Authenticity Evaluation of Tea-Based Products

Helena ČÍŽKOVÁ, Michal VOLDŘICH, Jana MLEJNECKÁ and František KVASNIČKA

Department of Food Preservation and Meat Technology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic

Abstract

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This study proposes a method for estimating the content of tea in tea-based products. Broadly, this methodology is based on selecting chemical markers of the quality and authenticity of tea (and, therefore, of tea-based products), optimising the relevant analytical methods, and determining variations (type, origin, quality) among samples of green and black leaf teas. The contents of the selected markers (theobromine, caffeine, theanine, and total polyphenols) in tea-based products (iced teas, instant teas) were determined. Then, the dry tea content in these products, expressed as the equivalent amount of dry tea leaves, was calculated on the basis of two sets of mean data for these analytes: average literature data, and the mean experimental data obtained with our samples of leaf tea. With regards to the latter, we propose to use of the following averages for caffeine, theobromine, theanine, and polyphenols: for 100 g of black tea-based products 1.9 g, 0.13 g, 1.3 g, and 9.1 g, respectively; for 100 g of green tea-based products 1.9 g, 0.16, 1.7 g, and 14.7 g, respectively. Using this method, we found that approximately one third of the analysed samples failed to meet the tea extract levels required by German and Austrian legislations, while about 10% did not comply with the less demanding Czech legislative requirements.

Keywords: tea; tea-based products; authenticity; quality

Due to its taste, together with its refreshing and mildly stimulant effect, tea, produced from the leaves of *Camelia sinensis*, is the most widely consumed beverage in the world. In addition, the positive health effects of tea have been shown since ancient times. Tea consumption has been implicated in lowering the relative risk of stomach, esophageal, and lung cancer, as well as in reducing the incidence of stroke, lowering the atherogenic index and improving liver functioning (Higdon & Frei 2003; Basu & Lucas 2007; Khan & Mukhtar 2007).

The main types of tea are distinguished by the type of the fermentation processing they undergo; green tea is not fermented, black tea is almost completely fermented, and Oolong tea is only partially fermented. Tea leaves and the infusions prepared usually vary greatly in quality, not only according to the type, but also due to the variety, geographic origin, processing technology used

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and other factors. Apart from sensory evaluation, chemical analysis is the most reliable method for evaluating the quality of tea and for confirming the presence of tea in infusions. Several studies (Horie & Kohata 1998; Liang *et al*. 2003) have identified chemical markers that positively correlate with the quality of tea, the most important of these being the following: caffeine and theobromine contents, and catechin and free amino acid contents and compositions (Table 1).

Caffeine, the compound responsible for tea stimulant effect, together with the minor tea alkaloids theobromine and theophyline, all make a vital contribution to the quality of tea, such as its briskness (Yao *et al*. 2006). The dry weight of tea contains about 3% caffeine, which, depending on the type, brand, and brewing method, corresponds to 30–90 mg per 0.25 l cup.

Theanine (1-glutamyl-gama-ethylamide), which, out of food products, appears only in tea, comprises 0.7–2% of the dry matter which makes it the predominant free amino acid in tea. High grade teas contain higher amounts of this compound (Aucamp *et al*. 2000).

The antioxidant activity of tea extracts is primarily attributed to the role of catechins which, as the most abundant polyphenols in tea, constitute up to 25% of its dry weight. The major catechins in tea are catechin, epicatechin, epigallocatechin, and epicatechin gallate, together with their fermentation products, the theaflavins and thearubigins. While dry green and black teas contain comparable amounts of polyphenols, in green tea these consist mainly of catechins; the content of catechins in green tea being approximately 3.5 times higher than in black tea. As tea leaves are fermented, the amounts of theaflavins and thearubigins increase, while catechins content decreases. Polyphenols are generally responsible for slightly bitter and astringent flavour, and while theaflavins and the thearubigins for the brownish colour of the black tea infusions (Aucamp *et al*. 2000; Peterson *et al*. 2005).

In recent times tea-based products such as iced teas and instant teas have become more and more popular, combining the advantages of the traditional leaf infusions, such as the taste, refreshment, stimulation, and health promotion effect, with the benefit of a convenient preparation. In addition to a certain amount of tea extract, these products consist mainly of sugars, organic acids, and artificial flavourings. However, as in the case of leaf tea, the quality of these tea-based beverages is likewise based on the content and composition of the tea extract used in their production.

The requirements for the quality of tea-based beverages are determined by individual national standards and directives, some of which are summarised in Table 2. Contrary to the legislative requirements of the other countries described in this table, Czech legislation currently neither prescribes the content of tea (or 'tea extract') in tea-based products, nor the methodology to be used in its evaluation. Czech law requires only the "presence" of tea extract in relevant products,

Table 1. Composition of tea (in mg/100 g)

Tea	Theanine	Caffeine	Theobromine	Total catechins	Epigallocatechin-3-gallate	References
Green	1 100	3 800	x	12 700	7 900	Aucamp *et al*. (2000)
Black	700	2 100	x	2 300	1 100	
Green				13 500	8 900	Peterson *et al*. (2005)
Black				4 100	1 300	
Green	1 100	2 100		14 000	6 000	Horie and Kohata (1998)
Green	1 700	3 700				Yao *et al*. (2006)
Black	1 200	3 900				
Green	1 800	3 400			10 300	Zhu *et al*. (2004)
Green		2 700	300	10 800		Yang *et al*. (2007)
Black		2 200–3 900	50–200		1 100	Fernandez *et al*. (2002)

x not detected

Table 2. Important qualitative parameters of tea and tea-based products according to various legislative requirements

Product	Parameter	Czech Republic*	Slovakia**	Germany***	Austria****
Tea	total ash (max., %)	8	8	1 *****	1*****
	water extract (min., %)	25	25	x	32
	moisture (max., %)	10	10	8	8
	caffeine in decaffeinated tea (max.)	x	0.4 g/100 g	0.4% in dry matter	0.4% in dry matter
Tea-based products	tea extract (dry matter) in beverage (min.)	x	x	1.2 g/l	0.12 %
	caffeine in dry matter/beverage (min.)	x	x	1.5%/x	1.5%/40 mg/l
	caffeine in decaffeinated tea extracts (max.)	x	1.2 g/100 g	1.2%	1.2%

*Czech Food Code No. 110/1997 Col. and decree No. 330/1997 Col. as amended

**The Food Code of the Slovak Republic, chapter 24

***German Food Code, BAnz. No. 66a from 29. 4. 1999

****Austria Food Code, decree B31

*****Insoluble in acid

but fails to specify exactly what this means, simply stating that tea extract is "a water extract of tea leaves attendant to beverage preparation". In fact, dry tea leaves are typically extracted with aqueous solvents, preferably using a counter current procedure at atmospheric pressure and high temperature, although low temperature, microwave, ultrasound, and ethanol extractions have all been also described in the literature (Jingming *et al*. 2004). Subsequently processed into the required final form (powder, granules, liquid) for commercial purposes, the product should retain the characteristic colour, aroma, and flavour of tea leaf and should be characterised by specific chemical-physical parameters (moisture; contents of total polyphenols, catechins, caffeine). However, Jingming *et al*. (2004) and Gramza-Michałowska *et al*. (2007) discovered that the extract composition is strongly influenced by the method of processing and purification; i.e. that standard extraction and ultrasound extraction produce an increase in the chemicals content of water extracts as well as in the total polyphenols/ascorbic acid ratio, while ethanol extraction positively influences the total polyphenol content and, consequently, the antioxidant activity of green tea extracts.

The aim of this study is to propose a method for estimating the tea content in tea-based products. This methodology involves the following steps:

1. Selection of chemical markers of the quality and authenticity of tea and tea-based products.
2. Selection of the relevant analytical methods.
3. Determination of variations in type, origin and quality among green and black leaf teas, and evaluation of their possible markers.
4. Analyses of samples of tea-based drinks for their theobromine, caffeine, theanine and total polyphenol contents.
5. Calculation of tea content according to both average literature data and the mean experimental data obtained with the samples of leaf tea.

MATERIALS AND METHODS

Samples. Twenty six samples of leaf tea (11 green teas, 15 black teas) and twenty six tea-based beverages (22 ice teas and 4 instant tea drinks) were randomly sampled and used in this study. The names, origins, and quality declarations and the names, producers and minimal tea extract contents declared by the producer are given in Table 3 in the alphabetical order for leaf teas and tea-based beverages, respectively. All samples were purchased on the open market.

Determination of chemical markers of the quality and authenticity of teas and tea-based products. Sample preparation: Both tea leaves (0.5 g) and instant tea drinks (5 g) were mixed with 100 ml of boiling water and extracted in an ultrasonic bath for 10 minutes. The samples were then filtered through Whatman No. 1 filter paper and a micro filter (0.45 μm). The liquid tea-based products (ice teas) were analysed directly.

Table 3. Name, origin and quality declaration of leaf tea samples and name, producer and minimal tea extract content declared by producer of tea-based beverages (in an alphabetical order)

	Name	Origin	Quality
Leaf tea			
Green tea	Gao qioo yin feng	China Yunnan	1
	Green tea Rohiny	India, Darjeeling	x
	Hai bei Tu Zu	China Ahui	1
	Huang shan	China Ahui	3
	Huo shan huong ya	China Ahui	2
	Che Thái ngueyuthou	Vietnam	x
	Kyoto Gyokuro	Japan	1
	Rolling Clouds	China Yunnan	2
	Sencha HCO	Japan	3
	Sencha KKH2	Japan	2
	Yau Zu Cha	China Yunnan	3
Black tea	Bukhial	India, Assam	3
	Dhelakot	India, Assam	1
	F 0011	China Yunnan	1
	F 0044	China Yunnan	3
	F 100	China Yunnan	2
	Guransa	Nepal	1
	Hajua	India, Assam	2
	Himalaya blend	India, Darjeeling	3
	Margarets Hope	India, Darjeeling	1
	Mist Valley	Nepal	3
	Puttabang	India, Darjeeling	2
	Sakhira	Nepal	2
	Special OP	Vietnam	x
	Tea Bank	Ceylon	1
	Yalta	Ceylon	2
	Name	Producer	Minimal tea extract content
Tea-based bevarages			
Instant tea drinks	Instant lemon tea drink	Laurens Spethmann GmbH & Co.	1.80%
	Ice tea lemon	Artifex Instant s. r. o.	x
	Instant lemon tea drink	AHOLD Czech Republic, a. s.	x
	Tang tea	Lagris a. s.	x
Ice tea	Aqua HIT ice tea lemon	HBSW a. s.	4.2 g/l
	AQUILA Tea lemon	Karlovarské minerální vody a. s.	x
	ARO Ice tea lemon	Poděbradka a. s.	x
	Good water Green tea lemon	HBSW, a. s.	5 g/l
	Good water Tea lemon	HBSW a. s.	4.2 g/l
	Green tea lemon	Karlovarské minerální vody a. s.	x
	Ice black tea lemon EURO Shopper	Ahold Czech republic, a. s.	0.30%
	Ice green tea	B.I.O. Trading, spol. s r. o.	x
	Ice green tea EURO Shopper	Ahold Czech republic, a. s.	0.30%
	Ice tea lemon	Fontea, a. s.	x
	Ice tea lemon	General Bottlers CR, s. r. o.	x
	Ice tea peach	Hermann Pfanner Getränke GmbH	x
	Ice tea-black tea lemon	B.I.O. Trading, spol. s r. o.	x
	Lipton Ice tea green tea	General Bottlers CR, s. r. o.	0.16%
	Nativa Green tea lemon	Rauch Fruchtsäfte	1.5 g/l
	Nativa White tea Yuzu	Rauch Fruchtsäfte	x
	Nestea apple with green tea	Coca-cola Beverage	x
	Nestea Ice tea lemon	Coca-cola Beverage	x
	Pfanner Green tee, lemon, cactus	Hermann Pfanner Getränke GmbH	x
	Pfanner Yellow tea Lemon – Physalis	Hermann Pfanner Getränke GmbH	1.2 g/l
	Sunday Green tea Limeta	Poděbradka, a. s.	x
	SunDay Ice tea lemon	Poděbradka, a. s.	x

x not declared

An HPLC (Gynkotek) system equipped with a Phenomenex Luna C18 column (250 × 4.6 mm; 5 μm) coupled to a guard column (20 × 4 mm) was used for the separation of caffeine and theobromine. The mobile phase consisted of methanol:water mixture (30:70, v/v). The following parameters were applied: flow rate 1 ml/min; injected volume 20 μl; column temperature 25°C; monitored wavelength 280 nm.

For the determination of theanine, electrophoresis was carried out using an HP^{3D} CE system with UV detection. An uncoated fused-silica capillary column with an internal diameter of 50 μm, outer diameter of 375 μm, and total length of 37 cm was used. The operating temperature was 30°C, the running buffer was borate buffer at pH 8.5, the current of 10 μA was applied, and the wavelengths of 200, 254 and 280 nm were used for the detection.

The total polyphenol content in the samples was determined according to the Folin-Ciocalteu colorimetric assay (Escarpa & Gonzales 2001). Tea extracts, and beverages directly, were mixed with 0.5 ml of 2N Folin-Ciocalteu reagent. Saturated sodium carbonate solution (10 ml) was added to the mixtures which were then shaken. The absorbance of the reaction mixtures was measured at 750 nm after 1 hour. To construct the standard curve, gallic acid was used as the standard compound in a concentration range of 75–125 μg/ml.

Data analysis. The tests in the presented paper were carried out in duplicates with each sample, and the mean values are presented. The accuracy of the methods was evaluated using four replicate analyses of both leaf tea and tea-based beverages samples; the relative standard deviations obtained were always lower than 6% for all the analytes followed. Cluster analysis and principal component analysis were carried out using Statistica Cz 7 software.

RESULTS AND DISCUSSION

Chemical composition of leaf tea samples

Table 4 shows that theobromine, caffeine, theanine, and polyphenols contents varied greatly from sample to sample. The caffeine contents in 100 g of the fifteen black leaf tea samples ranged from 1506 mg to 2534 mg, with a mean of 1909 mg, while those of the eleven green leaf tea samples ranged from 1130 mg to 2467 mg, with a mean of 1886 mg. This distribution corresponds to the literature data showing that the caffeine content of tea is strongly affected by the variety, season and stage of plucking (Fernandez *et al*. 2002). The caffeine/theobromine ratio derived ranged from 7.1 to 36.0 (average 25.6) for the black teas, and from 6.1 to 28.1 (average 17.0) for the green teas. This ratio is useful not only for evaluating the identity and authenticity of tea, but also for providing the proof of the addition of undeclared caffeine to tea-based products. The determined concentrations of theobromine and caffeine corresponded generally to the legislative limits. Closer correlations can be found in literature by differentiating according to variety and origin; for example, Chinese teas from the Fujian province gave an average ratio of 20 (Fernandez *et al*. 2002), whereas values lower than 10 were obtained for high grade teas from the Guangshou region (Yang *et al*. 2007).

The average values and variability obtained with the quality markers were used as the basis for our evaluation of the tea content in tea-based products.

Table 4. Chemical composition of green and black teas

Marker (mg/100 g)	Tea	Number of samples	Mean	Min.	Max.	SD
Theobromine	green	11	161	39	384	112
	black	15	129	22	242	72
Caffeine	green	11	1886	1130	2467	368
	black	15	1909	1506	2534	252
Theanine	green	11	1652	877	2641	544
	black	15	1261	720	2132	423
Polyphenols	green	11	14663	7389	23477	5179
	black	15	9116	3925	18612	3499

Relationship between chemical composition of tea-based products and tea content

The majority of recent works evaluating the quality and authenticity of tea-based products are based not only on the evaluation of sensory parameters (taste, flavour, colour of infusion), but increasingly also on the determination of specific chemical markers (Stancher & Celabrese 1999; Plastenjak *et al.* 2001; Liang *et al.* 2003). Table 5 presents the analyte concentrations determined within our study for twenty-two ice teas and four instant tea drinks. Due to the high degree of variability among the compositions of the tea leaves sampled, as well as possible processing effects, it was necessary to conduct analyses of more than one chemical marker of the tea content if potential outliers were to be excluded.

The caffeine/theobromine ratio in tea-based products varied from 0.5 to 100, with values below 4 (samples 32, 1N, 4N, 10N, 16N, 17N) indicating off-grade or decaffeinated tea extract and values above 50 suggesting an unauthorised addition of caffeine to the product (samples 33, 34, 35). However, for the majority of ice teas analysed, the ratios correlated with the limits (6.1 to 36) derived for the leaf teas.

Because the precise meaning of "tea extract content" is not specifically defined in Czech legislation, in this study we chose to express 'tea content' as the equivalent amount of dry tea (leaves). The mean tea content of the tea-based products samples was calculated according to the content of each analyte by comparison with:

- the published average values for the individual analytes (theobromine 0.18 g/100 g; caffeine 2.1 g/100 g; theanine 1.8 g/100 g; total polyphenols 12.7 g/100 g) (Aucamp *et al.* 2000; Fernandez *et al.* 2002; Zhu *et al.* 2004),
- the average values for the individual analytes as obtained in our analyses of the leaf tea samples, as described above, i.e. calculated according to the following equations:

$$\text{Black tea content (\%)} = \tfrac{1}{4}\left(\frac{C_{\text{caffeine}}}{1.9} + \frac{C_{\text{theobromine}}}{0.13} + \frac{C_{\text{theanine}}}{1.3} + \frac{C_{\text{polyphenols}}}{9.1}\right)$$

$$\text{Green tea content (\%)} = \tfrac{1}{4}\left(\frac{C_{\text{caffeine}}}{1.9} + \frac{C_{\text{theobromine}}}{0.16} + \frac{C_{\text{theanine}}}{1.7} + \frac{C_{\text{polyphenols}}}{14.7}\right)$$

where: C – concentration of the respectivel analyte (g/100 g) for the black and green tea-based products

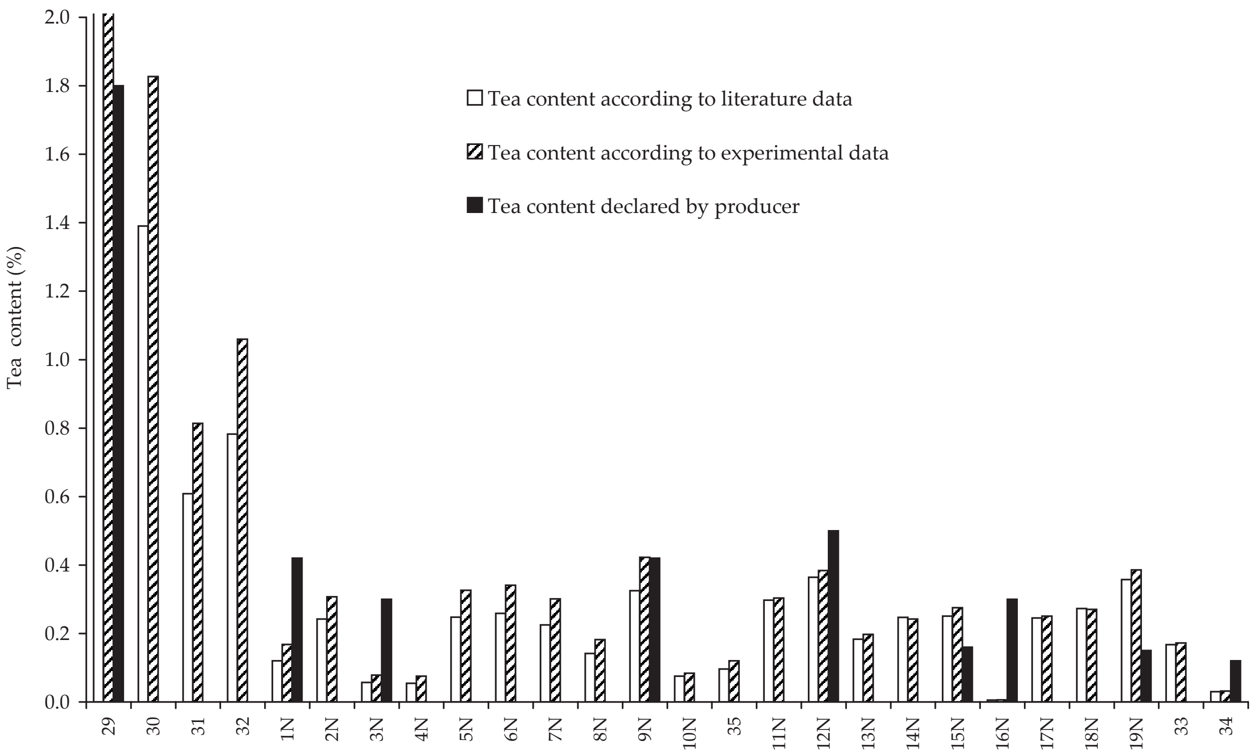

Figure 1. Tea content in tea-based products

Table 5. Contents of caffeine, theobromine, theanine and total polyphenols in tea-based products, together with the calculated tea content

Sample	Theobromine	Caffeine	Theanine	Total polyphenols	Tea content* (%)	
	(mg/100 g)				literature data	experimental data
29	13	148	150	1006	6.6	8.8
30	6	31	15	0	1.4	1.8
31	4	9	0	0	0.6	0.8
32	4	6	428	0	0.8	1.1
1N	4	5	38	52	0,1	0.2
2N	6	81	18	419	0.2	0.3
3N	3	4	8	37	0.1	0.1
4N	3	3	6	27	0.1	0.1
5N	5	56	43	467	0.2	0.3
6N	7	54	25	558	0.3	0.3
7N	8	39	43	62	0.2	0.3
8N	3	41	15	273	0.1	0.2
9N	11	82	15	504	0.3	0.4
10N	36	11	9	218	0.1	0.1
35	0	38	10	372	0.1	0.1
11N	4	32	75	1018	0.3	0.3
12N	13	62	25	801	0.4	0.4
13N	4	62	23	190	0.2	0.2
14N	3	12	51	1296	0.2	0.2
15N	6	91	35	33	0.3	0.3
16N	26	3	0	2	0.0	0.0
17N	10	5	14	893	0.2	0.3
18N	4	56	12	1299	0.3	0.3
19N	6	125	65	311	0.4	0.4
33	0	5	82	475	0.2	0.2
34	0	4	14	69	0.0	0.0

*Where one of the four measured markers was an outlier, it was not taken into account in the determination of the tea content in the respective sample (e.g. theanine in sample 32, and theobromine in samples 10N and 16N)

Figure 1 presents the declared and estimated contents of tea in our samples of tea-based beverages. The differences between the estimates obtained using the two different calculation methods varied from 0 to 20%, and, with the correlation between these methods being quite high (R^2= 0.999,) it appears that both methods can be used to estimate the tea extract content.

Principal component analysis was applied to the data matrix. Figure 2 plots the scores obtained for the first and third principal components (PC1, PC3) of the tea-based products. These two principal components accounted for 70% of the total variance. Theanine was the descriptor with the highest contribution to PC1. The set of the samples can be divided into 3 groups:

1. Samples with a minimal concentration of all markers, i.e. with no or very low tea content (31, 32, 34, 3N, 4N, 16N).
2. Suspect samples, in which at least one marker was outlying, i.e. the calculation of the tea content was inaccurate.

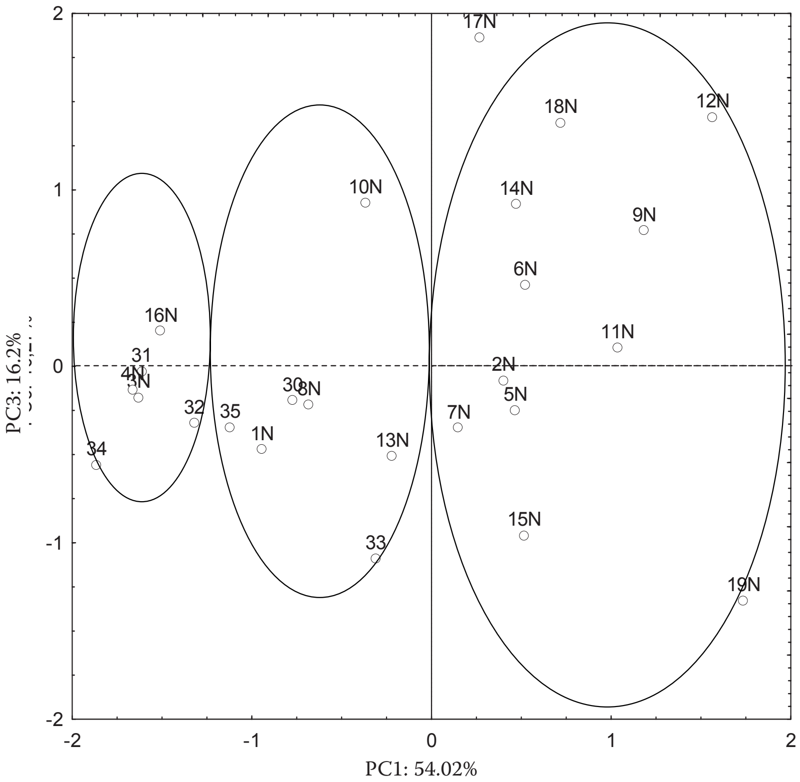

Figure 2. Plot of PCA scores for the set of tea-based products

3. Samples in which all markers were present, i.e. the markers were in a good correlation with the tea content.

The grouping is not very distinct, but it can be observed that the samples with a low orindistinguishable tea content are on the left (negative) side of PC1, while the superior samples are on the right (positive) side.

The compositions of the analysed samples of tea-based beverages were then compared with the legislative requirements of various countries. Of the 26 samples, 19 (73%) were found to contain at least 0.12% tea, and, therefore, to be in accordance with German and Austrian legislations. Of the 22 ice teas, 2 (10%) failed to comply with the less strict Czech legislative requirements; the nonconforming samples either containing no tea extract at all or containing an amount below the detection threshold of the analytical methods used.

CONCLUSION

The content of tea in tea-based beverages can be estimated according to the caffeine, theobromine, theanine, and total polyphenols contents in these products. Due to its stability and low variability within the analysed set of samples (1.7 g/100 g and 1.3 g/100 g for green and black teas, respectively), the amino acid theanine appears to be a particularly promising marker of the tea quality and authenticity. However, to obtain an accurate estimation of the tea content, the values of several analytes must be determined. The mean values used in the calculation of the tea content often vary widely in the literature, however, based on our experiments, we propose the use of the following averages for caffeine, theobromine, theanine, and polyphenols: for the black tea-based products 1.9 g/100 g, 0.13 g/100 g, 1.3 g/100 g, and 9.1 g/100 g, respectively; for the green tea-based products 1.9 g/100 g, 0.16 g/100 g, 1.7 g/100 g, and 14.7 g/100 g, respectively. These data correspond to the published values but should be verified at the time of use, to take into account potential changes in the quality of teas used in the production of tea-based beverages. Approximately one third of the analysed samples failed to contain the tea extract levels required by German and Austrian legislations, while 10% were not in compliance with the less demanding Czech legislative requirements.

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Corresponding author:

Ing. Helena Čížková, Ph.D., Vysoká škola chemicko-technologická, Fakulta potravinářské a biochemické technologie, Ústav konzervace potravin a technologie masa, Technická 3, 166 28 Praha 6, Česká republika
tel.: + 420 220 443 014, fax: + 420 220 444 404, helena.cizkova@vscht.cz