Approaches to Wine Aroma: Release of Aroma Compounds from Reactions between Cysteine and Carbonyl Compounds in Wine

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Under conditions close to those of wine, that is, low pH, aqueous medium, and low temperatures, this work examines the role of carbonyl (acetoin and acetol) and dicarbonyl (glyoxal, methylglyoxal, diacetyl, and pentane-2,3-dione) compounds associated with cysteine in the formation of odorous products. In particular, thiazole, 4-methylthiazole, 2-acetylthiazole, and trimethyloxazole and two sulfur and oxygenated heterocyclic compounds, 2-furanmethanethiol and thiophene-2-thiol, are examined. For thiophene-2-thiol, the reactional mechanism is proposed. Attempts were made to detect these compounds in wines from various origins. Certain molecules were identified for the first time in wine.
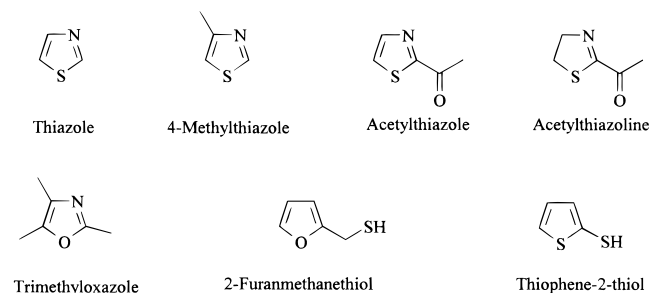
INTRODUCTION

Cysteine is one of the most remarkable amino acids from a chemical point of view. The thiol function carried by the side chain is suitable to form dimerous units for the formation of sulfur and disulfur bridges of the tertiary structure of proteins. In musts and wines, cysteine is present in variable amounts and is more or less metabolized by the various microorganisms. We were interested in the specific involvement of cysteine in the genesis of wine flavors. Recently, Tominaga et al. (1998) showed that cysteine could be involved in the varietal flavors of Sauvignon wine. Some flavor compounds were found in the Sauvignon must in the form of *S*-cysteine conjugate precursors. The work presented here deals with the involvement of this amino acid in the chemical reactions occurring in wine, more or less early in or during the aging process. These reactions may contribute to the typical flavor of certain wines.

According to Strecker, cysteine degradation leads to the formation of small very reactive molecules such as hydrogen sulfide, ammonia, or ethanal. These molecules of low molecular mass could be reagents in the aromatic heterocyclic compound formation studied in this work. In addition, the nitrogen-containing function of cysteine is an electrodonor, which is very reactive toward the electrophilic carbonyl functions. Many carbonyl and α-dicarbonyl compounds are present in wines; their origins have been studied in our laboratory and their contents specified (de Revel and Bertrand, 1994). The α-hydroxyketones and the α-dicarbonyls present characteristic and remarkable odors. Previous work in our laboratory showed that some carbonyl compounds placed in solution with an amino acid reacted, even if kept in a reducing medium and under soft conditions similar to those of in-bottle wine aging (low pH, low temperature, and aqueous medium). The reaction products presented odors close to those developed during Maillard

Chart 1. Heterocyclic Compounds Studied

Thiazole   4-Methylthiazole   Acetylthiazole   Acetylthiazoline

Trimethyloxazole   2-Furanmethanethiol   Thiophene-2-thiol

reactions (Pripis-Nicolau et al., 2000). The present study led us to identify new odorous compounds from model solutions with cysteine. All of them were known products of the Maillard reaction. This reaction occurs in the agrofood industry and leads to roasted food flavors, but in our mild conditions its mechanisms are poorly known. Among all of the compounds identified in the solutions (substituted alkyl- and acetylpyrazines, furanes, thiophenes, and thiazoles), five of the most abundant and most odorous were identified in wines and their contents specified: thiazole, 2-acetylthiazole, trimethyloxazole, 2-furanmethanethiol, and thiophene-2-thiol (Chart 1).

MATERIALS AND METHODS

Materials. Cysteine, carbonyl compounds (glyoxal, methylglyoxal, diacetyl, pentane-2,3-dione, acetoin, acetol, and ethanal), and compounds used for the identification of the final products alkylpyrazines, thiazoles, alkylthiazoles, 2-acetylthiazole, 2-acetyl-2-thiazoline, 2-furanmethanethiol, and aldehydes were purchased from Sigma Aldrich Chemical Co.; trimethyloxazole, thiophene-2-thiol, and 2,4-dimethylthiazole were purchased from Lancaster. Inorganic reagents and solvents were all commercial products of analytical grade. The mixture of a carbonyl compound and an amino acid in an aqueous ethanolic solution (12% volume), red wine or white wine, was prepared in stoichiometric conditions (20 mM) and adjusted to pH 3.5 with 1 N H_3PO_4 and 1 N NaOH (Pripis-

Table 1. Heterocyclic Compounds in Wines

		thiazole	4-methylthiazole	trimethyloxazole	2-acetylthiazole	thiophene-2-thiol
Alsace	no. of values	5	5	5	5	9
(white wines)	range (μg/L)	0−1	0−10	0−4	0.7−2	0−4
Burgundy	no. of values	8	8	8	8	11
(white wines)	range (μg/L)	0−19	0−1	0−2	0−7	0−1
Burgundy	no. of values	5	5	5	5	8
(red wines)	range (μg/L)	0−7	0−3	0−7	0−0.7	0−0.6
Champagne	no. of values	7	7	7	7	8
	range (μg/L)	0−23	0−0	0−5	0−3	0−4
Provence/Languedoc	no. of values	3	3	3	3	4
(white wines)	range (μg/L)	0−1	0−0	0−0	0−0.6	0−0
Provence/Languedoc	no. of values	3	3	3	3	4
(red wines)	range (μg/L)	0.4−3	0−0.6	0−0	0−5	0−1
Medoc/Graves	no. of values	6	6	6	6	7
(red wines)	range (μg/L)	0.6−2	0−6	0−7	0−7	0−0.3
Graves	no. of values	2	2	2	2	3
(white wines)	range (μg/L)	1.1−1.2	0−0	0−0	0−0	0−2
Pomerol/Saint Emilion	no. of values	7	7	7	7	9
(red wines)	range (μg/L)	0.9−14	0−0	0−1	0−14	0−5
botrytized wines	no. of values	4	4	4	4	7
	range (μg/L)	0−3	0−11	0−7	0−12	0−3
fortified wines	no. of values	4	4	4	4	6
	range (μg/L)	3−34	0−0	0−0	0−13	0−7

Nicolau et al., 2000). The solutions were stored at 25 °C in the dark and under nitrogen during a 4-week storage period. Flavor modifications were examined daily, and H_2S determination was performed. Heterocyclic compounds were determined after the storage period. To study the effect of temperature on reactions and flavor modifications, solutions containing cysteine and methylglyoxal were stored at 10, 20, and 40 °C.

Analytical Procedures. After the addition of the internal standard, each reaction mixture (50 mL) was extracted by various solvents and was analyzed by GC-FPD, GC-NPD, and GC-MS (Pripis-Nicolau et al., 2000).

GC-FPD Analysis. Two gas chromatographs (Hewlett-Packard) were coupled with a flame photometric detector (FPD). The least volatile sulfur compounds were determined according to the method of Pripis-Nicolau et al. (2000) by an initial gas chromatograph. H_2S was determined by a second gas chromatograph (Hewlett-Packard) also according to the method of Pripis-Nicolau et al. (2000).

GC-NPD Analysis. For the quantitative determination of thiazole derivatives, pyrazines, and trimethyloxazole, a gas chromatograph (Hewlett-Packard) was coupled with an NPD detector; separation was carried out with an HP5 column (50 m × 0.32 mm, 0.52 μm). The oven temperature was programmed from 60 to 200 °C at a rate of 2 °C/min. The final isothermal time was 20 min. The carrier gas was helium U, the splitless time was 20 s, and the split vent was 30 mL/min. The internal standard was N,N-diethylacetamide. Calibration curves were established by analysis of five different concentrations of pure products between 0.1 and 100 μg/L.

GC-MS Analysis. A first gas chromatograph (Hewlett-Packard) was coupled with a mass spectrometer (HP 5972; electronic impact, 70 eV; eMV, 2.7 kV). The column was a BP 21 (SGE), (50 m × 0.25 mm, 0.25 μm). The oven temperature was programmed from 40 to 220 °C at a rate of 2 °C/min, the initial step lasting 1 min and the final step lasting 20 min. The carrier gas was helium (1.5 mL/min). The injector was a splitless system: the splitless time was 20 s and split vent 30 mL/min. A qualitative determination of 2-acetylthiazole, 2-acetyl-2-thiazoline, and 2-acetylthiazolidine was done in the selected ion monitoring (SIM) mode selecting ions of m/z 89, 103, 112, 127, and 129; trimethyloxazole ions of m/z 111 and 139; thiazole, 2-methylthiazole, 2,4-dimethylthiazole, and 2,5-dimethylthiazole ions of m/z 58, 85, 99, 113, and 114; and pyrazine, methylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, and tetramethylpyrazine ions of m/z 80, 81, 94, 108, 122, and 136. For the other heterocyclic compounds, a second gas chromatograph (Hewlett-Packard) was coupled with a mass spectrometer (HP 6890; electronic impact, 70 eV; eMV, 2.7 kV). The column was a BP1 (50 m × 0.32 mm, 0.2 μm). The oven temperature was programmed from 40 to 220 °C,

the initial step lasting 1 min, a rate of 2 °C/min to 100 °C and at a rate of 5 °C/min to 220 °C, and the final step lasting 30 min. The carrier gas was helium (1.5 mL/min). The injector was a splitless system. A quantitative determination of thiophene-2-thiol and 2-furanmethanethiol was done in the SIM mode selecting ions of m/z 71 and 116 for thiophene-2-thiol and m/z 81 and 114 for 2-furanmethanethiol, and m/z 115 for the internal standard (N,N-diethylacetamide). Quantitative determination was done by comparison with a standard solution containing pure thiophene-2-thiol and pure 2-furanmethanethiol at the concentration of 1 μg/L.

HPLC Analysis. Amino acid analysis was carried out by reversed-phase HPLC using a Hewlett-Packard (HP 1050) liquid chromatograph. Samples were submitted to automatic derivatization with o-phthaldialdehyde (OPA) in the presence of 2-sulfanylethanol and a second derivatization with iodoacetic acid (IDA) for specific determination of sulfur amino acids. Solvents and gradient conditions were described by Anocibar Beloqui (1998). Separations were performed with two octadecyl Lichrocart cartridges mounted in series containing an RP 18 Lichrospher column and the same type of precolumn. Detection was done by a fluorometric detector (Jasco-821-FP) at λ_{ex} = 356 and λ_{em} = 445, and the data were acquired on an HP Chemstation. α-Dicarbonyl compound analysis was carried out by a type C_{18} phase HPLC using a Varian 5000 liquid chromatograph as described by de Revel et al. (2000). Samples were derived with o-diaminobenzene at pH 8. For wine analysis the quinoxalines were extracted by dichloromethane (2 × 5 mL, 5 min) at pH 2. After drying and the addition of 5 mL of methanol, 20 μL was injected. The quinoxalines were detected by spectrophotometry at λ = 313 nm.

Determination of Odor Descriptors. These were generated by four trained tasters (researchers from the laboratory) by smelling standard solutions in different dilutions in glasses (AFNOR). A data bank of odors was constituted. The odors of the model solutions at different times were compared to the data bank.

Determination of Olfactory Perception Thresholds. For each compound detected in wine, thiazole, 2-acetylthiazole, trimethyloxazole, 2-furanmethanethiol, and thiophene-2-thiol, the odor threshold was determined as the minimum concentration below which 50% of the tasters failed to smell the difference from the control by the triangle test at five concentrations in mineral water. Smelling of the solutions placed in glasses corresponding to AFNOR standards was done by a 20-person jury (Boidron et al., 1988).

Wines. Seventy-two wines from different regions of France and Europe were analyzed and are shown in Table 1.

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RESULTS AND DISCUSSION

Study of New Odorous Aromatic Heterocyclic Compounds. The model solutions containing cysteine and carbonyl compounds developed odors that can be defined according to three families of descriptors: odors of the type "popcorn" or "roasted"; odors of the type "herbaceous" or "potato"; and odors of the type "sulfur" or "rotten eggs". In addition, the odors developed by the solutions with an α-dicarbonyl compound (glyoxal, methylglyoxal, diacetyl, and pentane-2,3-dione) were always more intense and complex than those developed in the presence of ethanal or a hydroxy ketone (acetoin and acetol). The study of the model solution composition after 4 weeks of reaction led to the identification of many nitrogen-containing, sulfur-containing, and oxygenated heterocyclic compounds.

Alkylpyrazines were identified in the solutions of cysteine and dicarbonyl compounds. In the presence of methylglyoxal, there appeared 2,5-dimethylpyrazine and 2,6-dimethylpyrazine: the first has an "earthy" or "raw potato" odor (Chastrette et al., 1997); the second has a "roast" or "chocolate" odor (Maga, 1982). Trimethylpyrazine, which has a "roasted hazelnut" odor (Chastrette et al., 1997), was encountered with the addition of diacetyl. 5-Diethyl-3,6-dimethylpyrazine and 2,6-diethyl-3,5-dimethylpyrazine, which have odors of "roast" and "nut" (Maga, 1982), were identified in the solutions of pentane-2,3-dione. Koehler et al. (1971) determined the odor threshold for smelling alkylpyrazines, which vary from 10 to 100 mg/L. Among these, 2,6-dimethylpyrazine has a threshold of 54 mg/L and tetramethylpyrazine a threshold of 10 mg/L. Thus, the direct influence of these molecules on wine flavor is undoubtedly weak, so they were not studied further in this work.

Methylthiazoles. In the model solutions containing α-diketone (diacetyl or pentane-2,3-dione) and cysteine, there appeared various methylthiazoles, in particular 4-methylthiazole, 2,4-dimethylthiazole, and 2,5-dimethylthiazole. 4-Methylthiazole has a "green hazelnut" odor, its threshold being 55 µg/L. 2,4-Dimethylthiazole has "oxidized beer", "roasted red meat", or "coffee" odors. 2,5-Dimethylthiazole has "meat" or "hazelnut" odors. These molecules could not be detected in the wines.

Thiazole was formed in all of the solutions including glyoxal or methylglyoxal. Thiazole has an odor of "popcorn" and "peanut". Its odor threshold was 38 µg/L. Therefore, this molecule could be detected in the wines. The wines from Pomerol and Saint-Emilion showed some correlation ($R = 0.7$) between thiazole and cysteine levels (Figure 1). The maximum concentration encountered was 14 µg/L for a Pomerol wine of 15 years of age (Table 1); however, these compounds rarely exceeded 4 µg/L for wines of >10 years age. In addition, the fortified wines such as Port or Banyuls had high levels of thiazole, up to 34 µg/L for a 40-year-old Port bottled in 1968 and 6 µg/L in old Banyuls (*Hors d'Age*). On the other hand, these wines after a long aging presented very low levels in cysteine: 0.5 mg/L in Banyuls and 0 mg/L in Port wine. These wines have specific manufacturing and aging processes (fortified with alcohol, high sugar concentration, and long and oxidative aging) that could explain the contents encountered. Moreover, the wine odor impact could be important in Tawny Port.

2-Acetylthiazole was one of the molecules synthesized in the greatest amounts in the model solutions, and all
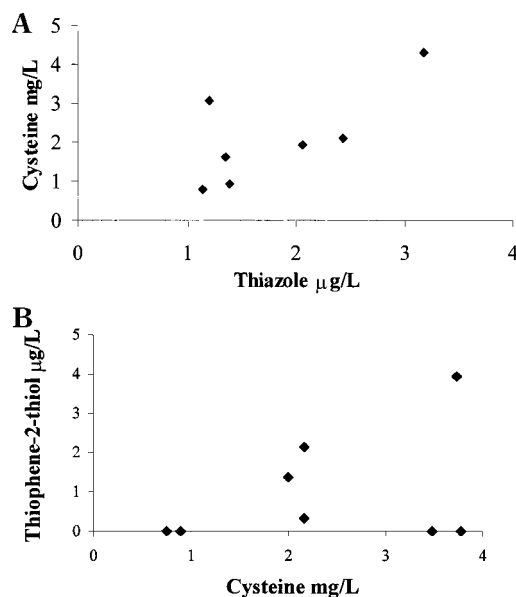
Figure 1. (A) Relationship between cysteine and thiazole in Pomerol and Saint-Emilion wines. (B) Relationship between cysteine and thiophene-2-thiol in Champagne.

of the solutions containing cysteine were concerned. This molecule is characterized by a persistent "roasted hazelnut" odor, and its threshold in water was 3 µg/L. The wines from Pomerol and Saint-Emilion presented higher 2-acetylthiazole levels on average than the other wines studied (Table 1). Nine wines from these regions were analyzed; they contained >3 µg/L on average, which is the odor threshold value in water. White Burgundy wines, Champagne, and Alsace contained on average between 1.4 and 1.8 µg/L of 2-acetylthiazole. On the other hand, red wines from Médoc, Burgundy, and Provence wines and fortified wines had <1 µg/L of 2-acetylthiazole. This molecule was identified for the first time in wine. The frequency of occurrence of 2-acetylthiazole and its concentrations were variable, but it is obvious that among the nitrogen-containing heterocyclic compounds, it was the molecule most frequently observed at concentrations with olfactory activity values (OAV is the quotient of the concentration divided by the odor threshold in water) >1. This molecule could play a role in wine flavor. Small quantities of 2-acetyl-2-thiazoline were detected in the wines richest in 2-acetylthiazole, which thus strengthened the assumption that 2-acetyl-2-thiazoline could be the reduced precursor of 2-acetylthiazole. A mode of formation was proposed by Griffith and Hammond (1988) for the origin of the aromatic components of Swiss cheese.

Trimethyloxazole, which was detected in all of the α-diketone solutions, has an aggressive "very ripe fruit" odor. Its odor threshold in water was 17 µg/L. Trimethyloxazole was the only non-sulfur heterocyclic compound that we found in wines. It is most frequently met in wines from the Pessac-Léognan, Bordeaux, and Médoc regions (Table 2). All of these vineyards are located in the same geographical area, and the wines are vinted according to very similar processes. Sixty-seven percent of the wines resulting from this area contained between 2 and 7 µg/L of trimethyloxazole. However, trimethyloxazole was detected in some red and white wines from other areas (Table 1): 38% of the Burgundy wines analyzed contained between 1.5 and 3 µg/L. Finally, 4 µg/L of trimethyloxazole was found in a late harvest 1995 wine from the Alsace region and 1

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Table 2. Trimethyloxazole Levels in Different Wines

wine origin	trimethyloxazole (μg/L)
red wines	
Saint Julien 1994	7
Saint Julien 1994	2
Pessac-Léognan 1994	6
Bordeaux 1994	0
Saint Estèphe 1994	4
white wines	
Bordeaux 1983	0
Barsac 1983	7
Graves 1996	0
Pessac-Léognan 1996	2

μg/L in a Saint-Emilion wine (great vintage of 1994) and in a Champagne-rosé wine. The other wines analyzed did not contain trimethyloxazole. This molecule has never been mentioned before in wine. This oxygenated and nitrogen-containing heterocyclic compound was described by Vernin and Metzger (1981) as the product of the condensation of α-aminoketone, resulting from the degradation of an amino acid according to the Strecker mechanism, and from acetaldehyde. Acetaldehyde (or ethanal) can be abundant in wine (up to 100 mg/L), although it is in the form of a bisulfite combination.

2-Furanmethanethiol was detected only in α-diketone solutions and only when they were kept at 40 °C. Its organoleptic descriptor is "roasted coffee" or "burned rubber" at high levels. The organoleptic importance of 2-furanmethanethiol could be very great because of its odor threshold, estimated at 1 ng/L. According to our tests, only the wines from Pomerol and Saint-Emilion, the *Crus Classés* of Burgundy wines, and some Champagnes presented 2-furanmethanethiol (Table 1). The average contents reached 350 ng/L for the wines of Pomerol and Saint-Emilion, 70 ng/L for the wines of Burgundy, and 10 ng/L for Champagnes. These contents mean that the OAV index is 350, 70, and 10, respectively, for these three types of wines. We could not detect 2-furanmethanethiol in any other wines. Schieberle (1993) defined 2-furanmethanethiol as the product of reaction between sugars or derivatives of sugars and cysteine or hydrogen sulfide. This furan-type sulfur molecule was also cited in wine by Blanchard et al. (1999), who attributed its presence in wine to oak wood.

Thiophene-2-thiol was observed in the solutions with each one of the carbonyl compounds studied and when cysteine was kept at 25 °C. This sulfur aromatic heterocyclic compound is very odorous. Its threshold in water was ~0.8 μg/L. It has an odor of "burned", "burned rubber", or "roasted coffee". It was detected in wines for the first time. Champagne and some Pomerol and Saint-Emilion wines were among the richest in thiophene-2-thiol. It was detected at levels of 1.2 μg/L for Pomerol and Saint-Emilion wines and 1 μg/L for Champagne wines, thus giving OAV indices of 1.7 and 1.4, respectively. Aging of wines from Pomerol and Saint Emilion is done in oak barrels. Thus, like 2-furanmethanethiol, thiophene-2-thiol could be released by wood in still wines. Eight Champagne wines were analyzed. For five wines, the thiophene-2-thiol levels were bound to the cysteine contents (Figure 1B); three others contained no or very low levels of thiophene-2-thiol despite cysteine concentrations ranging between 2 and 4 mg/L. The cysteine concentration of the wines could be a determining factor for this molecule. Thiophene-2-thiol was described by Shu et al. (1985) in a study of the pH effect on thermal degradation of cysteine in aqueous solution.

Scheme 1. Hypothesis for Formation of Thiophene-2-thiol from Cysteine (Ho, 1996)
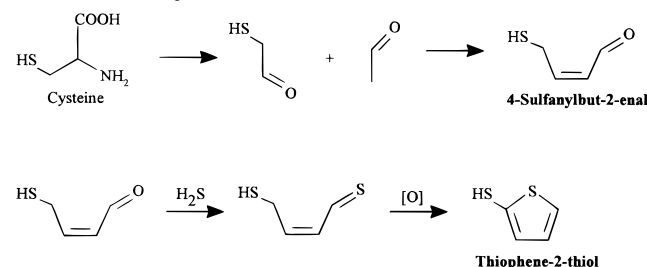
**Table 3. Influence of Temperature on Cysteine/Methylglyoxal Model Solution Odors[a]**

temp, °C	odor	days					
		2	5	7	9	11	15
10	sulfurous	*	**	**	****	***	**
	roasted		*	*	**	**	***
20	herbaceous	*	*	*	**		
	sulfurous	***	***	***	***	****	***
	roasted	*	*	*			
	pop corn				**		
40	herbaceous	**					
	sulfurous		***	***	***	***	***
	roasted		**	**	**	*	*
	carmel	*					

[a] *, weak; **, moderate; ***, intense; ****, very intense.

This molecule was one of the most abundant products when experimentation was conducted at pH 2.2; in addition, its formation is favored by the presence of carbonyl compounds. Ho (1996) proposed a protocol of cysteine decomposition leading to acetaldehyde and 2-sulfanylethanal. By condensation of two aldehydes, 4-sulfanylbuten-2-al was formed, which, in the presence of hydrogen sulfide, could lead to thiophene-2-thiol by cyclization (Scheme 1). This sulfur heterocyclic compound was identified in wine for the first time. The search for the origin of these molecules is underway in our laboratory.

All of the molecules studied in this work are known to be products of the Maillard reaction (Shibamoto and Russell, 1976; Farmer and Mottram, 1989; Schieberle and Hofmann, 1996). They all present odorous notes close to those developed in roasted food. The presence of thiazoles, pyrazines, and trimethyloxazole has already been reported by Pripis-Nicolau et al. (2000) as reactions between amino acids and carbonyl compounds under wine conditions. The heterocyclic compounds detected in model solutions could also exist in wine. The contents of seven compounds, that is, thiazole, 4-methylthiazole, 2-acetylthiazole, 2-acetyl-2-thiazoline, trimethyloxazole, 2-furanmethanethiol, and thiophene-2-thiol (Scheme 1), were specified for wines from various areas of France and Europe, with various types of cultivars and which are vinted according to various methods (Table 1).

Temperature Influence. Model solutions with cysteine and methylglyoxal were kept at 10, 20, or 40 °C. The notes developed are shown in Table 3. At 10 °C, there was a regular development of the sulfur notes, arising from the presence of hydrogen sulfide due to the decomposition of cysteine according to the Strecker mechanism. At the same time, weak "roasted" notes appeared. However, the odors remained fairly simple. At 20 °C, the "hydrogen sulfide" note was great on the second day of reaction, but its intensity increased very
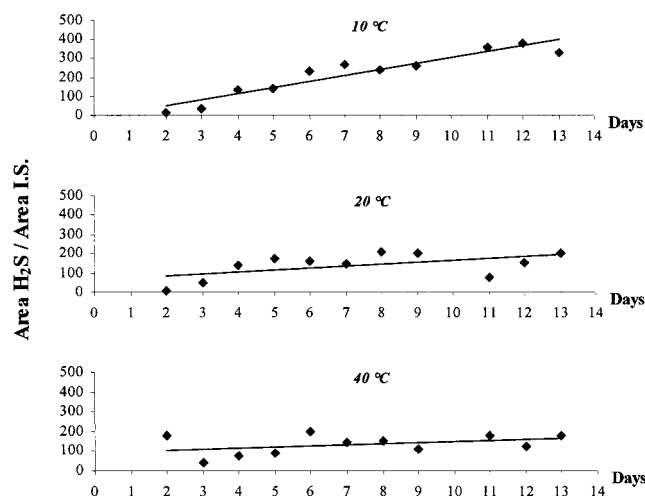
Figure 2. Evolution of H_2S in the headspace of cysteine/methylglyoxal solutions stored at 10, 20, and 40 °C.

little with time. Figure 2 shows the daily results of H_2S contained in the headspace. At 20 °C, the H_2S level tended to stabilize. On the other hand, at 10 °C, it continued to increase. Part of the hydrogen sulfide produced at 20 °C might be reused in chemical reactions which could induce the "roasted", "popcorn", and "herbaceous" notes appearing at this temperature. At 40 °C, the intensity of the "hydrogen sulfide" note was equivalent and even lower than the perceived intensity at 20 °C. Moreover, H_2S quantified in the headspace confirmed these observations. However, at 40 °C, the balance between the liquid and vapor phases shifted in favor of the latter. Therefore, by supposing that cysteine produced at least as much hydrogen sulfide at 40 °C as at other temperatures, the quantity of hydrogen sulfide measured in the headspace should be greater, yet the opposite was observed. Thus, consumption of hydrogen sulfide by chemical reaction is greater at 40 °C than at 20 °C. Figure 3 shows that on the second day, the hydrogen sulfide content was greater in the solution kept at the highest temperature. The phenomenon was reversed when the reaction time increased. In addition, "caramel" and "popcorn" notes appeared in the solutions at 20 and 40 °C. These odors are close to those developed during the roasting of food. Hydrogen sulfide could be a breakdown product of cysteine and a reagent in the formation of the odorous compounds responsible for the "roasted" "popcorn", and "caramel" notes.

Influence of Medium. A comparative sensory study of the model solutions and white or red wines was carried out. The influence of the matrix seemed to be rather low on the variety of the nuances perceived but was quite high on their intensity. In wine, the sensory threshold of the molecules responsible for the "roasted" and "popcorn" notes could even be higher. This phenomenon was noted with most of the molecules. In white wine, the "popcorn" and "roasted" notes described during the Maillard reactions developed more quickly than in red wine. In the case of pentane-2,3-dione solutions, the "popcorn" note, which was well identified previously in the synthetic medium, was perceptible in the white wine supplemented as of the third day, whereas it was necessary to wait >6 days in red wine. When the reaction time increased, the odors developed in the white and red wine solutions were equivalent. Simultaneous analysis of the "light" sulfur compounds of the various mixtures in the three media (white wine, red

wine, and model solution) over the same reaction time did not reveal any notable difference. Nevertheless, the red wine solutions contained lower quantities of hydrogen sulfide in their headspace than the other solutions. This was perhaps due to combinations.

This work confirms the high reactivity of cysteine with carbonyl and dicarbonyl molecules and its particularly interesting role in the genesis of flavors. We now show the presence of thiazole, 4-methylthiazole, 2-acetylthiazole, and trimethyloxazole and that of two sulfured and oxygenated heterocyclic compounds, 2-furanmethanethiol and thiophene-2-thiol. These molecules could play an important part because of their very low olfactory thresholds. Hydrogen sulfide is an intermediate in the formation of these odorous heterocyclic compounds. Molecules with "roasted coffee", "roasted hazelnut", "popcorn", "burned", and "roasted" notes were found in various wines. Among these, we found 2-acetylthiazole in many wines, thiazole particularly in Pomerol and Saint-Emilion wines and in fortified wines, trimethyloxazole in Graves and Medoc wines, 2-furanmethanethiol in some Champagne and in the great vintages of Burgundy, and thiophene-2-thiol particularly in Champagne, Pomerol, and Saint-Emilion wines. In some cases there seemed to be a correlation between cysteine and thiophene-2-thiol levels in Champagne wines and cysteine and thiazole in Pomerol and Saint-Emilion wines. These molecules described for the first time in wines could play a great part in their flavor.

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