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Linseed-Sunflower Meal Co-extrudate as a Functional Additive for Animal Feed – extrusion Optimization

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ABSTRACT

The presented study shows a simple way for optimization of extrusion process, which was used for deterioration of cyanogenic glycosides – antinutritive components of linseed, with minimum damage of essential Alpha-Linolenic Acid (ALA) at the same time. Extrusion of the material was done on a laboratory single screw extruder. Content of Hydrogen cyanide (HCN) as a measurement of cyanogenic glycosides in produced co-extrudate and fatty acid composition were determined, together with basic chemical analyses, which were done in accordance with AOAC methods. Statistical analysis showed that HCN content in the product was the most dependent (P= 0.0002) on quadratic influence of moisture content of starting material. The highest HCN content (126 mg kg^{-1}) was measured at the lowest moisture content (7%) and the lowest screw speed (240 rpm). Low moisture content caused weak volatilization of HCN along with the evaporating water, which was intensified with higher values of moisture content. However, increase in moisture content from 11.5 to 16% slightly increased the amount of present HCN, due to the lower material viscosity. Extrusion process caused some changes in fatty acid composition, but even the highest degradation of ALA did not exceed 4%. Linear and quadratic influence of moisture content on ALA reduction was significant (P< 0.05), as well as quadratic influence of screw speed. Specific attention has to be paid to selecting appropriate levels of screw speed and moisture content of the material which contains linseed, in order to achieve both detoxification of linseed and preservation of essential fatty acids.

Keywords: Cyanogenic Glycosides, Essential fatty acids, Extrusion, Linseed, Sunflower meal.

INTRODUCTION

Functional food is food which shows beneficial effects on health and wellbeing of humans or animals consuming it, over the satisfaction of basic nutritional requirements. A commonly used definition explains functional food as ‘food that can be satisfactorily demonstrated to beneficially affect one or more target functions in the body, beyond adequate nutritional effects, in a way relevant to an improved state of health and well-being and/or reduction of risk of disease’ (Contor, 2001). Its development is a serious scientific task, which demands interdisciplinary research and cooperation between research institutions and industry scientists.

Linseed (*Linum usitatissimum*) is an annual or biannual plant, one of the most useful crops, that has been cultivated as a commercial plant in over thirty countries all over the world (Gabiana, 2005). The seeds, containing about 40% of oil, have long been used in human and animal diet. Recently, there has been a growing interest in linseed oil due to the high concentration of Linoleic

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(LA, 18:2, n-6) and especially α-Linolenic Acid (ALA, 18:3, n-3), representatives of omega-6 (n-6) and omega-3 (n-3) PolyUnsaturated Fatty Acids (PUFAs) (Beare-Rogers *et al.,* 2001). Moreover, linseed is the richest oilseed source of ALA (Juárez *et al.,* 2010). Cereals and oilseeds, or meals commonly used in animal diet predominantly contain a low level of n-3 FAs (Csengeri, 1996). Therefore, intensive inclusion of linseed in animal nutrition could have a significant role in improvement of Fatty Acid (FA) composition of feed.

In spite of its favourable FA composition and high nutritive value, usage of linseed in animal diet is limited, due to the fact that it contains antinutritive components. Most of them can easily be overcome, but Cyanogenic Glycosides (CGs) present serious threat to animal health and major limitation of linseed application in animal nutrition (Ivanov *et al.,* 2012). The reason for toxicity of CGs lies in the release of Hydrogen Cyanide (HCN) due to the action of a *β*-glucosidase enzyme (oxynitrilase) (Vetter, 2010). Thus, their level in food or feed is expressed through content of HCN (mg) per kg of examined material. Different heat treatments, such as autoclaving, pelleting, microwave roasting, or extrusion are usually used for reducing the content of cyanogenic glycosides in linseed (Feng *et al.*, 2003; Wu *et al.*, 2008, Kumar *et al.,* 2015).

A major problem during extrusion of materials rich in fats is lubrication and limited expansion of the produced extrudates. Another disadvantage which occurs is the separation of the oil from solid phase, thereby changing a nutritional composition of produced extrudate, as is the case with the extrusion of linseed (Ivanov *et al.*, 2012). In order to overcome the aforementioned problem, oil crops are often added to another raw material, usually a protein component, which shows good ability of oil adsorption. There are well known examples of co-extruding the protein rich materials with linseed in the literature, and pea is mainly used as an optional component (Ivanov *et al.*, 2012, Htoo *et al.*, 2008; Thacker *et al.*, 2004). Nevertheless, sunflower meal is a protein rich, relatively cheap by-product from edible oil production, which shows great ability of oil adsorption (2.05 cm^3 g^{-1}), and it is widely spread especially in Eastern Europe (Ivanov *et al.*, 2012; Lević and Sredanović, 2012). Therefore, this paper is showing an assay to produce co-extrudates based on linseed and sunflower meal, since this combination promised a good result, but it was not used yet.

The aim of the presented study was to optimize linseed–sunflower meal co-extrusion in order to produce functional additive for improvement of FA composition of animal feed, and consequently FA composition of animal products. The basic criterion in optimization process was obtaining safe product, harmless for animal health, which will not lose its valuable characteristic. In this case, the most important characteristic of the product was its favourable FA composition reflected in high content of essential *n*-3 ALA FA.

MATERIALS AND METHODS

Material

The linseed and sunflower meal used for production of co-extrudate originated from Serbia. Linseed indigenous sort “Ljupko”, with the following chemical composition: Protein (22.52%), fat (37.62%), ash (3.79%) and crude fibres (6.55%) expressed on dry matter (dm), was cultivated in the valley of the River Beli Timok (South-Eastern Serbia). Before processing, linseed was cleaned up and impurities were removed. Sunflower meal was produced in the local oil factory in Vojvodina, northern Serbian province, and it contained 38,46% of protein, 1.98% of fat, 6.65% of ash and 12.09% of crude fibre (on dm).

Extrusion Process

All materials were milled on laboratory hammer mill (ABC Engineering, Serbia) with sieve openings of 4 mm. The two compounds were mixed in 50:50 (w/w) ratio in double-shaft paddle mixer - steam conditioner (Muyang SLHSJ0.2A, China). Higher contents of linseed in mixture caused separation of the linseed oil from solid phase of co-extrudate, therefore the produced functional additive did not have constant and controlled quality. It was also impossible to control production parameters and technological process in such conditions. Separated linseed oil caused lubrication effect, therefore achieved temperatures during extrusion could not exceed 55ºC. Lower contents of linseed in the mixture were not appropriate for the experiment, since the main goal of this examination was to produce feed additive for improvement of FA composition and increase of essential FAs in animal feed. Water and steam were added into the material during conditioning, in order to adjust starting moisture content of the mixture at desirable level. Extrusion of the mixture was done on a single screw extruder (OEE 8, AMANDUS KAHL GmbH and Co., KG Germany) shown in Figure 1, with L/D ratio 8.5:1. Extrusion parameters were set according to the levels determined in applied experimental design. Extruded product was dried in a fluid bed dryer/ cooler (FB 500×200, AMANDUS KAHL GmbH and Co., KG Germany) for 10 minutes at the temperature of 25°C and a material flow rate of 18 kg h^{-1}.

Temperature of extrusion process was measured at the die opening by pt 1,000 temperature probe produced by Institute of Microelectronic Technologies and Single Crystals, Serbia.

Basic chemical analyses

Moisture content of the samples was determined using gravimetrical AOAC Method 950.46, also known as "oven dry" method, and crude ash was done using standard AOAC Method 942.05. For determination of crude protein, Kjeldahl Method was used according to AOAC 978.04 Method, crude fibres were determined by AOAC 978.10 Method (AOAC, 2000), and total fat content by Soxhlet procedure, as explained in AOCS Method Ba 3-38 (AOCS, 2001).

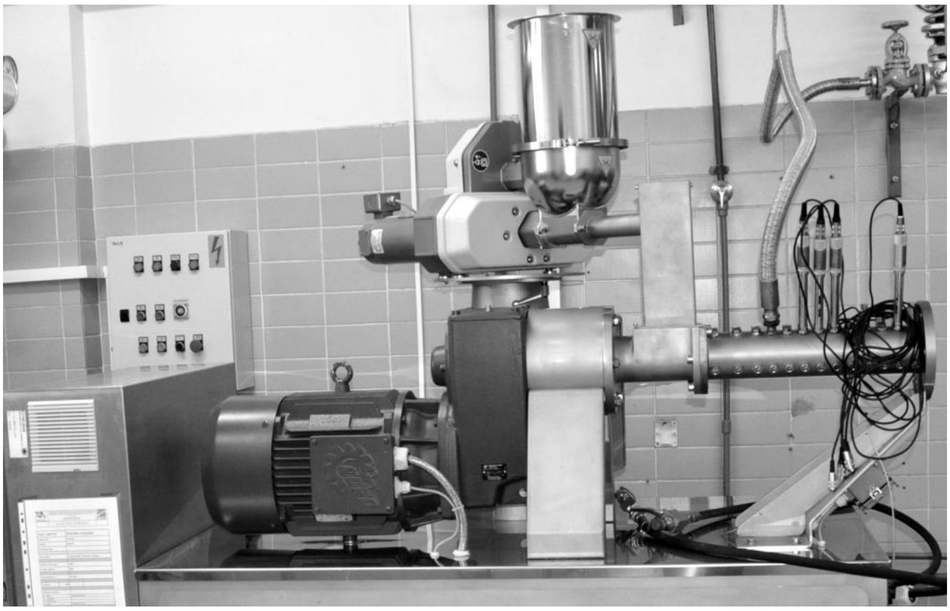

Figure 1. Extruder used in the experiment.

Fatty Acid Analysis

Supercritical Fluid Extraction (SFE) with CO_2 was used for extraction of lipids from the samples, since it showed good results as a preparative technique for FA analysis. LECO TFE-2000 fat analyzer was used for SFE, using CO_2 with 99.995% purity. Extraction conditions were adjusted as explained in the paper of Ivanov *et al.* 2012. FA methyl esters were prepared from the extracted lipids by transmetilation method that use 14% wt boron trifluoride/methanol solution (Karlović and Andrić, 1996; Ivanov *et al.* 2012). Obtained samples were analyzed by a Gas Chromatographer Agilent 7890A system (Agilent Technologies, CA, USA) with Flame Ionization Detector (GC-FID), equipped with fused silica capillary column (DB-WAX 30 m, 0.25 mm, 0.50 um) and helium as a carrier gas. The FAs peaks were identified by comparison of retention times with retention times of standards from Supelco 37 component FA methyl ester mix and with data from internal data library, based on previous experiments and FA methyl ester determination on GC-MS.

Determination of HCN in Co-extrudate

Determination of HCN was done according to AOAC official method 915.03, part B, (alkaline titration method). Silver Nitrate ($AgNO_3$) standard solution was used for titration. The volume of $AgNO_3$ standard solution consumed during the titration was recorded[20]. Equation (1) was used to calculate the HCN content in the sample:

$$X = C \times V \times 54 \times \frac{dilution}{aliquot} \times \frac{1000}{m} \qquad (1)$$

Where, X is the content of cyanide (including the HCN) in the sample (mg kg^{-1}); m the mass of the sample (g); C the concentration of $AgNO_3$ standard solution (mol L^{-1}); and V is the consumed volume of $AgNO_3$ standard solution (mL).

Removal Rate (RR) of HCN from the co-extrudate was calculated by the Equation (2):

$$RR = \left(1 - \frac{HCNcontent\_after\_extrusion}{HCNcontent\_before\_extrusion}\right) \times 100 \qquad (2)$$

Experimental Design and Optimization of the Extrusion Process

Response Surface Methodology (RSM) was a useful tool to identify and quantify how the inputs affect the outputs in a complex process (Mostafaei *et al.,* 2013). The operational conditions were planned according to Box-Behnken Experimental Design (BBD). This design was chosen because it does not include combinations of parameters in which all factors are at their highest or lowest levels (Ferreira *et al.*, 2007), thus avoiding extreme conditions of extrusion. The effects of moisture content of starting material (%), screw speed of the extruder (rpm), feeding rate (kg h^{-1}) and total die openings' area (mm^2) on content of HCN (mg kg^{-1}) and relative content of ALA (% w/w) in co-extrudate were examined. The ranges of each of the four variables were defined in order to obtain necessary data for extrusion optimization, as shown in Table 1. All measurements were limited with

Table 1. Independent experimental factors and their levels for BBD.

		Coded factor's level		
Experimental factor	Symbol	-1 (Low)	0 (Center)	+1 (High)
Moisture content of starting material (%)	X_1	7	11.5	16.0
Screw speed (rpm)	X_2	240	390	540
Loading capacity (kg h^{-1})	X_3	16	24	32
Total die opening's area (mm^2)	X_4	19.8	39.6	59.4

equipment's possibilities and capacities. A total of 27 different combinations, according to the selected experimental plan, were conducted in random order, each set of experiments was repeated three times, and the average value was taken.

Statistical analysis of experimental data was performed using STATISTICA software version 10 (Statsoft, Tulsa, OK, USA). The Analysis Of Variance (ANOVA) was used to detect significant factors in a multi-factor model, at the confidence level of 95%. The significance of the parameters influence on observed responses was determined by *p-value* and *t-value*, while the adequacy of the model was evaluated by coefficient of determination (R^2), model *P-* and *F-values*. Since BBD is used for fitting obtained data to second-degree polynomial model (3), this model was applied for description of the responses (Y) (Lazić, 2004):

$$Y = \sum b_i X_i + \sum b_{ii} X_{ii}^2 + \sum b_{ij} X_{ij} + b_o \qquad (3)$$

Where, X_i are the independent experimental factors, b_0 - intercept, b_i – linear effect of the factors, b_{ii} - the quadratic effect of the factors and b_{ij} - the interaction effect of the factors.

The graphical representations of the above equation in the form of response surface plots were used to describe the individual and cumulative effect of the two variables on the observed response, while the others were set at their central level.

Optimal values of the extrusion process parameters were determined using desirability function method (Trautmann and Weihs, 2006), and the calculations were performed in software Design-Expert 8.1 (StatEase, Inc., USA). Each of estimated responses was transformed to an individual desirability value (from 0 to 1). The overall desirability of the examined process was calculated as geometric mean of the individual desirability functions (Jeong and Kim, 2009). The experimental and predicted values of responses were compared in order to determine the validity of the model.

RESULTS

Linseed – sunflower meal co-extrudate had the following chemical composition (% of dm): Protein (30.31%), ash (5.09%), crude fibres (10.12%), and total fat (20.57%). Therefore produced functional supplement can be considered both as energy or protein source in feed, because of its relatively high content of both protein and fat. FA compositions of used linseed and sunflower meal are shown in Table 2.

Results obtained by varying independent factors of extrusion process according to BBD are shown in Table 3. Content of HCN ranged between 126 and 25.42 mg kg^{-1}, while relative content of ALA ranged between 45.39 and 49.10%.

A second-order polynomial model was generated to evaluate and quantify the influences of independent variables of extrusion on responses obtained after experiments. The results of the statistical analyses are presented in Table 4. The coefficients in the table are related to actual variables. As it can be seen from the results of calculated *P-value* and *t-value*, linear and quadratic influences of moisture content of starting material (P= 0.01 and P= 0.0002, respectively) and screw speed (P= 0.04 and

Table 2. Fatty acid composition of linseed and sunflower meal used in the experiment.[a]

FA	% Of individual FA in total FA	
	Linseed	Sunflower meal
C14:0	0,04 ± 0,03	0,2 ± 0,01
C16:0	5,30 ± 0,06	5,98 ± 0,03
C16:1	0,08 ± 0,01	0,4 ± 0,01
C18:0	4,31 ± 0,02	4,28 ± 0,03
C18:1 n9	23,02 ± 0,10	25,69 ± 0,09
C18:2 n6	16,94 ± 0,13	63,15 ± 0,11
C18:3 n3	50,31 ± 0,11	0,3 ± 0,02
SFA	9,65	10,64
MUFA	23,10	26,09
PUFA	67,25	63,45
PUFA/SFA	6,97	5,96

[a] Results are presented as mean±standard deviation. MUFA: MonoUnsaturated FA; PUFA: PolyUnsaturated FA, SFA: Saturated FA.

Table 3. Observed values for content of HCN and ALA in co-extrudate.

Coded level of experimental factors				Responses	
Moisture (%)	Screw speed (rpm)	Loading capacity (kg h^{-1})	Total die oppenings area (mm^2)	Content of HCN (mg kg^{-1})	Content of ALA (% w/w)
0	-1	1	0	98.46	47.78
0	1	1	0	80.01	49.10
0	-1	-1	0	84.13	47.97
0	1	-1	0	72.58	47.96
0	0	0	0	53.52	48.78
0	0	0	0	53.22	48.71
0	0	0	0	53.78	48.76
1	0	0	-1	96.62	48.08
1	-1	0	0	94.65	46.68
1	1	0	0	92.65	46.76
1	0	1	0	83.69	47.97
1	0	-1	0	97.00	47.81
1	0	0	1	91.43	47.94
0	0	-1	-1	30.33	48.14
0	0	1	-1	39.94	48.06
0	1	0	-1	40.85	47.45
0	-1	0	-1	77.32	47.75
0	0	-1	1	25.42	48.38
0	0	1	1	64.69	48.43
0	-1	0	1	94.96	47.79
0	1	0	1	49.36	48.58
-1	0	0	-1	94.59	46.32
-1	-1	0	0	126.00	47.07
-1	1	0	0	96.61	45.39
-1	0	1	0	97.10	48.09
-1	0	-1	0	50.99	47.75
-1	0	0	1	96.04	48.20

Table 4. Regression equation coefficients for modelled responses.

	HCN content			Relative content of ALA		
Coefficient	Value	*P–value*	*t– value*	Value	*P–value*	*t–value*
Intercept						
b_0	278.9901	0.140051	1.58018	38.57300	0.000025	6.60736
Linear						
b_1	-32.9112	0.011968	-2.95785	1.29255	0.004276	3.51323
b_2	-0.7329	0.049626	-2.18301	0.01073	0.352884	0.96647
b_3	7.7966	0.262877	1.17476	-0.14511	0.520935	-0.66127
b_4	0.5182	0.830027	0.21940	0.08319	0.307752	1.06519
Interactions						
b_{12}	0.0101	0.405832	0.86153	0.00065	0.121992	1.66397
b_{13}	-0.4126	0.086215	-1.86901	-0.00126	0.865791	-0.17267
b_{14}	-0.0186	0.838062	-0.20886	-0.00567	0.078646	-1.92215
b_{23}	-0.0063	0.363972	-0.94363	0.00028	0.228831	1.26805
b_{24}	-0.0008	0.778876	-0.28718	0.00009	0.323262	1.03014
b_{34}	0.0468	0.369247	0.93293	0.00020	0.907850	0.11822
Quadratic						
b_{11}	1.7173	0.000283	5.05220	-0.05415	0.000421	-4.81794
b_{22}	0.0009	0.009928	3.05840	-0.00004	0.003699	-3.59208
b_{33}	-0.0290	0.792309	-0.26925	0.00121	0.738884	0.34116
b_{44}	-0.0120	0.507505	-0.68312	-0.00055	0.363456	-0.94468

P= 0.009, respectively) had statistically significant impact on HCN content in co-extrudate, while their interaction showed a tendency to significance, since its *p-value* was above 0.05 (P= 0.08) but *t-value* indicated significance (t= 1.87). Linear and quadratic influence of moisture content (P= 0.004 and P= 0.0004, respectively) and quadratic influence of screw speed (P= 0.003) had a significant impact on ALA content. Intercept had the lowest *P-value* and the highest *t-value* (P< 0.0001, t= 6.60736) in this case, which means that there were even changes in the content of ALA, the values did not differ much from each other (Đurić *et al.*, 2004). Loading capacity and total die openings' area did not significantly (P> 0.05) affect examined responses, but interaction between moisture content and total die openings area showed tendencies to statistical significance (P= 0.07 and *t*= -1.922).

Table 5 presents ANOVA results of selected responses. Relatively high values of coefficients of determination (R^2) for both responses indicated good fit of experimental data to Equation (3). As suggested in literature, R^2 greater than 0.8 implies that the model fits the data well. The models *F-value* was 44.19303 for HCN content and 14,916.12 for relative content of ALA, which indicated that models were significant for selected responses. According to Lazić's studies, the model is significant if the *F-value* is higher than 2.6869, for applied experimental design (Lazić, 2004). Also, low *P-values* implied statistical significance of the models.

The final goal of RSM was optimization of extrusion process, in order to ensure reduction of HCN presence in feed and to minimize destruction of ALA as favourable *n*-3 FA. Equal importance (5) was assigned to both responses, and optimal extrusion conditions are given in Table 6. Optimization process was successfully performed, which was proven by high overall desirability function (0.917). The value of overall desirability function ranges between 0 and 1, and it should be as higher as possible (Jeong and Kim, 2009). The optimal values of extrusion parameters were:

Table 5. ANOVA of the modelled responses.[a]

Response	Source								
	Residual			Model					
	DF	*SS*	*MS*	*DF*	*SS*	*MS*	*F*-value	*P*-value	R^2
HCN	12	3032.20	252.69	15	167504.90	11166.99	44.19303	< 0.000001	0.82
ALA	12	3.32	0.276	15	61812.06	4120.804	14916.12	< 0.000001	0.82

[a] *DF*: Degree of Freedom; *SS*: Sum of Squares, *MS*: Mean Squares.

Table 6. Results of optimization of extrusion process and validation of model adequacy through co-extrudate production.

	Responses		Optimal extrusion conditions				
	HCN	ALA	Moisture (%)	Screw speed (rpm)	Loading capacity (kg h^{-1})	Total die openings area (mm^2)	Overall desirability function
Importance	5	5					
Goal	Minimize	Maximize					
Range	25.42-161	47.1-49.1	10.85	422.64	16	59.40	0.979
Predicted values	27.58	48.71					
Measured values	29.02	48.43					
Absolute error of the model	1.44	0.28					
Relative error of the model (%)	4.96	0.57					

Moisture content (10.85%), screw speed (365.35 rpm), loading capacity (16 kg h^{-1}), and total die openings' area (59.40 mm^2) (Table 6).

In order to validate the obtained results, optimal extrusion conditions were applied in practice. Looking at Table 6, it can be concluded that absolute errors of the model were low (1.04 mg kg^{-1} for HCN content, and 0.34% for ALA content), as well as relative errors, especially for ALA content (0.69%). Since content of HCN in non-processed mixture was 189.12 mg kg^{-1}, *RR* of HCN under optimal conditions was 81.96%.

Comparative results of FA compositions of unprocessed linseed-sunflower meal mixture and produced co-extrudate showed that there was no significant ($P> 0.05$) degradation of ALA in the material after extrusion. Changes of all FAs were statistically insignificant ($P> 0.05$), which is additional benefit of extrusion process. ALA content decreased for less than 1% (49.12% before, and 48.43% after extrusion).

DISCUSSION

The presence of CGs, anti-nutritive substances of linseed, was the main reason why this functional supplement was subjected to extrusion treatment. Figure 2 presents the influence of moisture content of starting material and screw speed on HCN reduction in co-extrudate. Looking at Figure 2, it is clear that the highest HCN content (126 mg kg^{-1}) was measured at lowest moisture content and lowest screw speed. Low screw speed caused relatively low extrusion temperature (63.80°C) and pressure, and low moisture content caused low volatilization of HCN along with evaporating water. Under such conditions, detoxification was the least efficient. As already mentioned, presence of water is a basic prerequisite for effective and permanent removing of CGs. According to some authors, dry heating is insufficient to provide reliable detoxification of linseed products (Oomah *et al*., 1993). High temperature during dry heating induces hydrolysis of CGs by increasing β-glucosidase activity, but HCN formed after the process remains in linseed products (Feng *et al.*, 2003). Since even the lowest temperatures during extrusion process were high enough for evaporation of HCN, increase in moisture content caused better detoxification reflected in lower HCN content. Evaporated HCN was permanently removed, so re-synthesis of CGs was prevented. Similar results were presented by Liang *et al.* (2002) who investigated the influence of moisture content of material on rapeseed detoxification.

Nevertheless, with increase of moisture content from 11.5 to 16%, a slight increase in HCN content was observed (Figure 2). Increase in moisture content above 11.5% caused a decrease of material viscosity (Morken *et al.*, 2012), which resulted in lower extrusion temperature (from 57.52 to 79.18°C) and pressure and higher mass flow through the extruder barrel. Consequently, material was shortly exposed to all influences in the extruder barrel and CGs reduction was less effective.

The lowest HCN content (25.42 mg kg^{-1}) was measured at lowest loading capacity and intermediate values of screw speed (Table 3). Such conditions sufficiently ensured high temperature (116.02°C), pressure and forces of

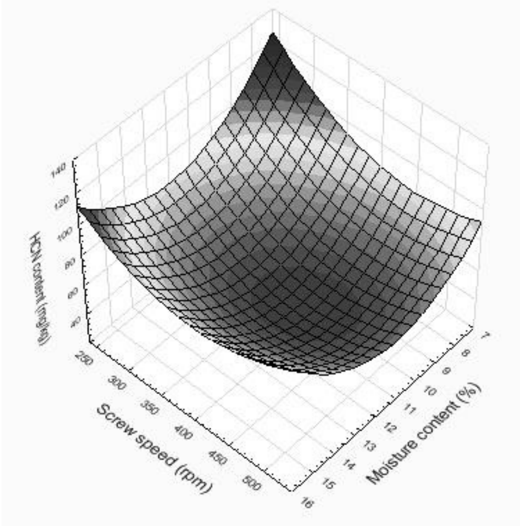

Figure 2. Influence of moisture content of starting material and screw speed on HCN content in co-extrudate.

friction for CGs destruction, while treated material was retained in extruder barrel long enough to ensure reaction between endogenous enzymes and substrate. Increasing of screw speed resulted in HCN content decrease, due to the better contact between CGs and *β*-glucosidase (Wu *et al.*, 2008). Higher temperature and pressure were achieved during extrusion by increasing screw speed (Morken *et al.*, 2012), which promoted detoxification. However, by increasing screw speed to its highest level (540 rpm), HCN content slightly increased, since mass throughput was faster, resulting in shorter exposure of the material to high temperature and other influences in extruder barrel. Additionally, drastic increase in screw speed induces very high extrusion temperatures, which may lead to the fast loss of β-glucosidase activity due to the thermal deactivation of enzyme (Wu *et al.*, 2008). Glucosidase in linseed products deactivated by high temperature could be replaced by the glucosidase formed by gut microflora of fed animals, which enables the release of HCN form GCs in the animal's body (Majak *et al.*, 1990). Wu and his co-workers showed the same trend in their experiments. According to their results, HCN content rapidly decreased by increasing the twin-screw extruder screw speed from 100 to 140 rpm, which was followed by a slow decrease in HCN content when screw speed was accelerated from 140 to 190 rpm. Finally, further increase in screw speed from 190 to 210 rpm caused a decrease in *RR* of HCN (Wu *et al.*, 2008).

Influences of total die openings' area and loading capacity on HCN content in co-extrudate as well as their interactions were not statistically significant (P> 0.05). However, the highest HCN content was determined at the maximum total die openings' area and loading capacity, with constant intermediate values of screw speed and moisture content of the material. The reason for that lies in fast throughput of the material and very short time of material exposure to extrusion conditions. Another reason was decrease of temperature (70.38°C) and pressure because of reduced resistance to the passage of the material through the die.

As mentioned earlier, linear and quadratic influence of moisture content on ALA reduction during the extrusion was significant (P< 0.05), as well as quadratic influence of screw speed (P< 0.05). Since ALA is essential FA, a very important aspect of extrusion optimization was to avoid, or at least minimize ALA destruction. Generally, extrusion does not have great influence on fat in material, but the potential changes in fat component cannot be neglected. PUFAs might be considered as the most susceptible to changes during extrusion due to their high reactivity and unstable structure (Riaz, 2000). Mild oxidation of fat components can occur because of high temperatures and intensive mechanical treatment of material (Ilo *et al.*, 1999). Porosity of extrudates and thickness of air cell walls, thus an increased contact area for oxygen, may also be of a definite importance for oxidation (Zademowski *et al.*, 1997).

The highest relative content of ALA would be determined at intermediate values of all parameters. At low values of moisture content, temperatures in extruder barrel were higher, therefore causing destruction of double bonds in ALA. The lowest ALA content was measured at the highest screw speed and the lowest moisture content, and it counted 45.39%. Increase in screw speed increased pressure, temperature and forces of shearing, causing mechanical degradation of double bonds of ALA. On the other hand, the highest values of moisture content in combination with extrusion temperature could induce enzymatic degradation of triglycerides under activity of endogenous lipase followed by oxidation of unsaturated FAs in the presence of high moisture content (O'Connor *et al.*, 1992), which could also lead to degradation of ALA. Thus, the lowest degradation of ALA is assumed to be at intermediate levels of all parameters. Despite of the statistically significant influence of several parameters on ALA content, maximal decrease of relative ALA content was 3.73% (from 49.12% in unprocessed mixture to 45.39%) under extreme conditions of lowest moisture content and highest screw speed. Hence, optimization of extrusion process offered the possibility to produce non-toxic functional supplement with high content of *n*-3 FA.

The overall desirability function was remarkably high and it counted 0.917 in this experiment, as presented in Table 5. Theoretically, HCN content in co-extrudate was supposed to be 33.08 mg/kg and the relative content of ALA was supposed to be 48.77% under predicted optimal conditions of extrusion (Table 5). In that case, the relative content of ALA would be reduced for only 0.35%, while *RR* from the co-extrudate would be 82.51%. After applying optimal conditions in practice, slightly different results of responses were obtained. Relative content of ALA was 48.43%, while the content of HCN in co-extrudate was 34.12 mg kg^{-1} (Table 5). Nevertheless, low values of absolute and relative errors indicated that optimal conditions were adequately chosen. Relative error for *RR* of HCN was 0.67%.

After production of co-extrudate under optimal conditions, FA compositions of untreated mixture and processed functional supplement were compared in order to examine the eventual changes occurred during extrusion process. Decrease of relative content of ALA, which counted only 0.69%, was insignificant (P= 0.21), as well as all other changes in FA contents (P> 0.05). Relative content of oleic acid (C 18:1 n-9) increased from 24.11 to 25.49%, as well as relative content of stearic acid (from 4.30 to 4.89%). This was probably the consequence of degradation of double bonds in ALA and linoleic acid (C 18:2 n-6). Similar results were presented by Žilić *et al.* (2010) who noticed that soybean extrusion caused a decrease in the content of ALA which was more evident with higher extrusion temperatures, while oleic acid content increased. As presented in their work, ALA content of soybean cultivar "Bosa" dropped from 7.6 to 6.8%, 6.4, 7 and 7.2% after dry extrusion on temperatures of 100, 125, 140 and 150°C, respectively. Decrease of ALA content was also registered in treated soybean cultivar "ZPS 0.15". At the same time, increase of MonoUnsaturated FA (MUFA) oleic acid (C 18:1 n-9) and Saturated FA (SFA) stearic acid (18:0) contents was registered. Nevertheless, the authors presented a slight increase in linoleic acid (C18:2 n-6).

Results of the presented experiment pointed out the importance of understanding extrusion process in production of animal feed. We have described a simple method for optimization of extrusion, which was, in this case, applied on preparation of linseed-sunflower meal co-extrudate. By optimization, the goal of the investigation was achieved: feed supplement, safe for animal health with preserved functional component (ALA), was successfully produced. *RR* of HCN content was 81.95%, while ALA content insignificantly decreased for less than 1%. Used optimization method was verified in practice, and experimental results agreed well with the theoretics. Produced supplement can be used in animal nutrition with no negative effects, but its amount in diets has to be carefully determined depending on age and species of bred animals.

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محصول فرایند کواکسترود (هم روزن رانی) کنجاله بذر کتان-آفتابگردان به عنوان یک افزودنی کاربردی برای خوراک دام- اکستروژن

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چکیده

مطالعه حاضر یک راه ساده برای بهینه سازی فرایند اکستروژن ارائه می نماید که جهت تخریب گلیکوزیدهایسیانوژنیک– ترکیبات ضد مغذی دانه کتان با حداقل آسیب به اسید لینولنیک ضروری(ALA) بطور همزمان، مورد استفاده قرار گرفته است. فرایند اکستروژن به وسیله ی یک اکسترود تک ماردون در آزمایشگاه انجام شد. میزان هیدروژن سیانید (HCN) به عنوان براوردی از میزان گلیکوزیدهایسیانوژنیک تولید شده از فرایند کواکسترود و همچنین ترکیب اسید چرب، به وسیله ی آنالیزهای شیمیایی پایه ، مطابق با روش هایAOAC ، اندازه گیری شد. آنالیزهای آماری نشان داد که میزان HCN در محصول بیشترین وابستگی را بصورت درجه دوم به رطوبت مواد اولیه دارد. بیشترین میزان HCN(۱۲۶ mg/kg) در کمترین میزان رطوبت (۷٪) و کمترین سرعت ماردون (۲۴۰ دور در دقیقه) اندازه گیری شد. رطوبت کم باعث تبخیرضعیفHCN همراه باتبخیرآب می شود، که این امر با مقادیر بالاتر رطوبت تشدید می شود. اگرچه ،افزایش رطوبت از ۱۱.۵٪ به ۱۶٪ به دلیل کاهش ویسکوزیته ی مواد اندکی میزان HCN موجود را کاهش می دهد. فرآینداکستروژن باعث برخی تغییرات در ترکیب اسیدچرب شد اما حتی بالاترین میزان تخریب ALA از ۴٪ هم تجاوز نکرد. تاثیر خطی و درجه دو رطوبت بر کاهش ALA مانند اثر درجه دو سرعت ماردون معنادار بود. برای سم زدایی بذر و حفظ همزمان اسیدهای چرب ضروری می بایست نسبت به انتخاب مقادیر مناسب سرعت ماردون و رطوبت موادی مانند بذر کتان، توجه خاص نمود.