

- der Bundesforschungsanstalt für Fischerei: Heenemann Verlagsgesellschaft mbH, Berlin, 1979; Vol. II, p 281.
- Kühl, J.; Nittinger, J.; Siebert, G. *Arch. Fischereiwiss.* 1978, 29, 99.
- Lovern, J. A.; Olley, J.; Pirie, R. *Fish. News Int.* 1964, 3, 310.
- McElroy, J. K. "Advances in Fish Science and Technology"; Connell, J. J., Ed.; Fishing News Books, Ltd.: Farnham, U.K., 1980; p 314.
- Miller, E. L. *FAO Fish. Rep.* 1970, No. 92, 41.
- Naumann, K.; Bassler, R., Eds. "Die chemische Untersuchung von Futtermitteln. Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten, Methodenbuch 3"; Verlag J. Neumann-Neudamm: Melsungen, 1976.
- Okutani, K. *MIT Sea Grant Rep. MITSG* 1978, Abstract No. 43.
- Pfeffer, E.; Becker, K. *Arch. Fischereiwiss.* 1977, 28, 19.
- Pfeffer, E.; Meske, C. *Z. Tierphysiol., Tierernaehr. Futtermittelkd.* 1978, 40, 74.
- Reinacher, E. *Inf. Fischwirt.* 1978, 25, 67.
- Reinacher, E. *Arch. Fischereiwiss.* 1979, 29, 129.
- Roach, A. G.; Sanderson, P.; Williams, D. R. *J. Sci. Food Agric.* 1967, 18, 274.
- Sahrhage, D.; Schreiber, W.; Steinberg, R.; Hempel, G., Eds. *Arch. Fischereiwiss.* 1978, 29 (Suppl. 1).
- Schulz, E.; Petersen, V. *Kraftfutter* 1978, 61, 130.
- Spackman, D. H.; Stein, H. W.; Moore, S. *Anal. Chem.* 1958, 30, 1190.
- Spies, J. R.; Chambers, D. C. *Anal. Chem.* 1948, 20, 30.
- Spies, J. R.; Chambers, D. C. *Anal. Chem.* 1949, 21, 1249.
- Spindler, M.; Pohl, Tanner H., personal communication, 1979.
- Tegge, G.; Bolling, H. *Staerke* 1959, 11, 162.
- Tiews, K.; Koops, H.; Gropp, J.; Beck, H. "Finfish Nutrition and Fishfeed Technology"; Halver, J. B.; Tiews, K., Eds.; Schriften der Bundesforschungsanstalt für Fischerei: Heenemann Verlagsgesellschaft mbH, Berlin, 1979; Vol. II, p 219.
- Vens-Capell, B.; Horstmann, H. *J. Oesterr. Fisch.* 1978, 31, 35.
- Vogt, H.; Harnisch, S.; Krieg, R.; Torges, R.-H.; Rauch, H.-W. *Arch. Geflügelkd.* 1980, 44, 141.
- von Lukowicz, M. "Finfish Nutrition and Fishfeed Technology"; Halver, J. B.; Tiews, K., Eds.; Schriften der Bundesforschungsanstalt für Fischerei: Heenemann Verlagsgesellschaft mbH, Berlin, 1979; Vol. II, p 293.
- Winter, E. *Z. Lebensm.-Unters. -Forsch.* 1963, 123, 205.

Hartmut Rehbein

Institut für Biochemie und Technologie
Bundesforschungsanstalt für Fischerei
Palmaille 9
2000 Hamburg 50, Federal Republic of Germany

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Thiazoles, Oxazoles, and Oxazolines Identified in the Volatile Flavor of Roasted Peanuts

Volatile flavor compounds were isolated from 70 kg of roasted peanuts by a specially designed apparatus. The isolated volatile flavor compounds were subjected to extensive gas chromatographic fractionation, and the pure fractions obtained were identified by infrared and mass spectrometry. Eight thiazoles, seven oxazoles, and three oxazolines were identified. All of them, except 4-methylthiazole, were new to the roasted peanut flavor.

The volatile flavor compounds of roasted peanuts have been studied by many researchers. Publications by Pattee et al. (1965), Mason et al. (1966, 1967), Brown et al. (1968), Walradt et al. (1971), and Johnson et al. (1971a,b) have described the isolation of an aroma complex from roasted peanuts and identification of many of its constituents. In a recent review, Van Straten (1977) stated that a total of 279 compounds had been identified.

Thiazoles, oxazoles, and oxazolines in food flavor have been receiving increased attention recently. Most thiazoles, oxazoles, and oxazolines possess some unique and potent sensory properties. For instance, 2-isobutylthiazole isolated from tomato flavor (Viani et al., 1969) was described as having a strong green odor resembling that of tomato leaf. 2-Isopropyl-4,5-diethyl-3-oxazoline, a synthetic flavor, possesses a typical cocoa aroma (Ohloff and Flament, 1979). However, only three thiazoles, namely, thiazole, 4-methylthiazole, and benzothiazole (Walradt et al., 1971), and no oxazoles and oxazolines have been reported in roasted peanut flavor.

The present paper reports the identification of eight thiazoles, seven oxazoles, and three oxazolines in the volatile flavor compounds of roasted peanuts.

EXPERIMENTAL SECTION

Material Used. Freshly roasted peanuts (kept at -20 °C) were used for isolation of the volatile compounds.

Florunner peanuts were roasted in a commercial roaster to meet the Neotec color reflection of 25.0 ± 0.3 (green). Experienced tasters confirmed that the roasted peanuts had a typical roasted peanut aroma and flavor.

Isolation of Volatile Flavor Compounds (VFC). The VFC were isolated from 70 kg of roasted peanuts by using the apparatus described by Chang et al. (1977). The principle of the apparatus is the removal and subsequent condensation of the volatile compounds in the headspace of the roasted peanuts. Nitrogen gas was used to remove the VFC from roasted peanuts. Seven kilograms of roasted peanuts was used for each isolation which lasted 48 h. A total of 70 kg of roasted peanuts was used. The total condensate collected in the traps cooled with dry ice and acetone was treated in a manner similar to that described by Herz and Chang (1966). The ethyl ether extract of the condensate was dried with anhydrous sodium sulfate and then concentrated to a volume of 50 mL with the use of a 30-plate Oldershaw column. It was finally concentrated to a volume of 5 mL with a 200-plate spinning band still.

Fractionation of the Flavor Isolate. The initial preparative gas chromatography of the isolated volatile roasted peanut flavor compounds was performed on a Perkin-Elmer Sigma 3 gas chromatograph equipped with a flame ionization detector, fitted with a $1/8$ in. o.d. \times 12 ft stainless steel column, packed with 10% OV-17 on 80-100-mesh Chromosorb W. The flow rate was 30 mL/min

Table I. Thiazoles, Oxazoles, and Oxazolines Identified in the Volatile Flavor of Roasted Peanuts

	new to roasted peanut flavor	structural status ^a	mass spectra data, <i>m/e</i> (rel intensity), or ref
thiazoles			
2-methylthiazole	yes	lit.	Vitzthum and Werkhoff (1974a)
4-methylthiazole	no	lit.	Vitzthum and Werkhoff (1974a)
5-methylthiazole	yes	lit.	Vitzthum and Werkhoff (1974a)
5-butylthiazole	yes	pro.	98 (100), 71 (60), 126 (38), 83 (36), 43 (34), 59 (32), 57 (21), 84 (20), 141 (7); M = 141
2-isopropyl-4,5-dimethylthiazole	yes	lit.	Buttery and Ling (1974)
4-butyl-2,5-dimethylthiazole	yes	lit.	Buttery et al. (1973)
2-propyl-4,5-diethylthiazole	yes	pro.	154 (100), 155 (88), 59 (35), 168 (34), 41 (33), 43 (30), 85 (25), 112 (24), 141 (23), 183 (21); M = 183
2,4-dimethyl-5-ethylthiazole	yes	lit.	Vitzthum and Werkhoff (1974a)
oxazoles			
4,5-dimethyloxazole	yes	lit.	Vitzthum and Werkhoff (1974a)
2-acetyloxazole	yes	pro.	111 (100), 43 (82), 68 (79), 69 (78), 41 (44), 39 (25), 42 (15), 112 (11); M = 111
2,4,5-trimethyloxazole	yes	lit.	Vitzthum and Werkhoff (1974a)
5-butyloxazole	yes	pro.	96 (100), 110 (98), 55 (87), 42 (80), 82 (58), 43 (55), 125 (55); M = 125
2-pentyloxazole	yes	pro.	42 (100), 97 (42), 110 (41), 111 (40), 41 (35), 59 (26), 139 (25), 124 (23); M = 139
2-ethyl-5-butyloxazole	yes	pro.	110 (100), 153 (34), 43 (31), 124 (30), 82 (29), 39 (19), 98 (18), 42 (16), 55 (15); M = 153
2,4-diethyl-5-propyloxazole	yes	pro.	138 (100), 43 (47), 139 (42), 84 (28), 152 (27), 41 (27), 167 (23); M = 167
oxazolines			
2-methyl-3-oxazoline	yes	pro.	43 (100), 31 (62), 55 (41), 29 (32), 85 (30), 42 (28), 58 (24), 70 (22); M = 85
2,4-dimethyl-3-oxazoline	yes	lit.	Mussinan et al. (1976)
2,4,5-trimethyl-3-oxazoline	yes	lit.	Chang et al. (1968)

^a lit. = matched with mass spectrum reported in the literature. pro. = structure proposed from interpretation of mass spectrum.

with a column temperature which was programmed from 40 to 240 °C at a rate of 3 °C/min. The chromatogram was divided into 24 broad fractions. Each broad fraction was accumulatively collected according to the method of Thompson et al. (1978).

Each of the 24 broad fractions was subjected to a second fractionation using a 1/8 in. o.d. × 12 ft stainless steel column packed with 10% SP-1000 on 80-100-mesh Chromosorb W. Some selected subfractions were chromatographed for a third time by using a 1/8 in. o.d. × 12 ft stainless steel column packed with 10% OV-101 on 80-100-mesh Chromosorb W.

Identification of the Gas Chromatographic Fractions. The gas chromatographic fractions were identified by the combination of infrared and mass spectrometry, according to the procedure reported previously (Smouse and Chang, 1967). A Du Pont 21-490 mass spectrometer with a jet separator interfaced with a Varian Moduline 2700 gas chromatograph fitted with an FID detector and a 1/8 in. o.d. × 12 ft stainless steel column packed with 10% OV-101 on 80-100-mesh Chromosorb W was used. The flow rate was 30 mL/min and the column temperature was programmed to provide maximum resolution of each fraction analyzed.

RESULTS AND DISCUSSION

Eight thiazoles, seven oxazoles, and three oxazolines were identified in the VFC isolated from 70 kg of roasted peanuts. Most of the thiazoles and oxazoles identified have never been reported before as volatile flavor components of roasted peanuts (Table I).

Numerous thiazoles were identified in volatiles of a wide variety of foods such as meat (Chang and Peterson, 1977), coffee (Vitzthum and Werkhoff, 1974a), and potato

products (Buttery and Ling, 1974; Coleman et al., 1981). However, in the volatiles of roasted peanuts only three thiazoles, namely, thiazole, 4-methylthiazole, and benzothiazole, were reported (Walradt et al., 1971). The present study identified eight thiazoles; among them 2-isopropyl-4,5-dimethylthiazole and 2-propyl-4,5-diethylthiazole had a pleasant nutty aroma and could be important to the flavor of roasted peanuts. The mechanism for the formation of thiazoles in foods would be the interaction of sulfur-containing amino acids with carbohydrates or carbonyls.

The occurrence of oxazoles has been reported only in five food systems, namely, coffee (Stoffelsma et al., 1968; Vitzthum and Werkhoff, 1974b), cocoa (Vitzthum et al., 1975), barley (Harding et al., 1978), potato (Coleman et al., 1981), and meat products (Peterson et al., 1975; Chang et al., 1977; Chang and Peterson, 1977). It is of interest to note that oxazoles have been reported only in foods which have been treated with heat. The formation of oxazoles might result from the Strecker degradation of aminoketones which can come from the condensation of α -dicarbonyl compounds with amino acids (Vitzthum and Werkhoff, 1974b). Most oxazoles identified in roasted peanut flavor were characterized as having green, nutty odors. 2-Acetyloxazole was described as nutty and popcorn-like. 2,4,5-Trimethyloxazole and 2,4-diethyl-5-propyloxazole have nutty, sweet, and green aromas. 4,5-Dimethyloxazole and 5-butyloxazole contributed a green, sweet, and vegetable note to the total flavor.

The first oxazoline, 2,4,5-trimethyl-3-oxazoline, was reported by Chang et al. (1968) in the volatiles of boiled beef. So far, oxazolines have been found to occur only in meat aroma (Hirai et al., 1973; Mussinan et al., 1976) and thought to have a certain importance in the development

of the flavor of cooked beef. Mussinan et al. (1976) suggested that 2,4,5-trimethyl-3-oxazoline can form in heated meat systems by the thermal interaction and rearrangement of the compounds, ammonia, acetaldehyde, and acetoin. Alternatively, a study of the Strecker degradation shows that 2,4,5-trimethyl-3-oxazoline might be formed from alanine and 2,3-butanedione (Rizzi, 1969; Ohloff and Flament, 1979). Among the three oxazolines identified in the volatiles of roasted peanuts, 2-methyl-3-oxazoline has never been reported in food flavor. 2,4,5-Trimethyl-3-oxazoline has woody and green aromas. 2-Methyl-3-oxazoline and 2,4-dimethyl-3-oxazoline were described as nutty and sweet.

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LITERATURE CITED

- Brown, B. A.; Lonigsbacher, K. S.; Ellison, F. E.; Mann, G. E. *J. Food Sci.* 1968, 33, 595.
- Buttery, R. G.; Ling, L. C. *J. Agric. Food Chem.* 1974, 22, 912.
- Buttery, R. G.; Ling, L. C.; Lundin, R. E. *J. Agric. Food Chem.* 1973, 21, 488.
- Chang, S. S.; Hirai, C.; Reddy, B. R.; Herz, K. O.; Kato, A.; Sipma, G. *Chem. Ind. (London)* 1968, 1639.
- Chang, S. S.; Peterson, R. J. *J. Food Sci.* 1977, 42, 298.
- Chang, S. S.; Vallese, F. M.; Hwang, L. S.; Hsieh, O.; Min, D. B. *S. J. Agric. Food Chem.* 1977, 25, 450.
- Coleman, E. C.; Ho, C.-T.; Chang, S. S. *J. Agric. Food Chem.* 1981, 29, 42.
- Harding, R. J.; Wren, J. J.; Nursten, H. E. *J. Inst. Brew.* 1978, 84, 41.
- Herz, K. O.; Chang, S. S. *J. Food Sci.* 1966, 37, 937.
- Hirai, C.; Herz, K. O.; Pokorny, J.; Chang, S. S. *J. Food Sci.* 1973, 38, 393.
- Johnson, B. R.; Waller, G. R.; Burlingame, A. L. *J. Agric. Food Chem.* 1971a, 19, 1020.
- Johnson, B. R.; Waller, G. R.; Foltz, R. L. *J. Agric. Food Chem.* 1971b, 19, 1025.
- Mason, M. E.; Johnson, B.; Hamming, M. C. *J. Agric. Food Chem.* 1966, 14, 454.
- Mason, M. E.; Johnson, B.; Hamming, M. C. *J. Agric. Food Chem.* 1967, 15, 66.
- Mussinan, C. J.; Wilson, R. A.; Katz, I.; Hruza, A.; Vock, M. H. *ACS Symp. Ser.* 1976, 26, 133.
- Ohloff, G.; Flament, I. *Fortschr. Chem. Org. Naturst.* 1979, 36, 231.
- Pattee, H. E.; Beasley, E. O.; Singleton, J. A. *J. Food Sci.* 1965, 38, 123.
- Peterson, R. J.; Izzo, H. J.; Jungermann, E.; Chang, S. S. *J. Food Sci.* 1975, 40, 948.
- Rizzi, G. P. *J. Org. Chem.* 1969, 34, 2002.
- Smouse, T. H.; Chang, S. S. *J. Am. Oil Chem. Soc.* 1967, 44, 509.
- Stoffelsma, J.; Sipma, G.; Kettenes, D. K.; Pypker, J. *J. Agric. Food Chem.* 1968, 16, 1000.
- Thompson, J. A.; May, W. A.; Paulose, M. M.; Peterson, R. J.; Chang, S. S. *J. Am. Oil Chem. Soc.* 1978, 55, 897.
- Van Straten, S. "Volatile Compounds in Food", 4th ed.; Central Institute for Nutrition and Food Research: Zeist, The Netherlands, 1977.
- Viani, R.; Bricout, J.; Marion, J. P.; Muggler-Chavan, F.; Reymond, D.; Egli, R. H. *Helv. Chim. Acta* 1969, 52, 887.
- Vitzthum, O. G.; Werkhoff, P. *J. Food Sci.* 1974a, 39, 1210.
- Vitzthum, O. G.; Werkhoff, P. *Z. Lebensm.-Unters.-Forsch.* 1974b, 156, 300.
- Vitzthum, O. G.; Werkhoff, P.; Hubert, P. *J. Food Sci.* 1975, 40, 911.
- Walradt, J. R.; Pittet, A. O.; Kinlin, T. E.; Murlaidhara, R.; Sanderson, A. *J. Agric. Food Chem.* 1971, 19, 972.

Min-Hsiung Lee¹
Chi-Tang Ho
Stephen S. Chang*

Department of Food Science

Cook College

New Jersey Agricultural Experiment Station

Rutgers, The State University of New Jersey

New Brunswick, New Jersey 08903

¹Present address: Department of Agricultural

Chemistry

National Taiwan University

Taipei, Taiwan, Republic of China

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Preliminary Results of in Vitro Propagation of Guayule

The propagation of whole guayule plants from tissue culture is desirable but has not yet been achieved. For facilitation of this effort, conditions which optimized tissue culture growth using MS media were investigated. In addition, preliminary results of the use of a chlorophenoxy derivative of triethylamine as a possible growth hormone are presented. The derivative was shown to alternately produce shoot or callus formation, exclusive of each other, as a function of concentration.

Guayule, *Parthenium argentatum* Gray, was first established in tissue culture by Bonner (Bonner and Arreguin, 1950) as a means to study the effects of various chemicals and extracts on rubber production. The propagation of whole guayule plants from tissue culture, however, has not been accomplished, and our efforts have been directed toward this end as a means of increasing rubber production through genetic manipulation. Initial experiments designed to define the optimal growth conditions for guayule revealed significantly different responses to

alterations in media and hormone levels. In an attempt to stimulate rubber production in guayule, Yokoyama et al. (1977) sprayed juvenile plants with 2-(3,4-dichlorophenoxy)triethylamine (TEA derivative). This treatment resulted in increased isoprenoid levels in the plant tissue. Since its effect was pronounced on juvenile guayule plants, it appeared worthwhile to ascertain its effect on in vitro cultures. In the process of examining these possible effects, interesting hormonal properties were discovered. A suppression of callus formation occurs at a 10 mg/L concen-