Importance of Cellular Constituents to Cottonseed Meal Protein Quality

WILDA H. MARTINEZ,[1] LEAH C. BERARDI,[1] VERNON L. FRAMPTON,[1]
HAROLD L. WILCKE,[2] DARYLE E. GREENE,[2] AND ROBERT TEICHMAN[2]

To determine the relative importance of cellular constituents to the nutritive value of cottonseed meals, three meals differing in chemical content were prepared from glandless cottonseed by extracting: free lipids, phospholipids from portions of the lipid-free material, and both phospholipids and carbohydrates. Treatments consisted of autoclaving each meal in the presence and absence of pure gossypol or of gossypol contained in a fraction of isolated cottonseed pigment glands. In a 4-week feeding test with weanling rats, the unautoclaved meals were equal in nutritive value to the soybean meal control. Autoclaving reduced the protein efficiency ratio (PER) of all meals, but less in the meals from which the phospholipids and carbohydrates were removed. The binding of gossypol and its effects on protein quality were dependent on both its physical state and the other cellular constituents of the meal. Within a series, PER was significantly correlated with the ε-free lysine content. However, the processing history of the cottonseed meal must be considered in any comparison of ε-free lysine values as criteria of protein quality.

A previous study investigated (Martinez *et al.*, 1961) the relative importance of gossypol and raffinose to the binding of lysine and the impairment of the nutritive value of the protein of cottonseed meal by exhaustively extracting glandless cottonseed meal with 80% ethanol, adding back appropriate amounts of raffinose and gossypol, and autoclaving to simulate the production of cottonseed meal. Under such conditions, autoclaving in the presence of raffinose and gossypol produced no greater binding of lysine or impairment of growth than autoclaving with raffinose alone. However, no treatment brought about so great a destruction of lysine as that caused by autoclaving the unextracted defatted glandless meal. Thus other factors in addition to gossypol and raffinose were implicated in the reactions with the lysine of cottonseed.

To augment the validity of the previous conclusions and to extend the range of constituents examined, the present experiment was designed to investigate the importance of the phospholipids, carbohydrate, and gossypol constituents to the nutritive quality of cottonseed meal. Selective extraction, rather than exhaustive extraction and readdition, was used to provide defatted meals from which only the phospholipid or both the phospholipid and carbohydrate constituents were removed. These meals, produced from glandless cottonseed, were autoclaved in the presence and absence of gossypol pigments and evaluated by chemical and animal assay.

Experimental

CM72. Glandless cottonseed was hulled, flaked, cold-hexane extracted, desolventized at room temperature, and sieved through a 30-mesh screen. These procedures removed the major portion of the hull and the freely extractable lipid.

CM72-CM. Portions of CM72 (225 grams) were extracted with six 1-liter quantities of chloroform-methanol (2 to 1 by volume), followed by three 1-liter quantities of diethyl ether, and allowed to equilibrate with atmospheric moisture. This produced a meal from which the phospholipid and the green pigments of the seed had been removed.

CM72-A. Additional portions of CM72 (225 grams) were extracted with 20 1-liter quantities of 80% aqueous ethanol, followed by three 1-liter quantities of diethyl ether, and allowed to equilibrate to atmospheric moisture. This procedure removed phospholipids, major carbohydrates, free amino acids, green pigments, and flavanoids.

G. Gossypol–acetic acid was hydrolyzed to free gossypol which was then recrystallized three times from ether xylene.

GP. Isolated cottonseed pigment glands were extracted to provide a water-dispersible fraction GP (Figure 1). Multiple portions of GP were obtained by mechanically shaking 5-gram lots of pigment glands in 100 ml. of water for 10 minutes and filtering through a coarse sintered glass funnel by gravity. The residues were further extracted successively with a 100-ml. and three 30-ml. portions of water, and the combined water extracts were dried by lyophilization.

GR. The water-indispersible residues were combined and dried similarly.

Crystalline gossypol (G) and the water-dispersible fraction of pigment glands (GP) were added to portions of each of the meals (CM72, CM72-A, CM72-CM) in

Pigment Glands	
	Exhaustive extraction with water
Dispersible	Residue
Fraction GP	Residue GR
80% by weight	19% by weight
21% gossypol	8% gossypol
2% nitrogen	8% nitrogen

Figure 1. Fractionation of pigment glands

[1] Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, New Orleans, La.

[2] Ralston Purina Co., Checkerboard Square, St. Louis, Mo.

appropriate amounts to provide approximately 1% gossypol in the meal. The materials were dry-mixed in a Hobart mixer for 20 minutes, spread in trays to a height of 0.5 inch, exposed to the atmosphere overnight, then autoclaved for 20 minutes at 122° C., 18 p.s.i., and vacuum-dried for 3 minutes followed by exhaust drying for 15 minutes while the autoclaving temperature slowly dropped. Fraction GR was combined in a like manner with CM72 to provide approximately a 1% concentration of gossypol in the meal. After autoclaving, the unreacted gossypol in each treatment was reduced to minimum levels by successive extraction with diethyl ether at room temperature.

Appropriate portions of each meal (CM72, CM72-CM, CM72-A) were autoclaved in the same manner without the addition of any gossypol pigments. The ϵ-lysine content of the meals was determined by the method of Rao *et al.* (1963). The Kjeldahl procedure was used for nitrogen determination. Gossypol contents of the meals and rations were determined by the procedure for use with mixed feeds (Pons and Hoffpauir, 1957) as well as by recommended methods (American Oil Chemists' Society, 1946–58).

The meals were evaluated for protein quality essentially according to the method of Derse (1960). Dextrose, however, was substituted for the recommended sucrose in the basal diet, as seen in Table I, to provide a more desirable texture to the ration. In subsequent evaluations of the method, dextrose was an adequate substitute (Derse, 1965).

Because of the dry powdery nature of the resulting meals and to ensure optimum feed intake and minimum feed wastage, each of the prepared rations was combined with water at 37° C. until it was of a doughy consistency. The dough was spread to a depth of 1 inch on aluminum foil, cut into 1-inch cubes which were allowed to dry at room temperature for 24 hours, and then stored under freezing conditions until needed.

One hundred twenty-six male weanling rats were allotted to individual cages according to a randomized block design with 9 rats assigned to each of 14 treatments. Feed intake and total protein consumption per animal were calculated to a moisture-free basis.

Table I. Composition of Basal Diet

Ingredient	
Protein source	A
Cottonseed oil, refined	$8 - \left(\frac{A \times \%\text{ ether extract}}{100}\right)$
Salt mixture (USP XIV)[a]	$5 - \left(\frac{A \times \%\text{ ash}}{100}\right)$
Cellulose	$1 - \left(\frac{A \times \%\text{ crude fiber}}{100}\right)$
Vitamin mixture[b]	2.2
Dextrose q.s.	100

$$A - \frac{9.09 \times 100}{N \times 6.25}$$

[a] Salt mixture supplies in mg. per 100 grams of diet: $CuSO_4 \cdot 5H_2O$, 0.39; $FeC_6H_5O_7 \cdot 5H_2O$, 76.41; $MnSO_4$, 1.01; $AlNH_4(SO_4)_2 \cdot 12H_2O$, 0.46; KI, 0.21; NaF, 2.54; $CaCO_3$, 343.0; $Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$, 541.5; CaH_2PO_4, 564.0; $MgCO_3$, 176.0; $MgSO_4$, 191.5; KCl, 623.5; K_2HPO_4, 1094.0; NaCl, 385.5.

[b] Vitamin mixture supplies per 100 grams of diet: Vitamin A, 2000 I.U.; vitamin D, 200 I.U.; vitamin E, 10 I.U.; choline, 120 mg.; *p*-aminobenzoic acid, 10 mg.; inositol, 10 mg.; niacin, 10 mg.; calcium pantothenate, 6.6 mg.; riboflavin, 2 mg.; thiamine hydrochloride, 2 mg.; pyridoxine hydrochloride, 2 mg.; menadione, 5 mg.; folic acid, 0.2 mg.; biotin, 0.05 mg.; and vitamin B_{12}, 0.003 mg.

Results and Discussion

The interpretation of complex reactions on the basis of model or semipurified reaction systems is always subject to verification in the whole material, particularly with reactions which involve cellular structures and membranes and complex pigment mixtures, such as those found in cottonseed. For this reason, selective extraction, rather than exhaustive extraction and readdition, was chosen as the method for this experiment.

Glandless cottonseed, which has been hulled, flaked, and cold-hexane-extracted, provides a material (CM72) that retains a large portion of the cell structure, the protein, carbohydrates, phospholipids, and flavonoids of the original seed. The cells of the cottonseed, according to recent cytological studies (Yatsu, 1965) are filled throughout with specific structures termed aleurones or protein bodies which contain the major portion of the protein of the cell. These protein bodies are embedded with globoids and surrounded by a mesh-like reticulum of spheresomes which are thought to be associated with the phospholipid and freely extractable lipid of the seed. In CM72 therefore, only that lipid which was freely extractable by hexane was removed, and the protein remained localized in the presence of the carbohydrate, phospholipid, and flavonoid constituents of the seed.

Chloroform-methanol extraction is one of the classic procedures for the isolation of the phospholipids of membranes and tissues. Such an extraction of defatted cottonseed should disrupt the various membranous structures of the cell which contain phospholipids and remove most of the phospholipids. In addition, chloroform-methanol extraction removes the nongossypol green pigments of the seed. The defatted cottonseed meal, after chloroform-methanol extraction, provided a material (CM72-CM) which contained the proteins, the carbohydrates, and the water-soluble flavonoids minus the phospholipids.

The previous experiment (Martinez *et al.*, 1961) demonstrated that exhaustive extraction with 80% ethanol removed the phospholipids, the flavonoids, the free amino acids, and the major carbohydrate constituents of the seed. It did not remove either the phytin or a high molecular weight pentosan which may or may not be chemically associated with the protein. Such an extraction produced a meal (CM72-A) from which both the phospholipid and major carbohydrates were removed.

In addition to the normal complement of cell types and constituents, "glanded" cottonseed contains discrete entities called pigment glands. These glands, which are dispersed throughout the cotyledon, isolate gossypol from the seed tissue. Gossypol comprises 20 to 40% of the weight of the isolated gland. The gland

wall contributes an additional 16% of the weight. The remaining constituents found in association with the gossypol, except for a minor protein fraction (1 to 2%), have yet to be characterized (Martinez and Berardi, 1963).

During the moist cooking phase of the normal processing of glanded cottonseed, the pigment glands rupture and exude their contents over the surrounding cellular constituents. Since the major portion of this exudate is not gossypol, it was deemed necessary to investigate the reactivity of recrystallized gossypol as well as both the aqueous dispersion (GP) and residue of pigment glands (GR).

The gossypol contents of the meals (CM72, CM72-CM, and CM72-A), after dry mixing with recrystallized gossypol or pigment gland fractions, after autoclaving, and after exhaustive extraction with ether are recorded in Table II. The loss in "total" gossypol which occurred on autoclaving is also included. Nearly 50% of the crystalline gossypol (G) was bound to the whole meal (CM72) by the dry mixing, free-steam autoclaving procedure. In the absence of the phospholipids of the meals (CM72-CM, CM72-A), very little of the original 1% of crystalline gossypol reacted to give the "bound" form.

The difference between these data and those of the previous experiment (Martinez *et al.*, 1961) on the binding of gossypol to glandless meal denotes another parameter of this reaction—i.e., the physical state of the gossypol in addition to the meal (dry mixing *vs.* acetone solution of the gossypol).

In the previous experiment, crystalline gossypol, incorporated as an acetone solution, produced a 50% ether-insoluble binding of the gossypol to the alcohol extracted meal by mixing alone. This occurred in the absence of phospholipids and without autoclaving. The present experiment demonstrated that crystalline gossypol, incorporated by dry mixing, did not react with the meal, even under conditions of moist autoclaving in the absence of the phospholipid constituents of the meal.

The significance of the meal constituents and the physical state of the gossypol to the "binding" of gossypol with the meal is complicated further by the results obtained with the pigment gland fractions. Under the same conditions of dry mixing and autoclaving used with crystalline gossypol, the gossypol of fraction GP reacted with all meals virtually to the same extent irrespective of the constituents present in the meal. Whether this reaction was due to the presence of constituents other than gossypol in this water-dispersible fraction, which achieved the same effect as the phospholipids of the meal—i.e., intimate contact of the gossypol with the meal constituents in the presence of moist heat—or whether it was due to a particularly reactive, complexed, or associated form of gossypol to be found in this fraction cannot be ascertained from these data. The present analytical procedures failed to indicate any major differences in the type of gossypol present in this fraction. Ninety-three per cent of the gossypol of fraction GP analyzed as "free" gossypol.

The analytical procedures also failed to account completely for the gossypol. In each preparation, after autoclaving, some portion of the gossypol was unaccounted for by the analytical procedures. The greatest loss occurred when meal CM72 and the pigment gland extract GP were mixed and autoclaved. The oxalic acid hydrolysis procedure provided an even poorer gossypol recovery from autoclaved meals than the mixed feed procedure. However, the mixed feed and the official AOCS procedures for "free" gossypol gave comparable results.

The dual role of gossypol as a toxic constituent and as a determinant of cottonseed protein quality has been well documented (Altschul *et al.*, 1958). To eliminate the toxic effects of "free" gossypol, each of the meals containing gossypol was exhaustively extracted with diethyl ether. The nature of the complexed or "lost" gossypol which was unaccounted for by the analytical procedures, its solubility in ether, and its biological activity were not ascertained. However, the whole meal (CM72) plus GP, the treatment which had the greatest loss in gossypol on autoclaving, produced no

Table II. Gossypol Contents of Meal Preparations[a]

Meal	Crystalline Gossypol, %				Fraction GP, %				Fraction GR, %			
	Total	Free	Bound	Lost	Total	Free	Bound	Lost	Total	Free	Bound	Lost
CM72												
Mixed	1.08	1.10	...	...	1.05	1.03	0.02	...	1.20	0.95	0.25	...
Autoclaved	1.01	0.57	0.44	6.5	0.67	0.11	0.56	32.6	0.93	0.27	0.66	22.5
Extracted	0.45	0.02	0.43	...	0.71	0.09	0.62	...	0.83	0.13	0.70	...
CM72-CM												
Mixed	1.15	1.24	...	...	1.05	1.02	0.03	...				
Autoclaved	0.97	0.98	...	15.7	0.77	0.21	0.56	26.7				
Extracted	0.18	0.02	0.16	...	0.70	0.14	0.56	...				
CM72-A												
Mixed	1.22	1.26	...	...	1.05	1.05	...	...				
Autoclaved	1.09	0.90	0.19	10.7	0.86	0.27	0.59	18.1				
Extracted	0.16	0.01	0.15	...	0.78	0.17	0.61	...				

[a] All gossypol contents calculated on a moisture-free basis.

symptoms of gossypol toxicity discernible by feed consumption or autopsy when fed to weanling rats for 4 weeks at a 20% protein level. Certain pertinent chemical characteristics of the meal treatments and the data from the feeding trial are summarized in Table III.

The results for the three unautoclaved meals (CM72, CM72-CM, and CM72-A) demonstrated that the growth response of rats to glandless cottonseed meal was comparable to that produced by the casein and soybean meal controls. Of the three cottonseed meals, the alcohol-extracted meal gave the highest live weight. This was consistent with the protein intake.

Autoclaving markedly lowered the weight gains, PER values, and ϵ-free lysine contents of the three meals. The removal of the phospholipids (CM72-CM) failed to reduce significantly the effects of autoclaving. CM72-A, which contained neither the major carbohydrates nor the phospholipid constituents of the meal, had a significantly higher PER than that of the autoclaved whole meal (CM72). This verified the importance of the carbohydrate constituents to the deleterious effects of heat on protein quality.

The PER of the autoclaved CM72-A was, however, significantly lower than that of the unautoclaved meal. This reduction and the reduction in ϵ-free lysine content were probably due to the reaction of those residual carbohydrates which may be chemically associated with the protein and/or to the interaction of the side chains of amino acid constituents of the protein.

Autoclaving each of the meals in the presence of 1% gossypol caused no significant change in PER or ϵ-free lysine content beyond that of autoclaving alone. Though the gossypol did not react with the meal in the presence of the carbohydrates (CM72-CM+G) this does in part support the results of the previous experiment.

Gossypol is known to possess pronounced antioxidant properties (Bickford *et al.*, 1954). It is possible that the unusually high protein intake and weight gain of the rats on CM72-CM+G were due to the presence of the gossypol during autoclaving which, perhaps, prevented some reaction, or that autoclaving produced a factor common to CM72-CM and CM72-CM+G which was removed from CM72-CM+G by the ether extraction of the "free" gossypol. The fact that there was no significant change in the ϵ-free lysine content of meal CM72+G when nearly 50% of the crystalline gossypol did react with the whole meal suggests that under these conditions the gossypol did not react with the ϵ-free lysine residues of the cottonseed protein.

Autoclaving in the presence of the pigment gland fractions (GP, CR) which contained approximately 1% gossypol reduced ϵ-free lysine content, weight gain, and PER of each meal beyond those obtained on autoclaving alone. The PER values of autoclaved CM72A and CM72A+GP differ significantly at the $P = 0.05$ level. The other autoclaved meals differ from their pigment gland containing counterparts (CM72+GP, CM72-CM+GP) at the $P = 0.01$ level. The greatest reduction in all their characteristics was obtained with the whole glandless meal (CM72+GP). The importance of the phospholipids to this reaction was demonstrated by the significant increase in PER of CM72-CM+GP (phospholipids removed). When both the carbohydrates and phospholipids are extracted, there is an additional numerical increase in the value of these characteristics.

These results are in direct contrast to the results obtained with crystalline gossypol in both the previous and the present experiments, where the reduction, due to autoclaving in the presence of gossypol and raffinose is no greater than that obtained with raffinose alone. The gossypol of the pigment glands under the conditions of this experiment is far more reactive than crystalline gossypol, and this reaction is detrimental to the protein quality of the meal. Though this reaction is reflected in a lowering of the ϵ-free lysine content of the meal, this decrease varies with the meal constituents present as does PER. Yet the "bound" gossypol content of the three meals is essentially constant. This indicates that the gossypol must have reacted with other constituents in addition to the ϵ-free lysine groups of the cottonseed protein and that these constituents could be different in each meal.

When the GR fraction of the pigment glands was combined with CM72, the gossypol binding after autoclaving was numerically the highest, but the reduction in ϵ-free lysine was not as great as that obtained with the GP fraction of the pigment glands. Since the GR fraction consisted primarily of the unextractable gossypol in association with the pigment gland wall which itself contains 8% nitrogen (Martinez and Berardi, 1963), little can be concluded as to the nature of the lysine and gossypol binding in this treatment. A comparison of the feed consumption, gossypol intake, ϵ-free lysine contents, and PER of CM72+G, CM72+GR, and CM-72-CM+GP suggests that some portion of the bound gossypol may have retained physiological activity.

In a multiple regression of PER on ϵ-free lysine content of the meal and ϵ-free lysine consumed, the ϵ-free lysine content appears to be the more important value. In every case, the sum of squares attributable to the regression was numerically greater for ϵ-free lysine content than for ϵ-free lysine consumed. This was true for each of the three groups and for each of the three sources of variability in a joint analysis of variance of the 108 sets of observations. In no case does the inclusion of ϵ-free lysine consumed, as a second independent variable, add a significant amount to the multiple regression. This is true for the three meals and also for the three sources of variability in the joint analysis of variance.

The regression coefficients of PER on ϵ-free lysine content of the meal and on ϵ-free lysine consumed for the three meals and their treatments are included in Table III. Each regression is highly significant and there are significant differences among them at $P < 0.01$. This would seem to indicate that the efficiency of the utilization of the lysine of cottonseed protein differs with the presence or absence of certain cellular constituents. A more acceptable explanation lies perhaps in an examination of the ϵ-free lysine determination. The precision of the method is well established (Rao *et al.*, 1963). El-Nockrashy (1965) has suggested, however, that the presence of carbohydrates in the reaction mixture can

Table III. Summary of Feeding Data

Meal	Treatment: None	Autoclaved	G, Gossypol added autoclaved	GP, Pigment gland extract added autoclaved	GR, Pigment gland residue added autoclaved	Regression coefficient PER on ε-free lysine
CM-72						
ε-free lysine content[a]	3.7	3.4	3.3	2.8	3.3	1.519[b]
Feed consumption	403	362	307	296	295	
Gossypol intake						
Free	0.000	0.000	0.012	0.047	0.065	
Total	0.000	0.000	0.126	0.231	0.268	
Protein intake	32.2	31.6	26.0	25.8	25.9	
ε-free lysine intake	1.19	1.07	0.86	0.72	0.85	2.477[b]
Weight gain	75.3	49.7	42.5	23.6	29.6	
PER	2.34	1.57	1.64	0.91	1.06	
Duncan multiple range test[c]	bc	ef	def	g	g	
CM72-CM						
ε-free lysine content	3.9	3.4	3.4	3.1		1.203
Feed consumption	380	297	378	317		
Gossypol intake						
Free	0.000	0.000	0.011	0.067		
Total	0.000	0.000	0.068	0.276		
Protein intake	29.8	25.2	34.5	26.8		
ε-free lysine intake	1.16	0.86	1.17	0.83		1.656
Weight gain	71.1	42.4	62.1	37.6		
PER	2.38	1.68	1.79	1.43		
Duncan multiple range test	ab	de	de	f		
CM72-A						
ε-free lysine content	4.1	3.8	3.7	3.5		0.953
Feed consumption	421	380	342	324		
Gossypol intake						
Free	0.000	0.000	0.003	0.062		
Total	0.000	0.000	0.051	0.240		
Protein intake	36.0	34.6	31.1	28.1		
ε-free lysine intake	1.48	1.31	1.15	0.98		1.081
Weight gain	79.2	63.2	55.6	45.7		
PER	2.19	1.84	1.79	1.61		
Duncan multiple range	bc	d	de	def		
Soybean meal						
Feed consumption	363					
Protein intake	30.7					
Weight gain	65.1					
PER	2.13					
Duncan multiple range	c					
Casein						
Feed consumption	358					
Protein intake	30.7					
Weight gain	80.2					
PER	2.59					
Duncan multiple range	a					

[a] ε-free lysine content of meals and meal treatments in g./16 g. of N. All other values are average values in grams per rat with nine rats per treatment for 28 days.
[b] CM72-GR excluded.
[c] PER means without a common letter are significantly different at $P = 0.01$.

contribute to the negative error. It is possible that the presence and absence of the carbohydrate constituents in CM72, CM72-CM, CM72-A are the major reason for the difference between the ε-free lysine values of the three autoclaved meals. Indeed, if one substitutes the 4.1 ε-free lysine value for CM72 and CM72-CM in the regression analysis, the coefficients become 1.08 and 0.947, respectively, and the regression coefficients no longer differ from each other.

Conclusions

Rather than an absolute value which determines the protein quality of the meal, the ε-free lysine value is indicative of protein quality—one that reflects the results of the reactions and interactions which have taken place during processing. Therefore, the processing history of the cottonseed meal must be considered in any comparison of ε-free lysine values as criteria of protein quality.

Acknowledgment

The authors express their appreciation to E. Fred Schultz, Jr., for his contributions to this paper.

Literature Cited

Altschul, A. M., Lyman, C. M., Thurber, F. H., in "Processed Plant Protein Foodstuffs," A. M. Altschul, Ed., p. 469, Academic Press, New York, 1958.
Am. Oil Chemists' Soc., "Official and Tentative Methods of Analyses," 2nd ed., rev. to 1963, V. C. Mehlenbacher and T. H. Hopper, Eds., 1946–58.
Bickford, W. G., Pack, F. C., Castillon, L. E., Mack, C. H., *J. Am. Oil Chemists' Soc.* **31,** 91 (1954)
Derse, P. H., *J. Assoc. Offic. Agr. Chemists* **43,** 38 (1960).
Derse, P. H., *J. Assoc. Offic. Agr. Chemists* **48,** 847 (1965).
El-Nockrashy, A. S., *Staerke* **17,** 89 (1965).
Martinez, W. H., Berardi, L. C., *Proc.* Seed Protein Conf., p. 299, U. S. Dept. Agr., Southern Regional Res. Lab., New Orleans, La., January 1963.
Martinez, W. H., Frampton, V. L., Cabell, C. A., J. AGR. FOOD CHEM. **9,** 64 (1961).
Pons, W. A., Jr., Hoffpauir, C. L., *J. Assoc. Offic. Agr. Chemists* **40,** 1068 (1957).
Rao, S. R., Carter, F. L., Frampton, V. L., *Anal. Chem.* **35,** 1927 (1963).
Yatsu, L. J., *Cell. Biol.* **25,** 193 (1965).

Received for review July 11, 1966. Accepted January 9, 1967. Division of Agricultural and Food Chemistry, 148th Meeting, ACS, Chicago, Ill., August/September 1964.