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ORIGINAL PAPER

OIL CONTENT AND COMPOSITION IN SEEDS OF CAMELINA SATIVA AND CRAMBE ABYSSINICA CULTIVARS

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ABSTRACT

The aim of the study was to evaluate seed oil content and oil composition in five Camelina sativa and four *Crambe abyssinica* cultivars as an interaction of a genotype and the climatic conditions in Lithuania. The studied species differed in the seed oil content, glucosinolate content in oil and oil fatty acid composition. The oil content was 22.1-42.5% in dry matter of camelina seeds and 23.4-36.6% in crambe seeds. The glucosinolate content varied from 61.8 to 68.6 µmol g^{-1} in oil of crambe, and from 8.6 to 30.5 µmol g^{-1} in oil of camelina. The oil content in seeds and glucosinolate content in oil of each species differed between the research years but not between the cultivars. The glucosinolate content in oil correlated significantly with the oil content in dry matter of seeds. However, this correlation was negative for camelina (r = 0.59, p = 0.045) and positive for crambe (r=0.86, p=0.007). Camelina oil was characterised by the dominance of linolenic acid (34.8-41.6%), while crambe oil was predominated by erucic acid (53.0-60.9%). The second most abundant fatty acid in oil of both species was oleic acid, whose amount did not differ between the species. At the same time, the content of all other studied fatty acids differed significantly between both species. Our study indicated that variation in seed oil content, glucosinolate content and fatty acid composition in each studied species was much less affected by inheritable features of cultivars than by meteorological conditions in the research years.

Keywords: oil composition, glucosinolate, fatty acid, erucic acid, linolenic acid.

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INTRODUCTION

Increasing prices and environmental impacts of fossil fuels have made the production of biofuels reach unprecedented volumes over the last 15 years (POPP et al. 2014). Oil crops are the base for biodiesel production, and rapeseed is the most common feedstock in Europe (THAMSIRIROJ, MURPHY 2010, ZALECKAS et al. 2012). The development of biofuel production has led to the competition between food and non-food sectors, the prices of rapeseed and oils started to rise, all along increasing the costs of biofuels (BAIER et al. 2009). This has stimulated interest in alternative feedstocks, including new species of oilseed crops. Due to the growing demand for oilseeds, two alternative oilseed crops, camelina (Camelina sativa (L.) Crantz) and crambe (Crambe abyssinica Hochst. ex R.E. Fries), have been identified as primary candidates for the future European bio-based economy (RIGHINI et al. 2016). According to the previous research, these species need low input management, demonstrate good agronomic performance and wide environmental adaptability, do not have particularly demanding requirements concerning the soil, preceding crop, fertilisation and other cultivation conditions (BERTI et al. 2011, RIGHINI et al. 2016).

Camelina oil, which falls within the group of oils prone to drying out, is used in the environmentally friendly industries making polymers, varnishes and paints (SCHULTE et al. 2013, OBOUR et al. 2016). The fatty acid methyl esters produced from camelina oil have a high iodine value, and therefore could be used as fuel for diesel engines in mixtures with methyl esters produced from animal fat or used as frying oil (ZALECKAS et al. 2012). Camelina is regaining prominence as a health product owing to the high content of polyunsaturated fatty acids such as omega-3 and alpha-linoleic acid (WARAICH et al. 2013). Unlike most wild-type *Brassicaceae*, camelina shows a relatively low glucosinolate content (RIGHINI et al. 2016).

The high content of erucic acid in seeds of crambe is a specific trait of the species. Oil of crambe, containing up to 65% of erucic acid, makes it suitable for several bio-based productions such as lubricants and plasticisers. Therefore, crambe oil is an excellent raw material to produce biodiesel (FALASCA et al. 2010). Moreover, the remaining crambe and camelina biomass feedstocks could be converted and extracted into various valuable products. The high essential amino acid content of camelina meal makes it potentially suitable for use in poultry and other animal feeed rations (CHERIAN 2012). Furthermore, camelina and crambe straw can be an alternative to other types of biomass, both for direct combustion, gasification, and in the production of second-generation biofuels (KRZYŻANIAK et al. 2020).

Due to a variety of agricultural technologies, ecological and climatic factors as well as meteorological conditions in different countries, the performance of crop plants could be highly different. The results of some previous research have shown that the seed harvest, the quantity and quality of camelina oil depend on both genotype and agro-climatic conditions (ZUBR, MATTHÄUS 2002, JIANG et al. 2014, OBOUR et al. 2016). However, the research on the effect of environmental and inheritable factors on the quantity and quality of crambe oil is insufficient. Our study aimed to evaluate seed oil content and composition in different cultivars of camelina and crambe as an interaction of genotype and climatic conditions in Lithuania.

MATERIALS AND METHODS

Four spring and one winter camelina cultivars, and four crambe cultivars were used in the study. Seeds of summer cultivars of camelina and all crambe cultivars were sown in early May, while winter cultivar of camelina was sown in early September at the Experimental Station of Vytautas Magnus University Agriculture Academy, on a medium textured Calc(ar)i-*Endohypogleyic Luvisol* (IUSS Working Group WRB, 2014), neutral: pH-KCl 7.6, containing 266 mg kg⁻¹ of mobile phosphorus, 134 mg kg⁻¹ of mobile potassium and 2.8% of humus. The field was ploughed, and the seedbed was prepared by a cultivator and a harrow.

The experiment consisted of three replicates in a randomised design. The size of each sample plot was 2 m², space between plots was 0.5 m. Seeds were hand-planted, and seedlings at the eight-leaf stage were hand-thinned to achieve final plant stands of 90-100 plants m⁻² for camelina and 45-50 plants m⁻² for crambe. The plots were hand-harvested at full maturity. The phenological development stages were performed using the BBCH-scale of camelina (MARTINELLI, GALASSO 2011).

Representative average samples were used to determine seed oil content. Seeds were ground so that at least 90% of the sample passed a sieve of 0.3 mm mesh size. The sample weight was determined by aiming to achieve a target of extracting 0.5-1.5 g fat; so up to a 5 g sample at 1 mg precision was weighed into a beaker. The protein of the sample was digested by 100 ml boiling hydrochloric acid (4 mol 1^{-1}) to break the lipo-protein and lipo-cellulose bonds. The liquid was quickly brought to boiling and simmered with reduced heating capacity for about 1 h. At the end of the hydrolysis, the digestion mixture was diluted to the double amount with cold water. It was then immediately filtered through pleated filter, which was moistened with water. The beaker and the condenser were rinsed at least three times with hot water (40°C). The filter also was rinsed until the indicator paper became a neutral reaction to the water. The filter with the sample was then placed on a watch glass and dried for about 1.5 h at $102\pm2^{\circ}$ C in a drying oven.

After cooling, the filter was placed in an extraction thimble and covered with cotton wool. Any remaining fat traces on the watch glass were taken up with some cotton wool, dampened with extraction agent, and put inside the extraction thimble as well. After adding petrol ether (40-60°C), the sample was extracted in a Soxterm Multistat/SX PC (Gerhardt GmbBH and Co. KG, Germany) automatically using the hand-picked programme. The duration of hot extraction was 40 minutes. Total time of fat extraction was 2 h and 5 min. After the programme was run, the extraction beakers were dried in a drying chamber, kept in an upright position for 75 min at $103\pm2^{\circ}$ C. Extraction beakers were weighed at 1 mg precision after cooling in desiccators. The fat content was calculated from the difference between the weight of the extraction beaker at the end and the start of the analysis. Each sample was analysed at least twice. The deviation between the double determinations was no more than 0.1%.

In order to determine fatty acids, the oils were extracted by the FOLCH et al. (1957) method and methylated with a 2% solution of sodium methylate (NaOMe), after CHRISTOPHERSON, GLASS (1969). The mixture of fatty acid methyl esters was analysed on a gas chromatographer GC-2010 Shimadzu with a hydrogen flame detector, using an Alltech capillary column ATTM-FAME (30 m, ID: 0.25 mm). The change of the column temp. was programmed from 150°C (starting from 3 min) to 240°C, rate of temp. increase was 4°C min⁻¹. Fatty acids were identified by the duration of retention compared to duration of fatty acid retention in mixtures of known composition. The amount of fatty acids (% of total amount of acids) was calculated with a chromatographic data processing programme GCsolution (Shimadzu, Kyoto, Japan).

The NIRS method was used to determine the glucosinolate content $(\mu mol g^{-1})$ in the Chemical Research Laboratory of the Lithuanian Research Centre for Agriculture and Forestry. Intact seed samples were scanned on a monochromator NIR Systems model 6500 (Perstorp Analytical, USA) equipped with a spinning module using a small ring cup. The reflectance spectra (log 1 R⁻¹) from 400 to 2500 nm were recorded, and quality components of seeds were predicted by equations developed. The glucosinolate content was calculated by multiplying the respective component concentration by particular seed yield.

The climate of Lithuania is between maritime and continental climates, with wet winters and moderate summers. The meteorological conditions during the growing season of camelina and crambe differed between the research years (Table 1).

The nonparametric Mann-Whitney U test was used to analyse the difference between species and years. The Kruskal-Wallis test was used to analyse the difference between cultivars. The relationship between oil content in seeds and glucosinolate content in oil as well fatty acid composition was analysed using the Spearman's rank correlations. The effect of species, cultivars and meteorological conditions (sum of precipitation, mean temperature) during the periods of phenological development stages: vegetative, generative and fruit ripening, on oil and glucosinolate content was analysed using

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Table	1

Month	Average air temperature (°C)				Total precipitation (mm)					
	2008	2009	2010	2015	MA#	2008	2009	2010	2015	MA
April	8.8	8.9	7.4	7.1	6.7	32.1	8.6	58.5	46.3	48.1
May	12.3	12.7	13.7	11.1	12.6	35.5	42.0.	94.8	43.8	47.2
June	16.0	14.8	16.7	11.4	15.6	83.2	107.4	127.4	16.4	67.2
July	18.1	18.4	21.9	17.4	17.6	43.0	83.8	101.0	2.4	83.0
August	17.9	16.9	19.7	20.3	17.1	99.3	87.5	6.9	72.4	73.2

Meteorological conditions during the growing season of Camelina sativa and Crambe abyssinica

[#] MA – multi-annual average (1974-2012)

the multiple stepwise regression. Although some variables were not normally distributed, a regression model yielded normal distributed residuals. PCA based on standardised data was performed to summarise the relative differences between species and among cultivars in relation to their fatty acid composition and to determine the contribution of fatty acids to these differences. Twelve fatty acids analysed in all oil samples in 2009–2015 were used in PCA. The PCA axes with eigenvalues higher than 1.0 were estimated. All statistical analysis was conducted using the software Statistica 10.0 (StatSoft Inc.).

RESULTS

The oil content in dry matter of camelina seeds varied from 22.1% to 42.5% (Figure 1). Winter cultivar Penziak did not fall outside the range of this species' characteristic. The oil content in crambe seeds varied in a slightly narrower range, from 23.4 to 36.6%, and was significantly lower than the oil content in camelina seeds (Z=2.11, p=0.035).

The averaged oil content in the dry matter of seeds of camelina and crambe cultivars varied from 33.1% to 36.1% and from 27.9% to 30.5%in camelina and crambe cultivars, respectively. No significant differences between the investigated cultivars of each species were found (p>0.05). The multiple regression result revealed that plant species, sum of precipitation during generative phase, mean temperatures during ripening and generative phases had a significant effect on oil content (Table 2). However, the impact of precipitation and mean temperatures were opposite. The increase of precipitation during the generative phase had a negative effect on the oil content, while an increase of mean temperatures during the ripening and generative phases had a positive impact.

The glucosinolate content varied from 61.8 to 68.6 μ mol g⁻¹ in oil of crambe and from 8.6 to 30.5 μ mol g⁻¹ in oil of camelina and differed significantly







Values followed by different letters are significantly (p<0.05) different

Table 2

Results of multiple regressions relating the variation in oil content in seeds of *Camelina sativa* and *Crambe abyssinica*

Source of variation	b*	t
Sum of precipitation during the generative phase	-0.53	-3.39*
Mean temperature during the ripening phase	0.49	2.76*
Species	-0.53	-4.45*
Mean temperature during the generative phase	0.33	2.10
Mean temperature during the vegetative phase	-0.27	-1.20

* p<0.05

between species (p<0.001; Figure 2). The smallest glucosinolate content in oil of camelina was found in the winter cultivar. However, the difference between spring and winter cultivars was insignificant (p = 0.139). The content of glucosinolate in oil was higher in 2009 than in 2010 for camelina but did not differ between the years for crambe (Figure 2).

The multiple regression result revealed that a plant species had the highest effect on the variation of the glucosinolate content in oil, while a cultivar had a lower albeit also significant effect. However, differences between cultivars for each species were insignificant (p>0.05). The glucosinolate content in oil was significantly affected by the amount of precipitation in the generative phase (Table 3). By analogy to the oil content, an increase of precipitation had a significant negative effect on the glucosinolate content. The glucosinolate content in oil correlated significantly with the oil content





Table 3

Results of multiple regressions relating the variation in glucosinolates content in the seed oil of Camelina sativa and Crambe abyssinica

Source of variation	b*	t
Species	1.01	31.63*
Sum of precipitation during the generative phase	0.03	0.32
Cultivar	0.06	2.38*
Sum of precipitation during the ripening phase	-0.33	-3.94*
Mean temperature during the ripening phase	0.09	2.34*

* p<0.05

in dry matter of seeds. However, this correlation was negative for camelina (r_s =-0.59, p=0.045) and positive for crambe (r_s =0.86, p=0.007).

Camelina oil was characterised by the predominance of linolenic acid (36.6% on average). In comparison, crambe oil was predominated by erucic acid (57.7% on average) and did not fall outside ranges reported by other authors (Table 4). The second most abundant fatty acid in oil of both species was oleic acid, which amount did not differ between species. At the same time, the content of all other studied fatty acids differed significantly between both species (p<0.001).

The PCA reduced values of oil composition to two principal components, which accounted for 93.77% of the total variance in fatty acid composition of camelina and crambe seed oil. The first component (PC1) explained 85.23% of the total variance and strongly correlated with most of the fatty

Table 4

Fatty acid	C. sativa	C. abyssinica
Palmitic C16:0	5.0-6.2	1.6-2.3
Palmitoleic C16:1ω9	0-0.1	0-0.3
Stearic C18:0	2.0-2.9	0.6-0.8
Oleic C18:1ω9	14.3-18.6	14.6-16.1
Linoleic C18:2ω6	16.7-20.7	7.9-10.6
Linolenic C18:3ω3	34.8-41.6	5.3-10.2
Arachidic C20:0	1.2-1.9	0.7-1.0
Gondoic C20:1ω9	13.2-16.6	1.5-3.1
Behenic C22:0	0.2-0.5	1.6-2.3
Erucic C22:1ω9	2.1-4.1	53.0-60.9
Lignoceric C24:0	0.1-0.3	0.7-0.9
Nervonic C24:1ω9	0.7-0.9	1.4-1.7

Fatty acid composition in percentages of total fatty acids of *Camelina sativa* and *Crambe abyssinica* seed oil

acids, while the second component accounted for 8.54% and negatively correlated with oleic acid (Figure 3). PC1 correlated positively with six fatty acids (arachidic, gondoic, linoleic, linolenic, palmitic and stearic), whose higher amounts were found in camelina oil, and negatively with five fatty



Fig. 3. PCA score plots of *Crambe abyssinica* and *Camelina sativa* based on the composition of 12 fatty acids. Labels with fatty acids display the eigenvector scores on PC1 and PC2, with the highest score presented at the top of the lists

acids (behenic, erucic, lignoceric, nervonic and palmitoleic), whose higher amounts were found in crambe oil. PC1 effectively separated samples of both species, while PC2 correlated negatively only with the amount of oleic acid, which did not correlate with the amounts of the other fatty acids.

The PCA based on the fatty acid composition of camelina seed oil reduced 12 variables to two principal components, which explained 73.71% of the total variance. The first component (PC1) negatively correlated with most fatty acids, namely behenic, palmitoleic, arachidic, lignoceric, gondoic and stearic, while PC2 directly correlated with linolenic acid and negatively with oleic and linoleic acids (Figure 4). The significant differences between



Fig. 4. PCA score plots of 4 cultivars of *Camelina sativa* based on the composition of 12 fatty acids. Labels with fatty acids display the eigenvector scores on PC1 and PC2, with the highest score presented at the top of the lists

camelina cultivars were found only in the amounts of two fatty acids, namely palmitic and linolenic and only for winter cultivar Penziak, which differed from all spring cultivars in the lowest content of palmitic acid (p<0.05) and from two spring cultivars (Svalof and Prophet) in a higher content of linolenic acid (p<0.05). However, there was no difference in the fatty acid concentration between camelina spring cultivars.

The PCA reduced 12 variables of crambe oil composition to two principal components, which accounted for 75.99% of the total variance. The first component (PC1) directly correlated with linoleic, linolenic and palmitic acids, while negatively correlated with lignoceric, behenic, erucic and palmitoleic acids. PC2 negatively correlated with stearic and arachidic acids (Figure 5). There were no differences between cultivars in the amounts of fatty acids (p>0.05, Kruskal-Wallis test) in each species. Accordingly, samples of crambe,



Fig. 5. PCA score plots of 4 cultivars of *Crambe abyssinica* based on the composition of 12 fatty acids. Labels with fatty acids display the eigenvector scores on PC1 and PC2, with the highest score presented at the top of the lists

as well as camelina cultivars, were scattered in the PCA ordination diagrams, while samples of the same year often formed distinct groups (Figures 4-5).

DISCUSSION

Considerable variation caused by a genotype as well as a genotype by the environment interaction and the combined effects of the external factors has been found in the camelina oil content ranging from 30% to 49% (ZUBR 2003, VOLLMANN, EYNCK 2015, STOLARSKI et al. 2018). According to VOLLMANN et al. (2007), seed quality characteristics are considerably modified by the environmental conditions, and the oil content is similarly influenced by the effects of the growing season both for high and low oil content genotypes. Also, the oil content in crambe seeds is affected by genotypes or the environmental conditions and varies from 28.3 to 38.6% (WANG et al. 2000, LALAS et al. 2012, LARA-FIOREZE et al. 2013, STOLARSKI et al. 2018). The highest values of the oil content in crambe seeds (42-47%) have been reported by ZANETTI et al. (2009). The oil content in seeds of summer and winter cultivars was highly variable and showed no regularities in both our study and literature data (ZUBR 1997, KARČAUSKIENĚ et al. 2014).

The meteorological conditions (air temperature and precipitation) during the growing season of camelina and crambe differed between years. The present results support the suggestion that the increase of mean temperatures during the generative and seed ripening phases has a positive impact on the seed oil content. A similar positive effect of mean daily temperatures during camelina seed development has been found by KIRKHUS et al. (2013), while, according to JIANG et al. (2014), the effect of temperatures on camelina seed oil content is diverse and depends on a geographical location. Our research showed that an increase of precipitation during the generative phase had a negative effect on the seed oil content. Similarly, the negative impact of precipitation has been recorded by KRZYŻANIAK et al. (2019), while JIANG et al. (2014) have not observed any correlation between precipitation and seed quality during seed development.

The glucosinolate content varies from 52.1 to 82.2 μ mol g⁻¹ in crambe seed oil (LAZZERI et al. 1994, WANG et al. 2000) and from 10 to 40.0 μ mol g⁻¹ in camelina seed oil (Büchsenschütz-Nothdurft et al. 1998, Gugel, Falk 2006). This variation in camelina strongly depends on weather conditions during the growth period and on the genotype origin (KIRKHUS et al. 2013). Same as our results, LESSMAN (1975) has reported significant difference in the glucosinolate content in crambe oil between cultivation years, but not between cultivars. Meteorological conditions have a significant effect on the glucosinolate content in spring rape as well, and its greatest content has been found in dry and warm years (JENSEN et al. 1996, ADOMAS 2004). Ssame as our results, no difference in the glucosinolate content between camelina winter and summer cultivars has been revealed by BÜCHSENSCHÜTZ-NOTHDURFT (1998). The results of this study demonstrated that correlation of the glucosinolate content in oil and oil content in seeds was negative for camelina and positive for crambe. However, LESSMAN (1975) and AZAM et al. (2013) have not found any significant correlation between these characteristics in Brassica napus and Crambe abyssinica, respectively.

MANDAL et al. (2002), who studied some species of the family *Brassicaceae*, found a negative correlation between oleic and erucic acid concentrations. However, no significant correlation was found between these acids in our study. Moreover, oleic acid was one of two fatty acids whose content did not correlate with the content of any other fatty acid in crambe oil. It is evident that variation of fatty acid composition in camelina and crambe seed oil is much less affected by inheritable traits of cultivars than by meteorological conditions of different years. The effect of growth conditions on the variation in the fatty acid content of camelina and crambe has also been found by other researchers (ZUBR, MATTHÄUS 2002, ZANETTI et al. 2009).

CONCLUSIONS

The studied species, *Camelina sativa* and *Crambe abyssinica*, differed in seed oil content, glucosinolate content in oil and oil fatty acid composition. The oil content was 22.1-42.5% in dry matter of camelina seeds and 23.4-36.6% in crambe seeds. The glucosinolate content varied from 61.8 to 68.6 μ mol g⁻¹ in oil of crambe and from 8.6 to 30.5 μ mol g⁻¹ in oil of camelina. Camelina oil was characterised by the predominance of linolenic acid (34.8-41.6%), while crambe oil was predominated by erucic acid (53.0-60.9%). The second most abundant fatty acid in oil of both species was oleic acid, whose amount did not differ between species.

Our study indicated that variation in seed oil content, glucosinolates content and fatty acid composition in each studied species was much less affected by inheritable traits of cultivars than by meteorological conditions of different years.

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