Occurrence of Tetrahydro-β-carboline-3-carboxylic Acids in Commercial Foodstuffs

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The occurrence of tetrahydro-β-carboline-3-carboxylic acids (THβC-3-COOHs) in foodstuffs was investigated. Spectral and chromatographic data showed the occurrence of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) in foodstuffs. The content of THβC-3-COOHs given as a sum of both THCA and MTCA was as follows: soy sauce, 94−517 mg/L; seasoning, 0.75−32.8 μg/g; table wine, 1.7−6.6 mg/L; beer, 0.3−17.9 mg/L; cider, 0.06−0.2 mg/L; liquor, 0−7.6 mg/L; wine vinegar, 3.9−9.7 mg/L; cider vinegar, 0.19−1 mg/L; yogurt, 0.05−0.15 μg/g; cheese, 0−3.4 μg/g; soft drinks, 0−0.45 mg/L; fruit juices, 0.1−5.1 mg/L; smoked fish, 0.08−0.4 μg/g; and bread, 0.16−3 μg/g. Usually MTCA was the major substance within THβC-3-COOHs, but bread and smoked fish contained more THCA. Experiments in which foodstuffs were spiked with formaldehyde and acetaldehyde proved the chemical formation of THCA and MTCA, respectively. It is concluded that the exogenous intake of these substances during the human ingestion of foods may be partially responsible of the reported endogenous presence of THβC-3-COOHs in the human biological tissues and fluids.

Keywords: *Tetrahydro-β-carboline-3-carboxylic acids; β-carbolines; L-tryptophan; foods; drinks; content; formation*

INTRODUCTION

1,2,3,4-Tetrahydro-β-carbolines (THβC) (also known as 1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indoles) are formed from indole ethylamines and aldehydes or α-keto acids through Pictet−Spengler chemical condensation. 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acids (THβC-3-COOHs) are produced by reaction of tryptophan and aldehydes (Figure 1). This reaction readily occurs even under mild conditions, and the rate is temperature and pH dependent (Herraiz and Ough, 1993).

Research in the last decade has pointed out the occurrence of THβCs and β-carbolines under physiological conditions in biological tissues and fluids (Myers, 1989; Rommelspacher et al., 1991; Adachi et al., 1991b). These compounds can function as neurotransmitters or neuromodulators via their effect on the monoamine oxidase and have been reported to form endogenously in the brain (Buckholtz, 1980; Myers, 1989). Furthermore, they have been increasingly implicated in alcoholism (Cohen and Collins, 1970; Beck et al., 1982; Rommelspacher and Schmidt, 1985; Bosin et al., 1986; Myers, 1989; Rommelspacher et al., 1990). Administration of THβC to rats has been reported to significantly alter alcohol consumption (Tuomisto et al., 1982). Tetrahydro-β-carbolines can also be postulated as possible precursors of β-carboline-3-carboxylate, a benzodiazepine receptor antagonist (Braestrup et al., 1980).

1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) has been described as a precursor of mutagenic *N*-nitroso compounds when tested in the Ames test using *Salmonella typhimurium* (Wakabayashi et al., 1983, 1984). Valin et al. (1985) studied the mutagenicity of some THβCs after a nitrosation reaction. THβC could react with nitrite already in foods, the mouth, or the stomach, giving rise to mutagenic compounds. Salvi and Choughuley (1990) and Sen et al. (1991, 1995) reported the nitrosation of some food-related THβC-3-COOHs.

MTCA may be implicated in the etiology of the eosinophilia−myalgia syndrome (EMS) associated with the ingestion of impure L-tryptophan that occurred in the United States in 1989 (Sakimoto, 1990; Adachi et al., 1991b). Initially, one impurity, identified as 1,1′-ethylidenebis(tryptophan) (EBT) was suggested to cause the disease (Mayeno et al., 1990; Belongia et al., 1990). However, EBT is converted to MTCA in acid solution such as the gastric fluid (Sakimoto, 1990; Ito et al., 1992). Recently, Brenneman et al. (1993) have reported that the diastereoisomer (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ((1*S*,3*S*)-MTCA) may cause neuronal death, playing a role in the etiology of some of the neurophatic features of L-Trp-EMS. Their results show that (1*S*,3*S*)-MTCA exhibits a pharmacological action on neuronal survival. In this regard full delineation of the biological activities and toxicity of the tetrahydro-β-carboline derivatives is important (Brenneman et al., 1993).

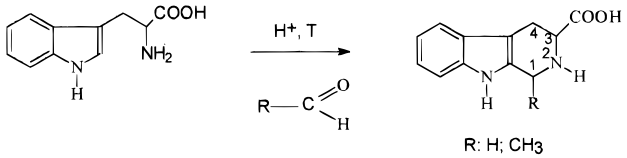

Figure 1. Scheme of the Pictet−Spengler reaction between L-tryptophan and aldehydes giving rise to 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THβC-3-COOH) (R = H and CH_3 corresponding to formaldehyde and acetaldehyde, respectively).

The occurrence of THβCs has also been described in several foodstuffs. THβC-3-COOHs were reported in soy sauce (Wakabayashi et al., 1983), smoked foods including fish, meat, and cheese (Papavergou and Clifford, 1992; Sen et al., 1995), and beer and wine (Bosin et al., 1986; Adachi et al., 1991a; Herraiz et al., 1993). Herraiz et al. (1993) determined the content of THβC-3-COOHs in table, sparkling, sherry, and port wines and in distillates and grape juices. Ways to reduce their content, by controlling the chemical and technological factors that influence their formation, have been suggested (Herraiz and Ough, 1993). Other THβCs, such

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as 6-hydroxy-1-methyl-1,2,3,4-THβC, were reported in beer and wine (Beck et al., 1983), while 1-phenyl-THβC-3-COOH and 1-salicyl-THβC-3-COOH have been reported in smoked foods (Papavergou and Clifford, 1992).

Owing to the very diverse biological and pharmacological actions of these compounds, it is necessary to accomplish extensive research considering nutritional, technological, and pharmacological approaches. The ingestion of foods containing high levels of THβC-3-COOHs could increase the level of these compounds in the human diet. Thus, hypothetically, an important part of THβC-3-COOHs detected in biological tissues could arise from the human diet. In this regard, the aim of this paper is to study the occurrence and the content of various THβC-3-COOHs in several common foodstuffs and beverages. The relative content of these compounds and possible chemical and technological factors influencing their formation in foodstuffs are also discussed.

MATERIALS AND METHODS

Reference Compounds. MTCA (ca. 98% by HPLC) was purchased from Sigma (St. Louis, MO). It contains a 16:1 diastereoisomeric mixture of (1*S*,3*S*)-MTCA and (1*R*,3*S*)-MTCA of known absolute configuration (Yamada and Akimoto, 1969; Brossi et al., 1973; Adachi et al., 1991a). The mixture of (−)-(1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ((*SS*)-MTCA) (major component) and (−)-(1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ((*RS*)-MTCA) (minor component) was also synthesized from L-tryptophan and acetaldehyde according to Brossi et al. (1973). In the same manner, 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (ETCA) and 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA) were prepared from L-tryptophan and propionaldehyde or formaldehyde, respectively, based on the procedure of Brossi et al. (1973). Data of NMR, MS (probe sample), and GC−MS (trifluoroacetyl and methoxycarbonyl methyl ester derivatives) were consistent with the structures of the synthesized compounds (Herraiz and Ough, 1994; Herraiz and Sanchez, 1996).

Samples Analyzed for THβC-3-COOHs. One hundred twenty-two different commercial samples of foodstuffs and beverages (Tables 1 and 2) were analyzed for THβC-3-COOHs. The samples were collected from local supermarkets and were from both national and imported origin.

Isolation of THβC-3-COOHs in Foodstuffs and Beverages. (a) Isolation of THβC-3-COOHs in beverages and liquid samples followed a previously published procedure (Adachi et al., 1991a; Herraiz et al., 1993). Thus, aliquots of 5−10 mL of beer, wine, vinegar, centrifuged juice, sangria, or liquor were spiked with 0.5 mL of ETCA solution (5 mg/L), used as internal standard (IS). Soy sauces were previously diluted (1:15) with HPLC water. Semicarbazide (Sigma) was added to the samples at 1 mg/mL to prevent artifactual formation (Bosin et al., 1986). The samples were acidified to pH 2 with 1 N HCl and loaded onto benzenesulfonic acid-derivatized silica gel SCX columns (Bond Elut, 3 mL size, Varian, Harbor City, CA). SCX columns were previously conditioned with 6 mL of methanol and 6 mL of 0.1 N HCl. The samples passed at a flow rate of 1 mL/min using a vacuum manifold. Then, the columns were immediately washed with 6 mL of 0.1 N HCl, 2 mL of methanol, and 6 mL of HPLC water and rinsed carefully with 2 mL of 0.4 M phosphate buffer, pH 9.1. THβC-3-COOHs were eluted with a 4 mL mixture containing methanol and 0.4 M phosphate buffer (1:1) adjusted to pH 9.1. The eluates were injected into the HPLC.

(b) Samples (3−10 g aliquot) of sauces, others than soy sauce, cheese, milk, yogurt, bread, cured ham, smoked fish, canned cucumber, and canned olive, were homogenized using an Ultra-Turrax homogenizer with a known volume of 0.6 M $HClO_4$ (up to 20 mL) containing 1 mg/mL semicarbazide. The solution was immediately centrifuged (5100*g*) at 0−5 °C for 20 min. Then, the supernatant was filtered and a 5−10 mL aliquot used for solid phase extraction of THβC-3-COOHs as described above.

Preparation of *N*-(Methoxycarbonyl)-THβC-3-COOH Derivatives (N-MC-THβC-3-COOHs). Standards of *N*-(methoxycarbonyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (N-MC-THCA), *N*-(methoxycarbonyl)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (N-MC-(1*S*,3*S*)-MTCA and N-MC-(1*R*,3*S*)-MTCA), and *N*-(methoxycarbonyl)-1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (N-MC-ETCA) were prepared from the corresponding THβC-3-COOH standards following the procedure of Bosin and Jarvis (1985).

Aliquots of 0.5 mL of THβC-3-COOHs extracted from foods and beverages were evaporated with a stream of He up to ca. less than 0.2 mL and derivatized with methyl chloroformate ($ClCOOCH_3$) (Aldrich Chemical Co.) according to Bosin and Jarvis (1985); e.g., to the samples were added 0.5 mL of 1 M phosphate buffer, pH 7, 50 μL of a 10 mg/mL semicarbazide solution, and 50 μL of $ClCOOCH_3$. The mixture was vortexed for 1 min and allowed to stand for 5 min. Then, 0.25 mL of saturated sodium carbonate solution was added to the samples followed by the addition of 50 μL of $ClCOOCH_3$. The samples were vortexed and allowed to stand for 10 min. Finally the samples were placed under a He stream and injected into the HPLC.

Chromatographic and Quantitative Analyses of THβC-3-COOHs. (a) Analysis of THβC-3-COOHs by HPLC was carried out according to Herraiz et al. (1993) with minor modifications. The HPLC system (Hewlett Packard, Santa Clara, CA) consisted of a 1050 high-performance liquid chromatograph with a 1046A fluorescence detector coupled to a 3365 Series II HP Chemstation and a Rheodyne type injector (loop 20 μL). Separation of THβC-3-COOHs was performed using a 150 mm × 3.9 mm, 5 μm, Nova-pak C18 column (Waters, Milford, MA). Fluorescence detection was set at 270 nm for excitation and at 343 nm for emission. Chromatographic conditions were as follows: 50 mM ammonium phosphate buffer adjusted to pH 3 with phosphoric acid (buffer A) and 20% of A in acetonitrile (buffer B). Gradient programmed from 0% to 40% B in 10 min and then 100% B at 12 min and allowed to stand for 3 min. The column was equilibrated with solvent A before the next injection. The flow rate was 1 mL/min, and the oven temperature was 40 °C. THβC-3-COOHs eluted within 10 min.

Quantitative analysis of THβC-3-COOHs was calculated from calibration curves obtained with standard samples of known concentration ranging from 0 to 2.6 mg/L (THCA) and from 0 to 4.6 mg/L (MTCA) with five levels of concentration. These standard samples were carried through the entire cleanup procedure. Calibration graphs were constructed by plotting the peak area ratios of THCA and MTCA relative to ETCA (IS) against THCA and MTCA concentration. The concentration of THβC-3-COOHs in foodstuffs was calculated from the peak area ratios of each sample by reference to the calibration curves. Recoveries of THβC-3-COOHs were estimated by comparing the peak areas of extracted and nonextracted samples spiked with standards. The detection limit and the reproducibility of the analysis were also estimated.

(b) Analysis of N-MC-THβC-3-COOHs was performed with the same HPLC equipment, columns, and HPLC eluents. Chromatographic conditions for elution were linear gradient from 0% to 32% B in 8 min and then 90% B at 18 min; flow rate, 1 mL/min; injection volume, 20 μL; and column temperature, 40 °C.

(c) Confirmation of the identity of the isolated THβC-3-COOHs was accomplished by HPLC retention time and coinjection with known standards using the two analytical methods previously mentioned: with and without derivatization with methyl chloroformate. In addition, the fluorescence spectra of the HPLC peaks were compared with those of authentic THβC-3-COOHs in order to confirm the presence of these compounds in food samples. Eluting peaks at the same retention time as authentic THβC-3-COOHs were trapped into the flow cell of the fluorescence detector by stopping the solvent pump, and, subsequently, the excitation and emission spectra were obtained.

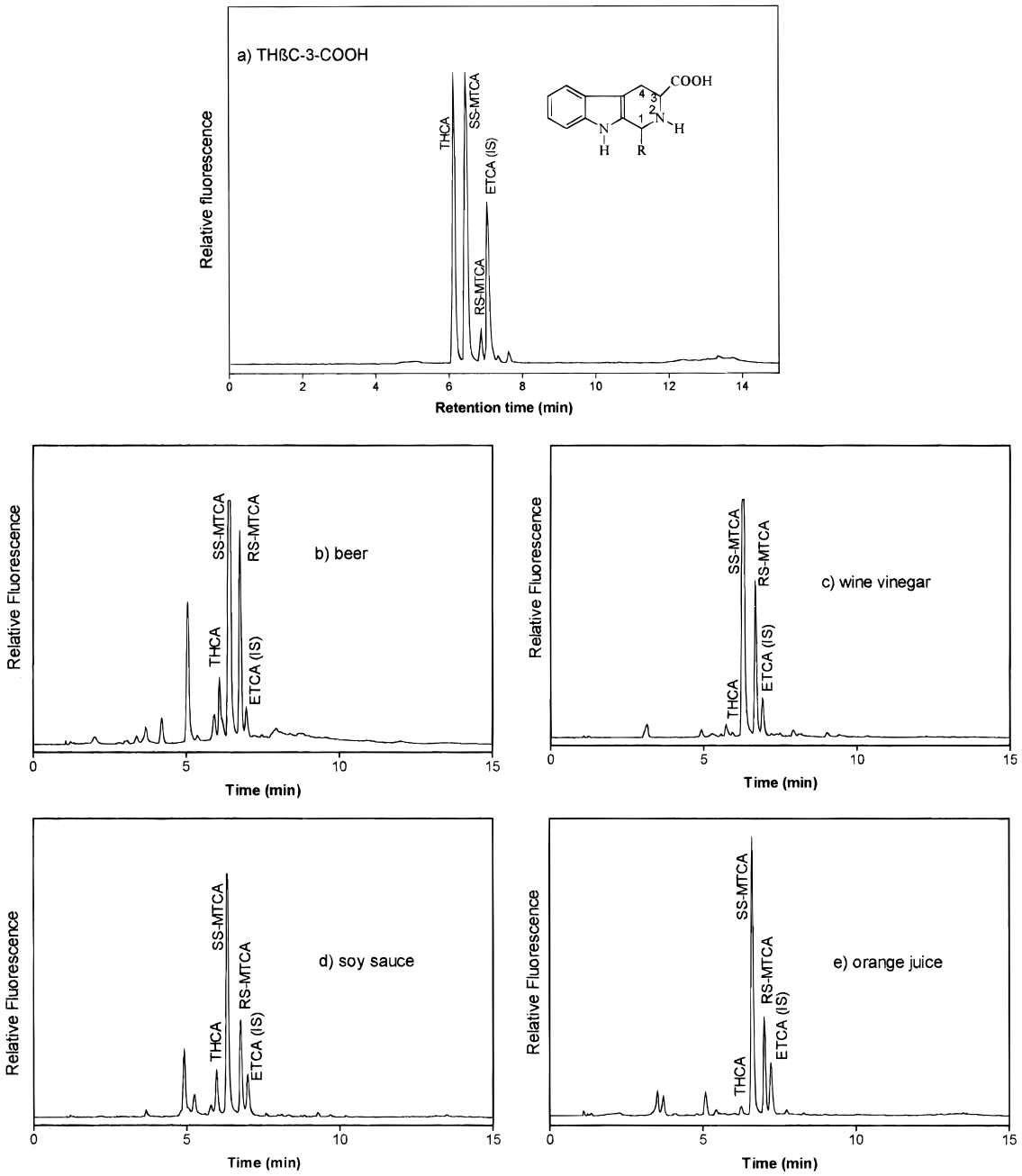

Figure 2. RP-HPLC chromatograms of 1,2,3,4-THβC-3-COOHs under the analytical conditions used for their separation and quantitative analysis: (a) THβC-3-COOH standards and (b−e) THβC-3-COOHs isolated from real samples of foodstuffs. THCA, 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; (*SS*)-MTCA, (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; (*RS*)-MTCA, (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; and ETCA, 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, as internal standard (IS).

Any possible artifactual formation during the isolation procedure was checked using blank and control samples. Also, homogenization of some foodstuffs was carried out at basic pH (100 mM borate buffer, pH 9) to assess any possible change in the calculated content of THβC-3-COOHs with respect to acidic pH. Direct HPLC injection of filtered liquid samples of foodstuffs was also accomplished before the sample cleanup to test the actual presence of THβC-3-COOHs in foodstuffs.

Formation of THβC-3-COOHs in Foodstuffs Spiked with Aldehydes. Additional evidence for the occurrence and possible formation of these compounds in foodstuffs was obtained by spiking food samples with aldehydes and determination of any further increase of THβC-3-COOHs: (a) Tubes containing 5 mL of a sample of beer were separately added with acetaldehyde at 0, 20, 50, and 100 mg/L, whereas similarly other tubes (5 mL) were added with 0, 5, 10, and 20 mg/L formaldehyde. After 2 days at 30 °C the samples were analyzed for THβC-3-COOHs. (b) In the same manner, tubes with 10 mL of a sample of commercially available orange juice were separately added with acetaldehyde at 0, 20, 50, and 100 mg/L and other tubes at 0, 20, 50, and 100 mg/L formaldehyde. After 2 days at 30 °C the samples were centrifuged and analyzed for THβC-3-COOHs. (c) Similarly, tubes containing 10 mL of yogurt were separately added with 0, 20, 50, and 100 mg/L acetaldehyde or 0, 20, 50, and 100 mg/L formaldehyde. The tubes were kept at 30 °C for 2 days, subsequently added with 1 mg/mL semicarbazide and 0.6 M $HClO_4$, centrifuged, and analyzed for THβC-3-COOHs.

RESULTS

Chromatographic Analysis of THβC-3-COOHs. Figure 2a shows an HPLC chromatogram of THCA, (*SS*)-MTCA, (*RS*)-MTCA, and ETCA under the analytical conditions used for their separation and quantitative

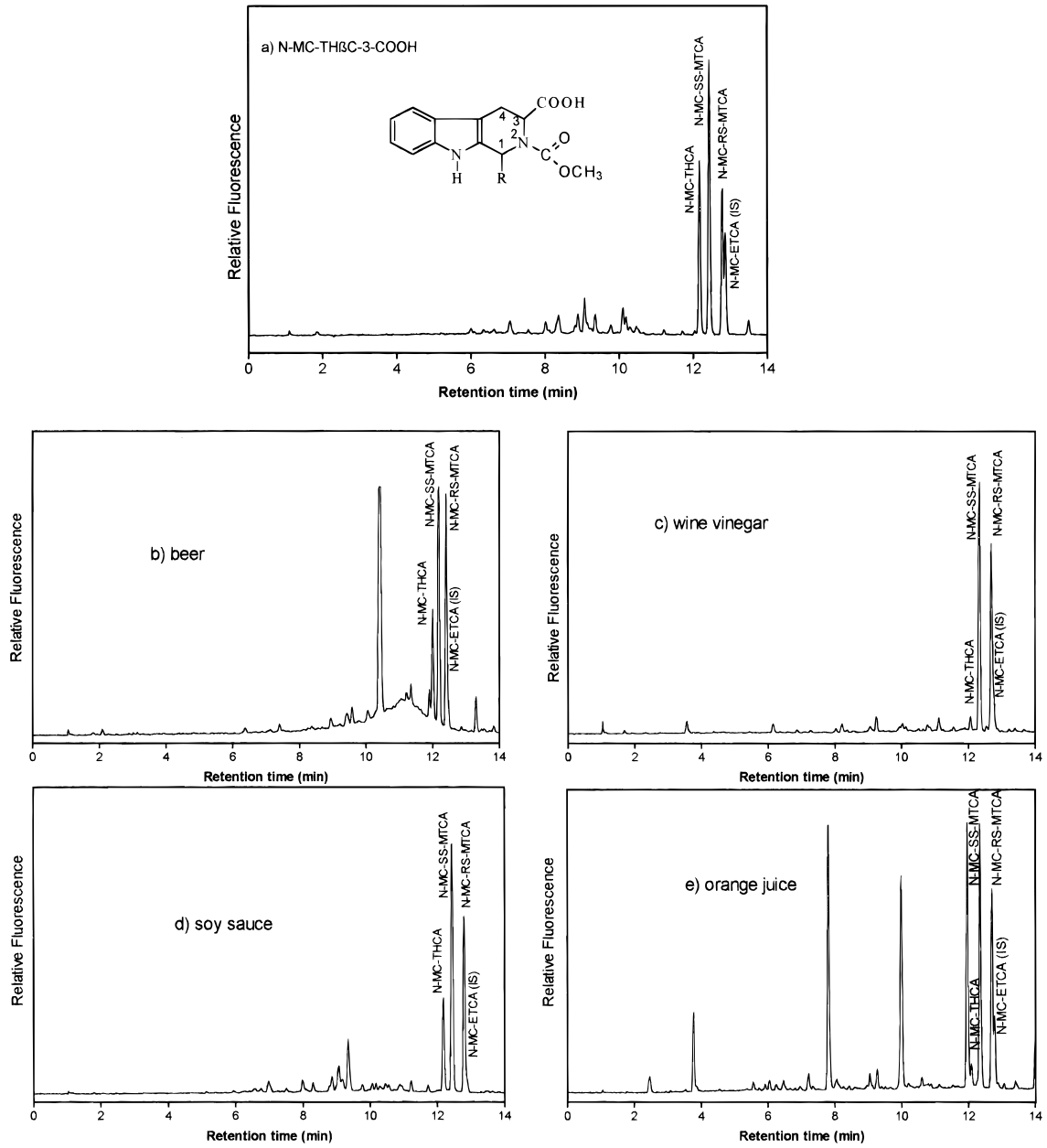

Figure 3. RP-HPLC chromatograms of *N*-(methoxycarbonyl)-1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (N-MC-TH*β*C-3-COOH) obtained by derivatization of TH*β*C-3-COOHs with methyl chloroformate: (a) *N*-(methoxycarbonyl)-TH*β*C-3-COOH standards and (b−e) *N*-(methoxycarbonyl)-TH*β*C-3-COOHs synthesized from TH*β*C-3-COOHs isolated from foodstuffs.

analysis in foodstuffs. A good separation is achieved using a C18 reversed phase Nova-pak column (Waters). (1*S*,3*S*)- and (1*R*,3*S*)-MTCA diastereoisomers are assigned following previous reports in which the 1*S*,3*S* isomer elutes earlier than the 1*R*,3*S* isomer (Wakabayashi et al., 1983; Bosin et al., 1986; Adachi et al., 1991a; Herraiz et al., 1993). Solid phase extraction using benzenesulfonic acid SCX columns allows a rapid, clean, and selective extraction of TH*β*C-3-COOHs from foods and biological samples (Adachi et al., 1991a; Herraiz et al., 1993; Herraiz and Ough, 1993, 1994). RP-HPLC chromatograms of TH*β*C-3-COOHs isolated from various foodstuffs are presented in Figure 2b−e.

TH*β*C-3-COOHs have been previously identified by MS, GC−MS, or HPLC−MS in several foods and beverages such as wine and alcoholic beverages (Bosin et al., 1986; Adachi et al., 1991a; Herraiz and Ough, 1994), beer (Bosin et al., 1986), smoked foods including meat, fish, and cheese (Papavergou and Clifford, 1992; Sen et al., 1995), soy sauce (Wakabayashi et al., 1983), and sake (Adachi et al., 1991a). Additionally, Herraiz and Sanchez (1996) have identified these compounds in numerous foodstuffs by GC−MS. Therefore, in this paper, identification was assigned by retention times, fluorescence spectrum, and cochromatography analysis in which authentic TH*β*C-3-COOHs were added to the extracted samples of foodstuffs and resulted in an increase of the TH*β*C-3-COOH peaks in the samples. Additional confirmation of the presence of these compounds in foods was also carried out through derivatization with methyl chloroformate (Bosin and Jarvis, 1985; Bosin et al., 1986). The amine group of these compounds readily reacts with methyl chloroformate, and the resulting N-MC-TH*β*C-3-COOHs derivatives exhibit a higher HPLC retention time than TH*β*C-3-COOHs (Figure 3a). Figure 3b−e shows chromatograms of the isolated TH*β*C-3-COOHs in foods following derivatization with methyl chloroformate. N-MC-TH*β*C-3-COOHs obtained from foods coeluted with the corresponding synthesized standard compounds.

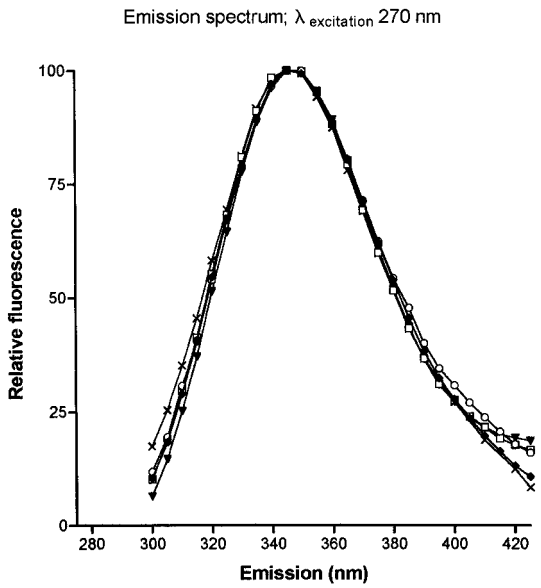

Figure 4. Emission fluorescent spectra obtained from the HPLC peaks of (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ((*SS*)-MTCA) following excitation at 270 nm. (*SS*)-MTCA standard (×) and (*SS*)-MTCA in foodstuffs: beer (□), wine vinegar (◆), soy sauce (▼), and orange juice (○).

The fluorescence spectra of HPLC peaks in real samples were compared with those of authentic standards to ensure that detected and quantified peaks corresponded to THβC-3-COOHs. The fluorescence pattern (excitation and emission) of THCA, (*SS*)-MTCA, and (*RS*)-MTCA peaks, detected in foods, was similar to those of standards. Indeed, they exhibited an emission maximum around 343−345 nm following excitation at 270 nm. Figure 4 shows the emission profile of (*SS*)-MTCA in standard and foodstuffs.

Occurrence of THβC-3-COOHs in Foodstuffs. Tables 1 and 2 report the content of THβC-3-COOHs in commercial foodstuffs. Most of the foods and drinks analyzed contain THCA, (1*S*,3*S*)-MTCA, and (1*R*,3*S*)-MTCA. Nevertheless, their content varies largely between foodstuffs and even between samples within a single food or beverage. High-alcohol beers (8.5−12%, v/v, of ethanol) have a significantly ($p < 0.05$) higher amount of THCA, (*SS*)-MTCA, and (*RS*)-MTCA than classical beers (ca. 5%, v/v, of alcohol) and beers without alcohol (less than 1%, v/v). The concentration of THβC-3-COOHs found in table wines is not really different from that reported previously (Herraiz et al., 1993). The content of THβC-3-COOHs in cider is significantly smaller ($p < 0.05$ for MTCA) than in beer and wine. Liquors had an extremely variable concentration ranging from undetectable, or a very low amount (apple liquor), to 5.7, 2.4, and 1.0 mg/L for (*SS*)-MTCA in those made from blackberry, cassis, and cherry, respectively. The content observed in commercial "sangria" is not significantly different from that of wines, which is what should be expected because it is made basically from wines (wine, sugar, and fruit juices). Commercial vinegars made from wine or cider contain greater amounts of THβC-3-COOHs than wines or cider, themselves. The content of THβC-3-COOHs in wine vinegar is significantly higher ($p < 0.05$) than in cider vinegar.

Soft drinks contained very small amounts or no THβC-3-COOHs at all. It seems that only soft drinks in which a pulp fruit was used (orange or lemon) during the elaboration process contain trace amounts. The occurrence of THβC-3-COOHs in bottled grape musts and packed fruit juices is noticeable. Commercial

Table 1. Content of 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic Acids in Commercial Alcoholic and Nonalcoholic Drinks and Foodstuffs[a]

		THCA (mg/L)			(*SS*)-MTCA (mg/L)			(*RS*)-MTCA (mg/L)			alcohol
sample	*N*	*X*	SD	range	*X*	SD	range	*X*	SD	range	(%, v/v) range
beer	4	0.116	0.08	0.03−0.25	0.476	0.162	0.306−0.59	0.13	0.05	0.076−0.2	<1
beer	9	0.124	0.075	nd−0.24	0.535	0.39	0.25−1.54	0.159	0.127	0.068−0.48	4.8−5.4
beer	6	0.413	0.279	0.17−0.84	6.54	3.47	1.8−13.1	1.95	1.04	0.57−3.93	8.5−12
wine	9	0.013	0.01	nd−0.03	3.37	1.30	1.38−5.24	0.97	0.37	0.38−1.40	12−13
cider	4	0.007	0.008	nd−0.02	0.078	0.035	0.046−0.14	0.021	0.011	0.011−0.041	4−5.6
liquor	8	0.05	0.074	nd−0.23	1.166	1.89	nd−5.71	0.35	0.54	nd−1.62	15−25
sangria	2	0.018	0.008	0.01−0.03	2.85	1.22	1.63−4.08	0.88	0.31	0.57−1.21	7−10
wine vinegar	4	0.05	0.04	0.01−0.12	5.76	1.64	3.09−7.5	1.6	0.456	0.84−2.06	
cider vinegar	2	0.003	0.003	nd−0.01	0.48	0.32	0.16−0.81	0.13	0.098	0.034−0.23	
grape must	2	0.02	0.002	0.018−0.022	0.88	0.32	0.56−1.2	0.252	0.082	0.17−0.34	
soft drink	8	0.002	0.003	nd−0.09	0.062	0.096	nd−0.288	0.014	0.023	nd−0.069	
fruit juice	9	0.06	0.04	nd−0.12	1.55	1.35	0.12−3.93	0.455	0.387	0.03−1.10	

[a] *N*: number of samples. *X*: mean. SD: standard deviation. nd: not detected. 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; and (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, (*RS*)-MTCA.

Table 2. 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic Acid Content in Commercial Samples of Foodstuffs

		THCA (μg/g)			(*SS*)-MTCA (μg/g)			(*RS*)-MTCA (μg/g)		
sample	*N*	*X*	SD	range	*X*	SD	range	*X*	SD	range
soy sauce[a]	3	26.3[a]	30.68[a]	2.16−69.6[a]	184.8[a]	125.8[a]	72−360.5[a]	46.46[a]	29.48[a]	20−87.6[a]
seasoning	4	1.45	1.19	0.03−3.18	11.33	8.99	0.62−24.0	2.73	2.04	0.15−5.59
cheese	13	0.18	0.237	nd−0.87	0.281	0.53	nd−2.06	0.063	0.135	nd−0.5
yogurt	6	0.013	0.004	0.007−0.02	0.078	0.027	0.04−0.11	0.016	0.004	0.009−0.02
milk	8	nd			nd			nd		
bread	9	0.75	0.754	0.12−2.51	0.113	0.095	0.025−0.327	0.075	0.064	0.013−0.21
smoked fish	4	0.21	0.133	0.082−0.399	0.002	0.0034	nd−0.008	nd		
cured ham	3	0.13	0.09	0.04−0.25	0.21	0.19	0.04−0.48	0.099	0.08	0.02−0.21
pickled cucumbers	3	1.32	0.13	1.1−1.4	0.35	0.03	0.31−0.38	0.11	0.006	0.1−0.11
canned olives	2	0.042	0.017	0.025−0.06	0.008	0.008	nd−0.017	nd		

[a] Concentration given in mg/L. nd: undetected.

orange juices ($n = 4$) had the highest amount, averaging 0.09, 2.9, and 0.85 mg/L for THCA, (*SS*)-MTCA, and (*RS*)-MTCA, respectively. Analyzed packed grape juice, peach + grape juice, pineapple juice, and banana juice also contained these compounds, although always in less than 1 mg/L as a sum of THβC-3-COOHs. Apple juice showed the lowest amount among the juices analyzed with nd, 0.12, and 0.03 mg/L for THCA, (*SS*)-MTCA, and (*RS*)-MTCA, respectively.

Soy sauces exhibit the highest amount among the foodstuffs analyzed. Seasonings made from sources other than soy bean contain high levels of THβC-3-COOHs as well, with the highest content detected in tabasco sauce. THβC-3-COOHs occur in lactic fermentation products such as cheese and yogurt. Except for some blue and smoked cheeses, most cheeses contain very low or undetectable amounts. Yogurt samples contain a low, though very similar, content of THβC-3-COOHs. In contrast, THβC-3-COOHs were not found in commercial packed milk in any case (whole, skim, powdered, and soy milk).

Interestingly, THCA was the major THβC-3-COOH found in bread. Within bread samples, toasted bread ($n = 4$) had the greatest content of THCA (1.34 μg/g on average). THCA was also found in smoked fish with an average concentration of 0.2 μg/g and in canned cucumber.

The analytical method used for quantitative determination of THβC-3-COOHs had a detection limit of less than 4 ng/g (3 times background). Reproducibility of the method was fairly good. The relative standard deviation (%RSD) was 1, 1.3, and 0.7 for THCA, (*SS*)-MTCA, and (*RS*)-MTCA in wine vinegar ($n = 8$) and 5.7, 5.6, and 5.6 for THCA, (*SS*)-MTCA, and (*RS*)-MTCA, respectively, in bread ($n = 7$). Estimated recovery was obtained by comparing the HPLC peak areas of extracted and nonextracted samples of six foodstuffs (wine, beer, wine vinegar, cider vinegar, orange juice, and yogurt) spiked with THβC-3-COOHs at 0.95, 1.66, and 0.5 mg/L for THCA, MTCA, and ETCA, respectively. The absolute recoveries were 92 ± 8.5% (THCA), 96 ± 3% (MTCA), and 96 ± 4% (ETCA). These values agree with previous results using this method (Adachi et al., 1991a; Herraiz et al., 1993).

The absence of any significant artifactual formation during the isolation procedure was considered by several ways. Blank samples of tryptophan (50 mg/L) carried through the entire analytical procedure did not yield THβC-3-COOHs. Control samples (20 mL) containing tryptophan (200 μg) and aldehydes (100 μg) carried through the sample cleanup did not give significant artifactual formation when analyzed promptly. Since the formation of THβC-3-COOHs is highly pH dependent (Herraiz and Ough, 1993), an additional proof for the absence of artifacts was obtained by isolation of THβC-3-COOHs from various foodstuffs (bread, blue cheese, and yogurt) at different pHs (acidic and borate buffer, pH 9). Similar concentrations were obtained following homogenization at both pHs, which indicates the absence of artifacts. On the other hand, the direct HPLC injection of filtered samples of fruit juice, vinegar, wine, cider, beer, yogurt, liquor, and seasoning also showed the actual presence of these substances in foodstuffs.

Formation of THβC-3-COOHs in Foodstuffs Spiked with Aldehydes. Several experiments in which foodstuffs were spiked with formaldehyde or acetaldehyde and kept for 2 days at 30 °C were carried out to test further formation of THβC-3-COOHs from L-tryptophan and aldehydes in foods (Figure 5a−f). Beer highly increases the content of THCA following addition of formaldehyde, whereas MTCA in both stereoisomers rises with the addition of acetaldehyde. Orange juice gives a substantial increase of THCA when formaldehyde is added. Similarly, an increase of MTCA in both stereoisomers is obtained in those samples added with acetaldehyde. In the same manner, yogurt shows a notable increase of THCA and MTCA after addition of formaldehyde or acetaldehyde, respectively. Figure 5 shows that the reaction rate is faster to form THCA than MTCA, in agreement with previous results (Herraiz and Ough, 1993).

DISCUSSION

This paper shows the presence of THβC-3-COOHs in foods. Indeed, it confirms that THβC-3-COOHs are a usual constituent in the human diet. The fact that most of the foodstuffs examined contain THβC-3-COOHs suggests that their formation through the Pictet−Spengler chemical condensation between tryptophan and aldehydes (Figure 1) may readily occur in plants, animals, and also during food production, processing, and storage.

Acetaldehyde and formaldehyde react with tryptophan even under mild conditions to provide THβC-3-COOHs (Herraiz et al., 1993; Herraiz and Ough, 1993; Herraiz, unpublished results). Thus, foodstuffs with free precursors available for further reaction will release these compounds. Obviously, the reaction progresses more easily in beverages than in solid foods. Our previous results showed that factors such as pH and temperature determine the reaction rate to form THβC-3-COOHs from available L-tryptophan and aldehydes. Low pH highly favors the reaction rate, as does high temperature. Longer storage time before consumption will also increase the amount of THβC-3-COOHs if tryptophan remains. The concentration of precursors, especially tryptophan but also aldehydes, is a decisive factor. Nevertheless, only a very low concentration of aldehydes may be sufficient to release THβC-3-COOHs if tryptophan is available for reaction in an acidic environment. The type of carbonyl compound may also influence the yields of the corresponding THβC-3-COOHs. Thus, reaction rate of tryptophan with formaldehyde is faster than with acetaldehyde (Herraiz and Ough, 1993). The same deduction can be obtained from Figure 5, and this is in agreement with the work of Whaley and Govindachary (1951) showing different rates during the Pictet−Spengler reaction depending on the aldehydes.

It should be expected that more aldehydes are in fermented and maturated foodstuffs, and consequently, more THβC-3-COOHs are in those products. The ranges of MTCA in beer and wine agree with those reported previously (Bosin et al., 1986; Adachi et al., 1991a; Herraiz et al., 1993, Herraiz and Ough, 1993). The content of THCA in beer is up to 12 times lower than that in Bosin et al. (1986), whereas it is similar to that reported by Adachi et al. (1991a) and Sen et al. (1995). The exact reason for this is not clear; however, it might be attributable to the different processing conditions and the nature of the products (Sen et al., 1995). Additionally, this paper includes data from very low- and high-alcohol beers that were not studied before. High-alcohol beer should have more aldehydes to react with available tryptophan providing higher levels of

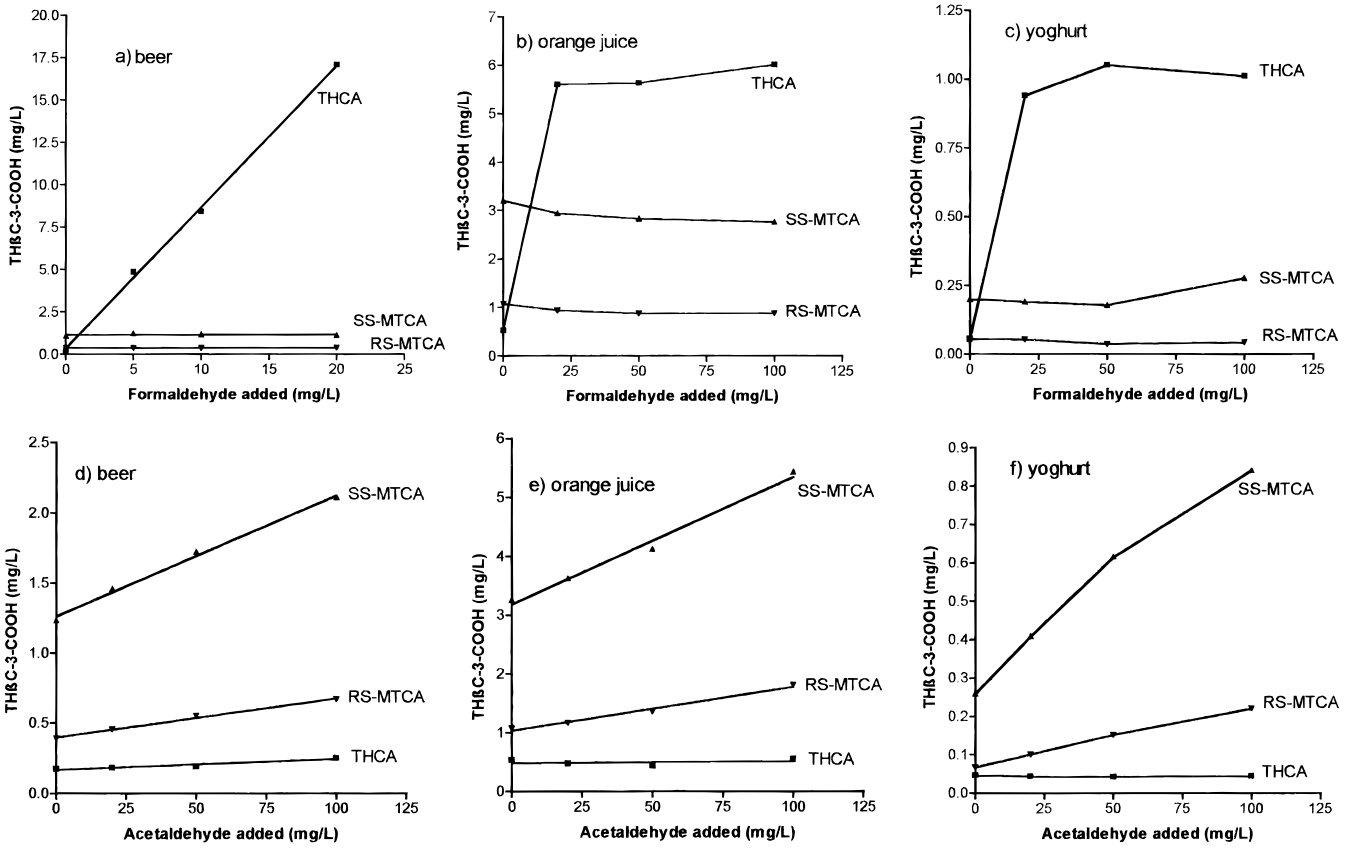

Figure 5. Formation of THβC-3-COOHs in beer, orange juice, and yogurt spiked with formaldehyde (a−c) or acetaldehyde (d−f). Samples were kept at 30 °C for 48 h before analysis for THβC-3-COOHs.

these compounds than classical and low-alcohol beers. Despite its high content of tryptophan, beer has a significant higher pH than wine and, therefore, a lower reaction rate to give MTCA. This fact would explain the low content of MTCA in classical beer compared to wine. The concentration of these compounds could increase during maturation, storage, or aging in beer and wine processing.

As far as we know, no data of THβC-3-COOHs are previously reported in cider, "sangria", wine vinegar, cider vinegar, carbonated soft drinks, bottled grape musts, or packed fruit juices. The differences in the THβC-3-COOHs content between cider and wine or beer probably arise from differences in the content of tryptophan in the raw products. Acetic fermentation in vinegar production seems to rise the content of these compounds. Fruit juices should contain a small amount of these compounds owing to their expected low level of aldehydes. However, the content observed in fruit juices, especially in orange juices, is surprisingly not so low. In this regard, it must be pointed out that the whole chemical reaction readily progresses if tryptophan is available, and low content of aldehydes occurs in an environment with favorable conditions, i.e., low pH and/or high temperature. Usually, the molar concentration of acetaldehyde in foodstuffs is higher than that of tryptophan. Therefore, tryptophan should play a major role in the formation of THβC-3-COOHs.

Soy sauces have been previously noticed to contain high concentrations of these compounds (Wakabayashi et al., 1983; Adachi et al., 1991a; Sen et al., 1995); however, other sauces also contain these compounds in an appreciable amount. Generally, lactic fermentation products such as cheese and yogurt contain a smaller content of THβC-3-COOHs than alcoholic or acetic fermentation products. A lower content of precursors in those products and, above all, the unfavorable conditions for reaction would explain this behavior. In this study, we were unable to detect THβC-3-COOHs in cow's milk and soy milk, in contrast with Adachi et al. (1991a) who detect concentrations lower than 1 μg/kg in cow's milk.

Usually the content of MTCA is higher than THCA. This fact must be certainly due to the higher amount of acetaldehyde compared to formaldehyde in foodstuffs. Some exceptions are bread, smoked fish, and canned cucumber that contain more THCA. Previously, Papavergou and Clifford (1992) and Sen et al. (1995) have reported a higher amount of THCA than MTCA in cured smoked meats.

Technological factors during elaboration, processing, or storage, such as smoking treatment, maturation and ripening, fermentation, or heating, and oxidative processes could enrich the concentration of THβC-3-COOHs in foodstuffs. Since chemical reaction in the presence of precursors occurs easily even under mild conditions, the best way to decrease or eliminate these substances is to reduce or avoid the presence of precursors. Previously, it was reported that using SO_2 in winemaking may decrease the formation of THβC-3-COOHs since it combines with acetaldehyde (Herraiz and Ough, 1993). Low temperature and high pH could also reduce the level of THβC-3-COOHs.

This paper highlights the occurrence of an exogenous intake of THβC-3-COOHs in humans. Thus, the endogenous presence of these compounds in biological materials (Myers, 1989; Rommelspacher et al., 1991; Adachi et al., 1991a) could arise, at least in part, from

the exogenous consumption of foods besides certain endogenous formation, itself. Endogenous formation could also occur in the stomach that provides a pH low enough to allow a rapid reaction (Herraiz, unpublished results). The present findings and those reported in the last few years evidence that this is an important field for investigation by nutritionists and food scientists alike since THβC-3-COOHs may have health consequences (Anonymous, 1991).

CONCLUSION

Two 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (THCA and MTCA) occur in many commercial foodstuffs and beverages ranging from undetectable amount to hundreds of milligrams/kilogram. Among the foodstuffs containing more THβC-3-COOHs are seasonings, alcoholic and acetic fermented products (beer, wine, and vinegar), orange juices, bread, and lactic fermented products (cheese and yogurt). The content of MTCA is usually greater than THCA; however, bread and smoked fish exhibit a higher content of THCA. An exogenous intake of THβC-3-COOHs occurs during food ingestion. Consequently, the endogenous presence of β-carbolines in biological tissues could be, at least in part, a result of their ingestion during food intake.

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