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Extraction of active ingredients from green tea (*Camellia sinensis*): Extraction efficiency of major catechins and caffeine

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Abstract

The effect of different extraction set-ups that influence the extraction efficiency of catechins and caffeine from green tea leaves (variety Fanning Belas, China) were studied using different aqueous and pure solvents (acetone, ethanol, methanol, acetonitrile, water), different temperatures (60, 80, 95 and 100 °C) and times (5–240 min). Raw extracts were analysed for contents of major catechins (EC, EGC, ECG, EGCG), caffeine, proanthocyanidins and flavonols (myricetin, caempherol, quercetin). Starting material was found to contain 191 g major catechins/kg material, 36 g caffeine/kg material and 5.2 g flavonols/kg material on a dry mass basis. The content of major catechins in green tea extracts varied from approximately 280–580 g/kg dry extract, with extraction efficiencies of major catechins varying from 61% to almost 100%. Content of caffeine in extract was in the range of 75 g/kg, where its extraction efficiency varied from 62% to 76%. Average extraction yield was 30% with exceptions when using pure acetone and acetonitrile, where extraction yield was about 3%. Contents of flavonols and proanthocyanidins were in the ranges 6–20 and 12–19 g/kg, respectively. Different extraction procedures with water were also investigated and optimal conditions determined: maximum achieved extraction efficiency of catechins with water was obtained at 80 °C after 20 min (97%) and at 95 °C after 10 min of extraction (90%). Degradation of catechins was observed at higher extraction temperatures and with prolonged extraction times. Using a lower ratio of solvent to material, extraction efficiencies were increased by applying a multi-step extraction procedure. Optimal extraction procedure was then performed using decaffeinated green tea leaves, which were obtained by high-pressure extraction with CO_2, when 98% of caffeine was selectively isolated without significant impact on valuable catechins.

Keywords: Green tea (*Camellia sinensis*); Solvent extraction; Raw and decaffeinated green tea extract; Catechins; Caffeine

1. Introduction

Tea (*Camellia sinensis*), originated in China, dates back several thousand years. There are two major kinds of tea, black tea and green tea, and they both contain caffeine (1–5%) with small amounts of other xanthine alkaloids also present. Tea composition varies with climate, season, tea variety, and age of the leaf. Tea also contains large amounts of tannins or phenolic substances (5–27%) consisting of catechin (flavanol) and gallic acid units, with those in green tea being higher than those in black tea (Leung & Foster, 1996). In general, fresh green tea leaves contain 36% polyphenols, among which catechins prevail. Pharmacological properties of tea are due primarily to its alkaloids (caffeine) and catechins, which are divided into four primary compounds (Fig. 1), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate

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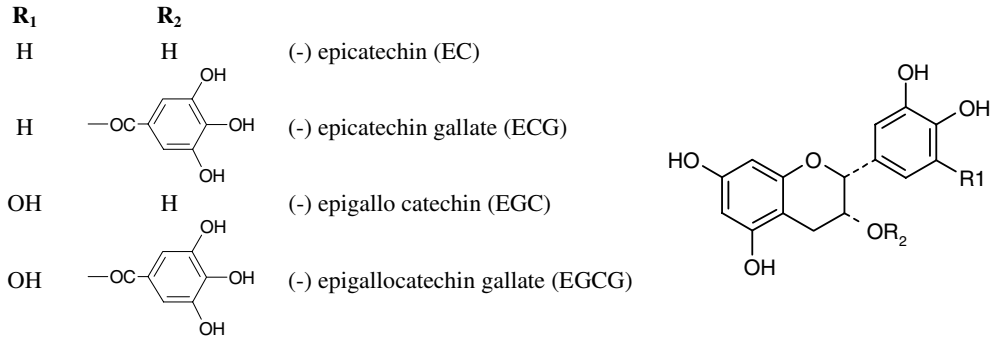

Fig. 1. Chemical structures of major catechins.

(EGCG), and four secondary compounds, catechin (C), catechin gallate (CG), gallocatechin (GC), and gallocatechin gallate (GCG). EGCG is the predominant catechin present in green tea leaves (48–55% of total polyphenols) (Ho, Chen, Wanasundara, & Shahidi, 1997).

Other components present in tea include fats (4–16%), amino acids, sterols, vitamins, minerals, flavour and aroma chemicals, proteins, triterpenoids and others (Leung & Foster, 1996; Lunder, 1992, chap. 9). Several reports have attributed, to green tea, chemo-preventive and therapeutic properties (McKay & Blumberg, 2002; Sueoka et al., 2001). It has also been shown that tea possesses antimutagenic, anticarcinogenic and anticlastogenic effects (Gupta, Saha, & Giri, 2002; Kuroda & Hara, 1999). For these reasons, green tea has become an attractive research material in the past 15 years.

Green tea active ingredients, mainly catechins and caffeine, are usually isolated by extraction with organic solvents, where extraction conditions (tea and solvent type, temperature, time, pH, ratio of solvent to material.) variously influence the extraction efficiency and quality of obtained extracts. Additionally, during extraction the epimerization of major catechins can occur (Komatsu et al., 1993; Wang & Helliwell, 2000; Yoshida, Kiso, & Goto, 1999). In order to obtain caffeine-free products of green tea, decaffeination processes are nowadays used and these include extraction with methylene chloride (Hartmann & Baumberger, 2000), ethyl acetate or supercritical carbon dioxide (Chang, Chiu, Chen, & Chang, 2000; Lack & Seidlitz, 1992, chap. 5). The quality of decaffeinated material, obtained with these solvents, varies in the amount of isolated caffeine, remaining catechins and solvent residue. An advantage of high-pressure extraction of green tea with CO_2 is that a decaffeinated material with almost the same amount of catechins as the starting material can be obtained.

The authors in this study investigated solvent extractions of a lower grade green tea (variety Fanning Belas, China), which is used in teabags. Different solvents (water, acetone, methanol, ethanol, acetonitrile) and aqueous solvent mixtures (25, 50, 80 vol%), extraction temperatures (70–100 °C) and times were varied in order to investigate the extraction efficiency of major catechins (EC, ECG, EGC, EGCG) and caffeine. Green tea extracts were additionally analyzed for contents of three flavonols (myricetin, caempherol, quercetin), and proanthocyanidins. Different extraction procedures were also conducted with water and optimal extraction temperature and time determined. A multi-step extraction procedure was performed in order to investigate the change of extraction efficiency by changing the ratio of solvent to material. A decaffeination process with supercritical CO_2 was done at company NATEX Prozesstechnologie GesmbH (Ternitz, Austria) and furthermore, residue material extracted with water in order to obtain a water-soluble caffeine-free green tea extract enriched in major catechins.

2. Materials and methods

2.1. Materials

Dry and ground green tea leaves (Fanning Belas, China) were supplied by RAPS GmbH & Co. KG. Reference substances, caffeine and major catechins, were purchased from Sigma Chemical Co.: caffeine (CO750), EGCG 80% (E4268), EC98% (E4018), ECG 98% (E3893), EGC 98% (E3768). Reference flavonols were myricetin 95% (Fluka, 70050), caempherol 96% (Fluka, 60010) and quercetin 99% (Acros, 174070250). All solvents used for analytical and extraction purposes were purchased from Merck.

Sieve analysis of ground material was performed in order to determine the average particle size, and moisture content of samples was measured by a Mettler Toledo DL31 Karl Fischer Titrator.

2.2. Analytics

A method for simultaneous *quantification of catechins and caffeine* in green tea samples was developed and

supplied by RAPS GmbH & Co. KG (Otto, 2001). The analytical method involved HPLC, wherein, quantifications of caffeine, EC, ECG, EGC and EGCG were performed via calibration curve. The HPLC system consisted of a Varian 9012 HPLC pump and a Varian 9065 diode array detector.

Quantification of proanthocyanidins was obtained after a hydrolysis reaction of proanthocyanidins to anthocyanidins in a reaction mixture with *n*-buthanol and concentrated hydrochloric acid (Bae et al., 1993; McMurrough & McDowell, 1978; Porter, Hrstich, & Chan, 1986). Repetition of the method was achieved with addition of Fe(III) salt to the reaction mixture. Twenty grams of extract were weighed in a 10 ml flask and diluted with distilled water to the mark. Two milliliters of solution were transferred to a 25 ml flask and 20 ml of a mixture of *n*-buthanol:conc. HCl, containing $FeSO_4 \cdot H_2O$ (77 mg/500 ml) added. The reaction mixture was incubated for 15 min at 95 °C, after which the sample was cooled to ambient temperature and analysed on UV Varian Cary 1E spectrophotometer at 540 nm against a reference sample. The absorbance of each sample solution was measured three times in two parallel trials. The standard deviation value was about 3%.

Quantification of flavonols (myricetin, quarcetin, caempherol) in green tea samples was obtained by an HPLC method (Wang & Helliwell, 2001) the HPLC system consisted of a solvent delivery system, constaMetric 3000, a spectromonitor 3100 variable length detector (LDC Analytical), and an HP 3396 Series II integrator. Sample preparation for green tea leaves consisted of weighing 1 g of green tea leaves in a round-bottom flask and adding 40 ml of 60 vol% ethanol, and 5 ml of 6 M HCl. The reaction mixture was thermostatted for 2 h at 95 °C and constant stirring was applied. The hydrolysed mixture was cooled and filtered (0.45 μm) into a 50 ml flask, diluted with 60 vol% ethanol to the mark, and twice analysed. The standard deviation value was about 2%. Fifty milligrams of green tea extract were weighed into a 20 ml flask and diluted with distilled water to the mark. Sixteen milliliters of solution were transferred to a round-bottom flask and 24 ml of absolute ethanol added with 5 ml of 6 M HCl. Hydrolysis and further dilution were performed as described for green tea leaves.

2.3. Extraction procedures

2.3.1. Extraction with different solvents at constant temperature

2.3.1.1. Different solvents, 2 h extraction. Twenty grams of green tea leaves were extracted with 400 ml of solvent (ratio 20 ml:1 g) in a round-bottom flask equipped with a condenser. Solvents used were acetone (25%, 50%, 80%, 100%), methanol (25%, 50%, 80%, 100%), ethanol, (25%, 50%, 80%, 90%, 96%, 100%), acetonitrile (50%, 100%) and water. Temperature of extraction for aqueous solvents was kept at boiling point temperature and, for water, 70, 85 and 100 °C. The extraction mixture was constantly stirred with a magnetic stirrer. After 2 h of extraction, the extraction mixture was cooled, vacuum filtered (0.45 μm) and the solvent evaporated under vacuum at 40 °C.

2.3.1.2. Water, different extraction times. In order to investigate higher ratios of solvent to material (40 ml:1 g), 2 g of raw material were extracted with 80 ml of water in the same manner as described above by maintaining constant temperature at 80 and 95 °C. Different extraction times, using water as solvent, were investigated.

2.3.2. Extraction with water at different starting temperature

2.3.2.1. Single extraction step. Hundred milliliters of distilled water (60, 80 and 95 °C) were poured over 1 g of green tea leaves in a flat-bottom flask and stirred for different time intervals from 1 to 120 min, without maintaining constant temperature. Extract solution was consequently cooled during this time and its temperature was monitored. The extraction mixture was then filtered (0.45 μm) and directly analyzed by HPLC. Two milliliters of extract solution were evaporated to dryness and concentration of dry matter determined as an average from triple trials.

2.3.2.2. Multiple extraction steps. A multi-step extraction procedure, at optimal determined starting temperature and time, was performed using 2 g of green tea leaves and 80 ml of water (ratio 40 ml:1 g), thus decreasing the ratio of solvent to material compared to the procedure from Section 2.3.2.1. Four subsequent extracts were obtained in dry form and analyzed for major catechins and caffeine.

2.3.3. Decaffeination and further processing of green tea

Thousand grams of green tea leaves were extracted with CO_2 by the company NATEX Prozesstechnologie GesmbH (Ternitz, Austria) in a high pressure extraction pilot plant (Lack & Seidlitz, 1992, Lack, Seidlitz, & Glanz, 5; Lack et al., 2001). The tea leaves were moistened up to 15–50 wt% with water, and extracted with wet CO_2 at 225–350 bar and 50–80 °C. Decaffeinated green tea was dried in an air oven at 40 °C to a humidity content of 4%, analysed for major catechins, and stored in a cool and dry place until further processing. For obtaining a decaffeinated green tea extract enriched in catechins, 150 g of decaffeinated green tea leaves were extracted with 1400 ml of water in a

Table 1
Caffeine, major catechins and flavonols in green tea leaves, variety Fanning Belas

Active ingredient	Content (g/kg dry leaves)
Caffeine	36.0
Catechins	
Epicatechingallate	15.2
Epigallocatechin	46.0
Epigallocatechin gallate	129
Epicatechin	0.9
Major catechins	191
Flavonols	
Myricetin	0.8
Quercetin	1.8
Kaempherol	2.6

multi-step extraction procedure as described in Section 2.3.2.2.

3. Results and discussion

3.1. General

Table 1 contains data of analysis of starting green tea material (variety Fanning Belas, China), where content of major catechins and caffeine on dry mass basis were 191 g catechins/kg material and 36.0 g caffeine/kg material, respectively. EGCG represents 67.5% of major catechins in green tea leaves. Total flavonols (myricetin, quarcetin, caempherol) contribute 5.2 g of flavonols per kg of dry green tea leaves. Median particle size and average particle diameter were determined to be 720 and 715 μm, respectively. Moisture content of raw material was 3.96%.

3.2. Extraction of green tea leaves with different solvents at constant temperature

3.2.1. Different solvents, 2 h extraction

Table 2 contains results of extraction yields, extraction efficiencies of major catechins and caffeine and contents of flavonols and proanthocyanidins in green tea extracts obtained with different solvents at constant temperature and ratio 20 ml:1 g. Extraction yield and efficiency of extraction were calculated, regarding the mass of extract on dry mass basis (residual humidity less than 6%), and mass of isolated active ingredient, respectively. Extraction yields were in the range 28–36%, with exceptions using pure acetone and acetonitrile, where extraction yield was about 3%. The lowest extraction efficiencies, for both caffeine, and major catechins, were thus obtained with pure acetone and acetonitrile. Efficiency of extraction for major catechins with aqueous

Table 2
Extraction of green tea with different solvents (T = const., t = 2 h, ratio 20 ml:1 g): extraction yield, extraction efficiency of caffeine and major catechins, content of catechins, flavonols and proanthocyanidins in dry extracts

Solvent	Vol (%)	Temperature (°C)	Extraction yield (%)	Extraction efficiency (%)		Content (g/kg dry extract)		
				Caffeine	Catechins	Catechins	Flavonols[a]	Proanthocyanidins
Acetone	25	Boiling point	35.2	75.6	98.5	534	8.7	17.7
	50		33.5	72.1	99.3	565	15.8	19.0
	80		31.9	67.5	95.2	569	18.8	18.7
	100		3.2	6.9	4.6	272	5.8	13.5
Methanol	25	Boiling point	30.9	66.3	79.7	492	13.9	16.3
	50		32.5	69.0	81.1	476	16.4	15.7
	80		30.3	62.0	77.9	491	16.5	15.9
	100		33.4	72.8	87.7	501	16.9	14.7
Ethanol	25	Boiling point	30.3	63.5	62.5	394	16.2	14.9
	50		32.8	68.9	76.4	445	15.9	15.7
	80		34.5	73.8	89.1	493	17.8	15.6
	90		30.0	65.7	74.5	474	15.8	14.3
	100		28.2	68.5	77.2	522	10.7	13.4
Acetonitrile	50	Boiling point	36.0	72.8	99.8	530	15.5	18.5
	100		2.9	9.2	8.1	534	6.8	13.4
Water	–	70	29.2	56.0	65.9	430	17.4	16.2
	–	80[b]	36.5	81.5	84.4	448	9.1	16.9
	–	85	30.4	63.8	61.3	385	17.0	16.8
	–	95[b]	43.0	89.1	57.2	258	13.4	13.7
	–	100	30.1	57.0	37.3	237	11.4	12.0

[a] Sum of myricetin, quercetin and kaempherol contents.
[b] Ratio solvent:material = 40 ml:1 g.

solvents was, on average, 80% for aqueous and pure methanol and above 95% for acetone aqueous solvents. Extremely high extraction efficiency (99.8%) of catechins was achieved with 50 vol% of acetonitrile. Efficiency of extracting caffeine with investigated aqueous solvents was in the range 56–75%, with exceptions using pure acetone or acetonitrile. Extraction efficiency of major catechins and caffeine using water at 85 °C was in the range 61–64%.

Fig. 2 shows the overall content of major catechins (EC, ECG, EGC, EGCG) in obtained extracts on a dry mass basis. It can be seen that extract obtained with pure water at 85 °C contained around 385 g major catechins/kg dry extract while, using investigated aqueous solvents, the content increased with increasing vol% up to approximately 560–580 g major catechins/kg dry extract. By increasing the water content in aqueous mixtures of ethanol and methanol the content of major catechins in dry extract decreases, whereas highest catechin contents were obtained with pure solvents. Unlike methanol and ethanol, pure acetone does not sufficiently extract the major catechins (272 g major catechins/kg dry extract). EGCG contributed up 57–68% of total major catechins in obtained dry extracts, and was confirmed to be the predominant catechin compound.

The contents of proanthocyanidins and flavonols (myricetin, quercetin, caempherol) in extracts varied from 13.4 to 19.0 g proanthocyanidins/kg dry extract and from 5.8 to 18.8 g flavonols/kg dry extract; highest values were obtained with water at 85 °C, 50% acetone and 80% ethanol. Extraction with water was also performed at 70 and 100 °C, where dry extracts contained 430 g of major catechins/kg and 237 g/kg, respectively. Extraction yields (30%) and content of caffeine in water extracts (68–69 g/kg), using the ratio 20 ml:1 g, were almost constant with temperature. Besides a lower content of catechins in water extract obtained at 100 °C, the contents of proanthocyanidins and flavonols were also lower compared to extract obtained at 70 and 85 °C and thus; 12.0 g proanthocyanidins/kg dry extract and 11.4 g flavonols/kg dry extract. With prolonged heating at 100 °C, half of the catechins were destroyed together with investigated flavonols.

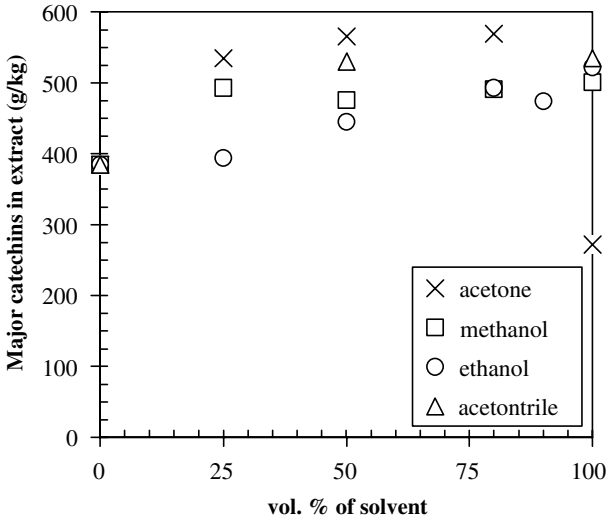

Fig. 2. Extraction of green tea with different solvents (T = const., t = 2 h, ratio 20 ml:1 g): content of major catechins in dry green tea extracts.

3.2.2. Water, different extraction times

Shorter extraction times, with a ratio of 40 ml:1 g, were, furthermore, investigated using water as solvent and constant extraction temperatures of 80 and 95 °C. Table 2 also contains results of 2 h extraction runs in comparison to results obtained with water at a ratio 20 ml:1 g. It can be seen that, by increasing the amount of solvent, the extraction yields, as well as the extraction

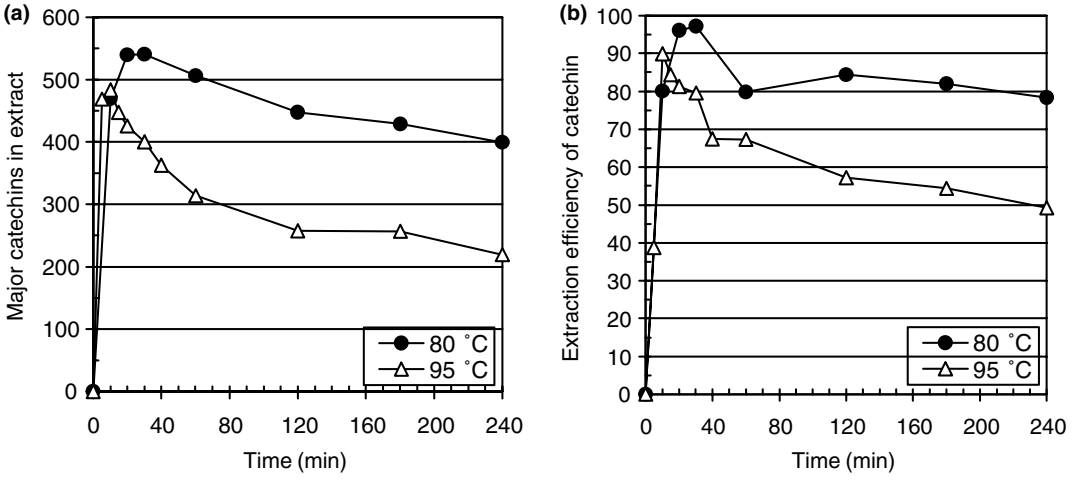

Fig. 3. Extraction of green tea with water (T = const., ratio 40 ml:1 g): (a) content of major catechins in dry extract and (b) extraction efficiency of major catechins.

efficiencies of caffeine and major catechins were increased. Fig. 3(a) presents the contents of major catechins in dry extracts as a function of extraction time and temperature. The content of major catechins reached a maximum after 20 min at 80 °C and after 10 min at 95 °C; with prolonged extraction time it decreased to 400 and 220 g catechins/kg dry extract at 80 and 95 °C, respectively. This is due to the degradation of major catechins, which is expressed more at higher temperature (95 °C compared to 80 °C). Taking into account extraction yields on a dry mass basis, the extraction efficiency of major catechins was calculated and results are shown in Fig. 3(b). Highest extraction efficiency (96–97%) was obtained at 80 °C after 20–30 min of extraction. Extraction efficiency of caffeine was in the range 75–85% and approximately constant after 10 min of extraction at both temperatures. Content of proanthocyanidins in dry extract at 80 °C was approximately constant and in the range 15–17 g proanthocyanidins/kg dry extract. At 95 °C, content of proanthocyanidins decreased from 17 g/kg at 10 min to 14 g/kg at 40 min and remained constant up to 240 min of extraction. No specific trend for content of flavonols was observed, whereas the content varied from 9 to 20 g flavonols/kg dry extract.

3.3. Extraction of green tea with water at different starting temperatures

3.3.1. Single extraction step

Regarding the preparation procedure for green tea beverage, a study was conducted employing different temperatures of hot water and different time intervals. Study was focussed on the content of major catechins and caffeine in the extract solution with no insight of the aroma, smell and taste. The cooling rate of extract solution was monitored and temperature decreased as shown in Fig. 4. Temperatures varied mostly during

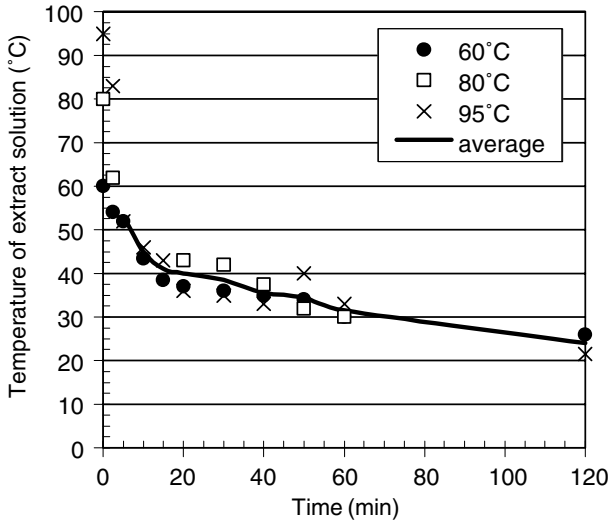

Fig. 4. Single-step extraction of green tea with water at different starting temperature (T_s) and ratio 100 ml:1 g: cooling rate of extract solution during extraction at different time intervals.

the first 2.5 min while, after 5 min of extraction, the values were comparable at all three starting temperatures (60, 80, 95 °C) and average values were calculated with standard deviation being ±2%. After 5 min of extraction, temperature of extract solution was around 50 °C, which then decreased to 40 °C in 20 min, and 30 °C was reached after 1 h. Dry matter of extract solutions reached maximum values from 3.1 to 3.6 mg/ml after 30 min of extraction and remained approximately constant up to 120 min. During the first 30 min of extraction, dry matter increased with increasing starting temperature at constant time interval due to increased mass transfer. Extract solutions were directly analyzed after the elapsed time interval and results are given as mg active ingredient per 100 ml of solution as a function of temperature and time (Fig. 5). Fig. 6 presents results given as g of active ingredient per kg of dry extract,

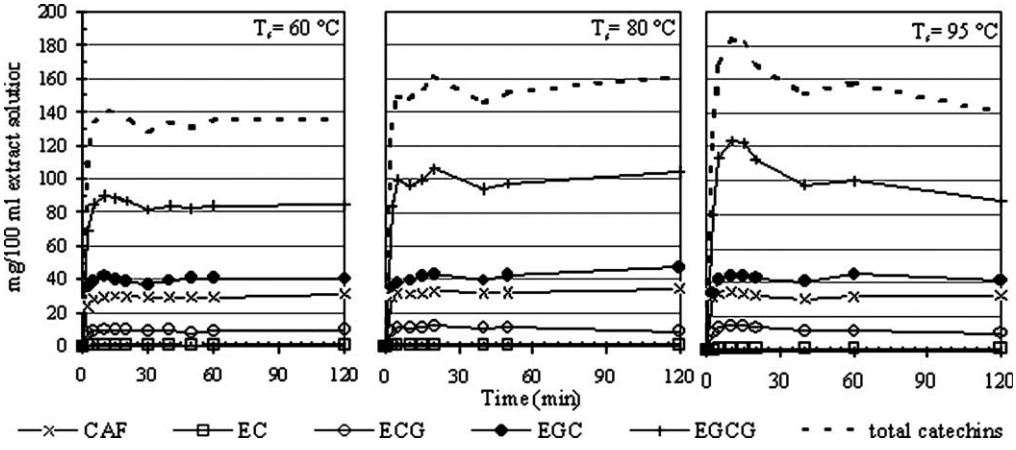

Fig. 5. Single-step extraction of green tea with water at different starting temperatures (T_s) and ratio 100 ml:1 g: contents of major catechins and caffeine in green tea extract solution (mg/100 ml) as a function of extraction time.

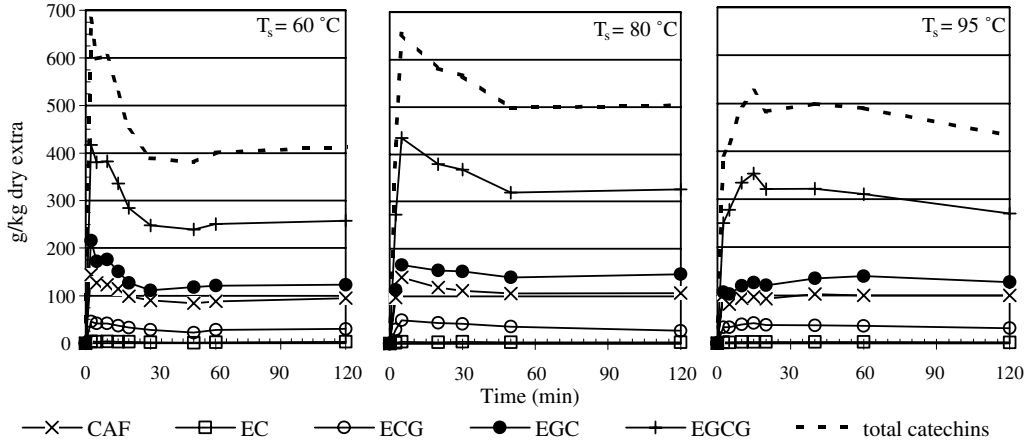

Fig. 6. Single-step extraction of green tea with water at different starting temperatures (T_s) and ratio 100 ml:1 g: contents of major catechins and caffeine in dry green tea extract (g/kg) as a function of extraction time.

which were obtained on the basis of dry matter determination of extract solutions.

The content of caffeine in extract solution was approximately constant after 2.5 min (30 mg/100 ml) at starting 80 and 95 °C whereas at 60 °C, an extraction of 15 min was required to reach this constant value. Content of major catechins increased up to approximately 10–20 min at all three investigated temperatures, and it increased with increasing temperature, the highest value being obtained at 95 °C and 10–15 min (183 mg/100 ml). Maximum content of major catechins in dry extract, on the other hand, decreased with increasing temperature. Content of major catechins in dry extract decreased from the maximum value of 682 to approximately 400 g/kg at 60 °C after 30 min, and from 654 to 500 g/kg at 80 °C after 50 min, and further remained constant up to 120 min. At 95 °C the content of major catechins decreased from a maximum value of 525–500 g/kg after 20 min and, after the prolonged time of 60 min started decreasing to 420 g/kg in 120 min. During this study, it was confirmed that contents of major catechins decreased after a certain time at elevated starting temperature due to their degradation. These results can be related to findings from published data of other authors (Komatsu et al., 1993; Wang & Helliwell, 2001), which indicate an epimerization of catechins above 80 °C, as well as oxidation and degradation. Content of caffeine in dry extract reached a constant value of approximately 100 g caffeine/kg extract after 30 min of extraction at all three investigated starting temperatures.

By decreasing the ratio of solvent to material (ml:g), a decrease in extraction efficiency of catechins and caffeine was observed. This was confirmed by extracting green tea leaves for 10 min at starting temperature of 95 °C (optimal parameters) at the applied ratio 40 ml:1 g instead of 100 ml:1 g, as described in results in previous paragraphs. The contents in dry extract were 414 g of major catechins/kg and 71.4 g caffeine/kg, which were by 17% for catechins and 24% for caffeine lower than extract obtained at the ratio 100 ml:1 g. Extraction efficiency for catechins and caffeine were therefore 61–64%, compared to 80% obtained at higher ratio.

3.3.2. Multiple extraction step

In order to increase the extraction efficiency, a multi-step extraction procedure, under the same conditions, was investigated, where the residue material from the first extraction step was once again extracted with water for 10 min at a starting temperature of 95 °C. This was repeated, altogether, four times, and results of analyses of extracts obtained in each step are shown in Table 3. Extraction yields drastically

Table 3
Multi-step extraction of green tea with water at ratio 40 ml:1 g (T_s = 95 °C, t = 10 min)

Extr. step	Content (g/kg dry extract)					Yield (%)	Cumulative amount (g/kg raw material)		Cumulative extraction efficiency[a] (%)	
	EGC	ECG	EC	EGCG	CAF		Catechins	CAF	Catechins	CAF
1	126	47.8	1.2	239	71.4	30.0	124	21.4	65.1	59.4
2	108	59.0	1.1	243	75.5	11.4	156	27.2	81.9	75.5
3	68.5	91.6	1.1	420	54.5	4.0	170	28.5	88.9	79.2
4	42.3	127.6	0.1	484	34.0	1.3	174	28.7	91.3	79.7

[a] Regarding the starting material, which contained 191 g catechins and 36 g caffeine per kg of raw material.

decreased with each extraction step, while overall content of major catechins in dry extract increased: contents of EGC and EC, however, decreased but increasing content of EGCG prevailed. The decreasing trend for EC and EGC may be due to the fact that they are precursors of theaflavin, one of the important oxidation-derived components in black tea (Nakabayashi, Ina, & Sakata, 1994) and under exposed conditions form intermediary products of theaflavin. Cumulative amounts of isolated catechins and caffeine were calculated, based on 1 kg of starting material and taking into account experimental losses of 3%, which occurred due to the multiple extraction procedure (filtrations and evaporations). Cumulative extraction efficiency of major catechins and caffeine, calculated on the basis of its cumulative amount in extract, increased up to 91.3% for catechins and 79.7% for caffeine after four subsequent extraction steps. Each subsequent extract was enriched in major catechins.

3.4. Extraction of decaffeinated green tea with water

Decaffeination of green tea leaves was performed by NATEX Prozesstechnologie GesmbH (Ternitz, Austria) (Lack et al., 2001). Caffeine, with a purity of 97%, was thereby selectively extracted without significantly affecting the polyphenolic compounds present. Decaffeinated green tea leaves contained 17% less catechins than the starting material. This was thought to be due to prior moistening and drying of material after processing with CO_2, and consequently low solubility of major catechins in aqueous solvent media. Dry decaffeinated green tea leaves contained 157 g major catechins and 0.7 g caffeine/kg dry material. Thus, 98% of caffeine was isolated from green tea leaves. The amount of flavonols in green tea leaves after decaffeination decreased by 10%, and hence from 5.2 to 4.6 g flavonols/kg dry material.

Decaffeinated green tea was, furthermore, extracted with water for 10 min at starting temperature of 95 °C, as determined for optimal extraction conditions in previous sections. Contents of major catechins in subsequent dry extract were lower than those of raw material. This is due to several reasons. First, this experiment was performed at much lower ratio of solvent to material, 9.3 ml:1 g compared to 40 ml:1 g, and additionally on a larger scale, 150 g compared to 2 g of green tea leaves. Greater amount of extract solution required longer times for filtration and drying, thus exposing isolated active ingredients to higher degradation risk in water solution. It was concluded that a green tea extract with approx. 31 wt% of major catechins and less than 0.1 wt% caffeine is produced during the first extraction step with a 23% yield, and a 38% extraction efficiency. After four extraction steps the cumulative extraction efficiency of major catechins of 65.3% was reached.

4. Conclusions

A lower grade green tea (variety Fanning Belas, China), containing 191 g major catechins/kg and 36 g caffeine/kg, was extracted with different solvents and employing different extraction conditions in order to obtain a green tea extract enriched in major catechins and known content of caffeine. By adjusting the extraction parameters, solvent, ratio of solvent to material, extraction temperature and time, green tea extracts with approx. 270–650 g of major catechins/kg dry extract can be obtained. This study showed the tendency of catechins to degrade during prolonged extraction, as well as after applying higher extraction temperatures. In respect to extraction temperature and time, it is advisable to use either a combination of high temperature (95 °C) and short extraction time (5–10 min), or lower temperature (60 or 80 °C) and longer extraction time (20 min) in order to avoid great degradation of catechins during extraction and thus obtain extracts with highly enriched catechin contents.

On the other hand, content of caffeine was mainly constant, and in the range of approx. 70–100 g caffeine/kg dry extract. A higher ratio of solvent to material (100 ml:1 g) gave better results in terms of higher contents of major catechins in extract. Similar results were, however, obtained by using lower ratios (40 ml:1 g or 9 ml:1 g) and a multi-step extraction procedure. As a result, raw and decaffeinated green tea extracts, with approx. 65 wt% of major catechins, 3 wt% of caffeine, and 44 wt% of major catechins and 0.1 wt% caffeine, respectively, were obtained. Cumulative extraction efficiency, using water as a solvent, was in the range 64–97% for major catechins and 61–85% for caffeine.

Extracts were also analysed for contents of proanthocyanidins and flavonols (myricetin, quercetin, kaempherol), and were found to be present in minor quantities compared to major catechins, from 6 to 20 g/kg extract.

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