Crambe Seed Processing: Decomposition of Glucosinolates (Thioglucosides) With Chemical Additives[1]

L.D. KIRK, G.C. MUSTAKAS, E.L. GRIFFIN, JR., Northern Regional Research Laboratory,[2] Peoria, Illinois 61604 and
A.N. BOOTH, Western Regional Research Laboratory, Albany, California 94706

ABSTRACT

Crambe meal was cooked with a variety of bases and metal salts to study decomposition of the undesirable glucosinolate (thioglucoside), *epi*-progoitrin. Salts of iron and copper were preferred because they were the most active decomposers and because they did not reduce the lysine content as did the alkalies. An unsaturated hydroxy nitrile, representing about 25 mole per cent of the decomposed *epi*-progoitrin, was the major reaction product left in the cooked meal. A thionamide product, representing about 7 mole per cent of the decomposed *epi*-progoitrin, was also observed in meals cooked with metallic salts. The thionamide was relatively unstable in moist, hot crambe meal, especially at basic pH, and may therefore be an intermediate in a complex decomposition path. Rats fed ferrous sulfate-treated crambe meal as 30% of a protein sufficient diet gained 70% compared with a basal control. Enlargement of thyroid, liver and kidneys was about 1.5 times that of the control organs. A crambe meal heated under the same conditions but without ferrous sulfate and fed at the same diet level caused 100% mortality within two weeks.

INTRODUCTION

Crambe abyssinica Hochst. ex R.E. Fries has been recommended as a new oilseed crop for the U.S. (1). The refined triglyceride oil is well suited as a mold lubricant in the continuous casting of steel and the isolated erucic acid is desired by the plastics industry (2-5). Although first processed on a plant scale in 1964 (6), the annual acreage of crambe in the U.S. remains small and confined at present to Indiana and Louisiana. Crambe is also grown in Canada where total seeding in 1970 was 6000 acres. Interest in this oilseed is continuing; "Prophet," a new high yielding variety of crambe, was recently announced by K.J. Lessman, Agronomy Department, Purdue University (private communication, 1968).

In view of the very favorable demand for crambe oil the defatted meal must be considered as a byproduct of the extraction process even though it represents nearly 70% of the seed weight. Maximum utilization of this meal as an animal feed will improve the economics of crambe processing and encourage acceptance of this seed as a new crop. Certain constituents in the meal, however, limit its palatability to animals and render it toxic to nonruminants. This laboratory has been engaged in identifying these factors, modifying the meal by a variety of process treatments and testing the products in feeding experiments.

Crambe meal was reported to be toxic to both rats (7) and chicks (8); *epi*-progoitrin, a naturally occurring glucosinolate, was identified as the main and perhaps only source of this toxicity (9,10). Several treatments based on either decomposition or removal of *epi*-progoitrin have been reported for improving the feeding value of crambe meal (7,10-15).

The *epi*-progoitrin decomposition treatments reduce meal toxicity and can be incorporated into the desolventizing-toasting step of conventional oilseed processing. The best of these methods involves the use of chemical additives which promote the thermal decomposition of *epi*-progoitrin. A meal cooked with soda ash to decompose glucosinolates produced 4 week growth in chicks equivalent to the control when fed at 20% of diet. The same meal produced a 6 week growth in rats of 82% that of the control when fed at 30% of diet (14). The meal was not, however, in either case fed under conditions to test its protein quality, and later analyses indicated a lysine content of only 3.3% of the protein compared to a chick requirement of 5% and a rat requirement of 4.3% (16). Meals cooked with and without the soda ash additive were fed to ruminants at the University of Nebraska (13,17). Although no evidence of toxicity was observed during or at the conclusion of these trials, crambe meal was observed to be less palatable to cattle than was soybean meal. Initial palatability studies indicated that this acceptability problem could be largely overcome when the crambe meal was pressure-cooked with soda ash. This improvement was less pronounced in subsequent 90 day finishing trials employing commercially prepared meals cooked at atmospheric pressure. In the latter trials the soda ash-cooked crambe meal performed as well as soybean meal if the quantity fed did not exceed one-half of the protein supplement.

[1]Presented at AOCS Meeting, Houston, Texas, May 1971.

[2]Northern Marketing and Nutrition Research Division, ARS, USDA.

TABLE I

Analyses of Crambe Seed and Meal

Constituent	Whole seed, %	Dehulled seed, %	Defatted meal, %
Moisture	7.1	4.6	7.0
Crude fat	33.3	45.6	0.2
Protein (N x 6.25)	17.1	24.2	44.7
Crude fiber	14.0	3.1	5.7
Ash	5.3	4.2	7.8
NFE	23.2	18.3	34.6

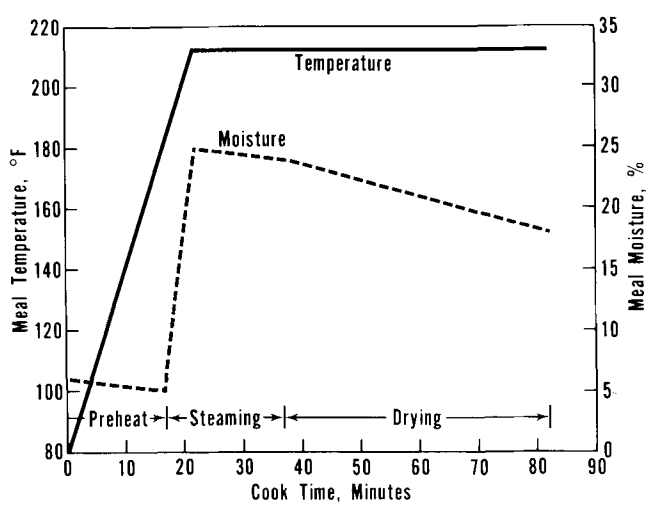
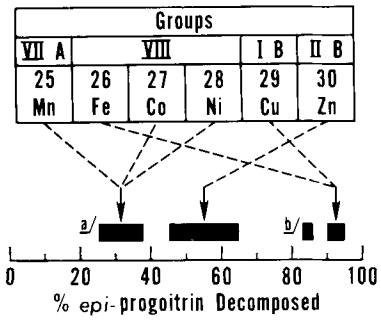


FIG. 1. Temperature, moisture-time profile of a standard crambe cook.



a/ Decomposition of this magnitude is also obtained without additive.
b/ Decomposition obtained with soda ash and other bases.

FIG. 2. Effectiveness of additives for promoting *epi*-progoitrin destruction when added to a standard cook at 4% of the meal weight.

Crambe meal toxicity can be completely removed by water extraction (14,15) of the *epi*-progoitrin. This approach involves a departure from conventional processing however and several problems must be solved before it can be considered economical for producing feed meal.

This paper is concerned with *epi*-progoitrin decomposition methods which, although they have not removed all toxicity, do offer a possibility of producing a crambe meal that can provide protein for nonruminant diets. Chemical additives consisting of metal salts of period 4 of the periodic table or base compounds represented by sodium carbonate, calcium hydroxide or sodium hydroxide were evaluated on the basis of cost, effectiveness in decomposing *epi*-progoitrin and effect on available lysine. Finally, meals cooked with the additive best meeting the above criterion and a meal cooked without additive were evaluated in rat feeding studies.

EXPERIMENTAL PROCEDURES

Materials and Methods

The crambe seed was grown in 1966 in Oregon (Table I). Dehulled (pericarp or pod removed), defatted, dry meal contained 10.6% *epi*-progoitrin, 0.64% glucosinolate precursors of volatile isothiocyanate and 0.11% organic nitrile. Approximately 94 mole per cent of the glucosinolates present in the meal were *epi*-progoitrin or the *epi*-progoitrin-derived nitrile. Available lysine content was 5.6% of protein.

The control ration used in the rat feeding studies was Purina Lab Chow. Experimental diets were formulated by substituting crambe meal for the control ration obtaining diets of either 15% crambe, 85% control ration or 30% crambe, 70% control ration.

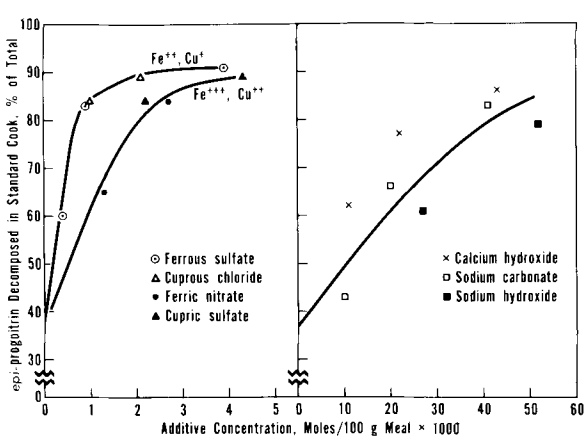


FIG. 3. Relative activity of acid and base additives.

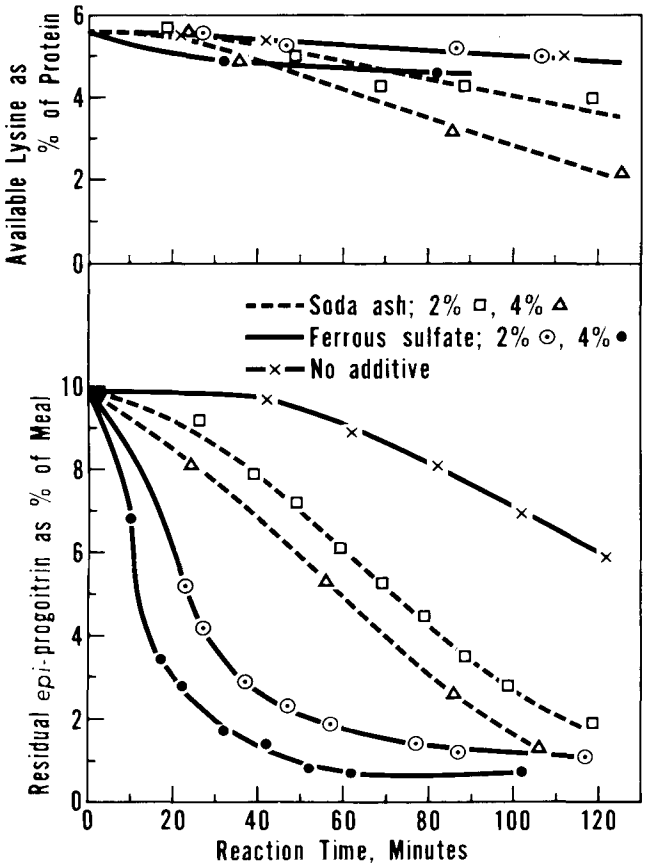


FIG. 4. Destruction of lysine accompanying the decomposition of *epi*-progitrin during standard moist cooks with and without additives.

Analysis for *epi*-progoitrin of meals containing active enzyme was conducted by enzymatic conversion to oxazolidinethione as reported earlier (14) except for changes in the *epi*-progoitrin extraction step. A pH 7 buffer solution replaced the distilled water for extracting *epi*-progoitrin from soda ash-treated meals. Also where enzyme was inactive the glucosinolate in all cooked meals was isolated at room temperature rather than with boiling water. These precautions were followed to minimize decomposition of *epi*-progoitrin during analysis. Nitrile analysis was performed by the method of Daxenbichler et al. (18), total glucosinolate by the method of McGhee et al. (19) and volatile isothiocyanate according to Wetter (20). Thionamide was determined by the following procedure: Two grams of meal containing approximately 10 mg of thionamide was extracted three times with 20, 20 and 10 ml, respectively, of distilled water at room temperature. Extracts recovered by centrifuging were combined and adjusted to 50 ml total volume. A 1-ml aliquot was extracted twice with 50 ml of methylene chloride. The methylene chloride extracts were combined, diluted to 100 ml total volume and optical density was read at 270 mμ. Thionamide content was determined as suggested by Austin et al. (21) from the relationship

$$C_{(\text{moles/liter})} = \frac{\text{optical density}}{1.12 \times 10^4}$$

Analysis for unsaturated nitrile and thionamide were also

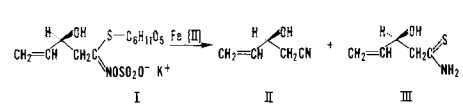


FIG. 5. Nonenzymatic degradation of *epi*-progoitrin (I) to nitrile (II) and thionamide (III).

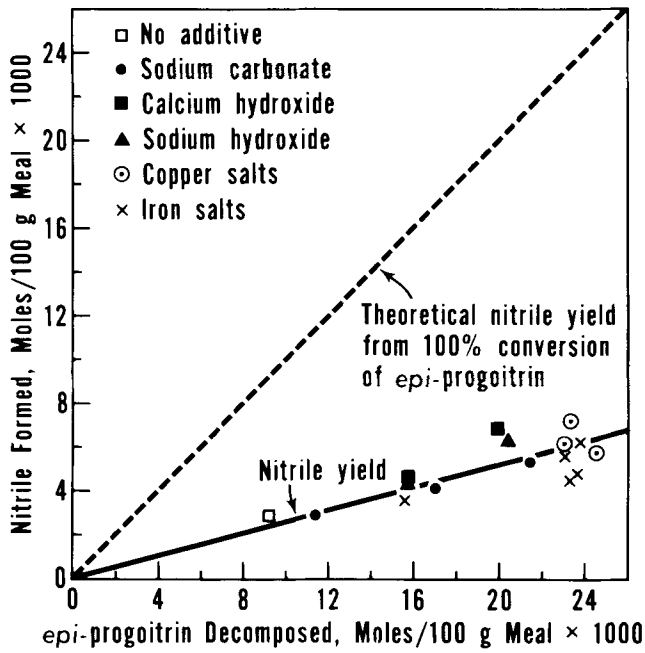


FIG. 6. Theoretical and actual yields of nitrile from *epi*-progoitrin decomposed with various additives in the standard cook.

conducted by the gas liquid chromatography (GLC) method of Daxenbichler et al. (22).

Available lysine was determined by the method of Rao et al. (23). Total amino acids were obtained by hydrolyzing the meal protein and analysis on a Spinco MS amino acid analyzer (24). Other analyses followed official AOCS methods (25). Rat feeding experiments were conducted at the Western Marketing and Nutrition Research Division, Albany, California.

Equipment and Procedure

Seed was cracked with 6 in. diameter corrugated rolls and the hulls aspirated at the feed and discharge ends of a 0.093 in. round hole shaker screen. The dehulled cracked seed was metered to a jacketed paddle conveyor in which it was heated to 210 F in 15 min. Moisture vapor was removed through vents in the conveyor headspace so that seed entering at 5% moisture was discharged to the continuous screw press at 3% moisture. The press (Fugi Bunka Co. Ltd., Tokyo, Japan) was operated without recycling oil to cool the barrel. However, cake and oil temperature did not exceed 200 F. About two-thirds of the oil was removed in pre-pressing and the expeller cake contained about 21% oil. This cake was cooled and stored for batch extraction.

The cooled cake was charged to a 25 gal screen-bottomed tank and the oil extracted in eight contacts with hexane at 140 F. The extracted meal was desolventized at room temperature.

The extracted meal was charged in 8.5 lb batches to a jacketed 8 gal capacity cooker equipped with meshing rod agitation system, spray head for introduction of water and steam sparge coil (26). When ferrous sulfate, cuprous chloride, ferric nitrate, cupric sulfate, calcium hydroxide, or sodium carbonate were used they were added to the charged meal as a finely ground powder. Only sodium hydroxide was added as a water solution during the moisture adjustment step later in the cook. The quantity of chemical added varied from 0.1% to 4% of the meal weight. Cooking or toasting of the meal generally followed a controlled temperature-moisture time profile (Fig. 1) hereafter referred to as a "standard cook." The meal was first heated to 185 F with indirect steam. Live steam was then added through the sparge coil and as the meal temperature

TABLE IV

Amino Acid Composition of Crambe Meals

Amino acid[a]	Quantity of amino acids in crambe meal, g/16 g N		
	Before cooking	Cooked without $FeSO_4$	Cooked with $FeSO_4$
Lysine			
Total	6.3	5.4	5.5
Available	5.7	5.6	5.1
Histidine	2.6	2.3	2.5
Arginine	6.7	6.2	6.2
Aspartic	6.9	6.7	6.5
Threonine	5.0	4.7	4.6
Serine	4.3	4.1	4.2
Glutamic	17.9	16.9	16.9
Proline	5.2	5.4	6.9
Glycine	6.0	5.7	5.3
Alanine	4.3	4.4	4.6
Cystine	2.8	2.3	1.5
Valine	4.7	4.2	4.4
Methionine	1.8	1.5	1.5
Isoleucine	3.9	3.6	3.8
Leucine	6.3	6.1	6.3
Tyrosine	2.9	2.7	2.9
Phenylalanine	3.9	3.2	3.7

[a]Total amino acids by Moore and Stein System (20).

TABLE II

Stability of Thionamide and Nitrile in Crambe Meal

Time at 212 F, min	Thionamide decomposition, %		Nitrile decomposition, %	
	pH 4-5	pH 8-9	pH 4-5	pH 8-9
30	33	100	9	17
60	62	—	23	26

TABLE III

Composition of Crambe Meals Prepared for Rat Feeding

Crambe cooked	Proximate analyses, % of meal						Content of *epi*-progoitrin and decomposition product, % of meal		
	Moisture	Protein	Fat	Fiber	Ash	NFE	*epi*-Progoitrin	Nitrile	Thionamide
Without additive	6.0	47.8	0.7	4.2	8.1	33.2	6.3	0.39	0.05
With 0.5% $FeSO_4 \cdot 7H_2O$	6.4	47.8	0.7	4.3	8.3	32.5	0.6	0.85	0.16
With 1.0% $FeSO_4 \cdot 7H_2O$	7.2	47.0	0.7	4.2	8.4	32.5	0.5	0.83	0.16

TABLE V

Results of Feeding Crambe Meals to Rats

Meal fed	Body weight		Organ wt/100 g body weight		
	g	% of control	Thyroid, mg/100 g	Liver, g/100 g	Kidneys, g/100 g
Trial No. 1, crambe fed at 30% of diet[a]					
Crambe without additive	All dead in 2 wk	—	—	—	—
Crambe with 0.5% $FeSO_4 \cdot 7H_2O$	222 ± 15	69.8	7.6 ± 1.0	5.32 ± 0.11	1.10 ± 0.04
Crambe with 1% $FeSO_4 \cdot 7H_2O$	223 ± 9	70.1	7.7 ± 0.7	5.61 ± 0.17	1.12 ± 0.03
Control meal	318 ± 29	100.0	5.7 ± 1.0	3.48 ± 0.11	0.71 ± 0.04
Trial No. 2, crambe fed at 15% of diet[b]					
Crambe without additive	119 ± 7	67.2	8.1 ± 1.6	6.42 ± 0.70	1.03 ± 0.04
Control meal	177 ± 12	100.0	7.4 ± 1.4	3.24 ± 0.23	0.69 ± 0.03

[a]Groups of six male rats (Fischer strain) were used for each meal tested.

[b]Groups of six female rats (Fischer strain) were used for each meal tested.

rose rapidly to 195 F, water was introduced to adjust the moisture content to 25%. The water added in this manner avoided the formation of "pasty" meal which occurred if the water was added at a lower temperature. Preheating with steam also inactivated the native enzyme system in crambe, avoiding an enzymatic degradation of *epi*-progoitrin. After steaming for 20 min the live steam was stopped and the charge dried for 45 min and discharged. This standard cook procedure was varied to study the effect of cooking on available lysine content by extending the drying period from 45 to 90 min.

Stability of thionamide and nitrile in moist hot crambe meal at the natural acid pH of the meal or at basic pH was determined by measuring the decomposition of these compounds under simulated cook conditions. Crambe meals containing maximum levels of either compound and free of residual *epi*-progoitrin were adjusted to 25% moisture (by addition of sodium hydroxide where necessary), placed in sealed metal containers and held in a boiling water bath for either 30 or 60 min. To prepare a meal high in thionamide for this purpose, the procedure of Austin et al. (27) was followed in which ferrous sulfate was reacted with a water slurry of crambe meal at room temperature and the mixture dried at 180 F. This meal contained no residual *epi*-progoitrin and about 0.6% thionamide. The "high nitrile" meal for this study was prepared by a standard cook with 1% cuprous chloride. This meal contained 1.24% of unsaturated nitrile.

RESULTS AND DISCUSSION

Destruction of *epi*-Progoitrin

Additives were chosen from two classes of compounds known to be effective in promoting glucosinolate decomposition: (a) Metal salts of period 4, periodic table (27,28), and (b) basic compounds represented by sodium carbonate, calcium hydroxide and sodium hydroxide. In this paper the metal salts are designated "acidic additives" while the second group is designated "basic additives" in accord with the pH obtained in water solution.

Salts of iron, copper and, to a lesser extent, zinc promoted *epi*-progoitrin destruction in the standard moist cook while the other related metals had no catalytic effect (Fig. 2). All base additives were also effective in decomposing *epi*-progoitrin.

Ferrous or cuprous salts were more effective than were ferric or cupric salts (Fig. 3). In order to compensate for the wide molecular weight ranges of the hydrated salts, the additive concentration is reported in molar concentration rather than as weight per cent. Since zinc salts were of much lower activity than the salts of iron or copper, they were not included in this evaluation.

Little difference in activity was observed among the basic additives when considered on a molar basis although they can be listed as $Ca(OH)_2 > Na_2CO_3 > NaOH$. The activity of the bases was much lower than that of the metal salts and appeared to depend solely upon the particular bases' ability to raise the pH of the system.

Ferrous sulfate and calcium hydroxide at approximately one cent per pound are the most economical acid and base additive respectively. Since a ferrous sulfate level of 0.5% of the charge weight is as effective as any of the bases at a 4% level, this iron salt is the most economical of all additives tested. Toxicity of the additives is, of course, also an important factor in determining choice. In this regard both soda ash and ferrous sulfate have been added to cottonseed meal without apparent toxic effects (29,30). Ferrous sulfate-treated rapeseed meals have also been successfully incorporated into the diets of mice and swine (31).

Protein Quality

A decline in protein quality was observed during the toasting of crambe meal similar to that reported with mustard meal (32). Lysine was destroyed during heating, presumably through a "browning" reaction with reducing sugars in the meal. The problem was most serious with base additives (Fig. 4). Although only data for Na_2CO_3 are reported, all bases tested caused similar destruction of lysine. Since lysine is an essential amino acid any loss represents a reduction in meal quality for nonruminants. In comparing the two classes of additives therefore the acid class offers the best opportunity for producing a nonruminant feed from crambe meal by the glucosinolate decomposition method. Meals containing approximately 1% *epi*-progoitrin and retaining at least 5% available lysine in the protein were produced by a standard cook with ferrous sulfate.

Decomposition Products of *epi*-Progoitrin

Two nonenzyme-derived products of *epi*-progoitrin decomposition were identified by Austin et al. (27) in aqueous *epi*-progoitrin solutions and crambe meal slurries in the presence of ferrous ion. These compounds are the thionamide (III) and nitrile (II) illustrated in Fig. 5. The decomposition of *epi*-progoitrin by this procedure differs from that of the enzymatic decomposition method which

yields three nitriles (33,34). Austin found that the maximum yield of thionamide and nitrile together did not exceed about 60 mole per cent of that theoretically obtainable from the *epi*-progoitrin (I) decomposed. The molar yield of each compound from the *epi*-progoitrin varied with reaction conditions and was approximately 26% nitrile and 34% thionamide at 203 F. No other byproducts of the *epi*-progoitrin decomposition were identified.

Decomposition of *epi*-progoitrin with ferrous sulfate in the present study was accomplished at 212 F in a moistened meal system rather than in the water slurry employed by Austin. The yield of nitrile was roughly 25% of the *epi*-progoitrin decomposed, even though the level and type of additive varried (Fig. 6). This nitrile was the same compound and obtained in the same yield as Austin's. In contrast to Austin's results however basic cooked meals contained no thionamide, and ferrous sulfate-cooked meals contained a small amount (7 mole per cent of the decomposed *epi*-progoitrin). As in Austin's work, the remaining decomposed *epi*-progoitrin was unaccounted for.

The stability of thionamide and nitrile in moist hot crambe meal was measured at acid and base pH to determine if decomposition of these compounds could account for the imbalance noted between *epi*-progoitrin and its reaction products. The nitrile in either pH range was quite stable to heat when compared to thionamide (Table II). The instability of thionamide at basic pH explains why thionamide was not found in meal cooked with base additives. Presumably then, *epi*-progoitrin was decomposed to nitrile and thionamide during moist cooking and the thionamide in particular further decomposed to as yet unidentified products. No other products formed from the *epi*-progoitrin were identified.

While this manuscript was in preparation a U.S. patent was granted to Youngs et al. for the use of ferrous sulfate in the catalytic decomposition of progoitrin (an isomer of *epi*-progoitrin) in rapeseed meal (31). These workers using conditions similar to those reported here for the cooking of crambe meal reported the formation of nitrile but not thionamide as progoitrin decomposition products. They also indicated a quantitative yield of nitrile from the progoitrin decomposed in rapeseed meal compared to our 25% yield of nitrile from the *epi*-progoitrin of crambe meal. This may represent a distinct advantage for application of the iron treatment to crambe meal since formation of the nitrile is undesirable.

Rat Feeding Tests

Crambe meals cooked by the standard cook technique (Fig. 1) without additive (meal no. 1). with 0.5% ferrous sulfate (meal no. 2). and with 1% ferrous sulfate (meal no. 3) were prepared for a 90 day rat feeding study.

Decomposition of *epi*-progoitrin was promoted by ferrous sulfate so that glucosinolate content was reduced by more than 90% when this additive was included in the cook (Table III). The meal cooked without additive contained about 60% of the initial *epi*-progoitrin. The reduction in *epi*-progoitrin content was accompanied as expected by a rise in nitrile content corresponding to about 1/4 mole of nitrile for each mole of *epi*-progoitrin decomposed. A slight increase in thionamide also accompanied the reduction in *epi*-progoitrin. All crambe meals were similar in proximate analyses and amino acid content (Table IV).

The control ration contained sufficient protein so that blends of 30% crambe test meal to 70% control ration could be fed without reflecting changes in protein quality of the crambe meals.

Feeding results including autopsy data are summarized in Table V. The crambe meal in a standard cook without ferrous additive caused death of all rats within 14 days when fed at 30% of diet. When the level was reduced to 15% in the second test the rats responded about the same as did the rats fed the ferrous sulfate meals at twice the level (30%). This response was true both in growth and in body organ enlargement. Kidney, liver and thyroid enlargement ranged from 1.3 to 1.6 times the control for the ferrous sulfate-treated meals. All organ weights from rats consuming experimental meals were significantly heavier than controls (P 0.05) with the exception of thyroid weights of female rats fed crambe meal without additive. All three cooked crambe meals caused excretion of a yellow pigment which was quite evident in the wood shavings used as litter.

Two lesions related to crambe ingestion were detected in tissues of rats fed the meal cooked without ferrous sulfate. These were primary hyperplasia of the bile ducts and pancreatic acinar cell hyperplasia. None of the tissues of rats fed the iron-treated meals exhibited the liver bile duct hyperplasia, but the pancreatic hyperplasia was detected in some tissues of rats fed these meals.

The results of feeding creambe meal cooked without additives at 15% and 30% of diet are consistent with those of VanEtten (33). The additive-cooked meals were much less toxic than VanEtten's meals which were prepared through an enzymatic decomposition of *epi*-progoitrin. The quantity of nitrile found in each type of meal was the same; however the enzymatic method of decomposition produced two *epi*-thio nitriles in addition to the (S)-1-cyano-2-hydroxy-3-butene nitrile formed by the additive decomposition method. These differences in nitrile composition may explain the reduced toxicity. Since the nitriles represent less than half the *epi*-progoitrin decomposed in each meal, other products of the reaction not yet identified may account for toxicity differences between preparation methods.

There was very little difference between responses of rats fed 0.5% and 1.0% ferrous sulfate, indicating that the ferrous sulfate itself fed under these conditions was not toxic.

Although the ferrous sulfate-treated meals resulted in a substantial reduction in growth when fed to rats, these meals were a definite improvement over meal cooked without ferrous sulfate. The growth was somewhat lower than reported earlier for a soda ash-cooked meal; however in the case of ferrous sulfate a meal is obtained that contains adequate lysine for both rats and chicks.

ACKNOWLEDGMENTS

Analyses were performed by J.E. McGhee, L.T. Black, F.B. Alaksiewicz, P. Schulz, J.F. Cavins and M.E. Daxenbichler.

REFERENCES

1. Bruun, J.H., and J.R. Matchett, JAOCS 40:1-5 (1963).
2. Greene, J.L., Jr., E.L. Huffman, R.E. Burks, Jr., W.C. Sheehan and I.A. Wolff, J. Polym. Sci. 5:391-394 (1967).
3. Nieschlag, H.J., I.A. Wolff, T.C. Manley and R.J. Holland, Ind. Eng. Chem. Prod. Res. Devel. 6:120-123 (1967).
4. Chang, S., T.K. Miwa and I.A. Wolff, J. Polym. Sci. 5:2547-2556 (1967).
5. Nieschlag, H.J., W.H. Tallent, I.A. Wolff, W.E. Palm and L.P. Witnauer, Ind. Eng. Chem. Prod. Res. Devel. 6:201-204 (1967).
6. Mustakas, G.C., G. Kopas and N. Robinson, JAOCS 42:550A (1965).
7. Van Etten, C.H., M.E. Daxenbichler, J.E. Peters and I.A. Wolff, J. Agr. Food Chem. 13:24-27 (1965).
8. Hesketh, H.R., C.R. Creger and J.R. Couch, Poultry Sci. 42:1276 (1963).
9. Daxenbichler, M.E., C.H. VanEtten and I.A. Wolff, Biochemistry 4:318-323 (1965).
10. Tookey, H.L., C.H. VanEtten, J.E. Peters and I.A. Wolff, Cereal Chem. 42:507-514 (1965).
11. Kirk, L.D., G.C. Mustkas and E.L. Griffin, Jr., JAOCS 43:334-336 (1966).
12. Kirk, L.D., G.C. Mustakas and E.L. Griffin, Jr., Ibid. 43:550-555 (1966).
13. Mustakas, G.C., L.D. Kirk and E.L. Griffin, Jr., Ibid. 45:53-57 (1968).
14. Mustakas, G.C., L.D. Kirk and E.L. Griffin, Jr., Abstr. Papers,

Paper No. 54, 42nd Meeting, AOCS, New York, October 1968.
15. Van Etten, C.H., M.E. Daxenbichler, A.N. Booth, D.J. Robbins and I.A. Wolff, Abstr. AGFD No. 53, 158th Meeting, ACS, New York, September 1969.
16. National Acad. Sci.-Nat. Res. Counc., Publ. 827:7 (1960).
17. Lambert, John, D.C. Clanton, I.A. Wolff and G.C. Mustakas, J. Anim. Sci. 47:601-607 (1970).
18. Daxenbichler, M.E., C.H. VanEtten and I.A. Wolff, Biochemistry 5:692-697 (1966).
19. McGhee, J.E., L.D. Kirk and G.C. Mustakas, JAOCS 42:889-891 (1965).
20. Wetter, L.R., Can. J. Biochem. Physiol. 33:980 (1955).
21. Austin, F.L., C.A. Gent and I.A. Wolff, Can. J. Chem. 46:1507-1512 (1968).
22. Daxenbichler, M.E., G.F. Spencer, R. Kleiman, C.H. VanEtten and I.A. Wolff, Anal. Biochem. 38:374-382 (1970).
23. Rao, S.R., F.L. Carter and V.L. Frampton, Anal. Chem. 35:1927-1930 (1963).
24. Spackman, D.H., W.H. Stein and S. Moore, Ibid. 30:1190-1205 (1958).
25. American Oil Chemists' Society, "Official and Tentative Methods," including additions and revisions (1969).
26. Mustakas, G.C., L.D. Kirk, V.E. Sohns and E.L. Griffin, Jr., JAOCS 42:33-37 (1965).
27. Austin, F.L., C.A. Gent and I.A. Wolff, J. Agr. Food Chem. 16:752-755 (1968).
28. Austin, F.L., and C.A. Gent, Chem. Commun., 71 (1967).
29. Heywang, B.W., Poultry Sci. 36:715-718 (1957).
30. Cavanagh, G.C. (Ranchers Cotton Oil), U.S. Patent 2,934,431 (1960).
31. Youngs, G.C., H.R. Sallens and J.M. Bell, U.S. Patent 3,560,217 (1971).
32. McGhee, J.E., L.D. Kirk and G.C. Mustakas, JAOCS 41:359-362 (1964).
33. Van Etten, C.H., W.E. Gagne, D.J. Robbins, A.N. Booth, M.E. Daxenbichler and I.A. Wolff, Cereal Chem. 46:145-155 (1969).
34. Daxenbichler, M.E., C.H. VanEtten and I.A. Wolff, Phytochemistry 7:989-996 (1968).

[Received May 20, 1971]