Table III. Hyperplastic Changes in Rats Fed Sesamol

Type of Lesion	No. of Males	No. of Females	Dietary Level of Sesamol	Days on Diet
Lesions in Rats Fed Commercial Sesamol 400 Days				
Papillomatous foci in bladder	1		0.06%	
	2		0.13%	
Adenoma of lung		1	0.03%	
Glandular tumor of stomach		1	1.0%	
Uterine polyp		1	1.0%	
Ovarian lipoma		1	0.5%	
Lesions in Rats Fed Pure Sesamol 400–634 Days				
Papillomatous foci in bladder	1		1.0%	400
Benign uterine polyp		1	0.03%	632
		1	0.06%	634
		1	0.25%	634
Adenoma of pancreas		1	0.25%	634
Adrenal medullary nodule		1	0.5%	632
Adenoma of adrenal cortex		1	0.25%	634
Mammary adenomas		1	0.016%	576
		1	0.03%	576
		1	0.125%	578
		1	0.25%	578
Subcutaneous lipoma	1		0.016%	576
Fibrosarcoma of ovary		1	0.5%	410

that the malignancy of the lesion was apparently limited.

The livers and other organs showed no changes which could be ascribed to the sesamol. Table III shows the types and frequency of hyperplastic changes in animals receiving pure sesamol for 400 to 634 days.

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OFF-FLAVORS IN PROCESSED CROPS

Relationship between Pyrrolidonecarboxylic Acid and an Off-Flavor in Beet Puree

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Pyrrolidonecarboxylic acid, presumably formed from the decomposition of glutamine, was found in off-flavored beet puree at concentrations greater than 200 mg. per 100 grams of puree. In essentially neutral solution, this acid has a decidedly unpleasant flavor, and off-flavor scores for beet puree correlated well with the acid concentration. When pyrrolidonecarboxylic acid is added to beet puree as the ammonium salt, a taste panel could detect significant flavor differences when the concentration is altered by about 50 mg. per 100 grams of puree. Its contribution to off-flavor in processed food is not uncommon.

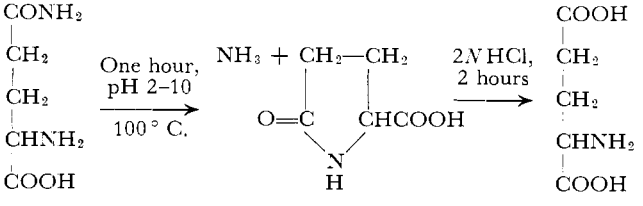
RECENTLY attention was drawn to an off-flavor in some garden beet purees that was variously described as bitter, metallic, medicinal, phenolic, and even burnt. The product showed a delayed flavor reaction, which was also described as cumulative and lingering. This off-flavor was not present in the raw beets as received from cold storage but occurred after processing, which included holding the puree at around 200° F. for some time before filling into jars and retorting for 45 minutes at 245° F. No apparent discoloration accompanied the incidence of off-flavor, but the pH was lower and the titratable acidity higher. Silicic acid column chromatography (*14*) of bitter and nonbitter beet purees revealed the presence of formic and pyrrolidonecarboxylic acid (PCA). The formic acid concentration was essentially the same—10 mg. per 100 grams of puree—but 130 mg. of PCA were found in the nonbitter, whereas 230 mg. were present in the off-flavored sample.

The occurrence of PCA in stored and processed biological materials resulting from glutamine degradation has been observed. Ellfolk and Synge (*6*) found that PCA formed in rye grass stored at −20° C. Goodban, Stark, and Owens (*8*) report that in the formation of molasses during the manufacture of beet sugar there is the "well-known conversion of glutamine to PCA." Rice and Pederson (*14*) found PCA in stored tomato juice in concentrations ten times that of freshly processed juice, and suggested that a relation may exist between the formation of PCA and other signs of tomato juice deterioration such as the formation of off-flavors and off-odors. Jodidi (*9*) isolated PCA from Alaska peas oxidized with bichromate.

Glutamine is found in a number of plants processed for food—for example, members of the families Solanaceae, Cruciferae, Umbelliferae, Cucurbitaceae, and Chenopodiaceae (*3, 16*). Specific examples are the potato (*17, 19*) and tomato (*16, 21, 23*), cabbage, carrots and cucumbers (*16*), and beets (*16, 21–23*). One feature of plants

which store glutamine is the relatively high concentrations which may be attained. For example, Vickery, Pucher, and Clark (*23*) found that beets grown with ammonium salts may accumulate 2% glutamine (dry basis) in the field and 5.4% in sand culture. After cold storage, the glutamine content may be higher (*5*, *22*). Thompson and Steward (*19*) found that glutamine may account for 50% of the soluble nitrogen of the potato variety Sebago.

The chemical reactions of glutamine have been reviewed by Archibald (*1*). One of the most specific reactions of glutamine is the ease with which it decomposes to ammonia and PCA (*1*, *21*). Upon boiling an aqueous solution at pH 2 to 10, ammonia is liberated, the ring closes, and PCA is formed.



Glutamine PCA Glutamic acid

At pH 6.5, 99% of the glutamine is converted in 1.5 hours (*1*). If PCA is refluxed for 2 hours in 2*N* acid, it is quantitatively converted to glutamic acid (*12*). These reactions have been used to measure both glutamine (*12*) and PCA (*8*).

In the present study, the effect of PCA upon the flavor of beet puree has been evaluated, and a routine procedure for measuring PCA has been developed based upon existing methods of analysis. A number of samples of beet puree varying in intensity of off-flavor have been examined for their PCA content and varying quantities of ammonium pyrrolidonecarboxylate have been added to normal beet puree to establish the lower levels at which its taste can be perceived.

Experimental

Preparation of PCA. Crystalline pyrrolidonecarboxylic acid was prepared as a standard in analysis of PCA in beet puree samples and for addition to purees when confirming its flavor effect by taste tests. One hundred grams of L-glutamic acid were carefully heated to melting at 148° C. (*15*). After cessation of bubbling (water evolution), and just prior to a rapid temperature rise exceeding 150° C., the melt was quickly triturated and dissolved in boiling ethyl alcohol. Hexagonal crystals of PCA were obtained upon cooling the solution overnight at 1° C. and recrystallizing it from methyl ethyl ketone—melting point 158–9° C.; $[\alpha]_D^{20} = -8.1$; and yield, 20 gram. Although this material was apparently partially racemized, it did not differ in flavor when compared with L-pyrrolidonecarboxylic acid prepared in smaller yield according to the method of Beecham (*2*), melting point 162–3° C., $[\alpha]_D^{20} = -11.1$. Both samples in essentially neutral solution (pH 5 to 6) had an unpleasant medicinal, phenolic, or bitter flavor. Moreover, they showed a delayed and lingering flavor reaction.

Extraction of PCA from Beet Puree. To extract PCA from beet tissues and to avoid the possibility of degrading intact glutamine to PCA, the method of Pucher and Vickery (*11*, *12*) was followed with a Widmark liquid-liquid extraction apparatus (*11*), operated at room temperature. The beet puree (normally 50 grams) was diluted with 25 ml. of water, 10 ml. of 20% sodium sulfate was added, and the pH was carefully adjusted to 2.5. The puree was then washed into a 500-ml. extraction flask (*11*), covered with ethyl acetate, and the extracted acids were collected in 40 ml. of 0.5*N* sodium bicarbonate. Nineteen hours were adequate for the complete extraction of PCA at levels normally found in beet purees. Analyses of an extract of a glutamine solution adjusted to pH 2.5 revealed negligible decomposition of glutamine during the extraction.

Determination of PCA. An aliquot of the bicarbonate solution containing the beet acids was hydrolyzed with 2*N* hydrochloric acid to convert the PCA to glutamic acid (*8*, *13*), which was then determined by the quantitative paper chromatographic method of Thompson, Zacharius, and Steward (*18*, *20*). Ten to 20 μl. of hydrolyzate were spotted on large sheets of Whatman No. 1 filter paper. This size of aliquot was normally found to give 25 to 100 γ of glutamic acid in the unknowns. To locate and calculate the concentration of glutamic acid, aliquots of a standard glutamic acid solution containing 50 γ were spotted at the same time on the filter paper. The chromatograms were developed for 38 hours in the butanol phase of a 4 to 1 to 5, v./v. butanol, acetic acid, water mixture.

After drying the chromatograms, glutamic acid spots were located by drawing the paper through a trough of 2% alcoholic ninhydrin and heating it at 65° C. for 0.5 hour. Ninhydrin-reacting areas of equal dimensions and corresponding to the standard glutamic acid spot were cut from the chromatograms, the blue color was eluted with 50% ethyl alcohol, and its absorbance was read at 570 mμ with a Beckman Model DU spectrophotometer. The PCA concentration was determined by comparing the absorbance of the glutamic acid spots with the absorbance of the spots of the glutamic acid standards and multiplying the glutamic acid found by 0.88. In all cases, only one blue spot was present in measurable concentration on the chromatograms, a factor justifying the use of one-dimensional chromatography. A yellow area, presumed to be proline, was usually observed on chromatograms of both the hydrolyzed and unhydrolyzed extracts.

Taste Panel Evaluation. To establish a relationship between the PCA content of the beet puree and the intensity of off-flavor, 12 purees having PCA concentrations ranging from 110 to 203 mg. per 100 grams of puree were submitted to a taste panel for evaluation of intensity of off-flavor. The panel consisted of 15 to 20 members who scored three samples at each sitting with a scoring system of 0 to 10 points where a score of 10 indicated the worst off-flavor. The average panel score was used to denote the relative intensity of off-flavor in each sample.

In a second series of taste tests, the contribution of PCA to the off-flavor was measured by adding varying quantities of PCA to samples of a puree having a normal flavor. For this purpose solutions of PCA that had been adjusted with 1*N* ammonium hydroxide to pH 5.3 were added to samples of a normal-flavored beet puree—PCA content, 114 mg. per 100 grams of puree, pH 5.3—so that the final concentration of PCA ranged from 117 to 430 mg. per 100 grams of puree. The consistency of the control samples was adjusted by stirring in equivalent amounts of distilled water. The treated and control samples were then evaluated for flavor differences by the triangle-intensity test of Davis and Hanson (*4*). Of the three samples presented to the panel at each sitting, one was different and two were identical and panel members were asked to select the odd sample and to indicate whether it had more or less off-flavor.

The object of using two taste tests was to demonstrate that the off-flavor, in addition to correlating with the concentration of PCA was, at least in part, caused by high concentrations of PCA.

Results

The concentration of PCA was found to vary directly with the intensity of off-flavor in beet purees. Average panel scores from the ranking flavor evaluation of a series of beet purees varying in their degree of off-flavor have been plotted in Figure 1 against the PCA concentration. The correlation coefficient of 0.90 is significant at the 1% level.

When varying amounts of PCA were added to samples of a normally flavored puree for off-flavor intensity evaluation by the triangle-intensity test, the panel selected the treated purees. The average panel scores from the triangle-intensity tests were then related in Figure 2 to the logarithm of the PCA concentration. In this method of taste-testing, the lower panel scores correspond to an increasing ability to detect flavor differences.

In concentrations approaching or exceeding 200 mg. per 100 grams of puree, PCA may cause an off-flavor in beet puree, and differences in PCA concentration of about 50 mg. per 100 grams of puree may cause a significant flavor difference.

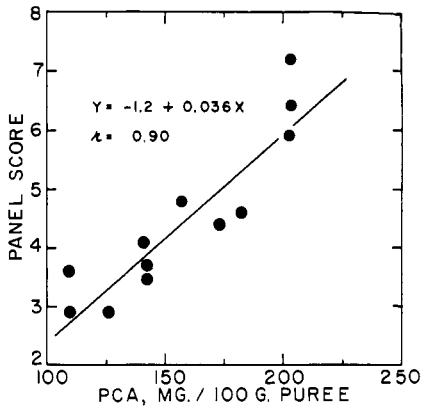


Figure 1. Relation between off-flavor scores for beet puree and concentration of PCA

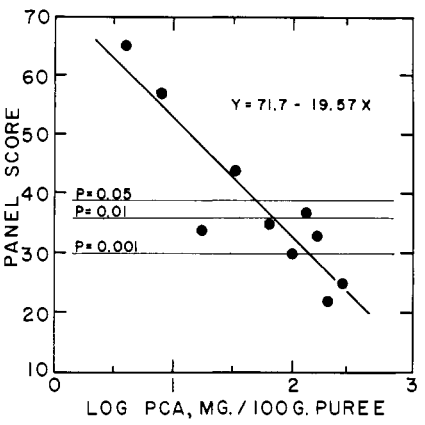


Figure 2. Effect of PCA, added to beet purees as the ammonium salt, upon the triangle-intensity score

Discussion

Taste tests made on aqueous solutions of ammonium, sodium, and potassium salts of PCA showed that they possess unpleasant flavors which might all be described in such terms as bitter and medicinal. Glutamine, the main probable precursor, is essentially tasteless.

Another source of PCA may be glutamic acid (*1, 7, 24*), but the relatively rigorous conditions required for the closing of the lactam ring make glutamic acid an unlikely source of PCA in processed foods. Moreover, this reaction provides no free ammonia. The free ammonia concentration (*13*) was found to parallel the occurrence of PCA in processed purees, and this may be considered as evidence that the probable precursor of PCA in processed beets is glutamine. The assumption that glutamine is broken down during processing to PCA and ammonia, with subsequent loss of buffering capacity, would explain the higher acidity initially observed in the off-flavored samples.

Other Products. The occurrence and contribution of PCA to off-flavor in processed foods may not be uncommon. Higher concentrations of PCA have been found, by the authors, in off-flavored dehydrated potato granules—510 mg. in the off-flavored sample in comparison with 210 mg. per 100 grams (dry-weight basis) of normal flavored granules. The PCA content of off-flavored carrot puree was found to be 50 mg. in contrast with 5 mg. per 100 grams of normal carrot puree. Pederson and Christensen (*10*) have also found an increase of from 70 to 150 mg. of PCA per 100 ml. of juice when comparing normal and off-flavored sauerkraut.

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MILK ANALYSIS

Determination of Manganese in Milk

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The low level of manganese—0.02 to 0.06 γ per ml.—in the presence of a high level of calcium—up to 0.1%—makes the determination of manganese in milk difficult because of turbidity interference by calcium sulfate.

Richards (*2*), using the periodate method with visual estimation, removed the calcium sulfate by precipitation in 33% (v./v.) sulfuric acid. The subsequent evaporation of the acid and the repeated filtrations which may be required to clarify the solution make the method inconvenient, especially with large samples. Fairly large samples, as high as 500-ml., may be required to give accurate results with the periodate method using most ab-