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Identification and Occurrence of Tryptamine- and Tryptophan-Derived Tetrahydro-β-carbolines in Commercial Sausages

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The identification and occurrence of tetrahydro-β-carbolines were studied in different kinds of commercial sausages including cooked, fresh, dry-fermented, and ripened sausages, such as salamis and Spanish chorizo, salchichon, fuet, and morcilla, both smoked and unsmoked. Four compounds were identified in several sausages by high-performance liquid chromatography−mass spectrometry (HPLC-MS): 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**1**), 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid diastereoisomers (**2a**,**b**), 1,2,3,4-tetrahydro-β-carboline (**3**), and 1-methyl-1,2,3,4-tetrahydro-β-carboline (**4**). The latter two (**3** and **4**) are now reported for the first time in meat products. The presence and occurrence of tetrahydro-β-carbolines were highly variable depending on each particular sample of sausage, and it did not follow a single specific pattern. The concentration range taken as a sum of the four carbolines varied from undetectable levels to 33 μg/g, with the highest content found in ripened, dry-fermented, and smoked sausages (salami, chorizo, and morcilla) and the lowest in cooked sausages (Frankfurt). Formation of tetrahydro-β-carbolines might occur during elaboration and the ripening process from a chemical condensation between tryptophan or tryptamine and aldehydes (formaldehyde and acetaldehyde). Smoked samples had higher concentrations of formaldehyde-derived 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**1**) and 1,2,3,4-tetrahydro-β-carboline (tryptoline) (**3**) than those unsmoked. Also, **1** and **3** were more concentrated in the outer part of the sausage, likely to be in contact with smoke. It is concluded that some dry-fermented and/or smoked sausages may be significant dietary sources of tetrahydro-β-carbolines.

KEYWORDS: Tetrahydro-β-carbolines; sausages; fermented meats; smoking; tryptophan; tryptamine

INTRODUCTION

1,2,3,4-Tetrahydro-β-carboline (THβCs) (tetrahydro-9H-pyrido-[3,4-b]indole) are naturally occurring tricyclic indole derivatives produced in foods from indolethylamines and aldehydes or α-ketoacids through a Pictet−Spengler condensation (*1*−*3*). Numerous reports have shown the occurrence of β-carbolines in biological tissues and fluids (*4*−*8*). These compounds might function as inhibitors of the monoamine oxidase and the monoamine uptake and release and bind to several brain receptors (*4*, *5*, *9*, *10*). Other researchers have focused on their potential toxicological effects, and in this regard, β-carbolines could be endogenously bioactivated to give neurotoxins (*11*, *12*), and some may be endogenous substrates of the polymorphic cytochrome P450 CYP2D6 (*13*). THβCs are not mutagenic (*14*) but, upon nitrosation, may afford nitrosated compounds in foods (*15*, *16*). Nitrosation of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2a**,**b**) provided potential mutagenic compounds (*17*, *18*). Tetrahydro-β-carboline-3-carboxylic acids are also oxidized to the fully aromatic β-carbolines norharman and harman (*19*) that may exhibit further biological activity and comutagenic activity in the presence of aromatic amines (*20*−*22*). On the other hand, these compounds might exert actions as antioxidants and free radical scavengers (*23*, *24*).

Because of the biological interest of THβC, our research effort has pursued to shed light on the occurrence of these compounds in foodstuffs. They appear to be naturally occurring substances produced during food production, processing, and storage (*2*, *19*). Our results and those from others point to the diet as a significant environmental source of β-carbolines in humans. Indeed, dietary sources can provide THβCs that might lately accumulate in biological tissues and fluids (*25*). Thus, tryptophan-derived THβCs such as **2a**,**b** and 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**1**) usually appear in many commercial foods (*2*, *19*, *26*). Furthermore, individual THβCs may appear in foodstuffs in a manner that appears to be specific to the food and processing involved (*1*−*3*, *15*, *19*, *26*−*29*).

Only a few attempts have been made so far to study the occurrence of THβCs in meat products. Compound **1** was the main β-carboline detected in several smoked products including

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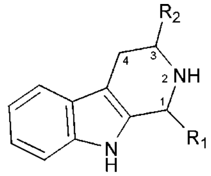

R_1	R_2	Comp.
H	COOH	1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**1**)
CH_3	COOH	1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (1*S*,3*S* isomer: **2a**; 1*R*,3*S* isomer: **2b**)
H	H	1,2,3,4-tetrahydro-β-carboline (**3**)
CH_3	H	1-methyl-1,2,3,4-tetrahydro-β-carboline (**4**)
CH_2OH	COOH	1-hydroxymethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (*cis* isomer) (**5**)
CH_2CH_3	COOH	1-ethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (IS)

Figure 1. Tetrahydro-*β*-carboline molecules found in sausages.

sausages (*15*, *30*, *31*), and it may also appear in raw and cooked meat and fish (*20*). Fermented sausages are products of complex composition because of the many ingredients and technological variables involved during manufacturing (*32*). Sausages are also cured meats rich in nitrosating agents. The aim of the present research was to study the occurrence of TH*β*Cs in different types of commercial sausages, including cooked, fermented, and ripened sausages, both smoked and unsmoked, as well as to accomplish the chemical identification by high-performance liquid chromatography−mass spectrometry (HPLC-MS) of this class of compounds in those products. The results obtained below show that several TH*β*Cs may appear in sausages in variable amounts. They include tryptamine-derived TH*β*Cs that are reported for the first time in those products. Their possible formation as a result of a reaction between tryptophan and/or tryptamine and aldehydes produced during manufacturing or storage of sausages is discussed.

MATERIAL AND METHODS

Reference Compounds and Commercial Samples of Sausages. TH*β*C molecules used in this work are illustrated in **Figure 1**. Compounds **1**, **2a**,**b**, and 1-ethyl-1,2,3,4-tetrahydro-*β*-carboline-3-carboxylic acid (ETCA) were synthesized as reported previously (*33*, *34*). 1,2,3,4-Tetrahydro-*β*-carboline (**3**) and 1-methyl-1,2,3,4-tetrahydro-*β*-carboline (**4**) were synthesized from tryptamine and formaldehyde or acetaldehyde, respectively. NMR, MS, and gas chromatography (GC)-MS (trifluoroacetyl and methoxycarbonyl methyl ester derivatives) data were consistent with the structures of the synthesized compounds (*33*, *34*).

A total of 89 different commercially available sausages from different origins (local and imported) and manufacturers were purchased locally in Spain and Greece. They included fresh, cooked sausages (Frankfurt) and cured and dry-fermented sausages, and some of them were labeled as smoked sausages. Briefly, the samples studied were fresh cooked sausages, salamis from different origins, Spanish fermented sausage called chorizo, Spanish sausage called morcilla, and Spanish dry-fermented sausages called salchichon and fuet. Chorizo, a popular dry-fermented sausage in Spain, is made of minced meat (generally pork) mixed with fat, red pepper (paprika), spices, garlic, and curing additives (salt, nitrate, nitrite, sugar, and ascorbates) stuffed into natural or artificial casings, fermented, and ripened at low temperature and controlled humidity (*32*). The variety chorizo of Pamplona is similar to chorizo in the technological process but with a particle size reduction to 3 mm and a high sausage diameter (55−80 mm) (*35*). Salchichon is similar to chorizo in composition but uses black pepper instead of garlic and paprika. Both products (chorizo and salchichon) are usually subjected to fermentation (20−24 °C, 48 h) and ripening (12−15 °C) for more than a month. Fuet is a type of dry salchichon in its composition but with a smaller diameter (less than 3 cm) and is fermented and ripened at 14−15 °C for 12−15 days (*36*). Morcilla is a sausage that contains animal blood as an essential ingredient in addition to meat and spices and may be cured and ripened. Chorizo, salami, and morcilla may suffer a smoking process during manufacturing, which is usually indicated on the label. Many variables are involved during the manufacturing process of fermented sausages that may change from product to product, brand to brand, and region to region (*32*, *37*).

Isolation of TH*β*Cs from Sausages and Chromatographic Analysis. TH*β*Cs were isolated by SCX solid phase extraction as described previously (*2*). Briefly, sausages were minced and ground, and about 3−5 g of them was added to 10−20 mL of 0.6 M $HClO_4$ containing 1 mg/mL semicarbazide (Sigma), homogenized in an Ultra-Turrax, and centrifuged (12 000 rpm, 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 an internal standard (IS) and passed through Bond Elut, 500 mg/3 mL size benzenesulfonic acid-SCX columns (Varian, Harbor City, CA) using a vacuum manifold. Washing of SCX columns was carried out with 0.1 M HCl (6 mL), methanol (2 mL), water (6 mL), and 0.4 M phosphate buffer, pH 9.2 (2 mL). Elution was done with a mixture of 0.4 M phosphate buffer (pH 9.2):methanol (1:1) (6 mL) and subsequently injected into reversed phase (RP)-HPLC.

The analysis of TH*β*Cs by RP-HPLC was carried out as previously described (*2*). A 150 mm × 3.9 mm, 4 *μ*m, 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 90% B at 18 min. The flow rate was 1 mL/min, the column temperature was 40 °C, and the injection volume was 20 *μ*L. Fluorescence detection was set at 270 nm for excitation and 343 nm for emission. Quantitation was calculated from calibration curves (area ratio vs concentration) constructed from TH*β*Cs solutions of known concentration and analyzed through the entire SCX procedure and using ETCA as an IS. This analytical method showed a good reliability (*2*, *20*). Confirmation of the identity of isolated TH*β*Cs was established by coelution with authentic standards. Also, fluorescence spectra of the HPLC peaks were compared with those of reference compounds to ensure that quantified peaks correspond to those expected. Several samples containing TH*β*Cs were also analyzed by HPLC-MS for chemical identification as indicated below.

RP-HPLC-MS Analysis. Samples of sausages were isolated for TH*β*Cs by SCX extraction as above. The eluting fractions corresponding to phosphate buffer + methanol (1:1) containing the TH*β*Cs were concentrated under a stream of nitrogen and subsequently injected into the HPLC-MS. Chemical identification was accomplished by HPLC-MS on a 3.9 mm × 150 mm Novapak C18 column, 4 *μ*m (Waters), by using an HPLC-MSD series 1100 (Hewlett-Packard) (electrospray positive ion mode). Eluents: A, formic acid (0.25%); B, 0.25% formic acid in acetonitrile. The gradient was programmed from 0 to 20% B in 30 min. The flow was 0.6 mL/min, the cone voltage was 50 V, the capillary voltage was 4000 V, and the mass range was 50−650 amu. Authentic standards of the compounds in **Figure 1** were also chromatographed under the same conditions to compare both spectra and retention time in order to confirm the presence of TH*β*Cs in sausages.

RESULTS

Numerous samples of commercial sausages of different types and brands were subjected to SCX solid phase extraction and analyzed by RP-HPLC-fluorescence (**Figure 2**). Some of the sausages, although by no means all of them, showed chromatographic peaks that coeluted with authentic standards of TH*β*Cs: i.e., **1**, **2a**,**b**, **3**, and **4**. A further study was accomplished in order to prove the presence of these carbolines in sausages by HPLC-MS. This was needed because of the lack of enough data about the chemical identification by MS of *β*-carbolines in meat products other than those reported by Sen et al. (*15*) and Papavergou and Herraiz (*31*). SCX-extracted samples of sausages exhibiting chromatographic peaks by HPLC-fluorescence were analyzed by HPLC-MS under electrospray ionization (ESI) (**Figure 3**). The presence of four TH*β*Cs, **1**, **2a**,**b**, **3**, and **4**, was demonstrated in several samples of sausages, such as salamis and Spanish chorizos, salchichon, and morcilla. The

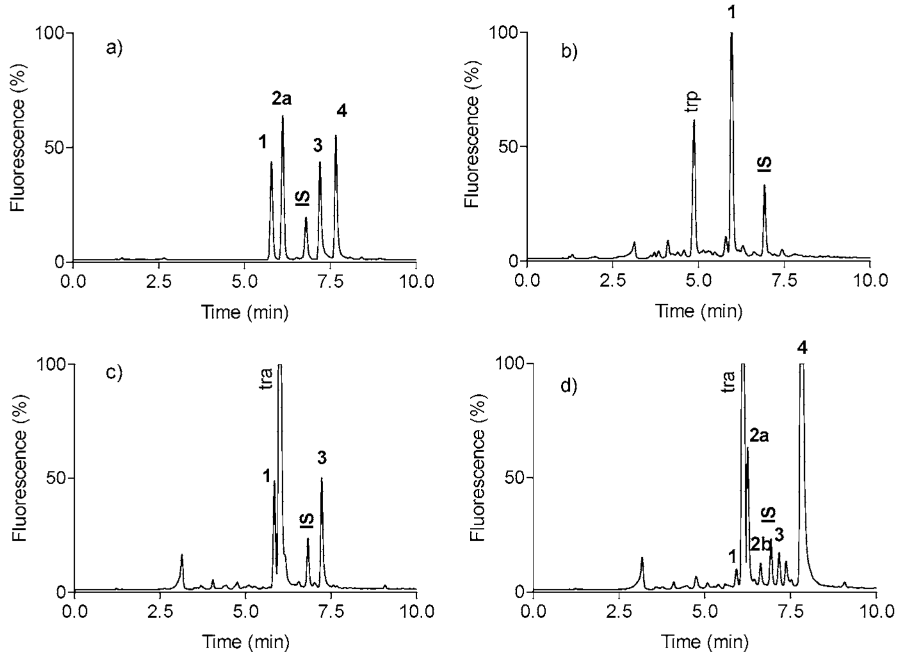

Figure 2. HPLC chromatograms of tetrahydro-β-carbolines from standards (**a**) and SCX-extracted samples of a Greek smoked salami (**b**), a smoked chorizo (**c**), and a sample of chorizo of *Pamplona* (**d**). Tetrahydro-β-carbolines are as in **Figure 1**, tryptophan (trp) and tryptamine (tra).

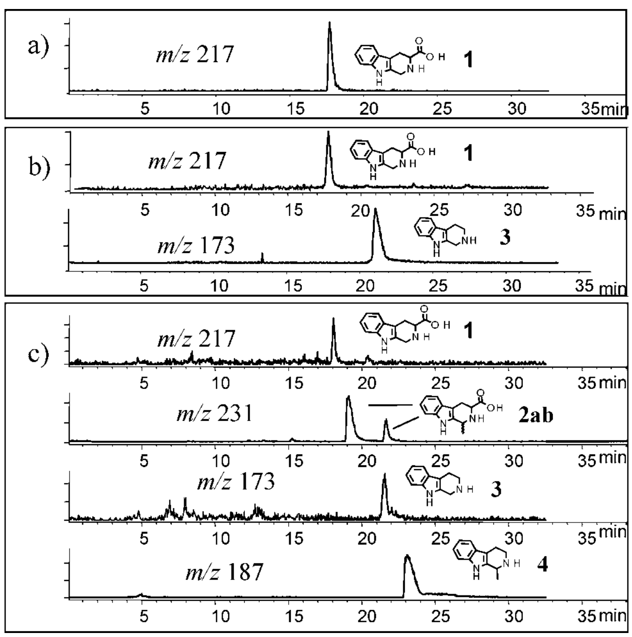

Figure 3. RP-HPLC-MS (ESI) of some extracted samples of sausages: a Greek smoked salami (**a**), a smoked salami (**b**), and a sample of chrorizo of *Pamplona* (**c**).

compounds afforded the corresponding trace ions and mass spectra dominated by the protonated molecular ions at *m/z* 217 $(M + H)^+$ (**1**), 231 $(M + H)^+$ (diastereoisomers **2a**,**b**), 173 $(M + H)^+$ (**3**), and 187 $(M + H)^+$ (**4**) and a loss of 29 amu for tryptolines **3** and **4** and 73 amu for tetrahydro-β-carboline-3-carboxylic acids **1** and **2a**,**b** due to the retro-Diels−Alder fragmentation (**Figure 4**). This was also confirmed by chromatographic analysis of the corresponding standards. Two of these carbolines, i.e., tryptoline (**3**) and 1-methyl-tryptoline (**4**), are novel compounds identified in sausages. Furthermore, some smoked salamis contained traces of 1-hydroxymethyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**5**) as previously reported (*15*, *30*). The presence of each individual THβC was very much dependent on each particular sausage, and usually, the four compounds were not present altogether in the same sample, perhaps with the only exception of **1**.

The occurrence of these THβCs in sausages is given in **Table 1**. A first noticeable fact is the highly variable content of THβCs among samples of different groups of sausages but also between samples within the same group. Regarding the individual samples analyzed, the whole amount taken as the sum of the four THβCs ranged from an undetectable amount to 28.7 μg/g that was found in a sample of smoked salami or 33.4 μg/g in a sample of chorizo of Pamplona. Regarding the different selected groups summarized in **Table 1**, the highest concentration of carbolines was found in chorizo, salami, and morcilla, whereas the lowest was found in salchichon, fuet, and also in cooked (Frankfurt) or country sausages. Compound **1** appeared in most samples of sausages, although with a varying content depending on the sample, and reached a highest value of 16 μg/g in a sample of smoked chorizo. The highest content of **1** was found in smoked and ripened dry sausages (morcilla, chorizo, and salami), which contained more than 10-fold the content of those unsmoked. When **2a**,**b** appeared in sausage, its concentration was generally lower than **1** with the exception of several samples of chorizo of Pamplona and some individual samples of unsmoked chorizo, salami, and a singular sample of salchichon. By comparing smoked vs nonsmoked sausages, there was not a definitively higher concentration of the carboline **2a**,**b** in smoked samples. As far as the tryptoline **3** is concerned, the highest average amount and concentration range was found in salamis, particularly those smoked ones, but it also appeared in several samples of chorizo and morcilla. THβC **3** appears to occur more often in smoked samples and very low or generally undetectable levels of it were generally found in salchichon, cooked sausages, and fuets. The carboline **4** was found in some dry-fermented and smoked sausages such as chorizos, salamis, and morcillas. The highest relative amount of **4** was observed in some ripened chorizo of Pamplona and also appeared in various salamis, morcillas, and a singular sample of salchichon. The compound **4** was not present in a much higher amount in smoked samples than those unsmoked ones. As indicated above,

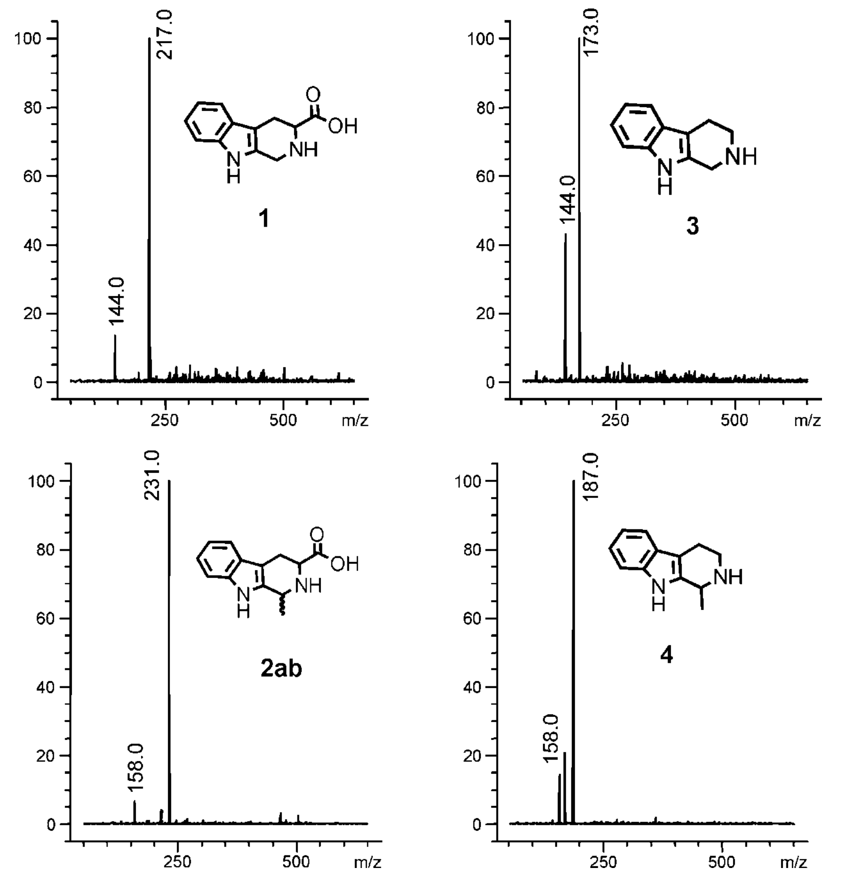

Figure 4. Mass spectra (ESI) of tetrahydro-β-carbolines: **1** (from smoked salami), **3** (from smoked chorizo), and **2a**,**b** and **4** (from a sample of chorizo).

Table 1. Concentrations (μg/g) of Tetrahydro-β-carbolines in Several Kinds of Sausages (Cooked, Ripened, Smoked, or Unsmoked)

		1		**2a**		**2b**		**3**		**4**	
sample	*N*	X ± SD	range	X ± SD	range	X ± SD	range	X ± SD	range	X ± SD	range
smoked salami	9	3.8 ± 2.1	0.09–7.2	0.3 ± 0.3	nd–0.96	0.1 ± 0.09	nd–0.2	3.7 ± 7.8	nd–23	0.33 ± 0.66	nd–1.6
unsmoked salami	16	0.35 ± 0.3	0.01–0.9	0.48 ± 0.96	nd–2.9	0.1 ± 0.2	nd–0.6	0.17 ± 0.2	nd–0.8	0.68 ± 1.6	nd–4.9
total salami	25	1.6 ± 2.1	0.01–7.2	0.4 ± 0.78	nd–2.9	0.11 ± 0.16	nd–0.63	1.4 ± 4.9	nd–22.6	0.56 ± 1.3	nd–4.9
smoked chorizo	5	6.2 ± 6.3	1.3–15.8	0.28 ± 0.27	nd–0.6	0.11 ± 0.08	nd–0.2	1.7 ± 1.4	0.6–4.0	0.02 ± 0.04	nd–0.1
unsmoked chorizo	18	0.64 ± 0.34	0.1–1.3	0.15 ± 0.17	nd–0.7	0.04 ± 0.05	nd–0.22	0.05 ± 0.13	nd–0.56	0.06 ± 0.18	nd–0.7
Pamplona chorizo[a]	8	1.3 ± 1.8	0.4–5.74	3.34 ± 5.8	nd–16.6	0.77 ± 1.4	nd–4	0.35 ± 0.3	nd–0.6	7.21 ± 10.9	nd–29.1
total chorizo	31	1.7 ± 3.2	0.1–15.8	0.98 ± 3.1	nd–16.6	0.26 ± 0.74	nd–4	0.40 ± 0.8	nd–4.0	1.9 ± 6.1	nd–29.1
smoked morcilla	2	14.9 ± 1.3	14–15.8	0.70 ± 1.0	nd–1.4	0.14 ± 0.2	nd–0.28	1.0 ± 0.1	1–1.1	0.44 ± 0.5	0.1–0.8
unsmoked morcilla	4	1.1 ± 0.4	0.6–1.6	0.15 ± 0.12	nd–0.3	0.06 ± 0.04	nd–0.08	0.34 ± 0.4	nd–0.9	0.08 ± 0.09	nd–0.17
total morcilla	6	5.7 ± 7.2	0.6–15.8	0.34 ± 0.5	nd–1.4	0.08 ± 0.1	nd–0.28	0.58 ± 0.49	nd–1.1	0.20 ± 0.29	nd–0.78
salchichon	9	0.45 ± 0.5	0.09–1.8	0.12 ± 0.15	nd–0.3	0.05 ± 0.05	nd–0.14	0.08 ± 0.15	nd–0.37	0.01 ± 0.02	nd–0.06
salchichon	1	0.37		2.63		0.57		0.51		9.9	
fuet	5	0.09 ± 0.05	0.04–0.16	nd		nd		nd		nd	
cooked sausages[b]	7	0.04 ± 0.04	nd–0.13	0.002	nd–0.01	nd		nd		nd	
country sausages[c]	5	0.28 ± 0.28	0.05–0.8	0.07 ± 0.09	nd–0.22	0.03 ± 0.04	nd–0.11	nd		nd	

[a] Pamplona chorizo may be slightly smoked although it was not stated on the label (*35*). [b] Cooked and smoked sausages of Frankfurt style. [c] Usually boiled and smoked products. nd, undetectable amount or below detection limit. Tetrahydro-β-carbolines **1**, **2a**,**b**, **3**, and **4** are as in **Figure 1**. X values may be affected by the variability among samples.

5 (not included in **Table 1**) only seemed to appear in various smoked salamis (*15*, *30*) within a range of nd−0.5 μg/g.

One of the main differences regarding the content of carbolines in sausages appears to be the smoking process. We further studied the presence of carbolines in two different parts of several smoked sausages containing THβCs. For that, we considered the outer part (approximately up to 1 cm deep from the surface) and the inner part of a taken section of the sausage (**Table 2**). The results showed that the outer part of smoked sausages, likely to be in contact with woodsmoke, contained a significantly ($p < 0.05$, paired *t*-test; inner vs outer parts) higher amount of **1** than the inner counterpart (from 1.7- to 4.5-fold). Moreover, smoked samples that contained a high level of **3** showed a much higher amount of this carboline in the outer part (up to 9.6-fold) than in the inner counterpart. In contrast, the other two THβCs, **2a**,**b**, and **4** appear to be more concentrated in the inner parts, suggesting that their occurrence was less linked to the smoking process as compared to **1** and **3**.

DISCUSSION

The above results point out the occurrence of several THβCs in sausages of different types: cooked, fermented, ripened, smoked, and unsmoked. Two of these compounds, **3** (tryptoline)

Table 2. Concentrations (μg/g) of Tetrahydro-β-carbolines Measured in the Outer[a] and Inner Parts of the Same Slice of Various Individual Commercial Smoked Sausages Containing Tetrahydro-β-carbolines

	1	2ab	3	4
salami-1 outer	7.3	1.2	13.7	0.67
salami-1 inner	2.5	2.4	2.3	3.18
salami-2 outer	6.6	1.3	13.5	0.09
salami-2 inner	2.4	1.2	1.4	1.07
salami-3 outer	5.7	0.7	0.09	nd
salami-3 inner	1.2	0.89	0.05	0.045
salami-4 outer	9.7	0.4	nd	nd
salami-4 inner	2.6	0.2	nd	nd
salami-5 outer	1	2.02	0.4	2.0
salami-5 inner	0.47	3.83	0.3	5.4
chorizo-1 outer	1.85	nd	2.94	nd
chorizo-1 inner	1.0	nd	1.76	nd

[a] Usually the outer part was taken up to approximately 1 cm deep from the surface of the sausage. Not detectable or below the detection limit (nd).

and **4** (1-methyl-tryptoline), are reported for the first time in those products. As shown from the concentration range and standard deviation in **Table 1**, the first noticeable fact is the great variation observed among the samples. This variation suggests that the concentration of THβCs in different sausages must depend on a variety of factors involved in the elaboration process. These factors include smoking, fermentation and microorganisms involved, ripening and storage (temperature, time, and humidity), artisanal production, and many others like the raw meats used, ingredients used for flavoring, spices, etc. This would explain the absence of any clear specific pattern of concentration among different groups of samples. Moreover, a particular characteristic of the sausage manufacturing process is the different ingredients and formulations that are used to achieve a characteristic flavor in commercial sausages and that may vary from region to region and brand to brand. These formulations include spices and condiments, some of which have been found to contain free tryptophan, tryptamine, and THβCs (Herrraiz, unpublished results) and therefore might contribute although to a minor extent to the occurrence of THβCs in sausages. However, a major part of THβCs is expected to occur through the so-called Pictet−Spengler chemical condensation from indoleamino acids (tryptophan) and indoleamines (tryptamine), both occurring in sausages, and aldehydes. A scheme of this is illustrated in **Figure 5**. Thus, tryptophan present in raw meats or formed during proteolysis might react with formaldehyde and/or acetaldehyde released during elaboration, ripening, and/or storage or, as a result of the activity of the microorganisms involved in fermentation, giving rise to the corresponding tetrahydro-β-carboline-3-carboxylic acid compounds. Alternatively, tryptamine produced during the decarboxylation of tryptophan by microbial fermentation will produce the corresponding THβCs (tryptolines) lacking the carboxylic acid in the tetrahydropyrido ring, either THβC **3** from formaldehyde and **4** from acetaldehyde. Moreover, from the chromatograms obtained in this study, we observed that the presence of tryptamine-derived THβCs **3** and **4** usually appeared in sausages that exhibited a high level of tryptamine. In contrast, samples with low or no tryptamine usually did not contain these THβCs. This is in line with the characteristic pattern of occurrence of these compounds depending on the amino acid and amine occurring in the sample, as recently reported in fruit products (*24*).

Compound **1** is a major β-carboline in smoked foods (*31*), in agreement with the results by Papavergou and Clifford (*30*) and others (*2*, *15*, *20*). The analysis of the outer and interior parts of several selected samples of smoked products presented in **Table 2** suggests a likely formation of **1** from formaldehyde arising from the smoke. Thus, the outer part of smoked salamis and chorizos, expected to be in direct contact with smoke, contained a much higher amount of **1** than the inner counterpart. Consequently, this particular β-carboline is produced during or after the smoking process with the involvement of formaldehyde, a naturally occurring carbonylic compound in wood smoke (*38*). Indeed, formaldehyde easily reacts with tryptophan in foods affording **1** (*2*, *19*). Furthermore, the same is expected to occur when free tryptamine, previously formed from enzymatic decarboxylation of tryptophan (**Figure 5**), reacts with wood-smoke formaldehyde affording the corresponding **3**. This explains the high amount of **3** encountered in various smoked sausages as well as its higher presence in the outer part of smoked sausages as compared to the inner part, as occur also with **1**. However, the existence of **1** and **3** in some unsmoked products suggests that other sources of formaldehyde in addition to smoking are likely during manufacturing and ripening of sausages.

Compound **2a**,**b** is a major β-carboline detected in many foods (*2*, *19*) because of the reaction between tryptophan and acetaldehyde. This compound was detected in several dry-fermented sausages, several unsmoked sausages, and chorizo of Pamplona, the latter containing the highest amount of this carboline. Compounds **2a**,**b** and **4**, both of which are likely produced with the participation of acetaldehyde (**Figure 5**), were not higher in smoked sausages nor in their corresponding outer parts as compared with inner counterparts. Then, they are not specifically linked to smoking and other factors should be

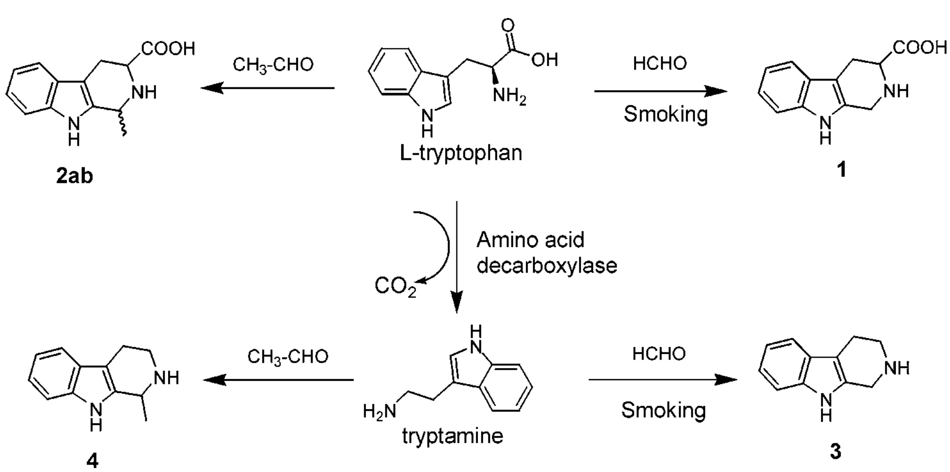

Figure 5. Scheme proposed for the formation of tryptophan- and tryptamine-derived tetrahydro-β-carbolines in sausages.

involved. One of these factors might be the fermentation and ripening process that would produce acetaldehyde from carbohydrates, which is able to react with tryptophan or tryptamine. Indeed, this reaction would be favored under the acidification process occurring during ripening of fermented sausages (*39*). This could be an important factor in long-ripened dry-fermented sausages having a relatively low pH, such as chorizo of Pamplona (pH around 4.6) (*35*). The opposite will occur in short-ripened dry-fermented sausages such as fuets, which have a higher pH (pH around 5.5−6.0) (*40*, *41*). Aldehydes might also form in meats through chemical oxidative reactions during ripening (*42*) or could be added within the ingredients during formulation. Nevertheless, other additional factors involved in the formation of these compounds in sausages should not be ruled out completely.

In conclusion, the present paper reports the identification and occurrence of several THβCs in different kinds of commercial sausages. This is in line with the likely formation of different molecules of THβCs during food production, processing, and storage. It is difficult, however, to clarify the specific contribution of sausages to the dietary intake of β-carbolines because of the great variability among samples. Nevertheless, in those fermented and ripened sausages with a high content of THβCs, this contribution may be substantial. While relatively low levels were found in raw unsmoked meat (pork and beef) and fish (*20*), those heavily smoked and dry-fermented and ripened sausages may contain a relatively high amount of THβCs, as shown in this paper and others (*15*, *20*, *30*, *31*). In addition to the known THβCs **1**, **2a**,**b**, and **5**, we have now reported the presence of novel tryptolines in sausages: **3** and **4** arising from the tryptamine occurring in fermented meats (*41*).

These and previous results on the presence of this class of heterocyclic THβCs in foods point out dietary intake as a likely explanation of their further presence and possible accumulation in tissues and biological fluids. Although several studies have already considered the possible mutagenicity, toxicity, or neuroactivity, and recently the antioxidant effects of THβCs, a complete delineation of the biological activity and/or toxicity of these compounds remains to be accomplished. In this regard, an interesting point linked to the presence of THβCs in cured sausages (nitrite-treated) is a reaction giving potential undesirable *N*-nitroso compounds (*15*−*18*).

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