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Effect of water composition on aluminium, calcium and organic carbon extraction in tea infusions

Aurélie Mossion [a,b], Martine Potin-Gautier [b], Sébastien Delerue [a,b], Isabelle Le Hécho [b], Philippe Behra [a,*]

[a] Université de Toulouse, UMR 1010 Chimie Agro-Industrielle (LCA), ENSIACET, Institut National Polytechnique de Toulouse, INRA, 118 Route de Narbonne, 31077 Toulouse Cedex 04, France
[b] Laboratoire de Chimie Analytique, Bio-Inorganique et Environnement (LCABIE), UMR 5254 IPREM, Université de Pau et des Pays de l'Adour/CNRS, Avenue de l'Université, BP 576, 64012 PAU Cedex, France

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Abstract

Tea infusion is the second world wide consumed beverage. In this study, total aluminium and calcium concentrations, total organic carbon and total polyphenol content were determined and compared with respect to the origin of tea leaves, their particle size (broken or whole leaves) and also the mineral composition of four waters. It appeared that the higher the mineral content, the lower the extraction yield of aluminium, total organic carbon and total polyphenols. This could be due to calcium uptake by leaves. Calcium present in mineral waters could be complexed with pectins present in cell walls thus leading to a decrease in the element extraction.

Keywords: Tea infusion; Water composition; Extraction mechanism

1. Introduction

Since several years, tea consumption has been raised due to all scientific papers reporting that tea has benefit impact on human health due to its mineral and organic composition (Huang, Ho, & Lee, 1992; Trevisanato & Kim, 2000; Zuo, Chen, & Deng, 2002). Actually, tea infusions contain major inorganic elements and organic compounds among which calcium, magnesium, potassium, phosphate, fluoride, catechins and alkaloids.

Tea infusion composition depends on several parameters such as temperature (Khokhar & Magnusdottir, 2002), duration of brewing (Xie, Von Bohlen, Klockenkaemper, Jian, & Guenther, 1998), leaf–water ratio (Astill, Birch, Dacombe, Humphrey, & Martin, 2001), and also water composition. The latter one influences both extraction mechanisms from leaves and complexation phenomena taking place in infusions. Indeed, previous studies performed with water containing a high amount of calcium, 1.46 g L^{-1}, have showed that extraction rates of theaflavins and caffeine are decreased compared to ultra pure water (Spiro & Price, 1987a, 1987b; Spiro, Price, Miller, & Arami, 1987c). Moreover, both calcium and magnesium appear to be the major elements (Chao & Chiang, 1999a, 1999b) involved in tea cream and scum formation (Spiro & Jaganyi, 1993). Tea cream is a precipitate formed as tea cools down and is due to complexation between caffeine and theaflavins or thearubigins. This phenomenon is controlled by several parameters such as leaf–water ratio, pH and extraction temperature (Chao & Chiang, 1999a) and is favoured by calcium addition (Jöbstl, Fairclough, Davies, & Williamson, 2005). Tea scum is defined as surface film composed of calcium, hydrogenocarbonates and organic matter. It appears only in infusions prepared with

* Corresponding author. Tel.: +33 (0)562 885 667; fax: +33 (0)562 885 730.
E-mail address: Philippe.Behra@ensiacet.fr (P. Behra).

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hard water and is produced by the oxidation of organic compounds induced to the presence of calcium carbonate (Spiro & Jaganyi, 1994a, 1994b). Calcium is of particular interest since tea leaves can uptake between 1 and 2.5 mg Ca g^{-1} leaves brewed in water containing 10–150 mg Ca L^{-1} (Anderson, Hollins, & Bond, 1971). Anderson et al. (1971) have shown that the effect of this uptake on polyphenol and caffeine extraction is negligible, which is against Spiro and Price's results (1987a, 1987b).

On the other hand, *Camellia sinensis* (tea tree) is an aluminium accumulating plant (Stagg & Millin, 1975). Due to its strong affinity to organic matter, aluminium is highly extracted during brewing. Total aluminium contents in tea infusions range between 2 and 6 mg L^{-1} depending on leaf origin and infusion parameters (Erdemoglu, Pyrzyniska, & Gucer, 2000a). Therefore, tea infusions could represent a primary source of aluminium daily uptake for consumers. Knowing that organically bound aluminium should be less toxic than free one (Driscoll, Baker, Bicogni, & Schofiled, 1980), many studies have focused on its fractionation and some others on its speciation according to the definitions given by IUPAC (Templeton et al., 2000). Those works have shown that, in tea infusions, aluminium is mainly bound to organic matter, the nature of which is not exactly known (Flaten, 2002). For a minor part, aluminium could be bound to fluoride (Erdemoglu, Turkdemir, & Gucer, 2000b) or oxalate (Flaten, 2002).

The present work aims at investigating the influence of the mineral composition of water and of origin of tea leaves, particle size (broken or whole leaves) on total aluminium and calcium concentrations, total organic carbon (TOC) and total polyphenol contents (TPC) in tea infusions. Some elementary mechanisms such as complexation or diffusion were assumed in order to describe extraction mechanisms from tea leaves.

2. Material and methods

2.1. Chemicals, teas and waters

Ultra pure water prepared using a Milli-Q system (Model Gradient A10, Millipore) was used throughout the experiments. Nitric acid (68%) and hydrogen peroxide (30%) were analytical grade (VWR Prolabo). A single element 1.000 g L^{-1} stock standard solution of aluminium (SCP Science) and a multi-element standard solution containing 100 mg L^{-1} of calcium and also Sb, As, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, Se, Tl, Ti, V, Zn, Li, Sr (CPI-International) were used. Standards were prepared daily. Buffer solutions at pH 4.01 and 7.01 (Fisher Bioblock Scientific) were used to calibrate pH-meter. Anions were analysed after calibration with a seven anion standard solution containing 10 mg L^{-1} of NO_2^- and Br^-; 20 mg L^{-1} of F^-; 100 mg L^{-1} of Cl^-, SO_4^{2-} and NO_3^- (Dionex).

Teabags containing broken leaves were purchased in a supermarket for the Lipton Yellow® (LS) and Lipton Lemon® (LC). Bags were removed before brewing. Loose teas with whole leaves were purchased from a French tradesman: two Darjeeling teas from oaks (M6) and Namring (M8) and two green teas from two different areas of China, one tea from Hu Bei province (M2) and one from Guang Xi province (M5).

Five different waters (Table 1) were used to prepare infusions, the ultra pure water (A) and four mineral waters: two different weakly mineralised ones (B and C), and two highly mineralised ones (D and E). Water E was only used to test tea scum formation. Waters A–C were considered to be unbuffered with respect to pH contrary to waters D and E.

2.2. Preparation of tea infusions

Tea infusions were prepared according to the advices given by tradesmen as follows: 600 g of water at 95 °C was added to the indicated mass of leaves (Table 2) in a clean tea pot. After 3 min, the infusions were filtrated with a sieve (Nylon) for removing large particles and leaves. Glass flasks were used for organic matter analyses and polyethylene flasks for inorganic compound analyses. When necessary, infusions were filtrated through 0.45 μm cellulose acetate filters (Nalgene); those samples are named with "F" added after tea name. The so called blank water followed the same procedure without tea leaves.

Infusions were weighted after 20 min cooling to estimate infusion volume assuming that tea infusion density is 1 (after measurement $\rho = 0.999 \pm 0.001$ at 20 °C with either waters A or D). Brewed leaves were oven-dried at 40 °C for 24 h before digestion and analysis.

For evaluating the impact of leaf size, M5 leaves were ground using a mortar and a pestle in agate before brewing. The infusion obtained was called M5 broken leaves.

2.3. Particle size measurements

Particle size distribution was evaluated using a scanner (Epson Perfection 3200 photo) and image analyse software Powdershape 4.3 (Innovative Sintering Technologies).

Measure mask parameters were the following ones:

- $100 \leqslant$ diameter $\leqslant 100{,}000$ μm;
- $1 \leqslant$ shape factor $\leqslant 10{,}000$;
- $0 \leqslant$ Feret ratio $\leqslant 1$;
- $0 \leqslant$ convexity $\leqslant 1$.

The shape factor is the ratio between the perimeter of the object and the perimeter of the surface equivalent circle. The Feret ratio is the ratio between the "maximum size", *i.e.*, the maximum distance between two parallel lines which squeezes the object and the "sieve size", *i.e.*, the minimum distance between two parallel lines which squeezes the object. Convexity is the ratio between the filled surface of the object and the surface of the convex enveloping object.

Table 1
Composition of waters

Waters		pH	Anions (mg L^{-1})					Cations (mg L^{-1})				Ionic strength[d] (mmol L^{-1})
			Cl^-	HCO_3^-	F^-	NO_3^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	K^+	
A	N.H.[a]	5.7–6.5	n.d.[c]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
	H.[b]	5.7–6.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
B	N.H.	6.1	2.4 ± 0.1	4.9 ± 0.1	n.d.	2.19 ± 0.03	2.16 ± 0.02	1.2 ± 0.1	0.4 ± 0.1	2.8 ± 0.5	0.9 ± 0.1	0.30 ± 0.03
	H.	7.1	2.9 ± 0.1	2.3 ± 0.1	n.d.	2.72 ± 0.03	2.70 ± 0.02	1.4 ± 0.1	0.5 ± 0.1	2.2 ± 0.5	0.6 ± 0.1	0.30 ± 0.03
C	N.H.	6.7	14.4 ± 0.2	78 ± 1	0.18 ± 0.01	6.3 ± 0.2	8.0 ± 0.2	13.2 ± 0.2	8.6 ± 0.2	18.8 ± 0.5	7.1 ± 0.1	2.93 ± 0.06
	H.	8.1	15.5 ± 0.1	66 ± 0.3	0.15 ± 0.01	7.5 ± 0.5	8.7 ± 0.4	14.4 ± 0.1	9.5 ± 0.2	18.8 ± 0.5	7.5 ± 0.1	3.01 ± 0.05
D	N.H.	7.2	10.3 ± 0.1	281 ± 4	0.20 ± 0.08	5.3 ± 0.4	338 ± 1	200 ± 1	45 ± 1	7.7 ± 0.5	2.5 ± 0.1	23.4 ± 0.2
	H.	8.0	11.0 ± 0.2	156 ± 1	0.14 ± 0.08	5.5 ± 0.4	359 ± 3	151 ± 8	47 ± 1	7.7 ± 0.5	2.5 ± 0.1	20.5 ± 0.6
E	N.H.	7.4	11.6 ± 0.1	330 ± 2	0.07 ± 0.05	8.7 ± 0.2	17.8 ± 0.5	92 ± 1	12.6 ± 0.5	4.1 ± 0.5	0.8 ± 0.1	9.1 ± 0.1
	H.	8.5	12.6 ± 0.1	213 ± 1	0.16 ± 0.05	9.3 ± 0.2	19.5 ± 0.5	64 ± 3	13.2 ± 0.5	4.3 ± 0.5	0.9 ± 0.1	6.8 ± 0.2

[a] N.H.: not heated water.
[b] H.: heated water.
[c] n.d.: not detected.
[d] Estimated from major ion analyses.

Table 2
Water/leaf ratios for preparing infusions

Teas	Water/leaf ratio (m:m, %)
Lipton Yellow (LS)[a]	0.6
Lipton Lemon (LC)[a]	
Darjeeling oaks (M6)	1.7
Namring (M8)	2
Green teas (M2, M5)	2.5

[a] After removing bag.

Tea leaves could be assimilated to fibres. Length is represented by maximum size and width is sieve size. Measurements were done in triplicates. For length and width, average, median and asymmetry coefficient were calculated.

2.4. Digestion procedure of tea infusions and leaves

Tea infusion (10 g) or 0.3 g of leaves were weighted in Teflon (PTFE) vessels. Three mL of HNO_3 and 2 mL of H_2O_2 were added. Vessels were introduced in pressure bombs, which were closed and put in a microwave oven. Samples were heated from 20 °C to 200 °C in 20 min. They were then kept at 200 °C for 15 min before cooling down to room temperature. This method has been optimised previously for plants at LCABIE (Pau). PTFE vessels were rinsed with ultra pure water and the resulting solutions were precisely adjusted to 35 g ultra pure water.

Blanks were prepared following the same procedure but without leaf or infusion.

2.5. Total polyphenol measurements

Total polyphenol content was determined using Folin–Ciocalteu's method (Montreau, 1972). In a graduated flask, 0.5 mL tea extract, 5 mL Folin–Ciocalteu's reagent (Panreac) and 10 mL sodium carbonate (20%, Panreac) were added and then adjusted to 100 mL with ultra pure water. The solutions were allowed to stand at 70 °C for 10 min before cooling down and absorbance measurement at 700 nm using a spectrophotometer. TPC was expressed in grams equivalent to the standard used (*i.e.*, gallic acid, GA, Panreac) per litre of aqueous solution noted g eq GA L^{-1}. Analyses were performed in triplicate.

2.6. Instrumentation

Leaves were digested using a Multiwave 3000 (Anton Paar).

Anions in water were measured using a Dionex ICS-2000 system. Samples were passed through a AG18 guard and AS 18 analytical columns (Dionex) with a flow rate of 1.0 mL min^{-1}. The gradient employed was as follows: 0–1 min, 23 mM NaOH; 1–8 min, linear gradient 23–40 mM; 8–12 min, linear gradient 40–52 mM; 12–13 min, linear gradient 52–23 mM; 13–21 min, 23 mM. The injection volume was 25 µL. The suppressor current was set at 129 mA.

Aluminium and calcium concentrations were analysed using an ICP-AES (inductively coupled plasma-atomic emission spectrophotometry) instrument (Panorama, Jobin-Yvon). The following conditions established after optimisation of the instrument parameters are: Rf. power: 1000 W; plasma gas flow rate: 15 L min^{-1}; nebulizer gas flow rate: 0.1 L min^{-1}; nebulizer: cross-flow type; sample uptake: 1.0 mL min^{-1}.

Dissolved organic carbon (DOC) was measured using a TOC-5000A (Shimadzu) after filtration through 0.45 µm cellulose acetate filter (Nalgene) by difference between total carbon and inorganic carbon.

The spectrophotometer used for total polyphenol measurement was a 8452A (Hewlett Packard). pH measurements were carried out after cooling down of samples

using a 330 pH-meter (WTW) with a combined Sentix 50 electrode (WTW).

Quantification limits (QL) were calculated from 10 blank measurements and according to the given Eq. (1) (IUPAC, 1987)

$$QL = \frac{10\sigma}{k}, \quad (1)$$

where k is the slope of the calibration curve and σ the standard deviation of the blank signal. QL values for Al, Ca, inorganic carbon, organic carbon and total polyphenol content were 0.2, 0.01, 0.5, 0.6 and 0.6 mg L^{-1}, respectively.

2.7. Extraction yield calculations

Tea infusion mass was lower than initial water mass due to loss of water by evaporation during heating (about 8%) and by absorption by leaves, especially in the case of whole leaves (decrease in 7% *vs.* 1% for broken leaves). This variation in mass was taken into account for calculating extraction yield, R

$$R = \frac{[x] \times V}{M} \times 100, \quad (2)$$

where $[x]$ is the experimental concentration in filtrated or digested infusions, V the infusion volume and M the theoretical mass, *i.e.*, mass calculated considering the complete extraction of elements from leaves during brewing. Errors were estimated by adding relative errors of concentration measurements, volume and theoretical mass.

3. Results

3.1. pH

Values of pH of waters and tea infusions are reported in Table 3. pH rises for blank value after heating, except ultra pure water A. In this specific case, pH before and after heating is unstable and varies quickly between 5.7 and 6.5. Variations are mainly due to CO_2 dissolution and very low ion concentration in the water. For the other waters, the increase in pH after heating can be due to both the loss of the CO_2 gas dissolved in water and the loss of hydrogenocarbonate as CO_2 gas following the reaction (3)

$$2HCO_3^- \leftrightarrow CO_{2(g)} + CO_3^{2-} + H_2O \ (\Delta H^\circ > 0). \quad (3)$$

Table 3
Influence of water and of tea used on pH of tea infusion

#	Waters		Black teas				Green teas	
	Blank	Heated blank	LS	LC	M6	M8	M2	M5
A	5.7–6.5	5.7–6.5	4.6	4.2	5.4	5.4	5.6	5.7
B	6.1	7.1	5.3	4.6	5.8	5.4	5.8	6.0
C	6.7	8.1	6.8	5.8	6.5	6.1	6.3	6.3
D	7.2	8.0	7.3	7.2	7.2	7.8	7.1	7.3
E	7.4	8.5	7.3	7.3	7.4	7.5	6.9	7.4

pH values of tea infusions strongly depend on the buffer capacity of waters (Table 3). Black tea infusions made with broken or whole leaves have pH values lower than green tea infusions with non-buffered waters A–C. For LC infusions, with the presence of lemon, pH is even lower compared to LS infusions. The calcium-carbonated system present in waters D and E buffers pH values between 7.1 and 7.8 even for lemon tea infusion (Table 3). These results are consistent with those reported by Xie et al. (1998). These authors have suggested that this could be due to the degree of fermentation, *i.e.*, oxidation, of organic compounds which leads to an increase in phenolic and carboxylic groups.

3.2. Particle sizes of teas

Particle sizes of tea leaves are reported in Table 4. Size distributions are asymmetric with positive skew for all tea leaf types so average values are above median ones except for M2 leaves. Whole leaves are 3–9 times longer than broken leaves LS and LC. Manually-ground M5 leaves are in average 10 times smaller than whole M5 leaves. Observations of M5B leaves showed that leaf cells, which have a fibrous aspect, were less damaged after manually grinding than LS and LC leaves.

3.3. Aluminium and calcium concentrations in tea leaves

Aluminium and calcium concentrations measured in the present study are reported and compared to literature data in Table 5. The concentrations are in the same order of magnitude but depend on the geographical origin (*i.e.*, China or Darjeeling) of leaves and type (*i.e.*, green or

Table 4
Particle sizes of tea leaves measured by image analysis

	μm	LS	LC	M6	M8	M2	M5	M5B
Length	Average size	805	1431	4647	5251	6966	5146	496
	Median	639	1410	4522	4794	7200	4124	372
	Skewness	1.7	0.5	0.5	0.52	0.3	1.2	2.2
Width	Average size	469	814	1666	2056	2006	1770	187
	Median	402	832	1649	1931	2041	1550	99
	Skewness	0.3	1.0	0.8	0.6	0.4	1.1	2.5

Table 5
Total aluminium and calcium contents (mg g^{-1}) of tea leaves

	Tea	Al	Ca	Reference
Black tea	LS	0.73 ± 0.02	3.8 ± 0.6	This study
	LC	1.0 ± 0.1	5.03 ± 0.04	This study
	M6	0.40 ± 0.02	3.65 ± 0.01	This study
	M8	0.93 ± 0.05	4.9 ± 0.4	This study
	Black tea	0.90 ± 0.01	4.5 ± 0.2	Odegard and Lund (1997)
	Black tea	0.81 ± 0.06	4.55 ± 0.07	Matsuura et al. (2001)
Green tea	M2	0.44 ± 0.02	3.29 ± 0.07	This study
	M5	0.54 ± 0.01	4.20 ± 0.04	This study

black) of tea. The concentrations of aluminium range from 0.4 to 1.0 mg g^{-1}, those of calcium from 3.3 to 5.0 mg g^{-1}.

3.4. Organic carbon in tea infusions

3.4.1. Dissolved organic carbon (DOC)

Extracted organic matter ranges from 0.3 to 0.5 g L^{-1} in broken tea leaves and from 1.0 g L^{-1} to 2.3 g L^{-1} for whole leaves. Infusions prepared with water B have nearly the same composition as those prepared with water A. The presence of 1.4 mg L^{-1} of calcium does not have any effect. Decrease in organic matter extraction is significant for waters C and D. For comparing extractions, results were expressed as mass of DOC with respect to one gram of leaves (Fig. 1). In that case, organic carbon contents are similar for black (from 40 to 110 mg g^{-1}) and green teas (from 38 to 80 mg g^{-1}) with a slight increase for broken leaves compared to whole ones. The decrease due to water mineralisation is more significant for tea with broken leaves than with whole ones (Fig. 1). Finally, organic carbon extracted during brew up decreases when water mineralisation increases.

3.4.2. Total polyphenol content

Total polyphenol contents were measured in liquors brewed up with M8 and analysed without filtration. The values obtained are 1.20 ± 0.02 g eq GA L^{-1} (water A), 1.11 ± 0.03 g eq GA L^{-1} (water B), 1.00 ± 0.06 g eq GA L^{-1} (water C) and 0.70 ± 0.06 g eq GA L^{-1} (water D). Those values are consistent with the DOC ones which are 1.78 ± 0.03 g C L^{-1} (water A), 1.34 ± 0.06 g C L^{-1} (water B), 1.39 ± 0.05 g C L^{-1} (water C) and 1.25 ± 0.02 mg C L^{-1} (water D).

3.5. Aluminium concentrations in tea infusions

Aluminium concentrations in infusions range from 0.06 to 3.24 mg L^{-1} depending on water used (Table 6). For ultra pure water, values are below those reported in literature especially for whole leaves. But authors used different experimental conditions to prepare infusions: either a teabag containing 2 g of powdered leaves in 200 mL of water (Odegard & Lund, 1997) or 2 g of powered leaves in 100 mL of water (Matsuura, Hokura, Katsuki, Itoh, & Haraguchi, 2001).

Extraction yields calculated according to relationship (2) were applied to aluminium (Fig. 2). In the present study,

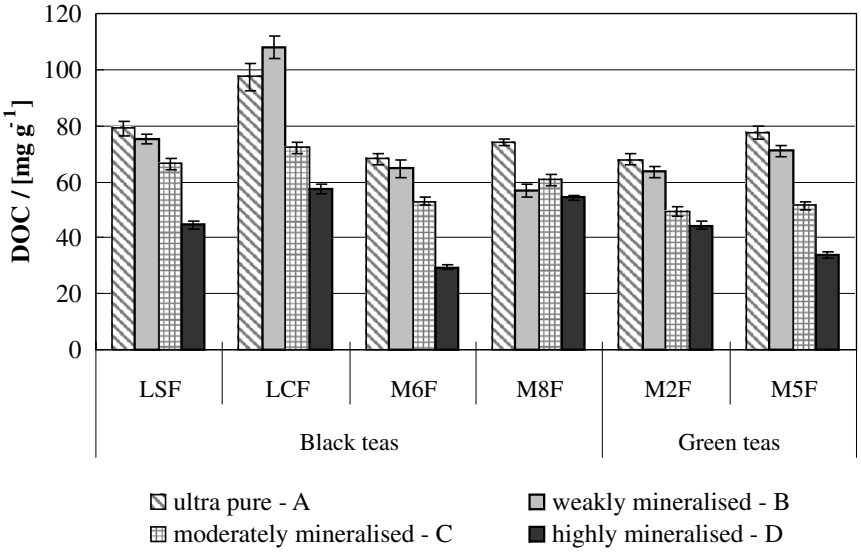

Fig. 1. Effect of water composition on dissolved organic carbon (DOC) content extracted from tea leaves per g of brewed leaves (mg C g^{-1}).

Table 6
Effect of water composition on total aluminium concentrations in infusions of black or green teas

Water	Aluminium (mg L^{-1})[a]					
	Black teas				Green teas	
	LS	LC	M6	M8	M2	M5
A	1.8 ± 0.2	1.83 ± 0.05	0.95 ± 0.05	2.47 ± 0.05	0.74 ± 0.06	3.24 ± 0.05
B	1.4 ± 0.1	1.42 ± 0.07	0.94 ± 0.04	2.45 ± 0.05	0.72 ± 0.02	2.80 ± 0.02
C	1.7 ± 0.2	1.84 ± 0.05	0.27 ± 0.05	1.79 ± 0.06	1.26 ± 0.07	1.50 ± 0.2
D	1.0 ± 0.1	1.92 ± 0.05	0.06 ± 0.05	1.51 ± 0.05	0.18 ± 0.06	0.89 ± 0.07

[a] Differences in the confident interval are due to variability of digestion.

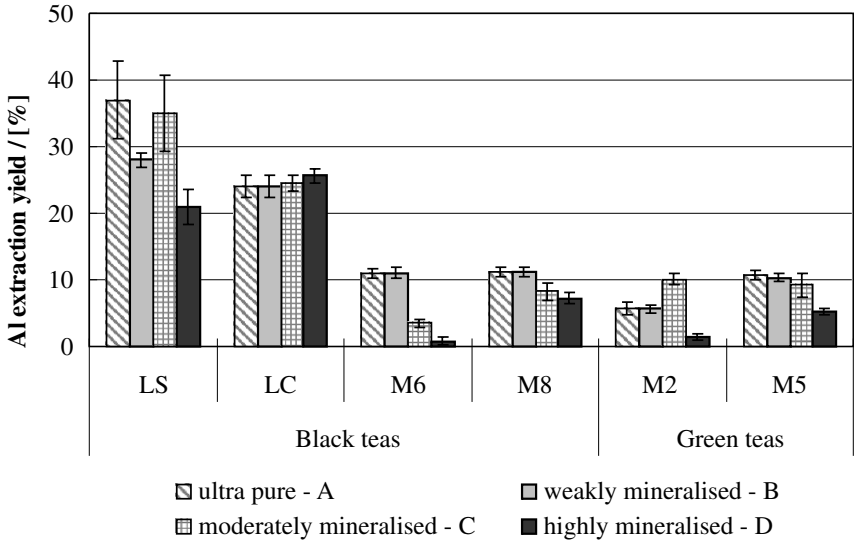

Fig. 2. Aluminium extraction yields in digested infusions.

aluminium is poorly extractable using whole leaves ($5\% < R < 11\%$). Al extraction is more efficient with broken leaves ($20\% < R < 40\%$). Grinding of leaves increases extraction yield by a factor of 1.5 (Fig. 3) but those yields are still lower than those obtained with LS and LC broken leaves. Except for infusions prepared with LC leaves, for all the other teas aluminium extraction depends strongly on water mineralisation. The largest decrease is observed when increasing mineral content. For LC leaves, water mineralisation does not seem to influence extraction which could be due to the presence of citric acid.

3.6. Calcium concentrations in tea infusions

Fig. 4 represents calcium concentrations in the different tea infusions. For each water used, blank water calcium concentration is reported and has to be compared to infusion ones.

Calcium concentrations in infusions range from 0.65 ± 0.07 to 2.6 ± 0.2 mg L^{-1} with ultra pure water (Fig. 4a) and corresponds to extraction yields smaller than 5% (data not shown). These results are consistent with previous studies by Odegard and Lund (1997) and Matsuura et al. (2001) who have reported values of 2.5 ± 0.6 mg L^{-1} and 4.03 ± 0.02 L^{-1}, respectively. With water B (containing 1.4 mg Ca L^{-1}), calcium concentrations range from 2.38 ± 0.1 to 4.0 ± 0.1 mg L^{-1} including calcium present in water. If calcium blank values are subtracted, extraction yields are equivalent to those obtained with ultra pure water. Grinding of leaves leads to an increase in extraction yield (Fig. 5) lower than the increase observed for aluminium (Fig. 3).

Heating water D leads to a decrease in the calcium content due to its precipitation as $CaCO_{3(s)}$ (Table 1). For waters C and D, calcium concentrations in infusions are lower than initial calcium water concentration in filtrated infusions (data not shown) as well as in digested ones (Fig. 4). Those decreases in calcium concentrations are more significant with the highest mineralised water D (from 10 to 70 mg L^{-1}) than with water C (from 3 to 10 mg L^{-1}) except for infusions prepared with broken leaves in water D. After calculating mass balance using 4 g of M8 leaves with 200 mL of water, it appears that, with water D, 1 mg of calcium is uptaken per g of brewed leaves (Table 7).

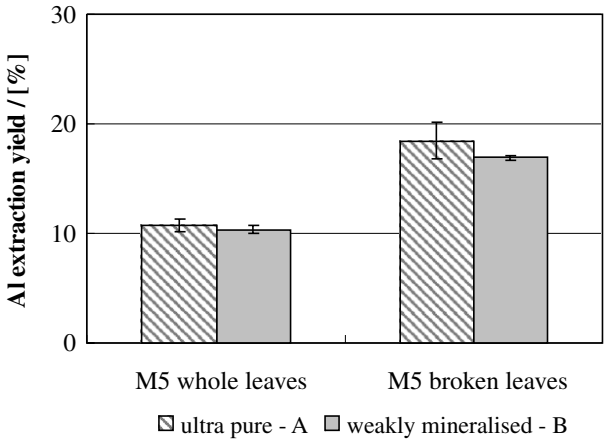

Fig. 3. Aluminium extraction yields with whole and broken M5 tea leaves.

3.7. Tea scum formation

Scum was formed in the presence of waters D and E due to the presence of calcium, hydrogenocarbonate and organic matter. Before heating, water E has the same composition as the water used by Spiro and Jaganyi (1994a, 1994b) who have not reported if given values are considered before or after heating water. As shown in the previous paragraph, heating highly mineralised water leads to a decrease in calcium and hydrogenocarbonate contents. Finally, after heating, water E contains 64 mg Ca L^{-1}

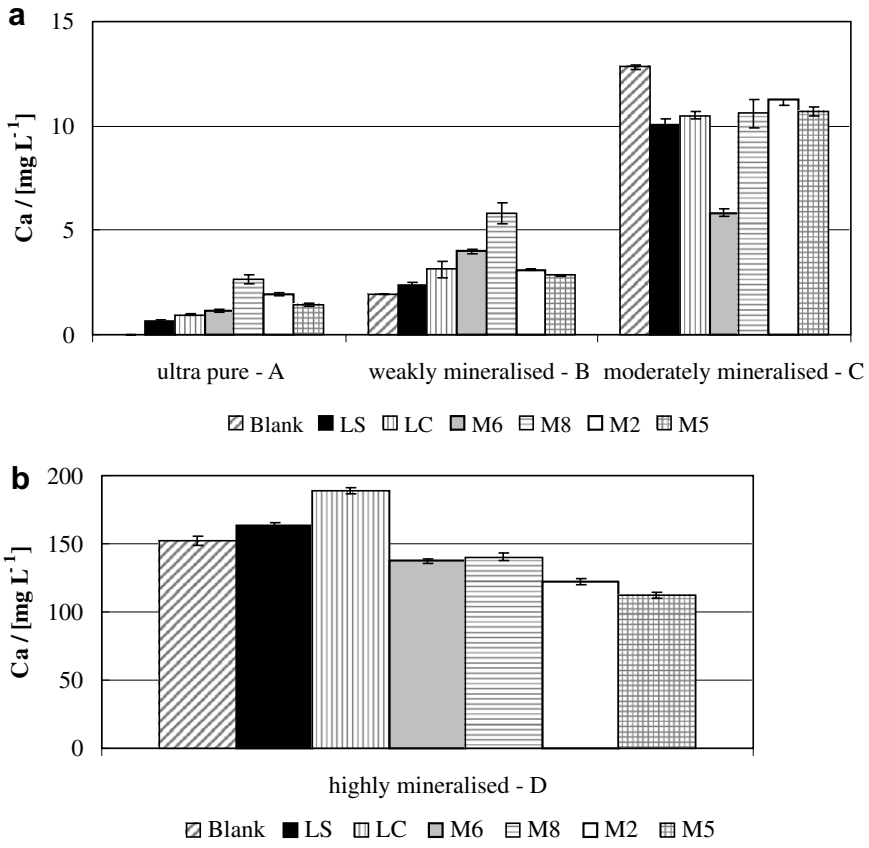

Fig. 4. Calcium concentrations in digested infusions with waters: (a) ultra pure – A, weakly mineralised – B, moderately mineralised – C and (b) highly mineralised – D.

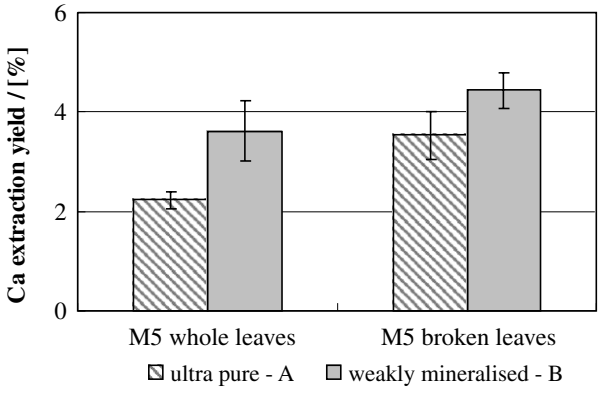

Fig. 5. Calcium extraction yields with whole and broken M5 tea leaves.

and 213.5 mg HCO_3 L^{-1}, which represents decreases in 30% and 35%, respectively. After heating, water D contains about 150 mg Ca L^{-1} and 156 mg HCO_3 L^{-1}, which represents 50% more calcium than in the study carried out by Spiro and Jaganyi (1994a, 1994b) but 50% less hydrogenocarbonate.

4. Discussion

Particle size seems to play a role in chemical extraction. Indeed, broken leaves and manually-ground M5 leaves always have extraction yields higher than whole ones. But yield from manually-ground leaves are always lower than those of broken ones. The highest yield could be due to manufacture effect. In fact, whole leaves are manufactured following the orthodox process resulting in less cellular damages than the crush tear and curl (CTC) process often used for teabag leaves (Goodsall, Hodges, Jones, Mawson, & Stabler, 1999). So, CTC leaves brew faster than whole ones. This result is consistent with leaf pictures performed during size measurement showing a strong difference between shapes of leaves.

On the other hand, water composition plays an important role in chemical extraction from tea leaves. Concerning calcium behaviour, its uptake by tea leaves, around

Table 7
Influence of water composition on mass balance of calcium in leaves and water before and after brewing of M8 tea

Calcium amount (mg)		Waters			
		A	B	C	D
Before brewing	In leaves	19 ± 1	19 ± 1	19 ± 1	19 ± 1
	In water	0	0.32 ± 0.04	2.4 ± 0.4	28.8 ± 0.4
	Total	19 ± 1	19 ± 1	21 ± 1	48 ± 1
After brewing	In leaves	14.0 ± 0.8	19.2 ± 0.8	18.8 ± 0.4	23.2 ± 0.8
	In infusion	0.44 ± 0.04	1.2 ± 0.4	2.00 ± 0.04	26.4 ± 0.8
	Total	14.4 ± 0.8	20 ± 1	20.8 ± 0.4	50 ± 2

1 mg g^{-1} leaves, is consistent with the work of Anderson et al. (1971). Moreover, extraction yields with ultra pure water are lower in the present study (water A) compared to Anderson et al. (1971) one. This phenomenon could be due to differences in cell structure or leaf treatment, CTC *vs*. orthodox. Indeed, it seems that this uptake is not diffusion-controlled because it takes place with water C although its calcium content is lower than calcium content of leaves, contrary to water D.

In most of speciation studies, infusions are filtrated through 0.45 μm membranes prior to analyses what leads to separation of precipitate and large colloids containing calcium and organic carbon on the filters before analyses. This treatment could cause the decrease in DOC and calcium in infusions, mainly for infusions prepared with highly mineralised waters because of cream formation enhancement by calcium addition (Jöbstl et al., 2005). The decrease in DOC would be however consistent with TPC measurements performed without any filtration confirming the role of water composition in organic matter extraction. But, TPC data, which corroborate Spiro and Price data (1987), are not consistent with Anderson et al. (1971) ones. The differences observed between Anderson et al. (1971) and the present work would be due to the analytical method used. Nowadays, Folin–Ciocalteu's method is considered as the most sensitive one for TPC analyses in tea compared to ferrous tartrate method, Lowenthal's method and α,α′-dipyridyl method (Yoshino, Sugiura, Shinohara, & Hirota, 2004). In 1971, Folin–Ciocalteu's method did not exist yet so that Anderson et al. used the Lowenthal's method. Spiro and Price (1987a) have observed the decrease in caffeine and theaflavin extraction using a water with an ionic strength of 0.11 mol L^{-1} prepared by addition of $CaCl_2$. Although, water D, used in the present work, has a lower ionic strength of 0.021 mol L^{-1}, decrease in organic matter extraction is confirmed. Moreover, we show that similar behaviour exits for inorganic compounds.

Some studies have reported on the cellular distribution of aluminium and calcium in tea leaves (Carr, Lombi, Kupper, McGrath, & Wong, 2003). Aluminium as well as calcium preferentially accumulates in cell walls of epidermis of fresh young leaves that are generally used for high grade tea. On the other hand, calcium seems to be present in the spongy mesophyllic tissue as crystals, their exact nature of which is not known. Those differences in compartmentation in tea leaf could explain why aluminium is more extractable than calcium.

Extraction mechanism of organic and inorganic compounds from tea leaves seems to follow two steps (Spiro & Price, 1987b): first, water is up taken by leaves; second, elements and molecules diffuse from tea leaves to the infusion. In the case of brewing tea in highly mineralised water, calcium uptake by leaves could take place during the first step and could be complexed by pectins present in cell wall as suggested by Spiro et al. (1987c). This complexation could modify cell wall properties. Indeed, calcium is well known to modulate gelification of pectins (Capel, Nicolai, Durand, Boulenguer, & Langendorff, 2006; Kawabata, Sawayama, Nakahara, & Kamata, 1981). Those modifications could then limit the extraction of organic but also inorganic compounds. It would be thus interesting to investigate where and how calcium is trapped. For aluminium, decrease in pH due to the presence of weak acids would increase its extraction from leaves due to competition between aluminium and proton for complex formation and presence of more soluble cation species of aluminium mainly for pH below 5 (Sigg, Behra, & Stumm, 2006).

Compared to the earliest studies where scum has been obtained by keeping the infusions at 80 °C for 60 min, scum formation is observed during the cooling process in the present work. A calcium content as low as 64 mg L^{-1} is sufficient to lead to scum formation and hydrogenocarbonate should not be in excess compare to calcium to observe it.

5. Conclusion

Analysis of aluminium, calcium and organic carbon in infusions prepared using broken black leaves as well as whole black and green leaves showed an effect of water mineralisation on their extraction. The higher the mineralisation, the lower the extraction yields of organic matter and aluminium. Calcium is taken up from highly mineralised water and assumed to be complexed with pectins in cell walls thus explaining the decrease in extraction yield. Further work about calcium fixation sites in leaves is needed to corroborate the hypothesis.

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