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ORIGINAL ARTICLE

Determination of biogenic amines in infusions of tea (*Camellia sinensis*) by HPLC after derivatization with 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl)

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Abstract The reagent 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl) was used for the pre-column derivatization of the biogenic amines (BAs) cadaverine (Cad), histamine (Him), octopamine (Ocp), phenylethylamine (Pea), putrescine (Put), spermidine (Spd), spermine (Spm), tyramine (Tym) and the internal standard 1,6-diaminohexane (Dhx). The resulting Fmoc-derivatives were resolved by high-performance liquid chromatography on a Superspher© C_{18} column using a binary gradient generated from sodium acetate and acetonitrile. For quantification, the fluorescence of derivatives was used at 263 nm excitation and 313 nm emission wavelength. This approach was applied to free BAs extractable with boiling water from 14 black, 5 green, 1 Oolong, and 1 instant tea. Infusions were prepared by adding 35 ml boiling water to one gram of tea and extracted for 20 min. In the Oolong tea and two black teas, no BAs could be detected. Limits of detection were 0.07–1.0 pmol for BAs at signal-to-noise ratio 3:1. Besides most abundant Tym and Spm lower quantities of Pea, Put, and Spd were detected, albeit not in all teas. Quantities of Tym ranged from 16 to 431 μg Tym/L infusion (1.1–25.3 μgTym/g tea) and 31 to 319 μg Spm/L infusion (1.5–16.9 μg Spm/g tea). In none of the teas, Him was detected. Owing to the low amounts of free BAs in tea infusions, no health risks are to be expected even on consumption of large quantities of tea as beverage.

Keywords Tea infusions · High-performance liquid chromatography · Polyamines · 9-fluorenylmethyl chloroformate · Risk assessment

Abbreviations

BA(s)	Biogenic amine(s)
PA(s)	Polyamine(s)
Cad	Cadaverine, pentane-1,5-diamine
Dhx	Hexane-1,6-diamine (internal standard)
Him	Histamine, 2-(1*H*-imidazol-4-yl)ethanamine
Pea	Phenylethylamine, phenylethan-2-amine
Ocp	octopamine, (*R,S*)-4-(2-amino-1-hydroxy-ethyl) phenol
Put	Putrescine, butane-1,4-diamine
Spd	Spermidine *N*-(3-aminopropyl)butane-1,4-diamine
Spm	Spermine, *N,N'*-bis(3-aminopropyl)butane-1,4-diamine
Tym	Tyramine, 3-(2-aminoethyl)phenol
HPLC	High-performance liquid chromatography

Introduction

Biogenic amines are low molecular weight alkaline nitrogenous compounds, which are naturally occurring in plants, animals and microorganisms and exert biological

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effects. They comprise the aromatic monoamines Pea, Tym, and Ocp, the heterocyclic Him, and the polyamines having at least two amine groups such as the diamines Put and Cad, the triamine Spd, and the tetramine Spm.

In plants the PAs Put, Spd and Spm are ubiquitous and, consequently, have been detected in edible plants such as vegetables and fruits or juices made thereof. Plant PAs are involved in growth and development processes, as well as defence mechanisms against biotic and abiotic stress such as pathogen attack or drought (Edreva 1997; Groppa and Benavides 2008).

In animals and human beings, PAs are required for cell proliferation and have also been detected at higher concentrations in tumor tissues in comparison to normal cells. For an entrance to the related literature see Agostinelli and Igarashi (2007). Therefore, Cipolla et al. (2007) assessed the contents of Put, Cad, Spm, and Spd in 233 current foods and beverages to reduce and control PA uptake in prostate carcinoma patients.

Despite the fact that BAs are also endogenous components of mammalian cells, adverse effects on human beings have been established for certain compounds when consumed with food or beverages. Him is a vasoactive substance that can cause pseudoallergic reactions, hypotension, headache and abdominal pains. A large dietary Tym intake has been associated with hypertension and migraine and can also induce brain haemorrhage and heart failure. Pea acts as releasing agent for the neurotransmitter dopamine and abnormally low physiological concentrations result often on clinical depression, whereas abnormally high concentrations have a strong correlation with schizophrenia (Potkin et al. 1979; Shalaby 1996; Zaman et al. 2009).

In a detailed study by Til et al. (1997) the acute and subacute toxicity of Tym, Spd, Spm, Put and Cad were examined in experimental rats following oral and intravenous administration. A relatively low oral toxicity of $\geq$2 g/kg body weight (BW) was found for Tym, Cad and Put, and of 0.6 g/kg BW for Spd and Spm. Intravenous administration of the BAs caused a decrease of blood pressure with the exception of Tym where an increase was found.

Exogenous BAs are directly absorbed from food in the intestine and alcohol can increase the absorption rate. Furthermore, synergistic effects of BAs resulting in potentiation of physiological effects have to be considered. Although BAs are broken down in the human body by the oxidative deamination catalyzed by monoamino oxidase (MAO), overload of the enzyme system, abnormal MAO deficiency, or therapeutical use of MAO inhibitors as antidepressant drugs have to be taken into account.

It has been pointed out that the intake of dietary PAs is of importance for health and disease (Agostinelli and Igarashi 2007; Agostinelli et al. 2010; Bardóz and White 1999; Beutling 1996; Binh et al. 2010; Shalaby 1996; Soda 2010), but that data on BA and PA contents in foods are limited and dispersed in the literature and that comprehensive databases should be established (Ali et al. 2011; Bardócz et al., 1995; Eliassen et al. 2002; Kalač and Krausová 2005; Nishibori et al. 2007; Zoumas-Morse et al., 2007).

However, quantities of BAs in foodstuffs can considerably vary and drastically increase as results of microbial spoilage or controlled microbial fermentation of raw materials (Askar and Treptow 1986; Bardócz and White, 1999; Stratton et al. 1991). Examples are seafood, meat, ripened cheeses, alcoholic fermented beverages such as wine and beer, or Oriental fermented foodstuffs (Busto et al. 1996, 1997; Hernández-Jover et al. 1997; Hornero-Méndez and Garrido-Fernández 1994; Joosten 1988; Kirschbaum et al. 1999; 2000; Silva and Gloria 2002; Stratton et al. 1991; Zaman et al. 2009).

Hence, sensitive and reliable methods for the detection, separation and quantification of BAs in foods and drinks are required. For the analysis of non-volatile BAs, liquid chromatographic methods are preferred. Since most PAs occurring in food neither exhibit satisfactory absorption in the ultraviolet or visible range nor show fluorescence, their determination is carried out preferably by HPLC using a large number of chromophoric or fluorescent chemical derivatizing reagents (Bockhardt et al. 1996; Koski et al. 1987; Lu et al. 1991; Merali and Clarkson 1996; Bellagamba et al. 1997; Molnár-Perl 2005a, b). Advanced methods use liquid chromatography combined with mass spectrometry (Cipolla et al. 2007; Gaboriau et al. 2003, 2005; Kirschbaum et al. 2000). For a survey of the extraction procedures and HPLC methods used for the determination of BAs, we refer to the literature (Karovičová and Kohajdová 2005; Molnár-Perl 2005b; Mengerink 2005).

We have used the fluorescent reagent 2-naphthyloxycarbonyl chloride (NOC-Cl) for the separation of amino acids (Brückner and Lüpke 1995) and determination of BAs in fruit juices, wines, vinegars and salmon (Kirschbaum et al. 1997) and the UV-absorbing *p*-nitrobenzyloxycarbonyl chloride (PNZ-Cl) for the determination of biogenic amines in a large number of beers, wines, and vinegars (Kirschbaum et al. 1999).

The UV-absorbing reagent 3,5-dinitrobenzoyl chloride (DNBZ-Cl) was used for the HPLC separation of amino acids (Brückner and Lüpke 1995) and determination of BAs in fermented fish products such as Anchovy paste and fermented fish sauce, Oriental fermented foods such as Soy sauce and Korean Miso, and lactic fermented cabbage juice (Kirschbaum et al. 2000).

However, among the abundance of pre-column derivatization reagents suitable for the HPLC determination of BAs, 9-fluorenylmethoxycarbonyl chloride, also known as 9-fluorenylmethyl chloroformate (Fmoc-Cl) has the

advantage of fast derivatization of the amines using a fully automated instrument. Further benefits are good separation of the derivatives on common C_{18} columns and sensitive detection owing to their fluorescence (Lu et al., 1991; Kirschbaum et al. 1994; Bellagamba et al. 1997; Ho 2005; Bauza et al. 2007).

Although numerous foods and beverages have been investigated for the presence of BAs, only few deal with tea or its aqueous infusions (Kakkar and Nagar 1997; Neumann and Montag 1983; Okamoto et al. 1997; Palavan-Ünsal et al. 2007; Serenkow and Projsser 1960, 1961). This is surprising since the aqueous extract of leaves and buds of *Camellia sinensis* (L.) O. Kuntze is the most popular drink in many countries that is consumed regularly and, in part, in large amounts. Therefore, we optimized and applied a sensitive HPLC method (Kirschbaum et al. 1994) for the quantitative determination of free BAs in tea infusions used as beverage.

In this context we briefly report on the kinds of teas analyzed. Basically, one has to distinguish among infusions prepared from green tea or from black tea. Briefly, green tea represents the dried young leaves and buds of cultivars and varieties of the plant *Camellia sinensis* usually subjected to heat or steam treatment and fast drying to destroy endogenous plant enzymes. Black tea is also made from leaves and buds of *Camellia sinensis* which are subjected to a sequence of procedures such as weathering, destruction of plant tissues by various rolling, crushing and/or tearing processes followed by enzymic maturation, and final drying. Destruction of the plant tissues results in the release of an abundance of enzymes being responsible for oxidation and degradation processes leading also to the formation of acidic polyphenols and characteristic color compounds. This non-microbial process is called tea fermentation. Oolong tea is semi-fermented tea resulting from a gentle and very short fermentation process. Since an abundance of various tea processing technologies are applied to a myriad of different tea cultivars growing in various countries, regions and altitudes, and leaves are harvested at various growth stages and seasons, a vast number of teas and brand names are on the market. Common instant teas are hot-water extracts mainly of black teas, followed by concentration and spray or freeze drying to provide a water-soluble powder. In recent years ready-to-use beverages named 'Ice Tea' became very popular. For details of tea production and processing, we refer to the literature (Bokuchava and Skobeleva 1979/80; Wickremasinghe 1978; Willson and Clifford 1992).

In tea plants, like in other plants, BAs are synthesized by biochemical pathways such as enzymic decarboxylation of amino acids, transamination of amino acids, or amination of aldehydes or ketones (Galston and Sawhney 1990). Consequently, BAs are also expected to occur in processed tea and beverages made thereof.

To provide data on the kinds and quantities of BAs occurring in representative tea infusions, we analyzed hot-water extracts of processed tea leaves as commonly consumed as beverages.

Materials and methods

Sources of tea samples and treatment for analysis

Tea samples were purchased from special tea shops and used without further treatment. Specification of teas (country and region of origin, trade names, types of teas and further characterization) are listed in Table 1.

Chemicals

All chemicals used were of highest purity available. From Merck were purchased: acetonitrile (MeCN), 9-fluorenylmethoxycarbonyl chloride (9-fluorenylmethyl chloroformate, Fmoc-Cl), glycine (Gly), and boric acid (H_3BO_3). The hydrochlorides of Him, Cad, and Dhx were purchased from Sigma-Aldrich (Steinheim, Germany). The hydrochlorides

Table 1 Tea samples analyzed for biogenic amines listed according to country/region of origin

No	Origin	Trade name	Type	Characteristics
01	Assam	Khongea	Green	–
02	Assam	Heeleakah	Black	Second flush
03	Assam	Orangajuli	Black	First flush
04	Ceylon	Kalupahani	Green	–
05	Ceylon	#23	Black	Highgrown
06	Ceylon	Blairlomond	Black	Highgrown
07	Darjeeling	Ambootia	Black	Fist flush
08	Darjeeling	Jungpana	Black	First flush
09	Darjeeling	Namring	Black	First flush
10	Darjeeling	Loher Garh	Black	Second flush
11	Darjeeling	Margret's Hope	Black	Second flush
12	Darjeeling	Risheehat	Black	Second flush
13	Darjeeling	Seeyok	Black	Second flush
14	Darjeeling	Balasun	Black	Autumnal
15	China	Chun Mee	Green	–
16	China	Messmer grüner Tee	Green	–
17	China	Yunnan Tuocha	Green	–
18	China	Ti Kuan Yin	Oolong	–
19	China	Keemun	Black	–
20	China	Yunnan Tuocha	Black	–
21	Instant tea	Nestlé Nestea	Instant	Soluble extract
				–

Ceylon, Sri Lanka; Assam, Darjeeling (India); Oolong, semi-fermented tea; – not defined

of Tym, Put, Spd, Pea, Spm, and DL- Ocp were from Fluka (Deisenhofen, Germany). Acetone, acetic acid (HOAc), *n*-butanol (*n*-BuOH), potassium hydroxide (KOH), and sodium hydroxide (NaOH) were from Carl Roth (Karlsruhe, Germany). Double distilled water from a quartz was still used for preparing all aqueous solutions.

Standard of biogenic amines

10 mM solutions of BAs (40 mM Him) in 0.1 M HCl served as standards. Suitable concentrations for calibration (100, 50, 20, 10, 5, and 2.5 µM; four-fold for Him) were prepared by appropriate dilution with 0.1 M HCl. As internal standard, 1 mM Dhx in 0.1 M HCl was used. The coefficients of determination ranged from 0.9948 (Ocp) to 0.9984 (Pea) and the limits of detection for Fmoc-derivatives of BAs ranged from 0.07 pmol (Tym) to 1.0 pmol (Him) at a signal-to-noise ratio of 3:1. Limits of quantification were 10 µM (Him), 5 µM (Spm), and 2.5 µM for the other amines.

Preparation of sodium acetate and borate buffers and reagents

For preparing the sodium acetate (NaOAc) buffer, HOAc (0.1 mol) in water (950 ml) was adjusted to pH 7.0 by titration with NaOH (20%, w/v). The buffer was filled up to 1 L in a volumetric flask by addition of water, and passed through a membrane filter (RC 55, pore size 0.45 µm, Schleicher and Schuell, Dassel, Germany).

Potassium borate buffer was prepared from boric acid (33.43 g, 0.5 mol) in water (950 ml) by titration with KOH (20%, w/w) to pH 8.5 and final adjustment to 1 L with water. As derivatizing reagent 3 mM Fmoc-Cl in acetone was used. As scavenger reagent, 20 mM glycine in water was used. A dilution buffer was prepared by mixing 0.1 M NaOAc buffer (pH 7.0) and MeCN (1:1, v/v).

Preparation of tea infusions

To quantify polyamines extractable with water, tea infusions were prepared referring to International Organization for Standardization: ISO 3103 entitled 'Tea–Preparation of liquor for use in sensory test' (ISO 3103). To tea leaves (1.00 g), 1 mM Dhx (250 µl, internal standard) and boiling double distilled water (35 ml) were added. After 20 min the infusion was filtered, and the cooled filtrate filled up in a volumetric flask with water to 50 ml. Measured pH ranged from 4.86 to 5.37 (3 black teas measured) and 5.41 to 5.50 (3 green teas measured). (Note: only single extraction of tea leaves was performed since in second extracts with hot water no polyamines could be detected by HPLC. This is also in agreement with common practice for preparing tea as beverage). To an aliquot (40 ml) of this extract, *n*-BuOH (5 ml) was added and the mixture was evaporated to dryness using a vacuum rotary evaporator. The remaining residue was dissolved in 0.1 M HCl (2.5 ml) and stored at −25°C. Aliquots of 20 µL were used for analyses (see below).

Chromatography

For pre-column derivatization of BAs, the reagent Fmoc-Cl and modifications of the analytical procedures described previously were used (Lu et al. 1991; Kirschbaum et al. 1994).

For HPLC a LaChrom® instrument, comprising a Model L-7100 pump with low-pressure gradient-former, L-7250 programmable autosampler, L-7612 solvent degasser, L-7300 column oven, L-7480 fluorescence detector with 12 µl flow cell, was used. The system was controlled by the D-7000 HPLC System Manager including chromatography data station software (all from Merck-Hitachi, Darmstadt, Germany, and Tokyo, Japan). The column used was a LiChroCART® Superspher® 60 RP-8 column (250 × 4 mm i.d., particle size 4 µm; Merck) equipped with a LiChrospher® guard column (4 × 4 mm i.d., particle size 5 µm; Merck). The column was heated at 40°C and the derivatized Fmoc-amines were detected by their fluorescence at an emission wavelength of 313 nm and excitation at 263 nm.

Automated derivatization procedure and chromatographic conditions

Aliquots of tea infusions or standards of BAs (20 µl), 0.5 M borate buffer (200 µl) and 3 mM Fmoc-Cl (200 µl) were drawn by the autosampler and mixed in a reaction vial. After 3 min at ambient temperature, the scavenger reagent glycine (20 mM, 50 µl) was added. After 3 min, to an aliquot of the reaction mixture (80 µl) dilution buffer (320 µl) was added and 20 µl of this mixture was injected into the column.

A binary gradient consisting of 0.1 M NaOAc (pH 7.0) (eluent A) and MeCN (eluent B) was used at a flow-rate of 1.2 mL/min. The gradient started with 60% A and 40% B and changed to 10% A and 90% B within 80 min. Under these chromatographic conditions Pea, Put, Him, Ocp, Cad, Tym, Spd, Spm and the internal standard Dhx, could be separated.

Results and discussion

Fourteen black teas, five green teas, one Oolong tea, and one instant tea, harvested in different regions and processed

and fermented under varying conditions, were analyzed for BAs (see Table 1). The internal standard Dhx was added prior to extraction to control retention behavior of the HPLC column and to evaluate matrix effects of tea leaves on polyamines.

Biogenic amines in tea infusions

Aqueous tea infusions were prepared and analyzed for their contents of BAs. Selected chromatograms of black tea no. 13 and green tea no. 16 in comparison to the HPLC of a standard mixture are shown in Fig. 1.

Since tea is consumed as a beverage, quantities of BAs were calculated in µg BA/L aqueous infusion prepared under standard conditions (see experimental part).

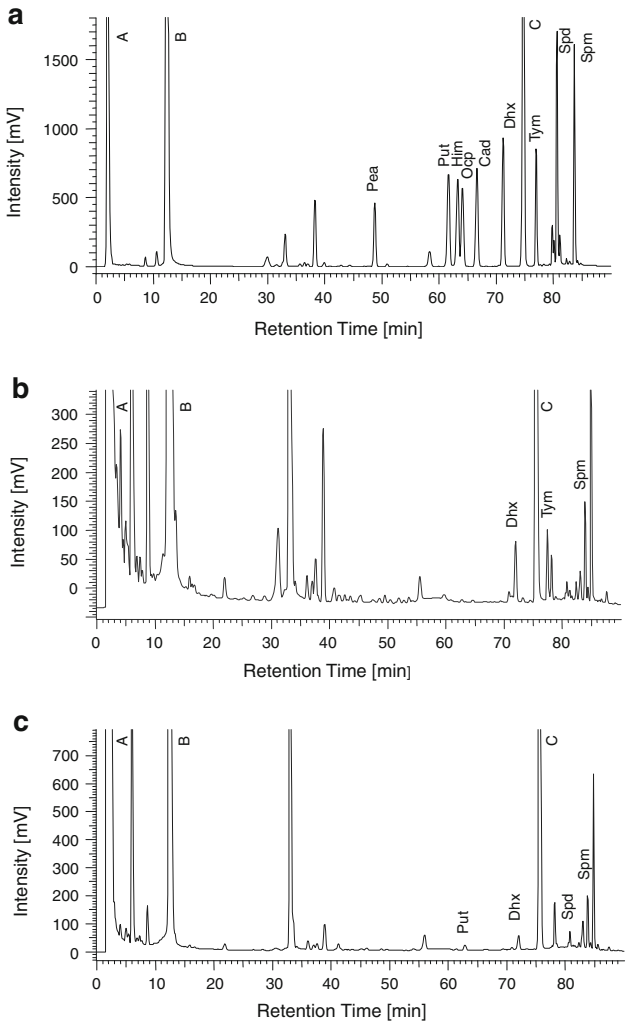

Fig. 1 Polyamines derivatized with Fmoc-Cl. **a** standard of polyamines (100 µM; His 400 µM), **b** aqueous extract of black tea no. 13, **c** aqueous extract of green tea no. 16; *A* Fmoc-Gly (from scavenger), *B* 9-fluorenylmethanol, *C* from reagent (probably carbamate). Peaks not assigned are unknown compounds. For chromatographic conditions, see experimental part

However, since preparation of tea infusions very much depend on consumer customs, and to compare our data to those published in the literature, the quantities of BAs in the teas have also been calculated as µg BA/g tea leaves. Data are compiled in Table 2.

No BAs were detectable in infusions of a black tea from Darjeeling (no. 10) and an Oolong tea from China (no. 18). Further, since among the teas analyzed Cad was detected only in sample no. 19 (but no other BAs were detected in this tea), the teas nos. 10, 18 and 19 are not included in Table 2.

The aqueous infusions generally contained very low amounts of BAs. In most extracts only Put, Spd, Spm, and Tym were detected. None of the twenty one aqueous infusions examined (instant tea included) contained Him or Ocp. Pea was measured in black teas no. 14 and no. 20 and in the instant tea no. 21. Variable amounts of Put (19–83 µg/L) could be detected in all green teas, but only in 3 black teas. Nine out of 21 teas contained Spd (23–93 µg/L), 12 teas contained Spm (31–337 µg/L), and 10 teas contained Tym (27–187 µg/L). Tym was detected in all teas from Assam and Ceylon, in 3 out of 8 teas from Darjeeling, and in none of the black or green teas from China. Largest amounts of Tym were detected in black tea no. 13 from Darjeeling (187 µg/L) and black tea no. 5 from Ceylon (103 µg/L). Total quantities of BAs in black teas ranged from 0 to 401 µg/L (no BAs detected in teas no. 10 and no. 18) and in green teas from 83 to 431 µg/L. In Darjeeling black teas, the sum of BAs in the first flush teas nos. 7–9 (16–31 µg/L) were much lower than those in the second flush teas nos. 11–13 (158–392 µg/L). This is attributed to the larger amounts of Spm in teas nos. 11–13 (158–205 µg/L) in comparison to teas no. 7 (31 µg/L), 8 and 9 (no Spm detected). First flush teas are harvested at the end of February to the end of April following spring rains, whereas second flush teas are harvested at the beginning of June to the beginning of July and 'autumnals' after the rainy season from the beginning of October to mid December.

In dried green tea leaves, only Spd, Spm, Put and Tym occurred in significant amounts. The presence of PAs like Spd, Spm and Put was expected to occur due to their function as growth factors in plants (Kakkar and Nagar 1997; Smith 1985). These amines have a common pathway for their biosynthesis with ornithine as the precursor amino acid. Therefore, their concentrations are highest in compartments with high cell division rates (Smith 1985; Galston and Sawhney 1990).

In the course of the manufacturing process of green tea the freshly harvested leaves are heated as soon as possible to inactivate the enzymes completely. Because no further biochemical processes take place after heating, the content of non-volatile BAs in green teas is assumed to be similar

Table 2 Quantities of hot-water extractable, free biogenic amines (BA) in black teas (BT), green teas (GT) and instant tea (IT) calculated for (a) tea leaves [μg BA/g dry leaves] and (b) determined in tea infusions prepared thereof [μg BA/L tea infusion]

	No.	Pea		Put		Spd		Spm		Tym		Total	Total
		a	b	a	b	a	b	a	b	a	b	a	b
BT	02	–	–	–	–	–	–	–	–	2.8	56.1	2.8	56.1
	03	–	–	–	–	1.4	26.9	–	–	3.1	62.3	4.5	89.2
	05	–	–	0.8	15.3	2.4	48.8	16.9	336.9	5.2	103.1	25.3	401.0
	06	–	–	–	–	–	–	–	–	2.0	40.6	2.0	40.6
	07	–	–	–	–	–	–	1.5	30.6	–	–	1.5	30.6
	08	–	–	0.8	15.6	–		16.9	–	–	–	0.8	15.6
	09	–	–	–	–	1.1	22.5	–	–	–	–	1.1	22.5
	11	–	–	–	–	–	–	7.2	143.1	1.9	37.5	9.1	180.6
	12	–	–	–	–	–	–	7.9	158.1	–		7.9	158.1
	13	–	–	–	–	–	–	10.3	205.0	9.4	186.9	19.7	391.9
	14	0.8	16.3	–	–	–		3.1	61.9	1.4	28.8	5.3	107.0
	20	1.4	28.1	0.6	12.5	1.6	32.5	2.7	54.4	–	–	6.3	127.5
GT	01	–	–	2.9	58.9	1.7	34.1	3.1	61.1	1.3	26.9	9.0	181.0
	04	–	–	2.6	51.8	2.4	47.4	7.8	154.7	1.8	36.0	14.6	289.9
	15	–	–	0.9	18.8	4.7	93.1	16.0	319.4	–	–	21.6	431.3
	16	–	–	1.9	38.8	3.1	61.9	11.6	231.3	–	–	16.6	332.0
	17	–	–	4.2	83.1							4.2	83.1
IT	21	0.9	18.8	–	–	2.7	53.8	–	–	1.7	33.1	5.3	105.7

Addition of 35 (+15) ml boiling water to 1.00 g tea (=20 g tea/L infusion; see experimental part), filtration after 20 min; (–) not detected or below limit of quantification; for specification of numbers see Table 1; not included are teas no. 10 and no. 18 (no BAs detected) and tea no. 19 (Cad only detected but no other BAs, see text); for chromatograms of no. 13 and no. 16 see Fig. 1; data are mean of 2 analyses

to their concentrations in fresh tea leaves (based on dry weight).

From Table 2 it is obvious that all green teas contain Put, Spd, and Spm, but only no. 1 and 3 contain Tym. Quantities of Tym in black teas (if present) are larger in comparison to green teas. BAs in black teas show greater diversity but some are absent in certain teas. This is explained by oxidative enzymic and chemical processes in the course of processing. Since in black teas quantities of the amino acid tyrosin are much higher in comparison to green teas (Neuman and Montag 1983), enzymic decarboxylation of tyrosin might lead to the formation of Tym.

To summarize, inspection of Table 2 allows selection of teas lacking completely or partly certain BAs or enriched in selected BAs. Our results should also be assessed in the following with regard to the scarce data on BAs in tea infusions.

Using benzoyl chloride for the derivatization of PAs and HPLC for the separation of derivatives Kakkar and Nagar (1997) determined the distribution and changes in free, perchloric acid extractable PAs in fresh tea leaves during winter dormancy. They report 7–26 μg Put/g tea leaves, 28–89 μg Spd/g tea leaves, and 20–35 μg Spm/g tea leaves [quantities of polyamines were calculated from Fig. 1 shown in Kakkar and Nagar (1997)]. Using a dedicated amino acid analyzer and a special programm for BAs, Neuman and Montag (1983) determined amines in acidic extracts of Assam and China Keemun black teas. The authors detected that per gram tea contained: 185 μg (109 μg) Spm, 28 μg (46 μg) Spd, 0 μg (0.4 μg) Him, 0.02 μg (0.2 μg) Put, 0.03 μg(1 μg) Cad, and 29 μg (20 μg) propylamine (rounded values; Keemun tea in round brackets).

Okamoto et al. (1997), using HPLC and *o*-phthaldialdehyde (OPA) for quantification, analyzed hot-water extracts of teas for BAs (ratio dry tea leaf to water 1:50, g/v; 85°C for 4 min). Single black, green and Oolong teas were analyzed for Put, Spd, Spm and Cad/Him (but not for the other BAs). Per gram dry leaves contains: in black tea 1.1 μg Put, 1.7 μg Spd, and <2 μg Spm; in green tea 1.5 μg Put, 4.6 μg Spd, and <1 μg Spm (no Cad and Him), in Oolong tea 2.1 μg Put, and 1.5 μg Spd (no Spm, Cad, Him). From total hydrolysates of tea leaves authors realized that water-extractable free Put and Spd in black tea represent only 15 and 9% of total amounts, in green tea 3.1 and 3.8%, and in Oolong tea 7 and 3%, respectively.

Nishimura et al. (2006) analyzed 227 food and drinks for the presence of PAs using HPLC and derivatization with OPA. The authors determined PAs in hot-water extracts (1 g tea leaf and 5 mL water) of a single black and

green tea. Quantities of BAs per gram tea were (green tea in parenthesis: 0.5 (1.6) μg Put, 1.2 (3.3) μg Spd, 2.2 (3.8) μg Spm, 2.7 (3.9) μg Cad.

Taking into account the few tea samples analyzed by these authors and the different analytical approaches used, the data are in excellent agreement with our results presented in Table 2.

Risk assessment of biogenic amines in tea

To estimate the risk of a possible intoxication related to the intake of tea, the following assumptions are made: for the preparation of tea, according to standard protocols (ISO 3103 or BS 6008), 2.8 g of tea leaves are extracted with 140 ml boiling water. Thus, for preparing 1.0 L of tea infusion 20 g of tea leaves are extracted. This corresponds to 1 L of infusion prepared in our experiment (1 g in 50 mL water). The uptake of Spd, Spm and Put in quantities shown in Table 2 is not considered as risk for health. For Him and Tym toxicological relevance has been reported and physiological effects for Pea and other PAs (Beutling 1996; Joosten 1988; Lüthy and Schlatter 1983; Shalaby 1996; Til et al. 1997). However, Him was not detected in any of our aqueous extracts. Pea was detected in none of the green teas and only in 2 out of 13 black teas in amounts not exceeding 28 μg/L.

Tym was detected in 10 out of 21 tea samples, but quantities were far below 100 μg/L with the exceptions of tea no. 5 (103 μg/L) and no. 13 (187 μg/L). It has been reported that humans suffering from monoaminoxidase-insufficiency have a toxicity threshold of 6 mg Tym (Lüthy and Schlatter 1983). However, approaching this amount would require consumption of 32 L tea infusion prepared from tea no. 13 which is not realistic. Although synergistic effects of BAs have to be taken into account, total amounts of BAs, not exceeding 441 μg/L in the aqueous infusions, are too low to bear the risk of intoxication. The quantities in tea infusions are much lower in comparison to other beverages.

For example in previous work we detected, among other BAs, amounts of 17,500 μgTym/L in a Lambic beer, 15,100 μgTym/L in a Rioja red wine, and 29,600–68,700 μg Tym/L in fermented cabbage (sauerkraut) juices (Kirschbaum et al. 1999).

Furthermore, data are frequently difficult to compare and interpret since BAs in intact or processed plants or foodstuffs made thereof occur to a lower extend in the free state. The majority is represented by low-molecular weight conjugates, and high-molecular weight, unsoluble adducts or conjugates in which basic BAs are tightly bound to acidic polyphenols, proteins, or nucleotides of the tea leafs. Differentiation among these forms requires extra analytical expenditures (Kakkar and Nagar 1997). With regard to tea as beverage, however, only BAs soluble in hot-water are of dietary importance. Addition of DAHx to 4 representative teas resulted in a recovery of only 15 ± 5% in aqueous extracts. This indicates strong binding of free PAs, being present as polycations (Agostinelli et al. 2010) in weakly acidic tea infusions This observation is in principle agreement with a report of Ingles et al. (1985). The authors found recoveries of only 25% Him and 55% Tym added as internal standards to dark chocolate. Cocoa, like tea, is rich in BA-binding polyphenols.

Treatment of tea leaves with strong acids such as trichloroacetic acid, perchloric acid, or 0.1 M HCl increases the quantities of extractable BAs (Kakkar and Nagar 1997). These extraction procedures, commonly used for seafood, meat products or cheese (Karovičová and Kohajdová 2005; Moret and Conte 1996) are of no relevance, however, for the preparation of common tea beverages since the leaves are not consumed. A remarkable exception is the suspension of tea powder employed in the Japanese tea ceremony.

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