 g of samples in the vessels were placed in heating blocks at 120 °C with 20 L h^{-1} air flow and 60 ml of ultra-pure water (0.055 µs). Due to the lack of a standard, as an antioxidant in edible oils, 1 and 5 g of powdered rosemary leaves were added to the 100 g of sunflower oil and were kept for 24 h. Experiments were performed in triplicate. The average and standard deviation values of the test results were calculated and given.

Accordingly, the results of oxidation stability analysis of RSO were compared with the powdered rosemary leaves added oil samples.

For isolation of the essential oil, 50 g of dried rosemary leaves were extracted with 500 ml of distilled water by a neo-Clevenger type apparatus. Hydro-distillation was performed for three h. The obtained essential oils were dried over anhydrous sodium sulphate and stored in amber vials at +4°C.

2.4.GC/FID-MS Analyze method

For identifying the components of the essential oil, a gas chromatography (GC) system (Agilent Technologies, 7890B) equipped with a flame ionization detector (FID) and coupled to a mass spectrometry (MSD) detector (Agilent Technologies, 5977A) was used. An HP-Innowax column (Agilent 19091N-116: 60 m×0.320 mm internal diameter and 0.25 µm film thickness) was used to separate the compounds. The samples were analyzed with the column held initially at 70 ºC after injection with 5 min hold time, then increased to 160 ºC with 3 ºC min^{-1} heating ramp. Finally, the temperature was raised to 250 ºC with 6 ºC min^{-1} heating ramp with 5 min hold time. The carrier gas was helium (99.99% purity) with 1.3 mL min^{-1} flow. The injection volume was set 1 µL (20 µL essential oil was solved in 1 mL n-Hexane) with 8.20 minutes solvent delay time. The injection was performed in split mode (40:1). Detector, injector, and ion source temperatures were 270 °C, 250 °C, and 250 °C, respectively. MS scan range was (*m/z*): 35-450 atomic mass units (AMU) under electron impact (EI) ionization of 70 eV (Soltanbeigi, 2020).

Retention indices were calculated against n-alkanes (C7-C30/Sigma-Aldrich) on HP-Innowax column by GC/FID system (Agilent Technologies, 7890B) under the same conditions. The compounds of the essential oils were identified by comparing retention indices and mass spectra by computer library database of US National Institute of Standards and Technology (NIST), Wiley libraries, other published mass spectra (Adams, 2007). Relative abundance (% area) calculated based on the ratio between the peak area of each compound and the sum of areas of all compounds.

3. Results and Discussion

3.1. Radical scavenging activity (%)

The results of the DPPH method using various solvent extractions of 0.001 g ml^{-1} rosemary are shown in Table 1. When the results were evaluated statistically, methyl alcohol and 2-propanol solutions had the highest and lowest effects on radical scavenging activity, respectively.

Table 1. Radical scavenging activities (%) and standart deviation of rosemary in various solvents

Extraction	Radical scavenging activity (%)
Ethyl alcohol	7.88±0.03
Methyl alcohol	10.60±0.06
Acetone	9.12±0.04
Ethyl ether	3.78±0.01
2-propanol	2.19±0.01

Because of the methyl alcohol was best solvent, absorption concentration graph of rosemary by using methyl alcohol solvent was indicated in Figure 1. Through graphic, the EC50 value of rosemary was determined as approximately 3.35 mg ml^{-1}.

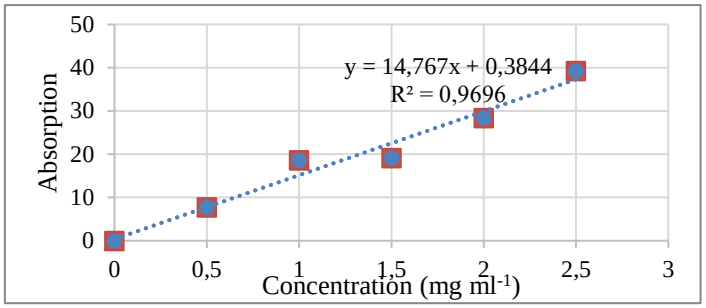

Figure 1. Absorption concentration graph of methyl alcohol extract of rosemary at 517 nm

3.2. Rancimat analysis

The induction period obtained from Rancimat analyses were given in table 2. The results showed that the average induction period of RSO was 1.57 h. The induction period was increased to 1.68 h by adding 1 g 100 ml^{-1} rosemary to the weight of RSO. The raising rosemary amount from 1 to 5 g 100 ml^{-1} increased the induction period to 1.79 h. Improving the stability of RSO to oxidation and increased shelf life was observed by adding different amounts of rosemary leaves powder.

Table 2. Average induction period and standart deviation of RSO, 1% rosemary added RSO, 5% rosemary added RSO under rancimat conditions, at 120 ˚C.

ID of the sample	IP, Induction Period
RSO	1.57±0.02
1% rosemary added RSO	1.68±0.02
5% rosemary added RSO	1.79±0.00

3.3. GC/FID-MS Analysis

Based on the chromatographic analysis, 1,8-Cineole (15.18%) was the most major constituent of rosemary essential oil. Camphor (13.59%), Borneol (11.39%), Germacrene D (11.12%), and Carvacrol (11.05%) were identified as the other main components. α-Pinene (6.01%), p-Cimene (3.07%), dl-Limonene (2.94%), Camphene (2.51), α-Terpineol (2.27%), and Linalool (2.06%) were also determined above 2% (Table 3).

Table 3. The essential oil compounds (%) of rosemary

RI	Compositions	%	RI	Compositions	%
1020	Tricyclene	0.122	1681	α-Humulene	0.326
1032	α-Pinene	6.01	1684	Z-Citral	0.242
1070	α-Fenchene	0.155	1698	γ-Muurolene	0.099
1080	Camphene	2.513	1703	α-Terpineol	2.275
1121	β-Pinene	0.127	1708	Borneol	11.393
1136	Verbenene	0.507	1719	Germacrene D	11.12
1158	δ-3-Carene	0.663	1731	cis-8-Methylbicyclo[4.3.0]non-7-ene	0.214
1168	β-Myrcene	0.178	1739	α-Citral	0.109
1188	Camphogen	0.295	1744	Carvone	0.26
1210	dl-Limonene	2.943	1765	β-Citronellol	0.153
1220	1,8-Cineole	15.189	1772	Campholaldehyde	0.218
1262	3-Octanone	0.109	1782	3-ethylidene-1-methylcyclopentene	0.097
1281	p-Cimene	3.078	1797	Homomyrtenol	0.992
1305	o-Isopropenyltoluene	0.116	1827	Santolina triene	0.158
1434	α-Thujone	1.133	1837	cis-Carveol	0.181
1438	β-Methylisoallylbenzene	0.099	1840	cis-Calamene	0.618
1447	p-Cymenene	0.615	1846	Geraniol	1.116
1451	1-Octen-3-ol	0.576	1852	p-Cymen-8-ol	0.32
1453	β-Thujone	0.335	1921	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	0.543
1478	cis-Linalool oxide	0.17	1934	Piperitenone	0.399
1501	Octa-2,4,6-triene	0.318	1971	1,3-Cyclopentadiene, 5,5-dimethyl-1-ethyl-	0.098
1530	Camphor	13.595	1985	Dehydro-carvenolide	0.095
1550	Linalool	2.068	1995	Caryophyllene oxide	0.203
1559	Pinocamphone	0.444	2019	Methyl eugenol	0.191
1581	Pinocarvone	0.222	2044	Humulene oxide II	0.14
1590	α-Fenchyl acetate	1.439	2091	Veridiflorol	0.867

1605	exo-methyl-camphenilol	0.106	2172	Thymol	0.148
1609	4-Terpineol	0.894	2175	Eugenol	0.111
1626	(-)-Verbenone	0.085	2189	t-Muurolol	0.314
1641	(1R)-(-)-Myrtenal	0.083	2199	1-[4-(2-Methylpropyl) phenyl] prop-2-en-1-one	0.127
1645	L-(-)-Menthol	0.082	2219	Carvacrol	11.054
1663	trans-Pinocarveol	0.204	2449	15-Crown-5	1.075
1678	α-Terpineol	0.27			

RI: Retention indices calculated against n-alkanes (C7-C30) on HP-Innowax column.

4. Conclusions and Recommendations

The synthesis of secondary metabolites in plants is affected by endogenous and exogenous factors. The production and quality of essential oils in plants are directly dependent on genetic, climatic, agronomic management, post-harvest practices (harvesting time, isolation method, etc.), as well as storage conditions (Soltanbeigi and Sakartepe, 2020). According to Hcini et al. (2013), 1,8-cineol (33.08-37.75%), camphor (13.55-18.13%), α-pinene (8.58-9.32%), α-terpineole (6.79-8.17%), camphene (5.07-5.58 %), borneol (4.08-5.48%), limonene (3.19-3.04%), and p-cymene (2.42-3.11%) were identified as the major components of *R. officinalis*. According to current results, 1,8-Cineole (15.18%) was the most major constituent of rosemary essential oil. Camphor (13.59%), Borneol (11.39%), Germacrene D (11.12%), and Carvacrol (11.05%) were identified as the other main components. The EC50 value of rosemary was determined as approximately 3.35 mg ml^{-1} by the DPPH method. According to the results, methyl alcohol was identified as the best solvent for DPPH experiments. At the same time, the maximum inhibition value reached 39.22%. Based on the results of Rancimat analysis, it can be concluded rosemary leaves may have positive effects on oxidation stability as a natural antioxidant to increase the shelf life of edible oils. The shelf life of RSO increased significantly by adding rosemary leaves. The present study results are consistent with numerous studies on the oxidative stability of edible oils by adding different medicinal plants (Tinello and Lante, 2020; Türk Baydir et al., 2021). The strong inhibitory effect of rosemary leaves extract (0.02%) on lipid oxidation and soybean lipoxygenase activity has been proved (Chen et al., 1992). In another study, by adding 0.1% of rosemary extract, the antioxidant potential of ghee increased in terms of radical scavenging activity (DPPH assay) without affecting sensory and physicochemical properties. The induction period of ghee increased significantly by adding rosemary extract compared with the control sample (Hussain Abdullah et al., 2010). According to the results of this study, rosemary plant has a certain amount of antioxidant content and can be used to prevent oxidation of oils. It is thought that this antioxidant effect may be due to the essential oil components it contains. Among the limitations of our study, the toxic effect of the rosemary plant was not taken into account in our study. The potential of antioxidant activities of other medicinal and aromatic plants on edible oils can be test to preserve and promote human health.

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Author Contributions

The authors declare that they have contributed equally to the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest between them.

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