Comparison of Geographical and Varietal Effects on the Peanut Volatile Profile by Peak-Ratio Analysis

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Differences in the volatile profiles of 13 different peanut varieties from selected geographical locations were studied. Qualitative differences in the profiles were not observed. Quantitative differences in the profile components were determined by percent composition and by peak-ratio analysis. Major differences found were between peanuts grown in and those grown outside the United States; foreign varieties had higher average values for pentane and lower values for methanol and hexanal. Peak-ratio data were found to be more sensitive indicators of the observed differences. The possible influence of genetic parentage on the volatile profiles is discussed, with the suggestion that this technique might be developed to aid the plant breeder in selecting early crosses for future quality potential.

Previous investigations indicated that hexanal is the backbone component of raw peanut flavor, that the volatiles of peanuts are quantitatively influenced by maturity and storage, and that quantitative and qualitative changes are produced in the volatiles by changes in mechanical curing conditions. These results have led to the establishment of an aromagram for raw peanuts which has been used to study the cause of spin-blanching off-flavor and to detect high-temperature curing off-flavor in peanuts (Pattee and Singleton, 1971; Singleton *et al.*, 1971).

Other workers have reported that variety and geographical location have a significant influence on the volatiles of various commodities, such as coffee (Biggers *et al.*, 1969), bananas (McCarthy *et al.*, 1963), oranges (Wolford *et al.*, 1963), potatoes (Self, 1963), tobacco (Swain *et al.*, 1966), tomatoes (Nelson and Hoff, 1969), and peppermint oils (Smith and Levi, 1961) with later statistical treatment by Hawkes and Wheaton (1967) and spearmint oils (Smith *et al.*, 1963). The differences found have been used to differentiate between selected varieties of coffee and to predict the quality of their various blends (Biggers *et al.*, 1969) and to postulate the geographical origin of peppermint oils (Hawkes and Wheaton, 1967). Most of these studies found that the differences were quantitative rather than qualitative. Thus, in order to establish "significant" differences or comparisons, it may be necessary to use statistical methods such as discriminant analysis of peak ratios as used by Powers and Keith (1968) and Biggers *et al.* (1969). In the present study, however, the complexity of the data precludes such a statistical approach and the use of other statistical evaluations of ratio data is mandatory in order to establish "significant" differences in the values obtained. The objectives here are to compare profiles of major marketed peanut varieties from some of the major peanut-producing areas in the world and to evaluate the influence of geographical locations within the United States on selected U. S. varieties.

EXPERIMENTAL PROCEDURE

Materials. All peanut varieties from outside the United States were shipped, as soon as possible after harvest and curing, *via* air freight to Washington, D. C., for plant quarantine inspection and handling. A common port-of-entry enabled all foreign samples to be handled by the same procedures. The United States samples were obtained from the U. S. peanut variety testing program to minimize the variation due to cultural practices, within variety differences, and harvesting and curing procedures. The source, type, and variety of peanuts used in this study are given in Table I. All samples were stored unshelled at 7° ± 2° and 60% relative humidity ±5%. Analysis of the samples began approximately 6 months after storage to eliminate the variation in the volatile profile due to storage (Pattee *et al.*, 1971). Approximately 8 months were required to complete the analysis of the samples.

Oil Analysis. A 20-g sample of peanut meal was extracted with 300 ml of chloroform : methanol (2 : 1; v/v) for 24 hr in a Soxhlet apparatus. The extract was evaporated to dryness in a vacuum oven at 25° and 635 mm. The residue was weighed and the percent of oil was calculated on a dry weight basis.

Preparation of Sample and Component Isolation. Duplicate 500-g peanut samples were analyzed for each variety. Each sample was shelled and subdivided into 100-g batches, frozen in liquid N_2, and macerated with 2 vol of water in a Waring Blendor for 1 min. The macerated sample was transferred to a 12-l. flask attached to a high-vacuum manifold and the volatile components were isolated according to the procedure of Pattee *et al.* (1970a).

Gas–Liquid Chromatography and Data Collection. Volatile components in 5-ml samples of vapor were separated on a Model 1840-10 Aerograph gas chromatograph equipped with dual-flame ionization detectors. Columns and gas chromatographic conditions were as follows: a $^1/_8$ in. × 6 ft stainless steel column packed with 60–80 mesh Chromosorb 102 programmed from 125 to 200° at 4°/min; a $^1/_8$ in. × 10 ft stainless steel column program packed with 15% Carbowax 20M on 60–80 mesh AWDMCS-treated Chromosorb W operated isothermally at 70°. Digital data and glc profiles used in this study were obtained from the Chromosorb 102 column. Data collection, manipulations, and storage procedure were essentially the same as described by Pattee *et al.* (1970b).

Experimental Design. A nested sampling scheme was used in which two distillations were made on subsamples from each location and then two injections were made from each distillation. At two U. S. locations, several varieties were obtained. These *location-variety* combinations are termed "varieties" in the discussion of the analysis of variance.

Statistical Analyses of Data. The peak ratios were generated from the raw peak-area data, even though variation due to extraction, injection, and instrument separation and detec-

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Table I. Percent Distribution of Volatile Components from all Varieties and Locations

Variety	Location	Type	Percentage of all volatiles measured[a]					
			Methanol	Acetaldehyde	Ethanol	Acetone	Pentane	Hexanal
Colorado-Manfredi	Argentine	Valencia	8.7	23.8	0.5	0.6	66.2	0.1
Natal Common	South Africa	Spanish	18.7	9.9	0.4	0.6	70.3	0.1
Red Spanish	Australia	Spanish	7.6	12.9	0.1	1.1	78.0	0.3
Virginia Bunch	Australia	Va. Bunch	9.8	28.4	0.6	1.3	59.4	0.1
Shulamith	Israel	Va. Bunch	6.8	21.2	0.6	3.1	67.9	0.4
Tainan No. 9	Taiwan	Spanish	26.6	13.9	2.1	2.2	49.9	4.7
Argentine	Va., USA	Spanish	36.8	20.8	1.0	1.0	38.9	0.8
NC-2	Va., USA	Va. Bunch	30.3	32.1	3.1	1.9	29.2	2.6
Early Runner	Va., USA	Va. Runner	36.2	24.8	1.5	3.1	31.8	1.8
56R	Va., USA	Va. Runner	37.8	38.6	2.1	0.9	18.6	0.9
Florispan	Va., USA	Va. Runner	34.3	30.1	2.1	0.7	30.5	1.4
Pearl	Va., USA	Spanish	53.2	14.2	1.4	0.6	28.7	1.3
Argentine	Georgia, USA	Spanish	29.1	46.2	1.3	0.7	21.3	1.1
NC-2	Georgia, USA	Va. Bunch	14.5	42.4	1.2	3.0	33.6	4.7
Early Runner	Georgia, USA	Va. Runner	15.7	32.9	1.0	5.0	41.5	3.0
56R	Georgia, USA	Va. Runner	20.2	42.8	2.2	1.8	18.3	1.4
61R	Georgia, USA	Va. Runner	19.8	52.3	2.6	2.6	19.4	2.1
Argentine	Oklahoma, USA	Spanish	32.8	41.0	2.1	1.2	20.6	0.7
LSD 05			7.3	13.7	2.0	2.0	11.4	0.3

[a] Average of two distillations and two injections.

tion could have been reduced by the use of ratios generated from the percent distribution data. We feel that the use of such a transformation places a bias on the data that cannot be readily evaluated.

The peak-ratio data were treated by analysis of variance according to the nested classification scheme. This analysis of variance provided estimates of the components of variance due to varieties, distillations, and injections. Effects of distillations and varieties were tested for significance. Where the F ratio for varieties was significant, least significant differences were calculated for comparing pairs of variety means and these are reported for the percent distribution data.

RESULTS AND DISCUSSION

In this report we have used statistical analysis of both percent-area distribution and peak-ratio data to focus on the differences that occur in the volatile profiles of peanuts due to variety and geographical location. Differences due to distillation and injection were found to be nonsignificant by analyses of variance.

Literature dealing with the effect of variety on volatiles from various fruits, vegetables, and seeds is not extensive; however, there is a consensus that only quantitative changes are attributable to variety. These observations are supported by the present results. Although peanut varieties were from diverse sources, environmental conditions, and genetic backgrounds, no qualitative differences were found among profiles of the isolated volatiles. Many significant quantitative differences were found, and through the use of ratio transformations of the data other less apparent differences have been shown.

Foreign Varieties. Among varieties from outside the United States, Tainan No. 9 from Taiwan differed most in profile distribution (Table I). Percent of methanol was significantly higher than in all other varieties and pentane was significantly lower except in Virginia Bunch (Australia). Other significant differences, using a percent distribution comparison, are found between Natal Common and all other varieties in methanol, Virginia Bunch being higher in acetaldehyde than Natal Common and Red Spanish. Red Spanish differed from Colorado Manfredi and Virginia Bunch in pentane concentration.

When peak ratios are made using the peak-area measurement and the significantly different ratios are determined, the differences among varieties become more apparent. In presenting significant ratios, the peak numbers given in the tables are identified as follows: (1) methanol, (2) acetaldehyde, (3) ethanol, (4) acetone, (5) pentane, and (6) hexanal. The number of ratio comparisons possible between two varieties with six components is 30. When Tainan No. 9 was compared with the other varieties (Table II), the average number of significantly different ratios was 14, or nearly 50%. The components involved in 33% or more of the significant ratios were methanol (35%), acetaldehyde (37%), ethanol (35%), and hexanal (44%). It should be noted that pentane, one of the significant percent-distribution compounds, is not among the compounds contributing substantially to the significant ratio values. Other variety comparisons showing major differences were Shulamith *vs.* Red Spanish (ten significant ratios) and Shulamith *vs.* Natal Common (15 significant ratios). In the first comparison, ethanol (60%), methanol (40%), and acetone (40%) were the major components associated with the significant ratios. In the latter comparison, methanol (60%) and acetone (33%) were the major ratio components.

Although most varietal comparisons had seven or more significantly different ratios, the Virginia Bunch variety was very similar to the Colorado Manfredi and Shulamith, with only two and three ratios, respectively, significantly different. The similarity here might be considered surprising, since the Shulamith is a large-seeded Virginia-type peanut while the Colorado-Manfredi is a small Spanish-type peanut. The Virginia Bunch is a runner-size Virginia-type peanut. Comparison of Spanish-type peanuts *vs.* Virginia-type peanuts suggests that, in general, peanut type has little influence on the volatile profile distribution. This probably results from the general crossbreeding of varietal types that has taken place to produce optimum quality seeds for commercial processing and consumption.

U. S. Varieties. Comparison of data from selected major U. S. peanut varieties shows the close genetic relationship of these varieties. The Pearl variety also grown at Holland, Va., is a poor-quality, low-roasting-flavor-potential variety which was included to indicate the effect of this quality defect on the volatile profile. Comparison of the major varieties grown at the Virginia location, using the component percent distribu-

Table II. Identification of Significant Peak Ratios from Comparison of Foreign Varieties[a]

Comparison	Peak combinations used for form ratios														
	2/1	3/1	4/1	5/1	6/1	3/2	4/2	5/2	6/2	4/3	5/3	6/3	5/4	6/4	6/5
Colorado-Manfredi *vs.*															
Red Spanish		R			R	R	R	*		*	*	R	*		
Natal Common	*R	R		*			R	*				R			
Shulamith			*R				*R					R	*		
Virginia Bunch							R						*		
Tainan No. 9	*R			*	*R	*R	*R		*		*	*R	*	*	*
Red Spanish *vs.*															
Natal Common	R			*	R	R				*	*		*		
Shulamith	*	*R	*			R				*	*	R	*		
Virginia Bunch		R		*	R	R		*		*	*R				
Tainan No. 9	R	*R		*	*	*R			*	*	*		*	*	*
Natal Common *vs.*															
Shulamith	*	*	*	*	*			*		*	*		*	R	
Virginia Bunch	*R	R				R		*			*		*		
Tainan No. 9	R	*R			*R	R		*	*			*	*	*	*
Shulamith *vs.*															
Virginia Bunch			*		*R										
Tainan No. 9	*R		*	*	*	*R			*	*	*	*		*R	*
Virginia Bunch *vs.*															
Tainan No. 9	*R			*	*R	*R	*		*		*	*R		*R	*

[a] * = ratio significant at the 5% level. R = reverse ratio significant at the 5% level.

Table III. Identification of Significant Peak Ratios from Comparison of Virginia-Grown Varieties[a]

Comparison	Peak combinations used to form ratios														
	2/1	3/1	4/1	5/1	6/1	3/2	4/2	5/2	6/2	4/3	5/3	6/3	5/4	6/4	6/5
Argentine *vs.*															
NC-2		*R	R		*						R		*	*	*
Early Runner			R										*R		
56R		R		R			R	R		R	R				
Florispan		R			R		R			R				R	R
Pearl	R		R	R										*	
NC-2 *vs.*															
Early Runner														*	
56R			R	R	*		R	R							
Florispan			R		*		R						*	*	
Pearl	R	*R	R	R	*								*	*	*
Early Runner *vs.*															
56R			R	R			*R	R		*R					
Florispan			R				*R			*R			*R	*R	
Pearl	R		R	R						R			*R	*	
56R *vs.*															
Florispan				R				R						*	
Pearl	R	R	R				R	R			R			R	
Florispan *vs.*															
Pearl	R	R	R	R			R								

[a] * = ratio significant at the 5% level. R = reverse ratio significant at the 5% level.

tion data (Table I), indicated that only hexanal served as a major distinguishing component. Only one comparison, Argentine *vs.* 56R, was nonsignificant for this component. Among the other components, only 56R shows a difference. This occurs in pentane, where its percentage was less, and in methanol where its percentage was higher than that of NC-2. The poor-quality variety Pearl was found to be higher in methanol and lower in acetaldehyde than most of the other varieties. Its intermediate level of hexanal was different from all varieties except Florispan.

The Georgia location, however, produced more significant differences in the distribution patterns. In methanol distribution Argentine was significantly higher than all other varieties. In acetaldehyde distribution Argentine and 61R were significantly higher than Early Runner. While in acetone distribution Early Runner was significantly higher than all other varieties, in pentane distribution it was higher than Argentine, 56R, and 61R. The hexanal distribution indicated that all varieties were significantly different from each other.

Comparisons by peak-ratio analysis showed that the U. S. varieties tended to be more uniform than foreign varieties. Within the Virginia location, the maximum number of significant ratios in a comparison of any two varieties was 9, as compared to the maximum of 16 for the foreign varieties (Table III). Within the Georgia location, the maximum number of significant ratios was 13 (Table IV). At the Virginia location, acetone (56%), methanol (32%), and pentane (32%) were the primary components involved in the significant ratios. Hexanal, although significantly different in percent distribution, was involved in only 22% of the significant ratios. Pearl, the poor-quality variety, accounted for 32 significant ratios or 37% of all significant ratios within the Virginia loca-

Table IV. Identification of Significant Peak Ratios from Comparison of Georgia-Grown Varieties[a]

Comparison	Peak combinations used to form ratios														
	2/1	3/1	4/1	5/1	6/1	3/2	4/2	5/2	6/2	4/3	5/3	6/3	5/4	6/4	6/5
Argentine *vs.*															
NC-2	*		R	R	*		R	R	*	R		*	R		*
Early Runner			*R	R	*		*R	R	*	*R			R	*	
56R			R				R							*	
61R		*	R		*		R				R		R	*	*
NC-2 *vs.*															
Early Runner					*		*					*		*	*
56R					*						R	*		*	*
61R				R	*			R			R	*		*	
Early Runner *vs.*															
56R							*	R			R	*			
61R	NONE														
56R *vs.*															
61R		*		R	*		*	R	*	*	R	*			

[a] * = ratio significant at the 5% level. R = reverse ratio significant at the 5% level.

Table V. Identification of Significant Peak Ratios from Comparisons of Similar Varieties Grown in Virginia and Georgia[a]

Comparison	Peak combinations used to form ratios														
	2/1	3/1	4/1	5/1	6/1	3/2	4/2	5/2	6/2	4/3	5/3	6/3	5/4	6/4	6/5
NC-2	*		*	*	R							*		R	*
Early Runner	*		*	*R						*					
56R			R	*			R						R		

[a] * = ratio significant at the 5% level. R = reverse ratio significant at the 5% level.

Table VI. Identification of Significant Peak Ratios from Comparison of Argentine Variety Grown at Three Different Locations[a]

Comparison	2/1	3/1	4/1	5/1	6/1	3/2	4/2	5/2	6/2	4/3	5/3	6/3	5/4	6/4	6/5
Virginia *vs.* Georgia	R				R		R	*R						*R	R
Virginia *vs.* Oklahoma		R	R	R				*R			*		*		
Georgia *vs.* Oklahoma							R							R	

[a] * = ratio significant at the 5% level. R = reverse ratio significant at the 5% level.

tion. The finding of such a dominant difference in a variety of different qualities might be expected, since Biggers *et al.* (1969) also demonstrated significant differences between varieties of coffee with high and low quality. Just how the inferior quality factors bring about the changes in the volatile profile is not known.

Study of the volatile profile of several poor quality peanut varieties might help to establish relationships between the peanut volatile profile and quality. Comparison of volatile profiles of new breeding lines of peanuts against the standard aromagram might then be utilized to eliminate poor quality lines early in the breeding program, thereby saving considerable time in the development of new commercial varieties.

Within the Georgia location there were 64 significant ratios, as compared to 53 for the Virginia location. Acetone (41%), hexanal (41%), and pentane (33%) were the three components most often found. However, pentane probably could not be considered as a significant contributor to these ratios, since methanol, ethanol, and acetaldehyde contributed 30, 28, and 25%, respectively. Within the varieties grown in Georgia, Argentine expressed the most differences and accounted for 35 significant ratios or 55% of all significant ratios.

Location Effects. The varieties for which we can compare location effects are Argentine, NC-2, Early Runner, and 56R. Within a variety type the effect of location on the percent distribution of each component (Table I) generally was the same for all varieties, *i.e.*, the Georgia location had lower methanol and ethanol and higher acetaldehyde, acetone, pentane, and hexanal than the Virginia location. The exception was 56R, where ethanol and pentane components showed slight reversals. The differences in methanol and hexanal distribution were significant for all varieties. The Spanish-type Argentine variety showed significant differences in acetaldehyde, pentane, and hexanal distribution. In Georgia the trend was toward higher acetaldehyde, ethanol, and hexanal, and toward lower methanol, acetone, and pentane. An Argentine variety from Oklahoma showed a similar trend in the major components and a slight reversal in acetone and hexanal.

Of the Bunch and Runner-types, using peak ratios, NC-2 showed the greatest difference due to location. The components primarily associated with the significant ratios were methanol (65%), pentane (41%), and acetone (35%) (Table V). The influence of location on the peak ratios of Argentine is shown in Table VI. Peanuts grown in Oklahoma and Georgia are subjected to conditions which are generally very warm and dry during the latter part of the growing season and high temperatures may still prevail at the time of harvest. In the Virginia area, the mean average temperature was 8° less than in the Georgia area during July, August, and September for that year, while the mean average temperature for Oklahoma was only 3° higher than that for Georgia. The similarity between Georgia and Oklahoma is also expressed in the lack of significant peak ratios, even though peanuts are generally harvested during late August and early September in Georgia and late September and October in Oklahoma.

Foreign *vs.* Selected U. S. Varieties. Varieties from the Holland, Va., location were used as the U. S. source for com-

Table VII. Identification of Significant Peak Ratios from Comparison of Varieties Grown at Holland, Va., and Foreign Locations[a]

Comparison	Peak combinations used to form ratios														
	2/1	3/1	4/1	5/1	6/1	3/2	4/2	5/2	6/2	4/3	5/3	6/3	5/4	6/4	6/5
Argentine *vs.*															
Colorado-Manfredi	*R	R	R	*R		R			R		*	R	*		R
Natal Common		R		*R	R			*			*		*		
Red Spanish	R	R	R	*R		R		*		*	*	*			
Virginia Bunch	*R	R	R	*R		R			R		*	R		*R	
Shulamith	*R	*R	*R	*R			*			*	*			R	
Tainan No. 9		R	R		*R	*	*R		*			*		*	*
NC-2 *vs.*															
Colorado-Manfredi	*			*R	*R	*	R		*R		*R	*R	*R	*	*R
Natal Common	R	*R		*R	*R			*R	*		*R		*R	*	*
Red Spanish		*R		*R		*R		*R		*R	*R		*	*	*
Virginia Bunch	*R	R	R	*R		R			R		*	R		*R	
Shulamith	*		*	*R		*		*		*R	*R			*R	*
Tainan No. 9					*			R	*					*	
56R *vs.*															
Colorado-Manfredi	*		R	*R	R	R		R	R	R	*R	R	*	*	*R
Natal Common	R	R		*R	R		R	*R		R	*R		*	*	*
Red Spanish		R	R	*R		R	R	*		*R	*R	*	*	*	*
Virginia Bunch	*		R	*R	R	R	R	R	R	R	*R	R		*R	*
Shulamith	*		*R	*R			*R	*R		*R	*R			*R	*
Tainan No. 9			R	R	*	*	*R	*R	*	R	R	*		*	*
Early Runner *vs.*															
Colorado-Manfredi	*			*R	R	R	*R	R			*	R	*R		*R
Natal Common		R		*R	R			*			*		*R		*
Red Spanish		R		*R		R		*		*	*		*R		*
Virginia Bunch	*			*R	R	R			R		*	*R	*R	R	*
Shulamith	*	R	*	*R				*			*		*	R	*
Tainan No. 9				R	*	*			*					*	
Florispan *vs.*															
Colorado-Manfredi	*		R	*R	R	R	R		R		*	R	*	*	*R
Natal Common		R	R	*R	R		R	*R		R	*			*	*
Red Spanish		R	R	*R		R	R	*R		*R	*	*		*	*
Virginia Bunch	*		R	*R	R	R	R		R	R	*	R		*R	*
Shulamith	*		*R	*R			*R			*R	*			*R	
Tainan No. 9			R	R	*	*	*R		*	R		*			*

[a] * = ratio significant at the 5% level. R = reverse ratio significant at the 5% level.

parison with foreign varieties. Percent distribution comparisons of the components show major differences in the methanol, pentane, and hexanal distributions (Table I), while acetaldehyde shows a significant difference among a relatively few variety comparisons. The very high level of pentane in the foreign varieties seems to be a general characteristic of these sources, but an explanation for the difference is not really available. Oil contents of the foreign varieties ranged from 45 to 52%, which was similar to the range for U. S. varieties. No correlation between pentane level and oil content was observed.

Comparison of the foreign varieties with selected U. S. varieties by peak-ratio analysis illustrates the highly significant difference between all foreign and U. S. varieties. Out of 900 possible comparisons, 373 were significant (Table VII). The U. S. variety having the greatest difference was 56R, with 51% of the ratios being significantly different. This 56R difference is consistent across all foreign varieties, with the lowest comparison difference being 14 for Tainan No. 9 and three comparisons with the highest difference of 16. All components seemed to contribute uniformly to the difference, with all but pentane in a 31% ± 3 range. Pentane was involved in 45% of the significant ratios. Although all varietal comparisons showed major differences which possibly resulted from environmental growing conditions and differences in harvesting and curing techniques by the individual growers, the much higher difference exhibited by 56R might reflect the absence of any Spanish-type percentage. All other varieties in these comparisons have in common a Spanish-type parentage. In the specific case of Shulamith *vs.* Florispan, the pollen donor was Florispan and the Shulamith variety was taken as a selection from a Florispan–Florigiant cross (Goldin, 1970). However, the close parentage linkage did not prevent major differences from appearing between the two varieties. The varieties with the least difference by peak-ratio analysis were NC-2 *vs.* Tainan No. 9 and Early Runner *vs.* Tainan No. 9. Only four and five significant peak-ratio differences, respectively, were found for these comparisons. Tainan No. 9 was the variety which showed the greatest difference when compared to the other foreign varieties and had the lowest number of significant peak-ratio differences when compared with the U. S. varieties. These observations suggest that the environmental conditions under which Tainan No. 9 was grown, the methods of handling and curing, and the physiological maturity of the lot analyzed were most closely related to those for the U. S. varieties.

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Spectrophotometric Cysteine Analysis

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Procedures have been developed for estimating cystine content of proteins by reducing disulfide bonds with mercaptoethanol and selectively alkylating the generated sulfhydryl groups with 4-vinylpyridine or 2-vinylquinoline. 4-Vinylpyridine reacts with cysteine to produce *S*-β-(4-pyridylethyl)-cysteine (Pe–cysteine), which can be determined in protein hydrolysates either by ion-exchange chromatography or ultraviolet spectrophotometry. Pe–cysteine cannot be determined spectrophotometrically in the intact protein, since the absorptivity varies with size and composition of the protein. 2-Vinylquinoline reacts with liberated sulfhydryls under conditions similar to those for 4-vinylpyridine approximately half as fast to produce *S*-2-(2-quinolylethyl)-L-cysteine (Qe–cysteine). Qe–cysteine absorbs at 318 nm with a molar absorptivity of 10,000 in 0.1 *N* acetic acid; since it is not affected by composition or size, spectrophotometric analysis can be made on solutions of the intact protein, as well as on its hydrolysate.

Demonstrated cystine and cysteine loss during hydrolysis of some natural products has led to the development of several special hydrolyses involving special handling, derivatization, or modification to protect them. Oxidation with performic acid (Schram *et al.*, 1954) and alkylation with iodo acetate (Crestfield *et al.*, 1963) or acrylonitrile (Weil and Seibles, 1961) produce a derivative stable to acid hydrolysis (6 *N* HCl at 100° for 24 hr). These procedures are acceptable in some specific instances, but incomplete reaction or recovery (Moore, 1963), interfering side reactions (Cavins and Friedman, 1967), and analytical difficulties (Kalan *et al.*, 1965) render them not universally acceptable. At present, performic acid oxidation coupled with chromatographic determination as cysteic acid is the most widely used alternate procedure for sulfur amino acids.

Cystine and cysteine contents of protein can be estimated by reducing the disulfide bonds with an excess of mercaptoethanol, followed by selective alkylation of the generated sulfhydryls with either 4-vinylpyridine or 2-vinylquinoline. Alkylation with 4-vinylpyridine yields *S*-β-(4-pyridylethyl)-L-cysteine (Pe–cysteine) in the protein hydrolysate, whereas 2-vinylquinoline yields *S*-2-(2-quinolylethyl)-L-cysteine (Qe–cysteine). Pe–cysteine can be determined in protein hydrolysates either chromatographically (Friedman *et al.*, 1970) or spectrophotometrically (Friedman and Krull, 1969; Wu *et al.*, 1971). Qe–cysteine can be determined spectrophotometrically in protein solutions or hydrolysates (Krull *et al.*, 1971).

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EXPERIMENTAL SECTION

S-β-(4-Pyridylethyl)-L-cysteine. In 50 ml of nitrogen-saturated deionized water was dissolved 2.00 g (0.0165 mol) of L-cysteine (Nutritional Biochemicals Corp.). Under an atmosphere of nitrogen, 2.3 ml (0.017 mol) of triethylamine and 1.75 ml (0.0165 mol) of 4-vinylpyridine were added. The reaction mixture was magnetically stirred under a nitrogen atmosphere for 24 hr and then clarified by filtration. A white crystalline material precipitated from the solution when it was rotary evaporated at 40°. The Pe–cysteine was recrystallized from 95% ethanol as fluffy needles, mp 210–212°, with decomposition.

S-2-(2-Quinolylethyl)-L-cysteine. This derivative was prepared like Pe–cysteine, except that 2.4 ml (0.017 mol) of 2-vinylquinoline replaced the 4-vinylpyridine. The product was recrystallized from a mixture of 80 ml of water and 60 ml of ethanol, mp 219–220°.

Reduction and Alkylation of Proteins. One gram of protein was dissolved in 100 ml of 8 *M* urea, pH 7.5 Tris buffer (Krull *et al.*, 1971), and saturated with nitrogen to remove dissolved oxygen. Mercaptoethanol (100 mol excess over total disulfide) was added under nitrogen and the mixture was stirred for 16 hr at room temperature. The free sulfhydryl