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Highlights

- Ethyl lactate, water and their mixtures were studied for decaffeinating green tea.
- Solubility of caffeine in pure and mixed solvents was studied at 298 K.
- Pressurized liquid extraction was accomplished comparing the different solvents.
- The solubility in the mixed solvents was considerably higher than in pure solvents.
- Extracts with higher caffeine/catechins selectivity were obtained with ethyl lactate.

Pressurized liquid extraction of caffeine and catechins from green tea leaves using ethyl lactate, water and ethyl lactate + water mixtures

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Abstract

Ethyl-lactate (ethyl 2-hydroxy-propanoate) is a bio-renewable agrochemical solvent, very suitable and environmental benign for food applications, permitted by the U.S. Food and Drug Administration as pharmaceutical and food additive. In previous work, the authors demonstrated that pressurized liquid extraction (PLE) using ethyl lactate is a suitable alternative to remove caffeine from vegetal materials, e.g. green coffee beans and green tea leaves. The solubility of caffeine in ethyl lactate + water mixtures, at ambient temperature and pressure, exhibits a substantial increase for 60:40 % ethyl lactate + water mixtures (data reported in this work). This result motivated the analysis of the effect of the ethyl lactate + water mixtures for the decaffeination target.

Furthermore, in the case of green tea, the removal of caffeine reducing the extraction of catechins is desirable due to the adverse effects of caffeine on health, while catechins are high valued functional food ingredients. Thus, the use of ethyl lactate, water and ethyl lactate + water mixtures to attain this objective, i.e. the removal of caffeine form green tea leaves minimizing the extraction of catechins, was studied in this work.

PLE was carried out in the temperature range 373-473 K and using different ethyl lactate + water mixtures. Extraction yield and recovery of key bioactive compounds (caffeine and monomeric catechins) were determined and compared, and the caffeine/catechins selectivity of the different solvents employed was estimated.

High extraction yields were obtained with a mixture containing 25:75 % of ethyl lactate + water, with values around 1.5 and 3.5 times higher than, respectively, the yields obtained with water and ethyl lactate. Yet, pure ethyl lactate proved to be the most selective solvent to extract caffeine from green tea leaves, minimizing the co-extraction of catechins, with a caffeine/catechins selectivity of 2.8 to 5.5 in the range 373 - 423 K. At these temperatures, with short extraction times (20 min) the recovery of caffeine is in the range 53-76 % but only 26-36 % of catechins present in the tea leaves were removed.

Keywords: Green Tea; Caffeine; Catechins; Ethyl Lactate; Pressurized liquid extraction.

Introduction

Tea is one of the most popular beverages in the world, is obtained from the leaves of the plant *Camellia sinensis* and green tea is one of the most widely consumed types. Green tea leaves contain several bioactive compounds, including methylxanthine alkaloids and polyphenols.

Caffeine is the most abundant alkaloid in green tea, being its amount in fresh leaves around 2-5 % mass of the dry weight (Perva-Uzunalic et al., 2006; Park et al., 2007a). The effects of caffeine as stimulant of the central nervous system are well known. Some adverse effects are derived from its consumption, including sleep deprivation, tachycardia, abortion and miscarriages. Similarly to caffeine, theophylline and theobromine stimulates the central nervous system, but its amount in green tea is lower than 0.5 % mass of the dry weight (Engelhardt, 2010; Zhao et al., 2011).

Regarding phenolic compounds, green tea contains large amounts of these compounds (up to 30 % mass of tea solids) and catechins are the major phenolic constituents (Wei et al., 2011; Chen et al., 2006; Engelhardt, 2010). Catechins are flavonoids (flavan-3-ols) which are composed primarily of epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Catechins contribute to the taste of the tea and important pharmacological properties have been assigned to their consumption, such as antioxidant, anticancer, anti-inflammatory, antibiotic and antiviral effects (Rusak et al., 2008; Härdtner et al., 2012; Singh et al., 2010; Osterburg et al., 2009; Song et al., 2005; Yang et al., 2011). Thus, a selective extraction of caffeine is desirable in the manufacturing of healthy caffeine-free green tea.

The current commercially available methods for decaffeinating green tea leaves have been solvent based extraction, using chlorinated solvents like chloroform or methylene chloride, but these solvents present high toxicity. Ethyl acetate, other solvent commercially used for decaffeination, present much lesser toxicity than chlorinated solvents, but its use also diminish the quantity of catechins in the tea leaf (Engelhardt, 2010; Dong et al., 2011). In this regard, Perva-Uzunalic et al. (2006) studied the extraction efficiency of catechins and caffeine using other different liquid solvents, such as acetone, methanol, ethanol, acetonitrile as well as water. By using water, the author reached caffeine and catechins recoveries of 56-89 % and 61-84 % respectively, at temperatures from 343-368 K and 2 hours of extraction time.

Supercritical fluid extraction (SFE) has become a commercial alternative to toxic solvents. Several works about the use of supercritical CO_2 for decaffeination of tea have been reported, in which a prior treatment with water or ethanol takes place. For example, Park et al. (2007a) studied the decaffeination of green tea using CO_2 and ethanol as cosolvent at 300 bar and 343 K, attaining a caffeine recovery around 80-96 % and a recovery of EGCG (the major catechin present in green tea) in the range of 46-74 % (caffeine/EGCG recovery ratio ≈ 1.3-1.7). In another contribution (Park et al., 2007b), the authors employed the same extraction conditions but water as cosolvent, and in this case the caffeine/EGCG recovery ratio was lower (around 1.1). Huang et al. (2007) tested pressures from 200 to 300 bar and temperatures of 313-333 K using also water as cosolvent. Practically complete decaffeination was attained (95.6 % of caffeine was removed from the green tea leaves) and the caffeine/catechins recovery ratio was 4.8. Nevertheless, it should be taken into account the small content of caffeine (9.92 mg/g) contained in the green tea utilized in their experiments. Kim et al. (2008) achieved a recovery ratio of 2.6 at 400 bar, 313 K and 7 % of water as cosolvent, but only 54 % of caffeine extraction yield could be reached. In conclusion, effective decaffeination can be achieved using SFE, but catechins are also significantly co-extracted, reducing the value of green tea as a functional healthy drink.

Ethyl lactate (ethyl 2-hydroxypropanoate) is an agrochemical and economically viable alternative to traditional liquid solvents, and it is fully biodegradable, non-corrosive, non-carcinogenic and non-ozone depleting. Ethyl lactate is recognized GRAS (generally recognized as safe) and due to its low toxicity was approved by the U.S. Food and Drug Administration (FDA) as pharmaceutical and food additive. These characteristics have increased the attention to the use of ethyl lactate as a green solvent for the food industry. Several reported potential applications are related with the extraction of carotenoids from different plant matrix (Ishida and Chapman, 2009; Strati and Oreopoulou, 2011), the extraction of γ-linolenic acid from Spirulina (Golmakani et al., 2012) and with the fractionation of edible oil compounds (squalene and tocopherol) (Hernández et al., 2011; Vicente et al., 2011).

The authors have studied recently (Villanueva et al., 2013) the pressurized liquid extraction (PLE) of caffeine from vegetal materials, e.g. green coffee beans and green tea leaves, and demonstrated that ethyl lactate is a suitable solvent for decaffeination. PLE was selected as

extractive method due to its faster extraction, higher yields and reduction of amount of solvent required in comparison with conventional solid-liquid extraction. Particularly, the removal of caffeine from green tea using ethyl lactate PLE was studied in the temperature range of 373-473 K, but no analysis regarding the co-extraction of catechins was informed.

In this work, the analysis of both caffeine and catechins PLE from green tea leaves using ethyl lactate, water and ethyl lactate + water mixtures is reported. Ethyl lactate + water mixtures were employed because it was observed (ambient temperature and pressure) that the solubility of caffeine in ethyl lactate increases in the presence of water. In fact, the solubility of caffeine in ethyl lactate + water mixtures shows a maximum for mixtures containing around 40 % mass of water (data measured in this work). This solubility behaviour leads to presume that decaffeination can be improved using ethyl lactate + water mixtures. Yet, since preservation of catechins in the vegetal material is desirable, the recovery of both caffeine and monomeric catechins in the different extracts were determined, and the caffeine/catechins selectivity of the different solvents employed was calculated and compared.

2. Material and methods

2.1 Samples and reagents

"Gunpowder" green tea (*Camellia sinensis*) leaves were acquired in a Spanish market. The green tea leaves were ground in a cooled knife mill using liquid nitrogen (particle size smaller than 250 μm).

Ethyl lactate (≥ 98 % purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) was obtained from Lab-Scan analytical sciences (Gliwice, Polonia). Formic acid (≥ 98 % purity) was obtained from Merck (Darmstadt, Alemania). Sea sand was obtained from Panreac Química S.A.U. (Barcelona, España).

Standards: (+)-catechin (≥ 99 % purity), (-)-epigallocatechin (≥ 95 % purity), (-)-epicatechin gallate (≥ 97.5 % purity) and (-)-epigallocatechin gallate (≥ 98 %) were purchased from Extrasynthèse (Genay, Lyon, Francia). (-)-epicatechin (≥ 90 % purity), caffeine (≥ 99 % purity), theophylline (≥ 99 % purity) and theobromine (≥ 99 % purity) were purchased from Sigma-Aldrich.

The total content of caffeine and catechins in the vegetal material was measured. For this purpose, 200 mg of green tea leaves were extracted at 343 K with 20 mL of an aqueous

ethanol solution (30 % v/v) (Park et al., 2007a) in a Stuart Orbital S150 shaker apparatus (Bibby Scientific Limited Stone, UK) during 4 h. Then, the solvent was renewed and successive extraction cycles of 4 h were accomplished. The amount of caffeine recovered after two successive cycles were, respectively, 26.7 mg/g (first cycle) and 0.3 mg/g (second cycle). Regarding catechins, the corresponding yields were 84.9 and 0.1 mg/g. Thus, the content of caffeine and catechins in the vegetal material were determined to be, respectively, 27 mg and 85 mg per g of green tea leaves.

2.2 Solubility measurements

The solubility of caffeine in the liquid ethyl lactate + water solvent were measured at 298 K and atmospheric pressure as a function of the ethyl lactate / water ratio. For all solutions studied, the liquid solvent and caffeine in excess were placed into glass vessels (10 ml) with a magnetic stir bar. The vessels were put inside a water bath heated by a magnetic hotplate stirrer (RCT classic IKAMAG® safety control. IKA Works GmbH & Co. KG, Staufen, Germany) which was used to agitate the samples during 24 h under fixed temperature, controlled by an electronic contact thermometer with probe (VWR VT-5 VWR International, LLC West Chester, Pensilvania, USA) with an accuracy of 0.2 K. After reaching the equilibrium, stirring was stopped and the vessels were left still for more than 48 hours to allow a complete phase separation. Samples of clear saturated liquid solution (100 μL) were taken using a micropipette, filtered through a 0.20 μm filters and placed into glass vials. Samples were appropriately diluted for HPLC analysis.

2.3 Pressurized liquid extractions

Extractions were carried out in an Accelerated Solvent Extraction system ASE 350 from Dionex Corporation (Sunnyvale, CA, USA) equipped with a solvent controller unit. A detailed explanation of the equipment and experimental procedure is given elsewhere (Villanueva et al., 2013).

Experiments were carried out at 323, 373, 423 and 473 K when ethyl lactate (EL) or water (W) were utilized. Moreover, two different temperatures (373 and 398 K) were selected when using 0:100 %, 25:75 %, 50:50 %, 75:25 % and 100:0 % EL:W mixtures. Around 1 g of solid sample and 1 g of sea sand (used as dispersant) were employed in each experimental assay.

Several preliminary tests were carried out in order to select the extraction time that was fixed at 20 min. All extracts produced were stored under refrigeration until analysis.

2.4 Identification and quantification of caffeine and catechins

Analysis were performed with an Accela (Thermo Electron Corporation, San Jose, CA) equipped with an ACE 3 C18-AR column (150×4.6 mm, 3 μm particle size) (Advanced Chromatography Technologies, Aberdeen, UK) equipped with a DAD detector and triple quadrupole mass spectrometer (TSQ-Quantum, Thermo Electron Corporation, San Jose, CA) with an ESI (Electrospray Ionization) interface. Based on the method of Pelillo et al. (2002), the composition of the mobile phase was (A) 0.5 % (v/v) formic acid in water (B) 0.3 % (v/v) formic acid in acetonitrile. The column temperature was maintained at 308 K, with a flow rate of 0.6 mL/min. The mobile phase gradient employed was as follows: initial 100 % A, 30 min 0 % A, 32 min 100 % A, 37 min 100 % A. Spray voltage and sheath gas pressure was set in 5000 and 35 psi respectively and the capillary temperature was 623 K. Mass analyzer was set simultaneously in full scan and SRM (Single Reaction Monitoring) modes. In this case, SRM experiments were done using 1 precursor ion and 1 daughter ion, operating in positive mode. Table 1 shows the SRM transitions selected automatically among the most abundant ions and the corresponding collision energies. The amount of target compounds in the different samples was calculated by triplicate from a calibration curve of standards.

3. Results and discussion

3.1 Solubility of caffeine in ethyl lactate + water mixtures

Table 2 shows the solubility of caffeine in ethyl lactate + water mixtures measured in this work at 298 K and atmospheric pressure. The table includes the values obtained for the pure solvents, namely ethyl lactate and water. Previous data available in the literature about the solubility of caffeine in water corresponds to values of 2.2 % mass at 298 K (Bustamante et al., 2002). On the other side, the solubility of caffeine in ethyl lactate was previously reported by the authors (Manic et al., 2012) resulting in 3.2 % mass at 303 K. Thus, previous data from the literature satisfactory agree with the solubilities measured in this work (2.0 and 3.0 % mass at 298 K for, respectively, water and ethyl lactate).

The values given in Table 2 correspond to the average value ($\bar{x}$) of experiments carried out by duplicate. Standard deviations (*SD*) were calculated according to the following equation:

$$SD = \sqrt{\frac{1}{2}\left[\left(x_1 - \bar{x}\right)^2 + \left(x_2 - \bar{x}\right)^2\right]} \quad (1)$$

being x_1 and x_2 the corresponding values obtained in each of the duplicate experiments. The average relative deviation (*ARD*):

$$ARD = \frac{1}{N}\sum \frac{SD_i}{\bar{x}_i} \quad (2)$$

was 1.7 % for the N experiments.

The solubility of caffeine in the mixed solvent ethyl lactate + water is considerably higher than the corresponding solubility in pure ethyl lactate or water, as can be clearly observed in Table 2. That is, the addition of water to the ethyl lactate solvent can substantially increase caffeine solubility, attaining a maximum around 8 % mass for a mixed solvent comprising 40 % mass of water. This solubility value is around 2.7 fold higher than the solubility in pure ethyl lactate and 4.0 fold higher than in water.

3.2 Pressurized liquid extraction of green tea leaves

3.2.1 PLE extraction using ethyl lactate or water

Preliminary studies were carried out in order to set the extension of the static extraction experiments. Sequential steps of 10 min were carried out at the highest temperature explored (473 K) using ethyl lactate or water. Around 15-20 % of the yield obtained during the first step was recovered in the second step, while yields lower than 2 % were attained in a third step. Thus, extraction time was set to 20 min in order to proceed with the study of (i) the effect of temperature and (ii) the use of ethyl lactate + water mixtures on the extraction of caffeine from green tea leaves. In this study, considering the co-extraction of the valuable bioactive catechins, the concentrations of EC, ECG, EGC, EGCG and (+)-catechin in the extracts were also determined. Additionally, two other alkaloids present in green tea leaves, theophylline and theobromine were identified and quantified.

First, the PLE of green tea leaves using pure ethyl lactate or water was accomplished in the temperature range of 323-473 K. The corresponding results are given in Table 3. The *ARD* calculated according to Eq. (2) were 5.5 % and 7.6 %, respectively, for the extraction yield and the concentration (% mass) of the target compounds in the extracts.

As can be observed in Table 3, higher extraction yields were obtained with water, but the increase of yield with temperature is particularly noteworthy when ethyl lactate is employed as extractive solvent (see Figure 1).

Concerning the analysis of bioactive compounds, caffeine was the main alkaloid extracted and the concentration of theophylline and theobromine were lower than 0.2 % mass.

In the case of ethyl lactate, the increase of temperature increases the concentration of both, caffeine and catechins, attaining a maximum around 373-423 K and then decreasing (see Table 3). When water is employed as solvent, the concentration of caffeine in the extracts is almost constant while catechins concentrations decrease with increasing temperature. Figure 2 compare the recovery of caffeine (Figure 2a) and catechins (Figure 2b) for each solvent employed. The recovery of caffeine increases with temperature and attain values higher than 75 % at temperatures higher than 423 K. In this regard, almost complete decaffeination was achieved using ethyl lactate at 473 K (92 % of caffeine was removed). On the other side, the recovery of catechins increase up to 423 K and then decrease, denoting the possibility of thermal degradation. This effect is especially pronounced when water is used unlike ethyl lactate. Thus, for both solvents, extraction temperature lower than 423 K is recommended in order to avoid thermal degradation of catechins. Furthermore, ethyl lactate can extract similar amounts of caffeine than water at temperatures around 373-423 K, while considerable lower amounts of catechins are extracted from the green tea leaves (see Figure 2).

The selectivity (S) of each solvent towards the extraction of the two types of solutes, caffeine (1) and catechins (2), was calculated according to:

$$S = k_1/k_2 \tag{3}$$

were k_1 and k_2 are, respectively, the distribution coefficient of caffeine and catechins, which were calculated considering the concentration (% mass) of the solute in the extract (y_i^{ext}) and in the residual vegetal material (x_i^{sol}), that is:

$$k_i = y_i^{ext}/x_i^{sol} \tag{4}$$

The y_i^{ext} values for caffeine and catechins are given in Table 3, and the x_i^{sol} concentrations in the extracted vegetal material were calculated according to:

$$x_i^{sol} = \frac{m_i^{sol}}{(1-Y/100)} \quad (5)$$

being m_i^{sol} the non-extracted solute per unit mass of raw material employed and Y the corresponding extraction yield (see Table 3). The non-extracted solute was calculated as the difference between the initial solute content in the raw material (27 mg/g of caffeine and 85 mg/g of catechins, respectively) and the mass of solute extracted ($y_i^{ext} \cdot Y$).

The calculated selectivity of water and ethyl lactate towards the extraction of caffeine and catechins is reported in Table 3. The selectivity of ethyl lactate is higher than the selectivity of water over the whole range of studied temperatures. The large increase of selectivity values observed at 473 K for both solvents (particularly for water) should not be taken into account due to the fact that although at this temperature almost the whole caffeine was removed, thermal degradation of catechins was produced, as noted above. In this respect, the selectivity calculated at 423 K may be also overestimated since the thermal stability of catechins at this temperature is not confirmed.

3.2.2 PLE extraction using ethyl lactate + water mixtures

Considering the solubility measurements and the results obtained from the PLE-temperature dependence study, the extraction of green tea leaves using ethyl lactate (EL) + water (W) mixtures (instead of pure solvents) was accomplished. Extraction temperatures were selected in order to avoid catechins thermal degradation (373 and 398 K) and different EL:W ratios (0:100 %, 25:75 %, 50:50 %, 75:25 % and 100:0 %) were employed.

Table 4 shows the extraction yield, concentration of caffeine and catechins and solvent selectivity for both temperatures studied. As can be observed, larger yields were obtained with the mixed solvent in comparison with the pure solvents. The addition of water to ethyl lactate significantly increased the PLE yield at both temperatures investigated. Nevertheless, regarding the concentration (mass %) of the target compounds in the extracts, the addition of water results in a decrease in the concentration of both caffeine and catechins, with values closer to those obtained using pure water. Consequently, the caffeine/catechins selectivity was not improved with respect to the values obtained with pure ethyl lactate. The highest selectivity attained was 2.8, at 373 K and with 50:50 % ethyl lactate + water, which is the same value obtained using pure ethyl lactate at the same temperature. Nevertheless, it should

be pointed out that with the 50:50 % solvent, better decaffeination (higher caffeine recovery) could be achieved.

The recoveries of caffeine and catechins are depicted in Figure 3 as a function of the EL:W ratio and considering the two different temperatures tested. In general, higher recoveries were attained for the mixed solvent in comparison with the pure solvents. However, as mentioned before, the caffeine/catechins selectivity for all EL:W ratios employed were not higher than the selectivity obtained with pure ethyl lactate.

Table 5 shows the concentration of each catechin in the extracts (% mass normalized). Similar concentrations were obtained for the identified catechins (EC, (+)-cat, ECG, EGCG, EGC) despite the solvent employed (ethyl lactate, water or mixtures ethyl lactate + water). For all extracts obtained, EGCG was the most abundant catechin obtained in the extracts (around 50-60 % of total catechins) under all extraction conditions, followed by EGC and ECG (around 20 % each).

Conclusions

PLE using pure ethyl lactate is a potential suitable alternative to remove caffeine from natural matter, and in the case of green tea leaves, the co-extraction of catechins is minimized in comparison with the extraction using other liquid solvents or supercritical CO_2. For example, at 373 K the caffeine/catechins selectivity was 2.8 (no thermal degradation of catechins was observed at this temperature) and the recovery of caffeine and catechins were, respectively, 53 % and 26 %. Then, the caffeine/catechin recovery ratio was 2.0, higher than those obtained in this work using water or those reported by Perva-Uzunalic et al. (2006) using other solvents. In comparison with $SCCO_2$ extraction, previous work (Park et al., 2007a; Park et al., 2007b) reported caffeine/catechins recovery ratios in the range 1.3-1.7 with suitable decaffeination (80-96 % caffeine removal). At higher temperatures (423-473 K), ethyl lactate PLE can remove 76-92 % of caffeine, but the higher caffeine/catechins selectivity obtained cannot be confirmed due to possible thermal degradation of catechins.

On the other side, with the ethyl lactate + water mixtures, higher caffeine recoveries were obtained but the recoveries of catechins were increased even more and thus, selectivity factors were not improved with respect to ethyl lactate, and were similar to those obtained with pure water. Nevertheless, it should be taken into account that these recoveries are 1.4-

1.8 (EL:W ratios around 75:25 %) higher than those obtained using water, and can be considered a good alternative to recover bioactives from green tea leaves.

The results presented in this work show the effective use of ethyl lactate, water and ethyl lactate + water mixtures to remove caffeine form green tea leaves. The co-extraction of catechins was also examined since these substances are the most important bioactives of green tea. The best caffeine/catechins selectivity in the decaffeination process was obtained using pure ethyl lactate.

In addition to caffeine and catechins, other bioactive compounds (proanthocyanidins, quercetin and kaempferol glycosides, theanine) could be present in the extracts produced using the solvents studied in this work. Furthermore, it should be considered also the co-extraction of lipids, favored with 100 % ethyl lactate, and carbohydrates, highly soluble in water, which also might be extracted. This work establishes the basis of further studies, including the analysis of the co-extraction of these compounds and their impact on the organoleptic quality of the infusion that can be produced after decaffeination.

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FIGURE CAPTIONS

Figure 1. Increase with temperature of the extraction yield ($Y_{(T)}/Y_{(323\,K)}$)) in the PLE of green tea leaves using (●) ethyl lactate or (▲) water.

Figure 2. Recovery (mass extracted / initial mass of the compound in vegetal matter x 100) of (a) caffeine and (b) catechins as a function of temperature in the PLE of green tea leaves using (●) ethyl lactate or (▲) water. Initial mass of caffeine and catechins determined in the raw material: 27 mg/g and 85 mg/g of green tea, respectively.

Figure 3. Recovery (mass extracted / initial mass of the compound in vegetal matter x 100) of (●) caffeine and (▲) catechins as a function of the EL:W ratio for the PLE of green tea leaves at (a) 373 K and (b) 398 K.

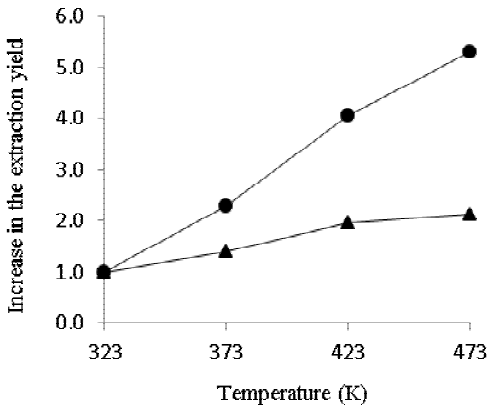

Figure 1. Increase with temperature of the extraction yield ($Y_{(T)}/Y_{(323\,K)}$)) in the PLE of green tea leaves using (●) ethyl lactate or (▲) water.

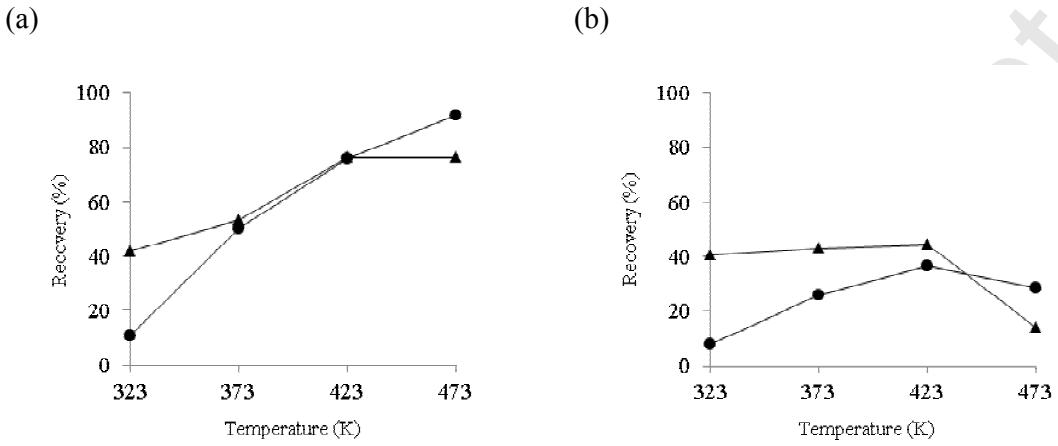

Figure 2. Recovery (mass extracted / initial mass of the compound in vegetal matter x 100) of (a) caffeine and (b) catechins as a function of temperature in the PLE of green tea leaves using (●) ethyl lactate or (▲) water. Initial mass of caffeine and catechins determined in the raw material: 27 mg/g and 85 mg/g of green tea, respectively.

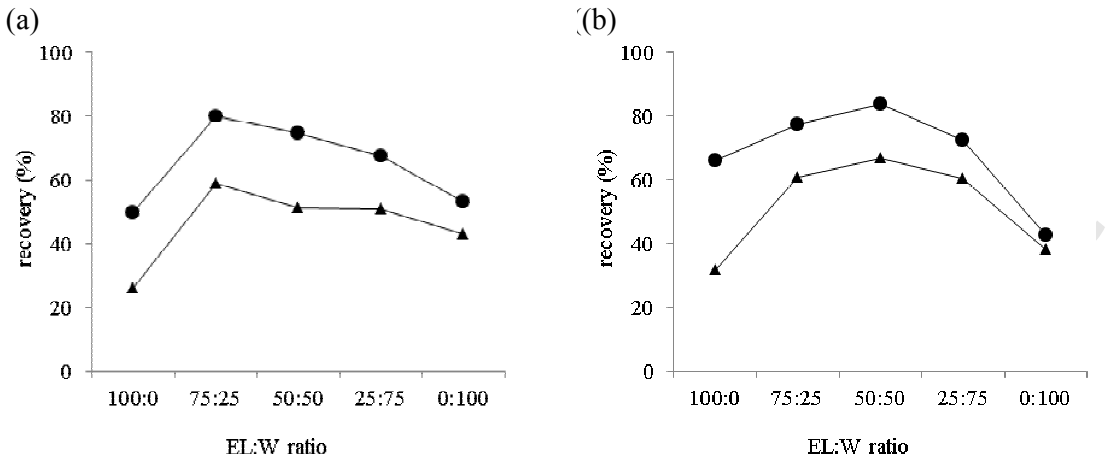

Figure 3. Recovery (mass extracted / initial mass of the compound in vegetal matter x 100) of (●) caffeine and (▲) catechins as a function of the EL:W ratio for the PLE of green tea leaves at (a) 373 K and (b) 398 K.

Table 1. Single Reaction Monitoring (SRM) transitions and the corresponding collision energies selected for the analysis of the main catechins and alkaloids of green tea.

Compound	Precursor ion	Daughter ion	Collision energy (V)
(+)-catechin	290.971	138.987	20
Epicatechin	290.971	138.987	20
Epigallocatechin	306.932	138.989	15
Epicatechin gallate	443.029	123.034	32
Epigallocatechin gallate	459.035	138.999	23
Caffeine	195.066	138.055	19
Theophylline	181.068	124.062	18
Theobromine	181.147	163.006	9

Table 2. Solubility of caffeine in ethyl lactate (EL) + water (W) mixtures at atmospheric pressure and 298 K**.**

Water content in the EL+W solvent (% mass)	Solubility (% mass of caffeine)
0	2.97 ± 0.08
9.6	5.94 ± 0.07
19.3	7.80 ± 0.07
39.0	8.04 ± 0.08
49.1	7.87 ± 0.08
59.1	6.86 ± 0.07
69.0	5.99 ± 0.09
79.2	4.56 ± 0.07
89.6	3.15 ± 0.05
100	2.00 ± 0.08

Table 3. Extraction yield (Y = mass extracted / mass of raw material x 100), concentration of key bioactives* (% mass) and caffeine/catechins solvent selectivity (Eq. 5) corresponding to the PLE of green tea leaves. Extraction time: 20 min.

Solvent		Temperature (K)			
		323	373	423	473
Ethyl lactate					
	Yield (Y)	3.9	10.8	16.0	20.8
	Caffeine (% mass)	7.4	12.5	12.8	11.9
	Catechins (% mass)	16.5	20.5	19.3	11.7
	Selectivity	1.5	2.8	5.5	27.4
Water					
	Yield (Y)	20.6	30.0	40.4	43.8
	Caffeine (% mass)	5.5	4.8	5.1	4.7
	Catechins (% mass)	16.7	12.2	9.4	2.7
	Selectivity	1.1	1.5	4.0	19.9

* *Catechins: (+)-catechin, (-)-EC, (-)-EGC, (-)-ECG, (-)-EGCG. Theobromine concentrations lower than 0.2 % mass. Theophylline: below quantification level.*

Table 4. Extraction yield (Y = mass extracted / mass of raw material x 100), concentration of key bioactives* (% mass) and caffeine/catechins solvent selectivity (Eq. 5) corresponding to the PLE of green tea leaves using as solvent different ethyl lactate (EL) + water (W) mixtures. Extraction time: 20 min.

Temperature		EL:W ratio				
		100:0	75:25	50:50	25:75	0:100
373 K						
	Yield (Y)	10.8	37.2	39.6	42.4	30.0
	Caffeine (% mass)	12.5	5.8	5.1	4.3	4.8
	Catechins (% mass)	20.5	13.5	11.0	10.2	12.2
	Selectivity	2.8	2.7	2.8	2.0	1.5
398 K						
	Yield (Y)	19.9	41.0	50.3	65.2	37.3
	Caffeine (% mass)	9.0	5.1	4.5	3.0	3.1
	Catechins (% mass)	13.6	12.6	11.3	7.9	8.7
	Selectivity	4.2	2.2	2.6	1.7	1.2

* *Catechins: (+)-catechin, (-)-EC, (-)-EGC, (-)-ECG, (-)-EGCG. Theobromine concentrations lower than 0.2 % mass. Theophylline: below quantification level.*

Table 5. Concentration of the different catechins (% mass normalized) corresponding to the PLE of green tea leaves using as solvent (a) ethyl lactate, (b) water and (c) ethyl lactate (EL) + water (W) mixtures. Extraction time: 20 min.

Solvent	Compound				
(a) Ethyl lactate		T (K)			
		323	373	423	473
	(+)-cat.	0.8	1.9	1.9	2.1
	EC	9.0	9.5	11.0	9.4
	EGC	17.6	22.4	16.0	17.4
	ECG	20.3	20.5	25.9	28.1
	EGCG	52.3	45.8	45.2	43.0
(b) Water		T (K)			
		323	373	423	473
	(+)-cat.	0.7	1.5	1.7	2.0
	EC	10.6	8.4	7.7	2.3
	EGC	26.9	23.4	20.7	23.3
	ECG	13.4	12.9	23.8	22.9
	EGCG	48.3	53.8	46.1	49.5
(c) EL + W					
		EL:W ratio			
T (K)		100:0	75:25	50:50	25:75	0:100
373						
	(+)-cat.		1.7	2.3	2.1	
	EC		6.7	8.0	7.0	
	EGC		22.9	16.8	20.4	
	ECG		15.5	20.2	17.3	
	EGCG		53.2	52.8	53.2	
398						
	(+)-cat.	2.2	2.0	2.1	2.8	2.1
	EC	8.2	6.8	6.4	6.4	5.9
	EGC	25.9	22.6	20.3	15.2	17.7
	ECG	16.5	16.4	16.5	15.5	14.3
	EGCG	47.3	52.3	54.7	60.0	60.0