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Short communication. Variability of fatty acid and mineral content in linseed (*Linum usitatissimum)* lines from a range of European sources

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Abstract

Linseed (*Linnum usitatissimum)* has recently gained popularity as a health food product. It has high levels of fatty acids and minerals, giving it characteristics beneficial for functional foods. This research is a comparative analysis of the fatty acid and mineral content of 23 European linseed lines. The levels of seven fatty acids were analysed using an Agilent 6890 N GC. Alfa-linolenic acid (ALA) (C18:3, n-3) was the most predominant, ranging from 49.4 to 56.4%, followed by oleic (C18:1, n-9; 19.8 to 28.8%), linoleic (C18:2, n-6; 10.8 to 16.0%), palmitic (C16:0, 4.1 to 6.2%) and stearic (C18:0, 3.3 to 7.1%) acids. In contrast arachidonic (C20:0) and gadoleic (C20:1) acids were only found at trace levels. One-way ANOVA test showed significant differences between the lines in terms of saturated and unsaturated fatty acid content ($p < 0.05$). A negative correlation ($r = –0.74$) exists between levels of ALA and oleic acid. The levels of ten minerals (Ca, Mg, Na, K, P, Cu, Fe, Mn, Zn and B) were also determined and showed significant variability between lines. The results can be used to assist variety selection in targeted breeding programs.

Additional key words: fatty acid composition; functional foods; mineral content; omega 3 acids.

Resumen

Comunicación corta. Variabilidad en el contenido de ácidos grasos y minerales en diferentes líneas de origen europeo de lino (*Linum usitatissimum*)

La semilla de lino (*Linnum usitatissimum)* o linaza ha ganado recientemente popularidad como producto alimenticio, ya que sus altos niveles de ácidos grasos y minerales le proporcionan las características beneficiosas de los alimentos funcionales. Esta investigación es un análisis comparativo del contenido de ácidos grasos y minerales de 23 líneas europeas de linaza. Se analizaron los niveles de siete ácidos grasos utilizando Agilent 6890 N GC. El ácido alfa-linolénico (ALA, C18:3, n-3) fue el más predominante (49,4-56,4%), seguido del oleico (C18:1, n-9; 19,8-28,8%), linoleico (C18:2, n-6; 10,8-16,0%), palmítico (C16:0; 4,1-6,2%) y esteárico (C18:0; 3,3-7,1%). Solamente se encontraron trazas de los ácidos araquidónico (C20:0) y gadoleico (C20:1). La prueba de ANOVA con un solo factor, para el contenido de ácidos grasos saturados o insaturados, mostró diferencias significativas entre líneas ($p < 0,05$). Existe una correlación negativa ($r = –0,74$) entre los niveles de ALA y ácido oleico. En las diferentes líneas se determinó también una variabilidad significativa entre los niveles de diez minerales (Ca, Mg, Na, K, P, Cu, Fe, Mn, Zn y B). Estos resultados pueden ser utilizados para asistir a la selección de variedades en programas de mejora.

Palabras clave adicionales: ácidos omega 3; alimentos funcionales; composición ácidos grasos, contenido en minerales.

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Abbreviations used: ALA (α-linolenic acid), DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), ω-3 (omega-3).

Linseed, *Linnum usitatissimum,* has been cultivated for eight to ten thousand years and has long been valued for its medicinal properties, its oil (used in cooking and cosmetics) and its fibre used for producing linen, rope, sail cloth and paper making (Sahi and Leitch, 1994). More recently linseed has been valued for its pleasant, nutty taste, unique nutrient profile, and health benefits. Its oil content, although affected by cultivation conditions, is generally found at levels of 38 to 47%. In addition to high oil content, its fatty acid composition increases its importance as a source of essential nutrients. The composition of linseed oil is made up of the saturated palmitic and stearic acids, the monounsaturated oleic acid, the doubly unsaturated linoleic acid and the triply unsaturated α-linolenic acid (ALA) as well as other minor acids. Unique among plants is linseeds high levels of ALA, a member of the essential omega-3 (ω-3) fatty acids, and the lignan. Linseed also provides protein and dietary fibre. These qualities mean that it is often used as a supplement in functional food products (Nuernberg *et al*., 2005; Valencia *et al*., 2006).

Among the most important of ω-3 fatty acids are: eicosapentaenoic acid (EPA), docosahexaenoic acid, (DHA) and ALA. EPA and DHA are mostly found in fish oil, whereas ALA is obtained from plant oils, the highest levels being found in linseed. DHA can also be synthesised in small amounts, from ALA, by animals. Omega-3 acids have been shown to be effective in reducing the risk of chronic diseases such as coronary heart disease, kidney disease, diabetes mellitus, high blood pressure, rheumatism and certain types of cancer (breast, prostate and colonic). They have also been proven to act in strengthening the immune system (Jelinska *et al*., 2003; Morris and Vaisey-Genser, 2003). It has been shown that regular inclusion of foods containing linseed in the diet may improve plasma lipids in subjects with hypercholesterolemia (Ridges *et al*., 2001) and decrease cholesterol levels as well as suppressing cancer, thrombosis and allergic reactions (Chan *et al*., 1991; Hirano *et al.,* 1991; Pokorny *et al.,* 2001). The ω-6 acids, of which linoleic and arachidonic acid are members, although essential for the human diet have been shown to be harmful in excess and where the modern Western diets typically have ratios of ω-6 to ω-3 in excess of 10 to 1, the optimal ratio is thought to be 4 to 1 or lower (Simopoulos, 2002). Linseed oil therefore offers this lower ratio and is increasingly being used as a food supplement in different food formulations such as baked products, flakes, yoghurt and bread. A review of current research shows that the mineral content of linseed has not been greatly considered with respect to human nutrition. Although mineral elements are found in low concentrations in most foods, they are an essential part of nutrition and have important functional roles in the human body (Fennema, 1996). The levels of these beneficial compounds and other constituents of linseed, such as biomass and lignin content, have been shown to be more dependent on genetic disposition than growing conditions (Wakjira *et al*., 2004; Zimmermann *et al*., 2006). In this research, two important constituents of linseed, fatty acids and minerals, were analysed in order to compare the makeup of the different lines. The results can be used to facilitate the enhancement of desired characteristics through targeted crop breeding programmes.

Twenty-three linseed samples were obtained from four countries (Bulgaria, Germany, Hungary and the Czech Republic) and planted at the University of Ankara, Faculty of Agriculture, Field Crops Department (39°57' N, 32°52' E, 860 m asl) in 2006. The soil conditions were clay-loam with good drainage, mildly alkali, limey with low levels of salt, medium levels of phosphorous, rich in potassium and poor in organic matter (water-holding capacity of 54%; pH 7.54; total NaCl 0.062%; $CaCO_3$ 8.51%; total N 0.17%; organic matter 1.13%). The soil was fortified with P_2O_5 (5.5 kg da^{-1}) and K_2O (250 kg da^{-1}). Seeds were sown in April in single rows 2 m long using 2 g of seed, with 30 cm between rows. Each sample was sown in triplicate and seeds harvested from these crops on reaching maturity. The sample origins, line numbers and names of the seed materials are as follows: Czech Republic (Norman); Hungary (Beladi, Nynke, Pinacle); Germany (L.C.S.D., Leuwarden, Polen I, Rembrandt, Cascade Amer., Cascade D.H., Verum, Lila, Izolda, Vnii19, Bewing, Lin 1771/91, Lin 1772/90, Lin 1780/91, Lin 1793/92, Lin 1794/92, Lin 1835/93, Lin 1839/93); Bulgaria (Fasad).

In order to determine mineral content approximately 0.1 g of ground sample was put into a burning cup and 4 mL pure HNO_3 was added. The samples were incinerated in a speedwave MWS-2 microwave oven (Berghof, Germany) with three different treatments: 100°C, 10 min, 70% power; 160°C, 12 min, 70% power; 100°C, 10 min, 40% power. The ash obtained was dissolved in water and further diluted to a standard volume with water. Concentrations were determined with an ICP-OES (Perkin Elmer Optima 2100 DV). The working conditions of the ICP-OES were set as follows - RF

Power: 1.3 kW (axial); plasma gas flow rate (Ar): 15 L min^{-1}; pomp flow rate (Ar): 1.5 L min^{-1}; auxiliary gas flow rate (Ar): 0.2 L min^{-1}; copy time: 3 s; delay time: 35 s; replicates: 2 (Boss and Fredeen, 2004). To extract oil the seeds (approximately 1 g) were ground and added to 75 mL of hexane. The mixture was processed using FOSS Soxtec 2055 apparatus. The extraction included boiling, rinsing, recovery and drying steps. Extraction was carried out twice to ensure complete oil retrieval. The extraction times were: boiling: 15 min; rinsing: 30 min; recovery: 10 min; drying time: 5 min. After determination of the dry matter oil yield, fatty acids were esterified as methyl esters and analysed by Agilent 6890 N GC equipped with a DB-23 capillary column (60 m × 0.25 μm) and flame ionization detector. The carrier gas was helium, at a flow rate of 1.2 mL min^{-1}. Injector and detector temperatures were both 250°C. Column temperature was initially maintained at 165°C for 15 min and then increased at a rate of 5°C min^{-1} to 200°C, where it was also maintained for 15 min. Samples of 1 μL were injected by auto sampler and in the split mode (1:50). The fatty acid identification was performed by retention time comparisons with the corresponding fatty acid methyl ester standards all purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). The content (percentage by weight) of fatty acids was calculated from their corresponding integration data.

The relative levels of individual fatty acids found in lines are given in Table 1. The distribution of saturated to unsaturated fatty acids ranged from 8.7: 91.3% (Norman) to 13.0: 87.0% (Lin 1794/92). The results of one-way ANOVA test show significant differences between linseed lines in terms of the total saturated and unsaturated fatty acid levels ($p < 0.05$). These results are in agreement with that of other researchers who found an average distribution of 9.3: 90.7% amongst 9 cultivars (Lukaszewicz *et al*., 2004). Amongst the saturated fatty acids palmitic and stearic acids are found at higher levels with palmitic ranging from 4.1% (Cascade) to 6.2% (Lin 1793/92) and stearic ranging from 3.3% (Norman) to 7.1% (Lin 1772/90). Arachidonic acid is only found

Table 1. Composition of fatty acids (%) in European linseed lines

Line	Saturated acids				Unsaturated fatty acids				
	Palmitic C16:0	Stearic C18:0	Arachidonic C20:0	Total	Oleic C18:1	Linoleic C18:2	ALA C18:3	Gadoleic C20:1	Total
Norman	5.23	3.34	0.13	8.70	24.66	14.71	51.74	0.20	91.30
Beladi	5.83	4.21	0.15	10.19	23.14	14.11	52.41	0.15	89.81
Nynke	5.11	3.94	0.17	9.22	23.42	13.44	53.69	0.23	90.78
Pinacle	5.62	4.20	0.14	9.95	21.22	14.42	54.27	0.14	90.05
LCSD	5.41	4.37	0.17	9.95	20.66	13.42	55.80	0.16	90.05
Leuwarden	4.29	5.56	0.23	10.08	25.69	13.06	50.97	0.21	89.92
Polen	5.36	4.79	0.16	10.31	20.49	14.14	54.90	0.16	89.69
Rembrandt	4.72	5.33	0.19	10.23	20.66	14.78	54.15	0.18	89.77
Cascade Amer	4.17	4.69	0.17	9.03	22.50	14.11	54.17	0.20	90.97
Cascade DH	4.07	4.78	0.15	9.00	22.32	14.19	54.30	0.19	91.00
Verum	4.61	4.86	0.20	9.67	23.84	12.92	53.38	0.19	90.33
Lila	5.72	5.07	0.19	10.99	21.34	16.00	51.56	0.12	89.01
Izolda	4.11	5.99	0.19	10.29	20.85	14.11	54.60	0.16	89.71
Vnii19	4.25	4.47	0.18	8.91	28.79	10.84	51.22	0.24	91.09
Bewing	5.80	4.39	0.17	10.36	24.28	13.68	51.55	0.14	89.64
Lin 1771/91	5.51	4.87	0.17	10.55	20.02	14.14	55.16	0.13	89.45
Lin 1772/90	5.58	7.10	0.25	12.93	24.39	12.53	49.99	0.15	87.07
Lin 1780/91	5.58	4.23	0.15	9.97	21.53	11.94	56.42	0.15	90.03
Lin 1793/92	6.23	5.06	0.18	11.47	22.92	12.53	52.96	0.13	88.53
Lin 1794/92	5.72	7.01	0.25	12.99	20.74	11.32	54.84	0.11	87.02
Lin 1835/93	5.44	5.33	0.18	10.95	19.83	15.00	54.09	0.13	89.05
Lin 1839/93	5.94	5.83	0.19	11.96	24.83	13.74	49.35	0.13	88.04
Fasad	5.70	4.58	0.18	10.47	22.29	12.93	54.18	0.14	89.53
SD[1]	0.66	0.89	0.03		2.14	1.19	1.85	0.04	

[1] SD: standard deviation.

at trace levels ranging from 0.1 to 0.3%. These levels are within the range of other reported levels of palmitic acid at 5.3% and stearic acid at 3.3% (Van Ruth *et al*., 2001). These acids are the precursors of linolenic acid and their levels correlate negatively with it. The rate of accumulation of linolenic acid is dependent on cultivar and growing conditions (Wakjira *et al*., 2004). Amongst the unsaturated fatty acids, ALA was found at highest levels ranging from 49.4 (Lin 1839/93) to 56.4% (Lin 1780/91). The next highest content was of oleic acid which ranged from 19.8 (Lin 1835/93) to 28.8% (Vnii19). Linoleic acid, was found at levels ranging from 10.8 (Vnii19) to 16.0% (Lila). Finally gadoleic acid was found at trace levels ranging from 0.1 to 0.2%.

Reported levels of linoleic are generally in agreement with those found in this research whereas oleic acid levels are generally lower and linolenic acid levels higher. Lukaszewicz *et al.* (2004) found a much greater range between cultivars. In research on 11 linseed varieties, Hettiarachy *et al.* (1990) reported values which are the closest to levels found in the lines investigated in this research. A comparison between Ethiopian varieties of linseed and introduced Canadian cultivars revealed that the exotic varieties have higher oil yield and lower ALA content, a quality desirable for cooking oil (Wakjira *et al*., 2004). The lines in this research show slightly lower levels of linolenic acid, suggesting that they too can be useful for development as sources of cooking oil. Linoleic acid, a member of the ω-6 acids, is less susceptible to oxidation than the essential ω-3 acids and therefore oil with a high content has a longer shelf life although it does not have the same nutritional value.

Analysis of the correlation between different fatty acid levels (Table 2) shows a strong negative correlation between the levels of ALA ($r = -0.74$) and linoleic ($r = -0.46$) and their precursor oleic acid, as well as between palmitic and gadoleic ($r = -0.75$) acids. There are strong positive correlations between the levels of stearic and arachidonic ($r = 0.88$) and oleic and gadoleic ($r = 0.58$) acids. Similar comparisons made by other researchers also showed high negative correlations between ALA and its precursor oleic suggesting that the rate of conversion of the precursor affects the levels of ALA found in the oil (Lukaszewicz *et al*., 2004).

Mineral composition of lines is shown in Table 3. Of the major minerals the highest average levels were found for K followed by P, Mg, Ca and Na respectively. Amongst the minor elements highest levels were shown by Fe followed by Mn, Zn, B and Cu. The range and standard deviations for each mineral show P to have the greatest values followed by K with the remaining minerals falling into the same order as they did for average values. A one-way ANOVA test results showed that, apart from Cu, there are statistically significant differences between lines ($p < 0.05$). Therefore there is within these varieties of linseed sufficient differentiation to allow the exploitation of a range of desired qualities.

In conclusion, the results show that there is considerable variability in fatty acid and mineral contents between linseed lines in agreement with other research. As all replicates were grown simultaneously under the same conditions in the same soil it is more likely that these differences are due to the cultivars genetic make-up, rather than conditions of cultivation, and therefore will be best exploited through targeted breeding programs. Linseed oil can be used for a variety of purposes and therefore the objective of the crop breeding program will determine which varieties are selected. For instance, linseed oil from varieties that show promisingly high levels of the desired ALA, such as Lin 1780/92, are also more susceptible to oxidation. Therefore combining these varieties with those that have higher levels of linoleum acid, such as Lila, to provide an optimal balance between the two acids can be attempted to produce a linseed oil with a higher shelf life that is more suitable as a food supplement. In this research lines richer in key minerals have been identified and it would be interesting to ascertain whether

Table 2. Correlation coefficients (r) between fatty acid contents of European linseed lines

Palmitic	0.01	–0.03	–0.23	0.01	–0.09	–0.75
	Stearic	0.88	–0.11	–0.24	–0.21	–0.39
		Arachidonic	0.13	–0.40	–0.32	–0.15
			Oleic	–0.46	–0.74	0.58
				Linoleic	0.01	–0.18
					ALA	–0.12
						Gadoleic

Table 3. Mineral composition (mg kg^{-1}) of European linseed lines

Line	Ca	Mg	Na	K	P	Cu	Fe	Mn	Zn	B
Norman	1,710.69	3,243.08	643.40	9,398.81	6,375.56	9.38	66.16	30.63	29.65	20.53
Beladi	1,695.19	3,081.14	741.99	9,294.27	6,404.24	8.47	40.22	27.97	35.50	20.59
Nynke	1,349.91	3,112.62	821.45	7,820.35	5,786.55	11.89	37.80	21.84	28.33	23.22
Pinacle	1,829.70	3,465.34	835.14	8,560.62	7,137.10	11.20	52.02	29.78	37.22	18.51
LCSD	1,660.43	3,543.68	810.17	7,827.14	5,913.10	9.82	67.71	26.45	19.78	24.92
Leuwarden	2,231.54	4,192.42	932.99	9,233.04	8,548.54	14.18	64.88	51.96	39.65	25.16
Polen	1,696.62	3,736.79	853.01	8,682.64	7,510.92	13.93	68.90	41.64	33.09	18.91
Rembrandt	2,058.17	4,073.56	843.58	9,608.35	8,297.14	14.77	98.80	46.81	42.13	22.34
Cascade Amer	2,159.05	3,643.27	621.30	9,351.80	7,664.81	14.11	40.77	28.82	40.73	21.10
Cascade DH	2,472.34	3,665.40	765.15	9,062.64	7,248.63	16.18	65.54	36.34	44.88	24.07
Verum	1,613.29	3,767.93	692.33	8,626.36	7,295.19	12.69	137.36	31.78	38.38	19.41
Lila	1,940.27	3,167.01	308.01	7,913.45	5,699.24	18.05	41.97	34.00	41.15	29.32
Izolda	1,784.86	3,826.95	603.26	9,200.41	8,317.59	12.90	64.19	31.45	38.98	19.69
Vnii19	1,760.76	3,226.83	790.48	9,508.44	6,903.28	21.66	46.66	29.16	50.35	22.46
Bewing	1,573.24	3,259.60	743.47	8,805.62	6,226.42	9.45	39.47	28.42	38.54	20.30
Lin 1771/91	2,099.89	3,048.49	768.25	7,757.24	4,948.93	6.96	259.05	25.24	18.32	23.89
Lin 1772/90	1,909.34	3,367.34	593.50	8,492.45	7,010.97	10.90	34.51	25.39	30.59	18.46
Lin 1780/91	1,876.18	3,284.07	612.40	9,054.69	6,540.12	11.89	157.40	35.96	30.49	15.48
Lin 1793/92	1,817.73	3,463.13	649.51	8,678.54	6,310.64	8.46	82.17	18.34	25.29	0
Lin 1794/92	2,248.01	3,547.76	415.42	10,068.65	6,189.46	11.02	147.35	25.72	29.08	24.00
Lin 1835/93	1,858.56	3,475.57	558.12	7,480.33	5,993.03	11.94	56.20	25.87	26.70	16.31
Lin 1839/93	1,571.65	3,432.75	551.30	8,611.98	6,374.25	6.92	28.19	19.54	29.61	18.57
Fasad	1,814.92	3,425.98	697.61	8,661.04	6,629.02	9.28	39.89	22.05	38.46	20.47
Min	1,349.91	3,048.49	308.01	7,480.33	4,948.93	6.92	28.19	18.34	18.32	0
Max	2,472.34	4,192.42	932.99	10,068.65	8,548.54	21.66	259.05	51.96	50.35	29.32
Range	1,122.43	1,143.93	624.98	2,588.32	3,599.61	14.74	230.86	33.61	32.04	29.32
Average	1,857.93	3,480.47	689.21	8,769.51	6753.25	12.00	75.53	30.22	34.21	20.33
SD[1]	259.5	301.7	147.5	671.5	906.5	3.5	53.8	8.2	7.9	5.4

[1] SD: standard deviation.

these levels have any effect on the stability of the oil fraction of linseed powder.

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