Chemical Evaluation of Defatted *Vernonia galamensis* Meal

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The defatted meal of *Vernonia galamensis* is rich in crude protein (43.76 ± 0.17%) with methionine as the first limiting amino acid and lysine the second when calculated based on the FAO Provisional Scoring Pattern. The carbohydrate fraction (6.57%) is rich in sucrose (2.36%), fructose (1.90%) and glucose (0.77%). Levels of the macrominerals, calcium (11.08 mg/g), potassium (14.18 mg/g) and magnesium (6.90 mg/g) not only meet nutritional requirements but are also higher than in contemporary oilseeds. The level of phosphorus is high (644 mg/g), perhaps accounting for the fairly high phytate (25.42 ± 0.06 mg/g) content, although this value is slightly less than for American Association of Cereal Chemists (AACC) standard wheat bran (31.42 ± 0.03 mg/g).

Residual oil content of defatted meal was found to be approximately 0.5%, but could be higher depending on the method of extraction. Lipid analysis of the residual oils shows vernolic acid (76.06 ± 1.80%) as the major component of the saponifiables (97.74 ± 0.20%). C18:2 (11.64 ± 0.69%), C16:0 (2.22 ± 0.12%), C18:0 (2.63 ± 0.11%), C18:1 (6.58 ± 0.19%), and C20:0 (trace) were all identified.

Sterols constitute the major (94.65 ± 0.08%) component of the unsaponifiables (2.06 ± 0.16%) with β-sitosterol (32%) and Δ^5-avenasterol (30%) being the major constituents. Cholesterol was fairly low (4.6%). Phytochemical screening for other possible toxicants did not reveal significant levels of antinutritional components.

In addition to the agronomic potential of *Vernonia galamensis* (1), several articles (2-7) have discussed the potential of *V. galamensis* seed oil as a rich source of epoxy acid for use in manufacturing plastic formulations, protective coatings and numerous other products. It has been suggested that a natural epoxy oil source such as *V. galamensis* could make a significant contribution toward supplying the 45-68 million kg of epoxy oils used annually in the United States (2). While most of the efforts have focused primarily on the direct application of the oil, *V. galamensis* oil recently has been shown by Ayorinde and his co-workers to be a good source of dibasic acids which are important precursor acids used in many industrial applications (8,9). The high level of interest in the oil (38-42% of the seed) centers on its important vernolic (*cis*-12,13-epoxyoctadec-*cis*-9-enoic) acid content (72-80%).

A by-product of the oil extraction is the defatted meal which, if not adequately handled, could pose an environmental problem in the future. The present preliminary paper reports the evaluation of defatted *V. galamensis* meal to identify its chemical properties as a preliminary step to its possible utilization in the animal feed industry.

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MATERIALS AND METHODS

The defatted meal was from the 1987 crop grown in Chirezi, Zimbabwe. The meal was ground to a fine powder and the residual oil extracted with chloroform-methanol as the solvent. The oil fraction was placed under a laboratory hood to allow evaporation of the solvent and then was gravimetrically determined. The meal was also transferred to the hood and allowed to dry for 24 hr for subsequent chemical analysis.

Meal analysis. The meal was analyzed for moisture, ash and crude protein by standard methods recommended by AOAC (1980) (10). Carbohydrate was calculated by difference based on the total seed composition.

Mineral Analysis. The mineral content (except phosphorus) was determined by the standard AOAC (1980) wet ashing method. Analysis and quantification of the minerals were performed with an Atomic Absorption Spectrophotometer (Perkin-Elmer Model 5000) (11). Phosphorus was determined by the colorimetric method described by Allen (12), with some slight modification.

Phytate and tannin analysis. The phytate level was determined by the method described by Harland and Oberleas (13). Tannin was estimated qualitatively.

Sugar analysis. Defatted meal (ca 10 g) was homogenized in hot 80% ethanol and refluxed for two hr. The homogenate was cooled, filtered and dried under a stream of nitrogen in a 70°C water bath. The filtrate was diluted to 5 ml with double distilled deionized water and analyzed by the gas chromatography method described by Iverson and Bueno (14).

Preparation and analysis of protein hydrolyzates for amino acid determination. Protein hydrolyzates were prepared by the method described by Stein and Moore (15). The amino acid composition of the protein hydrolyzates was analyzed with a Pico-Tag high-pressure liquid chromatograph (Waters Associates, Milford, MA) equipped with a reverse phase C 18 analytical column (15 × 0.4 i.d.) with the column temperature set at 38°C. The detector (Model 440 uv) was set at 254 nm. Mobile phase: Eluant A: 60 ml acetonitrile and 940 ml of 0.14M sodium acetate, pH of 6.40, containing 0.05% triethylamine; Eluant B: 60% acetonitrile and 40% water by volume. The Waters 840 Data and Chromatography Control Station was used for data collection.

Preparation and analysis of fatty acid methyl esters and sterol butyrates of residual oil. Fatty acid methyl esters and sterol butyrate derivatives were prepared as described by Sheppard *et al.* (16). In both cases, analyses were performed by gas chromatography: Perkin-Elmer Sigma 3B, with 6 in × 4 mm i.d. pyrex column packed with 1% SE-30 on 100/120 mesh Gas-Chro Q. (Applied Sciences Laboratory, State College, PA). Operating conditions for fatty acids: column temperature programmed for 180°C for 30 min, 3°/min for 10 min, and subsequently maintained at 210° for 26 min. Additional conditions existed: injector and detector temperatures—300°C, hydrogen flow rate—20 ml/min, air flow rate—30 ml/min, and inlet pressure—18 psig. Operating conditions for sterol analysis were identical to the fatty acid analysis except that the

column was set and maintained at 250°C as opposed to being varied. Minor flow rate adjustments were necessary to elute cholesterol in ca 15 min. Identification was performed by comparing relative retention times (min) of sample with authentic standards. Further identification was by examination of fragmentation patterns from gas chromatography-mass spectrometry analysis as described by Brumley *et al.* (17).

The method described by Sheppard *et al.* (16) was used to quantify the fatty acids. Quantification of the sterols was done by preparing standard curves for each identified peak. The process involves injecting (in duplicate) three different amounts of the working standard into the GC. Average response in area is graphically plotted against the amount for each quantity of standard injected. Response plot of the standard must bracket the peak area of sterol found in the samples. The concentration of unknown is calculated from the linear response plot.

RESULTS AND DISCUSSION

V. galamensis is an oil (39%) and protein (44%) seed (Table 1). The low value for carbohydrate (lower than the nutritional requirements for a balanced ration) would require supplementation for the meal to be considered dietarily satisfactory. The free monosaccharides in the meal (Table 2) amount to 6.6%, which is only an approximation because minor constituents such as saponins and phospholipids may also be in this fraction. The levels of the minerals (Table 3) detected in defatted *V. galamensis* meal are high, and in some cases, higher than values for comparable oilseeds (18). With the exception of the carbohydrate content, the proximate and mineral profiles of the meal compare favorably with data published for seed meals used in the animal feed industry (19).

TABLE 1

Defatted Meal Analysis[a]

	%
Moisture	5.5
Crude protein	43.4[b]
Carbohydrate	6.6[c]
Ash	5.3
Residual oil	0.8[d]

[a]Mean value of duplicate determinations.
[b]N × 6.25.
[c]Calculated by difference.
[d]What remained following 24 hr extraction of crude fat.

TABLE 2

Sugar Content of Defatted Vernonia Meal[a]

	%
Glucose	0.77
Fructose	1.90
Sucrose	2.36
2 Unidentified peaks	1.57

[a]Reported as a percent of the meal (using the value 6.6%).

TABLE 3

Mineral Content of Defatted Vernonia Meal[a]

	mg/g
Calcium	11.08 ±0.63
Potassium	14.18 ± 0.35
Magnesium	6.90 ± 0.33
Iron	0.20 ± —
Copper	Trace[b]
Manganese	0.27 ± —
Zinc	Trace
Sodium	0.36 ± —
Phosphorus	644.04 ± 0.21

[a]Mean value of 4 determinations ± SD.
[b]Trace = less than 0.10 mg/g.

TABLE 4

Fatty Acid Composition of Residual Oil from Defatted Vernonia Meal[a]

	%
C12:0	Trace[b]
C14:0	Trace
C14:1	Trace
C16:0	2:20 ± 0.12
C16:1	Trace
C17:0	Trace
C18:0	2.63 ± 0.11
C18:1	6.58 ± 0.19
C18:2	11.64 ± 0.69
C18:3	0.44 ± —
C20:0	Trace
$C_{30}H_{50}$	0.16 ± —
Vernolic acid	82.51 ± 0.70

[a]Mean value of 4 determinations ± SD.
[b]Trace = less than 0.10%.

Residual oil left in the meal interfered with the sugar analysis. Because none of the extraction processes used to remove the oil from the seed will be 100% efficient, it is important to characterize the residual oil with respect to its nutritive quality. Lipid analysis (Table 4) of the residual oil presented the same fatty acid profile as previously reported values (2). Vernolic acid remained as the dominant component. The total concentration of the essential fatty acids (12%) is lower than what van Niekerk and Burger (20) found in edible oils like soybean (60%) and peanut (36%) oils. This would suggest the need for supplementation with an external, more nutritious oil source to balance the essential fatty acids in the meal. The sterols are reported in Table 5. Cholesterol appeared as a small constituent (64 mg/100 g oil). β-sitosterol and another sterol that was identified as Δ^5-avenasterol (relative retention time = 1.14, β-sitosterol = 1) based on the work of Itoh *et al.* (21) occurred as dominant sterols. However, in line with the general pattern for phytosterols, brassicasterol was not detected. Cholesterol may not constitute a nutritional risk as the high concentration of β-sitosterol (which can function as its competitive inhibitor) will render it chemically and functionally inert (22). The level of residual oil varies considerably depending on the extraction technique employed to remove the oil (unpublished results). This aspect of the extraction process and its impact on the quality of the resulting defatted meal are being investigated further.

TABLE 5

Sterol Composition of Defatted Vernonia Meal[a]

	mg/100g oil	% Total sterol
Cholesterol	63.61 ± 0.69	4.6
Campesterol	223.02 ± 3.11	16.0
Stigmasterol	230.69 ± 1.54	16.5
β-sitosterol	453.30 ± 2.43	32.4
Δ^5-avenasterol	418.24 ± 4.79	29.9
Δ^7-stigmastenol	7.20 ± 0.42	0.5

[a]Mean value of 4 determinations ± SD.

TABLE 6

Weight Percent Amino Acids in Protein from Defatted Vernonia Meal[a]

Essential	wt %	Non-essential	wt %
Threonine	4.4	Aspartic acid	10.3
Lysine	5.7	Serine	5.5
Isoleucine	4.1	Glutamic acid	21.6
Leucine	8.0	Glycine	7.6
Phenylalanine	5.3	Alanine	4.6
Tyrosine	3.6	Histidine	2.3
Methionine	2.1	Arginine	8.6
Valine	5.1	Cystine	1.4

[a]Mean value of 2 determinations.

TABLE 7

Weight Percent Amino Acids in Protein from Defatted Vernonia Meal—Whole Wheat and Soybean

Essential	*V. galamensis*[a]	Whole wheat[b]	Soybean[b]
Threonine	4.4	3.0	3.9
Lysine	5.7	3.0	6.5
Trytophan	—	—	1.3
Isoleucine	4.1	3.4	4.6
Leucine	8.0	6.9	7.9
Phenylalanine + tyrosine	8.9	7.8	8.2
Methionine + cystine	3.5	4.2	2.6
Valine	5.1	4.6	4.9
Histidine	2.3	2.4	2.6
Chemical score[c]	76	64	49
Limiting Amino acid[d]	methionine	lysine	methionine

[a]Mean value of duplicate determinations.
[b]Ref. 19.
[c]The ratio of the essential amino acid content in a sample protein in comparison to the recommended FAO-WHO level for that amino acid expressed as a percentage. Lowest percentage is the chemical score when using the FAO Provisional Scoring Pattern (23).
[d]Essential amino acid showing the lowest chemical score which represents the most deficient essential amino acid in the protein.

TABLE 8

Antinutritive Components in Defatted Vernonia Meal[a]

	mg/g
Phytate	25.42 ± 0.06
Tannins	—
Polyphenols	—

[a]Mean value of 2 determinations ± SD.

The amino acid profile of *V. galamensis* defatted meal is shown in Table 6. One of the nutritional attributes of the meal is the superiority of its essential amino acid profile (Table 7) over that of wheat and its very close similarity to that of soybean meal (19).

Phytate (25.42 mg/g) and the tannin, which was estimated qualitatively, are presented in Table 8. Phytate values for soybean (6.02 mg/g) and safflower (47.70 mg/g) are presented for comparison. The high concentration of phytate in the meal may suggest that phytic acid would occur as the insoluble calcium salt in the meal, thereby lowering the dietary availability of the calcium. This can be balanced with a calcium supplement. Although no digestibility studies have been carried out, these results nevertheless indicate the potential nutritive qualities of the meal. Further work is in progress to include bioavailability of the minerals in nutrition as well as digestibility studies of the meal.

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