Spearmint hydrodistillation by-products: antioxidant activity and effects on lipid oxidation in sunflower and flaxseed oils

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Summary
In this study, spearmint hydrodistillation by-products (residual water and residual leaves material) were studied regarding their antioxidant potentials. Extraction of rosmarinic acid from the leaves to the residual water reached its maximum (7.04 $g \cdot l^{-1}$) at a distillation time of 150 min. The extracts obtained from residual water and residual leaves material were examined with several in vitro antioxidant activity-related assays including β-carotene bleaching activity, forced oxidation test and radical-scavenging activity to determine the antioxidative activities of extracts. Only residual water displayed dose-dependent antioxidative effects on β-carotene linoleate system with activity of 66 % at 1 $g \cdot l^{-1}$. The extracts added to the flaxseed and sunflower oils at the level of 1 $g \cdot l^{-1}$ exerted pro-oxidative effects on the oils in an accelerated test at 70 °C, whereas residual water had no observable positive or negative effects on the oxidation status of the oils. The results indicated that residual water and residual leaves material might be a candidate for enrichment and/or recovering of rosmarinic acid.

Keywords
spearmint; hydrodistillation; antioxidant activity; sunflower oil; flaxseed oil

Mentha spicata L. (spearmint) is an important medicinal and aromatic plant from the Lamiaceae family. Plants of this family have been recognized for their bioactive potentials such as antioxidant, antimicrobial and antidiabetic [1]. Spearmint is one of the functional medicinal plants in the *Mentha* genus, which involves 25–30 species [2]. Spearmint contains secondary metabolites such as terpenoids, essential oils and polyphenols that exhibit health benefits [3]. Among these metabolites, the main volatile compound in spearmint oil is carvone, forming 49–77 % of total essential oil [4], while the main phenolic compound in its leaves is rosmarinic acid, found between 7.1 $g \cdot kg^{-1}$ and 14.3 $g \cdot kg^{-1}$ [5]. Besides traditional medicine, spearmint leaves have been used in various foods as a flavouring agent for many years. Additionally, it is also grown for its essential oil, which is applied in cosmetic (e.g. in perfumes and make-up products), traditional medicine (e.g. treatment of cough, fever, obesity and digestive disorders) and food industries (e.g. as food preservatives and additives) [6]. To obtain essential oils of aromatic plants, various separation techniques are used, which involve steam distillation, supercritical solvent extraction, techniques involving ultrasonication, solvent-free microwave extraction, cold pressing, natural deep eutectic solvents, pulsed electric fields, subcritical water extraction and extraction by ionic liquids. Hydrodistillation is by far the most used technique both on laboratory and industrial scale [7]. In hydrodistillation, a mixture of water and aromatic plant is subjected to heat treatment to boil the water together with essential oil below their individual boiling points [8]. Vaporized components, water and essential oil, are then naturally separated because of their immiscibility after cooling the mixture in the collection vessel [9]. Distillation processes, regardless of the technique used, generate massive amounts of residuals compared to the obtained amount of essential oils at the end of the process. In the context of hydrodistillation, the technique has some drawbacks as volatiles above the boiling points of the mixture of essential oil and water can only be obtained in small amounts. Therefore, various components

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may remain as non-distilled in significant amounts in residual water and in the remaining biomass [8]. In literature, although there is no consensus on the naming of residuals remaining after hydrodistillation of the plant material, the residuals are mostly referred as distilled leaves (residual leaves material), residual water and hydrosol [10]. All residual parts may contain significant amounts of components after distillation of various aromatic and medicinal plants, for instance, the biomass after distillation was reported to contain cellulose, lignin, hemicellulose, whereas hydrosols and residual waters may contain bioactive compounds including polyphenols and terpenes [7]. Essential oils have antimicrobial, antioxidant and anti-inflammatory properties when incorporated into certain foods [11, 12]. Residual water and residual leaves material from the distillation of spearmint essential oil are examples of hydrodistillation by-products that may have various biological activities. Therefore, they can be suitable for recovery of bioactive components for food and pharmaceutical industries [13–15].

Oxidation of foods is a well-known and broadly studied phenomenon. It is regarded a major cause of quality loss that might occur at food processing operations and during storage. Numerous studies reported the protective effects of antioxidants including phenolics on oxidation of lipids. However, only a few studies [16, 17] reported the pro-oxidant effects of these compounds on lipid systems.

The objectives of the present study were to determine in vitro antioxidant potentials of by-products from spearmint hydrodistillation and use of the extracts of the by-products in the prevention of oil oxidation under forced oxidation conditions.

MATERIAL AND METHODS

Materials

Spearmint (*Mentha spicata* L.) leaves were provided in dry form from a local herb seller (Kayseri, Turkey). All chemicals were of analytical grade from Sigma-Merck (St. Louis, Missouri, USA). Sunflower oil (Orkide, Turkey) and flaxseed oil (Krk, Turkey) were purchased from a local market.

Hydrodistillation

Dry plant material (50 g) was distilled with water (500 ml) by using Clevenger apparatus (İldam Cam, Ankara, Turkey). Throughout the hydrodistillation process (4 h), distilled water was recycled into the extraction flask continuously. Spearmint residual water (SRW) that remained in the distillation flask together with plant material (spearmint) was sampled every 30 min to monitor the composition of SRW. For sampling, at the end of each period, SRW was withdrawn from the extraction flask with the help of a glass pipette, then these samples were transferred into tubes. Residual leaves material (DSL) that remained in the distillation flask together with SRW was dried in an oven at 50 °C after finishing the hydrodistillation process and separating SRW from DSL by filtration (cellulose filter, pore size of 50 μm; Macherey-Nagel, Düren, Germany). The dried DSL was ground into fine particles (approximately 500 μm) using a blender (Waring, Staufen, Germany) and then stored at room temperature (25 ± 2 °C) until analysed for a maximum of 3 months.

Extraction of phenolics

The extraction of phenolic compounds from SRW and DSL was carried out by a sequential extraction (Fig. 1). Five grams of DSL or of untreated dry leaves (SL) were extracted with 100 ml of a mixture of methanol and water (1 : 1, v/v) for 1 h at 60 °C in a shaking water bath at 1.7 Hz. The resulting mixture was filtered through a cellulose filter and the filtrates were collected. The extracts were coded as DSL_{MW} for methanol-water extract from DSL and SL_{MW} for methanol-water extract from SL, respectively. SRW (30 ml) was extracted with ethyl acetate (3 × 30 ml) in a separation funnel, decanted and coded as SRW_{EA}. DSL (50 g) was subjected to Soxhlet extraction for 6 h with ethyl acetate and the extract was coded as DSL_{EA}.

Spray drying

SRW and maltodextrin (glucose equivalent of 15–17) at equal dry matter amounts (1 : 1, w/w) were mixed by stirring (170 Hz, 5 min). This mixture was fed to a spray dryer model B290 (Buchi Labortechnik, Flawil, Switzerland) at 140 °C inlet temperature, 8 $ml \cdot min^{-1}$ feed flow rate and 600 $l \cdot h^{-1}$ drying air feed rate. The resulting spray dried spearmint residual water (SDRW) was collected and stored at 4 °C until analysed for a maximum of 3 months.

HPLC analysis of rosmarinic acid

The content of dominant phenolics in spearmint extracts was determined by a high performance liquid chromatography system equipped with a photodiode array detector (HPLC-DAD). Separations were done with a C18 analytical column (Brisa LC2, 150 mm × 4.6 mm, particle size 5 μm; Teknokroma, Barcelona, Spain) using

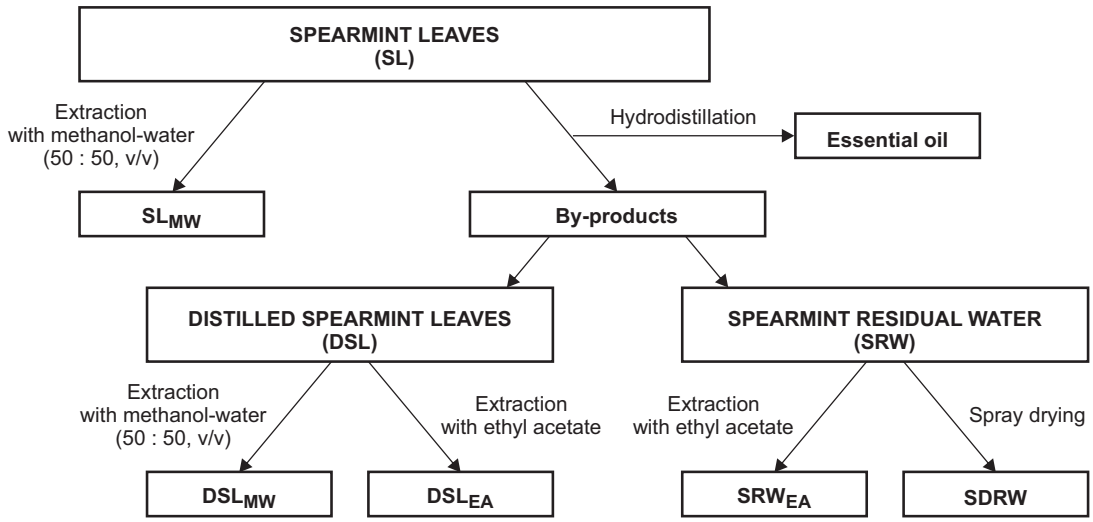

Fig. 1. Schematic diagram of the extraction process.

a mobile phase consisting of methanol (A) and 2% (v/v) acetic acid (B). The elution program was 25 % A for 5 min, 25–75 % A between 5 min and 35 min, 75–90 % A between 35 min and 38 min, and 90–25 % A between 38 min and 40 min. The flow rate was 0.7 ml·min^{-1}, while the column temperature was 25 °C. The spearmint extracts were filtered through a 0.45 μm polytetrafluoroethylene membrane filter before the injection (20 μl) into the column. Rosmarinic acid was quantified by external standard method at 329 nm.

Total phenolics concentration

Total phenolics concentration (*TPC*) was determined by a spectrophotometric method [18]. The extract containing water and methanol was diluted with water to reach the absorbance value lower than 1.000. The extracts in ethyl acetate or ethanol were evaporated to dryness under vacuum at 40 °C and then dissolved in and diluted with water. Diluted extract (400 μl) was added to 2 ml of 10-fold diluted Folin-Ciocalteu's reagent and 1.6 ml of 0.75 g·l^{-1} Na_2NO_3. The mixture was vortex-mixed and incubated at room temperature (25 ± 2 °C) in darkness for 60 min. After that, absorbance was read at 765 nm. The results were expressed as gallic acid equivalents (GAE).

Total flavonoids concentration

Total flavonoid concentration (*TFC*) was determined according to the method of Zhishen et al. [19]. The extracts were similarly diluted as indicated in the above section to reach absorbance value lower than 0.500. Briefly, 4 ml of distilled water and 1 ml of the diluted extract were mixed in a tube. Then, 300 μl of 0.5 g·l^{-1} $NaNO_2$ was added to the mixture and incubated for 5 min. After that, 300 μl of 1 g·l^{-1} $AlCl_3$ was added to the mixture and incubated for 1 min. Finally, 2 ml of 1 mol·l^{-1} NaOH and 2.4 ml of distilled water were added to tubes. Absorbance was measured at 510 nm. The results were expressed as catechin equivalents (CE).

Antioxidant activity

Radical-scavenging activity with 2,2-diphenyl-1-picrylhydrazyl) ($DPPH^{\cdot}$) radical was determined by a spectrophotometric method [20]. The extracts were diluted in a similar manner as indicated in previous sections to reach absorbance value lower than 0.700. Briefly, 0.1 ml of extract was added to 3.9 ml of the DPPH radical solution (0.025 g·l^{-1} in methanol), and the mixture was vortex-mixed. The mixture was incubated at room temperature (25 ± 2 °C) in darkness for 30 min. Absorbance was measured at 515 nm and the results were expressed as Trolox equivalents (TE).

Radical-scavenging activity with 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{+}$) was determined by a spectrophotometric method [21]. The extracts were diluted as stated previously to reach absorbance value lower than 0.700. Initially, a 2.45 mmol·l^{-1} of ABTS radical solution was prepared by dissolving the ABTS radical in 5 ml of 12.25 mmol·l^{-1} $K_2S_2O_8$ solution and then adjusting the volume to 25 ml with distilled water. This solution was kept in darkness for 12–16 h. The absorbance of ABTS

radical solution was adjusted to 0.700 ± 0.002 with 0.2 mol·l^{-1} phosphate buffer (pH 7.4). A volume of 20 ml of extract was added to 2 ml of ABTS radical solution. The test tube was incubated at room temperature (25 ± 2 °C) in darkness for 6 min. Absorbance was measured at 734 nm and the results were given as TE.

β-Carotene-bleaching activity was tested by the method of SINGH et al. [22]. A mixture was prepared containing 2 mg β-carotene, 20 mg linoleic acid, 200 mg Tween-40 and 200 μl chloroform. Chloroform was evaporated by purging nitrogen gas and distilled water (10 ml) with oxygenated water (30 ml) were added to the solution. Four millilitres of this β-carotene solution and 200 μl extract (diluted with ethanol to provide antioxidant activity between 10 % and 70 %) were added to a test tube and then the mixture was vortex-mixed. The test tube was incubated at 60 °C in a shaking water bath for 120 min. Absorbance was measured at 470 nm and the results were expressed as antioxidant activity (*AA*) in percent.

Preparation of spearmint extracts

Effects of SRW, SDRW and DSL_{MW} on oil oxidation were determined. For this purpose, SRW and DSL_{MW} were transferred to flasks and the solvents of them were evaporated at less than 40 °C. The dry extracts were re-solubilized in ethanol to the same volume. This was necessary to help solubilize the ethanolic solutions of the extracts in oil samples. The resulting ethanolic extracts (SRW_E, $SDRW_E$, DSL_E) were used to study the possible prevention of oxidation of oil samples. The final concentrations of the extracts in oil samples were adjusted to a fixed concentration for all oil samples (1 000 mg dried extract per 1 l of corresponding oil). After that, the oil samples were placed in an oven at 70 °C for forced oxidation. The same procedure was repeated for the control without the extract. Total exposure times to forced oxidation were 15 days and 7 days for sunflower oil and flaxseed oil, respectively.

Peroxide value and *p*-anisidine value

The peroxide and *p*-anisidine value were determined according to AOCS method Cd 8-53 [23] and spectrophotometric AOCS Cd 18-90 method [24], respectively.

Statistical analysis

Experimental data were compared using one-way analyses of variance (ANOVA) with SPSS 10.0.1 package program (SPSS, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Total phenolics and rosmarinic acid concentrations in SRW during distillation are presented in Fig. 2. *TPC* of SRW at 120 min was higher than the values at of other studied points, whereas rosmarinic acid reached the highest concentration at 150 min ($p < 0.05$). *TPC* and HPLC-DAD results indicated that penetration of various phenolics from spearmint to SRW may be different for different classes of phenolics. Moreover, although the thermal load was harsh on phenolics during hydrodistillation (approximately 100 °C for 240 min), certain phenolics (e.g. rosmarinic acid) were quite stable under these circumstances. This

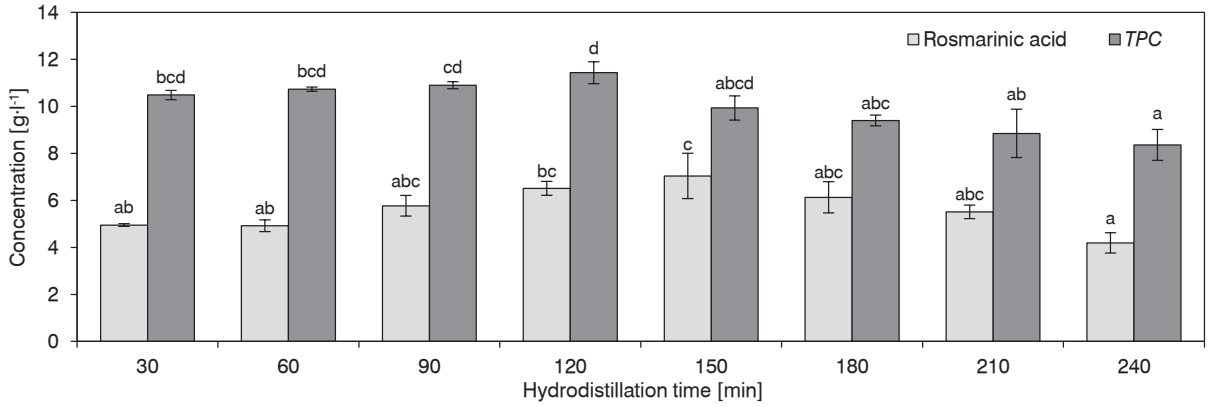

Fig. 2. Rosmarinic acid and total phenolics concentrations of spearmint residual water during hydrodistillation.

The values represent mean ± standard deviation ($n = 3$). The same letters in the same column indicate no difference ($p > 0.05$) according to Tukey's test.
TPC – total phenolics concentration (expressed as gallic acid equivalents).

Tab. 1. Antioxidant properties of spearmint extracts.

Extract	*TPC* [g·l^{-1}]	Rosmarinic acid [g·l^{-1}]	Antioxidant activity [g·l^{-1}]	
			AA_{ABTS}	AA_{DPPH}
SRW	10.73 ± 0.09 ^{c}	4.92 ± 0.25 ^{c}	14.41 ± 0.44 ^{c}	21.33 ± 0.15 ^{d}
SL_{MW}	10.51 ± 0.16 ^{c}	5.20 ± 1.41 ^{c}	6.75 ± 0.57 ^{b}	5.63 ± 0.39 ^{b}
DSL_{MW}	6.11 ± 0.08 ^{b}	2.32 ± 0.55 ^{b}	2.27 ± 0.18 ^{a}	2.67 ± 0.06 ^{a}
SRW_{EA}	6.88 ± 0.64 ^{b}	2.37 ± 0.97 ^{b}	15.61 ± 0.59 ^{c}	18.83 ± 0.22 ^{c}
DSL_{EA}	2.99 ± 0.39 ^{a}	0.11 ± 0.01 ^{a}	2.65 ± 0.09 ^{a}	2.23 ± 0.01 ^{a}

The values represent mean ± standard deviation (n = 3). The same letters in superscript in the same column indicate no difference (p > 0.05) according to Tukey's test.
TPC – total phenolics concentration (expressed as gallic acid equivalents). AA_{ABTS} – antioxidant activity determined by ABTS method (expressed as Trolox equivalents), AA_{DPPH} – antioxidant activity determined by DPPH method (expressed as Trolox equivalents).
SRW – spearmint residual water at 60 min, SL_{MW} – spearmint leaves extracted with methanol-water (50 : 50, v/v), DSL_{MW} – residual leaves material extracted with methanol-water (50 : 50, v/v), SRW_{EA} – ethyl acetate extract of spearmint residual water, DSL_{EA} – ethyl acetate extract of residual leaves material.

might be either due to the protective or synergistic effects of total extraction environment on rosmarinic acid or stable nature of rosmarinic acid itself [25]. Although various phenolics displayed different stability, VERGARA-SALINAS et al. [26] indicated high stability of thyme phenolics (including rosmarinic acid) under conditions of high pressure (10 342 kPa), temperature (100 °C) and time (30 min). Various distillation times for extraction of essential oil from spearmint were reported in the literature [27–29]. A study by BENYOUSSEF et al. [30] evaluated the yield of spearmint essential oil depending on extraction time and they reported that more than 96 % of the essential oil was extracted when 90 min hydrodistillation was applied. However, as determined in the present study, extraction of phenolics (rosmarinic acid as a representative of them) into SRW was not comparable to the yield of essential oil. The concentration of main phenolic of spearmint leaves, rosmarinic acid, could reach up to 2.32 g·l^{-1} in the extracts obtained from DSL (Tab. 1). This indicated that DSL contained significant amounts of antioxidant compounds, specifically, the main phenolic of spearmint (rosmarinic acid). Above studies indicated that DSL and SRW might be rich in rosmarinic acid, known for bioactive properties such as antioxidant, antimicrobial and antiinflammatory properties [25].

In one part of the present study, we focused on antioxidant properties of SRW to elucidate its potential. Both antioxidant tests, DPPH and ABTS, displayed almost the same trend, as a gradual increase in antioxidant capacity was observed com-

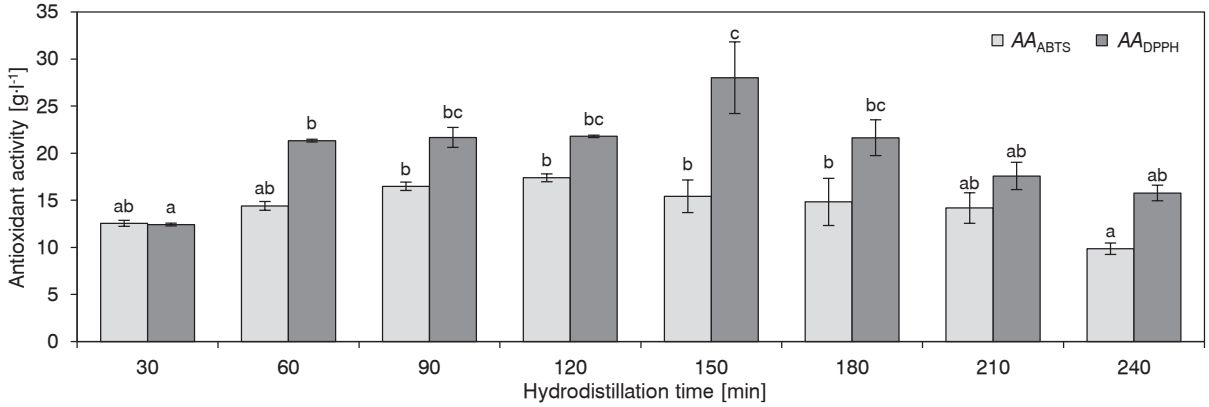

Fig. 3. Antioxidant activity of spearmint residual waters.

The values represent mean ± standard deviation (n = 3). The same letters in the same column indicate no difference (p > 0.05) according to Tukey's test.
AA_{ABTS} – antioxidant activity determined by ABTS method (expressed as Trolox equivalents), AA_{DPPH} – antioxidant activity determined by DPPH method (expressed as Trolox equivalents).

pared to starting conditions during hydrodistillation up to a certain point in which SRW reached the highest points at 120 min in terms of AA_{ABTS} and rosmarinic acid concentration. The other antioxidant activity assay (DPPH) displayed a similar trend as ABTS, however, the highest points of AA_{DPPH} were determined at 150 min. Extending the hydrodistillation time to 240 min resulted in gradual decrease in rosmarinic acid concentration and antioxidant activity scores (Fig. 2, Fig. 3). This might be due to the combined effects of temperature and time (thermal load), which enhances the extraction yield to some degree. However, extreme thermal load might also be detrimental as some labile components, such as phenolics, may undergo degradation. The trends in Fig. 2 and Fig. 3, displaying *TPC*, rosmarinic acid and antioxidant activities (ABTS and DPPH) of SRW, were quite similar. Especially, the trends of rosmarinic acid in Fig. 2 and DPPH in Fig. 3 displayed almost the same pattern in such a way that an increase was observed till 150 min and then a gradual decrease was observed. Standard hydrodistillation times of 2–8 h is reported in scientific literature and applied in industry [8, 31]. This is advised to reach maximum yield of essential oil. However, our results indicated that the distillation time should be closely inspected and optimized if the aim is to obtain bioactive components in addition to the essential oils.

Tab. 1 summarizes the potentials of SRW, DSL and their sub-fractions (DSL_{MW}, SRW_{EA}, DSL_{EA}) obtained by methanol-water (50 : 50, v/v) or ethyl acetate. Among the fractions, SRW displayed the highest *TPC*, AA_{ABTS} and AA_{DPPH}. When SL as the starting material was extracted with methanol-water (producing SL_{MW}), its rosmarinic acid concentration was the highest among the fractions, whereas antioxidant capacities of SL_{MW} were lower than that of SRW. All subfractions obtained with methanol-water (DSL_{MW}) or ethyl acetate (DSL_{EA}) displayed certain scores in *TPC*, rosmarinic acid concentration and antioxidant activities, however, the scores of these subfractions were lower than that of SRW. It was possible to extract significant amount of antioxidants to SRW_{EA} indicating the suitability of the proposed way for further purification of SRW. Correlations among *TPC*, rosmarinic acid concentration, AA_{ABTS} and AA_{DPPH} are shown in Tab. 2. A positive significant correlation ($p < 0.01$) was observed between rosmarinic acid and *TPC*. This indicated that rosmarinic acid is a suitable representative of other phenolics found in spearmint leaves in order to observe the effects of thermal processing operations on the phenolics. However, weak correlations between rosmarinic acid, AA_{ABTS} and AA_{DPPH} indicated that the components exerting antioxidant activity display different behaviour under certain thermal conditions.

Distillation waste materials including SRW, DSL_{MW} and SDRW were selected based on their antioxidant properties for further evaluation of their potential. This was necessary for the next stage of the experiments as one of our aims was to study the potential of the extracts to prevent the oxidation in a β-carotene-linoleate model system. The properties of obtained ethanolic solutions of SRW_E, DSL_E and $SDRW_E$ are listed in Tab. 3. Statistically significant differences ($p < 0.05$) were observed among ethanolic solution of various by-products (SRW, DSL and SDRW) in *TPC*, *TFC* and antioxidant activity. SRW_E displayed the highest values in *TPC*, *TFC*, AA_{ABTS} and AA_{DPPH} tests. As expected, $SDRW_E$ displayed approximately half values compared to SRW_E since maltodextrin as a drying agent was added to SDRW at the same amount as its soluble solids. This dilution gave rise to the halving of the values. On the other hand, DSL_E displayed the lowest values that might be due to its weak scores in antioxidant activity assays (Tab. 1). No antioxidant activity was observed for DSL_E in β-carotene-linoleate model (Fig. 4). The dark colour of DSL_E

Tab. 2. Correlation of total phenolics concentration, rosmarinic acid concentration and antioxidant activity for spearmint residual water and spearmint leaves extracts.

	TPC	Rosmarinic acid	AA_{ABTS}	AA_{DPPH}
TPC	1	0.877**	0.540	0.527
Rosmarinic acid		1	0.147	0.122
AA_{ABTS}			1	0.975**
AA_{DPPH}				1

** – significance at $p < 0.01$.
TPC – total phenolics concentration, AA_{ABTS} – antioxidant activity determined by ABTS method, AA_{DPPH} – antioxidant activity determined by ABTS method.

Tab. 3. Properties of spearmint by-products added into oil samples at accelerated oxidation test.

Extracts	*TPC* [g·l^{-1}]	*TFC* [g·l^{-1}]	Antioxidant activity [g·l^{-1}]	
			AA_{ABTS}	AA_{DPPH}
SRW_E	10.26 ± 0.74 ^{c}	15.03 ± 0.53 ^{c}	3.24 ± 1.34 ^{c}	2.42 ± 0.77 ^{b}
DSL_E	2.99 ± 0.54 ^{a}	1.23 ± 0.16 ^{a}	0.47 ± 0.24 ^{a}	1.35 ± 0.54 ^{a}
$SDRW_E$	5.55 ± 0.65 ^{b}	6.31 ± 0.48 ^{b}	1.62 ± 0.54 ^{b}	1.85 ± 0.16 ^{ab}

The values represent mean ± standard deviation (n = 3). The same letters in superscript in the same column indicate no difference (p > 0.05) according to Tukey's test.
SRW_E – ethanolic solution of spearmint residual waters, DSL_E – ethanolic solution of residual leaves material, $SDRW_E$ – ethanolic solution of spray-dried spearmint residual water.
TPC – total phenolics concentration (expressed as gallic acid equivalents), *TFC* – total flavonoids concentration (expressed as catechin equivalents), AA_{ABTS} – antioxidant activity determined by ABTS method (expressed as Trolox equivalents), AA_{DPPH} – antioxidant activity determined by ABTS method (expressed as Trolox equivalents).

blurred the total test solution and caused negative outcome in final antioxidant activity results. SRW_E displayed a dose-dependent antioxidant activity. Similar dose-dependent activity was observed for $SDRW_E$ but the values of antioxidant activity was lower than for SRW_E.

Peroxide and *p*-anisidine values of two different oil samples during the storage period are presented in Fig. 5. Peroxide and *p*-anisidine values of flaxseed and sunflower oils without any added antioxidants at the beginning and during the progression of accelerated oxidation between 60–70 °C were in good agreement with previously reported studies [32–34]. The above cited studies reported pro-oxidative, antioxidative or no observable effects of plant-based antioxidants that were incorporated to the oils aiming to retard oxidation. In the present study, no antioxidative effects of added extracts (SRW_E, DSL_E, $SDRW_E$) were detected on flaxseed and sunflower oils. However, SRW_E displayed better results, at least no negative impact, compared to other extracts in the prevention of oxidation of flaxseed and sunflower oils. The peroxide and *p*-anisidine values of flaxseed and sunflower oils with added DSL_E were higher than those of their respective controls, indicating the pro-oxidative effects of DSL_E and $SDRW_E$ specifically after 2 days of accelerated storage. Even though ethanolic extracts of 3 different samples (SRW_E, DSL_E, $SDRW_E$) were incorporated into the oil samples at the same level (1 g·l^{-1}), the results for DSL_E were remarkable. This might be due to the different nature of antioxidants in SRW and DSL, which made the latter more pro-oxidative than the former. Although no positive results were obtained regarding the prevention of oxidation of flaxseed and sunflower oils, SRW might be suitable to enrich the products that are weak in phenolics and antioxidants. It should be noted that the accelerated or forced oxidation experiments between 40 °C and 80 °C are commonly applied to determine the progression of oxidation in oil samples. However, the method has some drawbacks including the assumptions that oxidation mechanisms and antioxidant effects at elevated temperatures (40–80 °C) are the same as at room temperature [35]. This simplistic approach might lead to misinterpretation of the results, therefore, although it takes long time, oxidation status at ambient conditions might provide more realistic results. A similar study on soybean oil reported the pro-oxidant effect of α-tocopherol [16].

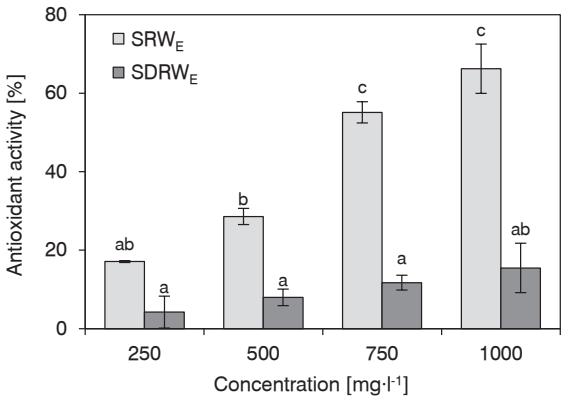

Fig. 4. Antioxidant activities of ethanolic solutions of spearmint distillation by-products obtained from β-carotene linoleate model system.

The values represent mean ± standard deviation (n = 3). The same letters in the same column indicate no difference (p > 0.05) according to Tukey's test.
Antioxidant activity was determined by β-carotene-linoleate model system.
SRW_E – ethanolic solution of spearmint residual waters, $SDRW_E$ – ethanolic solution of spray dried spearmint residual water.

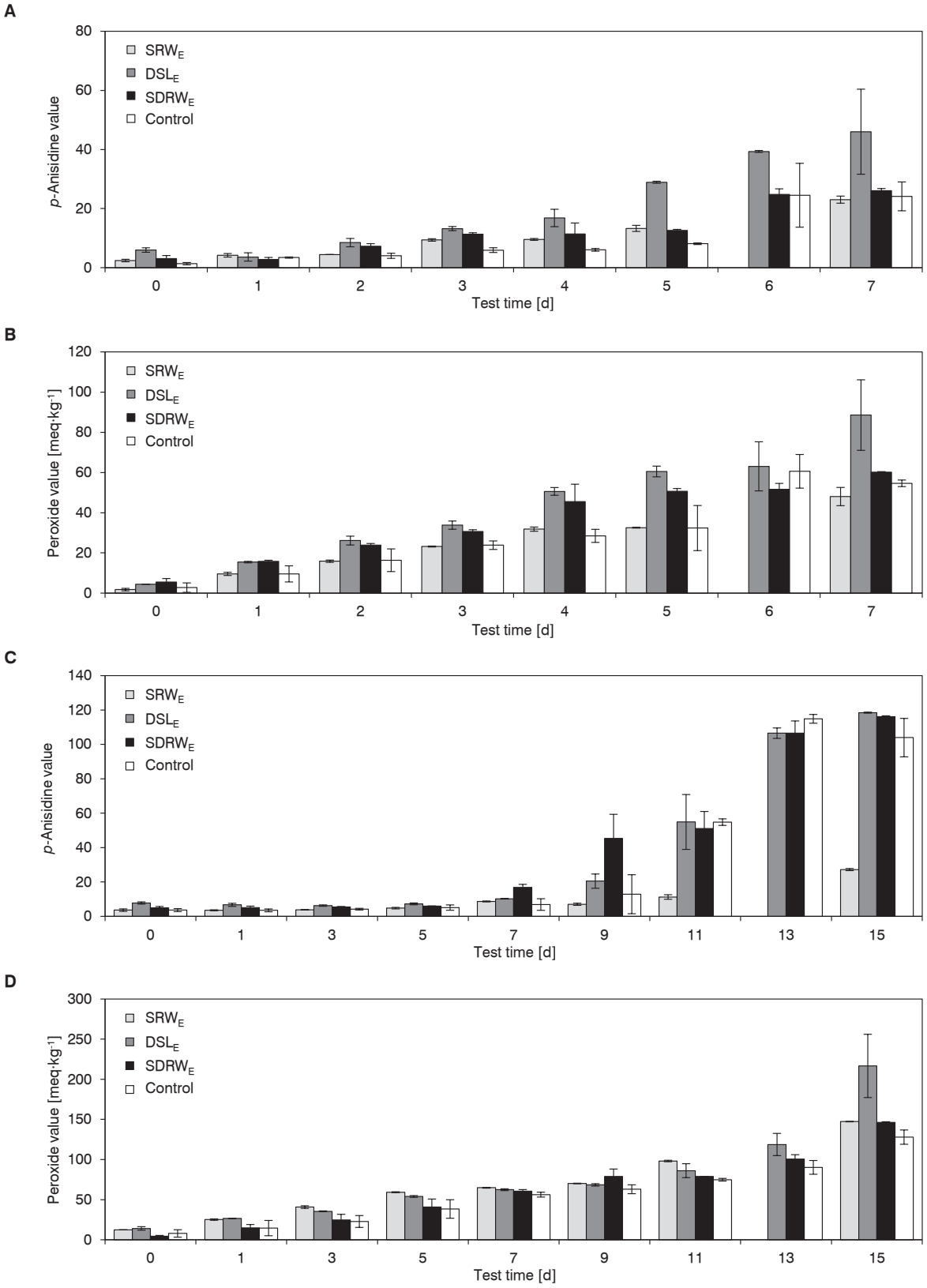

Fig. 5. Peroxide and *p*-anisidine values of flaxseed and sunflower oils during storage at 70 °C.

A – *p*-anisidine values of flaxseed oil, B – peroxide values of flaxseed oil, C – *p*-anisidine values of sunflower oil, D – peroxide values of sunflower oil.
SRW_E – ethanolic solution of spearmint residual waters, DSL_E – ethanolic solution of residual leaves material, $SDRW_E$ – ethanolic solution of spray-dried spearmint residual water, Control – oil without any added extract.

CONCLUSIONS

The results of the present study demonstrated that spearmint distillation by-products including SRW and DSL contained significant amounts of antioxidative compounds. Although both by-products were rich in antioxidants, residual water displayed better results than the residual leaves material in all tested parameters. The results also implied that residual water could be evaluated as a rich source of phenolics, specifically rosmarinic acid, for further use either directly in enrichment of food products or for recovering of pure rosmarinic acid. As the by-products are currently regarded as waste and treated thereof, using them as a source of antioxidants might provide economic and environmental benefits.

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