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Genotypic Variation on Oil Content, Fatty Acid Composition and Phenolic Compounds in Linseed (*Linum usitatissimum* L.)

Keten Tohumunun (*Linum usitatissimum* L.) Yağ İçeriği, Yağ Asidi Kompozisyonu ve Fenolik Bileşikleri Üzerine Genotipik Çeşitliliğin Etkisi

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Key Words:

Linseed, cultivar variety, total oil, fatty acids, SDG lignan, phenolics, flavonoids

ABSTRACT

Linseed is used as a functional food ingredient in food products because of its valuable bioactive compounds. In this study, the effect of cultivar variety on total oil content, fatty acid composition, level of α-linolenic acid, SDG (secoisolariciresinol diglucosid) lignan, phenolic (free and esterified) and flavonoid (free and esterified) content of linseeds (*Linum usitatissimum* L.) cultivated in Turkey was investigated. Also, it was aimed to determine the correlations between the bioactive compounds investigated in this research. The results of this study showed that cultivar variety had a significant effect on these bioactive compounds ($p<0.05$). Total oil contents changed from 37.55 to 45.07% and the level of α-linolenic acids were found between 46.33 and 55.47% in linseed oil samples. The highest total phenolic content was determined as 1209.20 mg ferrulic acid/100 g in brown coloured linseeds whereas, the highest total flavonoids was calculated as 33.65 mg luteolin/100 g in yellow coloured linseeds. Besides, SDG lignan content of linseeds changed from 5.87 to 23.84 mg/g in defatted linseed flour. On the other hand, strong correlations were obtained between percentages of unsaturated fatty acids and α-linolenic acids ($r=0.98$), SDG lignan and total phenolic contents ($r=0.98$) and also between total flavonoid content and α-linolenic acid level ($r=0.93$) of linseeds.

Anahtar Sözcükler:

Keten tohumu, genotipik çeşitlilik, toplam yağ, yağ asitleri, SDG lignan, fenolikler, flavonoidler

ÖZET

Keten tohumu içerdiği değerli biyoaktif bileşiklerden dolayı gıda ürünlerinde fonksiyonel gıda bileşeni olarak kullanılmaktadır. Bu çalışmada, Türkiye'de yetiştirilmiş keten tohumlarının (*Linum usitatissimum* L.) toplam yağ içeriğine, yağ asidi kompozisyonuna, α-linolenik asit düzeyine, SDG (sekoizolarikirezinol diglukosid) lignan, fenolik (serbest ve esterleşmiş), ve flavonoid (serbest ve esterleşmiş) içeriğine genotipik çeşitliliğin etkisi araştırılmıştır. Ayrıca, keten tohumunun yapısındaki bu biyoaktif bileşikler arasındaki korelasyon düzeylerinin belirlenmesi de amaçlanmıştır. Çalışmanın sonuçları genotipik çeşitliliğin söz konusu biyoaktif bileşikler üzerinde önemli bir etkisinin olduğunu göstermiştir ($p<0.05$). Keten tohumu yağlarında toplam yağ içeriği %37.55-45.07 arasında değişirken, α-linolenik asit düzeyleri %46.33-55.47 arasında bulunmuştur. En yüksek toplam fenolik madde kahverengi keten tohumlarında 1209.20 mg ferrulik asit/100 g olarak belirlenirken, en yüksek toplam flavonoid sarı keten tohumlarında 33.65 mg luteolin/100 g olarak saptanmıştır. Keten tohumlarının SDG lignan içeriği ise yağsız keten tohumu unlarında 5.87-23.84 mg/g arasındadır. Diğer taraftan, keten tohumlarının çoklu doymamış yağ asitleri ve α-linolenik asit içerikleri ($r=0.98$), SDG lignan ve toplam fenolik madde ($r=0.98$) ile toplam flavonoid ve α-linolenik asit içerikleri ($r=0.93$) arasında güçlü korelasyonlar elde edilmiştir

INTRODUCTION

Linseed is an economic oil seed in the world because of its high oil content (Oomah and Mazza, 1997) and also it is a valuable source of α-linolenic acid, lignans, phenolic acids and flavonoids with beneficial health effects (Oomah et al., 1995; Oomah et al., 1996; Meagher and Beecher 2000; Choo et al., 2007). The fatty acid composition of linseed oil is known to consist of high levels of α-linolenic acid ($C_{18:3}$) followed by linoleic ($C_{18:2}$) and oleic ($C_{18:1}$) acid (Wakjira et al., 2004; Krist et al., 2006; Choo et al., 2007; Bozan and Temelli, 2008). Linseed oil has the highest content of α-linolenic acid in comparison with other vegetable oils. The use of linseed oil for edible purposes, particularly as cooking oil, has been limited because of its instability, but it can be used as salad oil (Wakjira et al., 2004).

Linseed is the richest source of food lignans and contains lignans 75–800 times higher than other food sources (Muir and Westcott 2000). The major lignan present in linseed is secoisolariciresinol (SECO) (Westcott and Muir 2003), but free SECO is not found in linseed, rather it is present as the secoisolariciresinol diglucoside (SDG) which is converted to enterodiol and enterolactone which are the mammalian lignans in the gut (Adlercreutz, 2002). Linseed is reported to have a number of health benefits associated with its consumption. Especially, phytoestrogenic, anticarcinogenic, antioxidative and cardioprotective effects of linseed were attributed to SDG, phenolics, flavonoids and α-linolenic acid (Adlercreutz, 2002; Vanharanta et al., 2003; Collins et al., 2003; Lucas et al., 2004; Hosseinian et al., 2006).

Linseed is increasingly used as functional ingredient in food products because of its valuable bioactive compounds. (Madhusudhan et al., 2000; Johnsson et al., 2002; Medina, 2006). Bioactive compounds of linseed change with various factors such as cultivar, geographic location, climate, seeding time, stage of maturity, and storage conditions (Wanasundara and Shahidi 1994; Oomah et al., 1995; Oomah et al., 1996; Oomah et al., 1997; Thompson et al., 1997; Meagher et al., 1999). In this study, the effect of cultivar on bioactive compounds of linseed (*Linum usitatissimum* L.) was investigated. Total oil content, fatty acid composition, level of α-linolenic acids, SDG lignan, phenolic (free and esterified) and flavonoid (free and esterified) contents of seven linseed genotypes were aimed to be determined. Also, correlations between the amounts of these bioactive compounds of linseed genotypes were investigated.

MATERIAL and METHODS

Materials

Seven oil-type linseed cultivar (*Linum usitatissimum* L.), harvested on 2009 growing season, were supplied from National Gene Bank of Aegean Agricultural Research Institute in İzmir, Turkey. Seven linseed genotypes have been used in this study. Certificated cultivar TR 73572 (Sarı 85) was yellow in color and the others (TR 77705, TR 37060, TR 42077, TR 77418, TR 32204, TR 77416) were dark brown. Whole linseed was ground in a coffee grinder to pass a 1 mm screen before analyse.

The chemicals and reagents used were sodium carbonate (Na_2CO_3), n-hexane, hydrochloric acid (HCl), ammonium acetate, acetonitrile (Merck); potassium hydroxide (Supelco); sodium hydroxide (NaOH), sulphuric acid (H_2SO_4) (J. T. Baker), Folin-ciocalteau phenol reagent, methanol, luteolin (Sigma); ferrulic acid, 2-aminoethyl dipheylborinate (Fluka) and SDG standard (Bosco, Hong Kong). All the chemicals and solvents used were of analytical or HPLC grade.

Determination of moisture and total oil content

Moisture content and total oil content of linseed genotypes were determined using the air oven and Soxhelet method of AOAC (1998) respectively.

Determination of fatty acid composition

Linseed was finely ground and defatted twice with *n*-hexane under magnetic stirring at 20°C for 1 h. After filtration, n-hexane was removed on rotary evaporator. Fatty acid composition was determined using gas chromatography of fatty acid methyl esters (FAME). FAME were prepared according to the method of AOAC (1998). FAME was quantified on an Agilent 5890N gas chromatograph, (Agilent Technologies Inc., Wilmington, DE, USA) and a flame ionization detector. Separation was carried out on a DB23 capillary column (30m*250 µm, J. W. Scientific) with a film thickness of 0.25 mm. The FAME in n-hexane (2 µL) was injected into the column with a split ratio of 100:1. The injector and detector temperature were set at 250°C. The column temperature was programmed from 30 to 150°C at 20.0°C/min and then to 235°C at 6.0°C/min and was held at 230°C for 20 min (Łukaszewicz et al., 2004). Identification of fatty acids was carried out using a reference standard mixture FAME Q005 (Nu-Check Prep, Inc., Elysian, MN, USA) analyzed under the same operating conditions as those employed for FAME of the samples.

Determination of SDG lignan content

The procedure of Özkaynak Kanmaz and Ova (2013) was followed. Analysis of SDG was performed by using a HPLC-MS/MS system (API 4000) equipped with a Waters Model 600 pump, a 717 plus autosampler, an Agilent 1100 degasser, and a 996 photodiode array detector.

Determination of phenolic content

Free phenolics from 1 g of sample was extracted with 45 ml of 80% aqueous methanol in a shaker bath set at 40°C for 90 min and filtered. Then, the flask was allowed to cool to room temperature and diluted to a 50 ml volume with distilled water. The procedure of Özkaynak Kanmaz (2014) was followed for the extraction of esterified phenolics and spectrophotometric determination of phenolics.

Determination of flavonoid content

The procedure of Özkaynak Kanmaz (2014) was followed for the spectrophotometric determination of free and esterified flavonoids.

Statistical analysis

Data was interpreted by analysis of variance (ANOVA) with LSD test using SPSS software package. The statistical significance was evaluated at $p<0.05$ level.

RESULTS and DISCUSSION

Moisture and total oil content of linseeds

As shown in Table 1, all linseed genotypes had low moisture content changing from 5.58 to 6.48%.

Table 1. Moisture and total fat contents of linseeds.

Cultivars	Moisture (%)	Total fat (%)
TR 77705	6.48±0.01	42.29±1.55^{a}
TR 37060	6.23±0.02	38.73±1.18^{b}
TR 42077	6.46±0.00	37.55±1.43^{c}
TR 77418	6.31±0.01	38.71±1.03^{b}
TR 73572	5.58±0.01	45.07±1.30^{d}
TR 32204	6.24±0.03	37.89±1.23^{bc}
TR 77416	6.22±0.00	38.50±1.65^{bc}

Values are means±standard deviations of three (n=3) measurements
^{abcd} Values with different superscript letters within a column are significantly different at $p<0.05$

Total oil content of the linseed samples varied between 37.55 and 45.07% (Table 1) and significant variation was found between genotypes ($p<0.05$). Yellow-seeded flax cultivar TR 73572 and TR 77705 had higher total oil content than others. The highest oil content was found in cultivar TR 73572 as 45.07% followed by 42.29% in cultivar TR 77705 on dry matter basis. In studies conducted on linseed, the total oil content of genotypes varied between 29 and 48% (Green and Marshall, 1981; Batta et al., 1985; Eliasson et al., 2003; Wakjira et al., 2004).

Fatty acid composition of linseeds

The fatty acid composition and percentages of unsaturated fatty acids (UFA) of seven linseed oil were presented in Table 2. Cultivar had significant effect on levels of unsaturated fatty acid, α-linolenic and linoleic acid in linseed oil ($p<0.05$). As the percentage of unsaturated fatty acids were between 87.39 and 90.27%, α-linolenic acid levels changed from 46.37 to 55.47% in linseed oils respectively. There was a strong correlation ($r=0.98$) between levels of unsaturated fatty acids and α-linolenic acid of linseed genotypes. On the other hand, medium correlation ($r=0.76$) was found between level of α-linolenic acid and total oil content of linseeds, and also there was a medium correlation ($r=0.75$) between level of unsaturated fatty acids and total oil content of linseed samples.

Yellow-seeded cultivar TR 73572 and TR 77705 had higher level of unsaturated fatty acids and α-linolenic acid. The highest percentage of unsaturated fatty acids was found in cultivar TR 73572 as 90.27% followed by 89.39% in cultivar TR 77705. Also the highest level of α-linolenic acid was determined in cultivar TR 73572 as 55.47% followed by 54.56% in cultivar TR 77705. These amounts were significantly different ($p<0.05$) from the other genotypes. Results of total unsaturated fatty acids (85.90–91.61%) in linseed oils were in agreement with previous reports. Also, α-linolenic acid content in linseed oil varied between 47.00-60.42% of total fatty acids, depending on cultivation in the studies conducted by Wakjira et al. (2004), Krist et al. (2006), Choo et al. (2007) and Bozan et al. (2008).

The linoleic acid content of linseed oils were determined as 15.29-15.88% whereas oleic acids were changed from 18.95 to 25.47% with cultivar. TR 77416 had the highest level of oleic acid and it was significantly different ($p<0.05$) from other linseed oils. In the literature, linoleic and oleic acids in linseed oils were changed between 10.90–16.10% and 13.44–29.30%, respectively depending on cultivar (Wakjira et al.,2004; Krist et al., 2006; Choo et al., 2007; Bozan et al., 2008).

Yellow-seeded flax cultivar TR 73572 and TR 77705 in the current study had the highest level of

α-linolenic acid (55.47 and 54.56%) and the lowest level of oleic acid (18.95 and 19.30%). However, TR 77416 had the lowest level of α-linolenic acid (46.37%) and the highest level of oleic acid (%25.47). These results agree with Choo et al. (2007) who reported that there was a corresponding decrease in oleic acid with an increase in α-linolenic acid in linseed oil. Choo et al. (2007) reported that this may be due to a different linseed variety, origin and its corresponding environmental variation.

Table 2. Fatty acid composition (fatty acids %) of linseeds oils.

Fatty acids	Flaxseed cultivars						
	TR 73572	**TR 77705**	**TR 42077**	**TR 32204**	**TR 37060**	**TR 77418**	**TR 77416**
$C_{16:0}$	5.24 ±0.09^{a}	5.66±0.17^{b}	6.15±0.06^{c}	6.24±0.16^{b}	6.26±0.19^{d}	6.39±0.20^{e}	6.50±0.14^{f}
$C_{18:0}$	3.18±0.06^{a}	3.20±0.11^{b}	3.40±0.06^{c}	3.50±0.40^{d}	4.7±0.32^{e}	3.80±0.15^{f}	4.50 ±0.42^{g}
$C_{18:1}$	18.95±0.09^{a}	19.30 ±0.45^{b}	21.15±0.11^{c}	23.29±0.34^{d}	25.27±0.27^{e}	23.84±0.26^{f}	25.47±0.57^{g}
$C_{18:2}$	15.69±0.07^{a}	15.29±0.10^{b}	15.88±0.21^{c}	15.39±0.14^{d}	15.62±0.25^{e}	15.82±0.15^{f}	15.44±0.28^{d}
$C_{18:3}$	55.47±2.11^{a}	54.56±2.23^{b}	51.72±0.52^{c}	49.86±1.92^{d}	46.43±2.74^{e}	48.24±0.58^{f}	46.37±1.97^{e}
$C_{20:0}$	0.24±0.01	0.15±0.00	0.31±0.01	0.24±0.02	0.29±0.00	0.26±0.01	0.21±0.02
$C_{20:1}$	0.16±0.02	0.16±0.01	0.17±0.01	0.16±0.01	0.17±0.01	0.18±0.00	0.17±0.01
$C_{22:0}$	0.16±0.00	0.16±0.02	0.24±0.00	0.19±0.02	0.30±0.01	0.27±0.02	0.22±0.01
$C_{24:0}$	0.12±0.01	0.17±0.01	0.21±0.02	0.19±0.01	0.28±0.02	0.23±0.01	0.20±0.01
UFA	90.27^{a}	89.39^{b}	88.92^{c}	88.70^{c}	87.39^{d}	88.08^{e}	87.45^{d}

Values are means±standard deviations of three (n=3) measurements

^{abcdefg} Values with different superscript letters within a row are significantly different at $p<0.05$

UFA: Unsaturated fatty acids

SDG lignan content of linseeds

Lignans are assembled from the same simple phenolic precursors with lignins, wood fiber which the phenolic polymeric stuff in the woody cell walls of plants (Rouhi, 2000). Oilseeds, cereal brans, fruits and vegetables contain substantial quantities of plant lignans (Meagher and Beecher, 2000). However, Linseed has the highest concentration of plant lignans (Muir and Westcott 2000).

The amounts of SDG lignan present in linseed samples analyzed in this study varied between 5.87 and 23.84 mg/g defatted linseed flour on dry matter basis (Table 3) and differed significantly among genotypes ($p<0.05$). Cultivar TR 77705 had the highest amount of SDG lignan content and its amount was 2.1–4.1 times higher than other linseeds.

In the literature, cultivar had a significiant effect on SDG lignan content of linseed. Madhusudhan et al. (2000) determined SDG content as 12.9 and 14.3 mg/g whole linseeds. Johnsson et al. (2000) found SDG levels as 6.1–13.3 mg/g dry matter in whole linseeds. Wiesenborn et al. (2003) obtained the amount of SDG as 11.7–24.1 mg/g defatted linseed meal of genotypes. Eliasson et al. (2003) also reported that different samples of linseeds varied considerably in their content of SDG (11.9–25.9 mg/g). Beejmohun et al. (2007) found 16.1 mg SDG /g pressed linseed cake.

Table 3. SDG lignan content of linseeds.

Cultivars	SDG lignan (mg/g defatted flaxseed flour)
TR 77705	23.84±1.91^{a}
TR 37060	8.63±0.99^{b}
TR 42077	10.83±0.77^{c}
TR 77418	10.12±0.48^{c}
TR 73572	11.24±0.18^{c}
TR 32204	9.10±0.15^{c}
TR 77416	5.87±0.69^{d}

Values are means±standard deviations of three (n=3) measurements

^{abcd} Values with different superscript letters within a column are significantly different at $p<0.05$

Phenolic content of linseeds

Secoisolariciresinol and other phenolic compounds in linseed are present in bound forms with both glucosidic and ester bonds (Johnsson et al., 2002). So, in this study, free and esterified phenolics in linseed were analyzed separately and these forms were determined as ferrulic acid equivalent in defatted linseed flour (Table 4). The amounts of free,

esterified and total phenolics in the analyzed linseed samples differed significantly among genotypes (p<0,05). Esterified phenolics were the predominant ones in all linseed samples and accounted for 79.38–85.07% of the total phenolics and concentrations of esterified phenolics in the seven genotypes varied between 488.70 and 959.87 mg/100 g dry matter basis.

Table 4. Free, esterified and total phenolic contents of linseeds.

Cultivars	Phenolic contents (mg ferrulic acid/100 g defatted flaxseed flour)		
	Free	Esterified	Total
TR 77705	249.33±11.10[a]	959.87±38.20[a]	1209.20[a]
TR 37060	112.48±8.13[b]	617.03±31.59[b]	729.51[b]
TR 42077	125.52±6.62[b]	628.31±30.60[b]	753.83[b]
TR 77418	134.52±5.78[b]	624.27±26.53[b]	758.79[b]
TR 73572	160.91±9.40[c]	681.55±35.98[c]	842.46[c]
TR 32204	125.52±7.54[b]	618.12±37.77[b]	743.64[b]
TR 77416	85.78±3.63[d]	488.70±28.78[d]	574.48[d]

Values are means±standard deviations of three (n=3) measurements
[abcd] Values with different superscript letters within a column are significantly different at p<0.05

The antioxidant capacities of phenolic acids and their esters depend on the number of hydroxyl groups in the molecule. Therefore higher amount of esterified phenolic acids may play role to protect seeds from oxidative deterioration (Bozan and Temelli, 2008). In this study, total phenolics ranged from 574.48 mg/100 g for the cultivar TR 77416 to 1209.20 mg/100 g for the TR 77705 in this study. Although, highest free, esterified and total phenolic contents were found in cultivar TR 77705, lowest ones were determined in TR 77416 and these genotypes were significantly different (p<0.05) from the others. A strong correlation (r=0.98) was found between total phenolic and SDG lignan content of linseed genotypes and medium correlation (r=0.71) was determined between total phenolic content and of α-linolenic acid level of linseeds in the current study.

Wanasundara et al. (1994) determined total phenolics ranged from 130 to 220 mg ferrulic acid/100g linseed meal in different genotypes. On the other hand, Oomah et al. (1995) reported that linseed samples contained 800–1000 mg/100 g of total phenolics, about 500 mg/100 g of esterified phenolics and 300–500 mg/100 g of free phenolics as ferrulic acid equivalent in linseed and besides, the yellow-seeded flax cultivar had lower levels of total and esterified phenolics compared to the traditional brown-seeded type in the study. Bozan et al. (2008) reported that free, esterified and total phenolics were 383, 1287 and 1670 mg ferrulic acid in 100 g linseed respectively.

Flavonoid content of linseeds

Total flavonoid content of flaxseed samples in this study were presented as luteolin equivalent in defatted linseed flour (Table 5). Significant differences were found between free, esterified and total flavonoid contents of linseeds with cultivar in the current study (p<0,05). Predominant flavonoids were changed with cultivar. However, free forms of flavonoids were higher in genotypes TR 77705, 73572 and 42077, and also these genotypes contained the highest total flavonoids. Total flavonoid content of seven linseed genotypes ranged from 13.61 to 33.65 mg luteolin in 100 g dry matter basis.

Table 5. Free, esterified and total flavonoid contents of linseeds.

Cultivars	Flavonoid contents (mg luteolin/100 g defatted flaxseed flour)		
	Free	Esterified	Total
TR 77705	16.41±0.86[a]	11.58±0.66[a]	28.04[a]
TR 37060	7.94±0.46[b]	12.29±0.61[b]	20.23[b]
TR 42077	14.79±1.02[c]	11.17±0.73[b]	25.96[a]
TR 77418	8.16±0.33[b]	13.88±0.86[c]	22.04[c]
TR 73572	19.08±1.25[d]	14.57±0.79[c]	33.65[d]
TR 32204	8.39±4.12[b]	13.63±0.90[c]	22.02[c]
TR 77416	5.48±3.50[b]	8.13±0.34[d]	13.61[e]

Values are means±standard deviations of three (n=3) measurements
[abcde] Values with different superscript letters within a column are significantly different at p<0.05

Yellow-seeded cultivar TR 73572 had the highest free, esterified and total flavonoid contents (p<0.05) and lowest levels of all forms were determined for TR

77416. Cultivar TR 73572 had also the highest total oil content and α-linolenic acid level and there was a strong correlation (r=0.93) between total flavonoid contents and levels of α-linolenic acid whereas medium correlation (r=0.75) was found between total flavonoid and total oil content of linseed genotypes in the current study. Oomah et al. (1996) determined total flavonoids ranged from 35 to 71 mg luteolin in 100 g linseed ($p<0.05$). It was also reported that the yellow-seeded cultivar Omega had the lowest level of flavonoid compared to the brown-seeded type in the study.

CONCLUSIONS

The results of this study showed that cultivar had a significant effect on the total oil content, fatty acid composition, level of α-linolenic acid, SDG lignan, phenolic (free and esterified), and total flavonoid (free and esterified) contents of linseed ($p<0.05$). Esterified phenolics were the predominant form of phenolics in all linseed samples whereas predominant form for flavonoids changed with cultivar.

Yellow-seeded flax cultivar TR 73572 was found to have the highest total oil content, levels of unsaturated fatty acid and α-linolenic acid, and total flavonoid content. On the other hand, the highest total phenolic and SDG lignan contents were obtained for cultivar TR 77705. SDG lignan and total phenolic content of TR 77705 were 2.1 and 1.4 times higher than TR 73572 respectively and also TR 77705 linseed was the second rich source of total oil, α-linolenic acid and total flavonoids after TR 73572.

Strong correlation (r=0.98) was found between percantages of unsaturated fatty acid and α-linolenic acid of linseed genotypes. Besides, there was a strong correlation (r=0.98) between SDG lignan and total phenolic content and also, a strong correlation (r=0.93) was determined between total flavonoid content and level of α-linolenic acid of linseed samples.

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REFERENCES

Adlercreutz, H. 2002. Phyto-oestrogens and cancer. Lancet Oncology, 3: 364–373.

AOAC, 1998. Official Methods of Analysis of the Association of Analytical Chemists, Washington D. C., USA.

Batta, S. K., Ahuja, K. L., Laban, K. S. 1985. Variability in oil concent and fatty acid composition in linseed (Linum usitatissimum L.). Annals of Appplied Biology, 1: 80-85.

Beejmohun, V., Fliniaux, O., Grand, E., Lamblin, F., Bensaddek, L., Christen, P., Kovensky, J., Fliniaux, M. and Mesnard, F. 2007. Microwave-assisted extraction of the Main phenolic compounds in flaxseed. Phytochemical. Analaysis, 18: 275–282.

Bozan, B. and Temelli, F. 2008. Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. Bioresource Technology, 99: 6354-6359.

Choo, W. S., Birch, J., Dufour, J. P. 2007. Physicochemical and quality characteristics of cold-pressed flaxseed oils. Journal of Food Composition and Analysis, 20: 202-211.

Collins, T. F. X., Sprando, R. L., Black, T. N., Olejnik, N., Wiesenfeld, P. W., Babu, U. S., Bryant, M., Flynn, T. J., and Ruggles, D. I. 2003. Effects of flaxseed and defatted flaxseed meal on reproduction and development in rats. Food and Chemical Toxicology, 41: 819-834.

Çam, M., Hışıl, Y., Durmaz, G. 2009. Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. Food Chemistry, 112: 721-726.

Eliasson, C., Kamal-Eldin, A., Andersson, R., Åman, P. 2003. High-performance liquid chromatographic analysis of secoisolariciresinol diglucoside and hydroxycinnamic acid glucosides in flaxseed by alkaline extraction. Journal of Chromatography A, 1012: 151-159.

Green, A. G., and Marshall, D. R. 1981. Variation for oil quantity and quality in linseed (Linum usitatissimum). Australian Journal of Agricultural Research, 32(4): 599-607.

Hosseinian, F. S., Muir, A. D., Westcott, N. D., and Krol, E. S. 2006. Antioxidant Capacity of Flaxseed Lignans in Two Model Systems. Journal of the American Oil Chemists' Society, 83 (10): 835-840.

Johnsson, P., Kamal-Eldin, A., Lundgren, L. N., Åman, P. 2000. HPLC method for analysis of secoisolariciresinol diglucoside in flaxseeds. Journal of Agricultural and Food Chemistry, 48: 5216–5219.

Johnsson, P., Peerlkampa, N., Kamal-Eldina, A., Andersson, R. E., Anderssona, R., Lundgren, L. N., Åman, P. 2002. Polymeric fractions containing phenol glucosides in flaxseed. Food Chemistry, 76: 207–212.

Krist, S., Stuebiger, K., Bail, S., Unterweger, H. 2006. Analysis of volatile compounds and triacylglycerol composition of fatty seed oil gained from flax and false flax. European Journal of Lipid Science and Technology, 108: 48–60.

Łukaszewicz, M., Szopa, J., Krasowska, A. 2004. Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidants. Food Chemistry, 88: 225-231.

Madhusudhan, B., Wiesenborn, D., Schwarz, J., Tostenson, K., Gillespie, J. A. 2000. Dry mechanical method for concentrating the lignan secoisolariciresinol diglucoside in flaxseed. Lebensmittel-Wissenschaft und-Technologie, 33: 268-275.

Meagher, L.P., Beecher, G.R., Flanagan, V.P., Li, B.W. 1999. Isolation and characterization of the lignans, isolariciresinol and pinoresinol, in flaxseed meal. Journal of Agricultural and Food Chemistry, 47: 3173–3180.

Meagher, L. P. and Beecher, G. R. 2000. Assessment of data on the lignan content of foods. Journal of Food Composition and Analysis, 13: 935–947.

Medina, L. S. A. 2006. Phenolic Compounds: Their Role During Olive Oil Extraction and in Flaxseed-Transfer and Antioxidant Function. Doctorate thesis. University of Lleida Agronoöical, Forestal and Food Systems Doctorate Program Food Technology Department Lleida, Spain, 211p.

Muir, A.D., and N.D. Westcott. 2000. Quantitation of the Lignan Secoisolariciresinol Diglucoside in Baked Goods Containing Flax Seed or Flax Meal. Journal of Agricultural and Food Chemistry, 48: 4048–4052.

Oomah, B. D., Kenaschuk, E. O., Mazza, G. 1995. Phenolic Acids in Flaxseed. Journal of Agricultural and Food Chemistry, 43: 2016-2019.

Oomah, B. D., Mazza, G., Kenaschuk, E. O. 1996. Flavonoid content of flax seed. Influence of cultivar and environment. Euphytica, 90: 163-167.

Oomah, B. D. and Mazza, G. 1997. Effect of dehulling on chemical composition and physical properties of flaxseed. Lebensmittel-Wissenschaft und-Technologie, 30: 135–140.

Özkaynak Kanmaz, E. Ova, G. 2013. The Effective Parameters for Subcritical Water Extraction of SDG from Flaxseed (Linum usitatissimum L.) Using Accelerated Solvent Extractor. European Food Research and Technology, 237 (2): 159-166.

Özkaynak Kanmaz E. 2014. Subcritical Water Extraction of Phenolic Compounds from Flaxseed Meal Sticks Using Accelerated Solvent Extractor (ASE). Eur Food Res Technol, 238 (1): 85-91.

Popova, I., E., Hall, C., Kubátová, A. 2009. Determination of lignans in flaxseed using liquid chromatography with time-of flight mass spectrometry. Journal of Chromatography A, 1216: 217-229.

Thompson, L. U., Rickard, S. E., Cheung, F., Kenaschuk , E. O., Obermeyer, W. R. 1997. Variability in anticancer lignan levels in flax seed. Nutrition Cancer, 27: 26–30.

Vanharanta, M., Voutilainen, S., Rissanen, T. H., Adlercreutz, H., Salonen, J.T. 2003. Risk of cardiovascular disease-related and allcause death according to serum concentrations of enterolactone: Kuopio Ischaemic Heart Disease Risk Factor Study. International Archives of Medicine, 163: 1099–1104.

Wakjira, A., Labuschagne, M. T., Hugo, A. 2004. Variability in oil content and fatty acid composition of Ethiopian and introduced cultivars of linseed. Journal of the Science of Food and Agriculture, 84: 601–607.

Wanasundara, P.K.J.P.D., Shahidi, F. 1994. Alkanol ammonia water/hexane extraction of flaxseed. Food Chemistry, 49: 39–44.

Westcott, N.D. and A.D. Muir. 2003. Flax Seed Lignan in Disease Prevention and Health Promotion. Phytochemical. Reviews, 2: 401–417.

Wiesenborn, D., Tostenson, K., Kangas, N. 2003. Continuous abrasive method for mechanically fractionating flaxseed. Journal of the American Oil Chemists' Society, 80: 295–300.