TITLE: Nutritional characteristics of seeds of eighteen linseed cultivars from Serbia

AUTHORS: Dušica Čolović, Janoš Berenji, Alenka Levart, Jovanka Lević, Janez Salobir, Lato Pezo, Radmilo Čolović

Nutritional characteristics of seeds of eighteen linseed cultivars from Serbia

Dušica ČOLOVIC[1], Janoš BERENJI[2], Alenka LEVART[3], Jovanka LEVIC[1], Janez SALOBIR[3], Lato PEZO[4], Radmilo ČOLOVIC[1]

[1]Institute of Food Technology, University of Novi Sad
Car Lazar 1, 21000 Novi Sad, Serbia
E-mail: dusica.colovic@fins.uns.ac.rs

[2]Institute of Field and Vegetable Crops
Maksima Gorkog 30, 21000 Novi Sad, Serbia

[3]Institute of Nutrition, Biotechnical Faculty, University of Ljubljana
Groblje 3, SI-1230 Domžale, Slovenia

[4]Institute of General and Physical Chemistry, University of Belgrade
Studentski 12, 11000 Belgrade, Serbia

Abstract

The literature data about nutritional characteristics of linseed cultivars from a specific geographical area or country are rather scarce. This is the first study to document differences across autochthonous linseed cultivars in Serbia. Our paper is presenting chemical and mineral compositions of linseed cultivars, their fatty acid compositions and antioxidant capacity of lipid-soluble components, which might be interesting, especially due to the increasing trend of linseed usage in human diet. The examined linseed cultivars statistically differed ($p \leq 0.05$) in the content of protein (from 18.9% to 27.0%) and fat (from 34.1% to 40.7%). The highest content of α-linolenic acid (C18:3ω-3) was recorded in the cultivar labelled with No. 5 (61.1%), while cultivar No. 17 showed the most favourable ω-6:ω-3 ratio (0.16). The highest antioxidant capacity of lipid-soluble substances (ACL) was found in cultivar No. 18 – 392.4 μmol trolox kg^{-1} dry matter (d.m.). Cultivars with higher ACL value have stronger protection against oxidation of polyunsaturated fatty acids. Nevertheless, it seems that ACL is more affected by a cultivar, than by fatty acid composition. The aforementioned results could be useful for determination of shelf life of linseed, or its products, while the fatty acid composition of linseed seeds might be one of the criteria for the authentication of linseed origin. The presented results could be of great help in the future selection of the cultivars, depending on the purpose of linseed production.

Key words: α-linolenic acid, antioxidant capacity, fat, linseed, protein.

Introduction

Linseed is an annual or biannual plant of the *Linaceae* family, one of the most useful crops, that has been cultivated as a commercial plant in over fifty countries all over the world. In fact, its Latin name (*Linum usitatissimum*) means "useful". It has even come to be considered as the third most productive oil crop, after the sunflower and winter oilseed rape (Zajaç et al., 2013). This plant grows to a height up to 60 cm, with slim and very fibrous stems, leaves with three veins, up to 4 cm long and 4 mm wide, and has light blue flowers. It yields seeds, whose maturity occurs 30–60 days after flowering, and which are a rich source of both edible and non-edible oils (Rubilar et al., 2010). It was primarily grown (about 3000 B.C.) for medicinal purposes and for fibres which were used for making linen. Nowadays, linseed oil is mainly used, whether it comes to food or chemical industry. The seeds contain approximately 40% of oil, out of which more than 70% are unsaturated fatty acids (FAs). In addition to a large amount of oil, linseed contains approximately 20–30% of crude protein (Ivanov et al., 2012 b).

Canada is the main linseed producer, followed by China, the United States and India (Rubilar et al., 2010). The European Union is the world's largest consumer of linseed, which imports about two-

thirds of the world linseed trade (Oomah, 2001). Consequently, many countries in Western Europe, such as Britain, Ireland and Finland took steps to expand the amount of land for growing linseed (Sankari, 2000). Nowadays, linseed is predominantly used for the production of non-edible oil (Oomah, 2001), but recently there has been a growing interest in linseed oil as a functional food, due to the high concentration of linoleic (18:2 ω-6) and especially α-linolenic (18:3 ω-3) acid.

Raw linseed oil is dark yellow in colour, with strong specific smell and taste. This oil is well known as one of the most unsaturated vegetable oils with high content of α-linolenic acid, whose amount in total fat is more than 50% (Ivanov et al., 2012 a). The α-linolenic acid is a precursor of eicosapentaenoic acid and docosahexaenoic acid, ω-3 polyunsaturated FAs (PUFAs), responsible for the proper brain development in children, as well as for resistance to various allergies, autoimmune diseases, cardiovascular problems, and inflammation. These FAs are essential, because mammals, and therefore humans, cannot endogenously synthesize them and must adopt them exogenously from dietary sources (Sierra et al., 2008). The high quality of linseed oil is confirmed by the fact that the United States National Cancer Institute targeted linseed as one of the six plant materials for study as cancer-preventative foods. Linseed oil is a potent inhibitor of pro-inflammatory mediators even when used in domestic food preparation. This is a great advantage of linseed oil, which makes it suitable for the application in the development of novel anti-inflammatory therapies with or without pharmaceutical products for target populations (Oomah, 2001).

There are a lot of examples of positive linseed effects on animal health and wellbeing as well. The imunomodulating effects of ω-3 FAs combined with the potential hormonal effects of phytoestrogens may have a positive effect on sow productivity and the health of piglets. Due to the positive impact of the inclusion of ω-3 FAs in human diets, there is significant interest in enriching the ω-3 FA content of meat products made of beef and pork. Linseed can be included in poultry diet if used in proper proportions and formulated appropriately. The most popular commercial poultry product is omega-3 eggs. The inclusion of linseed oil in carp diet in the amount of 6% increased the α-linolenic acid content in carp fillets for approx. 6%, while eicosapentaenoic and docosahexaenoic acids contents increased from 0.06% to 0.19% and from 0.21% to 0.75%, respectively (Csengeri et al., 2013).

The aim of this study was to determine chemical and nutritional properties of eighteen native linseed cultivars cultivated in the northern Serbian province Vojvodina in order to detect some mutual characteristic and to determine whether any significant variations existed in their compositions. This is the first study to document differences across the autochthonous Serbian cultivars of linseed. The obtained results were compared with the same characteristics of linseed cultivars from other countries.

Materials and methods

Materials. The examined cultivars of linseed were developed in the year 2013 at the Institute of Field and Vegetable Crops in Novi Sad, Department for Alternative Crops in Bački Petrovac, Serbia. The linseed cultivars were labelled with numbers from 1 to 18. Cultivars labelled with numbers 2 and 9 were yellow linseed, while all other cultivars were brown linseed. The cultivars were developed in order to produce linseed with high yield of seeds and high oil content in seeds. Another aim was to modify fatty acid composition of the cultivars. Positive selection method was used for development of the cultivars. All of them were cultivated in Vojvodina, the Northern Province of Serbia. This plain covers an area between 440°38′ and 460°10′ of north latitude, and 180°10′ and 210°15′ of east longitude, with the total area of 21.506 km^2. It belongs to the same agro-climatic region as the chernozem zone of Russia and Ukraine. Moderate continental climate is prevalent (Manojlović et al., 2014). After harvesting, seeds were transported to the laboratory in polypropylene bags and held at room temperature. They were cleaned on an air screen cleaner to remove all impurities such as dust, dirt and immature and broken seeds.

Chemical analyses. Chemical analyses were performed at the Institute of Food Technology in Novi Sad, Serbia. Moisture content of linseed samples was determined using the gravimetrical AOAC method 950.46, and crude ash was measured using the standard AOAC method 942.05. For determination of crude protein, Kjeldahl method was used according to the AOAC 978.04 method with Kjeltec system (Foss Tecator AB, Sweden). Crude fibres were determined by AOAC 978.10 method (AOAC, 2000) on

an apparatus Fibertech 8000 (Foss Tecator AB, Sweden). The total fat analysis was performed with a Büchi 810 Soxhlet fat extraction apparatus Soxtec system HT, 1043 Extraction Unit (Foss Tecator AB, Sweden) in accordance with the manufacturer's procedure and AOCS method Ba 3-38 (AOCS, 2001). All analyses were done in triplicate.

Mineral content analyses. Mineral content analyses were performed at the Institute of Food Technology in Novi Sad, Serbia. The contents of calcium, magnesium, and potassium were determined according to SRPS EN ISO 6869:2008 method by atomic absorption spectrophotometry. The phosphorus content was determined by SRPS ISO 6491:2002 method using the spectrometric method. The analyses of all samples were done in duplicate.

Fatty acids analysis. The FA analysis was done at The Biotechnical Faculty, University of Ljubljana, Slovenia. Supercritical fluid extraction with CO_2 was used for the extraction of lipids from the samples, since it showed good results as a preparative technique for the FA analysis. The extraction was performed on LECO TFE-2000 fat analyzer (LECO Corporation, USA) and extraction conditions were adjusted as explained in the paper of Ivanov et al. (2012 a). FA methyl esters were prepared from the extracted lipids by the transmethylation method that uses 14% wt. boron trifluoride/methanol solution (Sigma Aldrich, USA), which is a recommended method for this type of substrates (Ivanov et al., 2012 a). The obtained samples were analyzed by a gas chromatograph with a flame ionization detector GC-FID (Agilent 7890A system, Agilent Technologies, USA), auto-injection module for liquid, equipped with a fused silica capillary column (DB-WAX 30 m, 0.25 mm, 0.50 um). The carrier gas was helium (purity >99.9997 vol. %, flow rate = 1.26 ml min^{-1}). The FAs peaks were identified by comparison of retention times with retention times of standards from Supelco 37 component FA methyl ester mix and with the data from the internal data library, based on previous experiments and FA methyl ester determination on GC with a mass spectrometer. The results were expressed as the mass of single FA or FA group (g) in 100 g of the total FAs (relative content).

Analysis of antioxidant capacity of lipid-soluble substances. The estimation of the antioxidant capacity in lipid-soluble substances (ACL) was done by the photochemiluminescence method using Photochem instrument (Jena Analitik, Germany) at the Biotechnical Faculty, University of Ljubljana, Slovenia. In the ACL assay, the photochemical generation of free radicals was measured with a sensitive detector by using chemiluminescence. Free radicals were produced from the luminol, which worked partly as a photosensitizer and partly as an oxygen radical detection reagent. Lipid-soluble antioxidants were measured with the ACL kit, according to the manufacturer's protocol (Jena Analytik, 2004). The working solution consisted of the following reagents: methanol (reagent 1) – 2.3 ml, buffer solution (reagent 2) – 200 μl, photosensitizer (reagent 3) – 25 μl. All reagents were mixed with a vortex for 20–30 s. The resulting solution was diluted with methanol in a ratio of 1:100 for preparation of trolox standard with the concentration of 100 μmol dm^{-3}, which was used for the construction of a calibration curve. Lipid soluble antrioxidants were extracted from linseed with hexane. After centrifugation, the hexane phase was transferred to a clean sample vial, stored in the dark on ice and analysed within the same day. Antioxidant capacities of the samples were determined by the extrapolation method. All measurements were done in duplicate.

Statistical analysis. Statistical analysis of the experimental data was performed using software *STATISTICA*, version 10 (StatSoft, USA). One way analysis of variance – *ANOVA* and Tukey's HSD comparison of means of samples were used for analyzing variations. Differences among means with probability $p \leq 0.05$ were accepted as representing statistically significant differences. Correlations between FAs and fat content pairs were evaluated at $p \leq 0.05$. Pattern recognition techniques (principal component analysis and cluster analysis) were applied to the experimental data (used as descriptors) to characterize and differentiate among the observed samples. Principal component analysis was used to discover the possible correlations among measured parameters, while cluster analysis was used to classify objects into groups.

Results and discussion

The chemical compositions of seeds of linseed cultivars are given in Table 1. The content of protein ranged between 18.9% and 27.0%, with a mean value of 21.8%. The fat content of seeds varied between 34.08% and 40.74%, and a mean value was 37.9%. The content of crude fibres ranged from 7.6% to 11.8%, and ash content ranged from 3.54% to 4.49%, which is in accordance with the literature data (Ivanov et al., 2012 a). Besides considerable fat content, linseed also had a relatively high content of protein - more than sunflower (16.0%) and rapeseed (19.1%), as shown by Sauvant et al. (2004), which is why it can be considered both as energy and protein source.

Table 1. Chemical composition of seeds (%) of investigated linseed cultivars

Number of linseed cultivar	Moisture	Ash	Protein	Crude fibre	Total fat
1	6.01 ± 0.07 a	4.49 ± 0.02 a	27.02 ± 0.02 a	9.52 ± 0.26 a	34.56 ± 0.03 b
2	6.04 ± 0.14 a	4.30 ± 0.02 a	25.91 ± 0.01 a	11.24 ± 0.23 a	34.66 ± 0.04 b
3	6.01 ± 0.09 a	4.28 ± 0.02 a	25.73 ± 0.23 a	11.85 ± 0.26 a	34.08 ± 0.03 b
4	5.78 ± 0.06 a	4.23 ± 0.02 a	21.15 ± 0.02 b	10.49 ± 0.26 a	38.35 ± 0.04 ab
5	5.39 ± 0.03 a	4.10 ± 0.01 a	22.02 ± 0.10 b	11.57 ± 0.26 a	39.09 ± 0.05 a
6	5.61 ± 0.21 a	4.09 ± 0.02 a	21.00 ± 0.02 b	11.36 ± 0.26 a	38.42 ± 0.05 ab
7	5.78 ± 0.06 a	4.11 ± 0.03 a	20.73 ± 0.02 b	12.49 ± 0.26 a	37.76 ± 0.04 ab
8	6.03 ± 0.01 a	4.26 ± 0.01 a	21.43 ± 0.08 b	9.95 ± 0.26 a	39.31 ± 0.03 a
9	5.73 ± 0.18 a	4.21 ± 0.01 a	20.52 ± 0.01 b	9.71 ± 0.26 a	37.65 ± 0.04 ab
10	5.72 ± 0.08 a	3.92 ± 0.02 a	22.19 ± 0.02 b	7.92 ± 0.26 b	40.29 ± 0.05 a
11	5.66 ± 0.06 a	3.81 ± 0.02 a	22.40 ± 0.02 b	8.37 ± 0.26 b	39.69 ± 0.06 a
12	5.91 ± 0.09 a	3.93 ± 0.01 a	21.57 ± 0.02 b	11.53 ± 0.26 a	38.66 ± 0.04 ab
13	5.57 ± 0.03 a	3.92 ± 0.02 a	18.93 ± 0.02 b	9.70 ± 0.26 ab	37.91 ± 0.08 ab
14	5.75 ± 0.01 a	4.15 ± 0.02 a	20.78 ± 0.11 b	9.12 ± 0.26 ab	37.09 ± 0.03 ab
15	5.45 ± 0.03 a	3.81 ± 0.01 a	20.37 ± 0.02 b	11.55 ± 0.26 a	39.93 ± 0.02 a
16	5.94 ± 0.01 a	3.85 ± 0.01 a	21.66 ± 0.02 b	9.26 ± 0.26 ab	36.30 ± 0.03 b
17	5.50 ± 0.04 a	3.54 ± 0.01 a	19.08 ± 0.02 b	7.56 ± 0.26 b	40.74 ± 0.04 a
18	5.79 ± 0.17 a	4.25 ± 0.02 a	19.56 ± 0.02 b	10.79 ± 0.26 a	37.91 ± 0.03 ab
Mean	5.76	4.07	21.78	10.22	37.91

Note. The values are represented as mean ± standard deviation (SD), n = 3; a-b – different superscripts within the same column indicate significant differences ($p \leq 0.05$).

A dendrogram of the chemical composition of linseed seeds with the use of complete linkage as an amalgamation rule and the city block (Manhattan) distance as a measure of the proximity between samples is shown in Figure 1. The dendrogram based on the descriptors data showed proper distinction between 18 linseed cultivars, creating four separate branches of the dendrogram. As shown in the figure, there were similarities in cultivars Nos. 1, 2 and 3 (with high moisture, ash and protein content compared to other samples), also in the cultivars Nos. 4, 5, 6, 7, 12 and 15 (with a high fibre content), cultivars Nos. 9, 13, 14, 16 and 18 (having average moisture, ash, protein and fibre content) as well as in cultivars Nos. 8, 10, 11 and 17 (with minimum of moisture, ash, protein and fibre content). However, no distinction was made on the basis of the fat content and all cultivars had the fat content higher than 34%, meaning that all cultivars could be characterised as oil linseed cultivars.

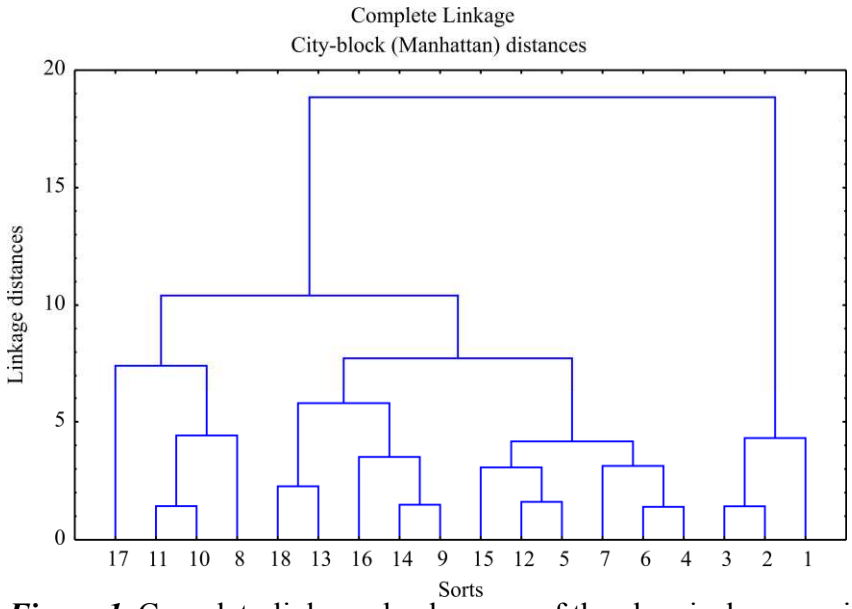


Figure 1. Complete-linkage dendrogram of the chemical composition in linseed seeds

By comparing the protein content between the examined cultivars and looking at Figure 1, it can be seen that the cultivars labelled with 1, 2 and 3 had a significantly ($p \leq 0.05$) higher content than other cultivars (labelled from 4 to 18). On the other hand, these cultivars had the lowest crude fat content among the investigated ones (34.6, 34.7 and 34.1 %, respectively). It seemed that with an increase in protein content, total fat content in linseed cultivars decreased, which was confirmed by the negative correlation coefficient $r = -0.71$, with $p = 0.0008$.

If the protein and crude fat content of the investigated linseed cultivars are compared with the linseed characteristics presented by Sauvant et al. (2004), it can be noticed that the protein contents are similar (21.8% and 22.6%, respectively), but the content of crude fat is higher in linseed samples from Serbia by approximately 5% (37.91% *vs* 32.7%). Fat content of linseeds examined in the paper of Sargi et al. (2013) ranged from 37.6% to 38.1%, while protein content ranged from 23.2% to 24.4%, which was more similar to the Serbian cultivars. The fat content of linseed can be altered through plant breeding methods, and it is affected by geography – cold nights improve the oil content and quality (Ganorkar, Jain, 2014). Khan et al. (2010) investigated chemical composition of six linseed cultivars from Pakistan. By comparing these results with the ones presented in this paper, it was found that the Pakistani linseed cultivars were richer in average in protein content (24.2% *vs* 21.8%) and had almost the same content of crude fat (37.8% *vs* 37.9%). An analysis of Canadian brown linseed showed that the average content of fat was 41%, which was slightly more than in the Serbian cultivars, while the reported average protein content was 20% (Morris, 2003). Martinchick et al. (2012) obtained the following results: the protein content varied from 20% to 30%, and the total fat content ranged from 35% to 45%.

Table 2 presents the seed mineral content of the examined linseed cultivars. As it can be seen from the results, all linseed cultivars were rich in magnesium (from 2729 to 4565.9 mg kg^{-1}) and potassium (from 6590.0 to 7978.4 mg kg^{-1}). However, significant differences among the cultivars occurred in the content of both minerals. The Ca content also widely ranged from 0.28% to 2.87%, and P content ranged from 0.53% to 1.13%. Cultivars Nos. 1, 2, 3, 14 and 15 showed a significantly lower ($p \leq 0.05$) Ca content (from 0.28% to 0.36%) than other cultivars.

Table 2. Mineral content of seeds of 18 linseed cultivars

Number of linseed cultivar	Ca %	P %	Mg g kg^{-1}	K mg kg^{-1}
1	0.36 a	0.93 a	3040.61 a	7945.00 a
2	0.36 a	0.70 a	3276.00 a	7235.00 a
3	0.31 a	0.85 a	3442.00 a	7423.00 a

4	2.87 c	0.88 a	4544.11 b	7553.13 a
5	0.35 a	0.72 a	3035.00 a	7316.00 a
6	1.65 b	0.86 a	4486.92 b	7328.34 a
7	2.42 c	0.82 a	4424.56 b	7978.44 a
8	0.74 ab	0.94 a	4480.04 b	7518.83 a
9	1.05 ab	0.92 a	4431.08 b	7365.35 a
10	1.00 ab	1.11 a	4290.89 b	7219.74 a
11	1.44 b	1.13 a	4472.02 b	7219.74 a
12	1.94 b	0.84 a	4213.48 b	7205.04 a
13	1.71 b	0.82 a	4430.02 b	6590.01 a
14	0.28 a	0.78 a	2729.00 a	7735 a
15	0.31 a	0.53 b	2729.00 a	7049 a
16	1.31 ab	0.78 a	4565.89 b	7109.73 a
17	1.25 ab	0.87 a	4401.08 b	7002.6 a
18	2.31 c	0.79 a	4508.92 b	7314.65 a
Mean	1.14	0.85	3972.3	7339.4

Note. a-b – different superscripts within the same column indicate significant differences ($p \leq 0.05$).

Linseed is generally rich in Mg and K, and the mean values were 3972.26 and 7339.36 mg kg^{-1}, respectively (Table 2). Some authors even represented the standpoint that the daily intake of 100 g of linseed meets the demands in human diet for these minerals. Magnesium has multiple roles in the human body, and some of its most important health benefits are regulating the relaxation/contraction of muscles, the production of proteins, and the production and transportation of energy throughout the body. Potassium is a very significant body mineral, important to both cellular and electrical function. It is one of the main blood minerals, which carries an electrical charge (potential). It controls the body's water balance together with Na, prevents stroke and aids in muscle contraction (Gupta, Gupta, 2014). Khan et al. (2010) reported the following mineral content of linseeds: the Ca content ranged from 0.31% to 0.46%, the Mg content varied from 0.07% to 0.10%, while the K content varied from 1.14% to 1.92%. According to Sauvant et al. (2004), average mineral content of linseed is: Ca – 3.8 g kg^{-1}, P – 6.1 g kg^{-1}, Mg – 3.6 g kg^{-1}, K – 7.2 g kg^{-1}, etc., which is similar to the Mg and K content presented in this paper. However, Ca and P content in Table 2 are remarkably lower than the results shown by Sauvant et al. (2004), which can be a consequence of cultivar characteristics, but also soil structure or environmental conditions during the maturation of linseed.

According to the results shown in Table 3, the FA composition of linseed oil is predominated by unsaturated (FAs) with eighteen C atoms (average content: oleic acid (C18:1) – 21.0%, linoleic acid (C18:2ω-6) – 14.0%, α-linolenic acid (C18:3ω-3) – 53.6%). Bean and Leeson (2002) reported the following mean percentages for these FAs: oleic acid – 18.5%, linoleic acid – 14.4% and α-linolenic acid – 57.1%. Myristic acid (C14:0) was not detected in all samples, and when detected, did not exceed 0.06%. Sauvant et al. (2004) also showed a low content of this FA (0.10%), while Bean and Leeson (2002) did not detect it.

Table 3. Fatty acid composition of extracted linseed oil

	Fatty acid content (% of total fatty acids)										
Number of linseed cultivar	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2ω-6	C18:3ω-3	SFA	MUFA	PUFA	ω-6: ω-3
1	0.00	4.58 a	0.20 a	19.43 a	17.99 b	14.83 a	42.97 c	24.01 a	18.20 ab	57.79 a	0.35 ab
2	0.00	4.89 a	0.08 a	5.96 b	20.77 ab	16.17 a	52.12 b	10.85 b	20.85 a	68.30 b	0.31 c
3	0.00	4.93 a	0.07 a	5.82 b	20.30 ab	15.88 a	52.99 b	10.76 b	20.38 a	68.87 b	0.30 b
4	0.00	5.31 a	0.08 a	4.08 b	21.03 ab	15.29 a	54.22 a	9.39 b	21.11 a	69.51 b	0.28 ab

5	0.00	5.42 a	0.11 a	3.81 b	16.33 b	13.27 a	61.06 a	9.23 b	16.44 b	74.33 c	0.22 b
6	0.05 a	5.75 a	0.07 a	4.32 b	20.94 ab	13.34 a	55.52 a	10.13 b	21.06 a	68.86 b	0.24 ab
7	0.05 a	6.29 a	0.13 a	5.78 b	21.72 ab	13.16 a	52.87 b	12.11 b	21.91 a	66.03 b	0.25 ab
8	0.05 a	5.91 a	0.09 a	4.32 b	22.43 a	14.91 a	52.29 b	10.27 b	22.57 a	67.20 b	0.29 b
9	0.06 a	6.42 a	0.15 a	3.65 b	21.02 ab	13.36 a	55.35 a	10.13 b	21.23 a	68.70 b	0.24 ab
10	0.05 a	5.71 a	0.08 a	4.78 b	22.02 a	14.44 a	52.92 b	10.53 b	22.16 a	67.36 b	0.27 b
11	0.05 a	5.77 a	0.10 a	4.49 b	20.88 ab	14.73 a	53.98 ab	10.31 b	21.02 a	68.71 b	0.27 ab
12	0.05 a	5.71 a	0.08 a	4.78 b	21.46 ab	14.09 a	53.82 ab	10.55 b	21.60 a	67.91 b	0.26 b
13	0.05 a	5.91 a	0.10 a	5.29 b	23.38 a	13.98 a	51.28 b	11.25 b	23.53 a	65.26 b	0.27 b
14	0.05 a	5.66 a	0.08 a	4.47 b	20.92 ab	13.13 a	55.69 a	10.18 b	21.05 a	68.82 b	0.24 b
15	0.04 a	5.93 a	0.11 a	4.89 b	22.67 a	13.84 a	52.52 ab	10.86 b	22.83 a	66.36 b	0.26 ab
16	0.05 a	6.28 a	0.10 a	3.98 b	21.44 ab	13.47 a	54.69 ab	10.31 b	21.59 a	68.16 b	0.25 a
17	0.05 a	6.15 a	0.10 a	4.53 b	22.56 a	9.18 b	57.45 a	10.72 b	22.70 a	66.62 b	0.16 a
18	0.04 a	6.05 a	0.09 a	4.48 b	20.17 ab	15.68 a	53.50 ab	10.57 b	20.30 a	69.17 b	0.29 a
Mean	0.05	5.70	0.10	5.49	21.00	14.04	53.62	11.23	21.14	67.66	0.26

Notes. The values are represented as mean, n = 3; a-c – different superscripts within the same column indicate significant differences ($p \leq 0.05$). C14:0 – myristic acid, C16:0 – palmitic acid, C16:1 – palmitoleic acid, C18:0 – stearic acid, C18:1 – oleic acid, C18:2ω-6 – oleic acid, C18:3ω-3 – α-linolenic acid; SFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid.

Cultivar No. 1 had significantly ($p \leq 0.05$) higher content of saturated the FAs (24.01%) in comparison with other investigated cultivars (from 9.23% to 12.11%). The content of monounsaturated FAs (MUFA) ranged from 16.4% (the cultivar No. 5) to 23.5% (the cultivar No. 13). Bayrak et al. (2010) reported in their paper average SFA content of 10.0% and MUFA content of 22.5%. Looking at the content of PUFA, it can be seen that cultivar No. 1 had a significantly lower ($p \leq 0.05$) PUFA content 57.8% than all other cultivars, while cultivar No. 5 had a significantly higher ($p \leq 0.05$) PUFA content (74.3%).

The FA composition of seed oils varies widely among different species. Usually, the occurrence of some specific FA is characteristic for particular plant families (Özcan et al., 2010). The most predominant FA in all examined linseed cultivars was α-linolenic acid, as expected, with the mean value of 53.6% (Table 3). The highest content of this FA was recorded in cultivar No. 5 (61.06%). Cultivar No. 1 a significantly differ ($p \leq 0.05$) from all other samples in lower content of α-linolenic acid (42.97%). It is noticeable that this cultivar contains significantly ($p \leq 0.05$) higher content of stearic acid, which was present in the amount of 19.4%. By comparing the obtained results with the ones presented in the paper of Bayrak et al. (2010), the difference in FA composition is evident. In the aforementioned paper, authors did not detect myristic acid and palmitoleic acid, while detecting arachidic and gondoic acid. Bean and Leeson (2002) obtained the results more similar to ours, except for the absence of myristic acid FA. The specific FA composition of the examined Serbian cultivars might be used as one of the benchmarks for the authentication of linseed origin.

As shown in the paper by Bean and Leeson (2002), the mean PUFA content of 23 examined linseed samples was 71.55%, which is in accordance with our results. However, El-Beltagi et al. (2007) reported lower values for the PUFA content (66.5%) in five linseed cultivars in Egypt. The FA composition of oil from linseed, especially content and composition of unsaturated FAs highly depends on climatic conditions. Early and late frosts, as well as high temperatures and drought damage seeds, causing a higher content of palmitic, linoleic and α-linolenic acid than it is in whole and undamaged seeds (Sediqi, 2012). Cool climatic conditions characteristic of Northern regions increase the content of linoleic and α-linolenic acid (Nykter, Kymäläinen, 2006). Steppe conditions with high humidity extend accumulation period of unsaturated fatty acids (Laza, Pop, 2012).

In linseed seeds ω-6:ω-3 ratio ranged from 0.16 (cultivar No. 17) to 0.35 (cultivar No. 1). It is a well known fact that modern human diet is imbalanced in fat, which means an insufficient intake of ω-3

FAs, and ω-6:ω-3 ratio from 20:1 to 15:1. Western diets are deficient in ω-3 FAs, while having extortionate amounts of ω-6 FAs in comparison with the diet on which humans evolved. The recommended values of the ω-6:ω-3 ratio should be at least less than 4:1 in the secondary prevention of cardiovascular disease, and a ratio of 2–3:1 for suppressing inflammation in patients with rheumatoid arthritis (Mc Daniel et al., 2013). Therefore, the FA composition of all investigated linseed cultivars can be considered as nutritionally desirable. Although there were significant differences in the ω-6:ω-3 ratio between the cultivars, all values were far below 4:1, or 2–3:1 (Table 3). Sargi et al. (2013) also found that linseed ω-6:ω-3 ratio is consistent with the recommended ratio for healthy balanced nutrition.

The average values for bilateral relationships between the oil content and FA composition of linseeds were subjected to correlation analysis (Table 4). The results revealed that myristic, palmitic and α-linolenic acids had significant ($p \leq 0.05$) positive effect on the oil content ($r = 0.530$, 0.581 and 0.473, respectively), while linoleic acid had significant ($p \leq 0.05$) negative effect ($r = -0.50$). Statistically significant positive correlation between α-linolenic acid and the oil content ($r = 0.37$), as well as negative correlation between linoleic acid and the oil content ($r = -0.21$) was also reported in the paper by Bjelková et al. (2012), but only in brown-seed cultivars with a high content of α-linolenic acid (more than 32.3% of total fatty acid). These data are in accordance with our results, since all cultivars examined in our experiment were brown-seed, with α-linolenic acid from 43.0% to 61.1%. The authors also investigated yellow linseeds and found positive linear correlation between the oil content and linoleic acid ($r = 0.62$) in cultivars with a low α-linolenic acid content. Another interesting information is that stearic acid had significant ($p \leq 0.05$) negative effect ($r = -0.82$) on content of essential α-linolenic acid. It means that with an increase in the stearic acid content, the content of α-linolenic acid generally decreased, which was especially obvious in cultivar No. 1, as already mentioned.

Table 4. Correlation coefficients between oil content and fatty acid content of extracted linseed oil

	Oil content	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2ω-6	C18:3ω-3
Oil content	1							
C14:0	0.530*	1						
C16:0	0.581*	0.867*	1					
C16:1	−0.23	−0.61*	−0.10	1				
C18:0	−0.50*	0.532*	−0.61*	0.742*	1			
C18:1	0.353	−0.52*	0.532*	−0.35	−0.39	1		
C18:2ω-6	−0.50*	0.445	−0.52*	−0.12	0.172	−0.21	1	
C18:3ω-3	0.473*	0.171	0.445	−0.52	−0.82*	−0.06	−0.45	1

* – indicate statistically significant correlation ($p \leq 0.05$); C14:0 – myristic acid, C16:0 – palmitic acid, C16:1 – palmitoleic acid, C18:0 – stearic acid, C18:1 – oleic acid, C18:2ω-6 – oleic acid, C18:3ω-3 – α-linolenic acid.

One of the main biochemical effects of free radicals is the initiation of lipid peroxidation. Therefore, the significance of lipid soluble antioxidants is crucial in the protection of lipid-rich food from fast oxidative deterioration. Since linseed is an oilseed, ACL was chosen as an appropriate criterion for determination of antioxidant capacity in linseed seeds, and the results are presented in Table 5. The highest ACL was recorded in cultivar No. 18 (392.40 μmol trolox kg^{-1} d.m.), and the lowest in cultivar No. 4 (136.44 μmol trolox kg^{-1} d.m.). The obtained results had a wide range of values, which were statistically different ($p \leq 0.05$) between themselves (Table 5). The cultivars with a higher ACL value had stronger protection against oxidation of PUFA. However, statistically significant correlation between ACL and PUFA, or α-linolenic acid content in cultivars could not be found. Correlation coefficient between the ACL and PUFA contents was $r = 0.04$, with $p = 0.84$, while $r = 0.006$ between ACL and α-linolenic acid, with $p = 0.99$. Russo and Reqqiani (2015) investigated antioxidant activity of eighteen linseed varieties and their results also showed significant differences at probability level 0.01. The authors concluded that oil linseed varieties are poorer in antioxidants than fibre varieties. They also suggested that if the oil and flour are both required products for industrial purposes or health, intermediate varieties of linseed which are richer in phenolic compounds than oil varieties would be recommended for use (Russo,

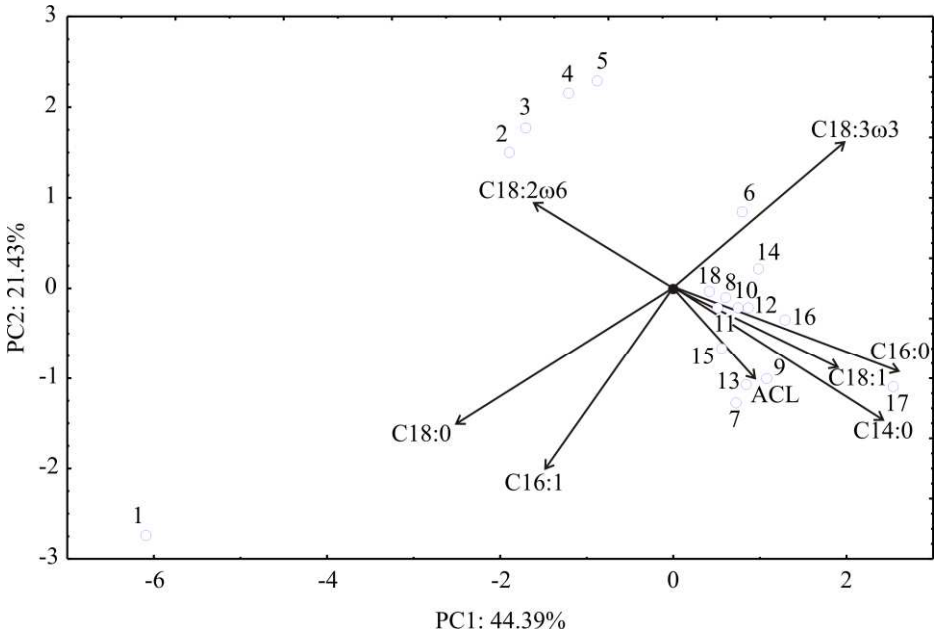
Reqqiani, 2015). Our results showed that there was no statistically significant correlation ($p > 0.05$) between oil content and ACL, probably due to the fact that all cultivars examined in this paper were oil cultivars.

Table 5. Antioxidant capacity in lipid-soluble substances (ACL) of linseed seeds

Number of linseed cultivar	ACL (μmol trolox kg^{-1} d.m.)	SD	CV
1	260.14 bcd	16.52	6.35
2	273.32 cd	9.23	3.38
3	273.55 cd	6.33	2.31
4	136.44 a	6.38	5.00
5	259.96 bcd	8.64	3.32
6	199.26 ab	9.59	4.81
7	266.28 cd	5.22	1.96
8	250.68 bc	4.50	1.80
9	227.65 bc	4.76	2.09
10	342.66 efg	10.45	3.05
11	343.47 efg	36.11	10.51
12	369.60 fg	15.55	4.21
13	319.69 def	21.46	6.71
14	319.73 def	12.82	4.01
15	288.46 cde	29.63	10.27
16	286.61 cde	1.07	0.37
17	340.75 efg	27.62	8.11
18	392.40 g	5.29	1.35
Mean	286.15		

n = 2; a-g – different superscripts within the same column indicate significant differences ($p \leq 0.05$); SD – standard deviation, CV – coefficient of variation.

The principal component analysis (PCA) was done taking into consideration the FA composition and ACL of all examined cultivars (Fig. 2). The points shown in the PCA graphics which are geometrically close to each other indicate the similarity of patterns that are represented by these points. The orientation of the vector describing the variable in factor space indicates an increasing trend of these variables, and the length of the vector is proportional to the square of the correlation values between the fitting value for the variable and the variable itself. The angles between corresponding variables indicate the degree of their correlations (small angles correspond to high correlations).



C14:0 – myristic acid, C16:0 – palmitic acid, C16:1 – palmitoleic acid, C18:0 – stearic acid, C18:1 – oleic acid, C18:2ω-6 – oleic acid, C18:3ω-3 – α-linolenic acid; SFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid

Figure 2. Principal component analysis (PCA) ordination of variables based on variable correlations

As it can be seen from Figure 2, there were similarities in cultivars Nos. 2, 3, 4 and 5 (with high C18:2ω-6 content), and cultivars Nos. 7, 9, 13, 16 and 17 (with a high content of myristic, palmitic and oleic acid and MUFA). Cultivars labelled with Nos. 8, 10, 11, 12, 14 and 18 were also similar (with an average FA content and ACL). However, the most interesting fact is that ACL did not show any significant correlation to PUFA, or the α-linolenic acid content, which might be assumed. Linseed cultivars with a high content of PUFA and α-linolenic acid are more susceptible to oxidation, so it could be reasonably expected that these cultivars have better natural protection against oxidation. Nevertheless, such conclusion cannot be made by looking at Figure 2. Obviously, ACL did not depend on FA composition of linseed. Some interesting results were presented in the paper by Sargi et al. (2013). These authors found that linseed cultivars with a higher content of ω-3 and ω-6 FA showed lower antioxidant capacity, although opposite dependence might be expected. Also, linseed products of cultivar No. 18 would probably have longer shelf life than products of other cultivars. The presented results could be of great help in the future selection of cultivars which depends on the purpose of linseed production. They may also be useful criteria for the manipulation and storage of linseed products.

Conclusions

1. The data showed that there were statistically significant differences in the chemical composition of seeds of the examined linseed cultivars, especially in the fat content (from 34.1% to 40.7%), as well as in the fatty acid (FA) composition. Negative correlation was found between the protein content and the fat content ($r = -0.71$, with $p = 0.0008$), meaning that cultivars with a higher content of protein had a lower content of fat, or vice versa.
2. Negative correlation was also found between FAs C18:0 and α-linolenic acid ($r = -0.82$, $p < 0.05$). Cultivars Nos. 5 and 17 can be considered as nutritionally most favourable, due to their high α-linolenic acid content (61.1% and 57.5%, respectively) and low n-6:n-3 ratio (0.22 and 0.16, respectively).
3. The seeds of all cultivars were generally rich in Mg and K. The mean values of the content of these minerals were 3972.3 and 7339.4 mg kg^{-1}, respectively.

4. Cultivar No. 18 had the best natural protection against oxidation (the antioxidant capacity of lipid-soluble components (ACL) = 392.40 μmol trolox kg^{-1} d.m.). However, ACL did not depend on the FA composition of linseed.

Acknowledgements

The authors thank Project of bilateral cooperation between Republic of Slovenia and Republic of Serbia "Influence of thermal treatments on antioxidative capacity, oxidative stability and nutritive value of animal feed" for financial support. The results presented in this paper are also a part of Research Project III46012 funded by Serbian Ministry of Education, Science and Technological Development.

References

AOAC Official Methods of Analysis. 2000 (17th ed.). Horwitz W. (ed.). AOAC International, Arlington, USA

AOCS Method Ba 3-38 2001. Official Methods and Recommended Practices (5th ed.). Firestone D. (ed.), Champaign, USA

Bayrak A., Kiralan M., Ipek A., Arslan N., Cosge B., Khawar K. M. 2010. Fatty acid compositions of linseed (*Linum usitatissimum* L.) genotypes of different origin cultivated in Turkey. Biotechnology and Biotechnological Equipment, 24 (2): 1836–1842

Bean L.D., Leeson S. 2002. Fatty acid profiles of 23 samples of flaxseed collected from commercial feed mills in Ontario. Journal of Applied Poultry Research, 11: 209–211

Bjelková M., Nôžková J., Fatrcová-Šramková K., Tejklová E. 2012. Comparison of linseed (*Linum usitatissimum* L.) genotypes with respect to the content of polyunsaturated fatty acids. Chemical papers, 66 (10): 972–976

Csengeri I., Čolović D., Rónyai A., Jovanović R., Péter-Szűcsné J., Sándor Z., Gyimes E. 2013. Feeding of common carp on floating feeds for enrichment of fish flesh with essential fatty acids. Food and Feed Research, 40 (2): 59–70

El-Beltagi H. S., Salama Z. A., El-Hariri D. M. 2007. Evaluation of fatty acids profile and the content of some secondary metabolites in seeds of different flax cultivars (*Linum usitatissimum* L.). General and Applied Plant Physiology, 33 (3–4): 187–202

Ganorkar P. M., Jain R. K. 2014. Effect of flaxseed incorporation on physical, sensorial, textural and chemical attributes of cookies. International Food Research Journal, 21 (4): 1515–1521

Gupta U.C., Gupta S.C. 2014. Sources and deficiency diseases of mineral nutrients in human health and nutrition: a review. Pedosphere, 24 (1): 13–38

Ivanov D., Čolović R, Lević J, Sredanović S. 2012 (a). Optimization of supercritical fluid extraction of flaxseed oil using RSM. European Journal of Lipid Science and Technology, 114 (7): 807–815

Ivanov D., Kokić B., Brlek T., Čolović R., Vukmirović Đ., Lević J., Sredanović S. 2012 (b). Effect of microwave heating on content of cyanogenic glycosides in linseed. Field and Vegetable Crop Research, 49 (1): 63–68

Jena Analytik. 2004. Determination of the antioxidative capacity (water soluble – ACW and lipid soluble – ACL) in PUFA oil. Reference No. BS_PLC_09_04_e|07/2004|Öz

Khan M., Laiq Sharif M., Sarwar M., Ameen S., Ameen M. 2010. Chemical composition of different varieties of linseed. Pakistan Veterinary Journal, 30 (2): 79–82

Laza A., Pop G. 2012. The influence of fertilization and seeding density on flax oil production quality. Research Journal of Agricultural Sciences, 44 (4): 96–102

Manojlović M., Bogdanović D., Lazić S., Nešić Lj. 2014. Fertility and depletion of land in border area. Poljoprivredni fakultet, Univerzitet u Novom Sadu, p. 1-59 (in Serbian)

Martinchik A. N., Baturin A. K., Zubtsov V. V., Molofeev V. 2012. Nutritional value and functional properties of flaxseed. Voprosy pitaniia, 81 (3), 4–10

Mc Daniel J., Ickes E., Holloman C. 2013. Benefitial n-3 polyunsaturated fatty acid levels and n-6:n-3 ratios after 4-week EPA + DHA supplementation associated with reduced CRP: a pilot study in healthy young adults. Modern Research in Inflammation, 2 (4): 59–68

Morris D. H. 2003. Flax: a health and nutrition primer (3rd ed.). Flax Council of Canada, Winnipeg, 140 p.

Nykter M., Kymäläinen H.-R. 2006. Quality characteristics of edible linseed oil. Agricultural Food Science, 15: 403–412

Oomah D. 2001. Flaxseed as a functional food source. Journal of Science of Food and Agriculture, 81: 889–894

Özcan M. M., Cesari İ., Arslan D. 2010. Physicochemical properties, fatty acid and mineral content of some walnuts (*Juglans regia* L.) types. Agricultural Science, 1: 62–67

Rubilar M., Gutiérrez C., Verdugo M., Shene C., Sineiro J. 2010. Flaxseed as a source of functional ingredients. Journal of Soil Science and Plant Nutrition, 10 (3): 373–377

Russo R., Reqqiani R. 2015. Phenolic and antioxidant activity in flax varieties with different productive attitude. International Food Research Journal, 22 (4): 1736–1739

Sankari H. S. 2000. Linseed (*Linum usitatissimum* L.) cultivars and breeding lin as shoot biomass producers. Journal of Agronomy and Crop Science, 184: 225–231

Sargi S. C., Silva B. C., Santos H. M. C., Montanher P. F., Boeing J. S., Santos Jùnior O. O., Souza N. E., Visentainer J. V. 2013. Antioxidant capacity and chemical composition in seeds rich in omega-3: chia, flax and perilla. Food Science and Technology, Campinas, 33 (3): 541–548

Sauvant D., Perez J.-M., Tran G. 2004. Table of composition and nutritional value of feed materials. Wageningen, The Nederlands and L'Institut National de la Recherche Agronomique (INRA), Paris, France, 304 p.

Sediqi M. N. 2012. Adaptability of oilseed species at high altitudes of Colorado and technology transfer to Afghanistan: MSc thesis, Colorado State University. Fort Collins, USA

Sierra S., Lara-Villoslada F., Comalada M., Olivares M., Xaus J. 2008. Dietary eicosapentaenoic acid and docosahexaenoic acid equally incorporate as docosahexaenoic acid but differ in inflammatory effects. Nutrition, 24: 245–254

SRPS EN ISO 6869: 2008. Determination of the contents of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc. Method using atomic absorption spectrometry. International Organization for Standardization, Institute for Standardization of Serbia, Beograd

SRPS ISO 6491:2002. Determination of phosphorus content – Spectrometric method. International Organization for Standardization, Institute for Standardization of Serbia, Beograd

Zajaç T., Olesky A., Stokłosa A., Klimek-Kopyra A., Kulig B. 2013. The development competition and productivity of linseed and pea-cultivars grown in a pure sowing or in a mixture. European Journal of Agronomy, 44: 21–33