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Abstract

Antioxidants are bioactive compounds that prevent lipid oxidation, and they can be either water-soluble or lipid-soluble, available in both natural and synthetic forms. Lipid-soluble antioxidants are particularly important for protecting fat-containing foods from spoilage, yet consumers increasingly prefer natural and minimally processed additives over synthetic ones. This study aimed to extract natural lipid-soluble antioxidants from rosemary leaves using soybean and canola oils as sustainable solvents, avoiding chemical solvents. Dried rosemary leaves were ground and added to the vegetable oils at 10% w/w, then mixed and heated at 110°C for 3 hours. Ethanol extraction was performed as a control. The resulting antioxidant solutions were filtered and analyzed by high-performance liquid chromatography (HPLC), confirming the presence of carnosic acid and carnosol—the main lipid-soluble antioxidants in rosemary. Canola oil extracts contained higher amounts of carnosic acid compared to soybean oil, while carnosol levels were similar. Moreover, vegetable oil extractions yielded higher carnosic acid content than ethanol extraction. To assess antioxidant efficacy, 2% (w/w) of the extracts were added to soybean oil and stored at 60°C for 22 days. The peroxide value (PV), anisidine value (AV), and Totox value of oils containing the extracts were significantly lower than those of the control oil. These results indicate that soybean and canola oils are effective and sustainable alternatives to chemical solvents for extracting lipid-soluble antioxidants from rosemary leaves.

Keywords: Antioxidants, Vegetable oils, Soybean oil, Rosemary, Oxidation

Introduction

Lipid oxidation occurs when a hydrogen atom is removed from a fatty acid, forming a free radical. Once this free radical is formed, oxygen present in the atmosphere reacts with the free radical to produce a peroxyl radical. This peroxyl radical continues to react with other lipids and compounds in the system, forming hydrogen peroxide, ketones, epoxides, aldehydes, and other compounds. Oxidation products lead to food spoilage, undesirable taste and odor, and the formation of toxic compounds (Cho et al., 2019).

Antioxidants have attracted significant attention due to their protective role in food and pharmaceutical products against oxidative degradation and pathological processes caused by oxidative stress. Investigating the antioxidant properties of plants and their derived compounds requires appropriate methods that consider the antioxidant mechanism of action and focus on reaction kinetics, including those involving antioxidants. Antioxidants are important in various parts of the body for protecting healthy cells and organs. They also help delay lipid oxidation in food systems (Golchin, 2020). These compounds can be either water-soluble or lipid-soluble. Water-soluble antioxidants assist in chelating metal ions in aqueous food emulsions (Lobo et al., 2010). Most antioxidants are added to foods to protect them from spoilage and discoloration caused by lipid oxidation. Therefore, this study focused on lipid-soluble antioxidants.

Rosemary (scientific name Salvia rosmarinus) belongs to the Lamiaceae family and is native to the Mediterranean region. It is a perennial shrub that remains green throughout the year and can grow to a height of 1 to 2 meters. This plant has slender branches with thick, leathery, and aromatic leaves and is considered an aromatic herb (Gholami et al., 2020). Among various plants, rosemary contains numerous water-soluble and lipid-soluble phenolic compounds. Due to its richness in antioxidants such as rosmarinic acid (RA), carnosic acid, and carnosol (CO), rosemary has been extensively studied and utilized in the food industry. Carnosic acid, a lipid-soluble antioxidant, is abundantly present in rosemary (Singleton, 2016).

Numerous studies have been conducted on the extraction of rosemary leaf extracts using chemical solvents. In a study by Alizadeh et al. (2013), the antioxidant effects of rosemary and savory extracts were compared with the synthetic antioxidant TBHQ on the oxidation process of oil during deep frying. This study proposed rosemary extract as a potential substitute for the synthetic antioxidant TBHQ. Muchkovska et al. (2020) investigated the impact of adding rosemary extract on the oxidative stability and quality of hemp oil. The aim of this research was to evaluate the antioxidant efficacy of rosemary extracts obtained using different solvents on the quality and stability of hemp oil. The results indicated that rosemary extract positively reduced oxidation and could serve as a natural alternative to synthetic antioxidants, especially for products with high fatty acid content. In a study by Ginzburg and Maleki (2020), high-oleic soybean oil, peanut oil, and cottonseed oil were used to extract lipid-soluble antioxidants from rosemary, and the conventional chemical solvent extraction method was employed for comparison. The results showed that all three oil-antioxidant solutions exhibited comparable antioxidant effects. Vegetable oils extracted 10 to 24% more antioxidant compounds compared to the chemical solvent extraction method (ethanol).

The conventional method used for extracting carnosic acid and other antioxidants from rosemary leaves involves the use of chemical solvents. The extracted antioxidants undergo treatments to remove the chemicals used during extraction; however, it is known that even with additional treatments, some chemical residues remain alongside the antioxidants, which is unacceptable to consumers. Moreover, the cost of using chemical solvents for extracting lipid-soluble antioxidants is high. The solvents must be removed, which is a time-consuming and expensive process. Once the solvent is removed, it cannot be reused for other purposes and is lost, as the common method of solvent removal is through evaporation (Huang & Li, 2023). Additionally, in chemical extraction

methods, the rosemary leaves used become contaminated with chemical solvents and cannot be reused, requiring disposal. To avoid these concerns, natural extraction processes are preferred. Since many antioxidants in rosemary leaves are lipophilic, a lipid source can be effective in antioxidant extraction; therefore, the use of vegetable oils is a promising solution for extracting antioxidants from rosemary leaves. Furthermore, consumers are familiar with vegetable oils and do not perceive them as harmful additives (Bertich et al., 2015). Overall, the aim of this study was to extract lipid-soluble antioxidants from rosemary leaves using vegetable oils without the use of chemical auxiliaries.

Materials and Methods

Sample Preparation

Fresh rosemary leaves were collected in early May 2023 from a private farm located in Behshahr city, Mazandaran province. To remove dust and surface contaminants, the samples were washed with water. Then, the samples were placed on a clean cloth to allow the water from washing to evaporate. The rosemary leaves were carefully separated from their stems, and the initial moisture content of the leaves was measured according to the standard moisture determination method AOCS Ai 2-75, using equation 3-1.

Equation 1

$$\text{Moisture content} = (\%) \ \frac{Weight\ loss}{Sample\ Weight} \times 100$$

An oven (Gosonic, GEO-433, Turkey) was used to dry the rosemary leaves at 50 ± 5°C. The sample weight was measured every hour at 50°C, and the weight loss due to moisture evaporation was recorded. After 5 hours, when the weight difference reached 0.1 g, the drying process was stopped. The dried samples were ground twice using a mill (Vidas, VI-3761B, Iran) at high speed for 7 seconds each time. The obtained dried rosemary leaf powder was placed in zip-lock polyethylene bags and stored at 4°C for further testing.

Measurement of Physicochemical Properties of Selected Vegetable Oils

As mentioned, the aim of this study was to use vegetable oils for the extraction of lipid-soluble antioxidants from rosemary leaves. In this study, two vegetable oils—soybean and canola—produced by Ghoncheh Oil Extraction Factory (Dasht-e Naz region, Mazandaran province), which underwent minimal processing and contained no added antioxidants, were used for extracting antioxidants from rosemary leaves. The viscosity of these oils was measured at 25°C, along with their acid value and density according to the standard methods of the American Oil Chemists' Society (AOCS). The iodine value was determined following the Vijs method, acidity was measured according to the Iranian National Standard No. 4178, and the refractive index at 40°C was measured using a refractometer according to Iranian National Standard No. 5108. Additionally, the percentage of saturated and trans fatty acids was analyzed using gas chromatography of fatty acid methyl esters according to Iranian National Standard No. 13126.

Analysis of Chemical Composition of Selected Vegetable Oils

The selection of oils was based on the expected fatty acid profile reported for vegetable oils, as different fatty acids may affect the efficiency of extracting lipid-soluble compounds. The oil analysis was performed by

fatty acid methyl esterification (FAME) followed by profiling of the derivatives using gas chromatography coupled with flame ionization detection (GC-FID) (Ginzburg & Maleki, 2020).

Evaluation of Oxidative Stability of Vegetable Oils

Measurement of Peroxide Value (PV)

The peroxide value (PV) of vegetable oils was measured using the official method 965.33 of the Association of Official Agricultural Chemists (AOAC) according to Equation 2.

Equation 2

$$PV = \frac{(S - B) \times N \times 1000}{M}$$

S – Volume of titrant used for the sample
B – Volume of titrant used for the blank
N – Normality of the titrant used
M – Mass of the sample

Measurement of p-Anisidine Value (AV)

The p-anisidine value (AV) was measured according to the American Oil Chemists' Society (AOCS) official method Cd 18-90 using Equation 3.

Equation 3

$$p - A.V. = \frac{25 \times (1.2As - Ab)}{m}$$

As – Absorbance of the vegetable oil solution after reaction with p-anisidine reagent
Ab – Absorbance of the vegetable oil solution (blank)
m – Mass of vegetable oil in grams

Measurement of Totox Value

This index is a qualitative indicator of the level of non-volatile carbonyl precursor compounds and the extent of developed oxidation products. It is used to evaluate the oxidative stability of oils and was calculated using Equation 4 (Farahmandfar, 2012).

Totox value = Anisidine value + (Peroxide value × 2) **Equation 4**

Measurement of Iodine Value

The iodine value (IV) was determined by the Wijs method according to the AOCS standard method Tg 14-64 using Equation 5.

Equation 4

$$IV = \frac{(B - S) \times M \times 12.691}{m}$$

S – Volume of titrant used for the sample
B – Volume of titrant used for the blank

M – Molarity of the titrant used
m – Mass of the sample

Extraction of Antioxidants from Rosemary Leaves
Extraction of Antioxidants from Rosemary Leaves Using Vegetable Oils

To perform the extraction of antioxidants from rosemary leaves using vegetable oils, the dried rosemary leaf powder (10% w/w) was completely immersed in vegetable oil at 110°C to ensure that the lipid crystals of the vegetable oils were melted and remained at the same temperature throughout the extraction process. The mixture of oil and ground rosemary was continuously stirred on a magnetic hot plate at this temperature for 3 hours. After heating, the samples were passed through a paper filter (20 µm pore size) to separate the solid residues from the solution. The oil–antioxidant solution was stored at 4°C for further tests on antioxidant efficacy, identification, and quantification (Ginzburg & Maleki, 2020).

Extraction of Antioxidants from Rosemary Leaves Using Chemical Solvent

To compare the extraction efficiency of the methods, extraction using a chemical solvent as a conventional method was performed. For this purpose, 6 g of dried rosemary leaf powder was mixed with 120 mL of 70% ethanol (solid-to-solvent ratio of 1:20). The extraction process was carried out by Soxhlet extraction at 70°C for 2 hours. The obtained extract was filtered using Whatman No. 2 filter paper and concentrated using a rotary evaporator (Toption evaporator, RE-5210, China) at 40°C. Finally, the concentrated extract was placed in a vacuum oven at 35°C for 2 hours to remove any remaining solvent. The dried extract was collected in a dark glass container and stored at 4°C until further use (Tavasoli et al., 2011).

Analysis of Antioxidants Extracted from Rosemary Leaves

The identification and quantification of carnosic acid and carnosol present in the extracted products were analyzed using high-performance liquid chromatography coupled with a photodiode array detector (HPLC-PDA) according to the method described by Ginzburg (2020).

Evaluation of the Effect of Extracted Antioxidants on Lipid Oxidation

The effect of antioxidant solutions extracted using soybean and canola oils on the oxidative stability of vegetable oil was evaluated by adding them to canola oil. Lipid oxidation in canola oil supplemented with 2% (w/w) rosemary antioxidant extract was examined. This sample, along with a control sample (pure canola oil without any additives), was incubated in triplicate at 60°C for 22 days (Ginzburg, 2020). Primary and secondary oxidation levels were assessed using peroxide value (PV), p-anisidine value (AV), and Totox value. To monitor changes in unsaturated fatty acid levels in the canola oil samples during oxidation, the content of double bonds was measured by iodine value determination.

Statistical Analysis

Statistical analysis of the data was performed using mean values with standard deviations for samples in triplicate. One-way ANOVA was conducted using SPSS software (version 26) to determine significant differences between treatments at a significance level of 0.05. Means were compared using Duncan's test at a 95% confidence level. Graphs were plotted using Microsoft Excel software (version 2022).

Results and Discussion

Moisture Content of Rosemary Leaves

The initial moisture content of the rosemary leaves was determined to be 71.41 ± 1.30%.

Characterization of the Initial Physicochemical Properties of the Selected Vegetable Oils

The physicochemical properties of soybean and canola oils were measured and are presented in Table 1.

Table 1 – Physicochemical Properties of Soybean and Canola Oils

Property	*Soybean Oil*	*Canola Oil*
Iodine Value	132.00 ± 3.00^{a}	117.67 ± 8.02^{b}
Anisidine Value	3.46 ± 0.22^{a}	3.30 ± 0.70^{a}
Density (g/ml)	0.915 ± 0.001^{a}	0.911 ± 0.001^{b}
Viscosity (cP)	50.09 ± 1.01^{b}	75.80 ± 0.80^{a}
Acid Value (mg KOH/g oil)	0.25 ± 0.05^{a}	0.16 ± 0.06^{a}
Peroxide Value (mmol O_2/kg)	2.70 ± 0.30^{a}	0.99 ± 0.27^{b}
Acidity (% w/w)	0.045 ± 0.005^{a}	0.043 ± 0.025^{a}
Refractive Index	1.467 ± 0.001^{a}	1.465 ± 0.003^{a}
Saturated Fatty Acid (%)	15.50 ± 0.50^{a}	7.00 ± 0.50^{b}
Trans Fatty Acid (%)	0.50 ± 0.20^{a}	0.65 ± 0.15^{b}

***Different letters in each row indicate statistically significant differences at the 0.05 level.**

The iodine value indicates the degree of unsaturation in oil and is expressed as grams of iodine absorbed per 100 grams of oil. A lower number of double bonds corresponds to a lower iodine value and, consequently, a lower susceptibility to oxidation (Farahmandfar, 2012). In this study, the iodine value of canola oil was significantly lower than that of soybean oil ($P<0.05$), suggesting that canola oil is more resistant to oxidation. The viscosity of soybean oil (50.09 ± 1.01 cP) was significantly lower than that of canola oil (75.80 ± 0.80 cP) ($P<0.05$), which is consistent with the fact that viscosity decreases with increasing double bonds. The peroxide value, which is used to measure the primary oxidation products, was also significantly lower in canola oil than in soybean oil ($P<0.05$), further supporting the greater oxidative stability of canola oil. A significant difference was observed in the density of the two oils, with canola oil exhibiting a lower value ($P<0.05$). However, no significant differences ($P>0.05$) were found between soybean and canola oils in terms of p-anisidine value, acid value, acidity, refractive index, and trans fatty acid content, indicating that they were similar in these characteristics.

Fatty Acid Profile of Vegetable Oils and Its Effects on Antioxidant Extraction

The GC-FID chromatograms of both soybean and canola oils are presented in Figure 1. The differences in peak areas and retention times indicate that the fatty acid profiles of these two vegetable oils are distinct from one another.

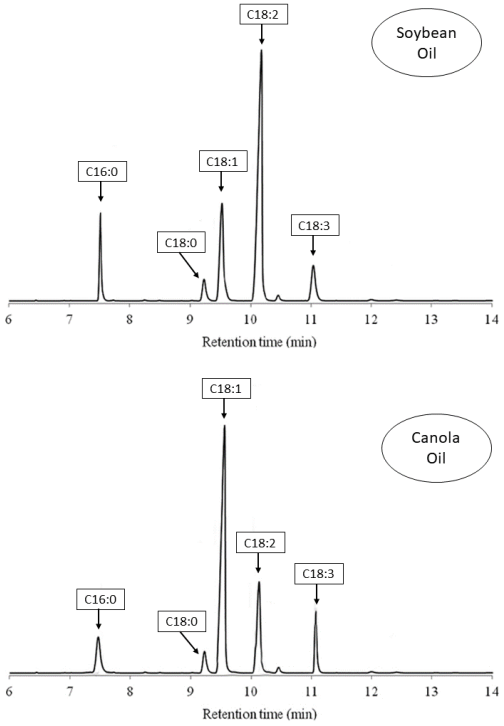


Figure 1. Fatty acid profiles obtained by gas chromatography coupled with flame ionization detection (GC-FID): Chromatograms of the vegetable oils used for extraction, (a) soybean oil and (b) canola oil.

The fatty acid profile results, expressed as percentages, are presented in Table 2. As observed, each oil differs in both the composition and proportion of fatty acids. Soybean oil is rich in linoleic acid (C18:2), with a content of 52.1%, which falls within the range reported by the Iranian National Standard No. 2392 (48–59%) for soybean oil. As shown in Table 4-2, the polyunsaturated fatty acid (PUFA) content of soybean oil (C18:2) is 2.4 times higher than the linoleic acid content in canola oil. Additionally, the saturated fatty acid content in soybean oil is higher than in canola oil. These variations in the fatty acid composition of soybean oil may render it a suitable solvent for extracting antioxidants from rosemary leaves, as they may help determine the role of saturated and polyunsaturated fatty acids in the extraction process. The predominant fatty acid in canola oil is oleic acid (C18:1), accounting for 59.1%, which lies within the reported range (51–70%) specified in Iranian National Standard No. 2-4935. Given the low oxidation rate of oleic acid, a monounsaturated fatty acid (MUFA), compared to polyunsaturated fatty acids, it exhibits greater oxidative stability. Moreover, the high solubility in oleic acid may support the potential of canola oil as an effective solvent (Cho et al., 2006). In addition, antioxidants dissolved in oleic acid are more effective at preventing lipid oxidation than when added directly to fat-containing foods (Hernandez, 2016). The only concern regarding the fatty acid profile of the oils lies in their susceptibility to lipid oxidation during the extraction process. Polyunsaturated fatty acids contain allylic hydrogens that require less activation energy for free radical abstraction compared to non-allylic hydrogens; thus, PUFAs undergo more oxidation than MUFAs and saturated fatty acids (Cho et al., 2006).

Table 2 – Fatty acid composition of vegetable oils (soybean and canola oils) used for the extraction of antioxidants from rosemary leaves.

Fatty Acid	(/.) Content	
	Soybean Oil	**Canola Oil**
Myristic Acid (C14:0)	0.1	0.07
Palmitic Acid (C16:0)	11.5	4.9
Palmitoleic Acid (C16:1)	0.11	0.2
Stearic Acid (C18:0)	4.5	2.5
Oleic Acid (C18:1)	21.1	59.1
Linoleic Acid (C18:2)	52.1	22
Linolenic Acid (C18:3)	8.3	8.5
Arachidic Acid (C20:0)	0.4	0.6
Gadoleic Acid (C20:1)	–	0.45
Behenic Acid (C22:0)	–	0.36
Erucic Acid (C22:1)	–	0.43

–Not detected; detection limit was above 0.1%

.

On the other hand, saturated fatty acids are more resistant to lipid oxidation (Cho et al., 2006), which may help prevent the high level of degradation observed in soybean oil during the extraction process. Therefore, to better understand the effect of the fatty acid profile of vegetable oils on the efficiency of antioxidant extraction from rosemary leaves, both soybean and canola oils were used after testing their oxidative stability.

Oxidative Stability Results of Vegetable Oils

The oxidative stability of the soybean and canola oils used for extraction was evaluated after subjecting the oils to a thermal treatment at 110°C for 3 hours, equivalent to the conditions used for antioxidant extraction. The results obtained are presented in Figure 2.

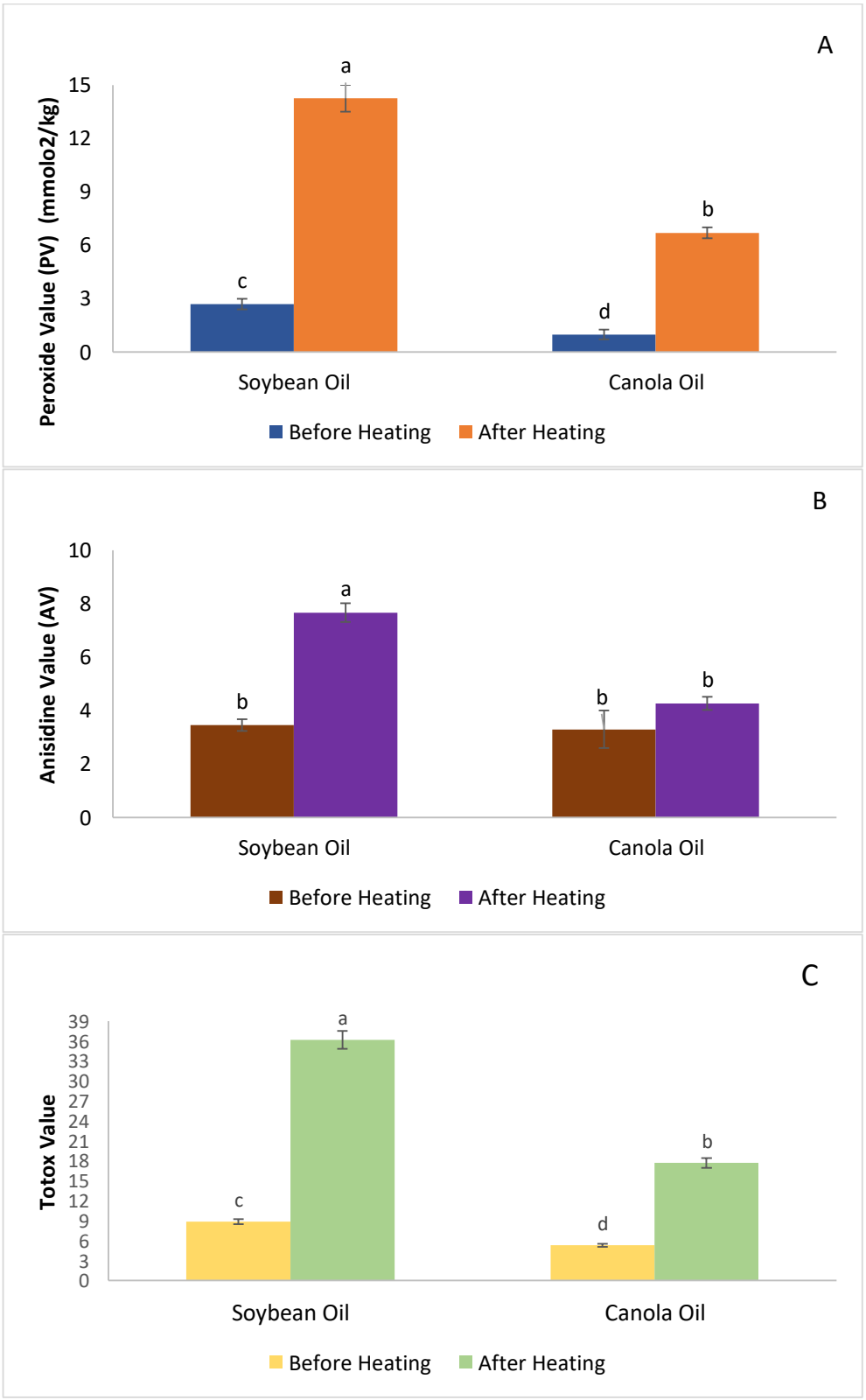


Figure 2 – Values of (A) peroxide value, (B) anisidine value, and (C) totox value of soybean oil (SO) and canola oil (CO), measured before and after the thermal process. Different English letters indicate statistically significant differences between the bars (P < 0.05)

According to the obtained results, the heating process increased the levels of oxidation-indicating parameters. However, its effects on the oxidation of the tested vegetable oils were not uniform. In soybean oil, a significant increase in hydroperoxides was observed after heating ($P < 0.05$). The peroxide value rose from 2.70 ± 0.30 to 14.26 ± 0.75 (mmol O_2/kg). In contrast, canola oil showed no significant increase in primary oxidation products compared to soybean oil after heating. These observations may be related to the higher content of PUFA or short-chain fatty acids in regular vegetable oils with low oleic acid content. The oleic acid percentages in canola and soybean oils were 60.9% and 23.3%, respectively (Farahmandfar, 2012). Similar results were reported by Ali et al. (2013) when comparing the thermal stability of high-oleic sunflower oil to regular sunflower oil. After heating at 185°C for 4 hours, the high-oleic sunflower oil produced significantly lower amounts of hydroperoxides, aldehydes, and ketones than the regular sunflower oil (Ali et al., 2013).

Comparison of secondary oxidation products of the oils is shown in Figure 2, part b. As expected, the anisidine value in canola oil, which contains higher oleic acid, changed less compared to the pre-heating sample, whereas the anisidine value in soybean oil increased significantly ($P < 0.05$). The increase in secondary oxidation products in soybean oil is due to its chemical composition. Soybean oil contains a relatively high amount of linoleic acid compared to canola oil, at 53.7% and 21.0%, respectively (Farahmandfar, 2012). Linoleic acid is highly susceptible to lipid oxidation (Cho et al., 2006). Therefore, during heating, secondary oxidation products form more rapidly in soybean oil than in canola oil.

The Totox value is a qualitative indicator of the amount of non-volatile carbonyl precursors along with developed oxidative compounds. According to the results shown in Figure 2, part c, the Totox value of soybean oil at the end of the heating process was higher than that of canola oil ($P < 0.05$). Although these observations confirm oxidation levels in the vegetable oils, the peroxide values remained below 15 milliequivalents per kilogram, and the anisidine index was below 10, which are the maximum acceptable limits for vegetable oils (Yi et al., 2013). Therefore, both oils are suitable for further research to identify the best oil for extracting fat-soluble antioxidants from rosemary leaves

Identification of antioxidants extracted using vegetable oils and chemical solvent

Figure 3 shows the HPLC chromatograms of vegetable oils after extracting antioxidant compounds from rosemary and compares them with the solution obtained from the chemical solvent extraction.

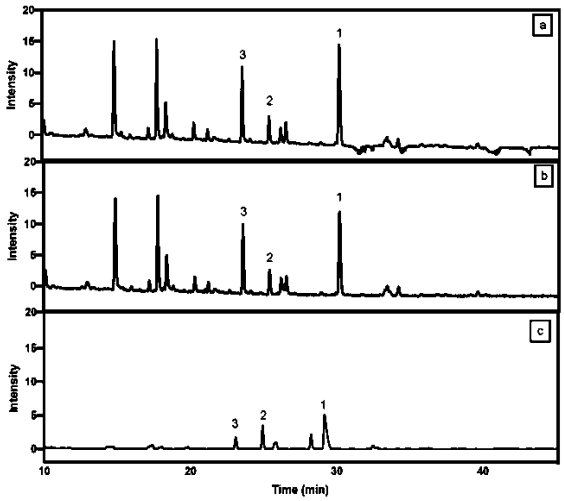


Figure 3 shows the high-performance liquid chromatography (HPLC) chromatograms of extracts obtained using (a) canola oil, (b) soybean oil, and (c) chemical solvent. The numbers represent carnosic acid (1), carnosol (2), and carnosic acid quinone (3).

According to the reports by Bentayeb et al. (2007), Masuda et al. (2002), and Ginzburg (2019), peaks 1, 2, and 3 correspond to carnosic acid, carnosol, and carnosic acid quinone, respectively. Comparison of Figures 3a and 3b shows no difference in the fatty acid profiles between the vegetable oils used. In other words, the type of oil does not affect the ability to extract fat-soluble antioxidants. However, for a more precise analysis, the peak areas and intensities should be examined. To this end, after clear separation and identification of the fat-soluble antioxidant compounds based on retention time and UV spectra according to the standards of the targeted compounds, standard calibration curves for carnosic acid and carnosol should be constructed. Due to limitations in this study, this was not performed.

Although the peak of carnosic acid quinone (peak 3) shows relatively high intensity, it was not further quantified due to limited resources and literature on this compound. Carnosic acid quinone is not commercially available as a standard for HPLC analysis and standard curve construction. Additionally, carnosic acid quinone lacks antioxidant activity; it is an intermediate degradation product of carnosic acid to carnosol and does not exhibit antioxidant properties (Masuda et al., 2002).

As shown in Figure 3, vegetable oils can extract higher amounts of carnosic acid from rosemary leaves compared to ethanol. This level of carnosic acid extraction by vegetable oils demonstrates the advantages of antioxidant extraction using vegetable oils over other techniques. Reported data by researchers indicate that supercritical fluid extraction and 70% methanolic extraction yield 35.70 mg/g and 14.76 mg/g of carnosic acid in rosemary, respectively, which is approximately 28 to 67% lower than the amounts obtained using vegetable oils (Tuna et al., 1997; Biki et al., 2000; Rodriguez-Rojo et al., 2012). The results obtained in this study are consistent with these previous reports.

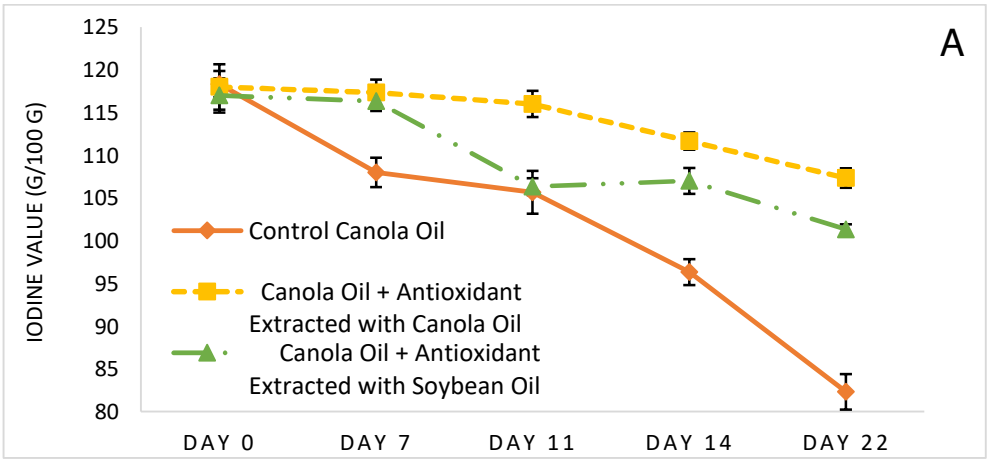
According to the chromatograms in Figure 3, the content of carnosol in the solvent extract is higher compared to the extract obtained using vegetable oils. Although the higher carnosol content might be related to the degradation of carnosic acid during chemical solvent extraction, further studies are required to clarify the increase of carnosol content in this method.

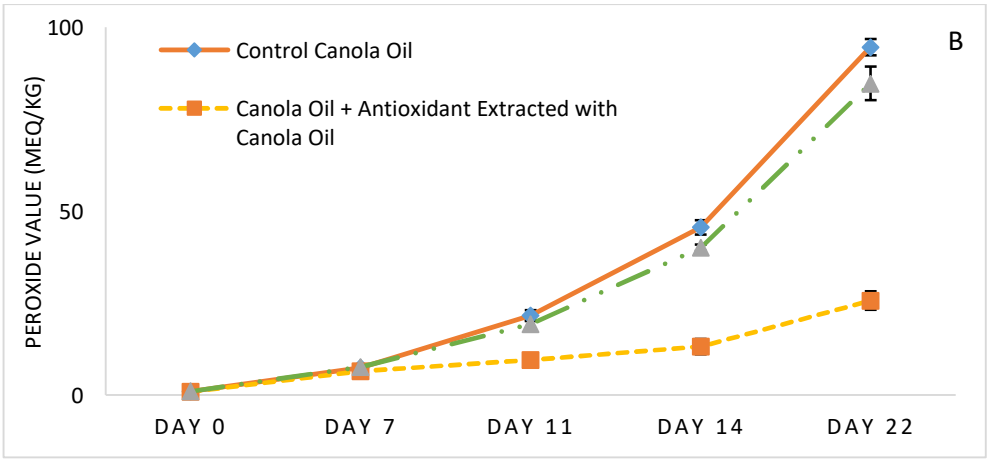
The carnosic acid quinone peak, as shown in Figure 3, is smaller in the chemical solvent extract compared to the vegetable oil extracts. However, as previously discussed, carnosic acid quinone is an intermediate product between carnosic acid and carnosol. Therefore, its smaller peak in the HPLC chromatogram of the chemical solvent extract compared to vegetable oils is because the ethanol extraction process induces more oxidation. Hence, more carnosic acid quinone was decomposed into carnosol in the ethanol extract.

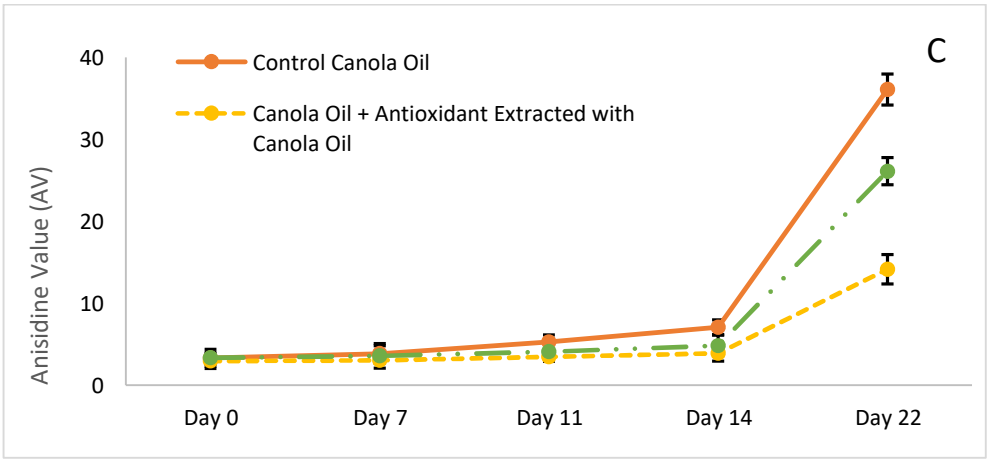
Effects of the Extracted Antioxidants on the Oxidative Stability of Canola Oil

As shown in Figure 4a, during the oxidation process, the level of unsaturated fatty acids in canola oil decreased in all samples. In the control sample, canola oil without any additives exhibited a significantly greater reduction in unsaturated fatty acids. The iodine value (IV) dropped from 118.33 ± 1.53 to 82.33 ± 2.08 g per 100 g in the control after 22 days of storage, compared to the canola oil treated with antioxidants extracted using soybean oil, which decreased from 117.00 ± 2.56 to 101.33 ± 1.15 g per 100 g, and the canola oil treated with antioxidants extracted using canola oil, which decreased from 118.00 ± 2.00 to 107.33 ± 0.58 g per 100 g, respectively. These changes in the IV of the oils over storage time were plotted, and the slopes of the curves were measured to compare oxidation rates. The slope of the control sample was significantly steeper than those of the antioxidant-treated oil samples. Specifically, the slope for the control sample was 8.37 per day, whereas for the canola oil samples containing antioxidants extracted with soybean and canola oils, the slopes were 4.07 and 2.70, respectively. These iodine values indicate a much higher

oxidation rate in the control samples compared to the others. Between the two antioxidant-oil combinations, the oxidation rate decreased more markedly in the canola oil sample containing antioxidants extracted with soybean oil. This can be explained by the fatty acid profile of soybean oil and its high linoleic acid content. Oils with higher linoleic acid content are more susceptible to oxidation compared to MUFAs and saturated fatty acids; upon oxidation, they form conjugated dienes (Cho et al., 2006).







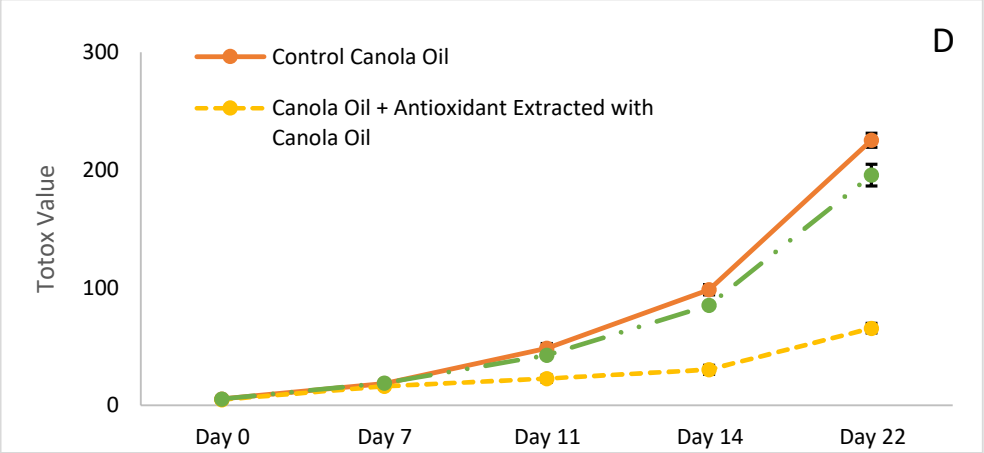


Figure 4 – Values of iodine value (A), peroxide value (B), anisidine value (C), and totox value (D) for canola oil (control) and canola oil with 2% (w/w) antioxidants extracted using canola oil and soybean oil, stored for 22 days at 60°C.

After seven days of storage, as shown in Figure 4b, the peroxide value (PV) slightly increased in all samples. After 11 days of storage, a significant increase in peroxide value was observed in the control sample compared to the canola oil samples containing extracted antioxidants. Similar findings were reported by Sadeghi et al. (2016). In their study, after storing soybean oil with and without added antioxidants for 6 days in the dark at 65°C, no significant change in peroxide value was observed compared to day one; however, after 12 days of storage, a significant increase in peroxide value was reported for canola oil without antioxidants compared to canola oil with added antioxidants (Sadeghi et al., 2016). As observed in Figure 4b, after 14 days of storage, the formation of hydroperoxides in canola oil containing antioxidants extracted with canola oil showed the least increase. The control sample exhibited the highest increase in hydroperoxide formation. In the canola oil sample with antioxidants extracted using soybean oil, although the increase in peroxide value was lower, it was very close to that of the control sample.

Moreover, after 22 days of storage, the peroxide value (PV) in canola oil without any additives was significantly higher (94.57 ± 2.25 mEq/kg). On the final day of storage, the PV levels in canola oil with antioxidants extracted using soybean oil and canola oil were 84.70 ± 4.56 and 25.67 ± 2.52 mEq/kg, respectively. To compare the efficiency of vegetable oils in antioxidant extraction, the slopes of the peroxide value curves were also measured and analyzed. The slope of PV increase in the control sample (22.53) was much steeper than that of canola oil with antioxidants extracted by soybean oil (19.98) and canola oil with antioxidants extracted by canola oil (5.62). A significant increase in hydroperoxide formation was observed in the control sample compared to canola oil with added antioxidant extracts. When comparing canola oils with the added extracted antioxidants based on the vegetable oils used for extraction, the oil containing antioxidants extracted with canola oil exhibited greater oxidative stability.

The same trend observed in the measurement of peroxide value (PV) was also seen in the anisidine value (AV). As shown in Figure 4c, the anisidine index increased more significantly in the control sample, while canola oil samples with extracted antioxidants exhibited a lower slope. The stability of the anisidine index until day 11 indicates that the oil had not undergone secondary oxidation at that time. Moreover, aldehydes and ketones previously formed interacted with other molecules in the system and thus were not accounted for in the AV measurement (McClements & Decker, 2008). Furthermore, after 22 days at 60°C, the AV in the control sample (canola oil without any added antioxidants) increased from 7.07 ± 0.90 (after 14 days of storage) to 36.07 ± 1.90. In contrast, canola oil containing antioxidants extracted using canola oil and soybean oil increased from 3.87 ± 0.95 and 4.83 ± 1.26 to 14.13 ± 1.80 and 26.52 ± 2.10, respectively. This lower AV level indicates that significantly more secondary oxidation products formed in the control sample

compared to the treated samples. These findings are consistent with those of Sadeghi et al. (2016), who reported higher levels of secondary oxidation (AV) in soybean oil stored at 65°C for 12 days without added antioxidants compared to soybean oil with added antioxidants.

Consistent with the PV results, the amount of secondary oxidation products formed in canola oil containing antioxidants extracted with canola oil was significantly lower than that in the canola oil sample containing antioxidants extracted with soybean oil and the control sample; in other words, the canola oil sample with antioxidants extracted using canola oil contained fewer ketones and aldehydes compared to the other two samples. These findings align with the results discussed in section 4-4 regarding the oxidative stability of the vegetable oils. Canola oil with antioxidants extracted using soybean oil showed a significant increase in secondary oxidation products formed after the heating process used for antioxidant extraction compared to canola oil with antioxidants extracted using canola oil. This could be due to the oxidative instability of soybean oil or differences in the antioxidant content extracted by canola oil compared to that extracted by soybean oil.

The results of the Totox value are presented in Figure 4, part (c). This value for the control sample after 22 days of storage was 225.20 ± 6.07, while for the canola oil samples containing antioxidants extracted with soybean oil and canola oil, the values were 195.50 ± 9.31 and 65.47 ± 4.11, respectively. As observed, the canola oil containing antioxidants extracted with canola oil exhibited the lowest Totox value among all samples. These findings are consistent with the results reported in section 4-4 regarding the oxidative stability of vegetable oils.

Conclusion

Vegetable oils are good alternatives to chemical solvents for extracting fat-soluble antioxidants from rosemary leaves. Vegetable oils primarily consist of fatty acids that trap and dissolve carnosic acid and other fat-soluble antioxidants present in rosemary leaves. Dried rosemary leaves were used as the raw material. Both vegetable oils used for antioxidant extraction (soybean oil and canola oil) can be employed as solvents for antioxidant extraction. Although canola oil extracted more carnosic acid than soybean oil, both vegetable oil extracts exhibited comparable antioxidant effects. Vegetable oils extracted higher amounts of carnosic acid compared to the chemical solvent; however, the chemical solvent extracted more carnosol than the vegetable oils. The results demonstrated that the use of vegetable oils for extracting fat-soluble antioxidants was successful; however, further research is required to improve and develop antioxidant–vegetable oil solvent systems.

In the HPLC chromatograms, numerous peaks other than those of carnosic acid and carnosol were observed. These peaks require further investigation to determine whether they originate from the antioxidant–vegetable oil solutions or from the sample preparation necessary for HPLC analysis. Additionally, these peaks should be analyzed to ascertain whether the compounds they represent are non-toxic and safe for human consumption. The volatile antioxidants present in the solutions have not been thoroughly examined. These volatile antioxidants were not measured in the antioxidant–vegetable oil solutions. For identification and quantification of volatile compounds in the solutions, the volatiles must be extracted from the oil, since oil cannot be directly injected into a gas chromatography system for analysis. Therefore, a more comprehensive evaluation of the obtained antioxidant–vegetable oil solutions should be conducted before asserting their antioxidant potency.

Additionally, the rosemary antioxidant–vegetable oil solutions appear green due to the presence of chlorophyll pigments and other non-antioxidant compounds in the solutions; therefore, the extracted antioxidant solution must be purified before use. Consumers and industry professionals are generally reluctant to use greenish mixtures that cause discoloration of the final product. It is preferable to employ a non-chemical method to remove residual colored compounds from the antioxidant–vegetable oil solutions. Moreover, these antioxidant–vegetable oil solutions have a strong odor derived from compounds extracted from rosemary leaves and should be deodorized before being added to food products. These aromatic

compounds from rosemary leaves are primarily volatile antioxidants present in the extract; thus, removing them may reduce some of the antioxidant effects of the solutions. Therefore, it is advisable to develop a method to deodorize the solutions without compromising their antioxidant activity.

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