

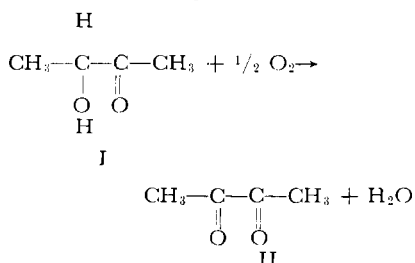
Nonenzymatic Formation of Acetoin in Canned Vegetables

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The concentration of combined 3-hydroxy-2-butanone (acetoin) and 2,3-butanedione (biacetyl) in spinach, asparagus, beets, and peas is 120 to 300 p.p.m. The acetoin level of blanched, frozen peas was increased from 260 to 340 p.p.m. by heating at 100° C. for 15 minutes; heating for 45 minutes caused the concentration of acetoin to drop to 285 p.p.m. The observed increase of acetoin during the short heating period is due to the thiamine-catalyzed conversion of pyruvic acid to acetoin. This reaction was demonstrated with model systems in the pH range and reactant concentration levels found in canned vegetables. There is no simple relationship between thiamine and acetoin contents in canned vegetables. The presence and concentration changes of acetoin during processing must be considered in any explanation of vegetable flavor.

DURING INVESTIGATIONS of flavor in canned vegetables, a study was made of the content and formation of the known flavor constituent, 3-hydroxy-2-butanone (I), more commonly called acetoin (8). This compound has been reported in corn and kidney beans (6), peas (4), and broccoli (2). There is no quantitative report on acetoin concentration levels in canned vegetables. Part of the role of acetoin in flavoring vegetables may lie in its ready autoxidation to biacetyl (II), a more volatile and intense flavor component.



Experimental

The peas used were portions of 2467 pounds of blanched, frozen, Perfection (canners) peas prepared on June 25, 1958, at Milton, Ore. The canned vegetables were commercial products purchased at a local market. All equipment used with the vegetable materials was sterilized by heat to avoid microbiological formation of acetoin.

Colorimetric Determination of Acetoin and Biacetyl. The method of Westerfeld (12) for the determination of acetoin was modified. The reproducibility of multiple analyses was influenced by the quality of the 1-naphthol used in the color reaction for biacetyl determination. To obtain consistent results 1-naphthol was purified each day analyses were run. The 1-naphthol was codis-

tilled with water, separated by filtration from the cold water suspension, and dried to constant weight over phosphorus pentoxide at reduced pressure. The color reaction was run on 5 ml. of distillate containing 1 to 7 γ of biacetyl as described by Westerfeld, except that 50 mg. of purified, solid 1-naphthol and 1.0 ml. of 2.5*N* sodium hydroxide were used in place of the 1-naphthol solution. A calibration curve was compiled from analyses of known concentrations of crystallized (from acetone) acetoin dimer. All analyses were run in duplicate or triplicate.

COMBINED ACETAIN AND BIACETYL IN CANNED VEGETABLES. The canned

vegetables were homogenized in a food blender. Weighed amounts of about 100 grams of the total can contents were diluted with distilled water to a final volume of 250 ml. A 10-ml. aliquot of the suspension was centrifuged to remove solids. The solids were washed three times with 10-ml. portions of water. The aqueous extracts were combined and diluted to 250 ml. The pH of this solution was measured and a 2-ml. aliquot taken for analysis of acetoin and biacetyl.

ACETAIN IN FROZEN, INTACT PEAS. The frozen peas were thawed for 4 hours at room temperature. They were treated with cold water, hot water, or buffer solutions, and separated by filtra-

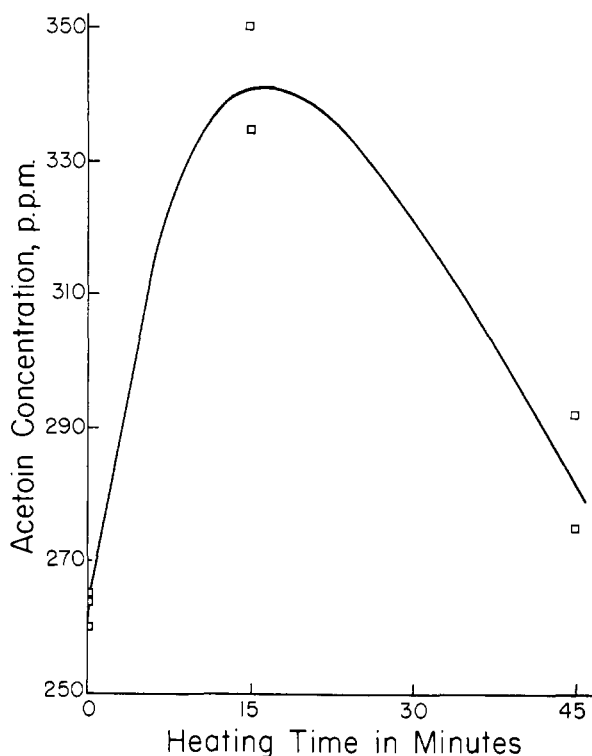


Figure 1. Change in acetoin content of peas at 100° C.

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Table I. Combined Acetoin and Biacetyl Concentration, Thiamine Content, and pH of Canned Vegetables

Vegetable	Acetoin + Biacetyl, Mg./100 G.	Thiamine, Mg./100 G. (9)	Average pH of Diluted Contents
Peas, green, sweet	30.4 ± 2.3	0.109	6.62
Beets, red, diced	27.9 ± 0.8	0.009	6.02
Spinach, leaf	12.2 ± 0.3	0.019	6.00
Asparagus, green, spears	12.6 ± 0.7	0.064	5.96

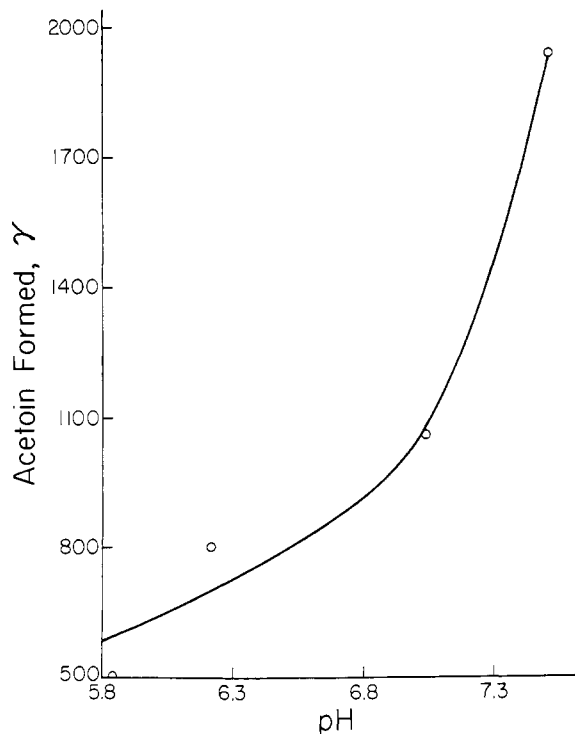


Figure 2. Acetoin formation from pyruvic acid (0.1M) and acetaldehyde (1.0M) catalyzed by thiamine (0.1M) as a function of pH at 35° C. for 24 hours

tion. The filtrate was diluted to 250 ml. the pH taken, and aliquots were assayed for biacetyl or acetoin.

ACETOIN IN HEATED, HOMOGENIZED PEAS. Fifty grams of frozen peas were thawed for 4 hours, 40 ml. of distilled water were added, and the container was heated in a boiling water bath for the times shown in Figure 1. The cooked peas were cooled, homogenized in a food blender, and made up to a volume of 100 ml. with distilled water. The subsequent operations were identical to those for canned vegetables.

Model Studies on Thiamine-Catalyzed Conversion of Pyruvic Acid to Acetoin. Pyruvic acid (0.1M), acetaldehyde (1.0M), and thiamine chloride hydrochloride (0.1M) solutions were used as previously described (7), except on a 10-fold scale. The reactions were run in a nitrogen atmosphere in incubators at 25° or 35° C. The 100° C. runs were made with a positive nitrogen pressure over the system; heat was supplied by a boiling water bath. The reaction mixtures were diluted to 250 ml. with water and 7-ml. aliquots were taken for the acetoin determination.

The experiments were conducted in a laboratory illuminated with daylight passing through flint glass windows; there was little possibility of ultraviolet light-catalyzed formation of acetoin (10).

Results and Discussion

Analyses of four canned vegetables for combined acetoin and biacetyl content are shown in Table I. The data are for duplicate analyses of total contents of single cans. These results show that acetoin (or biacetyl) is present in appreciable concentrations in a variety of vegetables. Acetoin is formed in the growing vegetable from pyruvic acid with pyruvic carboxylases (or carboligases) as catalysts (11). The prosthetic group of these enzymes is thiamine pyrophosphate. It might be expected that there would be a direct relationship between thiamine and acetoin contents in vegetables. The data in Table I show no such simple correlation. The acetoin content of vegetables is undoubtedly a complex function of pyruvic acid content, enzyme concentration, and pH. The major portion of acetoin is

Table II. Extraction of Acetoin from Thawed Peas

Treatment	Acetoin Extracted, γ/G. Fresh Weight
Extraction of intact peas with water at 25° C., thawed 4 hours	49
Extraction of frozen, intact peas with boiling water, heated 12 min.	84
With boiling 0.5M Na ₂ HPO ₄ at pH 8.7, heated 12 min.	200
Extraction of homogenized peas with water at 25° C., thawed 4 hours	260

probably formed by enzymatic processes in the preblanched vegetable.

The major effort in this study was devoted to the acetoin content of peas. In dilute solutions prepared from crystalline acetoin almost no acetoin is distilled out with water, under the conditions of the analysis. Extracts of frozen, intact peas showed no appreciable concentration of biacetyl. The amount of acetoin extracted from peas was influenced by the type of treatment employed (Table II). The first point of interest is the evidence of partial migration of acetoin through the skin of the intact pea. The membrane effect was influenced by the pH and ionic strength of the extracting aqueous solution. These data are pertinent to the question of the flavor of packaged peas prepared for household or institution consumption. About one third of the acetoin would be extracted into the cooking water of frozen peas, or into the liquid portion of thermally processed whole peas. The extracted acetoin would be oxidized rapidly to biacetyl, but the remaining two thirds of the acetoin would be present in the peas as eaten.

The changes in acetoin content of intact peas with heating are shown in Figure 2. During short heating periods the acetoin concentration increases; then with longer heating the free acetoin concentration decreases. The decrease is readily explained on the basis of the known high degree of reactivity of acetoin with functional groups of organic molecules (3). The increase of acetoin concentration presents a more interesting problem and can be explained by the nonenzymatic conversion of pyruvic acid to acetoin catalyzed by thiamine.

The formation of acetoin from pyruvic acid and acetaldehyde at pH 8.4 in the presence of thiamine was demonstrated in 1951 (7). The emphasis of subsequent research has been on the mechanism of thiamine action in enzymatic systems. The progress in this field and the latest proposal for the catalytic thiamine structure are presented in a

Table III. General Base Requirement in the Nonenzymatic Formation of Acetoin Catalyzed by Thiamine at pH 8.4 and 35° C. for 24 Hours

(Pyruvic acid (0.1M), acetaldehyde (1.0M), thiamine (0.1M))

Base	Thiamine	Acetoin Formed, γ
NaOH	Absent	250
	Present	1,500
Ba(OH) ₂	Present	850
CH ₃ CO ₂ K	Present	4,400
K ₂ HPO ₄	Present	10,000

recent publication (7). All the previous workers have run their experiments at pH 8.4 and used only one base, sodium hydroxide. Before the reaction could be accepted as the cause of the observed acetoin increase in heated peas, it was necessary to establish several criteria: general base requirement, appreciable rate of reaction at pH 5 to 7, and formation of acetoin from pyruvic acid at low concentrations.

The data in Table III clearly demonstrate the general base requirement for the thiamine-catalyzed formation of acetoin from pyruvic acid and acetaldehyde. The reaction is also influenced by the ionic strength of the solution. The amounts of potassium acetate and dipotassium hydrogen phosphate required to adjust the pH to 8.4 caused a marked increase in acetoin.

Figure 1 shows the data for the amount of acetoin formed at normal vegetable pH; the reaction proceeds at moderate rate under these conditions.

VEGETABLE VITAMINS

Loss of Vitamin C in Fresh Vegetables as Related to Wilting and Temperature

Fruits and vegetables furnish approximately 94% of the vitamin C in the diet of urban families in the United States. These foods are largely consumed fresh; temperature and humidity are primary factors in their preservation. Kale, collards, turnip greens, spinach, rape, cabbage, and snap beans were subjected to slow, moderate, and rapid wilting at 32°, 50°, 70°, or 75° F. Conditions favorable to wilting resulted in a more rapid loss of vitamin C. Vegetables that lose moisture readily and wilt appreciably tend to be affected more by humidity and to lose vitamin C more rapidly than those resistant to wilting. Even those that wilt most readily are affected much less by humidity than by temperature.

IF THE RECOMMENDED DAILY dietary allowances of the Food and Nutrition Board, National Research Council, 1953, are accepted as representing the relative amounts required, then vitamin C (ascorbic acid) is required in the diet in greater amounts than all the

Table IV. Formation of Acetoin in Dilute Pyruvic Acid ($1 \times 10^{-4}M$) and of Thiamine ($1.5 \times 10^{-5}M$) Solutions at pH 7.16

Time of Reaction, Hours	Temp., ° C.	Acetoin Formed, γ
0.0	25	2.6
0.5	100	7.3
1.0	100	6.0
24	25	3.0
48	25	3.0
216	25	3.5

The last question which needed to be answered was the formation of acetoin at the low concentration levels found in vegetables. The data in Table IV illustrate the results of this study at reported thiamine levels in peas and at reasonable pyruvic acid concentration. Very small amounts of acetoin are formed at room temperature even over long periods of time. Significant amounts of acetoin are formed by short heating at 100° C. Thiamine activity is rapidly lost in aqueous solutions at pH 7. Therefore, the 100° C. reaction would soon terminate because of thiamine destruction. Thiamine is much more resistant to heat in vegetable materials than in aqueous solutions (5). It would be expected that more acetoin would be formed in the vegetable during heating than is formed in aqueous solutions. The extent of formation would be limited by the amount of pyruvic acid available for reaction.

These studies demonstrate that acetoin can be formed in vegetable products

by a nonenzymatic reaction from pyruvic acid catalyzed by thiamine. The results make it desirable to consider the changes of acetoin content during processing, in order to preserve the normal contribution of this important factor to vegetable flavor.

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available if deficiencies are to be prevented.

Fruits and vegetables, including potatoes, furnish 94% of the vitamin C in the diet of urban families in the United States (2). These crops are often grown in districts far removed from the