# Determination of the Chemical Composition of Tea by Chromatographic Methods: A Review

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## Abstract

Despite the fact that mankind has been drinking tea for more than 5000 years, its chemical composition has been studied only in recent decades. These studies are primarily carried out using chromatographic methods. This review summarizes the latest information regarding the chemical composition of different tea grades by different chromatographic methods, which has not previously been reviewed in the same scope. Over the last 40 years, the qualitative and quantitative analyses of high volatile compounds were determined by GC and GC/MS. The main components responsible for aroma of green and black tea were revealed, and the low volatile compounds basically were determined by HPLC and LC/MS methods. Most studies focusing on the determination of catechins and caffeine in various teas (green, oolong, black and pu-erh) involved HPLC analysis.

Knowledge of tea chemical composition helps in assessing its quality on the one hand, and helps to monitor and manage its growing, processing, and storage conditions on the other. In particular, this knowledge has enabled to establish the relationships between the chemical composition of tea and its properties by identifying the tea constituents which determine its aroma and taste. Therefore, assessment of tea quality does not only rely on subjective organoleptic evaluation, but also on objective physical and chemical methods, with extra determination of tea components most beneficial to human health. With this knowledge, the nutritional value of tea may be increased, and tea quality improved by providing via optimization of the growing, processing, and storage conditions.

**Keywords:** tea, chemical composition, catechins, HPLC, GC, theaflavins

## 1. Introduction

Tea extracts have gained popularity as ingredients in dietary supplements and functional foods. Interest in tea chemical composition analysis has increased dramatically in the last decade due to an abundance of scientific data regarding the positive effect of tea on human health. Epidemiological and animal studies suggest that tea is protective against certain cancers, cardiovascular diseases, and neurodegenerative diseases (Yang & Koo, 2000; Mandel & Youdim, 2004; Butt et al., 2015). Tea has a complex chemical composition, containing over 2000 components. Green tea, oolong tea, and black tea differ in their phytochemical contents. Chromatographic methods are keys for the analysis of such complex multi-component mixtures in tea and widely used for identification, tea quality estimation and quantitative analysis of tea bioactive compounds. Overview of the different chromatographic methods used for tea analysis is provided here.

## 2. Primary Compounds Present in Tea and Their Structural Formulas

The structural formulas of the most biologically active components of tea will be discussed in this section. Unfortunately, the published literature, especially popular science, contains many inaccuracies and errors regarding the nature of the compounds conferring its biological activity to tea. A list of the main chemical compounds present in tea is provided in Table 1.

Table 1. Primary chemical compounds present in tea (analyzed by Chromatographic Methods)

| No. | Compound name | Notes: main representative elements |
|-----|---------------|-------------------------------------|
| 1. | Catechins | Flavanols: 12 catechins are indentified, including 8 occurring in significant quantity, i.e., (+)-catechin, (-)- epicatechin, (-)-gallocatechin, (-)-epigallocatechin, (-)-catechin gallate, (-)-epicatechin gallate, (-)-gallocatechin gallate, (-)-epigallocatechin gallate |
| 2. | Oxyaromatic acids | Gallic, caffeic, quinine, chlorogenic, n-coumaric acids |
| 3. | Flavonols | Quercetin, kaempferol, myricetin |
| 4. | Theaflavins | Theaflavin, theaflavin-3-O-gallate, theaflavin-3'-O-gallate, theaflavin-3-3'-O-gallate |
| 5. | Teagallins | Teagallin |
| 6. | Thearubigins | High-molecular weight polymers of catechin gallates with molecular weight from 1000 to 40000 Da |
| 7. | Pigments | Carotenoids and chlorophyll |
| 8. | Alkaloids | Caffeine, theophylline, theobromine |
| 9. | Sugars | Glucose, fructose, saccharose |
| 10. | Amino acids | Isoleucine, leucine, methionine, threonine, phenylalanine, glutamine, asparagine, alanine, serine, proline, histidine, glutamic acid, aspartic acid, theanine |
| 11. | Vitamins | C, α-, β-, γ-, δ-tocopherols, riboflavin |
| 12. | Dibasic acids | Succinic, malic, tartaric, citric, quinic, aspartic, glutamic, oxalic acids |
| 13. | Cations | $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $NH_4^+$, $Al^{3+}$ |
| 14. | Metals | Fe, Zn, Cu, Ni, Al |
| 15. | Lignans and triterpenoid saponins | Mixture of many compounds |

In particular it regards to catechins, the most useful components of tea and especially green tea. There are twelve catechins in total identified by chromatography, although only eight catechins are present in tea in significant quantity (Zeeb et al., 2000). However, most published studies focus specifically on a limited subset of catechins, with the most abundant catechins, epigallocatechin gallate and epigallocatechin, which constitute over 70% of the total amount of all catechins. The catechins ratios found in different tea grades will be specified in later sections. The structural formulas of the eight most widely known catechins are shown in Figure 1. The stereoisomers are catechin and epicatechin, gallocatechin and epigallocatechin, catechin gallate and epicatechin gallate.
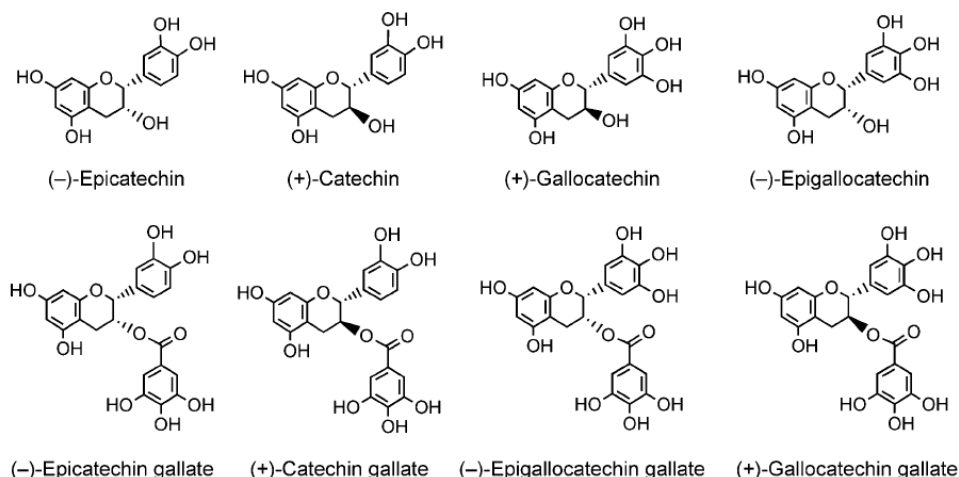
Figure 1. Eight major catechins found in tea

Black tea contains large amounts of theaflavins and thearubigins, which are condensation and polymerization products of catechins. Precursors of theaflavin are EC+EGC, precursors of theaflavin-3-gallate are EC+EGCG, precursors of theaflavin-3'-gallate are ECG+EGC, and precursors of theaflavin-3,3'-digallate are ECG+EGCG.

Structural formulas of theaflavins and other chemicals are shown in Figure 2. Theasinensin A was detected in Oolong tea. Oligomeric catechins presented in tea are thearubigins – 4"-O-methyl-EGCG and 4',4"-dimethyl-EGCG (Kartsova & Alekseeva, 2008).

Tea contains also significant amounts of gallic acid and caffeine, as well as small amounts of theobromine and theophylline (Figure 4) (Rio et al., 2004). Among amino acids, theanine, and gamma-aminobutyric (GABA) acid (Figure 5) are worth noting (Syu et al., 2008).
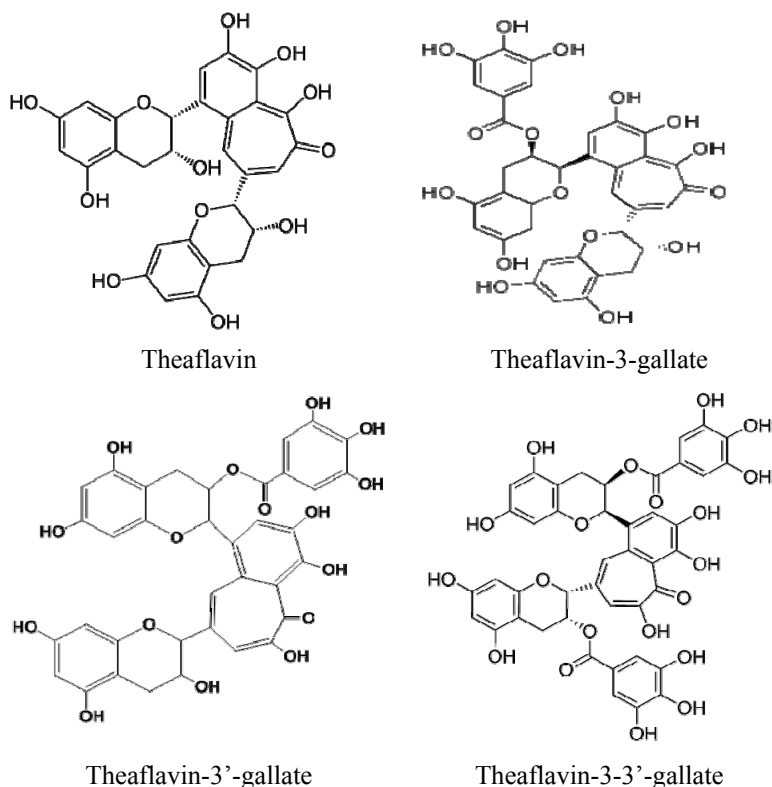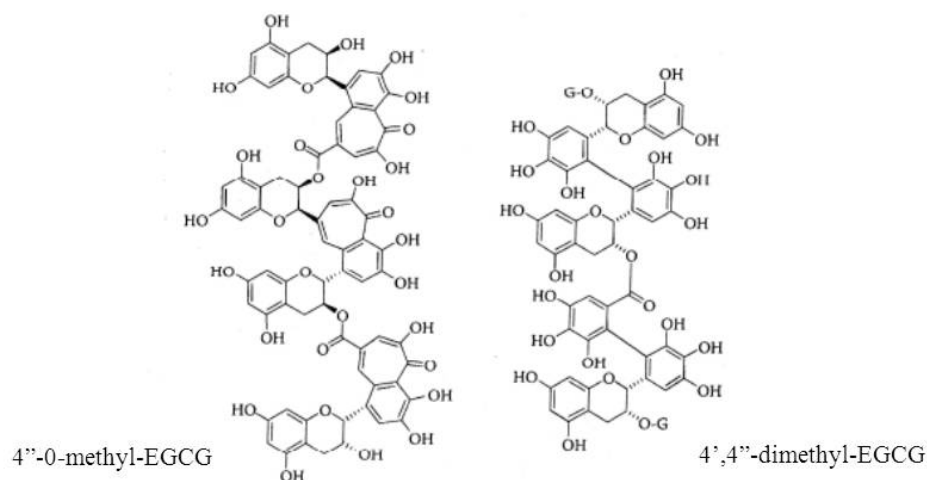


Theaflavin             Theaflavin-3-gallate

Theaflavin-3'-gallate         Theaflavin-3-3'-gallate
Figure 2. Structure formula of tea theoflavins

4"-0-methyl-EGCG 4',4"-dimethyl-EGCG

Oligomeric catechins

Figure 3. Structure of oligomeric catechins

Theophyline Theobromine

Caffeine
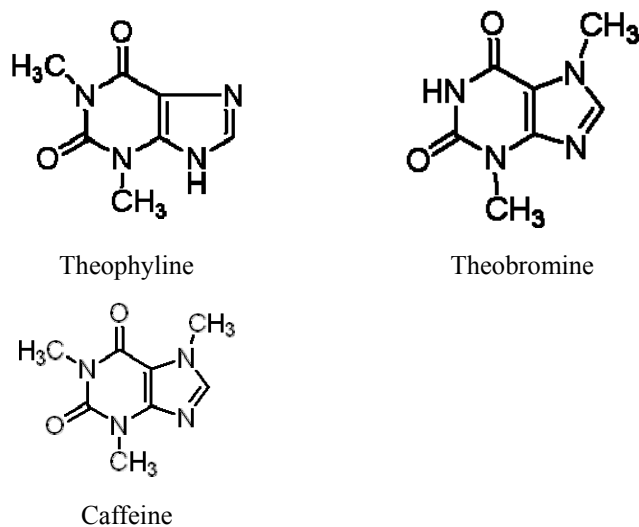
Figure 4. Structure of tea methylxanthins

Theanine ɤ -Aminobutyric acid (GABA)
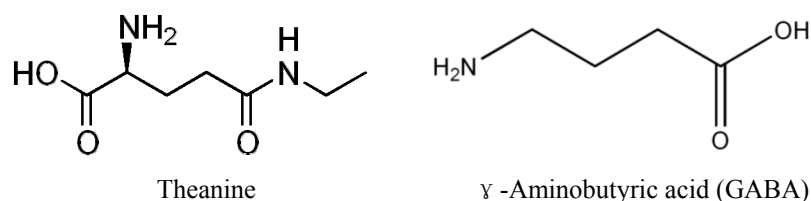
Figure 5. Structure formulas of tea Theanine and γ-Aminobutyric acid (GABA)

## 3. Chromatographic Methods Used to Determine the Chemical Composition of Tea

Chromatographic methods are keys for tea analysis, relying on the separation of individual components on dedicated chromatographic columns, with the separation based on the interaction of each component with the stationary phase of the column and the mobile phase. Once separated, each component is quantified at the column outlet with special detectors (including absorbance detector, diode array, electrochemical detection and mass spectrometer).

Chromatographic methods have been applied to identify and quantify a broad range of sample types, from gases to high-molecular weight compounds, as well as cation and anion mixtures. Unsurprisingly, chromatography and capillary electrophoresis are the primary methods used to analyze the components of tea (see Table 2).

In order to identify the separated tea compounds (i.e., to conduct qualitative analysis) gas chromatography with mass spectrometry and liquid chromatography with mass spectrometry have recently come into wide use (Table 1).

The low and non-volatile compounds of tea components are best analyzed using liquid chromatography with amperometric detector (AD) or liquid chromatograph with AD and absorbance detectors. Typical chromatogram of green tea is shown in Figure 6.
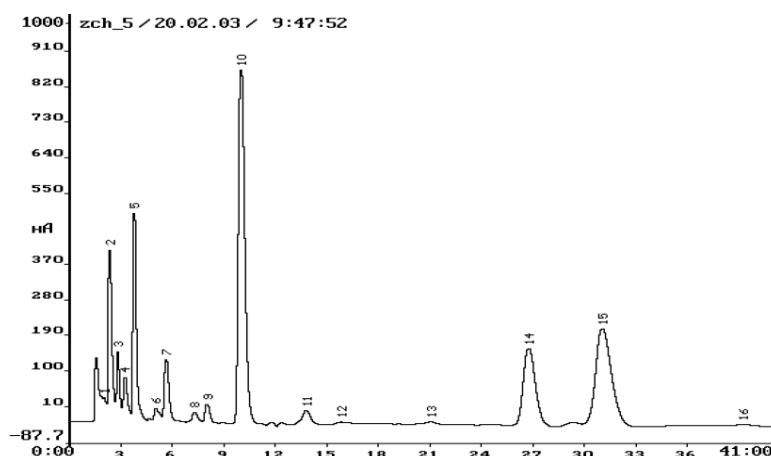


Figure 6. Chromatogram of green tea (brewing time 5 min) on a 150x4.6mm column with C18 Luna (5μ) eluted with acetonitrile and water; 2 – (-)- gallocatechin (GC), 5 – (-)- epigallocatechin (EGC), 10 – (-)- epicatechin (EC), 14 – (-)- epigallocatechin gallate (EGCG), 15 – (-)- epicatechin gallate (ECG) (Yashin et al., 2005-1

Table 2. Chromatographic methods used for the chemical analysis of tea

| No. | Methods | Notes: Specific Characteristics and Application | Reference |
|---|---|---|---|
| 1 | Gas chromatography (GC) | Volatile components which determine the aroma of tea.<br><br>Low volatile components in the form of derivatives, particularly in the form of trimethylsilyl derivatives. | Bondarivich et al., 1967; Pierce et al., 1969; Guth and Grosch, 1993. |
|  | Headspace GC |  | Page and Charbonneau, 1984: Baptista, Tavares et al., 1998; Hu, Ding et al., 2007 |
|  | SPME-GC | Vitamin K, volatile flavour compounds, | Baptista, Tavares et al., 1998; Baptista, da P. Tavares et al., 1999; Reto, Figueira et al., 2007; Wang, Lee et al., 2008 |
|  | GC-ECD | Pesticides, organochlorides, aroma precursors | Gu, Yao et al. , 2011; Li, Li et al., 2004; Huang and Huang, 2006; Mo, Zheng et al., 2009 |
|  | GC-FID | Metabolic fingerprinting |  |

| | | | |
|---|---|---|---|
| | Gas chromatography with mass spectrometry (GC-MS) | Used for identification of a range of compounds, including | Donovan et al., 1999; Kanrar et al. , 2010; Steiniger et al. , 2010; Xu et al., 2010; Tsumura et al. 1994; Li et al. 2004; Huang and Huang 2006; Cho et al., 2008; Schurek et al., 2008; Mo et al., 2009; Moinfar and Hosseini, 2009; |
| | | Pesticides, Essential oils, volatile compounds, metabolic fingerprinting, acrylamides | Hu, Liang et al., 2010; Clark and Bunch, 1997; Bilia, Flamini et al., 2002; Mizukami, Kohata et al., 2006; Pongsuwan, Fukusaki et al., 2007; Rawat, Gulati et al., 2007; Pongsuwan, Bamba et al., 2008; Ciecierska and Obiedzinski 2009; del Mar Caja, Preston et al., 2009 |
| 2 | Paper chromatography | Two-way chromatography with visual spectrophotometry. | Singh et al., 1999 |
| 3 | Thin layer chromatography | Separation of catechins on cellulose plates; chemically modified phases spread on plates. | Vovk et al., 2005; Fecka et al., 2001 |
| | | Methyl-xanthine, catechols, flavonoids | Cimpoiu, Hosu et al., 2010; Tirimann. As, 1973; Ligor, Kornysova et al., 2008; Kartsova and Alekseeva, 2009 |
| 4 | High performance liquid chromatography (HPLC): | | |
| | HPLC-UV | Determination of polyphenols, monosaccharides, flavonoids, caffeine | Rechner et al., 2002; Guillarme, Casetta et al. , 2010; Bramati et al., 2002; Pelillo et al., 2002; Bramati et al., 2003; Pelillo et al., 2004; Yamauchi et al., 2008; Cordero et al., 2009; Lv et al., 2009 |
| | HPLC-ECD | Selective determination of polyphenols, including catechins | Sano et al., 2001; Novak et al., 2010 |
| | HPLC-FD | Determination of (+)-catechin in tea | Ho et al., 1995 |
| | | Polycyclic hydrocarbons | Kayali-Sayadi et al., 1998 |
| | CL-HPLC | Determination of catechins in tea. | Nokagawa and Miyazawa, 1997 |
| | | Oxalic acid | Wu, He et al., 1998 |
| | HPLC-MS | Identification of theaflavins, etc. | Chen et al., 1998 |
| | | Pesticides | Kanrar, Mandal et al., 2010; Huang, Zhang et al., 2009 |

| | | | |
|---|---|---|---|
| | HPLC-MS | Cathechins, Flavonoids, polyphenols | Kuhnert, Clifford et al., 2010; Kiehne and Engelhardt, 1996; Kiehne, Lakenbrink et al., 1997; Umashankar, 2001; Pelillo, Biguzzi et al., 2002; Clifford, Stoupi et al., 2007; Venzie, Castro et al., 2007; Cordero, Canale et al. 2009 |
| | Ultra HPLC | catechins | Jiang, Zhang, 2014 |
| | Nano HPLC | polyphenols, methylxantines in tea | Fanali, 2013 |
| | Two dimensional UHPLC | analysis of green and black teas | Scopano, 2013 |
| | Simulated moving bed chromatography | epigallocatechin gallate | Wong, 2013 |
| 6 | High-speed countercurrent chromatography | Separation of black tea theaflavins. | Köhler et al., 2004 |
| | | Catechins, theflavins | Wedzicha, Lo et al., 1990; Degenhardt, Engelhardt et al., 2000; Baumann, Adler et al., 2001; Du, Jiang et al., 2001; Cao, Lewis et al., 2004 |
| 7 | Preparative HPLC and countercurrent chromatography | Isolation of pure epigallocatechin gallate from green tea. | Kim et al., 2002 |
| | | Polyphenols, alkaloids | Degenhardt, Engelhardt et al., 2000; Yuan, Chen et al., 2004 |
| 8 | Capillary electrophoresis | Separation green tea components. | Horie et al., 1997 |
| | | Amino acids, inorganic cations, trace elements, polyphenols and alkaloids | Li, Huang et al., 2010; Ning, Gong et al., 2010; Horie, Mukai et al., 1997; Arce, Rios et al., 1998; Horie, Yamauchi et al., 1998; Barroso and van de Werken, 1999; Carducci, Dabas et al., 2000; Horie and Kohata, 2000; Lee and Ong, 2000; Wright, Aucamp et al., 2001; Feng, Wang et al., 2003; Kartsova and Ganzha, 2006; Hsieh and Chen 2007; El-Hady and El-Maali 2008; Kodama, Ito et al., 2008; Lee, Jeon et al., 2008; Chi, Li et al., 2009; Li, Zhou et al., 2009 |
| 9 | Chiral chromatography | Separation of catechin optical isomers. | Kofink et al., 2007; Koenig, Evers et al., 1989; Stalcup, Ekborg et al., 1993; Kodama, Yamamoto et al., 2004; Gotti, Furlanetto et al., 2009 |
| 10 | Ion chromatography | Separation of cations and anions of dibasic acids. | Ding et al., 1997 |

| | | | |
|---|---|---|---|
| | Ion chromatography | Anionic minerals, organic acid, inorganic ions | Kuang, Miao et al., 2010; Ding, Chen et al., 1997; Kralj, Krizaj et al., 2005; Michalski, 2006; Kumar, Narayan et al., 2008 |
| 11 | Size-exclusion (molecular-sieve) chromatography | Separation of high-molecular weight compounds. | Flaten and Lund, 1997; Odegard and Lund, 1997; Odegard and Lund, 1997; Matsuura, Hokura et al., 2001; Kasai and Nakatsubo, 2006 |

Abbreviations: UV-ultraviolet detector, ECD-electrochemical detector, FD-fluorometric detector, CL-chemiluminescence, MS-mass spectrometry.

The first publications which presented chromatographic analysis of tea appeared over 30 years ago (Bondarovich et al., 1967; Pierce et al., 1969; Yashin et al., 2009; Hoeffer & Coggon, 1976). Large volumes of chromatographic data on various types of tea have been published, including numerous reviews and books (Bokuchava & Skobeleva, 1969; Bokuchava et al., 1980; Wickremasinghe, 1978; Finger et al., 1992; Balentine et al., 1997; Dalluge & Nelson, 2000; Yashin et al., 2005a; Yashin Ya. & Yashin A., 2004; Yashin et al., 2005b; Harbowy & Balentine, 1997; Hara et al., 1995). Tea constituents of the foremost importance have been determined, as listed in Table 1.

For analysis of tea the following standard methods have been released (OSHA Tea Analysis; ISO Standards; British Standard). The sensory method is used for tea quality determination (Adman, 2013). The quantitative analysis of green tea extracts can also be provided by ESI-MS without chromatography (Savic, 2014).

The following primary goals for tea chemical composition analysis can be identified:

– Determination of how the quantitative content of tea active components, particularly catechins, depends on the tea growing conditions;

– Determination of how tea quality depends upon its chemical composition, in order to optimize production technology;

– Objective assessment of tea quality based on the content of certain compounds or compound groups (despite of great achievements attained by physicochemical methods used for compositional analysis of tea, tea quality is primarily assessed organoleptically by tea tasters)

– Determination of health benefits of certain constituents found in tea—this knowledge can be used as part of strategies for disease prevention;

– Determination of bioavailability and bioaccessibility of the most beneficial components present in tea;

– Exploring the potential of tea and tea products as part of antioxidant treatment of oxidative stress.

Some of these objectives can be achieved by chromatographic methods. In some areas, satisfactory results have already obtained, whereas in others, the work is still at the initial stage.

### 3. Determination of Volatile Impurities in Tea by Gas Chromatography

Several volatile compounds contribute to the aroma of tea beverages, and are identified by GC-MS in conjunction with head-space analysis or solid-phase microextraction (SPME). The list of compound classes and the number of compounds indentified in the aroma of black tea by GC-MS is provided in Table 3 (Bokuchava & Skobeleva, 1986)

Table 3. Classes of compounds contained in aroma of black tea

| No. | Compound class | Number of identified compounds |
|---|---|---|
| 1. | Acids | 71 |
| 2. | Ketones | 57 |
| 3. | Aldehydes | 55 |
| 4. | Esters | 55 |
| 5. | Alcohols | 46 |
| 6. | Hydrocarbons | 37 |
| 7. | Pyridines | 23 |
| 8. | Pyrazines | 22 |
| 9. | Phenols | 19 |
| 10. | Amines and nitrogen-containing compounds | 18 |
| 11. | Lactones | 16 |
| 12. | Pyrroles | 10 |
| 13. | Furans | 9 |
| 14. | Thiazoles | 7 |
| 15. | Sulfides and sulfur-containing compounds | 5 |
| 16. | Oxazoles | 2 |
| 17. | Thiophenes | 1 |
| 18. | Other compounds | 14 |

Total: 467 compounds.

Approximately 500 volatile compounds total have been identified in tea extracts. Alcohols are considered as forming in tea leaves through biosynthesis. Most of the remaining volatile constituents of black tea are formed from their precursors (i.e., carotenoids, lipids, or amino acids) during the fermentation process.

Some of the most important compounds which affect tea aroma are listed below, and include monoterpene hydrocarbons, β-myrcene, limonene, n-cumene, terpinolene, sesquiterpene hydrocarbons, methylnaphthalenes, monoterpene alcohols (linalol, nerol, geraniol, citronellol, α-terpineol, 4-terpineol, menthol, borneol, and farnesol), sesquiterpene alcohols (α- and δ-cadinols, cedrol, etc.), terpenoid aldehydes (neral, geranial, citronellal, β-cyclocitral, safranal, etc.), aromatic aldehydes, ketones (2-heptanone, 5-isopropyl-2-heptanone, 3,5-octadiene-2-one, etc.), isoprenoid ketones (geranylacetone, α-ionone, β-ionone, 5,6-epoxy-β-ionone, 4-oxo-β-ionone, etc.), phenol derivatives (guaiacol, n-ethylguaiacol, vinylguaiacol, isoeugenol), thiophene, oxazoles (4,5-dimethyloxazole, and 2,4,5-trimethyloxazole), sulfur compounds (methanethiol, dimethylsulfide), and many others (Bokuchava & Skobeleva, 1986).

The volatile constituents in different grades of green tea are compared in article (Shimoda et al., 1995). Tea tasters use various definitions to assess tea aroma, for example, fresh, sharp, strong, aromatic, fragrant, vivid, deep, medium, delicate, fruity, berry-like, sweetish, burnt, wood-like, rustic, satiated, sultry, smooth, and many others.

The following compounds determine a typical aroma of green tea: D-nerolidol, 6-methyl-α-ionone, coumarone, indole and coumarin (Shimoda et al., 1995). 3-Hexene acid and methyl jasmonate along with aliphatic alcohols and aldehydes create the freshness of the green tea aroma. 3,5,5-Trimethyl-2(5H)-furanone and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone impart the depth to aroma. Linalool, 2,6-dimethyl-1,3,7-octatriene-6-ol, benzeneacetaldehyde, and 3-hexanyl hexanoate enhance the floral and fruity tones of the aroma. 1-Ethyl-1H-pyrrole-2-carboxaldehyde, 3-ethyl-4-methyl-1H-pyrrole-2,5-dione, 3-ethyl-3-methyl-2,5-pyrrolidinedione, coumarone, and coumarin contribute to a burnt and slightly sweet aroma. For identification of the volatile constituents, capillary column gas chromatography in temperature-programming

mode with flame ionization or mass spectrometry detection is primarily used (Heins et al., 1966).
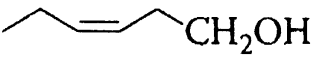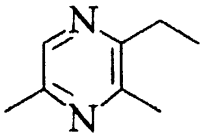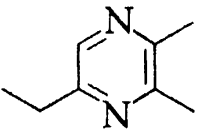
Structural formulas for the compounds which impart various aromas to green and black teas are provided in Tables 4 (Ho et al., 2009). Volatile components of dry tea leaves were mostly determined using static head-space analysis and GC-MS (Heins et al., 1966; Reymond et al., 1966). In particular, the aroma of Ceylon black tea was examined by means of GC-MS and head-space analysis with charcoal adsorption (Wickremasingle et al., 1973). Other analytical methods involved for aroma analysis included static headspace analysis (Witzthum & Werkhoff, 1978), as well as extraction with diethyl ether, steam distillation followed by extraction with isopentane and diethyl ether (Yamanishi et al., 1965), liquid-liquid extraction with pentane and dichloromethane, vacuum distillation, and separation by column liquid chromatography on silica gel columns (Yamanishi et al., 1972). Meanwhile, 57 compounds were identified in extracts by means of GC-MS and IR spectroscopy.
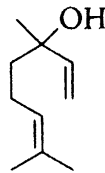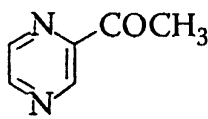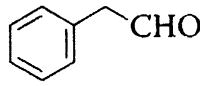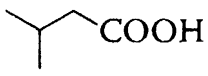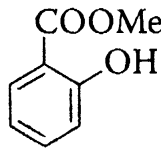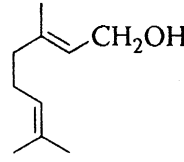
In some publications the teas are classified by aromaticity indices (Owuor et al., 1991). Aromaticity index is a ratio between the peak areas of two compound groups. The first group consists of twelve compounds with undesirable smell, including hexanal, 2,4-heptadienal, 3-hexenol, 2-hexenal, n-pentanol, n-hexanol, while the second group consists of twelve compounds which impart a pleasant aroma to tea, including linalool and its oxides, benzaldehyde, geraniol, α-terpineol, methyl salicylate, benzyl alcohol, β-ionone. These indices have been shown to be connected to the conditions of gathering tea leaves, the height of tea bushes, and types of nitrogen fertilizers. Moreover, Japanese researchers differentiate different tea tree clones by terpene indices (i.e., content of terpene compounds in tea aroma) (Owuor et al., 1987).
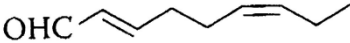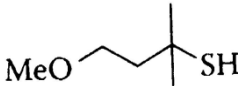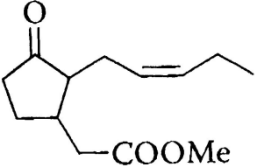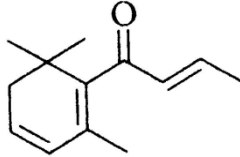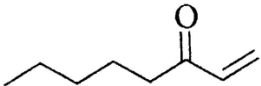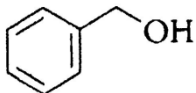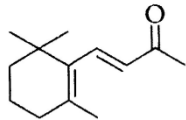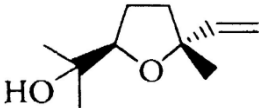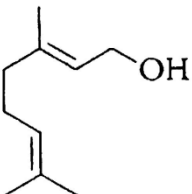
GC-MS was initially used for determining the difference in aromas of different tea grades (Mick and Shreier, 1984). However, during such analysis, decomposition of some unstable tea components was noted, in particular, h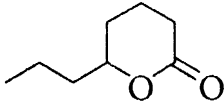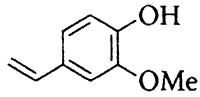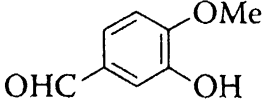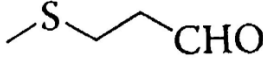exenal, which may distort the true composition of tea aroma (Kinugasa & Takaeo, 1990). Analysis of the enantiomers (optical isomers) of some tea compounds is an entirely new approach to analyzing tea aroma (Wekhoff et al., 1991).

Volatile compounds of green, black, oolong and white teas by dispersive liquid-liquid microextraction coupled with GC have been reported (Sereshti, 2013). For ranking of Japanese green tea (sen-cha) by volatile profiling is used GC-MS and multivariate analysis (Jumtee et al., 2013). The aroma of Pu-erth tea characterized using headspace – solid phase microextraction, combined with GC-MS and GC-olfactometry (Lu et al., 2012).

Table 4. Main volatile components determined in tea samples

| No. | Compound name | Structural formula | Type of aroma |
|-----|---------------|--------------------|---------------|
| **Green Tea** | | | |
| 1 | (Z)-3-hexenol |  | Greenery |
| 2 | (Z)-4-heptenal |  | Hay-like |
| 3 | 2-ethyl-3,5-dimethylpyrazine |  | Nut-flavored |
| 4 | 5-ethyl-2,3-dimethylpyrazine |  | Nut-flavored |

| 5 | Linalool | | Floral |
|---|----------|---|--------|
| 6 | 2-acetylpyrazine | | Roasted |
| 7 | Phenylacetaldehyde | | Sweetish, honey-like |
| 8 | 3-methylbutanoic acid | | Oily |
| 9 | Methyl salicylate | | Minty |
| 10 | Geraniol | | Floral |
| 11 | 5-octanolide | | Sweetish |
| 12 | 2-methoxy-4-vinylphenol | | Spicy |
| 13 | Vanillin | | Vanillin |
| **Balck Tea** | | | |
| 1 | Methional | | Potato-like |

| 2 | (E, Z) – 2,6 - nonadienal | | Cucumber-like |
| 3 | 4-methoxy-2-methyl-2-butanethiol | | Meaty |
| 4 | (Z)-methyl jasmonate | | Floral |
| 5 | β-damascenone | | Honey like |
| 6 | 1-octen-3-one | | Smell of mushrooms |
| 7 | Benzyl alcohol | | Scorched, weakly aromatic |
| 8 | β-ionone | | Floral, wood-like |
| 9 | Menalool oxide | | Sweetish, floral, creamy |
| 10 | Geraniol | | Sweetish, honey-like |

## 4. Determination of Amino Acids and Sugars in Teas by Anion Exchange Chromatography

Direct determination of amino acids and sugars in teas by means of anion exchange chromatography using a pulsed amperometric detector with a gold electrode has been described in (Ding et al., 2002). A 250 × 0.2 mm separation column with AminoPAC PA10 at 30 °C was employed. The gradient elution was performed with a

mixture of deionized water, 0.25 μM sodium hydroxide, and 1.0 μM sodium acetate as a mobile phase (eluent). The mixture composition changed over time according to a definite principle. The flow rate was 0.25 ml/min. The root mean square deviation (RMSD) was 4.6%. The detection limit was within the range of 0.12-4.9 pmol, linear range was three orders of magnitude with a correlation coefficient of 0.99.

The samples were prepared as follows: 0.5 g of tea leaves were placed in a flask and filled with hot water (80 °C), brewed for 30 min, then filtered through a membrane filter with a pore size of 0.45 μm. The solution without suspensions was diluted 25 times and analyzed by chromatography.

The results of sugar analysis in Longjing tea and Bi Luo Chun tea are provided in Table 5. The total content of sugars is approximately 12 mg/g of dry tea leaves; saccharose (sucrose) was found in the largest amount among all sugars.

Theanine (non-protein amino acid) was found in a relatively large amount, about 11 mg/g; the aggregate amount of all 14 remaining amino acids was also about 11 mg/g. Five of 14 amino acids are indispensable (Table 6). Theanine deserves separate consideration. Theanine (γ-glutamylethylamide) structure is shown in Figure 6. The content of theanine in different grades of tea varied within the range of 1.5-3% of dry weight. Tea primarily contains L-theanine and small amounts of D-theanine. Theanine easily dissolves in water but does not dissolve well in ethanol or esters. L-theanine is a weak amino acid, stable in acidic medium at pH=3. Theanine significantly contributes to the taste of green tea. Green tea is typically characterized by four tastes: bitterish, astringent, sweetish, and piquant. The sweetish, piquant tastes arise via the presence of theanine. That is why the content of theanine is one of the most important indicators of the tea quality in world markets. The best Chinese grades of green tea and Japanese Gyokuro tea contain up to 2% theanine.

Table 5. Content of Sugars in Green Tea Determined by HPLC with Amperometric Detector (Ding et al., 2002)

| No. | Name of sugars | Content in dry tea, mg/g | |
| --- | --- | --- | --- |
| | | Longjing | Bi Luo Chun |
| 1. | Saccharose | 7.8 | 9.9 |
| 2. | Glucose | 1.3 | 0.9 |
| 3. | Fructose | 0.8 | 0.9 |

Table 6. Content of amino acids in green tea determined by HPLC with pulsed amperometric detector (Ding et al., 2002)

| No. | Amino acids | Content, mg/g | |
| --- | --- | --- | --- |
| | | Longjing | Biluochun |
| 1. | Isoleucine | 0.10 | — |
| 2. | Leucine | 0.32 | 0.21 |
| 3. | Methionine | 0.10 | — |
| 4. | Threonine | 0.27 | 0.23 |
| 5. | Phenylalanine | 0.26 | 0.13 |
| 6. | Glutamine | 2.24 | 3.11 |
| 7. | Asparagine | 0.3 | 0.25 |
| 8. | Alanine | 0.33 | 0.51 |
| 9. | Serine | 0.69 | 0.55 |
| 10. | Proline | 0.20 | 0.11 |
| 11. | Histidine | 0.30 | 0.35 |
| 12. | Glutamic acid | 3.05 | 3.13 |
| 13. | Aspartic acid | 2.21 | 2.67 |
| 14. | Tyrosine | 0.36 | 0.30 |
| 15. | Theanine | 10.73 | 9.91 |

## 5. Application of High Performance Liquid Chromatography (HPLC) for the Determination of non-Volatile Compounds in Tea

*5.1 Analytical Methods:*

HPLC is the most frequently used methods to determine catechins, alkaloids, theaflavins, and thearubigins in teas. Thearubigins are high-molecular weight compounds with molecular weight varying from 1000 to 40,000 (Haslam, 2003). HPLC is also used to determine phenolic acids (such as gallic and caffeic acids, etc.), flavonols (such as quercetin, kaempferol, and myracetin), lignans, triterpenoid saponins, pigments (chlorophyll and carotenoids) in tea. Among various HPLC methods, reversed-phase chromatography on $C_{18}$ bonded phase columns is used most often; while $C_8$ bonded phase columns are used less often.

Comparative selectivity separation and yield sequence was studied on 150 ×4.6 mm columns filled with various bonded phases, such as $C_{18}$ perfluorophenyl and nitrile phases (Blythe & Rereira, 2006). Yield sequences of catechins of the standard mixture were as follows:

$C_{18}$ – C, EGCG, EC, GCG, ECG, and CG;

Perfluorophenyl phase – C, EC, EGCG, GCG, ECG, and CG;

Phase with a CN group – C, EC, EGCG, ECG, GCG, and CG.

In particular, switching from C18 to perfluorophenyl phase changed the order of peaks 2 and 3; while switching from C18 to CN-rich phase changed the order of peaks 2 and 3, 4 and 5.

In various research papers, the 100, 150, or 250 mm columns with an inner diameter of 3, 3.9, 4, 4.6, or 5mm were used to determine catechins and other constituents in tea. C18 reversed-phase sorbents of the following companies were employed: Hypersil ODS, Zorbax, Cosmosil, Nucleosil, Water, TSKgel, Lichrosorb, Tosoh, and Wakosil. Selected mobile phases (eluent) were: water-acetonitrile, water-methanol, and water-isopropanol. Apart from isocratic elution, the gradient elution is often used. A broad range of detection systems are utilized with HPLC, including UV detectors, diode array spectrophotometers, electrochemical detectors (ECD), such as amperometric and coulometric detectors, mass spectrometry detectors, and fluorometric detectors. Absorbance, SPD and ECD detectors are used most often. Absorption regions at 210, 230, 254, and 370-280nm are used to determine catechins, with evidence showing that when determining five catechins with electrochemical detectors, the sensitivity is 1000 times compared to using a UV detector (Umegaki et al., 1996), while others have shown ECD to be 300 times more sensitive than UV detectors, particularly, for determining catechins (Kumamoto et al., 2000). Meanwhile, amperometric detectors register catechins at potentials of 400mV and 900mV, with maximum signal observed at 750-800 mV (Long et al., 2001). However, in some cases, it is recommended to use 500-600 mV in order to eliminate concurrent oxidation compounds (Umegaki et al., 1996).

*5.2 Analyte Extraction:*

Aqueous extracts at 80-100°C are used most frequently, followed by other solvents and their mixtures, such as ethanol, methanol, and esters. Black tea extracted for 2, 8, and 16 hours with different solvents, namely, absolute acetone, N,N-dimethylformamide (DMF), ethanol, methanol, and their 50% aqueous solutions (Turkman et al., 2007) – with varying recovery depending on the solvent used (Table 7). The total polyphenol content and antioxidants, as well as antibacterial activities, were determined in extracts. Polyphenol content varied within the range of 0.44-114 mg/g of dry tea, with regard to gallic acid, depending on the solvent and extraction time (Table 7).

Table 7. Extraction of polyphenols from tea using different solvents (mg/g)

| Extraction time, min | Methanol | | Ethanol | | Acetone | | Dimethylformamide, | |
|---|---|---|---|---|---|---|---|---|
| | 50% | > 99% | 50% | > 99% | 50% | > 99% | 50% | > 99% |
| 2 | 58.4 | 8.6 | 74.4 | 0.5 | 114.0 | 0.44 | 95.6 | 18.6 |
| 8 | 62.9 | 22.8 | 85.4 | 1.9 | 92.8 | 1.4 | 105.0 | 44.1 |
| 18 | 68.7 | 23.1 | 86.3 | 2.1 | 98.2 | 1.6 | 109.4 | 49.1 |

Aqueous acetone and DMF have shown the best results, whereas pure acetone was least effective. When antibacterial activity was studied, staphylococcus was sensitive to all tea extracts except the methanolic extract.

The impact of temperature on the catechin extraction at 100, 80, and 60 °C for 5 min was studied, as well as

extraction duration (USDA Database for Flavonoid Content, Release 2.1, 2007). Catechin content increases by 30-40% if the extraction was carried out for 10 min. EGCG extraction is the most dependant on temperature – with 60% of EGCG extracted at 80 °C and only 50% at 60 °C. Meanwhile, extraction of catechin was least dependant on temperature – with 98% and 85% extracted at 80 °C and 60 °C respectively.

The conditions for the extraction of catechins from green tea using hot water have been optimized in (Vuong et al., 2012). Method GuEhERS has been applied for extraction pesticides in teas (Lozano et al., 2013; Zhai, 2014) and planar solid phase extraction for analysis pesticides residue in tea by LC-MS (Oellig & Schwack, 2013)

*5.3 Determination of Non- Volatile Compounds*

Averaged catechins content (USDA Database, 2007; Arts et al., 2000; Bronner & Beecher, 1998; Ding et al., 1992; Hertog et al., 1993; Khokhar & Magnusdottir, 2002; Lee & Ong, 2000; Price et al., 1998; Price & Spitzer, 1993; Sakakibara et al., 2003; Steinhaus & Engelhardt, 1989; Wang & Helliwell, 2001) were provided in Table 8; there were 68 different grades of green tea, 83 grades of black tea, and 7 oolong teas involved in analyses. Mean value of all catechins in 68 grades of green tea was 12% of dry weight, 3.3% in 83 grades of black tea, and 5.2% in oolong. Among the 68 grades of green tea, the minimum and maximum values for some catechins were different by an order of magnitude, i.e., 10 times. Maximum content of catechins in green tea was 33.1% of dry weight.

Table 8. Content of flavonoids (% dry weight) in the leaves of green tea, oolong tea, and black tea (mg/g) (USDA Database, 2007; Arts et al., 2000; Bronner & Beecher, 1998; Ding et al., 1992; Hertog et al., 1993; Khokhar & Magnusdottir, 2002; Lee & Ong, 2000; Price et al., 1998; Price & Spitzer, 1993; Sakakibara et al., 2003; Steinhaus & Engelhardt, 1989; Wang & Helliwell, 2001)

| No. | Type of flavonoids | Type of tea | | |
| --- | --- | --- | --- | --- |
| | | Green | Oolong | Black |
| Flavan-3-ols | | | | |
| 1 | (-)-Epicatechin | 8.1 | 2.5 | 2.6 |
| 2 | (-)-Epicatechin-3-gallate | 14.9 | 6.3 | 6.9 |
| 3 | (-)-Epigallocatechin | 20.6 | 7.5 | 9.6 |
| 4 | (-)-Epigallocatechin-3-gallate | 71.1 | 34.1 | 11.2 |
| 5 | (+)-Catechin | 0.6 | 0.3 | 1.4 |
| 6 | (+)-Gallocatechin | 0.0 | 0.0 | 0.9 |
| 7 | Theaflavin | 0.04 | 0.1 | 1.6 |
| 8 | Theaflavin-3,3'-digallate | 0.0 | 0.2 | 1.7 |
| 9 | Theaflavin-3'-gallate | 0.04 | 0.0 | 1.6 |
| 10 | Theaflavin -3-gallate | 0.01 | 0.0 | 1.3 |
| 11 | Thearubigins | 1.31 | 0.0 | 59.2 |
| Flavonols | | | | |
| 12 | Kaempferol | 1.5 | 0.02 | 1.3 |
| 13 | Myricetin | 1.1 | 0.0 | 0.4 |
| 14 | Quercetin | 2.6 | 0.02 | 2.0 |

A study focusing on the content of six catechins, purine alkaloids, and gallic acid was determined by HPLC in 45 tea grades from China, Japan, and Taiwan (Lin et al, 1998). Separation was performed on a 250 × 4.6 mm column with Cosmosil C18-MS, with particle size of 5μ eluted with methanol, redistilled water, and formic acid (19.5:80.2:0.3), the dose of 20 μ. The results were registered by UV detector at a wavelength of 280 nm. RMS deviation varied in the range of 4-10%. The averaged results of measurements taken for 15 grades of green tea from China, 13 grades of green tea from Japan, 9 grades of oolong tea, and 7 grades of Pu-erh tea are provided in Table 9.

Table 9. Content of catechins, gallic acid, caffeine, theophylline, and theobromine in green tea, oolong tea, black tea, and pu-erh tea, in mg/g (Lin et.al., 1998)

| No. | Tea grade | Catechins | | | | | | Total amount of catechins | Alkaloids | | | Gallic acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | EGCG | EGC | ECG | EC | C | GCG | | Caffeine | Theo-phylline | Theo-bromine | |
| 1. | Green tea from China (mean value for 15 teas) | 134 | 4.4 | 29 | 5.5 | 0.2 | 2.6 | 180 (130-220) | 77.3 | 0.8 | 6.0 | 5.2 |
| 2. | Green tea from Japan (mean value for 13 teas) | 131.4 | 8.8 | 19.5 | 8.7 | 0.2 | 3.8 | 178 (90-240) | 76.8 | 0.6 | 3.1 | 2.3 |
| 3. | Oolong tea (averaged value for 9 teas from China and Taiwan) | 49.7 | 3.5 | 8.4 | 2.7 | 0.1 | 0.9 | 65.1 | 63.8 | 0.4 | 2.8 | 4.5 |
| 4. | Black tea (85% fermentation) | 3.0 | 1.9 | — | 0.4 | — | | 5.2 | 160.3 | — | 2.6 | 18.3 |
| 5. | Pu-erh tea (Mean value for 7 teas) | 1.5 | 1.9 | 0.3 | 0.9 | — | 0.1 | 4.9 | 77.0 | 0.3 | 6.3 | 14.9 |

Of particular interest, the aggregate values of the six catechins were very close for Chinese and Japanese teas, as well as the caffeine content. For some components, the average level of EGCG, C, and gallic acid were also similar. The content of EGC, EC, and GCG catechins was higher in Japanese green teas, whereas content of ECG, gallic acid, theophylline, and theobromine was higher in Chinese green teas. Oolong tea contains less catechins than green teas, and the Pu-erh tea contains only a small amount of catechins. The amount of gallic acid significantly increases during fermentation process due to its liberation from catechin gallates.

The total content of catechins significantly decreases during the fermentation process. This issue was studied with TTE tea, a tea variety cultivated in Taiwan which is highly resistant to various insect infestations. This tea variety was used to make green tea, paochong tea, oolong tea, and black tea with a known percent of fermentation. The content of catechins changes depending on the fermentation degree in ten times.

One simple and quick method to determine the four primary catechins, caffeine, and gallic acid in tea using HPLC with a diode array detector (Khokhar & Magnusdottir, 2002). Extraction was carried out thrice, with a 30% methanol solution. The separation was performed in a gradient mode on a 4.5 mm × 25 cm column with $C_{18}$ Alltech adsorbosil, 5 µ, for 20 min. Methanol, acetate with an aqueous buffer were used as an eluent. UV detection was performed mainly at 280 and 360 nm wavelength (Table 10). The yield sequence of the compounds was as follows: gallic acid (GA), epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin (EC), epicatechin gallate (ECG) and caffeine.

The aggregate content of catechins stayed within the range of 100-123 mg/g (10-12.3%) in green tea; 40-63 mg/g (4-6%) in oolong tea; 15.3 mg/g (1.53%) in black tea, and 12.8 mg/g (1.28%) in Pu-erh tea. As fermentation degree increases, the content of gallic acid increases from 0.4 to 5.5 mg/g, the content of caffeine varies within the range of 19-26 mg/g (1.9-2.6%) of dry weight (except for Fujion oolong tea).

Five catechins and caffeine were determined by HPLC-MS (with chemical ionization) with preparative concentration by means of solid-phase microextraction on capillaries coated with polypyrrole. Catechins were determined in both positive and negative ionization modes (Wu et al., 2000). Detection limits of catechins were under 0.5 ng/ml and caffeine about 0.01 ng/ml. When concentration is employed, the detection limits are lower by an order of magnitude.

The determination results for five primary catechins and caffeine in green tea, oolong tea, and black tea, both pure and aromatized with fruits, flowers, or spices are provided in Table 11.

The table shows the results of measurements taken after brewing tea for 5 and 10 min. In all cases, the content of catechins at brewing time of 10 min is higher approximately by 30% compared to the brewing time of 5 min. The aggregate content of catechins varies within the range of 68.5 to 101 mg/g of dry weight in green teas (i.e., approximately 7-10%), approximately 50 mg/g (5%) in oolong teas, and approximately 10-12 mg/g (1-1.2%) in aromatized black teas.

The content of caffeine is about 30-40 mg/g (3-4%) in green teas, 26 mg/g (2.6%) in oolong teas, and 15-33 mg/g (1.5-3.3%) in black teas.

Table 10. Content of catechins, gallic acid, and caffeine in various tea grades (Zuo et al., 2002)

| | Tea grade | Content, mg/g of dry tea | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | EGC | EGCG | EC | ECG | Total amount of catechins | Gallic acid | Caffeine |
| 1. | Meifoo green tea | 27.7 | 52.7 | 10.3 | 21.8 | 112.5 | 0.74 | 26.8 |
| 2. | Shanghai green tea | 30.8 | 51.1 | 7.25 | 11.3 | 100.45 | 0.37 | 23.0 |
| 3. | Hongzhou Lung Ching green tea | 37.6 | 62.4 | 6.6 | 16.3 | 122.9 | 1.84 | 28.5 |
| 4. | Jasmine green tea | 27.6 | 54.2 | 6.9 | 15.8 | 104.6 | 1.13 | 29.6 |
| 5. | Fujian oolong tea | 10 | 22.2 | 2.63 | 6.06 | 40.89 | 1.42 | 7.44 |
| 6. | Jiangxi oolong tea | 15.9 | 28.2 | 2.96 | 6.45 | 63.51 | 1.67 | 18.7 |
| 7. | Fujian black tea | 5.71 | 3.79 | 1.36 | 4.45 | 15.31 | 2.06 | 21.6 |
| 8. | Pu-erh tea | 6.23 | 1.99 | 3.24 | 1.32 | 12.78 | 5.53 | 22.4 |

Table 11. Content of catechins and caffeine in various teas determined by HPLC-MS (Wu et al., 2000)

| | Tea grade | Brewing time, min | Content in mg/g of dry tea | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | EGC | C | EC | EGCG | ECG | Total amount of catechins | Caffeine |
| 1. | Chinese green tea | 10 | 23.3 | 1.0 | 10.1 | 52.2 | 14.3 | 100.9 | 39.5 |
| | | 5 | 15.2 | 0.7 | 8.7 | 43.1 | 9.0 | 76.6 | 30.4 |
| 2. | Lung Ching green tea | 10 | 13.6 | 0.9 | 8.0 | 37.0 | 9.8 | 69.3 | 29.4 |
| | | 5 | 10.2 | 0.6 | 6.3 | 31.0 | 7.8 | 55.9 | 22.9 |
| 3. | Jasmine green tea | 10 | 9.2 | 2.2 | 11.5 | 31.5 | 14.1 | 68.5 | 29.4 |
| | | 5 | 6.9 | 1.6 | 8.5 | 22.5 | 10.4 | 49.9 | 20.7 |
| 4. | Tie Guan Yin oolong tea | 10 | 8.8 | 0.7 | 5.5 | 27.8 | 6.3 | 49.1 | 26.5 |
| | | 5 | 6.2 | 0.5 | 3.5 | 20.0 | 5.9 | 36.0 | 20.5 |
| 5. | Black tea | 10 | 5.2 | 1.0 | 8.7 | 18.8 | 8.4 | 42.1 | 33.8 |
| 6. | Black tea (Krasno-rozovy), aromatized | 10 | 0.7 | 0.4 | 1.3 | 6.0 | 3.7 | 12.1 | 38.9 |
| 7. | Orange Pekoe black tea (Tetley), aromatized | 10 | 0.8 | 0.3 | 0.9 | 4.2 | 3.4 | 9.5 | 14.9 |

A method for simultaneous determination of all polyphenols in vegetables and teas is proposed in work (Sakakibara et al., 2003). 100 polyphenols which occur most often in significant quantities in these products were selected; these include benzoic and cinnamic acids, flavonoids (flavones, flavonols, flavonone, isoflavones, catechins, theaflavins, chalcones, anthocyanins, anthraquinones, etc.). The retention parameters were determined on a 250x4.6mm column with $C_{18}$ Capcell pek (5 μ) at 35 °C in gradient mode (eluted with methanol–sodium phosphate–water), and optimal wavelengths for diode array spectrophotometer, slope of the calibration curves, and detection limits were selected. Using the data obtained above, polyphenols content was determined in 63 vegetables, fruits, and teas. The measurement data for some polyphenols found in black teas from Japan, oolong tea from China, and black tea from Kenya as well as total content of catechins, total content of all polyphenols, and the portion of catechins in all polyphenols is provided in Table 12.

Table 12. Content of polyphenols in green teas from Japan, oolong tea from China, and black tea from Kenya, in mg/g (Karori et al., 2007)

| No. | Polyphenols | Green tea | | Oolong tea | Black tea |
|---|---|---|---|---|---|
| | | Gyokuro | Sencha | | |
| 1. | Catechin | 8.72 | 2.78 | 2.07 | 1.58 |
| 2. | Gallocatechin | 2.27 | 14.60 | 9.99 | – |
| 3. | Catechin gallate | 0.32 | – | 0.45 | 1.15 |
| 4. | Gallocatechin gallate | 4.47 | 3.75 | 2.97 | 2.71 |
| 5. | Epicatechin | 23.60 | 58.00 | 6.65 | 20.10 |
| 6. | Epigallocatechin | 80.60 | 179.00 | 49.10 | 9.19 |
| 7. | Epicatechin gallate | 14.00 | 23.50 | 8.94 | 8.23 |
| 8. | Epigallocatechin gallate | 91.70 | 149.00 | 53.80 | 10.20 |
| 9. | Theaflavin | – | – | 0.27 | 3.10 |
| 10. | Theaflavin-3-gallate | – | – | 0.26 | 4.30 |
| 11. | Theaflavin-3'-gallate and theaflavin-3,3'-gallate | – | – | 0.70 | 9.60 |
| 12. | Kaempferol-3-O-glucoside | 2.68 | 1.82 | 0.83 | 3.05 |
| 13. | Kaempferol-3-O-rutinoside | 0.12 | – | 0.60 | 2.53 |
| 14. | Kaempferol glycoside | 2.50 | 2.59 | 0.43 | 1.15 |
| 15. | Quercetin-3-O-rhamnoside | 0.30 | 1.16 | 1.39 | 6.33 |
| 16. | Quercetin glycoside | 1.50 | 7.69 | 1.98 | – |
| 17. | Myricetin-3-O-rutinoside | 0.98 | 5.17 | 2.08 | 2.08 |
| 18. | Gallic acid | 1.54 | 2.54 | 13.30 | 17.90 |
| | Total content of all polyphenols | 235.30 | 451.60 | 153.81 | 101.20 |
| | Total content of catechins | 225.68 | 430.63 | 133.97 | 53.16 |
| | The portion of catechins in Total amount of polyphenols | 96% | 95% | 87% | 52% |

Five primary catechins and caffeine were determined in 31 commercial tea samples from China, Taiwan, and Japan by means of HPLC in boiling water extracts and 75% ethanol extracts (Table 13) (Lin et al., 2003). According to their total content of catechins and epigallocatechin gallate (EGCG), various teas were classified in the following order: green tea > green tea (fresh leaves)> oolong tea > black tea > Pu-erh tea. Extracts obtained using 75% ethanol solution showed a higher level of EGCG and total content of all catechins. Meanwhile, when ranked according to their caffeine content, the teas produced from the same tea bush but fermented to different degrees were arranged in the following sequence: Black tea > oolong > Green tea > fresh tea leaves.

The level of flavonols and flavones intake by a healthy population in the USA is 20-22 mg per day (Sampson et

al., 2002). In food products and beverages, the flavonols are primarily quercetin, kaempferol, and myricetin, and flavones (apigenin and luteolin). Tea and onions are primary sources of these flavonols and flavones both for men and women. Black teas (Lipton, Tetley, and Salada) were found to contain 26.0 mg/kg of quercetin, approx. 4.0 mg/kg of kaempferol, and 19-21 mg/kg of myricetin. New method (RP-HPLC) developed for determination of quercetin in green tea has recently been published (Savic et al., 2013). The proportion of tea with regard to total flavonol and flavone intake is 26-35%.

In Great Britain, black tea is the primary source of polyphenols among regular tea drinkers (Khokhar and Magnusdottir, 2002). Six grades of black tea purchased in Great Britain were analyzed specifically for this purpose. The overall antioxidant activity of the selected teas was determined by means of TEAC, ORAC and FRAPS methods; individual polyphenols were identified by HPLC. Epigallocatechin gallate, epicatechin gallate, teagallin, four theaflavins, quercetin-3-rutinoside, and 4-caffeoylquinic acid were identified. Thearubigins made 75-82% of all polyphenols. On average, one cup of brewed tea (one tea bag) was found to contain 262 mg of GAE, 65 mg of which were individual polyphenols. The antioxidant potential of individual tea phenolics from Kenyan green and black teas were provided using an on-line high-performance liquid chromatography (Stewart et al., 2005).

Table 13. Content of epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), catechin (C), epicatechin (EC), and total amount of all catechins in various grades of tea from China, Taiwan, and Japan, in mg/g of dry tea (W – hot water extracts, A – alcoholic extract, 75%)

| No. | Type of tea, country | Tea name | EGCG | | EGC | | ECG | | C | | EC | | Total catechins | |
|-----|----------------------|----------|------|------|-----|-----|-----|-----|---|---|----|----|-----------------|---|
| | | | W | A | W | A | W | A | W | A | W | A | W | A |
| 1. | Green, China | Xian-Zhe-Zhu-Jian | 24.47 | 29.38 | 26.29 | 20.88 | 2.54 | 9.98 | 1.00 | 0.50 | 2.54 | 1.94 | 59.65 | 62.68 |
| 2. | Green, China | E-Mei-Shan - Snow Bud | 14.74 | 14.90 | 37.20 | 28.06 | 3.14 | 5.51 | 1.10 | 0.49 | 3.70 | 2.49 | 59.88 | 51.45 |
| 3. | Green, China | Lu-Shan Ckoud (D) | 32.08 | 47.34 | 83.83 | 77.58 | 7.80 | 11.68 | 1.39 | 2.41 | 6.37 | 6.59 | 136.47 | 145.60 |
| 4. | Green, China | Lu-Shan Ckoud (K) | 30.64 | 43.48 | 40.11 | 18.81 | 8.68 | 11.95 | 1.70 | 1.82 | 3.93 | 2.91 | 85.06 | 78.97 |
| 5. | Green, Taivan | Longjing | 31.17 | 48.18 | 67.31 | 70.98 | 5.18 | 9.37 | 0.98 | 0.81 | 4.14 | 4.29 | 108.78 | 133.63 |
| 6. | Green, Japan | Decoct Tea | 21.19 | 35.76 | 83.62 | 83.07 | 3.45 | 6.79 | 0.64 | – | 5.69 | 5.93 | 114.59 | 131.55 |
| 7. | Green, Japan | Decoct Tea | 21.30 | 36.95 | 82.44 | 94.72 | 3.64 | 6.43 | 0.96 | – | 6.05 | 6.54 | 114.39 | 144.64 |
| 8. | Оолонг, China | Xiao-Hong-Pao | 7.85 | 30.68 | 56.28 | 87.67 | 0.96 | 5.10 | 2.47 | 3.03 | 3.44 | 5.03 | 71.00 | 131.51 |
| 9. | Oolong, China | Rock Tea | 3.69 | 14.67 | 47.02 | 43.83 | 0.49 | 2.90 | 1.78 | 2.08 | 3.15 | 2.95 | 56.13 | 66.43 |
| 10. | Oolong, China | Xiao-Hong-Pao | 13.15 | 28.10 | 38.39 | 72.74 | 3.16 | 9.22 | 1.41 | 2.58 | 5.12 | 5.53 | 61.23 | 118.17 |
| 11. | Oolong, Taiwan | Ping-Lin | 10.49 | 27.74 | 78.54 | 99.64 | 1.19 | 4.23 | 0.48 | 0.61 | 4.01 | 5.25 | 94.71 | 137.47 |
| 12. | Oolong, Taiwan | Shi-Zhuo-Zheng Lu | 7.76 | 29.23 | 73.43 | 115.00 | 1.06 | 4.75 | 0.50 | 0.84 | 3.89 | 6.23 | 86.64 | 156.05 |
| 13. | Black, Taiwan | Lipton black | 2.42 | 4.14 | 10.83 | 61.78 | 1.93 | 3.77 | – | – | 1.17 | 1.06 | 16.35 | 70.75 |
| 14. | Pu-erh, China | Xiao-Huang-Yin | 5.96 | 4.79 | 13.82 | 5.78 | 9.56 | 10.08 | 1.42 | 0.72 | 4.87 | 2.58 | 35.63 | 23.95 |

An intake level of catechins, caffeine, and total polyphenols via tea was determined in Great Britain (GB). Tea was purchased in typical supermarkets both as tea bags and in bulk. Tea was prepared with boiling water and brewed for 5 minutes. The measurement results are provided in Table 14 (Khokhar & Magnusdottir, 2002).

Table 14. Total polyphenols, total catechins and caffeine content in tea (Khokhar et al., 2002)

| No. | Type of tea | Content, mg/g of dry tea | | |
|-----|-------------|--------------------------|---|---|
| | | Total amount of polyphenols | Total amount of catechins | Caffeine |
| 1. | Green tea | 87-106.2 | 51.5-84.3 | 11-20 |
| 2. | Black tea | 80.5-134.9 | 5.6-47.5 | 22-28 |
| 3. | Fruit tea | — | 8.5-13.9 | — |

A daily intake of three cups of tea a day (200 ml each brewed with 1% of tea leaves by weight), equates to 405.5 mg, 92.7 mg, and 61.5 mg of catechins from green, black, and fruit tea respectively. Meanwhile, this equates to 92-146 mg of caffeine daily with the same amount of tea.

Average content of catechins in different types of teas is provided. Range of catechin content in tea: green tea – 16-30, oolong – 8-20, black – 3-10 (in % of dry weight).

The published literature provides some conflicting information regarding the total content of polyphenols in green and black teas, with some researchers reporting 67.7 mg/g of polyphenols in black tea and 62.3 mg/g in green tea (Hoff & Singletion, 1977); with others reporting a higher content of polyphenols in green tea compared to black tea, namely, 95.4 mg/g and 80.1 mg/g respectively (Manzocco et al., 1998). This difference may be related to many factors, such as type of tea, storage conditions, extraction conditions, etc.

The primary phenolic components of black tea are theaflavins, thearubigins, and theabrownins. These compounds were measured in 56 grades of tea purchased in Australian surpermarkets (Yao et al., 2006). The content of theaflavins varied within the range of 0.29-1.25%. Low content of theaflavins suggests incomplete fermentation and/or long storage period. Solubility of thearubigins and theabrownins in tea bags was between 82 and 92%. This indicates differing permeability of tea bags.

Theaflavins are the first oxidation products of catechins and catechin gallates during the fermentation process. Theaflavins can be further oxidized forming more highly polymerized molecules, thearubigins; yet further oxidation creates condensed theabrownins, which presumably are thearubigins bonded with proteins.

Thearubigins in general are characterized as fractions insoluble in ethyl acetate which are separated by butanol into two fractions, both soluble and insoluble (Roberts et al., 1956). Theaflavins impart an astringent taste to black tea, and a bright golden color to brewed black tea. Meanwhile, thearubigins contribute a reddish color and full rich taste, theabrownins impart a dark brown color and negatively affect the quality of tea. Theaflavins and thearubigins should not be present in pure green teas, or should be present only in very small amounts.

However, in some green teas purchased in Australian supermarkets theaflavins and thearubigins were detected in quantities close to those contained in black teas. This fact may indicate that some production and/or storage protocols for these green teas were breached. Therefore, the analysis of tea polyphenols contained in the final product may be used to control shortcomings of production processes and violation of storage conditions.

For assessment of black tea quality, we suggest consideration of the proportion of theaflavins concentration to the thearubigin concentration. Fresh good black tea must contain over 1% of theaflavins and approximately 10% of thearubigins, and their ratio must be greater than 0.1. These indicators were measured for the most popular grades of black tea (Hara et al., 1995). Tea tasters confirm the high quality of those teas in which the ratio of theaflavins to thearubigins is greater than 0.1. Therefore, the above ratio could be used for assessment of tea quality as well as for ensuring the right production technology and storage conditions.

As early as 1972, it was determined that black tea contains 5% unoxidized polyphenols and 25% oxidized (Sanderson, 1972). Brewed black tea contains only 4.5% simple polyphenols and 15% oxidized polyphenols by total weight of dry tea, i.e., the total content of polyphenols is 19.5%.

It was determined that the total content of polyphenols in Kenyan black tea is 20% (5% simple unoxidized polyphenols, 2% theaflavins, and 13% thearubigins) (Obanda et al., 1997). Many Indian teas were found to contain an average of 20% polyphenols (Dev Choudhury & Goswami, 1983).

The total content of polyphenols in green teas ranged between 21-33% (average 25%).

The difference in the content of polyphenols, both in black and green teas, is connected with the place where they were grown: tea from India and Sri Lanka usually has higher total polyphenols content than teas from China. An overall aggregate content of polyphenols in two grades of green tea from Sri Lanka was determined as 26% and 34%, whereas Chinese teas had only 21% and 23% (Owuor et al., 1987).

Therefore, the total content of polyphenols, in addition to the aromaticity index, can be used to assess the origin and quality of tea (Owuor et al., 1987) the content of amino acids (Hara et al., 1995) and caffeine (Owuor and Chavanji, 1986). Low total polyphenols content in some teas, both black and green, may indicate that these teas were stored for too long. In the case of black teas it can also suggest their incomplete fermentation.

## 6. Conclusion

As follows from this article, the composition of the same type of tea may vary significantly depending on the place where it was grown (i.e., soil, climate, height, precipitation, etc.), production technology, storage conditions, etc.

The composition of green and black teas accounting for possible variations is provided in Table 15 (Robb and Brown, 2001; Peterson et al., 2005)

Table 15. The variation in composition of green and black teas

| No. | Compound | Content | |
|-----|----------|---------|---|
| | | Green tea | Black tea |
| 1. | Catechins | 10-30% | 3-10% |
| 2. | Theaflavins | 0 | 2-6% |
| 3. | Thearubigins | 0 | 10-20% |
| 4. | Phenolic acids | 2% | 1% |
| 5. | Flavonols | 2% | 1% |
| 6. | Other polyphenols | 3-6% | 3-10% |
| 7. | Caffeine, theobromine, and theophylline | 3-6% | 3-6% |
| 8. | Amino acids | ~10 mg/g | ~5 mg/g |
| 9. | Theanine | 2% | – |
| 10. | Peptides, proteins | 6% | 6% |
| 11. | Organic acids | 2% | 2% |
| 12. | Mono- and disaccharides | 11 mg/g | 11 mg/g |
| 13. | Mineral substances | 10-13% | 10-13% |

The total content of polyphenols can serve as an indicator of tea quality.

The presence of theaflavin and thearubigin in green tea indicates breach of production technology and storage conditions (these components should not be present in true green tea).

For assessment of black tea quality, we suggest consideration of the proportion of theaflavins concentration to the thearubigin concentration. High-quality black tea must contain over 1% of theaflavins and approximately 10% of thearubigins, and their ratio must be greater than 0.1. Tea tasters confirm the high quality of black tea only at such ratio of theaflavins to thearubigins.

Therefore, the analysis data on aggregate polyphenols as well as their individual constituents may be used for the overall assessment of tea quality, production technology, and storage conditions. Many analytical methods have been developed for the analysis of bioactive compounds in tea, including catechins, theaflavins, thearubigins, and methyl xanthines. HPLC methods are by far the most common procedures employed due to its separation power and its capabilities of coupling to different highly sensitive detectors such as ESD and MS. LS-MS is most powerful tool for studying tea bioactive components in complex biological matrices (Wong et al., 2009).

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