GLUCOSINULATES CONTENT IN CAMELINA (*Camelina sativa* (L.) Crantz) SEEDS AND OILCAKES WITH REGARD TO PRODUCTION LOCATION

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Abstract
The main product of camelina (*Camelina sativa* (L.) Crantz) is its high nutritional oil, but also its oilcakes contain high levels of proteins, carbohydrates, vitamins, minerals, and low level of phytochemicals such as glucosinolates (GLS) compared with other Brassica species. The aim of this study was to evaluate different camelina cultivars (Danish cultivars Vega and Hoga, German cultivars Calena, organically produced Calena (Bio Calena) and Ligena, and Slovenian autochthonous cultivar) grown in year 2012 on four different locations in Slovenia to find out the chemical composition of seeds and oilcakes as a by-product in oil production that could be used as feed for animals. The content of glucosinolates did not preclude the use of seeds and/or camelina oilcake in animal nutrition. Content of a particular GLS in seeds and consequently in oilcakes was strongly influenced by both environmental conditions during the growing period and by botanical origin. In seeds GLS-10 (6.9–28.5 mmol/g) was the most dominant GLS in all samples. The relative amount of GLS-10 (glucocamelinin) among all GLS was from 59 to 70 %. The second one was GLS-9 (glucoarabin; 1.3–15.3 mmol/g) followed by GLS-11 (11-(metilsulfonil) undecilglucosinolate; 1.5–7.6 mmol/g). The total amount of GLS in the samples ranged from 13.0 to 48.9 mmol/g (mean 24.8 mmol/g). At cultivars Vega and Hoga there was significantly lower content of GLS in oilcakes in comparison to seeds, while in all other cases the contents are comparable.
Key words: *Camelina sativa*, false flax, glucosinolates, environmental conditions, cultivars, seeds, camelina oil, oilcakes, chemical analyses, nutritional values

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VSEBNOST GLUKOZINULATOV V SEMENU IN POGAČAH RIČKA (*Camelina sativa* (L.) Crantz) GLEDE NA LOKACIJO PRIDELAVE

Izvleček
Glavni pridelek ričkа (*Camelina sativa* (L.) Crantz) je njegovo zelo hranilno olje, medtem ko tudi oljne pogače, ki ostanejo po stiskanju olja in se lahko uporabijo kot krma za živali, vsebujejo veliko beljakovin, ogljikovih hidratov, mineralov, vitaminov in malo fitokemikalij, kot so glukozinulati (GLS), v primerjavi z drugimi vrstami iz družine Brassicaceae. Namen študije je bil preučiti kemično sestavo semena in oljnih pogač različnih sort ričkа, pridelanih pri enakih agrotehnoloških razmerah v poljskem poskusu v letu 2012 na štirih različnih lokacijah v Sloveniji. Vključene sorte ričkа so bile: danski sorti Vega in Hoga, nemški sorti Calena, ekološko pridelana Calena (Bio Calena) in Ligena ter slovenska avtohtona sorta. Vsebnost glukozinolatov ne izključuje uporabe semena in/ali oljnih pogač ričkа v prehrani živali. Na vsebnost posameznih GLS v semenu in oljnih pogačah so zelo vplivale tako pedoklimatske razmere kot sorta. V vseh vzorcih semen je bil prevladujoč GLS-10 (6,9–28,5 mmol/g). Relativna vsebnost GLS-10 (glucocamelinin) med vsemi GLS je bila od 59 do 70 %. Na drugem mestu je bil GLS-9 (glucoarabin; 1,3–15,3 mmol/g), sledil je GLS-11 (11-(metilsulfonil) undecilglucosinolate; 1,5–7,6 mmol/g). Skupna količina GLS v vzorcih je bila od 13,0 do 48,9 mmol/g (povprečje 24,8 mmol/g). Pri sortah Vega in Hoga je bila značilno nižja vsebnost GLS v oljnih pogačah v primerjavi s semenom, medtem ko je bila pri drugih sortah primerljiva.
Ključne besede: *Camelina sativa*, navadni riček, glukozinolati, okoljske razmere, sorte, semena, ričkovo olje, oljne pogače, kemijske analize, prehranska vrednost

1 INTRODUCTION

Camelina sativa is an underexploited member of the Brassicaceae family commonly known as false flax, gold of pleasure, and leindotter (Zubr, 1997). Archaeological excavations in Europe and Scandinavia suggest that this plant was an important oil crop 2000 years ago (Hatt, 1937). Since then it had been characterized as a weed species throughout Europe, but it was revived as a minor crop on a small scale in Europe and the Balkans in the early 20th century where camelina oil was used as a dietary oil, in herbal medicine (for stomach ulcers, treatment of burns, wounds, eye inflammations, and as a tonic) and for technical purposes (paints, lubricant oil) (Zubr, 1997; Loebe,1845; Rode, 2002).
Recently, interest in camelina oil has been renewed in some parts of Central and Northern Europe as a rich source of essential n-3 α-linolenic acid, and in parts of North America and Australia as the feedstock for biodiesel manufacture as the crop is low-input and hence economical to produce (Zubr, 1997; Pilgeram, 2007; Ghamkhar, 2010).
The main product of *C. sativa* is the oil. The high nutritional value of this oil results from its high content of oleic, linoleic , α-linolenic and gondoic acid, and its

low content of erucic acid. Camelina oil also contains a high content of tocopherols and phenolic compounds, making it more stable toward oxidation than highly unsaturated linseed oil (Zubr, 1997; Abramovič, 2007; Hrastar, 2009). Camelina oilcake contains high levels of proteins, carbohydrates, minerals, vitamins, and low level of phytochemicals such as glucosinolates (GLS) (Zubr, 2010; Matthäus, 2000) and its use in the EU was legalized by EU Commission Directive in 2008 (EC, 2008).

Camelina seed and oilcake can be exploited as a protein and n-3 fatty acid rich ingredient in fodder mixtures for animals and it can be used as forage for rabbits, pigs, and ruminants (Schuster, 1998; Peiretti, 2007).

The aim of this study was to evaluate different camelina cultivars grown on different locations in Slovenia at the same agrotechnology to find out the most appropriate one for our environment, to get the information about the agronomic properties as well as chemical composition of seeds and oilcakes. The yield of investigated cultivars with regard to location was already evaluated and represented by Čeh et al. (2012). In this paper glucosinolates content in their seeds and oilcakes are examined; this is a chemical group of metabolites which were interesting to analyse, because in the past they were one of the reason to ban oilcakes as feed.

2 MATERIAL AND METHODS

2.1 Investigated cultivars

In the experiment two Danish cultivars were included: cv. Vega and cv. Hoga, two German cultivars Calena and Ligena as well as organically produced Calena (Bio Calena) and Slovenian autochthonous.

2.2 Field experiments

Field experiment was conducted in year 2012 on four different locations in Slovenia with regard to soil type and also geographically (in Prekmurje region: Rakičan /medium heavy soil/ and Murska Sobota /light soil/, in Savinjska valley (Žalec): Savinjska valley 1 /heavy soil/ and Savinjska valley 2 /medium heavy soil/) as a block trial with six camelina cultivars in four replications. The sizes of individual plots were 36 m^2 (6 m x 6 m).

Sowing was done on 30th March 2012 in Savinjska valley and on 19th April in Prekmurje, the seed rate was 6 kg/ha. Before sowing fertilization was performed according to soil analyse results. Nitrogen was fertilized at sowing (60 kg/ha N) and before flowering (30 kg/ha N) in the form of calcium ammonium nitrate (CAN). Field was not irrigated. Harvest was done in the time of technological maturity on 10th July.

2.3 Weather conditions

There was a lack of rainfall from autumn 2011 until March 2012. The drought endangered the growth of most crops in spring. From April to June, rainfalls were relatively well distributed. Then, again, a lack of rainfall was detected; in Žalec there was only 13 mm of rain from 15th June to 12th July. All the growth season was characterized by above average temperatures at all investigated locations (Agrometeorological …., 2012).

2.4 Chemical analyses

All organic impurities in the seeds were manually removed prior analyses. Moisture content in seeds was determined by standard gravimetric method by drying at 105 °C to the constant mass.

Determination of glucosinolates in seeds was performed immediately after harvest, while in oilcakes it was done after the cold pressing. In both cases the applied methodology was the same as follows.

Soxhlet extraction of the milled (10 g) *Camelina sativa* seeds was carried out in Soxhlet apparatus for 6 h using hexane (boiling point at 69 °C) as solvent to remove all fats from the sample. Defatted seed powder was stored at 20 °C until further analysis on glucosinolates (GLS). All analyses were made in duplicates. The extraction method described in Hrastar et al. (Hrastar, 2012) was based on extraction of GLS with hot methanol used as a solvent. The defatted seed powder (0.25 g) was transferred into a 10 mL tube with screw top. Three milliliters of 70 % methanol and 5 μmol of sinigrin (C2 Bioengineering ApS) as internal standard were added. The content was mixed and the tube was placed in a 70 °C hot water bath for 10 min, during which the samples were mixed for several times. Afterwards, the solution was centrifuged (at 4000 rpm, 10 min). Supernatant was transferred to a 10 mL flask. The defatted seed powder was re-extracted twice with 3 mL of 70 % methanol and centrifuged. Supernatants were combined and diluted to 10 mL. Two milliliters of solution were filtered through 0.45 μm filter, transferred into a HPLC vial and stored at -20 °C until further analysis.

For GLS quantification, a high-performance liquid chromatography (HPLC) method described in Hrastar et al. (Hrastar, 2012) was used. Two microliters of GLS containing extract solution was run on an Agilent 1200 Series HPLC system (Palo Alto, CA, USA) at 1 mL/min. The column was a Zorbax Eclipse XDB-C18, 150 mm x 4.6 mm; particle size 5 μm (Agilent). The GLS were detected with DAD detector at 229 nm. The mobile phase used was an aqueous 0.005 M tetrabutylammonium bisulfate (phase A) versus methanol (phase B) for a total running time of 45 min. The gradient changed as follows: 100 % A for 10 min, then in 25 min to 35 % A/65 % B, followed by 100 % B for 5 min. Afterward the column was equilibrated at 100 % A for 5 min. GLS were identified by comparing

to external standards which were a generous gift of Berhow, M. A. (USDA). Quantification was done based on a calibration curve of freshly prepared pure glucoiberin (C2 Bioengineering ApS, Denmark) in 10 mL flask spiked with 5 μmol of sinigrin. The content of each GLS was back calculated and expressed in micromoles per gram (μmol/g) of dry seed. All the chemicals used were of HPLC or analytical grade (Fluka, Sigma–Aldrich).

3 RESULTS AND DISCUSSION

In plants about 120 different GLS's, a group of plant secondary metabolites, are known to occur naturally; their hydrolytic and metabolic products can act as chemoprotective or toxic agents (Mithen, 2000). When using camelina oilcakes as animal fodder, the affect of GLS in camelina oilcake can be considered as comparable or rather smaller than the effect of GLS in rapeseed products (Matthäus, 2000). GLS's are found in the highest concentrations in the Brassicaceae family. GLS-9 and GLS-10 were also identified in other species, e.g., *Arabis alpina*, *Capsella bursa-pastoris* (Daxenbichler, 1991), while GLS-11 was detected in *Camelina sativa* only. As confirmed by Matthäus (2000) camelina seeds contain significant levels of three GLS's; GLS-9 or glucoarabin (9-(methylsulfinyl) nonylglucosinolate), GLS-10 or glucocamelinin (10-(methylsulfinyl) decyl-glucosinolate), and GLS-11 (11-(methylsulfinyl) undecylglucosinolate). They are located in the non-oil part of the seed. A HPLC chromatogram of all three GLS's in *Camelina* seed of different cultivars from our field experiment is shown in Figure 1. The upper chromatogram in the figure corresponds to the mixture of standard compounds and the lower one to the real sample extract. Retention times were 12.3, 13.6 and 14.9 min for GLS-9, GLS-10 and GLS-11 respectively.

In Table 1 the results of determination of GLS in seeds of different camelina cultivars grown at four different locations in our field experiments are presented. GLS-10 (6.9–28.5 mmol/g) was the most dominant GLS in all samples. The relative ammount of GLS-10 among all GLS vary from 59 to 70 %. The second one was GLS-9 (1.3–15.3 mmol/g) followed by GLS-11 (1.5–7.6 mmol/g). The total amount of GLS in the samples ranged from 13.0 to 48.9 mmol/g (mean 24.8 mmol/g). From the results it can be seen that in average the highest GSL content was in the seed samples from Prekmurje region (Rakičan and Murska Sobota).

After the analysis of camelina seeds, samples of the same cultivars from different locations were mixed together to get the average – a representative sample for a particular cultivar, which was after dry pressed. In oilcakes we determined the content of GLS. The results are presented in Table 2. It could be seen that only in at cv. Vega and cv. Hoga we got significantly lower content of GLS in oilcakes in comparison to seeds, while at all other cultivars the contents were comparable.

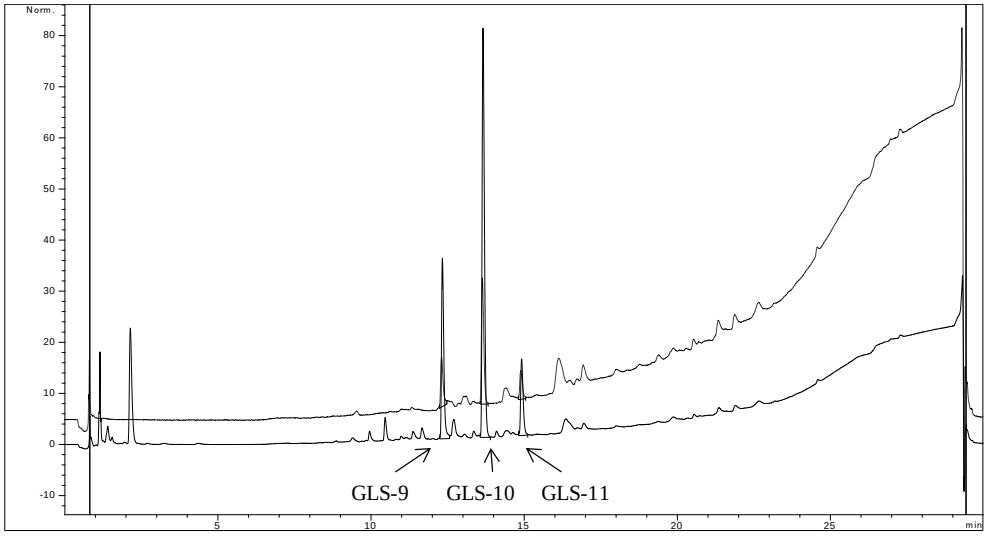

Figure 1: HPLC chromatogram of camelina seed extract from our field experiment (lower line) and standard solution of GLS (upper line). GLS-9 (glucoarabin), GLS-10 (glucocamelinin) and GLS-11 were identified as significant GLS.
Slika 1: HPLC kromatogram ekstrakta ričkovih semen (spodnja črta) in standardne raztopine glukozinolatov (zgornja črta). Identificirani so bili trije prevladujoči glukozinolati: GLS-9 (glukoarabin), GLS-10 (glukokamelinin) in GLS-11.

Table 1: Content of glucoarabin (9), glucocamelinin (10) and 11-(metilsulfonil) undecilglucosinolate (11) in seeds of different cultivars, planted in 2012.
Preglednica 1: Vsebnost glukoarabina (9), glukokamelinina (10) in 11-(metilsulfonil) undecilglukozinota (11) v semenu rička različnih sort, posejanih v sortnih poskusih v letu 2012.

c (µmol/g)	Savinj. valley 1			Savinj. valley 2			Rakičan			Murska Sobota		
Cultivar	**9**	**10**	**11**	**9**	**10**	**11**	**9**	**10**	**11**	**9**	**10**	**11**
Vega	1.3	11.0	3.4	1.8	13.7	4.2	5.2	23.9	5.6	3.6	16.5	4.0
Hoga	1.9	15.1	5.0	3.0	22.2	7.6	4.4	23.3	7.1	5.3	23.4	7.0
Calena	5.1	11.7	2.4	5.2	12.8	3.0	4.9	9.9	1.9	10.5	20.9	3.9
Bio Calena	3.6	7.7	1.7	8.1	18.0	3.8	5.7	11.3	2.1	5.5	10.7	1.9
Ligena	2.9	6.9	1.5	7.1	16.6	3.7	15.6	28.1	5.2	4.9	10.4	1.9
Slo. autocht.	5.5	12.4	2.2	4.1	9.2	1.6	4.7	9.2	1.6	15.3	28.5	4.5

Table 2: Content of glucoarabin (9), glucocamelinin (10) and 11-(metilsulfonil) undecilglucosinolate (11) in oilcakes of different camelina cultivars.
Preglednica 2: Vsebnost glukoarabina (9), glukokamelinina (10) in 11-(metilsulfonil) undecilglukozinota (11) v pogačah različnih sort rička.

Cultivar	Averaged samples from all growing sites together		
	9	**10**	**11**
Vega	2.5	12.5	3.5
Hoga	3.0	14.4	4.0
Calena	6.5	14.0	2.7
Bio Calena	6.1	13.4	2.5
Ligena	6.6	14.8	2.9
Slo. avtocht.	6.8	15.5	2.8

4 CONCLUSIONS

Analyses showed the expected results according to the available literature data, as in the seeds as well as in the oilcakes. The content of glucosinolates does not preclude the use of seeds and/or camelina oilcake in animal nutrition. Content of a particular GLS in seeds and consequently in oilcakes is strongly influenced by both climate conditions during the growt period and by botanical origin.

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5 LITERATURE

Agrometeorological portal of Republic Slovenia (http://agromet.mkgp.gov.si/APP/Home/METEO/-1), (Cited: 15. October 2012)

Abramovič H., Butinar B., Nikolič V. Changes occurring in phenolic content, tocopherol composition and oxidative stability of Camelina sativa oil during storage. *Food Chem.* 2007; 104, 903–909.

Čeh B, Štraus S, Hladnik A, Oset Luskar M, Čremožnik B. Pridelek rička (*Camelina sativa* (L.) Crantz) glede na lokacijo in sorto. *Hmeljarski bilten/Hop Bulletin.* 2012; 19: 88-99.

Daxenbichler M.E., Spencer G.G., Carlson D.G., Rose G.B. Glucosinolate composition of seeds from 297 species of wild plants. *Phytochemistry.* 1991; 30, 2623–2638.

EC Commission Directive: 2008/76/EC of 25 July 2008, amending Annex I. Off. J. Eur. Union. 2008, L 198/37.

Hatt G. In: Udvalget for Folkeoplysnings Fremme, Koebenhavn, Denmark 1937.

Hrastar R., Petrišič M.G., Ogrinc N., Košir I.J. Fatty acid and stable carbon isotope characterization of Camelina sativa oil: Implications for authentication. *J. Agric. Food Chem.* 2009; 57, 579–585.

Hrastar R., Abramovič H., Košir I.J. In situ quality evaluation of Camelina sativa landrace, *Eur. J. Lipid Sci. Technol*. 2012; 114, 343–351.

Loebe W. In: Loebe, W. (Ed.), Om Olievaerterne, deres Dyrkning og Behandling samt om Oliepresning og Olierensning, Vorlaget af den Re´eske Boghandel, Aalborg, Denmark 1845, pp. 66–73.

Matthäus B., Zubr J. Variability of specific components in Camelina sativa oilseed cakes. *Ind. Crops Prod*. 2000; 12, 9–18.

Mithen R.F., Dekker M., Verkerk R., Rabot S., Johnson I.T. Review: The nutritional significance, biosynthesis and bioavailibility of glucosinolates in human foods. *J. Sci. Food Agri*. 2000; 80, 967–984.

Peiretti P.G., Mussa P.P., Prola L., Meineri G. Use of different levels of false flax (Camelina sativa L.) seed in diets for fattening rabbits. *Livest. Sci*. 2007; 107, 192–197.

Rode J., Study of autochthon Camelina sativa (L.) Crantz in Slovenia. *J. Herbs Spices Med. Plants.* 2002; 9, 313–3185.

Schuster A., Friedt W. Glucosinolate content and composition as parameters of quality of Camelina seed. *Ind. Crops Prod*. 1998; 7, 297–302.

Zubr J., Oil-seed crop: Camelina sativa. *Ind. Crop. Prod*. 1997; 6, 113–119.

Zubr J., Carbohydrates, vitamins and minerals of Camelina sativa seed. *Nutr. Food Sci*. 2010; 40, 523–531.