Formation of Flavor Components by the Reaction of Amino Acid and Carbonyl Compounds in Mild Conditions

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This work describe products of reactions between four α-dicarbonyl compounds (diacetyl, pentan-2,3-dione, glyoxal, and methylglyoxal) or two α-hydroxy ketones, (acetoine and acetol) and amino acids present in wines. The results shows the formation of odorous products or strong-smelling additives resulting from the Maillard and Strecker reaction in a primarily aqueous medium, at low temperature and low pH ($\sim$pH 3.5) of the wine. GC/FID, GC/FPD, GC/NPD and GC/MS techniques were used. The olfative characteristics of the products are described. In the presence of sulfur amino acids and in particular cysteine, many products were formed with a heterocycle production such as pyrazines and methylpyrazines, methylthiazoles, acetylthiazoles, acetylthiazolines, acetylthiazolidines, trimethyloxazole, and dimethylethyloxazoles. These various compounds present odors of sulfur, cornlike, pungent, nut, popcorn, roasted hazelnut, toasted, roasted, and ripe fruits. The chemical conditions of the model reactions are specified. The influence of temperature and pH on the reactions in the presence of cysteine were also studied.

Keywords: *Amino acids; cysteine; carbonyl compounds; Strecker degradation; volatile heterocycle compounds; flavor*

INTRODUCTION

In musts and wines, the amino acids represent the most important form of total nitrogen. Moreover, by their polyfunctional character, the amino acids have a great chemical reactivity with respect to the carbonyl compounds, in particular with sugars, according to the Maillard reaction. This well-known reaction occurring in the agrofood industry is generally described like a dry favored reaction and at high temperature, leading to the intermediaries of Amadori and Heyns evolving by rearrangement and retroaldolization into α-dicarbonyl compounds. This is made possible by a greater

reactivity of the carbonyl functions, with more electrophiles than those of the sugars, in an aqueous medium and at ambient temperature. Finally, a nucleophilic addition of the amine function of amino acids can occur. Such a study is justified because, after alcoholic and malolactic fermentations, α-dicarbonyl compounds are encountered in wines in oxydoreduction equilibrium with the reduced forms, i.e., the α-hydroxy ketones and the α-diols. Diacetyl is a well-known example and is described in the literature (de Revel and Bertrand, 1994). Nucleophilic addition to the carbonyl of α-dicarbonyl compounds and α-hydroxy ketones of wines may occur during bottling (sometimes at high temperature), or during self-keeping because increases in temperature favorable to the reaction may take place.

Finally, there is a sensorial impact of certain products resulting from Strecker degradation i.e., pyrazines from

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Table 1. Identified Reaction Products and Principal Odors Detected in the Synthetic Amino Acid Solutions in the Presence of α-Dicarbonyl Compounds

substrates	odor[a]	reaction products
cysteine		
glyoxal	rotten eggs***, sulfur**, cabbate**, roasted*	H_2S, methanetriol, carbon disulfide, 2-methylthiazole, pyrazine
methylglyoxal	popcorn***, hazelnut**, toasted*, roasted*	H_2S, 2-acetylthiazole, 2-acetyl-2-thiazoline,2-acethylthiazolidine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine
diacetyl	toasted***, sulfur**, cabbage**, pungent*	methanethiol, tetramethylpyrazine, trimethyloxazole
pentan-2,3-dione	sulfurous and catty***, toasted**, roasted**	methanethiol, carbon disulfide, 2,5-diethyl-3,6-dimethylpyrazine, 2,6-diethyl-3,5-dimethylpyrazine, 2,5-dimethy-4-ethyloxazole, 2,4-dimethyl-5-ethyloxazole
methionine		
glyoxal	potato***, cabbage**	methanethiol, dimethyl disulfide, methional
methylglyoxal		
diacetyl		
pentan-2,3-dione		
valine		
glyoxal	cheese**	2-methylpropanal
methylglyoxal		
diacetyl		
pentan-2,3-dione		
leucine		
glyoxal	amylic**	3-methylbutanal
methylglyoxal		
diacetyl		
pentan-2,3-dione		
isoleucine		
glyoxal	fruity**	2-methylbutanal
methylglyoxal		
diacetyl		
pentan-2,3-dione		
phenylalanine		
glyoxal	floral**	benzaldehyde, phenylacetaldehyde
methylglyoxal		
diacetyl		
pentan-2,3-dione		

[a] Odors: *weak, **moderate, and ***intense.

α-aminocetone precursor and odorant aldehydes containing a carbon atom on less than the initial α-amino acid. Many authors have studied the reactivity of the amino acids to carbonyl functions at high temperature. Some of the products present a particularly low threshold, such as thiazoles, oxazoles, pyrans, pyrazines, pyridines, furans, etc. (Rizzi, 1972; Vernin and Metzger, 1981; Chi-Kuen Shu, 1998). However, the formation of these molecules is poorly understood regarding the physicochemical conditions close to those of the wine: low pH, low temperature, and in the presence of water. Moreover, the data are sparse. Griffith and Hammond (1988) described a study of the reaction between a carbonyl compound and an amino acid in model solutions at a temperature of 25 °C but at high pH. They showed the presence of aldehydes and heterocycles of pyrane type, pyrazine type, and 2-acetylthiazol. Few authors have reported these types of reaction in wine. Hasiba (1978) underlined the presence of Amadori compounds in Japanese white wines. Maujean (1989) described the thermal origin of volatile sulfur products in Champagne wines stored at 25 °C in the dark, which can be formed by Strecker degradation of sulfur amino acids.

We therefore used a model reaction to study the reactivity of 14 amino acids with respect to 6 carbonyl compounds present in wine: glyoxal, methylglyoxal, diacetyl, pentan-2,3-dione, acetoin, and acetol.

MATERIALS AND METHODS

Materials. Amino acids (arginine, phenylalanine, lysine, cysteine, methionine, proline, valine, leucine, isoleucine, glycine, serine, alanine, γ-aminobutyric acid, and glutamic acid),

carbonyls (glyoxal, methylglyoxal, diacetyl, pentane-2,3-dione, acetoin, and acetol), and compounds used for identification of final products (alkylpyrazines, alkylthiazoles, 2-acetylthiazole, 2-acetyl-2-thiazoline, trimethyloxazole, and aldehydes) were purchased from Sigma Aldrich Chemical Co. Inorganic reagents and solvents were all commercial products of analytical grade.

The mixture of carbonyl compound and amino acid in a hydro alcoholic solution (12%) was prepared in stoichiometric conditions (20 mmol/L) and adjusted to pH 3.5 with H_3PO_4 (1/3) and 1 M NaOH. The solutions were stored at 25 °C in the dark. Modifications of the flavor of these mixtures were examined during a 4-week storage period.

Analytical Procedures. Each reaction mixture (50 mL) was extracted by various solvents such as ethyl acetate (2 × 5 mL), ethyl ether (2 × 5 mL), and a mixture of ethyl ether and hexane (50/50 v/v) (5 mL + 2 × 2 mL) after 7, 14, and 30 days. The extracts (2 μL) were analyzed by GC/FPD, GC/NPD, GC/FID, and GC/MS. The measurement of amino acid degradation was performed by HPLC.

GC/FPD Analysis. The gas chromatograph (Hewlett-Packard) was coupled with a flame photometric detector (FPD). The column was a Carbowax CW 20M (50 m × 0.25 mm, 0.25 μm). The oven temperature was kept at 40 °C for 5 min and programmed at a rate of 3 °C/min to 200 °C, with the final step lasting 20 min. The carrier gas was hydrogen (1,5 mL/min); the splitless time was 50 s, and the split flow rate was 30 mL/min. The flow rate of hydrogen in the flame was 75 mL/min, and a mixture of nitrogen/oxygen (80/20) at 80 mL/min was used. The makeup gas was nitrogen at 20 mL/min.

GC/NPD or FID Analysis. The gas chromatograph (Hewlett-Packard) was coupled with NPD or FID detectors; separation was carried out with a column HP5 (60 m × 0.32 mm, 0.12 μm). The oven temperature was programmed from 60 to 200 °C at a rate of 2 °C/min. The final isothermal time

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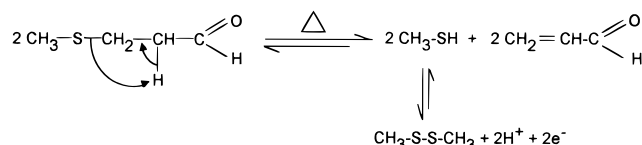*J. Agric. Food Chem.,* Vol. 48, No. 9, 2000 **3763**

Figure 1. Formation of methanthiol and dimethyl disulfide by the retro-Michael reaction.

was 20 min. The carrier gas was helium U, the splitless time was 50 s, and the split vent was 30 mL/min.

GC/MS Analysis. The 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, with the final step lasting 20 min. The carrier gas was helium "Aga 5.6" (1.5 mL/min). The injector was a splitless system: the splitless time was 20 s, and the split vent was 30 mL/min. A quantitative determination of 2-acetylthiazol and acetyl-2-thiazoline was done in SIM mode selecting ions of m/z = 58, 83, 99, 101, 127, 129. Ions m/z = 99, 127 were chosen for quantification of 2-acetylthiazol; ions m/z = 101, 129 for acetyl-2-thiazoline, and ions m/z = 58, 83 for the internal standard (octan-3-ol).

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 derivation with orthophthaldialdehyde (OPA) in the presence of 2-sulfanyl-ethanol, and a second derivation with iodoacetic acid (IDA) for specific determination of sulfur amino acids. Solvents and gradient conditions were as described by Anocibar Beloqui (1998). Separations were performed with two octadecyl Lichrocart cartridges mounted in series containing a RP 18 Lichrospher column and the same type of precolumn. Detection was done by a fluorimetric detector (Jasco-821-FP) λ excitation = 356, λ emission = 445, and the data was acquired on a HP chemstation.

RESULTS AND DISCUSSION

Principal Odors Detected and Identified Reaction Products in the Reaction Mixtures. Concerning

the model reaction with α-hydroxy ketones (acetoin, acetol) and the majority of amino acids, few olfactive changes and few compounds with organoleptic impact appeared during the reaction time. However, olfactive modifications were noted with valine, methionine, and cysteine, respectively, the cheesy notes, the potato and cabbage notes, and the rotten eggs notes. These olfactive impressions could be associated with the 2-methylpropanal observed with valine, with methanethiol, dimethyl disulfur and methional for methionine, and with hydrogen sulfide and methanethiol for cysteine. 3-Methylbutanal and 2-methylbutanal were also identified when leucine and isoleucine, respectively, were used.

On the other hand, in the reaction mixture of amino acid and α-dicarbonyl, interesting changes were observed in the presence of sulfur amino acids (Table 1). For the other amino acids involved, changes were also noted but only in the model reactions containing valine, leucine, isoleucine, and phenylalanine. There was a release of cheesy, amyl, fruity, and floral notes with valine, leucine, isoleucine, and phenylaldehyde, respectively. The products identified (Table 1) were aldehydes formed by Strecker degradation. Thus, we identified 2-methylpropanal, 3-methylbutanal, 2-methylbutanal, and phenylacetaldehyde in the presence of valine, leucine, isoleucine, and phenylalanine, respectively. In the presence of the latter, a low level of benzaldehyde was also noted. Strecker degradation of the amino acids also led to the formation of the very reactive α-aminoketones, which are precursors of the formation of pyrazines by cyclic duplication. However, we did not identify these pyrazines. Pyrazines are more easily synthesized in neutral or basic pH (Shibamoto and Yeo, 1994; Meynier and Mottram, 1994).

With all model reactions containing methionine, there was a release of potato and cabbage notes with different intensities. These potato odors detected in the presence of methionine were associated with a large formation
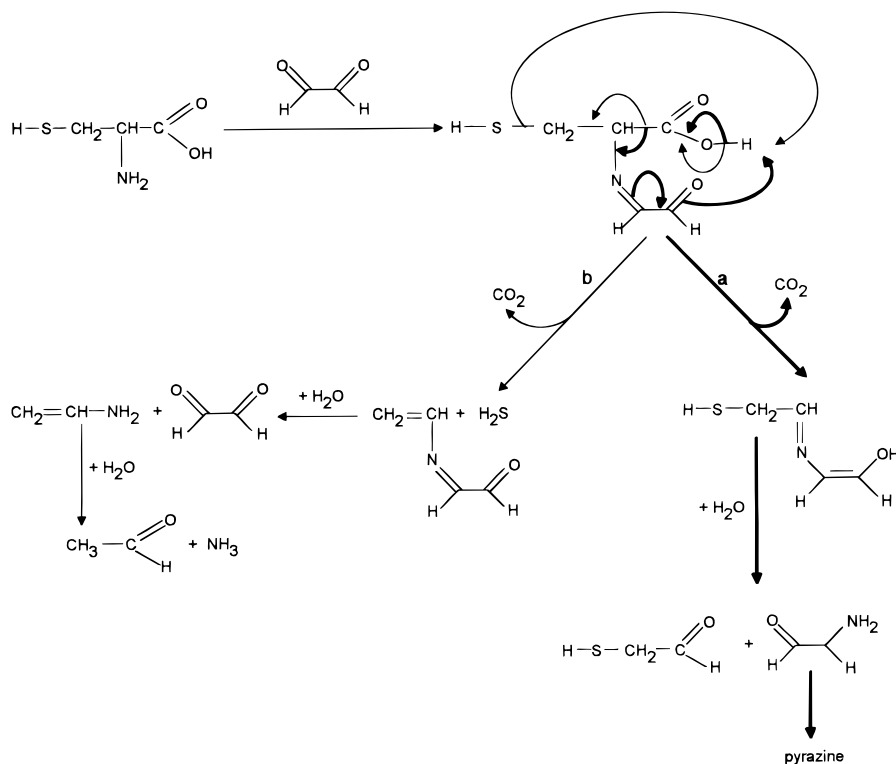

Figure 2. Strecker degradation of cysteine (a) and proposed mechanism for the production of hydrogen sulfide, acetaldehyde and ammonia (b) in the presence of glyoxal.
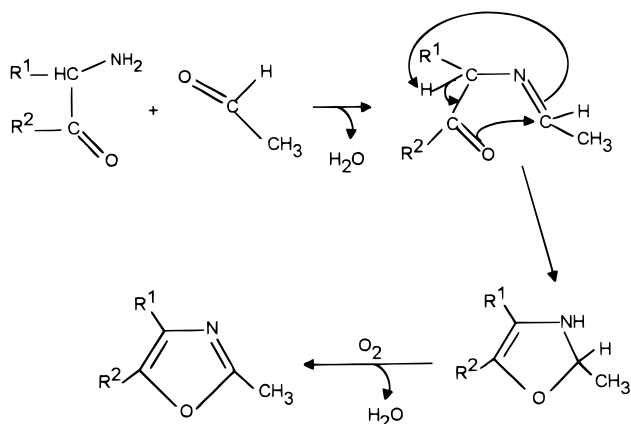
Figure 3. Possible pathway for the formation of the alkyloxazoles starting from α-amino ketone and acetaldehyde.

of methional, and we also detected methanthiol and dimethyl disulfide. The latter two compounds arose from methional which is unstable by retro-Michael reaction (Figure 1).With the cysteine model reaction, more complex and varied odors appeared (Table 1). Thus in the presence of methylglyoxal, very intense popcorn and roasted odors were noted in the first hours. After approximately four weeks, these odors gave way to a roasted hazelnut note. The solution with pentane-2,3-dione had an intense sulfurous and catty odor, which disappeared after one week to be replaced by roasted and toasted notes. In the presence of diacetyl and glyoxal, the mixture presented sulfur type odors, which evolved to roasted and toasted notes.

The greater wealth of olfactive perceptions noted in the reaction mixture with cysteine (Table 1) compared to methionine could be explained by the larger number of sulfur compounds identified such as hydrogen sulfide (with glyoxal and methylglyoxal), methanethiol (except with methylglyoxal), carbon disulfide (with glyoxal and pentane-2,3-dione) but also many heterocycles such as thiazoles, acetylthiazole, oxazoles, and pyrazines. An explanation of the difference between the two sulfur amino acids could be that cysteine in addition to the Strecker degradation (formation of aldehyde and of the α-aminoketone) can undergo a decarboxylation reaction, leading to the release of hydrogen sulfide and the formation of Schiff base, which by hydrolysis leads to acetaldehyde and ammonia. We present a possible

pathway of the release of these molecules in the presence of glyoxal (Figure 2b), compounds already observed by Vernin and Metzger (1981) in the presence of α-diketones. This competitive reaction of Strecker degradation (Figure 2a) is possible only with cysteine. Finally, the release of the acetaldehyde by cysteine explains the formation of the alkyloxazoles identified by us in the presence of α-diketones. Indeed, with diacetyl, we also identified the formation of trimethyloxazole and with pentane-2,3-dione (asymmetrical α-diketone) the formation of two oxazole isomers: 2,5-dimethyl-4-ethyloxazol and 2,4-dimethyl-5-ethyloxazol. We propose the formation of these oxazoles starting from α-aminocetone and the acetaldehyde released by cysteine (Figure 3). From the olfactive point of view, trimethyloxazole presents melon and very ripe kiwi notes. The same odor type with more aggressive nuances and pungent notes characterizes the other two oxazoles. Alkyloxazoles are added in the case of the glyoxal-cysteine model reaction to the formation of the 2-methylthiazol, which again implies the presence of acetaldehyde. On the other hand, the alkylthiazoles could not be detected (FPD and NPD) with diacetyl and pentan-2,3-dione, whereas olfactive controls indicated the presence of characteristic roasted and toasted notes. With regard to the cysteine–methylglyoxal model reaction, we noted the formation of 2-acetylthiazole and its intermediates, 2-acetyl-2-thiazoline and 2-acetylthiazolidine. 2-Acetyl-2-thiazoline and the 2-acetylthiazol are very often cited as being the products of the Maillard reaction in the presence of cysteine (Haufmann and Schieberle 1995; Meynier and Mottram, 1995). These molecules are known for their popcorn, roast meat, and roasted hazelnut descriptors and are involved in many food aromas. The formation of 2-acetylthiazol was also identified by Griffith and Hammond (1988) in model methylglyoxal–cysteine solutions but at high pH. These authors proposed a pathway in which the 2-acetylthiazolidine seemed to be an intermediary. In addition to these products identified with cysteine, there are the alkylpyrazines resulting from the Strecker reaction (Figure 2a). Indeed, pyrazine was formed with glyoxal, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine were formed in the presence of methylglyoxal, tetramethylpyrazine was formed in the presence of diacetyl, and two other alkylpyrazine isomers, 2,5-diethyl-3,6-dimethylpyrazine and 2,6-diethyl-3,5-dimethylpyrazine,
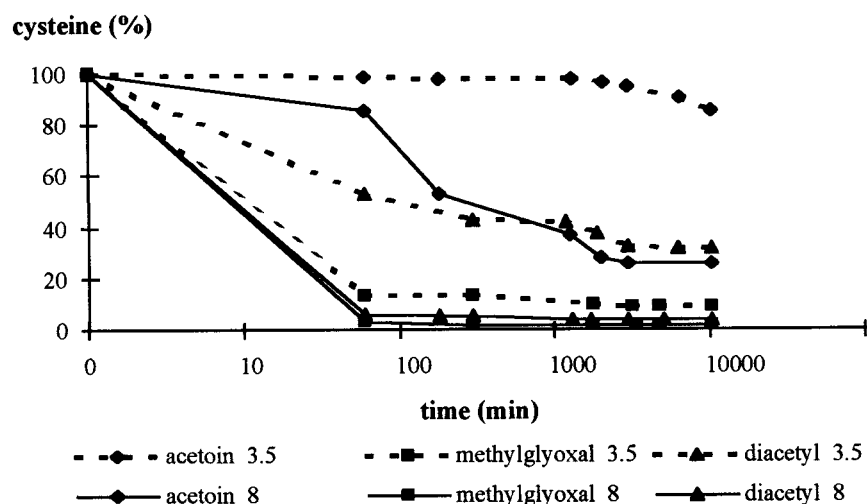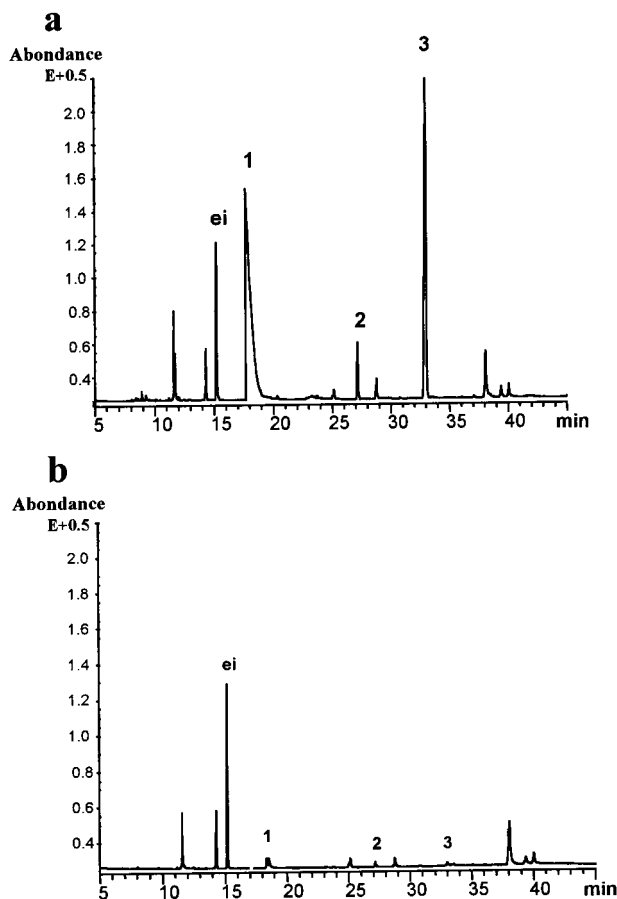

Figure 4. pH influence on cysteine degradation with different carbonyl compounds.

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1: acetic acide; 2: 2-acetylthiazol; 3: 2-acetyl-2-thiazoline; ei: octan-3-ol.

Figure 5. Chromatograms (GC/FID) of the cysteine-methylglyoxal model reaction at pH 3.5 (a) and at pH 8 (b).

Figure 6. Cysteine degradation and production of volatile compounds in the presence of methylglyoxal at 25 (a) and 10 °C (b).

were formed with pentan-2,3-dione. The alkylpyrazines identified were characterized by odors of cornlike, nut, hazelnut, roast, pungent, and chocolate. The presence of these types of molecule is often cited by various authors (Rizzi, 1972; Valisek et al., 1976; Maga, 1982; Tressl et al., 1983) as being the products of reactions at high temperatures between amino acids and α-dicarbonyls or reducing sugars.

Kinetic Study of Cysteine Degradation with Three Carbonyl Compounds According to the pH of the Models Reactions. Because of the organoleptic value of the products formed, we studied the rate of decomposition of cysteine in the presence of various carbonyl compounds (methylglyoxal, diacetyl, and acetoin) at two different pH's (3.5 and 8) and at a temperature of 25 °C. The results are presented in Figure 4. At pH 8 (pH known to favor this type of reaction), the decomposition of cysteine was faster than at pH 3.5 and was encountered in the presence of methylglyoxal and diacetyl. On the other hand, the rate was relatively slow in the presence of acetoin, particularly at pH 3.5. In addition, at the two pH conditions, in the presence of methylglyoxal, differences in odor and color were noted. At pH 3.5, the solution released popcorn and roasted hazelnut notes, and a yellow-brown color. At pH 8, rotten eggs, cabbage, and roasted meat notes were released, and the solution color was yellow straw. Concerning the reaction products, greater quantities of 2-acetylthiazole and 2-acetyl-2-thiazoline were formed at pH 3.5 (Figure 5a). These results indicate that
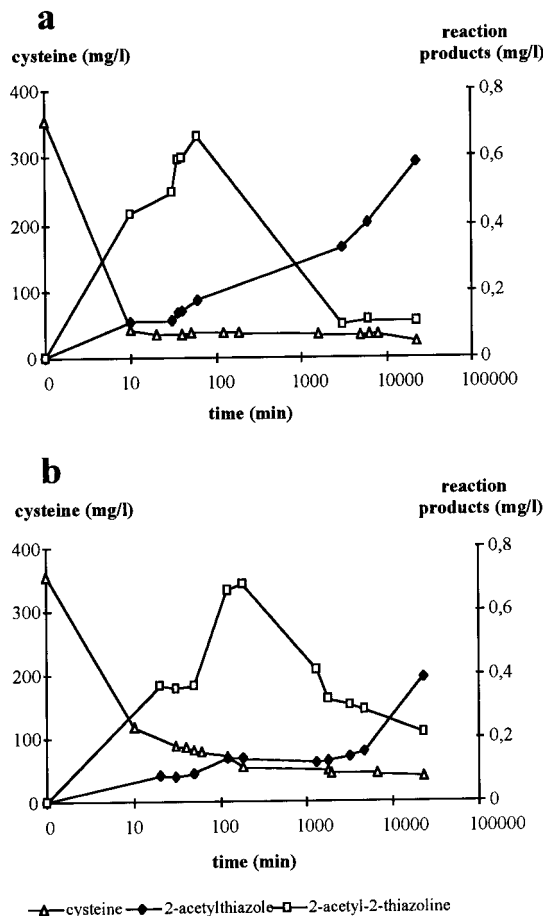
during this reaction, an acid pH close to that of wines is unexpectedly more favorable to the formation of 2-acetylthiazole and 2-acetyl-2-thiazoline.

Additionally, a comparison was made of the reaction between cysteine and methylglyoxal at a low concentration (2 mM) at two temperatures, 10 and 25 °C. This model system was chosen for the speed of the reaction at pH 3.5 and for the organoleptic importance of the 2-acetylthiazol and its intermediates. At 25 °C, cysteine degradation was greater (Figure 6a). After 10 min, 2-acetyl-2-thiazoline presented a characteristic curve of intermediate reaction, and a maximum appeared after 60 min. This molecule was oxidized in 2-acetylthiazole which continued to increase regularly. At 10 °C (Figure 6b), the reaction slowed a little. 2-Acetyl-2-thiazoline formation reached a maximum at 180 min (3 h later a at 25 °C). The formation of 2-acetylthiazol was also delayed.

CONCLUSION

This work shows the importance of the possible reactions arising from carbonyl compounds in the genesis of aromatic products even under difficult conditions of low pH and low temperatures, i.e., conditions encountered during the conservation and aging of wine. The most interesting molecules were produced in the presence of the sulfur amino acids, in particular cysteine, with the production of heterocycles such as pyrazines, alkylpyrazines, methylthiazoles, acetylthiazole, acetylthiazoline, acetylthiazolidine, trimethylox-

azole, and dimethylethyloxazoles. These various compounds generated notes of sulfur, popcorn, hazelnut, toasted, roasted, and ripe fruits which are very interesting for their complexity. Among these reactions, the formation of 2-acethylthiazol and 2-acetyl-2-thiazoline was most interesting, because they occur rapidly with an acid pH at low temperature and have great olfactive interest. With cysteine, these reactions could occur in wine just after alcoholic or malolactic fermentations, particularly the latter by being at the origin of the formation of dicarbonyl compounds such as diacetyl. In addition, during the reactions in the presence of sulfur amino acids, we identified volatile sulfur compounds such as hydrogen sulfide, methanthiol, dimethyl disulfide, and carbon disulfide. Due to their off-flavor character and particularly low threshold, these compounds can lead to olfactive defects.

LITERATURE CITED

Anocibar Beloqui, A. Contribution to the study of sulphur compounds of the red wines. Ph. D. Thesis Number 611, University Victor Segalen Bordeaux 2, Bordeaux, France, 1998.

Chi-Kuen, S. Pyrazine formation from amino acids and reducing sugars, a pathway other thanstrecker degradation. *J. Agric. Food Chem.* **1998**, *46*, 1515−1517.

Griffith, R.; Hamond, G. Generation of Swiss cheese flavor components by the reaction of amino acids with carbonyl compounds. *J. Dairy Sci.* **1988**, *72* (3), 604−613.

Hashiba, H. Isolation and identification of Amadori Compounds from miso, white wine and sake. *Agric. Biol. Chem.* **1978**, *42* (9), 1727−1731.

Hofmann, T.; Schieberle, P. Evaluation of the key odorants in a thermally treated solution of ribose and cysteine by aroma extract dilution analysis. *J. Agric. Food Chem.* **1995**, *43*, 2195−2198.

Maga, J. A. Pyrazine in foods: an update. In *CRC Critical Reviews In Food Science and Nutrition*; Furia, T. E., Ed; CRC: Boca Raton, FL, 1982; Vol. 16, pp 1−48.

Maujean, A. Goûts anormaux dans les moûts et les vins en relation avec la présence de produits soufrés volatils. *Revue des Œnologues.* **1989**, 31−32.

Meynier, A.; Mottram, D. S. The effect of pH on the formation of volatile compounds in meat-related model systems. *Food Chem.* **1995**, *52*, 361−366.

de Revel, G.; Bertrand, A. Dicarbonyl compounds and their reduction products in wine. Identification of wine aldehydes. In *Trends in Flavour Research Proceedings of the 7th Weurman Flavour Research Symposium*, 15−18 June 1993; Maarse, H., van der Heij, D. G., Eds: Elsevier: Zeist, The Netherlands, 1994; pp 353−361.

Rizzi, G. P. A mechanistic study of alkylpyrazine formation in model system. *J. Agric. Food Chem.* **1972**, *20*, 1081−1085.

Shibamoto, T.; Yeo, H. Flavor in the cysteine-glucose model system prepared in microwave and conventional ovens. *Am. Chem. Soc., Symp. Ser.* **1994**, *543*, 457−465.

Tressl, R.; Helak, B.; Grunewald, K. G.; Silwar, R. Formation of flavour components from proline, hydroxyproline and sulphur containing amino acids. In *International Symposium on Food Flavors*; Adda, J., Richard, H., Eds; Tech. et Doc. Lavoisier, Paris, France, 1983; pp 207−230.

Velisek, J.; Davidek, J.; Cuhrova, J.; Kubelka, V. Volatile heterocyclic compounds in the reaction of glyoxal with glycine. *J. Agric. Food Chem.* **1976**, *24* (1), 3−7.

Vernin, G.; Metzger, J. La chimie des arômes: les hétérocycles. *Bull. Soc. Chim. Belg.* **1981**, *90*, 553−587.