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AT: HPLC analysis, graphs and tables preparation, PCA and statistical analysis; GS: seeds delivery, data interpretation, manuscript revision; MC: GC analysis and data interpretation; DL: GC samples preparation, data interpretation, revision of the manuscript; JK: PC isolation, revision of the manuscript, and data discussion; RS: idea of the experiments, data analysis and interpretation, manuscript writing

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ORIGINAL RESEARCH PAPER

Tocochromanols and fatty acid composition in flax (*Linum usitatissimum* L.) accessions

Agnieszka Trela[1], Grażyna Silska[2], Marek Chyc[3], Dariusz Latowski[4], Jerzy Kruk[4], Renata Szymańska[1]*

[1] Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Reymonta 19, 30-059 Kraków, Poland
[2] Institute of Natural Fibres and Medicinal Plants, Wojska Polskiego 71b, 60-630 Poznań, Poland
[3] Department of Environmental Protection, State Higher Vocational School in Tarnów, Mickiewicza 8, 33-100 Tarnów, Poland
[4] Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

* Corresponding author. Email: renata.szymanska@fis.agh.edu.pl

Abstract
Flax, *Linum usitatissimum*, cultivars are grown throughout the world. Flax oil is a dietary source of polyunsaturated fatty acids, vitamin E, as well as phospholipids, sterols, and phenolic acids. Linseed plays a pivotal role in protecting cells from oxidative damage associated diseases, i.e., atherosclerosis, neurodegenerative disorders, cancer, and inflammation. In this study, two groups of *L. usitatissimum* seeds were used to evaluate and compare the content and composition of tocochromanols (vitamin E) and fatty acids. Group I included accessions originating from Poland and the Ukraine, while Group II encompassed worldwide flax cultivars (such as from the United States, Argentina, and Italy). A comparison of the tocochromanol profiles showed a higher content in Group I, although there were no significant differences in tocopherol content and composition between the genotypes within this group. All accessions in Groups I and II contained γ-tocotrienol and plastochromanol-8, which confirms the high nutritional value of flaxseeds. The composition of fatty acids varied depending on the varieties, with linolenic acid showing the greatest discrepancy. Based on the tocochromanol content and fatty acid composition, we conducted a principal component analysis (PCA) and cluster analysis, which revealed a greater similarity among the accessions in Group I. An analysis of the tocochromanol and fatty acid composition of flaxseeds is important from an agronomic and medicinal perspective and can be used to select the most appropriate flax cultivar.

Keywords
fatty acids; flaxseeds; PCA; vitamin E

Introduction

Linum encompasses 200 species, but only *Linum usitatissimum* L. (linseed and fiber) is cultivated. This species probably comes from the Mediterranean region, but it is widely cultivated for oil, fiber, and other industrial uses [1,2]. According to FAOSTAT, the annual global production of linseed oil is estimated to amount to 600 thousand tons. In Poland, linseed has been cultivated for centuries, but its production does not exceed 0.1% of the world harvests. Accessions of flax and hemp are collected by the Institute of Natural Fibers and Medicinal Plants [3,4]. In recent years, the area in which linseed is cultivated has increased because of the growing interest in the valuable health properties of linseed oil. Linseed products can be used in different branches of industry, namely, food, feed, and fibers. For decades, *L. usitatissimum* has occupied an important position in science, which has become even more prominent since 2012 when the flax genome sequence was published [5].

Linum usitatissimum is divided into three common types of cultivars: linseed, combined, and fiber. They differ in length, seed size, and number of primary panicle branches [2]. Although linseed can be grown throughout the world, the most favorable conditions prevail in a moderate climate. A moderate temperature and a minimum annual rainfall of 600–800 mm Hg H_2O are needed. Linseed is climate sensitive, which means that the climate influences its harvest. For example, flax seedlings are cold resistant but sensitive to high temperatures [2]. In addition to these factors, the yield depends on the genotype and biotic and abiotic stresses, as well as on agronomic treatments and cultivation methods [6]. This is reflected in the morphological features and biochemical and molecular changes in the flaxseeds and oils. The quality of linseed seeds and oils depends upon their fatty acid composition and other compounds, which in turn determine their use in industry. Mature flaxseeds are a source of a fat (varies between 35% and 46%), proteins (18–25%), and carbohydrates (23–30%). The content of these compounds depends largely on genotype and environmental conditions [7–9]. Linseed oil, which yields about 35–50% of the seed weight, is a dietary source of polyunsaturated fatty acids (PUFA), vitamin E, as well as phospholipids, sterols, phenolic acids, among other things [2]. Linseed oil content is comparable to sunflower oil fraction (more than 45%), but considerably higher than that in other oily seeds such as safflower (~24%), rapeseed (~40%), or soybean (~22%) [10]. Flax oil is mainly represented as a triacylglycerol fraction, which contains a large amount of linolenic acid (ω-3), linoleic (ω-6), and oleic acid. The proportion of these acids varies within a wide range; linolenic acid varies from 30% to as much as 70% of the total fatty acid content [11,12]. While the proportion of linoleic acid exceeds 20%, that of oleic acid is about 30%. Moreover, only a small proportion of saturated acids like palmitic and stearic acids can be detected (~7%) [1]. The seeds of flax accessions from the Polish Flax Collection (compiled by the Institute of Natural Fibres and Medicinal Plants) were found to be very rich sources of α-linolenic acid (ALA, C18:3) [13]. A study of 16 *L. usitatissimum* genotypes showed that the α-linolenic acid content ranged from 50.9% (LG-0,1-96 breeding line) to 59.2% (Bukoz variety). The content of oleic acid varied between 9.6% (CVT-LC-36) and 14.5% (Puławski variety), while that of linoleic acid (LA, C18:2) ranged from 19.2% (Bukoz) to 26.4% (CVT-LC-36) [13]. In studies on nine varieties of flaxseed, the fatty acid composition was as follows: α-linolenic acid, from 48.9% (Royale) to 59.9% (Alfonso Inta); linoleic acid, from 12.4% (Tabare) to 17.1% (AC Mc Duff); oleic acid, from 17.1% (Alfonso Inta) to 26.7% (Royale), of the total fatty acids [13].

Vitamin E is a family of related compounds (named tocochromanols), which show antioxidant properties [14]. They are divided into saturated tocopherols, unsaturated tocotrienols, and plastochromanol-8 (a homolog of a γ-tocotrienol). The best-studied function of vitamin E is its antioxidant properties, mostly as a chain-breaking agent that protects cell components against damage caused by reactive oxygen species [15]. Considering the relevance of vitamin E to human biology, it was assumed that α-tocopherol had the strongest antioxidant activity, followed by β-, γ-, and δ-homologs [16]. However, compared to α-tocopherol, tocotrienols have stronger antioxidant activity in in vivo and in vitro models [17]. In contrast to the fully substituted chromanol ring in α-tocopherol, the γ-homolog has an unsubstituted 5-position and, thus, is able to trap reactive nitrogen species synthesized during inflammation [14]. Oxidative stress has been linked to aging and numerous diseases, such as cancer, cardiovascular disease, and inflammation. According to the literature data, the tocopherol content of flax is estimated to be 300–700 mg/kg, with γ-tocopherol as a major fraction (more than 70%) [18]. Tocotrienols are also detected. Linseed oil prevents the development and spread of cancers, cardiovascular diseases, vascular problems, inflammatory disorders, and allergic reactions [19–22]. In addition to its preventative properties, linseed oil plays a pivotal role in protecting cells from oxidative damage [2]. Several clinical research studies on humans have suggested the beneficial effect of a high linseed oil (high α-linolenic acid) diet in reducing markers of oxidative stress and inflammation associated with the risk of many common chronic diseases (e.g., atherosclerosis) [23].

Considering the increasing use of linseed and linseed oil worldwide and limited scientific data on Polish linseed varieties, the aim of this study was to determine the tocochromanols and fatty acid content and composition of 11 flax varieties originating from Poland (nine fibre, two linseed) and three foreign local varieties (known as

Group I). We compared them with a second group of flax genotypes (Group II), which included six linseed accessions, six combined genotypes, and one accession of flax representing a fiber type of use. Such comparisons can be helpful for introducing new varieties (breeding material). Thus, our results provide data that are of physiological and nutritional interest.

Material and methods

Plant material

Two groups of flax genotypes were used. The first group (Group I) encompassed 11 genotypes native to Poland, two accessions from the Ukraine, and one flax genotype received from the Czech collection. The second group (Group II) consisted of 12 varieties of linseed from across the world: fiber genotypes (Atena, Temida, Luna, Artemida, Selena, Sara, Nike, Modran, Jan) and linseed (Jantarol, Bukoz), and three native genotypes (2010/0030, 2010/0031, Lino de Pedre). The accessions in Group I were obtained from the Polish Institute of Natural Fibers and Medicinal Plants (FAO code POL026), and are the result of cross breeding. The lists of Multi-Crop Passport Descriptors (MCPD V.2.1) are given in the supplementary material (Tab. S1, Tab. S2). The local varieties (2010/0030, 2010/0031) were collected from native locations in the Ukraine. Of the *L. usitatissimum* genotypes in Group II, the accessions analyzed were Bachmalskij 1056 (Russia), Betta 88 (Hungary), Buck 2/34 (Argentina), Capace (Italy), C.I.1128 (USA), and the combined accessions were AL-1200 (Uruguay), AL-CV-3 (Brazil), Barnaulskij 120 (unknown country of origin), Bison Boyena C.I.389 (USA), C.I.917 (USA), Entre Rios 15445 (Argentina), and fiber flax: Blenda 44205 (Portugal). All seeds were collected by the Polish Institute of Natural Fibers and Medicinal Plants and deposited in the Polish Gene Bank (Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, Poland).

Tocochromanols extraction and analysis

To analyze for tocochromanols, 80 mg of flaxseeds were precisely weighed and ground in a cold mortar with 1.5 mL of acetonitrile/methanol/water mixture (J. T. Baker, USA) (72:8:1, v/v). Next, the content was transferred to an Eppendorf tube, and centrifuged in a bench-top centrifuge (Eppendorf, Hamburg, Germany) for 3 min (10,000 *g*). The supernatants were transferred to new tubes and analyzed using high-performance liquid chromatography (HPLC). To analyze for plastochromanol-8, the seeds were extracted with a methanol/hexane mixture (J. T. Baker, USA) (17:1, v/v) under the conditions described above.

The HPLC measurements were conducted using a setup equipped with a PU-4180 pump, an FP4020 fluorescence detector (excitation–emission detection at 290–330 nm), and ChromNav software purchased from Jasco (Tokyo, Japan). We also used a C18 reverse-phase column Nucleosil 100 (250 × 4 mm, 5 µm) (MZ-Analysentechnik, Mainz, Germany) and a Rheodyne injector (Berlin, Germany) with 100-µL loop. All separations were performed with isocratic mobile phases. To analyze for tocopherols and tocotrienols, acetonitrile/methanol/water (72:8:1, v/v) was used as an eluent at a flow rate of 1.5 mL/min. To analyze for plastochromanol-8, a methanol/hexane (17:1, v/v) mixture at the same flow rate was used.

To identify and calculate the tocochromanol concentrations, high purity tocopherol and tocotrienol standards (α, 99.9%; β, 99.5%; γ, 99.5%; δ, 99.9%) (Merck-Millipore, Darmstadt, Germany) were used. Pure tocotrienol standards (~99.5%) were purchased from Calbiochem (Merck-Millipore). The plastochromanol-8 standard was synthesized from natural plastoquinone according to the method described by Gruszka and Kruk [24]. All stock solutions of the standards were prepared in absolute ethanol.

The tocochromanols were identified based on the retention time of the peaks in the extract samples and compared with the peaks of the standards. The concentration of tocochromanols (mg/100 g) was calculated based on the peak areas from the fluorescence detector with the corresponding peaks of the standard solutions.

Extraction of fatty acids and analysis

The fatty acids were extracted by homogenizing the seeds (43–62 mg) frozen in liquid nitrogen. These homogenates were suspended in 0.20 mL of toluene and transferred into screw-capped glass test tubes. Subsequently, 1.50 mL of methanol and 0.30 mL of 8.0% HCl solution were added. The test tubes were vortexed and then incubated at 45°C overnight to turn the fatty acids extracted into fatty acid methyl esters (FAMEs). After cooling to room temperature, the FAMEs were extracted by adding 1 mL of hexane and 1 mL of water [25]. The test tubes were vortexed, and then the hexane layers were analyzed using gas-liquid chromatography (GC). The *n*-hexane extracts were analyzed chromatographically on an Agilent 6890N gas chromatograph (Agilent Technologies, USA) equipped with a flame ionization detector (FID) and capillary column. Certified reference material 37 FAME MIX (Supelco, CRM 47885) and an internal standard (biphenyl) were used to identify and quantify the fatty acid profiles. The analyses were conducted using a gas chromatograph equipped with a split/splitless injector. An ionic liquid fused silica capillary column (IL100, Supelco, 28884-U) (30 m × 0.25 mm ID × 0.2 mm film thickness) with matrix 1,9-di(3-vinylimidazolium) nonane bis(trifluoromethanesulfonyl)imide phase was operated under the following programmed conditions: 50–240°C at 3°C min^{-1} in 30 min (detector and injector temperatures of 240°C), injection volume and mode of 0.5 μL and split (10:1), and helium 6.0 as carrier gas (velocity 40 cm min^{-1}). FAME was identified by comparison with the standard mixture (Sigma-Aldrich) and its time of retention.

Statistical analysis

The data were statistically evaluated using one-way analysis of variance (ANOVA). Significant differences between means were determined using Tukey's HSD multiple comparison test in STATISTICA 13.3 (TIBCO Software Inc., USA). A significance level of $\alpha = 0.05$ was selected. In addition, the relationship between linseed cultivars examined was calculated using principal component analysis (PCA) and hierarchical cluster analysis. First, the Pearson's linear correlation coefficients were calculated between the parameters. Based on the correlation matrix, PCA was conducted to obtain further complementary information on the structure of the data. The cluster analysis was performed using the group average method with Euclidean distance measure. Both analyses were carried out using the software package OriginPro 2018 (OriginLab, Northampton, USA).

The tocochromanol data shown in the tables and graphs are mean values ± standard deviation (*SD*) of triplicate measurements ($n = 3$).

Results

Tocochromanol content and composition

As shown in Tab. 1, total tocopherol content in Group I ranged from 15.4 mg/100 g seeds (in Artemida) to 22.2 mg/100 g (in the Jan variety). The mean tocopherol value was 19.6 mg/100 g seeds. Among the tocopherols, the γ-homolog predominated in all samples, with Jan, 2010/0030, Atena, and 2010/0031 having the highest values. α-Tocopherol constituted no more than 0.2% of the total tocopherol content (Tab. 1). There were no differences between tocopherol content and composition in linseed, combined genotypes (Bukoz, Jantarol, 2010/0030, 2010/0031, Lino de Pedre), and fiber varieties (Tab. 1).

Overall, the total content of tocopherols in cultivars across the world was lower (the mean value was 18.2 mg/100 g seeds) than that in local varieties (the mean value was 19.6 mg/100 g). Seeds with a total tocopherol content ranging above 20.0 mg/100 g were only found in two genotypes, C.I.917 and C.I.1128 (originating from the USA) (Tab. 2). Among these forms, γ-tocopherol predominated. The content of the remaining homologs was much lower, where α-tocopherol and δ-tocopherol accounted for

Tab. 1 Tocopherol content and composition in accessions of flax (Group I).

Accessions	Tocopherols (mg/100 g)			
	α	γ	δ	Total
Atena	0.12 ±0.01 ^{a,b}	21.12 ±0.76 ^{b}	0.29 ±0.01 ^{b,c,d,e}	21.53 ±0.78 ^{b}
Temida	0.13 ±0.001 ^{a,b}	19.53 ±0.75 ^{a,b}	0.26 ±0.02 ^{a,b,c}	19.92 ±0.77 ^{a,b}
Luna	0.16 ±0.03 ^{b}	18.00 ±1.80 ^{a,b}	0.26 ±0.02 ^{a,b,c,d}	18.42 ±1.84 ^{a,b}
Artemida	0.07 ±0.01 ^{a}	15.12 ±0.97 ^{a}	0.23 ±0.02 ^{a,b}	15.42 ±0.99 ^{a}
Selena	0.14 ±0.009 ^{a,b}	20.22 ±0.62 ^{a,b}	0.26 ±0.008 ^{a,b,c,d}	20.62 ±0.64 ^{a,b}
Sara	0.19 ±0.01 ^{b}	19.45 ±1.10 ^{a,b}	0.33 ±0.03 ^{c,d,e}	19.97 ±1.12 ^{a,b}
Nike	0.16 ±0.006 ^{b}	17.70 ±0.63 ^{a,b}	0.28 ±0.02 ^{b,c,d,e}	18.14 ±0.64 ^{a,b}
Modran	0.14 ±0.03 ^{a,b}	18.04 ±1.77 ^{a,b}	0.26 ±0.03 ^{a,b,c}	18.44 ±1.82 ^{a,b}
Jan	0.15 ±0.01 ^{a,b}	21.74 ±1.35 ^{b}	0.35 ±0.03 ^{e}	22.24 ±1.38 ^{b}
Bukoz	0.20 ±0.02 ^{b}	17.82 ±0.59 ^{a,b}	0.25 ±0.01 ^{a,b}	18.27 ±0.61 ^{a,b}
Jantarol	0.07 ±0.008 ^{a}	19.82 ±0.36 ^{a,b}	0.20 ±0.004 ^{a,b}	20.09 ±0.36 ^{a,b}
2010/0030	0.12 ±0.01 ^{a,b}	21.44 ±1.65 ^{b}	0.18 ±0.01 ^{a}	21.74 ±1.68 ^{b}
2010/0031	0.12 ±0.02 ^{a,b}	20.92 ±0.31 ^{b}	0.35 ±0.003 ^{d,e}	21.39 ±0.31 ^{b}
Lino de Pedre	0.14 ±0.02 ^{a,b}	18.18 ±0.92 ^{a,b}	0.27 ±0.01 ^{b,c,d,e}	18.59 ±0.95 ^{a,b}

Different letters in the same column indicate a statistically significant difference (α = 0.05).

Tab. 2 Tocopherol content and composition in worldwide accessions of flax (Group II).

Accessions	Tocopherols (mg/100 g)			
	α	γ	δ	Total
A1200	0.08 ±0.01 ^{a}	18.21 ±0.36 ^{a,b,c,d}	0.24 ±0.008 ^{b,c,d}	18.53 ±0.37 ^{a,b,c,d}
AL-CV-3	0.07 ±0.001 ^{a}	18.40 ±1.08 ^{a,b,c,d}	0.26 ±0.02 ^{c,d}	18.73 ±1.11 ^{a,b,c,d}
Bachmalskij 1056	0.12 ±0.02 ^{a,b}	19.57 ±0.83 ^{b,c,d}	0.22 ±0.009 ^{b,c,d}	19.91 ±0.86 ^{b,c,d}
Barnaulskij 120	0.09 ±0.008 ^{a,b}	16.65 ±0.60 ^{a,b,c,d}	0.20 ±0.02 ^{a,b,c}	16.94 ±0.61 ^{a,b,c,d}
Betta 88	0.13 ±0.013 ^{a,b,c}	15.47 ±1.03 ^{a,b,c}	0.18 ±0.01 ^{a,b}	15.78 ±1.04 ^{a,b,c}
Blenda 44205	0.09 ±0.009 ^{a,b}	15.08 ±0.68 ^{a}	0.21 ±0.008 ^{a,b,c}	15.38 ±0.69 ^{a}
Buck 2/34	0.15 ±0.004 ^{b,c,d}	17.02 ±0.69 ^{a,b,c,d}	0.21 ±0.01 ^{b,c}	17.38 ±0.70 ^{a,b,c,d}
Entre Rios 15445	0.08 ±0.002 ^{a}	15.28 ±0.64 ^{a,b}	0.14 ±0.003 ^{a}	15.50 ±0.64 ^{a,b}
Bison Boyena C.I.389	0.17 ±0.02 ^{c,d}	19.39 ±1.03 ^{a,b,c,d}	0.29 ±0.02 ^{d,e}	19.85 ±1.06 ^{b,c,d}
C.I.917	0.19 ±0.01 ^{d}	20.59 ±0.76 ^{d}	0.34 ±0.009 ^{e}	21.12 ±0.78 ^{d}
C.I.1128	0.18 ±0.01 ^{c,d}	19.73 ±0.97 ^{c,d}	0.26 ±0.01 ^{c,d}	20.17 ±0.99 ^{c,d}
Capace	0.14 ±0.01 ^{b,c,d}	18.56 ±1.21 ^{a,b,c,d}	0.24 ±0.02 ^{b,c,d}	18.53 ±0.37 ^{a,b,c,d}

Different letters in the same column indicate statistically significant difference (α = 0.05).

about 2.0% and 1.0% of the total tocopherol content, respectively (Tab. 2). Among the tocotrienol homologs, only the gamma-form was detected (Fig. 1, Fig. 2). The 26 *L. usitatissimum* accessions displayed variations in γ-tocotrienol content. In Group I, the γ-tocotrienol content was the highest in Atena, Jan, and 2010/0031 (0.11, 0.10, and 0.10 mg/100 g, respectively; Fig. 1).

The lowest amount of γ-tocotrienol was detected in Artemida and Jantarol (Fig. 1). In Group II, the γ-tocotrienol content was similar across all samples, with the exception of C.I.1128 genotype, which had the highest value (0.13 mg/100 g) (Fig. 2). Plastochromanol-8 was detected in all varieties (Fig. 3, Fig. 4). Overall, the plastochromanol-8 content in Group I was slightly lower (1.29–2.76 mg/100 g) than that in Group II (2.04–3.20 mg/100 g). The Polish cultivars with the highest plastochromanol-8 values

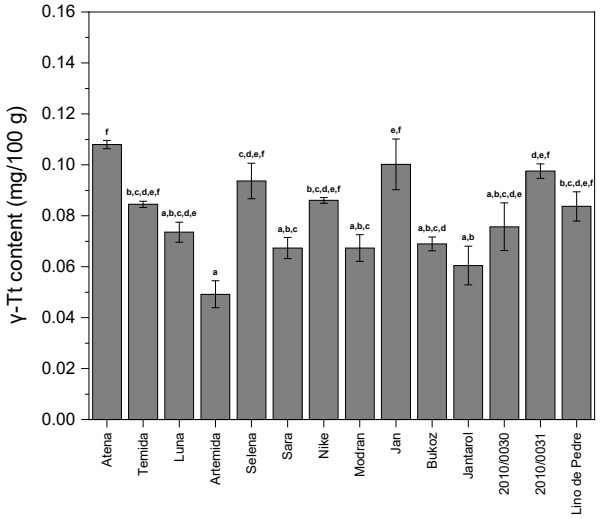

Fig. 1 Gamma-tocotrienol (γ-Tt) content in accessions of flax (Group I). Different letters indicate significant differences (Tukey's HSD multiple comparison test, α = 0.05).

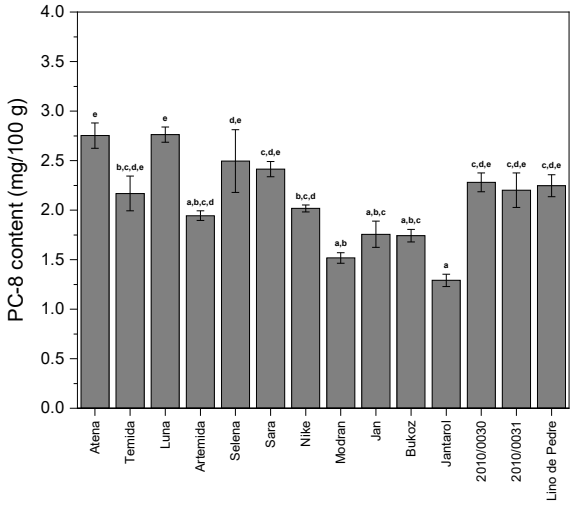

Fig. 3 Plastochromanol-8 (PC-8) content in accessions of flax (Group I). Different letters indicate significant differences (Tukey's HSD multiple comparison test, α = 0.05).

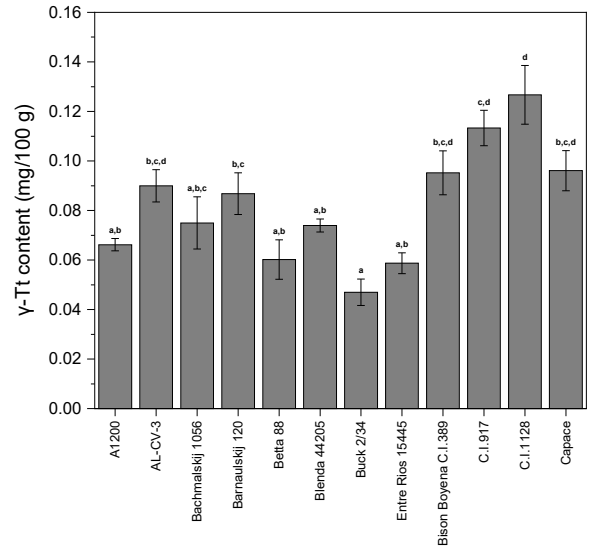

Fig. 2 Gamma-tocotrienol (γ-Tt) content in varieties of flax from across the world (Group II). Different letters indicate significant differences (Tukey's HSD multiple comparison test, α = 0.05).

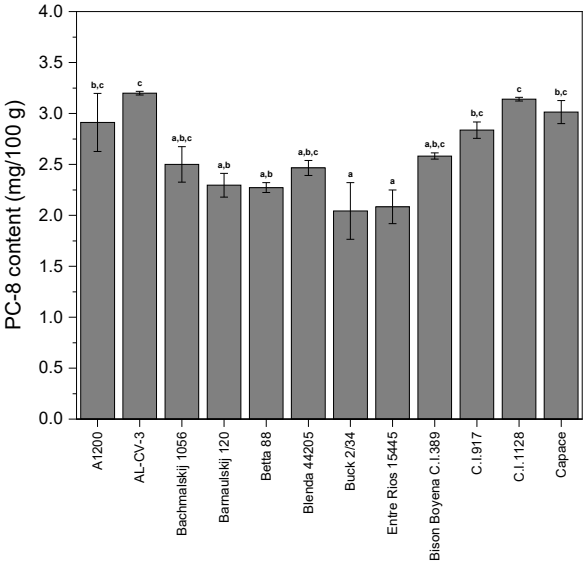

Fig. 4 Plastochromanol-8 (PC-8) content in worldwide accessions of flax (Group II). Different letters indicate significant differences (Tukey's HSD multiple comparison test, α = 0.05).

were Atena, Luna, Selena, and Sara (Fig. 3), while those with the highest values from across the world were AL-CV-3, C.I.1128, C.I.917, and Capace (Fig. 4). In most cases, the varieties with a high plastochromanol-8 content also had an elevated γ-tocotrienol concentration (Fig. 4, Fig. 5).

Fatty acid composition

The fatty acid composition of the linseed samples varied depending on the varieties (Tab. 3, Tab. 4). The biggest differences were reported for oleic, linoleic, and linolenic acids in both groups. In Group I, the oleic acid content in the Nike and Lino de Pedre varieties was the highest (26%) (Tab. 3). The lowest oleic acid content was found in the Atena and Temida cultivars (14% and 15%, respectively; Tab. 3). The linoleic acid content in the flax varieties ranged from 14% (Selena, Bukoz, Lino de Pedre) to 18% (Luna). Linolenic acid showed the most variation, i.e., between 59% and 58% in Atena,

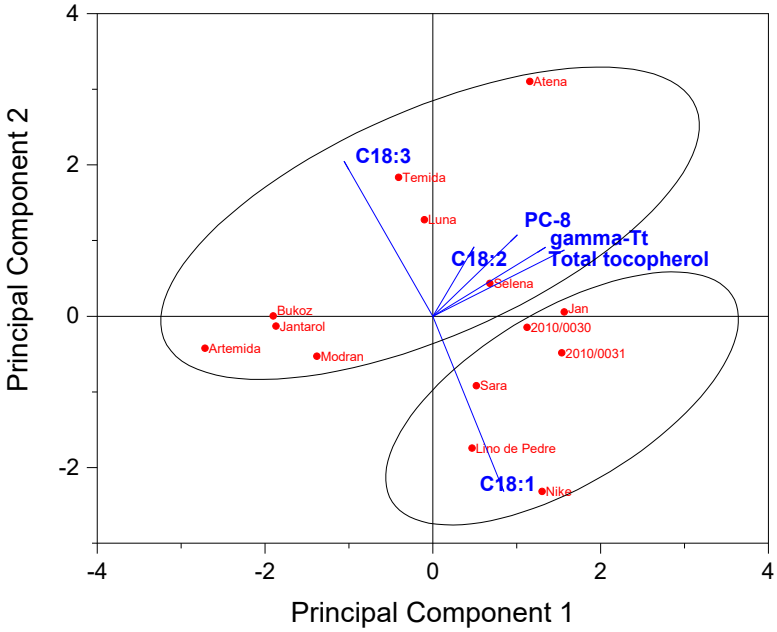

Fig. 5 Biplot representation of PCA for local varieties of flax (Group I).

Tab. 3 Total fatty acid composition (%) in accessions of flax (Group I).

Accessions	Fatty acids (%)				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Atena	6	4	14	17	59
Temida	6	3	15	17	58
Luna	6	3	18	18	56
Artemida	6	5	18	16	56
Selena	6	5	20	14	55
Sara	7	4	23	16	50
Nike	6	6	26	17	44
Modran	6	3	20	16	55
Jan	6	3	22	17	52
Bukoz	7	3	18	14	58
Jantarol	6	3	18	15	57
2010/0030	6	4	22	17	51
2010/0031	6	5	23	15	51
Lino de Pedre	6	3	26	14	51

Temida, and Bukoz to 44% in the Nike variety (Tab. 3). This corresponds to a previous study conducted by Silska [13]. The saturated acid content was about 10% (~6% for palmitic and ~4% for stearic acid) (Tab. 3). Compared to Group I, Group II showed a different pattern for unsaturated fatty acid content (Tab. 4). The content of oleic acid was higher in Group II (the mean value was 23.4%) than that in Group I (20.2%), and ranged between 17% in C.I.1128 and 27% in AL-CV-3 and Blenda 44205 (Tab. 4). However, the linoleic acid content was lower in accessions from the flax collection than those from Group I. Blenda 44205, Entre Rios 15445, and C.I.1128 had the highest linoleic acid content (17%), while Buck 2/34 had the lowest (13%). The linolenic acid content ranged from 47% (Blenda 44205) to 57% (C.I.1128) (Tab. 4). The mean value for linolenic acid was 51.5%, which was significantly lower than the mean value for flax genotypes in Group I (53.8%). The saturated fatty acid content was similar in both groups, accounting for approx. 10% (Tab. 3, Tab. 4).

Tab. 4 Total fatty acid composition (%) in accessions of flax from across the world (Group II).

Accessions	Fatty acids (%)				
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
A1200	7	4	21	14	54
AL-CV-3	7	4	27	14	48
Bachmalskij 1056	7	4	25	14	50
Barnaulskij 120	7	3	24	15	51
Betta 88	7	3	25	14	52
Blenda 44205	7	3	27	17	47
Buck 2/34	7	4	24	13	51
Entre Rios 15445	6	3	23	17	51
Bison Boyena C.I.389	6	3	24	16	51
C.I.917	7	3	21	16	53
C.I.1128	7	3	17	17	57
Capace	6	3	23	15	53

Principal component and cluster analysis

Principal component analysis (PCA) was carried out using the composition of the three fatty acids (oleic, linoleic, and linolenic), as well as the total tocopherol, γ-tocotrienol, and plastochromanol-8 content of each linseed cultivar in each group (I and II) as variables. The PCA results were visualized using biplots (Fig. 5, Fig. 6).

In Group I, the first three components represented 83.68% of the total variance (34.15%, 31.80%, and 17.73%, respectively). The first component was essentially explained by the total tocopherol and γ-tocotrienol content while the second was explained by the composition of the oleic and linolenic acids. The composition of the linolenic and oleic acids had opposite sign loadings, indicating that the second component could distinguish between linseed samples with a high linolenic acid and low oleic acid content. Therefore, the samples were placed into two main groups (Fig. 5), which included subgroups according to the total tocopherol and γ-tocotrienol content. For example,

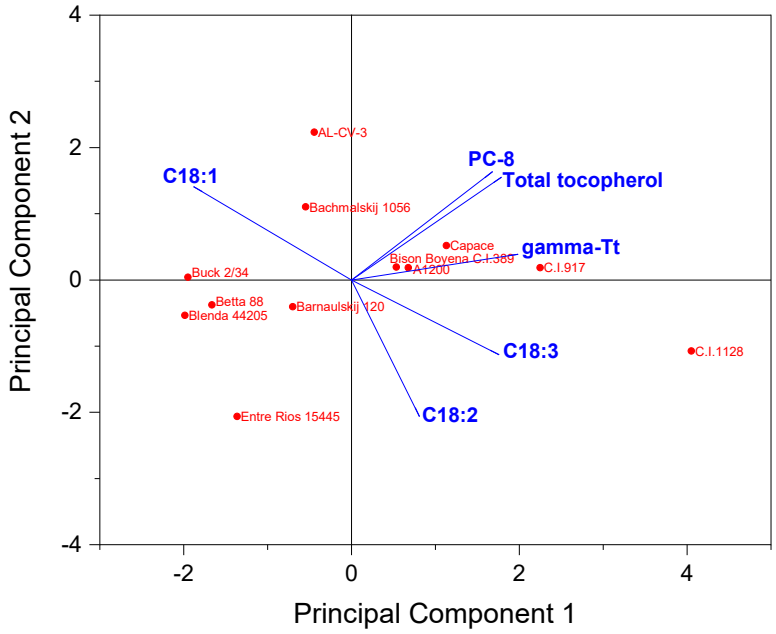

Fig. 6 Biplot representation of PCA for worldwide accessions of flax (Group II).

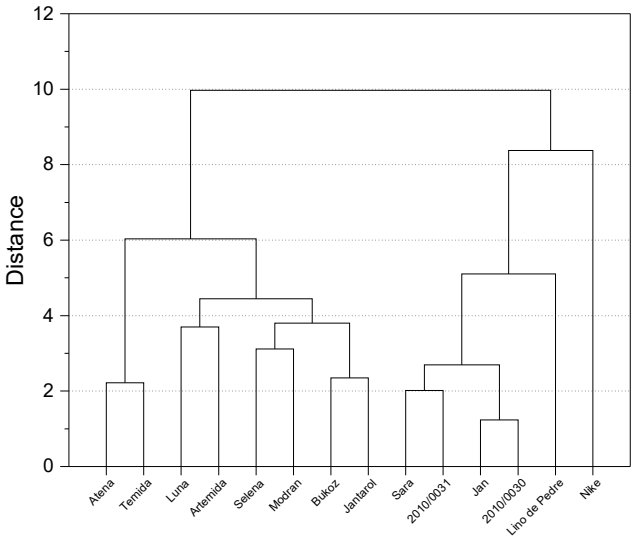

Fig. 7 Dendrogram derived from a cluster analysis of 14 flax accessions native to Poland and the Ukraine (Group I).

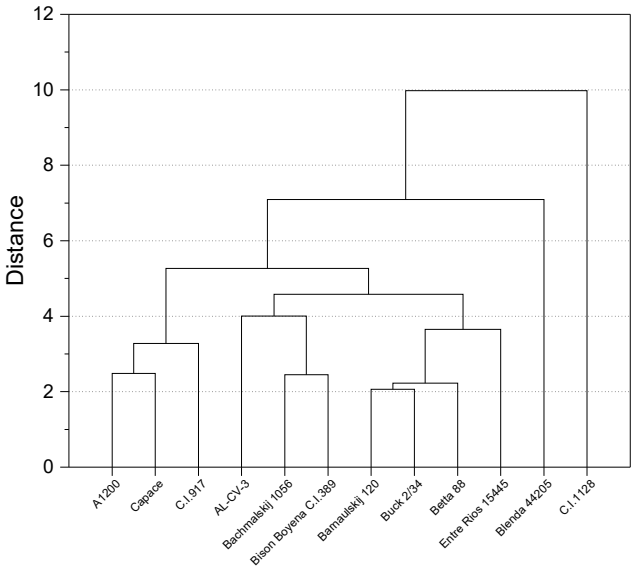

Fig. 8 Dendrogram derived from a cluster analysis of 12 flax accessions from across the world (Group II).

Jan, 2010/0030, and 2010/0031 were characterized by a high total tocopherol and γ-tocotrienol content while Sara, Lino de Pedre, and Nike had lower concentrations of these compounds.

In Group II, the first three components contributed 92.58% to the total variance (55.83%, 19.05%, and 17.70%, respectively) (Fig. 6). The first component was explained by the total tocopherol and γ-tocotrienol content, as well as the composition of the linolenic and oleic acids. The second component was mainly represented by the linoleic acid and plastochormanol-8 content. The linseed varieties were widely dispersed across the biplot in Group II (Fig. 6), showing no prominent groupings. Only the C.I.1128 genotype, which has the highest linolenic acid content, as well as a high total tocopherol and γ-tocotrienol content, was separated from the other genotypes in the group.

In order to visualize the similarities between the flax varieties, we conducted a cluster analysis (Fig. 7, Fig. 8). The dendrograms were obtained using the same data that were used in the PCA. Fig. 7 shows local flax varieties (Group I) arranged into two clades. The first clade encompassed the Atena, Temida, Luna, Artemida, Selena, Modran, Bukoz, and Jantarol cultivars, whereas the second clade included the Sara, 2010/0031, Jan, 2010/0030, Lino de Pedre, and Nike cultivars (Fig. 7). The clade pattern suggests that the similarities are an effect of their common origins (i.e., native to Poland and the Ukraine) including, among other things, climate, biotic, and abiotic circumstances. Interestingly, the first clade included varieties mostly obtained using the crossbreeding method (Fig. 7).

The second clade included those three cultivars that were obtained from native standings (Lino de Pedre, 2010/0030, and 2010/0031). Our conclusions are supported by the results presented in the second cladogram (Fig. 8). The clade obtained for cultivars from across the world (Group II) did not show any distinct groupings (Fig. 8). Considering that they originated from different regions (different longitude, latitude, climate, etc.), the tendency for them to group on a biochemical level did not occur.

Discussion

The content and composition of tocochromanols and fatty acids depends strictly on the flax variety and origin. Our results showed variability in the lipid-soluble compounds in both of the analyzed flax groups, which is consistent with results obtained in other studies. Oomah et al. [26] reported 96–98% of γ-tocopherol and lower proportions of α-tocopherol in eight flax cultivars. Likewise, Bozan and Temelli [27] reported that γ-tocopherol was the main tocopherol (79.4 mg/100 g oil), which accounted for 95% of the total tocopherols in Turkish local flaxseeds varieties. The differences in tocopherol types and content between our study and those reported in other studies may be due to differences in genotypes, solar radiation, and soil types. Velasco and Goffman [18] suggested that this kind of pattern (γ-tocopherol domination, low α-tocopherol content, and the presence of plastochromanol-8 together with fatty acid composition) identifies important chemotaxonomic parameters for the genus *Linum* sect. *Linum*. They analyzed 288 accessions of 24 *Linum* species. The values they obtained for the tocopherol content of *L. usitatissimum* accessions were slightly lower (10.2–16.9 mg/100 g seed) than those presented in our study. Zou et al. [28] compared the tocopherol content

in the oils of linseed and fiber flax of 13 Chinese varieties. Their results are consistent with our study results and indicate that the tocopherol content is similar in both types of cultivars (the average content of γ-tocopherol in fiber flax was 33.8 ±0.4 mg/100 g of oil, while that in the oil of linseed genotypes was 34.2 ±0.5 mg/100 g of oil). These results suggest that fiber flaxseeds also contain oil of a good quality, i.e., with a high proportion of unsaturated fatty acids and high vitamin E content.

Plastochromanol-8 is a less known tocochromanol homolog, and might be a stronger antioxidant than tocopherols. Velasco and Goffman [18] also reported the presence of plastochromanol-8 in *L. usitatissimum* accessions, but its level was twice as high as the average value reported in the present study (2.6 to 7.2 mg/100 g seeds). The presence of vitamin E homologs in flaxseeds has important health benefits. In addition, they protect flax fatty acids against oxidation, thus, fulfilling their antioxidant potential [14].

The composition of fatty acids in flax has an important agronomic significance. The polyunsaturated fatty acid (PUFAs) levels found in our study were similar to those reported in other studies [1,28,29]. An analysis of five linseed varieties of German origin (collected by the seed herbarium in Turkey) showed variations in their fatty acid content. Among the oil samples analyzed, linolenic acid ranged between 47.8% and 59.9%, linoleic acid 10.2–13.4%, and oleic acid 17.0–24.8% [1]. These authors also showed a large discrepancy in tocopherol content, which was not observed in our study. An analysis of seven Romanian linseed cultivars showed that linolenic acid in oil dominates (ranging from 47.5% to 64.1%). Interestingly, the fatty acid profile varied not only among cultivars but also within the same cultivars collected in different years, suggesting that climate had a significant influence (mainly hydrothermal conditions) [29]. This phenomenon is also supported by a study on 198 Ethiopian linseed cultivars of flax [30], as well as nine Kazakhstan varieties [31]. Zou et al. [28] compared several Chinese varieties of linseed and fiber flax and showed great variation in linolenic acid content in these varieties. The analysis of the tocochromanol and fatty acid composition of flaxseeds is important from a physiological and nutritional perspective. A high oil content with a large amount of polyunsaturated fatty acids, as well as a considerable amount of vitamin E (especially γ-tocopherol and plastochromanol-8), makes linseed a valuable material. Biochemical analysis (fatty acid profiling and vitamin E content) can be further used to estimate and select the most appropriate flax cultivar for certain applications, i.e., nutritional usage. It is also worth recognizing that flax adapts to regional climatic circumstances and environmental effects because the content of these compounds can even vary between flax accessions from the same region.

To compare natural resources and products, multivariate analysis techniques are often used to identify naturally clustering groups based on the similarities between samples. PCA has been widely used for this purpose. The results of the PCA in the present study, which showed origin-dependent clustering in the first group, conform to the results reported in several other studies. Anastasiu et al. [29] performed a PCA to classify Romanian linseed cultivars according to the degree of oil unsaturation in terms of weather conditions and seasonal response. You et al. [32] analyzed a flax core collection totaling 391, which represented a broad range of geographical origins, different improvement statuses, and two morphotypes. They performed, among other things, PCA analysis, which included seed and fiber quality as well as disease resistance traits in flax. This cluster contained accessions originating from different geographical regions, indicative of a weak relationship between trait performance and geographical origins [32]. There is consensus that linseed yields and the biochemical quality of their seeds vary greatly across the universe and are strongly influenced by climatic conditions [33].

Conclusions

We compared tocochromanol content and composition in two groups consisting of local and worldwide flax varieties. Although Group I had a higher tocochromanol content than that in Group II, there were no differences in tocopherol content and composition among the genotypes within Group I. All accessions analyzed contained a high amount of γ-tocotrienol and plastochromanol-8, which confirms the high nutritional value of

flaxseeds. The fatty acid composition varied depending on the varieties, with linolenic acid showing the greatest discrepancy. Analyzing the composition of tocochromanols and fatty acids in flaxseeds is important from a physiological and nutritional perspective. Biochemical analysis (fatty acid profiling and vitamin E content) can be further used to estimate and select the most appropriate flax cultivar. The PCA and cluster analysis indicated that flax might adapt to regional climatic conditions and environmental effects.

Supplementary material

The following supplementary material for this article is available at http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.3636/0:

Tab. S1 Passport data of flax accessions in Group I.

Tab. S2 Passport data of flax accessions in Group II.

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