Mechanistic Studies on Thiazolidine Formation in Aldehyde/Cysteamine Model Systems

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A mechanism was proposed to elucidate the formation of a thiazolidine in aldehyde/cysteamine model systems. Buffer dramatically promotes thiazolidine formation from formaldehyde and cysteamine. Phosphate tends to stabilize the primary carbocation formed, and this may lead to completion of the cyclization by attack of the amino nitrogen on the activated carbon. Protic solvent, by removing the water molecule, further enhances thiazolidine formation. Redox reaction catalyzed by phosphate ions results in the conversion of thiazolidine to the corresponding thiazoline through hydride transfer.

Keywords: *Cysteamine; aliphatic aldehyde; formaldehyde; thiazoline; thiazolidine; redox reaction*

INTRODUCTION

The volatile carbonyl compounds, especially aldehydes, are important contributors to rancid and unpleasant flavors in various lipid-containing foods. They are formed from oxidation or decomposition of lipid during the storage of food. Because of their detrimental effects to the nutritional value of food or their potential carcinogenic property, it is necessary to measure the levels of volatile aldehyde compounds in food.

Quantitative analysis of highly volatile and reactive aldehydes such as formaldehyde and acetaldehyde has been the subject of several studies (Gray, 1978; Frankel, 1987). Most commonly used analysis methods for these aldehydes involved derivatization with 2,4-dinitrophenylhydrazine (Stanley et al., 1975; Caporaso and Sink, 1978). However, this derivatization requires a strong acidic condition which may cause undesirable reactions such as decomposition of trimethyl oxide, carbohydrate, lipid, and protein.

Recently, a thiazolidine derivative method for the determination of trace aldehydes in foods and beverages has been developed (Hayashi et al., 1986; Miyashita et al., 1991). This method is based on the reaction of volatile carbonyl compounds with cysteamine (2-aminoethanethiol) to form stable thiazolidine derivatives under mild conditions (room temperature and neutral pH). The thiazolidine derivatives formed were subsequently determined by gas chromatography. However, the formation pathways of thiazolidines are not yet well documented.

Yasuhara and Shibamoto (1991) reported that it was difficult to obtain consistent results from replicate experiments on formaldehyde and acetaldehyde analysis. Only limited amounts of formaldehyde and acetaldehyde were able to be trapped by cysteamine. They attributed this mainly to the high volatility of formaldehyde (bp −19 °C) and acetaldehyde (bp 20.8 °C). It is well documented that a Schiff base formed between the amino group of cysteamine and the aldehyde group of formaldehyde (Hayashi et al., 1986). In our previous studies, a significant combined effect of buffer and protic solvent on Schiff base formation was observed (Huang, 1997). The objective of this experiment is, therefore, to investigate mechanistically the formation of thiazolidine between cysteamine and aldehydes.

EXPERIMENTAL PROCEDURES

Materials. Formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, and valeraldehyde were purchased from Aldrich Chemical Co. (Milwaukee, WI). Disodium hydrogen phosphate, sodium dihydrogen phosphate dihydrate, sodium carbonate, sodium hydrogen carbonate, trisodium citrate dihydrate, citric acid monohydrate, sodium acetate, and sodium hydroxide were of chemical grade and obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Ethanol, chloroform, and cysteamine were purchased from Sigma Chemical Co. (St. Louis, MO).

Reaction Procedure. Reaction mixtures were composed of 0.6 g of cysteamine and ~0.02 g of aldehydes (formaldehyde, 0.667 mmol; acetaldehyde, 0.455 mmol; propionaldehyde, 0.345 mmol; butyraldehyde, 0.278 mmol; and valeraldehyde, 0.233 mmol), dissolved in 50 mL of deionized water or buffer solutions. Buffers involving carbonate, phosphate, citrate, and acetate were used, and the final pH value of the reaction mixture was adjusted to the pH equivalent to the pK_a value of each salt with sodium hydroxide; the mixtures were then made up to a final volume of 100 mL. To study the effect of buffer capacity on thiazolidine formation, different proportions of anion phosphate (0.025, 0.05, 0.1, and 0.2 M) and an initial pH of 7.2 were used to replace the aqueous medium described previously. Potassium chloride was utilized to maintain a constant ion strength for all three model systems following the method of Reynolds (1959). All of the reactions were run at 25 °C for 30 min, and then 1 mL of tributylamine (0.2 mg/10 mL chloroform) was added as an internal standard for GC analysis. The reaction mixtures were extracted two times with 10 mL of chloroform each time, and the extract was made up to 25 mL with chloroform. After drying over Na_2SO_4, 10 μL of the chloroform extract was subjected to GC analysis.

Effect of Ethanol Content on Thiazolidine Formation. Different percentages of ethanol (0, 20, 40, 60, and 80%) in

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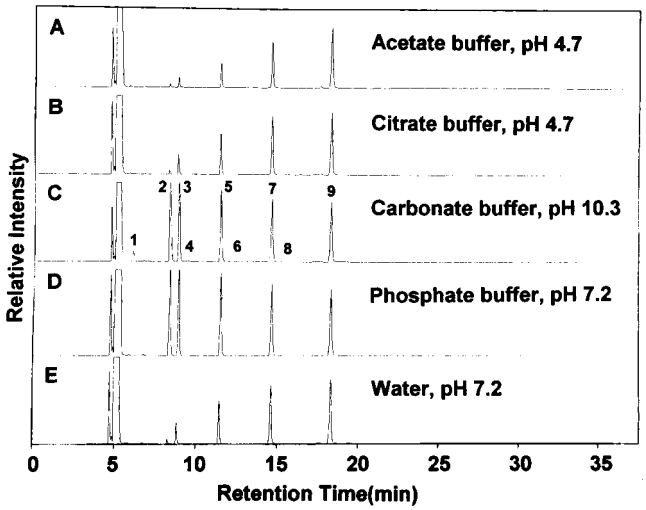

Figure 1. Gas chromatogram of derivatives from aldehydes: peak 1, 2-methylthiazoline from acetaldehyde; peak 2, thiazolidine from formaldehyde; peak 3, 2-methylthiazolidine from acetaldehyde; peak 4, 2-ethylthiazoline from propionaldehyde; peak 5, 2-ethylthiazolidine from propionaldehyde; peak 6, 2-propylthiazolidine from butyraldehyde; peak 7, 2-propylthiazolidine from butyraldehyde; peak 8, 2-butylthiazoline from valeraldehyde; and peak 9, 2-butylthiazolidine from valeraldehyde.

phosphate buffer, 0.2 M, pH 7.2, were used as reaction medium to investigate the combined effect of buffer and solvent on thiazolidine formation. The reacted mixtures were extracted with chloroform as described previously. Thiazolidines were quantified against an internal standard (tributylamine).

Gas Chromatography. A HP 5890 A gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a fused silica column (60 m × 0.32 mm i.d., film thickness, 0.25 μm; SPB-1 Supelco Co.) and a flame ionization detector was used to analyze the chloroform extracts. The operation conditions were as follows: injector and detector temperatures, 250 °C; extrapure helium carrier flow rate, 2.0 mL/min; temperature program, 70−180 °C at 4 °C/min.

Gas Chromatography/Mass Spectrometry. GC/MS analysis was accomplished by using a HP 5890 A gas chromatograph coupled to a 5972 mass selective detector. Mass spectra were obtained by electron ionization and chemical ionization at 70 eV and a source temperature of 250 °C. The filament emission current was 1 mA, and the spectra were recorded and analyzed with HP G111034C MS Chemstation software installed in an IBM personal computer. The operation conditions were the same as those used in the GC analysis described above.

RESULTS AND DISCUSSIONS

GC Separation of Thiazolidines. Under the experimental conditions described, complete resolution was obtained for a standard mixture composed of formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, and valeraldehyde that was reacted with cysteamine. Elution order increased according to chain length as shown in Figure 1. The structures of all thiazolidines were confirmed by both EI and CI GC/MS. The mass spectra are listed in Table 1. The mass spectra of the thiazolidines were in good agreement with the findings of Yasuhara and Shibamoto (1989). The series of thiazolines were characterized in the same model system. These compounds eluted before the corresponding thiazolidine. The mass spectra revealed compounds 2 mass units less than the corresponding thiazolidines. The mass spectra are similar in pattern for all of the thiazolines except the one derived from

Table 1. MS Spectra of Thiazolidine and Thiazoline

no.	compound	RI[a]	MS
1	2-methylthiazoline	804	M^+ = 101 (65), 69 (46), 60 (100), 59 (80), 45 (70)
2	thiazolidine	816	M^+ = 89 (95), 59 (23), 43 (100)
3	2-methylthiazolidine	889	M^+ = 103 (80), 88 (95), 56 (100), 43 (75)
4	2-ethylthiazoline	924	M^+ = 115 (50), 89 (23), 69 (26), 60 (100), 59 (63), 45 (60)
5	2-ethylthiazolidine	983	M^+ = 117 (15), 88 (100), 70 (35), 56 (20), 43 (10)
6	2-propylthiazoline	1041	M^+ = 129 (5), 114 (7), 101 (68), 88 (18), 60 (100), 59 (50), 45 (30)
7	2-propylthiazolidine	1081	M^+ = 131 (25), 88 (100), 70 (40), 61 (55), 56 (70) ,43 (57)
8	2-butylthiazoline	1123	M^+ =143 (5), 114 (13), 101 (100), 88 (12), 69 (8), 60 (64), 59 (35), 45 (24)
9	2-butylthiazolidine	1182	M^+ = 157 (5), 142 (2), 128 (8), 114 (22), 101 (100), 88 (4), 68 (4), 60 (74), 59 (24), 46 (2)

[a] Kovats index.

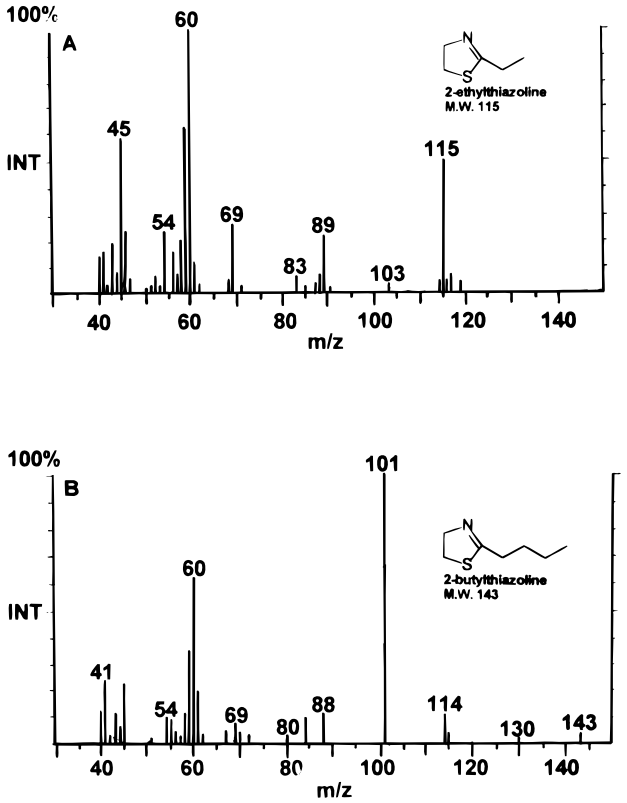

Figure 2. Mass spectra of thiazolines from aldehydes: (A) 2-ethylthiazoline from propionaldehyde; (B) 2-butylthiazoline from valeraldehyde.

propionaldehyde (Figure 2). The McLafferty rearrangement takes place on the longer side chain containing an *n*-propyl or longer alkyl group, giving a characteristic peak of *m*/*z* 101. Two volatile compounds, 2-acetyl-2-thiazoline and 2-acetylthiazole, were characterized in a heated aqueous L-cysteine solution (Sheldon and Shibamoto, 1987). These authors postulated that these two acetyl derivatives were the dehydrogenation products of 2-acetylthiazolidine. A significant amount of 2-acetylthiazolidine has also been observed in the reaction mixture of aldehydes and cysteamine by Hayashi et al. (1986). An interesting redox reaction similar to that reported by Huyghues-Despointes and Yaylayan

Table 2. Effect of Phosphate Buffer Concentration on Thiazolidine Formation[a]

	thiazolidine concn (mM) at phosphate buffer concn of				
	0.025 mM	0.05 mM	0.1 mM	0.2 mM	0 mM
thiazolidine	0.020 (2.9)[a]	0.066 (9.9)	0.168 (24.8)	0.278 (41.6)	0.009 (1.3)
2-methylthiazolidine	0.042 (9.3)	0.101 (22.1)	0.179 (39.4)	0.264 (57.9)	0.025 (5.4)
2-ethylthiazolidine	0.071 (20.4)	0.116 (33.7)	0.169 (48.9)	0.253 (73.2)	0.065 (18.8)
2-propylthiazolidine	0.088 (31.5)	0.121 (43.6)	0.164 (59.1)	0.243 (87.5)	0.076 (27.2)
2-butylthiazolidine	0.094 (40.3)	0.127 (54.3)	0.167 (71.1)	0.267 (99.7)	0.087 (37.2)

[a] Values are means of triplicates. [b] Molar recovery % = mol of corresponding thiazolidine formed/mol of aldehyde in reaction mixture.

Table 3. Effect of Various Buffer Salts on Thiazolidine Formation[a]

	thiazolidine concn (mM) with buffer of		
	acetate (pH 4.7)	citrate (pH 4.7)	carbonate (pH 10.2)
thiazolidine	0.014 (2.2)	0.009 (2.2)	0.330 (49.5)
2-methylthiazolidine	0.048 (10.4)	0.047 (10.4)	0.263 (57.7)
2-ethylthiazolidine	0.108 (39.3)	0.116 (33.6)	0.243 (73.2)
2-propylthiazolidine	0.195 (69.9)	0.212 (76.3)	0.243 (87.6)
2-butylthiazolidine	0.245 (100)	0.267 (100)	0.258 (100)

[a] Values are means of triplicates. [b] Molar recovery (%) = mol of corresponding thiazolidine formed/mol of aldehyde in reaction mixture.

(1996) was observed in the formation of thiazoline from thiazolidine. The buffer salt may serve as a hydride acceptor.

Effect of Phosphate Concentration on Thiazolidine Formation. Formation of thiazolidines was affected dramatically by the concentration of phosphate buffer as shown in Table 2. A limited amount of unsubstituted thiazolidine was detected in the model system (pH 7.2) without phosphate. Concentrations of individual alkylthiazolidines increased with increasing chain length of the alkyl group. The molar recoveries of the five thiazolidines formed from the corresponding aldehyde and cysteamine were found to be quite low. They were 1.3, 5.5, 18.8, 27.2, and 37.3% for unsubstituted thiazolidine, 2-methylthiazolidine, 2-ethylthiazolidine, 2-propylthiazolidine, and 2-butylthiazolidine, respectively. The reactivity of the aldehydes increased with increasing alkyl chain length as shown in Figure 1. Quantitative data obtained in this experiment revealed that phosphate was an effective buffer system for the formation of an thiazolidine. Addition of phosphate buffer (0.2 M) resulted in 30.9-, 10.6, 3.9-, 3.2-, and 3.1-fold increases for unsubstituted thiazolidine, 2-methylthiazolidine, 2-ethylthiazolidine, 2-propylthiazolidine, and 2-butylthiazolidine, respectively (Figure 1D) as compared with that in aqueous system at pH 7.2 (Figure 1E). The molar recovery for all five thiazolidines increased with increasing phosphate concentration from 0.025 to 0.2 M as shown in Table 2. This observation correlates well with the finding of Yasuhara and Shibamoto (1995). They reported that aliphatic aldehydes larger than C3 (C3−C5) possess higher activity toward cysteamine than those of formaldehyde and acetaldehyde in the preparation of thiazolidine from various aldehyde and cysteamine.

Effect of Various Buffer Systems on Thiazolidine Formation. Significant buffer effect on the formation of thiazolidines in model systems was observed. Carbonate exhibited a tendency similar to that of phosphate, which is shown in Table 3 and Figure 1C. The molar recoveries of the thiazolidines were 49.5, 57.8, 70.4, 87.6, and 110.7% for unsubstituted thiazolidine, 2-methylthiazolidine, 2-ethylthiazolidine, 2-propylthiazolidine, and 2-butylthiazolidine, respectively. However, less significant buffer effects were found for those aldehydes with carbon number >2. Acetate and citrate buffers enhance thiazolidine formation only slightly as compared with carbonate and phosphate buffer salts (Figure 1A,B). The effectiveness increased with increasing chain length of the alkyl group on an aldehyde molecule. Acetate showed 1.56-, 1.92-, 1.66-, 2.60-, and 2.82-fold increases for unsubstituted thiazolidine, 2-methylthiazolidine, 2-ethylthiazolidine, 2-propylthiazolidine, and 2-butylthiazolidine, respectively. Citrate exhibited a similar tendency, as shown in Table 3. Generally, the order of the effectiveness is as follows: carbonate > phosphate > acetate > citrate. These data are in good agreement with those reported by Sauders and Jervis (1966) and Bell and Wetzel (1995). Since higher pH value (carbonate buffer, pH 10.3) may promote a significant amount of the unexpected thiazoline formation, phosphate was chosen for further experiment.

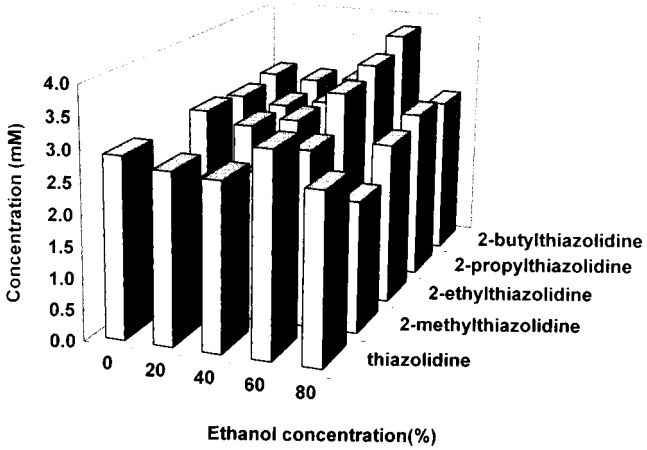

Figure 3. Effect of ethanol on the formation of thiazolidines in aldehydes/cysteamine model system.

Effect of Protic Solvents on Thiazolidine Formation. An interesting combined solvent and buffer effect on thiazolidine formation was observed. As shown in Figure 3, a 1.13-fold increase of the unsubstituted thiazolidine and 1.30-, 1.19-, 1.27-, and 1.31-fold increases of 2-methylthiazolidine, 2-ethylthiazolidine, 2-propylthiazolidine, and 2-butylthiazolidine, respectively, were observed when 60% ethanol in phosphate buffer was utilized as the reaction medium. More ethanol did not enhance thiazolidine formation. Similar combined solvent and buffer effects on the formation of 2-methylthiazolidine, 2-ethylthiazolidine, 2-propylthiazolidine, and 2-butylthiazolidine were observed as well, as shown in Figure 3. These findings are in good agreement with that reported by Rosen et al. (1985) and Huang et al. (1996). Schiff base formation was significantly promoted by replacing part of the aqueous medium by

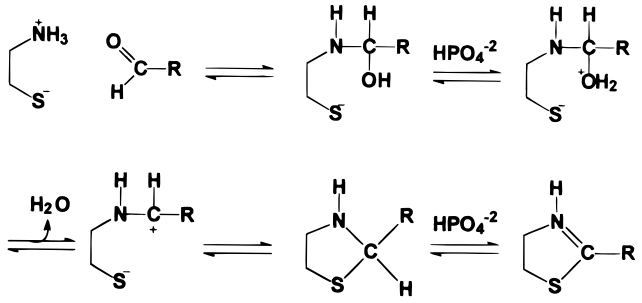

Figure 4. Proposed formation mechanism for thiazolidines and thiazoline from aldehydes and cysteamine.

methanol. Protic solvent is believed to absorb the water molecule released during Schiff base formation.

Proposed Mechanism for Thiazolidine Formation. The proposed mechanism for the formation of thiazolidines is shown in Figure 4. At pH 7.2, cysteamine exists predominantly in the zwitterionic form. The amino nitrogen ($pK_a = 10.7$) on cysteamine can attack the carbonyl group on an aldehyde. For the aldehydes with carbon number >2, the nucleophilic amino group attacks the positively induced carbonyl carbon to form a hemiketal. Protonation of the hydroxyl group leads to the formation of a good leaving group, H_2O, and gives a secondary carbocation. Another nucleophilic attack of the thiolate on the carbocationic carbon leads to the formation of a substituted thiazolidine. It is well documented that the secondary carbocation is more susceptible to nucleophilic attack. Since the primary carbocation is particularly unstable, its existence as an intermediate in chemical reactions has never been demonstrated. For formaldehyde, the formation of a primary carbocation was expected. An anionic phosphate ion tends to stabilize the primary carbocation and leads to the completion of unsubstituted thiazolidine formation.

ABBREVIATIONS USED

bp, boiling point; i.d., internal diameter; GC/MS, gas chromatography/mass spectrometry; CI, chemical ionization; EI, electric ionization.

LITERATURE CITED

Bell, L. N.; Wetzel, C. R. Aspartame degradation in solution as impacted by buffer type and concentration. *J. Agric. Food Chem.* **1995**, *43*, 2608−2612.

Caporaso, F.; Sink, J. D. Lipid-soluble carbonyl compounds of ovine adipose tissue. *J. Food Sci.* **1978**, *43*, 1379−1381.

Frankel, E. Secondary products of lipid oxidation. *Chem. Phys. Lipids* **1987**, *44*, 73−85.

Gray, J. I. Measurement of lipid oxidation: a review. *J. Am. Oil Chem. Soc.* **1978**, *55*, 539−546.

Hayashi, T.; Shibamoto, T. Analysis of methyl glyoxal in foods and beverages. *J. Agric. Food Chem.* **1985**, *33*, 1090−1093.

Hayashi, T.; Reece, C. A.; Shibamoto, T. Gas chromatographic determination of formaldehyde in coffee via thiazolidine derivative. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 101−105.

Huang, T. C. Combined effects of buffer and solvent on tetramethylpyrazine formation in a 3-hydroxy-2-butanone/ammonium hydrixide system. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1013−1015 .

Huang, T. C.; Fu, H. Y.; Ho, C. T. Mechanistic studies of tetramethylpyrazine formation under weak acidic conditions and high hydrostatic pressure. *J. Agric. Food Chem.* **1996**, *44*, 240−246.

Huyghues-Despointes, A.; Yaylayan, V. A. Retrol-aldol and redox reactions of Amadori compounds: mechanistic studies with variously labeled D-[^{13}C]glucose. *J. Agric. Food Chem.* **1996**, *44*, 672−681.

Miyake, T.; Shibamoto, T. Quantitative analysis of acetaldehyde in foods and beverages. *J. Agric. Food Chem.* **1993**, *41*, 1968−1970.

Miyashita, K.; Kanda, K.; Takagi, T. A simple and quick determination of aldehydes in autoxidized vegetable and fish oils. *J. Assoc. Off. Anal. Chem.* **1991**, *68*, 748−751.

Reynolds, T. M. Chemistry of non-enzymic browning. VI. Effect of bisulphite, phosphate and malate on the reaction of glycine and glucose. *Aust. J. Chem.* **1959**, *12*, 265−274.

Rosen, L.; Woods, J. W.; Pigman, W. Reactions of carbohydrates with nitrogenous substances. VI. The amadori rearrangement in methanol. *J. Am. Chem. Soc.* **1958**, *80*, 4697−4702.

Saunders, J.; Jervis, F. The role of buffer salts in non-enzymic browning. *J. Sci. Food Agric.* **1966**, *17*, 245−249.

Sheldon, S. A.; Shibamoto, T. Isolation and identification of volatile chemicals formed in aqueous L-cysteine solution with a UV light. *Agric. Biol. Chem.* **1987**, *51*, 2473−2477.

Stanley, J. B.; Brown, D. F.; Senn, V. J.; Dollear, F. G. Mass spectral characterization of 2,4-dinitrophenylhydrazones of 24 saturated aldehydes and ketones found in foods. *J. Food Sci.* **1975**, *40*, 1134−1137.

Yasuhara, A.; Shibamoto, T. Determination of physical and spectral data on thiazolidines for trace aldehyde analysis. *Agric. Biol. Chem.* **1989**, *53*, 2273−2274.

Yasuhara, A.; Shibamoto, T. Determination of volatile aliphatic aldehydes in the headspace of heated food oil by determination with aminoethanethiol. *J. Chromatogr.* **1991**, *547*, 291−298.

Yasuhara, A.; Shibamoto, T. Quantitative analysis of volatile aldehyde formed from various kinds of fish flesh during heat treatment. *J. Agric. Food Chem.* **1995**, *43*, 94−97.

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