



Effect of Sprouting on Chemical, Fatty Acid Composition, Antioxidants and Antinutrients of Flaxseeds

M. A. M. Abd-El-hady^{1*} and M. E. I. Elsorady¹

¹*Department of Oils and Fats Research, Food Technology Research Institute, ARC Giza, Egypt.*

Authors' contributions

This work was carried out in collaboration between both authors. Author MAMA designed the study, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Author MEIE performed the statistical analysis, and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2020/v19i130231

Editor(s):

(1) Dr. Samaila James, Federal University of Technology, Minna, Nigeria.

Reviewers:

(1) Gehan Fawzy Abdel Raouf Kandeel, National Research Centre, Egypt.

(2) S. Miltonprabu, University of Madras, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/62319>

Original Research Article

Received 25 August 2020
Accepted 30 October 2020
Published 20 November 2020

ABSTRACT

The objective research investigated the effect of flaxseed sprouting on chemical composition, fatty acid composition, antioxidants and flaxseed antinutrients during the four-day sprouting period. For attempts to reduce flaxseed levels of some antinutritional factors, such as cyanogenic glucosides, and improve nutrient palatability and availability, the sprouting technique has been used. After 4 days of sprouting, the dry matter content of the seeds was decreased by 5.54%. Significant decreases in oil content were observed during the sprouting period, but there were increases in protein, fibre, P, Ca, Fe and Zn content. During sprouting, the content of cyanogenic glucosides as antinutrients has decreased, thus increasing the nutritional quality and the economic demand for flaxseed sprouts. Increases were also found in the value of free fatty acids, peroxide and saponification. In addition, unsaponifiable matter has been reduced. Among fatty acids, while linoleic and oleic were increased during the sprouting period, linolenic was decreased. The results revealed that in extracted oils, total phenols and antioxidant activity decreased during and at the end of sprouting, whereas flavonoids, carotenoids and chlorophylls increased.

*Corresponding author: Email: mahmoudelkholy15@gmail.com;

Keywords: Antioxidant activity; antinutrients; chemical composition; flaxseed; flaxseed oil; sprouting.

1. INTRODUCTION

Flaxseed (*Linum usitatissimum*) is one of the oldest crops, cultivated for oil and fiber. It was used for medical purposes in ancient Egypt and Greece, and as an energy source [1]. Flaxseed has nutritional and functional properties because it provides us with oil rich in omega-3, soluble dietary fibers, lignans, vitamin E, proteins and carbohydrates to satisfy basic needs of human diet and health maintenance [2]. Flaxseed has high content of α -linolenic acid, an essential fatty acid, usually greater than 50% of the fatty acid composition. However, α -linolenic acid is sensitive to oxidation and therefore, flaxseed oil is usually cold-pressed from the seed [3]. Other bioactive compounds of flaxseed are from the class of phenolic compounds, including lignans, flavonoids and phenolic acids [2], [4].

Currently, due to their estrogenic, antioxidant activity, anti-inflammatory activity, safety from some forms of tumour and cardiovascular diseases and even type 2 diabetes, flax lignans (mainly secoisolariciresinol diglucoside, SDG) are in focus [4],[5],[6],[7]. While flaxseeds are an excellent nutrient source, the use of raw flaxseeds is limited because they contain low levels of adverse healthy compounds such as cadmium, cyanogenic glycosides and trypsin inhibitors [2].

Some antinutrients, such as cyanogenic glucosides, were found in flaxseed. They are secondary plant metabolites consisting of α -hydroxynitrile aglycone and a moiety of sugar [8]. Cyanogenic glucosides are converted to hydrogen cyanide (HCN) after plant tissue damage (by chewing or technical processing) by a two-step process [8]: first, cyanogenic glucosides are decomposed into cyanohydrins (α -hydroxynitrile) and sugars by β -glycosidase; second, cyanohydrins can decompose (spontaneously or in an enzymatic reaction catalyzed by hydroxynitrile lyase) and form HCN. After acid hydrolysis, cyanide hydrogen may also be released [9]. Due to their ability to connect ions such as iron, copper or manganese, both HCN and its anion type (CN⁻) are toxic to animals and humans; ions are functional groups of enzymes, particularly those of the respiratory cytochrome chain. Cyanide exposure can lead to fatal, acute intoxication. Chronic intoxication, however, has also been observed; long-term exposure to cyanide released from a rich-in-

cyanogenic diet has been shown to be responsible for human central nervous syndrome [10],[11]. The cyanogenic glucoside level in flaxseed depends on the variety of plants, climate, season, and type of soil [12]. Official analysis in the EU (Belgium) showed a total cyanide equivalent of up to 338 mg per kilogramme of flaxseed and meal cakes [8]. It is important to improve the control of the quantity of cyanogenic glucosides in popular food / feeding materials (e.g. flaxseed by-products and cassava chips) and the manufacturing effect on their quality, according to the EFSA recommendation [8].

There is a misconception among consumers about the consumption of raw flaxseeds, considering their good nutritional profile. This is due to the existence of cyanogenic glycosides, an antinutritional compound that is a potent respiratory inhibitor that interferes with the absorption of iodine and phytic acid, interfering with the availability of minerals by forming complexes [13].

The toxic cyanogen glycosides should be eliminated during the processing of flaxseed. An extraction process has suggested the elimination of cyanogenics from flaxseeds [14],[15]. However, the extraction fails to eliminate cyanogenics completely, but extracts beneficial flaxseed meal ingredients.

Sprouting is an ancient habit embraced thousands of years ago by ancient Egyptians [16], a natural method of increasing bioavailability and decreasing cyanide poisoning of raw flaxseeds, and flaxseed sprouts are easily digestible, contain bioactive nutrients and are often referred to as miracle foods [17] [18]. Germination is a technical application that is commonly used for its ability to reduce seed levels of antinutritional factors and increase nutrient palatability and availability [19]. To date, cereals and pulses have been the most widely used sprouts. Reports on sprouts of soybean [20] and canola [21] examined the ability of crop seeds as sprouts for human nutrition. Sprout intake reduced the risk of cancer due to the presence of higher concentrations of phytochemicals that protect health.

In order to resolve the antinutrients, various researchers have embraced the sprouting technique. Therefore, attempts to sprout

flaxseeds have been made to investigate improvements in their biochemical structure. In the literature on these aspects of sprouts of Egyptian flaxseed genotypes, however, scanty and scattered information is available. The objective of this study was to investigate the effect of sprouting on chemical, fatty acid composition, antioxidants and antinutrients of flaxseeds.

2. MATERIALS AND METHODS

2.1 Preparation of Flaxseed Sprouts

Flaxseeds (*Linum usitatissimum*., Giza 12) were obtained from the Fiber Crops Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. The preliminary experimental process for flaxseed sprouting was achieved on plates with covers and the observation of the end sprouting stage and entry into the germinating stage (the presence of leaves) under the Egyptian environmental condition was on the 5th day, Fig. (1). Flaxseeds were then incubated on plates with covers for the sprouting process at room temperature ($20 \pm$

2°C) for 4 days in the dark due to the presence of leaves on the 5th day. The sprouted flaxseeds were dried at 40°C for further study, grounded and packed in tight air bags.

2.2 Proximate Analysis

For their proximate composition, the raw and sprouted flaxseeds were evaluated. The content of moisture, protein, fat, ash and fibre was determined according to AOAC [22]. Finally, the carbohydrate content was calculated by differences. All determinations were in three replicates.

2.3 Minerals Analysis

Minerals including, calcium, magnesium, iron and zinc were determined using an Atomic Absorption Spectrometry (Perkin Elmer Model 4100 ZL) according to procedure of the AOAC [22], while sodium and potassium were determined using flame photometer. On the other hand, using the colorimetric method, phosphorus was measured by the ascorbic acid technique.

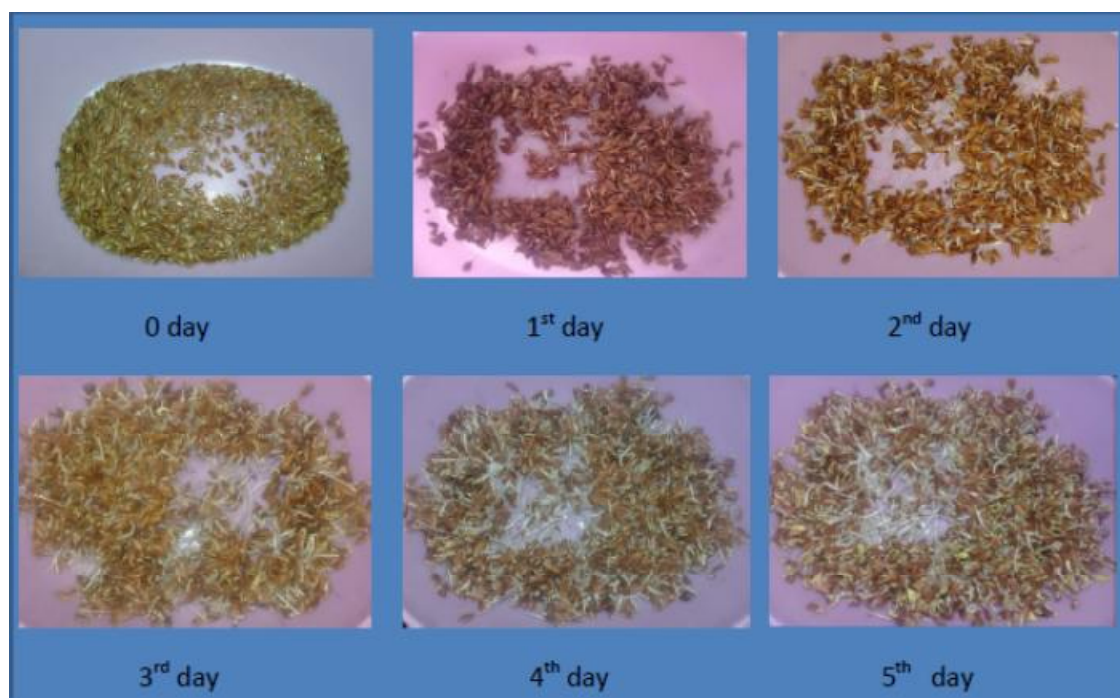


Fig. 1. Flaxseed sprouts

2.4 Cyanogenic Glycosides Content

Cyanogenic glycosides were also analyzed using AOAC method [22]. In a Kjeldahl flask, about 20 g of flaxseed meal was transferred, and then 200 ml of water was added and combined with the sample. The solution was subsequently distilled after 2 h. Distillate was collected in a flask containing 20 ml 2.5% NaOH solution (0.5 g in 20 ml H₂O), until distilled to a definite volume. 8 ml 6M NH₄OH and 2 ml 5% KI solution were added to the distillate before titration with 0.02 M AgNO₃ using a microburette. HCN was calculated as follow: HCN (mg) = ml of 0.02 M AgNO₃ × 1.08.

2.5 Physicochemical Parameters

Free fatty acid (FFA) (as oleic acid, %), peroxide value (meq. O₂ / Kg oil), refractive index (RI) (20°C), conjugated dienes, conjugated trienes, saponification values and unsaponifiable matter of extracted oil from raw and sprouted flaxseeds were carried out according to AOCS [23].

2.6 Determination of Fatty Acids Composition

Fatty acids of flaxseed oils were determined using of Gas Chromatography (Agilent 6890) (GC) apparatus. All oil samples have been measured in triplicates and the average value has been recorded according to the methods of Cossignani [24].

2.7 Pigment Content

Chlorophyll and carotenoids in flaxseed oils were determined calorimetrically as previously described by Minguez-Mosquera [25].

2.8 Phytochemicals

The extraction and determination of total phenols and flavonoid contents of flaxseed oils were

determined according to the method of Gutfinger [26].

2.9 Antioxidant Activity

Total phenols, as methanolic extracts, of flaxseed sprouts have been measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical- scavenging assay according to the method described by Gorinstein [27]. In 2.8 mL of DPPH solution (0.1 mM in methanol), an aliquot of 0.2 mL methanol sample extract was added, vigorously and then still in the dark for 30min. At 517 nm, the absorbance was immediately measured using spectrophotometer. The ability to scavenge the radical DPPH has been calculated with the following equation:

$$\text{Inhibition percentage} = (\%) = [(A_0 - A_1) / A_0] \times 100$$

Where A₀ is the absorbance of the control, A₁ is the absorbance in the presence of sample.

2.10 Statistical Analysis

Using SPSS version 16.0 of the program, statistical analyses were performed.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of Flaxseeds During Sprouting

Table (1) shows the proximate compositions of flaxseeds during sprouting. There were significant differences in moisture, oil, protein, fiber, ash and carbohydrate content in flaxseed during sprouting. The moisture content of flaxseeds was 6.31% at zero time then increased to 11.50% after 4 days of sprouting. These results agreed with Herchi [28] and Wang [29], while did not agree with Kajla [30] and Kaur [31].

Table 1. Approximate composition of flaxseeds during sprouting

Characteristics	Sprouting period (days)				
	0	1 st	2 nd	3 rd	4 th
Moisture	6.31±0.03 ^a	9.32±0.02 ^b	10.83±0.13 ^c	11.20±0.10 ^d	11.50±0.05 ^e
Oil	29.60±0.10 ^e	26.40±0.05 ^d	23.51±0.11 ^c	22.10±0.04 ^b	20.35±0.56 ^a
Proteins	20.10±0.10 ^a	20.62±0.12 ^b	20.85±0.10 ^c	20.90±0.15 ^c	20.96±0.11 ^c
Fibers	11.75±0.15 ^a	12.20±0.05 ^b	12.46±0.06 ^c	12.75±0.04 ^d	12.79±0.01 ^d
Ash	3.80±0.05 ^a	3.99±0.07 ^c	3.90±0.05 ^{bc}	3.75±0.06 ^a	3.81±0.01 ^{ab}
Carbohydrates	28.47±0.42 ^b	27.47±0.31 ^a	28.44±0.45 ^b	29.30±0.39 ^b	30.59±0.73 ^c

*a-e different superscripts indicate significant differences (p<0.05)

There was significant reduction in oil content of the sprouted flaxseeds when compared to the flaxseeds at zero time (29.60%) (Table 1), this trend is in agreement with that reported by Herchi [28], Kajla [30] and Narina [32]. The oil is the main source of energy during sprouting [33]. Results also revealed that protein content has slightly increased from 20.10% at zero time to 20.96% during sprouting. This might be due to synthesis of hydrolytic enzymes during germination [20]. Similar trends in protein content in flaxseeds were recorded [28],[30],[34].

As a result of the sprouting of flaxseed, the fibre content increased, reaching 12.97 percent (Table 1). These may be due to the degradation of the nutrients. Similar results reported by Kaur [31]. Germination could be an effective way of improving the fiber content in foods [35],[36].

The data in Table (1) indicates that the ash and carbohydrate contents were 3.80 and 28.47% at zero time, which increased and decreased during sprouting, respectively. The changes during sprouting are greatly associated with the activation of some endogenous enzymes making sprouted foods higher in nutritional quality compared to non-sprouted seeds [37].

3.2 Effect of Sprouting on Minerals Content in Flaxseeds

Table (2) shows that all macro minerals were decreased during sprouting, with the exception of phosphorus and calcium, as well as micro minerals (Fe, Zn) that were increased. These findings agreed with Narina [34]. This indicates that flax sprouts could be healthier due to availability of essential micronutrients (Fe and Zn) and can be used as potential alternative in place of ground flax seed for human consumption, reflecting a rise in demand for the economy.

Changes in the mineral content of sprouts can be associated with a change in the composition of specific amino acids to simpler amino acids, along with the production of non-protein amino acids [38] [34]. The increase in mineral content in germinated flaxseed might be due to hydrolysis of complex organic compounds by endogenous enzymes and release more nutrients leaving the antinutrients to leach into the germination medium [30]. Due to respiration during the sprouting process, this is a loss in total dry matter, an increase in total protein, a decrease in starch, an increase in sugars, and a slight increase in some vitamins and minerals [39]. In addition, phytic acid in the seed is depleted during germination due to the activity of phytase enzymes, resulting in increased trace mineral availability compared to dry seeds [40].

3.3 Effect of Sprouting on Cyanogenic Glucosides Content in Flaxseeds

Cyanogenic glucosides, as antinutrients, are nitrogenous secondary plant metabolites derived from amino acids. It induces chronic effects that occur in the nervous system and is seen in populations that consume large levels of cyanate in foods [41]. Fig. (2) shows effect of sprouting of flaxseed on cyanogenic glycosides content. The findings showed that the content of cyanogenic glycosides was decreased from 140.20 to 41.20 mg / kg after 4 days from sprouting. Reduction (%) was increased with sprouting approximately 70%. These findings agreed with Kajla [30], who suggested that cyanogenic glycosides content in raw flaxseed varieties ranged from 421.20 to 559.15 mg / kg and observed a decrease in the content of cyanogenic glycosides in germinated flaxseed varieties. Wanasundara [17] reported that cyanogenic glycosides in germinated flaxseeds decreased by about 70%.

Table 2. Effect of Sprouting on minerals content in flaxseeds

Minerals (mg/kg)	Sprouting period (days)				
	0	1st	2nd	3rd	4th
Macro minerals:					
Phosphorus (P)	3100.25	3345.10	3521.54	3862.40	4125.35
Calcium (Ca)	1210.12	1217.25	1225.42	1236.30	1245
Magnesium (Mg)	3846.47	3584.12	3112.85	2759.64	2530.114
Potassium (K)	511.24	486.52	467.58	443.23	421.36
Sodium (Na)	302.51	274.14	243.69	210.36	175.32
Micro minerals					
Iron (Fe)	50.14	59.35	65.78	72.69	80.23
Zinc (Zn)	40.51	48.61	57.10	65.42	71.54

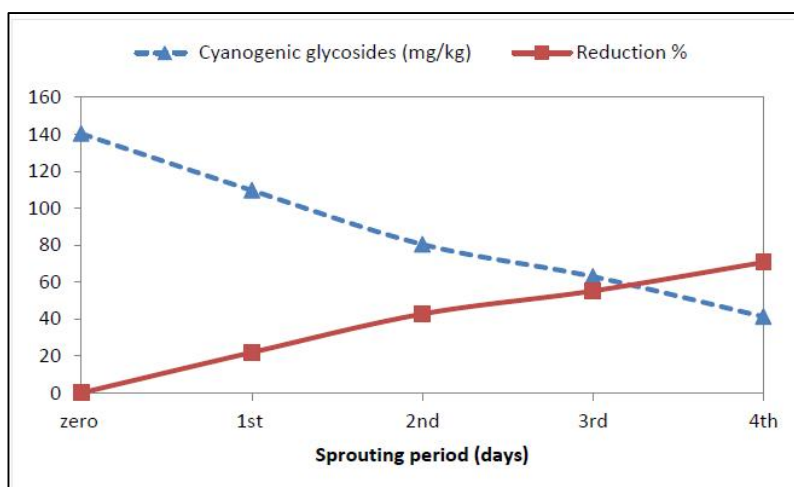


Fig. 2. Effect of Sprouting on cyanogenic glucosides content in flaxseeds

During germination, the absence of cyanogenic glycosides decreases the risk of developing HCN, thus enhancing the nutritional quality of flaxseed sprouts, reflecting an increase in economic demand.

3.4 Effect of Sprouting on Some Physical and Chemical Characteristics of Extracted Oils Form Flaxseeds

With regard to physical and chemical characteristics of extracted oils form sprouting flaxseeds, (Table 3), results revealed that there was significantly decrease in refractive index and unsaponifiable matter with Sprouting (1.4733-1.4722) and (1.08-0.89%), respectively. This may be associated with a decrease in the content of oil during sprouting. On the other side, FFA, peroxide value, CD, CT, TBA value and Saponification value increased with sprouting. FFA increased from 0.36 to 1.16% (as oleic acid). This agreed with Herchi [28] and Wanasundara [42]. The high levels of free fatty acids have been found in flax sprouts [43]. During the germination period of oilseeds, lipolytic activity is very high and, depending on the plant species, the lipase may be located in the membrane of the lipid bodies or in other cellular compartments [44]. The lipolytic activity in flaxseeds increased during germination [42],[45].

Data in Table (3) indicated that peroxide value increased from 1.90 to 2.69 (meq O₂/kg oil) after 4 days of sprouting. These values were lower than 15 meq O₂/kg oil, which is the cold pressed and virgin oils' highest limit of peroxide content Codex Alimentarius Commission Standard. CD,

CT and TBA increased during sprouting and ranged between (1.72-1.91), (0.22-0.32) and (0.52-0.78 mg malonaldehyde/kg oil), respectively. These results agreed with Herchi [28]. Conjugated diene (CD) and conjugated triene (CT) seem to be important parameters for the determination of primary product of oil oxidation [46]. TBA test measures a secondary product (malonaldehyde) of oil oxidation.

Saponification value (SV) is an indication of the molecular weights of triglycerides in oil [47]. Saponification values were significantly increased from 192.62 to 196.84 (mg KOH/g) after 4 days of sprouting (Table 3). The lower FFA, peroxide value, CD, CT and TBA values of sprouted flaxseed oil indicated that the oil has a better quality and suitable as edible oil.

3.5 Effect of Sprouting on Fatty Acid Composition of Extracted Oils Form Flaxseeds

Data in Table (4) indicated fatty acid composition of flaxseeds during sprouting for 4 days. The main unsaturated fatty acids were C18:3, C18:1 and C18:2, while the most saturated fatty acids were C18:0 and C16:0. By observing in during sprouting, C16:0 was increased from 7.80 to 7.91% on the first day of germination and then decreased and increased, even C16:1 had the same pattern. Narina [32] suggested that C16:0 had decreased in flax sprouts. There was no significance difference in the first 2 days of sprouting and there was a significance difference in the 3rd and 4th day of sprouting.

Table 3. Some physical and chemical characteristics of extracted oils form sprouting flaxseeds

Characteristics	Sprouting period (days)				
	0	1 st	2 nd	3 rd	4 th
Refractive index (at 20° C)	1.4733±0.0001 ^e	1.4729±0.0001 ^d	1.4726±0.0001 ^c	1.4724±0.0001 ^b	1.4722±0.0001 ^a
FFA % (as oleic acid)	0.36±0.02 ^a	0.56±0.04 ^b	0.78±0.05 ^c	0.96±0.04 ^d	1.16±0.05 ^e
Peroxide value (meq O ₂ /kg oil)	1.90±0.05 ^a	2.11±0.04 ^b	2.42±0.09 ^c	2.50±0.09 ^c	2.69±0.09 ^d
CD ^{232nm}	1.72±0.02 ^a	1.78±0.04 ^b	1.82±0.02 ^{b c}	1.86±0.03 ^{cd}	1.91±0.04 ^d
CT ^{268nm}	0.22±0.02 ^a	0.25±0.02 ^a	0.28±0.01 ^b	0.31±0.01 ^{b c}	0.32±0.02 ^c
TBA value (mg malonaldehyde/kg oil)	0.52±0.03 ^a	0.62±0.06 ^b	0.69±0.05 ^c	0.72±0.03 ^{cd}	0.78±0.03 ^d
Saponification value (mg KOH/g)	192.62±0.73 ^a	193.66±0.20 ^{ab}	194.62±0.78 ^{bc}	195.68±0.76 ^{cd}	196.84±0.73 ^d
Unsaponifiable matter (%)	1.08±0.02 ^d	1.01±0.02 ^c	0.96±0.02 ^b	0.93±0.02 ^b	0.89±0.03 ^a

*a-e different superscripts indicate significant differences ($p < 0.05$)

Results revealed that $C_{18:3}$ decreased from 53.59% at zero time to 48.75% after 4 days of sprouting (Table 4). Also, $C_{20:1}$ decreased during sprouting. On the other side, $C_{18:2}$, $C_{18:1}$ and $C_{20:0}$ increased during sprouting. These results agreed with Wanasundara [42], Narina [32] and Kaur [31]. Fatty acid composition of flaxseed oil extracted from flax sprouts agreed with Codex Alimentarius Commission (1999) Standard.

Data also showed that PUSFA and $C_{18:3}/C_{18:2}$ decreased during sprouting while $C_{18:1}/C_{18:2}$ increased. Narina [32] indicated that PUSFA decreased in flax sprouts. The ratio of n-3 and n-6 fatty acids is important for human health. Flaxseed oil has a very desirable omega-3: omega-6 ratio of 1: 0.3 [48]. According to the Codex Alimentarius Commission (1999) Standard, the fatty acid composition of flaxseed

oil extracted from flax sprouts were still within the limits for fatty acid composition of flaxseed oil.

3.6 Effect of Sprouting on Phytochemicals of Extracted Oils Form Flaxseeds

Phytochemicals are important plant secondary metabolic products produced during the growth of plants [37]. There was a significance difference in total flaxseed oil phenols extracted from flaxseed sprouts, i.e. total phenols reduced from 89.66 to 62.46 mg/100 g oil (Table. 5). Similar findings were found by Herchi [28]. Gujral [49] cited that the decrease in total phenol content during germination is related to enzymatic activity. Randhir [50] reported that the germination causes the total phenolic content in Green Mung to decrease.

Table 4. Fatty acid composition of flaxseed oils extracted form Sprouting flaxseeds

Fatty acids %	Sprouting period (days)					Codex standard (1999)
	0	1 st	2 nd	3 rd	4 th	
$C_{16:0}$	7.80±0.05 ^d	7.91±0.04 ^e	7.32±0.02 ^a	7.48±0.02 ^b	7.73±0.03 ^c	4.0-11.3
$C_{16:1}$	0.15±0.04 ^b	0.16±0.01 ^b	0.13±0.01 ^{ab}	0.09±0.01 ^a	0.10±0.01 ^a	ND-0.5
$C_{18:0}$	3.48±0.06 ^a	3.45±0.03 ^a	3.57±0.06 ^{ab}	3.66±0.04 ^b	4.47±0.13 ^c	2.0-8.0
$C_{18:1}$	19.13±0.16 ^a	20.85±0.05 ^b	21.27±0.05 ^c	21.34±0.03 ^c	21.84±0.04 ^d	9.8-36.0
$C_{18:2}$	15.45±0.05 ^a	16.13±0.08 ^b	16.40±0.12 ^c	16.43±0.06 ^c	16.71±0.10 ^d	8.3-30.0
$C_{18:3}$	53.59±0.17 ^e	51.07±0.06 ^d	50.85±0.14 ^c	50.57±0.06 ^b	48.75±0.13 ^a	43.8-70.0
$C_{20:0}$	0.19±0.01 ^a	0.21±0.01 ^a	0.27±0.02 ^b	0.30±0.01 ^c	0.36±0.01 ^d	ND-1.0
$C_{20:1}$	0.22±0.02 ^c	0.21±0.01 ^c	0.20±0.02 ^c	0.13±0.02 ^b	0.05±0.01 ^a	ND-1.2
Σ SFA*	11.47±0.10 ^b	11.57±0.06 ^b	11.15±0.07 ^a	11.43±0.06 ^b	12.56±0.11 ^c	
Σ USFA**	88.53±0.10 ^b	88.43±0.06 ^b	88.85±0.06 ^c	88.56±0.06 ^b	87.44±0.11 ^a	
MUSFA***	19.50±0.12 ^a	21.22±0.05 ^b	21.60±0.06 ^c	21.56±0.05 ^c	21.99±0.04 ^d	
PUSFA****	69.03±0.21 ^d	67.21±0.02 ^c	67.25±0.09 ^c	67.00±0.00 ^b	65.45±0.10 ^a	
$C_{18:1}/C_{18:2}$	1.24±0.02 ^a	1.29±0.01 ^b	1.30±0.01 ^b	1.30±0.01 ^b	1.31±0.01 ^b	
$C_{18:3}/C_{18:2}$	3.47±0.00 ^d	3.17±0.02 ^c	3.10±0.03 ^b	3.08±0.02 ^b	2.92±0.02 ^a	
USFA/ SFA	7.72±0.08 ^b	7.64±0.05 ^b	7.97±0.05 ^c	7.74±0.04 ^b	6.96±0.07 ^a	

*a-e different superscripts indicate significant differences ($p<0.05$); * SFA: Saturated Fatty Acids. ** USFA: Unsaturated Fatty Acids. *** MUSFA: Monounsaturated Fatty Acids. **** PUSFA: Polyunsaturated Fatty Acids

Table 5. Phytochemicals of flaxseed oils extracted from Sprouting flaxseeds

Phytochemicals	Sprouting period (days)				
	0	1 st	2 nd	3 rd	4 th
Total phenols (mg/100 g oil) as ferulic acid equivalents	89.66±1.07 ^e	80.11±1.36 ^d	74.44±1.10 ^c	68.31±0.74 ^b	62.46±0.56 ^a
Total flavonoids (mg/100 g oil)	11.48±0.38 ^a	13.29±0.43 ^c	14.10±0.37 ^d	12.52±0.17 ^b	12.27±0.16 ^b
Chlorophyll (mg/kg oil)	4.75±0.11 ^a	5.89±0.07 ^b	6.04±0.07 ^b	6.63±0.12 ^c	7.12±0.07 ^d
Carotenoids (mg/kg oil)	2.34±0.11 ^a	4.19±0.07 ^b	5.36±0.12 ^c	6.12±0.07 ^d	6.46±0.12 ^e
Antioxidant activity (%)	40.28±0.60 ^e	38.78±0.28 ^d	35.39±0.41 ^c	32.38±0.17 ^b	29.32±0.46 ^a

*a-e different superscripts indicate significant differences ($p<0.05$)

Due to L-phenylalanine changes to cinnamic acid during growth under the effect of phenylalanine ammonia lyase (PAL) catalyzation [51], the total flavonoid content increased on the first and second days and then dropped on the third and fourth days. From then on, many phenolic components such as caffeic acid, ferulic and others are synthesized. These can later be converted into tannins, flavonoids and other compounds. These phytochemicals have antioxidant properties.

Both chlorophylls and carotenoids are considered to have an important role in keeping the quality of edible oils, mainly due to their action as photo-sensitizers or singlet oxygen quenchers, respectively [52]. The contents of chlorophylls and carotenoids in flaxseed oils extracted from sprouting flaxseeds were increased from 4.75 and 2.34 to 7.12 and 6.46 (mg/kg oil), respectively after 4 days of sprouting (Table 5). Carotenoids content in flaxseed oils varied from 2 to 11.5 (mg/kg oil) [53].

Antioxidant activity with higher values indicating greater antioxidant activity was expressed as a percentage of DPPH radical scavenging activity. The antioxidant activity decreased from 40.28% of the zero time to 29.32% of the fourth day (Table. 5) during sprouting at an approximately constant rate. The percentage of antioxidant activity during and at the end of sprouting shows steadily decreased values due to the decrease in total phenol levels, this decrease in antioxidant activity is not equal to a substantial decrease in total phenol levels due to an increase in the levels of flavonoids and carotenoids (Table. 5), which serves as a control in the dropdown in antioxidant activity. It has been found that the scavenging activity of plant constituents relates to polyphenolic compounds [54]. Polyphenols have a positive correlation with antioxidant activity, more the quantity of polyphenols extracted, more the activity of antioxidants [55].

4. CONCLUSION

During sprouting, the chemical composition of flaxseeds was changed; oil content was decreased while protein and fibers were increased. Flaxseed sprouts could be healthier due to availability of essential micronutrients (Fe and Zn) and reduction in cyanogenic glucosides. The absence of cyanogenic glycosides during germination, from a nutritional and economic point of view, reduces the risk of developing HCN and thus improves the nutritional quality of

flaxseed sprouts, reflecting the rise in economic demand. It is remarkable that flaxseed oils extracted from sprouts contained significantly higher FFA, PV. The predominant fatty acids of flaxseed oil extracted from flaxseed sprouts were C_{18:3}, C_{18:2} and C_{18:1} and also were within the limits fixed by CODEX. Moreover, during flaxseed sprouting, the phytochemical content was slightly decreased.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rubilar M, Gutiérrez C, Verdugo M, Sineiro J. Flaxseed as a source of functional ingredients. *J. Soil Sci. Plant Nutr.* 2010;10 (3):373–377.
2. Bernacchia R, Preti R, Vinci G. Chemical composition and health benefits of flaxseed. *Austin J Nutri Food Sci.* 2014;2 (8):1045.
3. Choo WS, Birch EJ, Dufour JP. Physicochemical and stability characteristics of flaxseed oils during pan-heating. *J Amer Oil Chem Soc.* 2007;84:735–740. DOI: 10.1007/s11746-007-1096-7
4. Ganorkar PM, Jain RK. Flaxseed—a nutritional punch. *Int Food Res J.* 2013; 20:519–525.
5. Toure A, Xueming X. Flaxseed lignans: Source, biosynthesis, metabolism, antioxidant activity, bioactive components and health benefits. *Compr Rev Food Sci Food Saf.* 2010;9:261–269.
6. Katare C, Saxena S, Agrawal S, Prasad GBKS, Bisen PS. Flaxseed: A potential medicine food. *J Nutr Food Sci.* 2012;2:120–127.
7. Kajla P, Sharma A, Sood DR. Flaxseed—a potential functional food source. *J Food Sci Technol;* 2014. DOI: 10.1007/s13197-014-1293-y
8. EFSA (European Food Safety Authority). Opinion of the scientific panel on contaminations in the food chain on a request from the commission related to cyanogenic compounds as undesirable substances in animal feed. *EFSA J.* 2007; 434:1–67
9. Kobaisy M, Oomah BD, Mazza G. Determination of cyanogenic glucosides in flax by barbituric acid-pyridine, pyridine-

- pyrazolone, and high-performance liquid chromatography method. *J Agric Food Chem.* 1996;44:3178–3181
10. WHO (World Health Organization) Toxicological evaluation of certain food additives and natural occurring toxicants. In: Report of the 39th meeting of the Joint FAO/WHO Experts Committee on Food Additives (JECFA) Food Additives Series 30, WHO, Geneva. 1993;299–337
 11. Simeonova FP, Fishbein L. Hydrogen cyanide and cyanides: Human health aspects. Concise international chemical assessment document 61. WHO, Geneva; 2004.
 12. Oomah BD, Mazza G, Kenaschuk EO. Cyanogenic compounds in flax. *J Agric Food Chem.* 1992;40:1346–1348.
 13. Deng GF, Lin X, Xu XR, Gao LL, Xie JF, Li HB. Antioxidant capacities and total phenolic contents of 56 vegetables *Functional Foods.* 2013;5:260-266. Available:<https://doi.org/10.1016/j.jff.2012.10.015>
 14. Wanasundara PKJPD, Amarowicz R, Kara MT, Shahidi F. Removal of cyanogenic glycosides of flaxseed meal. *Food Chemistry.* 1993;48:263–266.
 15. Varga TK, Diosady LL. Simultaneous extraction of oil and antinutritional compounds from flaxseed. *Journal of American Oil and Chemical Sciences.* 1994;71:603–607.
 16. Abdallah MMF. Seed sprouts, a pharaohs heritage to improve food quality. *Arab University Journal of Agriculture Sciece.* 2008;16:469-478.
 17. Wanasundara PKJPD, Shahidi F, Brosnan ME. Changes in flax (*Linum usitatissimum*) seed nitrogenous compounds during germination. *Food Chem.* 1999a;65:289–295.
 18. Meyerowitz S. Sprouts, the miracle food: the complete guide to sprouting. Sproutman Publication, Great Barrington, Mass. 01230, USA, 8th edition; 2010.
 19. Vidal-Valverde FJ, Sierra I. New functional legume foods by germination: Effect on the nutritive value of beans, lentils and peas. *Eur J Food Res Technol.* 2002;215:472–477.
 20. Bau HM, Villaume C, Nicolas JP, Mejean L. Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (*Glycine max*) seeds. *J Food Sci Agric.* 1997;73:1-9.
 21. Bhardwaj HL, Hamama AA. Effect of cultivar and growing location on the mineral composition of canola sprouts. *Hort Sci.* 2009;44:508-511.
 22. AOAC. Official methods of analysis (19th ed). Association of official analytical chemists AOAC. Benjamin Franklin Station Washington, DC., USA; 2005.
 23. AOCS. Official and tentative methods of the American oil chemists society (5th ed.). American Oil Chemists Society, 35 East Wacker Drive, Chicago, Illinois, USA; 1998.
 24. Cossignani L, Simonetti MS, Damiani P. Biocatalyzed acidolysis of olive oil triacylglycerols with 9c, 11t and 10t, 12c isomers of conjugated linoleic acid *Eur. Food Res. Technol.* 2005;220:267-271.
 25. Minguez-Mosquera MI, Rejano L, Gandul B, Sanchez AH, Garrido J. Color-pigment correlation in virgin olive oil. *J Am Oil Chem Soc.* 1991;68:332–336.
 26. Gutfinger T. Polyphenols in olive oils. *J. Am. Oil Chem. Soc.* 1981;58:966– 968. Available:<http://dx.doi.org/10.1007/BF02659771>
 27. Gorinstein S, Cvikrova M, Machackova I, Haruenkit R, Park YS, Jung ST. Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. *Food Chem.* 2004;84:503–510. Available:[http://dx.doi.org/10.1016/S0308-8146\(03\)00127-4](http://dx.doi.org/10.1016/S0308-8146(03)00127-4)
 28. Herchi W, Bahashwan S, Sebei K, Ben Saleh H, Kallel H, Boukhchina S. Effects of germination on chemical composition and antioxidant activity of flaxseed (*Linum usitatissimum* L) oil. *Grasas Aceites.* 2015; 66(1):e057. Available:<http://dx.doi.org/10.3989/gya.0463141>.
 29. Wang H, Qiu C, Abbasi A, Gu Chen G, You L, Li T, Fu X, Wang Y, Guo X, Liu R. Effect of germination on vitamin C, phenolic compounds and antioxidant activity in flaxseed (*Linum usitatissimum* L.). *International Journal of Food Science and Technology.* 2015;50:2545–2553.
 30. Kajla P, Sharma A, Sood DR. Effect of germination on proximate principles, minerals and antinutrients of flaxseeds. *Asian J. Dairy & Food Res.* 2017;36(1):52-57
 31. Kaur A, Kaur R, Bhise S. Baking and sensory quality of germinated and ungerminated flaxseed muffins prepared from wheat flour and wheat atta. *Journal of*

- the Saudi Society of Agricultural Sciences. 2020;19:109–120.
32. Narina SS, Hamama HA, Bhardwaj HL. Fatty Acid Composition of Flax Sprouts. *Journal of Agricultural Science*. 2013;5(4): 75-79.
DOI:10.5539/jas.v5n4p75
 33. Graham IA. Seed storage oil mobilization. *Annu Rev Plant Biol*. 2008;59:15–142. Available:<http://dx.doi.org/10.1146/annurev.arplant.59.032607.092938>.
 34. Narina SS, Hamama HA, Bhardwaj HL. Nutritional and mineral composition of flax sprouts. *Journal of Agricultural Sciences*. 2012;4(11):2012.
 35. Chinma CE, Adewuyi O, Abu JO. Effect of germination on the chemical, functional and pasting properties of flour from brown and yellow varieties of tiger nut (*Cyperus esculentus*). *Food Research International*. 2009;42:1004–1009. Available:<https://doi.org/10.1016/j.foodres.2009.04.024>
 36. Rumiya, James AP, Jayasena V. Effect of germination on the nutritional and protein profile of Australian Sweet Lupin (*Lupinus angustifolius* L.). *Food and Nutrition Sciences*. 2012;3:621–626.
 37. Zhang G, Xu Z, Gao Y, Huang X, Yang T. Effects of germination on the nutritional properties, phenolic profiles, and antioxidant activities of buckwheat. *Journal of Food Science*. 2015;80. H1111–H1119. Available:<https://doi.org/10.1111/1750-3841.12830>
 38. Finney PL. Effect of germination on cereal and legume nutrient changes and food or feed value: A comprehensive review. *Recent Advances in Phytochemistry Volume 17 Mobilization of Reserves in Germination*. Plenum press, New York and London. 1982;228-307.
 39. Lorenz K. Cereal sprouts: Composition, nutritive value, food applications *Critical Reviews in Food Science and Nutrition*. 1980;13(4):353-385.
 40. Van Hofsten B. Legume sprouts as a source of protein and other nutrients. *J. Am. Oil Chemists' Soc*. 1979;56:382.
 41. Tarpila A, Wennberg T, Tarpila S. Flaxseed as a functional food. *Curr Top Nutraceut R*. 2005;3:167-188.
 42. Wanasundara PKJPD, Wanasundara UN, Shahidi F. Changes in flax (*Linum usitatissimum* L.) seed lipids during germination. *JAOCS*. 1999b;76(1):41–48.
 43. Mattioli S, Dal Bosco, A., Martino M, Ruggeri S, Marconi O, Sileoni V, Falcinelli B, Castellini C, Benincasa P. Alfalfa and flax sprouts supplementation enriches the content of bioactive compounds and lowers the cholesterol in hen egg. *Journal of Functional Foods*. 2016;22:454–462
 44. Borgston B, Brockman HL. *Lipases*. Amsterdam, Elsevier; 1984.
 45. Younis ME, Hasaneen MNA, Nemet-Alla MM. Plant growth metabolism and adaptation in relation to stress conditions iv. Effects of Salinity on Certain Factors Associated with the Germination of Three Different Seeds High in Fats, *Annals Botany*. 1987;60:337–344.
 46. Yoon SH, Kim SK, Shin MG, Kim KH. Comparative study of physical methods for lipid oxidation measurement in oils. *J. Am. Oil Chem. Soc*. 1985;62(10):1487- 1489.
 47. Muhammad N, Bamishaiye E, Bamishaiye O, Usman L, Salawu M, Nafiu M, Oloyede O. Physicochemical properties and fatty acid composition of cyperus esculentus (tiger nut) tuber oil. *Bioresour. Bull*. 2011; 5:51-54.
 48. Bhardwaj H, Hamama A, Narina S, Parry J. Effect of consumption of ground wholeseed flax on human blood traits. *Journal of Agricultural Science*. 2012;4(8): 106-111.
 49. Gujral HS, Angurala M, Sharma P, Singh J. Phenolic content and antioxidant activity of germinated and cooked pulses. *Int. J. Food. Prop*. 2011;14:1366–1374.
 50. Randhir R, Lin Y, Shetty K. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochem*. 2004;39:637–646. Available:[http:// dx.doi.org/10.1016/S0032-9592\(03\)00197-3](http://dx.doi.org/10.1016/S0032-9592(03)00197-3)
 51. Nkhata SG, Ayua E, Kamau EH, Shingiro J-B. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Sci Nutr*. 2018;6:2446–2458. Available:<https://doi.org/10.1002/fsn3.846>
 52. Tuberoso IGC, Kowalczyk A, Sarritzu E, Cabras P. Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. *Food. Chem*. 2007;103:1494–1501.

53. Daun JK, Barthet VJ, Chornick TL, Duguid S. Structure, composition, and variety development of flaxseed. *Flaxseed in Human Nutrition* (2ndEdn), AOCS Press, USA. 2003;1-40.
54. Siger A, Nogala-Kalucka M, Lampart-Szczapa E. The content and antioxidant activity of phenolic compounds in cold-pressed plant oils. *Journal of Food Lipids*. 2008;15(2008):137–149.
55. Anwar F, Przybylski R. Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). *Acta Sci. Pol. Technol. Aliment*. 2012;11:293-301.

© 2020 Abd-El-hady and Elsorady; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/62319>