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Effectiveness of different processing methods in reducing hydrogen cyanide content of flaxseed

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Abstract: A study was conducted to determine the effectiveness of reducing the hydrogen cyanide (HCN) content of flaxseed (FS) by processing. FS was processed by oven heating, single or repeated pelleting alone or in a mix with corn or other ingredients, autoclaving, and microwave roasting. The comparative effectiveness in reducing HCN in FS by these processes was monitored through HCN measurements by alkaline titration. The HCN content was 377 mg kg^{-1} in raw feed-grade FS and 139 mg kg^{-1} in a human food-grade FS. All processing methods tested significantly ($p < 0.05$) reduced the HCN content of FS. Autoclaving FS reduced its HCN content by 29.7%. Microwave roasting of FS reduced the HCN content by 83.3%. Because of the 5.7% water loss recorded after 4 min of FS roasting, this reduction could be related to more evaporation of the newly formed HCN. Pelleting FS once reduced HCN content by 13.3%, and three and six repeated pelleting processes reduced HCN content by 29.0% and 54.9% respectively. When FS was pelleted in a mix with 50% corn, the HCN reduction was even greater. After pelleting six times, HCN reduction reached 63.8%. However, the greatest reduction in HCN content was 73.8%, and was obtained when FS was mixed with several ingredients and pelleted twice. The HCN reduction could be the result of deactivation of the glycosidase, or the evaporation of HCN formed from cyanogenic glycosides. The HCN reduction increased as the number of pelletings and the temperature of the pelleted product increased. The greater and prolonged exposure to a higher temperature by several pelletings seems to promote a greater HCN reduction. The appropriate processing of FS is essential for the use of this oilseed in animal feeding.

Keywords: flaxseed; processing methods; autoclaving; microwave roasting; pelleting; hydrogen cyanide

INTRODUCTION

It is scientifically well established that proper processing is required before linseed meal can be satisfactorily utilized in diets for monogastric animals. Kratzer[1,2] reported that water soaking of linseed meal overcame its growth depression effect when used in chick diets at levels over 5%. Other researchers[3] reported that water-soaked linseed meal did not cause depressed growth when used at levels up to 30% in chick diets, whereas 4.5% untreated linseed meal did. The product of wet autoclaving of linseed meal yielded similar responses in performance.[4] One of the benefits of processing is the reduced HCN content in linseed meal.[5] As it is well recognized that flaxseed or linseed (*Linum usitatissimum* L) meal contains relatively high levels of hydrogen cyanide (HCN), a potent respiratory inhibitor, it will be of great interest to know the HCN status in processed flaxseed products. In addition, by assessing the effectiveness of the method of processing flaxseed to reduce the HCN content, then the relative cost of each one may be the deciding factor in the method of choice.

Recognizing the unavoidable need to process linseed meal, the same principle can be applied to flaxseed, but unfortunately there is very limited information on the subject because its use as a feed resource is relatively recent. The present study intended to establish the effectiveness of various processing methods in removing HCN from flaxseed to be used in animal feeding.

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MATERIALS AND METHODS

Processing of flaxseed

Two lots of commercially available brown flaxseed purchased from the local feed market, one feed grade and one human food grade, were used in this study. They were submitted to different processing methods that included autoclaving, microwave oven roasting, pelleting, and grinding. Water soaking of flaxseed was also evaluated. Raw canola seed was also included as a reference material.

Autoclaving was performed with a steam sterilizer (Barnstead Still & Sterilizer Co, Boston, MA, USA) at 16.5 kg cm^{-2} at 120 °C for 15 min. During autoclaving, flaxseed was placed as a 3 cm thick layer on a stainless-steel tray. Microwave processing was performed with a household microwave oven with 750 W output, under the operating frequency of 2450 MHz (Kenmore, manufactured by Goldstar Co Ltd, Korea). 200 g of flaxseed were laid on a 20×20 cm^2 plastic tray and cooked under maximum output for 4 min.

Pellets are formed with high pressure and increased temperature. Tissue disruption when ingredients are passed through the pellet die may encourage the enzymatic formation of HCN, while cooling the pellets could help the removal of HCN. By controlling these two procedures, one may achieve the efficient removal of HCN from flaxseed. The pelleting process in this study was performed using a California laboratory pellet mill, or California Pellet Mill (CPM), Master Model (California Pellet Mill Co, 1114 E Wabash Av, Crawfordsville, IN 47933, USA). The die diameter of the pellet machine was 0.31 cm. Flaxseed was pelleted either one, three or six times, alone or mixed with 50% corn. The temperature reached during pelleting was recorded by measuring the pellet temperature at the pelleter's outlet. The temperature was obtained with a Raytek non-contact infrared thermometer (Raytek, TS-2, USA). When flaxseed was pelleted more than once, the pelleted feed was laid on the concrete floor for about 15 min to cool.

Grinding of flaxseed was accomplished using a domestic coffee grinder (Black & Decker Canada Inc, Brockville, Ontario). Water soaking consisted of adding 100 g of flaxseed to 200 ml of water in a 400 ml beaker and letting stand for 2 h.

Chemical analysis

The detection of cyanogenic glycosides in flaxseed can be performed by two methods. One is the quantitative determination of individual cyanogenic glycosides by high-performance liquid chromatography (HPLC). The other is the colorimetric titration of HCN, which is used normally for the comparative study between samples or cultivars.

The HCN content in flaxseed in this study was determined by alkaline titration.[6] 20 g of air-dried flaxseed were ground with a coffee grinder and transferred to a Kjeldahl flask. 200 ml water was added and mixed with the sample. After 2 h, the solution was then distilled. Distillate was collected in a flask containing 20 ml 2.5% NaOH solution (0.5 g in 20 ml H_2O), until distilled to a definite volume. 8 ml 6 M NH_4OH and 2 ml 5% KI solution were added to the distillate before titration with 0.02 M $AgNO_3$ using a microburette. HCN was calculated as: HCN (mg) = ml of 0.02 M $AgNO_3/1.08$.

Statistical analysis

The General Linear Models procedure of SAS[7] was used for the statistical analysis. The statistical significance of the differences between least-squares means of HCN in the raw flaxseed and the processed ones were determined by student's t test. The statistical significance was accepted when $p < 0.05$.

RESULTS

Processing of flaxseed by autoclaving, microwave roasting, and pelleting (with and without other ingredients) significantly reduced the HCN content of flaxseed. The ground raw flaxseed had an HCN content of 377.0 mg kg^{-1}. When flaxseed was pelleted once with a CPM laboratory pellet mill, HCN was reduced by 13.3%, a statistically significant ($p <$ 0.049) reduction. Repeated pelleting reduced HCN further, either when flaxseed was pelleted alone or when in a mixture with corn. When flaxseed was pelleted with other ingredients, as should be the case in a commercial pelleting operation, the HCN content in the flaxseed was reduced to 98.9 mg kg^{-1}, which was 73.8% less than that in ground raw flaxseed. The process of oven heating also reduced the HCN content, but to a lesser extent. Among all the processing methods tested, flaxseed processing by microwave oven seemed to be the most effective in reducing HCN content, which lowered it to 63.5 mg kg^{-1}. Among the pelleting treatments, the repeated pelleting in a mix with corn yielded the lowest HCN content.

The temperature of the feed at the time of leaving the pellet mill outlet ranged from 30 to 56 °C. This value increased with repeated pelleting, and the highest temperature was recorded when flaxseed was pelleted in a mix with other ingredients.

Water soaking of whole flaxseed was not adequate, due to the slurry effect of the outer layer mucilage, which prevented complete drying of the sample. Hence, no HCN measurement was performed for the flaxseed after water soaking. The results obtained in the assessment of the processing methods are shown in Table 1.

DISCUSSION

Hydrogen cyanide content in flaxseed

Cyanogenic glycosides are glycosides of aldehyde or ketone cyanohydrin. Their presence serves for the plant's self-protection, as plants are static and animals are mobile.[8] When the plant tissue is damaged,

Table 1. Effects of processing on hydrogen cyanide content in flaxseed

Ingredient and processing	DM (%)	HCN[a] (mg kg^{-1})	Comparison with raw flaxseed		Pellet temperature[b] (°C)					
			Reduction	Pr > *t*	1st	2nd	3rd	4th	5th	6th
Flaxseed, raw	93.3	377.0								
Autoclaved	89.4	265.0	−29.7	0.0001						
Microwave	87.6	63.5	−83.2	0.0001						
Pelleted once		327.0	−13.3	0.0494	31.5					
Pelleted three times	91.7	267.5	−29.0	0.0001	29.0	31.7	33.9			
Pelleted six times	91.9	170.0	−54.9	0.0001	29.0	31.0	33.5	35.5	37.0	38.0
Pelleted once with 50% corn		292.3	−22.5	0.0001	34.0					
Pelleted three times with 50% corn		264.5	−29.8	0.0001	34.0	36.0	37.1			
Pelleted six times with 50% corn	90.0	136.5	−63.8	0.0001	33.7	36.0	37.0	38.1	39.5	40.3
Pelleted twice in a mixed diet with other ingredients		98.8	−73.8	0.0001	48.5	56.0				
Oven heated[c] A		316.0	−16.2							
Oven heated[c] B		291.0	−22.8							
Human grade		138.8		0.0001						
Canola		68.0		0.0001						
SEM		7.41								

[a] Values are least squares means of five measurements for each sample; HCN content of flaxseed in compounded feed had been converted back to 100% flaxseed basis.
[b] Pellet temperatures were taken when the pelleted feed was coming out of the outlet of the pellet machine. They were the average of three measurements of each pellet preparation following the order from first to the last pelleting process.
[c] Flaxseed was oven heated for 10 min and 20 min for A and B respectively at 130 °C. The values were the average of two measurements.

cyanogenic glycosides are hydrolysed and HCN as the hydrolysed product is released. The liberation of HCN from cyanogenic glycosides is an inhibitor of cytochrome oxidase, and consequently a cell respiration inhibitor. The minimum lethal dose to man is quoted to be between 0.5 and 3.5 mg kg^{-1} body weight.[9] Over 1000 species of plants are known to produce HCN,[9] and flaxseed obviously is one of them.

There are four forms of cyanogenic glycosides in flaxseed: linamarin, linustatin, lotaustralin and neolinustatin. Linustatin and neolinustatin are diglycosides, and linamarin and lotaustralin are monoglycosides.[10] Flaxseed contains a very low level of linamarin, but considerable amounts of the diglycosides linustatin and neolinustatin.[11] Young flax contains mainly the monoglycosides, linamarin, and lotaustralin, at levels as high as 90% of total cyanogenic glycosides. Older flax contains about 30% of the diglycosides linustatin and neolinustatin.[12]

The reported values of HCN in flaxseed vary greatly, due to cultivar variation, differences in detection methods, or as their expression in different compounds. Mandokhot and Singh[4] reported values of 470–490 mg kg^{-1}. Rosling[13] reported that the cyanide (CN^-) content varied from 4 to 12 mmol kg^{-1} (104 to 312 mg kg^{-1}). Bhatty[14] reported the average HCN content of seven western Canadian grown flaxseed cultivars being 79 to 98 µg kg^{-1} seed by using the (barbituric acid–pyridine reaction) colorimetric method. Chadha *et al*[15] detected 124–196 mg kg^{-1} of CN^- by using HPLC. These values are much less than HCN found in cassava, which is 2450 mg kg^{-1}.[16] The present study determined the HCN content of raw flaxseed at 377 mg kg^{-1}, which is in general agreement with reported values.

The effectiveness of processing in reducing the hydrogen cyanide content

Pelleting

After being processed, the level of HCN found in flaxseed depends on the enzymatic hydrolysis of the cyanogenic glycosides, or the activity of glycosidase. The hydrolysis reactants, cyanogenic glycosides as a substrate and the enzyme glycosidase are kept apart when the seed is intact.[9] The disruption of the seed tissue during ingestion by animals or processing makes this enzymatic reaction possible and HCN could be formed. Once formed, HCN must be released from the seed or flax products. HCN is volatile (boiling point 26 °C) and can readily diffuse through plant tissue.[17] It is known that, to guarantee the successful removal of HCN from cassava during processing, running water or steam must be provided. Obviously, this contaminated water and vapour could be toxic.[16] This knowledge on cassava detoxification can be applied to flaxseed processing by pelleting.

The present study tested repeated pelleting and pelleting together with other ingredients. It was expected that, by altering the condition of pelleting, the pellet temperature could be raised to maximize the formation of HCN. This assumption was formulated since steam was not available on the experimental pellet machine and, therefore, the real pellet temperature would be lower than those attained under commercial conditions. When steam is provided, as in commercial feed pelleting, repeated pelleting may not be necessary. Our results demonstrated that increased temperature was achieved by repeated pelleting and caused a further reduction of HCN.

Some researchers reported that heat processing of flaxseed did not change its HCN content.[11] This could be due to the fact that the HCN formed after cyanogenic glycoside hydrolysis remain in flax products, or the deactivation of glycosidase in plant tissue could be replaced by the glycosidase formed by gut microflora.[18–20] It is reasonable to state that effective flaxseed processing requires more than just cyanogenic glycoside hydrolysis or deactivation of glycosidase in tissue alone. That may also partly explain the ineffectiveness of dry heating in improving the feeding value of flaxseed for chicks.[21,22]

The high oil content in flaxseed and the low storing temperature (4 °C) of the flaxseed used in this experiment prior to pelleting may have contributed to the relatively low temperature, 30 to 56 °C, recorded immediately after pelleting. High oil content reduces the friction of the feed passing through the die. Such a condition would not happen under commercial conditions, as the lipid content in a compounded animal feed would normally not exceed 6%, and the temperature would mostly be between 65 and 80 °C, or higher.[23] If steam were provided for preconditioning, the beneficial effects of both increased temperature and moisture would promote glycosidase enzymatic reaction and perhaps result in a greater removal of the HCN formed.

Autoclaving

Autoclaving is one kind of heat processing under high pressure. The poisoning potential of flaxseed cyanogenic glycoside may be reduced as the result of inactivation of glycosidase by high temperature. This is supported by reported results of HCN reduction after autoclaving,[4,5] as well as by the results of the present study. Besides high temperature, autoclaving differs from dry heating because of the presence of vapour. Even though autoclaving for 30 min reduced the HCN level in linseed meal from 470–490 mg kg^{-1} to negligible amounts, water played a role in improving the feeding value of linseed meal, as has been reported previously.[4] Mandokhot and Singh[4] Deshmukh *et al*[5] observed that when linseed meal was soaked first and then autoclaved, which was defined as wet autoclaving, chicks performed better than on linseed meal processed by dry autoclaving. The benefits of vapour may be tissue moisturizing, which may encourage enzymatic reaction, or its HCN carrying capacity, as seen in pelleting. The present study showed that both autoclaving and dry oven heating reduced the HCN content of flaxseed, although autoclaving showed greater effectiveness. Our findings are in agreement with previously reported studies.[4] High pressure may be an additional benefit for autoclaving, namely the possible destruction not only of toxic chemicals, such as cyanogenic glycosides, but also other anti-nutritional factors present in flaxseed. Furthermore, other enzymes produced by the gut microflora could replace the process of hydrolysation catalysed by the enzyme glycosidase. This has been reported previously by several researchers.[18–20] We have observed that birds fed flaxseed autoclaved for a short period and at a low temperature (13.4 kg cm^{-2}, 110 °C for 7.5 min) showed no improvement in performance.[24] In fact, the response was slightly negative, whereas autoclaving for a longer period of time and at a higher temperature (16.5 kg cm^{-2}, 120 °C for 15 min) showed a positive response in performance. This seems to indicate that there is a destruction of the toxic chemical rather than only the deactivation of the glycosidase.

Microwave

Microwave processing is an interaction process between microwaves and the processed material. Microwaves lie between radio-wave frequencies and infrared frequencies. They are reflectable, absorbable and transmittable. The interaction between microwave and material generates heat that is used for material processing.[25] This heat generation is from within the processed material itself, induced by the 'cold' microwave, in contrast to the heat obtained from the outer layer as in conventional oven heating. It is fast and efficient, because of the high penetrating power,[26] and it has been used extensively in the material and food industry. It is worth using in food processing for the inactivation of some enzymes and anti-nutritional factors. There have been examples of polyphenolosidase polyphenoloxidase inactivation in mushroom,[27] inactivation of the enzyme lipase and lipoxygenase in cereal bran, germ and soybean,[28] inactivation of trypsin–chymotrypsin inhibitor of Blackgram[29] (*Phaseolus mungo* L), and reduction of the thermolabile antinutritive constituents in soybean, such as trypsin inhibitor and urease.[30] Similar effects could be achieved by flaxseed microwave roasting.

In the present study, microwave roasting achieved the highest level of HCN reduction in flaxseed among all the processing methods tested. The HCN was decreased from 377 mg kg^{-1} to 63.5 mg kg^{-1}, a reduction of 83.2%. This reduction could be the result of the heat deactivation of glycosidase, the evaporation of HCN after formation from hydrolysis, or both. The evaporation of HCN was supported by the 5.7% water loss in flaxseed after 4 min microwave roasting, which was higher than the 3.9% by autoclaving, and the 1.4% obtained after pelleting six times. One explanation for this water loss is the strong heat induction power of microwaves. Together with the rearrangement of charge groups induced by microwave–material interaction, heat induces great formation of HCN. The vaporization of this volatile HCN is then possible, which would result in the greatest reduction of HCN among all processing methods tested.

The application of microwave roasting in soybean revealed that this processing causes no major changes in the main nutrients and fatty acid profile. Yoshida *et al*[31] studied the tocopherol distribution and oxidative stability of oils prepared from the hypocotyl of soybean roasted in a microwave oven. The oil characters in hypocotyls changed slightly in carbonyl value, and anisidine value, with increased roasting time. Compared with the original levels, more than 80% of tocopherols remain after 20 min roasting. More recently, researchers[32] from the same laboratory reported that microwave heating (2450 MHz for 6, 12, or 20 min) significantly increased free fatty acids and 1,3- and 1,2-diacylglycerols. There were significant differences ($p < 0.05$) in fatty acid distributions when soybeans were treated for 12 min or more in a microwave. The influence of microwave processing on nutrient composition other than HCN removal merits more research.

The effect of various processing methods on flaxseed protein quality associated to the likelihood of Maillard reactions and subsequent reduction of lysine availability was not evaluated. However, it is possible to predict a greater heat-damaging effect as temperature and exposure time of the treatment increases.[33]

CONCLUSIONS

The present study demonstrates that a series of flaxseed processing methods reduce the HCN content in flaxseed. The effectiveness of the degree of HCN reduction varies among the methods. Microwave processing and the pelleting with other ingredients produced the greatest reductions, whereas dry oven heating is less effective. The result of reduced HCN content could be the result of glycosidase inactivation, the vaporization of HCN after it is formed from cyanogenic glycosides hydrolysis, or the destruction of the cyanogenic glycoside compounds. Pelleting would be of the greatest potential in commercial production of feed containing flaxseed.

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