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SEED MEAL DETOXICATION

Seed Meal from *Crambe abyssinica*

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Compositional characteristics of crambe seed meal are reported which are significant to its utilization. The hexane-extracted meal contains 9 to 11% thioglucosides, determined by a sulfate procedure described in detail. These thioglucosides are extractable with acetone or methanol containing 20 to 25% water, which removes one fifth to one fourth of the meal solids. Extraction of defatted meal with aqueous acetone removed much of the toxic or unpalatable material which was present in both the whole and the hexane-extracted seed meal as determined by rat feeding experiments. Nitrogen solubility of the hexane-extracted meal plotted as a function of pH gave two minima at which 40 and 42% of the nitrogen are in solution, only 12% of which was accounted for as nonprotein nitrogen.

ABYSSINIAN kale (*Crambe abyssinica* Hochst ex R. E. Fries) is a member of the Cruciferae family, related to rape and mustard. It is of interest as a possible farm crop grown as an industrial raw material because the acids from the seed oil glycerides contain 54 to 61% erucic acid (*19*). The crude protein content of the hexane-extracted seed meal without pericarp (pod) ranges from 49 to 55% and has an amino acid composition indicative of good nutritional quality (*8*). However, the seed also contains thioglucosides (*3*) that yield on enzymatic hydrolysis isothiocyanates, oxazolidinethiones, and possibly other similar products which, if present in sufficiently large amounts, can cause meals to be unpalatable, toxic, or both to farm animals (*2*).

Information is given here on the total thioglucoside content of defatted crambe seed meal and the extent of thioglucoside removal from the meal by selected solvents. Rat feeding tests are used as a guide for evaluating the presence of toxic or growth-inhibiting materials in seed, pericarp, and selected solvent-extracted meals. Solubilities of nitrogenous meal components in different solvents and amino acid compositional data are also included.

Experimental

Seed Origin and Meal Preparation. Mature seed grown in Texas (1961 planting) was selected for the experimental work. The sample as received contained 22% pericarp (pod). After removal of the pericarp the seed contained 46% oil which was removed by cold hexane (b.p. 30° to 60° C.) extraction of the flaked seed, leaving a seed meal, ground to pass a 100-mesh screen, containing 0.2 to 0.8% residual oil and 49% crude protein. The product had a yellow color, pleasant odor, and bitter taste. Additional crambe accessions from Montana, Nebraska, and Texas (1962 plantings) were also used to obtain some measure of variability in composition.

Estimation of Thioglucosides. Total thioglucosides were estimated by measuring the sulfate ion formed by myrosinase hydrolysis of the thioglucosides in water extracts. One gram of air-dry meal in a 125-ml. Erlenmeyer flask was placed in a boiling water bath. After 5 minutes, 30 ml. of boiling water were put in the flask, which was held at above 90° C. for 10 minutes. After gentle shaking for 10 minutes the solids were removed by centrifuging. The supernatants, including three thorough 10-ml. washes of the solids, were made to 50-ml. volume. Two 20-ml. samples of the extract were pipetted into 50-ml. flasks and 4.0 ml. of 0.1*M*, pH 6.0, citrate buffer were added to each. The thioglucosides in one flask were enzymatically hydrolyzed by addition of 15 mg. of white mustard myrosinase prepared according to Schwimmer (*13*). After standing overnight at room temperature both samples were analyzed for sulfate ion by a method similar to that reported by Fritz and Yamamura (*6*). Each solution was passed through a 5- to 6-cm. × 0.9-cm. diameter column of IR 120-H, 20- to 50-mesh ion exchange resin followed by distilled water washes to make a volume of 50 ml. Twenty-milliliter aliquots from each were made to about 100-ml. volume with absolute alcohol. Five milliliters of standard 0.005*M* sulfuric acid in 80% ethanol were added to the aliquot without myrosinase. After addition of 6 drops of 0.2% aqueous thorin indicator, each solution was titrated to matched end points with 0.005*M* barium perchlorate in 80% ethanol.

The method may be carried out without using a comparison sample to which no myrosinase has been added. However, titration of the sulfate in the comparison sample provides information on thioglucoside hydrolysis in the seed meal which may occur before analysis. Normally this titration was low. Calculation of total thioglucosides was based on the total sulfate titrated from the aliquot to which myrosinase was added. To estimate oxazolidinethione-forming thioglucosides an aliquot of the water extract was buffered to pH 7 and the thioglucosides were converted by mustard myrosinase. After 3 hours at 38° to 40° C. the oxazolidinethione formed was extracted into peroxide-free ether and estimated according to the method of Ettlinger and Thompson (*4*).

Prior to development of the above method oxazolidinethiones and volatile isothiocyanates formed from the thioglucosides were estimated by the methods of Wetter (*22, 23*) for measurement of these substances in rapeseed. Some of the data in the tables were obtained by these earlier methods.

Amino Acid Analysis, Nitrogen Solubility, and Organic Sovent-Extraction Tests. Acid hydrolysis and ion exchange chromatographic analysis for amino acids were carried out as previously reported (*20*). After alkaline hydrolysis tryptophan was estimated by starch column chromatography (*9*). Solubility of nitrogenous components of the meal in aqueous solutions was measured as previously described (*18*).

The meal was extracted with organic solvents and with aqueous organic solvents as follows. A 10-gram sample of the meal was extracted three times, first with 100 ml. of the solvent, followed by two 50-ml. portions. The solvent and meal were shaken in a 250-ml. centrifuge bottle for 1 hour and then centrifuged; the supernatant was decanted.

Feeding Experiments. Young rats, five per group, at a starting weight of 40 to 54 grams were individually caged and fed *ad lib.* the following basal ration (*1*): 73% yellow corn meal, 10% crude casein, 10% linseed oil cake meal, 3% U.S.P. cod liver oil, 2% dehydrated alfalfa meal, 1.5% bone ash, and 0.5% sodium chloride. The material tested was substituted at the expense of the entire basal ration.

Results and Discussion

Thioglucoside Content. Results from analyses of defatted seed by the Wetter methods are given in Table I. Comparison of these results with those for rapeseed (*22, 23*) by the same method show that thioglucosides from crambe seed form the same or smaller amounts of volatile isothiocyanates, depending on the rapeseed variety, and larger amounts of oxazolidinethiones.

Total thioglucosides from crambe seed by the Wetter methods, calculated as progoitrin, ranged from 4.0 to 5.2% by weight of the meal. Analysis of the same accessions by measurement of enzymatically formed sulfate ion gave thioglucoside contents calculated as progoitrin ranging from 9.2 to 11.0%. Myrosinase hydrolysis by the Wetter procedure was carried out at pH 4.0. Recent work (*4, 14*) has shown that quantitative mustard oil formation by myrosinase is more sensitive to pH change than is the formation of sulfate ion (*12*). We have found that oxazolidinethione formation from myrosinase hydrolysis of a purified preparation of crambe thioglucosides reaches a maximum plateau at pH 5.5 to pH 7, the highest pH tested in current studies. Measurement of oxazolidinethione-forming thioglucosides by enzyme conversion at pH 7 and volatile isothiocyanate-forming thioglucosides by the Wetter method (Table I) accounts for 90 and 10%, respectively, of the total thioglucosides obtained by the sulfate titration.

Agreement between amounts of thioglucosides determined by analyzing for specific hydrolysis products and by the sulfate titration procedure indicates that the sulfate ion was derived from thioglucosides and not from other sulfate esters which might be in the seed meal. The results also show that the larger total thioglucoside content obtained by sulfate titration is more accurate than that obtained by the Wetter methods, as previously reported for crambe seed (*3*). Titration of both preformed sulfate and total sulfate after myrosinase hydrolysis as well as oxazolidinethione from hot water extracts of crambe seed preparations offers a convenient means of following thioglucoside conversions as related to various processing or experimental treatments.

Analysis of pericarp separated from the seed showed that it contained only traces of thioglucosides.

Removal of Thioglucosides by Solvent Extraction. In the aqueous acetone, methanol, and ethanol solvent mixtures tried (Table II), a composition containing 20 to 30% water was required to remove over 90% of the thioglucosides that give oxazolidinethiones. Under these conditions, 20 to 34% of the meal solids were extracted. Extractions with the following solvents removed little if any thioglucosides: methanol and acetone mixed in different proportions; ethyl acetate–water, 39 to 1; ethyl acetate–ethanol, 7 to 3; 99% 2-propanol; and 99% methyl ethyl ketone.

The acetone-water 4 to 1 or 3 to 1 solvent mixture removed almost all the thioglucosides with minimum extraction of total solids, and the extracted meal was high in crude protein. The more detailed analysis (Table I) of acetone-water (4 to 1) –extracted meal suggests that it would be a good source of protein for feed or food, provided that the amino acids are nutritionally available and that unpalatable, toxic, and growth-inhibitory substances are not present. For seed protein, both lysine and methionine are high. The product contains more methionine than reported for soybean meal (*11*) and slightly less lysine.

Table I. Composition of Air-Dry[a] Crambe Seed Meals Extracted with Hexane and with Hexane Followed by Acetone-Water (4:1 V./V.)

Component	Hexane-Extracted[b] Av.	Hexane-Extracted[b] Range	Hexane- and Aqueous Acetone–Extracted[c]
Crude protein (N × 6.25), %	49.6	52.0–48.6	56.3
Sulfur, %	2.2	...	1.2
Phosphorus, %	1.0	...	0.3
Thioglucosides as progoitrin, %[d]			
Total by sulfate titration	10.1	11.0–9.2	0.7
Thioglucosides forming volatile isothiocyanates	1.0	1.3–0.8	0.1
Thioglucosides forming oxazolidinethiones at pH 7	9.1	9.7–8.0	0.1
Volatile isothiocyanates, mg./g. meal[e]	2.5	3.5–2.0	0.4
Oxazolidinethione, mg./g. meal[f]			
By Wetter method (*23*)	10.0	11.2–9.4	0.3
By conversion at pH 7.0 (see text)	27.5	29.3–24.0	0.4
Nitrogen as amino acids, %	72.5	76.–70.	84.1
Nitrogen as ammonia, %	14.1	18.–12.	11.2
Lysine, g./16 g. N	5.2	6.0–4.8	6.3
Methionine, g./16 g. N	1.6	1.8–1.5	2.0
Cystine, g./16 g. N	2.6	...	2.8
Tryptophan, g./16 g. N	1.2	...	...

[a] Moisture content of air-equilibrated meal ranged from 5.8 to 8.8%. [b] Nitrogen and amino acid analyses, except for cystine and tryptophan, are averages from five accessions; remaining analyses are based on one to five determinations. [c] Averages of analyses from two preparations from one accession, Texas 1961 crop. [d] Calculated as progoitrin anhydrous potassium salt. [e] Calculated as butenylisothiocyanate. [f] Calculated as vinyloxazolidinethione.

Table II. Solvent Extractions of Defatted Crambe Seed Meal to Remove Thioglucosides

Extraction, Aqueous Solvent	% V./V.	Insoluble Meal Recovered: Wt. of starting meal, %	Crude protein[a] (N × 6.25)	Oxazolidinethione, mg./g. recovered from meal[b]
None		100.0	48.4	10.0
Acetone	95	93.8	52.4	11.5
Acetone	90	89.0	53.4	8.5
Acetone	80	80.0	57.4	1.4
Acetone	75	76.2	58.0	0.9
Methanol	70	66.1	53.2	1.3
Ethanol	75	72.8	60.4	3.8

[a] Calculated on basis of meal containing 7.0% moisture. [b] Calculated as vinyloxazolidinethione. Analysis by Wetter procedure (*23*).

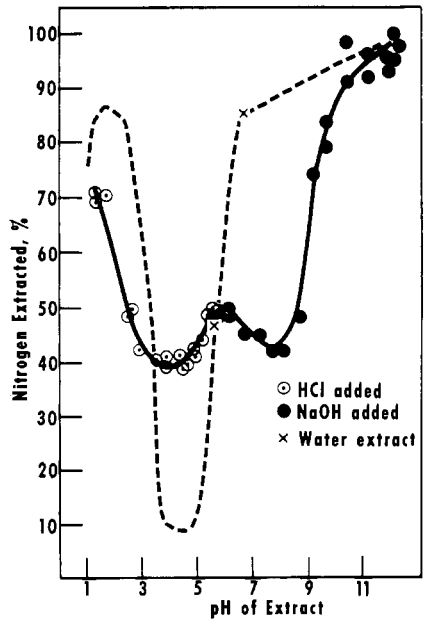

Figure 1. Solubility of nitrogen in meal as a function of pH

——— Crambe seed meal
– – – – Soybean meal (*15*)

Comparison of analyses of the hexane-plus aqueous acetone–extracted meal with those from the meal extracted with hexane (Table I) shows that the additional aqueous acetone extraction either removed a large amount of nitrogenous material that was not derived from proteins or amino acids or that it removed material that prevented quantitative acid hydrolysis of the protein to amino acids. Of the total nitrogen in the hexane-extracted meal, 72.5% was amino acid nitrogen. After aqueous acetone extraction, 84.1% of the nitrogen was from amino acids. The amino acid and ammonia nitrogen was calculated from complete ion exchange chromatographic analysis, except for tryptophan. The additional amino acid nitrogen found in the aqueous acetone–extracted samples was rather evenly distributed among all the amino acids. Complete amino acid composition is not given in Table I for the five accessions of hexane-extracted meal because the analytical values were not markedly different from those previously reported (*8*).

Solubility of Nitrogenous Meal Components. Average of duplicate experiments with aqueous extractants gave percentages of the total nitrogen solubilized as follows: 0.03*M* NaOH, 96%; 0.5*M* NaCl, 75%; 0.5*M* Na_2HPO_4, 74%; water, 48%; 70% aqueous ethanol, 24%; and 0.8*M* trichloroacetic acid, 12%. More nitrogen is solubilized by salt solutions from *C. abyssinica* seed meal than from a majority of those species examined by Smith *et al.* (*18*). These data suggest that, on the basis of classification by solubility, large amounts of the proteins are globulins. The greater nitrogen solubility in 70% alcohol than in 0.8*M* trichloroacetic acid suggests the presence of small amounts of prolamine-type protein.

Figure 1 shows that at the pH of solubility minima for crambe about four times as much nitrogen was in solution as for soybeans, and a higher pH was required to obtain maximum solubility. Solubility data of this type have been reported for seed meals from cotton, peanuts (*5*), navy beans (*10*), safflower (*21*), radish (*18*), linseed (*17*), sunflower (*16*), Alaska peas, tepary beans, and wheat (*15*). Solubility curves obtained, except for radish, were like that from soybeans. Radish and crambe, both members of the crucifer plant family, have similar solubility curves.

At minimum solubility (pH 3.5 to 4.5), 40% of the nitrogen was solubilized. Extraction with 0.8*M* trichloroacetic acid indicates 12% of the nitrogen is nonprotein nitrogen. On the basis of these data, at least 26% of the protein is soluble at all hydrogen ion concentrations tested.

Feeding Experiments. The results show (Table III) that the seed meal after extraction of the oil is the source of growth-inhibiting substance(s) which also cause death of the animals when fed at high levels. Defatted crambe seed meal gave a similar growth response when fed to chicks (*7*). Some growth inhibition caused by feeding the pericarp was probably due to its high fiber content.

Six rats fed the hexane and aqueous acetone–extracted meal at the 23% level gave an average growth response of 65.7 grams in 28 days in comparison with 69.7 grams for the controls. This limited feeding experiment indicated that the aqueous acetone removed much of the toxic and growth-inhibiting material. Since the feeding experiments were designed to test for toxicity (*1*), the results gave no information as to the nutritional quality of the seed protein. Future experiments are planned to test the nutritional quality of the protein in crambe meal as more complete information on composition becomes available and is used as a basis for meal treatment procedures.

Table III. Growth and Survival Obtained with Crambe Seed Meal by Rat Feeding Tests

Additive to Basic Diet (*1*)		Mean Wt. Gain, G.
Meal	%	(5 Male Rats/189 Days)
None (control)	..	262
Defatted soybean	30	313
Full-fat crambe	5	254
	15	134
	25	(All died in 42 days)
Hexane-extracted crambe	5	238
	15	(All died in 90 days)
	25	(All died in 21 days)
Crambe pericarp	30	203[a]
Hexane- and aqueous acetone–extracted crambe	23	66[b]

[a] Discontinued after 119 days, at which time weight gain of control animals was 248. [b] Discontinued after 28 days, at which time weight gain of control animals was 70.

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NUTRITIONAL VALUE OF FOODS

Vitamin C in Canned Pineapple Products at the Retail Level

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Vitamin C analyses were run on 266 samples of canned pineapple juice and 279 of solid pack pineapple at the retail level during the winter of 1962–63, collected in 40 states in the continental United States. The effect of market location appeared to relate to the average temperatures of the various regions, the higher temperatures lowering the vitamin C mean values to as low as 6.6 mg. per 100 ml., and the cooler locations favoring as high as 9.3 mg. as a mean content in the juice. On solid pack items the range for locations was 3.0 to 8.9 mg. per 100 ml. Companies varied in the level of vitamin C in their products, especially for pineapple juice, where the range was from 6.1 to 9.8 mg. per 100 ml. The products in supermarkets had somewhat more vitamin C than those of the same age in neighborhood groceries; the product in the latter stores was generally 3 to 4 months older. Age exerted a modest influence on vitamin C content. The products were all at least 6 months old, and some were in marketing channels for 55 months. A typical mean value of 7.8 mg. per 100 ml. for juice and 6.4 mg. per 100 ml. for canned pineapple at the point of consumption is indicated by this study.

ONLY meager information on the vitamin C (ascorbic acid) level in canned pineapple products at the point of purchase has appeared in the literature. Perhaps the most thorough was the report of Teply *et al.* (*3*) in the series of studies of the nutritive value of canned foods. This paper reported on the vitamin C content of 26 cans of sliced pineapple and 17 cans of pineapple juice. The former ranged from 0.94 to 5.85 mg. per 100 grams, averaging 3.59. The juice ranged from 5.90 to 10.60, averaging 7.87 mg. per 100 grams.

Both length of time in storage and storage temperatures may affect the vitamin C level of the products after they have left the cannery. In turn, these may vary in different parts of the country and even with different kinds of stores. Some authoritative publications (*4, 5*) summarizing information on the nutrient content of food products give the vitamin C value of canned pineapple juice as approximately 9 mg. per 100 grams or 9.3 mg. per 100 ml. of juice. It is not clear whether the vitamin C value refers to the product after canning or at the time of consumption after a normal shelf life. This study was undertaken to provide information on the range in content of vitamin C in pineapple as it is offered on the retail shelf.

Experimental

Budget, distance, and time argued against an extensive and completely representative sampling. The plan decided upon was to obtain shelf samples from each of two stores in a major marketing center located in each of 48 continental states. Sales personnel representing the Hawaiian pineapple companies agreed to do the actual can purchasing, identifying, packing, and shipping of the selected material to Honolulu for subsequent chemical analysis.

The instructions were kept to a minimum for obvious reasons. In a city, the instructions were to obtain samples from a major supermarket and from a small (neighborhood) store. In a store, buyers were instructed to select from the retail shelf a can of each of four brands of No. 1 or No. $1^1/_4$ pineapple slices (or other comparable solid pack) and a can of each of four brands of No. 2 or No. 211 cylinder pineapple juice. The cities selected were left to the discretion of the personnel handling the area.

Forty-one locations distributed in 40 states geographically well dispersed comprised the final sample. No claim is made or implied that this constitutes a truly representative sample, but it does offer information not otherwise available.

Four rather obvious sources of variation existed in the data as collected: company that prepared the pack, location where sample was procured, type of store in which displayed, and time elapsed since the product was sealed in the can. Least squares procedures, as described by Kempthorne (*2*), were applied to estimate the magnitudes of these factors. Prior to analysis the vitamin C values were transformed using $\log_{10}(X + 1)$, where X represents vitamin C in milligrams per 100 ml., which ensured the assumption of additivity in the model.

In the original sample, some cans packed in Taiwan, Malaya, Texas, and Mexico were obtained along with those

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