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Research / Araştırma

THE INFLUENCE of GERMINATION TIME on SDG LIGNAN, PHENOLIC and FLAVONOID CONTENTS of FLAXSEED (*LINUM USITATISSIMUM* L.) SPROUTS

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Abstract

In this study, the effect of germination time on SDG (secoisolariciresinol diglucoside) lignan, phenolic and flavonoid contents of brown and yellow flaxseeds (*Linum usitatissimum* L.) and their sprouts was investigated. SDG lignan content of brown and yellow flaxseeds were obtained as 13.76 and 6.17 mg/g dw respectively whereas, germination resulted in a noticable reduction of SDG lignan in seeds. 13-old-day brown flaxseed sprouts had the highest SDG lignan content (0.72 mg/g dw) whereas, the highest SDG lignan content was determined as 0.37 mg/g in yellow flaxseed sprouts at 11 days. Free and esterified phenolics and flavonoids were increased after with germination and during germination (5-13 days) and cultivar had a significant effect (P<0.05). Total phenolic content of brown and yellow flaxseeds increased 4.2-fold and 7.3-fold after germination of 13 days respectively, similarly total flavonoid content increased 33.5-fold and 26.8-fold. The percentage of esterified phenolics decreased with germination in seeds and represented 66.11 and 67.02% of total phenolics in brown and yellow flaxseed sprouts at 5 days whereas, the percentage of free flavonoids in seeds increased with germination and reached to 77.35 and 71.11% in brown and yellow flaxseed sprouts respectively.

Keywords: Flaxseed, flaxseed sprouts, germination, germination time, cultivar variety, SDG lignan, phenolics, flavonoids.

KETEN TOHUMU FİLİZLERİNİN SDG LİGNAN, FENOLİK ve FLAVONOİT İÇERİKLERİNE ÇİMLENDİRME SÜRESİNİN ETKİSİ

Özet

Bu çalışmada, kahverengi ve sarı keten tohumlarının (*Linum usitatissimum* L.) ve filizlerinin SDG (sekoizolarikirezinol diglukosit) lignan, fenolik ve flavonoit içeriğine çimlendirme süresinin etkisi araştırılmıştır. Kahverengi ve sarı keten tohumlarında SDG lignan miktarı sırasıyla 13.76 ve 6.17 mg/g olmasına karşın çimlendirme işlemi tohumların SDG lignan içeriğinde önemli bir azalmaya neden olmuştur. 13 günlük kahverengi keten tohumu filizleri en yüksek SDG lignan (0.72 mg/g) içeriğine sahipken sarı keten tohumu filizlerinde en yüksek SDG lignan 0.37 mg/g olarak 11. günde saptanmıştır. Keten tohumlarının yapısındaki serbest ve esterleşmiş fenolikler ve flavonoitler çimlendirme işleminden sonra ve 5-13 günlük çimlendirme süresince artmıştır ve kültürel çeşitlilik istatistiksel anlamda önemli bulunmuştur (P<0.05). Kahverengi ve sarı keten tohumlarının toplam fenolik içeriği 13 günlük çimlendirme işleminden sonra sırasıyla 4.2 ve 7.3 kat artmış olup benzer şekilde toplam flavonoit içerikleri de 33.5 ve 26.8 kat artmıştır. Keten tohumlarında esterleşmiş fenoliklerin oranı çimlendirme işlemi ile düşerek 5 günlük kahverengi ve sarı keten tohumu filizlerinde toplam fenoliklerin %66.11 ve 67.02'sini oluşturduğu saptanmıştır. Buna karşın, tohumlardaki serbest flavonoitler çimlendirme işlemi ile artarak 13 günlük kahverengi ve sarı keten tohumu filizlerinde sırasıyla %77.35 ve 71.11 oranlarına ulaşmıştır.

Anahtar kelimeler: Keten tohumu, keten tohumu filizi, çimlendirme, çimlendirme süresi, kültürel çeşitlilik, SDG lignan, fenolikler, flavonoitler.

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INTRODUCTION

Flaxseed is generally used as a functional ingredient in food industry because of its valuable omega-3 fatty acids, and lignans especially SDG lignan (1-3). Also, flaxseed is a natural source of major phytochemicals such as phenolic acids and flavonoids (4, 5). Flaxseed is the richest source of food lignans and contains lignans 75–800 times higher than other foods (2). These bioactive compounds in flaxseed have antioxidant (6-8), phytoestrogenic (9, 10) and anticarcinogenic effects (11, 12).

Seed sprouts are also considered healthy ingredient in functional foods (13). Sprouting is the practice of soaking, draining and leaving seeds until they germinate and begin to sprout. This method has been known for a very long time, mainly in the Eastern countries such as China and Japan (14). It has been identified as an inexpensive and effective technology for improving the nutritional quality of seeds (15-18). Sprouts are used as functional ingredient in many different foods including breakfast items, salads, soups, pasta, and baked products (17, 19, 20).

Different seeds such as legumes (bean, pea, lentil, soybean), grains (rye, wheat, barley, oats), some vegetables (alfalfa, radish) and oilseeds (flaxseed, sesame) can be sprouted for human consumption. Flaxseed sprouts are produced and consumed whereas there are not sufficient studies as much as other seed sprouts. Also, in the literature, there is no information about SDG lignan, phenolics and flavonoids in flaxseed sprouts and also the effect of cultivar and long period of germination time. The present study was undertaken to investigate the influence of germination time (5-13 days) on SDG lignan, free, esterified and total phenolic and flavonoid contents of brown and yellow flaxseeds (*Linum usitatissimum* L.) and their sprouts.

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 color and the other seed, TR 77705 was dark brown. Seeds were germinated in a growth chamber for 13 days in dark at 20±1 °C and approximately %78±2 relative humidity (21). 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.

The chemicals and reagents used in the study were sodium carbonate, hydrochloric acid (Merck); Folin-ciocalteau phenol reagent, methanol, luteolin (Sigma); ferrulic acid, 2-aminoethyl dipheylborinate (Fluka); sodium hydroxide, sulphuric acid (J. T. Baker), methanol, (Sigma) and SDG (secoisolariciresinol diglucoside) standard (Bosco, Hong Kong). 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 (22).

Determination of SDG lignan content

The procedure of Özkaynak Kanmaz (2014) was followed (23). 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 these samples were extracted with 45 ml of 80% aqueous methanol in a shaker bath set at 40 °C for 90 min and filtered. Then, the flasks 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 (24).

Determination of flavonoid content

Flavonoid analysis was occured in free and esterified phenolic extracts. The procedure of Özkaynak Kanmaz (2014) was followed for the spectrophotometric determination of free and esterified flavonoids (24).

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

SDG lignan content of flaxseeds and their sprouts

Flaxseed, flaxseed meal and flaxseed flour have the highest concentration of plant lignans. In addition cereals, cereal brans, a few oilseeds, fruits and vegetables contain substantial quantities of plant lignans (2). In the current study, germination time and cultivar had a noticable effect on SDG lignan content in flaxseeds and their sprouts. SDG lignan content of flaxseeds differed significantly (P<0.05) between cultivars with brown seed (13.76 m/g dw) having 2.5 times higher value than yellow seed (6.17 m/g dw). Germination resulted in a noticable reduction of SDG lignan in brown and yellow flaxseeds as 99.93 and 96.92% after 5 days (Table 1). However, a significant increase (2.8 and 3.7 times) was reported in free phytoestrogens, genistein and daidzein content of soybean seed after germination of 96 h (14). Lignans are also one of the major classes of phytoestrogens but 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 (25). In the literature, it was reported that degradation of carbohydrates during germination is required to provide the energy or protein synthesis in plant growth. These findings may be an answer for the significant decrease in SDG lignan content of flaxseed with germination at 5 days (26).

Germination from 5 to 13 days, SDG lignan content of sprouts increased but several variations were observed during different days of germination (Table 1). Minimum value was obtained at 5 and 9 days for brown seed sprouts whereas, yellow seed sprouts had minimum value at 7 days. The highest SDG lignan content was determined as 0.72 mg/g dw on dry matter at 13 days in brown flaxseed sprouts but, 0.37 mg/g dw was observed in yellow seed sprouts at 11 days.

Phenolic content of flaxseeds and their sprouts

The results of this study indicated germination time and cultivar had a significant (P<0.05) effect on free, esterified and total phenolics. Brown flaxseeds (697.83 mg/100 g dw) had significantly (P<0.05) higher total phenolic content than yellow seeds (462.77 mg/100 g dw). After germination of 5 days, a noticable increase (4.3 and 2.4 times respectively) was shown in the total phenolic content of yellow and brown flaxseeds and also, 5-old-day yellow seed sprouts (2008.95 mg/100 g dw) contained significantly (P<0.05) higher value than brown seed sprouts (1675.67 mg/100 g dw). In addition, total phenolic content of sprouts increased during germination from 5 to 13 days. A significant increase (1.7 and 1.8 times respectively) was observed in phenolics with germination from 5 to 13 days and the total concentration of phenolics reached to 3396.57 and 2945.24 mg/100 g dw in yellow and brown seed sprouts respectively (Figure 1).

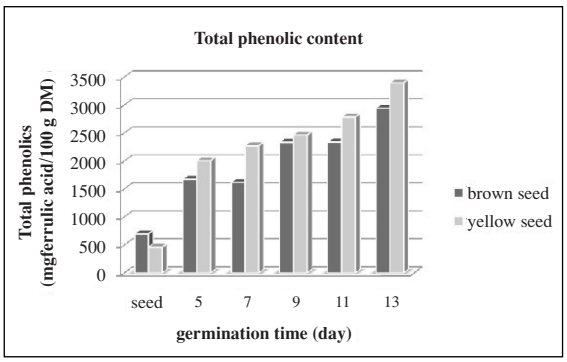

Figure 1. Comparison of total phenolic content of brown and yellow flaxseeds and their sprouts.

It was shown that total phenolic contents of yellow and brown seeds increased 7.3 and 4.2 fold with germination of 13 days respectively. Also in the literature, it has been has been reported that

Table 1. SDG lignan 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***
SDG lignan (mg/g DW)	Brown flaxseed	13.76±1.10^{a}	0.01±0.00^{b}	0.08±0.00^{c}	0.01±0.00^{b}	0.07±0.00^{d}	0.72±0.00^{e}
	Yellow flaxseed	6.17±0.11^{a}	0.19±0.00^{b}	0.05±0.00^{c}	0.12±0.00^{d}	0.37±0.00^{e}	0.31±0.00^{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

the phenolics of seeds increased with germination. Fernández-Orozco et al. (2006) found that the total phenolic acids of lupin seeds increased 53% as compared with lupin sprouts after germination of nine days (27). Also, total phenolics of soybeans increased 1.5-2 fold after germination and increased with increasing germination time from 1 to 4 days (28). In addition, it was reported that total phenolic contents increased 2 fold in amaranth, quinoa and wheat sprouts and 4 fold in buckwheat sprouts with short time germination (29). Besides, Pérez-Balibrea et al. (2011) reported that phenolic acids of broccoli seeds increased 2-6 fold with germination of 3 days (30). Also, a significant increase (35-60 times) was determined in the polyphenol contents of quinoa sprouts after germination of 4 days in the darkness (20). Besides, the cultivar had a significant effect on phenolic content of soybean and broccoli sprouts (30, 31).

With germination of 5 days, a considerable increase (P<0.05) was observed in the free and esterified phenolic contents of two flaxseed cultivars. However, the increase in free phenolics of seeds was noticably higher than esterified phenolics and this increase was significantly (P<0.05) higher in yellow seeds. After germination of 5 days, 6.5 and 3.1 fold increase was found in the free phenolics of yellow and brown seeds respectively and similarly 3.8 and 2.2 fold increase was determined in the esterified phenolics of seeds. Yellow seed sprouts (571.68 and 1437.27 mg/100 g dw respectively) had significantly (P<0.05) higher free and esterified phenolic contents than brown seed sprouts (451.80 and 1223.87 mg/100 g dw respectively) at 5 days (Table 2).

Also during germination from 5 to 13 days, free and esterified phenolics significantly (P<0.05) increased in 5-old-day flaxseed sprouts. Free phenolics increased (2.0 and 2.2 times respectively) in 5-old-day yellow and brown seed sprouts and 1.6 fold increase was determined in the esterified phenolics of sprouts after 13 days for each cultivar. When flaxseeds were compared with 13-old-day sprouts, free phenolics increased more than esterified phenolics in seeds and yellow seed sprouts had higher phenolics than brown seeds at 13 days. Free and esterified phenolics increased 12.7 and 6.1 fold in yellow seeds and reached to 1120.25 and 2276.32 mg/100 g dw respectively after 13 days. However, free and esterified phenolics increased 6.9 and 3.5 fold in brown seeds and calculated as 998.27 and 1946.97 mg/100 g dw respectively (Table 2).

There were few differences between the percentages of free and esterified phenolics in flaxseeds and their sprouts (Figure 2). Esterified phenolics were significantly higher than free phenolics in seeds (P<0.05). The esterified phenolics represented 79.38 and 80.90% of the total phenolics in brown and yellow flaxseeds respectively. Esterified phenolics were also significantly higher than free phenolics in 5-old-day flaxseed sprouts P<0.05).

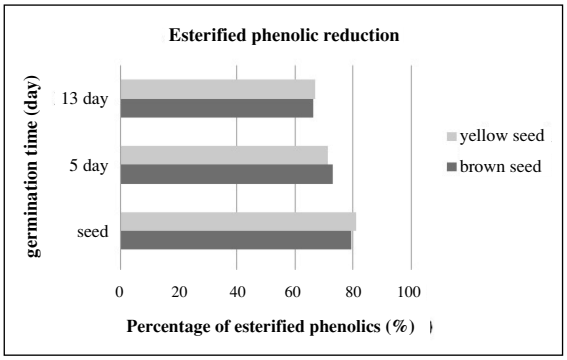

Figure 2. Change in the percentage of esterified phenolic acids with germination and from 5 to 13 days germination.

With germination (at 5 days) esterified phenolics decreased 73.04 and 71.54% in brown and yellow seed sprouts respectively. Also from 5 to 13 days, the

Table 2. Free and esterified phenolic content (mg ferrulic acid/100 g DM) of flaxseed and sprouts of brown (TR 77705) and yellow (TR 73572) seed cultivars.

Phenolic acids	Cultivar	Germination time (day)					
		seed	**5***	**7***	**9***	**11***	**13***
free	Brown flaxseed	143.89±10.07^{a}	451.80±19.25^{a}	509.12±31.06^{a}	684.31±35.45^{b}	711.74±29.61^{b}	998.27±62.29^{c}
	Yellow flaxseed	88.39±5.75^{a}	571.68±28.64^{b}	630.63±39.10^{b}	707.43±29.85^{c}	876.18±43.02^{d}	1120.25±60.72^{e}
esterified	Brown flaxseed	553.94±27.70^{a}	1223.87±61.32^{b}	1109.73±46.50^{b}	1648.95±76.68^{c}	1624.94±66.95^{c}	1946.97±78.66^{d}
	Yellow flaxseed	374.38±29.20^{a}	1437.27±74.59^{b}	1643.68±88.43^{c}	1754.29±95.26^{c}	1908.38±91.22^{c}	2276.32±117.23^{d}

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

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

* Hull-free sprouts

percentage of esterified phenolics significantly (P<0.05) decreased in brown and yellow seed sprouts and represented 66.11 and 67.02% of total phenolics at the end of the germination time respectively (Figure 2).

Flavonoid content of flaxseeds and their sprouts

Figure 3 illustrated the total flavonoid content (expressed as mg luteolin/ 100 mg on dry matter) of brown and yellow flaxseeds and their sprouts. The results of this study showed germination time and cultivar had a significant (P<0.05) effect on free, esterified and total flavonoids. As total flavonoid content of brown flaxseeds was calculated as 16.15 mg/100 g dw, yellow seeds contained 18.36 mg/100 g dw and the cultivars differed significantly (P<0.05). A considerable increase (14.3 and 11.2 times respectively) was obtained in the total flavonoids of brown and yellow flaxseeds with germination of 5 days, and total flavonoids calculated as 231.17 and 205.22 mg/100 g dw in 5-old-day sprouts respectively. Also, total flavonoid contents of sprouts increased during germination (5-13 days).

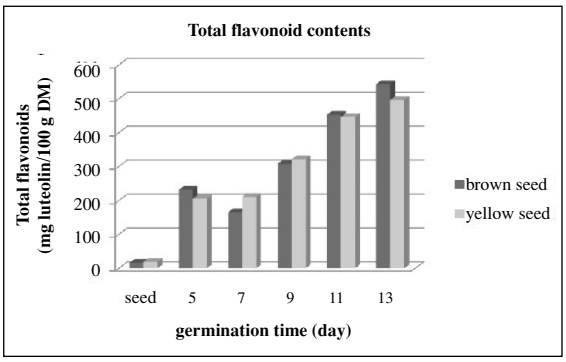

Figure 3. Comparison of total flavonoid content of brown and yellow flaxseeds and their sprouts.

After germination of 13 days, total flavonoid content of brown flaxseeds increased 33.5 times and reached to 543.22 mg/100 g dw in brown seed sprouts. On the other hand, total flavonoid content of yellow seed sprouts increased 26.8 fold and reached to 495.95 mg/100 g dw after 13 days. Also in the literature, it was reported that the flavonoid contents of seeds increased with germination. Lin and Lai (2006) reported that total flavonoid of soybeans increased with germination of one day and increased with increasing sprouting time up to 4 days (28). Also, a noticable increase was determined in the amounts of total isoflavone of soybean seeds after germination of 5 days and isoflavone levels widely varied depending on the varieties (32). Besides, it was reported that flavonoids of broccoli seeds increased 2-3 fold with germination after 3 days whereas, flavonoids decreased during sprouting from 3 to 7 days and significant differences was found between broccoli cultivars (30). On the other hand, it was also reported that the isoflavonoid contents of 5-old-day soybean sprouts were 50-100 times higher than seeds and the cultivar had significant effect on isoflavonoid content of seeds and sprouts (31).

With germination (at 5 days), a noticable increase was obtained in free and esterified flavonoid contents of flaxseeds. The increase in free flavonoids of seeds was higher than esterified flavonoids and this increase was significantly (P<0.05) higher in brown seeds. Brown seed sprouts had significantly (P<0.05) higher free flavonoids than yellow seed sprouts at 5 days. Free and esterified flavonoids increased 15.9 and 12.1 fold in brown seeds and reached to 150.52 and 80.65 mg/100 g dw respectively after germination of 5 days. Also free and esterified flavonoids increased 11.9 and 10.2 fold in 5-old day yellow seeds and calculated as 123.73 and 81.49 mg/100 g dw respectively (Table 3).

Table 3. Free and esterified flavonoid content (mg luteolin/100 g DM) of flaxseed and sprouts of brown (TR 77705) and yellow (TR 73572) seed cultivars.

Flavonoids	Cultivar	Germination time (day)					
		seed	**5***	**7***	**9***	**11***	**13***
free	Brown flaxseed	9.47±0.64^{a}	150.52±8.10^{b}	106.08±7.75^{c}	204.68±12.59^{d}	380.44±19.25^{e}	420.17±17.82^{f}
	Yellow flaxseed	10.36±0.52^{a}	123.73±6.40^{b}	126.88±7.82^{b}	221.81±11.93^{c}	315.97±19.40^{d}	352.65±18.30^{e}
esterified	Brown flaxseed	6.68±0.53^{a}	80.65±4.15^{b}	59.73±3.63^{c}	103.18±5.17^{d}	72.13±4.39^{e}	123.05±5.64^{f}
	Yellow flaxseed	8.00±0.82^{a}	81.49±5.01^{b}	83.80±4.27^{b}	99.77±6.07c	130.37±6.65^{d}	143.30±8.90^{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

Also during germination (5-13 days), free and esterified flavonoids of sprouts significantly (P<0.05) increased. In brown seed sprouts, free and esterified flavonoids increased 2.8 and 1.5 fold respectively and similarly 2.9 and 1.8 fold increase was found in yellow seed sprouts after 13 days. When flaxseeds were compared with 13-old-day sprouts, free and esterified flavonoids of brown seeds increased 44.4 and 18.4 fold and reached to 420.17 and 123.05 mg/100 g dw in 13-old day sprouts respectively. However, free and esterified flavonoids of yellow seeds increased 43 and 17.9 fold and obtained 1120.25 and 2276.32 mg/100 g dw respectively (Table 3). Effect of cultivar was significant (p<0.05) for free and esterified flavonoids.

There were few differences between the percentages of free and esterified flavonoids in flaxseeds and their sprouts (Figure 4). Free flavonoids were significantly higher than esterified flavonoids in seeds and also 5-old-day sprouts (P<0.05). Free flavonoids represented 58.64 and 56.43% of the total flavonoids in brown and yellow flaxseeds respectively. With germination, this group increased 65.11 and 60.29% in brown and yellow seed sprouts after 5 days. Also, the percentage of free flavonoids in sprouts increased from 5 to 13 days and represented 77.35 and 71.11% of total flavonoids at 13 days respectively (Figure 4).

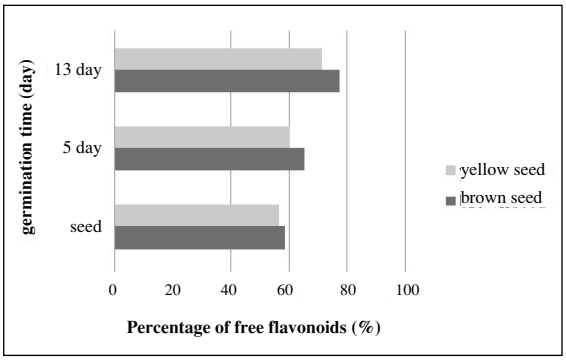

Figure 4. Change in the percentage of esterified flavonoids with germination and from 5 to 13 days germination.

CONCLUSIONS

Based on these results, it was concluded that germination and germination time had a significant (P<0.05) effect on SDG lignan, free and esterifed phenolics and flavonoids of brown and yellow flaxseeds and their sprouts. SDG lignan contents showed a considerable decrease in brown and yellow flaxseeds with germination process after 5 days whereas, total phenolic and flavonoid contents of flaxseeds rapidly increased with germination of 5 days. Especially, free and esterified flavonoid contents of seeds increased 10.2-15.9 fold after germination of 5 days. Also a noticable increase was determined in the phenolic and flavonoid contents of flaxseed sprouts during germination from 5 to 13 days. Besides, cultivar had a significant (P<0.05) effect on phenolics. Also, maximum SDG lignan contents were obtained in brown and yellow seed sprouts after germination of 13 and 11 days respectively and cultivars were significantly different (P<0.05). Selection of the right flaxseed cultivar combined with a suitable germination time could provide good source of phenolics and flavonoids from flaxseed sprouts. It is a good way to use flaxseed sprouts as a functional ingredient in food industry and also they can be used to have flavour salads and sandwiches as well as additional components of bread at home.

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