Content of biogenic elements and fatty acid composition of fenugreek seeds cultivated under different conditions

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) is a medicinal plant that has been recognized for its numerous health benefits throughout the centuries. The species is a rich source of biogenic elements, and it has a favorable composition of fatty acids. This study evaluated the effect of agrotechnological factors on the chemical composition of fenugreek seeds. The experiments conducted in north-eastern Poland had a fractional factorial design with 54 plots. A total of five agrotechnological factors were tested: seed inoculation with *Rhizobium meliloti*, sowing date, row spacing, weed control, and protection against fungal pathogens. The chemical composition of fenugreek seeds was influenced mainly by sowing date, row spacing and plant protection. Fenugreek seeds grown in north-eastern Poland contained 26.0% protein and 4.8% oil. Delayed sowing increased N content (9.2%) and decreased the content of P (8.8%), K (5.1%) and Mg (2.8%). An increase in row spacing from 15 cm to 45 cm promoted the accumulation of Fe (31%). Agrotechnological factors induced the greatest variations in the composition of saturated fatty acids (mean difference of up to 14.5%), followed by monounsaturated (up to 9.5%) and polyunsaturated fatty acids (up to 4.5%). Total unsaturated fatty acids accounted for 80% of the fatty acid profile, with a predominance of essential fatty acids in oil: linoleic acid (37.9%) and α-linolenic (28.2%) acid. Sowing date and weed control were responsible for up to 3.1%-4.5% of differences in concentrations of essential fatty acids between treatments.

Key words: Agrotechnological factors, biogenic elements, fatty acids, medicinal crop, *Trigonella foenum-graecum*.

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INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) belongs to the botanical family *Papilionaceae*. Its native geographic range is the area extending from Iran to northern India, but it is presently cultivated also in other regions of the world (Zakia et al., 2014; Bieńkowski et al., 2016). Fenugreek is grown mostly for its seeds, seldom for straw as cattle forage, and fresh fenugreek leaves are consumed in some cuisines, including Indian. This species has been mainly used in medicine for centuries. Fenugreek seeds contain chemical compounds with medicinal properties, and in the past, they were consumed by pregnant women (Taloubi et al., 2013). Fenugreek seeds, leaf extractions, roots and stems have scientifically proven antidiabetic, anticarcinogenic, antimicrobial, and other health-promoting properties (Chauhan et al., 2011; Khorshidian et al., 2016). It is worth noting that fenugreek seed fibers, which are composed mostly of non-starch polysaccharides (saponins, tannin, pectin, and others), lower the rate of glucose absorption in the intestines and regulate blood sugar levels. On account of those properties, fenugreek seeds are recommended for diabetes diets (Srinivasan, 2006). Fenugreek seeds contain chemical compounds which are highly valued in the cosmetics industry. Akhtar et al. (2010) reported that cream bases and cream formulations containing fenugreek seed extract substantially improved skin elasticity, hydration and skin's ability to resist photo-aging.

Fenugreek seeds are a natural source of vitamins such as thiamine, biogenic elements such as Fe, Si and Na, and a rich source of P and S (El Nasri and El Tinay, 2007). In the research conducted by Kochhar et al. (2006), fenugreek seeds contained 25.8% crude protein and 6.53% oil. Seed DM had the following chemical composition: 3% ash, 6.28% crude fiber and 58.13% total carbohydrates. El Nasri and El Tinay (2007) estimated the protein content of fenugreek seeds at 28.4%, crude fiber at 9.3% and crude fat at 7.1%. The fatty acid profile was dominated by unsaturated acids: oleic, linoleic, and alpha-linolenic acids which accounted for 16.3%, 50.0% and 24.4% of total fatty acids, respectively. The unique mineral and organic properties of fenugreek are exploited in the production of functional and nutritional foods as well as nutraceuticals and cosmetics (Hooda and Jood, 2005; Lubbe and Verpoorte, 2011).

The profile and content of chemical compounds in fenugreek seeds may be differentiated by production technology and growing conditions. The most important agrotechnological factors and postharvest processes determine the chemical composition of seeds and the shelf life of the resulting products by preserving

vitamins, enzymes, flavonoids and the structure of components responsible for essentiality, aroma, color and moisture content. Those quality properties of fenugreek seeds and products are largely determined by soil moisture content (Hussein and El-Dewiny, 2011) and agronomic factors such as sowing date, sowing density, fertilization and plant protection during the growing season (Wierzbowska and Żuk-Gołaszewska, 2014; Żuk-Gołaszewska et al., 2015; Zapotoczny et al., 2015).

The objective of this study was to determine the impact of agrotechnological factors on the chemical composition of fenugreek seeds.

MATERIALS AND METHODS

Origin of seeds

Fenugreek seeds were obtained in a field experiment conducted in 2009 in northeastern Poland (53°43' N, 20°24' E). The experiment had a fractional factorial design, and treatments were randomly assigned to 54 plots. Five agrotechnological factors were tested: (A) seed inoculation with *Rhizobium meliloti* bacteria (0: no, 1: yes); (B) sowing date (0: earliest possible date, 1: delayed by 10 d, 2: delayed by 20 d); (C) row spacing (0: 15 cm, 1: 30 cm, 2: 45 cm); (D) weed control (0: mechanical, 1: chemical); (E) protection against fungal pathogens (0: seeds not dressed, chemical plant protection, 1: seeds dressed, no chemical plant protection, 2: seeds dressed, chemical plant protection). The experiment was set up on Haplic Cambisol (Eutric) soil of quality class IVa with a light loam overlay (IUSS Working Group WRB, 2015). The soil was characterized by slightly acidic pH, moderate content of P and K, and low content of Mg (Bieńkowski et al., 2016).

Laboratory analyses

Harvested seeds were cleaned, dried to 12 ± 0.5% moisture content. Seed samples (0.5 g) were mineralized in concentrated sulfuric acid (VI) with the use of hydrogen dioxide as oxidant. Total N content was determined calorimetrically with hypochlorite (Baethen and Alley, 1989). Phosphorus content was determined by the vanadium-molybdenum method; K, Ca and Na concentrations were analyzed by atomic emission spectroscopy (AES), and Mg content was measured by atomic absorption spectroscopy (AAS) (Żuk-Gołaszewska et al., 2015). The content of micronutrients was determined in seed samples (0.5 g) mineralized in a mixture of perchloric and nitric acid with the addition of hydrochloric acid. Mineralized seeds were transferred to 50 cm^3 flasks and supplemented with water. Micronutrient concentrations were measured by AAS in a Shimadzu spectrophotometer. The composition of the identified fatty acids (Table 1) was determined by the chromatographic separation method modified by Żegarska et al. (1991).

Fat was extracted by the Soxhlet method (AOAC, 2005). Fatty acids were separated and determined by gas chromatography in a gas chromatograph (CP-3800, Varian, Walnut Creek, California, USA). Fatty acid methyl esters (FAME) were prepared according to the modified Peisker method (methanol:chloroform:concentrated sulfuric acid, 100:100:1, v/v) (Żegarska et al., 1991). The resulting FAMEs were stored in sealed tubes and were analyzed by gas chromatography-flame ionization detection (GC-FID; column: 50 m × 0.25 mm × 0.25 μm). The temperature of the GC injection port was set to 225 °C in split mode (split ratio 50:1) with helium as the carrier gas at a constant flow rate of 1.2 mL min^{-1}. Detector temperature was 250 °C and column temperature was 200 °C. Fatty acids were identified by comparing their retention times with those of pure FAME standards (Sigma-Aldrich, St. Louis, Missouri, USA) and peaks in the analyzed samples. The relative content of fatty acids was expressed as the percentage of the total surface area of all fatty acids detected in each sample.

Table 1. Composition of fatty acids identified in fenugreek seeds.

Symbol	Systematic name	Common name
Saturated fatty acids (SFAs)		
$C_{12:0}$	Dodecanoic acid	Lauric acid
$C_{14:0}$	Tetradecanoic acid	Myristic acid
$C_{15:0}$	Pentadecanoic acid	
$C_{16:0}$	Hexadecanoic acid	Palmitic acid
$C_{17:0}$	Heptadecanoic acid	Margaric acid
$C_{18:0}$	Octadecanoic acid	Stearic acid
$C_{20:0}$	Eicosanoic acid	Arachidic acid
$C_{22:0}$	Docosanoic acid	Behenic acid
Monounsaturated fatty acids (MUFAs)		
$C_{12:1}$	Dodecenoic	Linderic acid
$C_{16:1}$	Hexadecenoic acid	Palmitoleic acid
$C_{17:1}$	Heptadecenoic acid	
$C_{18:1}$	Octadecenoic acid	Oleic acid
$C_{20:1}$	Eicosenoic acid	Gadoleic acid
Polyunsaturated fatty acids (PUFAs)		
$C_{18:2}$ Essential fatty acid (EFA)	Octadecadienoic acid	Linoleic acid
$C_{18:3}$ Essential fatty acid (EFA)	Octadecatrienoic acid	Alpha-linolenic acid
$C_{20:2}$	Eicosadienoic acid	

Statistical analyses

The parameters describing the chemical properties of fenugreek seeds were analyzed by factorial and multivariate ANOVA. Nonsignificant higher-order interactions constituted the experimental error. Data were arranged in two groups of variables to estimate the impact of agrotechnological factors and interactions on the chemical properties of seeds: the "biogenic elements" group describing the content of micronutrients and macronutrients, and the "fatty acids" group describing the fatty acid composition of seeds. In MANOVA, the effect size of agrotechnological factors and interactions was measured with the use of partial eta-squared: $\eta^2_p = SS_{effect}/(SS_{effect}+SS_{error})$, where SS_{effect} is the sum of squares for the effect of interest and SS_{error} is the error term associated with this effect. In factorial ANOVA, the effect size η^2 was measured as the ratio between the variance of a factor or interaction (SS_{effect}) and the total variance of a given chemical property (SS_{total}). All analyses were performed at a significance level $p < 0.05$ in the Statistica package (Dell Inc, Round Rock, Texas, USA).

RESULTS AND DISCUSSION

Main and interaction effects of agrotechnological factors – effect size

The analyzed agrotechnological factors had a varied influence on the main effects and interaction effects of the analyzed chemical properties of fenugreek seeds. Multivariate ANOVA (MANOVA) revealed that sowing date (B) contributed to differences in the content of biogenic elements and fatty acids. The second factor which was responsible for significant variations in the fatty acid profile of fenugreek seeds was weed control (D) (Table 2). In the group of main effects and interaction effects of biogenic elements, the main effects of sowing date (B) (70.1%) and row spacing (C) (43.6%) and the Row spacing × Weed control interaction effect (DE) (39.8%) were chiefly responsible for variations in the analyzed agrotechnological factors (or interactions) and experimental error (η^2_p). The effect size of fatty acids was significantly greater than the effect size of biogenic elements. Weed control (D) (94.1%), sowing date (B) (86.6%), Sowing date × Weed control interaction (BD) (85.5%) and Row spacing × Weed control interaction (CD) (80.2%) were characterized by the greatest effect size.

Factorial ANOVA revealed that sowing date differentiated the content of N, P, K, saturated fatty acids – myristic acid $C_{14:0}$, palmitic acid $C_{16:0}$, stearic acid $C_{18:0}$, arachidic acid $C_{20:0}$, monounsaturated oleic acid $C_{18:1}$, and essential fatty acids – linoleic acid $C_{18:2}$, and alpha-linolenic acid $C_{18:3}$ (Table 3). Weed control significantly differentiated the concentrations of fatty acids – lauric acid $C_{12.0}$, stearic acid $C_{18:0}$, arachidic acid $C_{20:0}$, heptadecanoic acid $C_{17:1}$ and linoleic acid $C_{18:2}$, whereas the date of Sowing × Weed control interaction (BD) also influenced the content of N and fatty acids – palmitoleic acid $C_{16:1}$, linoleic acid $C_{18:2}$, alpha-linolenic acid $C_{18:3}$ and eicosadienoic acid $C_{20:2}$. The concentrations of alpha-linolenic acid $C_{18:3}$, an exogenous essential fatty acid, were determined by seed and plant protection against fungal pathogens.

Table 2. Wilk's statistic in MANOVA and partial eta-squared (η^2_p) for the content of biogenic elements and fatty acids in fenugreek seeds.

	Biogenic elements			Fatty acids		
Factor/ Interaction	Wilk's statistic	p-value	η^2_p	Wilk's statistic	p-value	η^2_p
A	0.660	0.454	0.340	0.396	0.796	0.604
B	0.089	0.000	0.701	0.018	0.043	0.866
C	0.318	0.159	0.436	0.069	0.434	0.737
D	0.814	0.848	0.186	0.059	0.016	0.941
E	0.402	0.360	0.366	0.080	0.515	0.717
A×B	0.415	0.394	0.356	0.049	0.267	0.780
A×C	0.463	0.532	0.320	0.079	0.508	0.719
B×C	0.322	0.860	0.270	0.011	0.594	0.687
A×D	0.702	0.566	0.298	0.358	0.721	0.642
B×D	0.489	0.606	0.301	0.021	0.059	0.855
C×D	0.661	0.936	0.187	0.039	0.190	0.802
A×E	0.568	0.797	0.247	0.070	0.441	0.736
B×E	0.316	0.847	0.273	0.022	0.866	0.622
C×E	0.161	0.259	0.398	0.016	0.746	0.656
D×E	0.706	0.970	0.160	0.097	0.625	0.689

A: Seed inoculation, B: sowing date, C: row spacing, D: weed control, E: antifungal protection.

Biogenic elements

In the group of the examined agrotechnological factors, sowing date (B) and row spacing (C) significantly influenced the chemical composition of fenugreek seeds. On average, 1 kg DM contained 41.6 g N, 18.6 g K, 7.17 g P, 3.00 g Ca, 2.12 g Mg, 0.960 g Na and 0.234 g Fe (Table 4). The protein content of seeds from 54 experimental plots, determined by multiplying the N content by an N-to-protein conversion factor, ranged from 22.2% ($A_1B_0C_2D_1E_1$) to 29.7% ($A_1B_2C_1D_0E_1$), with an average of 26.0%. Our results were approximately 9% higher than the values reported by Rahmani et al. (2014) and Singh et al. (2013), where protein percentages ranged from 21.28% to 22.58% and

Table 3. Significant main effects and interaction effects of chemical properties of fenugreek seeds determined by ANOVA.

Source of variation	Biogenic elements	Fatty acids
A		$C_{14:0}$, $C_{20:0}$
B	N, P, K, Mg	$C_{14:0}$, $C_{16:0}$, $C_{20:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$
C	Fe	$C_{16:1}$, $C_{20:1}$
D		$C_{12:0}$, $C_{18:0}$, $C_{20:0}$, $C_{17:1}$, $C_{18:2}$
E		$C_{14:0}$, $C_{22:0}$, $C_{17:1}$, $C_{18:3}$
A×B		$C_{18:0}$, $C_{20:0}$, $C_{16:1}$
A×C		$C_{17:0}$, $C_{12:1}$, $C_{16:1}$, $C_{20:1}$
B×C		$C_{16:1}$
A×D	N	
B×D	N	$C_{16:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:2}$; crude fat
C×D		$C_{16:1}$, $C_{20:1}$
A×E	P	$C_{16:0}$, $C_{16:1}$, $C_{20:1}$
B×E		$C_{16:1}$
C×E	N, Fe	$C_{16:0}$
D×E		$C_{16:1}$, $C_{20:1}$

A: Seed inoculation, B: sowing date, C: row spacing, D: weed control, E: antifungal protection.

Table 4. Content of biogenic elements in fenugreek seeds subjected to different treatments of agrotechnological factors.

Factor	Level	N	K	P	Ca	Mg	Na	Fe
		g kg^{-1} DM						
A	0	41.3	18.5	7.13	2.98	2.13	0.980	0.233
	1	41.8	18.8	7.22	3.02	2.11	0.940	0.235
B	0	40.1b	19.6a	7.30a	2.90	2.13a	0.956	0.236
	1	40.8b	18.1b	7.29a	3.02	2.15a	0.937	0.244
	2	43.8a	18.2b	6.93b	3.07	2.07b	0.986	0.222
C	0	40.8	18.3	7.10	2.99	2.10	0.946	0.202c
	1	42.4	19.1	7.14	3.02	2.12	0.935	0.235b
	2	41.4	18.5	7.28	2.99	2.14	0.999	0.265a
D	0	41.8	18.9	7.18	2.98	2.11	0.952	0.238
	1	41.3	18.3	7.17	3.02	2.12	0.967	0.230
E	0	41.3	18.8	7.25	3.01	2.12	0.994	0.229
	1	41.2	18.8	7.17	2.93	2.11	0.925	0.254
	2	42.2	18.3	7.11	3.06	2.13	0.961	0.219
Mean		41.6	18.6	7.17	3.00	2.12	0.960	0.234

A: Seed inoculation with *Rhizobium meliloti* (A0: no, A1: yes); B: sowing date (B0: earliest date, B1: 10 d delay, B2: 20 d delay); C: row spacing (C0: 15 cm, C1: 30 cm, C2: 45 cm); D: weed control (D0: mechanical, D1: chemical); E: antifungal plant protection (E0: non-dressed seeds, chemical protection, E1: dressed seeds, no chemical protection, E2: dressed seeds, chemical protection).
Values with the same letter do not differ significantly in Tukey's test.

from 18.1% to 24.63%, respectively. In the other study by Kochhar et al. (2006), protein concentrations were nearly identical to those noted in our study, and 9% higher than those reported by El Nasri and El Tinay (2007).

When sowing was delayed by 20 d (from B0 to B2), the N content of fenugreek seeds increased by 9.2% (from 40.1 to 43.8 g kg^{-1} DM), whereas P concentrations decreased by 8.8% (from 19.6 to 18.2 g kg^{-1} DM), K by 5.1% (from 7.30 to 6.93 g kg^{-1} DM) and Mg by 2.8% (from 2.13 to 2.07 g kg^{-1} DM). An increase in row spacing from 15 cm (C1) to 45 cm (C3) contributed to the accumulation of Fe whose content increased by 31.1% (from 0.202 to 0.265 g kg^{-1} DM).

The presence of a relationship between the mineral status of seeds and agronomic and environmental factors was reported by other authors (Hassanein et al., 2012; Abou-Shleel, 2014; Żuk-Gołaszewska et al., 2015). In the work of Al-Jasass and Al-Jasser (2012), production and environmental factors differentiated the percentage of protein (12.91 ± 0.4%) and content of K (603 ± 15.0 mg 100 g^{-1}), Mg (42 ± 5.0 mg 100 g^{-1}), Ca (75 ± 9.0 mg 100 g^{-1}) and Fe (25.8 ± 1.2 mg 100 g^{-1}) in fenugreek seeds. In a study by Ahmed et al. (2012), different fertilizers (organic and bio-fertilizers) significantly increased protein content from 19.9% to 23.8% and oil content from 10.22% to 12.85%. Abou-Shleel (2014) found that sowing dates exerted a significant influence on the chemical composition and active ingredients of fenugreek seeds. In this study, seeds sown on the first (earliest) date were characterized by the highest content of protein and lipids. The authors have attributed this effect to air temperature during seed maturation which increased the plants' respiration rates and, consequently, decreased the accumulation of chemical components. Our results are consistent with the findings of Hassanein et al. (2012). In another study by Żuk-Gołaszewska et al. (2015), fenugreek was exposed to water deficit stress, and higher rates of K fertilizer significantly increased the content of crude protein (by 3.2%-5.4%) and K (by 7%-8%). Hussein and El-Dewiny (2011) demonstrated that soil water deficit reduced concentrations of N, P and Cu, increased the content of Fe, and stabilized K content of fenugreek seeds across water deficit variants.

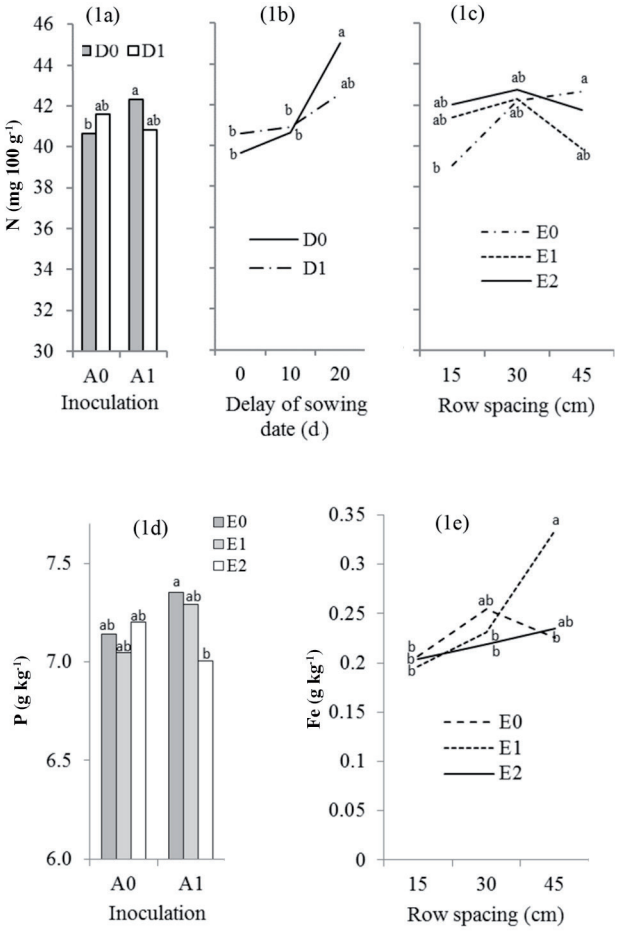
Significant two-factor interactions are presented in Figure 1. Non-inoculated seeds (A0) grown in plots with chemical weed control (D1) contained more N than seeds grown in plots with mechanical weed control, whereas a reverse relation was observed in inoculated seeds (Figure 1a). Delayed sowing led to higher N accumulation in seeds from treatments subjected to mechanical weeding than from plots with chemical weed control (Figure 1b). Regardless of fungicide application, the increase in row spacing from 15 to 30 cm increased the N content of seeds. A further increase in row spacing to 45 cm stabilized or decreased N concentrations (Figure 1c).

A similar trend was noted in the content of Fe in agrotechnological variants with seed dressing (E0) and seed dressing combined with fungicide application (E3). In plots subjected to antifungal plant protection only (E1), Fe accumulation increased significantly with the highest row spacing of 45 cm (Figure 1d). Inoculated seeds from treatments subjected to seed dressing only or chemical protection only were more likely to accumulate P, whereas the P content of seeds grown in plots with seed dressing and antifungal plant protection (E3) was significantly lower than in the variant without inoculation (Figure 1e).

Fatty acids

In our study, the total oil content of fenugreek seeds ranged across agrotechnological variants from 3.37% to 5.82%, with an average of 4.77% (SE = 0.077). In a Canadian experiment investigating different fenugreek cultivars grown on semiarid soil in three experimental fields, the lipid content of seeds fluctuated widely from 5.8% to 15.2% (Ciftci et al., 2011). Abdelgani et al. (1999) attributed the significant increase in the oil content of fenugreek seeds to inoculation with *Rhizobium* strains. The results of the

Figure 1. Influence of significant interactions between agrotechnological factors on the accumulation of N (1a: Inoculation × Weeding, 1b: Sowing date × Weeding, 1c: Row spacing × Fungicide treatment), Fe (1d: Row spacing × Fungicide treatment) and P (1e: Inoculation × Fungicide treatment).



Mean values with the same letter do not differ significantly.
A: Seed inoculation with *Rhizobium meliloti* (A0: no, A1: yes); B: sowing date (B0: earliest date, B1: 10 d delay, B2: 20 d delay); C: row spacing (C0: 15 cm, C1: 30 cm, C2: 45 cm); D: weed control (D0: mechanical, D1: chemical); E: antifungal plant protection (E0: non-dressed seeds, chemical protection, E1: dressed seeds, no chemical protection, E2: dressed seeds, chemical protection).

cited studies support the observation that agrotechnological factors and environmental conditions may strongly differentiate the oil content of fenugreek seeds.

The oil content and fatty acid profile of fenugreek seeds were relatively stable across all agrotechnological variants. The content of the identified fatty acids can be arranged in the following descending order: linoleic acid $C_{18:2}$ 37.9%, α-linolenic acid $C_{18:3}$ 28.2%, oleic acid $C_{18:1}$ 13.3%, palmitic acid $C_{16:0}$ 13.1%, stearic acid $C_{18:0}$ 3.8%, arachidic acid $C_{20:0}$ 1.4%, followed by acids with less than 1% content ($C_{22:0}$ 0.82%, $C_{17:0}$ 0.43%, $C_{20:1}$ 0.27%, $C_{15:0}$ 0.19%, $C_{14:0}$ 0.18%, $C_{17:1}$ 0.15%, $C_{20:2}$ 0.082%, $C_{16:1}$ 0.07%, $C_{12:1}$ 0.04%, $C_{12:0}$ 0.02%) (Table 5). The fenugreek seeds analyzed by Ciftci et al. (2011) were characterized by a similar content of the major fatty acids, but significant differences were reported in the concentrations of: linoleic acid $C_{18:2}$ 45.1%-47.5%, α-linolenic acid $C_{18:3}$ 18.3%–22.8%, oleic acid $C_{18:1}$ 12.4%-17.0%, palmitic acid $C_{16:0}$ 9.8%-11.2% and stearic acid $C_{18:0}$ 3.8%-4.2%. Srinivasan (2006) identified the following fatty acids in fenugreek seeds: oleic acid $C_{18:1}$ 35.1%, linoleic acid $C_{18:2}$ 33.7%, α-linolenic acid $C_{18:3}$ 13.8%, palmitic acid $C_{16:0}$ 9.6%, stearic acid $C_{18:0}$ 4.9% and arachidic acid $C_{20:0}$ 2%.

In the group of the analyzed agrotechnological factors, sowing date and weed control had the greatest influence on the fatty acid content of fenugreek seeds. Delayed sowing increased the concentrations of myristic acid $C_{14:0}$, stearic acid $C_{18:0}$, oleic acid $C_{18:1}$, α-linolenic acid $C_{18:3}$, and arachidic acid $C_{20:0}$, but decreased the content of palmitic acid $C_{16:0}$ and linoleic acid $C_{18:2}$. Seeds collected from treatments with mechanical weed control (D0) were characterized by a higher content of saturated fatty acids – lauric acid $C_{12:0}$, stearic acid $C_{18:0}$, arachidic acid $C_{20:0}$ and heptadecanoic acid $C_{17:1}$, and a lower content of essential fatty acid $C_{18:2}$. Seeds inoculated with *R. meliloti* contained similar amounts of fatty acids but less myristic acid $C_{14:0}$ in comparison with unprotected treatments.

Seeds from treatments with greater row spacing were more abundant in MUFAs – palmitoleic acid $C_{16:1}$ and gadoleic acid $C_{20:1}$, whereas seeds from treatments with full antifungal protection (E2) were characterized by higher levels of heptadecenoic acid $C_{17:1}$, α-linolenic acid $C_{18:3}$ and behenic acid $C_{22:0}$.

The variations in the fatty acid profile of fenugreek seeds grown in different treatments were relatively low, but percentage changes in the concentration of individual fatty acids ranged from -14.5% to 10.2% for margaric acid $C_{17:0}$ (Table 6). Significant variations were observed in the content of lauric acid $C_{12:0}$ (-8.6% to 6.8%), linderic acid $C_{12:1}$ (-9.5% to 7.8%), eicosadienoic acid $C_{20:2}$ (-10.0% to 8.2%) and behenic acid $C_{22:0}$ (-7.8 to 10.8%). The above results suggest that agrotechnological factors modified the fatty acid profile of fenugreek seeds. The concentrations of margaric acid $C_{17:0}$ (-6.6%) and linderic acid $C_{12:1}$ (-4.7%) varied significantly in seeds inoculated with *R. meliloti*. Delayed sowing reduced the content of margaric acid $C_{17:0}$ from -2.3% to -14.5% and eicosadienoic acid $C_{20:2}$ from -2.0% to -5.5%, and increased the content of myristic acid $C_{14:0}$ to 7.4% and lauric acid $C_{12:0}$ to 6.8%. Greater spacing between rows increased the concentrations of margaric acid $C_{17:0}$ from -14.0% to 10.2% and eicosadienoic acid $C_{20:2}$ from -10.0% to 8.2%, and decreased the content of linderic acid $C_{12:1}$ from 7.8% to -9.5%.

In comparison with mechanical weed control (D0), herbicide use (D1) induced the greatest decrease in the content of unsaturated fatty acids – lauric acid $C_{12:0}$ (-8.6%), heptadecenoic acid $C_{17:1}$ (-6.8%), behenic acid $C_{22:0}$ (-6.3%) and stearic acid $C_{18:0}$ (-6%). Antifungal protection led to the most significant changes in the concentrations of eicosadienoic acid $C_{20:2}$ (from -7.8% to 10.8%) and heptadecenoic acid $C_{17:1}$ (from -7.8% to 5.6%). Agrotechnological factors differentiated the content of saturated fatty acids – lauric acid $C_{12:0}$ and margaric

Table 5. Average fatty acid content of fenugreek seeds across the analyzed agrotechnological variants.

		SFA							MUFA					PUFA		
Variant	$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	$C_{20:0}$	$C_{22:0}$	$C_{12:1}$	$C_{16:1}$	$C_{17:1}$	$C_{18:1}$	$C_{20:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:2}$
A0	0.0246	0.184a	0.191	13.1	0.448	3.77	1.44	0.803	0.0410	0.0699	0.146	13.3	0.270	37.9	28.1	0.082
A1	0.0253	0.179b	0.188	13.1	0.419	3.79	1.44	0.832	0.0390	0.0687	0.146	13.3	0.270	37.9	28.2	0.082
B0	0.0246	0.176b	0.190	13.4a	0.459	3.70b	1.41b	0.795	0.0392	0.0686	0.140	13.0b	0.268	38.1a	28.1b	0.085
B1	0.0244	0.180b	0.192	13.1b	0.449	3.77ab	1.44ab	0.829	0.0396	0.0689	0.147	13.4a	0.265	38.4a	27.6b	0.082
B2	0.0260	0.189a	0.185	12.8c	0.392	3.86a	1.46a	0.828	0.0412	0.0704	0.150	13.5a	0.276	37.2b	28.8a	0.080
C0	0.0243	0.181	0.195	13.2	0.463	3.75	1.44	0.816	0.0393	0.0665b	0.152	13.2	0.262b	38.0	28.0	0.086
C1	0.0257	0.182	0.186	13.0	0.398	3.77	1.43	0.830	0.0424	0.0706a	0.145	13.3	0.270ab	37.8	28.4	0.077
C2	0.0250	0.181	0.187	13.1	0.439	3.80	1.44	0.807	0.0383	0.0709a	0.140	13.4	0.277a	37.9	28.1	0.083
D0	0.0261a	0.182	0.187	13.1	0.422	3.90a	1.47a	0.844	0.0410	0.0687	0.151a	13.4	0.272	37.6b	28.2	0.084
D1	0.0239b	0.181	0.191	13.1	0.444	3.66b	1.40b	0.791	0.0390	0.0699	0.141b	13.2	0.268	38.2a	28.1	0.080
E0	0.0244	0.183	0.188	13.2	0.423	3.78	1.44	0.784b	0.0401	0.0706	0.151a	13.3	0.273	38.2	27.8b	0.080
E1	0.0246	0.177	0.191	13.2	0.432	3.78	1.43	0.868a	0.0385	0.0672	0.139b	13.4	0.273	37.8	28.1ab	0.084
E2	0.0260	0.184	0.189	13.0	0.445	3.77	1.44	0.801ab	0.0413	0.0702	0.147a	13.2	0.264	37.7	28.6a	0.082
Mean	0.0250	0.182	0.189	13.1	0.433	3.78	1.44	0.818	0.0400	0.0693	0.146	13.3	0.270	37.9	28.2	0.082

SFA: Saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
A: Seed inoculation with *Rhizobium meliloti* (A0: no, A1: yes); B: sowing date (B0: earliest date, B1: 10 d delay, B2: 20 d delay); C: row spacing (C0: 15 cm, C1: 30 cm, C2: 45 cm); D: weed control (D0: mechanical, D1: chemical); E: antifungal plant protection (E0: non-dressed seeds, chemical protection, E1: dressed seeds, no chemical protection, E2: dressed seeds, chemical protection).
Values with the same letters do not differ significantly in Tukey's test.

Table 6. Treatment differences in the fatty acid content of fenugreek seeds.

Fatty acid	Percentage differences between means across treatments (e.g. the value in column A1-A0 is (A1-A0)/A0*100)												
	A1-A0	B1-B0	B2-B0	B2-B1	C1-C0	C2-C0	C2-C1	D1-D0	E1-E0	E2-E0	E2-E1	Min.	Max.
$C_{12:0}$	2.8	-0.9	5.9	6.8	6.0	2.9	-2.9	-8.6	1.0	6.4	5.4	-8.6	6.8
$C_{14:0}$	-2.5	2.6	7.4	4.7	0.7	0.1	-0.6	-0.8	-3.3	0.1	3.5	-3.3	7.4
$C_{15:0}$	-1.7	1.1	-3.0	-4.1	-4.4	-4.0	0.4	2.0	1.8	0.4	-1.3	-4.4	2.0
$C_{16:0}$	-0.2	-2.1	-4.4	-2.4	-1.5	-0.6	0.9	0.2	-0.1	-1.2	-1.1	-4.4	0.9
$C_{17:0}$	-6.6	-2.3	-14.5	-12.6	-14.0	-5.3	10.2	5.2	2.1	5.0	2.8	-14.5	10.2
$C_{18:0}$	0.6	2.0	4.3	2.3	0.6	1.4	0.8	-6.0	0.2	-0.1	-0.3	-6.0	4.3
$C_{20:0}$	-0.2	1.8	3.5	1.7	-0.5	-0.1	0.3	-4.5	-0.9	-0.3	0.6	-4.5	3.5
$C_{22:0}$	3.6	4.3	4.1	-0.2	1.7	-1.1	-2.8	-6.3	10.8	2.2	-7.8	-7.8	10.8
$C_{12:1}$	-4.7	0.9	5.1	4.1	7.8	-2.4	-9.5	-4.7	-4.1	3.0	7.4	-9.5	7.8
$C_{16:1}$	-1.8	0.4	2.6	2.3	6.1	6.5	0.4	1.9	-4.8	-0.5	4.5	-4.8	6.5
$C_{17:1}$	-0.3	5.3	7.0	1.6	-4.2	-7.5	-3.4	-6.8	-7.8	-2.7	5.6	-7.8	7.0
$C_{18:1}$	-0.5	2.5	3.8	1.3	0.4	1.2	0.8	-1.3	0.1	-1.0	-1.2	-1.3	3.8
$C_{20:1}$	0.1	-1.0	3.1	4.1	2.7	5.7	2.9	-1.6	0.0	-3.2	-3.2	-3.2	5.7
$C_{18:2}$	-0.1	0.9	-2.1	-3.1	-0.6	-0.5	0.1	1.5	-1.0	-1.3	-0.4	-3.1	1.5
$C_{18:3}$	0.4	-1.9	2.5	4.5	1.4	0.3	-1.1	-0.3	1.0	2.8	1.8	-1.9	4.5
$C_{20:2}$	0.3	-3.6	-5.5	-2.0	-10.0	-2.6	8.2	-3.8	5.7	3.7	-1.9	-10.0	8.2

A: Seed inoculation with *Rhizobium meliloti* (A0: no, A1: yes); B: sowing date (B0: earliest date, B1: 10 d delay, B2: 20 d delay); C: row spacing (C0: 15 cm, C1: 30 cm, C2: 45 cm); D: weed control (D0: mechanical, D1: chemical); E: antifungal plant protection (E0: non-dressed seeds, chemical protection, E1: dressed seeds, no chemical protection, E2: dressed seeds, chemical protection).

acid $C_{17:0}$, and unsaturated fatty acids – linderic acid $C_{12:1}$, heptadecenoic acid $C_{17:1}$ and eicosadienoic acid $C_{20:2}$. Relatively low variations in the concentrations of pentadecanoic acid $C_{15:0}$, palmitic acid $C_{16:0}$, palmitoleic acid $C_{16:1}$, stearic acid $C_{18:0}$, oleic acid $C_{18:1}$, linoleic acid $C_{18:2}$, α-linolenic acid $C_{18:3}$, arachidic acid $C_{20:0}$ and gadoleic acid $C_{20:1}$ were noted between treatments, regardless of the agrotechnological factors. In the study Chatterjee et al. (2010) the fatty acid profile was dominated by unsaturated acids, namely oleic, linoleic and linolenic acids accounting for 16.3%, 50% and 24.4%, respectively of the total fatty acids. The mean values of seed yield, and oil content, and fatty acid content (saturated, monounsaturated and polyunsaturated fatty acids) of fenugreek seeds are presented in Table 7. The profiles of fatty acid groups were relatively stable across treatments. Sowing date and weed control were the only agrotechnological factors that exerted a significant influence on seed properties. In the treatment with chemical weed control, seed yield was higher, the concentrations of saturated fatty acids were somewhat reduced, and the content of polyunsaturated fatty acids was somewhat higher. Delaying sowing date led to a decrease in the oil and SFA content and a minor increase in the MUFA content of fenugreek seeds.

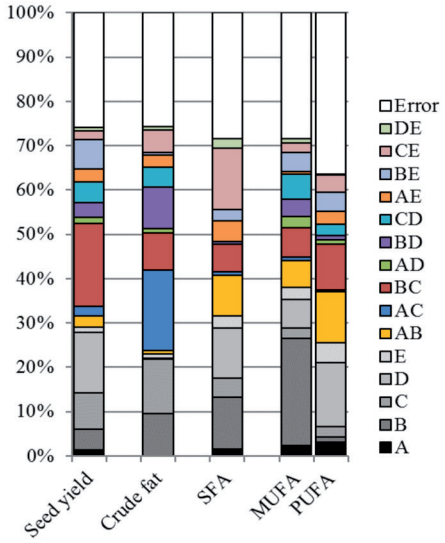
The results reported by Ciftci et al. (2011), Ali et al. (2012), Suliema et al. (2008) and Al-Jasass and Al-Jasser (2012) univocally confirmed that fenugreek seed oil is a rich source of polyunsaturated fatty acids, including essential fatty acids. In a study by Ali et al. (2012), α-linolenic acid was the major PUFA (42.5%), oleic acid was the main MUFA (20%), and palmitic acid was the main SFA (10.5%). In the work of Al-Jasass and Al-Jasser (2012), the

Table 7. Influence of agrotechnological factors on the mean values of seed yield, oil content and percentage content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

Agrotechnological factor	Treatment	Seed yield	Oil	SFA	MUFA	PUFA
		kg ha^{-1}	% of DM	% in oil		
Inoculation	A0	823.9	4.77	20.0	13.9	66.1
	A1	822.0	4.76	20.0	13.8	66.2
Sowing date	B0	858.9	5.01a	20.2a	13.5b	66.3
	B1	835.9	4.67b	20.0ab	13.9ab	66.1
	B2	774.2	4.63b	19.8b	14.1a	66.2
Row spacing	B0	883.7	4.53	20.1	13.8	66.1
	B1	768.2	4.76	19.9	13.8	66.3
	B2	817.1	5.01	20.0	13.9	66.1
Weed control	B0	765.3b	4.78	20.1a	13.9	65.9b
	B1	880.7a	4.75	19.8b	13.7	66.4a
Antifungal protection	B0	803.4	4.72	20.0	13.9	66.1
	B1	847.4	4.74	20.1	13.9	66.0
	B2	818.2	4.84	19.9	13.7	66.4
Overall mean		823.0	4.77	20.0	13.8	66.2

Values with the same letters do not differ significantly in Tukey's test.
A: Seed inoculation with *Rhizobium meliloti* (A0: no, A1: yes); B: sowing date (B0: earliest date, B1: 10 d delay, B2: 20 d delay); C: row spacing (C0: 15 cm, C1: 30 cm, C2: 45 cm); D: weed control (D0: mechanical, D1: chemical); E: antifungal plant protection (E0: non-dressed seeds, chemical protection, E1: dressed seeds, no chemical protection, E2: dressed seeds, chemical protection).

Figure 2. Effect size (eta-squared) of agrotechnological factors and interactions in ANOVA on the concentrations of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids relative to seed yield and the crude fat content of fenugreek seeds.



A: Seed inoculation, B: sowing date, C: row spacing, D: weed control, E: antifungal protection.

total content of unsaturated fatty acids was determined at 92.99%. Fenugreek seeds analyzed by Suliema et al. (2008) contained 82.3% unsaturated fatty acids, and were most abundant in linoleic acid (43.2%), followed by α-linolenic acid (22%) and oleic acid (16.7%). Total saturated fatty acids accounted for 17.7% of total fatty acids, and palmitic acid was the dominant SFA (11.0%).

The effect size (eta-squared) of agrotechnological factors and interactions had a significantly varied effect on the concentrations of saturated and unsaturated fatty acids in fenugreek seeds (Figure 2).

The main agrotechnological factors which contributed to the overall variation in the concentrations of SFAs were sowing date, weed control and the Row spacing × Weed control interaction. The observed differences in the content of MUFAs were attributed to sowing date, and the variations in the content of PUFAs – to weed control, Inoculation with *R. meliloti* × Sowing date and Sowing date × Row spacing interactions. In comparison with seed yield and crude fat content, agrotechnological factors had a similar contribution to the overall variations in the concentrations of SFAs and MUFAs at approximately 71%, whereas the effect size of PUFAs was significantly lower at around 63%. Those results indicate that agrotechnological factors are more likely to induce variations in the concentrations of SFAs and MUFAs than PUFAs.

CONCLUSIONS

The results of this study indicate that variations in the concentrations of biogenic elements and fatty acids in fenugreek seeds are caused mainly by a specific combination of agrotechnological factors in a farming system. The most influential agrotechnological factors were sowing date, row spacing and plant protection. The seeds of fenugreek plants grown in the humid continental climate of north eastern Poland contained 26.0% protein and 4.8% oil. They had similar protein content and lower oil content than seeds grown in the Mediterranean region and subtropical climates of Asia Minor.

The effect size of agrotechnological factors had a much smaller influence on variations in the concentrations of biogenic elements than fatty acids. The greatest effect sizes for biogenic elements were associated with the variation induced by sowing date (B) (70.1%), followed by row spacing (C) (43.6%) and the Row spacing × Weed control (DE) interaction (39.8%). The main contributors to variations in the fatty acid content of fenugreek seeds were weed control (D) (94.1%), sowing date (B) (86.6%), Sowing date × Weed control (BD) interaction (85.5%) and Row spacing × Weed control (CD) interaction (80.2%). Sowing delayed by 20 days increased N concentrations (by 9.2%) and decreased P (8.8%), K (5.1%) and Mg (2.8%) concentrations in fenugreek seeds. An increase in row spacing from 15 to 45 cm promoted the accumulation of Fe by 31%.

Agrotechnological factors modified the fatty acid profile of fenugreek seeds and induced the greatest variations in the SFA content of seeds. Agrotechnological factors were responsible for significant differences in the average content of margaric acid C17:0 (14.5%), behenic acid C22:0 (10.8%), eicosadienoic acid C20:2 (10.0%) and lauric acid C12:0 (8.6%). The variations in the content of EFAs (linoleic C18:2 and α-linolenic C18:3) across experimental treatments (up to 3.1-4.5%) were attributed mainly to

sowing date and plant protection. Agrotechnological factors had a greater impact on the concentrations of SFAs and MUFAs than PUFAs. Fenugreek seeds were particularly abundant in linoleic acid (37.9%) and α-linolenic acid (28.2%). The overall PUFA content of seeds was determined at 80.0%. Sowing date influenced the total content of SFAs and MUFAs, whereas weed control was responsible for variations in the total content of SFAs and PUFAs.

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