Chemical and Technological Factors Determining Tetrahydro-β-carboline-3-carboxylic Acid Content in Fermented Alcoholic Beverages

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Chemical and technological factors influencing the formation of tetrahydro-β-carboline-3-carboxylic acid in fermented alcoholic beverages were studied in model solutions and during alcoholic fermentation of grape juices. 1*S*,3*S* and 1*R*,3*S* diastereoisomers of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) were formed from tryptophan when acetaldehyde was released by the yeast during growth and alcoholic fermentation. Low pH and high temperatures of storage accelerated the reaction between tryptophan and acetaldehyde to give MTCA. Sulfur dioxide reacted with both acetaldehyde and tryptophan, restricting the amount of MTCA formed, during both alcoholic fermentation and storage. L-Tryptophan and acetaldehyde available for the reactions determined the level of tetrahydro-β-carboline-3-carboxylic acids in the wines. Yeast absorbed tryptophan rapidly in the first days of fermentation, minimizing the formation of tetrahydro-β-carboline-3-COOH. The reaction rate to form 1,2,3,4-tetrahydro-β-carboline-3-COOH (THCA) after reaction of tryptophan with formaldehyde was much faster than for MTCA. THCA may also be formed at the highest storage temperatures.

INTRODUCTION

Tetrahydro-β-carbolines (THβC) are synthesized as products of the reaction between indole amines and aldehydes. The condensation between tryptophan and aldehydes forms tetrahydro-β-carboline-3-carboxylic acids. Figure 1 shows the reactions that take place concerning tryptophan and aldehydes and the wine relationships that are pertinent.

THβC have been reported as possible neurotransmitters or neuromodulators and have been related with alcoholism (Buckholtz, 1980; Bosin et al., 1986; Myers, 1989). Alcohol consumption increased in rats following the infusion of 1-methyl-1,2,3,4-tetrahydro-β-carboline (Toumisto et al., 1982).

1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid has been reported as being a precursor of mutagenic *N*-nitroso compounds (Wakabayashi et al., 1983). Valin et al. (1985) studied the mutagenicity of some THβC after nitrosation reaction. It has been postulated that THβC could react with nitrite already in foods, in the mouth, or in the stomach, giving rise to mutagenic compounds. Salvi and Choughuley (1990) reported on the nitrosation of some food-related tetrahydro-β-carboline-3-carboxylic acids to form mutagenic compounds.

Endogenous formation of THβC after ingestion of alcohol has been pointed out (Myers 1989). THβC have also been reported in different foodstuffs such as cheese, soy sauce, smoked foods, and also human milk (Adachi et al., 1991; Papavergou and Clifford, 1992) and in alcoholic fermentation products such as sake, beer, wine, and distillates (Beck and Holmstedt, 1981; Beck et al., 1983; Bosin et al., 1986; Adachi et al., 1991; Herraiz et al., 1993). The ingestion of these products could increase the level of THβC in the human diet.

Herraiz et al. (1993) reported the concentration ranges of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-COOH (*SS*-MTCA and *RS*-MTCA) as well as 1,2,3,4-tetrahydro-β-carboline-3-COOH (THCA) in different wines and distillates. The same authors suggested that chemical and technological factors during the winemaking process should influence the formation of tetrahydro-β-carboline-3-COOH. The aim of this paper is to study the factors determining the content of tetrahydro-β-carboline-3-carboxylic acids in fermented alcoholic beverages. To this goal, decisive variables such as tryptophan and acetaldehyde profiles, grape variety, pH, temperature, storage time, yeast, and sulfur dioxide are considered in order to understand and control the formation of the THβC compounds during the winemaking process.

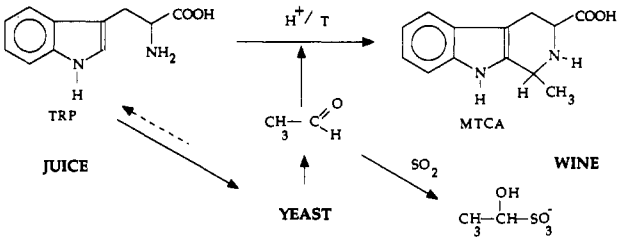

Figure 1. Proposed scheme for formation of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-COOH (MTCA) during alcoholic fermentation.

MATERIALS AND METHODS

Tetrahydro-β-carboline-3-carboxylic Acid Used as Reference. 1-Methyl-1-2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) and L-tryptophan were purchased from Sigma. The racemic mixture of (-)-(1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (*SS*-MTCA) and (-)-(1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (*RS*-MTCA) was prepared from L-tryptophan and acetaldehyde (Jacobs and Craig, 1936; Brossi et al., 1973). 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid (THCA) was synthesized in the same manner from L-tryptophan and formaldehyde, as was 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid from L-tryptophan and propionaldehyde. Acetaldehyde, formaldehyde, and propionaldehyde were obtained from Fisher, Baker Chemicals, and Kodak, respectively. Confirmation of structures was carried out by ^{1}H NMR and ^{13}C NMR and by GC–MS of the corresponding *N*-trifluoroacetyl carboxylic acid methyl ester derivatives.

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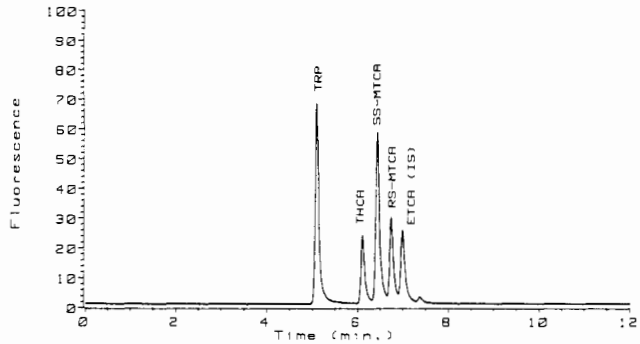

Figure 2. HPLC chromatogram of a standard solution containing tryptophan (Trp), 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA), (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (*SS*-MTCA), (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (*RS*-MTCA), and 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (ETCA). ODS column; flow, 0.45 mL/min; solvent A, 50 mM ammonium phosphate (pH 3); solvent B, 20% v/v of A in acetonitrile; program, 95% A to 40% A from 0 to 10 min, 100% B at 12 min; fluorescence detection, 270 nm for excitation and 343 nm for emission.

Samples Analyzed for Tetrahydro-β-carboline-3-COOH. Ethanolic model solutions with tryptophan and acetaldehyde and/or formaldehyde were analyzed in duplicate for tetrahydro-β-carboline-3-COOH content to establish the factors influencing the formation of these compounds:

(*A*) To determine the effect of pH, 50-mL quantities of a solution of 25 mg/L tryptophan (10% v/v ethanol) were adjusted at the corresponding pH of 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, or 10, previous to the addition of acetaldehyde at 50 mg/L. Samples were attemperated at 25 °C.

(*B*) To determine the effect of storage temperature, bottles containing 50 mL of the above ethanolic solution (at 25 mg/L tryptophan and 50 mg/L acetaldehyde, pH 3.5) were stored at the following temperatures −2, 12, 25, 40, and 80 °C.

(*C*) To check the influence of the addition of sulfur dioxide, the same ethanolic solution was treated with potassium metabisulfite ($K_2S_2O_5$) to concentrations of 0 (control), 25, 50, 100, and 200 mg/L SO_2.

(*D*) Finally, the effect of time of storage in the presence of tryptophan and acetaldehyde or formaldehyde on the formation of THβC was also determined for MTCA and THCA.

The formation of tetrahydro-β-carboline-3-carboxylic acid in grape juice fermentation was studied using the following procedure. Grapes from the varieties Semillon, Sauvignon blanc, Chardonnay, Petite Shiraz, Cabernet Sauvignon, Chenin blanc, Muscat blanc, Thompson Seedless, Pinot noir, and Carmine were harvested in the vintage of 1992 from the Department of Viticulture and Enology, University of California, Davis, vineyard. The grapes were crushed and the juices rapidly frozen to −20 °C to avoid any formation of tetrahydro-β-carboline-3-COOH. No sulfur dioxide was used in the preparation. Duplicate fermentations of the grape juices in 100-mL quantities were carried out in 250-mL Erlenmeyer flasks at 17 °C with active dry yeast *Saccharomyces cerevisiae* (Montrachet) (Red Star, Universal Food Corp., Milwaukee). Inoculations were at 1×10^6 cells/mL, calculated from known viable yeast count of the dry yeast. Five-milliliter samples were taken throughout and after the fermentation to determine the test compound. Final wines were racked at day 17 and stored at 4 °C in the refrigerator.

To determine the influence of sulfur dioxide added before fermentation, a Sauvignon blanc grape juice was used. Five-hundred-milliliter Erlenmeyer flasks with 300 mL of grape juice were previously adjusted with tryptophan at 25 mg/L and diammonium phosphate, 2 g/L. Then, sulfur dioxide was added to give final concentrations of 75 and 150 ppm of SO_2 before the addition of the active dry yeast as previously described. Fermentations were carried out at 17 °C in duplicate. In addition, a Chardonnay grape juice known to produce a wine with a high concentration of tetrahydro-β-carboline-3-COOH was fermented in test tubes containing 15 mL of juice and 0 (control), 25, 50, 100, or 150 mg/L of sulfur dioxide. The fermentation was carried out at 17 °C in duplicate.

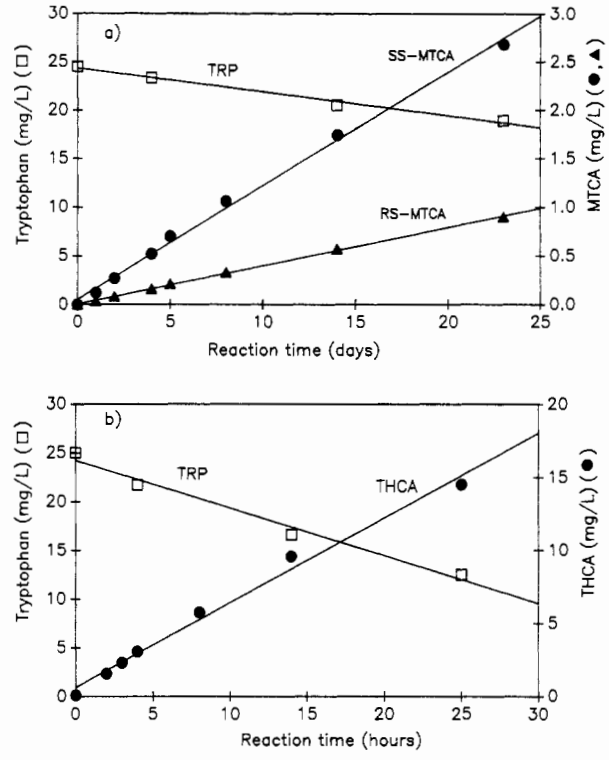

Figure 3. Dependence of MTCA (a) and THCA (b) formation and tryptophan removal with time. Ethanol solution (10% v/v) containing 25 mg/L tryptophan and 50 mg/L acetaldehyde (for MTCA) or 50 mg/L formaldehyde (for THCA) (pH 3.5) was kept at 25 °C. Points are average of duplicates.

Isolation and Analysis of Tetrahydro-β-carboline-3-COOHs. For grape juices and wines, aliquots of 5 mL were spiked with 1 mL of the internal standard, 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (ETCA) solution (10 μg/mL). Semicarbazide hydrochloride (Sigma) was added at 1 mg/mL to avoid any formation of artifacts as previously indicated (Bosin et al., 1986). The sample was acidified to pH 2–2.5 with some drops of 1 N hydrochloric acid and loaded onto 3-mL volume SCX cartridges (Bond Elut Varian, Harbor City, CA) (Adachi et al., 1991). The column conditioning procedure and the elution of tetrahydro-β-carboline-3-carboxylic acid have been described (Adachi et al., 1991; Herraiz et al., 1993). Ethanolic model solutions were not passed through SCX columns but were analyzed by injection onto the HPLC column immediately after the internal standard (ETCA) and the semicarbazide were added.

A Hewlett-Packard (Santa Clara, CA) 1090 HPLC apparatus with a fluorescent detector (Hewlett-Packard 1046A) and a Hewlett-Packard 9000 Chem-Station, for data processing, were used for analysis of the tetrahydro-β-carboline-3-carboxylic acids. An ODS Hypersil microbore column (200 × 2.1 mm, 5-μm particle size, Hewlett-Packard) was used for the separation of the tetrahydro-β-carbolines. Column oven temperature was kept at 40 °C for all separations. Fluorescence detection was carried out at 270 nm for excitation and 343 nm for emission. Chromatographic conditions for elution of those compounds, as well as calibration curves for quantitative results, were established as per Herraiz et al. (1993).

Identification of tetrahydro-β-carboline-3-COOHs in the HPLC chromatograms was by retention time and co-injection with known standards. Identification in wines was carried out by GC–MS of the *N*-trifluoroacetyl carboxylic acid methyl ester derivatives of tetrahydro-β-carboline-3-carboxylic acid by both total ion and single ion monitoring.

Analysis of Tryptophan and Acetaldehyde. Tryptophan was analyzed by direct injection of the sample after addition of ETCA as internal standard and following the same chromatographic procedure reported for tetrahydro-β-carboline-3-COOHs. Acetaldehyde analysis was carried out by gas chromatographic analysis (Herraiz et al., 1993).

Table I. Formation of MTCA and THCA from Tryptophan and Acetaldehyde at Different Temperatures of Storage[a]

	SS-MTCA (mg/L)					*RS*-MTCA (mg/L)					THCA (mg/L)				
days	−2 °C	12 °C	25 °C	40 °C	80 °C	−2 °C	12 °C	25 °C	40 °C	80 °C	−2 °C	12 °C	25 °C	40 °C	80 °C
2	0.03	0.10	0.27	0.52	1.9	0.0	0.03	0.08	0.16	0.95	0.0	0.0	0.0	0.0	0.093
5	0.09	0.28	0.70	1.3	4.0	0.02	0.08	0.21	0.48	2.06	0.0	0.0	0.0	0.0	0.34
8	0.15	0.47	1.08	2.0	5.8	0.03	0.14	0.34	0.77	2.96	0.0	0.0	0.0	0.0	0.52
14	0.27	0.83	1.82	3.3	7.7	0.06	0.25	0.60	1.27	3.97	0.0	0.0	0.0	0.017	0.687
23	0.44	1.3	2.8	4.6	9.0	0.11	0.36	0.95	1.81	4.67	0.0	0.0	0.013	0.032	1.2

[a] Model solution containing 25 mg/L Trp and 50 mg/L acetaldehyde in 10% v/v ethanol (pH of model solution was 3.5). Results are the average of duplicates. (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (*SS*-MTCA); (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (*RS*-MTCA); 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA).

RESULTS

Formation of tetrahydro-β-carboline-3-carboxylic acid was studied in ethanolic model solutions containing tryptophan, acetaldehyde, and/or formaldehyde. Figure 2 shows a HPLC chromatogram of the standards utilized in this study. As shown in Figure 3a, the condensation reaction between tryptophan and acetaldehyde by both stereoisomers was time dependent at the same temperature and pH. THCA formation rate is also time dependent and faster than that of MTCA (Figure 3b). Moreover, although not shown in Figure 3, if acetaldehyde and formaldehyde were both present at the same concentration in model solutions with tryptophan, no significant amount of MTCA and predominantly THCA were produced due to the faster formation rate of THCA. Formation of both MTCA and THCA was accompanied by a reduction in the content of tryptophan as shown in Figure 3. 1,2,3,4-Tetrahydro-β-carboline-3-COOH content increases with the amounts of precursors (Herraiz et al., 1993), and controls that lacked one or both precursors, aldehydes or tryptophan, did not form tetrahydro-β-carbolines.

Reaction rate increases with the temperature of storage (Table I). The highest formation of MTCA in both stereoisomers was produced when solutions of tryptophan and acetaldehyde were stored at 42 and 80 °C, whereas very low amounts were formed at −2 and 12 °C. Some formation of THCA occurred at higher temperatures. THCA was not a product of degradation of MTCA as demonstrated when a solution of MTCA was kept at 80 °C for several days. Formation of trace amounts also occurred when a tryptophan solution was heated without added acetaldehyde or formaldehyde.

Formation of MTCA increased rapidly at lower pH (Figure 4). Below pH 3 the reaction rate is very fast, whereas at pH 4 and higher the reaction is very slow; above pH 6 practically no formation of MTCA could be detected in 20 days at 25 °C. Calculating the amount of MTCA produced on average per day as a sum of both isomers, approximately 820 days are needed to reach 10 mg/L at pH 5, 227 days at pH 4, 24 days at pH 3, 6.4 days at pH 2, and 5.7 days at pH 1.5 (Figure 5).

Sulfur dioxide significantly decreases or avoids the formation of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-COOH (Table II). Sulfur dioxide reacts with acetaldehyde to form hydroxyethanesulfonate (Ough and Amerine, 1988), avoiding further reaction of acetaldehyde with tryptophan. In addition, sulfur dioxide reacted with tryptophan, decreasing its concentration in the ethanolic solution (Figure 6). During heating at 80 °C for several days, the reaction rates of the adduct hydroxyethanosulfonate and the free aldehyde and sulfur dioxide (between free ⇄ bound) increased. As the free acetaldehyde reacted with the available tryptophan, more free acetaldehyde was released more rapidly at the elevated temperature. This explains the increase in MTCA again, as long as tryptophan was still available (solutions with 25 and 50 ppm of sulfur dioxide).

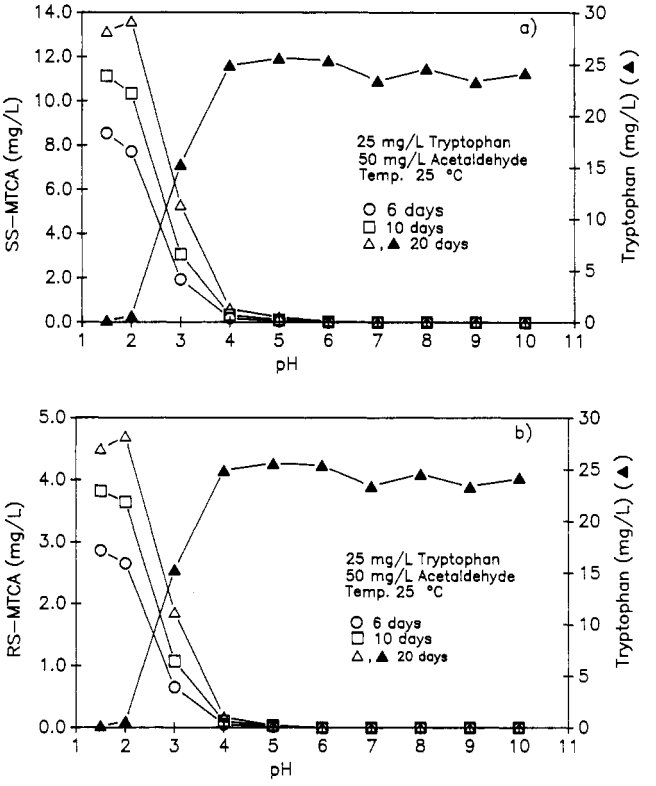

Figure 4. Formation of *SS*-MTCA (a) and *RS*-MTCA (b) from tryptophan (Trp) and acetaldehyde depending on the pH. Ethanol solution was 10% v/v with tryptophan at 25 and 50 mg/L acetaldehyde with storage at 25 °C. Points are average of duplicates.

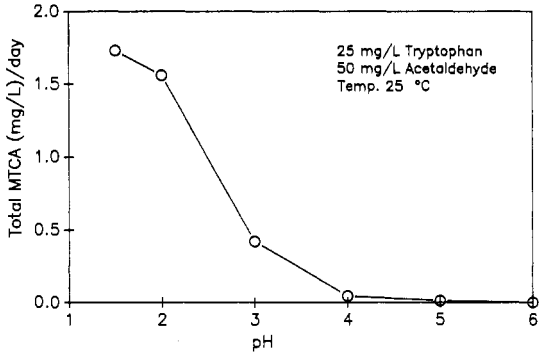

Figure 5. MTCA as sum of both stereoisomers produced per day vs the pH (same solution as in Figure 3). Points are average of the analysis after 6 days and 10 days.

Formation of tetrahydro-β-carboline-3-COOHs was followed during the fermentation of grape juices. Table III lists tryptophan and tetrahydro-β-carboline-3-COOH concentrations in the grape juices and in the resultant wines. Slight amounts of MTCA were found in grape juices, probably as a consequence of reaction with some acetaldehyde already present. Generally, formation of MTCA was produced during the fermentation as a result of the

Table II. Formation of MTCA from Tryptophan and Acetaldehyde in the Presence of SO_2[a]

	SS-MTCA (mg/L)				RS-MTCA (mg/L)				Trp (mg/L)			
days	[SO_2] = 0 mg/L	[SO_2] = 25 mg/L	[SO_2] = 50 mg/L	[SO_2] = 100 mg/L	[SO_2] = 200 mg/L	[SO_2] = 0 mg/L	[SO_2] = 25 mg/L	[SO_2] = 50 mg/L	[SO_2] = 100 mg/L	[SO_2] = 200 mg/L	[SO_2] = 0 mg/L	[SO_2] = 25 mg/L	[SO_2] = 50 mg/L	[SO_2] = 100 mg/L	[SO_2] = 200 mg/L
4	0.54	0.016	0.0	0.0	0.0	0.15	0.0	0.0	0.0	0.0	23.0	23.9	18.5	9.1	4.3
8	1.08	0.05	0.0	0.0	0.0	0.34	0.0	0.0	0.0	0.0	21.4	22.7	14.6	2.7	0.9
14	1.87	0.12	0.0	0.0	0.0	0.60	0.0	0.0	0.0	0.0	20.4	22.4	11.4	0.8	0.3
21	2.60	0.21	0.0	0.0	0.0	0.90	0.0	0.0	0.0	0.0	19.0	22.8	10.5	0.5	0.0
21 + 1[b]	3.47	0.62	0.11	0.0	0.0	1.32	0.23	0.04	0.0	0.0	17.0	14.8	3.6	0.0	0.0
21 + 5[c]	5.51	2.13	0.58	0.0	0.0	2.38	0.99	0.24	0.0	0.0	13.2	6.1	1.4	0.0	0.0

[a] Model solution with 25 mg/L Trp and 50 mg/L acetaldehyde in 10% v/v ethanol stored at 25 °C (pH of model solution was 3.5). Results are the average of duplicates. SS-MTCA and RS-MTCA are as in Table I. [b] One day at 80 °C after 21 days at 25 °C. [c] Five days at 80 °C after 21 days at 25 °C.

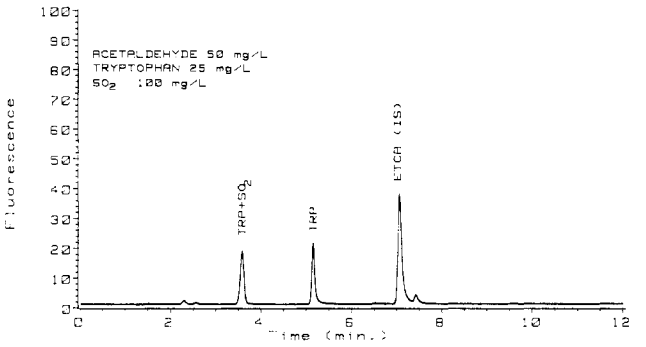

Figure 6. HPLC chromatogram of an ethanol solution (10% v/v) containing 25 mg/L tryptophan, 50 mg/L acetaldehyde, and 100 mg/L sulfur dioxide (pH 3.5) stored at 25 °C for 8 days. TRP+SO_2 was a peak detected only in the presence of tryptophan and sulfur dioxide.

formation of acetaldehyde by the yeast. Most of the wines studied had low concentrations of MTCA compared to our previous results (Herraiz et al., 1993). This could result from the different conditions of fermentation. Nevertheless, Chardonnay wines reached 5.15 and 1.42 mg/L of *SS*-MTCA and *RS*-MTCA, respectively (Figure 7).

As expected, a higher concentration of tryptophan in the grape juice (Chardonnay and Thompson Seedless) resulted in more MTCA in the wine. However, big differences could be found depending upon the grape juices. Figure 8 shows the changes in the concentration of tryptophan, acetaldehyde, and *SS*-MTCA during fermentation. Tryptophan decreased rapidly during the first period of fermentation (3 days) in four of the grape juices. However, tryptophan remained at a relatively high concentration in Chardonnay juice until late in the fermentation. Thus, only Chardonnay wine fermentation had enough tryptophan to give rise to a very high level of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-COOH after reaction with acetaldehyde being formed during the alcoholic fermentation.

As previously shown in model solutions, sulfur dioxide decreased MTCA production during storage. Sulfur dioxide addition before the fermentation also decreased the level of MTCA in the wine. Fermentation of a Sauvignon blanc grape juice in the presence of 75 and 150 ppm of SO_2 gave a significant reduction in MTCA formation but did not prevent its formation (Figure 9). Yeast growth and fermentation were delayed in the presence of SO_2, and no MTCA was formed until the beginning of the yeast growth and fermentation when the excretion of acetaldehyde occurred. As before, tryptophan was rapidly taken up by the yeast. Analysis of acetaldehyde in the final wines gave 116 mg/L for control, whereas 55 mg/L was seen with 150 ppm of SO_2 added. A similar trend of decreasing MTCA was observed when a Chardonnay grape juice was fermented with an increasing amount of sulfur dioxide (Figure 10).

DISCUSSION

In a previous paper, Herraiz et al. (1993) reported the range of concentration of tetrahydro-β-carboline-3-COOH in grape juices, wines, and distillates. On average, total contents of tetrahydro-β-carboline-3-COOH for grape juices and red, white, sparkling, sherry, and port wines were 0.23, 1.3, 2.9, 3.9, 4.8, and 6.8 mg/L, respectively, whereas distillates, other than old brandy from sherry, did not have measurable tetrahydro-β-carboline-3-COOH. The results reported here highlight the factors that are determinant.

Although formation of tetrahydro-β-carboline-3-COOH is time dependent, the reaction between tryptophan and

Table III. Concentration (Milligrams per Liter) of Acetaldehyde, Tryptophan, and Tetrahydro-β-carboline-3-COOH in Grape Juices and Corresponding Wines[a]

	grape juices			wines					
	Trp	*SS*-MTCA	acetaldehyde	Trp	THCA	*SS*-MTCA	*RS*-MTCA	acetaldehyde	pH
Muscat blanc	7.5	0.19	nd[b]	0.0	0.0	0.24	0.07	217	3.15
Chardonnay	30.3	0.48	9.8	1.0	0.02	5.15	1.42	197	3.45
Semillon	12.5	0.09	nd	0.0	0.0	0.26	0.07	191	3.5
Carmine	2.0	0.02	nd	0.0	0.0	0.024	0.0	220	3.1
Chenin blanc	1.5	0.0	nd	0.0	0.0	0.02	0.0	147	3.0
Petite Shiraz	6.8	0.27	9.4	0.43	0.0	0.41	0.12	86	3.4
Thompson Seedless	27.6	0.34	16.0	0.19	0.003	0.72	0.19	246	3.2
Pinot noir	2.3	0.18	nd	0.0	0.0	0.22	0.06	230	2.9
Cabernet Sauvignon	0.3	0.04	nd	0.0	0.0	0.04	0.0	80	3.2
Sauvignon blanc	2.0	0.42	16.7	0.01	0.004	0.66	0.18	132	3.2

[a] *SS*-MTCA, *RS*-MTCA, and THCA are as in Table I. [b] nd, not determined.

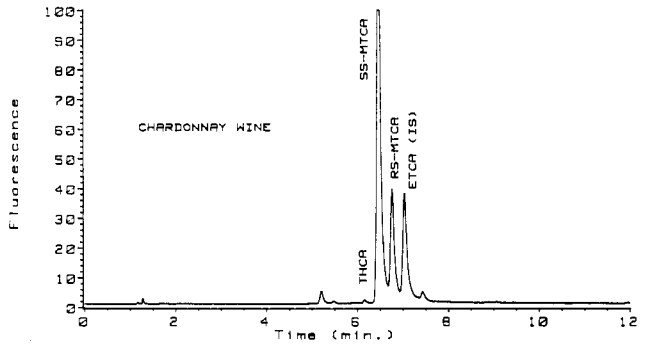

Figure 7. HPLC chromatogram of tetrahydro-β-carboline-3-COOH in Chardonnay wine.

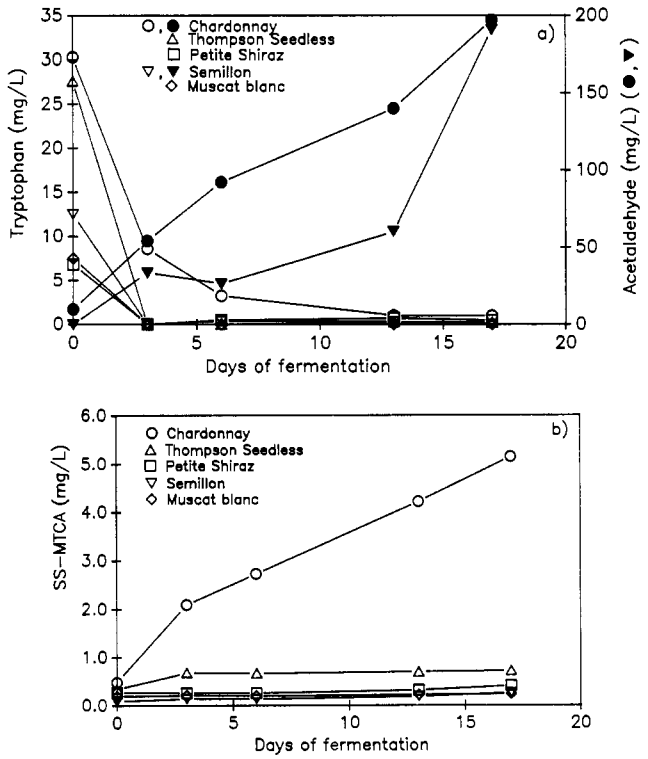

Figure 8. Content of tryptophan and acetaldehyde (a) and *SS*-MTCA (b) during fermentation of grape juices with *S. cerevisiae*.

aldehydes is relatively fast at room temperature. So, after fermentation, only small differences in the content between aged and young wines, kept at room temperature after several months, are expected. Storage at higher temperatures, as may happen in sherry and port wines, could accelerate the formation of those compounds if tryptophan and acetaldehyde are present. Lower temperatures decrease the reaction rates. The reaction rate is faster to form THCA than MTCA, but formaldehyde concentration in wine is much lower than acetaldehyde and, consequently,

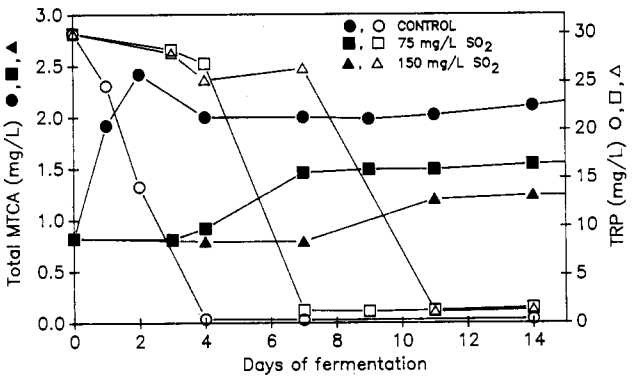

Figure 9. Content of tryptophan and MTCA as sum of both isomers and tryptophan during fermentation with *S. cerevisiae* of a Sauvignon blanc grape juice in the absence or presence of sulfur dioxide. Points are average of duplicates.

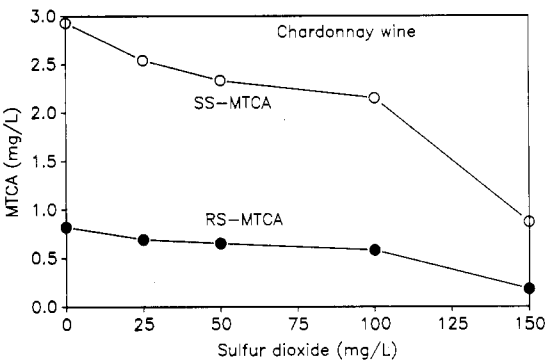

Figure 10. MTCA content in a Chardonnay wine fermented with *S. cerevisiae* in the presence of increasing amounts of sulfur dioxide. Points are average of duplicates.

THCA is found at a lower concentration than MTCA (Adachi et al., 1991; Herraiz et al., 1993).

Low pH sharply increases the reaction rate, indicating that condensation between tryptophan and acetaldehyde is highly acid catalyzed. The higher average content of MTCA in white wines, compared to red wines, could be a result of lower pH in white grape juices and white wines than in red wines. Neutral or basic pH that would increase nucleophilic attack on acetaldehyde does not cause cyclization of tetrahydro-β-carboline-3-COOH. It might indicate that *in vivo* formation, after consumption of ethanol, should not proceed rapidly if one considers the physiological pH. Minor amounts could form in the stomach with its relatively low pH, but at blood pH level any formation would be extremely slow. Part of the tetrahydro-β-carbolines detected after consumption in the blood plasma and urine of alcoholics or healthy humans (Rommelspacher and Schmidt, 1985; Myers, 1989; Adachi et al., 1991) could come from the ingestion of foods and beverages containing those compounds.

Tryptophan concentration in the grape juice and its pattern of uptake and release by the yeast determine the content of tetrahydro-β-carboline-3-COOHs in wine. Rapid formation of acetaldehyde when tryptophan is still available is also a major factor.

Amino acid content of the juice decreases early in the fermentation and increases slightly at the end in the final wine (Kluba et al., 1978; Herraiz and Ough, 1993). In this regard, uptake and excretion of tryptophan by yeasts will depend on the amino acid composition of the grape juice as well as fermentation conditions and yeast strain. Acetaldehyde formation will also depend upon fermentation conditions. Winemaking practices, such as "sur lie" aging in sparkling wines, which favors autolysis of the yeast, or sweet wine production in which fermentation is not concluded could increase tryptophan and acetaldehyde in the wine and therefore increase the amount of tetrahydro-β-carboline-3-COOH.

Sulfur dioxide, traditionally used as a preservative and antioxidant in winemaking, reduces the formation of these compounds. Bisulfite anion (HSO_3^-) reacts with aldehydes to form hydroxysulfonates, minimizing formation of tetrahydro-β-carboline-3-COOH during storage if tryptophan remains. Sulfur dioxide used before fermentation reacts with free acetaldehyde produced by yeasts, giving hydroxyethanesulfonate adduct at wine pH (Ough and Amerine, 1988). The equilibrium is very much in favor of the bound form. Using no sulfur dioxide before the fermentation produces wines of relatively higher free acetaldehyde, enabling the reaction to give tetrahydro-β-carboline-3-COOH. In this regard, sherry and dessert wines have generally more acetaldehyde than table wines and more tetrahydro-β-carboline-3-COOH as well (Herraiz et al., 1993). On the other hand, SO_2 could react with tryptophan, removing it from the medium. Sundberg (1970) reported the nucleophilic attack of bisulfite present in ethanolic solution on position 2 of the indole ring, whereas Yang (1973) reported the oxidative destruction of tryptophan in the presence of sulfite. In this work, a chromatographic peak was evidenced after reaction of sulfur dioxide and tryptophan.

Finally, small amounts of tetrahydro-β-carboline-3-COOH are expected in the juices since tryptophan and small amounts of acetaldehyde, either from the whole grape or produced during crushing, are present. Adachi et al. (1991) reported some amounts of tetrahydro-β-carboline-3-COOH in raw materials still to be fermented. Most distillates should not have these compounds due to the inability of tetrahydro-β-carboline-3-COOH and tryptophan to distill.

CONCLUSIONS

Formation of 1,2,3,4-tetrahydro-β-carboline-3-COOH is time dependent and increases substantially with the concentration of tryptophan and aldehydes. Such formation is acid catalyzed, increasing sharply at lower pH, whereas little or no formation occurred at pH higher than 6. Higher temperatures of storage favor MTCA formation, whereas lower temperatures substantially decrease the reaction rate.

The content of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-COOH in wine depends essentially on the pattern of uptake of tryptophan by the yeasts along with the release of acetaldehyde during the alcoholic fermentation. Excretion of tryptophan at the end of the fermentation could also be a second factor. To reduce the level of THβC in wines, tryptophan and acetaldehyde content could be controlled before, during, and after fermentation. Grape variety, fermentation conditions, additions, and other variables such as yeast strain could be chosen to reach this goal.

Sulfur dioxide reduces the amount of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-COOH in wine by reacting with acetaldehyde excreted by the yeasts as well as with tryptophan. Sulfur dioxide removes precursors that condense to give rise to tetrahydro-β-carboline-3-COOH.

Normal wines have a lower content of THCA relative to MTCA because of the very low content of formaldehyde in wine, despite the reaction rate being much faster to form THCA than MTCA.

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Received for review January 19, 1993. Accepted March 22, 1993.