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Effect of Combination of Physical Pretreatment on Toxic Factors and Nutritional Value of Canola Meal

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ABSTRACT

The aim of this study was to determine the effect of combination of soaking and heat treatment on nutritional characteristics of canola meal and certain anti nutritional factors. Canola meal was soaked in water for 12 and 24 hours (h) followed by heating at 80°C and 100°C for 30 min and 60 min respectively. Crude protein and fat content of canola meal increased significantly ($P<0.05$) with soaking for 24 h followed by heating at 100°C. Crude fiber content reduced with increasing soaking time and heat treatment. Glucosinolate and erucic acid content reduced significantly ($P<0.05$) with soaking at 24 h followed by heat treatment for 60 min irrespective of the temperature. Results of this study indicate that combination of high temperature heating (100 °C for 60 min) and soaking (24 h) may be necessary to reduce the toxic compounds and improve nutritional characteristics of canola meal.

KEYWORDS: Anti Nutritional Factors, Canola meal, Erucic acid, Glucosinolate, Soaking

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INTRODUCTION

The scarcity generated due to competition for feed ingredients between human and animals and the elevated prices of protein rich ingredients for animal feed emphasize the need for comprehensive studies on all potential protein sources to support efficient livestock production (Ghazalah et al., 2022). Moreover, the declining profitability of the livestock/ poultry industry, driven by rising costs of key raw feedstuffs, has compelled nutritionists to seek more affordable cheaper alternative protein sources. This paves way towards utilization of certain unconventional feeds in animal feeding. However, the efficient use of relatively low-cost unconventional feed ingredients depends on the chemical and physical properties for sustainable animal production (Sinha et al., 2020).

Canola, belonging to the family of Brassica, is a registered name for a type of rapeseed which is highly valued as a component of animal feed (Woyengo et al., 2014). Canola is generally used to prepare the canola oil, and the canola meal is produced as a byproduct of rapeseed during oil extraction process. The presence of high protein content and a well balanced composition of desirable amino acid are favoring canola meal to be a good animal feed. The distribution of amino acid in canola meal is similar to that present in soyabean meal. Canola meal is a rich source of various vitamins and minerals, particularly sulfur, selenium, phosphorus, and B vitamins. It contains less than 2% erucic acid in the total fatty acids of its oil and less than 30 µmoles of glucosinolates per gram (Ravichandran et al., 2008). But, the presence of some anti-nutrients in canola meal limits its potential as a protein supplement. The main antinutritional factors in canola meal are dietary fibre, glucosinolates, phytic acid, and phenolic compounds. But, it is mostly the presence of glucosinolate in canola meal which marks the limitation for its usage in animal feeding. Also, the high fiber content of canola meal restricts its quality and interferes with the digestibility of meal in non ruminant diet.

Glucosinolates are plant secondary metabolites, classified into aliphatic and aromatic glucosinolates, depending on involvement of amino acids for synthesis. Glucosinolates in general play a role in plant defense against insects and diseases; and has anti cancer properties (Sønderby et al., 2010). Glucosinolates themselves are non toxic; however their degradation products are toxic to animal body

(Sharma and Nagra, 2009). High glucosinolate levels and their metabolites, produced through enzymatic breakdown, can have harmful or toxic effects (Cartea et al., 2008).

To minimize the harmful effects on animals and improve incorporation rates in their feed, various processing techniques can be employed. Physical, chemical, and microbiological methods can be used to reduce the glucosinolate (Gls) content. The aim of the present study was to identify combination of various physical techniques which could be beneficial in minimizing the deleterious components present in canola meal and enhancing its nutritional characteristics.

MATERIALS AND METHODS

The GSC07 canola cultivar variety samples were procured from farmer's demonstration unit. The canola meal was obtained after oil extraction by double pressed expeller processing methods. The canola meal samples (10 g) were soaked in water (ratio of meal: water 1:10, w/v) at room temperature (24 °C) for 12 and 24 hours, and then drained by using a vacuum filter. The samples were heated in oven at 80 and 100°C for 30 min and 1 hour based on the treatment (Table1). Each treatment was conducted in 3 replicates.

Table 1. Details of physical treatment of canola meal

Treatment	Soaking time (hrs)	Heating time (min)	Heating temp (^{0}C)
1	12	30	80
2	12	30	100
3	12	60	80
4	12	60	100
5	24	60	80
6	24	60	100
7	24	30	80
8	24	30	100
9	Control	Control	Control

Chemical composition

Proximate analysis: Following different treatments each canola meal sample was analyzed for dry matter (DM), organic matter (OM),ash, crude protein (CP), crude fiber (CF) and ether extract (EE) as per AOAC (1995) procedures.

Estimation of Glucosinolate: Spectrophotometric estimation of glucosinolate was done using methanolic extract prepared by homogenizing 0.1 g defatted seed meal in a 2 ml vial with 80% methanol and centrifuged at 3000 rpm for 4 min. The supernatant collected was incubated at room temperature for 1 hour, absorbance was measured at 425 nm using a spectrophotometer. Total glucosinolates was calculated (Mawlong et al., 2017) by putting OD of each sample taken at 425 nm into the predicted formula:

$$y = 1.40 + 118.86 \times A_{425}$$

Determination of fatty acid methyl ester (erucic acid) was performed with a GC system (Russo et al., 2021) CHROMPACK CP3800, with auto sampler 8200 CX and flame ionization detector. Helium was used as a carrier gas with a flow rate of 1 ml/min. The split ratio was set to 1:20 and injector temperature was 280°C.

STATISTICAL ANALYSIS

The data were analyzed by using one way ANOVA with Tukey's post hoc testing to compare experimental groups test using SPSS (2010) computer package. For all statistical analyses, where the probability values were less than 0.05, were considered as significant.

RESULT AND DISCUSSION

Effect on Chemical composition

Combination of soaking and heat treatment did not result in significant (P>0.05) increase in the moisture content of canola meal, with dry matter content ranging from 91.7% to 95.0% (Table 2). The meal generally have an ability to absorb and retain water and oil helping to improve binding of the structure, enhance flavor retention and reduce moisture and fat losses of food products (Sreerama et al., 2008). However, soaking and heating of canola meal alone also showed no significant change in the dry matter content when heated at 127°C for 15 min in a preheated oven (Aleid et al., 2025). The findings are consistent with those found in heat treated canola meal at 90, 110, 120 and 150° C by Piotr et al., 2020.

Heat treatment is one of the most common methods to reduce ruminal protein degradation and increase post ruminal availability (Sniffen et al., 1992). Water soaking of feedstuffs is beneficial in reducing the antinutritive factors but may also result in loss of soluble protein content (Widharna et al.,2012).Combination of physical treatment significantly improved the protein percentage of canola meal as compared to the untreated group. The author reported an increase in protein percentage by 25% when canola meal was soaked in water for 24 hours over control. This rise in protein percentage when canola meal was soaked in water for an extended period (24 hrs), might be due to the leaching of non-nitrogenous soluble materials into the water (Widharna, 2012). Additionally, heating at higher temperatures (100°C) led to an increase in protein levels, likely due to enhanced synthesis of protease enzymes (Bau et al., 1997). According to Burakowska et al. 2019, degradable fractions of crude protein in CM decreased linearly with increasing temperature of heat treatment which was much higher (110°C) than that used in present experiment. Similarly, protein solubility decreased linearly from 85% to 81%, 61%, 52% and 40% after the toasting (100°C) time increased from 30 min to 60 min (Jensen et al., 1995). The combination of pretreatment in the current study also significantly reduced glucosinolate levels, which may have ensued in enhanced protein levels.

Table 2. Effect of physical pretreatment on proximate composition (%) of canola meal

Treatment	Dry Matter	Protein	Ether Extract	Crude Fiber	Ash
1	94.3±0.33	38.21^{b}± 0.15	11.57^{c}±0.04	11.00^{b}±0.21	5.94±0.18
2	95.0±0.88	39.54^{cde}±0.29	10.63^{b}±0.08	12.60^{c}±0.18	6.12±0.13
3	93.5±0.29	40.2^{de} ±0.15	8.77^{a} ±0.10	11.67^{b}±0.44	6.12±0.07
4	94.0±1.53	39.3^{cd}±0.14	10.20^{b}±0.15	11.77^{b}±0.15	6.29±0.12
5	93.0±0.58	39.3^{cd}±0.15	12.77^{d}±0.15	12.17^{b}±0.60	6.13±0.14
6	94.0±0.58	40.3^{de}±0.15	11.87^{c}±0.09	11.00^{b}±0.06	6.11±0.07
7	93.2±0.60	39.1^{bc}±0.28	13.73^{e}±0.12	12.53^{c}±0.38	5.77±0.13
8	93.3±0.44	40.3^{de}±0.33	13.96^{e}±0.03	11.56^{b}±0.08	6.30±0.36
Control	91.7±0.33	32.0^{a}±0.29	10.40^{b}±0.08	13.10^{d}±0.59	6.52±0.29

Values with different superscript varied significantly (p< 0.05)

Ether extract percentage in untreated and treated canola meal ranged from 8.77 to 13.96%. Significant change (p<0.05) in ether extract was recorded with different soaking time and heat temperature. Increased oil content was recorded in canola meal soaked in water for 24 hours indicating that increased soaking time might promote hydrolysis of fatty acids (Mohamadzadeh et al., 2009). However, maximum

level of fat percentage was recorded when combination of soaking (24 hours) and heat (100°C) pretreatment was applied suggesting increased soaking time and heat, raised the levels of free fatty acids (Mohamadzadeh et al., 2009). The increase in levels may also be associated with heat dissociation of the proteins and denaturation by heat pretreatment, unmasking the non-polar residue from the interior of the protein molecules and enhancing fat absorption (Kinsella, 1976). Contrary, Piotr et al., 2020 reported slightly less fat content for heat treated canola meal owing to higher heating temperatures (150°C).

The effect of different pre treatment on the crude fiber levels compared to control sample is shown in Table 1. The CF content ranged from 13.10 to 11.00 % in untreated and treated canola meal respectively recording significant reduction in CF levels. Both soaking and heat treatment had significant impact on fiber content of canola meal as significantly low fiber levels were recorded in canola meal exposed to 12 h and 24 h of soaking alongwith heating temperature of 100°C.

It has been reported that commercial rapeseed meal contains 12.1% crude fiber, most of which is derived from hulls. Mohamadzadeh et al., 2009 recorded that soaking rapeseed for 100 min followed by hot air drying reduced the crude fiber content significantly correlating the high correlation efficiency of the treatment applied. Also, application of heat has long been deployed in animal feed industry to remove anti-nutritional factors and improve nutrient availability and palatability of animal feed (Allan and Booth 2004).

Physical pretreatment did not alter the ash content in canola meal samples, with ash content ranging from 5.77% to 6.52%. These values are consistent with those found in other varieties of canola meal. Since ash analysis indirectly reflects the mineral content in feed, the lack of significant change suggests that mineral content is not impacted by either moisture or heat treatment in canola meal. This supports the results of Widharna et al. (2012), who reported that pretreatment with heat and moisture did not alter the ash content.

Effect on glucosinolates and erucic acid

The nutritionally undesirable glucosinolates followed by erucic acid in seed meal of rapeseed–mustard cultivars prevalent in India are very high and range between 43 to 57% and 150–240 µmol g-1, respectively (NBPGR, 2003).The data presented in Table 3 indicates that in present study, glucosinolate content of untreated canola variety sample was 77 µmol/g, while glucosinolate content of treated samples varied from 58.04 to 64.95 µmol/g, demonstrating 24% reduction in glucosinolate levels following soaking and heating treatments. Furthermore, significant differences were observed among the treatments, with shorter soaking times resulting in greater glucosinolate reduction (24%) compared to longer soaking times. This finding aligns with Widharna et al. (2012), who recommended shorter soaking times to effectively reduce glucosinolate levels. Tripathi and Mishra (2007) reported heating of canola meal from 10 to 60 min at 100^0 C did not reduce the glucosinolate content significantly whereas heating in combination of moisture reduced glucosinolate content by 18.8%.

Table 3. Glucosinolates and erucic acid of canola meal

Treatment	1	2	3	4	5	6	7	8	Control
Glucosinolate (µmol g−1)	61.11^{b}± 0.20	61.42^{b} ±0.47	58.59^{a}±0 .30	58.04^{a}±0 .09	58.53^{a}±0 .29	60.53^{b}± 0.26	63.30^{c}±0 .44	64.95^{d}± 0.07	77.00^{e}±0 .56
Erucic acid (%)	24.76^{ab}± 0.43	25.91^{b}± 0.39	23.62^{a}±0 .19	23.15^{a}±0 .60	28.20^{c}±0 .44	24.08^{ab}± 0.87	23.41a± 0.36	22.57^{a}±0 .58	33.00^{d}± 0.63

Values with different superscripts varied significantly ($p< 0.05$)

Soaking canola meal in water facilitates the leaching of toxic substances, including glucosinolates, followed by cell lysis and diffusion (Baenas et al., 2020). The glucosinolate levels remained lower and stable when heating was conducted for 1 hour, regardless of temperature. This is consistent with the findings of Nugrahedi (2015), who reported that prolonged processing time increases cell lysis and thermal degradation, leading to greater glucosinolate losses. However, Jensen et al. (1995) noted that heating beyond 60 minutes can reduce both the quantity as well as quality of protein in addition to decreasing glucosinolate content. The decrease in glucosinolates due to thermal and enzymatic degradation has been documented in different studies (Wennberg et al., 2006).

The erucic acid content in both treated and untreated canola meal samples ranged from 22.57% to 33%. A significant decline in erucic acid levels was observed in the treated groups compared to the untreated samples. Additionally, notable differences were recorded among the various treatment groups. Treatments with lower glucosinolate content tended to have lower erucic acid levels, although in some cases, lower erucic acid levels were accompanied by higher glucosinolate content. Zhang et al. (2020) found that erucic acid content is positively correlated with the degradation products of glucosinolates.

Prolonged heating increases the rate of fatty acid auto-oxidation, leading to a decline in erucic acid content (Alireza, 2010). Dawodu et al. (2015) reported a 2–3% increase in free fatty acids when temperatures reached 250°C, which strongly supports the findings of this study. High temperatures also enhance the solubility of targeted phenolic compounds in canola meal (Li and Guo, 2016). Khajali and Slominski. (2012), also reported that higher erucic acid consumption is associated with the myocardial lesions in laboratory animals.

CONCLUSION

The results presented here indicated that glucosinolate content of canola meal was decreased as the soaking time and heating increased. Following the glucosinolate content of canola meal, protein and crude fiber levels were altered by combination of physical treatment. These finding and observed change in anti-nutritive factors propose that, a combination of soaking and heating effect had potential for improving nutritional value of canola meal.

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