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INFLUENCE OF TWIN-SCREW HOT EXTRUSION ON LINOLENIC ACID RETENTION IN FLAXSEED MEAL

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Flaxseed *(Linum usitatissimum* L.*)* provides multiple nutritional benefits including high quality protein, dietary fiber and is the most abundant source of α-linolenic acid ($C_{18:3}$). This study focuses on the effect of twin-screw hot extrusion on α-linolenic acid retention in full-fat flaxseed meal. The ranges of processing variables selected using Box-Behnken design were: barrel exit temperature (BET) of 120-140ºC; screw speed (SS) of 200-400 rpm; feed rate (FR) of 1-2 kg/h and feed moisture (FM) of 20-30%. The amount of α-linolenic acid retention in extruded samples ranged from 89.2% to 99.3%. Optimal operating conditions were established; BET (121ºC), SS (388 rpm), FR (1 kg/h) and FM (22.2%) for maximum (99.9%) retention of α-linolenic acid. This effect was mainly dependent on BET and FM ($p \leq 0.01$), whereas SS and FR imparted a lesser effect ($p > 0.05$). The results of this study demonstrated that the twin-screw hot extrusion can be successfully explored to produce fatty meals with significant fatty acids retention for commercially food or feed purposes.
Keywords: Hot extrusion; Twin screw; Flaxseed meal; α-Linolenic acid; RSM

INTRODUCTION

The flaxseed (*Linum usitatissimum* L.) is one of the world's oldest domesticated crops, which is cultivated as either an oil or fiber crop. The seed of flax known as "flaxseed" has a little resurgence for breakfast drinks, specialty breads, muffins and other bakery products in Europe and Asia. The principal attributes of flaxseed in human nutrition are the lipids, dietary fiber, protein and abundant α-linolenic acid ($C_{18:3}$) content (Pradhan *et al.*, 2010). The world flaxseed production remained static about 2.5 million tones as compared with other oilseed crops and represents 1% of total world oilseeds supply (FAOSTAT, 2009). Intakes of flax omega-3 fatty acids have been shown to have a favorable impact on serum lipids, fatty acids composition, cholesterol, platelet function and blood pressure in humans (Gebauer *et al.*, 2006; Yashodhara *et al.*, 2009).

Extrusion processing focus on high temperature and short time has long been employed in human and pet food products development. Seeds with a high content of lipid are generally used to produce snacks and breakfast cereals, whereas ready-to-use extruded fatty meals are not largely available on the commercial scale. This is due to the technological drawbacks such like oil percolation at the die and the hygienic condition of the extruder plant involved in processing whole seeds having lipid content >16% (De Pilli *et al.*, 2005). The fatty acids contents such as α-linolenic, linoleic and oleic fatty acids show more vulnerable attraction towards degradation under high temperature thermal processing (Camire *et al.*, 1990; Ilo *et al.*, 2000).

Extrusion cooking modelling for quality changes involves numerous process input parameters and multiple product output properties. The response surface methodology is a mathematical and statistical approach which has been widely used for optimizing the response of multivariate parameters during modeling of hot extrusion processing (Ganjyal *et al.*, 2003; Shihani *et al.*, 2006). The objective of this study was to investigate the influence of hot extrusion of full-fat flaxseed at different barrel exit temperatures, screw speeds, feed rates and feed moisture on the retention of α-linolenic acid using response surface methodology.

MATERIALS AND METHODS

The flaxseed (cv. *Chandni*) was procured from Oilseeds Research Institute, Faisalabad, Pakistan. Seeds were cleaned to remove any debris or field dirt and stored in sealed polyethylene bags at 5 ± 1 ºC.

***Extrusion Processing*:** Twin-screw extruder, APV co-rotating, with a barrel diameter of 19 mm and barrel length (BL) to diameter (LD) ratio of 25:1 (Model MP19T2-25, APV Baker, Grand Rapids, MI) was used for the production of full-fat flaxseed meal. Heating of the barrel was controlled by electric heating elements jacketing the barrel. Heating was divided into five zones along the length of the barrel, with Zone-1 nearest the feed section and Zone-5 nearest the barrel exit end. Flaxseed moisture content was adjusted to the required level during extrusion cooking by injection of water into the barrel using a Brook Crompton E2 Metripump (Hudders Field, England). Regular flaxseed was fed using a K-TRON K2M twin-screw volumetric feeder (K-

TRON, Pittman, NJ). The FR for different treatments was calculated with the relation of feeder speed (FS). The FS was set at different rates (50, 100, 200, 300, or 400 rpm) for 1 min time schedule. The average material collected during different FS was used for development of regression equation and FR was determined accordingly.

The regression equation developed for water pump calibration was:

$$Y = 0.9373x - 1.2972 \quad (R^2 = 0.9894)$$

The regression equation developed for FR determination from FS was:

$$Y = 0.068x + 5.0494 \quad (R^2 = 0.9871)$$

The formula developed to obtain required seed moisture content (%) during extrusion runs on the basis of % water pump stroke was:

$$M_cW_t = \frac{M_F(M_{Cs}) + x}{M_S + x}$$

The extrusion system can be operated under various processing conditions to achieve multitude of final product qualities. Medium shear twin-screw extruder classification was used in the following experiment. The extruder die was removed during the processing of full-fat flaxseed under different extrusion runs to avoid oil percolation and hygienic condition of extruder plant. The extruded samples were cooled down to room temperature and placed in sealed polyethylene bags for analysis of α-linolenic acid content.

Fatty acids Profile**:** The fatty acids profile of flaxseed oil was carried out according to the method described in AOCS (1998) Method No. Ce 1f-96. Briefly, the oil sample (50 µl) was methylated in the presence of 4 mL KOH (1 M) at room temperature for 1 h in order to produce fatty acids methyl esters. The resultant methyl esters were extracted with GC grade n-hexane and immediately analyzed through Gas Chromatograph (Varian 3900) apparatus equipped with an auto sampler, flame-ionization detector (FID) and supelco wax column (30 m x 0.25 µm film coating). The samples (1 µL) were injected with Helium (1 mL/min) as a carrier gas onto the column, which was programmed for operating conditions such as column oven temperature 160 °C @ 0 minutes with subsequent increase of 3 °C/min until 180 °C. The column oven temperature was increased from 180 °C to 220 °C @ 1 °C/min and was held for 7.5 min at 220 °C. Split ratio was 50% with injector 240 °C and detector 250 °C temperatures. The peak areas and total fatty acids composition were calculated for each sample by retention time using Varian Chem Station software.

α-Linolenic acid Retention**:** The retention of α-linolenic acid in flaxseed samples obtained as a result of different extrusion runs was calculated according to expression given below:

$$\alpha\text{-Linolenic acid retention \%} = \frac{\text{The content of } \alpha\text{-linolenic acid after extrusion}}{\text{The content of } \alpha\text{-linolenic acid in raw material}} \times 100$$

Experimental Design and Statistical Analysis**:** A four factors and three levels multiple regression analysis was carried out to achieve the maximal information about dependent variable from a minimal number of possible experiments using Box-Behnken design (Table 1) and analysis of experiments was carried out according to Montgomery (2008).

Table 1. Coded and actual levels of independent variables used for optimization of twin-screw hot extrusion as determined by the Box-Behnken Design (BBD)

Independent variable	**Coded levels**		
	-1	**0**	**1**
Barrel exit temperature (ºC)	120	130	140
Screw speed (rpm)	200	300	400
Feed rate (kg/h)	1.0	1.5	2.0
Feed moisture (%)	20	25	30

For better accuracy and simplification of result interpretation, the coded multiple regression coefficients were used and reconverted into original values at the end of experiment. Each experiment was performed in triplicate and the average values were taken as response, Y. The experimental data was fitted to second order polynomial response model and regression coefficients of linear, quadratic and interaction terms were obtained. The analysis of variance (ANOVA) with 95% confidence level was then employed for α-linolenic acid response variable in order to test the model significance and suitability using MATLAB® (Ver. 7.9.0) software (Mathworks, Inc., Natick, USA). The significance of all terms in the polynomial model was analyzed statistically by computing mean square at probability (p) of 0.01 or 0.05.

RESULTS AND DISCUSSION

The concentration of α-linolenic acid found in tested raw flaxseed samples was 55.4%. The combinations of independent variables for hot extrusion optimization and α-linolenic acid retention in extruded flaxseed meal have been shown in Table 2. The results indicated that α-linolenic acid content in extruded flaxseed samples ranged from 89.2% to 99.3%. The extrusion run point 3 exerted maximum retention (99.3%) of α-linolenic acid content. The lowest α-linolenic acid retention (89.2%) was for extrusion run 15.

The coefficient of determination (R^2) is defined as the ratio of the explained variation to the total variation (Wang *et al.*, 2008). This value indicates the relevance of the dependent variables in the model and the small value of R^2 shows the poor relevance of the dependent variables. R^2 computed for α-linolenic acid retention was found 74.28% and S = 2.082 which indicates the validity and proficiency of the applied model equation.

The effects of process variables were regressed and respective regression equations were developed. The second-order polynomial model equation obtained for α-linolenic acid retention is given as under:

$$Y = 98.167 - 2.092x_1 + 0.283x_2 - 1.492x_3 - 0.867x_4 - 0.200x_1x_2 - 1.025x_1x_3 - 1.100x_1x_4 - 0.050x_2x_3 - 2.650x_2x_4 - 0.650x_3x_4 - 1.475x_1^2 - 0.675x_3^2 - 1.563x_4^2$$

Where Y is the response, x_1 = BET (ºC), x_2 = SS (rpm), x_3 = FR (kg/h) and x_4 is FM (%)

Table 2. Percent values of α-linolenic acid retention in extruded flaxseed samples as determined by the Box-Behnken Design (BBD)

Extrusion run	Independent variable				α-Linolenic acid retention, %
	[1]BET (ºC)	[2]SS (rpm)	[3]FR (kg/h)	[4]FM (%)	
1	0	0	1	-1	96.6
2	0	-1	0	1	98.3
3	-1	0	0	-1	99.3
4	0	1	0	-1	96.6
5	1	-1	0	0	94.1
6	0	0	-1	1	96.8
7	1	1	0	0	95.4
8	-1	0	0	1	97.3
9	0	0	0	0	98.1
10	-1	-1	0	0	94.9
11	0	-1	-1	0	97.8
12	0	1	0	1	94.1
13	0	0	0	0	98.3
14	0	0	1	1	91.5
15	1	0	0	1	89.2
16	0	1	-1	0	96.8
17	-1	0	-1	0	98.8
18	1	0	0	-1	95.6
19	1	0	1	0	90.5
20	-1	1	0	0	97.0
21	0	1	1	0	94.6
22	-1	0	1	0	98.0
23	0	-1	0	-1	90.2
24	0	0	0	0	98.1
25	1	0	-1	0	95.4
26	0	0	-1	-1	99.2
27	0	-1	1	0	95.8

[1]BET = Barrel exit temperature (ºC); [2]SS = Screw speed (rpm); [3]FR = Feed rate (kg/h); and [4]FM = Feed moisture (%)

The second-order polynomial regression equations developed as a function of independent variables in terms of coded factors for α-linolenic acid retention are given as under:

$$Y1 = 759.141 - 10.125x_1 + 0.038x_1^2$$
$$Y2 = 121.186 - 0.227x_2 + 0.0004x_2^2$$
$$Y3 = 133.653 - 58.347x_3 + 18.461x_3^2$$
$$Y4 = 182.693 - 7.529x_4 + 0.149x_4^2$$

Where, Y = response; Y1 = barrel exit temperature, °C; Y2 = screw speed, rpm; Y3 = feed rate, kg/h; Y4 = feed moisture, %

The exploration and optimization of a fitted response model equation may produce poor or misleading results, unless the model presents the linear, quadratic and mutual interaction by good fit statistical analysis. The statistical testing of the model was done in the form of variance by fitting of the experimental data and results have been summarized in Table 3. The mean square values were obtained by dividing the sum of squares of each of the two sources of variation (model and error variance) by the respective degree of freedom. The linear and quadratic model effects were observed more significant as compared to interaction effects ($p \le 0.01$).

Table 3. ANOVA of the predicted second-order polynomial model for α-linolenic acid retention in extruded full-fat flaxseed meal samples

Source of Variation		df	Mean Square	p value
	Intercept	14	10.69*	0.0642
Linear	BET	1	52.5***	0.0047
	SS	1	0.96^{NS}	0.6472
	FR	1	9.72^{NS}	0.1618
	FM	1	25.52**	0.0326
Interaction	BET:SS	1	0.16^{NS}	0.8515
	BET:FR	1	4.84^{NS}	0.3135
	BET:FM	1	4.2^{NS}	0.3463
	SS:FR	1	28.09**	0.0262
	SS:FM	1	0.01^{NS}	0.9626
	FR:FM	1	1.69^{NS}	0.5458
Quadratic	BET:BET	1	11.6^{NS}	0.1293
	SS:SS	1	11.41^{NS}	0.1323
	FR:FR	1	13.02^{NS}	0.1101
	FM:FM	1	2.43^{NS}	0.4704
Residual		12	4.37	-
Lack of Fit		10	5.25***	0.0025
Pure Error		2	0.013	-

***Significant at 0.01 level ; **Significant at 0.05 level
*Significant at 0.1 level ; ^{NS} Non-Significant

The optimum extruder conditions for the maximum retention of α-linolenic acid in full-fat flaxseed can be obtained by varying two independent parameters and fixing the two variables at the coded zero level. The mutual impact of BET and SS on α-linolenic acid retention, by setting the FR and FM at 1.5 kg/h and 25%, respectively, is shown in Fig. 1A. It can be concluded from the data shown in Table 2 that BET and SS mutually impart an effect on α-linolenic acid retention. The maximum predicted value of α-linolenic acid retention (98.2%) can be found with the BET of 120 ºC and SS of 400 rpm. Increasing the BET at low SS decreased the

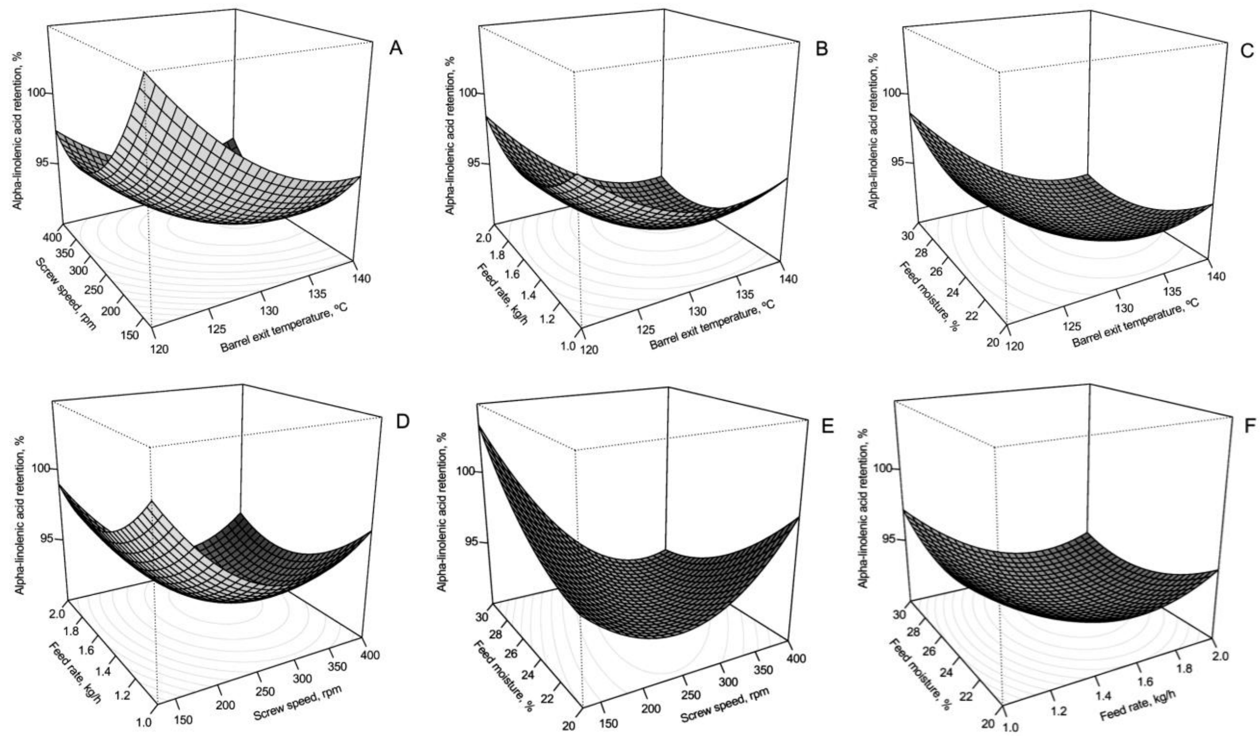

Figure 1. Mutual interaction effect of barrel exit temperature (BET, ºC); screw speed (SS, rpm); feed rate (FR, kg/h); and feed moisture (FM, %) on α-linolenic acid retention during flaxseed meal production at: (A) FR 1.5 kg/h & FM 25%; (B) SS 300 rpm & FM 25%; (C) SS 300 rpm & FR 1.5 kg/h; (D) BET 130 ºC & FM 25%; (E) BET 130 ºC & FR 1.5 kg/h; and (F) BET 130 ºC & SS 300 rpm

α-linolenic acid retention in extruded flaxseed. Twin-screw extrusion at a temperature of 180 °C and screw speed 300 rpm did not show change in free polyenoic fatty acids and α-linolenic acid composition of oat flour extrudates (Zadernowski *et al.,* 1997). However, Manthey *et al.* (2002) reported apparent decline in α-linolenic acid contents for flaxseed based spaghetti during extrusion processing. The content of linolenic acid dropped (by 6 to 12%) in extruded full-fat soya bean samples processed at a temperature of 120 ˚C as reported by Zilic *et al.* (2010). The decrease in α-linolenic acid retention may be attributed to holding time effect on feed material, low rotation of screws and increased barrel temperature (Gómez *et al.,* 1996). The transition of α-linolenic acid content to more saturated state and conversion of double bonds configuration from cis to trans after disruption of seed cell walls at higher barrel temperature also led towards destruction of essential fatty acids activity (Camire *et al.,* 1990; Grela *et al.,* 1999). Maximum predicted value of α-linolenic acid retention (94.5%) can be found with the BET of 120ºC and FR of 1 kg/h by fixing the SS and FM at 300 rpm and 25%, respectively (Fig. 1B). A remarkable retention of α-linolenic acid content was noted at low feed rate and low barrel temperature which is supported by the study of Wicklund and Magnus (1997) who found a non-significant effect of extrusion temperature (150°C) on recovery of α-linolenic acid in sifted oat flour. The alteration in fatty acids composition during thermal treatment of raw materials may be due to lipolytic activity, interactions between lipids and other constituents or processing conditions. The extrusion of raw and pre-conditioned linseed at 120 °C showed non-significant reduction in α-linolenic acid contents (Akraim *et al.,* 2006). In the present study, when setting the SS and FR at 130 rpm and 1.5 kg/h, respectively, the maximum α-linolenic acid retention (98.8%) could be obtained at BET 120°C and FM 20% (Fig. 1C). It can be substantiated from the results that the mutual influence of BET and FM is slightly significant. It is also obvious from the results that increasing the BET at high FM negatively influenced α-linolenic acid retention during extrusion cooking of flaxseed.

As shown in Fig. 1D, when the BET and FM were fixed at the zero coded level, i.e., 130°C and 25%, respectively, the prediction of maximum α-linolenic acid retention (97.6%) was found at SS 400 rpm and FR 1 kg/h. The mutual influence of SS and FR was found to be significant. It is also obvious from the results that decrease in rotation of screws with increased feed rate negatively influenced α-linolenic acid retention. The extrusion of flaxseed at low rotation of screw with high feed rate may increase residence time in extruder barrel which negatively influenced retention of α-linolenic acid. The reduced feed rate led towards significant retention of α-linolenic acid content which is supported by the study of Kong *et al.* (2008) who found non-significant differences in *α*-linolenic acid contents among salmon fillet treatments before and after lab-scale twin screw extrusion cooking. The sorghum-maize-soy blends prepared at extruded high barrel temperature did not show significant changes in oleic, linoleic and linolenic fatty acids content when compared with raw feed material (Nicole *et al.*, 2010). The maximum predicted α-linolenic acid retention value was found at SS (400 rpm) and FM (20%) when BET and FR were set at their coded value, i.e., 130ºC and 1.5 kg/h as presented in Fig. 1E. An increase in FM with reducing SS negatively influenced the retention of α-linolenic acid. Fig. 1F displays the maximum α-linolenic acid retention (98.2%) found at FR 1 kg/h and FM 20% when the BET and SS were fixed at the zero coded level, i.e., 130°C and 300 rpm, respectively. It is also noteworthy that increasing the feed rate with increasing moisture content reduced the retention of α-linolenic acid in full-fat flaxseed.

Conclusion**:** The results of this study demonstrated that hot extrusion processing at different barrel temperatures; screw speed, feed rate and feed moisture did not showed gradual decrease in the retention of *α*-linolenic acid. The amount of α-linolenic acid retention in extruded flaxseed meal samples ranged from 89.2 to 99.3%. Based on the results of this study, it can be concluded that hot extrusion can be successfully used for oilseed meals production with significant fatty acids retention for commercially food or feed purposes.

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REFERENCES

Akraim, F., M.C. Nicot, P. Weill and F. Enjalbert. 2006. Effects of preconditioning and extrusion of linseed on the ruminal biohydrogenation of fatty acids. In vivo studies. Anim. Res. 55:83-91.

AOCS. 1998. Official Methods and recommended Practices of AOCS, 5th edn. American Oil Chemists Society. Champaign, IL, USA.

Camire, M.E., A. Camire and K. Krumhar. 1990. Chemical and nutritional changes in food during extrusion. Crit. Rev. Food Sci. Nutr. 29:35-57.

De Pilli, T., C. Severini, A. Baiano, A. Derossi, A. Arhaliass and J. Legrand. 2005. Effects of operating conditions on oil loss and properties of products obtained by co-rotating twin-screw extrusion of fatty meal: preliminary study. J. Food Engg. 70:109-116.

FAOSTAT. 2009. World Linseed Production and Import/Export Data," FAO Database: Food and Agriculture Organization of the United Nations. [Online]. Available at http://apps.fao.org/default.htm/.

Ganjyal, G.M., M.A. Hanna and D.D. Jones. 2003. Modeling selected properties of extruded waxy maize cross-linked starches with neural networks. J. Food Sci. 68:1384-1388.

Gebauer, S.K., T.L. Psota, W.S. Harris and P.M. Kris-Etherton. 2006. n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am. J. Clin. Nutr. 83:1526-1535.

Gómez A.C.A., B.F. Martínez, C.J.D. Figueroa, F.C.A Ordorica and H.J. González. 1996. Chemical and nutritional changes during preparation of whole corn tortillas prepared with instant flour obtained by extrusion process. Arch. Latinoam. Nutr. 46:315-319.

Grela, E.R., S.K. Jensen and K. Jakobsen. 1999. Fatty acid composition and content of tocopherols and carotenoids in raw and extruded grass pea (*Lathyrus sativus* L.). J. Sci. Food Agric. 79:2075-2078.

Ilo, S., R. Schoenlechner and E. Berghofe. 2000. Role of lipids in the extrusion cooking processes. Grasas y Aceites. 51:97-110.

Kong, J., M.P. Dougherty, L.B. Perkins and M.E. Camire. 2008. Composition and consumer acceptability of a novel extrusion-cooked salmon snack. J. Food Sci. 73: 118-123.

Manthey, F.A., R.E. Lee and C.A. Hall. 2002. Processing and cooking effects on lipid content and stability of alpha-linolenic acid in spaghetti containing ground flaxseed. J. Agric. Food Chem. 50:1668-1671.

Montgomery, D.C. 2008. Response surface methods and designs. p440. In: Design and Analysis of Experiments, Wiley, New York, USA.

Nicole, M., H.Y. Fei and I.P. Claver. 2010. Characterization of ready-to-eat composite porridge flours made by soy-maize-sorghum-wheat extrusion cooking process. Pak. J. Nutr. 9:171-178.

Pradhan, R.C., V. Meda, S.N. Naik and L. Tabil. 2010. Physical properties of Canadian grown flaxseed in relation to its processing. Int. J. Food Prop. 13:732–743.

Shihani, N., B.K. Kumbhar and M. Kulshreshtha. 2006. Modeling of extrusion process using response surface methodology and artificial neural networks. J. Engg. Sci. Tech. 1:31-40.

Wang, Y., D. Li, L.J. Wang, Y.L. Chiu, X.D. Chen, Z.H. Mao and C.F. Song. 2008. Optimization of extrusion of flaxseeds for in vitro protein digestibility analysis using response surface methodology. J. Food Engg. 85:59-64.

Wicklund, T and E.M. Magnus. 1997. Effect of extrusion cooking on extractable lipids and fatty acid composition

in sifted oat flour. Cereal Chem. 74:326-329.
Yashodhara, B.M., S. Umakanth, J.M. Pappachan, S.K. Bhat, R. Kamath and B.H. Choo. 2009. Omega-3 fatty acids: a comprehensive review of their role in health and disease. Postgrad. Med. J. 85:84-90.
Zadernowski, R., H. Nowak-Polanska, T. Wicklund and L. Fornal. 1997. Changes in oat lipids affected by extrusion. Nahrung. 41:224-227.
Zilic, S.M., S.S. Sobajic, D. Snezana, M. Drinic, B.J. Kresovic and M.G. Vasic. 2010. Effects of heat processing on soya bean fatty acids content and the lipoxygenase activity. J. Agric. Sci. 55:55-64.