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Original research paper

DETOXIFICATION OF LINSEED-SUNFLOWER MEAL CO-EXTRUDATE – PROCESS PREDICTION

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ABSTRACT: For many years, linseed has been attracted a great attention in animal nutrition because of its exceptionally favourable fatty acid composition and high content of essential α-linolenic acid. However, the presence of antinutritive components, cyanogenic glycosides, limits its inclusion in the animal's diet. Several ways of linseed detoxification were observed in literature, emphasizing extrusion as one of the most effective processes.

In the presented study, the application of Artificial Neural Network (ANN) has been observed, as a tool for prediction of process influence on the deterioration of cyanogenic glycosides during the extrusion process of linseed-sunflower meal co-extrudate. The content of hydrogen cyanide (HCN) was determined according to the AOAC method as an indicator of cyanogenic glycosides in the produced co-extrudate. Extrusion of the material was performed on a laboratory single screw extruder. The performance of ANN model was compared with experimental data in order to develop rapid and accurate method for prediction of HCN content in co-extrudate.

According to the experimental results, the highest HCN content (126 mg/kg) was determined at the lowest moisture content (7%) and the lowest screw speed (240 rpm). With the increase of moisture content and temperature during extrusion, the content of HCN drastically decreased. The ANN model showed high prediction accuracy (r^2> 0.999), which indicates that the model could be easily and reliably applied in practice.

Keywords: *extrusion, antinutritive components, cyanogenic glycosides, artificial neural network*

INTRODUCTION

Many antinutritional and toxic factors occur in conventional seeds cultivated for the purposes of feed production. The role of these factors in plants is to defend seeds against environmental vagaries and thus help to protect them (Kumar and Sharma, 2008). These factors, though good for the plant, cause deleterious effects or could be even toxic to animals. When it comes to linseed, cyanogenic glycosides (CGs) are the main limitation of linseed unrestricted usage in animal nutrition (Čolović et al., 2016). The biological role of CGs in plants is most probably protection against different phytopatogens. Several studies have shown that CGs can act either as feeding deterrents or phagostimulants, depending on the insect species (Sun et al., 2018). The reason for toxicity of CGs lies in the release of hydrogen cyanide (HCN) due to the action of a β-glucosidase enzyme (Čolović et al., 2016; Sun et al., 2018). Thus, their level in food or feed is expressed through the content of HCN (mg) per kg of the examined material. European Food Safety Authority (EFSA) recommended following limits of HCN content in animal feed (mg HCN equivalents/ body weight per day): pigs – 2.9 mg/kg per day, poultry – 2.8 mg/kg per day, ruminants (on the basis of goat studies) – 0.4 mg/kg per day. There is no literature data on tolerated levels of HCN in fish feed (EFSA, 2006; Ivanov et al., 2012).

Heat treatments commonly used for linseed detoxification are autoclaving, pelleting, microwave roasting, extrusion, etc. (Čolović et al., 2015). Several authors have proposed a detoxification method of linseed that involved boiling with water or a distillation of linseed powder (Wu et al., 2007). However, most of the mentioned detoxification techniques cannot be applied in feed production, thus the extrusion process seems to be a convenient solution for easy implementation at industry level. Before application in real production, extrusion detoxification of linseed has to be examined in laboratory conditions in order to determine the effects of extrusion parameters on processed material and to predict the results of the detoxification process, if possible.

A major problem during extrusion of materials rich in fats are 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. 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 (Čolović et al., 2016).

Recently, mathematical modelling has been increasingly used for the study of the given systems. Artificial Neural Network (ANN) models have gained momentum for modelling and control of extrusion processes (Li and Bridgwater, 2000; Cubeddu et al., 2014; Sovány et al., 2016). ANN models are recognized as a good modelling tool since they provide the empirical solution to the problems from a set of experimental data, and are capable of handling complex systems with non-linearities and interactions between decision variables (Shankar et al., 2007; Fan et al., 2013). The developed empirical models show a reasonable fit to experimental data and successfully predict extrusion processes (Deng et al., 2018; Altarazi et al., 2018). Nonlinear models are found to be more suitable for real process simulation.

The specific objective of this study was to investigate the effect of extrusion process parameters (moisture, screw speed, loading capacity and total die opening area) on the content of hydrogen cyanide (CHCN) in processed co-extrudate of linseed and sunflower meal. The performance of the ANN was compared with the experimental data in order to develop rapid and accurate method for prediction of CHCN.

MATERIALS AND METHODS

Extrusion process

The linseed and sunflower meal used for production of co-extrudate originated from Serbia. Linseed indigenous sort "Ljupko" was cultivated in the valley of the river Beli Timok. Before processing, linseed was cleaned up and impurities were removed.

Sunflower meal was produced in the local oil factory in Vojvodina and it contained 38.46% of protein, 1.98% of fat, 6.65% of ash and 12.09% of crude fibre (on dry matter).

All materials were milled on a laboratory hammer mill (ABC Engineering, Serbia) with sieve openings of 4 mm. The two compounds were mixed in 50:50 (w/w) ratios in double-shaft paddle mixer - steam conditioner (Muyang, SLHSJ0.2A, China) in order to avoid the separation of oily phase. Mentioned ratio was chosen after preliminary tests, since our goal was to add the lowest proportion of sunflower meal which will prevent linseed oil separation. Water and steam were added into the material during conditioning, in order to adjust starting moisture content of the mixture at a desirable level. Extrusion of the mixture was done on a single screw extruder (OEE 8, AMANDUS KAHL GmbH and Co., KG, Germany), with L/D ratio 8.5:1. Extrusion parameters were set according to the levels determined in the 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. Temperature of extrusion process was measured at the die opening by "PT 1000 temperature probe" produced by Institute of Microelectronic Technologies and Single Crystals, Serbia.

HCN content determination

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. Equation (1) was used to calculate the HCN content in the sample:

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

Where, X is the content of cyanide expressed through HCN content in the sample (mg/kg); m is the mass of the sample (g); C is the concentration of $AgNO_3$ standard solution(mol/l); and V is the consumed volume of $AgNO_3$ standard solution (ml).

Experimental design and ANN modelling

The operational conditions were planned according to Box-Behnken's experimental design for four parameters. According to general recommendations for ANN design, prior to ANN modelling, the second order polynomial model was derived, coupled with the analysis of variance (ANOVA), in order to check the significant effect of the input variables over the output, as well as to justify the later use of the ANN model by the coefficient of determination (r^2). 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), loading capacity (kg/h) and total die openings' area (mm^2) on the content of HCN (mg/kg) 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.

A multi-layer perceptron model (MLP) consisted of three layers (input, hidden and output) was used in this investigation. Such a model has been proven as a quite capable of approximating nonlinear functions (Hu and Weng, 2009) giving the reason for choosing it in this study. The number of hidden neurons for optimal network was ten and the Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm was used for ANN modelling. This algorithm was used to speed up and stabilize convergence (Basheer and Hajmeer 2000).

Coefficients associated with the hidden layer (weights and biases) were grouped in matrices W_1 and B_1, while the coefficients associated with the output layer were grouped in matrices W_2 and B_2.

W_2 and B_2. The representation of the neural network could be written using matrix notation (Y is the matrix of the output variables f_1 and f_2 are transfer functions in the hidden and output layers, respectively, and X is the matrix of input variables) (Kollo and von Rosen, 2005):

$$Y = f_1(W_2 \cdot f_2(W_1 \cdot X + B_1) + B_2) \tag{2}$$

The weights of matrices W_1 and W_2 were determined during the ANN learning cycle, which updated them using optimization procedures to minimize the error between network and experimental outputs (Trelea, et al., 1997; Pezo, et al., 2013), calculated according to the sum of squares (SOS), while the coefficients of determination were (r^2) used as parameters to check the performance of the obtained ANN model.

After the best behaved ANN was chosen, the model was implemented using an algebraic system of equations to predict HCN content in co-extrudate.

The numerical verification of the developed models was tested using coefficient of determination (r^2), reduced chi-square (x^2), mean bias error (MBE), root mean square error (RMSE) and mean percentage error (MPE).

A neural network was tested using sensitivity analysis, to determine whether and under what circumstances obtained model might result in an ill-conditioned system (Taylor, 2006). The infinitesimal amount (+0.0001%) has been added to each input variable, in 10 equally spaced individual points encompassed by the minimum and maximum of the training data. These signals were normally distributed with a constant intensity and frequency.

RESULTS AND DISCUSSION

The results of proximate analysis of HCN content in co-extrudate are presented in Figure 1. HCN content varied significantly, implying that fitting of the experimental data can be performed using ANN modelling.

Determination of the appropriate number of hidden layers and the number of hidden neurons in each layer is one of the most critical tasks in ANN design. The number of neurons in a hidden layer depends on the complexity of the relationship between inputs and outputs. As this relationship becomes more complex, more neurons should be added (Ćurčić et al., 2015).

The optimum number of hidden neurons was chosen upon minimizing the difference between predicted ANN values and desired outputs, using Sum of Squares (SOS) during testing as a performance indicator. Used multi-layer perceptron models (MLPs) were marked according to Stat Soft Statistica's notation.

MLP was followed by a number of inputs, number of neurons in the hidden layer, and the number of outputs. According to ANN performance (Table 2), it was noticed that the optimal number of neurons in the hidden layer for HCN content in co-extrudate calculation was 10 (network MLP 4-10-1), when obtaining high values of r^2 (0.999995) and low values of SOS (0.002909). The quality of the model fit was tested and the residual analysis of the developed model was presented in Table 2. The ANN model had an insignificant lack of fit tests, which means the model satisfactorily predicted the HCN content in co-extrudate. A high r^2 is indicative that the variation was accounted for and that the data fitted the proposed model satisfactorily (Montgomery, 1984).

Table 3 presents the elements of matrix W_1 and vector B_1 (presented in the bias row), and Table 4 presents the elements of matrix W_2 and vector B_2 (bias) for the hidden layer, used for calculation in Equation 1.

Optimal network, used for prediction of HCN content was able to predict reasonnably well the output for a broad range of the process variables (coefficients of determination reached 0.999995 for HCN content prediction). The predicted values were very close to the experimental (target) values in most cases, in terms of r^2 value of ANN model.

The mean and the standard deviation of residuals have also been analysed. The mean of residuals for an ANN model was equal to 0.000, and the standard deviation was $6.04\cdot10^{-3}$. The skewness showed minimal deviations from a normal distribution, -0.277, while the kurtosis showed almost neglecting the difference in "peakedness" compared to normal distribution, 13.000). The evaluated values of coefficient of determination (0.999995), the mean relative percent error ($4.0\cdot10^{-2}$), the root mean square error ($7.6\cdot10^{-2}$) and the reduced chi-square ($6.0\cdot10^{-3}$) showed a good fit of the model to experimental results.Therefore, it was confirmed that obtained ANN model was statistically significant and in agreement with experimental results.

In order to validate the ANN model and to test its fitting to the experimental results, three independent experiments were performed. Table 5 shows the model validation results. The predicted values obtained by the ANN model were comparable to the values obtained in the experiment. Low coefficients of variation (CV) for all process variables (<10%) indicated the adequacy of ANN model.

Table 1.
Independent experimental factors and their levels for Box-Behnken's experimental design

Code	W	SS	LC	TD
	(%)	(rpm)	(kg/h)	(mm^2)
-1	7	240	16	19.8
0	11.5	390	24	39.6
+1	16	540	32	59.4

W - moisture; SS - screw speed; LC - loading capacity; TD - total die opening area

Table 2.
The results of ANN performance

Network name	Training performance	Training error	Training algorithm	Error function	Hidden activation	Output activation
MLP 4-10-1	0.999995	0.002909	BFGS 132	SOS	Logistic	Identity
"Goodness of fit" of the model		χ^2	RMSE	MBE	MPE	
		$6.0\cdot10^{-3}$	$7.6\cdot10^{-2}$	$-4.5\cdot10^{-11}$	$4.0\cdot10^{-2}$	
Residual analysis		Skew.	Kurt	Mean	StDev	Var.
		-0.277	13.000	0.000	0.078	$6.04\cdot10^{-3}$

Performance term represents the coefficients of determination, while error terms indicate the lack of data for the ANN model. r^2 - **coefficient of determination, χ^2- reduced chi-square, MBE - mean bias error, RMSE - root mean square error, MPE- mean percentage error, Skew. - skeweness, Kurt - kurtoisis, StDev - standard deviation of residuals, Var. - variation of residuals*

Table 3.
Elements of matrix W_1 and vector B_1 (presented in the bias row)

Node	1	2	3	4	5	6	7	8	9	10
W	-1.26	-0.22	-0.51	-4.26	-4.20	-1.00	1.41	-7.87	2.82	-0.81
SS	3.32	-3.03	-3.30	-0.40	-11.25	-1.90	-3.55	-3.96	-5.96	-2.13
LC	5.55	1.86	-1.50	1.58	-2.52	1.01	2.93	3.83	-0.88	0.58
TD	1.51	0.17	3.72	-5.14	-2.39	0.72	-0.55	0.39	2.27	0.75
Bias	1.73	-1.25	-0.12	1.81	1.15	-0.79	-0.71	1.43	-1.38	-0.16

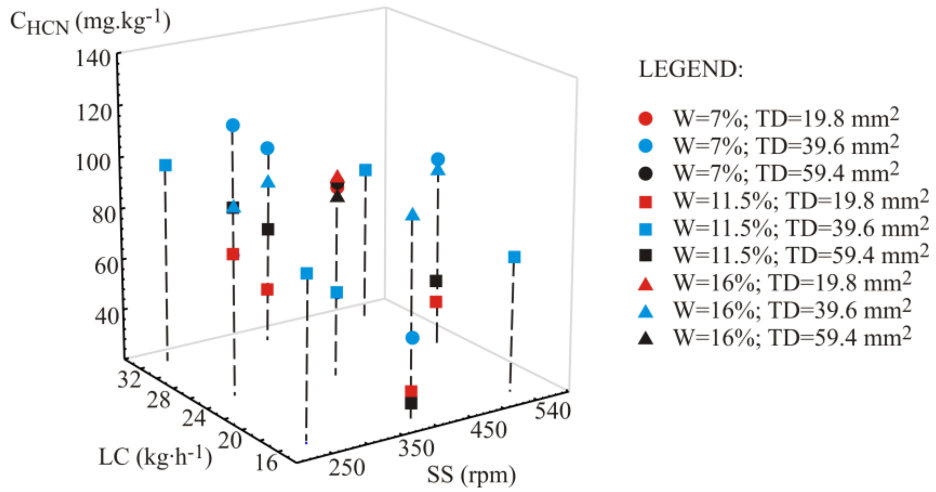
Table 4.
Elements of matrix W_2 and vector B_2 (presented in the bias column)

Node	1	2	3	4	5	6	7	8	9	10	Bias
CHCN	2.05	-2.65	-1.15	-1.01	3.24	-0.27	0.63	1.70	1.64	-0.57	-1.30

Table 5.
Predicted and observed responses at optimum conditions

No.		*W*	*SS*	*LC*	*TD*	C_{HCN}
1	ANN predicted	11,5	390	16	20	33.28
	Experiment					35.04
	St. dev.					1.25
	CV					3.56
2	ANN predicted	11,5	390	16	59,4	24.73
	Experiment					23.33
	St. dev.					0.99
	CV					4.24
3	ANN predicted	11,5	390	30	20	36.05
	Experiment					38.40
	St. dev.					1.66
	CV					4.33

W - moisture; SS - screw speed; LC - loading capacity; TD - total die opening area; C_{HCN} - HCN content in co-extrudate



W - moisture content; SS - screw speed; LC - loading capacity; TD - total die opening area

Figure 1. Results of proximate analysis of HCN content in co-extrudate (n=27 runs)

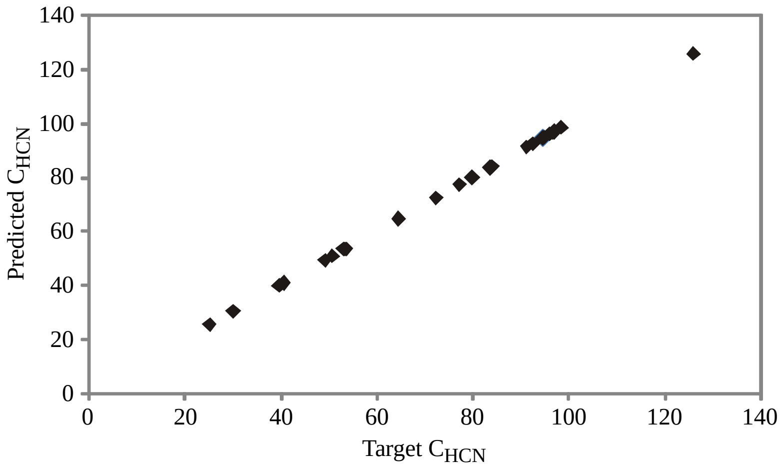


Figure 2. Comparison of experimentally obtained HCN content

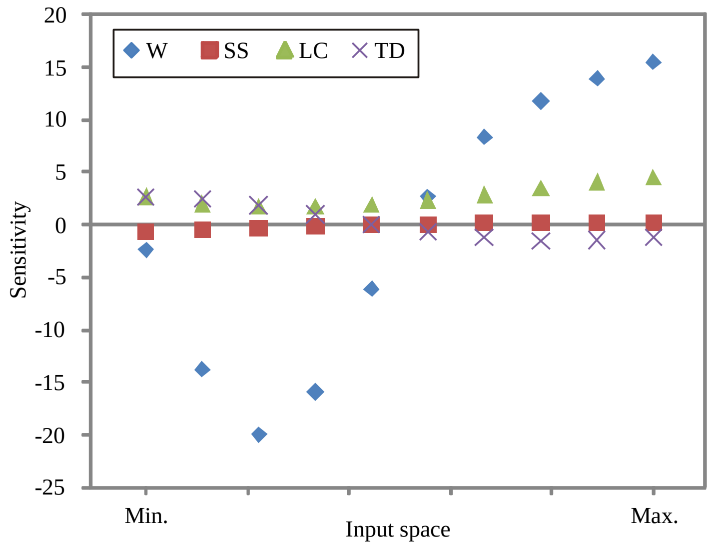


Figure 3. Sensitivity analysis - the influence of the input over the output variables

In order to assess the effect of changes in the outputs due to the changes in the inputs, a sensitivity analysis was performed. The greater effect observed in the output implies that greater sensitivity is presented with respect to the absolute value of the input (Montaño and Palmer, 2003; Taylor, 2006; Pezo et. al., 2013; Turanyi and Tomlin, 2014). Sensitivity analysis has been performed to test an infinitesimal change in an input value in 10 equally spaced individual points, ranged by the minimum and maximum of the observed assay, in order to explore the changes in observed outputs. It is also used to test the model sensitivity and measurement errors.

Sensitivity analysis is used to show the influence of the inputs, but it also shows the importance of an input variable at a given point in the input space (Saltelli et al., 2010). The influence of the input over the output variables, i.e. calculated changes of output variables for infinitesimal changes in input variables, is shown in Figure 3. The obtained values corresponded to level of experimental errors, and also showed the M, SS, LC and TD influence on HCN content in co-extrudate. According to Figure 3, CHCN was mostly influenced by M.

CONCLUSIONS

ANN-based model could be effectively used for predictive purposes, modelling and optimization, according to the results shown above. The model was developed for prediction of HCN content in co-extrudate for a wide range of input variables, which makes it suitable for unsteady conditions of feed production. Taking into account that a considerable amount and wide variety of data were used in the present work to obtain the ANN model, and considering that the model turned out to yield a sufficiently good representation of the experimental results, it shows a great potential for application in practice. The prediction ANN model showed high prediction accuracy, according to gained results. ANN model was statistically significant and in agreement with experimental results.The increase of moisture content during the extrusion process induces a noticeable decrease of the content of HCN.

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ДЕТОКСИФИКАЦИЈА КОЕКСТРУДАТА ЛАНЕНОГ СЕМЕНА И СУНЦОКРЕТОВЕ САЧМЕ – ПРЕДВИЂАЊЕ ПРОЦЕСА

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Сажетак: Већ дуги низ година ланено семе привлачи велику пажњу у исхрани животиња због свог изузетно повољног маснокиселинског састава и високог садржаја α-линелонске киселине. Ипак, његова примена у је ограничена због присуства антинутритивних материја – цијаногених гликозида. До сада је у литератури обрађена неколицина поступака за детоксификацију ланеног семена, а екструдирање се истиче као најефикасније међу њима. У приказаном експерименту, испитивана је примена вештачких неуронских мрежа са циљем да се предвиди утицај процеса на разарање цијаногених гликозида током поступка екструдирања коекрудата ланеног семена и сунцокретове сачме. Као индикатор количине присутних цијаногених гликозида у производу одређиван је садржај цијановодоничне киселине (HCN), у складу са AOAC методом. Екструдирање материјала изведено је на лабораторијском једнопужном екструдеру. Функционисање модела вештачке неуронске мреже упоређено је са експерименталним резултатима како би се развио брз и тачан метод за предвиђање садржаја HCN у ко-екструдату. Како су екстеприментални резултати показали, највиши садржај HCN(126 mg/kg), измерен је при најнижем садржају влаге (7%) и најмањој брзини обртања пужа екстурдера (240 обртаја/ минуту). Са порастом садржаја влаге и температуре током екструдирања, садржај HCN је нагло опадао. Модел вештачких неуронских мрежа показао је високу тачност предвиђања (r^2> 0.999), што указује на то да би модел могао врло лако да буде примењен и у пракси.

Кључне речи: *екструдирање, антинутритивне компоненте, цијаногени гликозиди, вештачке неуронске мреже*

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