Occurrence of 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic Acid and 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid in Fruit Juices, Purees, and Jams

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1,2,3,4-Tetahydro-β-carboline-3-carboxylic acid (THCA) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA) as two diastereoisomers (1*S*,3*S* and 1*R*,3*S*), occur in fruit juices, juices and fruit purees for infants, and jams. Concentrations in commercially prepared fruit juices were in the ranges 0.01−1.45, 0.02−9.1, and 0.01−2.48 μg/g for THCA, 1*S*,3*S*-MTCA, and 1*R*,3*S*-MTCA, respectively. The content was higher in citrus juices (orange, grapefruit) than in other juices (grape, apple, pineapple, peach, banana, pear, tomato). Commercially prepared infant juices contained 0.14, 0.52, and 0.16 μg/g on average, for THCA, 1*S*,3*S*-MTCA, and 1*R*,3*S*-MTCA, respectively. Commercial infant fruit purees averaged 0.04, 0.35, and 0.11 μg/g for THCA, 1*S*,3*S*-MTCA, and 1*R*,3*S*-MTCA. Jams and marmalades averaged 0.23, 0.76, and 0.25 μg/g for THCA, 1*S*,3*S*-MTCA, and 1*R*,3*S*-MTCA, respectively. The reported endogenous presence of these substances in humans could be influenced by their exogenous ingestion in the diet. This is the first specific report on THCA and MTCA in fruit products.

Keywords: *Tetrahydro-β-carboline-3-carboxylic acid; tetrahydro-β-carbolines; β-carbolines; alkaloids; tryptophan; aldehydes; fruit juices; infant purees; jams*

1. INTRODUCTION

1,2,3,4-Tetrahydro-β-carbolines (THβCs) are naturally occurring tricyclic indole derivatives produced from indole−ethylamines and aldehydes or α-ketoacids through Pictet−Spengler condensation (Whaley and Govindachari, 1951). Similarly, 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (THβC-3-COOHs) arise from a condensation between L-tryptophan and aldehydes. This latter reaction readily occurs in foods and is temperature and pH dependent (Herraiz and Ough, 1993).

Research in the past two decades has pointed out the occurrence of THβCs and β-carbolines (βCs) under physiological conditions in biological tissues and fluids (McIsaac, 1961; Buckholtz, 1980; Airaksinen and Kari, 1981; Melchior and Collins, 1982; Myers, 1989; Rommelspacher et al., 1991; Adachi et al., 1991; Callaway et al., 1994). THβCs and βCs might function as neuromodulators via effects on monoamine oxidase, monoamine uptake, and benzodiazepine receptor binding (Buckholtz, 1980; Braestrup et al., 1980). Simultaneously, THβCs and βCs were increasingly studied in relation with alcoholism (Cohen and Collins, 1970; Myers and Melchior, 1977; Tuomisto et al., 1982; Rommelspacher and Schmidt, 1985; Beck et al., 1987; Myers, 1989; Adachi et al., 1993). Collins and coworkers have reported that N-methylated THβCs and βCs are endogenous neurotoxins (Collins and Neafsey, 1985; Matsubara et al., 1992). 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA), the corresponding tryptophan−acetaldehyde condensation product, is a precursor of mutagenic *N*-nitroso compounds (Wakabayashi et al., 1983; Higashimoto et al., 1996), shows cytogenetic effects (Fujie et al., 1990), and can cause neuronal cell death (Brenneman et al., 1993). Several βCs may also exhibit genotoxic potential (de Meester, 1995). Considered altogether, a full delineation of the biological activity and possible toxicity of THβCs and βCs is desirable and still needed.

Despite research on THβCs started long ago, the widespread presence of MTCA and 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (THCA) in commercial foods and drinks has been recently proved (Herraiz, 1996, 1997; Herraiz and Sanchez, 1997; Gutsche and Herderich, 1997). MTCA and THCA were initially associated with fermentation (Wakabayashi et al., 1983; Bosin et al., 1986; Adachi et al., 1991; Herraiz et al., 1993; Herraiz and Ough, 1993) or smoking treatments (Papavergou and Clifford, 1992; Sen et al., 1995). However, their generalized presence in foodstuffs suggests they can be naturally occurring substances chemically produced during food production, processing, and storage. Obviously, dietary sources may provide THβCs and surely influence their content in biological tissues and fluids (Herraiz, 1996; Tsuchiya et al., 1996).

This paper reveals the occurrence of two tetrahydro-β-carbolines, MTCA and THCA, in commercial fruit juices, juices and purees for infants, and jams. This is the first specific survey on THβC-3-COOHs in those products. A brief discussion on the chemical and technological factors influencing their formation is included.

2. MATERIALS AND METHODS

Reference Compounds. MTCA was purchased from Sigma (St. Louis, MO). The diastereoisomeric mixture of MTCA [1*S*,3*S*-MTCA and (−)-(1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, 1*R*,3*S*-MTCA], THCA,

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Table 1. Concentrations of THβC-3-COOHs in Commercial Fruit Juices

		THCA (μg/g)			*SS*-MTCA (μg/g)			*RS*-MTCA (μg/g)		
fruit juice	*N*[a]	*X*[b]	SD[c]	range	*X*	SD	range	*X*	SD	range
orange	15	0.12	0.06	0.03−0.25	2.34	2.26	0.51−9.1	0.65	0.59	0.18−2.48
concentrated orange	10	0.13	0.06	0.08−0.25	2.02	2.57	0.51−9.1	0.59	0.69	0.18−2.48
squeezed orange	5	0.09	0.05	0.03−0.14	2.97	1.48	1.07−5.0	0.78	0.35	0.31−1.29
grapefruit	3	0.10	0.07	0.05−0.18	1.59	1.62	0.53−3.4	0.48	0.46	0.18−1.02
grape (must)	2	0.05	0.03	0.03−0.07	1.26	0.82	0.68−1.8	0.37	0.22	0.22−0.53
apple	6	0.04	0.04	0.01−0.11	0.24	0.22	0.04−0.57	0.07	0.07	0.01−0.16
peach	3	0.02	0.002	0.018−0.023	0.11	0.02	0.09−0.14	0.04	0.013	0.03−0.05
pineapple	4	0.17	0.14	0.07−0.31	0.24	0.07	0.18−0.31	0.10	0.03	0.08−0.14
pear	2	0.04	0.04	0.01−0.07	0.04	0.03	0.02−0.06	0.015	0.01	0.01−0.02
peach + grape	3	0.04	0.02	0.03−0.06	0.37	0.36	0.12−0.78	0.12	0.11	0.04−0.25
tomato	2	0.92	0.36	0.66−1.2	1.24	0.15	1.1−1.35	0.41	0.04	0.38−0.48
grape + pineapple	1	0.12			0.6			0.2		
mandarin	1	0.16			2.75			0.77		
kiwi	1	0.07			0.40			0.12		
banana	1	0.04			0.21			0.09		
mixed fruit	1	0.11			1.24			0.39		
carrot	1	1.45			0.26			0.11		

[a] Each sample represents a different brand. Samples are both juices (orange, apple, peach, peach + grape, mandarin, pineapple, grape, tomato) and nectars (carrot, peach, kiwi, pineapple, banana, and pear). [b] Mean. [c] Standard deviation.

Table 2. Concentrations of THβC-3-COOHs in Commercial Infant Juices and Purees

	N	THCA (μg/g)	*SS*-MTCA (μg/g)	*RS*-MTCA (μg/g)
juices				
apple	2	0.055 ± 0.02	0.03 ± 0.0004	0.009 ± 0.0008
apricot	1	0.50	1.18	0.36
mixed fruit[a]	1	0.09	1.03	0.31
mixed fruit[b]	1	0.1	0.42	0.14
mixed fruit[c]	1	0.09	0.44	0.16
total infant juice[d]	6	0.14 ± 0.17	0.52 ± 0.49	0.16 ± 0.15
purees				
apple	1	0.03	0.10	0.023
peach + strawberry	1	0.11	0.39	0.105
apple + banana + orange	1	0.03	0.75	0.26
apple + mandarin + pear	1	0.04	0.40	0.12
mixed fruits[e]	1	0.008	0.1	0.027
total purees[d]	5	0.04 ± 0.04	0.35 ± 0.27	0.11 ± 0.10

[a] Apple, orange, pear, and banana. [b] Grape, pear, apricot, and orange. [c] Grape, orange, and banana. [d] Mean values ± standard deviation. [e] Peach, apple, banana, apricot, and orange juice.

Table 3. Concentrations of THβC-3-COOHs in Commercial Jams and Marmalades[a]

jams and marmalades	THCA (μg/g)	*SS*-MTCA (μg/g)	*RS*-MTCA (μg/g)
apricot a	0.10	0.30	0.08
apricot b	0.42	0.85	0.31
cherry	0.06	0.1	0.02
orange	0.36	1.28	0.48
bitter orange	0.25	0.92	0.30
sweet orange	0.30	0.72	0.24
lemon	0.13	0.24	0.07
plum	0.18	0.54	0.16
strawberry	0.31	0.66	0.20
red currant + raspberry	0.2	1.98	0.67
total ($n = 10$)[b]	0.229 ± 0.116	0.76 ± 0.555	0.25 ± 0.2

[a] Jams contained 30−50% fruit. [b] Values are means ± standard deviation (SD).

and 1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (ETCA) were obtained according to the method of Brossi et al. (1973). NMR, MS, and GC/MS (trifluoroacetyl and methoxycarbonyl methyl ester derivatives) data were consistent with the structures of the synthesized compounds (Herraiz and Ough, 1994; Herraiz and Sanchez, 1997; Herraiz, 1997).

Samples Analyzed. Commercial samples of fruit juices, including juices made from concentrate juice, nectars, and squeezed-direct pressed fruit-(fresh and refrigerated) fruit juices, commercial juices and purees for infants (i.e., baby foods), and jams and marmalades (Tables 1−3) were purchased in local supermarkets and were of both local and imported origins. Packages were both from carton and glass for fruit juices and from glass jars for infant juices, purees, jams, and marmalades.

Isolation of THβC-3-COOHs. Free THβC-3-COOHs were isolated using SCX solid phase extraction following a previously described cleanup procedure (Adachi et al., 1991; Herraiz et al., 1993; Herraiz, 1996). (A) Fruit juice (20 mL) was added with 1 mg/mL semicarbazide (Sigma) and centrifuged (5100*g*, 0−5 °C) for 10−15 min. An aliquot of supernatant (5.5 mL) was spiked with 0.5 mL of ETCA solution (5 mg/L) used as internal standard (IS), acidified with three drops of 1 N HCl, and slowly passed through benzenesulfonic acid SCX columns (Bond Elut, 500 mg/3 mL size; Varian, Harbor City, CA) using a vacuum manifold. (B) Infant purees and jams (5 g) were homogenized using an UltraTurrax homogenizer with 20 mL of 0.1 M HCl containing 1 mg/mL semicarbazide and centrifuged (5100*g*, 10−15 min, 0−5 °C). An aliquot of supernatant (5.5 mL) was added to 0.5 mL of ETCA solution (5 mg/L) and SCX solid phase extracted. Elution of THβC-3-COOHs from SCX columns was carried out according to the method of Herraiz (1996), and extracted samples were injected into RP-HPLC.

Chromatographic Analysis. The analysis of THβC-3-COOHs by RP-HPLC and fluorescence detection was carried out as previously described (Herraiz, 1996). A 150 mm × 3.9 mm, 5 μm, Nova-pak C18 column (Waters, Milford, MA) was used for separation. Chromatographic conditions were as follows: 50 mM ammonium phosphate buffer (pH 3) (buffer A) and 20% of A in acetonitrile (buffer B). The gradient was programmed from 0% (100% A) to 32% B in 8 min and then to 90% B at 18 min. The flow rate was 1 mL/min, the column temperature was 40 °C, and the injection volume was 20 μL.

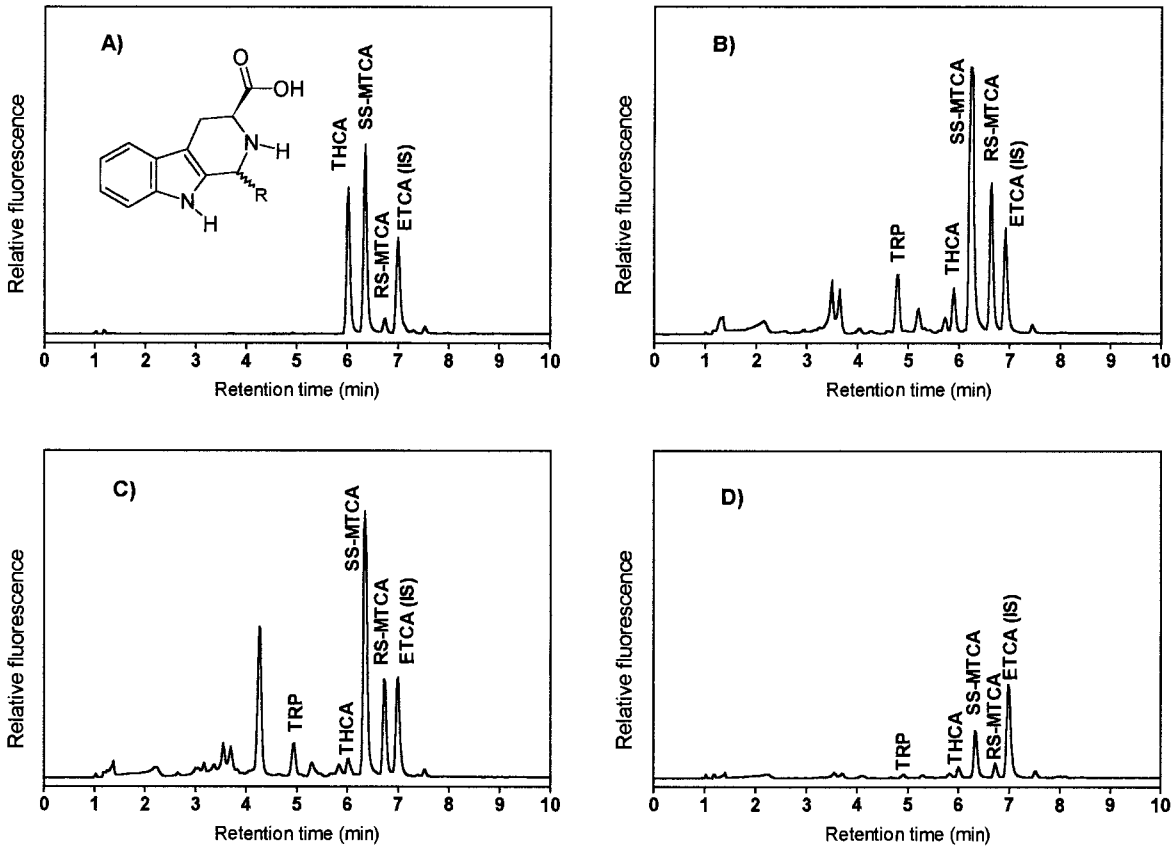

Figure 1. Representative HPLC chromatograms of THβC-3-COOH standards (A) and those isolated from orange juice (B), infant multifruit juice (apple, orange, pear, banana) (C), and orange marmalade (D): THCA, *SS*-MTCA, *RS*-MTCA, ETCA as IS, and L-tryptophan (TRP).

Fluorescence detection was set at 270 nm for excitation and at 343 nm for emission.

Quantitation was obtained from calibration curves constructed from synthetic solutions of THCA and MTCA reference compounds analyzed through the entire procedure. Blanks and control samples (100 mg/L L-tryptophan dissolved in 50 mM phosphate buffer, pH 3) did not give artifacts during isolation and analysis. Confirmation of the identity of isolated THβC-3-COOHs was established by HPLC retention times and coelution with authentic standards. Also, fluorescence spectra of the HPLC peaks were compared with those of reference compounds. For this, eluting peaks corresponding to THβC-3-COOHs were trapped in the flow cell of the fluorescence detector by stopping the solvent pump, and excitation and emission spectra monitored.

THβC-3-COOH gives rise to *N*-methoxycarbonyltetrahydro-β-carboline-3-carboxylic acid (N-MC-THβC-3-COOH) through reaction with methyl chloroformate ($ClCOOCH_3$) (Bosin and Jarvis, 1985; Herraiz, 1996). For this, aliquots (0.5 mL) of SCX-isolated fractions evaporated under a He stream up to ~0.2 mL were derivatized and extracted as previously described (Bosin and Jarvis, 1985; Herraiz, 1996). The samples redissolved in phosphate buffer 0.4 M/methanol (1:1), pH 10, were injected into RP-HPLC as above.

Formation of THβC-3-COOH in Juices Spiked with Aldehydes. Additional evidence for the occurrence and possible formation of THCA and MTCA in fruit products was investigated by exogenous addition of formaldehyde and acetaldehyde and determining any further increase of THβC-3-COOHs. (A) Tubes containing 10 mL samples of orange juice, banana nectar, peach + grape juice, and carrot nectar were separately added with acetaldehyde at 0 (control), 25, 50, and 100 mg/L, whereas similarly other tubes (10 mL) were added at 0 (control), 12.5, 25, and 50 mg/L formaldehyde. (B) In the same manner, tubes with 5 g aliquots of baby puree (made from a mixture of banana, apple, and orange) were separately added with acetaldehyde at 0 (control), 50, 100, and 200 μg/g, on the one hand, and at 0, 25, 50, and 100 μg/g formaldehyde, on the other. The samples were kept in a bath at 30 °C for 70 h, except for fruit puree (96 h), and vortexed (every ~8 h) before analysis.

3. RESULTS

Chromatographic Analysis. Recently, we have reported the chemical identification and characterization of THCA and MTCA in several foods, including fruit juices, by GC/MS (Herraiz, 1997; Herraiz and Sanchez, 1997). The present research revealed that SCX-extracted fruit juices and fruit-derived products (i.e., jams, infant purees) gave chromatographic peaks coeluting with authentic standards of THCA and MTCA (1*S*,3*S*-MTCA and 1*R*,3*S*-MTCA) (Figure 1). It has been reported that a good chromatographic separation avoids possible coelution of isomeric THβC (Gutsche and Herderich, 1998). In this case, no interfering peaks were observed and fluorescence detection increased the selectivity and specificity of the analysis. Furthermore, the HPLC peaks coeluting with standards of THCA and MTCA were trapped into the flow cell of the fluorescence detector and fluorescence spectra monitored. Excitation and emission profiles from THCA and MTCA peaks detected in juices and jams were very consistent with those from authentic standards (Figure 2).

The reagent methyl chloroformate ($ClCOOCH_3$) has been used in THβC analysis (Bosin and Jarvis, 1985; Herraiz, 1996). The resulting *N*-methoxycarbonyl derivatives (N-MC-THβC-3-COOH) exhibit fluorescence and higher HPLC retention times than parent compounds. Samples of fruit-derived products isolated for

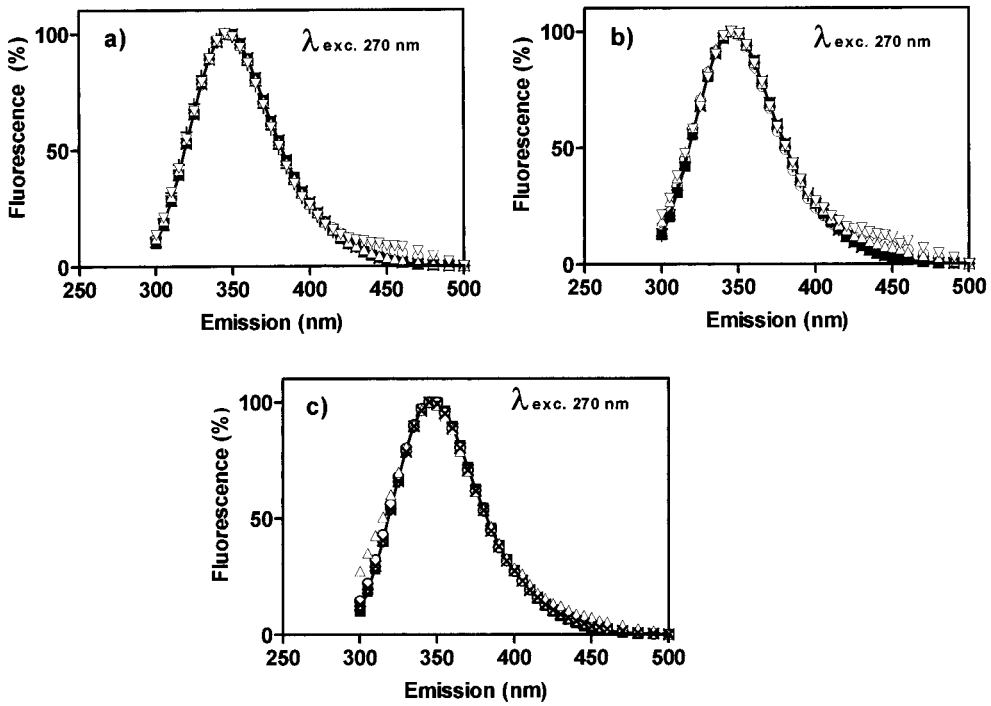

Figure 2. Emission spectra (excitation at 270 nm) for HPLC peaks trapped into the detection cell: *SS*-MTCA (a) and *RS*-MTCA (b) from authentic standards (■) and from apple juice (△), infant apricot juice (○), squeezed orange juice (×), grapefruit juice (+), grape juice (*), and orange marmalade (▽); THCA (c) from THCA standard (■) and from apple juice (△), tomato juice (×), apricot juice (○), and carrot juice (◇).

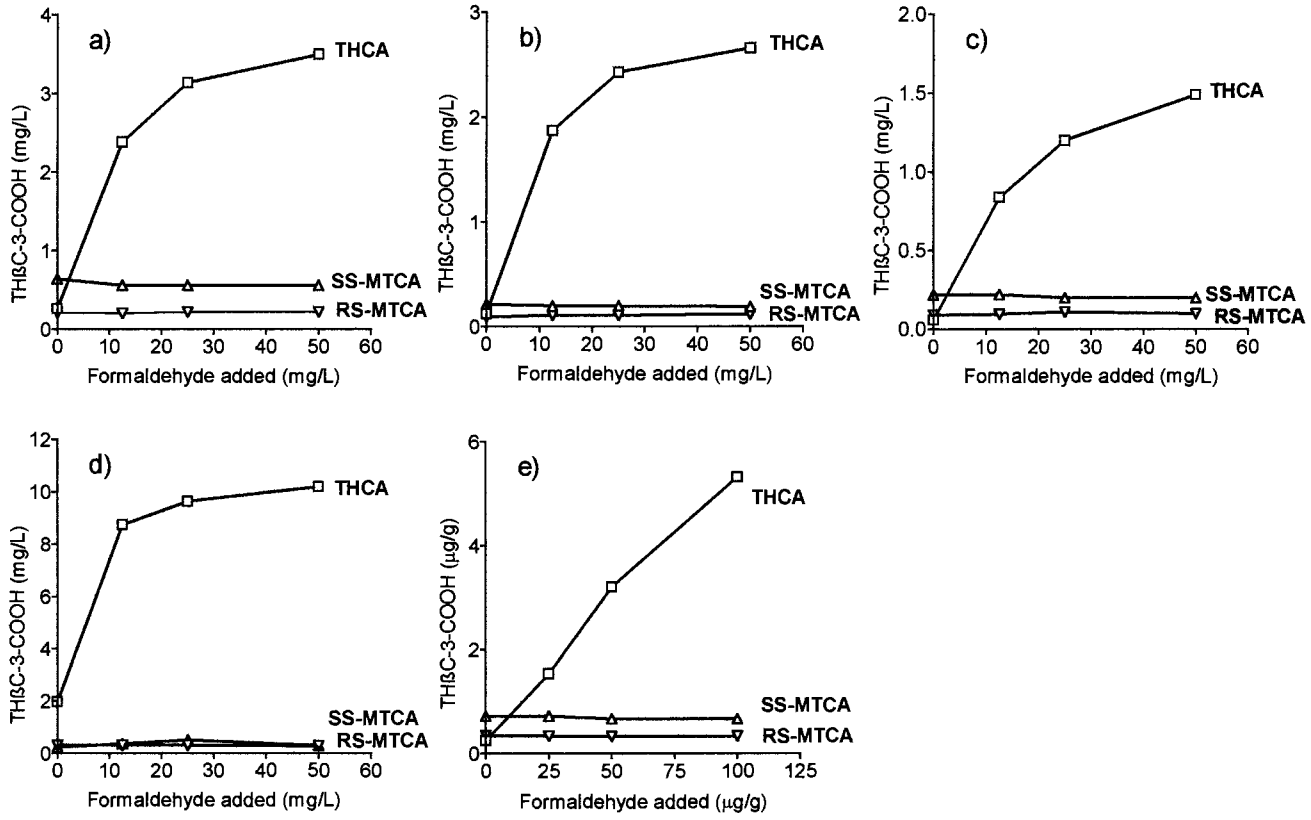

Figure 3. Formation of THβC-3-COOHs in orange juice (a), banana nectar (b), peach + grape juice (c), carrot juice (d), and infant puree (apple, 52%; banana, 28%; orange, 20%) (e) spiked with formaldehyde. Samples were kept at 30 °C for 70 h (a−d) and 96 h (e) and vortexed every ∼8 h.

THβC-3-COOHs reacted with methyl chloroformate, giving rise to peaks corresponding to *N*-methoxycarbonyl derivatives that coeluted with authentic standards in cochromatography injections (results not shown).

Occurrence of THβC-3-COOHs in Fruit-Derived Products. The occurrence of THβC-3-COOHs in juices is summarized in Table 1. The content varied largely between juices and among groups of juices. Citrus

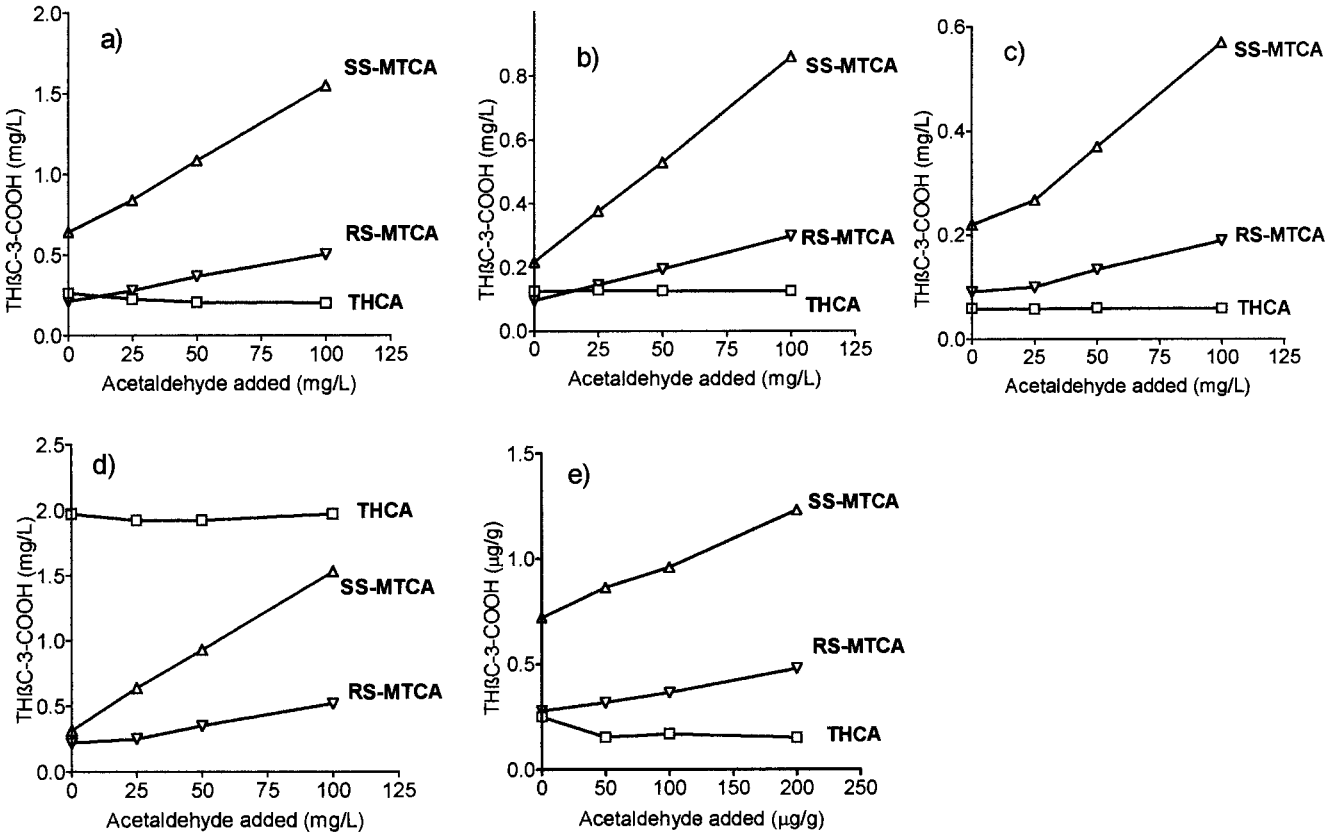

Figure 4. Formation of THβC-3-COOHs in orange juice (a), banana nectar (b), peach + grape juice (c), carrot juice (d), and infant puree (banana, 28%; apple, 52%; orange, 20%) (e) spiked with acetaldehyde. Samples were kept at 30 °C for 70 h (a−d) and 96 h (e) and vortexed every ~8 h.

juices (orange, mandarin, grapefruit) exhibited the highest level of THβC-3-COOHs. Orange juices gave average contents of 0.12 μg/g (THCA), 2.34 μg/g (*SS*-MTCA), and 0.65 μg/g (*RS*-MTCA). The content of those orange juices made from concentrate was not significantly different ($p < 0.05$) from that of squeezed orange juices (fresh and refrigerated). THβC-3-COOHs were also found in fruit juices made from grape (must), tomato, apple, kiwi, pineapple, banana, and carrot. The lowest levels were detected in peach and pear nectars. THβC-3-COOHs were widely distributed in commercial fruit juices regardless if the juices were from concentrate, squeezed, fresh (refrigerated), or nectar. Also, they occurred in infant fruit juices (Table 2). Those from apples exhibited the lowest content of THCA and MTCA. THβC-3-COOHs were found in commercial baby fruit purees, as well (Table 2). Their average concentrations were 0.04, 0.35, and 0.11 μg/g for THCA, *SS*-MTCA, and *RS*-MTCA, respectively. Table 3 lists the content determined in jams, averaging 0.23, 0.76, and 0.25 μg/g for THCA, *SS*-MTCA, and *RS*-MTCA, respectively. Usually, MTCA was the major THβC-3-COOH encountered in juices and jams. As reported for other foodstuffs (Adachi et al., 1991; Herraiz, 1996), MTCA appeared as a mixture of two diastereoisomers (1*S*,3*S*) and (1*R*,3*S*).

Formation of THβ-3-COOHs. Juices and purees spiked with formaldehyde or acetaldehyde evidenced a subsequent increase of occurring THβC-3-COOHs. Addition of formaldehyde triggered the formation of THCA, whereas MTCA remained unchanged (Figure 3). The addition of acetaldehyde to the samples generated a notable increase of 1*S*,3*S*-MTCA and 1*R*,3*S*-MTCA diastereoisomers (Figure 4). The reaction rate was faster for the formation of THCA than for the formation of MTCA. Formation of THCA and MTCA occurred with a simultaneous decrease of L-tryptophan (L-tryptophan peak area in chromatograms decreased up to 40% with addition of acetaldehyde and 90% with formaldehyde) (data not shown). Similar results were obtained previously in wines and foodstuffs (Herraiz et al., 1993; Herraiz and Ough, 1993; Herraiz, 1996).

4. DISCUSSION

These results reveal, for the first time, the widespread occurrence of THCA and MTCA (diastereoisomers 1*S*,3*S* and 1*R*,3*S*) in fruit juices, fruit-derived products such as jams, and infant foods (juices, and purees). This finding agrees with the extended presence of THβC-3-COOH in foods and drinks (Herraiz, 1996). The levels of THβC-3-COOHs found in fruit juices, especially citrus juices, and jams are noticeable, if compared with those in wines or beers (Bosin et al., 1986; Adachi et al., 1991; Herraiz et al., 1993; Herraiz, 1996). In previous studies we have reported that both acetaldehyde and formaldehyde react with tryptophan under conditions (pH, temperature) found in food systems to provide THβC-3-COOHs (Herraiz and Ough, 1993; Herraiz, 1996). The same appeared to occur here with fruit products. This suggests that free aldehydes might release THβC-3-COOHs after reaction with tryptophan in mild acidic environments such as those of fruit-derived products. In this regard, it is accepted that acetaldehyde and formaldehyde are natural constituents of fruits and vegetables (Feron et al., 1991). Those and previous results suggest that formation of THβC-3-COOHs through Pictet−Spengler chemical condensation be-

tween tryptophan and aldehydes may occur during food production, processing, and storage. However, we cannot rule out other sources leading to these compounds in foodstuffs.

Tryptophan may undergo complex transformations giving rise to possible antinutritional and toxic manifestations (Friedman and Cuq, 1988). Occurrence of THβC-3-COOH in commonly ingested foods such as fruit-derived products and others (Herraiz, 1996) strongly suggests the exogenous intake of THβC-3-COOHs (probably up to several milligrams per day) by humans during food and drink consumption. This may influence the endogenous presence of THβCs in tissues, organs, and fluids (Buckholtz, 1980; Melchior and Collins, 1982; Myers, 1989; Rommelspacher et al., 1991; Adachi et al., 1991; Brossi, 1993; Manabe et al., 1996). Indeed, accumulation in vivo could arise, at least in part, from food-ingested THβCs or, eventually, from those formed endogenously through ingested precursors. In this regard, experiments in rats have shown absorption and accumulation of MTCA in many organs including the brain (Fukushima et al., 1991; Nagahara and Kumagai, 1991; Ogawa et al., 1993). Although, in the past few years, several studies have considered the possible mutagenicity, toxicity, or neuroactivity of THβCs and βCs (Buckholtz, 1980; Airaksinen and Kari, 1981; Wakabayashi et al., 1983; Fujie et al., 1990; Brenneman et al., 1993; Higashimoto et al., 1996), there is still a great need for a full delineation of their biological activity and/or toxicity.

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