

Comparison of bioactive compounds, antioxidant activity, fatty acid composition and phenolic compounds of three rapeseed varieties

Özden Öztürk¹
Mehmet Musa Özcan^{2✉}
Nurhan Uslu²

¹Department of Field Crops, Faculty of Agriculture, Selçuk University, 42079 Konya, Turkey

²Department of Food Engineering, Faculty of Agriculture, Selçuk University, Konya, Turkey

In this study, differences in oil contents, bioactive properties (total phenol, total flavonoid, total chlorophyll, antioxidant activity), fatty acids and polyphenols of rapeseed and oils harvested in Turkey (Yarma-Karatay-Konya) were investigated. The chlorophyll-a and chlorophyll-b amounts of seeds changed between 1.45 (Sygenta Linus) and 4.45 µg/g (PR44W29) to 0.07 (Neptune) and 4.74 µg/g (Sygenta Linus), respectively. Total chlorophyll contents of rapeseed varieties changed between 1.30 (PR44W29) and 2.37 mg/kg (Neptune). Total phenol and flavonoid amounts of rapeseed samples varied between 54.82 (Neptune) and 75.69 mgGAE/100g (Sygenta Linus) to 111.31 (Neptune) and 138.21 mg/100g (Sygenta Linus), respectively. Antioxidant activity values of rapeseeds were measured between 2.84 (Neptune) and 3.30 mmol/kg (Sygenta Linus). Oleic, linoleic, linolenic, and palmitic acids were found in abundant amounts in rapeseed oils. Oleic and linoleic acid amounts of rapeseed oils were found between 60.97 (Neptune) and 63.96% (Neptune) to 19.02 (Sygenta Linus) and 22.52% (Neptune), respectively. While gallic acid contents of rapeseeds vary between 15.44 (Neptune) and 23.27 mg/100g (Sygenta Linus), gallic acid amounts of rapeseed oils were identified between 14.10 (Neptune) and 19.15 mg/100g (Sygenta Linus).

Keywords: rapeseed, variety, oil, chlorophyll, bioactive compounds, fatty acids, polyphenols, minerals

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1. INTRODUCTION

Rapeseed (*Brassica napus oleifera* L.) is a good source of edible raw materials, and it is an annual plant [1]. The importance of seeds, fruits and vegetables has been emphasised recently due to some of their functional and bioactive properties [2]. Its oil is considered one of the healthiest cooking oils due to its nutritional value, abundant unsaturated fat, and beneficial fatty acids. In addition, rapeseed oil contains nutritionally appropriate essential fatty acids in a ratio of 2:1 (linoleic/ linolenic) [3]. It is thought that this ratio rapeseed oil in essential fatty acids can be consumed as part of a healthy diet. Sinapic acid and its derivatives such as sinapine, sinapoylglucose, phenylindon and 4-vinylsyringol trimer are the main antioxidant components of rapeseed oil [4]. In addition, these polyphenolics found in rapeseed oil can also affect the sensory properties of the oil [5]. Phenolic compounds, commonly found in plants, are the main source of primary antioxidants [6]. Phenolic compounds formed as secondary metabolites in plants have an important role as defence compounds in plants [7]. It has been reported that the phenolic component content of rapeseed in oilseeds is higher than other seeds, and rapeseed phenolic compounds are used as powerful antioxidants in foods and in the cosmetic and pharmaceutical field [8]. Rapeseed oil is known to have high nutritional value because it contains many active biological compounds such as phytosterols, essential fatty acids, tocopherol, phenolic compounds, β-car-

✉ CORRESPONDING AUTHOR:
Dr Mehmet M Özcan
mozcan@selcuk.edu.tr
Tel: +0.332.2232933

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otene [9, 10]. Variety, geographical location, growing conditions and processing techniques are among the factors that affect the chemical composition of rapeseed oil [8, 11]. Rapeseed is the seed obtained from a plant also known as rapeseed or canola in Turkey. Rapeseed is a product mostly used in the production of edible oil. Significant efforts have been made in recent years to develop models for rapeseed. Several species of Cruciferae are currently cultivated as oil crops, such as *B. napus*, *Brassica campestris* [12]. The aim of current study was to compare differences in oil contents, bioactive properties (total phenol, total flavonoid, total chlorophyll, antioxidant activity), fatty acids and polyphenols of rapeseed and oils harvested in Turkey (Yarma-Karatay-Konya) in 2020

2. MATERIAL AND METHODS

2.1. MATERIAL

Rapeseeds were harvested in the Konya (Yarma-Karatay) district in Turkey in July 2020. After the seeds were harvested, they were cleaned of any foreign matter such as leaves, stems, soil, foreign seeds and dried at room temperature. Air dried rapeseeds were stored in a glass jar in the refrigerator until analysis.

2.2 METHODS

2.2.1 Moisture content

The moisture amounts of rapeseed samples were measured at 105°C using an oven until a constant weight.

2.2.2 Chlorophyll content of rapeseed

After each ground rapeseed sample (5 g) was stirred with 25 ml of acetone, it was sonicated for 10 minutes. The mixture was filtered on filter paper and then taken into a measuring balloon. The volume of the flask was made up with acetone:water (80:20, v/v) mixture. Absorbance values of the samples were reported for chlorophyll a and chlorophyll b at 663 nm and 645 nm, respectively [13].

$$\text{Chlorophyll a} = \frac{((12.7 \times 663) - (2.69 \times 645)) \times V}{(1000 \times m)}$$

$$\text{Chlorophyll b} = \frac{((22.9 \times 645) - (4.68 \times 663)) \times V}{(1000 \times m)}$$

(V: Volume of flask, m: Amount of sample)

2.2.3. Chlorophyll content of rapeseed oil

The chlorophyll contents of rapeseed oil samples were determined by spectrophotometer and measured at 670 nm [14].

$$\text{Chlorophyll (mg/kg)} = \frac{(A_{670} \times 10^6)}{(613 \times 100 \times d)}$$

(A: Absorbance, d: Thickness of cuvette)

2.2.4. Oil content

Rapeseed cleaned of foreign matter was ground through a 0.5 mesh sieve in a laboratory mill. After weighing approximately 10 g of ground rapeseed into a Soxhlet cartridge, the cartridge was placed in the Soxhlet chamber. The oil of each sample was obtained with petroleum ether in the Soxhlet for 5 hours at 50°C. After petroleum ether was then evaporated by a rotary evaporator at 50°C, the amount of remaining crude oil was calculated gravimetrically [15].

2.2.5. Extraction procedure

Seed powders were extracted according to methods described by Jakopic et al. [16] with some changes. 15 ml of methanol was added to the ground samples (5 g). After the solution was stored in an ultrasonic water-bath for 1 h, it was centrifuged at 6000 rpm for 10 minutes and the supernatant was filtered through a 0.45 µm membrane filter. After the n-hexane (15 ml) solution was added, it was stirred using a vortex apparatus. Then, a separating funnel was used to separate the methanol and hexane layer in the sample. Later, the extracts obtained after evaporation were dissolved in 10 ml of methanol [16].

Seed oils were extracted according to Durmaz and Gökmen [17]. Oil sample (1 g) was mixed with 5 ml of methanol: water (70:30, v/v). The mixture was vortexed for 1 min, followed by centrifugation at 6000 rpm for 5 min, and then the supernatant was removed. These steps were repeated twice, and the extract was washed three times using 2 ml of n-hexane, and the solution was filtered prior to injection.

2.2.6. Total phenolic content

The Folin-Ciocalteu method stated by Yoo et al. [18] was used to determine total phenolic contents of seed extracts. After 1 ml Folin-Ciocalteu and 10 ml of 7.5% Na₂CO₃ were added to extract, mixture was stirred using the vortex. The deionised water was added until 25 ml and sample and kept in the dark for 1 h. The absorbance values of samples were recorded at 750 nm. The findings were described as mg gallic acid equivalent/100 g (dw).

2.2.7. Total flavonoid content

For the total flavonoid contents of samples, then 0.3 ml of NaNO₂, 0.3 ml of AlCl₃ and 2 ml of NaOH, respectively, were added to 1 mL rapeseed extract, and vigorously mixed using a vortex. Then it was kept in the dark for 15 min. The absorbance value of each sample was determined at 510 nm using a spectrophotometer. The results obtained are stated as mg quercetin (QE)/100g [19].

2.2.8. Antioxidant activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to determine the free radical scavenging activity of extracts [20]. After 2 ml of a methanolic solution of DPPH was added to the extract, it was mixed using a Vortex

and then kept in dark for 30 min. After this, the absorbance value of each sample was recorded at 517 nm. The results are described as mmol trolox (TE)/kg.

2.2.9 Fatty acid composition

Gas chromatography (Shimadzu GC-2010) equipped with a flame-ionisation detector (FID) and a capillary column (Tecnocroma TR-CN100, 60 m × 0.25 mm, film thickness: 0.20 µm) was used to determine the fatty acid methyl esters of rapeseed oil esterified according to ISO-5509 (1978) method. The temperature of the injection block and detector was 260°C. 1.51 ml/min flow rate, 80 ml/min and 1/40 were set for mobile phase, total flow and split rates were nitrogen, respectively. Column temperature was set 120°C for 5 minutes and increased 240°C at 4°C/min and held 25 minutes at 240°C.

2.2.10. Determination of phenolic compounds

The chromatographic separation of phenolic constituents with High Power Liquid Chromatography equipped with a PDA detector and an Inertsil ODS-3 (5 µm; 4.6 × 250 mm) column was carried out. A mixture of 0.05% acetic acid in water (A) and acetonitrile (B) with the flow rate of 1 ml/min at 30°C was used as a mobile phase. 20 µl was considered as the injection volume. The peaks were obtained at 280 using a PDA detector.

2.2.11. Tocopherol content

Tocopherol content was identified according to methods stated by Spika et al. [21] with some changes. After 1g oil was stirred in 3 ml of *n*-hexane, mixture was filtered using a filter (0.45 µm). HPLC (Shimadzu) equipped with PDA detector and LiChroCART Silica 60 (4.6 × 250 mm, 5 µ; Merck, Darmstadt, Germany) column was used to detect tocopherols in rapeseed oil. The flow rate of the mobile phase and the injection volume were 0.9 ml/min and 20 µl, respectively. 295 and 330 nm with PDA detector were used to obtain the peaks. The total running time per sample was 30 min.

2.3. STATISTICAL ANALYSIS

Analysis of the results was performed using Minitab-16 statistical program. The means of significant

variation sources were compared to Tukey Test. The significance level is given as $p < 0.05$ unless otherwise stated. The analyses were repeated 3 times ($n=3$).

3. RESULTS AND DISCUSSION

3.1. THE CHEMICAL PROPERTIES, BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITIES OF SOME RAPESEEDS

The chemical properties of three rapeseed varieties are presented in Table I. No significant differences between the moisture contents of rapeseed samples were observed. While chlorophyll-a contents of seeds change between 1.45 µg/g (Sygenta linus) and 4.45 µg/g (PR44W29), chlorophyll-b amounts of rapeseed samples were determined between 0.07 (Neptune) and 4.74 µg/g (Sygenta Linus). Also, total chlorophyll amounts of rapeseed oils were measured between 1.30 (PR44W29) and 2.37 mg/kg (Neptune). The oil amounts of rapeseeds were determined between 35.70% (PR44W29) and 37.70% (Sygenta Linus). While total phenol amounts of rapeseed samples vary between 54.82 mgGAE/100g (Neptune) and 75.69 mgGAE/100g (Sygenta Linus), antioxidant activity values of rapeseeds were measured between 2.84 mmol/kg (Neptune) and 3.30 mmol/kg (Sygenta Linus). As seen, total flavonoid contents of rapeseeds were measured between 111.31 (Neptune) and 138.21 mg/100g (Sygenta Linus). In general, the chemical and bioactive features of the "Sygenta Linus" rapeseed were high compared to other two rapeseed varieties. Total phenol and flavonoid contents of rapeseeds showed a relationship with the antioxidant activity values of rapeseeds. Rapeseed oil contained 1.31 mg CAE/100g total phenol [22]. The total phenol amounts of rapeseed oils changed between 40.3 and 412.3 mgGA/kg [11]. Findings exhibited some fluctuations compared to results of previous studies. These fluctuations can be presumably due to variety, genetic, climatic factors, growing conditions, harvest time, extraction system and solvent type used.

3.2. THE FATTY ACID COMPOSITIONS OF THREE DIFFERENT RAPESEED OILS

The fatty acid compositions of the oils of three different rapeseed varieties (PR44W29, Neptune and Sygenta

Table I - Some chemical and bioactive properties of rapeseed

Sample	Moisture content (%)	Chlorophyll a (µg/g seed)	Chlorophyll b µg/g seed)	Oil content (%)
PR44W29	7.22 ± 0.69*	4.45 ± 0.00A**	3.55 ± 0.00A	35.70 ± 0.80
Neptune	7.36 ± 0.17	1.63 ± 0.00B	0.07 ± 0.00B	37.45 ± 0.55
Sygenta Linus	7.06 ± 0.39	1.45 ± 0.00B	4.74 ± 0.00A	37.70 ± 0.20
Sample	Total phenolic content (mg/100g)	Total flavonoid content (mg/100g)	Antioxidant activity (mmol/kg)	Chlorophyll content (mg/kg oil)
PR44W29	71.77 ± 3.32A	125.36 ± 3.55B	3.11 ± 0.01B	1.30 ± 0.01C
Neptune	54.82 ± 1.53B	111.31 ± 0.34C	2.84 ± 0.03C	2.37 ± 0.01A
Sygenta Linus	75.69 ± 3.10A	138.21 ± 4.56A	3.30 ± 0.00A	1.57 ± 0.02B

* values within each column followed by different letters are significantly different at $p > 0.05$

** values within each column followed by different letters are significantly different at $p < 0.01$

Table II - Fatty acid composition of rapeseed oils

Fatty acids (%)	PR44W29	Neptune	Sygenta Linus
Palmitic	4.49 ± 0.08b	5.08 ± 0.00a	4.93 ± 0.12ab
Stearic	5.36 ± 1.64*	1.36 ± 0.00	1.93 ± 0.02
Oleic	61.93 ± 1.09	60.97 ± 0.00	63.96 ± 0.13
Linoleic	19.06 ± 0.38B**	22.52 ± 0.02A	19.02 ± 0.02B
Arachidic	0.52 ± 0.02ab***	0.41 ± 0.00b	0.55 ± 0.04a
Linolenic	8.38 ± 0.12B	9.30 ± 0.04A	9.33 ± 0.07A
Behenic	0.26 ± 0.02	0.24 ± 0.01	0.28 ± 0.03
Erucic	-	0.14 ± 0.01	-

* values within each row followed by different letters are significantly different at p>0.05

** values within each row followed by different letters are significantly different at p<0.01

*** values within each row followed by different letters are significantly different at p<0.05

-: Not detected

Linus) are presented in Table II. Results showed some changes depending on rapeseed oil (Fig. 1). Oleic, linoleic, linolenic, and palmitic acids were the vast fatty acids of rapeseed oils. While oleic acid amounts of rapeseed oils are identified between 60.97% (Neptune) and 63.96% (Neptune), linoleic acid contents of seed oils were detected between 19.02% (Sygenta Linus) and 22.52% (Neptune). As saturated fatty acids, palmitic acid amounts of oil samples were identified between 4.49% (PR44W29) and 5.08% (Neptune) while stearic acid amounts of oil samples vary between 1.36% (Neptune) and 5.36% (PR44W29). Other fatty acids were found at minor levels. Oleic, arachidic, linolenic and behenic acid amounts of Sygenta Linus oil were high compared to other varieties. Rapeseed oil contains 1.5-6.0% palmitic, 0.5-3.1% stearic, 28% oleic, 11-23% linoleic and 5-13% linolenic acids [10]. Szydlowska-Czerniak et al. [23] reported that rapeseed oils contained 4.36-4.57% palmitic, 1.59-1.82% stearic, 61.89-64.21% oleic, 18.50-19.91% linoleic and 8.52-10.70% linolenic acids. Bozdoğan-Konuskan [24] determined 3.97% palmitic, 2.12% stearic, 63.68% oleic, 17.43% linoleic, 6.75% γ-linolenic, 2.82% eicosenoic and 1.93% erucic acids in rapeseed oil. It was determined that the dominant fatty acids obtained were like the literature and partial differences were found in their amounts. These differences may probably be due to factors such as location, variety, climatic factors, harvest time, growing conditions, fertilisation.

3.3. THE PHENOLIC CONSTITUENTS AND THEIR QUANTITATIVE VALUES OF RAPESEED AND OILS

The phenolic constituents and their quantitative values of rapeseed and their oils are shown in Table III. The abundant phenolic constituents of rapeseed and oils were gallic acid, followed by 3,4-dihydroxybenzoic acid, catechin and quercetin (Fig. 2). While gallic acid contents of rapeseeds vary between 15.44 (Neptune) and 23.27 mg/100g (Sygenta Linus), gallic acid amounts of rapeseed oils were identified between 14.10 (Neptune) and 19.15 mg/100g (Sygenta Linus). Also, while 3,4-dihydroxybenzoic acid amounts of rapeseed varieties were detected between 6.56 (Neptune) and 37.17 mg/100g (Sygenta

Linus), 3,4-dihydroxybenzoic acid amounts of rapeseed oils were identified between 3.83 (Neptune) and 15.45 mg/100g (PR44W29). Catechin amounts of rapeseed and oils were established between 1.49 (Neptune) and 42.17 mg/100g (Sygenta Linus) to 2.22 mg/100g (Neptune) and 19.00 mg/100g (Sygenta Linus), respectively. Also, while quercetin contents of rapeseed samples were detected between 2.32 (PR44W29) and 6.61 mg/100g (Sygenta Linus), quercetin amounts of rapeseed oils were detected between 0.18 (PR44W29) and 3.29 mg/100g (Sygenta Linus). In general, the highest amounts of phenolic components were determined in the "Sygenta Linus" rapeseed variety. In addition, significant differences were found compared to the amount of phenolic components of three different rapeseed oils. The possible reason for these differences in both rapeseeds and oils may be due to the genetic structure of the seeds, agricultural factors, climate and environmental conditions, sunning period, and maturation situation. In general, the phenolic components of the seeds were high according to rapeseed oils. This shows that the significant amount of phenolic components of rapeseeds remains in a cake after extraction. The vinylsyringol content of crude rapeseed oil was determined between 245 and 700 µg/g [25]. Rapeseed flour contained 639 µg/g phenolic acid [26]. Syringic acid amount of rapeseed oil was detected as 6.8 mg/kg [27]. Rapeseed oil contained 1.6 p-hydroxybenzoic acid, 0.3 caffeic acid, 13.1 p-coumaric acid, 5.6 ferulic acid, 236 µg/100 g sinapic acid [22]. The highest amount of sinapic acid was detected in the insoluble bound phenolic fraction of rapeseed meal, followed by ferulic, p-coumaric and o-coumaric acids in decreasing order [28]. In comparison both with the Merlot wine (mother vine) standard and the wines obtained from other two clone candidates, the Merlot wine of the clone candidate No. 022 was found to have the highest total content of all three examined components 1.89 ± 0.05 g/L (polyphenolics), 185.59 ± 5.00 mg/L (anthocyanins) and 1.11 ± 0.03 g/L (tannins), as well as six phenolic acids including gallic acid (25.49 ± 0.27 mg/L) [29]. The total phenolic content in 1 mg of the *Bryum moravicum* extract was equivalent to 356.44 ± 9.56 µg of ferulic

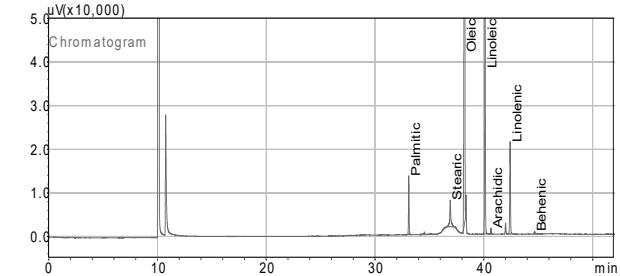
Table III - Phenolic compounds of seed and oil of rapeseed

Phenolic compounds (mg/100g)	Seed		Oil	
	PR44W29	Neptune	PR44W29	Neptune
Gallic acid	16.59 ± 0.25*	15.44 ± 2.61	23.27 ± 1.27	14.22 ± 0.24
3,4-Dihydroxybenzoic acid	7.54 ± 0.08B**	6.56 ± 1.94B	37.17 ± 4.50A	15.45 ± 4.16
Catechin	2.30 ± 0.04B	1.49 ± 0.99B	42.17 ± 3.07A	14.57 ± 3.58ab
Caffeic acid	1.04 ± 0.33B	0.30 ± 0.14B	6.64 ± 0.17A	0.39 ± 0.11
Syringic acid	0.70 ± 0.01b***	0.58 ± 0.02b	7.57 ± 1.74a	0.42 ± 0.01
Rutin	2.12 ± 0.56b	1.38 ± 0.00b	19.99 ± 3.43a	1.31 ± 0.45
p-Coumaric acid	0.29 ± 0.12B	0.30 ± 0.06B	3.15 ± 0.35A	0.48 ± 0.23
Ferulic acid	1.24 ± 0.24B	0.72 ± 0.11B	4.54 ± 0.35A	0.65 ± 0.02
Resveratrol	0.17 ± 0.02	0.21 ± 0.05	2.55 ± 0.93	0.24 ± 0.06
Quercetin	2.32 ± 0.37B	3.97 ± 0.24B	6.61 ± 0.21A	0.18 ± 0.01
Cinnamic acid	0.46 ± 0.41	0.48 ± 0.10	0.71 ± 0.23	0.48 ± 0.03
Kaempferol	0.50 ± 0.39	0.34 ± 0.03	0.54 ± 0.30	1.24 ± 0.27

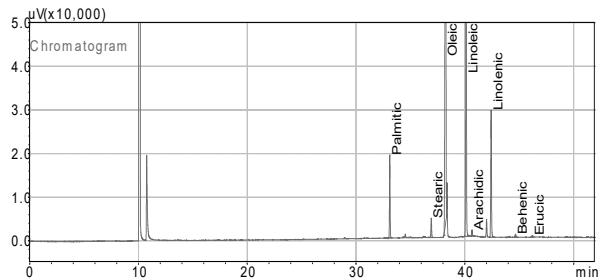
* values within each row followed by different letters are significantly different at p>0.05

** values within each row followed by different letters are significantly different at p<0.01

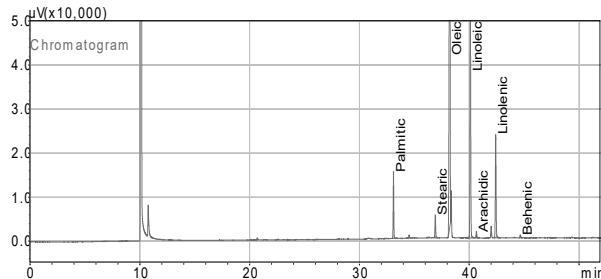
*** values within each row followed by different letters are significantly different at p<0.05



Sygenta Linus



Pionar



Neptune

Figure 1 - Fatty acid chromatograms of rapeseed oils

acid used as a standard [30]. Climatic factors such as temperature, precipitation, growing conditions and degree of maturity may have been effective on the phenolic components of rapeseed.

3.4 TOCOPHEROL CONTENTS OF RAPSEED OILS

Tocopherol amounts of rapeseed oil are illustrated in Table IV. DL- α -tocopherol amounts of rapeseed oil ranged between 8.56 mg/100g (PR44W29) to 8.98 mg/100g (Neptune), while β -tocopherol contents of the oil samples varied between 6.73 mg/100g (Sygenta Linus) and 7.62 mg/100g (PR44W29) (Fig. 3). Also, δ -tocopherol was detected only in Neptune (5.45 mg/100g) sample. Tocopherol amounts of cold-pressed rapeseed oils (line PN1 03/1i/14) and (line PN1 563/1i/14) were as 33.07 and 29.32 alpha-tocopherol, 0.11 and 0.08 beta-tocopherol, 39.27 and 35.67 gamma-tocopherol, 0.78 and 0.51 mg/100g delta-tocopherol [10], respectively. The initial tocopherol amount of the rapeseed oil was 610.5 mg/kg [31]. Gogolewski et al. [32] reported that crude rapeseed oils contained 19.68-22.79 mg/100g alpha-tocopherol, 28.22-35.24 mg/100g gamma-tocopherol, 0.91-1.50 mg/100g delta-tocopherol. Cold-pressed

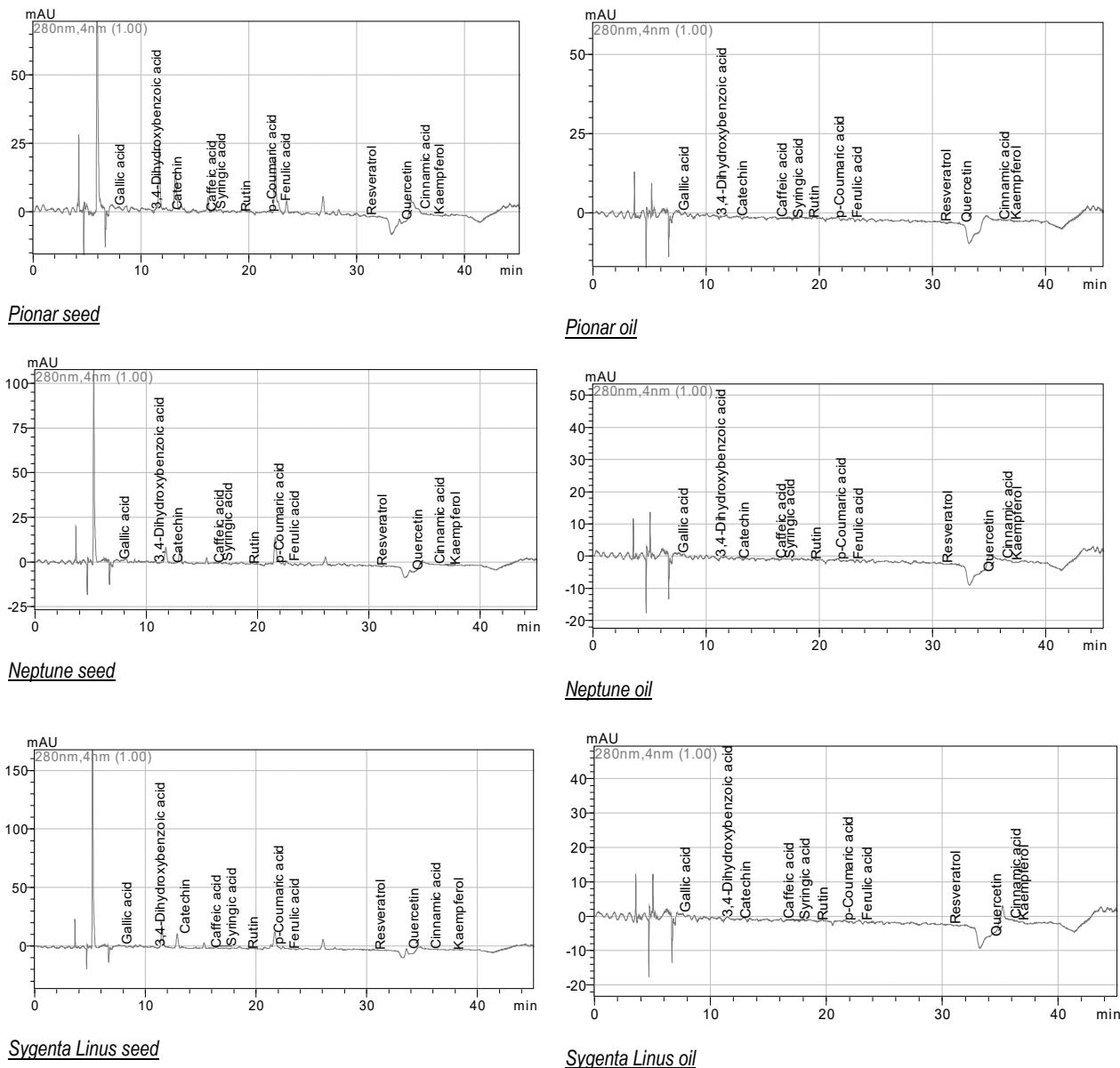


Figure 2 - Phenolic chromatograms of rapeseed and oils

rapeseed oil contained 181 mg/kg alpha-tocopherol, 244 $\beta+\gamma$ -tocopherol and 9.3 delta-tocopherol [33]. Tocopherols contribute to the oxidative stability of edible oils. α -Tocopherol is considered to have the highest nutritional value among all the different forms of tocopherol [33]. Gündüz Ergönül and Köseoğlu [34] determined 418.5 alpha-tocopherol, 64.3 beta-tocoph-

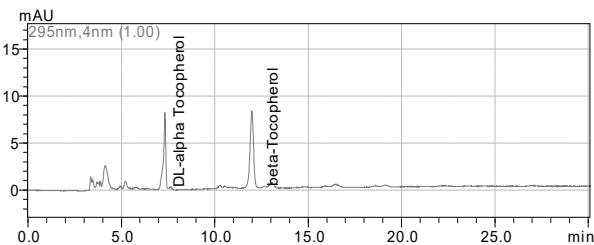
erol, 328.0 gamma-tocopherol, 12.0 mg/kg delta-tocopherol in crude rapeseed oil. The results obtained showed partially differences compared to results of previous studies [35-36]. These changes can be presumably due to variety, genetic factors, location, temperature, harvest time and analytical factors such as extraction and solvent type used.

Table IV - Fatty acid composition of rapeseed oils (mg/100g)

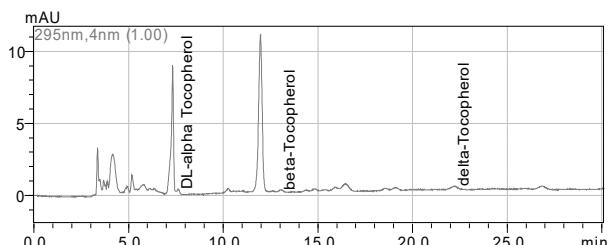
Tocopherols	PR44W29	Neptune	Sygenta Linus
DL- α -Tocopherol	8.56 \pm 0.02c*	8.98 \pm 0.03a	8.89 \pm 0.04b
β -Tocopherol	7.62 \pm 0.01a	7.08 \pm 0.02b	6.73 \pm 0.00c
δ -Tocopherol	-	5.45 \pm 0.06	-

* values within each row followed by different letters are significantly different at p<0.05

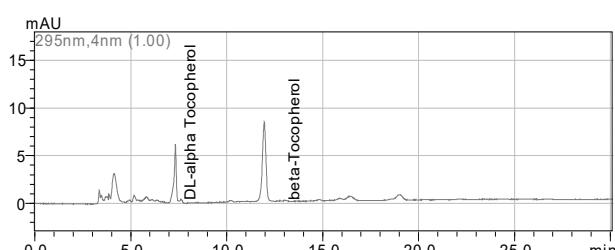
- : Not detected



Pionar oil



Neptune oil



Syngenta Linus oil

Figure 3 - Tocopherol contents of rapeseed oils

4. CONCLUSION

The chemical and bioactive features of the Syngenta Linus rapeseed were high compared to other two rapeseed varieties. Also, total phenol and flavonoid contents of rape seeds showed a relationship with the antioxidant activity values of rapeseeds. Results showed some changes depending on rapeseed oil samples. Oleic, linoleic, linolenic, and palmitic acids were the key fatty acids of rapeseed oils. The abundant phenolic constituents of rapeseed and oils were gallic acid, followed by 3,4-dihydroxybenzoic acid, catechin and quercetin. In general, the highest amounts of phenolic components were determined in the "Syngenta Linus" rapeseed variety. DL- α -tocopherol and β -tocopherol were detected in all the rape-seed oils studied.

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