Antioxidants in flour of the oilseed crop *Camelina sativa* (L.) Crantz

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Abstract

Forty-seven accessions of *Camelina sativa* (L.) Crants (41 spring and 6 winter forms) were analyzed for antioxidant activity, total phenolic content (TPC), flavonoids and proanthocyanidins. The antioxidant activity (AA%) was high in camelina accessions and with a significant variability among accessions and between spring and winter forms. The highest antioxidant activities have been observed in some spring accessions (CAM35, CAM173 and CAM268). TPC was high in camelina and significantly different among accessions. Antioxidant activity resulted highly correlated with TPC, while no correlation was observed with flavonoid and proanthocyanidin contents. Significant variability was observed among accessions but not between groups (winter and spring) for flavonoid and proanthocyanidin contents. These two classes of compounds showed a significant inverse correlation between them suggesting that their synthesis is in competition. The camelina cake can enrich the animal diet of antioxidant compounds (phenolics) and some spring accessions are more promising for future breeding programs.

Introduction

Camelina sativa (L.) Crantz also known as *false flax*, is an annual oilseed crop with both autumn and spring biotypes and belongs to the brassica family. The interest for this plant has grown recently because it is a low-input crop. In fact, it can grow well on marginal lands (semiarid, low fertile or saline soils). *Camelina sativa* is tolerant to cold and drought and it does not require much chemistry (fertilizers, herbicides and pesticides).[1] Its oil is highly unsaturated (>90%) with a profile rich in α-linolenic acid[2] which make it suitable for non-feed (jetfuel) or feed applications.[3]

Meal resulting from oil extraction has a remarkable potential economic value as an ingredient for animal nutrition.[4,5] Camelina flour contains 290-370 mg g^{-1} of crude protein with a favorable amino acid composition.[5] The use of camelina meal for animal feed is limited by the presence of glucosinolates. Usage limit is 10% in USA and 12% in Canada.[6,7] In the EU, the use has been legalized by Directive 2008/76/EC.[8] The glucosinolates allowed in EU was set at 1.5 mmol kg^{-1} of feed for monogastric animals. However, there is a great variability in glucosinolate content between camelina accessions and some accessions have more favorable glucosinolate contents.[9]

Camelina flour also brings a considerable amount of antioxidant compounds to diets.[10,11] In general, the oil extraction process leads to an increase in antioxidant activity and compounds related to this activity (phenolics, flavonoids and tannins).[3] In the present study, the variability inside a collection of 47 camelina accessions (41 spring and 6 winter biotypes) for antioxidant activity, phenolics, flavonoids and proanthocyanidins (tannins) was assessed in order to identify useful accessions for breeding programs aimed at camelina flour enriched in antioxidants.

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Materials and Methods

Reagents and plant materials

DPPH, Folin-Ciocalteu, caffeic acid, catechin, Na_2CO_3, $NaNO_2$ and $AlCl_3$ were purchased from Sigma-Aldrich (Milan, Italy). All organic solvents were analytical grade. Seeds of all the accesses of *Camelina sativa* L. were kindly provided by IPK genebank (Germany) with the exception of the PI650142, PI650146 and PI650168 accessions provided by USDA (USA), KARTNER, MORGENSONNE and ST. PERNITZEN accessions provided by Arche Noah genebank (Austria) and accession CAMELIA gifted by Panghea Natural and Chemical Innovation (Milan, Italy). For the origin of these accessions see Russo and Reggiani.[9] Only six accessions were winter forms (CAM37, CAM76, CAM132, PI650168, WILEDO, ZARJA SOCIALISMA) and therefore were exposed to low temperatures for a short time after germination. All varieties were reproduced in pots with commercial soil. After harvesting, the camelina seeds were ground in mortar and the fat extracted with hexane (defatted flour).

Antioxidant activity assay

Samples were prepared by extracting camelina flour with ethanol using a ratio of 1:10 (w/v). Antioxidant activity was determined as DPPH radical scavenging activity according to methodology described by Brand-Williams *et al.*[12] The reaction mixture included 50 µL of alcoholic extract or trolox (2-50 nmol), 300 µL of ethanol and 30 µL of 0.5 mM DPPH in ethanol. In the control, 50 µL of ethanol replaced the extract. In the blank, ethanol replaced the DPPH. When DPPH reacts with antioxidant compounds can donate hydrogen and in its reduced form change color. After 100 min of reaction, the samples were transferred to 96-well transparent plates and the 517 nm absorbance read through the Infinite M200 PRO microplate reader (Tecan Italia Srl, Cernusco sul Naviglio, Italy).

The antioxidant activity was expressed both as trolox equivalent antioxidant capacity (TEAC) and as scavenging activity percentage (AA%) according to this formula $AA\% = 100 - [(Abs_{sample} - Abs_{blank}) \times 100 / Abs_{control}]$,[13] where Abs_{sample}, ABS_{blank} and $Abs_{control}$ are absorbances of sample, blank and control.

Total phenolics, flavonoids and proanthocyanidins analyses

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu method.[14] Phenols were extracted from defatted flours with 80% ethanol at 70°C. The reaction mixture was composed by 100 µl of alcoholic extract or caffeic acid standard (50-400 µg) and 500 µL of Folin-Ciocalteu reagent (diluted 2-fold with distilled water). The samples were allowed to

stand at room temperature for 5 min and then 400 µL of 60 g L^{-1} of Na_2CO_3 solution were added. The test tubes were mixed and heated at 45°C for 15 min. The samples were allowed to rest for 30 min at room temperature in the dark and then the absorbance was measured at 765 nm. Eighty% ethanol was used as a blank and the results were expressed as caffeic acid equivalents per kg of dry weight (mg CAE g^{-1} DW).

Samples for flavonoid determination were extracted with methanol using a ratio of 1:10 (w/v). To 100 µL of alcoholic extract were added 400 µL of water, 30 µL 5% $NaNO_2$ and mixed. The tubes were allowed to stand for 5 min before that 30 µL of 10% $AlCl_3$ were added. After 1 min, to each sample were added 200 µL 1M NaOH and 240 µL of water. The reaction mixture was then centrifuged and allowed to stand for 15 min in the dark. The samples (300 µL) were transferred to 96-well transparent plates and the absorbance read at 510 nm against a blank (where sample was replaced with methanol). Flavonoid content was calculated from a standard curve of catechin (5-60 µg).

Proanthocyanidins (tannins) were extracted from defatted flours with 70% acetone twice (at 1:10 w/v ratio). The extracts were evaporated to dryness and resuspended in methanol. Proanthocyanidins were assayed by the vanillin method according to Butler *et al.*[15] using catechin as standard (50-200 µg). The absorbance of the samples and standards was read at 500 nm.

Statistical analysis

Statistical analysis was carried out using SPSS version 11.5 software. The extraction of each accession was performed in triplicate. For camelina flours, the model included the accession or the group (winter-springs biotypes) as the fixed effect and the sample as a random effect. Means, standard errors and Pearson's correlations were calculated and the mean separations were obtained by Tukey's range test ($P<0.05$).

Results and Discussion

Antioxidant activity

The antioxidant activity in camelina accessions expressed as TEAC and AA% is shown in Table 1. The ANOVA showed that the differences in TEAC and AA% among accessions are significant at 0.01 level. In general, antioxidant activity was extremely high in camelina flour with an AA% mean value of 92.8. This datum confirms that

Table 1. Trolox equivalent antioxidant capacity (TEAC) and antioxidant activity percentage (AA%) in 47 different accessions of *Camelina sativa*.

Accessions	TEAC°	AA%
BAVARIA	14.6±0.12 (cdefg)	93.9±0.4
CALENA	14.5±0.01 (cdefgh)	93.6±0.1
CAM7	14.4±0.17 (cdefgh)	93.2±0.6
CAM8	14.0±0.08 (gh)	91.9±0.3
CAM25	14.4±0.09 (cdefgh)	93.1±0.3
CAM29	14.4±0.14 (cdefgh)	93.3±0.5
CAM31	14.4±0.17 (cdefgh)	93.2±0.6
CAM34	14.6±0.04 (cdefg)	94.0±0.1
CAM35	15.6±0.12 (a)	97.2±0.4
CAM37*	12.9±0.20 (i)	88.1±0.7
CAM38	14.7±0.06 (cdefg)	94.1±0.2
CAM39	13.8±0.29 (h)	91.4±1.0
CAM58	14.4±0.09 (cdefgh)	93.1±0.3
CAM76*	10.9±0.14 (k)	81.1±0.5
CAM108	14.7±0.21 (cdefg)	94.4±0.7
CAM110	14.6±0.05 (cdefg)	94.0±0.2
CAM111	14.9±0.09 (bcde)	94.8±0.3
CAM116	12.8±0.08 (i)	87.8±0.3
CAM123	14.6±0.05 (cdefg)	93.9±0.2
CAM132*	15.0±0.05 (abcd)	95.2±0.2
CAM136	14.6±0.04 (cdefg)	94.0±0.2
CAM137	14.8±0.11 (cdef)	94.7±0.3
CAM170	14.7±0.10 (cdefg)	94.1±0.3
CAM171	14.3±0.14 (defgh)	92.9±0.5
CAM173	15.6±0.08 (a)	97.2±0.3
CAM174	14.1±0.09 (fgh)	92.2±0.3
CAM175	10.7±0.14 (k)	80.1±0.6
CAM187	13.8±0.29 (h)	91.4±1.0
CAM265	14.6±0.05 (cdefg)	94.0±0.2
CAM266	14.6±0.04 (cdefg)	94.0±0.1
CAM268	15.6±0.12 (a)	97.2±0.4
CAM269	12.9±0.20 (i)	88.1±0.7
CAM270	14.7±0.06 (cdefg)	94.1±0.2
CAMELIA	15.0±0.23 (abc)	95.4±0.7
KARTNER	14.3±0.01 (defgh)	92.8±0.1
LIGENA	11.9±0.08 (j)	84.6±0.3
LINDO	14.5±0.03 (cdefgh)	93.6±0.1
MORGENSONNE	14.4±0.10 (cdefgh)	93.3±0.3
PI650142	14.0±0.03 (gh)	92.0±0.1
PI650146	15.5±0.20 (ab)	96.8±0.6
PI650168*	14.7±0.21 (cdefg)	94.4±0.7
SOLEDO	14.7±0.09 (cdefg)	94.2±0.3
ST. PERNITZEN	14.9±0.09 (bcde)	94.8±0.3
UKRAJINSKAJA	15.1±0.06 (abc)	95.5±0.2
WILEDO*	14.2±0.15 (efgh)	92.7±0.5
WROCLAWSKA	14.6±0.27 (cdefg)	94.0±0.9
ZARJA SOCIALISMA*	14.7±0.05 (cdefg)	94.1±0.2
Mean	14.3±0.17	92.8±0.1
P accession	58.55§	63.19§
P group#	6.07^	6.17^

*Camelina winter forms; °Data are expressed as mmol Trolox g^{-1} DW ± SEM; #groups are winter and spring biotypes; §Significant at ≤0.01; ^Significant at ≤0.05; the lowercase letters in parentheses within the same row differ significantly by Tukey's range test (P≤0.05).

observed by Aziza *et al.*[11] In absolute terms, the antioxidant activity is higher than that described by Quezada and Cherian[3] with the DPPH assay (1.42-2.70 mmol TE g^{-1}) in camelina low-high fat meal and lower than that reported by Rahman *et al.*[16] for defatted meal. Moreover, The ANOVA showed significant differences at 0.05 level among groups (winter *vs* spring accessions) and this is due to the fact that the accessions with the highest antioxidant activities are all springs (CAM35, CAM173 and CAM268; letters a by the Tukey's range test).

Phenolic, flavonoid and proanthocyanidin contents

The TPC was determined in 47 camelina accessions by the Folin-Ciocalteu method. Table 2 shows that the TPC ranged from 9.3 to 18.1 mg CAE g^{-1} DW in camelina flour with an average of 13.6. These values are in the same range to that reported previously in camelina[16] and significantly higher than other species rich in phenolics[17-19] Significant differences at 0.01 level among accessions and groups were evidenced by ANOVA analysis. The highest phenolic contents were observed in CAM266, PI650146 and CAM58 (17.9, 18.1 and 17.5 mg CAE g^{-1} DW, respectively).

The ANOVA showed that the differences in flavonoids among accessions are significant at 0.01 level while no differences are present between groups (Table 2). The mean flavonoid content in camelina was 3.60 mg g^{-1} DW, which is lower than that reported by Aziza *et al.* and Rahman *et al.*,[11,16] but higher respect to Salminen *et al.*[20] The accessions BAVARIA, SOLEDO and ZARJA SOCIALISMA were the richest in flavonoids (letters a and b by the Tukey's range test).

Proanthocyanidins (flavan-3-ol based biopolymers) may be anti-nutritional factors when they are concentrated in plant tissues otherwise they may have an antioxidant function due to the presence of numerous phenolic groups. As their content is relatively low in camelina,[21,22] they can be more important as antioxidants than as anti-nutritional compounds. Significant differences at 0.01 level among accessions for proanthocyanidins were evidenced by ANOVA analysis but not between groups (Table 2). The mean proanthocyanidins content in camelina was 5.20 mg g^{-1} DW but many accessions have contents close to 3.60 mg g^{-1} DW as described by Rahman *et al.*[16] The accessions CAM58, CAM132, CAM170 and CAM171 showed the highest tannins contents (letters a and b by the Tukey's range test).

Table 2. Total phenolic content (TPC), flavonoids and proanthocyanidins in 47 different accessions of *Camelina sativa*.

Accessions	TPC°	Flavonoids#	Proanthocyanidins#
BAVARIA	13.6±0.02 (klm)	4.78±0.01 (a)	3.57±0.07 (rs)
CALENA	11.9±0.04 (u)	3.57±0.02 (hi)	3.66±0.08 (r)
CAM7	16.3±0.04 (e)	4.65±0.03 (b)	5.86±0.10 (efgh)
CAM8	16.3±0.02 (e)	3.89±0.04 (f)	3.78±0.07 (qr)
CAM25	16.1±0.10 (e)	2.95±0.01 (r)	5.70±0.02 (hi)
CAM29	16.0±0.05 (ef)	3.84±0.02 (fg)	4.40±0.03 (o)
CAM31	11.1±0.09 (vw)	3.39±0.01 (kl)	5.87±0.10 (efgh)
CAM34	11.4±0.07 (v)	3.18±0.02 (o)	6.02±0.06 (efg)
CAM35	14.8±0.06 (g)	4.00±0.05 (e)	6.41±0.15 (c)
CAM37*	9.3±0.06 (z)	3.36±0.01 (lm)	6.41±0.14 (c)
CAM38	15.0±0.12 (g)	4.51±0.01 (c)	6.08±0.10 (def)
CAM39	12.7±0.06 (s)	2.92±0.02 (r)	6.01±0.06 (efgh)
CAM58	17.5±0.06 (b)	3.47±0.02 (ijk)	6.98±0.01 (b)
CAM76*	13.4±0.06 (op)	3.54±0.01 (hi)	5.83±0.09 (efgh)
CAM108	9.8±0.07 (y)	3.51±0.01 (hij)	3.99±0.03 (pq)
CAM110	14.3±0.07 (hi)	4.24±0.02 (d)	4.00±0.04 (pq)
CAM111	11.9±0.11 (u)	2.64±0.02 (s)	3.55±0.03 (rs)
CAM116	13.1±0.07 (qr)	3.76±0.03 (g)	3.34±0.01 (s)
CAM123	13.8±0.02 (jkl)	4.48±0.03 (c)	3.53±0.10 (rs)
CAM132*	12.6±0.01 (st)	3.47±0.01 (ijk)	6.86±0.07 (b)
CAM136	12.7±0.06 (s)	3.14±0.03 (op)	5.73±0.01 (ghi)
CAM137	13.6±0.01 (klm)	2.97±0.03 (qr)	5.52±0.03 (ij)
CAM170	15.8±0.04 (f)	2.44±0.03 (t)	7.49±0.03 (a)
CAM171	14.8±0.04 (g)	2.95±0.03 (r)	7.04±0.03 (b)
CAM173	14.1±0.04 (ij)	2.39±0.01 (t)	6.01±0.06 (efgh)
CAM174	14.7±0.01 (g)	4.16±0.02 (d)	5.70±0.02 (hi)
CAM175	11.2±0.02 (vw)	4.45±0.03 (c)	6.12±0.01 (de)
CAM187	9.6±0.06 (y)	3.38±0.01 (kl)	3.52±0.01 (rs)
CAM265	13.7±0.06 (klm)	2.39±0.01 (t)	6.07±0.04 (def)
CAM266	17.9±0.05 (a)	3.33±0.02 (lm)	4.39±0.01 (o)
CAM268	11.9±0.08 (u)	3.78±0.01 (g)	6.37±0.02 (cd)
CAM269	12.4±0.05 (t)	3.28±0.04 (mn)	6.10±0.03 (def)
CAM270	14.4±0.03 (h)	3.55±0.02 (hi)	4.10±0.04 (p)
CAMELIA	13.4±0.06 (mn)	3.50±0.01 (hij)	3.83±0.06 (pqr)
KARTNER	13.9±0.05 (jk)	3.52±0.02 (hij)	5.29±0.05 (jk)
LIGENA	10.4±0.01 (x)	3.57±0.03 (hi)	4.50±0.04 (o)
LINDO	11.1±0.06 (vw)	3.60±0.02 (h)	4.37±0.03 (o)
MORGENSONNE	13.1±0.05 (qr)	3.50±0.02 (hij)	5.30±0.03 (jk)
PI650142	16.6±0.06 (d)	3.05±0.02 (pq)	4.83±0.10 (mn)
PI650146	18.1±0.03 (a)	3.20±0.04 (no)	4.78±0.02 (n)
PI650168*	13.3±0.04 (pq)	2.64±0.02 (s)	5.79±0.06 (fghi)
SOLEDO	12.1±0.08 (u)	4.78±0.01 (a)	4.92±0.06 (lmn)
ST. PERNITZEN	17.2±0.11 (c)	4.24±0.01 (d)	6.42±0.04 (c)
UKRAJINSKAJA	12.8±0.03 (rs)	3.59±0.01 (h)	4.44±0.04 (o)
WILEDO*	11.1±0.05 (w)	4.23±0.01 (d)	5.10±0.04 (klm)
WROCLAWSKA	13.5±0.03 (lmn)	3.42±0.01 (jkl)	5.14±0.06 (kl)
ZARJA SOCIALISMA*	13.1±0.02 (qr)	4.85±0.03 (a)	3.63±0.06 (rs)
Mean	13.6±0.32	3.60±0.10	5.20±0.16
P accession	1399.45^	869.23^	339.94^
P group§	9.49^	0.58	2.69

*Camelina winter forms; °Data are expressed as mg CAE g^{-1} DW ± SEM; #mg g^{-1} DW ± SEM; §groups are camelina winter and spring forms; ^Significant at ≤0.01; the lowercase letters in parentheses within the same row differ significantly by Tukey's range test (P≤0.05).

Table 3. Pearson correlation coefficients (r) for antioxidant activity and compounds in 47 accessions of *Camelina sativa*.

	TEAC	TPC	Flavonoids
TPC	0.292*	1	
Flavonoids	–0.118	–0.002	1
Proanthocyanidins	–0.024	0.161	–0.294*

*Correlation is significant at the 0.01 level.

Correlation between antioxidant activity and antioxidant compounds

In Table 3 are shown the Pearson correlation coefficients (r) among TEAC, TPC, flavonoids and proanthocyanidins. TEAC was significantly correlated (at 0.01 level) with TPC but not with flavonoids and proanthocyanidins, thus suggesting that phenolics are the main class of substances determining antioxidant activity. Flavonoid and proanthocyanidin contents were instead inversely correlated and this could indicate that the biosynthetic pathways of these compounds compete with each other.

Conclusions

The current study showed that the flours of camelina have a high antioxidant activity, which derives mainly from the high content of TPC, while the contribution of flavonoids and proanthocyanidins is probably modest. Therefore, interesting accessions for future breeding programs are those with higher TPC content (many of which are spring biotypes). Camelina enriches the animal diets as well as protein, omega-3 and tocopherols (the latter two contained in the residual oil of the meal) also of antioxidants. Moreover, the high content of phenolics as well as contributing to animal health may be important to avoid fat rancidity in camelina meals.

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