tion of *n*-propyl disulfide, allyl-*n*-propyl disulfide, and allyl disulfide. Allyl-*n*-propyl disulfide, if present, would have appeared as a peak following peak V of Figure 1. Addition of this compound to the oil produced a separate peak at the expected position.

The two different methods of isolation, carbon adsorption of the volatiles and isopentane extraction of steam distillates, yielded oils giving different patterns upon gas chromatography, but the same disulfides and trisulfides were found in each case. This establishes the fact that the trisulfides were not merely artifacts resulting from adsorption on carbon or subsequent desorption with ether. The poorly resolved peak following V in Figure 1,*A*, although appearing in the expected position for allyl-*n*-propyl disulfide, was shown not to contain any of this compound.

As explained in the experimental section, the five volatile carbonyl components were identified as dinitrophenylhydrazones.

The most important new findings are that allyl-*n*-propyl disulfide is not present in significant amounts; methyl as well as *n*-propyl derivatives occur; and substantial quantities of trisulfides corresponding to the disulfides are found.

The isolation of methyl as well as *n*-propyl sulfur derivatives is consistent with the findings of Fujiwara, Yoshimura, and Tsuno (*5*) who obtained evidence for the presence of methyl methanethiolsulfinate, *n*-propyl propanethiolsulfinate, and a mixed methyl *n*-propanethiosulfinate in freshly cut *Allium cepa*. These compounds are very labile and on decomposition will yield disulfides and thiolsulfonates. Significantly, these investigators could find no allyl thiolsulfinates in their onion samples. Virtanen and Matikkala (*13*) have recently demonstrated the presence of *S*-methyl-cysteine sulfoxide and *S*-propyl-cysteine sulfoxide from Finnish onions, and in this laboratory (*12*) a crude enzyme preparation from commercial onions was recently isolated, which decomposes both of these amino acids to thiosulfinates, pyruvate, and ammonia in a manner similar to that of alliinase from garlic.

Since enzymic breakdown of the sulfoxide amino acids produces thiolsulfinates which in turn decompose to thiolsulfonates and disulfides, both of which can further interact with thiols, the actual pattern of disulfides and trisulfides will probably vary with conditions of the enzyme reaction and with the method of extraction.

Although Semmler (*10*) presented evidence for the presence of allyl trisulfide in garlic oil based on elemental analysis of higher boiling fractions, the present work is believed to represent the first unequivocal isolation of pure, well-defined aliphatic trisulfides from plant sources. The authors have no explanation for the formation of these compounds. They are not artifacts of gas-liquid chromatography nor are they formed by a simple mercaptan-disulfide exchange. However, hydrogen sulfide or free sulfur may react with disulfides to yield polysulfides (*14*) and this may be the origin of these compounds in an onion macerate.

Acknowledgment

The authors thank Francis Jones for crystallographic examinations, and Glen Bailey and Edith Gong for infrared determinations. They are grateful to the Basic Vegetable Products, Inc., Vacaville, Calif., for supplying the onions used.

Literature Cited

(1) Carson, J. F., Wong, F. F., *J. Org. Chem.* **22,** 1725 (1957).
(2) *Ibid.*, **24,** 175 (1959).
(3) Challenger, F., Greenwood, D., *Biochem. J.* **44,** 87 (1949).
(4) Dateo, G. P., Clapp, R. C., MacKay, D. A. M., Hewitt, E. J., Hasselstrom, T., *Food Research* **22,** 440 (1957).
(5) Fujiwara, M., Yoshimura, M., Tsuno, S., *J. Biochem. (Tokyo)* **42,** 591 (1955).
(6) Grote, I. W., *J. Biol. Chem.* **93,** 25 (1931).
(7) Guenther, Ernest, "The Essential Oils," Vol. **VI**, p. 70, Van Nostrand, New York, 1952.
(8) Kohman, E. F., *Science* **106,** 625 (1947).
(9) Niegisch, W. D., Stahl, W. H., *Food Research* **21,** 657 (1956).
(10) Semmler, F. W., *Arch. pharm.* **230,** 434 (1892).
(11) *Ibid.*, p. 443.
(12) Schwimmer, S., Carson, J. F., Makower, R. U., Mazelis, M., Wong, F. F., *Experientia* **16,** 449 (1960).
(13) Virtanen, A. I., Matikkala, E. J., *Acta Chem. Scand.* **13,** 1898 (1959).
(14) Westlake, Jr., H. E., Lacquer, H. L., Smyth, C. P., *J. Am. Chem. Soc.*, **72,** 436 (1950).

Received for review May 23, 1960. Accepted October 21, 1960. Presented in part at the Joint Symposium of Analytical and Petroleum Chemistry, 132nd Meeting, ACS, New York, September 1957. Mention of specific products does not imply recommendation by the Department of Agriculture over other of a similar nature not mentioned. Work done at a laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

OFF-FLAVORS IN FOODS

Effect of Pyrrolidonecarboxylic Acid on Flavor of Processed Fruit and Vegetable Products

ABID A. MAHDI,[1] A. C. RICE,[2] and K. G. WECKEL

Department of Dairy and Food Industries, University of Wisconsin, Madison, Wis.

PYRROLIDONECARBOXYLIC ACID (PCA) in processed fruit and vegetable products is formed by the conversion of free glutamine during heat treatment and subsequent storage (*4*). Rice and Pederson (*8*) observed that a relationship might exist between the formation of PCA and the appearance of off-flavors and other undesirable changes in canned foods. Subsequent investigations by Shallenberger and coworkers (*10, 11*) revealed that off-flavors, described as bitter, medicinal, or phenolic, were significant in processed beet purees but not in raw beets, and that the intensity of off-flavor in beet purees varied directly with PCA concentration. An analysis of 22 processed fruits and vegetables, reported herein, revealed the presence of PCA in all products examined. The purpose of this study was to determine the effect of PCA on the flavor of several representative processed products.

[1] Present address, Wisconsin Alumni Research Foundation, Madison, Wis.
[2] Present address, Seneca Grape Juice Corp., Dundee, N. Y.

Methods

Determination of PCA in Commercially Processed Fruits and Vegetables. Commercially canned fruits and vegetables were purchased from local grocery stores. Drained weight and volume of brine were determined, after which the can contents were pureed in a Waring Blendor and filtered through Reeve Angel filter paper No. 202. PCA and glutamine were determined by column chromatography of the filtrate (*4*). Glutamine was determined only in high-acid foods, since it had been reported

The study was undertaken to determine the extent of formation of pyrrolidonecarboxylic acid (PCA) in processed foods and its effect on flavor. PCA was found in all processed fruits and vegetables examined; the concentration ranged from 0.008% in sweet corn to 0.26% in beets. PCA causes a detectable off-flavor when its concentration exceeds a certain level which varies with different products; 0.05% (w./v.) produces an effect in sweet corn and tomato juice. Greater concentrations are necessary in other products. In most processed fruits and vegetables, PCA is not a major factor in flavor deterioration.

Table I. PCA and Glutamine Content of Canned Fruits and Vegetables

Product	Mg./100 Grams Drained Weight	
	PCA	Glutamine
Beets[a]	260	..
Tomato juice[b]	210	7
Pumpkin	118	..
Asparagus	81	..
Carrots	76	..
White potatoes	70	..
Mushroom (buttons)	64	..
Onions	63	..
Wax beans	52	..
Peas	39	..
Dark red kidney beans	36	..
Snap beans	30	..
Sweet potatoes	20	..
Spinach	18	..
Sweet corn	8	..
Red raspberry	44	25
Sauerkraut	35	0
Dark sweet cherries (unpitted)	32	29
Pineapple	23	20
Peaches	17	0
Pears	10	5
Prunes	10	0

[a] Supplied by John Kelly, Horticulture Department, University of Wisconsin.
[b] Sample two years old, University of Wisconsin.

Table II. Effect of PCA on Flavor Preference of Sweet Corn and Green Beans

(Triangulation test)

PCA Level, %	PCA Added to Treated Sample, %	Level of Significance, %	
		Triangulation test	T-I[a]
Sweet Corn			
Control (0.008)	0.10	1[b]	0.10
Control (0.008)	0.05	N.S.[c]	1
Control (0.008) + 0.05 PCA	0.10	N.S.	1
Green Beans			
Control (0.036)	0.10	N.S.	5
Control (0.036)	0.05	N.S.	N.S.
Control (0.036) + 0.05 PCA	0.10	N.S.	N.S.

[a] Triangular-intensity (*1*).
[b] 5, 1, and 0.10%. Significance at level indicated.
[c] N.S. No significant difference.

that all glutamine in low-acid foods is converted to PCA during thermal processing (*4*).

Effect of PCA on Flavor. The effect of PCA on flavor of four canned food products was evaluated by the use of a trained taste panel, on the basis of the multiple comparison test (*5*) or the triangulation procedure (*3, 6, 7, 9*). Products to be tested were selected on the following basis:

Sweet Corn. A product that normally contains a very low concentration of glutamine (less than 10 mg. per 100 grams of drained weight).

Green Beans. A product that normally contains a moderate amount of glutamine (30 to 40 mg. per 100 grams of drained weight).

Beets. A product that normally contains a high amount of glutamine (over 100 mg. per 100 grams of drained weight) and, as a result of the high sterilization temperature employed, contains a large amount of PCA (*4*).

Tomato Juice. A product that normally contains a high amount of glutamine (over 100 mg. per 100 ml.) but, because of the mild sterilization temperature used, contains both glutamine and PCA (*4*).

Canned sweet corn and green beans were processed by commercial procedures. PCA concentration was adjusted by addition of crystalline PCA to the material before preparation of the slurry. It was possible to obtain beets of the same variety and the same seed lot, grown on two soil types (muck and upland sandy loam) at two locations in Wisconsin, and processed by standard commercial procedure. A complete history of these beets was available. The beet samples were evaluated by the tasted panel according to the multiple comparison test (*5*) using sample A with the lowest PCA content (102 mg. per 100 grams of drained weight) as a control. Three evaluations were made:

Slurries were prepared by blending beets and brine for 2 minutes in a Waring Blendor. All of the eight beet samples were evaluated.

The PCA content of samples A, C, and G was adjusted to that of sample H (185 mg. per 100 grams of drained weight). The four samples were slurried in the same way as above, and were evaluated by the same method.

The beets were not slurried, but were submitted to the panel as sliced beets. Only samples A, C, and G were available for this test.

The tomato juice was processed by the regular commercial procedure. Two samples were prepared that differed only in the time of sterilization (15 and 30 minutes) in boiling water. These were given to the taste panel for flavor evaluation using the triangulation procedure.

Results and Discussion

PCA Content of Fruits and Vegetables. PCA and glutamine contents of a number of commercially canned fruit and vegetable products are presented in Table I. The data indicate that all of the processed foods examined contained PCA, which varied from 0.008% (on the basis of drained weight) in sweet corn to 0.26% in beets. The greatest concentrations were found in beets, tomato juice, and pumpkin.

Effect on Flavor of Canned Foods. Foods of Low and Medium Glutamine Content. During initial investigation, the addition of 0.10% PCA to blended sweet corn which contained no PCA initially (*4*) caused a highly significant difference in flavor and 100% preference for the control or unmodified sample as evaluated by the multiple comparison test (*5*). As a result of the significant flavor difference resulting from addition of PCA to corn, further flavor evaluations were conducted. The effects of additions of PCA on flavor of sweet corn and green beans are indicated in Table II.

These results confirm those obtained with sweet corn using the multiple comparison test. Addition of 0.05% PCA to corn slurries did not produce a significant difference between the samples evaluated by the triangulation taste test. However, when the triangulation results were analyzed according to the triangular-intensity procedure (*1*), a highly significant difference was found. Addition of 0.05% PCA to slurries of green beans did not produce a significant difference in flavor regardless of the procedure of flavor evaluation used. The addition of 0.10% PCA to green beans produced a significant difference at the 5% level, when the triangular-intensity test was used. A preference rating of the corn and beans indicated the control was preferred over the treated

sample. The effect of level of added PCA on the flavor of beans was similar to that with peas; an off-flavor was produced only when the level exceeded 0.1% (*2*). Since a number of food products (such as sweet corn, green beans, and peas) normally have a low PCA content, PCA would not be expected to contribute significantly to flavor deterioration or off-flavor development.

FOODS OF HIGH GLUTAMINE CONTENT. *Beets.* Results of taste panel evaluations of beet slurries of the same beet variety but different PCA levels are presented in Table III. As the concentration of PCA increased, greater differences in flavor were observed. Significant differences were observed between samples with lesser and with greater levels of PCA. The flavor of the samples with lesser PCA content (control) was preferred over the samples with greater PCA content. To evaluate the possible influence of factors such as soil type, location, growing period, and harvest date on the PCA content and flavor of beets, a further evaluation was made. Slurries of A and C beets from muck soil were evaluated against slurries of G and H beets from sandy soil after the PCA content of all samples was adjusted to 0.185%. The results presented in Table III indicate a significant preference for muck soil samples A and C. Thus, equalization of the PCA level did not change the highly significant difference between the muck samples, A and C, and those from sandy soil, G and H. In both evaluations sample G was the least preferred, as indicated by the high percentage of nonpreference, 62.5 to 75.6%.

The third taste panel evaluation used sliced beets rather than slurries to determine whether the form in which the sample was presented influenced the results. Although only samples A, C, and G were available for this evaluation, the results were similar to those obtained previously. Sample G was still the least preferred, but the level of significance between samples A and C increased from 5.0 to 1.0%. A and C had the same PCA level and were grown on the same type of soil but in two different locations. These results indicate that the flavor of beets was influenced by components other than PCA.

Other physical and chemical characteristics of the beets used in the flavor evaluations are presented in Table IV. Samples G and H, which were grown on sandy soil, contained higher percentages of soluble and total solids, alcohol-insoluble solids, total nitrogen, and PCA than A and C, grown on muck soil. Although G had a long growing period (124 days), the values obtained for the various components compared closely with the values obtained for H, grown on the same soil, but for a shorter growing period. These values illustrate that soil type definitely has an effect upon the growing beets and that this is reflected on the flavor components as well. Although both samples A and C were grown on muck soil, A was most preferred. One difference noted between these samples was titratable acidity, sample A having apparently one half the acid content of C. This difference may be responsible, in part, for the significant preference for sample A.

Tomato Juice. The effect of PCA on the flavor of tomato juice is indicated in Table V.

The PCA content of treatment B (0.090%, w./v.) was greater than that of treatment A (0.065%, w./v.), as a result of the difference in heat sterilization time, 30 minutes for treatment B, 15 minutes for treatment A. An evaluation of the juices given the two treatments by a trained taste panel using the triangulation procedure indicated that sample A was significantly preferred over sample B; there was a highly significant difference between the two samples. Since the difference might be due to factors other than PCA level, the two samples were re-evaluated after the PCA level had been adjusted in both samples to 0.140% (w. v.). The taste panel did not detect any difference between the two treatments at the same PCA level, and neither treatment was preferred. Thus, equalization of PCA level masked, or minimized, any flavor difference caused by the longer period of heat sterilization. Addition of 0.05% (w./v.) PCA to tomato juice

Table III. Relation of PCA Content to Flavor Preference for Canned Beets

Treatment	PCA Content, %	Type of Soil	Multiple Comparison Test: Level of significance, %	Multiple Comparison Test: % non-preference	Multiple Comparison Test: Significance at 1% level
First Evaluation, Slurries					
A (control)	0.102	Muck	(Control)	18.7[a]	N.S.
B	0.104	Muck	N.S.[b]	37.5	S
C	0.113	Muck	5	54.2	S
D	0.137	Sand	5, 1	56.3	S
E	0.148	Sand	5	37.6	S
F	0.155	Sand	5, 1	43.8	S
G	0.178	Sand	5, 1	62.5	S
H	0.185	Sand	5, 1	54.2	S
Second Evaluation, Slurries					
A (control)	0.185 (adjusted)	Muck	(Control)	24.6[c]	S
C	0.185	Muck	N.S.	50.0	
G	0.185	Sand	5, 1	75.6	S
H	0.185	Sand	5, 1	55.2	S
Third Evaluation, Sliced					
A (control)	0.102	Muck	(Control)	32.0[d]	S
C	0.113	Muck	5, 1	55.2	S
G	0.178	Sand	5, 1	73.2	S

[a c,d] Minimum percentage of nonacceptable required for significance at 1% level. 20.1% in [a], 36 judgments; 10.7% in [c] and [d], 78 judgments.
[b] N.S. No significant difference.

Table IV. Physical and Chemical Characteristics of Beets Used in Flavor Evaluation

[Variety, Perfect Detroit Small Crown (Woodruff Seed Co.)]

Code	Soil Type	Location	Growing Period, Days	Fresh Beets, Solids: Soluble	Fresh Beets, Solids: Total	Fresh Beets, Solids: A.I.S.	Acidity of Slurry: pH	Acidity of Slurry: Total[a]	N_2: Fresh	N_2: Dry weight	Canned Beets: pH	Canned Beets: PCA, %/drained weight
A	Muck	Coleman	73	7.60	10.66	2.76	6.41	1.97	0.238	2.23	5.60	0.102
C	Muck	Madison	68	6.50	10.41	2.93	6.19	4.14	0.226	2.17	5.35	0.113
G	Sandy loam	Madison	124	10.30	13.98	3.21	5.92	3.16	0.375	2.68	5.50	0.178
H	Sandy loam	Madison	67	11.75	13.65	3.31	6.02	3.39	0.371	2.72	5.50	0.185

[a] Ml. 0.1*N* NaOH/20 g. beets (40 g. slurry of 1:1 of beets: H_2O).

Table V. Effect of PCA Content on Flavor Preference of Tomato Juice

(Triangulation procedure)

Controls, % (W/V.)			Treatments, % (W./V.)			Level of Significance, %	
Code	PCA added	Total PCA content	Code	PCA added	Total PCA content	Triangulation procedure	T-I value
A[a]	0	0.065	B[a]	0	0.090	1.0	0.10
A	0.075	0.140	B	0.050	0.140	N.S.[b]	N.S.
A	0	0.065	A	0.075	0.140	1.0	1.0
B	0	0.090	B	0.050	0.140	5.0	0.10
B	0	0.090	B	0.100	0.190	1.0	0.10
B	0.050	0.140	B	0.100	0.190	5.0	1.0

[a] A and B samples sterilized for 15 and 30 minutes, respectively, in boiling water.
[b] N.S. No significant difference.

produced a significant difference in flavor. Addition of greater amounts of PCA produced undesirable flavor and a greater level of significance between treated and untreated samples. The undesirable flavor was described by the taste panel as bitter, medicinal, chemical, and sour. This indicates that the PCA level has a great influence on the flavor of tomato juice. An increase in sterilization time will produce a greater amount of PCA, which in turn will adversely affect the flavor of the juice.

Literature Cited

(1) Davis, J. G., Hanson, H. L., *Food Technol.* **8,** 335 (1954).
(2) Lee, F. A., Shallenberger, R. S., *Food Research* **24,** 68 (1959).
(3) Lockhart, E. E., *Food Technol.* **5,** 428 (1951).
(4) Mahdi, A. A., Rice, A. C., Weckel, K. G., J. AGR. FOOD CHEM. **7,** 712 (1959).
(5) Mahoney, C. H., Stier, H. L., Crosby, E. A., *Food Technol.* **11,** 37 (Symposium) (1957).
(6) Peryam, D. R., *Ibid.*, **12,** 231 (1958).
(7) Peryam, D. R., Swartz, V. W., *Ibid.*, **4,** 390 (1950).
(8) Rice, A. C., Pederson, C. S., *Food Research* **19,** 106 (1954).
(9) Roessler, E. B., Warren, J., Guymon, J. F., *Ibid.*, **13,** 503 (1948).
(10) Shallenberger, R. S., Moyer, J. C., J. AGR. FOOD CHEM. **6,** 604 (1958).
(11) Shallenberger, R. S., Palleson, H. R., Moyer, J. C., *Food Technol.* **13,** 92 (1959).

Received for review May 11, 1960. Accepted September 30, 1960. Research supported in part by the Research Committee of the Graduate School, with funds provided by the Wisconsin Alumni Research Foundation.

COFFEE CONSTITUENTS

Isolation of Chlorogenic Acid and Its Isomers from Coffee

ERNEST SONDHEIMER and C. D. SZYMANSKI

Chemistry Department, State University College of Forestry at Syracuse University, Syracuse 10, N. Y.

J. W. CORSE

United States Department of Agriculture, Western Utilization Research and Development Division, Albany 10, Calif.

A method is presented for the sequential isolation of isochlorogenic, chlorogenic, and neochlorogenic acids, and another isomer designated "Band 510" from coffee beans. Isochlorogenic acid is removed first by extraction with butyl acetate, chlorogenic acid is then precipitated as the caffeine complex, and neochlorogenic acid and "Band 510" are separated by silicic acid column chromatography. The behavior of isochlorogenic acid during acid-base titrations was investigated.

ALTHOUGH CHLOROGENIC ACID was isolated from coffee in 1908 (*7*) and its structure firmly established in 1932 (*6*), interest in this compound is reviving. There is now good evidence for the existence of several isomers of chlorogenic acid and for their widespread occurrence (*12*). Possibly of greater interest is the fact that these compounds are closely related to shikimic acid, an intermediary in the biosynthesis of many aromatics, and that chlorogenic acid has been shown to inhibit the activity of indoleacetic acid oxidase (*10*) and potato phosphorylase (*11*). Also, further work is required to permit a complete structure assignment for the isomers of chlorogenic acid.

Methods for the individual isolation of these compounds have appeared in the literature. Chlorogenic acid, a major soluble constituent of green coffee beans, can be isolated as a crystalline solid from its potassium caffeine complex (*9*). Isochlorogenic acid has been obtained by Barnes, Feldman, and White (*1*) from coffee by extraction with *n*-butyl acetate as a noncrystalline solid. A preparation isolated by Uritani and Miyano (*13*) from sweet potatoes infected with black rot has been named pseudochlorogenic acid. This is a noncrystalline solid with properties that are very similar to isochlorogenic acid. Its existence as a bona fide isomer of chlorogenic acid has not been confirmed. Corse (*4*) has isolated crystalline neochlorogenic acid from peach purée by *n*-butyl alcohol extraction and countercurrent distribution. This isomer is widely distributed in plants, occurs in coffee beans, and seems to be the predominant isomer in members of the prunus family (*12*). A substance isolated from coffee beans by silicic acid column chromatography has been designated "Band 510" and appears to be a true isomer of chlorogenic acid (*12*). Combinations and modifications of the earlier isolation procedures have led to a method which permits the isolation of isochlorogenic acid, chlorogenic acid, Band 510, and neochlorogenic acid from coffee beans in one operation. The high chlorogenic acid content of coffee beans helps make this a convenient procedure. The present authors have also simplified