JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification and Quantitation of Key Aroma Compounds Formed in Maillard-type Reactions of Fructose with Cysteamine or Isothiaproline (1,3-Thiazolidine-2-carboxylic Acid)

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Fructose was reacted in the presence of either cysteamine (model A) or isothiaproline (model B) in aqueous buffer at 145 °C and pH 7.0. Application of an aroma extract dilution analysis on the bulk of the volatile compounds formed in model A revealed 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine (**19**), *N*-(2-mercaptoethyl)-1,3-thiazolidine (**16**), 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (**15**), and 2-acetyl-2-thiazoline (**11**) as the key aroma compounds among the 10 odorants detected. A similar set of aroma compounds was formed when isothiaproline was reacted (model B), but the flavor dilution factors were generally lower. Substitution of the buffer by silica gel/water (9 + 1 w/w) in both models and application of 150 °C for 10 min also gave the same key odorants from both thio compounds; however, under these conditions isothiaproline was the better precursor of, in particular, **19** and **11**. Quantitative measurements performed by means of stable isotope dilution assays revealed a significant effect of the pH on odorant formation. For example, in model A, formation of **19** as well as of **11** was suppressed at pH values <5.0. A clear maximum was, however, found for **19** at pH 7.0 (~1 mol % yield), whereas **11** increased with increasing pH from 7.0 to 9.0.

KEYWORDS: Fructose; cysteamine; 5-acetyl-3,4-dihydro-*2H*-1,4-thiazine; *N*-(2-mercaptoethyl)-1,3-thiazoline; 2-acetyl-2-thiazoline; stable isotope dilution assay

INTRODUCTION

The amino acid cysteine is well accepted in the literature as one of the most relevant precursors of very potent sulfur compounds formed during food processing, such as 2-mercaptomethylfuran (furfurylthiol), 2-acetyl-2-thiazoline, 2-mercaptopentan-3-one, or 2-methyl-3-furanthiol, when reacted with reducing sugards (Maillard reaction). Numerous volatiles have to date been identified in model experiments on carbohydrate/cysteine mixtures (*1*), and, recently, their aroma contribution has also been clarified (*2*, *3*).

The degradation of cysteine into flavor volatiles is assumed to start by a reaction with α-dicarbonyls, which are formed during carbohydrate degradation (*4*). Depending on the reaction parameters, the degradation may lead to quite different, very reactive intermediates, such as H_2S, cysteamine, or mercaptoethanal (**Figure 1**). The reaction of H_2S with either carbohydrates or intermediates formed therefrom has quite often been studied (*5*). However, although cysteamine has been used as a source to generate aroma-active thiazolidines for use in chocolate, smoky, and meat-like aromas (*6*, *7*), only a few research groups have reported on volatiles generated from a direct reaction of cysteamine with a carbohydrate.

The first study aimed at identifying volatiles formed in the reaction of cysteamine with glucose, acetaldehyde, or glyoxal succeeded in the identification of 24 compounds, most of them thiazolidines and other heterocycles (*8*). Additionally, some

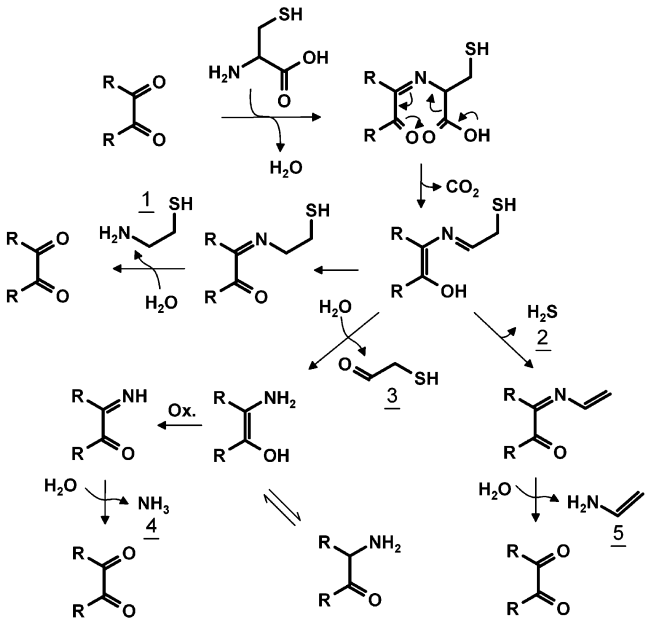

Figure 1. Different degradation pathways of cysteine as catalyzed by α-dicarbonyl compounds yielding either cysteamine (**1**), H_2S (**2**), mercaptoacetaldehyde (**3**), ammonia (**4**), or ethene amine (**5**).

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10.1021/jf0203186 CCC: $22.00
Published on Web 08/17/2002

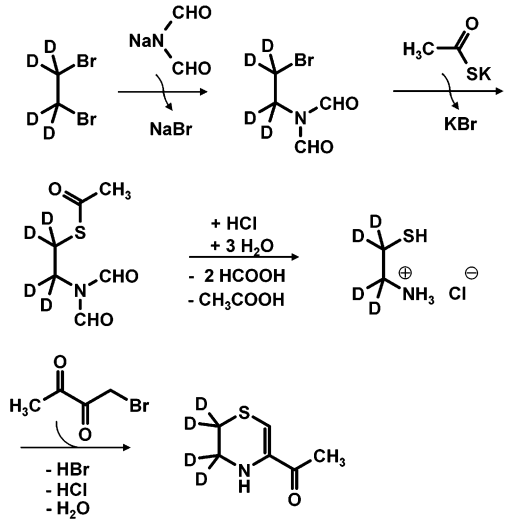

Figure 2. Synthetic route used in the preparation of deuterium-labeled 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine.

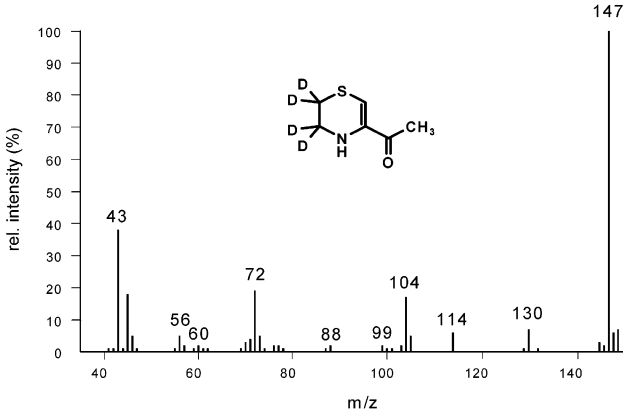

Figure 3. Mass spectrum (MS/EI) of the deuterium-labeled 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine.

polyhydroxy-thiazolidines were identified in the nonvolatile fraction of a thermally treated glucose/cysteamine model mixture (*9*). From the reaction of cysteamine with 2,3-butanedione (*10*−*12*), 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine has been reported as the main volatile component.

In a Maillard-type reaction, cysteamine might undergo further reactions with different oxo compounds to form 1,3-thiazolidines. Because it is known that glyoxylic acid reacts with cysteamine to form thiazolidine-2-carboxylic acid (*13*), a proline analogue (isothiaproline), this amino acid may also be formed in Maillard-type reactions involving cysteine. However, no data are available at present as to which compounds are formed from isothiaproline when reacted with carbohydrates.

The aim of the following study was, therefore, (i) to characterize the key odorants formed in mixtures of either cysteamine or isothiaproline with fructose by applying the aroma extract dilution analysis (AEDA), a tool to select odor-active compounds from the odorless volatiles (*14*), and (ii) to study the influence of the reaction parameters on the yields of some key aroma compounds by quantitative experiments.

MATERIALS AND METHODS

Chemicals. Cysteamine hydrochloride (2-aminoethane thiol) and glyoxylic acid (50% solution in water) were from Fluka (Neu-Ulm, Germany). D-Fructose was from Merck (Darmstadt, Germany). [1,1,2,2-$^2H_4$]Dibromoethane was from IC-Chemikalien (Ismaning, Germany). The respective reference compounds of the odorants identified were purchased from the sources following: compound **1** (Fluka, Neu-Ulm, Germany), compounds **4**, **11**, **15**, **17**, and **18** (Aldrich, Steinheim, Germany), and compound **5** (Merck, Darmstadt, Germany).

The following compounds were synthesized following the directions given in the literature cited: *N*-(2-mercaptoethyl)-1,3-thiazolidine (*15*), 5-acetyl- and 5-propionyl-3,4-dihydro-2*H*-1,4-thiazine (*10*), 2-propionyl-2-thiazoline (*16*), and *N*-(2-mercaptoethyl)-pyrrol (*17*).

Syntheses. *Thiazolidine-2-carboxylic Acid.* To a stirred solution of glyoxylic acid (50% solution in water, 0.05 mol) in ethanol (10 mL) was added 2-aminoethane thiol hydrochloride (5.68 g, 0.05 mol) dissolved in ethanol/pyridine (28 mL, 5 + 2 v/v). After stirring for 2 h, the crystals of the target compound formed were isolated by filtration and were air-dried. The material was recrystallized from 70% aqueous 2-propanol and dried in vacuo, yielding white prisms (5.3 g, 80% yield). The melting point was in agreement with the data reported in ref *13*.

[1,1,2,2-$^2H_4$]Aminoethane Thiol ([$^2H_4$]Cysteamine). The synthetic route used in the preparation of the target compound is shown in **Figure 2**. In the first step, [1,1,2,2-$^2H_4$]dibromoethane (25 g, 130 mmol), tributylhexadecylphosphonium bromide (6.6 g, 13 mmol), and sodium diformylamide (13 g, 135 mmol) were suspended in dry toluene (60 mL) and refluxed for 24 h at 60 °C. After filtration through a Büchner funnel and flushing of the residue with diethyl ether (total volume = 100 mL), the organic solution was concentrated to ~20 mL under vacuum (40 °C, 80 mbar). The target compound, *N*-(2-bromo-[1,1,2,2-$^2H_4$]ethyl)diformylamide, formed was purified by flash chromatography (column size = 40 × 2.5 cm) on silica 60 (50 g) in *n*-pentane. After elution with *n*-pentane (400 mL), the target compound was eluted with diethyl ether (300 mL), yield = 46%.

In the next step, a solution of the *N*-(2-bromo-[1,1,2,2-$^2H_4$]ethyl)-diformylamide (10.7 g, 58 mmol) and potassium thio acetate (12.5 g, 110 mmol) in methanol (100 mL) was refluxed for 3 h at 65 °C with stirring. The thio ester formed was directly hydrolyzed by adding concentrated hydrochloric acid (50 mL) and by further heating at 80 °C for 24 h under a gentle stream of nitrogen. The solvent mixture was evaporated at 80 °C under nitrogen and the residue extracted four times with a mixture of methanol/diethyl ether (50 mL; 1:1 by volume). After evaporation of the diethyl ether, the solution containing the [1,1,2,2-$^2H_4$]aminoethane thiol was made up to 100 mL with methanol using a volumetric flask.

1-Bromobutane-2,3-dione. To a refluxed solution of butane-2,3-dione (25 g, 0.29 mmol) in dichloromethane (50 mL) was very carefully added dropwise a solution of bromine (46 g, 0.29 mmol) in dichloromethane while reflux was maintained. The hydrobromic acid formed was removed by repeated extraction with an aqueous saturated NaCl solution (total volume = 300 mL). After evaporation of the dichloromethane, the residue was distilled under vacuum. The yellow-orange target compound was obtained in a yield of 48%: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 2.45 (s, 3H), δ 4.31 (s, 2H); ^{13}C NMR ($CDCl_3$, 360 MHz) δ 27.5 ($-CH_3$), 32.0 (Br−**C**H_2−CO), 192.5 (CH_3−**CO**), 199.0 (Br−CH_2−**CO**).

5-Acetyl-[2,2,3,3-$^2H_4$]-2,3-dihydro-2H-1,4-thiazine. To a stirred solution of [1,1,2,2-$^2H_4$]aminoethane thiol (14.5 mmol) in methanol (25 mL) was added dropwise a solution of bromodiacetyl (2.3 g, 14 mmol) in methanol (25 mL). After 1 h, solid, finely ground $NaHCO_3$ (28 mmol) was added to the mixture. After the carbon dioxide had evolved from the mixture upon magnetic stirring (5 min), water (150 mL) and an aqueous Na_2CO_3 solution (0.5 mol/L; 50 mL) were added. The target compound was isolated from the reaction mixture by extraction with diethyl ether (5 × 50 mL). The yield was determined gas chromatographically with flame ionization detection (FID) using acetylpyrazine as the internal standard. The calculated yield was ~50% of the theoretical value, and the purity checked by HRGC was 90%. The mass spectrum is shown in **Figure 3**.

N-(2-Mercaptoethyl)-[4,4,5,5-$^2H_4$]-1,3-thiazolidine. The synthesis was performed as described for the unlabeled compound (*15*), but using the synthesized [1,1,2,2-$^2H_4$]aminoethane thiol instead of the unlabeled cysteamine. The mass spectrum of the labeled intermediate [4,4′,5,5′-$^2H_4$]-1,3-thiazolidine is shown in **Figure 4**. The concentration was determined gas chromatographically with FID using *N*-(2-hydroxyethyl)morpholine as the internal standard.

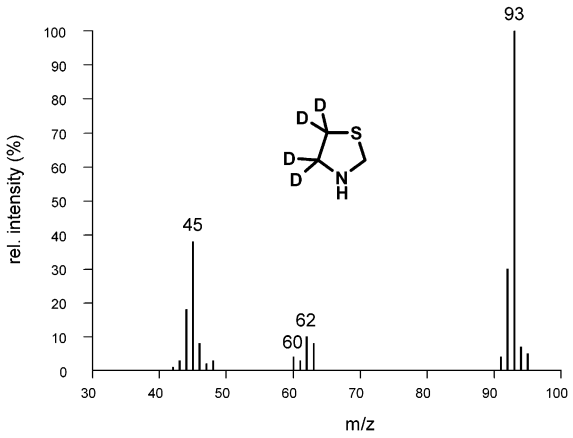

Figure 4. Mass spectrum (MS/EI) of the deuterium-labeled 1,3-thiazolidine.

[$^2H_4$]-2-Acetyl-2-thiazolidine was synthesized as previously reported (*18*).

Model Reaction. To simulate cooking conditions, either cysteamine (3.3 mmol; model A) or 1,3-thiazolidine-2-carboxylic acid (3.3 mmol; model B) was dissolved in phosphate buffer (100 mL, 0.1 mol/L, pH 7.0) and reacted with fructose (10 mmol) in a laboratory autoclave (type II, 200 mL total volume; Roth, Karlsruhe, Germany) by raising the temperature within 20 min from 20 to 145 °C.

To mimic dry-heating conditions, either cysteamine (3.3 mmol; model C) or 1,3-thiazolidine-2-carboxylic acid (3.3 mmol; model D) and fructose (10 mmol) were dissolved in a low volume of water (1.2 mL, pH 7.0) and mixed with silica gel (19.1 g). The mixture was transferred into eight glass vials (1 cm i.d., total volume = 10 mL), and the material was heated for 10 min at 150 °C in a metal block. In parallel, the reaction was also performed using wheat starch as the matrix.

Isolation of the Volatiles and Aroma Extract Dilution Analysis (AEDA). The volatile reaction products formed were isolated by extraction with diethyl ether and distillation under high vacuum as recently described (*19*). The distillates were concentrated at 40 °C by means of a Vigreux column (60 × 1 cm i.d.) and a microdistillation apparatus to exactly 1 mL. The odor-active compounds were evaluated by AEDA as previously described (*2*, *3*).

High-Resolution Gas Chromatography (HRGC)—Mass Spectrometry (MS). HRGC was performed with a type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) using the following capillaries: (30 m × 0.32 mm fused silica capillary, free fatty acid phase, 0.25 μm; J&W Scientific, Fisons Instruments) = FFAP; and (30 m × 0.32 mm fused silica capillary DB-5, 0.25 μm; J&W Scientific, Fisons Instruments) = DB 5. The samples were applied by the cold on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 60 °C, held for 1 min isothermally, then raised at 6 °C/min to 230 °C, and held for 15 min. The flow of the carrier gas helium was 1.5 mL/min. The FID was held at 220 °C. Linear retention indices (LRI) of the compounds were calculated from the retention times of *n*-alkanes. MS analysis was performed by means of an MAT 95S (Finnigan, Bremen, Germany) in tandem with the capillaries described above. Mass spectra in the electron impact mode (MS/EI) were generated at 70 eV and in the chemical ionization mode (MS/CI) at 115 eV with isobutane as reactant gas. For high-resolution MS (HRMS) the masses measured were corrected using perfluorokerosene as the reference.

Stable Isotope Dilution Assays/Quantification. After cooling, aliquots of the respective reaction mixture were spiked with known amounts of the three labeled internal standards [$^2H_4$]-5-acetyl-3,4-dihydro-2*H*-1,4-thiazine, [$^2H_4$]-*N*-(2-mercaptoethyl)-1,4-thiazolidine, and [$^2H_4$]-2-acetyl-2-thiazoline (5−10 μg, dissolved in ethyl ether depending on the amounts of the analytes present). The samples were extracted with diethyl ether (five times; total volume = 120 mL), the volatiles were isolated by a SAFE distillation (*19*), and the resulting distillate was separated by HRGC. The ion intensities of the respective molecular ions obtained by MS/CI were monitored as previously described for 2-acetyl-2-thiazoline (*18*). The calibration factors (CF) were determined in mixtures of equal amounts of unlabeled odorants and the corresponding labeled standards in ratios of 3:1 to 1:3 (by weight) by means of mass chromatography and were calculated using the equation $CF = (C_u I_L / C_L I_u)$, with C_u = concentration of the unlabeled compound, C_L = concentration of the labeled compound, I_L = intensity of the ion of the labeled compound, and I_u = intensity of the ion of the unlabeled compound. For mass chromatography, the capillary column was coupled to an ITD-800 ion trap detector (Finnigan, Bremen, Germany), running in the chemical ionization mode with methanol as reactant gas. Mass spectra were generated at 70 eV.

RESULTS AND DISCUSSION

Odorants Formed under Aqueous Reaction Conditions. In a first experiment, fructose was reacted with cysteamine under aqueous conditions (model A). The flavor extract prepared by solvent extraction followed by a SAFE distillation elicited a very intense roasty, popcorn-like aroma. By application of the ADA on a 100:1 concentration (100 mL of reaction mixture → 1 mL of extract), 10 odor-active areas were detected, among which compound **19** (**Table 1**) with an intense popcorn-like aroma exhibited by far the highest flavor dilution (FD) factor. On the basis of the data of the reference compound, **19** was identified as 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine, previously identified by us for the first time in a processed glucose/cysteine solution (*3*). Two additional odorants with high odor intensities were identified as *N*-(2-mercapotethyl)-1,3-thiazolidine (**16**) and the well-known caramel-like carbohydrate degradation product 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**15**). Evidence for the thiazolidine, which has not been reported prior to this work, is given separately (*15*). Somewhat lower FD factors were shown by 2-acetyl-2-thiazoline (**11**), 2-furfurylthiol (**4**), and ethane-1,2-dithiol (**3**). Compared to the previously studied glucose/cysteine mixture (*3*), in particular, ethanedithiol (**3**), *N*-mercaptoethylpyrrole (**10**), and *N*-(2-mercaptoethyl)-1,3-thiazolidine (**16**) were characterized as additional aroma contributors to the fructose/cysteamine model studied here.

Isothiaproline can be regarded as a condensation product of cysteamine and glyoxylic acid and is known to be formed from both intermediates in synthetic experiments. Reacting this "synthetic" amino acid with fructose (model B) led to an extract with a roasty, pretzel-like aroma. By applying AEDA, 12 odor-active areas were detected (**Table 1**; model B). The results of the identification experiments in combination with the FD factors revealed 2-acetyl-2-thiazoline (**11**) and 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine (**19**) as the most important aroma compounds in this mixture. Lower FD factors were found for 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**15**), 2-propionyl-2-thiazoline (**12**), and *N*-(2-mercaptoethyl)-1,3-thiazolidine (**16**). A comparison of these results to those obtained for cysteamine (model A) clearly showed a reduction in **19**, whereas **11** was enhanced.

Assuming that isothiaproline is cleaved during the thermal reaction as postulated in **Figure 5** and that the reaction pathways generating the same odorants in both systems are identical, the slow release of cysteamine from isothiaproline, which in turn might react with 2-oxopropanal to yield **11**, should favor the formation of this thiazoline, which is known to be unstable at higher temperatures (*18*).

Odorants Formed under Roasting Conditions. To simulate roasting conditions, both reactions were repeated, but water was replaced by silica gel/water (9 + 1 w/w) as the matrix. Furthermore, a higher temperature was immediately applied. Although silica is known to catalyze certain reactions, preliminary experiments revealed the same odorants when the reaction

Table 1. Most Odor-Active Compounds (FD ≥ 1) Formed in a Mixture of Cysteamine and Fructose (Model A) or Isothiaproline and Fructose (Model B) Reacted under Aqueous Conditions[a]

			retention index (RI) on		FD factor[d] in model	
no.	odorant[b]	odor quality[c]	DB-5	FFAP	A	B
1	butane-2,3-dione	buttery	<800	980	<1	4
2	pentane-2,3-dione	buttery	<800	1060	<1	4
3	ethane-1,2-dithiol	cabbage-like	815	1330	256	<1
4	2-mercaptomethylfuran	roasty, sulfury	908	1425	256	4
5	acetic acid	sour	<800	1430	<1	4
7	unknown	roasty, sulfury	1087	1620	16	<1
10	*N*-(2-mercaptoethyl)pyrrole	roasty, burnt	1110	1755	<1	1
11	2-acetyl-2-thiazoline	popcorn-like	1098	1760	256	4096
12	2-propionyl-2-thiazoline	popcorn-like	1205	1850	64	64
14	unknown	roasty, sulfury	1261	1980	64	<1
15	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	1080	2030	1024	256
16	*N*-(2-mercaptoethyl)-1,3-thiazolidine	roasty, popcorn-like	1325	2035	1024	64
17	4-hydroxy-5-methyl-3(2*H*)-furanone	caramel-like	1042	2120	64	16
19	5-acetyl-3,4-dihydro-2*H*-1,4-thiazine	popcorn-like	1372	2255	65536	4096
20	5-propionyl-3,4-dihydro-2*H*-1,4-thiazine	popcorn-like	1450	2315	<1	64

[a] Fructose (10 mmol) and the respective sulfur compound (3.3 mmol) were dissolved in phophate buffer (100 mL; pH 7.0; 0.1 mol/L) and reacted for 20 min at 145 °C in an autoclave. [b] The compound was identified by comparing it with the reference compound on the basis of the following criteria: retention index (RI) on the two stationary GC phases given in the table, mass spectra (MS/EI and MS/CI), as well as odor quality and odor threshold determined in the sniffing port. [c] Odor quality perceived in the sniffing port. [d] Flavor dilution (FD) factor.

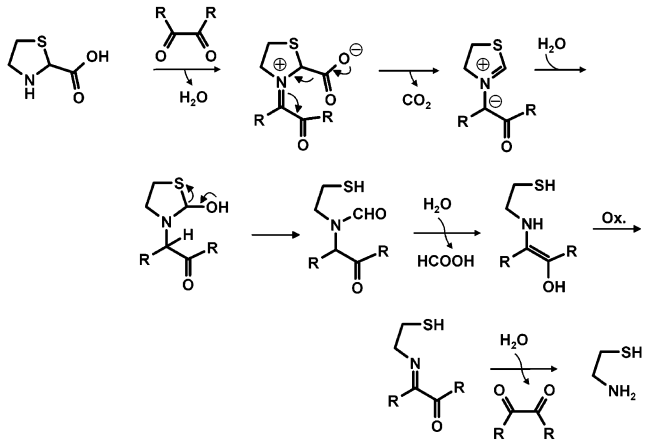

Figure 5. Hypothetical cleavage of isothiaproline into cysteamine as induced by α-dicarbonyls under roasting conditions.

was performed on, for example, wheat starch. However, because the degradation of starch gave additional odorants and, furthermore, the material formed could not be properly extracted, silica was used in the further studies.

In the flavor extract prepared from the thermally treated fructose/cysteamine mixture, 13 odorants were detected by GC−olfactometry (**Table 2**; model C). The results of the identification experiments in combination with the FD factors suggested 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine followed by 2-acetyl-2-thiazoline as the most odor-active compounds. Somewhat lower FD factors were determined for 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone and acetic acid. The same odorants also contributed most to the overall aroma of the fructose/isothiaproline mixture (**Table 2**; model D). However, in particular, the intensities (FD factors) of 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and 2-acetyl-2-thiazoline were higher compared to those in the fructose/cysteamine reaction (model C).

A comparison of the data with those found in the aqueous systems (cf. **Tables 1** and **2**) clearly showed that application of the "roasting" conditions favored, in particular, the generation of 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine from isothiaproline. On the other hand, the formation of *N*-(2-mercaptoethyl)-1,3-thiazolidine was inhibited, especially in the fructose/cysteamine system.

Quantitative Experiments. To get a clearer picture of the factors governing the formation of **11**, **16**, and **19**, labeled isotopomers of the two first mentioned odorants were synthesized and used in stable isotope dilution assays. For the quantitation of **11**, the method previously reported was used (*18*). Quantitation of **16** and **19** was developed in the present study.

In a first series of experiments, **16** was quantified in model A (aqueous fructose/cysteamine) with varying pH. The results showed (**Figure 6**) that a maximum of 55 μg of this very potent odorant was formed when the reaction was performed at pH 8.0. Below pH 5, however, the formation of the flavor compound was completely inhibited. According to the literature, the isoelectric point of cysteamine lies between 8.2 and 8.6 and drops with increasing temperature. so, obviously, the formation of **16** is favored at the isoelectric point of cysteamine.

The formation of **16** can be explained by a reaction of cysteamine with formaldehyde to form 1,3-thiazolidine, which in turn reacts with thiirane (**Figure 7**). Because the formation of thiirane from cysteamine by elimination of ammonia is most probable at the isoelectric point of the amine, this might explain the optimal formation of the odorant at pH 8.0.

Formation of **19** showed a similar behavior (**Figure 8A**). At pH 7.0, a maximum with a very high concentration was observed (2700 μg), whereas at pH values of 6 or 8, respectively, only 760 or 580 μg was generated. Below pH 5.0, **19** was not formed. Contrarily, **11** was preferably generated at pH values >7.0, whereas its formation was inhibited at pH <6.0 (**Figure 8A**).

Substitution of cysteamine by isothiaproline (model B) also showed a maximum for **19** (**Figure 8B**), but this maximum was shifted downward in pH compared to model A. Furthermore, the maximum concentration was by a factor of >15 lower than that from cysteamine. In contrast, the formation of **11** was not as much affected because the amount formed from isothiaproline at pH 8.0 (490 μg) was only lower by a factor of 2.8 than that from cysteamine (1400 μg) (cf. parts A and B of **Figure 8**).

In a further experiment the amounts of **11** and **19** formed in models C and D (roasting conditions) were determined. The

Table 2. Most Odor-Active Compounds (FD ≥ 1) Formed from a Mixture of Fructose and Cysteamine (Model C) or Fructose and Isothiaproline (Model D) Reacted under Roasting (Low Water) Conditions[a]

			retention index (RI) on		FD factor[d] in model	
no.	odorant[b]	odor quality[c]	DB-5	FFAP	C	D
1	butane-2,3-dione	buttery	<800	980	4	16
2	pentane-2,3-dione	buttery	<800	1060	<1	16
4	2-mercaptomethylfuran	roasty	980	1425	64	64
5	acetic acid	sour	<800	1430	256	64
6	3-dihydroxy-3-hexene-2,5-dione	caramel-like	975	1530	16	<1
8	butanoic acid	sweaty	820	1630	16	<1
9	2- and 3-methylbutanoic acid	sweaty	875	1665	16	<1
10	*N*-(2-mercaptoethyl)pyrrole	roasty, burnt	1110	1755	<1	4
11	2-acetyl-2-thiazoline	popcorn-like	1098	1760	1024	4096
12	2-propionyl-2-thiazoline	popcorn-like	1205	1850	64	64
13	2,3-dihydro-5-hydroxy-6-methyl-4*H*-pyran-4-one	caramel-like	1079	1860	16	256
15	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	caramel-like	1080	2030	256	4096
16	*N*-(2-mercaptoethyl)-1,3-thiazolidine	roasty, popcorn-like	1325	2035	4	16
18	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	seasoning-like	1115	2190	16	16
19	5-acetyl-3,4-dihydro-2*H*-1,4-thiazine	popcorn-like	1372	2225	16384	65536
20	5-propionyl-3,4-dihydro-2*H*-1,4-thiazine	popcorn-like	1450	2315	<1	256

[a] Fructose (10 mmol) and the respective sulfur compound (3.3 mmol) was dissolved in tap water (2.1 g), mixed with silica gel (19.1 g) and heated for 10 min at 150 °C. [b] The compound was identified by comparing it with the reference compound on the basis of the following criteria: retention index (RI) on the two stationary GC phases given in the table, mass spectra (MS/EI and MS/CI), as well as odor quality and odor threshold determined in the sniffing port. [c] Odor quality perceived in the sniffing port. [d] Flavor dilution (FD) factor.

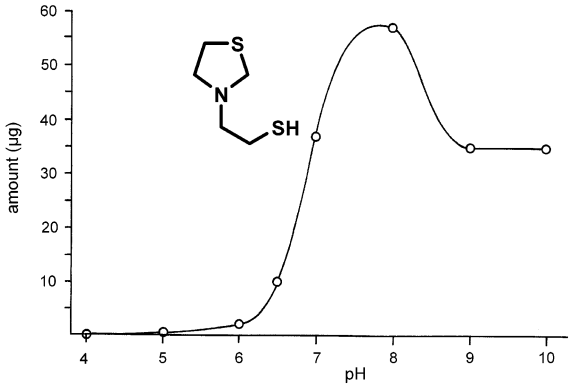

Figure 6. Influence of pH on the formation of *N*-(2-mercaptoethyl)-1,3-thiazolidine in model A (aqueous mixture of fructose/cysteamine).

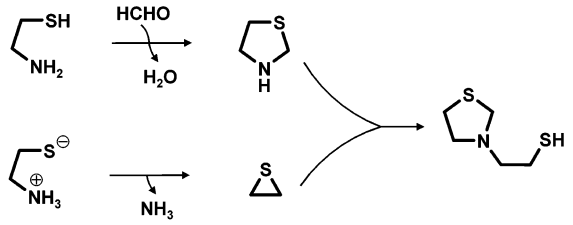

Figure 7. Proposed formation pathway leading to *N*-(2-mercaptoethyl)-1,3-thiazolidine.

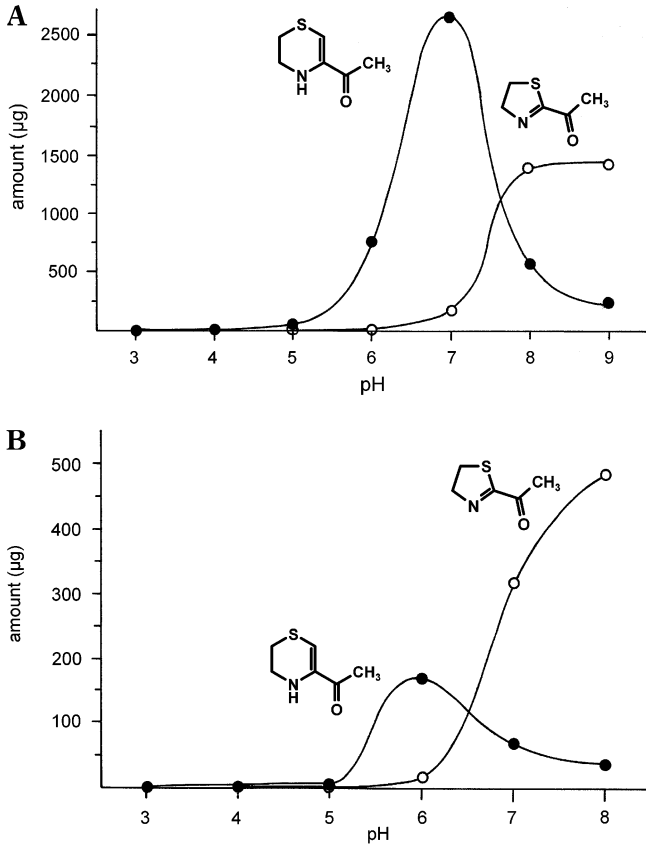

Figure 8. (A) Influence of pH on the formation of 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine (solid circles) and 2-acetyl-2-thiazoline (open circles) in model A (aqueous mixture of fructose/cysteamine). (B) Influence of pH on the formation of 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine (solid circles) and 2-acetyl-2-thiazoline (open circles) in model B (aqueous mixture of fructose/isothiaproline).

results showed that from both thio compounds very high amounts of, in particular, the thiazine (**19**) were formed either from cysteamine (3500 μg) or from isothiaproline (5400 μg), when reacted with fructose. Contrarily, the amounts of the thiazolidine (**11**) were low from cysteamine (240 μg) as well as from isothiaproline (250 μg). Compared to the highest concentrations determined in the respective aqueous models (cf. parts A and B of **Figure 8**), in particular, formation of **19** was favored under roasting conditions with isothiaproline being the better precursor. On the contrary, formation of **11** was favored under aqueous conditions with cysteamine as the better precursor.

2-Acetyl-2-thiazoline may be formed from fructose and cysteamine via the 4-deoxyosone (**Figure 9**). This pathway involves two retro-Aldol reactions eliminating formaldehyde and hydroxyacetaldehyde. Furthermore, an oxidation step must be assumed to enable the formation of **11**. Such oxidation steps are, however, likely to occur in Maillard-type reactions and have

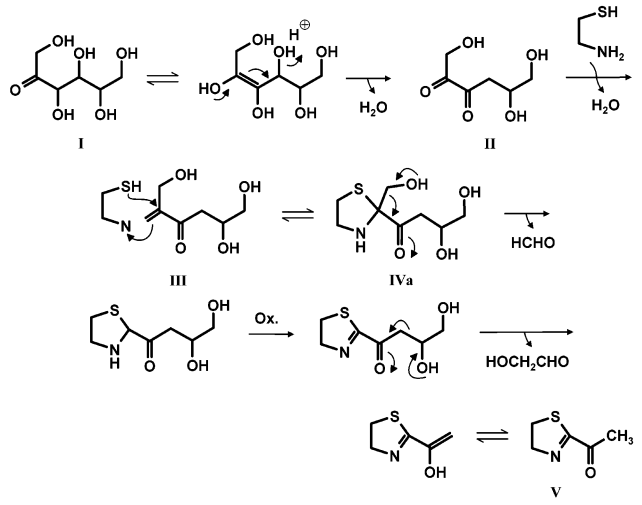

Figure 9. Hypothetical reaction pathway leading from the 4-desoxyosone of fructose (**II**) and cysteamine to 2-acetyl-2-thiazoline.

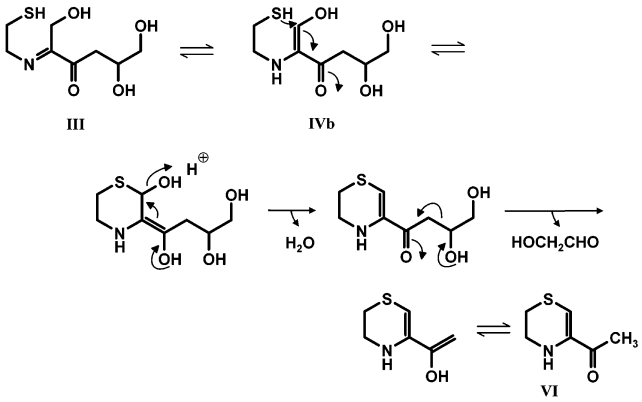

Figure 10. Hypothetical reaction pathway leading from the Schiff base of cysteamine and the 4-deoxyosone to 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine.

recently been proven in the generation of **11** from 2-oxopropanal and cysteamine (*18*).

It has been suggested that 5-acetyl-3,4-dihydro-2*H*-1,4-thiazine (**19**) is formed in Maillard-type reactions by a condensation of cysteine with butanedione (*2*), and the preparation of **19** has also been performed using cysteamine and 2,3-butanedione (*10*). As shown in **Figure 10**, a Schiff base formation of cysteamine with the 4-desoxyosone may also yield **19** involving a retro-Aldol cleavage and an elimination of hydroxyacetaldehyde.

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Received for review March 13, 2002. Revised manuscript received June 11, 2002. Accepted June 12, 2002.

JF0203186