Formation of Pyrrolidonecarboxylic Acid in Processed Fruit and Vegetable Products

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Pyrrolidonecarboxylic acid is produced in processed fruit and vegetable products during heating, both in the blanch and sterilization processes. Pyrrolidonecarboxylic acid and its two possible precursors, glutamine and glutamic acid, were measured by partition chromatography in cherries, peas, snap beans, Lima beans, beets, sweet corn, and tomato juice, before and after heat processing and during a 2-year storage period. Although both possible precursors were present in most products examined, only glutamine contributed to the pyrrolidonecarboxylic acid present after processing.

NUMEROUS UNDESIRABLE CHANGES occur in canned fruits and vegetables during processing and storage, including destruction of vitamins, loss of color, loss of flavor, and development of off-flavors. The presence of pyrrolidonecarboxylic acid (PCA) in certain processed vegetables (*5*, *8*, *13*, *15*) may be regarded as an additional example of a specific heat-induced chemical change, as the compound is not a constituent of the fresh or unprocessed product (*4*, *7*, *10*). Pyrrolidonecarboxylic acid contributes to off-flavor in beet purees and may be responsible for flavor deterioration of other processed foods as well (*14*).

Pyrrolidonecarboxylic acid may be formed from either glutamic acid or glutamine. Quantitative conversion of glutamine occurs in 1 to 2 hours at 212° F. within a pH range of 2 to 10, whereas a quantitative conversion of glutamic acid occurs in 4 hours at a temperature of 257° F., and a pH range of 3 to 4 (*1*, *16*). The mild conditions needed to transform glutamine would suggest that most of the PCA found in canned food products comes from this compound. However, the sterilization process (15 to 35 minutes, 240° to 250° F.), required for low acid canned foods is sufficiently drastic that some of the glutamic acid could also be transformed into PCA. As salts of glutamic acid may contribute to the desirable flavor of foods, loss of glutamic acid during processing could cause additional flavor deterioration. This might explain, in part, the value of adding monosodium glutamate to foods.

The present investigation was undertaken to determine the precursor or precursors of PCA in food products, and the extent to which PCA may occur in a variety of canned foods.

Methods

Samples. Canned peas (regular and Blair process), red, sour, pitted cherries (Montmorency), sweet corn, tomato juice, snap beans, beets, and Lima beans were obtained during the 1956 season in accordance with the following schedule:

NOT HEAT PROCESSED. Six cans were removed from the processing line after closure of the can and frozen immediately.

HEAT PROCESSED. Six cans were frozen immediately after heat sterilization. Thirty-six cans were obtained after completion of processing, and were stored at three temperatures, 40°, 72°, and 90° F. for periods of 1, 6, 12, and 24 months.

The thermal process received by each of the canned products is presented in Table I.

Analytical Procedure

Measurements of PCA, by partition chromatography, were made on the brine from the canned products. Glutamic acid and glutamine were converted to PCA which was then determined chromatographically. All values reported for glutamic acid, glutamine, and PCA are expressed as milligrams per 100 grams of drained weight of product.

The brine was drained from the canned product, measured volumetrically, and divided into three portions. The first portion was analyzed directly for PCA content. The second portion was adjusted to pH 6.7 and heated for 1 hour at 214° to 216° F. (*12*). This treatment converted over 99% of the glutamine, but only a negligible amount of glutamic acid to PCA. The PCA content of this portion, minus that of the first portion, gave the PCA equivalent of glutamine present in the sample. The third portion of brine was acidified to pH 3.3 with concentrated hydrochloric acid, and was heated for 4 hours at 260° F. (*6*, *11*, *17*). This treatment converted glutamine and over 94% of the glutamic acid to PCA. The PCA content of this portion, minus that of the second portion, gave the PCA equivalent of glutamic acid present in the sample.

A silicic acid column, 7 mm. inside diameter and requiring 6.5 grams of silicic acid, was used (*3*, *9*, *13*). The brine samples were prepared for chromatographic analysis by acidifying a measured portion to pH 1.0 with concentrated sulfuric acid, mixing 0.5 ml. with 0.75 grams of dry silicic acid, and adding the blend to the top of the column. A solvent schedule of 150 ml. of 5% *n*-butyl alcohol in chloroform (v./v.) followed by 200 ml. of 10% *n*-butyl alcohol in chloroform (v./v.) was employed. Pyrrolidonecarboxylic acid was eluted between the 26th and 30th fractions, with a peak at the 28th fraction.

Results

The results, presented in Table II, clearly indicate the relationship of PCA to glutamine in these foods. Glutamine was present in the brine of

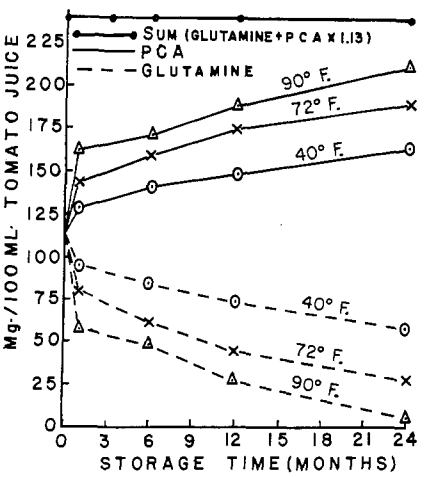

Figure 1. Relationship of glutamine, PCA, and summation of glutamine plus degraded glutamine (glutamine + PCA × 1.13) in tomato juice during 24 months of storage at the temperatures indicated

all products, before sterilization, except Lima beans, sweet corn, and Blair processed peas. After sterilization, PCA was present in all products except Lima beans and sweet corn. Thus, with the exception of Blair processed peas, all canned products that contained measurable quantities of glutamine prior to sterilization contained PCA following this step. Corn and Lima beans, which contained no glutamine initially, contained no PCA after sterilization.

That glutamine serves as the sole precursor of PCA was confirmed by using tomato juice and demonstrating, in Figure 1, that the summation of intact and degraded glutamine (PCA × 1.13) is a constant value, regardless of the storage time or temperature. The same relationship existed in canned cherries, and also in beets and peas when a slurry of the vegetable was analyzed.

Discussion

Although glutamic acid was present in all products examined, no relationship could be established between its concentration and the PCA observed. In most products, the glutamic acid concentration remained stable during both the processing and the storage periods. The glutamic acid concentration of tomato juice, as shown in Table II, remained constant throughout the processing and storage periods, and bore no relationship to the increasing PCA content during this period.

To further check the stability of glutamic acid under conditions similar to those encountered in the vegetable products during processing, 0.1% solutions of glutamic acid buffered at pH 5.5 and 6.0 with phosphate-borate buffers were heated for 40 minutes at 240° F. and then analyzed for PCA. No PCA was found, chromatographically or colorimetrically (*17*), at either pH. Thus, all the evidence indicates that glutamic acid does not contribute to PCA formation in these products.

The products could be separated into two groups, based on the sterilization temperature employed. In the first group, which included those low acid products processed at 240° F. or above, all of the glutamine was converted to PCA during sterilization. In the second group, which included those high acid products processed below 212° F., only a part of the free glutamine was converted to PCA during the sterilization, the remainder slowly undergoing conversion during storage.

Pyrrolidonecarboxylic acid was found in beets, tomato juice, and cherries prior to sterilization. Each of these products had undergone mild heat treatment before sterilization—e.g., the beets were steamed, prior to peeling, the hot break procedure was used with the tomato juice, and the cherries were steamed (exhausted), and some conversion of glutamine may have occurred during these operations. This was confirmed, for beets, by the analysis of extracts of whole beets before and after steaming. Pyrrolidonecarboxylic acid was not found in the extract before steaming, while 65 mg. were found in the extract of beets steamed for 20 minutes at 210° F. Thus, for the canned products examined, PCA did not occur in the unprocessed vegetable as such, but was formed only after the product had undergone some heat treatment.

Sweet corn and lima beans contained no PCA or glutamine throughout the storage period. To determine if this was characteristic of these items, additional samples of canned Lima beans and sweet corn were obtained from a local grocery and were analyzed for PCA content. Up to 8 mg. of PCA were found in the sample of corn, and 14, 8, and 0 mg. of PCA were found in the samples of Lima beans. Thus, the amount of PCA normally present in these two items is negligible.

Although, glutamic acid and glutamine were found in regular process peas, neither constituent was found in the brine of Blair process peas, prior to sterilization. Pyrrolidonecarboxylic acid appeared in the brine of the Blair process peas within one month of storage, and throughout the balance of the storage period remained at a level of

Table I. Process Time and Temperature of Canned Foods

Product	Process, ° F.	Process Time, Minutes
Peas		
Blair	260	7
Regular	240	35
Snap beans	240	25
Sweet corn	240	30
Beets	240	40
Lima beans	240	40
Tomato juice	206	20
Cherries	195	7

Table II. Pyrrolidonecarboxylic Acid, Glutamine, and Glutamic Acid Content[a] of Various Canned Foods before and after Sterilization and during Storage

Product		Before Sterilization	After Sterilization	Storage Interval: 1 Month			6 Months			12 Months			24 Months		
				40° F.	72° F.	90° F.	40° F.	72° F.	90° F.	40° F.	72° F.	90° F.	40° F.	72° F.	90° F
		Mg./100 G. Drained Wt.													
Snap beans	PCA	0	27	29	26	31	30	29	30	...	...	...	30	31	30
	Glutamine	27	0	0	0	0	0	0	0	...	...	...	0	0	0
	Glutamic acid	7	8	7	8	8	8	7	11	...	...	...	...	...	...
Peas, regular process	PCA	0	7	8	23	29	26	28	34	38	40	39	38	39	39
	Glutamine	8	0	0	0	0	0	0	0	0	0	0	0	0	0
	Glutamic acid	14	16	17	19	22	25	25	30	27	27	28	...	...	...
Peas, Blair process	PCA	0	0	4	5	7	11	15	13	...	14	14	15	15	15
	Glutamine	0	0	0	0	0	0	0	0	...	0	0	0	0	0
	Glutamic acid	0	36	36	37	39	38	38	39	...	37	38	...	...	...
Beets	PCA	4	64	78	88	97	77	89	111	105	99	111	106	102	108
	Glutamine	24	0	0	0	0	0	0	0	0	0	0	0	0	0
	Glutamic acid	5	5	8	8	8	9	9	10	8	8	8	...	...	...
Sweet corn	PCA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Glutamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Glutamic acid	13	23	27	27	31	27	31	32	34	31	38	...	...	...
Lima beans	PCA	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Glutamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Glutamic acid	0	13	13	13	14	13	14	14	15	15	16	...	...	...
Cherries	PCA	20	27	29	35	41	30	47	52	40	50	53	50	54	57
	Glutamine	47	40	35	23	20	36	14	12	23	11	9	9	4	2
	Glutamic acid	7	7	4	6	7	5	7	5	5	5	7	7	6	6
Tomato juice	PCA	49	115	132	144	164	141	161	173	150	176	190	164	190	210
	Glutamine	...	114	95	81	59	85	62	49	75	46	29	59	29	7
	Glutamic acid	210	210	210	210	210	210	210	210	210	210	210	210	210	210

[a] Determined from brine of canned products.

only 1/4 to 1/2 of that observed in regular process peas. To determine the cause of this difference, a lot of No. 3 sieve-size peas was divided into two portions. One portion was processed by the regular method, the other by the Blair process (*2*). Pyrrolidonecarboxylic acid content was found to be 28 mg. in the regular process peas, and 21 mg. in the Blair process peas. Thus, 25% less PCA was present in the lot processed by the Blair method. Evidently this loss resulted from the leaching of substantial amounts of free glutamine from the peas during the soaking and blanching steps (in sodium carbonate and calcium hydroxide solutions, respectively) of the Blair method.

Other factors, such as variety, also may affect the PCA level in peas. Samples of Blair and regular process peas were obtained from the 1958 pack of a processor (Canned Food, Inc., Waupun, Wis.) who used a special variety of peas for the Blair process. The regular pack, All Sweet type, contained 11 mg. while the special variety processed by the Blair method contained 19 mg. of PCA.

With products processed at a high temperature and containing low acid content, the apparent increase in PCA in the brine, as shown in Table II, was due to the gradual diffusion of PCA from the plant tissue. The rate of diffusion differed somewhat depending on the plant product and the storage temperature.

Conclusion

Low acid foods that normally are processed at high temperatures will contain PCA in an amount proportional to the glutamine content of the raw stock, and stoichiometrically related to the amount of glutamine decomposed. The probability of eliminating PCA seems remote, unless cultural practices, raw product storage conditions, and varieties are selected to minimize the free glutamine content in the raw product. High acid foods that normally require processing temperatures below 212° F., and short sterilization times will have only a part of the initial free glutamine converted to PCA during the processing operation. Conversion of the remaining glutamine will occur during storage, but the rate of conversion will be dependent on the storage temperature.

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SWEET POTATO DEHYDRATION

Interactions between Copper Ions and Sweet Potato Polyphenolase Oxidized Substrates

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THE POLYPHENOLASE OF THE SWEET POTATO is often dormant or inactive in the potato tissue, becoming active on injury of the plant tissue or on separation from the plant tissue. During the dehydration of sweet potatoes, the activation of the enzyme leads to the oxidation of natural substrates which discolor the potato tissue, unless certain practical procedures, which can minimize this discoloration, are followed (*1*).

The activation of the enzyme also leads to its reaction inactivation. Probably one of the causes of the phenomenon of reaction inactivation is the decrease in the effective concentration of the enzyme by the removal or binding of its metal prosthetic group, copper (*4*), during oxidation of the substrate. Joselow and Dawson (*7*), using radioactive copper-64 and relatively large quantities of enzyme, investigated the exchange reaction between the copper of ascorbic acid oxidase and ionic copper. They reported a significant amount of exchange when the enzyme was actively catalyzing the oxidation of ascorbic acid in an aerobic system. In a previous communication from this laboratory, using ion exchange methods, radioactive copper-64, and catalytic quantities of tyrosinase, free copper ions formed complexes with the oxidation products of the substrates (*2*).

This report presents experimental data, obtained with sweet potato polyphenolase and radioactive copper, which indicate the effects of reaction conditions on the formation of copper complexes with oxidation products of the substrates and possibly ionic copper exchange with enzymatic copper.

Materials

Sweet potatoes of the Unit I Porto Rico variety were obtained directly from a grower. The peelings and cortex were