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EFFECT OF DIVERSIFIED FERTILIZATION WITH NITROGEN, SULPHUR AND BORON ON FATTY ACIDS PROFILE IN OIL FLAX SEEDS*

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Abstract

Following strict standards imposed on raw materials for food production, the most important aim in plant cultivation is to obtain high quality yields with desirable chemical composition. It can be achieved, for example, through fertilization, which modifies the content and share of organic components (protein, fat) in crops. This study was conducted in 2009, 2010, 2011, and dealt with the effect of nitrogen, sulphur and boron fertilization on the content and chemical composition of fatty acids in light- and dark-coloured seeds of oil flax. The content of saturated acids, with the following number of carbon atoms C8, C10, C12, C16,C18, C22, and unsaturated acids with carbon atoms C16:1, C18:2 and C18:3, was especially modified by the weather conditions. In 2009, with the lowest mean values of precipitation and air temperatures during the plant growing season, the highest content of oleic acid (19.94%) and MUFA (20.1%) was determined. In turn, the highest air temperature and mean precipitation values in 2011 favoured a high content of linolenic acid (64.3%) and PUFA (78.5%)

Regardless of the year of flax cultivation, the dark-seed cultivar had a higher share of oleic acid $C_{18:1}$, *n*-9 in the sum of fatty acids (by about 6%), and a lower share of linolenic acid $C_{18:3}$, *n*-3 (by about 10%).

The cultivar with light-coloured seeds accumulated higher amounts of capric, palmitoleic, behenic acids, as well as 6.7% more of palmitic acid and 5.3% more of linolenic acid than the dark-seeded cultivar.

The three-year experiment demonstrated that the introduction of a nitrogen dose of 40 kg ha^{-1} was advantageous only to the accumulation of palmitic and stearic acids, while the simultaneous application of 15 kg B and 70 kg S ha^{-1} resulted in a decreased lignoceric acid content. The tested fertilization did not affect significantly the content of MUFA, PUFA and saturated fatty acids (SFA).

No statistically significant differences were determined in the total content of saturated fatty acids in flax oil of the examined cultivars. The dark-seeded cultivar presented a higher value of mono-unsaturated fatty acids, by 19.3%, while the light-seeded cultivar Oliwin contained 4.4% more of essential unsaturated fatty acids.

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The average content of saturated fatty acids (SFA) in flax oil reached the level of 8.24%. The percentage of Essential Fatty Acids (EFA = PUFA – polyunsaturated Fatty Acids) was about 74.9%, while the percentage of MUFA (Monounsaturated Fatty Acids) was nearly 17%.

In the experiment, the $C_{18:2}$ (*n*-6) to $C_{18:3}$ (*n*-3) ratio for the light-seeded cultivar was 0.24:1, compared to 0.32:1 for the dark -seeds variety. The ratio of saturated to unsaturated fatty acids reached 1:8.97 for the examined cultivars, being higher in the light-seeded cultivar.

Key words: flax oil, nitrogen, sulphur, boron, fatty acids, MUFA, PUFA, SFA.

WPŁYW ZRÓŻNICOWANEGO NAWOŻENIA AZOTEM, SIARKĄ I BOREM NA PROFIL KWASÓW TŁUSZCZOWYCH W NASIONACH LNU OLEISTEGO

Abstrakt

Ze względu na określone wymagania stawiane surowcom do produkcji żywności, istotnym zadaniem w uprawie roślin jest uzyskanie plonów o wysokiej jakości i pożądanym składzie chemicznym. Jednym ze sposobów jest nawożenie, które modyfikuje ilość i wzajemne proporcje związków organicznych (białka, tłuszczu) w badanych odmianach.

W latach 2009, 2010, 2011 badano wpływ nawożenia azotem, siarką i borem na zawartość i skład kwasów tłuszczowych w jasnych i ciemnych odmianach lnu oleistego. Zawartość kwasów nasyconych, o liczbie atomów węgla C8, C10, C12, C16,C18, C22 oraz nienasyconych C16:1, C18 o 1, 2 i 3 wiązaniach była w szczególności modyfikowana przebiegiem warunków pogodowych. W 2009 r. o najniższym poziomie opadów i średniej temperaturze powietrza w okresie wegetacyjnym roślin odnotowano najwyższą zawartość kwasu oleinowego (19,94%) i jednonienasyconych kwasów tłuszczowych (MUFA – Monounsaturated Fatty Acids) – 20,1%, a najwyższe temperatury powietrza i średnia ilość opadów atmosferycznych w 2011 r. sprzyjały wysokiej zawartości kwasu linolenowego (64,3%) i wielonienasyconych kwasów tłuszczowych (PUFA - Polyunsaturated Fatty Acids) – 78,5%.

Bez względu na rok uprawy wyższy udział kwasu oleinowego $C_{18:1}$, *n*-9 w sumie kwasów tłuszczowych stwierdzono u odmian o ciemnych nasionach (o ok. 6%), a jednocześnie niższy udział kwasu linolenowego $C_{18:3}$, *n*-3 (o ok. 10%).

Odmiana o jasnych nasionach kumulowała więcej kwasów kaprynowego, palmitooleinowego, behennowego oraz o 6,7% kwasu palmitynowego i o 5,3% więcej kwasu linolenowego, w porównaniu z odmianą o nasionach ciemnych.

W 3-letnim doświadczeniu wykazano, że zastosowanie dawki azotu w ilości 40 kg ha^{-1} okazało się korzystne w gromadzeniu jedynie kwasów palmitynowego i stearynowego, natomiast jednoczesne zastosowanie 15 kg B ha^{-1} i 70 kg S ha^{-1} spowodowało zmniejszenie zawartości kwasu lignocerynowego. Zastosowane nawożenie nie modyfikowało w istotny sposób zawartość MUFA, PUFA i nasyconych kwasów tłuszczowych (SFA – Saturated Fatty Acids).

Nie stwierdzono istotnych różnic w sumarycznej zawartość nasyconych kwasów tłuszczowych w oleju lnianym badanych odmian. Odmiana o ciemnych nasionach (Szafir) zawierała więcej jednonienasyconych kwasów tłuszczowych (o 19,3%), a odmiana o jasnych nasionach (Oliwin) – więcej niezbędnych nienasyconych kwasów tłuszczowych (o 4,4%).

Średnia zawartość nasyconych kwasów tłuszczowych (SFA) w oleju lnianym wynosiła 8,24%, NNKT- niezbędnych nienasyconych kwasów tłuszczowych (EFA = PUFA) stwierdzono ok. 74,9%, natomiast jednonienasyconych kwasów tłuszczowych (MUFA) ok. 17%.

Wykazano, że stosunek $C_{18:2}$ (*n*-6) do $C_{18:3}$ (*n*-3) wynosił u odmiany o jasnych nasionach 0,24:1, u odmiany ciemnonasiennej – 0,32:1, natomiast stosunek kwasów nasyconych do nienasyconych u badanych odmian kształtował się na poziomie 1:8,97 i był wyższy u odmiany jasnonasiennej.

Słowa kluczowe: olej lniany, azot, siarka, bor, kwasy tłuszczowe, NNKT, MUFA, SFA.

INTRODUCTION

Flax is a plant with a wide range of applications, including the chemical, cosmetics and food industries. The area cropped with oil flax is predicted to increase, owing to the increased demand for plant oils, the climate in Poland, suitable for oil flax cultivation, as well as the advantageous dietary properties of flax seeds (ZAJĄC et al. 2010). As oil flax seeds are used in bakery , consumption of bread introduces healthy fatty acids into the human organism. Flax oil contains unsaturated fatty acids (SFA),undesirable nutrients in the human diet, as well as unsaturated fatty acids (PUFA, MUFA), prone to oxidation (VERSCHUREN, ZEVENBERGEN 1990). Oxidation depreciates the nutritive value of fatty acids, first of all by decreasing their content of EFA (PUFA) and the content of soluble vitamins in fats (ŻBIKOWSKA, RUTKOWSKA 2008). Oxidizability of fats depends on their chemical composition. Therefore, determination of the composition of fatty acids, apart from the evaluation of their quality, can be useful as far as the applicability of fatty acids is concerned (ŻBIKOWSKA, RUTKOWSKA 2008).

Therefore the type of fat does significantly affect not only on physical and sensoric properties of products, but also on their health – promoting properties (ŻBIKOWSKA, RUTKOWSKA 2008). Thus, the important task in agricultural production is not only to obtain high yields, but also to provide the highest quality of raw material. The yield size, the content of fat and its quality is modified by fertilization (ZAJĄC et al. 2010).

Nitrogen is regarded as the most important yield – forming macroelement (CZUBA et al. 1999), yet too high doses of this element lead to the decrease in fat content in seeds (ZAJĄC 2005).

In the case of oil plants, it is crucial to provide them with sufficient amount of sulphur, which stimulates nitrogen absorption by plants and increases the quantity of FAT in seeds (KOZŁOWSKA-STRAWSKA, KACZOR 2003).

In modern cultivation of flax a significant role is also played by boron fertilization (DIEPENBROCK, PORKSEN 1992, ANTONIEWICZ, ZAJĄC 2005).

The deficit of boron becomes a serious problem on acid soils, where soil erosion brings about high lose of this chemical element resulting from washing it out. Boron is the basic microelement of all higher plants and a structural component (BOLANOS et al. 2004).

Additionally, B is one of the nutrients responsible for changes in the concentration and metabolism in vascular plants (BLEVINS, LUKASZEWSKI 1998).

In Poland, the research on comparison of productivity of bright – and dark – coloured seeds cultivars was conducted. The mentioned cultivars were assessed on the basis of their field – forming value, oil content and chemical composition of fatty acids (ZAJĄC et al. 2010). PIOTROWSKA , FUROWICZ 1999) proved that the light – coloured cultivars accumulated higher amount of FAT in seeds than the dark – seed ones.

In the working hypothesis it is assumed that application of diverse nitrogen fertilization, at simultaneous introduction of fertilization with boron and sulfur in the cultivars of dark and light seeds, will result in beneficial effect on the increase in linolenic and linoleic acid content in linseed oil, while their ration will achieve desirable values.

The aim of field and laboratory experiments was determination of the effect of nitrogen, sulphur and boron fertilization on fatty acids profile in oil flax cultivars of bright – coloured and dark – coloured seeds.

MATERIAL AND METHODS

In the years 2009-2011, in Experimental Station belonging to Department of Plant Cultivation in Pawłowice (eastern longitude 17°12′ and northern latitude 51°31′) field experiments, using the method of sub blocks in correlated "split – plot" system, in four replications and with four variable factors were carried out.

The first factor to be examined were flax cultivars Oliwin – bright – coloured seeds and Szafir – dark – coloured seeds. The second factor was nitrogen fertilization in the following doses: N_1 – 0 kg ha^{-1}, N_2 – 20 kg ha^{-1}, N_3 – 40 kg ha^{-1}, N_4 – 60 kg ha^{-1}. The third examined factor was combined fertilization with sulphur and boron S_1+B_1, S_2+B_2, S_3+B_3, where: S_1 – 30, S_2 –50, S_3 – 70 kg S ha^{-1} and B_1 – 5, B_2 – 10 and B_3 – 15 kg B ha^{-1}. In pre – sowing fertilization of oil flax, triple superphosphate in the amount of 40 kg P ha^{-1}, 60% potassium salt in the quantity of 70 kg K ha^{-1}, and 46% urea was applied. Ammonium nitrate was introduced in the form of top – dressing fertilization. Sulphur and boron fertilization was introduced in the stages of herringbone and budding

Fatty acids profile was determined using the method of gas chromography with flame – ion detection FID and with the use of column ZB-WAX (30 m × 0.25 mm i.d., 0.25 μm film thickness).The analysiswas done in the following conditions: the carrier gas was helium flowing at the speed of 20 m s^{-1}. The temperature in injection cell – 250°C, detector temperature – 270°C, column work temperature programme – 50°C for 1 min, temperature rise to 180°C at the speed of 15°C min^{-1}, temperature rise to 230°C at the speed of 7°C min^{-1}, temperature rise to 400°C at the speed of 30°C min^{-1}, temperature 400°C for 10 min. Total time of analyses equaled 33 min. Quantitative interpretation of chromograms was done through comparison of retention times of methyl esters of fatty acids of the examined sample with retention times of methyl esters standards for fatty acids, using Supelco Component FAME Mix C8 - C24.The obtained results were subjected to statistical analysis according to Statistica 8.0 PL (StatSoft Polska) Programme.

The soil in Pawłowice is classified as autogenic, brown soil, originating from light loam on medium loam, assigned to agriculturally usable good wheat complex of soil class III b. The soil richness in mineral components and its pH were determined at the Department of Plant Nutrition, Wrocław University of Environmental and Life Sciences. In the years of investigation, phosphorus content in the soil ranged from a medium to a very high level, potassium content was between a low and a very high range, magnesium – high to very high content, while pH range was slightly acidic (Table1)

The course of weather conditions in the years of investigation-distribution of temperature and rainfall for Pawłowice were shown in Tables 2 and 3.

Table1

The content of P, K, Mg (mg 100 g^{-1}) in the soil and pH (in 1 n KCl)

Years	pH	Mg	P	K
2009	5.74*a*	8.08*b*	6.93*b*	11.3*b*
2010	6.22*a*	15.0*a*	6.05*b*	17.3*a*
2011	5.87*a*	7.53*b*	8.88*a*	20.8*a*

Table 2

Climate characteristics according to Schmuck (1960)

Specification	Value
Average temperature of the vegetative period	14.5°C
Number of days of the temperature above 0°C	300
Number of days of the temperature above 15°C	95
Average number of ground frost - days more than	91
Annual sum of rainfall (mm)	500-600
Field works can start in	the second decade of April

The year 2009 characterized the lowest summary precipitation in growing period, ranging, approximately, 440 mm and the lowest mean value of monthly temperatures. In 2010 the highest summary precipitation amounting 540 mm at the lowest sum of mean monthly value of air temperatures equal 93°C was recorded. The hottest year in the course of three – year experiment proved to be 2011.

RESULTS AND DISCUSSION

Unique composition of flax oil was the subject of research for a number of authors (Zając et al. 2010). The content of particular fatty acids in flax oil, a considerable parameter from the nutrition point of view underwent

Table 3

Weather conditions in the years 2009-2011 (according to meteorological station in Wrocław-Swojec) for Pawłowice

Years	Average monthly day temperatures (°C)											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2009	-2.3	0.2	4.6	12.0	14.2	15.8	19.5	19.3	15.4	7.9	6.8	-0.4
2010	-5.9	-1.1	4.2	9.3	12.7	17.9	21.4	18.9	12.5	7.0	6.5	-5.2
2011	0.62	-1.62	4.39	11.91	14.81	19.1	18.19	19.27	15.49	9.34	3.81	3.89
Mean valutes of the years (1979--2008)	-0.5	0.1	3.4	8.7	14.2	17.1	18.9	18.4	13.8	9.3	3.9	0.8
Years	monthly sums of rainfall (mm)											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2009	34.6	46.8	48.3	30.9	67.6	141.7	134.2	53.5	12	76	32.5	51.9
2010	40.6	11	44.9	45.4	140.7	32.9	78.6	109.1	134.1	5.7	66.4	63.2
2011	35.6	10.5	45.2	27	49.4	95.7	170.9	78.9	30.4	42.6	0	48.7
Mean values of the years (1979--2008)	29.2	26.4	34.6	35.4	53.4	67.1	85.9	69.6	47.5	35.7	41.1	36.0

numerous analyses as far as human nutrition was concerned (Verschuren, Zevenbergen 1990, Gambuś et al. 2004, Juttelstad 2004, Żbikowska, Rutkowska 2008) and animal (Barowicz et al. 1997, Barowicz 2000, Barowicz , Breja 2000, Pietras et al. 2000, Sawosz et al. 2000, Niemiec et al. 2001, Barowicz et al. 2002, Cieślak et al. 2003, Jelińska et al. 2003, Borowiec et al. 2004, Micek et al. 2004, Brzóska 2005, Bhattacharya et al. 2006, Białek et al. 2009) The mentioned investigation also involved nutrition factors taking into account the division into brown - and yellow - seed cultivars (Barowicz et al. 1997, Saeidi, Rowland 1999, Barteczko et al. 2001, Borowiec et al. 2001*a*, Białek et al. 2009). The composition and content of flax seeds fat result from the effect of the environmental factor (Albrechtsen, Dybing 1973, Hocking et al. 1987, Diepenbrock, Porksen 1992, Diepenbrock et al. 1995, Piotrowska, Furowicz 1998, Casa et al. 1999, Grant et al. 1999, Aufhammer et al. 2000, Zając et al. 2001, 2002). In 2009, characterized by the lowest mean values of precipitation and air temperatures in growing period, the highest value of oleic acid (19.94%) and MUFA, ranging 20.1% was recorded, while the highest air temperature values and mean precipitation values were recorded in 2011, which favored high content of linolenic acid (64.3%) and PUFA (78.5%) – Tables 4 and 5. In the experiment by Zając et al.. (2001), no statistically

Table 4

Fatty acids content in oil flax seeds (means for the factors 2009-2011)

Specification	Caprylic acid	Capric acid	Lauric acid	Myristic acid	Palmitic acid	Palmi-toleic acid	Stearic acid	Oleic acid	Linoleic acid	Linole-nic acid	Ara-chidic acid	Behenic acid	Ligno-ceric acid
Years													
2009	0.324*a*	0.036*a*	0.013*b*	0.084	6.63*a*	0.091*b*	0.131*b*	19.94*a*	12.50*c*	59.19*b*	0.074*a*	0.152*a*	0.760*a*
2010	0.009*b*	0.026	0.032*a*	0.077	6.06*a*	0.118*a*	2.052*a*	16.93*b*	17.77*a*	56.79*b*	0.038*b*	0.057*b*	0.039*b*
2011	0.005*b*	0.034*a*	0.043*a*	0.120	4.41*b*	0.035*b*	2.300*a*	13.21*c*	14.24*b*	64.30*a*	0.037*b*	0.154*a*	0.031*b*
Cultivar													
Oliwin	0.089	0.031*a*	0.031	0.099	6.24a	0.098*a*	1,331*b*	14.86*b*	14.84	61.68*a*	0.046	0.162*a*	0.290
Szafir	0.136	0.028*b*	0.028	0.082	5.82b	0.064*b*	1.658*a*	18.53*a*	14.83	58.40*b*	0.053	0.080*b*	0.263
Fertilization N (kg ha^{-1})													
N1	0.099	0.033	0.039	0.088	5.93	0.103*a*	1.368*a*	16.00	14.89	60.92	0.055	0.126	0.308
N2	0.064	0.028	0.029	0.099	6.20	0.053*b*	1.171*b*	16.94	14.81	60.12	0.045	0.128	0.268
N3	0.119	0.033	0.023	0.081	5.93	0.085*a*	1.864*a*	16.38	14.86	60.04	0.041	0.129	0.327
N4	0.168	0.033	0.027	0.095	6.06	0.084*a*	1.575*a*	17.45	14.80	59.30	0.056	0.102	0,.202
Fertilization S + B (kg ha^{-1})													
S1+B1	0.134	0.034	0.028	0.075	6.19	0.080	1.322	16.49	15.29	59.82	0.049	0.128	0.317*a*
S2+B2	0.109	0.032	0.032	0.085	6.08	0.078	1.589	16.65	14.44	60.34	0.042	0.130	0.325*a*
S3+B3	0.094	0.030	0.029	0.112	5.82	0.085	1.573	16.95	14.78	60.13	0.058	0.105	0.187*b*

Table 5

Sum of SFA, MUFA and PUFA in oil flax seeds
(means for the factors 2009-2011)

Specification	SFA	MUFA	PUFA
Years			
2009	8.200	20.10a	71.70c
2010	8.385	17.06b	74.56b
2011	8.121	13.34c	78.54a
Cultivar			
Oliwin	8.334	15.04b	76.62a
Szafir	8.137	18.63a	73.24b
Fertilization N (kg ha^{-1})			
N1	8.042	16.16	75.81
N2	8.037	17.04	74.92
N3	8.546	16.56	74.90
N4	8.317	17.58	74.10
Fertilization S + B (kg ha^{-1})			
S1B1	8.278	16.62	75.11
S2B2	8.426	17.80	74.78
S3B3	8.003	17.09	74.91

significant differences in fatty acids content between subsequent years of flax cultivation were reported.

The composition of fatty acids is strictly connected, among others, with a genetic factor (Turner 1991, Dribnenki, Green 1995, Froment et al. 1998, Sankari 2000, Borowiec et al. 2001, Zając et al. 2001, Gambuś et al. 2003, Diederichsen, Raney 2006, Zając et al. 2012). In the experiment by the author, Oliwin cultivar accumulated higher amounts of capric, palmitoleic, behenic acids, as well as more by 6.7% of palmitic acid and by 5.3% of linolenic acid. Similar results were obtained by Borowiec et al. (2001) and Froment et al. (1998), while Zając et al. (2001) did not report statistically significant differences between the examined cultivars, regarding the content of fatty acids. The only exception was the content of oleic acid (differences determined between cultivars) and of palmitic acid (differences determined between the years of cultivation). The dark – seed cultivar, in the experiment by Zając et al. (2001), characterized higher content of oleic acid (25.0%) than the light – seed cultivar (19.0%). The content of oleic acid, in the experiment by the author, was lower than the values obtained by Froment et al. (1998) and by Zając et al. (2001).

Statistically significant differences in summary content of saturated fatty acids in flax oil of the examined cultivars were not determined. Yet those differences were proved for the content of MUFA and PUFA, which amounted, average, 16.8% and 74.9% respectively. The dark – seeds cultivar, Szafir, featured higher value of mono – unsaturated fatty acids, by 19.3%, while the light – seeds cultivar, Oliwin- proved to contain higher content of indispensable unsaturated fatty acids by 4.4%.

The fertilization of plants with sulphur (Krzywy et al. 2001), boron (Shorroks 1997, Blevins, Lukaszewski 1998, Bolanos et al. 2004, Heidarabadia et al. 2011) or nitrogen (Aufhammer et al. 2000, Zając, Kulig 2001, Zając et al. 2001) had a diverse impact on the quality and content of fat, although the analysis of fatty acids composition, resulting from simultaneous application of sulphur, boron and nitrogen fertilization, was not taken into account as an object of research, probably because of high cost of such analyses. In three – year – lasting experiment it was possible to prove that introduction of nitrogen dose 40 kg ha^{-1} was advantageous only in the case of accumulation of palmitic and stearic acids, while combined application of 15 kg B ha^{-1} and 70 kg S ha^{-}resulted in decreased value of lignoceric acid content. The introduction of fertilization did not significantly affect the content of MUFA, PUFA and saturated fatty acids (SFA).

In the experiment by Zając et al. (2001), regardless the year of flax cultivation, the dark – seed cultivar characterized higher share of oleic acid $C_{18:1}$, *n*-9 in the sum of fatty acids (by about 6%), at lower share of linolenic acid $C_{18:3}$, *n*-3 (by about 10%). The yellow – seed cultivar featured more beneficial relation between the quantity of saturated and unsaturated fatty acids, as well as $C_{18:2}$ (*n*-6) and $C_{18:3}$ (*n*-3) ratio. In the experiment by the author, $C_{18:2}$ (*n*-6) to $C_{18:3}$ (*n*-3) ratio was for Oliwin cultivar – ranged 0.24:1 and 0.32:1 – for Szafir cultivar. The ratio of saturated to unsaturated fatty acids amounted 1:8.97 for the examined cultivars and it was higher for Oliwin cultivar.

CONCLUSIONS

1. High content of stearic acid occurred in the hottest year, 2010, while the wet year, 2009, favored accumulation of palmitic and linoleic acid.

2. Bright – colour seeds cultivar of oil flax in comparison to dark – colour seeds, characterized higher content of C10:0, C16:0, C16:1, C18:3 i C22:0 by 19%, while the content of stearic and oleic acid were of lower values.

3. Nitrogen fertilization, with the dose amounting 40 and 60 kg ha^{-1}, did advantageously effect on the content of palmitic and stearic acid. High doses of sulphur and boron brought about the decrease in lignoceric acid in the fat made from oil flax.

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