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# DETERMINATION OF CHEMICAL PROPERTIES AND ANTIOXIDANT EFFECT OF *SALVIA OFFICINALIS* L.

Ayşegül Türk Baydir*[1], Amir Soltanbeigi[2], Rukiye Saygili Canlidinç[3], Mehmet Selçuk Erdoğan[4]

[1] Food Control Research and Application Center, Afyon Kocatepe University, AFYONKARAHISAR
[2] Afyon Health Sciences University Faculty Of Pharmacy, AFYONKARAHISAR
[3] Department of Chemistry, Faculty of Arts and Sciences, Dumlupınar University, KÜTAHYA
[4] Department of Chemistry, Faculty of Arts and Sciences, Dumlupınar University, KÜTAHYA

## Abstract

In this study the essential oil component of Salvia officinalis which was grown in Afyonkarahisar/Turkey were analyzed by GC-MS/FID. According to results, the major components of essential oil were α-thujone 19.89%, camphor 15.72%, borneol 12.86%, 1,8-cineole 12.06%. The total phenolic content of the plant leaves was calculated as 0.324g 100g-1 in terms of caffeic acid. According to DPPH analysis of the inhibition value of 0.01 g ml-1 sage methyl alcohol extraction was reported as 76.91%. In addition, while the refined sunflower oils induction period was 1.36h at rancimat conditions at 120 ºC, it was 2.17h when 2% S. officinalis was added and waited one week. In other words, sage increases the oxidation stability of refined sunflower oil and extends shelf life. For this purpose, it is concluded that sage can be used as natural antioxidant for refined sunflower oil.

**Key Words:** DPPH, essential oil, oxidation stability, phenolic content, rancimat, *Salvia officinalis*

## 1. Introduction

The $^3O_2$ molecule reacts with lipid radicals and causes autooxidation called free radical chain reaction. The formation of hydroperoxides by the reaction of unsaturated fats with oxygen is called free radical chain. While hydroperoxides formed in the first step of oxidation are stable at room temperature and in the absence of metal, in the presence of high temperature or metal, transforms into secondary reaction components such as ketone, aldehyde, carboxylic acid.. (Choe & Min, 2006). In the Rancimat device, the so-called induction period is the time until the formation of volatile acids, which are secondary reaction products. The induction period refers to the oxidation stability(Gertz & Kochha, 2001) The formation of primary and secondary oxidation products in oils is not desired, but it reduces the quality of the oil by changing the physical and chemical properties of the oil. This phenomenon is catalyzed by some external factors such as heat, light temperature. Antioxidants (AH) cause interruption of the chain reaction by interfering in the initial or development phase of the reaction (Frankel, 1985)For this reason, the use of artificial and natural antioxidants or substances with similar effects in oils and studies on this subject are quite common(Dıraman & Baydır, 2017; Duman et al., 2015; Yang et al., 2016). This situation guided and inspired our work.

Due to the disadvantages of synthetic antioxidants, there is increasing interest in scientific studies on the use of natural antioxidants in foods. In these studies, plant extract, extract or pure powder form can be used directly. *Salvia officinalis* (common sage) is an aromatic plant from the Labiatae / Lamiaceae family that is unique to the Middle East and Mediterranean regions and is currently produced all over the world. In folk medicine, *S. officinalis* has been used in the treatment of various disorders.(Ghorbani & Esmaeilizadeh, 2017). Carnosol, oleanolic acid and ursolic acid are some of the compounds found in the leaves. The gastrointestinal activity of the hydroalcoholic extract of *S. officinalis* is reported to be effective in the ethanol-induced gastric lesion model due to its carnasol content (Mayer et al., 2009). S. Officinalis plant is quite rich about phenolic acids and flavonoids. (Farhat et al., 2014)(Martins et al., 2014) (Khiya et al., 2019).. *S. officinalis* has been reported to compensate for learning and memory deficiencies caused by diabetes due to the rosmarinic acid content (Hasanein et al., 2016). Sage leaves

***Sorumlu Yazar (Corresponding Author):**
Ayşegül TÜRK BAYDIR, Afyon Kocatepe Üniversitesi Gıda Kontrol Uygulama ve Arş. Lab. Afyonkarahisar/Turkey

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used as herbal tea, medicine and spices are reported to have anti-inflammatory effect (Li et al., 2019). The essential components found in essential oil have been recorded as 1,8-cineole, viridiflorol, camphor, borneol, α-humulene, trans-thujone (Hassan et al., 2019; Hassiotis, 2018; Verma et al., 2015) Essential oil has been shown to exhibit anticandidal activity against all strains of C. albicans(Sookto et al., 2013). The antimicrobial effect of essential oil on *Bacilus subtillis* and *Staphylococcus epidermidis* bacteria has also been reported *(Wei et al., 2018)*.. The presence of valuable compounds such as α-thujone, β-thujone, camphor and sesquiterpenes in the essential oil cause this plant to exhibit anticancer properties and makes the studies related to this plant attractive (Russo et al., 2013). The antioxidant properties of *S. officinalis* are mentioned, but there are no studies on how they affect oxidation stability in edible oils. This is one of the original values of our study. Furthermore, since the percentages of the compounds in the plant content have been reported to vary depending on environmental factors, examination of sage which growing in Afyonkarahisar also adds a distinctive specificity(Russo et al., 2013).

## 2. Material and Method

### 2.1 Plant Material

*S. officinalis* plant material was obtained from the scientific project carried out in Afyonkarahisar Medicinal and Aromatic Plants Center in 2018. *S. officinalis* leaves dried by cabin dryer at 37 ºC for 72 h. and then it was ready for analysis.

### 2.2 % Radical Scavenging Activity

Radical scavenging activity was determined according to the DPPH method with some modifications (Brand-Williams et al., 1995; Locatelli et al., 2009). 1 ml of 0.01g / ml methyl alcohol extracts of sage mixed with 0.5 ml of 0.02g / 100ml dpph methyl alcohol solution and 15 minutes kept in the dark. The absorbance at 517 nm was read. 1 ml of methyl alcohol and 0.5 ml DPPH solution mixed and at 0. minutes 517 nm absorbance value was read and recorded as a control.

### 2.3 Phenolic Analysis

Phenolic analysis was performed according to folin-ciocelteu method. Methyl alcoholic solution of 1-0.5-0.25-0.125mg $ml^{-1}$ caffeic acid was prepared, distilled water, 10% $Na_2CO_3$ and 0.5N folin reagent were added and incubated for 30 minutes. 0.5 g of sage was extracted with 10 ml of methyl alcohol. Distilled water, 10% $Na_2CO_3$ and 0.5 N folin reagent were added to the extraction solution and kept for 30 minutes and the absorbance value was read at 760nm. Using the equation of the standard calibration curve caffeic acid equivalent was calculated.

### 2.4 Isolation of Essential Oils

*S. officinalis* leaves were dried by cabin dryer at 37 °C for 72 h. Then, 50 gr of leaves with distilled water (1:10) were subjected to hydro-distillation by using a Clevenger type apparatus for three hours.

### 2.5 GC-MS/FID Analysis

To identify the components of the extracted essential oils, 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) is used. An HP-Innowax column (Agilent 19091N-116: 60 m×0.320 mm internal diameter and 0.25 µm film thickness) was used for the separation of the compounds. Samples are analyzed with the column held initially at 70 ºC (after injection with 5 min hold time), and then increased to 160 ºC with 3 ºC $min^{-1}$ heating ramp. Finally, temperature was raised to 250 ºC with 6 ºC $min^{-1}$ heating ramp with 5 min hold time by using helium (99.999% purity) as carrier gas at 1.3 mL $min^{-1}$flow with 1 µl injection volume (20 µL essential oil was solved in 1 mL n-Hexane) and 8.20 min 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 (Figure 2). MS scan range was (m/z): 35-450 atomic mass units (AMU) under electron impact (EI) ionization (70 eV). Identification of the essential oils compounds area done by computer library search database of US National Institute of Standards and Technology (NIST), Wiley libraries.

### 2.6 Rancimat Analysis

Rancimat analyses were performed with the help of rancimat 743 device according to the standard rancimat method. In this method, the temperature is 120 °C, the sample amount is 3 g, the air flow is 20 L $h^{-1}$, the water amount is 60 mL. 0.055 µs ultra-pure water was used in the experiments. Refined sunflower oil produced in 2018 was used as oil. Since these plants are not used as an antioxidant in edible oils, 2% and 5% grams were added to sunflower oil and kept for 24 hours and one week. Rancimat analysis results were compared in their pure form. The experiments were performed in three replicates and the average of the test results was given.

## 3. Results

### 3.1. % Radical Scavenging Activity

Percentages calculated from the radical scavenging activity = (A_Control-A_Sample) / A_Sample x100 equation. According to the results of the experiment, the inhibition value of methyl alcohol extraction was reported as 76.91%.

### 3.2. Phenolic Analysis

The standard calibration curve obtained for phenolic analysis is given in Figure 1. The absorption value of sage was recorded as 0.5750. When the absorption value of sage extraction is written instead of y, the result is recalculated considering the dilution factor. The total phenolic content of sage was calculated as 0,324 g 100 $g^{-1}$ in terms of caffeic acid.

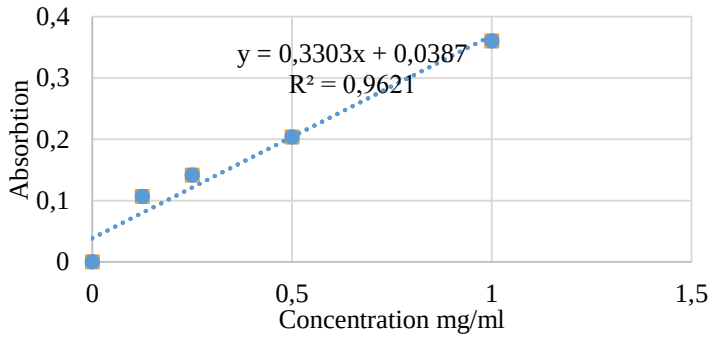


**Figure 1.** Calibration curve of caffeic acid standard

### 3.3. GC-MS/FID Results

The essential oil components of sage obtained by GC-MS/FID are given in Table 1.

**Table 1.** *S. officinalis* GC-MS/FID analysis results (RI: retention index, Pct total: % amounts)

| RT | Compound | (%) |
|---|---|---|
| 8.808 | α-Pinene | 4.01 |
| 9.843 | Camphene | 4.10 |
| 10.931 | β-Pinene | 1.86 |
| 13.769 | dl-Limonene | 1.59 |
| 14.146 | 1,8-Cineole | 12.07 |
| 22.729 | α-Thujone | 19.89 |
| 23.473 | β-Thujone | 5.20 |
| 26.580 | Camphor | 15.72 |
| 28.949 | Bornyl acetate | 3.31 |
| 29.664 | Caryophyllene | 3.70 |
| 32.434 | α-Humulene | 3.19 |
| 33.464 | Borneol | 12.86 |
| 47.540 | Veridiflorol | 4.21 |
| 58.886 | Epimanool | 1.01 |

### 3.4. Rancimat Analysis Results

The induction periods obtained under the rancimat conditions of refined sunflower oil and %2 and %5 sage added refined sunflower oils are given in table 2.

**Table 2.** Induction periods average of sunflower oil samples, %2 and %5 sage added and waited different times of refined sunflower oil samples

| | IP (h)(24 hour waited) | IP (h)(one week waited) |
|---|---|---|
| RSO | 1.36 | |
| 2% sage added | 1.58 | 2.17 |
| 5% sage added | 1.75 | 2.05 |

## 4.Discussion and Suggestions

A study done showed that natural products can improve the stability of rapeseed oil, especially when it is in a matrix rather than an extract or a pure compound. When the changes in induction time were tested at the end of one day and 1 week by adding some vegetable powders such as 1 gram beet, broccoli carrot celery, spinach tomato pea to 20 ml rapeseed oil, it was observed that it increased the induction time successfully (Tundis et al., 2017). It has been confirmed in another study that the sumac thyme mint nettle extracts increase the antioxidant properties and shelf life of refined corn oil. In this study, schaal oven test method was used instead of ransimat method(Baştürk et al., 2018). Another study showed that grape seed extract up to 600-800 ppm inhibited lipid oxidation in a similar way to BHT and could be used as a potential natural extract to improve the oxidative stability of sunflower oil (Poiana, 2012). Mulberry (*Morus indica* L.) leaves and powder compared to BHT rice bran oil has been tested in another study to be effective in inhibiting lipid oxidation (Roy et al., 2010). The Schaal oven test indicated that rosemary extract delays lipid oxidation of flaxseed oil (Wang et al., 2018). These studies inspired and guided our work.

The major components of essential oil were α-Thujone 19.891%, Camphor 15.717%, borneol 12.856%, 1.8-cineole 12.065%.In addition, camphene 4.103%, α-pinene 4.007%, β-thujone 5.2%, veridiflorol 4.209% are also in remarkable amounts. in a study done α-Thujone has been tested as the main component of sage and it is consistent with the results of our study(Moura-Alves et al., 2020). The highest antioxidant and phenolic contents of salvia officinalis were detected during the flowering period, and rosmarinic acid and phenolic diterpenes are highly abundant components(Farhat et al., 2014). The total phenolic content of sage was calculated as 0.324 g / 100g in terms of caffeic acid. The effects of natural antioxidant sources alternative to synthetic antioxidants, such as ginger turmeric powder, on the shelf life of oils have been investigated in scientific studies and give positive results(Dıraman & Baydır, 2017; Tinello & Lante, 2020). According to the results of the rancimat analysis, the average induction period of refined sunflower oil was 1.36h and the induction period when 2% sage added and waited 24 hours is 1.58. the induction period 2% sage added and waited one week is 2.17. The induction period 5% sage added to sunflower oil and waited one day and one week respectively are 1.75 and 2.05 . Shelf life under rancimat conditions increase when added sage as powder . Here, we can state that sage increases refined sunflower oils shelf life and oxidation stability and can be used as a natural antioxidant for this purpose.

## 5. Conclusion

In this study, the total antioxidant, phenolic properties and the essential oil composition of the s officinalis plant grown in Afyonkarahisar were determined. In addition, the positive effect of dried plant leaves on the oxidation stability of sunflower oil was confirmed by the Rancimat method and it was concluded that it can be used as a natural antioxidant source. In subsequent studies, it is thought that the antioxidant effect can be investigated by adding different amounts of plant powder or essential oil to different cooking oils at different holding times. Sensory analysis of the oil with added antioxidant source can also be planned. This study proves the accuracy of the conclusion that foods containing antioxidants slow down or prevent oxidation in edible oils. Our study is a guide for scientists doing research on natural antioxidants or s officinalis.

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