Characterisation of white tea - Comparison to green and black tea

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Abstract: An overview is given on the manufacture of the different types of tea along with the most important phenolics present in tea and methods of analysis. Compositional data are presented for green, white and black teas. A differentiation of green and black tea by using the ratio between total phenolics and sum of the major catechins seems to be feasible.

For white tea there is no general accepted definition. Possible approaches are geographic origin, the botanical variety and the manufacture or the appearance. The differentiation between green and white teas by the ratio mentioned above is not possible.

Zusammenfassung: Es wird eine Übersicht über die Teemanufaktur und die resultierenden Arten von Tee gegeben, begleitet von einer Übersicht über einige wichtige Inhaltsstoffe (Flavanole, Zusammenstellung analytischer Methoden zur Bestimmung von (Poly)phenolen und Daten über grüne, schwarze und weiße Tees). Derzeit scheint eine Differenzierung von grünem und schwarzem Tee über das Flavanol:Gesamtphenolverhältnis möglich.

Für weißen Tee gibt es derzeit keine allgemein akzeptierte Definition. Mögliche Ansätze für diese sind die geographische Herkunft, die botanische Varietät oder die Art der Herstellung. Eine Differenzierung durch das Flavanol:Gesamtphenolverhältnis von grünem und weißen Tee ist nicht realistisch.

1. Introduction

Tea is one of the most popular beverages worldwide. It is by definition derived solely and exclusively, and produced by acceptable processes, from the tender shoots of varieties of the species *Camellia sinensis* (Linnaeus) O. Kuntze, known to be suitable for making tea for consumption as a beverage.

All kinds of tea originate from *Camellia sinensis* (earlier called *Thea sinensis*) which has the two subspecies var. *sinensis* (China tea) and var. *assamica* (Assam tea).

Tea has an attractive aroma, good taste, and health-promoting effects, which makes it one of the most popular drinks in the world. As early as 3000 B.C., the Chinese used it as a medicinal drink and as a beverage because of his effective pharmaceutical activity. Tea plants are now widely cultivated (in Southeast Asia including China, India, Japan, Taiwan, Sri Lanka, and Indonesia and in central African countries).

The aim of this paper is to provide information on the chemical composition of all types of tea along with the methodology to be employed. Special attention is drawn to white tea which is now worldwide on the market. Instant teas have not been included although the market data for rtd (ready-todrink) beverages mostly using instant green, black or white tea is still growing.

2. Tea manufacture

From fresh tea leaf (*Camellia sinensis*) different types of tea are produced. Well known are the green tea, the Oolong tea and black tea. Common term for the conversion of tea phenolics in the manufacture of black and Oolong teas is fermentation. This conversion is accomplished by endogenous enzymes – mainly the polyphenol oxidase. Recently the term fermentation has been replaced by aeration. One of the reasons for this is due to the fact that in some tea-drinking muslim countries fermentation is kind of suspicious as people might think tea contains ethanol.

For the manufacture of green teas the relevant enzymes are deactivated by heat before rolling. As can be seen from Fig. 1 there are two different types of processing. Black and Oolong tea (and also puerrh teas which are not included in Fig. 1) are (after withering) mechanically treated to give rise to the enzyme conversion of flavanols. There is a notably difference between orthodox and CTC teas in most cases as the reaction is much stronger in macerated leaves.

2.1 White tea definition

Currently there is no general accepted definition of white tea and very little international agreement. What could such a definition be based on?



Fig. 1 Tea manufacture – major steps and corresponding types of tea.



R1	R2	R3	R4	config.	name
OH H OH H H H	H OH H OH galloyl galloyl galloyl	OH OH OH OH OH H	H H OH OH H OH H	2R, 3S 2R, 3R 2R, 3S 2R, 3R 2R, 3R 2R, 3R 2R, 3R 2R, 3R	(+)-catechin (–)-epicatechin (+)-gallocatechin (–)-epigallocatechin (–)-epicatechin gallate (–)-epigallocatechin gallate (–)-epigafzelechin gallate

- 1. The Chinese position: white tea is defined by the sub-species it is manufactured from (*Camellia sinensis var. khenghe bai hao* and *Camellia sinensis var. fudin bai hao*) found only in Fujian province and with minimal processing following traditional guide-lines. White tea is a seasonal crop (spring) with special sensory and health benefits.
- 2. Other producing countries define white tea by plucking standard *i.e.* only the bud or first leaves that are plucked and dried with minimal processing. In this way the delicate white leaf hairs are left intact lending the appearance of 'white tea'. It has to be noted that, if this definition would be generally accepted, it would exclude traditional Chinese white tea varieties such as Pai Mu Tan (White Peony).

So there are a few issues: firstly the appearance (liquor of white tea is very pale yellow in color, and mild tasting in cup) and

secondly the manufacture (minimal processing, just drying, no "fermentation"). There are health claims underway, such as: (a) white tea is lower in caffeine than green tea, (b) white tea is much higher in antioxidants, esp. catechins than green tea and (c) white tea has a higher anti-mutagenicity action compared to green tea. From compositional data available it can be stated that the first two claims are completely nonsense. Currently there is a great marketing and public interest for white tea. As there is a high demand but low supply it has sometimes been suspected that more white tea is now sold than is grown!

3. The key players – Phenolics in tea

Tea contains a wide range of phenolic compounds of which the most important are briefly mentioned. Tea's polyphenols include flavanols, flavandiols, flavonoids, and phenolic acids; these compounds may account for up to 30% of the dry weight of the tea leaves according to the literature.

Catechins (flavanols) are the most abundant group of phenolic compounds in fresh leaf and green tea. The major tea catechins are (-)-epigallocatechin 3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (-)-epicatechin 3-gallate (ECG), and (+)-catechin (C). Other, such as epiafzelechin and its gallate as well as acetylated catechins have also been identified (Hashimoto et al., 1987).

4. Flavanol related compounds

Tea is a natural source of proanthocyanidins which does occur in green teas in relevant amounts. Green tea is relatively rich in proanthocyanidins and lower in bisflavanols, while black tea has higher amounts of bisflavanols. This is in tune with the observation of Hashimoto et al. (1992) that especially the gal-



Fig. 3 Proanthocyanidins and Bisflavanols; a. EGC-4 β -8-EGCG: R₁=H, R₂=galloyl; EGCG-4 β -8-EGCG: R₁=R₂=galloyl; b. Bisflavanol A: R₁= R2 =galloyl.



Fig. 4 Theaflavins (Theaflavin: $R_1=R_2=H$; Theaflavin-3'-gallate $R_1=$ galloyl, $R_2=H$; Theaflavin-3-gallate $R_1=H$, $R_2=$ galloyl, Theaflavin-3,3'-gallate: $R_1=R_2=$ galloyl).

loylated proanthocyanidins are degraded during the so-called fermentation. There are at least 16 proanthocyanidins present in tea (Lakenbrink, 2000; Engelhardt et al., 2004). Not much information on the contribution of proanthocyanidins to the taste of tea beverages or to the health effects is currently available.

There are 2 important groups of oxidation products by tradition. The group of the theaflavins and related compounds comprise four major TFs and minor TF and related compounds (Lapczynski, 2000). The term theaflavins was set up by Roberts (1958) for orange reddish pigments in tea and the structures have been fully elucidated years later (Collier et al., 1973). TFs are formed from 2 catechin monomers by the action of polyphenoloxidase (EC 1.10.3.1). Tab. 1 shows examples for the precursors and the theaflavins.

Taking this into account it is quite clear that a (black) tea with a high concentration of catechins will be relatively low in respect to theaflavins and *vice versa*. For example Darjeelings

Tab. 1 Precursors of the major theaflavins.

Precursor A	Precursor B	Product
epicatechin	epigallocatechin	theaflavin
epicatechin	epigallocatechin gallate	theaflavin-3 gallate
epicatechin gallate	epigallocatechin	theaflavin-3' gallate
epicatechin gallate	epigallocatechin gallate	theaflavin-3,3' gallate

are low in theaflavins and high in catechin while the opposite is true for a typical Kenyan CTC tea.

The other "oxidation products" are the thearubigins which also got their name from Roberts (1958). Not much is known about the structures of TR. It is well known that the compounds are formed during the enzyme oxidation of flavanols in black tea manufacture. Not much is known about the chemistry of thearubigins except they are weakly acidic and in part responsible for the colour of (black) tea. No structures have been elucidated as yet, however, it has been suggested that B-Ring interflavonoid bond (2'-2' as present in the bisflavanols) might be a backbone of thearubigins or a fraction of these.

Flavonols (quercetin, kaempferol, and myricetin) are present in form of their O-glycosides. At least 14 different glycosides have been detected in tea (Engelhardt et al, 1992; Scharbert et al, 2004). There are mono-, di- and triglycosides present in tea, among those often the quercetin 3-rhamnoglucoside in the highest concentration. Flavonol glycosides (FOG) are interesting in terms of health benefits. According to the literature there are protective effects against coronary heart disease. Scharbert et al. (2004) stated that the flavonol glycosides are responsible for the astringency in black tea and not theaflavins or catechins. The FOG content is not very much affected by the enzyme transformation (fermentation).

Flavones are present in tea in form of their C-glycosides in much lower concentrations (factor of about 10). C-glycosides are not hydrolysed by acids or enzymes. As already mentioned tea flavonoids are claimed to be responsible for most of the positive health effects of tea. Those health effects are not subject of this paper, it is referred to a number of reviews dealing with those properties (Shankar et al., 2007; Fraser et al., 2007; Friedmann, 2007; Feng, 2006; Stangl et al., 2007; Gardner et al., 2007; Scalbert et al., 2005; Williamson and Manach, 2005). Briefly, catechins, especially epigallocatechin gallate (EGCG), theaflavins and flavonol glycosides are thought to be responsible for antioxidative properties in tea. Flavonol glycosides (FOG) have activities against myocardial infarct and stroke. The mechanism of action is subject to ongoing research. As regards black tea there is an ongoing debate about the influence of milk on health properties. Kyle et al. (2007) stated that the addition of milk does not affect the increase in



Fig. 5 Flavonols and a flavonol glycoside (Kaempferol: $R_1=R_2=H$; Quercetin: $R_1=$ OH, $R_2=H$; Myricetin: $R_1=R_2=OH$; Kaempferol-3-O- $[\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 3)- α -L-rhamnop

e.g. plasma antioxidant capacity. Lorenz et al. (2007) found that milk counteracted the favourable health effects of tea on vascular function.

Theanine (N-ethyl-L-glutamine) is a nonproteinogenic amino acid which was identified already in the 50ies of the last century. Due to possible health properties it has attracted a lot of interest again recently. According to the literature L-theanine might be neuroprotective and cognitive enhancing agent (Dimpfel et al., 2007; Nathan et al., 2006) and also reduce psychological and physiological stress (Kimura et al., 2007). It is worth to mention that the German BfR (Federal Institute for Risk Assessment) did not recommend the addition of isolated L-theanine to rtd beverages (BfR, 2003).

5. Methods of Analysis

There are two methods standardized on an international base only. The extraction affects the results dramatically. Both ISO methods mentioned below use the same extraction: ground tea is extracted twice using 70% methanol (aq.) at 70 °C. This is for most compounds kind of an exhaustive extraction. Moreover, the compounds are more stable in 70% methanol compared to water. Other types of extraction, e.g. reflux with water for one hour yields a degradation of the catechins (Kuhr, 1993). For studies aiming to get information about the constituents of consumer brews there are different concepts which will be mentioned below. The first is a method for the determination of total phenolics by the Folin-Ciocalteu-Assay (ISO, 2005a). Briefly, the reagent has phospho-tungstic acids as oxidants, which on reduction by readily oxidized phenolic hydroxy groups yield a blue colour (tungsten and molybdenum blue) with a broad maximum absorption at 765 nm, which can be measured by a spectrophotometer. The Folin-Ciocalteu phenol reagent reacts with a wide range of phenolic compounds. There is a kind of variation in response to individual phenolic compounds to the reagent, however, the use of gallic acid (as monohydrate) gives a reasonable measure for the total phenolic content of the tea analysed.

The second method is a HPLC method for the determination of the catechins (ISO2005b). In early days the calibration was based on pure catechins. This has at least two drawbacks: the standards are not quite cheap and it happened frequently that those were not really pure. Consequently the ISO method relies on the RRF (relative response factor) concept. The calibration is done using caffeine and relative response factors are used for the individual catechins. Those factors have been determined in an international ring test with pure catechins of known water content. Those factors are between 0.84 (gallic acid) and 11.24 (epigallocatechin). This concept enables labs worldwide to obtain reliable results for the catechins.

There are other useful methods for tea flavonoids:

- HPLC methods for flavonol and flavone glycosides (some after polyamide clean-up) have been published (Lakenbrink, 2000; Scharbert et al., 2004). As the concentration of flavonol O-glycosides is much higher compared with the flavone C glycosides a hydrolysis step to destroy the Oglycosides before measuring the flavone C-glycosides makes sense.
- Theaflavins by HPLC (Lapczynski, 2000).
- Proanthocyanidins and Bisflavanols (Lakenbrink, 2000; Engelhardt et al., 2004).
- Chlorogenic acids (Lakenbrink et al., 2000).

For the determination of theanine some HPLC methods in most cases require a pre- or postcolumn derivatisation as theanine is (nearly) transparent in the UV-region (Tippeswamy et al, 2006; Desai and Armstrong, 2004) and also TLC methods (Speer et al., 2006). Recently we developed a quite simple concept for the determination of theanine (Engelhardt and Simonides, 2007) in tea which is currently tested in a first international ring test. Briefly, a RP column capable to separate more polar compound is used with water as an eluent and detection at 210 nm is employed. Tea is extracted using hot water and used as such or after a passage through a polyamide column (to remove polyphenols). As the theanine content is relatively high the detection limit is good enough.

418 Y. Hilal and U. Engelhardt

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	Green tea	Average	Black tea	Average
Total polyphenols	10.1 – 22.2	17.0	8.3 – 24.8	16.5
Catechins	8.5 – 20.6	15.1	0.74 - 10.00	4.2
Caffeine	1.5 – 5.2	3.4	2.0 - 5.4	3.5
Theogallin	0.1 – 1.4	0.6	0.1 – 1.0	0.6
Gallic acid	0.01-0.19	0.09	0.16 - 0.60	0.26
Theaflavins	n.d.	n.d.	0.30 – 2.41	0.94
Flavonol glycosides *	0.28 – 0.95	0.64 (1.38)	0.24 - 0.87	0.47 (0.89)
Flavone C glycosides*	0.005 - 0.14	0.086 (0.16)	0.02 - 0.12	0.051 (0.09)

Tab. 2 Flavonoid and other data of teas from the German market. Data are given in g/kg. n.d. = not detected.

* Data are calculated as aglycones, in parentheses as glycosides.



Fig. 6 Catechins vs total phenolics in green and black teas.

6. Compositional data

There are a few problems with analytical data to be mentioned. Firstly, as already stated, the extraction procedures are often different. To gain an overview on the composition of teas from different origins it is necessary to ensure the authenticity of the origin (Engelhardt, 2007). It is not possible to differentiate green and black tea simply by the catechin data. It is true that the so-called fermentation decreases the content of catechin on the expense of theaflavins and thearubigins, however, a (black) Darjeeling sample can contain as much as 10% of catechins (sum of the major 5) which is more than in some green teas. Consequently the ISO option is a ratio between catechins and total polyphenols. The breakpoint to be set will probably be between 50-60% catechins. Currently the ISO SC 8 TC 15 working group on tea is collecting data on catechins and total phenolics for origin teas worldwide to set up a differentiation between green and black teas. Currently more than 400 origin teas are in the database. It is planned to publish those data in the near future to have a more solid basis. Another option is a ratio between catechins and theaflavins, however,

the ISO catechin method is capable to determine the theaflavin content but it has not been validated for that purpose as yet. Recently a paper has been published which employs the amino acid pattern as a possible criterion (Alcazár et al., 2007). Fig. 6 shows a snapshot which does contain about 50 green and black teas each from the German market. The methodology used is in principle the one described in the ISO methods. For those teas data for other flavonoids are also available as shown in Tab. 2 (Engelhardt et al., 2000).

It is worth mentioning that those data were selected to be representative for the German tea market. There might be a difference from other data as there were not too many real "hard fermented" CTC teas in the program. Data for proanthocyandins and bisflavanols in tea are scarce. Tab. 3 gives data for also a limited set of samples.

It worth to note that tea contains at least 3 coumaroyl- and caffeoylquinic acids each. The amounts are between 0.2 and 0.9%. The average amount of theanine in tea is around 1%. Based on 60 teas we found 0.15 - 1.2% in green and black tea samples. Speer et al. (2006) analysed more than 80 samples of different types of tea and their results were in between



Fig. 7 HPLC separation according to the ISO method of each one green, white and black tea (1: theogallin, 2: gallic acid, 3: theobromine, 4: epigallocatechin, 5: catechin, 6: caffeine, 7: epicatechin, 8: epigallocatechin gallate, 9: epicatechin gallate).

Tab. 3 Proanthocyanidins and bisflavanols in green (n=29) and black (n=9) teas (Engelhardt et al., 2004).

Tab. 4 Data on green and white teas are given in g/100 g.

	Green tea	Average	Black tea	Average
Proanthocyanidins (%)	0.13-1.89	0.85	0.10-0.98	0.50
Bisflavanols(%)	0.01-0.11	0.05	0.33-0.81	0.65
sum		0.90		1.15
proportion of bisflavanols (%)	0.9-12.7	5.9	40.2-77.1	59.7

	White tea	Average	Green tea	Average
Total polyphenols	16.23-25.95	21.54	13.7-24.7	19.18
total Catechins	7.94-16.56	13.22	9.89-17	12.95
caffeine	3.35-5.74	4.85	1.67-3.90	2.90
epigallocatechin gallate	5.23-9.49	8.00	4.40-9.6	6.75
epigallocatechin	0.24-2.64	1.11	1.94-4.07	2.84
flavonol glycosides *	0.06-1.44	0.61(1.25)	0.64-2.02	1.1(2.27)

* Data are calculated as aglycones, in parentheses as glycosides.

nearly nothing up to 2.25%. In white teas (n=8) we found 1.0 – 3.7% (mean 1.9%) which is higher compared to our other samples.

7. White and green tea

Based on a limited number of samples green and white teas (green and white around 30 samples each) are compared in the following section. In contrast to the data for green and black teas we tried to get authentic green and white teas as pairs from one area – consequently the green tea data are a bit different (Tab. 4).

It can be withdrawn from Tab. 4 that the ratio catechin/ total phenolics will not serve as a criterion for a differentiation of green and white tea. It is obvious that the caffeine content of white tea is often much higher. Theogallin might be higher in white teas compared with green (data not shown). In Fig. 7 chromatograms for the catechin determination of green, white, and black teas are shown. Those are not truly representative as a black Darjeeling looks much similar to a green tea than to a CTC black tea sample.

8. New flavonol glycoside

Most of the investigated white teas contain a new myricetin triglycoside, which was not found up to now yet in the tea (see Fig. 8, Peak 1). Analysis by HPLC and HPLC-ESI-MS showed the UV-spectrum of myricetin and a mass of 787, MS2 gave m/z of 316 for myricetin aglycone. The exact structural elucidation is currently carried out by NMR techniques.

9. Consumer brews

For the characterisation of origin teas it is crucial to have a uniform and possibly exhaustive extraction. However, an extraction with 70% methanol is not really common to the average consumer. It is certainly not possible to cover all consu-



Fig. 8 HPLC Separation of flavonol glycosides from a white one and a green tea.



Fig. 9 Average extraction efficiencies from a tea bag (2 min brewing) given in % of an exhaustive extraction. CQA: chlorogenic acids; FOG: Flavonol glycosides; FCG: Flavone C glycosides.

mer habits, but it is certainly necessary to employ an at least reproducible procedure. One of those is the "stir and squeeze" concept employed brewing studies with (black) tea bags (Lakenbrink et al., 2000). Briefly, teabags were brewed with hot water, stirred once using a spoon and – at the end of the brewing time – the bags was squeezed against beakers wall. A hexaplicate extraction was employed, and the extracts were combined to give the analytical sample. The figures found were compared to the ones determined after a methanol extraction according to the ISO protocol which was set as an exhaustive extraction. Fig. 9 shows the extraction efficiency of one tea bag products. The results for the other bags were quite similar. It has to be stressed that there are a few variables, such as the particle size, will have a strong influence on the extraction efficiency. Theanine was not involved into the brewing experiments these days. A more recent study showed that theanine as expected is very efficiently extracted. Most teas do have 10–20 mg per cup if brewed in a usual way (ca. 2 g tea, 200 mL of water) certainly depending on the theanine content of the leaves¹.

Communication of Engelhardt, U. H., Maiwald, B., Simonides, M. and Hilal, Y. (2007).

10. Literature

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