

Effect of pH on the Volatile Formation from the Reaction between Cysteine and 2,5-Dimethyl-4-hydroxy-3(2H)-furanone

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The effect of pH on the volatile components formed by the reaction between cysteine and 2,5-dimethyl-4-hydroxy-3(2H)-furanone was studied in a Parr bomb model system. pH values of 2.2, 5.1, and 7.1 represented points below, around, and above the isoelectric points of cysteine. At pH 2.2, the major components formed were 3-methyl-2-(2-oxopropyl)thiophene, 1,2,3-trithia-5-cycloheptene, 2,5-dimethyl-4-hydroxy-3(2H)-thiophenone, and 2,4-hexanedione. At pH 5.1, the major components formed were 3,5-dimethyl-1,2,4-trithiolane isomers and 2,5-dimethyl-4-hydroxy-3(2H)-thiophenone. At pH 7.1, various classes of heterocyclic compounds, including thiazoles, thiazolines, and pyrazines, were identified. All three of the reactions produced roasted and meaty aromas, but the reaction at pH 2.2 generated the best meat flavor in terms of the roasted and meaty notes evaluated.

2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF) is an important α -dicarbonyl, which has been identified in many food sources (Rodin et al., 1965; Tonsbeek et al., 1968) and used extensively in many food areas (Re et al., 1973; Shu et al., 1985b). Cysteine and other sulfur-containing amino acids are considered to be important contributors to the formation of various food flavors, especially to meat flavors (Hurrell, 1982; Ching, 1979). The reaction between cysteine and DMHF would, therefore, be expected to produce some interesting products chemically and organoleptically. We have previously reported the compounds identified in this reaction (Shu et al., 1986). In the present study, we report effect of pH on the volatile components and the aroma of the mixture generated from the reaction between cysteine and DMHF at 160 °C for 0.5 h in a closed system. The results of this study suggest that meat reaction flavor may be formed from the reaction of cysteine and DMHF, especially at low pH.

EXPERIMENTAL SECTION

Each solution was prepared by dissolving 0.05 mol of L-cysteine hydrochloride (Ajinomoto Co., Tokyo, Japan) and 0.05 mol of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (99%) (International Flavors and Fragrances, Union Beach, NJ) in approximately 450 mL of distilled water, titrating the solution to the appropriate pH value (2.2, 5.1, 7.1) with 10% Na₂CO₃, and diluting the sample to a final volume of 500 mL with distilled water. The solution was then placed in a 2-L Parr bomb (Parr Instrument Co., Moline, IL) and heated at 160 °C for 0.5 h. The reaction mass was subjected to a vacuum steam distillation, extraction, and concentration, and the concentrate was analyzed by gas chromatography-mass spectrometry (GC-MS) on fused silica columns (OV-1 and Carbowax 20M) as described previously (Shu et al., 1985a). Aroma properties of the volatile mixture from each reaction were evaluated by Dr. Manfred Vock, IFF Chief Flavorist.

RESULTS AND DISCUSSION

Table I shows the volatile components identified from the reaction of cysteine and DMHF at different pH levels.

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At pH 2.2 the major components from this reaction were 3-methyl-2-(2-oxopropyl)thiophene, 1,2,3-trithia-5-cycloheptene, acetone, 2,5-dimethyl-4-hydroxy-3(2H)-thiophenone, 2,4-hexanedione, and 3,5-dimethyl-1,2,4-trithiolane. 3-Methyl-2-(2-oxopropyl)thiophene, 2,5-dimethyl-4-hydroxy-3(2H)-thiophenone, and 2,4-hexanedione appear to be formed by the interaction of cysteine and DMHF (Shu et al., 1985c). 1,2,3-Trithia-5-cycloheptene is a cysteine degradation product (Shu et al., 1985a), and acetone can be derived from DMHF (Shu et al., 1985b). Cysteine degradation can also yield 3,5-dimethyl-1,2,4-trithiolane (Shu et al., 1985a), but this product may alternatively be formed by the reaction of H₂S, another cysteine degradation product, with acetaldehyde, which can be derived from either cysteine or DMHF.

At pH 5.1, 3,5-dimethyl-1,2,4-trithiolane accounted for 25% of the total volatiles. This compound is apparently formed easily when pH = pI and cysteine is in its neutral form. Other major components of the reaction at pH 5.1 were 2,5-dimethyl-4-hydroxy-3(2H)-thiophenone and various carbonyls.

At pH 7.1, several classes of compounds were identified, including thiazoles, thiazolines, thiolanes, furanones, cyclopentenones, pyrazines, oxazoles, and dithiazine. Pyrazines were identified only at this pH, suggesting that the amino group of cysteine was more reactive at pH 7.1 than at pH 2.2 or 5.1. Several factors indicated that secondary reactions occurred readily at this pH. First, a large number of compounds were identified in the GC profile of the volatile fraction, but none could be designed as a major reaction product. Second, 2,4-hexanedione and the thiophenones were not detected as products, and 3,5-dimethyl-1,2,4-trithiolane accounted for only 3% of the volatiles formed. Table II summarized the effect of pH on the volatiles formed by the reaction of cysteine and DMHF.

Table III shows the aroma properties of the volatiles formed from the reaction of cysteine and DMHF at different pH's. Comparison of the aroma properties of each volatile mixture indicates that the best meat flavor was produced by the reaction at pH 2.2, although all three of the reactions produced similar roasted/meaty characters. Correlation of the aroma data (Table III) with the chemical data (Tables I and II) indicates that a combination of the compounds identified, especially the major compounds listed in Table II, is responsible for the desirable meaty roasted flavor produced by the reaction.

Figure 1 shows the yields obtained from the reaction between cysteine and DMHF as well as from the degra-

Table I. Volatile Components Identified from the Reaction of Cysteine and DMHF at Different pH Values

components identified	GC area, %		
	pH 2.2	pH 5.1	pH 7.1
Acyclic Carbonyls			
acetaldehyde	T ^a	T	T
ethyl acetate		4.3	
acetol acetate	T	T	
acetoin acetate	T	T	
acetone	13.3	1.5	2.4
methyl ethyl ketone	T	4.8	5.4
2-pentanone		2.3	T
3-pentanone		2.4	
2,4-hexanedione	4.7	6.7	
2,3-pentanedione	T	T	
acetol			T
3-hydroxy-2-butanone			T
3-hydroxy-2-pentanone	1.1	3.6	1.4
2-hydroxy-3-pentanone	T	1.2	1.0
Cyclic Carbonyls			
2,5-dimethyl-4,5-dihydro-3(2H)-furanone			T
2,5-dimethyl-3(2H)-furanone	1.4	2.4	3.6
2,4,5-trimethyl-3(2H)-furanone	T	T	1.3
2,4-dimethyl-5-ethyl-3(2H)-furanone	T	T	
2-ethyl-2-cyclopenten-1-one			T
5-methyl-2-ethyl-2-cyclopenten-1-one			1.8
2-methyl-2-pentenolide			T
2-ethyl-2-pentenolide			1.2
Sulfur Compounds			
3,5-dimethyl-1,2,4-trithiolane	3.1	24.7	3.2
4,6-dimethyl-1,2,3,5-tetrathiane		T	
3,6-dimethyl-1,2,4,5-tetrathiane		T	
3-methyl-1,2,4-trithiane			T
2-thiophenethiol	2.2		
1-mercapto-2-propanone		T	
2-acetylthiophene			1.6
3-methyl-2-acetylthiophene			T
5-methyl-2-acetylthiophene			1.4
3,5-dimethylthiophene-3-carboxaldehyde			T
3-methyl-2-(2-oxopropyl)thiophene	23.0	T	
2-methyl-3-propionylthiophene	2.0		
tetrahydrothiophene	T		
thienothiophene	T	T	
2,5-dimethyl-4-hydroxy-3(2H)-thiophenone	6.0	5.8	
2,5-dimethyl-2,4-dihydroxy-3(2H)-thiophenone	T	T	
2,5-dimethyl-2-hydroxy-3(2H)-thiophenone	T	T	
3,4-dimethylisothiazole		T	
2,4-dimethyl-3-thiazoline			1.1
2,4,5-trimethyl-3-thiazoline			T
4,5-dimethyl-2-ethyl-3-thiazoline			T
thiazole		T	
2-methylthiazole	T	T	
2-acetylthiazole	T	T	7.8
2,5-dimethylthiazole		T	
2-methyl-5-ethylthiazole		T	
4,5-dimethyl-2-acetylthiazole			T
2,4,5-trimethylthiazole		T	T
2-thiazolyl ethyl ketone			4.6
1,2,3-trithia-5-cycloheptene	10.6		
Nitrogen Compounds			
pyrazine			T
2,5-dimethylpyrazine			4.4
2,6-dimethylpyrazine			1.9
2-methyl-5-ethylpyrazine			T
2-methyl-6-ethylpyrazine			T
2,3,5-trimethylpyrazine			2.0
2,5-dimethyl-3-ethylpyrazine			1.4
2,6-dimethyl-3-ethylpyrazine			1.4
2,6-diethyl-3-methylpyrazine			1.1
2,4,5-trimethylloxazole			T
2,3-dimethylpiperidine			T
2,4,6-trimethyl-1,3,5-dithiazine			T

^aT = trace, less than 1%.

dation of each reactant at different pH's (Shu et al., 1985a,b). From these data, the reaction between cysteine and DMHF appears to be more similar to cysteine degradation than to DMHF degradation. It is therefore likely

Table II. Summary of pH Effect on the Volatile Formation from the Reaction of Cysteine and DMHF

pH	class of compounds formed
2.2	(1) thiophenes and cyclic trisulfide dominating (2) 2,5-dimethyl-4-hydroxy-3(2H)-thiophenone and 2,4-hexanedione next dominating
	(3) 3,5-dimethyl-1,2,4-trithiolanes, low level (4) no pyrazines formed
5.1	(1) 3,5-dimethyl-1,2,4-trithiolanes dominating (2) 2,5-dimethyl-4-hydroxy-3(2H)-thiophenone next dominating
	(3) no pyrazines formed
7.1	(1) pyrazines, thiazoles, thiazolines formed (2) 3,5-dimethyl-1,2,4-trithiolanes, low level (3) no 2,4-hexanedione formed

Table III. Aroma Properties of the Volatiles Formed from the Reaction of Cysteine and DMHF at Different pH's

pH	aroma properties
2.2	roasted, meaty, bread crust
5.1	oniony, roasted, biting
7.1	solvent, roasted, burnt

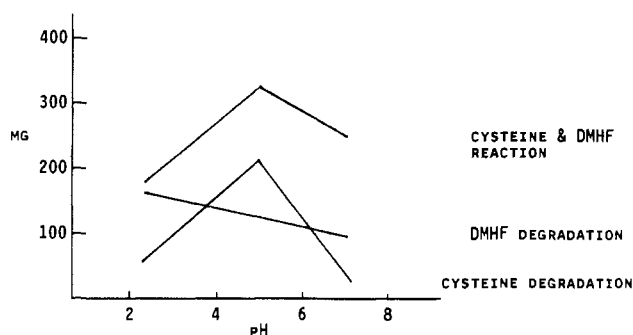


Figure 1. pH effect on the yield of volatiles formed from the reaction of cysteine and DMHF, DMHF degradation, and cysteine degradation.

that cysteine has a stronger influence on the reaction than DMHF.

ACKNOWLEDGMENT

New Jersey Agricultural Experiment Station Publication No. D-10205-7-86 supported by State Funds. We are indebted to International Flavors and Fragrances (R&D) for supplying the facilities and to Dr. Manfred Vock for his organoleptic evaluation during this study. We thank Joan B. Shumsky for her secretarial aid.

Registry No. DMHF, 3658-77-3; L-cysteine, 52-90-4; acetaldehyde, 75-07-0; ethyl acetate, 141-78-6; acetol acetate, 592-20-1; acetoin acetate, 4906-24-5; acetone, 67-64-1; methyl ethyl ketone, 78-93-3; 2-pentanone, 107-87-9; 3-pentanone, 96-22-0; 2,4-hexanedione, 3002-24-2; 2,3-pentanedione, 600-14-6; acetol, 116-09-6; 3-hydroxy-2-butanone, 513-86-0; 3-hydroxy-2-pentanone, 3142-66-3; 2-hydroxy-3-pentanone, 5704-20-1; 2,5-dimethyl-4,5-dihydro-3(2H)-furanone, 64026-45-5; 2,5-dimethyl-3(2H)-furanone, 14400-67-0; 2,4,5-trimethyl-3(2H)-furanone, 64880-73-5; 2,4-dimethyl-5-ethyl-3(2H)-furanone, 74902-72-0; 2-ethyl-2-cyclopenten-1-one, 2931-10-4; 5-methyl-2-ethyl-2-cyclopenten-1-one, 78210-64-7; 2-methyl-2-pentenolide, 72649-02-6; 2-ethyl-2-pentenolide, 85287-76-9; 3,5-dimethyl-1,2,4-trithiolane, 23654-92-4; 4,6-dimethyl-1,2,3,5-tetrathiane, 96504-25-5; 3,6-dimethyl-1,2,4,5-tetrathiane, 67411-27-2; 3-methyl-1,2,4-trithiane, 43040-01-3; 2-thiophenethiol, 7774-74-5; 1-mercaptopropane, 107-03-9; 2-acetylthiophene, 88-15-3; 3-methyl-2-acetylthiophene, 13679-72-6; 5-methyl-2-acetylthiophene, 13679-74-8; 3,5-dimethylthiophene-3-carboxaldehyde, 114719-67-4; 3-methyl-2-(2-oxopropyl)thiophene, 96504-26-6; 2-methyl-3-propionylthiophene, 100207-46-3; tetrahydrothiophene, 110-01-0; 2,5-dimethyl-4-hydroxy-3(2H)-thiophenone, 26494-10-0; 2,5-dimethyl-2,4-dihydroxy-3(2H)-thiophenone, 96504-28-8; 2,5-dimethyl-2-hydroxy-3(2H)-thiophenone, 96504-27-7; 3,4-dimethylisothiazole,

27330-46-7; 2,4-dimethyl-3-thiazoline, 60755-05-7; 2,4,5-trimethyl-3-thiazoline, 60633-24-1; 4,5-dimethyl-2-ethyl-3-thiazoline, 76788-46-0; thiazole, 288-47-1; 2-methylthiazole, 3581-87-1; 2-acetylthiazole, 24295-03-2; 2,5-dimethylthiazole, 4175-66-0; 2-methyl-5-ethylthiazole, 19961-52-5; 4,5-dimethyl-2-acetylthiazole, 7531-76-2; 2,4,5-trimethylthiazole, 13623-11-5; 2-thiazolyl ethyl ketone, 43039-98-1; 1,2,3-trithia-5-cycloheptene, 13005-82-8; pyrazine, 290-37-9; 2,5-dimethylpyrazine, 123-32-0; 2,6-dimethylpyrazine, 108-50-9; 2-methyl-5-ethylpyrazine, 13360-64-0; 2-methyl-6-ethylpyrazine, 13925-03-6; 2,3,5-trimethylpyrazine, 14667-55-1; 2,5-dimethyl-3-ethylpyrazine, 13360-65-1; 2,6-dimethyl-3-ethylpyrazine, 13925-07-0; 2,6-diethyl-3-methylpyrazine, 18138-05-1; 2,4,5-trimethylloxazole, 20662-84-4; 2,3-dimethylpiperidine, 5347-68-2; 2,4,6-trimethyl-1,3,5-dithiazine, 94944-51-1.

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Received for review July 18, 1986. Revised manuscript received February 5, 1988. Accepted March 11, 1988.

Effect of Solar Drying on Vitamin D₃ and Provitamin D₃ Contents in Fish Meat

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Vitamin D₃ (cholecalciferol) and provitamin D₃ (7-dehydrocholesterol) in fish meat were analyzed by high-performance liquid chromatography, and their contents in solar-dried fish meat were compared with those in fresh one. Moreover, the effects of peroxides and freshness on the nutritive constituents in fish meat were examined. Vitamin D₃ in fish meat was reduced by solar drying from 7.4 (mackerel) and 5.2 (saury) $\mu\text{g}/100\text{ g}$ to less than detection limits (1.4 $\mu\text{g}/100\text{ g}$). However, no marked changes in provitamin D₃ contents by the processing were observed. The vitamin D₃ was unstable against sunlight, but it was not affected by peroxides and freshness. These results suggest that vitamin D₃ in fish meat is not produced by solar radiation and sunlight plays a large part in the reduction of vitamin D₃.

It has long been known that a deficiency of vitamin D leads to rickets in children and osteomalacia in adults. In recent years, the number of patients with diseases peculiar to the aged, such as osteoporosis, has tended to increase with the advancing average span of human life. Recently, it was reported that the plasma $1\alpha,25\text{-(OH)}_2\text{-D}_3$ levels in osteoporosis patients decreased as the characteristic symptoms progressed (Heaney, 1977). Moreover, Orimo et al. (1982) indicated that relief of lumbago and an increase in bone density were produced by the administration of $1\alpha\text{-OH-D}_3$ to the patients. These findings suggest that vitamin D plays a very important role in the bone disease.

In foods vitamin D is a natural component and results from fortification with a source of vitamin D₂ or vitamin

D₃. A relatively small number of natural foods, such as fungi (Takeuchi et al., 1985), eggs (Parrish, 1979), and fishes (Higashi et al., 1961; Yamakawa et al., 1963; Takeuchi et al., 1984a), contain nutritionally significant quantities of vitamin D. There are many processed foodstuffs, especially in fishes. Considering the relation between the health of aged humans and the foods they consume, it is important to determine the changes in vitamin D content of fish meat by processing. However, there are few studies of vitamin D contents in the processed foodstuffs of fishes.

The present study was designed to determine the effect of solar drying on the contents of vitamin D₃ and provitamin D₃ in fish meat and to clarify the effects of sunlight, peroxides, and freshness on the nutritive constituents.

MATERIALS AND METHODS

Preparation of Samples. The fresh mackerels (average weight 402 g) and sauries (average weight 132 g) caught in the Pacific Ocean in February, 1986, were obtained from a fish market. To determine the effect of solar drying on vitamin D₃ and provitamin D₃ contents, mackerels and sauries of six fishes each were used. These fishes were cut

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