small: molecular weights of about 330–380 were reported (Greenberg and Shipe, 1979). The determination of TCA-soluble peptides and amino acids alone therefore gives only a partial picture of the effects of the enzyme. Only SDS–PAGE made it possible to ascertain whether endopeptidase splitting with formation of large-sized fragments occurred.

Globulins 4 and 6 are similar in amino acid, sugar, and protomer composition, but their different molecular weights (Duranti et al., 1981; Restani et al., 1981; Cerletti, 1983) and surface hydrophobicity (Bonomi et al., 1983) suggest that the protomers are assembled differently. The different effect of trypsin indicates different availability of specific sites for the enzyme. The temperatures at which the tertiary structure of these proteins starts to break up indicate a looser conformation for globulin 4 (Bonomi et al., 1983). This may explain why the native protein is split by trypsin with formation of large and small peptides and of free amino acids. Also, the effect of deglycosylation is more pronounced, as indicated by the amino acids and insoluble peptides produced. Amino acid liberation from nondeglycosylated globulin 4 develops stepwise: this may indicate that, once acted upon by trypsin, the protein undergoes rearrangement before again being available to the enzyme. A similar situation has been described by Fukushima (1968) for the 11s globulin from the soybean, when attacked by the proteinase of *Aspergillus soyae*.

Registry No. Trypsin, 9002-07-7.

LITERATURE CITED

Anantha Samy, T. S. *Arch. Biochem. Biophys.* **1967**, *121*, 703–710.
Bonomi, F.; Guerrieri, N.; Cerletti, P. In "Research in Food Science and Nutrition"; McLoughlin, J. V.; McKenna B. M., Eds.; Boole Press: Dublin, 1983; Vol. 2, pp 130–131.
Boonvisut, S.; Whitaker, J. R. *J. Agric. Food Chem.* **1976**, *24*, 1130–1135.
Cerletti, P. In "Developments in Food Proteins-2"; Hudson, B. J. F., Ed.; Applied Science Publishers, Ltd.: Barking, England, 1983; pp 133–171.
Cerletti, P.; Duranti, M.; Restani, P. *Qual. Plant.-Plant Foods Hum. Nutr.* **1983**, *32*, 145–154.
Christensen, T. B.; Birkeland, A. J. *IRCS Libr. Compend.* **1974**, *2*, 1054.
Christensen, T. B.; Vegarud, G. *IRCS Libr. Compend.* **1974**, *2*, 1311.
Chu, F. K.; Trimble, R. B.; Maley, F. *J. Biol. Chem.* **1978**, *253*, 8691–8693.
Churchill, L.; Hokin, L. E. *Biochim. Biophys. Acta* **1976**, *434*, 258–264.
Dixon, D. K.; Coherle, O. J. *J. Food Sci.* **1981**, *46*, 983–999.
Duranti, M.; Restani, P.; Poniatowska, M.; Cerletti, P. *Phytochemistry* **1981**, *20*, 2071–2075.
Eaton-Mordas, C. A.; Moore, K. G. *Phytochemistry* **1979**, *18*, 1775–1779.
Fukushima, D. *Cereal Chem.* **1968**, *45*, 203–224.
Gallardo, F.; Araya, H.; Pak, N.; Tagle, M. A. *Arch. Latinoam. Nutr.* **1974**, *2*, 183–189.
Gottshalk, A.; Fazakas de St. Groth, S. *Biochim. Biophys. Acta* **1960**, *43*, 513–519.
Greenberg, N. A.; Shipe, W. F. *J. Food Sci.* **1979**, *44*, 735–737.
Itzaki, R. F.; Gill, D. M. *Anal. Biochem.* **1964**, *9*, 401–409.
Kakade, M. L. *J. Agric. Food. Chem.* **1974**, *22*, 550–555.
Lamport, D. T. A. "The Biochemistry of Plants"; Preiss, J., Ed.; Academic Press: London, England, 1980; Vol. 3, pp 501–541.
Li, S. C.; Li, Y. T. *J. Biol. Chem.* **1968**, *243*, 3994–3998.
Li, S. C.; Lit, Y. T. *J. Biol. Chem.* **1970**, *245*, 5153–5160.
Long, C. "Biochemist"s Handbook"; Spon, E. and F., Ltd., London, England, 1968, p 41.
Lynch, C. J.; Rha, C. K.; Catsimpoolas, N. *J. Sci. Food Agric.* **1977a**, *28*, 971–979.
Lynch, C. J.; Rha, C. K.; Catsimpoolas, N. *Cereal Chem.* **1977b**, *54*, 1282–1285.
Marsh, J. W.; Denis, J.; Writon, J. C., Jr. *J. Biol. Chem.* **1977**, *252*, 7678–7684.
McCaldin, D. J. *Chem. Rev.* **1960**, *60*, 39–51.
McKelvy, J. F.; Lee, Y. C. *Arch. Biochem. Biophys.* **1969**, *132*, 99–110.
Restani, P.; Duranti, M.; Cerletti, P.; Simonetti, P. *Phytochemistry* **1981**, *20*, 2077–2083.
Romero, J.; Ryan, D. S. *J. Agric. Food Chem.* **1978**, *26*, 784–788.
Schwimmer, S. "Nutrition and Clinical Nutrition"; Marcel Dekker: New York, 1975; Vol. I, p 501.
Wachsmuth, E. D.; Fritze, I.; Pfleiderer, G. *Biochemistry* **1966**, *5*, 169–174.
Wang, F.-F. C.; Hirs, C. H. W. *J. Biol. Chem.* **1977**, *252*, 8358–8364.

Received for review March 26, 1984. Accepted October 15, 1984. This research was supported by C.N.R., Italy, Special Grant I.P.R.A. Subproject No. 3.

Volatile Constituents of Carambola (*Averrhoa carambola* L.)

Charles W. Wilson, III,* Philip E. Shaw, Robert J. Knight, Jr., Steven Nagy, and Marty Klim

Forty-one volatile components were identified from an extract of carambolas by using a capillary gas chromatograph-mass spectrometer combination. The most prominent aroma was grape-like, and methyl anthranilate was the most abundant component. The strong fruity aroma of the extract is probably due to the major esters and ketones in the extract.

Carambolas are consumed mostly as fresh fruit and have recently progressed from a dooryard ornamental to small commercial plantings of one or two selected cultivars (Brooks, 1983). The unique star-shaped fruit vary in color from white to dark yellow. The yellow varieties have more commercial appeal because the deeper yellow color is more attractive to the comsumer. The white varieties have been reported to be sweeter than the yellow varieties (Harler, 1983). Wagner et al. (1975) reported ascorbic and oxalic acid content, acidity, Brix, and taste panel evaluation for carambolas that were mostly of the yellow varieties. Flavor attributes ascribed to some of these were sweet, good, and apple-like, sour, tart, and apple-like, and sweet, good, and mild. Others have suggested carambolas have an apricot-like flavor (Harler, 1983). Little information on the chemical composition of carambolas is available, and to

U.S. Citrus & Subtropical Products Laboratory, U.S. Department of Agriculture, Agricultural Research Service, South Atlantic Area, Winter Haven, Florida 33883 (C. W.W. and P.E.S.), U.S. Subtropical Horticulture Laboratory, U.S. Department of Agriculture, Agricultural Research Service, South Atlantic Area, Miami, Florida 33158 (R.J.K.), and Florida Department of Citrus, Lake Alfred, Florida 33850 (S.N. and M.K.).

date, no reports on volatile flavor components of carambolas have been published. Information on chemical composition will be useful to plant breeders and horticulturists for plant breeding studies and to food processors and fresh product suppliers in selecting varieties for marketing and processing. The current study reports volatile components of carambola and discusses the importance of individual components as contributors to its flavor.

EXPERIMENTAL SECTION

Ripe carambolas (7.5 kg, cv. Golden Star) were obtained from the U.S. Subtropical Horticultural Research Station, Miami, Fl, and processed immediately. They were deseeded, mixed with an equal weight of deionized water, and blended in a high-speed blender. The puree was filtered under vacuum through filter aid to obtain about 8.5 L of aqueous filtrate, and the filter cake was extracted once with methylene chloride to remove residual volatile components. The solvent from the filter cake wash was used as part of the solvent to extract the aqueous filtrate. The aqueous filtrate was extracted in 1-L batches with 5 × 50 mL of methylene chloride and the extract dried over sodium sulfate. The combined extracts were concentrated to about 1 mL on a rotary evaporator at room temperature and 20 mmHg. The concentrated extract was kept at 4 °C.

Samples were chromatographed on a Hewlett-Packard 5840 gas chromatograph (GC) equipped with a 0.32 mm i.d. × 30 m bonded-phase nonpolar DB-5 fused silica capillary column (J & W Scientific, Rancho Cordova, CA). The oven temperature was held at 40 °C for 0.5 min, raised to 60 °C at 20 °C/min, and then programmed to 250 °C at 4 °C/min. The flow rate (H_2) was 38 cm/s. The flame ionization detector was at 350 °C. An injection port splitter was used at 250 °C with a split ratio of 100:1. Retention times were determined for each compound by using 0.02–0.1% solutions of authentic compounds, and mass spectral identifications were confirmed by coinjection of authentic compounds, which were obtained from commercial sources.

A gas chromatograph–mass spectrometer (GC–MS), either a Finnigan Model 4021 and/or a Kratos MS25 (DS-55 system), was used in the electron impact mode at 70 eV. The GC column used in both instruments was a 0.25 mm i.d. × 60 m DB-5 fused silica capillary column. Injection port temperatures were 250 °C. The carrier gas flows were 1 mL of helium/min for the Finnigan and 1 mL of hydrogen/min for the Kratos mass spectrometer. The oven temperature for the Finnigan GC–MS was programmed from 40 to 225 °C at 2 °C/min. For the Kratos GC–MS, the oven temperature was held at 40 °C for 0.5 min, increased to 60 °C at 20 °C/min, and then programmed to 250 °C at 4 °C/min. The injection port split ratio was 100:1. Mass spectral identifications were made by comparison with those published for the authentic compounds (American Society for Testing and Materials, 1969; Heller and Milne, 1978).

RESULTS AND DISCUSSION

The volatile constituents identified in a concentrated methylene chloride extract from an aqueous filtrate of macerated carambolas are listed in Table I. Also listed are their GC retention times and relative area percentages. Forty-one compounds were identified by GC–MS. Their identities were confirmed by comparison of GC retention times with those for authentic compounds upon coinjection with the concentrated methylene chloride extract.

The aroma of the concentrated extract compared to that of other tropical fruits, which usually possess a strong floral fruity aroma, was unique since several different aromas (grape-like, fruity, and aromatic) were perceived. Many of the compounds found in this carambola extract are common to other tropical fruits. Methyl anthranilate, a major component, has a distinct grape-like aroma, and its aroma is easily detected above the aromas of other volatile constituents in both the fresh fruit and solvent extract. The aromas of ethyl sorbate (also a major component), ethyl acetate, and ethyl butyrate have been described as warm fruity and somewhat ethereal (Arctander, 1969). Thus, these esters, in addition to the other esters, ethyl hexanoate, diethyl succinate, methyl salicylate, methyl benzoate, and ethyl benzoate, can provide a warm fruity flavor or aroma attribute (Arctander, 1969). The ketones acetophenone, *o*-methylacetophenone, and β-ionone, can also impart a warm fruity or ethereal-type flavor and aroma (Arctander, 1969). The aromas of 6-methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol have been described as oily–green, pungent–herbaceous, grassy, and diffusive with fresh and green fruity notes (Arctander, 1969). These major components in addition to *cis*- and *trans*-3-hexen-1-ol and hexanal probably contribute a green grassy flavor and aroma. However, the green grassy aroma associated with these components is not as noticeable in the solvent extract as it is in the fresh fruit.

Phenylethyl acetate and phenylethyl alcohol have been reported as constituents of several subtropical fruits (Van

Table I. Volatile Constituents of Carambola Fruit Extract

component	GLC RT, min	GLC rel area %[a,b]
acetaldehyde	1.48	1.60
ethyl acetate	2.22	1.20
2-methyl-1-propanol	2.39	0.20
1-pentanol	3.04	0.05
1-penten-3-ol	3.34	0.04
3-methyl-2-butanone	3.87	0.20
3-methyl-1-butanol	3.97	0.20
ethyl butyrate plus hexanal	5.12	6.41
cis-3-hexen-1-ol	6.56	0.80
trans-3-hexen-1-ol	6.83	1.20
hexanol	7.01	0.60
α-pinene	9.22	0.20
benzaldehyde	10.25	0.41
6-methyl-5-hepten-2-one	10.47	5.28
6-methyl-5-hepten-2-ol	10.59	5.00
β-binene	10.78	1.40
ethyl hexanoate	11.12	1.01
1,8-cineole	12.47	0.80
limonene	12.66	5.40
benzyl alcohol	13.14	0.60
octanol plus acetophenone	14.03	4.10
methyl benzoate	14.60	0.60
ethyl sorbate	15.16	14.51
phenylethyl alcohol	15.85	3.41
veratrole	17.04	1.40
borneol	18.08	3.20
diethyl succinate	18.23	1.20
o-methylacetophenone	18.65	2.55
4-terpineol	18.88	0.07
ethyl benzoate	18.91	0.60
methyl salicylate	19.08	0.80
ethyl nicotinate	19.93	0.80
benzothiazole	20.30	0.80
methyl anthranilate	20.83	21.20
carvone	20.94	0.80
phenylethyl acetate	21.46	4.61
diethyl glutarate	22.00	0.05
cinnamyl aldehyde	22.41	0.80
quinoline	24.55	0.50
cinnamyl acetate	28.29	0.41
β-ionone	29.53	1.01

[a] Methylene chloride solvent representing 92.5% of total peak area was excluded from integration calculations. [b] Unidentified components accounted for 3.98%.

Straten and de Vrijer, 1973; Shaw and Wilson, 1982). These compounds possess strong, floral rose-like aromas and are probably responsible for some of the fruity floral aroma of carambolas (Arctander, 1969). The aroma of borneol has been described as dry, woody, and camphoraceous (Arctander, 1969). Quinoline, benzothiazole, and ethyl nicotinate, which are present in low concentrations, probably contribute to the aromatic aroma. Benzothiazole has been reported in mangoes, cranberries, vegetables, and animal products, and quinoline has been reported in coconuts, cocoa, nuts, and beer (Van Straten and de Vrijer, 1973; MacLeod and Pieris, 1984). Ethyl nicotinate has not been reported in foods, but the related compound, methyl nicotinate, has been found in nuts and coffee (Van Straten and de Vrijer, 1973). Although carambolas have been reported as apricot-like or apple-like in flavor and several of the constituents of this fruit are common to both apples and apricots (Chairote et al., 1981; Flath et al., 1967), the extract we prepared did not possess an aroma characteristic of either of those two fruit.

The identity of forty-one volatile components of one major carambola selection (Robert Newcomb, cv.) has been established for the first time. Several of these components are not common constituents of other fruit. This study provides a basis for comparison of other carambola cultivars to aid plant breeders and horticulturists in selecting varieties for further marketing and processing.

Registry No. Acetaldehyde, 75-07-0; ethyl acetate, 141-78-6; 2-methyl-1-propanol, 78-83-1; 1-pentanol, 71-41-0; 1-penten-3-ol, 616-25-1; 3-methyl-2-butanone, 563-80-4; 3-methyl-1-butanol, 123-51-3; ethyl butyrate, 105-54-4; hexanal, 66-25-1; *cis*-3-hexen-1-ol, 928-96-1; *trans*-3-hexen-1-ol, 928-97-2; hexanol, 111-27-3; α-pinene, 80-56-8; benzaldehyde, 100-52-7; 6-methyl-5-hepten-2-one, 110-93-0; 6-methyl-5-hepten-2-ol, 1569-60-4; β-pinene, 127-91-3; ethyl hexanoate, 123-66-0; 1,8-cineole, 470-82-6; limonene, 138-86-3; benzyl alcohol, 100-51-6; octanol, 111-87-5; acetophenone, 98-86-2; methyl benzoate, 93-58-3; ethyl sorbate, 2396-84-1; phenylethyl alcohol, 60-12-8; veratrole, 91-16-7; borneol, 507-70-0; diethyl succinate, 123-25-1; *o*-methylacetophenone, 577-16-2; 4-terpineol, 562-74-3; ethyl benzoate, 93-89-0; methyl salicylate, 119-36-8; ethyl nicotinate, 614-18-6; benzothiazole, 95-16-9; methyl anthranilate, 134-20-3; carvone, 99-49-0; phenylethyl acetate, 103-45-7; diethyl glutarate, 818-38-2; cinnamyl aldehyde, 104-55-2; quinoline, 91-22-5; cinnamyl acetate, 103-54-8; β-ionone, 79-77-6.

LITERATURE CITED

American Society for Testing and Materials (ASTM). "Index of Mass Spectral Data"; ASTM: Philadelphia, PA, 1969.
Arctander, S. "Perfume and Flavor Chemicals"; Arctander, S., Ed.; Arctander, S.: Montclair, NJ, 1969.
Brooks, P., International Tropical Fruit Workshop, Homestead, FL, July 18–21, 1983.
Chairote, F.; Rodriguez, F.; Crouzet, J. *J. Food Sci.* **1981**, *46*, 1898.
Flath, R. A.; Black, D. R.; Guadagni, D. R.; McFadden, W. H.; Schultz, T. H. *J. Agric. Food Chem.* **1967**, *15*, 29.
Harler, C. *Fla. Grow. Rancher* **1983**, *76*, 9.
Heller, S. R.; Milne, G. W. A. "EPA/NIH Mass Spectral Data Base"; National Bureau of Standards, U. S. Department of Commerce: Washington, DC, 1978.
MacLeod, A. J.; Pieris, N. M. *Phytochemistry* **1984**, *23*, 361.
Shaw, P. E.; C. W., III. *J. Food Sci.* **1982**, *47*, 1743.
Van Straten, S.; de Vrijer, F. "Lists of Volatile Compounds in Foods"; Central Institute for Nutrition annd Food Research: Zeist, Netherlands, 1973.
Wagner, C. J., Jr.; Bryan, W. L.; Berry, R. E.; Knight, R. J., Jr. *Proc. Fla. State Hortic. Soc.* **1975**, *88*, 466.

Received for review June 15, 1984. Revised manuscript received October 29, 1984. Accepted November 13, 1984. Mention of a trademark or proprietary product is for identification only and does not imply a warranty or guarantee of the product by the U.S. Department of Agriculture over other products that may also be suitable.

Measurement of Amino Acid Racemization in Alkali-Treated Proteins Using an Immobilized D-Amino Acid Oxidase–Catalase Reactor

Si-Yin Chung,[1] Harold E. Swaisgood,* and George L. Catignani

An immobilized enzyme reactor system consisting of D-amino acid oxidase and catalase coimmobilized on porous succinamidopropyl-glass beads was examined with regard to its function in a method for determination of amino acid racemization in proteins. The extent of racemization was measured from the reduction in the amount of an amino acid, as determined by chromatographic analyses, following treatment of an acid hydrolysate with the immobilized enzyme reactor. Racemization rates resulting from alkaline heat treatments (0.2 N NaOH, 40 °C) of five proteins (soy isolate, wheat isolate, α-lactalbumin, bovine serum albumin, and β-lactoglobulin) indicated that, although the rates varied between proteins, the relative rate of phenylalanine racemization was constant and roughly double that for alanine. Racemization of tyrosine occurred at a rate similar to that of alanine; however, that for isoleucine, leucine, and valine was barely detectabl following a 20-h incubation.

A number of methods for determination of the extent of amino acid racemization in a protein has been reported. These include use of enzymes (L-lysine decarboxylase) and microorganisms (Provansal et al., 1975), gas chromatographic/mass spectrometric analysis following deuterium labeling (Liardon and Jost, 1981; Liardon and Hurrell, 1983), and tritium–hydrogen exchange techniques (Hayashi and Kameda, 1980). Most of these methods, however, require the use of sophisticated instrumentation not readily available to many laboratories. Racemization resulting from alkaline food processing conditions could seriously impair the digestibility of a protein. Therefore, we sought to develop a simplified technique for measuring

Department of Food Science, North Carolina State University, Raleigh, North Carolina 27695-7624.
[1]Present address: Thrombosis Research Center, Health Sciences Center, Temple University, Philadelphia, PA 19140.

0021-8561/85/1433-0201$01.50/0