Crambe Seed Processing: Removal of Glucosinolates by Water Extraction[1]

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ABSTRACT

A method is reported for removing *epi*-progoitrin, the major glucosinolate, from crambe seed meal. Defatted meal was cooked and water extracted or treated with soda ash and then water extracted. Although soda ash aided destruction and removal of glucosinolate factors, there was a 28% reduction in total lysine. In animal feeding tests designed to reflect differences due to toxic factors, soda ash treated and water extracted meals gave the best results. No toxicity was apparent in rats and chicks fed these meals in nutritionally adequate diets. The rat diet included 30% crambe meal for 90 days; the chick diet, 20% crambe meal for 4 weeks. Pathological examinations in both series showed no organ damage.

INTRODUCTION

Crambe abyssinica, a member of the family Cruciferae, has been identified as a potential new oilseed in a USDA screening program. The oil, which makes up roughly one-third of the seed weight, is high in erucic acid (55-60%). On the basis of this component crambe oil has industrial value (1).

Defatted crambe meal has a high protein content, and its amino acid composition suggests that it might be a valuable supplement in animal feeds (2-4). The feeding value of the meal is greatly impaired, however, by the presence of undesirable compounds known as glucosinolates. These compounds have been extensively investigated at the Northern Laboratory (5,6).

During crambe processing studies in 1962, we observed that crambe meals prepared by conventional methods were toxic to rats. In 1963, Hesketh and coworkers (7) reported that crambe meal added to a chick ration depressed growth and decreased feed efficiency proportional to the level of crambe in the ration. Also, thyroid glands enlarged. In 1965, workers at the Northern and Western Laboratories (8) reported defatted, dehulled crambe meal was fatal to rats consuming it for 90 days at a 15% diet level. The major glucosinolate, *epi*-progoitrin, was isolated and identified by Daxenbichler and coworkers (9). Degradation of *epi*-progoitrin by enzymes present in the seed itself can yield *R*-goitrin, a mixture of organic nitriles, or all these compounds, depending on temperature, water dilution, and enzyme activity (10). *epi*-Progoitrin, *R*-goitrin, and a nitrile fraction obtained by directed enzyme hydrolysis were isolated, fed to rats, and their toxicity reported (11). Austin, et al., (12) found that in the presence of ferrous ion in water solution, *epi*-progoitrin decomposed to a thionamide and to one of the nitriles. These identified toxic factors in crambe meals are shown in Figure 1.

In 1968, we improved palatability and reduced toxicity of the crambe feed meal by a soda ash process (13), whereby the glucosinolate fraction of the meal was destroyed. In these studies, *epi*-progroitrin was decomposed by the action of moist heat under basic conditions to products with an infrared (IR) absorption band at 4.4 μ, indicating a nitrile moiety. Thionamide was not formed under these conditions. Although this process was satisfactory for yielding a nontoxic protein supplement for ruminant animals, it did not give a meal that was completely nontoxic to nonruminants. We now have investigated a method for removing the toxic factors from crambe meal by water extraction. Rapeseed often has been water extracted (14-17). In our approach, glucosinolates were removed from defatted crambe protein meals rather than from whole seed.

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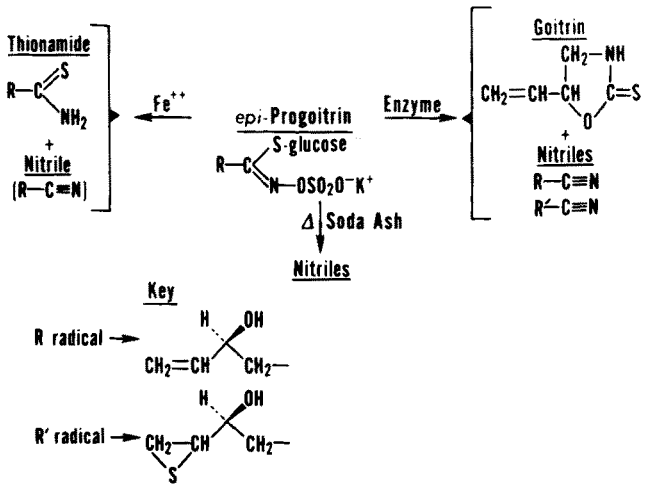

FIG. 1. Identified toxic factors in crambe meals.

EXPERIMENTAL PROCEDURES

Materials

Crambe seed was grown in Oregon under a private contract in 1966 in cooperation with the former Crops Research Division, ARS, USDA. Analyses of whole seed, dehulled seed (pericarp removed), and dehulled, defatted meal used for the detoxification tests are given in Table I.

Analytical Methods

Total glucosinolate, thioglucoside, was determined by the sulfate method of McGhee, et al., (6). Glucosinolate precursors of volatile isothiocyanates were determined by the method of Wetter (18), and *epi*-progoitrin glucosinolate was determined by enzymatic conversion to goitrin. In the last determination, a modified Wetter procedure (19) was used with the following modifications. One g of meal was extracted with boiling water to remove all glucosinolates, and a 2 ml samples of the water extract was enzyme converted to goitrin in 4 ml of pH 7 buffer containing 16 mg myrosinase enzyme and held 2 hr at 55 C. The goitrin which resulted from the enzyme conversion of the glucosinolates was extracted twice with methylene chloride, to remove goitrin, using 50 ml of solvent each time and adjusting final solvent volume to 100 ml. Optical density of the methylene chloride extract was read on a Beckman DB spectrophotometer at 5 mμ intervals from 210-280 mμ. A correction for background absorption was made (20) by subtracting the absorption reading at the intersection of lines drawn vertically through the absorption peak at 248 mμ and a baseline drawn tangent to the peak shoulders,

TABLE I

Analyses of Crambe Seed and Meal[a]

Assay	Whole seed[b] (%)	Dehulled seed[c] (%)	Dehulled, defatted source meal[d] for detoxification tests (%)
Moisture	7.1	4.6	6.8
Crude fat	33.3	45.6	0.4
Protein (N x 6.25)	17.1	24.2	44.8
Crude fiber	14.0	3.1	4.6
Ash	5.3	4.2	7.9
Nitrogen free extract	23.2	18.3	35.5

[a]"As is" basis.

[b]Hull content = 30%.

[c]Dehulled indicates pericarp or pod removed.

[d]Total glucosinolate content = 10.7%; nitrile content = 0.1%; *epi*-progoitrin content = 9.5%; volatile isothiocyanate precursors (calculated as sinigrin) = 0.60%.

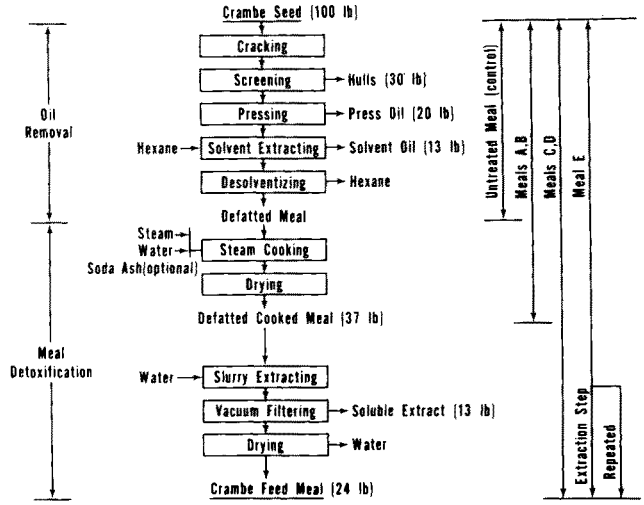

FIG. 2. Detoxification of crambe meal by a steam cooking water extraction process.

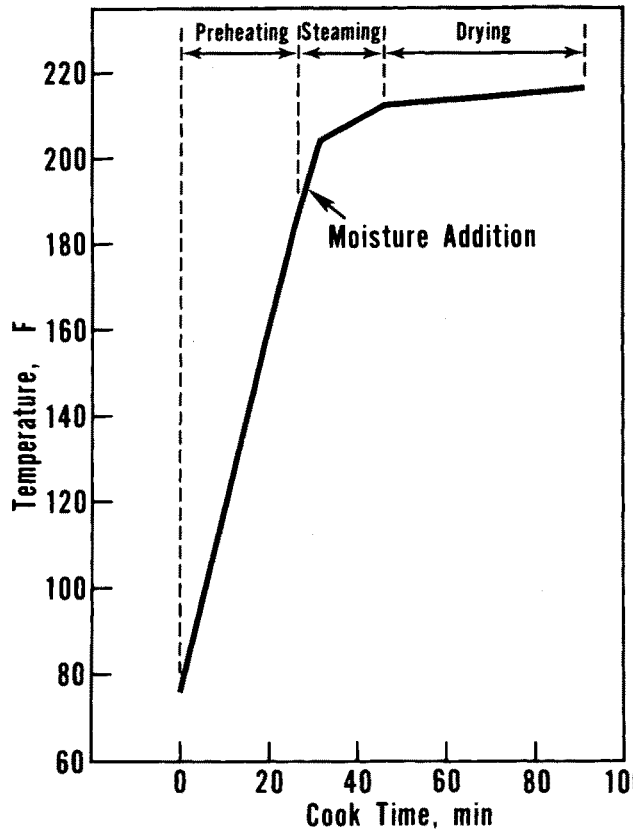

FIG. 3. Time-temperature profile of steam cooking process of dehulled, defatted crambe meal.

usually at 210 and 280 mμ. *epi*-Progoitrin was calculated as the sodium salt, mol wt 411.

Organic nitriles were measured by their IR absorption at 4.4 μ (21), and calculated as 1-cyano-2-hydroxy-3-butene, mol wt 97. The compounds so measured and calculated are referred to simply as nitriles. We did not isolate nor characterize them further.

Crude fat (22), moisture (23), ash (24), crude fiber (25), and protein analyses (26) followed AOCS Official Methods. Lysine was determined according to Spackman, et al., (27). Statistical means were compared by Duncan's method (28), and significant differences are defined here as those statistically different at the 95% level.

Equipment

The seed was cracked on 6 in. diameter rolls with 10 corrugations per inch. Dehulling equipment consisted of a shaker screen with provision for aspiration at the feed and discharge ends. Prepressing was conducted in a continuous 4 in. diameter screw press. A 2 stage, steam jacketed padded conveyor served as a preconditioner for the screw press. Solvent extraction was done batchwise in a steam jacketed, 50 gal, screen bottom tank. For atmospheric steam cooking, a 5 gal, jacketed, stainless steel vessel was equipped with a meshing rod agitation system, a spray nozzle, and a steam sparging coil (29).

Water extraction was performed in a 10 gal tank equipped with an agitator. The slurry then was filtered through a Büchner type unit consisting of a 22 in. diameter screen bottom tank with 60 mesh screen. The outlet under the screen was connected to a 10 gal receiver under vacuum.

Countercurrent water extraction of cooked crambe meal was carried out by a multiple batch process in standard laboratory glassware.

Procedure

Meal preparation: Oil removal, cooking, and water extraction (Fig. 2) were carried out to study the removal of toxic factors from crambe seed. Quantitative recoveries of oil, meal, and byproduct hulls appear in Figure 2 in brackets. The first 5 steps are conventional for dehulling and removing vegetable oil from high oil content seeds, and represent the initial preparation for all meals. The crambe seed was cracked on corrugated rolls at 0.025 in. clearance to free the pericarp or hull, which then was removed by screening and aspiration. The dehulled seed was heated to 200 F by indirect steam reducing moisture to 3% and then pressed to remove ca. two thirds of the oil. The remaining

TABLE II

Composition of Animal Diets

Ingredient	90-Day rat diet (%)	28-Day chick diet (%)	Basal control diets	
			Rat[a] (%)	Chick (%)
Crambe meal	30	20	-	-
Corn meal	51.2	27	73	33.8
Alfalfa meal	1.4	5	2	6.2
Linseed meal	7	-	10	-
Soybean meal	-	32.5	-	40.6
Fish meal	-	3	-	3.8
Casein	7	-	10	-
Cod liver oil	2.1	-	3	-
Corn oil	-	6	-	7.5
Bone meal	1.0	2	1.5	2.5
Limestone	-	2	-	2.5
Grit	-	2	-	2.5
Salt	0.3	0.5	0.5	0.6

[a]19.1% protein.

TABLE III

Analyses of Crambe Feed Meals

Meal identification	Added treatment	Meal analyses (%) moisture free basis					Toxin analyses (%)		Total lysine (g/16 g N)
		Protein	Fat	Fiber	Ash	NFE[a]	*epi*-Progoitrin	Nitriles	
"Untoasted" crambe meal (defatted)	None	48.1	0.4	4.9	8.5	38.1	9.5	0.10	6.2
A	Steam cook only	49.4	0.4	4.9	8.6	36.7	6.7	0.40	5.4
B	Soda ash + steam cook	48.6	0.4	4.9	12.8	33.3	1.7	0.70	3.3
C	Steam cook, water extracted	54.2	0.4	6.8	9.6	29.0	2.9	0.16	5.4
D	Soda ash + steam cook, water extracted	56.0	0.4	6.7	11.0	25.9	0.0	0.20	3.9
E	Soda ash + steam cook, water extracted (2 times)	59.1	0.4	9.1	11.0	20.4	0.0	0.10	3.9

[a]NFE = Nitrogen free extract.

oil in the press cake was removed by multiple batch extractions with hexane at 140 F. The hexane wet defatted meal then was air desolventized, untoasted, defatted crambe meal.

At this point, 4% soda ash, based on wt of defatted meal, was added as a dry powder to some meals. The meals then were cooked by a moist steaming and drying procedure typical of the so-called toasting operation used in commercial oilseed meal production. A typical time-temperature profile for the cooking step is shown in Figure 3. The cooking was initiated by heating the meal to 185 F under jacketed steam. Live steam then was admitted and room temperature water was added to the meal to raise its moisture content to 25%. Open steaming was continued for 20 min, followed by a 45 min dryout with only jacket steam. The hot meal then was air cooled and dried to a moisture content of ca. 10%.

To extract with water, the meal was slurried for 30 min in 3 parts of water at room temperature and separated on the 60 mesh screen. The cake then was washed on the screen with 9 parts of water and dried. For one experiment (Fig. 2, meal E), the water extraction step was repeated a second time before drying.

Animal feeding: Rat feeding studies were conducted at the Western Regional Research Laboratory. Weanling female rats of the Fisher strain were divided into 6 groups of 5 rats each in such a manner as to equalize average group wt. The animals were housed in individual screen bottom cages and offered feed and water ad libitum throughout the 90 day experimental period. Body wt and feed consumption data were gathered weekly, and at the conclusion of the experiment, all animals were sacrificed. Body organs of all animals were weighed and inspected, and particular attention was given to the thyroids, livers, and kidneys.

Rats were fed a basal diet of sufficient protein quality and quantity so that difference in their growth should reflect differences in toxic factors rather than protein quality variation. The experimental crambe meals were fed at a level of 30% of the total diet; composition of the diet is given in Table II. The diet with crambe meal contained 27% total protein.

Chick feeding studies were conducted at the Wisconsin Alumni Research Foundation, Madison, WI. Day old, white male broiler chicks were randomly allotted to 6 groups of 20 chicks each, 5 groups fed crambe meal, and one fed the control diet. The chicks were housed in Jamesway electrically heated battery brooders with experimental diets and water supplied ad libitum for 4 weeks. Body wt and feed consumption data were gathered weekly. At the conclusion of the experiment, 10 chicks from each treatment were selected randomly for sacrifice and gross necropsy. Appearance of liver, kidney, and gizzard lining was noted, and thyroid wt was recorded.

Crambe meals were fed in a nutritionally adequate basal diet so that the reduction in growth was attibutable only to toxic factors. The chick diet with crambe test meal at a 20% level (Table II) contained 30% total protein.

RESULTS AND DISCUSSION

Meal Composition

Proximate analyses of the meals fed in the animal studies are given in Table III. Composition of the dehulled, defatted meal before steam cooking and water extraction is included.

Steam cooking improved filtration. Meals A and B (Fig. 2; Table III) represent a variation in cooking procedure; meal A was produced by steam cooking only and meal B by the same procedure but in the presence of soda ash. Steam cooking only (meal A) decomposed about 30% of the *epi*-progoitrin and increased nitrile content. Steam cooking with soda ash (meal B) greatly increased glucosinolate destruction but yielded additional nitrile. The conversion of *epi*-progoitrin to nitrile for meals A and B is 30-50% of theoretical; conversion products other than nitriles have not been identified. Water extraction of steam treated meal by the simple slurry wash technique used here (meal C) reduced glucosinolate and nitrile contents, but still left a glucosinolate content 30% as high as in the untoasted meal. Water extraction of the soda ash cooked meal produced meals D and E, which were free of *epi*-progoitrin and had lower nitrile contents. A second water extraction (meal E) further reduced the nitrile content below that in meal D.

Addition of soda ash increased the ash content of meals B, D, and E, and decreased their lysine content ca. 30% below the value for corresponding treatments without soda ash. This reduction would impair the feeding quality of these meals for nonruminants somewhat, unless the treatment can be modified to lower the soda ash level or to minimize the degree of heat treatment.

Water extraction (meals C, D, and E) increased protein and fiber contents owing to the removal of water soluble carbohydrate and some of the added soda ash. A material balance of the process streams, starting with 100 parts by weight of whole crambe seed, showed a 13% loss of solids in the water extract. Basic ion exchange resins have been found effective in the recovery of glucosinolates from the water extract (L.D. Kirk, unpublished data), but market research will be required to find consumer uses for this byproduct. Water extraction of cooked crambe meal (not soda ash treated) also was evaluated as a countercurrent multiple batch process.

epi-Progoitrin was completely removed in a 60 min countercurrent extraction with water at room temperature, with a water:meal ratio of 3:1. After establishing equilibrium for the countercurrent stage operation, solids concentration of the extract was ca. 12.5%. Solids loss (13%) in the water extract was approximately the same as for water extracted meals C, D, and E. Protein content of the extracted meals was 59.0%, moisture free basis.

Animal Feeding Data

Growth retardant properties of processed crambe meals for rats correlated closely with the total *epi*-progoitrin plus nitrile content (Fig. 4). The action of soda ash decreased the amount of *epi*-protoitrin plus nitrile (meal B) and significantly improved growth. The 2 soda ash cooked, water extracted meals (D and E) gave wt gains not significantly different from the basal control diet. These meals contained no *epi*-progoitrin and 0.2% or less of nitriles.

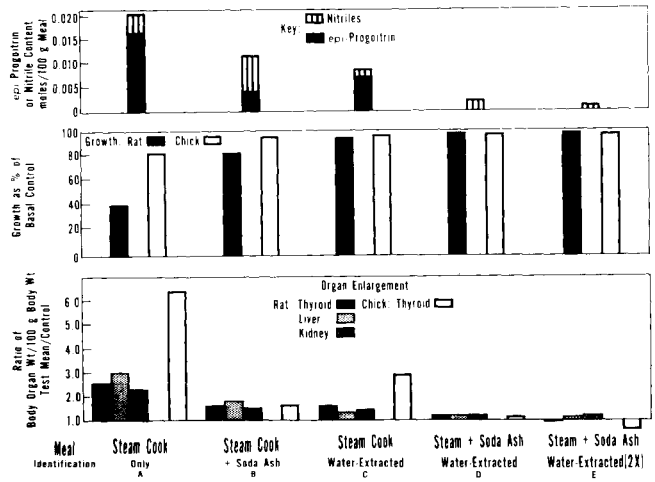

FIG. 4. Effect of feeding processed crambe meals on growth and toxicity to rats and chicks.

Chick wt gains on these feed meals also are shown in Figure 4 for comparison with rat data. Only with steam cooked meal A was there a statistically significant lower growth than with the control. Chicks were fed a lower level of crambe meal, 20% as compared to 30% for rats, and for a shorter period of time, 28 days as compared to 90 days for the rats. The lower level and shorter time of feeding could account for the lower toxicity effects observed over those in the rat feeding tests. Based on wt gains of both rats and chicks, there was no significant evidence of toxicity for the soda ash cooked, water extracted meals.

Toxicity also was evaluated by histological examination of body organs (Table IV). After 90 days of feeding, all rats were sacrificed, and liver, spleen, kidneys, heart, adrenal, and thyroid wts were determined. The livers, kidneys, and thyroids, which are known to be affected by feeding raw crambe, were closely examined by a pathologist for lesions and other abnormalities. The mean wt of key organs obtained upon autopsy per 100 g of body wt are given in Table IV. In Figure 4, bar graphs represent ratios of the percentage body organ wts of test animals to the percentage body organ wts of animals fed the basal control diet. Test meals yielded relative organ wts either above or below the control value.

Body organs of rats consuming the steam cooked meal (A) were significantly enlarged over those of rats consuming all other meals, both crambe and basal control. Meal A also produced the greatest number of lesions in liver and kidney. To a lesser extent, both soda ash cooked meal (B) and steam treated, water extracted meal (C) caused the same pathology. Rats fed the 2 soda ash cooked, water extracted meals (D and E) were free of significant liver and kidney lesions; and the more thoroughly extracted meal (E) produced no goitrogenic effect. There was relatively little dif-

TABLE IV

Histological Data from Rats

Meal identification	Toxic factor (%)[1]		Mean body wt	Mean organ wt (g/100 g body wt)				
	epi-Progoitrin	Nitrile	(g)	Thyroid[2]	Liver	Kidney	Heart	Adrenals[2]	Spleen
A	6.7	0.40	66 d[3]	24.4 a	9.71 a	1.56 a	0.54 a	43.0 a	0.42 a
B	1.7	0.70	144 c	14.9 b	5.63 b	1.00 b	0.41 b	22.8 c	0.27 b
C	2.9	0.16	158 b	15.0 b	4.16 c	0.93 bc	0.40 b	24.4 bc	0.26 bc
D	0	0.20	172 ab	11.1 cd	3.69 cd	0.82 de	0.38 bc	27.4 b	0.25 bc
E	0	0.10	172 ab	8.4 e	3.53 cd	0.84 cd	0.33 c	25.2 bc	0.25 bc
Basal control	--	--	175 a	9.2 de	3.20 d	0.68 f	0.33 c	25.2 bc	0.23 bc

[1]"As is" basis.

[2]Organ wts of adrenals and thyroids = mg/100 g body wt.

[3]Two means with no letter in common differ significantly at 95% level.

ference in body organ size of the rats fed soda ash cooked, water extracted meals when compared to the organs of rats fed the basal control diet. There was a difference in kidneys of rats fed soda ash cooked, thoroughly extracted meal (E) when compared to the control. A pigmentation of the kidney in these animals was, in the judgment of the pathologist, minor in nature, and not due to the crambe.

Pathology of the chicks was not explored as thoroughly as that of the rat. Half of each group of 20 chicks was sacrificed after 4 weeks. Visual inspection of livers and kidneys indicated no appreciable enlargment or damage. The thyroids were weighed, and results are compared in Figure 4 with similar rat data. There was a high correlation of thyroid enlargement with content of *epi*-progoitrin. The soda ash cooked, water extracted meals did not produce thyroid variation significantly different from that of the basal control diet. Pathology of chicks indicated that these meals were free of crambe toxicity and was in agreement with the rat pathology.

Because both rat and chick feeding data show that toxicity was closely related to the residual amounts of glucosinolates, its removal by water extraction should improve protein quality. The costs of water extraction and protein losses incurred in the process, however, will need to be balanced against nutritional quality of water extracted crambe meals.

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