Development of Off-Odors and Off-Flavors in Papaya Puree

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Papaya puree prepared by macerating papaya fruit without special treatment invariably develops off-flavors and off-odors due to enzymatic or microbial activity. The development of undesirable odors was investigated in papaya purees prepared in laboratory and pilot plant operations. Butyric, hexanoic, and octanoic acids and their methyl esters were found in purees prepared by commercial methods; these samples had strong off-odors and off-flavors. In an improved method for processing papaya puree, acidification and heat inactivation of enzymes prevented development of the compounds, which contributed to unpleasant odor and flavor.

Papaya (*Carica papaya*) is now one of the major export crops of Hawaii; most of the crop is exported in the fresh form. Since the adoption of uniform standards last year through a marketing order granted by the U. S. Department of Agriculture, increasing amounts of off-grade papayas have become available for processing. The development of off-flavors and off-odors has been a major obstacle to increasing the amount of papayas processed into puree. These off-flavors and off-odors have been described as "sulfury, butyric, acrid, pungent, sour, amine-like, and bitter."

Commercially frozen papaya puree, as now prepared, is not heat treated. Hence, off-flavors might be induced by reactions due to naturally occurring enzymes during processing and frozen storage. The puree is frozen in bulk containers of 25 lb or more and is subsequently allowed to thaw at room temperature for about 24 hr before being used in the preparation of other food products. Conceivably, off-flavors could arise from microbial action in the center of the puree mass prior to freezing and at the periphery of the mass during thawing.

In their work on papaya volatiles, Katague and Kirch (1965) reported the presence of numerous acetate esters, the corresponding free alcohols, and 2-heptanone. Several authors (Ettlinger and Hodgkins, 1956; Tang, 1971) reported the presence of benzyl isothiocyanate in macerated papaya pulp and seeds, and Tang (1971) has shown that, with maturity, the concentration of this component (or its glucosinolate precursor) decreases in the flesh and increases in the seeds.

The purpose of this study was to determine the nature and origin of off-flavors and unpleasant odors in papaya puree and to evaluate improved processing methods.

EXPERIMENTAL SECTION

Samples for Observation of Enzymatic and Microbial Activity. Fully ripe papayas, Solo variety, that were off-grade for fresh market were used to prepare puree. Whole fruits were steamed for 2 min to coagulate the milky latex in the peel and to inactivate enzymes in the peel. The fruits were halved and the seeds were removed. The fruit was then crushed in a papaya deflesher (Angara *et al.*, 1969), a device also known as a "crusher-scraper." The resulting pulp was separated from the skins in a centrifugal separator and passed through a paddle pulper fitted with a 0.027-in. screen. At this point the pulp was divided into two portions. One was passed immediately through a tubular heat exchanger in which it was heated to 210°F and held for 17 sec to inactivate enzymes, as indicated by a negative test for catalase. These samples were designated "−E." Part of this enzyme-inactivated material, −E, was then treated with penicillin G (100 units/g) (E. R. Squibb & Sons, Inc.) and aureomycin (100 μg/g) (Lederle Labs.) to inhibit microbial growth. This sample was assumed to be free of enzymes and microorganisms and was designated as "−E −MO." These purees then were transferred to 6-oz enamel-lined cans, sealed, frozen at −78° in a Dry Ice–acetone bath for 30 min, and then stored at −17.8°. The other half of the material from the heat exchanger was inoculated with about 1%, by weight, of spoiled puree, which contained an abundance of natural microflora. This inoculum contained a mixed flora of gram-positive cocci and bacilli. The inoculated sample was held at room temperature for 24 hr, then frozen in the same manner as above in 6-oz cans, and designated as "−E +MO." The unheated portion of the puree containing active enzymes was protected from microbial action by the addition of the two antibiotics as above. After 4 hr at room temperature, the puree was passed through the heat exchanger in the same manner as above to inactivate enzymes; the heat treatment was sufficient to inactivate catalase. This sample was designated as "+E4 −MO" to indicate 4 hr of enzyme activity and no microbial activity. These frozen puree samples (−E −MO, +E4 −MO, and −E +MO) subsequently were thawed for sensory evaluation.

Sensory Evaluation. Aromas of the thawed purees were evaluated by a panel of 11 members experienced in judging papaya products. A seven-point scoring scale was used on which: 1 = natural papaya aroma, absence of off-aroma and 7 = intense off-aroma. Samples were thawed with running tap water at room temperature just before serving. Each judge received 50 ml of puree in 8-oz wine goblets which were covered with petri dish covers. The panel evaluated the samples at four different sessions, resulting in 42 observations per treatment. The control (−E −MO) was compared with the enzyme-active sample (+E4 −MO) and with the microbially active sample (−E +MO).

Samples for Acidification Effect. Previous work at this laboratory had indicated that off-flavor development in puree was inhibited by citric acid (Brekke *et al.*, 1972a,b), possibly through inhibition of microbial growth and activity. To investigate this effect, puree was prepared as described above by steaming, removing seeds, separating pulp from peel, and passing the pulp through a paddle pulper. The enzymes in the puree were inactivated by heat in the tubular heat exchanger. A control sample was prepared by adding the antibiotic mixture of penicillin G and aureomycin. A microbially contaminated sample was prepared by adding 1%, by weight, of puree, which had a

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high level of microbial activity. The inoculated material was then divided into two portions. One was acidified to pH 3.55 and the other remained at its natural pH 5.2. The control, acidified, and nonacidified purees were incubated at room temperature (25°). Samples were withdrawn at 6-hr intervals and analyzed for pH, total acids, volatile acids, and bacterial activity.

Total titrable acidity and total volatile acidity were determined by the methods of the Association of Official Agricultural Chemists (1960); pH was determined with a glass-electrode pH meter. Bacterial activity was estimated by the methylene blue-reduction method of the Standard Methods for the Examination of Dairy Products (American Public Health Association, 1967). The samples to which methylene blue was added were incubated at room temperature (25°).

Volatiles from Fresh Fruit, Commercial Puree, and Improved-Process Puree. A study of the volatile odor and flavor constituents of papaya and processed papaya puree included comparisons of the chromatograms of volatiles from fresh fruit and from puree samples prepared in different ways.

A puree was made by commercial methods by comminuting the whole fruit in a pulper fitted with a 0.033-in. screen. The puree was not heated to inactivate enzymes and contained some pulverized peel and seeds.

The improved method for preparation of papaya puree (Brekke *et al.*, 1972) is summarized here and shown on the flow chart (Figure 1). Whole ripe papayas were steamed for 2 min; this coagulated the latex, inactivated enzymes in the skin, and softened the outer portion of the fruit. The fruit was spray cooled, sliced, and crushed in a deflesher or crusher-scraper (Angara *et al.*, 1969). Peel was separated from the pulp and seeds in a centrifugal separator. The intact seeds were separated from the pulp by means of a paddle pulper fitted with rubber paddles to prevent rupturing the seeds and sarco testa. The pulp was acidified with citric acid (in 50% solution) to pH 3.3–3.6, then pumped through a heat exchanger in which it was held at 205°F for 2 min, and then cooled to 85°F through the cooling section. It then went through a paddle finisher fitted with a 0.020-in. screen to remove specks and some fiber. The finished puree was poured into 25-lb paperboard containers with plastic bag liners and was frozen in a blast freezer at 0°F.

A modified version of the apparatus described by Forss *et al.* (1967) was used to concentrate the more volatile organic components of papaya fruit and puree. To recover volatiles from fresh papaya, fully yellow ripe fresh fruit were quartered without cutting into the seeds. The seeds were removed carefully to avoid rupturing the sarco testa, and the papaya skin or peel was removed with a knife. The flesh was cut into thin slices and about 2.7 kg was placed in the pot of the apparatus, which had been previously flushed with nitrogen. Two liters of distilled water was added to permit better stirring. For puree stripping, 6–8 kg of material was used. Distilled water was only added if the volume of a given batch of puree was too small for adequate stirring or temperature monitoring. The volatiles were stripped from the pot contents under vacuum at room temperature or lower, essentially in the manner described by Flath and Forrey (1972). The stripped volatiles were collected in a liquid nitrogen-cooled trap. After completion of the stripping, the trap contents were melted and the organic components were taken up in ether. Careful removal of the ether solvent left a concentrated solution of the organic volatiles for analysis. Typically, the yield from 6 kg was about 0.1 ml (4 ppm) of solution, at least three-fourths of which was ether. Yields might be increased by extending the stripping period.

Free Acids in Puree. Because the aroma of papaya puree made by commercial methods is reminiscent of the

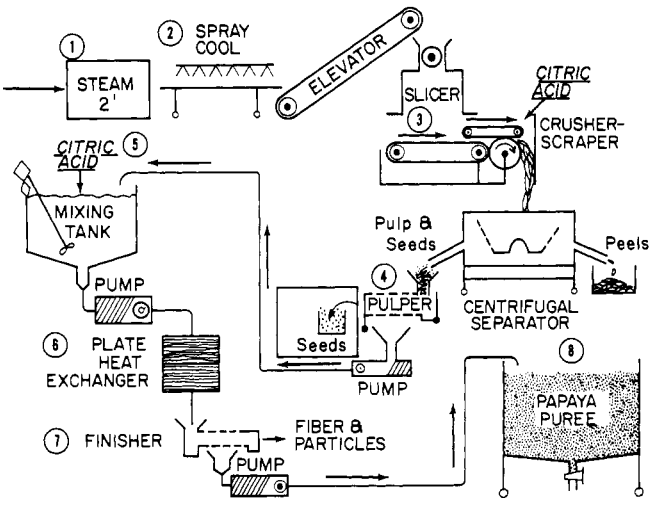

Figure 1. Flow chart for processing papaya puree by the improved method.

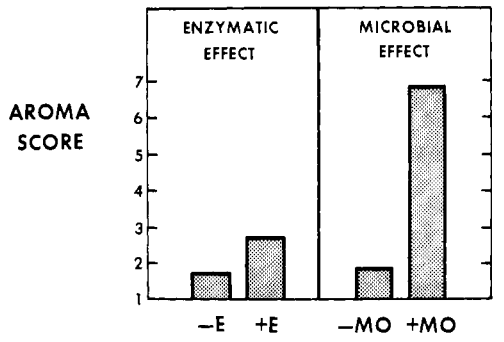

Figure 2. Aroma scores of enzymatically and microbially induced off-flavors: −E and −MO = free of enzymatic and microbial activity; +E = enzymatically active and free of microbial activity; +MO = microbially active and free of enzymatic activity.

lower fatty acids, the pot contents remaining after volatiles concentration were strained (puree solids tended to coagulate and remain in suspension in a fairly clear liquid) and the yellowish liquid was saturated with sodium chloride. This solution (3.6 l.) was extracted with ether (3×, total of 1.1 l.), and the ether extract in turn was washed with 1 *N* sodium hydroxide (50 ml) and then water (50 ml). The water wash was used to help break an emulsified layer remaining from the base wash. The two washes were combined, filtered, and washed with ether (30 ml). The basic aqueous layer was filtered, yielding a clear, pale yellow solution, which was acidified with dilute hydrochloric acid to give a cloudy mixture smelling strongly of lower carboxylic acids. The acids were taken up with ether (2×, 60 ml total) and then esterified with diazomethane (ethereal-ethanolic solution); the excess diazomethane was destroyed by addition of several drops of formic acid (methyl formate, bp = 31.5°). The solution was washed with saturated sodium bicarbonate solution and then concentrated by careful distillation of solvent, leaving 0.43 g of yellow solution.

Separation and Identification of Components. *Gas-Liquid Chromatography.* The equipment and operating parameters used for the separation of the aroma concentrates have been described (Flath and Forrey, 1972). Large-bore open-tubular columns coated with methyl silicone oil were used exclusively.

Component Identifications. Gas chromatography–mass spectrometry was used for component identifications. Tentative identifications were checked by comparison of

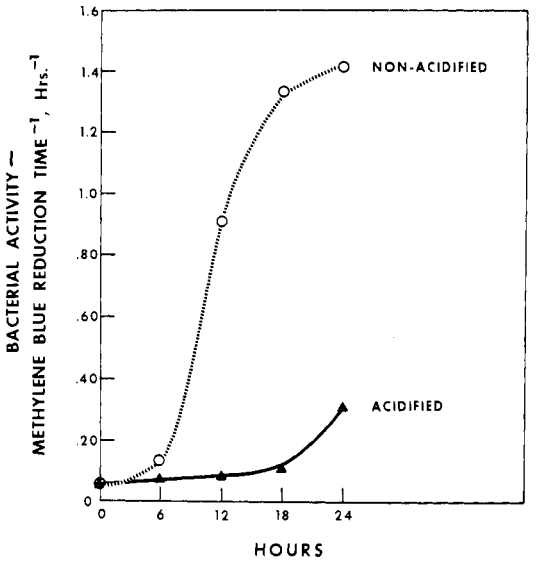

Figure 3. Bacterial activity of acidified and nonacidified purees.

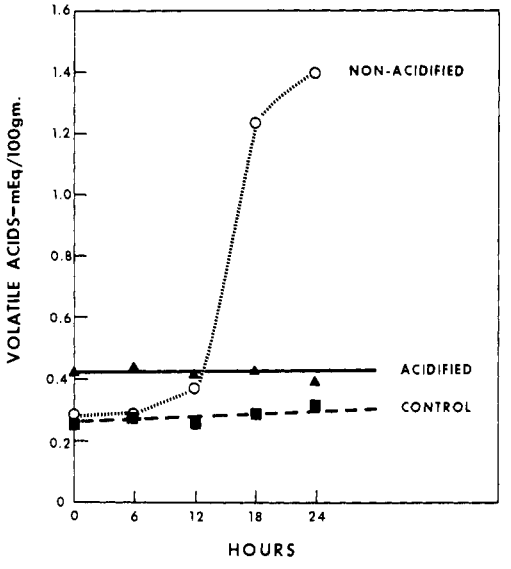

Figure 4. Bacterial production of volatile acids.

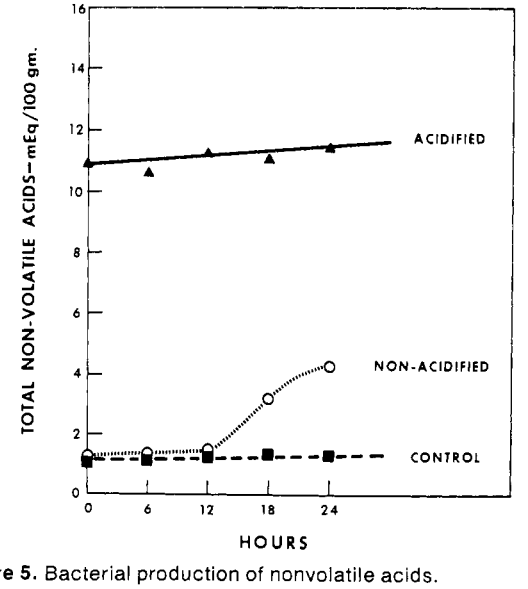

Figure 5. Bacterial production of nonvolatile acids.

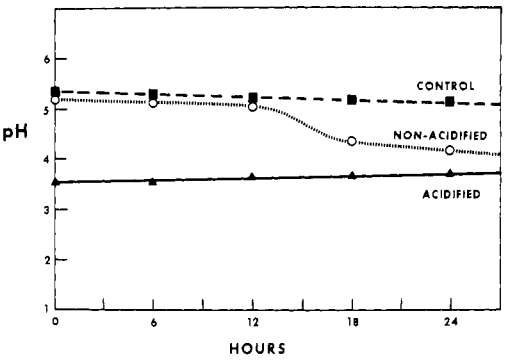

Figure 6. pH changes in acidified and nonacidified papaya purees.

Table I. Identity of Nonacidic Volatile Compounds in Figure 7

Chromatogram A (fresh fruit)	
Peak	Identity
1	Ethyl acetate
2	Butanol
3	Linalool oxide
4	Linalool oxide
5	Linalool
6	Benzyl isothiocyanate
Chromatogram B (puree from commercial method)	
Peak	Identity
7	Ethyl acetate
8	Butanol
9	Methyl butyrate
10	Methyl hexanoate
11	1,2,4-Trimethylbenzene
12	1,2,3-Trimethylbenzene
13	Linalool oxide
14	Linalool oxide
15	Linalool
16	Methyl octanoate
17	Phenyl acetonitrile
18	Methyl decanoate
19	Benzyl isothiocyanate
Chromatogram C (puree from improved method)	
Peak	Identity
20	Ethyl acetate
21	Hexanal
22	Heptanal
23	Benzaldehyde
24	Linalool oxide
25	Linalool oxide
26	Linalool
27	Phenyl acetonitrile
28	α-Terpineol
29	Benzyl isothiocyanate
30	BHT, artifact from ether solvent

the retention behavior of the gc peak with an authentic sample.

RESULTS AND DISCUSSION

Off-Odors due to Enzymatic and Microbial Activities. The sensory evaluations in the comparisons of the control (−E −MO) with the enzymatically active (+E4 −MO) and the microbially active (−E +MO) are shown in Figure 2. These results indicate that off-odor in papaya puree can result from both enzymatic and microbial activity. In both comparisons, the scores differed significantly at the 1% probability level.

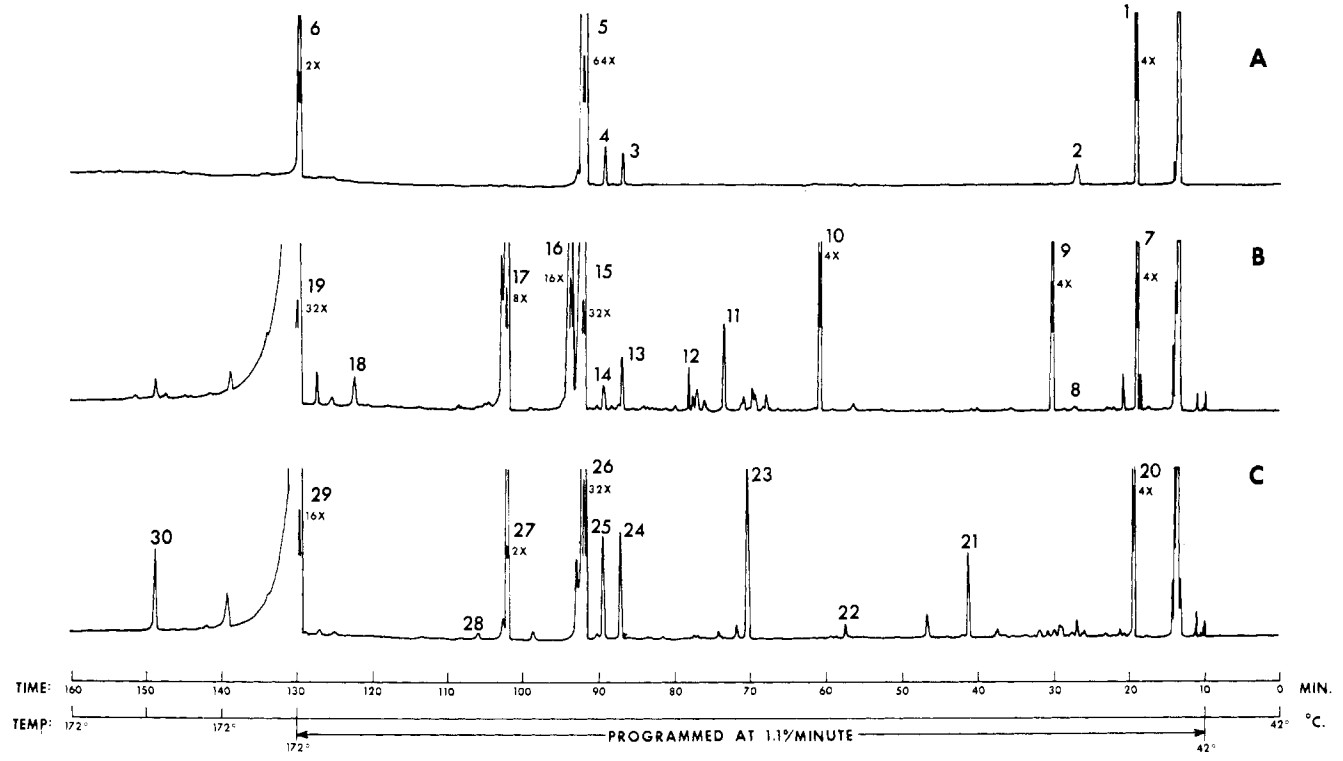

Figure 7. Chromatograms of nonacidic volatiles from fresh papaya (A), commercial method (B), and improved processing method (C).

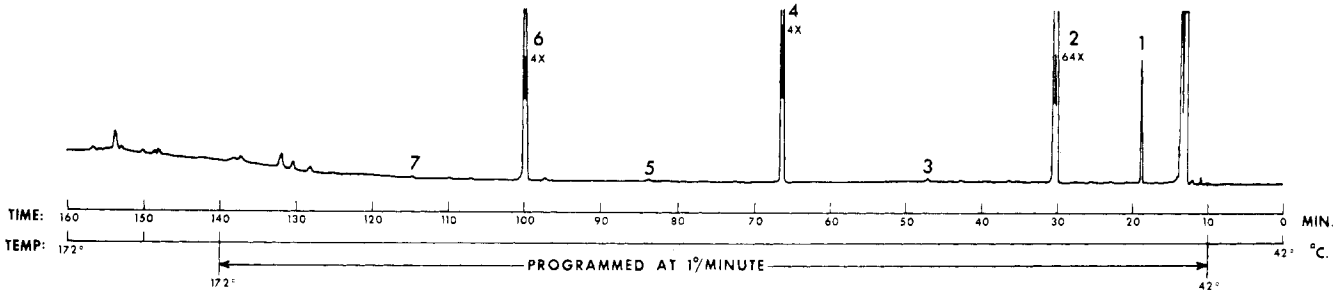

Figure 8. Chromatogram of the methyl esters of free acids extracted from puree prepared by the commercial method.

Acidification Effect. One effect of acidifying papaya puree to pH 3.55 is shown in Figure 3. Note that the nonacidified puree, originally at pH 5.2, showed an increase in bacterial activity at 6 hr and a rapid increase thereafter. In contrast, the bacterial activity in the acidified puree was retarded greatly; a significant increase occurred after a lag phase of 18 hr. Also, acidification of the puree suppressed the ability of the bacteria to produce acid, as shown in Figures 4–6. Volatile acids, nonvolatile acids, and pH levels remained essentially unchanged for the acidified puree, but after 12 hr showed large changes in the nonacidified puree. The decrease in pH can be attributed mainly to bacterial production of nonvolatile acids. As shown in Figure 5, nonvolatile acids in the acidified sample increased slightly and remained almost unchanged in the control. However, in the nonacidified sample, the nonvolatile acids increased about 3 mequiv/100 g in 24 hr; volatile acids increased about 1.1 mequiv/100 g of puree during 24 hr.

This inhibition of bacterial activity by acidification confirms earlier work (Brekke *et al.*, 1972a,b) on the effect of acidification on flavor quality of papaya puree. In the previous work, sensory evaluation revealed that after 24 hr at room temperature, nonacidified puree developed intense off-flavors, whereas acidified puree showed no flavor quality changes.

Gram stains of the nonacidified puree at 6-hr intervals showed a progressive increase of gram-positive diplococci at the initial stages of incubation, shifting to a predominance of gram-positive rods in the latter stages. Very few yeasts were observed at any time. The changes in pH, volatile, and nonvolatile acids correspond well with the increase in bacterial activity in the sample.

Comparison of Volatile Compounds from Fresh Fruit and from Purees Made by Commercial and Improved Processing Methods. In comparison with the aroma concentrates of the various puree samples, the fresh, ripe fruit concentrate contains relatively few components (Figure 7, A; Table I). The major odorous compound is linalool, with much smaller amounts of ethyl acetate, 1-butanol, two configurational isomers of the linalool oxides (2-methyl-2-vinyl-2-[2-hydroxy-2-propyl]tetrahydrofuran), and by benzyl isothiocyanate. Traces of numerous other components are present. A second volatiles concentration run, using slightly greener fruit, yielded a solution with slightly higher concentrations of certain minor components (Flath and Forrey, 1972).

In marked contrast to that of fresh fruit, the aroma concentrate of puree prepared by commercial methods contained a greater variety of compounds, and much more benzyl isothiocyanate relative to the linalool concentration (Figure 7, B). The major new compounds are the

Table II. Identity of Methyl Esters of Free Acids in Figure 8

Peak	Identity
1	Ethyl acetate, artifact from extraction
2	Methyl butyrate
3	Methyl pentanoate
4	Methyl hexanoate
5	Methyl heptanoate
6	Methyl octanoate
7	Methyl nonanoate

methyl esters of butyric, hexanoic, octanoic acids, and phenyl acetonitrile. Nitriles previously have been found associated with mustard oils (Challenger, 1959). The same acids, especially butyric, were extracted in the free form from the puree and were identified as their methyl esters (Figure 8; Table II) after treatment with diazomethane. Formation of the free acids probably is the major cause of off-odor in the puree product. In addition, there is evidence of traces of pentanoic, heptanoic, and nonanoic acids.

Puree prepared by the improved processing method differs from the fresh fruit puree in that it contains hexanal, heptanal, benzaldehyde, and traces of α-terpineol. These may be present in low concentration in the commercial puree and in fresh fruit, but have not yet been detected. The nonacidic volatiles found in puree prepared by commercial methods, but not found in the puree prepared by improved processing method, are the methyl esters of butyric, hexanoic, and octanoic acids.

SUMMARY

Off-flavor development in papaya puree can be of either microbial or enzymatic origin. Acidification of papaya puree to pH 3.5 was beneficial in retaining quality by reducing microbial growth. Acidification is recommended as part of an improved processing method. Data from the gc–ms study of papaya flavor components show that the unpleasant odorous compounds, butyric, hexanoic, and decanoic acids and their methyl esters, are present in purees with off-odors and off-flavors. They are not present in purees prepared by the improved processing method.

LITERATURE CITED

American Public Health Association, "Standard Methods for the Examination of Dairy Products," 12th ed., 1967, pp 129, 141.
Angara, F. S., Jr., Wang, J.-K., Agena, U., *Trans. Amer. Soc. Agr. Eng.* **12**, 745 (1969).
Association of Official Agricultural Chemists, "Methods of Analysis," 9th ed., 1960.
Brekke, J. E., Chan, H. T., Cavaletto, C. G., *Food Prod. Develop.* 6(6), 36 (1972a).
Brekke, J. E., Chan, H, T., Cavaletto, C. G., Research Bulletin No. 170, Hawaii Agricultural Experiment Station, University of Hawaii, 1972b.
Challenger, F., "Aspects of the Organic Chemistry of Sulfur," Butterworths, London, 1959, pp 120–132.
Ettlinger, M. G., Hodgkins, J. E., *J. Org. Chem.* **21**, 204 (1956).
Flath, R. A., Forrey, R. R., unpublished data, 1972.
Forss, D. A., Jacobsen, V. M., Ramshaw, E. H., *J. Agr. Food Chem.* **15**, 1104 (1967).
Katague, D. B., Kirch, E. R., *J. Pharm. Sci.* **54**, 891 (1965).
Tang, C.-S., *Phytochemistry* **10**, 117 (1971).

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Lipoxygenase-Mediated Pentane Production: Characterization of the System

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Pentane production was studied in a model system consisting of soybean lipoxygenase and linoleic acid. After an initial lag period of 3.5 min, there was a linear increase in pentane concentration up to 20 min. Pentane production increased linearly with the amount of enzyme between 35 and 75 μg of protein. The optimum linoleic acid concentration for pentane production was 2.1 m*M*, while higher levels were inhibitory. Lipoxygenase has pH optima at 7.1 and 9, while pentane production has a broad alkaline optimum with the maximum at 9. Purification of the enzyme on Sephadex G-150 and DEAE-Sephadex yields two lipoxygenase fractions with pH optima at 7 and 9, respectively. Only the pH 9 fraction produced pentane.

Pentane has only recently been identified as a constituent of plant products. Buttery *et al.* (1961) found pentane in dehydrated potatoes and Pattee *et al.* (1969) showed that pentane was a normal volatile constituent of raw peanuts. Horvat *et al.* (1964) first demonstrated pentane production in a model system by headspace analysis of the autoxidation products of methyl linoleate. Evans *et al.* (1967) formed pentane by the thermal decomposition of 13-hydroperoxylinoleic acid, which is the primary product of the reaction of crystalline soybean lipoxygenase (EC 1.13.1.13) with linoleic acid. Pattee *et al.* (1970) found a rough correlation between pentane content and lipoxygenase activity during the maturation of peanut seeds and postulated that lipoxygenase might be involved in pentane production. Meanwhile, Garssen *et al.* (1971) demonstrated that 13-oxotridecadienoic acid and pentane would be formed in an anaerobic system containing soybean lipoxygenase, 13-hydroperoxy linoleic acid, and linoleic acid.

We report here the partial characterization of a pentane-producing system containing linoleic acid as substrate and soybean lipoxygenase as catalyst.

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