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Glucosinolates and Sinapine in Camelina Meal

Roberto Russo, Remo Reggiani

Istituto di Biologia e Biotecnologia Agraria, CNR, Milano, Italy
Email: reggiani@ibba.cnr.it

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Abstract

Forty seven accessions of camelina (*Camelina sativa* L. Crantz) were analyzed for glucosinolates (GSLs) and sinapine in defatted meal. These antinutritional compounds are undesirable in camelina meal for use in animal feeding and therefore we show their variability to identify the best varieties for future breeding programs. Total GSLs ranged from 19.6 to 40.3 mmol Kg^{-1} dry weight (DW) with an average of 30.3 mmol kg^{-1} DW. Great variability has also been observed in the levels of individual GSLs (GSL1, GSL2 and GSL3), so that the content of GSL1 and GSL3 were not correlated to each other in the accessions of camelina. Five out of six winter forms of camelina showed low content of GSLs. Sinapine ranged from 1.09 to 4.75 g Kg^{-1} DW with an average of 2.57 g kg^{-1} DW. The sinapine content was not correlated with that of GSLs. The use of camelina meal is only limited by the presence of GSLs while sinapine content can be ignored in camelina varieties.

Keywords

Camelina sativa L. Crantz, Glucosinolates, Sinapine, Meal, Feeding

1. Introduction

In the last century, with the rapid growth of the industrial food animal production, an increasing demand for cheap sources of protein and essential nutrients grew for feed formulation. More than 95% of the meat and fresh dairy products available in Europe are produced in the EU itself [1]. However, if we take into account the origin of plant protein for animal production industry in the EU appears a completely different picture. Sixty-nine% of protein-rich feed materials is imported into the EU of which, for the soybean (the main source of essential amino acids), self-sufficiency is only 3% while soybean meal provides 64% of protein-rich feed materials [2]. It follows that the EU's livestock farming sector heavily depends on the amount of soybean meal used for meat unit produced: 232, 648 and 967 g kg^{-1} for beef, pork and poultry, respectively [3].

If Europe wants to increase self-sufficiency in the production of crop meals rich in protein, it should increase the use of alternative or new crops that contain protein levels comparable to soybean such as canola or flax. *Camelina sativa* (L.) Crantz is an ancient oilseed crop cultivated in eastern and central Europe since the Bronze Age [4]. Subsequently, camelina cultivation almost vanished and remained an underexploited oilseed crop. In recent decades, an interest has risen for this plant in Europe and North America as a suitable feedstock for biofuel. Its seed contains oil (35% - 43% on a dry weight [DW] basis) extremely rich in polyunsaturated fatty acid [5] [6] [7]. After oil extraction, the residual meal contains a lot of protein (330 - 400 g kg^{-1}) [8] which makes it a suitable source of vegetable protein for animal feeds [9]. From a nutritional point of view, camelina proteins, having a good content of sulfur amino acids, complement well those of legumes [10].

The use of camelina meal as ingredient in livestock rations is a critical factor to further increase the economic value of the plant. The exploitation of this by-product might reduce costs and promote environmental sustainability. In order to use camelina meal as feed, the presence of anti-nutritional compounds has to be considered. Being camelina a crucifer, the major antinutritional compounds that arouse attention are glucosinolates (GSLs) and sinapine. Isothiocyanates, thiocyanates, nitriles and epithionitrile are catabolic products of GSLs which are responsible for their high toxicity [11]. Sinapine is a choline ester of sinapic acid which is important in plants for the biosynthesis of lignin and flavonoids. However, sinapine has several undesirable properties as a constituent in animal feeds. It is a bitter substance that, if present in the diet of certain brown egg-laying hens at levels exceeding 1 g kg^{-1}, leads to a fishy odour or taste in the eggs [12]. Especially for the presence of GSLs, the European Food Safety Authority recommends limiting the total GSL content to 1 - 1.5 mmol kg^{-1} of feed for monogastric animals [11], while the US Food and Drug Administration approved inclusion in up to 10% of the weight of the total ration in the diets of beef cattle and poultry [13].

Despite various papers have described the content of GSLs and sinapine in camelina [14] [15] [16] [17], little is known about the overall variability for these anti-nutritionals in different accessions. In the present study, GSLs and sinapine contents were evaluated in a collection of 47 camelina accessions to assess the variability of these anti-nutritionals in order to identify the best ones for future breeding programs.

2. Material and Methods

2.1. Reagents and Plant Materials

DEAE-Sephadex A-25, sinigrin, sulfatase Type H1 were purchased from Sigma-Aldrich (Milan, Italy). All organic solvents were analytical grade. *Camelina sativa* L. seeds of 46 accessions were kindly provided by three genebanks: IPK (Germany), USDA (USA) and the Arche Noah (Austria). Camelia variety was

Table 1. List of 47 accessions of *Camelina sativa* with the country of origin.

Accession	Origin	Accession	Origin
BAVARIA[a]	Germany	CAM173[a]	Russia
CALENA[a]	Germany	CAM174[a]	Unknown
CAM7[a]	Kyrgyzstan	CAM175[a]	Sweden
CAM8[a]	Russia	CAM187[a]	Spain
CAM25[a]	Russia	CAM265[a]	Italy
CAM29[a]	Ukraine	CAM266[a]	Russia
CAM31[a]	Poland	CAM268[a]	Bulgaria
CAM34[a]	Russia	CAM269[a]	United Knigdom
CAM35[a]	Russia	CAM270[a]	Switzerland
CAM37[a]	Russia	CAMELIA[d]	Romania
CAM38[a]	Austria	KARTNER[c]	Austria
CAM39[a]	Austria	LIGENA[a]	Germany
CAM58[a]	Germany	LINDO[a]	Unknown
CAM76[a]	Russia	MORGENSONNE[c]	Austria
CAM108[a]	Poland	PI650142[b]	Denmark
CAM110[a]	Poland	PI650146[b]	Sweden
CAM111[a]	Russia	PI650168[b]	USA
CAM116[a]	Belgium	SOLEDO[a]	Unknown
CAM123[a]	Poland	ST. PERNITZEN[c]	Austria
CAM132[a]	Unknown	UKRAJINSKAJA[a]	Russia
CAM136[a]	Poland	WILEDO[a]	Unknown
CAM137[a]	Denmark	WROCLAWSKA[a]	Poland
CAM170[a]	Poland	ZARJA SOCIALISMA[a]	Russia
CAM171[a]	Unknown		

[a]IPK, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany; [b]USDA, United States Department of Agriculture, USA; [c]Arche Noah, The Austrian Seed Savers Association, Austria; [d]Panghea Natural and Chemical Innovation, Italy.

kindly gifted by Panghea Natural and Chemical Innovation (Milan, Italy). Origin of all varieties is shown in Table 1. All accessions were spring forms with the exception of six (CAM37, CAM76, CAM132, PI650168, WILEDO, ZARJA SOCIALISMA) who were winter forms. All the 47 varieties have been reproduced in pots with commercial soil. Winter forms were exposed to low temperatures for a short time after germination. After harvesting, the seeds were ground in mortar and the flours defatted with hexane before extractions. This defatted material (camelina meal) was subsequently used for the extraction of GSLs and synapine.

2.2. Extraction and Separation of GSLs

GSL extraction and assay basically follows the official HPLC method (ISO 9167-1) but adapted to camelina GSLs from Russo and Reggiani [16]. GSLs were extracted with 70% hot ethanol overnight and the sample centrifuged for 15 min

at 15,000 rpm. The supernatant were adsorbed onto a DEAE-Sephadex A-25 column (100 mg) having formate as counterion. The substances not retained by the resin were washed twice with 1 mL of 20 mM Na acetate at pH 4.0. Bound GSLs were desulfated by addition of 50 µL of sulfatase (500U) and the reaction in column was maintained at 37˚C overnight. Desulfo-GSLs were eluted from the DEAE column with 1 mL of ethanol and the samples dried at 65˚C. The samples were resuspended in 20% ethanol and filtered with 0.22 µm Costar Spin-X Centrifuge Tube Filter (Corning Incorporated, NY, USA) before HPLC analysis.

Desulfo-GSLs were separated by HPLC according to Russo *et al.* [18]. A calibration curve with desulfo-sinigrin was carried out and detection was at 229 nm.

2.3. Extraction and Separation of Sinapine

Sinapine was extracted from defatted flours with 70% methanol as recommended by Bjerg *et al.* [19] for 30 min at 75˚C and the samples were then centrifuged for 10 min at 15,000 rpm. The supernatant was diluted 1:1 with water (HPLC grade) and filtered with 0.22 µm Costar Spin-X Centrifuge Tube Filter (Corning Incorporated, NY, USA) prior to analysis.

The HPLC analysis for sinapine was basically the method described by Clausen *et al.* [20] slightly modified by us. Sinapine was separated by isocratic HPLC and detection at 330 nm. A 100 × 2.1 mm Waters Atlantis T3 C18 (2.6 µm) was used for separation. The mobile phase consisted of 13.5% acetonitrile in 10 mM Na acetate (pH 4.0). The flow rate was 0.275 mL min^{-1} and the peak of sinapine eluted at 4.5 minutes.

2.4. Statistical Analysis

The extraction of each variety was performed in duplicate and the HPLC analysis in triplicate. Means, standard deviations and Pearson's correlations were calculated with SPSS version 11.5 software.

3. Results and Discussion

3.1. GSLs in Camelina Accessions

In **Figure 1** are shown the levels of GSLs for 47 camelina accessions. As can be seen, total GSL content in the accessions was evenly distributed between 19.6 (CAM25) and 40.3 (CAM58) mmol Kg^{-1} DW, with an overall average of 30.3 mmol Kg^{-1} DW. This range of GSLs distribution between accessions is slightly higher to that described by Schuster and Friedt [17] (13.2 - 36.2 mmol kg^{-1} DW). Five of the six winter forms of camelina were below the general mean and four of them (CAM37, PI650168, WILEDO and CAM132) had among the lowest levels of GSLs. Also Schuster and Friedt [17] reported generally low GSL content in winter forms in comparison with spring varieties.

Many of the studies on the presence of camelina meal and GSLs in the diets were carried out on poultry. The threshold of tolerance of camelina meal in diets

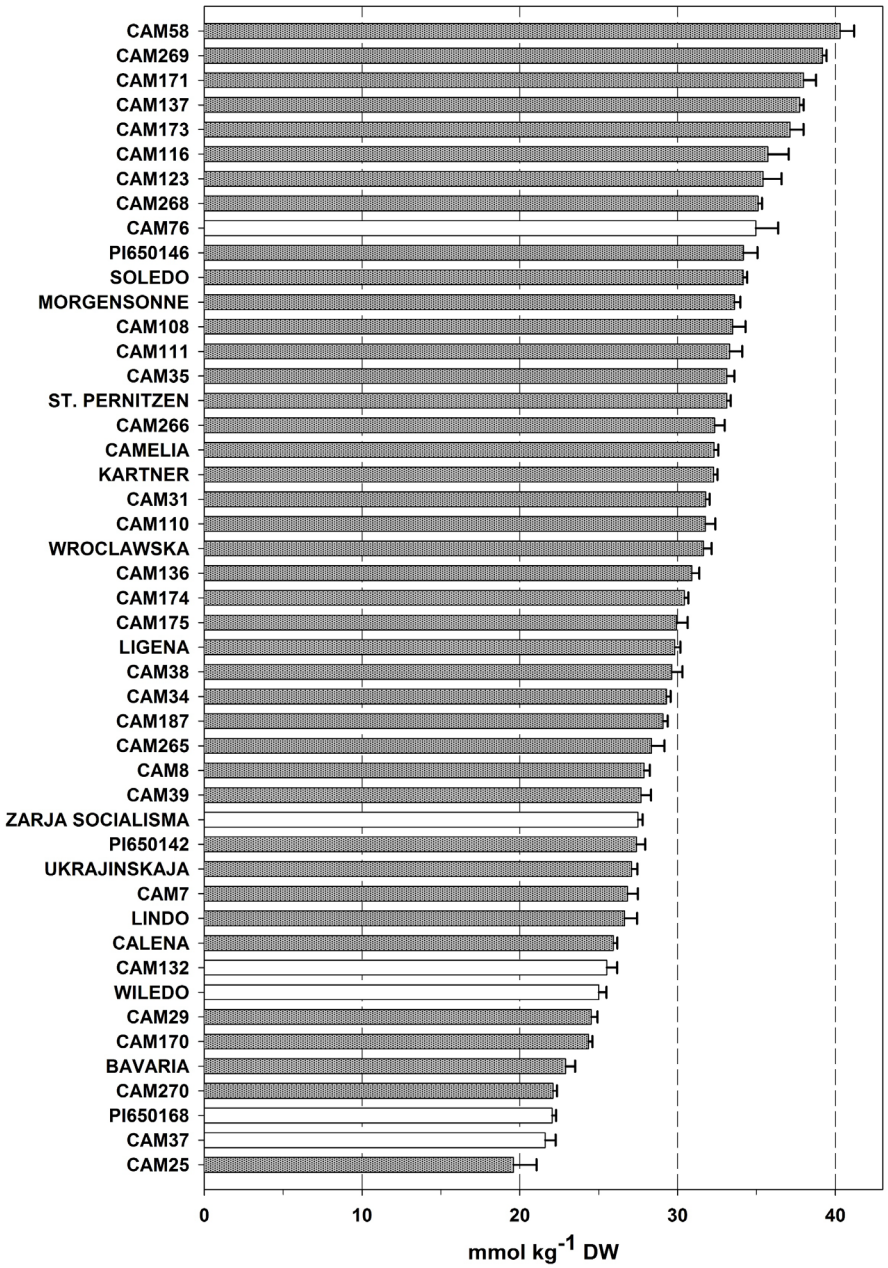

Figure 1. Levels of glucosinolates in 47 accessions of *Camelina sativa* (L.) Crantz. White bars are winter forms.

of birds seemed to be 10% [21] [22] [23]. In Particular, the poultry performance is compromised if GSLs are more than 2.5 mmol kg^{-1} in complete feeds [24]. This means that camelina meal could contain up to 25 mmol kg^{-1} (10 times is the dilution factor of GSLs in complete feeds) on a DW basis, or 29.4 mmol kg^{-1} in an expeller which may contain up to 15% of residual oil. Thus, virtually all accessions below the average can be safely used at 10% in the composition of feeds.

In camelina, there are 3 GSLs that can be easily separated by HPLC: 9-methyl-sulfinyl-nonyl-GSL (GSL1), 10-methyl-sulfinyl-decyl-GSL (GSL2) and 11-methyl-sulfinyl-undecyl-GSL (GSL3) [17] [18]. In **Table 2** are shown the contents of the

Table 2. Levels of GSL1, GSL2 and GSL3 in 47 camelina accessions. Low levels for a class of GSL are evidenced in bold.

Accession	GSL1	GSL2	GSL3
BAVARIA	7.00 ± 0.2	**13.5** ± 0.3	2.4 ± 0.2
CALENA	8.0 ± 0.1	**14.9** ± 0.3	3.0 ± 0.6
CAM7	9.0 ± 1.2	**14.9** ± 0.4	2.9 ± 0.2
CAM8	10.7 ± 0.1	15.6 ± 0.2	**1.6** ± 0.1
CAM25	**2.6** ± 0.3	**14.5** ± 1.0	2.6 ± 0.2
CAM29	7.1 ± 0.3	**14.6** ± 0.2	2.8 ± 0.1
CAM31	11.2 ± 0.1	17.5 ± 0.1	3.1 ± 0.0
CAM34	10.3 ± 0.4	16.2 ± 0.1	2.8 ± 0.1
CAM35	10.7 ± 0.0	17.6 ± 0.4	4.8 ± 0.1
CAM37[a]	**3.9** ± 0.4	**13.8** ± 0.0	3.9 ± 0.3
CAM38	9.8 ± 0.1	16.9 ± 0.4	3.0 ± 0.2
CAM39	8.8 ± 0.1	16.3 ± 0.6	2.6 ± 0.1
CAM58	13.3 ± 0.6	21.5 ± 0.3	5.5 ± 0.0
CAM76[a]	12.3 ± 0.1	18.6 ± 1.1	4.1 ± 0.3
CAM108	12.2 ± 0.3	17.0 ± 0.3	4.3 ± 0.3
CAM110	10.5 ± 0.3	17.9 ± 0.2	3.3 ± 0.2
CAM111	13.0 ± 0.3	17.6 ± 0.4	2.7 ± 0.1
CAM116	11.5 ± 0.6	18.8 ± 0.5	5.5 ± 0.3
CAM123	11.4 ± 0.17	18.4 ± 0.68	5.7 ± 0.7
CAM132[a]	6.6 ± 0.3	**14.7** ± 0.3	4.2 ± 0.2
CAM136	9.2 ± 0.1	18.9 ± 0.3	2.8 ± 0.1
CAM137	13.9 ± 0.1	20.6 ± 0.1	3.3 ± 0.1
CAM170	7.2 ± 0.6	14.6 ± 0.8	2.6 ± 0.2
CAM171	15.6 ± 0.1	19.3 ± 1.0	3.0 ± 0.1
CAM173	13.3 ± 0.0	19.5 ± 0.4	4.3 ± 0.5
CAM174	8.9 ± 0.3	16.6 ± 0.1	4.9 ± 0.1
CAM175	9.4 ± 0.2	17.4 ± 0.3	3.2 ± 0.2
CAM187	9.5 ± 0.1	16.9 ± 0.3	2.6 ± 0.1
CAM265	9.0 ± 0.0	15.9 ± 0.3	3.4 ± 0.2
CAM266	11.8 ± 0.3	18.1 ± 0.6	2.4 ± 0.0
CAM268	13.1 ± 0.3	18.5 ± 0.1	3.5 ± 0.2
CAM269	13.2 ± 0.0	19.9 ± 0.1	6.1 ± 0.1
CAM270	7.3 ± 0.6	**12.9** ± 0.5	**2.0** ± 0.2
CAMELIA	9.5 ± 0.1	18.4 ± 0.3	4.5 ± 0.1
KARTNER	11.1 ± 0.1	17.4 ± 0.1	3.7 ± 0.1
LIGENA	10.7 ± 0.0	16.5 ± 0.2	2.7 ± 0.0
LINDO	7.8 ± 0.2	15.6 ± 0.4	3.2 ± 0.2
MORGENSONNE	11.5 ± 0.2	18.1 ± 0.2	4.0 ± 0.0
PI650142	**3.5** ± 0.0	18.8 ± 0.0	5.0 ± 0.6
PI650146	10.5 ± 0.2	18.1 ± 0.5	5.7 ± 0.2
PI650168[a]	**1.0** ± 0.0	**15.0** ± 0.1	6.0 ± 0.1
SOLEDO	11.7 ± 0.1	18.2 ± 0.1	4.3 ± 0.1
ST. PERNITZEN	7.8 ± 0.1	21.3 ± 0.1	4.1 ± 0.0
UKRAJINSKAJA	8.3 ± 0.1	15.8 ± 0.1	3.0 ± 0.1
WILEDO[a]	9.1 ± 0.1	**14.7** ± 0.1	**1.3** ± 0.0
WROCLAWSKA	12.4 ± 0.4	17.2 ± 0.3	2.1 ± 0.2
ZARJA SOCIALISMA[a]	9.1 ± 0.0	**14.8** ± 0.1	3.6 ± 0.2

[a]Winter forms of camelina.

3 molecular species of GSLs in 47 accessions of camelina. The average content of the 3 GSLs was 9.7, 17.0 and 3.6 for GSL1, GSL2 and GSL3, respectively. We have evidenced in bold the values of GSL1, GSL2 GSL3 that are well below average. In particular it can highlight that CAM25, CAM37 and PI650168 were low for both GSL1 and GSL2. CAM25 and CAM37 showed similar levels of GSL1 and GSL3, while in PI650168, GSL1 content was very low and significantly lower than GSL3. The GSLs pattern in PI650168 was very similar to that described for *Camelina microcarpa* [18]. Accessions CAM270 and WILEDO showed low levels of both GSL2 and GSL3. Also the varieties low in GSL2 (BAVARIA, CALENA, CAM7, CAM29 and CAM132) might be considered interesting since GSL2 is often at least 50% of the total GSLs.

3.2. Sinapine in Camelina Accessions

In **Figure 2** are shown the contents of sinapine in 47 camelina accessions. The variability that is observed on the concentration of sinapine in flours is definitely higher than that observed on GSLs. The accession Kartner contains about 3.6 times more sinapine of accession CAM269. This variability is larger than that described by Matthäus and Zubr (1.7 - 4.2 g kg^{-1} DW) [14]. The average content of sinapine was 2.6 g kg^{-1} DW, with many accessions showing content between 2 and 3 g kg^{-1} DW. Nine accessions were well below 2 g kg^{-1} DW, of which only CAM270 also had a low content of GSLs. Winter accessions of camelina are not among those with low sinapine content.

The content of sinapine in camelina (0.1% - 0.5%) is lower than those of canola [25] [26] or other Brassicaceae [27]. Sinapine concentrations below 0.1% would be desirable to make the feeds containing cruciferous flour palatable to animals [28]. Considering the current limit of use of camelina meal in animal feed (up 10%), unpleasant effects of sinapine are to be excluded regardless from the camelina accession used.

3.3. Correlations between GSLs and Sinapine in Camelina Accessions

In a previous work [16], a negative correlation was found between total GSLs and sinapine analyzing only 12 accessions. **Table 3** shows that, with a greater number of accessions which makes the test more reliable, such a correlation is not verified (both on total and on individual GSLs). Total GSLs was significantly correlated with individual GSLs even if the value of r was lower towards GSL3. GSL1 and GSL3 were not correlated with each other and this is explained by the high variability that is observed on the content of these two compounds (**Table 2**). In fact, in most of the accessions the content of GSL1 > GSL3, but in some accessions the content was similar (CAM25, CAM37), while in others the content of GSL3 > GSL1 (PI650142, PI650168).

4. Conclusion

The use of camelina meal for feeding is limited by the presence of GSLs while the

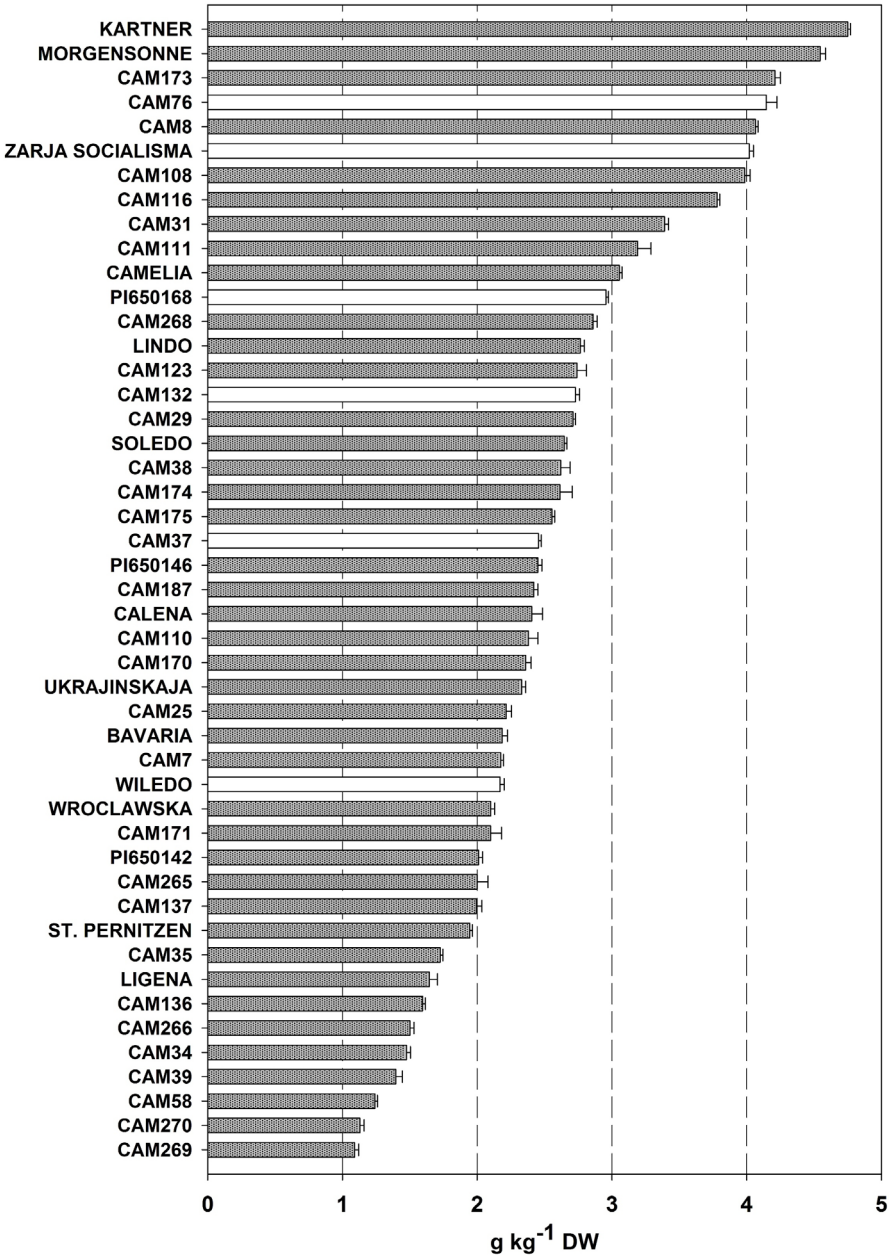

Figure 2. Levels of sinapine in 47 accessions of *Camelina sativa* (L.) Crantz. White bars are winter forms.

Table 3. Pearson correlation coefficient (*r*) among antinutritional compounds in 47 accessions of camelina.

Compounds	GSL1	GSL2	GSL3	Sinapine
Total GSLs	0.864**	0.900**	0.446**	0.117
GSL1	1	0.620**	0.021	0.141
GSL2		1	0.465**	0.001
GSL3			1	0.135
Sinapine				1

**Correlation is significant at the 0.01 level.

content of sinapine can be overlooked. Only one accession (CAM270) was low both as GSLs that as sinapine. This screening shows that there are many camelina accessions (both winter and spring forms) that can produce expellers to be safety used in animal feeds at 10%. GSL content in camelina it is not absolutely high and could be easily reduced at levels comparable with canola by conventional breeding. Thus, the amount of camelina meal in the diets would be increased and hence the content of omega-3 and antioxidants in foods of animal origin. The lack of correlation between GSL1 and GSL3 could be exploited to draw specific crosses between varieties to lower the content of both. We are adopting this last strategy in our laboratory and the results seem promising.

Acknowledgements

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