SHORT COMMUNICATION

Effect of pressing and combination of three storage temperatures and times on chemical composition and fatty acid profile of canola expellers

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Abstract

This experiment investigated the effects of combinations of three temperatures and three storage times on the chemical composition, fatty acid profile, and oxidative stability of canola expellers obtained from the cold-pressing extraction of oil. Canola seeds were single-crushed at moderate temperatures (60°C) during 3 pressing sessions. Nine samples (100±1 g) of each session were collected, inserted into sealed bags, stored at three temperatures (12, 24, 36°C) over 3 periods of time (10, 20, 30 d). Then, samples (100±1 g) of canola seeds collected before each pressing session and canola expellers collected before and after each storage time were analyzed for chemical composition, fatty acid profile, peroxide number and Kreis test. Before storage, the fatty acid profile of canola seeds and expellers differed significantly, except for myristic (P=0.18), palmitic (P=0.57), oleic (P=0.07), and α-linolenic acids (P=0.45). Compared to canola seeds, expellers showed greater content of saturated, poly-unsaturated, and n-6 fatty acids (P<0.01), but a lower content of mono-unsaturated fatty acids (P<0.01). Peroxide values were definitely (P<0.01) greater for expellers and averaged 4.22 and 4.11 mEq/kg fat before and after storage, respectively. The Kreis test was negative for all samples. Under different temperatures and times of storage, canola expellers showed to maintain a good oxidative stability, as highlighted by low peroxide values (<10 mEq/kg fat) and negative response for Kreis test. Canola expellers obtained by on-farm cold extraction, despite great oil residual (from 17 to 19% ether extract on dry matter basis), can be stored at farm without significant chemical and nutritional changes.

Introduction

Canola represents the trademark of a particular rapeseed with a low content of erucic acid (<5% by weight, for EU) and glucosinolates [<30 mmol/kg dry matter (DM), for EU]. In the last years an increasing number of farms have been equipping themselves with small-sized facilities for the mechanical extraction of oil from canola seeds. After the extraction, the oil is usually converted into biodiesel and used as fuel for tractors, whereas the resulting by-products (canola expellers) are included in ruminant diets as protein and fat sources. This productive cycle offers economical and environmental advantages, as it contributes towards reducing the need for fossil fuels in the agricultural industry and to recycling the resulting expellers as animal feeds (Baquero *et al.*, 2010; Esteban *et al.*, 2011). However, to date, information about the nutritional and energy value of canola expellers are still limited. The literature indicates that the oil content of canola expellers is extremely variable, with a fat percentage ranging from 8 to almost 30% DM, as the mechanical extraction is a poorly standardized and inefficient method (Leming and Lember, 2005; Spragg and Mailer, 2007; Thacker and Petri, 2009). Furthermore, canola oil, similarly to all vegetable oils, is characterized by a great degree of unsaturation and, thus, is susceptible to oxidation (O'Brien, 2008; Matthäus, 2012). This chemical profile could strongly reduce the possibility of storing canola expellers at the farm level and discourage their use in animal feeding. Thus, the identification of techniques to store canola expellers at the farm level could represent a topic of public and scientific interest. This experiment aimed at investigating the effects of pressing and combination of three storage temperatures and three storage times on the chemical composition, fatty acid profile, and oxidative stability of canola expellers obtained from on-farm cold-pressing extraction of oil.

Materials and methods

Experimental design and analytical procedures

Canola seeds were obtained by eight hybrid cultivars of canola: Dkexme, Excalibur, and Excel (Dekalb, Monsanto Agricoltura Spa, Lodi, Italy), PR46W10 (Pioneer Hi-Bred Srl, Cremona, Italy), Pulsar and Tissot (Società Italiana Sementi, Bologna, Italy), Toccata and Makila (Maisadour Semences Italia, Verona, Italy). Hybrids were grown and harvested at the pilot farm of the Veneto Agricultura Agency (Caorle, VE, Italy). After harvest, canola seeds were screened to remove extraneous material, artificially dried and all stored into the same silo until pressing for oil extraction. Three pressing sessions were conducted in three successive months, where canola seeds were single-crushed at moderate temperatures (60°C) using two presses equipped with a rotating screw shaft within an horizontal barrel (Mailca Srl, Modena, Italy). The two presses had an input flow rate of 120 kg/h of seeds, with a production of 40 kg/h of oil and 80 kg/h of expellers. After each pressing session, 9 samples of canola expellers (100±1 g) were randomly collected, inserted into sealed bags, and stored at three different temperatures (12, 24, and 36°C) over three periods of time (10, 20, and 30 d). This combination of temperatures and times was chosen to simulate environmental conditions occurring at the farm over different seasons of the year in the Po valley. The chemical composition and fatty acid profile of canola expellers, previously ground to 1 mm using a hammer mill (Pullerisette 19, Fritsch GmbH, Laborgeratebau, Germany), were determined before storage (t=0) and at the end of each storage time (10, 20, and 30 d). The same analyses were conducted on samples (100±1 g) of canola seeds collected before

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each pressing session, to detect possible effects of storage into the silo on chemical composition of seeds. Chemical analyses were performed in the same laboratory by the same technician. Samples were analyzed for dry matter (DM; #934.01; AOAC, 2003), crude protein (CP; #976.05; AOAC, 2003), ether extract (EE; #920.29; AOAC, 2003), ash (#942.05; AOAC, 2003), and neutral detergent fibre (aNDF), as suggested by Mertens (2002). The aNDF fraction, including residual ash, was determined with α-amylase and sodium sulphite using the Ankom^{220} Fibre Analyzer (Ankom Technology®, Macedon, NY, USA). The fatty acid profile was determined in duplicate using a Thermo Finnigan Spectra System AS3000 autosampler (Thermo Electron Corporation, Waltham, MA, USA) equipped with a H_2SO_4 0.0025 N Bio-Rad HPX-87H column (Bio-Rad Laboratories, Richmond, CA, USA). After extracting with chloroform and methanol (Folch *et al.*, 1957), the oil was analyzed for peroxide number, using conventional iodometric titration with thiosulfate (Peroxide value of oils and fats, #965.33; AOAC, 2003), and for the Kreis test (NGD, 1979).

Statistical analysis

Experimental data were subjected to analysis of variance using the general linear model procedure (PROC GLM) of SAS (2005). For the fatty acid profile provided by canola seeds and canola expellers before storage (t=0) the model included the effects of feed (canola seeds or canola expellers) and pressing session:

$$y_{ijk} = \mu + F_i + P_j + FP_{ij} + \varepsilon_{ijk}$$

where
y_{ijk}, single observation;
μ, overall mean;
F_i, effect of feed (i = 1 to 2);
P_j, effect of pressing session (j = 1 to 3);
ε_{ijk}, residual error.

Regarding the chemical composition and fatty acid profile of samples of canola expellers collected after the different periods of storage at varying temperatures, the model included the effects of pressing session, storage temperature, storage length, and the corresponding first-order interactions.

$$y_{ijkl} = \mu + P_i + T_j + L_k + PT_{ij} + PL_{ik} + TL_{jk} + \varepsilon_{ijkl}$$

where
y_{ijk}, single observation;
μ, overall mean;
P_i, effect of pressing session (i = 1 to 3);
T_j, effect of storage temperature (j = 1 to 3);
L_k, effect of storage length (k = 1 to 3);
PT_{ij}, PL_{ik}, TL_{jk}, first order interactions
ε_{ijkl}, residual error.

Significant differences were always accepted if P≤0.05.

Results and discussion

The proximate analysis of canola seeds and expellers after each pressing is given in Table 1. Chemical values provided by canola seeds conformed with tabulated data (NRC, 2001). However, the batch of seeds subjected to each pressing session differed for lipid (from 42.2 to 46.3% DM) and NDF content (from 25.8 to 27.6% DM). Magnitude of these differences suggests that seeds of different cultivars were not homogeneously distributed into the silo. After 3 pressing sessions canola expellers showed a similar CP content (from 30.6 to 30.9% DM), whereas ether extract content varied notably (from 17.5% to 19.1% DM), as consequence of variable composition of original seeds. The highest ether extract value (19.1% DM) was in line with the findings of Leming and Lember (2005), and intermediate between 13% DM as reported by Spragg and Mailer (2007) and 27% DM as observed by Thacker and Petri (2009). However, in relative terms, the lipid content found in this experiment must be considered to be high, and this demonstrates that the efficiency of oil extraction from canola seeds, using a small-size facility, was low. Literature indicates that processing conditions, and especially the number of pressings, can influence greatly the amount of residual oil in the expellers (Weigal, 1991; Glencross *et al.*, 2004). Currently, the common practice is to press seeds once or twice; the double pressing ensures a greater recovery of oil from seeds but also causes the generation of high temperatures inside the pressing system, thereby increasing the amount of free fatty acids and, hence, reducing the storability of oil (Matthäus, 2012). In the present study canola seeds were subjected to single-pressing, as the purpose was to evaluate the possibility of storing expellers with a high oil residual. Moreover, moderate temperatures (60°C) were applied for the extraction of oil, to avoid possible alterations of fatty acid profile. Before storage (t = 0), the fatty acid profile of canola seeds and expellers differed significantly, except for the content of myristic (C14, P=0.18), palmitic (C16, P=0.57), and α-linolenic acid (C18:3 n-3; P=0.45) (Table 2). Compared to the canola seeds, expellers showed significantly (P<0.01) greater content of saturated (SFA), poly-unsaturated (PUFA), and n-6 fatty acids. Conversely, the content of mono-unsaturated fatty acids (MUFA) was significantly lower (P<0.01) in the canola expellers compared to the seeds. Therefore, notable differences were found between the fatty acid profile of expellers and that of original seeds. Harris and James (1969) stated that the temperature controls the dehydrogenation of fatty acids by influencing the amount of available oxygen. More specifically, dehydrogenation is usually limited at increasing temperatures, as oxygen becomes less soluble and, hence, less available in the cell cytoplasm. On this basis, it could be hypothesized that the decrease of MUFA content in expellers in relation to the original seeds was due to the effect of temperature during the oil extraction process. The pressing session influenced significantly (P<0.05) only the SFA content and the ratio between the SFA and unsaturated fatty acids, thereby causing it to be higher in the second pressing session compared to the others. The results of this trial (Table 2) also revealed that the amount of peroxides markedly (P<0.01) increased passing from canola seeds to expellers (on average 1.60 *vs* 4.97

Table 1. Chemical composition of canola seeds and canola expellers before storage (t=0) in the three pressing sessions.

	Canola seeds Pressing session			Canola expellers Pressing session		
	1	2	3	1	2	3
Dry matter, %	94.1	93.8	94.1	92.4	92.2	92.5
Ether extract, % DM	42.2	46.3	44.7	17.5	19.1	18.7
Crude protein, % DM	20.6	20.3	20.5	30.7	30.6	30.9
Neutral detergent fibre, % DM	27.6	25.8	26.9	21.1	19.8	21.4
Ash, % DM	4.3	4.2	4.2	6.3	6.2	6.4
Non-structural carbohydrates, % DM	5.3	3.4	3.7	24.4	24.3	22.6

DM, dry matter.

mEq O_2/kg fat, for seeds and expellers, respectively). Läubli and Bruttel (1986) observed that the formation of peroxides is enhanced by temperature, so it could be hypothesized that the increased content of peroxides in the expellers was related to heat which was emitted from the pressing of the seeds. On the contrary, peroxide values were not influenced (P=0.21) by pressing session, because increasing values observed for seeds, passing from first to third pressing (from 1.29 to 2.05 mEq O_2/kg fat), were compensated by decreasing values found for expellers (from 7.13 to 3.24 mEq O_2/kg fat), and because these values were extremely variable (SEM=0.618). Magnitude of differences observed for peroxide values of seeds among pressing sessions seem to confirm that these differences could be mainly attributed to an heterogeneous distribution of seeds into the silo rather than to changes in chemical composition during the storage into the silo.

The lack of significant effects due to the pressing session on the fatty acid profile provides evidence that the cold extraction procedure had a satisfactory degree of standardization. On the other hand, the presence of notable effects due to the feed seems to suggest that the pressing procedure changed significantly the fatty acid profile of expellers compared to that of original seeds. To date, there has been relatively little investigation on the effects of processing conditions on the nutrient profile of expellers. Some authors have found that processing can affect the content of glucosinolates (Newkirk and Classen, 2002; Seneviratne *et al.*, 2011) and of amino acids in residual expellers (Seneviratne *et al.*, 2011), whereas, to our knowledge, no information is available on the possible effects on the fatty acid profile of these by-products.

Considering the canola expellers after the different storage conditions (Table 3), the pressing session affected significantly both the NDF (P<0.05) and the ash (P<0.01) contents, but these differences were presumably related to the fact that expellers derived from various canola cultivars. As expected, the temperature and time of storage affected significantly the DM content of canola expellers with a linear increase at increasing temperature and storage length. In the current work, the fatty acid profile of canola expellers (Table 4) appeared to fall in line with the literature (on average: C18:1, 55.5%; C18:2, 20.3%; C18:3, 6.3%). Deng and Scarth (1998) reported that the conventional canola oil contains about 6% SFA, 55-60% oleic acid, 20-26% linoleic acid, and 8-10% α-linolenic acid. The temperature and length of storage affected significantly only the PUFA content (P<0.05). During stor-

Table 2. Least Square means: effect of feed and pressing session on fatty acid profile of canola seeds and canola expellers before storage (t=0) in the three pressing sessions.

	Canola seeds Pressing session			Canola expellers Pressing session			SEM	P value	
								Feed	Pressing session
	1	2	3	1	2	3			
Fatty acids, g/100 g of fat									
C14:0	0.09	0.09	0.09	0.07	0.11	0.12	0.005	0.18	0.06
C16:0	7.63	7.69	7.38	8.10	8.23	8.37	0.045	0.57	0.93
C18:0	1.09	1.09	1.09	1.50	1.10	1.09	0.045	0.07	0.06
SFA	10.3	10.3	10.1	11.3	12.1	11.3	0.221	<0.01	<0.05
C18:1	59.9	59.4	59.5	56.1	56.1	56.0	0.532	<0.01	0.10
MUFA	64.5	64.1	64.4	62.0	62.1	62.0	0.341	<0.01	0.17
C18:2	19.1	19.3	19.3	20.4	19.4	20.4	0.163	<0.01	0.26
C18:3	6.2	6.1	6.2	6.0	6.0	6.0	0.010	0.61	0.07
PUFA	25.3	25.6	25.5	26.7	25.7	26.6	0.167	<0.01	0.21
n-6	19.0	19.2	19.2	20.8	19.6	20.8	0.215	<0.01	0.19
n-3	6.1	6.0	6.1	6.1	6.1	6.1	0.010	0.45	0.06
SFA/UFA	0.11	0.12	0.11	0.13	0.14	0.13	0.002	<0.01	<0.05
MUFA/PUFA	2.55	2.51	2.52	2.33	2.41	2.33	0.027	<0.01	0.40
n-6/n-3	3.14	3.17	3.10	3.38	3.20	3.38	0.034	<0.01	0.28
Peroxides, mEq O_2/kg fat	1.29	1.47	2.05	7.13	4.55	3.24	0.618	<0.01	0.21
Kreis test	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	--	--	--

SFA, saturated fatty acids; MUFA, monounsaturated fatt acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; Neg., negative.

Table 3. Least Square means: effect of pressing session, storage temperature, storage length, and their interactions on chemical composition of canola expellers.

	Pressing session			Storage temperature			Storage length			SEM	P value					
											S	T	L	S × T	S × L	T × L
	1	2	3	12°C	24°C	36°C	10 d	20 d	30 d							
Dry matter, %	92.8	92.8	92.8	92.1	92.3	94.0	92.5	92.8	93.0	0.162	0.91	<0.01	<0.01	0.21	0.20	<0.01
Ether extract, % DM	18.2	18.8	19.4	18.8	19.2	18.5	19.0	18.3	19.1	0.245	0.19	0.51	0.38	0.27	0.50	0.27
Crude protein, % DM	30.9	30.8	31.2	30.8	31.0	31.1	31.0	31.0	31.0	0.062	0.07	0.17	0.97	0.56	0.40	0.73
Neutral detergent fibre, % DM	21.4	20.5	21.1	21.3	21.2	20.6	20.9	20.9	21.2	0.130	<0.05	<0.05	0.34	0.14	0.32	0.38
Ash, % DM	6.21	6.21	6.41	6.29	6.31	6.30	6.29	6.31	6.31	0.016	<0.01	0.68	0.68	0.20	0.32	0.40
Non-structural carbohydrates, % DM	23.2	23.6	21.9	22.9	22.3	23.5	22.8	23.5	22.3	0.117	0.08	0.27	0.28	0.44	0.85	0.44

DM, dry matter; S, pressing session; T, storage temperature; L, storage length.

Table 4. Least Square means: effect of pressing session, storage temperature, storage length, and their interactions on fatty acid profile of canola expellers.

	Pressing session			Storage temperature			Storage length			SEM	P value					
	1	2	3	12°C	24°C	36°C	10 d	20 d	30 d		S	T	L	S × T	S × L	T × L
Fatty acids, g/100 g of fat																
C14:0	0.07	0.18	0.11	0.07	0.20	0.08	0.10	0.10	0.18	0.032	0.60	0.38	0.89	0.62	0.61	0.51
C16:0	7.4	7.5	8.0	7.8	7.7	7.4	7.9	7.9	7.3	0.21	<0.05	0.34	0.14	0.28	<0.01	0.16
C18:0	1.4	1.0	1.2	1.4	1.0	1.2	1.1	1.2	1.2	0.044	<0.05	0.41	0.86	0.49	0.53	0.30
SFA	11.1	11.1	11.1	10.8	11.0	11.4	11.0	10.4	11.3	0.215	0.99	0.59	0.38	0.32	0.80	0.67
C18:1	54.7	55.9	56.0	56.2	55.7	54.8	55.4	55.7	55.0	0.286	0.16	0.20	0.64	0.30	0.44	0.43
MUFA	61.8	62.1	62.0	62.2	62.0	61.7	61.9	62.3	61.7	0.163	0.81	0.53	0.66	0.26	0.87	0.67
C18:2	20.4	20.2	20.4	20.4	20.5	20.2	20.0	20.7	20.4	0.070	0.08	<0.01	<0.01	0.36	<0.01	<0.01
C18:3	6.4	6.2	6.2	6.2	6.3	6.3	6.2	6.3	6.5	0.067	<0.01	0.07	<0.05	0.24	0.53	<0.01
PUFA	27.1	26.7	26.8	26.9	26.9	26.8	27.0	27.2	26.9	0.096	0.20	0.88	<0.01	0.32	0.56	0.15
n-6	20.6	20.4	20.6	20.6	20.7	20.4	20.7	20.5	20.5	0.073	0.09	<0.01	<0.05	0.61	<0.01	<0.01
n-3	6.5	6.4	6.3	6.3	6.4	6.4	6.3	6.5	6.6	0.065	0.35	0.78	0.10	0.44	0.59	0.92
SFA/UFA	0.12	0.12	0.12	0.12	0.12	0.13	0.12	0.12	0.13	0.003	0.99	0.58	0.40	0.32	0.69	0.77
MUFA/PUFA	2.28	2.32	2.31	2.31	2.30	2.30	2.28	2.30	2.29	0.008	<0.05	0.62	<0.01	0.14	0.75	0.06
n-6/n-3	3.18	3.20	3.30	3.26	3.24	3.18	3.30	3.18	3.11	0.031	0.25	0.52	0.08	0.36	0.50	0.65
Peroxides, mEq O2/kg fat	4.99	4.31	3.38	5.69	4.21	2.79	4.12	4.17	3.66	0.374	<0.01	<0.01	0.06	0.30	<0.01	0.06
Kreis test	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	--	--	--	--	--	--	--

S, pressing session; T, storage temperature; L, storage length; SFA, saturated fatty acids; MUFA, monounsaturated fatt acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; Neg., negative.

age, peroxide values decreased linearly with the increase in storage temperature (5.69, 4.21, 2.79 mEq O_2/kg fat; P<0.01). Vázquez-Añón and Jenkins (2007) observed that peroxide values similar to those found in this experiment (3.5 mEq O_2/kg fat) did not impair rumen fermentation and microbial activity. The same authors hypothesized that peroxide values of 215 mEq O_2/kg fat could be sufficient to affect negatively the rumen function. On this basis, it could be concluded that canola expellers showed a good oxidative stability at all temperatures and times of storage. This pattern is also supported by the responses of the Kreis test, that was negative for all samples (Tables 3 and 4). In this experiment, the permanence of a good oxidative stability was presumably favoured by moderate temperatures used. In this respect, Koski *et al.* (2002) found that rapeseed oil stored at 70°C reached peroxide values of 70 mEq O_2/kg fat within only 4 days. Naczk *et al.* (1998) indicated that rapeseed and canola oil, compared to other oilseeds, have the greatest content of phenolic compounds with antioxidant properties (*i.e.*, sinapic acid and tocopherols), and this argument was supported by several studies (Bandoniene *et al.*, 2000; Koski *et al.*, 2002; Amarowicz *et al.*, 2003; Shen *et al.*, 2012). The presence of these compounds makes rapeseed and canola oil more persistent to the occurrence of the oxidative process (Naczk *et al.*, 1998). In the current work, canola expellers were not characterized for phenols, but it could be supposed that these substances, being heat-stable (Shen *et al.*, 2012), contributed to preserving the oxidative stability of expellers during storage under different conditions.

Conclusions

Under different temperatures (12, 24 and 36°C) and times of storage (10, 20 or 30 d), canola expellers obtained from cold extraction on farm did not show significant changes in chemical composition and fatty acid profile, and maintained a good oxidative stability, as highlighted by the low peroxide values (<10 mEq O_2/kg fat) and the negative response of the Kreis test. From these preliminary results it could be hypothesized that the storage of by-products derived from the productive cycle of biofuel is possible. Furthermore, canola expellers tested in this experiment, despite their great oil residual (from 17% to 19% EE on DM basis), could be potentially used as animal feeds, as the level of peroxides was definitely under the threshold limit that would have led to the impairment of the rumen function. However, the latter hypothesis should be validated *in vivo*.

References

Amarowicz, R., Raab, B., Shahidi, F., 2003. Antioxidant activity of phenolic fractions of rapeseed. J. Food Lipids 10:51-62.

AOAC, 2003. Official Methods of Analysis, 17th ed., Association of Official Agricultural Chemists, Gaithersburg, MD, USA.

Bandoniene, D., Pukalskas, A., Venskutonis, P.R., Gruzdiene, D., 2000. Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil. Food Res. Int. 33:785-791.

Baquero, G., Esteban, B., Rius, A., Riba, J.R., Puig, R., 2010. Small-scale production of straight vegetable oil from rapeseed and its use as biofuel in the Spanish territory. Energ. Policy 38:189-196.

Deng, X., Scarth, R., 1998. Temperature effects on fatty acid composition during development of low-linolenic oilseed paper (Brassica napus L.). J. Am. Oil Chem. Soc. 75:759-766.

Esteban, B., Baquero, G., Puig, R., Riba, J.R., Rius, A., 2011. Is it environmentally advantageous to use vegetable oil directly as biofuel instead of converting it to biodiesel? Biomass Bioenerg. 35:1317-1328.

Folch, J., Lees, M., Sloane-Stanely, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–507.

Glencross, B., Hawkins, W., Curnow, J., 2004. Nutritional assessment of Australian canola meals. II. Evaluation of the influence of the canola oil extraction method on the protein value of canola meals fed to the red seabream (Pagrus auratus, Paulin). Aquacult. Res. 35:25-34.

Harris, P., James, A.J., 1969. Effect of low temperature on fatty acid biosynthesis in seeds. Biochim. Biophys. Acta 187:13-18.

Koski, A., Psomiadou, E., Tsimidou, M., Hopia, A., Kefalas, P., Wähälä, K., Heinonen, M., 2002. Oxidative stability and minor constituents of virgin olive oil and cold pressed rapeseed oil. Eur. Food Res. Technol. 214: 294-298.

Läubli, M.W., Bruttel, P.A., 1986. Determination of the oxidative stability of fats and oils: comparison between the active oxygen method (AOCS Cd 12-57) and the Rancimat method. J. Am. Oil Chem. Soc. 63:792-795.

Leming, R., Lember, A., 2005. Chemical composition of expeller-extracted and cold-pressed canola meal. Agraarteadus 16:103-109.

Matthäus, B., 2012. Oil technology. In: S.K. Gupta (ed.) Technological innovations in major world oil crops, vol. 2. Springer Publ., New York, NY, USA, pp 27-92.

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85:1217-1240.

Naczk, M., Amarowicz, R., Sullivan, A., Shahidi, F., 1998. Current research developments on polyphenols of rapeseed/canola: a review. Food Chem. 62:489-502.

National Research Council, 2001. Nutrient Requirements of Dairy Cattle, 7th rev. ed. National Academy Press, Washington, DC, USA.

Newkirk, R.W., Classen, H.L., 2002. The effects of toasting canola meal on body weight, feed conversion efficiency and mortality in broiler chickens. Poultry Sci. 81:815-825.

Norme Grassi e Derivati, 1976. Metodo NGD C56-79. Stazione Sperimentale degli Oli e dei Grassi Ed., Milano, Italy.

O'Brien, R., 2008. Fats and oils formulating and processing for applications. 3rd ed. Formulating and processing for applications. CRC Press, Schulenburg, Texas, USA.

SAS, 2005. User's Guide, 5th ed. SAS Inst., Inc., Cary, NC, USA.

Seneviratne, R.W., Beltranena, E., Goonewardene, L.A., Zijlstra, R.T., 2011. Effect of crude glycerol combined with solvent-extracted or expeller-pressed canola meal on growth performance and diet nutrient digestibility of weaned pigs. Anim. Feed Sci. Tech. 170:105-110.

Shen, Z., Wijesundera, C., Ye, J.H., 2012. Effect of seed heat-treatment on the oxidative stability of canola oil body emulsions. Food. Nutr. Sci. 3:981-990.

Spragg, J., Mailer, R., 2007. Canola meal value chain quality improvement. Available from: http://www.australianoilseeds.com/__data/assets/pdf_file/0011/5798/AOF_Stage_2_Protein_Meal_Report.pdf

Thacker, P.A., Petri, D., 2009. The effects of canola or mustard biodiesel press cake on nutrient digestibility and performance of broiler chickens. Asian Austral. J. Anim. 22: 1531-1539.

Vázquez-Añón, M., Jenkins, T., 2007. Effects of feeding oxidized fat with or without dietary antioxidants on nutrient digestibility, microbial nitrogen, and fatty acid metabolism. J. Dairy Sci. 90:4361-4367.

Weigal, J., 1991. Oilseed meals and the effect of processing on their subsequent quality. In: D.J. Farrell (ed.) Recent advances in animal nutrition in Australia, University of New England Publ., Armidale, Australia, pp 317-322.