Identification of Alkyloxazoles in the Volatile Compounds from French-Fried Potatoes

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Twenty-four alkyloxazoles were identified in the volatile compounds from french-fried potatoes. This represents the largest number of oxazoles identified in a food system. Eleven of the alkyloxazoles are being reported for the first time as components of food. The synthesis and mass spectral data of these new oxazoles are reported.

INTRODUCTION

The flavor of potato has been extensively studied. Approximately 429 volatile compounds have been identified as components of potato or potato products (Carlin, 1983). These products include french-fried potatoes, baked potatoes, potato chips, and dehydrated potatoes.

The objective of our work was to characterize the volatile components of french-fried potato flavor. This paper reports the identification of 24 alkyloxazoles in the volatiles isolated from french-fried potatoes.

Heterocyclic compounds such as pyrazines and thiazoles have been reported as important contributors to the flavor of potato or potato products. The oxazoles are a relatively new class of flavor compounds that possess some unique and important sensory characteristics. Their occurrence in food has been reviewed by Maga (1978). 2,4,5-Trimethyloxazole and 5-acetyl-2,4-dimethyloxazole have been identified in baked-potato flavor (Coleman et al., 1981). They are the only oxazoles previously reported as components of potato or potato products.

EXPERIMENTAL SECTION

Synthesis of α-Bromo Ketones. α-Bromo ketones were synthesized according to the method of Catch et al. (1948), which involves direct bromination of the appropriate ketone. Two bromo ketones are formed when an unsymmetrical ketone serves as the reactant. The mixture of two bromides was taken through the oxazole synthesis, and the two isomeric oxazoles were separated by gas chromatography.

Synthesis of Alkyloxazoles. Alkyloxazoles were synthesized according to the method of Theilig (1953). A 1-equiv portion of α-bromo ketone was allowed to react with 2 equiv of amide. The distilled products were further purified by gas chromatography using a Beckman GC-5 fitted with a 12 ft. × $^1/_8$ in. stainless-steel column packed with 10% SP-1000 on 80/100-mesh Chromosorb W. The column flow rate was 30 mL/min. The column oven temperature was programmed from 50 to 230 °C at 5 °C/min.

GC Analysis of Synthesized Alkyloxazoles. Synthesized alkyloxazoles were analyzed using a Varian 3400 gas chromatograph fitted with a 60 M × 0.25 mm SPD-1

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fused silica capillary column (Supelco, Inc.). The column flow rate was 2 mL/min. The column oven temperature was held at 50 °C for 5 min and then programmed at 2 °C/min to a final temperature of 280 °C, which was held for 30 min. Linear retention indices were calculated according to the modified Kovats method described by Majlat et al. (1974). Normal paraffins were used as references (C_8–C_{25}, Altech Associates).

GC–MS Analysis of Synthesized Alkyloxazoles. A Du Pont 21-490 mass spectrometer interfaced with a jet separator to a Varian Moduline 2700 gas chromatograph was used for the GC–MS analysis of the synthesized alkyloxazoles. The GC was fitted with a 10 ft × $^1/_8$ in. stainless-steel column packed with 10% OV-101 on 60/80-mesh Chromosorb W. The column flow rate was 30 mL/min. The column temperature was held at 40 °C for 5 min and then programmed to 6 °C/min to a final temperature of 225 °C, which was held for 15 min.

Isolation of the Volatile Compounds from French-Fried Potatoes. Isolation of the volatile compounds from french-fried potatoes has been described by Carlin (1983). French-fried potatoes were prepared fresh in the laboratory. Approximately 16 lbs of Fri-al shortening (Intercon Co., Carteret, NJ) was placed in a fryer (Cecilware Model ELK-250 electric fryer; Long Island City, NY) and heated to 190 °C. A total of 24 lbs of authentic McDonald's frozen french-fried potatoes were fried for each isolation.

The volatile compounds were isolated from french-fried potatoes by using a headspace gas flushing apparatus previously described by Chang et al. (1977). The sample was maintained at approximately 72 °C during the isolation period. Each isolation lasted 48 h. The volatile compounds were isolated from a total of 312 lbs of french-fried potatoes. This constituted 13 individual isolations of 24 lbs of french fries each. The aqueous flavor isolated obtained from the isolation procedure was extracted with ethyl ether and combined with ether washings of the collecting traps. The ether solution was dried over anhydrous sodium sulfate and concentrated to a final volume of 115 mL on a 30-plate Oldershaw distillation column and a 200-plate spinning-band still.

Gas Chromatographic Fractionation of the Flavor Isolate. The french-fried potato flavor isolate was subjected to a systematic method of gas chromatographic fractionation in order to obtain relatively pure compounds for GC–MS analysis. The methodology has been previously described by Coleman et al. (1981). More than 300 fractions were generated from the GC fractionation of the flavor isolate.

GC–MS Analysis of Fractions. Fractions obtained from the gas chromatographic fractionation of the flavor isolate were analyzed by GC–MS as previously described for the analysis of synthesized oxazoles.

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Table I. Alkyloxazoles Identified in the Volatile Compounds from French-Fried Potatoes

alkyloxazole	I_K,[a] SPD-1	mass spec ref or data, m/z (rel intens)	approx rel concn
2,4,5-trimethyloxazole	826	ten Noever de Brauw et al. (1980)	40
2-methyl-4-ethyloxazole	807	Vitzthum and Werkhoff (1974a)	1
2,4-dimethyl-5-ethyloxazole	907	Vitzthum and Werkhoff (1974a)	1
2,5-dimethyl-4-ethyloxazole	b	ten Noever de Brauw et al. (1980)	1
2-ethyl-4,5-dimethyloxazole	886	ten Noever de Brauw et al. (1980)	1
2,4-dimethyl-5-propyloxazole	989	Ho et al. (1983a)	1
2-methyl-4-butyloxazole	993	Ho et al. (1983a)	1
2-isopropyl-4,5-dimethyloxazole	938	Ho et al. (1983a)	40
2,4-dimethyl-5-butyloxazole	1089	Ho et al. (1983b)	1
2-methyl-4-ethyl-5-propyloxazole	1067	Ho et al. (1983b)	30
2,5-dimethyl-4-butyloxazole	1082	111 (100), 110 (77), 43 (70), 69 (33), 42 (28), 153 (21), 41 (14), 70 (12), 27 (11), 39 (10), 112 (8), 55 (7), 68 (7), 124 (6), 138 (5), 154 (5); M_r = 153	1
2-methyl-4-pentyloxazole	1095	110 (100), 42 (33), 43 (24), 27 (22), 41 (20), 68 (12), 29 (11), 111 (10), 28 (9), 39 (9), 153 (9), 97 (7), 55 (6), 69 (5), 26 (4), 56 (4); M_r = 153	1
2-ethyl-4-methyl-5-propyloxazole	1071	124 (100), 41 (20), 29 (19), 27 (18), 56 (16), 42 (15), 39 (12), 153 (12), 28 (11), 57 (8), 125 (8), 43 (7), 55 (6), 26 (4), 68 (4), 40 (3), 54 (3), 82 (3); M_r = 153	20
2-isopropyl-4-methyl-5-ethyloxazole	b	ten Noever de Brauw et al. (1980)	10
2-butyl-4,5-dimethyloxazole	1096	111 (100), 110 (35), 43 (27), 41 (22), 42 (21), 68 (21), 153 (21), 27 (10), 69 (9), 55 (8), 125 (8), 39 (7), 112 (7), 124 (7), 29 (6), 70 (6), 138 (5), 28 (4), 54 (4); M_r = 153	1
2-isobutyl-4,5-dimethyloxazole	b	ten Noever de Brauw et al. (1980)	1
2-pentyl-4-methyloxazole	1090	97 (100), 27 (72), 42 (69), 41 (65), 29 (54), 110 (54), 55 (39), 68 (39), 28 (34), 39 (28), 43 (21), 111 (19), 124 (16), 54 (14), 26 (13), 153 (9), 40 (8), 67 (8), 83 (8), 98 (8); M_r = 153	10
2,4-diethyl-5-propyloxazole	b	Ho et al. (1983a)	10
2-pentyl-4,5-dimethyloxazole	1193	111 (100), 124 (60), 43 (28), 110 (27), 167 (27), 41 (24), 42 (19), 125 (19), 55 (18), 68 (18), 138 (18), 29 (9), 39 (8), 112 (8), 69 (7), 83 (7), 67 (6), 70 (6); M_r = 167	10
2-butyl-4,5-diethyloxazole	1292	29 (100), 139 (99), 41 (64), 57 (45), 27 (34), 39 (25), 28 (24), 56 (24), 166 (23), 124 (22), 152 (22), 55 (20), 69 (15), 82 (14), 83 (14), 43 (13), 181 (13), 84 (12), 138 (12), 140 (9), 42 (8), 53 (8), 54 (8), 153 (8); M_r = 181	1
2-butyl-4-propyl-5-methyloxazole	1285	139 (100), 29 (65), 41 (46), 57 (40), 27 (22), 124 (22), 166 (22), 152 (21), 56 (20), 39 (19), 28 (18), 55 (18), 69 (13), 181 (13), 138 (12), 82 (11), 83 (11), 84 (10), 43 (9), 140 (9), 153 (8), 54 (7), 42 (6), 53 (6), 40 (5); M_r = 181	1
2-pentyl-4-methyl-5-ethyloxazole	1263	125 (100), 138 (53), 28 (46), 43 (33), 139 (19), 41 (16), 181 (16), 152 (15), 124 (12), 55 (11), 82 (9), 69 (8), 166 (8), 27 (7), 29 (7), 126 (7), 39 (6), 56 (6), 83 (6), 110 (6), 42 (5), 44 (5), 54 (5), 98 (5); M_r = 181	1
2-hexyl-4,5-dimethyloxazole	1287	111 (100), 124 (50), 43 (45), 41 (40), 55 (35), 42 (30), 27 (28), 125 (28), 68 (22), 110 (21), 29 (17), 138 (14), 39 (13), 181 (11), 28 (10), 67 (8), 69 (8), 112 (8), 152 (8), 70 (6), 40 (5), 53 (5), 54 (5), 166 (5); M_r = 181	10
2-hexyl-4-methyl-5-ethyloxazole	1359	125 (100), 41 (53), 54 (47), 138 (47), 43 (45), 27 (41), 29 (41), 55 (41), 28 (26), 39 (21), 69 (15), 139 (15), 166 (15), 152 (12), 42 (10), 70 (10), 96 (10), 53 (9), 97 (9), 126 (9), 68 (8), 167 (8), 195 (8), 56 (6), 180 (6); M_r = 195	1

[a] Calculated Kovats indices. [b] Not synthesized.

RESULTS AND DISCUSSION

A total of 24 alkyloxazoles were identified in this study. This represents the largest number of oxazoles identified in a food system. Of the alkyloxazoles, 11 are being reported for the first time as components of food. Of the compounds identified, only 2,4,5-trimethyloxazole has been previously reported as a component of potato or potato products.

Compounds were identified by comparing their mass spectra and retention indices with those of reference compounds. Table I lists the mass spectral reference or tabulation for the oxazoles identified and includes the retention indices of oxazoles that were synthesized.

The oxazoles identified represented less than 0.01% of the total volatile compounds isolated from french-fried potatoes. Approximate relative concentrations of the oxazoles identified are included in Table I. This information is useful in formulating an oxazole fraction for use in a french-fried potato flavor.

Several of the oxazoles identified such as 2,4-dimethyl-5-propyloxazole, 2-methyl-4-butyloxazole, and 2,4-dimethyl-5-butyloxazole have a characteristic strong green, herbal, and vegetable-like aroma (Jin et al., 1984). This aroma characteristic is quite distinctive and was present in a large number of the fractions generated from the gas chromatographic fractionation of the french-fried

potato flavor isolate. It is probably an important part of the total french-fried potato flavor.

2-Pentyl-4-methyl-5-ethyloxazole has a strong buttery, sweet, and lactone-like flavor (Jin et al., 1984). It is probably an important contributor to the fried food aspect of french-fried potato flavor.

Oxazoles have been identified in only a few food systems, each of which has been subjected to heat treatment. The exact mechanism of oxazole formation is not known. They could possibly form through the Strecker degradation of amino ketones that result from the condensation of α-dicarbonyl compounds with amino acids (Vitzthum and Werkhoff, 1974a,b; Ho and Hartman, 1982). They might also form through reactions between amino acids (Ohloff and Flament, 1979).

2-Hexyl-4,5-dimethyloxazole, and other oxazoles identified, have long-chain alkyl substitutions at the 2-position of the oxazole ring. The involvement of lipid or lipid decomposition products in the formation of these compounds could account for the long-chain alkyl substitutions. Future papers will report the results of model system studies evaluating the formation of alkyloxazoles in french-fried potatoes.

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LITERATURE CITED

Carlin, J. T. Ph.D. Dissertation, Rutgers University, New Brunswick, NJ 08903, 1983.

Catch, J. R.; Elliot, D. F.; Hey, D. F.; Jones, E. R. H. *J. Chem. Soc.* 1948, 272.

Chang, S. S.; Vallese, F. M.; Hwang, L. S.; Hsieh, O. A.-L.; 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*.

Ho, C.-T.; Hartman, G. J. *J. Agric. Food Chem.* 1982, *30, 793*.

Ho, C.-T.; Jin, Q. Z.; Lee, M.-H.; Chang, S. S. *J. Agric. Food Chem.* 1983a, *31, 1984*.

Ho, C.-T.; Jin, Q. Z.; Lee, K. N.; Carlin, J. T.; Chang, S. S. *J. Food Sci.* 1983b, *48, 1570*.

Jin, Q. Z.; Hartman, G. J.; Ho, C.-T. *Perfum. Flavor.* 1984, *9*(4), 25.

Maga, J. A. *J. Agric. Food Chem.* 1978, *26, 1049*.

Majlat, P.; Erdos, Z.; Takacs, J. *J. Chromatogr.* 1974, *91, 89*.

Ohloff, G.; Flament, I. *Forsch. Chem. Org. Naturst.* 1979, *36, 231*.

ten Noever de Brauw, M. C.; Bouwman, J.; Tas, A. C.; La Vos, G. F. *Compilation of Mass Spectra of Volatile Compounds in Foods*; TNO: Zeist, The Netherlands, 1980.

Theilig, G. *Chem. Ber.* 1953, *86, 96*.

Thompson, J. A.; May, W. A.; Paulose, M. M.; Peterson, R. J.; Chang, S. S. *J. Am. Oil Chem. Soc.* 1978, *55, 897*.

Vitzthum, O. G.; Werkhoff, P. J. *J. Food Sci.* 1974a, *39, 1210*.

Vitzthum, O. G.; Werkhoff, P. J. *Z. Lebensm. Unters.-Forsch.* 1974b, *156, 300*.

Effects of Reduction with Dithiothreitol on Some Molecular Properties of Soy Glycinin

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The reduction of soy glycinin with 5 and 10 mM dithiothreitol (DTT) caused rupture of the disulfide bonds linking the acidic and basic subunits and the intramolecular bonds, respectively. The surface hydrophobicity as determined by *cis*-parinaric acid increased fourfold following treatment with 5 mM DTT while specific viscosity was doubled. Further reduction of disulfide bonds had little effect. The UV absorbance spectra decreased while the ionization of tyrosines above pH 9.5 was enhanced following reduction. The intrinsic fluorescence of glycinin was accentuated following reduction, and a red shift was observed, suggesting exposure of tryptophan to a more polar environment. The data are consistent with the initial dissociation of glycinin with 5 mM DTT while further reduction, with 10 mM DTT, resulted in some reassociation of the component subunits.

The major globulins of soybeans are comprised of glycinin (11S) and conglycinin (7S). Glycinin contains six disulfide-linked acidic (37 kDa) and basic (20 kDa) subunits, and both are transcribed as a single polypeptide (Neilsen, 1985). the component polypeptides show a high degree of homology (Nielsen, 1984). The subunits are packed as two identical apposed hexagons, each composed of three pairs of alternating acidic and basic subunits mostly joined by one disulfide bond with hydrophobic forces holding the adjacent pairs of subunits together. The opposing hexagonal layers associate by electrostatic forces and/or hydrogen bonding (Kinsella et al., 1985; Badley et al., 1975; Kitamura et al., 1976). Soy 11S globulin has about 5% α-helix and about 35% β-structure with the remainder being random coil (Catsimpoolas et al., 1970; Koshiyama and Fukushima, 1973; Jacks et al., 1973). Soy

11S contains 38–42 half-cysteine residues per molecule including two free sulfhydryl groups (Saio et al., 1971; Kella and Kinsella, 1985). In addition to the six intersubunit disulfide bonds linking the acidic and basic subunits, there are from zero to two intramolecular disulfide bonds in acidic subunits and from zero to one disulfide bond in basic subunits on the basis of amino composition (cysteine) of the subunits (Moreira et al., 1979; Utsumi, 1981; Iyengar and Ravenstein, 1981; Catsimpoolas et al., 1971; Okubo et al., 1969).

Molecular flexibility is an important attribute governing some functional properties, e.g. the surface-active properties of proteins (Graham and Phillips, 1976; Kinsella, 1981; Halling, 1981). Soy glycinin is a compactly folded molecule, the rigidity of which is stabilized by the disulfide bonds, and as such it has limited surface-active properties (Kinsella, 1979). However reduction of some of the disulfide bonds by "loosening" the tertiary structure of glycinin making it more flexible may improve its functional properties. In this study we report the effects of reduction

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