Biological Evaluation of Crambe Meals Detoxified by Water Extraction on a Continuous Filter[1]

E.C. BAKER, G.C. MUSTAKAS, Northern Regional Research Center, ARS, USDA, Peoria, IL 61604, **M.R. GUMBMANN,** and **D.H. GOULD,** Western Regional Research Center, ARS, USDA, Albany, CA 94710

ABSTRACT AND SUMMARY

Crambe meals prepared by water extraction on a continuous filter when fed to rats gave protein efficiency ratios that were equal to or higher than the casein control, indicating that the water washing produced a palatable, nutritious meal. In a 4-week chick-feeding study, crambe was fed at 20% of the total diet. The diets containing crambe had somewhat lower gains (83-87% of control) and feed efficiency (94-95%) compared to the basal control group. Livers and kidneys appeared normal for all groups. There was some very slight gizzard erosion in the crambe-fed group. In a 90-day rat-feeding study, water-washed crambe was fed at 30% of the total diet, and body and organ weights were determined. Growth was slightly less than with the 30% soy control. There were no significant differences among relative organ weights for all groups. Results of feeding studies in rats and chicks indicate that the process of water extraction on a continuous filter can successfully prepare crambe meals with greatly reduced toxicity.

INTRODUCTION

Crambe oil has been used in a variety of applications (1). Crambe meal has a high protein content with a well balanced amino acid pattern. However, its biological quality is diminished by the presence of glucosinolates which are sources of toxic compounds (2). In 1965, workers at the Northern and Western Centers demonstrated that an autolyzed unheated crambe meal that contained no *epi*-progoitrin, the major glucosinolate in crambe, and no (R)-goitrin, the expected hydrolysis product, when fed to rats proved fatal within 2 weeks (3). After extraction with acetone, the meal gave essentially normal growth (4). The alternate products from autolysis were conclusively identified as the nitriles described by Daxenbichler et al. (5,6). The isolated nitriles as well as crambe seed meals containing them were later shown by rat-feeding studies to be much more toxic than isolated *epi*-progoitrin or (R)-goitrin or meals containing them with the enzymes inactivated (7). Later reports show autolysis of other Cruciferae plants also forms organic nitriles instead of the expected mustard oils (8). The poor growth of mice fed Bronowski rapeseed meal, which is low in total glucosinolates, has been attributed to the organic nitriles formed during meal preparation (9). These discoveries demonstrate that nitrile formation, in addition to previously recognized mustard oils and goitrin, complicates detoxification of oilseed meals from the Cruciferae.

Earlier studies demonstrated improved palatability and nutritional quality for ruminants by chemically modifying defatted crambe meal with soda ash (10), ammonia (11), or ferrous sulfate (12). Many reports have appeared in the literature on extraction of glucosinolates from Cruciferae oilseeds. Rapeseed meats and meals have been water extracted (13-16) by numerous workers. Crambe meal has been extracted with water, aqueous acetone, or aqueous methanol (17-18). Previous work at the Northern Center demonstrated a method for removal of toxic factors from defatted crambe meal by batch extraction with water (19). More recently a pilot-plant process was demonstrated for the continuous water extraction of 92 to 96% of the glucosinolates from defatted crambe meal (20). Quantities of meal sufficient for rat and chick feeding studies were prepared by this process, and the results of these feeding trials are presented in this paper.

[1]Presented at the AOCS meeting, Chicago, September 1976.

TABLE I

Analyses of Crambe Meals Prepared for Feeding Trials

Item	I Unwashed	II Water-washed	III Water-washed
Moisture, %	3.0	5.2	4.4
Ash, %	8.5	9.6	10.5
Oil, %	0.7	0.8	0.8
Fiber, %	6.7	8.3	9.0
Protein (N x 6.25), %	47.9	50.7	48.0
Sucrose, %	9.6	1.2	11.0
Dextrose, %	2.9	0.5	0.4
Other carbohydrates (by difference) %	20.7	23.7	25.3
	100.0	100.0	100.0
Total glucosinolates, %[a]	5.6	0.6	0.3
Available lysine, %	5.1	5.0	4.6
Thioglucosidase activity	Negative	Negative	Negative
Free goition, %	0.06	0.04	0
Free nitrile, %	0.04	0.03	0.03

[a]As *epi*-progoitrin sodium salt.

MATERIALS

The defatted crambe meal was slurried and water washed on a 6 sq ft continuous pilot-plant filter (20). Two water-washed crambe meals were prepared containing different amounts of residual glucosinolates by varying the quantities of wash water on the continuous belt filter. The third meal used in the feeding trials was unwashed crisped crambe. Analyses of these three meals are shown in Table I.

METHODS

Total glucosinolates were determined on the crambe meals by enzymatic conversion to goitrin by a modified procedure of Wetter (21). Meal (1 g) was extracted with boiling water to remove all the glucosinolates. A 2-ml sample of the water extract was enzymatically converted to goitrin in 4 ml of pH 7 butter containing 16 mg of myrosinase and held 2 hr at 55 C. The enzyme-converted solution was extracted twice with methylene chloride to remove goitrin; 50 ml of solvent was used for each contact, and final solvent volume was adjusted to 100 ml. Optical density of methylene chloride extract was read on a Beckman DB spectrophotometer at 5 mμ intervals from 210-280 mμ. Goitrin was analyzed by a method similar to that of Appelqvist and Josefsson (22). Organic nitriles were determined by IR absorption (23). Glucosidase activity was tested by the method of VanEtten et al. (24). Available lysine was determined by the method of Rao et al. (25),

TABLE II

Amino Acid Composition of Soy, Casein, and Crambe Proteins

Amino acid	g/100 g Protein Casein[a]	g/16 g N			
		Defatted soy meal[b]	Unwashed crambe meal I	Washed crambe meal II	Washed crambe meal III
Essential					
Arginine	4.1	7.6	6.6	6.8	7.5
Histidine	3.1	2.2	2.5	2.6	2.9
Isoleucine	6.1	4.4	4.0	4.4	4.7
Leucine	9.2	6.7	6.4	6.8	7.2
Lysine	8.2	6.0	5.3	5.6	6.4
Methionine	2.8	1.4	1.9	2.1	2.2
Phenylalanine	5.0	4.5	4.0	4.2	4.7
Threonine	4.9	3.7	4.5	5.0	5.5
Valine	7.2	4.5	4.8	5.2	5.5

[a]See Ref. 35.
[b]See Ref. 36.

and nonprotein nitrogen by the method of Becker et al. (26). Amino acids were analyzed by the method of Benson and Patterson (27). Crude fat (28), moisture (29), ash (30), crude fiber (31), and protein analyses (32) followed Official AOCS methods. Statistical means were compared by Duncan's method (33).

EXPERIMENTAL PROCEDURES

Protein Efficiency Ratio (PER) Bioassay

PER (34) and rat-feeding studies were conducted at the Western Regional Research Center. Each of the three crambe test meals was fed to rats for 28 days at a level to provide 10% protein in the diet. A fourth group was fed a casein control diet. There were five male weanling rats per group, Sprague-Dawley strain, initial age of 21 days and at an initial weight of 53 g.

Ninety-Day Rat Feeding Study

Six diets were tested. Water-washed crambe meals at two levels of residual glucosinolates were tested against soybean meal, all at 30% of the diet. Unwashed crambe meal was fed at 5, 10, and 15% to establish a dose-response for a positive control. In addition to soy or crambe meal, the diets contained 7% casein, 5% corn oil, 4% salts fortified with zinc (125 mg/kg) and cobalt (6 mg/kg), 2.2% vitamin mixture (nutritional Biochemicals), dl-methionine to provide methionine levels equal to that in the 30% crambe diets, and corn meal to make 100%. Thirty-six weanling male Sprague-Dawley rats were assigned to the six experimental diets so as to obtain as nearly equal initial mean body weight as possible for each group. They were housed three to a cage and fed the experimental diets ad libitum for 90 days. Individual body weights and feed consumption per cage were determined once a week.

At the end of the test period, the animals were killed by exsanguination under ether anesthesia, and blood samples were obtained from the brachial artery for hematology and clinical chemistry.

Hematology determinations included erythrocyte and leukocyte counts (determined on a Coulter Counter), packed cell volume, hemoglobin, and differential leukocyte counts. Clinical determinations on blood plasma (determined on an Autoanalyzer-II) included the enzymes glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, and ornithine carbamoyl transferase. Also measured were plasma urea nitrogen, albumin, total protein, and total bilirubin. Urine samples were collected from individual rats a few days prior to autopsy for standard urinalysis.

At autopsy, animals were subjected to complete gross examination. Tissues were routinely fixed in a 10% phosphate buffered formalin. Following fixation, tissues were embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin.

Four-Week Chick Feeding Study

Day-old male broiler chicks were randomly allotted to four groups of fifteen chicks each. The control group was fed a corn-soy basal diet while the other three groups were fed the basal diet plus 20% of the crambe test meals. The diets were adjusted to 20% protein through the addition of ground yellow corn and 44% soy meal. Chicks were housed in electrically heated battery brooders with raised wire floors. Test diets and water were offered ad libitum for the duration of the study.

At the conclusion of the 4-week feeding period, ten chicks from each group were randomly selected and asphyxiated with carbon dioxide. They were then examined grossly: the liver and kidneys checked for abnormalities, thyroids removed and weighed, and the gizzards examined for erosion. The chick-feeding trials were conducted at WARF Institute, Inc. at Madison, Wisconsin.

RESULTS AND DISCUSSION

The crambe meals used in the feeding trials had protein contents of 47.9 to 50.7% which compare favorably to soybean meal at ca. 49% protein (Table I). Sucrose and dextrose contents were considerably reduced by the water-washing procedure. Total glucosinolates were reduced by 92 to 96% in the washed crambe meals. Available lysine, a good indicator of heat damage during processing, remained fairly high in the range 4.6 to 5.1% (Table I) compared to 5.5% in the starting defatted meal (15). Thioglucosidase activity in the meals was negative, effectively inactivated by the moist heat in the crisping step. Conseuqently, free nitrile and goitrin, products of enzymatic hydrolysis, were held to very low values (Table I). The amino acid contents of the crambe meals are compared to casein and soybean meal in Table II. In general, higher contents of essential amino acids, except arginine, were found in crambe meals compared to soybean meal, which comprises a recognized good-quality oilseed protein.

Protein Efficiency Ratio

Groups fed diets containing unwashed crambe meal had final mean body weights significantly lower than the groups fed the casein control diet (Table III). Final mean body weights of groups fed diets containing water-washed crambe were higher though not significantly different from the control group.

Groups fed diets containing unwashed crambe consumed

TABLE III

Protein Efficiency Ratios for Processed Crambe Meals

Protein source		Protein[a] source in diet (%)	Final mean body weight (g)	Total feed consumption (g)	PER[b] Actual	PER[b] Adjusted	Percent digestibility[c] Diet	Percent digestibility[c] Nitrogen
Casein control		11.4	140Aa[d]	293Ab	2.93Aa	2.50	95	91
I	Unwashed crambe	22.2	44Bb	116Bc	−0.80Bb	−0.68	95	90
II	Water-washed crambe	19.7	164Aa	373Aa	2.96Aa	2.53	91	74
III	Water-washed crambe[e]	20.8	167Aa	352Aab	3.19Aa	2.72	90	73

[a]All diets calculated to contain 10% protein.

[b]PER = Protein Efficiency Ratio. Weight gain/grams protein intake.

[c]Digestibility: diet = feed intake−fecal wt/feed intake x 100; nitrogen = N intake−fecal N/N intake x 100.

[d]Duncan's Multiple Range Test: means without a superscript letter in common are significantly different; P = <0.05 = lower case; P = <0.01 = upper case.

[e]Data on four rats.

TABLE IV

Growth and Feed Consumption of Rats Fed Crambe and Soy Meals (Cumulative Data to 85 Days)

Meal fed	Level (%)	Growth[a] Mean weight gain (g)	Growth[a] Mean weight gain % of control	Feed consumption[b] Mean per rat per day (g)	Feed consumption[b] Mean per rat per day % of control	Feed efficiency Weight gain/feed consumption Ratio	Feed efficiency Weight gain/feed consumption % of control
I Unwashed crambe	5	399BC	88.7	17.9AB	83.6	0.262A	104.4
I Unwashed crambe	10	324C	74.0	16.0B	74.8	0.240AB	95.6
I Unwashed crambe	15	285D	66.4	16.5B	77.1	0.207B	82.4
II Water-washed crambe	30	401B	89.1	19.0AB	88.8	0.248AB	98.8
III Water-washed crambe	30	394B	87.7	18.2AB	85.0	0.255A	101.6
Soy control	30	457A[c]	100.0	21.4A	100.0	0.251A	100.0

[a]Six rats per group.

[b]Significance of differences among means determined by Duncan's multiple range test; means without a superscript letter in common are significantly different, $P < 0.05$.

[c]Values are means of two groups of three rats each.

less than half that consumed by the control group. The water-washed crambe diets were consumed at a higher rate (20-27%) than the casein control.

Groups fed diets containing unwashed crambe had a negative weight gain and thus a negative PER value. Both groups on the diets containing water-washed crambe gave higher though not significantly different PERs compared to the control group.

It is interesting to note that the severely limited feed intake of unwashed crambe meal was accompanied by relatively high digestibility figures; whereas for the other two meals which were ingested in greater amounts and produced adequate growth, digestibility was less complete, especially for nitrogen (Table III). This might possibly be explained be comparing the nitrogen solubility index figures of 25 for unwashed crambe compared to 4 or less for the washed meals. The washing procedure removes a significant quantity of the soluble protein which might be presumed to be more readily digestible.

Ninety-Day Toxicity Study in Rats

Rats fed the two water-washed crambe meals at two different levels of residual glucosinolates did not display growth significantly different from each other although both meals produced a growth rate somewhat lower than the soy control (88-89% of control) (Table IV). The initial weekly feed consumption rate was significantly greater for the soy control and inversely related to the percent of unwashed crambe in the diet; however, these differences disappeared at 43 days and were not significantly different for the balance of the experiment. These differences were also evident in the overall mean feed consumption values shown in Table IV. Comparison of feed efficiency for both water-washed crambe meals at the 30% level vs. unwashed crambe at the 15% level indicates a toxic effect for unwashed crambe, as expected (Table IV). The feed efficiency of diets containing water-washed crambe meal was initially less than that of soy, but differences were never significant. This may reflect nutritional differences rather than any toxicity.

Body and organ weights at autopsy are given in Table V. Liver enlargement, relative to body weight, is evident from feeding unwashed crambe. There was a similar tendency with relative kidney, brain, and testes weights. The data suggest for these latter three organs that the relative organ weights may have been altered by growth retardation per se, rather than by a direct effect of crambe on the organs. Unwashed crambe also brought about decreased realtive spleen weight and distinct thyroid enlargement. There were no significant differences among relative organ weights of rats fed the soy control and washed crambe meals II and III.

Plasma enzymes and other blood constituents were measured to detect adverse effects on liver and kidney function. Plasma alkaline phosphatase increased in response to unwashed crambe in the diet; however, this enzyme is also present in tissues other than liver. Ornithine carbamoyl transferase, which is believed to be highly specific for liver damage, the two transaminases, and alkaline phosphatase showed no significant differences in activity between the soy control and either of the water-washed crambe samples. Differences in plasma urea nitrogen, albumin, total protein, and total bilirubin between the water-washed crambe diets and the soy control were not significant (except for slightly

TABLE V

Body and Organ Weights of Rats Fed Crambe and Soy Meals for 90 Days

Meal fed	Body weight (g)	Percent of body weight							
		Liver (%)	Kidneys (%)	Spleen (%)	Heart (%)	Testes (%)	Brain (%)	Adrenals (%)	Thyroids (%)
I Unwashed crambe, 5%	445^{Ba}	2.8^{C}	0.73^{AB}	0.15^{BC}	0.28^{A}	0.80^{AB}	0.45^{C}	10.8^{A}	8.8^{BC}
I Unwashed crambe, 10%	388^{C}	3.2^{B}	0.76^{A}	0.17^{AB}	0.28^{A}	0.87^{AB}	0.50^{B}	13.3^{A}	11.1^{AB}
I Unwashed crambe, 15%	348^{D}	3.8^{A}	0.77^{A}	0.14^{C}	0.27^{A}	0.94^{A}	0.55^{A}	12.9^{A}	13.6^{A}
II Water-washed crambe, 30%	461^{B}	2.8^{C}	0.71^{AB}	0.18^{AB}	0.29^{A}	0.87^{ABb}	0.44^{C}	12.4^{A}	7.9^{C}
III Water-washed crambe, 30%	447^{B}	2.7^{C}	0.68^{B}	0.19^{A}	0.29^{A}	0.74^{B}	0.46^{BC}	12.9^{A}	6.6^{C}
Soy control, 30%	516^{Ab}	2.6^{C}	0.69^{AB}	0.18^{AB}	0.30^{A}	0.72^{B}	0.43^{C}	13.0^{A}	7.5^{C}

[a]Duncan's multiple range test. Means without a superscript letter in common are significantly different $P < 0.05$. Data on six animals per group.

[b]Data on five animals.

TABLE VI

Results of 4-Week Feeding of Crambe and Corn-Soy Meals to Chicks[a]

Diet	Growth		Feed consumption		Feed efficiency	
	Mean weight gain		Mean per chick		Weight gain/feed consumption	
	(g)	% of control	(g)	% of control	Ratio	% of control
Corn-soy basal diet (CSBD) control	558	100.0	970	100.0	0.57	100.0
CSBD + 20% unwashed crambe I	461	82.6	881	90.8	0.52	91.0
CSBD + 20% water-washed crambe II	478	85.7	884	91.1	0.54	94.0
CSBD + 20% water-washed crambe III	457	81.9	834	86.0	0.55	95.3

[a]Fifteen chicks per group.

TABLE VII

Body and Thyroid Weights of Chicks Fed Crambe and Corn-Soy Meals for 4 Weeks

Diet	Mean body weight		Thyroid weight		Mg thyroid/100 g body weight	
	(g)	% of control	(mg)	% of control	Ratio	% of control
Corn-soy basal diet (CSBD) control	593^{Aa}	100.0	47^{B}	100.0	7.9^{B}	100.0
CSBD + 20% unwashed crambe I	526^{BA}	88.7	154^{A}	327.6	29.3^{A}	370.6
CSBD + 20% water-washed crambe II	539^{BA}	90.9	39^{BC}	82.9	7.2^{B}	91.6
CSBD + 20% water-washed crambe III	491^{B}	82.8	37^{C}	78.7	7.5^{B}	95.4

[a]Duncan's multiple range test. Means without a superscript letter in common are significantly different $P < 0.05$. Data on ten animals per group.

lowered total protein associated with the crambe II diet) and revealed no clear adverse effects. Slight increases in urea nitrogen, albumin, and total protein were associated with ingestion of unwashed crambe I diets.

No significant differences in hematologic and urinalysis data were noted between rats fed water-washed crambe and the soy control. Histologic evaluation indicated that diet-related lesions were confined to the kidneys, thyroids, and possibly pituitary glands. In the kidneys, nuclear enlargement (nephrocytomegalia) in the straight portion of the proximal convoluted tubule was observed to be related in incidence and severity to the amount of unwashed crambe meal in the diet. This lesion was found to occur to a minor extent in both washed crambe meal diets and soy control.

Thyroid lesions (described as follicular epithelial vacuolation, disorganization, and diminution in size) were also generally related in incidence and severity to the amount of unwashed crambe in the diet. A low incidence of vacuolation was evident in the washed crambe meal groups and absent in the soy control, while a low incidence of the remaining two thyroid lesions was present in all three dietary groups.

Vacuolation of anterior pituitary cells was observed in all groups, but was most pronounced in rats fed 15% unwashed crambe meal. Liver lesions as described in a previous crambe meal feeding study (7) were not evident in these animals.

TABLE VIII

Gizzard Erosion of Chicks Fed Crambe and Corn-Soy Meals for 4 Weeks

Diet	Gizzard erosion lesions	
	Absent	Present
Corn-soy basal diet (CSBD) control	10	0
CSBD + 20% unwashed crambe I	2	8[a]
CSBD + 20% water-washed crambe II	6	4[a]
CSBD + 20% water-washed crambe III	7	3

[a]Significantly different at 0.05 level (single tail).

Four-Week Chick Feeding Study

Chicks fed the three diets which contained crambe meals had slightly lower gains (82-86%) compared to the control basal group. Feed consumption was 86 to 91% of the control, and feed efficiencies were in the range of 91 to 95% of the control basal group (Table VI).

The group fed the unwashed crambe meal gave a significantly higher ratio of thyroid weight to body weight as compared to control basal group. Groups fed either water-washed crambe meals II or III gave ratios of thyroid weight to body weight that were not significantly different from

the control group (Table VII).

The group fed the unwashed crambe meal had the largest number of gizzard erosions and showed a significant difference from the basal control group (Table VIII). The groups fed the water-washed crambe meals also exhibited some gizzard erosion. However, the group fed the more thoroughly washed crambe meal III had gizzard erosion that was not significantly different from the control group.

ACKNOWLEDGMENTS

At the Northern Center, analyses were made by L.T. Black, J.D. Glover, F.B. Alaksiewicz, C.L. Harlan, J.E. McGhee, and D.E. Uhl. Amino acid analyses were made by J.F. Cavins. The pilot-plant equipment was operated by R.L. Brown and J. Kerr (deceased). At the Western Center, technical assistance in rat-feeding studies was contributed by D.J. Robbins, V.V. Horring, and G.M. Dugan. Chick-feeding studies were conducted by R.M. Bodden and E.S. Robaidek, Wisconsin Alumni Research Foundation.

REFERENCES

1. Neischlag, H.J., and I.A. Wolff, JAOCS 48:723 (1971).
2. VanEtten, C.H., and I.A. Wolff, in "Toxicants Occurring Naturally in Foods," Edited by F.M. Strong, National Academy of Sciences, Washington, DC, 1973, Chapter 10, pp. 210-223.
3. VanEtten, C.H., M.E. Daxenbichler, J.E. Peters, I.A. Wolff, and A.N. Booth, J. Agric. Food Chem. 13:24 (1965).
4. Tookey, H.L., C.H. VanEtten, J.E. Peters, and I.A. Wolff, Cereal Chem. 42:507 (1965).
5. Daxenbichler, M.E., C.H. VanEtten, W.H. Tallent, and I.A. Wolff, Can. J. Chem. 45:1971 (1967).
6. Daxenbichler, M.E., C.H. VanEtten, and I.A. Wolff, Phytochemistry 7:989 (1968).
7. VanEtten, C.H., W.E. Gagne, D.J. Robbins, A.N. Booth, M.E. Daxenbichler, and I.A. Wolff, Cereal Chem. 46:145 (1969).
8. Daxenbichler, M.E., C.H. VanEtten, and G.F. Spencer, J. Agric. Food Chem. (In press).
9. Josefsson, E.J., J. Sci. Food Agric. 26:1299 (1975).
10. Mustakas, G.C., L.D. Kirk, and E.L. Griffin, Jr., JAOCS 45:53 (1968).
11. Kirk, L.D., G.C. Mustakas, and E.L. Griffin, Jr., Ibid. 43:550 (1966).
12. Kirk, L.D., G.C. Mustakas, and E.L. Griffin, Jr., Ibid. 48:845 (1971).
13. Ballester, D., R. Rodrigo, J. Narouzi, C.O. Chichester, E. Yanez, and F. Monckeberg, J. Sci. Food Agric. 21:143 (1970).
14. Eapen, K.E., N.W. Tape, and R.P.A. Sims, JAOCS 46:52 (1969).
15. Sulsulski, F.W., F.S. Soliman, and R.S. Bhatty, Can. Inst. Food Sci. Technol. J. 5:101 (1972).
16. Tape, N.W., Z.I. Sabry, and K.E. Eapen, Ibid. 3:78 (1970).
17. VanEtten, C.H., M.E. Daxenbichler, and I.A. Wolff, Abstract AGFD 53, 158th National Meeting of the American Chemical Society, New York, September, 1969.
18. VanEtten, C.H., M.E. Daxenbichler, J.E. Peters, I.A. Wolff, and A.N. Booth, J. Agric. Food Chem. 13:24 (1965).
19. Mustakas, G.C., L.D. Kirk, E.L. Griffin, Jr., and A.N. Booth, JAOCS 53:12 (1976).
20. Baker, E.C., G.C. Mustakas, and V.E. Sohns, Ibid. 52(2):Abst. 25 (1975).
21. Wetter, L.R., Can. J. Biochem. Physiol. 35:293 (1957).
22. Appelqvist, L.A., and E.J. Josefsson, J. Sci. Food Agric. 18:510 (1967).
23. Daxenbichler, M.E., C.H. VanEtten, and I.A. Wolff, Biochemistry 5:692 (1966).
24. VanEtten, C.H., C.E. McGraw, and M.E. Daxenbichler, J. Agric. Food Chem. 22:483 (1974).
25. Rao, S.R., F.L. Carter, and V.L. Frampton, Anal. Chem. 35:1927 (1963).
26. Becker, H.C., R.T. Milner, and R.H. Nagel, Cereal Chem. 17:447 (1940).
27. Benson, J.V., Jr., and J.A. Patterson, Anal. Chem. 37:1108 (1965).
28. "Official and Tentative Methods of the American Oil Chemists' Society," Vol. I, Second edition, AOCS, Champaign, IL, 1964 (Revised to 1969), Method AC-3-44.
29. Ibid., Method AC 2-41.
30. Ibid., Method Ba 5-49.
31. Ibid., Method Ba 6-61.
32. Ibid., Method AC 4-41.
33. Duncan, D.B., Multiple Range and Multiple F Tests, Biometrics 11:1 (1955).
34. Committee on Protein Malnutrition, Food and Nutrition Branch, "Evaluation of Protein Quality," Washington DC, National Academy of Science–National Research Council, Publication No. 1100, 1963.
35. Gordon, W.G., W.F. Semmett, and M.J. Bender, J. Am. Chem. Soc. 72:4282 (1950).
36. Rackis, J.J., R.L. Anderson, H.A. Sasame, A.K. Smith, and C.H. VanEtten, J. Agric. Food Chem. 9:409 (1961).

[Received May 9, 1977]