

POLYPHENOL CONTENT IN GREEN, BLACK AND OOLONG TEA (*CAMELLIA SINENSIS* /L./ KUNTZE) INFUSIONS IN DIFFERENT TIMES OF TEA MACERATION*

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Spectrophotometrically with Folin-Ciocalteu's reagent total polyphenol content (TP) in tea infusions was estimated in six green tea sorts (Bai Mu Dan, Bancha Kyoto, Bi Luo Chun, Cui Ya Cha, Gyokuro Kyoto, Zhu Cha), six black tea sorts (Assam, C.T.C., Ceylon "Adam's Peak", Ceylon "Tiger River", Darjeeling "Himalaya" TGFOP, Pu Er and Yunnan) and in the oolong tea Wu Long. The selected sorts from the harvest represented different climate, altitude, the main regions of cultivation and different technologies of tea production. Polyphenol contents in the infusions were estimated after different times of elution in the range 1–60 min. Total polyphenol content increased with increasing time of elution statistically significantly with the exception of Wu Long tea. The increase of TP content in the first phase of maceration was very high (17.33–63.13 g.kg⁻¹ tea in 1st min, 31.28–92.43 g.kg⁻¹ tea in 5th min), at the end of the maceration it was already low (35.00–116.59 g.kg⁻¹ tea in 30th min and 44.55–118.75 g.kg⁻¹ tea in 60th min). The maceration of black tea sorts was quicker in comparison with green tea and half-fermented oolong tea. The highest TP content was estimated in the first and third minutes of maceration in Assam, C.T.C. tea (63.13 g.kg⁻¹ or 70.81 g.kg⁻¹ tea), in the fifth and eighth minutes in Gyokuro Kyoto tea (92.43 g.kg⁻¹ or 88.31 g.kg⁻¹ tea), in the tenth minute in Biluochun tea (90.91 g.kg⁻¹ tea). Green tea sorts were macerated slower, but the infusions contained comparable average amounts of TP in comparison with black tea sorts. Black teas could be divided into three groups: "high-mountains tea sorts" (Ceylon "Adam's Peak", Darjeeling "Himalaya" TGFOP) with higher contents of TP, "low-altitude tea sorts" (Ceylon "Tiger River", Yunnan) with lower TP contents and others, such as Assam, C.T.C. (very specific) or Pu Er. Green tea sorts could be divided into two groups: Bancha Kyoto, Bai Mu Dan and Cui Ya Cha with lower TP contents and slow extraction and Bi Luo Chun, Gyokuro Kyoto and Zhu Cha with higher TP contents and higher TP increase during their maceration. Very high correlation coefficients were found in Cui Ya Cha and Zhu Cha teas during their maceration (0.933) and in Bi Luo Chun and Bai Mu Dan teas (0.973). From the nearest neighbor clustering method results the close similarity between Ceylon "Tiger River" and Yunnan (similar with other teas) and between Pu Er and Wu Long teas (non similar with other teas) and on the other hand, the specific character of Gyokuro Kyoto and Assam, C.T.C. teas could be found.

black tea; green tea; oolong tea; total polyphenol antioxidant content; tea infusions; time of maceration

INTRODUCTION

Tea's beneficial health effects are thought to stem from polyphenols with antioxidant properties. Green tea contains polyphenols, which include flavanols, flavandiol, flavonoids, and phenolic acids (up to 30% of dry weight). Green tea contains catechins including (-)-epigallocatechin-3-gallate, (-)-epigallocatechin, (-)-epicatechin-3-gallate, and epicatechin. Catechins are present at about 10% of the dry basis of green tea, and half of that (5%) in black tea (Yamamoto et al., 1997). In addition, there are phenolic acids, such as gallic acid and characteristic amino acids, such as theanine contained. The fermentation process of black tea produces other groups of polyphenols known as theaflavins and thearubigins, which are the various oxidation products of the catechins. Both green and black teas also contain flavonol quercetin and its glycoside rutin. The other catechins contained are (+)-gallocatechin and (+)-catechin.

Tea catechins undergo many molecular changes during the course of processing that leads from fresh leaf to made tea. These yield many other classes of compounds found in black tea – theaflavins (Davis et al., 1995), theaflavates (Wan et al., 1997), theafulvins (Bailey et al., 1993, 1994), theacitrins (Davis et al., 1997) and thearubigins (Davis et al., 1995).

Total phenol content in leaves is estimated to 50–270 g.kg⁻¹. Among polyphenolic compounds tannins prevail in leaves (33–270 g.kg⁻¹) and from individual compounds L-gallocatechin-gallate > epigallocatechin-gallate > theasinensin-A > epicatechin-gallate and epigallocatechin > L-gallocatechin > epicatechin > L-epicatechin-gallate > (D, L)-gallocatechin and (+)-catechin > quercetin. Hertog et al. (1993) investigated contents of quercetin, kaempferol and myricetin in different types of tea infusions. In black tea infusions quercetin (10–25 mg.L⁻¹), kaempferol (7–17 mg.L⁻¹), and myricetin (2–5 mg.L⁻¹) were detected. Flavonoid levels in green tea were comparable to those in black tea.

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Epidemiological observations and laboratory studies have indicated that polyphenolic compounds present in tea may reduce the risk of a variety of illnesses, including coronary heart disease (M u k h t a r et al., 2000). Results from studies in rats, mice, and hamsters showed that tea consumption protects against lung, fore stomach, esophagus, duodenum, pancreas, liver, breast, colon, and skin cancers induced by chemical carcinogens. The inhibitory action of tea and tea components against cancer formation has been demonstrated in different models (Y a n g et al., 2000). Tea polyphenols, namely theaflavin, theaflavin-3-gallate, theaflavin-3,3'-digallate, (-)-epigallocatechin-3-gallate, and gallic acid reduce oxidative stress (L i n et al., 2000). Tea polyphenols have potent inhibitory effects (> 50%) on PMA-stimulated superoxide production. A m a r o w i c z et al. (2000) described antioxidative activity of (-)-epicatechin, antibacterial actions of tea polyphenols and free-radical scavenging action of catechin and related compounds. Results showed that tea polyphenols have strong bactericidal effect against pathogenic bacteria, but only a slight effect against beneficial bacteria, such as lactic acid bacteria. K a o et al. (2000) found that among green tea polyphenols especially (-)-epigallocatechin-gallate significantly reduced food intake, body weight, blood levels of testosterone, estradiol, leptin, insulin, insulin-like growth factor I, LH, glucose, cholesterol, and triglyceride, as well as growth of the prostate, uterus, and ovary.

MATERIAL AND METHODS

Tea samples: Twelve tea samples (Zhu Cha, Bi Luo Chun, Bai Mu Dan, Cui Ya Cha, Wu Long, Yunnan,

Bancha Kyoto, Gyokuro Kyoto, Darjeeling "Himalaya" TGFOP, Assam, C.T.C., Ceylon "Adam's Peak and Ceylon "Tiger River") were from the harvest of 2000 and Pu Er tea from the harvest of 1995. The samples were obtained from the Association of Tea Lovers (Good Tea Room, Ltd.). Characteristics of tea sorts are given in Table 1.

Preparation of samples – maceration: The samples (0.5 g) were poured with 200 mL of boiling distilled water and after maceration (1, 3, 5, 10, 15, 20, 30, 45, 60 min) the infusion was filtered through three layers of cellulose and a gauze layer. During the maceration longer than 15 min the infusions were agitated periodically in 15 min intervals. At the beginning of the maceration the temperature of infusion was approx. 90 °C and then the infusions were left to stand at the laboratory temperature (in 5th min 81 °C, 10th min 72 °C, 15th min 64 °C, 20th min 60 °C, 30th min 52 °C, 45th min 43 °C, and 60th min 39 °C, resp.).

Determination of total polyphenols: Modified method after L a c h m a n et al. (1998) was used. For the determination the 5 or 10 mL aliquots were pipetted into 50 mL volumetric flasks, 2.5 mL Folin-Ciocalteu's reagent (PENTA Chrudim, CZ) was added and then after five minutes 7.5 mL 20% Na₂CO₃ solution. After two hours standing the absorbancy of blue coloration was measured in the cuvettes 0.5 cm at $\lambda = 765$ nm against blank. TP content was expressed as mg of gallic acid contained in one kg of tea.

Statistical evaluation: The analytical software CORRELATION within Microsoft Excel and the clustering method – nearest neighbor (single linkage) – in Statgraphic program was used. Distance metric was expressed as squared Euclidean.

Table 1. Characterisation of analysed tea sorts

Tea Sort	Name	Origin and short characterisation
Green tea	Zhu Cha (Gunpowder)	China, province Zhejiang, Ning Bo region rich in fluoride (0.1–0.15 mg.kg ⁻¹)
Green tea	Bi Luo Chun (Bi Lu Chun, Pi Lo Chun, "Green Snail Spring")	China, province Tjiang-su and Dongting in Suzhou province, hand-made production
Green tea	Bai Mu Dan (Paj-Mu-Tan, "White peony")	China, mountain region Wu-I, province Fu-t'jien – rich in silver tips, "white tea"
Green tea	Cui Ya Cha (Cui-Ja-Ccha)	China, Huang Shan mountains, "yellow tea"
Green tea	Bancha Kyoto – bancha	Japan green tea, steamed, sun. dried and roasted mature big leaves plucked in June in Kyoto area
Green tea	Gyokuro Kyoto (Gjokuró) – gyokuro	Japan exclusive tea, cultivated on overshadowed plantages (2 weeks), rolled with needle – shaping, choicest tender dark green needles
Oolong tea	Wu Long (Wu-lung)	China, Wu-I mountains near the province Fu-t'jien (Fujian) and Guangdong
Black tea	Assam (Kundalimukh) – C.T.C., GFBOP	India, Assam region in the northeast part of Brahmaputra river, crushing – tearing – curling technology
Black tea	Ceylon "Adam's Peak" – OP	Sri Lanka, High Grown in Nuwara Eliya region – (Labookeli)
Black tea	Ceylon "Tiger River"	Sri Lanka, Low Grown Kandy
Black tea	Darjeeling "Himalaya" – TGFOP	India, Darjeeling region under Himalayas with wet and cold climate
Black tea	Yunnan (Jün-nan) – TGFOP	China, brown-red coloured, province Jün-nan
Black tea	Pu-Er (Pchu-er) – C	China, province Jün-nan, partially fermented, special technology

C.T.C. – Crushing – Tearing – Curling Technology, TGFOP – Tippy Golden Flowery Orange Pekoe, GFBOP – Golden Flowery Broken Orange Pekoe, C – Compressed, OP – Orange Pekoe, gyokuro – rolled with needle shaping, bancha – steamed

RESULTS AND DISCUSSION

Total polyphenol content (TP) was determined spectrophotometrically by Folin-Ciocalteu's reagent based on the reduction of phosphowolframic acid ($H_3P[W_3O_{10}]_4$) in alkaline solutions into phosphowolframic blue (based on $WO_2 \cdot nWO_3$). The absorbance of formed blue is proportional to the number of aromatic phenolic groups and it is used to their determination expressed as gallic acid as the calibrant (Singleton et al., 1999). Relative standard error of this method is about 1.96 %rel. There is very good accordance between results obtained by this method and antioxidant properties of plant resources of polyphenols, e.g. buckwheat (Holasová et al., 2001, 2002).

Some of the factors affecting the rate of infusion of tea solubles into aqueous solution have been widely studied – e.g. the nature of raw materials, the purity and temperature of water, the infusion time or the ratio tea-

leaf/water (Natarjan et al., 1962). The amount of extracted tea constituents as a function of time of maceration could be expressed in infusion curves (Spiro, Jago, 1982). In first 5–10 min the maceration was investigated in detail (time 3–5 min is usually recommended for tea preparation). The longer time periods of maceration (20, 30, 45 and 60 min) were used for the purpose to determine obtainable TP amounts from individual tea sorts and how high TP contents are contained in them.

The obtained results (average values from two parallel infusions and two parallel determinations from each infusion) have proved the individuality of every tea sort. Every tea sort differs from the others with the course of maceration as well as with the amount of extracted polyphenols (Table 2).

The course of extraction of TP from individual teas corresponded could be described by semi-logarithmic

Table 2. Content of total polyphenols in infusions of different tea sorts expressed as extracted from 1 kg tea

Tea	Tea sort	TP content (g.kg ⁻¹ tea)									
		1	3	5	8	10	15	20	30	45	60
Assam, C.T.C.	BT	63.13	70.81	83.65	87.83	85.68	54.46	59.51	48.15	92.68	99.74
Ceylon "Adam's Peak"	BT	28.83	49.25	46.87	63.94	65.38	79.27	73.32	91.10	89.55	91.17
Ceylon "Tiger River"	BT	26.55	44.52	53.66	52.73	61.80	67.58	66.83	62.42	67.45	74.71
Darjeeling "Himalaya" TGFOP	BT	35.00	55.31	68.80	65.06	61.43	83.13	87.37	105.20	104.31	101.28
Yunnan Hong	BT	28.33	41.71	40.53	63.64	64.50	63.05	63.40	65.79	75.22	66.11
Pu Er	BT	17.33	26.97	31.28	32.58	34.43	44.35	51.85	34.99	42.73	44.56
Wu Long	OT	16.50	22.03	33.29	44.91	45.46	55.57	42.23	35.96	39.19	44.82
Bancha Kyoto	GT	25.76	43.72	45.52	52.68	56.04	61.98	60.03	86.55	80.41	97.99
Cui Ya Cha	GT	18.64	34.97	48.06	38.31	59.28	64.64	72.86	85.85	60.38	107.02
Gyokuro Kyoto	GT	52.71	70.19	92.43	88.31	59.65	10.58	64.24	113.65	94.46	95.67
Bai Mu Dan	GT	17.36	30.21	34.85	32.89	60.91	56.78	64.44	80.52	88.80	83.66
Bi Luo Chung	GT	41.85	63.49	71.76	75.01	90.91	79.67	91.52	110.87	124.10	118.75
Zhu Cha	GT	36.21	36.79	61.67	62.17	69.42	82.49	99.70	116.60	85.50	109.10

BT – black tea, OT – oolong tea, GT – green tea

Table 3. Course of the extraction of polyphenols from individual teas

Tea	$y = a \ln(x) + b$	Regression coefficient (R^2)
Assam, C.T.C.	$y = 3197.9 \ln(x) + 66\ 858$	0.0481
Ceylon "Adam's Peak"	$y = 16\ 288 \ln(x) + 28\ 619$	0.9340
Ceylon "Tiger River"	$y = 10\ 491 \ln(x) + 32\ 543$	0.8672
Darjeeling "Himalaya" TGFOP	$y = 17\ 506 \ln(x) + 34\ 372$	0.9126
Yunnan Hong	$y = 10\ 654 \ln(x) + 31\ 554$	0.7905
Pu Er	$y = 6659.2 \ln(x) + 20\ 060$	0.6740
Wu Long	$y = 6368.0 \ln(x) + 22\ 652$	0.4647
Bancha Kyoto	$y = 16\ 405 \ln(x) + 21\ 538$	0.9050
Cui Ya Cha	$y = 18\ 187 \ln(x) + 15\ 177$	0.7743
Gyokuro Kyoto	$y = 10\ 113 \ln(x) + 59\ 340$	0.3705
Bai Mu Dan	$y = 18\ 830 \ln(x) + 9669.4$	0.9039
Bi Luo Chung	$y = 19\ 652 \ln(x) + 39\ 440$	0.8213
Zhu Cha	$y = 19\ 953 \ln(x) + 27\ 884$	0.8144

function (Table 3) and it is in good accordance with the data obtained by Astill et al. (2001). Total amounts of extracted polyphenols are influenced by extrinsic factors (pH, temperature and time of infusion) and intrinsic factors (regeneration of inactivated enzymes). We have chosen boiling distilled water for extraction with pH 6.8 in spite of the fact that solids extraction yield could be doubled when tea is extracted at pH 1.2 as Liang and Xu (2001) found. One of the reasons is the fact that H^+ encourages black tea cream particle formation by either releasing more solids into infusion or stimulating polyphenols to interact with polysaccharides and nucleophilic groups on protein in tea infusions. Yoshida et al. (1999) and Komatsu et al. (1993) found that at higher pH conditions (> 6) the amounts of the major catechins (EC, EGG, Ecg and EGCg) extracted from tea decreased and the amounts of minor catechins (C, GC, Cg and GCg) were increased due to their epimerisation. Epimerisation was much more proceeded during extraction with hot water (80 °C) as compared with extraction at 20 °C (Suematsu et al., 1995). The stability of polyphenols is affected especially in the range 4–8 (Zhu et al., 1997). But at the same pH value, extraction efficiencies varied, depending on the tea-water ratio and this is the reason why we used the same ratio tea-water 1 : 400 g/mL. We tried to estimate only the influence of time of maceration and sort of tea on TP extracted amounts in infusions and other factors remained constant. Also the temperature course during the preparation of infusions was the same, because as Komatsu et al. (1993) found in green tea infusion at temperatures below 95 °C, a turning point temperature on an Arrhenius plot was observed at 82 °C. The temperature above 80 °C in water solutions causes the epimerisation of catechins, as Seto et al. (1997) confirmed. This was very important because it is well known that preparation method, including the amounts of tea and water used, infusion, time and

amount of agitation, was shown to be a major determinant of the component concentrations of tea beverages as consumed (Astill et al., 2001). As investigated material we have used whole well defined teas and not the tea-bag materials and sieved fractions that affect kinetics and equilibrium of tea infusion and slows the infusion (Jaganyi, Mdletshe, 2000; Price, Spiro, 1985).

In the range 1–15 min of the maceration the effect of time of maceration was statistically significant in all tea sorts except of Assam, C.T.C. Enzymes contained in fresh tea's leaves are during tea production only inactivated and during the preparation of tea infusions the function of enzymes could be regenerated. In the fifth minute of maceration (Fig. 2) the average extracted polyphenol content in tea infusion from black tea represented 60.05% and from green tea 57.87% from total polyphenol content. The highest polyphenol contents after maceration for the period of sixty minutes were estimated in black tea Darjeeling "Himalaya" TGFOP and in three green teas: Bi Luo Chun, Zhu Cha and Cui Ya Cha. These results correspond to the data given by Astill et al. (2001) that green teas are richer in polyphenol (in average 17.5%) in comparison with black teas (14.4%). Oolong tea Wu-long was on the other hand low in polyphenols and the results found correspond well with the results reported by Hertog et al. (1993) who found that the obtained flavonoid amount in oolong tea was lesser in comparison with black tea. In great number of tea sorts significant correlation between time of maceration and the amount of extracted polyphenolic compounds was proved and found, esp. in green teas Bai Mu Dan, Banacha Kyoto, Bi Luo Chun (Table 3).

Statistical clustering method on the basis of nearest neighbor (single linkage) created 4 clusters from 13 tea samples as it is shown in Table 4 and 5 and Fig. 1. From the statistical evaluation could be seen the specific char-

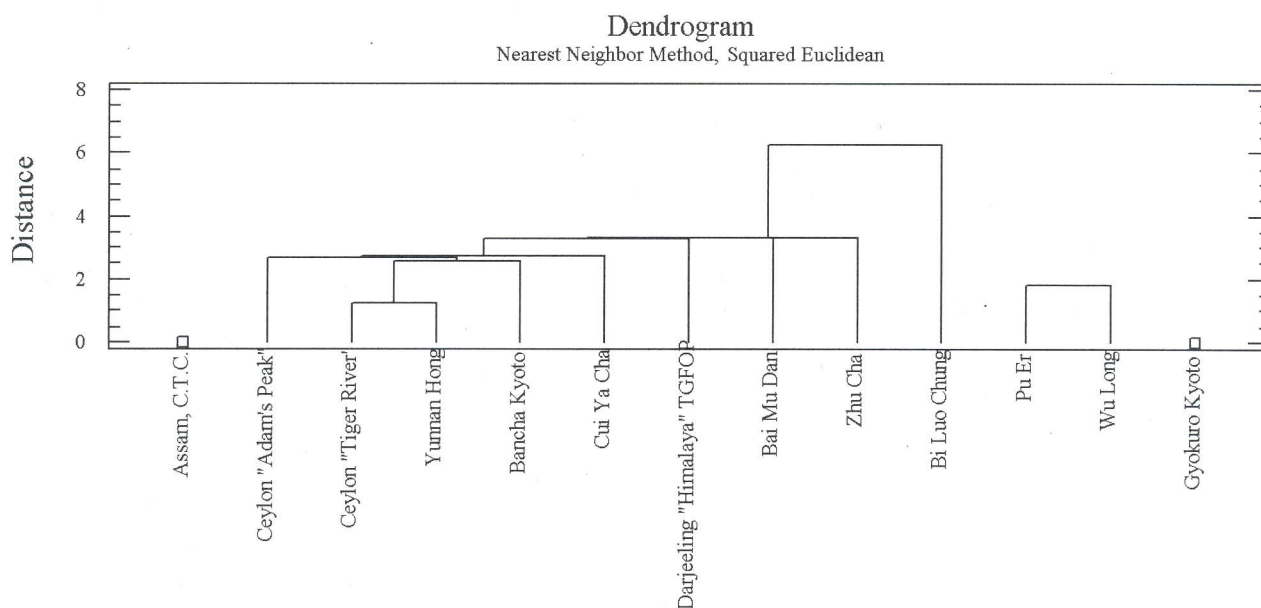


Fig. 1. Dendrogram of tea clusters

Table 4. Teas belonging to certain cluster

Row	Tea	Cluster Nr.
1	Assam, C.T.C.	1
2	Ceylon "Adam's peak"	2
3	Ceylon "Tiger River"	2
4	Darjeeling "Himalaya" TGFOP	2
5	Yunnan Hong	2
6	Pu Er	3
7	Wu Long	3
8	Bancha Kyoto	2
9	Cui Ya Cha	2
10	Gyokuro Kyoto	4
11	Bai Mu Dan	2
12	Bi Luo Chung	2
13	Zhu Cha	2

Table 5. Clusters – comprising the number of teas and total percentage

Cluster Nr.	Tea members	Percent
1	1	7.69
2	9	69.23
3	2	15.38
4	1	7.69

acter Gyokuro Kyoto and Assam, C.T.C. teas. Assam, C.T.C. differed from other teas with higher increase of TP at the beginning of maceration (after first minute of the maceration 63.13 g.kg^{-1} tea – the highest value among tested teas) and the correlation between the time of maceration and TP content was low (0.38). This atypical course of maceration in comparison with orthodox-manufactured black teas (rolling technology) is likely

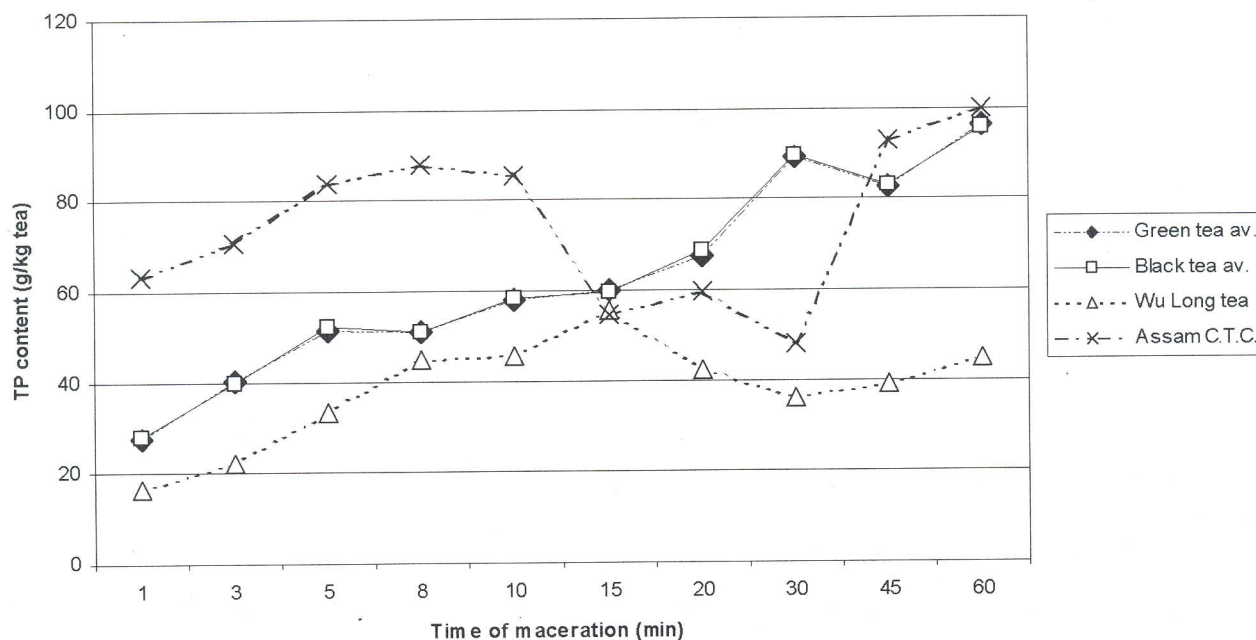
due to the technology crushing – tearing – curling used in its production and a function of the greater leaf disruption (Astill et al., 2001). On the other hand, from the dendrogram (Fig. 1) results the close similarity between Ceylon "Tiger River" and Yunnan (similarity with other teas) and between Pu Er and Wu Long teas non similar with other teas. The course of maceration of black, green and oolong teas is given in Fig. 2.

CONCLUSIONS

By the comparison of black tea sorts we can divide black teas into three groups: "high-mountains" (Ceylon "Adam's Peak", Darjeeling "Himalaya" TGFOP with higher total polyphenol contents and "lowland" teas (Ceylon "Tiger River", Yunnan) with lesser content of total polyphenols. The third group is represented by specific tea sorts, such as Assam, C.T.C. or Pu Er that could be comparable with oolong tea Wu Long. From oolong tea Wu Long and also Pu Er tea contained the lowest amount of total polyphenols extracted with the maximum at 15–20 min Assam, C.T.C. due to its technology showed the highest TP contents macerated in first minutes of maceration among teas tested.

The green teas could be divided into two groups: Bancha Kyoto, Bai Mu Dan and Cui Ya Cha teas that contain lesser amounts of TP and the process of their extraction is slower, and on the other hand, Bi Luo Chun, Gyokuro Kyoto and Zhu Cha contain higher amounts of TP and the increase of their contents in the infusions is higher.

Black tea sorts are extracted much quicker in comparison with green or oolong tea sorts. In the green tea sorts the liberation of polyphenolic compounds is gradual and in the comparison with black tea sorts the total TP amounts in the infusions are finally comparable.

Fig. 2. Comparison of average TP contents extracted from green, black and oolong teas (mg.kg^{-1}) at different times of extraction

Very high correlation coefficients were found in Cui Ya Cha and Zhu Cha teas during their maceration (0.933) and in Bi Luo Chun and Bai Mu Dan teas (0.973). From nearest neighbor clustering method results the close similarity between Ceylon "Tiger River" and Yunnan (similar with other teas) and between Pu Er and Wu Long teas (non similar with other teas) and on the other hand, specific character of Gyokuro Kyoto and Assam, C.T.C. teas could be found.

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REFERENCES

- ASTILL, C. – BIRCH, M. R. – DACOMBE, C. – HUMPHREY, P. G. – MARTIN, P. T.: Factors affecting the caffeine and polyphenol contents of black and green tea infusions. *J. Agric. Fd Chem.*, *49*, 2001: 5340–5347.
- AMAROWICZ, R. – PEGG, R. B. – BAUTISTA, D. A.: Antibacterial activity of green tea polyphenols against coli K 12. *Nahrung*, *44*, 2000: 60–62.
- BAILEY, R. G. – NURNSTEN, H. E. – McDOWELL, I.: The chemical oxidation of catechins and other phenolics: a study of the formation of black tea pigments. *J. Sci. Fd Agric.*, *63*, 1993: 455–464.
- BAILEY, R. G. – NURNSTEN, H. E. – McDOWELL, I.: A comparison of the HPLC, mass spectra, and acid degradation of theaflavins from black tea and proanthocyanidin polymers from wine and cider. *J. Sci. Fd Agric.*, *64*, 1994: 231–238.
- DAVIS, A. L. – CAI, Y. – DAVIES, A. P.: ^1H and ^{13}C NMR assignment of theaflavin, theaflavin monogallate and theaflavin digallate. *Magn. Reson. Chem.*, *33*, 1995: 549–552.
- DAVIS, A. L. – LEWIS, J. R. – CAI, Y. – POWELL, C. – DAVIES, A. P. – WILKINS, J. G. P. – PUDNEY, P. – CLIFFORD, M. N.: A polyphenolic pigment from black tea. *Phytochemistry*, *46*, 1997: 1397–1402.
- HARA, Y. – SHAHIDI, F. – HO, C. T.: Antibacterial actions of tea polyphenols and their practical applications in humans. In: SHAHIDI, F. – HO, C. T. (eds): *Phytochemicals and phytopharmaceuticals*. Champaign, USA, AOCS Press 1999: 214–221.
- HERTOG, M. G. L. – HOLLMAN, P. C. H. – van de PUTTE, B.: Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J. Agric. Fd Chem.*, *41*, 1993: 1242–1246.
- HOLASOVÁ, M. – FIEDLEROVÁ, V. – SMRČINOVÁ, H. – ORSÁK, M. – LACHMAN, J. – VAVREINOVÁ, S.: Buckwheat – the source of antioxidant activity in functional foods. *Food Res. Int.*, *35*, 2002: 207–211.
- HOLASOVÁ, M. – FIEDLEROVÁ, V. – RÉBLOVÁ, Z. – SMRČINOVÁ, H. – ORSÁK, M. – LACHMAN, J. – VAVREINOVÁ, S.: Antioxidant activity of buckwheat leaves. In: PFANNHAUSER, W. – FENWICK, G.R., KHOKHAR, S. (eds): *Biologically-Active Phytochemicals in Food*. London, The Royal Society of Chemistry 2001: 349–353.
- JAGANYI, D. – MDLETSHE, S.: Kinetics of tea infusion. Part 2: the effect of tea-bag material on the rate and temperature dependence of caffeine extraction from black Assam tea. *Food Chem.*, *70*, 2000: 163–165.
- KAO, Y. H. – HIIPAKKA, R. A. – LIAO, S. S.: Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. *Endocrinology – Philadelphia*, *141*, 2000: 980–987.
- KOMATSU, Y. – SUEMATSU, S. – HISANOBU, Y. – SAIGO, H. – MATSUDA, R. – HARA, K.: Studies on preservation of constituents in canned drinks. 2. Effects of pH and temperature on reaction-kinetics of catechins in green tea infusion. *Biosci. Biotech. Biochem.*, *57*, 1993: 907–910.
- LACHMAN, J. – HOSNEDL, V. – PIVEC, V. – ORSÁK, M.: Polyphenols in cereals and their positive and negative role in human and animal nutrition. In: *Proc. Conf. Cereals for Human Health and Preventive Nutrition*, Brno, MZLU, 1998: 118–125.
- LIANG, Y. R. – XU, Y. R.: Effect of pH on cream particle formation and solids extraction yield of black tea. *Food Chem.*, *74*, 2001: 155–160.
- LIN, J. K. – CHEN, P. C. – HO, C. T. – LIN SHIAU, S. Y.: Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells by theaflavin-3,3'-digallate, (-)-epigallocatechin-3-gallate, and propyl gallate. *J. Sci. Agric. Fd Chem.*, *48*, 2000: 2736–2743.
- MUKHTAR, H. – AHMAD, N. – HARPER, A. E.: Tea polyphenols: prevention of cancer and optimising health. *Am. J. Clin. Nutr.*, *71*, 6 Suppl., 2000: 1698S–1702S.
- NATARAJAN, C. P. – RAMAMANI, D. E. – LEELAVATHI, R. et al.: Studies on the brewing tea. *Food Sci. (Mysore)*, *11*, 1962: 321–332.
- PRICE, W. E. – SPIRO, M.: Kinetics and equilibria of tea infusion – theaflavin and caffeine concentrations and partition in several whole teas and sieved fractions. *J. Sci. Fd Agric.*, *36*, 1985: 1303–1308.
- SETO, R. – NAKAMURA, H. – NANJO, F. – HARA, Y.: Preparation of epimers of tea catechins by heat treatment. *Biosci. Biotech. Biochem.*, *61*, 1997: 1434–1439.
- SINGLETON, V. L. – ORTHOFER, R. – LAMUELA-RAVENTOS, R. M.: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: PACKER, L. (ed.): *Methods in Enzymology. Oxidants and Antioxidants. Part A*. San Diego, CA, Academic Press 299, 1999: 152–178.
- SPIRO, M. – JAGO, D. S.: Kinetics and equilibria of tea infusion. *J. Chem. Soc., Faraday Trans.*, *78*, 1982: 295–305.
- SUEMATSU, S. – HISANOBU, Y. – SAIGO, H. – MATSUDA, R. – KOMATSU, Y.: Studies on preservation of constituents in canned drinks. 5. A new extraction procedure for determination of caffeine and catechins in green tea. *Nippon Shokuhin Kagaku Kogaku Kaishi*, *42*, 1995: 419–424.

- YAMAMOTO, T. – JUNEJA, L. R. – CHU, D. C. – KIM, M.: Chemistry and Applications of Green Tea. Boca Raton/New York, CRC Press LLC 1997. 176 pp.
- YANG, C. S. – CHUNG, J. Y. – YANG, G. Y. – LI, C. A. – MENG, X. F. – LEE, M. J.: Mechanisms of inhibition of carcinogenesis by tea. In: Proc. 2nd Int. Conf. On Food Factors. BioFactors, 13, 2000: 1–4, 73–79.
- YOSHIDA, Y. – KISO, M. – GOTO, T.: Efficiency of the extraction of catechins from green tea. Food Chem., 67, 1999: 429–433.
- WAN, X. – NURSTEN, H. E. – CAI, Y. – DAVIS, A. L. – WILKINS, J. P. G. – DAVIES, A. P.: A new type of tea pigment – from the chemical oxidation of epicatechin gallate and isolated from tea. J. Sci. Fd Agric., 74, 1997: 401–408.
- ZHU, Q. Y. – ZHANG, A. – TSANG, D. – HUANG, Y. – CHEN, Z.: Stability of green tea catechins. J. Agric. Fd Chem., 45, 1997: 4624–4628.

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Obsah polyfenolů v zelených, černých a polofermentovaných druzích čaje (*Camellia sinensis* /L./ Kuntze) při různých dobách macerace.

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Spektrofotometricky s Folin-Ciocalteuovým fenolickým reagens byl stanoven obsah celkových polyfenolů (CP) v šesti druzích zeleného čaje (Bai Mu Dan, Bancha Kyoto, Bi Luo Chun, Cui Ya Cha, Gyokuro Kyoto, Zhu Cha), šesti druzích černého čaje (Assam, C.T.C., Ceylon „Adam’s Peak“, Ceylon „Tiger River“, Darjeeling „Himalaya“ TGFOP, Pu Er a Yunnan) a v oolong čaji (polofermentovaném) Wu Long. Vybrané druhy čaje reprezentovaly různé klimatické podmínky, nadmořskou výšku, hlavní pěstební oblasti a různé technologické postupy přípravy čaje. Obsahy polyfenolů byly v nálevech stanoveny po různé době macerace v rozmezí 1–60 min. Obsah celkových polyfenolů se statisticky významně zvyšoval s dobou macerace s výjimkou čajů Assam, C.T.C. a Wu Long. Nárůst obsahu celkových polyfenolů byl v první fázi macerace velmi vysoký (17,33–63,13 g.kg⁻¹ čaje v 1. min, 31,28–92,43 g.kg⁻¹ čaje v 5. min), na konci macerace byl již nízký (34,99–116,59 g.kg⁻¹ čaje ve 30. min a 44,56–118,75 g.kg⁻¹ čaje v 60. min). Macerace černých druhů čaje probíhala ve srovnání se zelenými čaji a polofermentovaným oolong čajem rychleji. Nejvyšší obsah CP byl zjištěn v 1. a 3. minutě macerace u čaje Assam, C.T.C. (63,13 g.kg⁻¹ čaje, resp. 70,81 g.kg⁻¹ čaje), v 5. a 8. minutě u čaje Gyokuro Kyoto (92,43 g.kg⁻¹ čaje, resp. 88,31 g.kg⁻¹ čaje), v 10. minutě u čaje Bi Luo Chun (90,91 g.kg⁻¹ čaje). Zelené druhy čaje se macerovaly pomaleji, avšak nálevy obsahovaly srovnatelná množství CP ve srovnání s černými druhy čaje. Černé čaje bylo možné rozdělit do tří skupin: „vysokohorské čaje“ (Ceylon „Adam’s Peak“, Darjeeling „Himalaya“ TGFOP) s vyšším obsahem CP, „nížinné čaje“ (Ceylon „Tiger River“, Yunnan) s nižším obsahem CP a ostatní jako Assam, C.T.C. (velmi specifický) nebo Pu Er. Zelené čaje bylo možné rozdělit do dvou skupin: Bancha Kyoto, Bai Mu Dan a Cui Ya Cha s nižším obsahem CP a pomalou macerací a Bi Luo Chun, Gyokuro Kyoto a Zhu Cha s vyšším obsahem CP a vyšším nárůstem CP v průběhu macerace. Během macerace byly stanoveny velmi vysoké korelační koeficienty podobnosti u čajů Cui Ya Cha a Zhu Cha (0,933) a Bi Luo Chun a Bai Mu Dan (0,973). Použitím metody tvorby clusterů na základě podobnosti byla zjištěna podobnost čajů Ceylon „Tiger River“ a Yunnan (podobnost s ostatními čaji) a Pu Er a Wu Long (odlišné od ostatních čajů), zatímco jako zcela specifické byly označeny čaje Gyokuro Kyoto a Assam, C.T.C.

černý čaj; zelený čaj; oolong čaj; obsah celkových polyfenolů; čajové nálevy; doba macerace

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