

Chemical characterization of fermented rapeseed meal produced using *Saccharomyces cerevisiae* and *Bacillus subtilis*

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Abstract

Fermented rapeseed meal (FRSM) is a protein-rich feed ingredient whose chemical composition is influenced by the characteristics of the raw material and fermentation process. The present study characterized the proximate composition, mineral profile, total glucosinolate content and total phenolic content of FRSM produced using *Saccharomyces cerevisiae* and *Bacillus subtilis*. The FRSM sample contained 90.54% dry matter, 38.40% crude protein, 1.20% ether extract, 6.50% crude fibre, 12.11% total ash and 41.79% nitrogen-free extract. Calcium and phosphorus contents were 0.91 and 0.93%, respectively. The total glucosinolate concentration was 16.00 $\mu\text{mol/g}$, while total phenolic content was 6.67 mg tannic acid equivalent/g on a dry matter basis. The crude protein and fibre concentrations were within the ranges reported for fermented rapeseed meal. The chemical profile indicates that FRSM provides a substantial protein fraction together with measurable glucosinolates and phenolic compounds, which are relevant when assessing its suitability as an alternative protein source in poultry nutrition. The findings provide chemical characterization of the FRSM used in the associated feeding study and establish its nutritional and selected anti-nutritional profile.

Keywords: Fermented rapeseed meal, *Saccharomyces cerevisiae*, *Bacillus subtilis*, glucosinolates, total phenolic content, proximate composition, mineral composition

Introduction

Rapeseed meal is an important protein-containing by-product of the oil extraction industry and has potential for use as an alternative protein source in livestock and poultry diets. Its utilization in poultry nutrition is influenced by its protein concentration, fibre content and the presence of anti-nutritional compounds, particularly glucosinolates. Glucosinolates and their hydrolysis products have long been considered important factors limiting the use of rapeseed products in animal nutrition (Tripathi and Mishra, 2007) [5]. The chemical composition of rapeseed-derived feed materials can vary considerably with rapeseed variety, cultivation conditions, processing and subsequent treatment. Such variation is also evident among fermented rapeseed meals, with differences reported in crude protein, fibre, ash and glucosinolate concentrations.

Fermentation has been investigated as an approach for modifying the nutritional characteristics of rapeseed meal. Microbial fermentation can alter the substrate through microbial growth and enzymatic activity and has been associated with changes in fibre and glucosinolate concentrations (Shi *et al.*, 2015) [4]. Fermented rapeseed meal has subsequently been evaluated as a protein source in poultry diets (Xu *et al.*, 2012; Wu *et al.*, 2021).

The chemical characterization of the final fermented product is important because crude protein concentration alone does not adequately describe its feeding value. Glucosinolates remain particularly relevant because their degradation products can exert adverse physiological effects in animals when present at high concentrations (Tripathi and Mishra, 2007) [5]. Phenolic compounds are also naturally present in rapeseed-derived materials and may contribute to their chemical and biological properties.

Therefore, the present study was undertaken to characterize the chemical composition of fermented rapeseed meal

produced using *S. cerevisiae* and *B. subtilis*. Proximate constituents, calcium, phosphorus, total glucosinolates and total phenolic content were determined to provide a chemical profile of the fermented feed ingredient.

Materials and Methods

1. Fermented rapeseed meal

The fermented rapeseed meal (FRSM) used in the study was obtained from M/S Abhay Cotex Private Limited, Jalna, Maharashtra, India. The product had been fermented using *Saccharomyces cerevisiae* and *Bacillus subtilis*. The FRSM was subsequently used as a feed ingredient in experimental work conducted at the College of Veterinary Science, Tirupati.

2. Proximate and mineral analysis

The proximate composition of FRSM was determined according to the procedures of AOAC (2007). Dry matter, organic matter, crude protein, ether extract, crude fibre, total ash, acid insoluble ash and nitrogen-free extract were determined. Calcium and phosphorus were analysed according to AOAC (2007).

3. Estimation of total glucosinolates

Total glucosinolates were estimated according to the method of McGhee *et al.* (1965). A 10 g sample of ground FRSM was immersed in 250 mL of boiling water for 5 min to deactivate enzymes and prevent glucosinolate degradation. The sample was filtered and the residue was washed three times with hot water. The combined filtrate was adjusted to 500 mL.

An aliquot of the extract was treated with silver nitrate and ethanol and refluxed for 45 min. After cooling, dilution and filtration, the solution was treated with nitric acid and ferric ammonium sulfate and titrated against 0.01 N potassium

thiocyanate. A blank determination was conducted with each analysis. Total glucosinolates were expressed as $\mu\text{mol/g}$.

4. Estimation of total phenolic content

Total phenolic content (TPC) was estimated using a modified Folin–Ciocalteu method based on Hossain and Shah (2015) [2]. Approximately 200 mg of sample was initially extracted with 10 mL diethyl ether containing 1% acetic acid. Following centrifugation, the remaining material was extracted with 70% aqueous acetone and incubated at 30°C for 2 h with shaking.

Tannic acid was used for preparation of the standard curve. The extract was reacted with Folin–Ciocalteu reagent and sodium carbonate and incubated at room temperature for 40

min. Absorbance was measured at 650 nm. Total phenolic content was calculated from the calibration curve and expressed as mg tannic acid equivalent (TAE)/g on a dry matter basis (Hossain and Shah, 2015) [2].

Results and Discussion

1. Proximate and mineral composition

The chemical composition of the FRSM is presented in Table 1. The sample contained 90.54% dry matter and 87.89% organic matter. Crude protein was 38.40%, while ether extract and crude fibre were 1.20 and 6.50%, respectively. Total ash was 12.11%, acid insoluble ash was 0.55% and nitrogen-free extract was 41.79%. Calcium and phosphorus concentrations were 0.91 and 0.93%, respectively.

Table 1: Chemical composition of fermented rapeseed meal

Parameter	FRSM
Dry matter (%)	90.54
Organic matter (%)	87.89
Crude protein (%)	38.40
Ether extract (%)	1.20
Crude fibre (%)	6.50
Total ash (%)	12.11
Acid insoluble ash (%)	0.55
Nitrogen-free extract (%)	41.79
Calcium (%)	0.91
Phosphorus (%)	0.93
Total glucosinolates ($\mu\text{mol/g}$)	16.00
Total phenolic content (mg TAE/g)	6.67

The crude protein concentration of 38.40% indicates that the FRSM provided a substantial protein fraction. This value was comparable with the 39.60% crude protein reported by Xu *et al.* (2012), 35.81% reported by Shi *et al.* (2015) [4] and 40.90% reported by Wu *et al.* (2021). Differences in the chemical composition of fermented rapeseed meal may arise from variation in rapeseed variety, growing conditions, processing and fermentation procedures.

The crude fibre concentration of 6.50% was toward the lower end of the range reported for FRSM. The literature compilation in the thesis showed crude fibre values ranging from 6.11 to 16.72%, with a mean of 10.15%. The relatively low fibre concentration observed in the present sample may be favourable from a poultry nutrition perspective because high fibre concentrations can limit the utilization of rapeseed-derived ingredients.

The calcium and phosphorus concentrations were 0.91 and 0.93%, respectively. These values were within the ranges reported for fermented rapeseed meal, with calcium reported from 0.61 to 0.91% and phosphorus from 0.34 to 1.19% in the literature compilation.

2. Total glucosinolate content

The total glucosinolate concentration of the FRSM was 16.00 $\mu\text{mol/g}$. Glucosinolates are characteristic secondary metabolites of rapeseed and represent an important chemical factor in the evaluation of rapeseed-derived feed ingredients (Tripathi and Mishra, 2007) [5].

The reported glucosinolate concentration in the present FRSM was near the upper end of the range reported for fermented rapeseed meal. Values of 0.3252–17.09 $\mu\text{mol/g}$, with a mean of 7.53 $\mu\text{mol/g}$, were compiled from previous studies. The present value therefore demonstrates that

measurable glucosinolates remained in the fermented product.

Fermentation can influence glucosinolate concentrations through microbial and enzymatic activity, although the magnitude of change depends on the substrate and fermentation conditions (Shi *et al.*, 2015) [4]. Consequently, the glucosinolate concentration of the final fermented material should be determined analytically rather than assumed from the use of a particular microbial culture.

The presence of glucosinolates is relevant when determining the inclusion level of FRSM in poultry diets because their hydrolysis products have been associated with effects on thyroid function, feed utilization and animal performance at excessive exposure (Tripathi and Mishra, 2007) [5].

3. Total phenolic content

The total phenolic content of the FRSM was 6.67 mg TAE/g dry matter. The Folin–Ciocalteu method provides an estimate of total reducing phenolic compounds expressed relative to the selected standard rather than quantifying individual phenolic compounds.

The value observed in FRSM was higher than the corresponding values recorded for maize and soybean meal analysed alongside the sample, which were 2.65 and 3.40 mg TAE/g, respectively. The FRSM value was 6.67 mg TAE/g.

Phenolic compounds form part of the naturally occurring chemical constituents of rapeseed-derived materials. Their concentration can be affected by processing and fermentation, and therefore determination of total phenolic content provides additional chemical information about the fermented ingredient.

4. Comparison with reported fermented rapeseed meal composition

The chemical composition observed in the present study was generally within the ranges reported for FRSM in previous investigations. The crude protein concentration of

38.40% was close to the reported mean of 39.42%, while crude fibre was lower than the reported mean of 10.15%. The calcium concentration of 0.91% was at the upper end of the reported range, whereas phosphorus at 0.93% was within the reported range.

Table 2: Comparison of selected chemical characteristics of the present FRSM with reported ranges

Parameter	Present FRSM	Reported range
Dry matter (%)	90.54	87.94–94.57
Crude protein (%)	38.40	34.00–44.63
Ether extract (%)	1.20	1.21–9.29
Crude fibre (%)	6.50	6.11–16.72
Total ash (%)	12.11	6.30–13.11
Glucosinolates (μmol/g)	16.00	0.3252–17.09
Calcium (%)	0.91	0.61–0.91
Phosphorus (%)	0.93	0.34–1.19

The differences between the present values and previously reported values highlight the chemical variability of fermented rapeseed meal. Such variability may arise from differences in the original rapeseed material, processing conditions and fermentation protocols. Therefore, chemical characterization of each fermented product remains important before its nutritional value is assessed.

Conclusion

The fermented rapeseed meal produced using *Saccharomyces cerevisiae* and *Bacillus subtilis* contained 38.40% crude protein, 6.50% crude fibre, 0.91% calcium and 0.93% phosphorus. The total glucosinolate concentration was 16.00 μmol/g, while total phenolic content was 6.67 mg TAE/g dry matter. The protein and fibre concentrations were within the ranges reported for fermented rapeseed meal, whereas the glucosinolate concentration was toward the upper end of the reported range. The results demonstrate that fermentation does not eliminate the need for chemical characterization of the final product. The measured proximate, mineral, glucosinolate and phenolic profile provides useful baseline information for evaluating this FRSM as a feed ingredient for poultry.

References

1. AOAC. Official Methods of Analysis of AOAC International. Association of Official Analytical Chemists, 2007:18th Edition.
2. Hossain MA, Shah MD. A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant *Murraya koenigii*. Arabian Journal of Chemistry, 2015;8(1):66–71.
3. McGhee JE, Kirk LD, Mustakas GC, Griffin EL. A rapid method for determination of glucosinolates in rapeseed. Canadian Journal of Plant Science, 1965;45(6):607–612.
4. Shi C, He J, Yu J, Yu B, Huang Z, Mao X, Zheng P, Chen D. Solid state fermentation of rapeseed cake with *Aspergillus niger* for degrading glucosinolates and upgrading nutritional value. Journal of Animal Science and Biotechnology, 2015;6(1):13.
5. Tripathi MK, Mishra AS. Glucosinolates in animal nutrition: A review. Animal Feed Science and Technology, 2007;132(1-2):1–27.
6. Wu Z, Chen J, Ahmed Pirzade S, Haile TH, Cai H, Liu G. The effect of fermented and raw rapeseed meal on

the growth performance, immune status and intestinal morphology of broiler chickens. Journal of Animal Physiology and Animal Nutrition, 2021;106(2):296–307.

7. Xu FZ, Zeng XG, Ding XL. Effects of replacing soybean meal with fermented rapeseed meal on performance, serum biochemical variables and intestinal morphology of broilers. Asian-Australasian Journal of Animal Sciences, 2012;25(12):1734–1741.