&Effect of Seed Maturity on Seed Oil, Fatty Acid and Crude Protein Content of Eight *Cuphea* **Species**

A.E. Thompson a,* and R. **Klelman b**

aU.S. Water Conservation Laboratory, ARS/USDA, 4331 E. Broadway Rd., Phoenix, AZ 85040, and bNorthem Regional **Research Center,** ARS/USDA, Peoria, IL 61604

Thirty-six lots of eight *Cuphea* **species grown at nine geographical locations from 1983 to 1985 were analyzed for seed weight, oil percentage, fatty acid and crude protein content. Twenty-two samples were separated into two distinct seed maturity groups and also analyzed. Seed maturity varied widely but had little effect on oil percentage, even though mature seeds were significantly heavier than less mature seeds. Lauric acid content generally increased and capric acid decreased with increasing seed maturity. Crude protein of whole seeds and defatted seed meal increased with increasing seed maturity. The net effect of harvesting** *Cuphea wrightii* **seeds at full maturity in comparison with that for less mature seeds was to increase seed weight by 12%, decrease capric acid by 3%, increase lauric acid by 2% and increase crude protein of whole seeds and defatted meal by 5% and 4%, respectively. Seed oil content was decreased by a statistically nonsignificant 1%. The effect of seed maturity was comparable for the other four lauric acid- and three capric acid-rich species, even though distinct species differences in all factors were measured. Location and environment contributed to some quantitative and qualitative changes, but these factors are not considered to be major sources of variation. It is concluded that variation in seed maturity does not present a major constraint to commercialization of** *Cuphea* **as a new, alternative source of lauric and other medium-chain fatty acids. The ultimate significance of these minor changes will depend upon relative yields, demands and values of the various seed components.**

The United States currently imports about 450,000 metric tons (MT) of coconut and palm kernel oils as the primary source of lauric acid and minor amounts of other medium-chain acids for the production of soaps, detergents, lubricants and related products. Essentially equal quantities of petrochemicals are converted and utilized annually to meet the total domestic demand for medium-chain fatty acids. In addition to the conventional uses of medium-chain fatty acids, research has demonstrated recently that caprylic and capric acids have potentially important applications in medical, nutritional and dietetic fields (1,2), which may increase future demands for them.

Research conducted by the ARS/USDA Northern Regional Research Center in Peoria, Illinois, in the early 1960s determined that seeds of *Cuphea* species contained from 16 to 42% oil, and that these oils had high levels of lauric and capric acid and other medium-chain fatty acids (3). The seed oils of 73 of the estimated 260 species sampled from 11 of the 12 sec-

tions of the genus have been analyzed recently for fatty acid composition (4-6). These studies revealed unparalleled diversity of fatty acid patterns within *Cuphea,* with lauric, capric and caprylic acids the predominant components.

Little effort was made to develop *Cuphea* as a new crop until a research program was started in the mid-1970s at the University of Göttingen in West Germany (7-9). In 1983, ARS/USDA initiated a major research effort to evaluate and develop improved *Cuphea* germplasm (10,11). In 1982, Frank Hirsinger moved his research from West Germany and started a domestication program at the University of California at Davis, California. This program was moved to Corvallis, Oregon, in 1983 when a unique, equallyfunded, three-way R & D effort, involving ARS/ USDA, Oregon State Agricultural Experiment Station, and member companies of the Soap and Detergent Association, was initiated to domesticate and commercialize production of *Cuphea.* Data on agronomic potential, seed composition and morphological descriptions of various species evaluated during the early phase of this program have been published (12,13).

Major constraints to successful commercialization of *Cuphea* are the indeterminate pattern of plant growth and flowering and the excessive shattering of seeds from the maturing seed pods. To date, no germplasm has been found or developed to correct these deficiencies, but major breeding and genetic efforts are in progress. As a consequence of these deficiencies, seeds collected at harvest vary widely in maturity. The objective of this research was to determine the effect of seed maturity on oil content and distribution of fatty acids within the seed oil, and on crude protein content of the seed meal. Such information should be of considerable value to current research as well as future producers and processors of *Cuphea* seed oils.

EXPERIMENTAL PROCEDURES

Thirty-six seed lots of eight *Cuphea* species grown at nine geographic locations from 1983 to 1985 were available in the ARS/USDA *Cuphea* working germplasm collection. Included were five species rich in lauric acid; *C. wrightii, C. tolucana, C. wrightii X C. tolucana, C. laminuligera,* and *C. lutea.* Capric acidrich species were represented by *C. leptopoda, C. paucipetala* and *C. procumbens.* Maturity of individual seeds, as indicated by a range of seed coat colors from green to nearly black, varied considerably within each seed lot.

A seed maturity score based upon seed coat color was constructed ranging from 1 (green) to 9 (dark brownish-black), with 5 an intermediate yellowishbrown color. Each seed lot was physically divided into two distinctly different maturity classes, desig-

^{*}To whom correspondence should be addressed.

nated as "green" or "mature." The two lots were weighed to determine the percentages of each class within the bulk seed samples. A 1000-seed weight determination was made on each sample. The relative maturity of each of the two maturity classes varied among the various seed lots, and each sublot was scored for maturity on the 1 to 9 scale.

A minimum of eight g of seeds was considered necessary to conduct accurate analysis for determination of moisture, oil, fatty acid and crude protein contents. Twenty two of the 36 seed lots had adequate amounts of seeds to obtain eight-g aliquots of "green" and "mature" plus eight g of the original bulk seed lot. The quantity of seeds of 11 lots was insufficient to obtain the eight-g samples for the two maturity groups. These lots were chemically analyzed only as bulk samples. However, a sample from each lot was divided into two maturity groups to determine the percentages in each class and to score for maturity on the 1 to 9 scale. Determinations also were made of 1000-seed weights for each seed lot. Three lots of C. *wrightii* were a uniform grayish color resulting from moisture exposure during harvest, which results in the extrusion and matting of coiled hairs from the seed coats (14). These wetted or weathered seed lots were given a special maturity score or designation of 10 because little or no normal seed coat color could be discerned. These three lots were chemically analyzed as bulk samples.

A total of 80 eight-g seed lots were analyzed. The seeds were ground in a Varco electric dry-food grinder. The ground seeds (3 g) were subjected to 130 C in a forced draft oven for three hr to determine moisture. An additional three-g sample was extracted in a Butt apparatus for six hr for gravimetric oil determination. A portion of the extracted oil was converted to methyl ester by reacting the oil with a 1% solution of sodium methoxide in methanol, at room temperature, for 30 min. The fatty acid methyl esters were extracted into heptane and injected directly into a Varian model 3700 gas chromatograph for fatty acid analysis. The ester mixture was injected onto a 1.8-m by 2-mm i.d. glass column packed with 3% LAC 2 R-446. The column oven was temperature programmed from 100 to 180 C at a rate of 4 C/min. Peak identification was made by comparison with known standards; quantitation was accomplished with programs on a Modular Computer Systems, Inc. model 32/85 computer. Nitrogen was determined by AOAC method 2.055 (15). Digestion and distillation were accomplished with the Tecator Nitrogen Analysis System.

RESULTS AND DISCUSSION

Nineteen of the 36 bulk seed lots analyzed for seed quality and fatty acid content were of one species, C. *wrightii* (Table 1). An additional three lots of C. *wrightii* that had been subjected to moisture exposure during harvest are also included. All of these seed lots are considered to be from a common source, having descended from an original seed collection designated as Graham 651. Seed maturity varied widely between lots. Two lots from Phoenix, Arizona, one produced in the field and the other in a greenhouse, had over 75%

of their seeds with a maturity score of 1.7 and 2.7 and were light green and yellowish green in color, respectively. Seed lots produced in Puerto Rico and California had relatively low quantities of green seed with a general maturity score of 6.9 to 7.3 and a medium brown color. One thousand-seed weights for fieldgrown seeds varied from 1.39 to 1.81 g. Seeds of three lots produced in a greenhouse at Phoenix were generally heavier, ranging from 1.77 to 2.44 g.

Regardless of the wide range of seed weight and maturity, oil percentages among the lots were relatively uniform, ranging from 30 to 35% with a mean of 33.6 \pm .32% and a coefficient of variation (C.V.) of only 4.1%. In general, the range of variability of the predominant fatty acids (lauric $54.0 \pm .54$ %, C.V. = 4.4%, and capric 34.7 \pm .46%, C.V. = 5.7%) was low and comparable to that of oil percentage for these seed lots. Relative variation in crude protein expressed as either whole seed percentage $(20.3 \pm .3\%, C.V. 7.7\%)$ or percentage of defatted seed meal (30.5 \pm .6%, C.V. = 7.9%) was only slightly larger than for oil and fatty acid percentages.

Exposure of the three seed lots of *C. wrightii* to moisture at harvest did not appear to significantly alter oil percentage, fatty acid content or crude protein percentage. For the two seed lots from Corvallis, Oregon, no real difference in seed contents was detected even though they had been differentially subjected to exposure of sunlight and heat (Table 1).

Obvious differences in seed oil percentage, relative amounts of lauric and capric acid, seed weight, and crude protein content exist among the eight species tested (Table 1). In general, the fatty acid contents and differences between the species closely approximate that previously reported (4). Differences in 1000 seed weight also correspond closely to the differences among species previously documented (12,13).

Twenty-two of the 36 seed lots were subdivided into two seed maturity groups, scored for seed maturity and analyzed for 1000-seed weight, seed oil percentage, capric and lauric acid content, and crude protein percentage of whole seed and defatted seed meal (Table 2). Eleven pairs of samples of *C. wrightii* from seven geographic locations are included. Means, standard errors and coefficients of variation are recorded for each of the 11 entries within each maturity group. A mean difference and standard error of the mean difference were calculated, and differences among the means compared statistically with a t test. As expected, the paired seed lots were highly significantly different with regard to seed maturity. This difference in seed maturity was clearly reflected in a difference of about 0.2 g in seed weight. Seed maturity had little effect on seed oil percentage. The more mature seeds had about 0.3% less oil content than the less mature samples.

Capric acid content of the 11 pairs of sampled C. *wrightii* was about 0.9% lower in mature seeds; conversely, lauric acid content was about 1.2% higher in mature seeds (Table 2). These mean differences were signficant only at the 5% level. The actual number of days for seed to mature from a rating of 3 to 6 probably varies somewhat under different environments but represents a relatively short time in terms

TABLE 1.

Quality Constituents and Fatty Acid Distributions of 36 Bulk Seed Lots of Eight Species Grown at Nine Locations from 1982-1985

aSeed maturity score based on seed coat color ranging from green (1) to yellowish-brown (5) to brownish-black (9). Three seed lots with uniformly gray color resulting from moisture exposure during harvest are designated with a score of 10.

bSeed for planting was produced from one seed lot (Davis, CA, 1983) and grown at various locations as part of the 1985 *Cuphea* Regional Adaptation Trial.

CSeed produced on plants seeded in the fall in contrast to **all** others produced as spring planted.

dSeed produced in a greenhouse on plants grown in pots.

eFrom same seed harvest. Second seed lot subjected to one month exposure to sunlight and increased temperature in a greenhouse in an attempt to minimize seed dormancy.

fSeed from distinctly different accessions and source.

TABLE 2.

Quality Constituents of Seed of Eight *Cuphea* **Species Grown at Seven Locations and Separated into Two Maturity Groups**

aSeed maturity score based on seed coat color ranging from green (1) to yellowish-brown (5) to brownish-black (9).

bSeed produced in a greenhouse on plants grown in pots.

cSeed for planting was produced from one seed lot (Davis, Ca, 1983) and grown at various locations as part of the 1985 *Cuphea* Regional Adaptation Trial.

dSeed from distinctly different accessions and source.

*,**,***Mean differences among paired samples significantly different from zero at the 0.05. 0.01 and 0.001 probability levels.

of elapsed days. Under field conditions, seeds of C. *wrightii* are exposed to the external environment and subject to shedding about 14 to 18 days after anthesis. The period of time for seeds that are initially green in color, to proceed from a yellowish color (maturity rating 3) to medium brown (maturity rating 6) represents only about 3 to 5 days under most field conditions. It appears that some changes in the lengthening of the carbon chain from 10:0 to 12:0 occur as seeds mature from stage 3 to 6 even though relatively small changes are detected in oil percentage.

The differences in seed quality constituents for the two maturity groups of the eight seed lots of the other four lauric acid-producing species were of a magnitude comparable to those of *C. wrightii,* with the following exceptions (Table 2). The mean 10:0 content of the two mature seed samples of *C. laminuligera* was about 1.2% higher than that for the less mature seed samples. This is in contrast to the observed general trend for reduction in 10:0 content associated with increased seed maturity. The only other species that appears to be responding similarly to *C. laminuligera* is the capric acid-producing species *C. procumbens,* which also had over 2% higher 10:0 in the mature seed sample.

The trend for a slight increase in 12:0 content in mature seed samples of *C. wrightii* appears to be reversed in the four other lauric acid-producing species. However, *C. lutea,* which has a 12:0 to 10:0 percentage ratio of about 39/28 rather than the mean ratio of 60/28 for the other lauric acid-producing species, was responsible for a major portion of this difference. In this respect, *C. lutea* appears to respond similarly to the three capric acid-producing species with a 12:0/10:0 ratio of about 2/88, which also produce slightly less 12:0 in their mature seed samples.

Highly significant increases in crude protein content of about 1% in both whole seeds and defatted meal of the *C. wrightii* samples are associated with increasing maturity and 1000-seed weight (Table 2). Similar but slightly smaller differences in crude protein content are also noted for the other species. Some species differences are apparent, with *C. laminuligera* having the highest mean content. However, additional evaluation under comparable conditions is needed to more accurately assess the relative yields of these species for seed proteins as well as for oil and fatty acid content,

Linear correlation coefficients were calculated among the means of all seven of the seed quality constituents of 11 pairs of *C. wrightii* samples grown at seven locations, and separated into two maturity groups (Table 3). Regressions were calculated for those instances where significant association among factors was measured.

Figure 1 presents the regression of seed maturity score on 1000-seed weight. The distribution clearly

FIG. I. Regression of seed maturity score on 1000-seed weight of *Cuphea wrightii* **seed samples separated on the basis of seed maturity.**

shows the differences in seed weight that are associated with seed maturity. However, the relatively small r^2 value (0.292) indicates that less than 30% of the variability in seed weight is accounted for by this association. Such a difference does have some economic significance. If one assumes a 1000 kg/ha. yield of mature seed with a mean maturity score of 6.4 and a 1000-seed weight of 1.69 g, harvesting seed at a maturity score of 3.5 (1000-seed weight = 1.58 g) would reduce the yield by 6.5% to around 935 kg.

The regression of capric *acid content* on content of lauric acid for the 11 paired samples of *C. wrightii* is

TABLE 3.

Linear Correlation Coefficients Among Seed Quality Constituents of 11 Pairs (n = 22) of *Cuphea wrightii* **Samples Grown at Seven Locations and Separated into Two Maturity Groups**

	1000- seed weight	Percent seed oil	Percent C10:0	Percent C12:0	Percent crude protein- whole seed	Percent crude protein- defatted meal
Seed maturity score	$.540**$	$-.039$	-325	.322	$.653***$.605**
1000-seed weight		.102	-131	$-.031$	$.684***$	$.691***$
Percent seed oil			.414	$-.278$	$-.119$.304
Percent C ₁₀ :0				$-.848***$	$-.587**$	$-.380$
Percent C12:0					.331	.213
Percent crude protein- whole seed						.908***

,*Significantly different from zero at the 0.01 and 0.001 probability levels.

FIG. 2. Regression of capric acid content on content of lauric acid in *Cuphea wrightii* **seed samples separated on the basis of seed maturity.**

depicted in Figure 2. The effect of seed maturity per se is not clearly evident, and is overshadowed by differences among the various locations. However, in most instances with specific paired samples, the decrease in capric acid associated with increased seed maturity is accompanied by a shift toward higher lauric acid content. The shift from the less mature seed with a mean capric acid content of 35.5% to the more mature seeds with 34.6% capric results in a concomitant increase in lauric acid from 53.5 to 54.4%. If one is willing to assume that the slight, but statistically nonsignificant, reduction in seed oil percentage from 34.0 to 33.7% associated with increased seed maturity is real, the quantity of lauric acid produced by each seed maturity group is essentially the same. However, because 1000-seed weight is significantly lighter in the less mature seed, approximately 1070 kg/ha, of seed would be needed to produce the same yield as 1000 kg of more mature seed.

A highly significant positive correlation was measured between seed maturity score and crude protein content of both whole seed and defatted seed meal $(r = .653$ and $.605$, respectively, Table 3). The regression of seed maturity score on crude protein content of whole seeds depicts this relationship in Figure 3. The distribution shows that differences in crude protein content are associated with seed maturity. The regression of 1000-seed weight on crude protein con-

FIG. 3. Regression of seed maturity score on crude protein content of whole seeds of *Cuphea wrightii* **seed samples separated on the basis of seed maturity.**

tent of whole seeds in Figure 4 shows a similar relationship. The correlations and regressions of seed maturity score and 1000-seed weight with crude protein content of the defatted seed meal are very similar to those involving crude protein content of the whole seed. In all instances, the r^2 values of these four relationships, which range from .37 to .48, indicate that over half of the observed variability in crude protein content is not accounted for by these correlations. Increased seed maturity, as characterized by seed maturity score and 1000-seed weight, has a positive and potentially economically significant influence on yield of crude protein in the whole seed or defatted seed meal of *C. wrightii.* In both instances, an increase in seed maturity score from 3.5 to 6.4 and an increase in 1000-seed weight from 1.54 to 1.72 results in an increase of about 1.0% and 1.6%, respectively, in crude protein content of whole seeds and defatted seed meal.

Crude protein content of whole seeds was found to be negatively correlated $(r = -.587)$ with capric acid content (Table 3). However, no statistically significant correlation was obtained for crude protein of whole seed with lauric acid content or with crude protein content of defatted meal with either capric or lauric acid content. The nature of the regression of capric acid content on crude protein content of whole

FIG. 4. Regression of 1000-seed weight on crude protein content of whole seeds of *Cuphea wrightii* **seed samples separated on the basis of seed maturity.**

seeds is displayed in Figure 5 where the effect of seed maturity is not readily apparent. However, in most instances with specific paired samples, the decrease in capric acid associated with increased seed maturity is accompanied by a shift toward higher crude protein content. The shift from less mature seeds with a mean capric acid content of 35.5% to the more mature seeds with 34.6% capric acid results in a concurrent increase in crude protein from 19.1 to 20.0%.

It is clearly apparent that species differences exist for seed weight, seed oil percentage, fatty acid and crude protein content. There is an indication that some genotype by environmental interactions exists for these characters. However, the extent of interaction is difficult to assess accurately from these data. The coefficients of variation of all the quality constitutents of the *C. wrightii* data are quite small when one considers the wide range of environments included in this experiment. All of the seeds utilized in the plantings at different locations and years originated from a common source. Nine of the seed lots, which were produced at six locations throughout the country as part of the 1985 Cuphea Regional Adaptation Trial, came from a single seed lot produced at Davis, California in 1983. Because *C. wrightii* is highly self pollinated, one must conclude that very little genetic variability exists within this population.

FIG. 5. Regression of capric acid content on crude protein content of whole seeds of *Cuphea wrightii* **seed samples separated on the basis of seed maturity.**

In all instances except two, the seed samples of the other seven species are from single accessions. The two accessions of *C. procumbens* and the accession of *C. lutea* grown in Arizona are distinctly different, and from different original seed collections. The two *C. lutea* seed samples grown in Oregon are from the same original source. Very small differences between these distinct accessions are noted, with the possible exception of crude protein content in *C. lutea* grown in Arizona. However, the increased protein content may be an artifact and reflect only an environmental difference since the Arizona sample was produced in a greenhouse. In general, seeds produced in the greenhouse tend to be heavier in seed weight and higher in crude protein content. The two paired seed maturity samples of *C. wrightii* having highest protein content, which were produced in a greenhouse in Arizona, are easy to identify in Figures 3, 4 and 5. The mean 1000-seed weight of three bulk seed samples of *C. wrightii* grown in a greenhouse in Arizona was 2.02 g (Table 1). In contrast, the two field-grown Arizona samples averaged only 1.62 g, exactly the same mean weight of the other 14 samples grown at eight other locations throughout the country. Mean crude protein contents of the three greenhouse-grown samples with heavier seed weight were 22.2% and 33.5% for whole seed and defatted meal, respectively (Table 1). The mean crude protein contents of the two

Arizona field-grown samples were only 19.2% and 28.7%, which were lower than the means for the other 14 samples (20.3% and 30.5% for whole seed and defatted meal, respectively). The only other valid comparison in this regard is between the two bulk seed samples of *C. tolucana* (Table 1). In this instance, the seeds from Arizona were produced in a greenhouse and had higher crude protein contents, but the seed weights were essentially the same. The three bulk seed samples of *C. wrightii* produced at Beltsville, Maryland, gave another indication of the effect of environment on protein content (Table 1). These samples, which came from different planting dates and harvests, tended to have higher mean crude protein contents than those observed for the whole population (21.9% and 33.1% for whole seed and defatted meal, respectively). In addition, the higher protein content came from seeds with a lower than average 1000-seed weight (1.5 g in comparison with 1.7 g for the total population).

In summary, harvesting *C. wrightii* seeds at full maturity (seed maturity score $= 6.4$) rather than at a less mature stage (seed maturity score = 3.5) can be expected to produce the following changes in seed quality constituents: 1000-seed weight, 12% increase; seed oil content, a nonsignificant decrease of 1%; capric acid content, 3% decrease; lauric acid content, 2% increase; and crude protein content of whole seeds and defatted seed meal, 5% and 4% increases, respectively. Comparable results were obtained for the other seven species evaluated. Although the oil percentages and fatty acid contents were not greatly affected by seed maturity, agronomic practices need to be attuned to harvesting and handling seeds with the highest possible degree of maturity. Initially, multiple vacuum harvesting was considered to be the most feasible method. Recently, once-over harvesting by cutting and swathing the plants, and subsequent field drying to allow seeds to mature with minimal shattering before combining has received attention. Such harvesting could result in a high percentage of green colored, less mature seeds. Excessively high amounts of less mature, green colored seed could cause some problems in oil extraction, and necessitate decolorization for certain end product usages.

In general, one may conclude that variation in seed maturity results in relatively small changes in seed oil quality and quantity and does not present a major constraint to commercialization of *Cuphea* as a new, alternative source of lauric and other medium-chain fatty acids. The ultimate economic significance of these minor quantitative and qualitative changes will depend upon the relative yields, demands and values attached to the various end product components of yield. The ultimate success in commercialization of *Cuphea* as a new industrial oilseed crop depends heavily upon genetic and plant breeding research to obtain determinate flowering, nonshattering cultivars. Continued agronomic research to develop improved cultural and harvesting systems designed to minimize loss of seed and variations in seed maturity also must continue to receive high priority.

ACKNOWLEDGMENTS

Cuphea seeds were produced at various locations by Frank Hirsinger, Steven J. Knapp, James M. Crane, Clinton C. Shock, John A. Yungen, Joy Adams, Frank Cline, W.C. Adamson, Karla Grasse, T. Austin Campbell, Francisco Vazquez and Antonio Sotomayor-Rios. Special thanks to Donna Thomas for oil and ester determinations, and to Warren Rayford for nitrogen analyses.

REFERENCES

- 1. Babayan, V.K., *J. Am. Oil Chem. Soc.* 58:49A (1981).
- 2. Bach, A.C., and V.K. Babayan, *Am. J. Clin. Nutr.* 36:950 (1982).
- 3. Miller, R.W,, F.R. Earle, I.A. Wolff, and Q. Jones, *J. Am. Oil Chem. Soc.* 41:279 (1964).
- 4. Graham, S.A., F. Hirsinger, and G. RSbbelen, *Am. J. Bot.* 68:908 (1981).
- 5. Wolf, R.B., S.A. Graham, and R. Kleiman, *J. Am. Oil Chem. Soc.* 60:27 (1983).
- 6. Graham, S.A., and R. Kleiman, *Ibid.* 62:81 (1985).
- 7. Hirsinger, F., *Angew. Botanik* 54:157 (1980).
- 8. Hirsinger, F., *Fette, Seifen, Anstrichm.* 82:385 (1980).
- 9. Röbbelen, G., and F. Hirsinger, in *Improvement of oil seeds and industrial crops,* International Atomic Energy Agency, Vienna, Austria, 1982, p. 161.
- Thompson, A.E., *HortScience* 19:352 (1984). 10.
- Thompson, A.E. *Econ. Bot.* 39:436 (1985). 11.
- Hirsinger, F,, and P.F. Knowles, *Ibid.* 38:439 (1984). 12.
- Hirsinger, F, J., *Am. Oil. Chem. Soc.* 62:76 (1985). 13.
- Stubbs, J.M., and A.R. Slabas, *Planta 155:392* (1982). 14.
- *Official Methods of Analysis,* Association of Official Analytical Chemists, 1984, p. 16. 15.

[Received June 24, 1987]