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The EFFECT of GERMINATION TIME on MOISTURE, TOTAL FAT CONTENT and FATTY ACID COMPOSITION of FLAXSEED (*LINUM USITATISSIMUM* L.) SPROUTS

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Abstract

In this study, moisture content, total fat content and fatty acid composition were evaluated in brown (TR 77705) and yellow (TR 73572) flaxseeds (*Linum usitatissimum* L.) and their sprouts (at 5–13 day). Total fat content of brown and yellow flaxseeds reduced 68.72 and 76.61% after 5 days germination whereas, moisture content of seeds significantly increased as 14 and 17 fold respectively (P<0.05). Total fat in 5-day-old brown and yellow seed sprouts were determined as 13.23 and 10.54% on dry matter respectively and similarly 3.20 and 3.36% in 13-day-old sprouts. Highest percentage of unsaturated fatty acid in brown and yellow seed sprouts were determined as 89,80 and 86.20% at 5 days and similarly α-linolenic acids were calculated as 52.58 and 45.15% respectively. The palmitic acids in noticably increased during germination and the highest levels were obtained as 18.96 and 16.73% in yellow and brown seed sprouts at 11 days respectively.

Keywords: Flaxseed, flaxseed sprouts, germination, germination time, cultivar variety, moisture, total fat, α-linolenic acid, fatty acids.

KETEN TOHUMU (*LINUM USITATISSIMUM* L.) FİLİZLERİNİN NEM ve TOPLAM YAĞ İÇERİĞİ ile YAĞ ASİDİ KOMPOZİSYONU ÜZERİNE ÇİMLENDİRME SÜRESİNİN ETKİSİ

Özet

Bu çalışmada, kahverengi (TR 77705) ve sarı (TR 73572) keten tohumlarının (*Linum usitatissimum* L.) ve 5-13 günlük filizlerinin nem ve toplam yağ içeriği ile yağ asidi kompozisyonu incelenmiştir. Kahverengi ve sarı keten tohumlarının toplam yağ içeriği 5 günlük çimlendirme işlemi ile sırasıyla %68.72 ve 76.61 oranında düşmüş olup tohumların nem içerikleri ise sırasıyla 14 ve 17 kat artmıştır. (P<0.05). Kahverengi ve sarı keten tohumlarının 5 ve 13 günlük filizlerinin toplam yağ içerikleri ise kuru madde bazında sırasıyla 13.23; 10.54% ve 3.20; 3.36% olarak saptanmıştır. Kahverengi ve sarı keten tohumu filizlerinin en yüksek doymamış yağ asidi içerikleri sırasıyla %89.80 and 86.20 olarak 5 günlük filizlerde elde edilmiş olup α-linolenik asit yüzdeleri de en yüksek oranda 5 günlük filizlerde (sırasıyla %52.58 and 45.15) bulunmuştur. Palmitik asit filizlenme işlemi sırasında önemli düzeyde düşme göstermiş olup en yüksek palmitik asit içerikleri 11 günlük sarı ve kahverengi keten tohumu filizlerinde sırasıyla %18.96 and 16.73 olarak saptanmıştır.

Anahtar kelimeler: Keten tohumu, keten tohumu filizi, çimlendirme, çimlendirme süresi, kültürel çeşitlilik, nem, toplam yağ, α-linolenik asit, yağ asitleri.

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INTRODUCTION

The fatty acid composition of flaxseed oil is known to consist of high levels of α-linolenic acid followed by linoleic and oleic acid (1). Flaxseed is used as raw material in functional foods because it is a valuable source of omega-3 fatty acids, secoisolariciresinol diglucoside (SDG) lignan and other phenolic compounds with beneficial health effects (2, 3) as antioxidant (4-6), phytoestrogenic (7, 8) and anticarcinogenic effects (9, 10).

Seed sprouts are valuable dietary supplement and also considered healthy ingredients in functional foods. Sprouting of various type of seeds has become popular in the world. Sprouts are used as functional ingredient in many different foods including breakfast items, salads, soups, pasta and baked products (11-14). Sprouting is the practice of soaking and leaving seeds until they germinate and begin to sprout. This practice is reported to be associated with improvement in the nutritive value of seeds (13, 15, 16). Also, the lipids, carbohydrates and storage proteins are broken down to smaller and more digestible nutrients during this complex metabolic process (17).

Flaxseed sprouts are produced and consumed whereas, there are not sufficient study as much as other seed sprouts. In general germination conditions, germination time and cultivar have pronounced to influence the formation of bioactive compounds in sprouts (12, 13, 18). In the literature, little information is available regarding the total fat and fatty acid composition in flaxseed sprouts. The present study was undertaken to investigate the influence of germination and germination time on moisture content, total fat content and fatty acid composition of brown and yellow flaxseeds and (*Linum usitatissimum* L.) their sprouts (5-13 days).

MATERIALS and METHODS

Materials

Two oil-type flaxseed cultivars (*Linum usitatissimum* L) were used in this study and they were supplied from National Gene Bank of Aegean Agricultural Research Institute in İzmir, Turkey. The seeds were cleaned and stored at room temperature without exposure to direct sunlight. Certificated cultivar TR 73572 (Sarı 85) was yellow in colour and the other seed, TR 77705 was brown. Seeds were germinated in a growth chamber for 13 days in dark at 20±1°C and approximately %78±2 relative humidity (19). Seeds and sprouts were washed twice a day to avoid microbial growth. Under the conditions in this study, 98–99% germination of flaxseed was achieved. Sprouts with out pericarps were harvested after 5, 7, 9, 11, and 13 days with hand. Germination time was determined after a pilot germination study. In this study, it was shown that the hulls of seeds falled after 5 days and the flaxseed sprouts were still alive and fresh up to 13 days.

The chemicals and reagents used in the study were n-hexane (Merck); potassium hydroxide (Supelco); fatty acid methyl esters (Fatty acid methyl esters) FAME Q005 (Nu-Check Prep, Inc., Elysian, MN, USA). All the chemicals and solvents used were of analytical or HPLC grade.

Determination of moisture and water content

Moisture and water content of flaxseeds and sprouts were determined using the air oven method (20).

Determination of total fat content

Total fat content of flaxseeds and sprouts were determined using the Soxhelet method (20).

Determination of fatty acid composition

Ground flaxseeds and blended sprouts were 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 of samples were determined using gas chromatography of fatty acid methyl esters (FAME). FAME were prepared according to the method of (20). 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 (21-Lukaszewicz et al., 2004). Identification of fatty acids was carried out using a reference standard mixture FAME Q005 and FAME of the samples were analyzed under the same operating conditions.

Statistical analysis

Data were interpreted by analysis of variance (ANOVA) with LSD test using SPSS (17.0) software package. The statistical significance was evaluated at $P<0.05$ level.

RESULTS and DISCUSSION

Moisture and water contents of flaxseeds and their sprouts

The flaxseed cultivars, TR 77705 (brown seed) and TR 73572 (yellow seed) had low moisture content as 6.48 and 5.58% respectively. The moisture content of seeds increased drastically with germination and reached to 91.37 and 91.95% in brown and yellow seed sprouts after 5 days respectively (Table 1). In the literature, it was also reported that the loss of dry matter occured as a result of oxidation and breakdown of the stored macromolecules such as lipids and proteins of flaxseeds during germination (22).

During germination, water content exhibited an almost linear upward trend with increasing germination time and water contents of brown and yellow seed sprouts were observed as 96.53 and 96.79% at the end of 13 days (Table 1). In the literature, a noticable increase in moisture and water contents of seeds and sprouts were observed in other studies with flaxseed, sesame and chickpea (12, 13, 17, 22-25).

Total fat content of flaxseeds and their sprouts

The effects of germination time and cultivar on total fat content of flaxseeds and their sprouts were significant ($P<0.05$) as shown in Table 2. Yellow flaxseeds had higher total fat content (45.07%) than brown flaxseeds (42.29%). Total fat contents of yellow and brown flaxseeds reduced 76.61 and 68.72% at the end of the 5 days germination respectively and a significant variation was found between cultivars ($P<0.05$). Also, a significant ($P<0.05$) decrease was observed during germination and total fat contents were determined as 3.36 and 3.20% in yellow and brown seed sprouts after germination of 13 days.

In the other study with flaxseed, it was also found that total fat significantly reduced with germination (22). It was also noted significant losses in total fat content of canola seeds during germination (15). Besides, degradation of total fat with germination was recognized in soybean and seasame sprouts (17, 26). On the whole, it was reported that degradation of reserve nutrients (lipids and carbohydrates) during germination is a process whose essential purpose is required to provide the energy or protein synthesis in plant growth (17).

Fatty acid composition of flaxseeds and their sprouts

In this study, α-linolenic (C18:3), linoleic (C18:2), and oleic (C18:1) acids were the predominant fatty acids and also α-linolenic acid was the major fatty acid in flaxseeds and their sprouts. Percentages of α-linolenic acid in brown and yellow flaxseeds were found as 54.56 and 55.47% respectively and similarly unsaturated fatty acids were 89.39 and 90.27% (Table 3).

Table 1. Moisture and water content of flaxseeds and sprouts of brown (TR 77705) and yellow (TR 73572) seed cultivars.

Composition	Cultivar	Germination time (day)					
		seed	**5***	**7***	**9***	**11***	**13***
%	Brown flaxseed	6.48±0.01	91.37±0.01	93.42±0.02	95.73±0.02	95.91±0.01	96.53±0.01
	Yellow flaxseed	5.58±0.01	91.95±0.01	94.94±0.01	95.69±0.01	96.18±0.03	96.79±0.01

Values are means±standard deviations of three (n=3) measurements
* Hull-free sprouts

Table 2. Total fat content of flaxseed and sprouts of brown (TR 77705) and yellow (TR 73572) seed cultivars.

	Cultivar	Germination time (day)					
		seed	**5***	**7***	**9***	**11***	**13***
Total fat	Brown flaxseed	42.29±1.55^{a}	13.23±0.73^{b}	9.88±0.83^{c}	7.26±0.77^{d}	3.41±0.95^{e}	3.20±0.56^{f}
(% DW)	Yellow flaxseed	45.07±1.30^{a}	10.54±0.35^{b}	6.37±0.22^{c}	6.73±0.67^{c}	3.57±0.37^{d}	3.36±0.58^{e}

Values are means±standard deviations of three (n=3) measurements
^{abcdef} Values with different superscript letters within a row are significantly different at p<0.05
* Hull-free sprouts

With germination process (after 5 days), there was a slight decrease (3.63%) in the level of α-linolenic acid in brown seeds whereas, a noticable decrease (18.61%) was obtained for yellow seeds. During germination (5-13 days), the percentage of α-linolenic acid reduced 32.76 and 41.54% in yellow and brown seed sprouts respectively and similarly unsaturated fatty acids reduced 12.31 and 12.78% (Table 3). In the literature, it was also reported that there was decrease in the percentage of α-linolenic acid and unsaturated fatty acids of flaxseed and sesame sprouts with germination (17, 22). Level of α-linolenic acids in brown and yellow seed sprouts was significantly different (52.58 and 45.15% respectively) at 5 days-in the initial stages of germination whereas, ended on an almost similar value (30.74 and 30.36% respectively) after germination of 13 days. However brown and yellow seed sprouts had significantly different level of unsaturated fatty acids as 78.32 and 75.59% at 13 days respectively (Table 3).

After germination of 5 days, a slightly increase was found in relative percentages of palmitic and stearic acids in flaxseeds. Also, there was a slightly increase in the level of oleic acid in brown flaxseeds whereas a noticeable increase (37.31%) was observed in the level of oleic acid for yellow seeds. During germination (5-13 days), several variations were observed in the percentages of linoleic acids at different days. The highest level of linoleic acid were observed 28.33% in yellow seed sprouts at the end of the 13 days whereas, brown seed sprouts had the highest level (31.52%) at 9 days. While the highest percentage of oleic acid was determined as 26.02% in yellow seed sprouts at 5 days, brown seed sprouts had the highest percentage (21.96%) at 13 days.

The level of palmitic acid in yellow and brown seed sprouts noticably increased during germination and the highest levels were obtained as 18.96 and 16.73% at 11 days respectively (Table 3). Also the percentage of stearic acids increased in sprouts during germination (5-13) and reached the maximum value at 11 days. In the another flaxseed sprout study, it was reported that flaxseed oils were rich in polyunsaturated fatty acids (α-linolenic and linoleic acids-77%) and monounsatureted fatty acids (oleic acids-12%) after germination of 8 days and contained only 10% of saturated (palmitic and stearic acids) fatty acids (22).

CONCLUSIONS

Moisture content of flaxseeds significantly increased with germination (P<0.05). Omega-3 fatty acids-α-linolenic acid was found to be the major fatty acid of flaxseeds and their sprouts. Total fat content of brown and yellow flaxseeds showed a considerable decrease with germination process after 5 days and also, level of α-linolenic acids and unsaturated fatty acids of seeds showed a significant decrease (P<0.05). With noticable reduction in total fat content, 5-old-day brown

Table 3. Fatty acid composition of flaxseed and sprouts of brown (TR 77705) and yellow (TR 73572) seed cultivars.

Fatty acids (%)	Cultivar	Germination time (day)					
		seed	**5***	**7***	**9***	**11***	**13***
C 16:0	Brown flaxseed	5.66±0.17^{a}	5.77±0.69^{a}	6.47±0.59^{b}	16.45±0.93^{c}	16.73±0.56^{d}	12.25±0.48^{e}
	Yellow flaxseed	5.24 ±0.09^{a}	7.11±0.78^{b}	7.66±0.53^{c}	18.60±0.34^{d}	18.96±0.65^{e}	15.62±0.47^{f}
C 18:0	Brown flaxseed	3.20±0.11^{a}	4.43±0.62^{b}	5.46±0.51^{c}	9.22±0.87^{d}	11.68±0.51^{e}	9.33±0.86^{f}
	Yellow flaxseed	3.18±0.06^{a}	6.70±0.84^{b}	7.89±0.88^{c}	8.76±0.59^{d}	10.50±0.68^{e}	8.78±0.55^{d}
C 18:1	Brown flaxseed	19.30 ±0.45^{a}	19.58±0.27^{b}	17.65±0.39^{c}	15.05±0.44^{d}	20.69±0.86^{e}	21.96±0.55f
	Yellow flaxseed	18.95±0.09^{a}	26.02±0.19^{b}	24.08±0.24^{c}	15.91±0.37^{d}	16.06±0.29^{d}	16.90±0.45^{e}
C 18:2	Brown flaxseed	15.29±0.10^{a}	17.64±0.36^{b}	23.77±0.68^{c}	31.52±0.23^{d}	23.84±0.49^{e}	25.62±0.64^{f}
	Yellow flaxseed	15.69±0.07^{a}	15.03±0.31^{b}	18.45±0.17^{c}	27.20±0.74^{d}	26.13±0.81^{e}	28.33±0.46^{f}
C 18:3	Brown flaxseed	54.56±2.23^{a}	52.58±1.48^{b}	46.55±1.32^{c}	27.77±0.68^{d}	26.77±0.37^{e}	30.74±0.77^{f}
	Yellow flaxseed	55.47±2.11^{a}	45.15±1.22^{b}	41.93±1.25^{c}	29.53±0.79^{d}	28.78±0.54^{e}	30.36±0.29^{f}
UFA	Brown flaxseed	89.39^{a}	89.80^{b}	87.97^{c}	74.34^{d}	71.30^{e}	78.32^{f}
	Yellow flaxseed	90.27^{a}	86.20^{b}	84.46^{c}	72.64^{d}	70.97^{e}	75.59^{f}

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

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

* Hull-free sprouts

UFA: Unsaturated fatty acids

flaxseed sprouts oil contained considerable amount of unsaturated fatty acids (89.80%) and α-linolenic acid (52.58%). During germination (5-13 days), brown and yellow flaxseed sprouts had the highest level of α-linolenic acid and unsaturated fatty acids and also total fat content after germination of 5 days and there was significant differences between cultivars (P<0.05). The level of palmitic acids noticably increased in yellow and brown seed sprouts during germination from 5 to 13 days. As a result, selection of brown flaxseed cultivar combined with germination of 5 days could provide better sources of α-linolenic acids from flaxseed sprouts.

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