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11 **Extraction of caffeine from natural matter using a bio-renewable**
12 **agrochemical solvent**
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Abstract

This paper reports experimental data on the pressurized liquid extraction of caffeine from green coffee beans and green tea leaves using ethyl-lactate (ethyl 2-hydroxy-propanoate). This solvent is a new bio-renewable agrochemical solvent, naturally produced by fermentation from corn derived feedstock, which has been recently considered as a very suitable and environmental benign solvent for food industrial applications.

Static extraction assays (one step during 10 min) were carried out in an Accelerated Solvent Extraction (ASE) system at three different extraction temperatures, namely 100, 150 and 200°C. Extraction yield and caffeine recovery were determined and compared with those obtained when using other liquid solvents, such as ethyl acetate or ethanol. High recovery of caffeine ($\approx 60\%$) was found in the extracts produced using ethyl lactate, which demonstrates the potential use of this green solvent for the extraction of caffeine from different vegetable sources.

Keywords: Coffee; Green Tea; Caffeine; Ethyl Lactate; Accelerated Solvent Extraction.

1. Introduction

Coffee plant belongs to the genus *Coffea* of the *Rubiaceae* family with more than 70 species, but only two of them have economic and commercial importance: the species Arabica (*Coffea arabica*) and Robusta (*Coffea robusta*) (Alonso-Salces et al., 2009). Arabica coffee beans are preferred by consumers and are considered of superior quality at the international market (Meinhart, et al., 2010).

Coffee is one of the most traded commodities, and is one of the most popular drinks in the world due to its unique flavor and sensory characteristics. Coffee beans are an important source of caffeine, which is the most common consumed alkaloid in the world. Depending on coffee variety, caffeine content in green beans is around 1-2 mass % (Ashihara and Crozier, 2001). Other active principles present in coffee beans are coffee oil, an ingredient of special interest for the cosmetic and pharmaceutical industries (Folstar, 1985), and phenolic acids (elagic, caffeic and chlorogenic acids) to which several biological properties have been attributed (Naidu et al., 2008; Brezova et al., 2009).

Another plant that contains caffeine is green tea (*Camellia sinensis*), which has been a much consumed drink in Asian countries over years. Nowadays, it is very popular all over the world, due to its recognized beneficial health effects. Green tea leaves contains caffeine, catechins, fats, amino acids, aroma chemical, vitamins and chlorophyll, among others (Stone et al., 1991). Indeed, the major bioactive components are caffeine and catechins. Caffeine content is around 20-40 mg/g while catechins are in the range of 190-260 mg/g (Park et al., 2007; Perva-Uzunalic et al., 2006). Catechins are recognized to be the beneficial bioactive compounds of green tea, including antioxidant, anticancer, anti-inflammatory, antibiotic and antiviral effects (Cai et al., 2002; Tedeschi et al., 2002; Cooper et al., 2005). Thus, the consumption of green tea is considerably increasing, so as products with green tea flavor such as beverages and ice-creams.

Unquestionably, supercritical CO₂ (SCCO₂) extraction has proved to be the most convenient commercial and environmental friendly technology for the removal of caffeine from green coffee beans (Zosel, 1978). SCCO₂ is selective for the caffeine, there is no associated waste treatment of a toxic solvent and extraction times are generally moderate. SCCO₂ coupled with a cosolvent, such as ethanol or water, was employed to extract caffeine form green tea leaves. By varying the extraction conditions, it is expected to maximize the amount of caffeine

1 extracted and minimize the co-extraction of bioactive catechins. Nevertheless, substantial
2 losses of catechins proved to be unavoidable (Kim et al., 2008; Park et al., 2007).
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4 Among liquid solvents traditionally employed for coffee decaffeination, benzene, chloroform,
5 trichloroethylene and dichloromethane have been used over years. However, when evidence
6 suggested that chlorinated solvents might be carcinogenic (Lynge et al., 1997) their use was
7 severely reduced.
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11 Extraction with water (definitely a green solvent) is also called “indirect extraction” since a
12 two-step process is required: coffee beans are first soaked in water, an organic solvent is
13 employed to selectively extract caffeine from water, and the caffeine-free water goes back in
14 contact with the beans and is evaporated (Clarke, 2003). This procedure strips away many of
15 the essential flavor and aroma substances. Ethyl acetate is much more selective for caffeine
16 and thus, extraction can be accomplished in a single-step contact process (“direct
17 extraction”). Since ethyl acetate presents much less health and environmental hazard than
18 chlorinated solvents, decaffeination of coffee beans using this solvent is often called “natural
19 decaffeination” despite the fact that the ethyl acetate employed was obtained from synthesis
20 and not from natural sources.
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30 As green coffee beans, the current commercially available methods for decaffeinating green
31 tea leaves have been solvent based extraction, using chlorinated solvents, ethyl acetate,
32 acetone, methanol, ethanol and acetonitrile. Effective decaffeination can be achieved using
33 these solvents but catechins are also significantly co-extracted, reducing the value of green
34 tea as a functional healthy drink.
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40 Ethyl lactate (ethyl 2-hydroxypropanoate) is an agrochemical and economically viable
41 alternative to traditional liquid solvents liquid solvents, and it is fully biodegradable, non-
42 corrosive, non-carcinogenic and non-ozone depleting. It was self-affirmed GRAS (generally
43 recognized as safe) and due to its low toxicity, was approved by the U.S. Food and Drug
44 Administration (FDA) as pharmaceutical and food additive. These characteristics have
45 increased the attention to the use of ethyl lactate as a green solvent for the food industry.
46 Several reported potential applications are related with the extraction of carotenoids from
47 different plant matrix (Ishida and Chapman, 2009; Strati and Oreopoulou, 2011), the
48 extraction of γ -linolenic acid from *Spirulina* (Golmakani et al., 2012) and with the
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1 fractionation of edible oil compounds (squalene and tocopherol) (Hernández et al., 2011;
2 Vicente et al., 2011).
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4 The solubility of caffeine in ethyl lactate has been reported by the authors in a recent
5 contribution (Manic et al., 2012). At 303 K the solubility was reported to be 3.2% by mass,
6 which is very similar to the values reported for the solubility of caffeine in water (Bustamante
7 et al., 2002). These solubility data motivated the present study: the potential use of ethyl
8 lactate as an environmentally friendly solvent to extract caffeine from natural matter. To the
9 best of our knowledge the extraction of green coffee beans and green tea leaves using ethyl
10 lactate is presented for the first time.
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21 **2. Material and methods**

24 **2.1 Samples and reagents**

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27 Green coffee beans (*Coffea arabica* variety) and green tea (*Camellia sinensis*) leaves were
28 acquired in a Spanish market. Water content in beans and leaves was determined to be,
29 respectively, 11.2% and 6.2%. The moisture content was determined by oven drying at 80°C
30 until a constant weight was obtained.
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35 The coffee beans were ground in a ceramic mortar using liquid nitrogen; particle sizes were
36 separated by using sieves with manual agitation. Two different sizes of particles were
37 employed in the experiments: the entire green beans and ground beans with particles in the
38 range of 500-1500 µm. The green tea leaves were ground in a manual knife mill using liquid
39 nitrogen; particle of sizes 200-500 µm were employed for the experiments and were
40 separated by using sieves with manual agitation.
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47 Ethyl lactate (≥ 98% purity) and ethyl acetate (≥ 99.7% purity) were obtained from Sigma-
48 Aldrich (St. Louis, MO, USA), and ethanol (99.5% v/v purity) from Panreac (Castellar del
49 Vallés, Barcelona, Spain). Caffeine standard (≥ 99.0% purity) was obtained from Fluka
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2.2 Accelerated Solvent Extraction (ASE)

Caffeine extraction was carried out in an Accelerated Solvent Extraction system ASE 350 from Dionex Corporation (Sunnyvale, CA, USA) equipped with a solvent controller unit. A scheme of the equipment is shown in Figure 1.

In the case of coffee beans samples, extractions were performed with three different liquid solvents (i.e. ethyl lactate, ethanol and ethyl acetate) at three different extraction temperatures (100, 150 and 200°C) using 3 g of solid sample. The extraction of caffeine from green tea leaves was performed at 100, 150 and 200°C, with ethyl lactate and ethanol as extractive solvents. In this case, each cell was filled with around 1 g of solid sample.

Several preliminary assays were carried out in order to analyze the effect of time in the extraction procedure. At 200°C, very similar yields were obtained employing 10 min and 20 min of static extraction, being the differences lower than 15%. In view of this, and in order to reduce the possibility of thermal degradation, a static extraction time of 10 min was selected to carry out all experiments.

The experimental procedure for both raw materials was as follows. The cells employed (10 ml capacity) were placed into an oven; each cell was filled with the corresponding amount of solid sample. After loading the sample into the extraction cell, the cell was filled with the corresponding solvent up to a pressure of 10 MPa (which ensures the liquid state of the three solvents employed at the three temperatures studied) and was heated-up to the desired temperature. Then, a static extraction continued for 10 min. with all the system valves closed. In order to prevent over-pressurization of the cell, a static valve was pulsed open and closed automatically when the cell pressure exceeded the set point. The solvent that escaped during this venting was collected in the collection vial. After extraction the cell was washed with the solvent and subsequently the solvent was purged from cell using N₂ gas until complete depressurization was accomplished.

The extracts were stored under refrigeration until they were dried. A rotavapor was used for partial elimination of solvent. Then, a thermo-block at 100°C was used to dry the samples to a constant weight. All experiments were carried out by duplicate. The dried samples obtained were stored at 4°C until analysis.

2.3 Identification and quantification of caffeine

A Varian ProStar Analytical HPLC (Agilent Technologies, Santa Clara, California, USA) with a ternary pump to create gradients, thermostatic controlled column oven, autosampler mode 410 with a 20 μ L sample loop and diode array detector was employed in the study. All the modules were controlled by PC with interface and HPLC Varian Star system control software.

The column employed was Microsorb-MV100 column C-18, 5 μ m (250 x 4.6 mm), fitted with a suitable pre-column. Based in the method of Sharma et al. (2005) the mobile phase adopted was (A) acetonitrile / (B) 0.1 % mass ortho-phosphoric acid in water with a flow rate of 0.8 mL/min and column compartment temperature of 35°C. The mobile phase gradient employed was as follows: initial 10% A, 15 min 30% A, 20 min 35% A, 22 min 20% A, 25 min 10% A. The amount of caffeine in the different samples was calculated from a calibration curve of caffeine standard. HPLC analysis was carried out by duplicate.

2.4 Determination of total caffeine content in green coffee beans

The beans were ground in a coffee mill using liquid nitrogen and particles separated using sieves and manual agitation. In this case, particles of small size (250-500 μ m) were selected in order to enhance the extraction of caffeine from solid matrix. Then, 3 g of sample were extracted with ethanol at 60°C in a Stuart Orbital S150 shaker apparatus (Bibby Scientific Limited, Stone, UK) during 80 h. Solvent was renewed at different intervals of time, and the extraction was finished when the caffeine extracted in the corresponding interval of time was around 2% of total caffeine extracted.

2.5 Determination of other bioactive substances in coffee extracts

Coffee oil. The content of lipid-type compounds present in the coffee beans extracts was obtained gravimetrically after hexane extraction. Base-catalyzed methanolysis (Vázquez et al., 2008) of these extracts was accomplished to determine the fatty acid profile. The GC-MS analyses (Agilent 7890A System - Agilent Technologies, Santa Clara, California, USA) were based on the method reported by Lu et al. (2004). The main FAMES present in the sample were identified by comparison with standard mass spectra from library (Wiley 229).

Total phenolic compounds (TPC). The presence of phenolic-type antioxidants was determined using the Folin-Ciocalteu reagent by the Singleton et al. (1999) method. The results were expressed as GAE (mg of gallic acid / g of sample). 3 mL of distilled water was mixed with 50 µL of sample or standard. Then, 250 µL of Folin-Ciocalteu reagent was added and the content of the tube was mixed thoroughly. After 3 min, 0.75 mL of Na₂CO₃ (20% mass) followed by 0.95 mL of distilled water was added and the mixture was allowed to stand for 2 h. The absorbance was measured at 760 nm.

3. Results and discussion

3.1 Extraction of caffeine from natural matter

Figure 2 shows the kinetic behavior of caffeine extraction from grounded coffee beans during 80 h of extraction in the Stuart Orbital S150 shaker apparatus, and renewing the solvent (ethanol) at different intervals of time. Taking into account these results, the content of caffeine in the green coffee beans was estimated to be 9.3 mg of caffeine / g coffee beans, which compares reasonably with the values reported for *Coffea arabica* variety in the literature (Ashihara and Crozier, 2001) (caffeine content in green beans ≈ 1 % mass).

Table 1 shows the yields obtained in the extraction of the entire and ground green coffee beans, using three different solvents, namely ethyl lactate, ethanol and ethyl acetate, at 100, 150 and 200°C. The caffeine concentration is also reported in the table for all samples collected. The average relative standard deviations of the N experiments (*ARSD*):

$$ARSD = \frac{1}{N} \sum \frac{SD_i}{\bar{x}_i}$$

were 9.4% and 7.7%, respectively, for extraction yield and caffeine content in the samples.

As expected, despite the solvent employed, extraction yield increased considerably with temperature. Further, the most significant increase of extraction yield with temperature was observed in the case of using ethyl lactate. In general, higher extraction yields were obtained when processing the ground beans than when employing the entire beans, although in the case of ethyl lactate at 200°C differences were not noteworthy.

Despite the solvent employed, the higher concentrations (mass % of caffeine in the extract) were obtained at 150°C. That is, although a narrow range of temperatures were explored, and for all solvents studied, it appears that the selectivity towards the extraction of caffeine increased with temperature up to a maximum and then decreased. Certainly, the higher concentrations of caffeine in the extracts were obtained with ethyl acetate, followed by ethanol and ethyl lactate. That is, among the solvents employed, ethyl acetate is definitely the most selective to extract caffeine from coffee beans.

Considering the value obtained for the content of caffeine in Arabica green coffee beans (9.3 mg / g beans) (Ashihara and Crozier, 2001), it can be concluded that high caffeine recovery was obtained (60%) using ethyl lactate at 200°C. These values are 30-40% higher in comparison to the values obtained with ethyl acetate, which is considered the greenest solvent for direct coffee decaffeination. Thus, ethyl lactate may be a viable ecological alternative liquid solvent for the extraction of caffeine from green coffee beans.

In order to test the capability of ethyl lactate to extract caffeine from other type of vegetable matter, the ASE extraction of green tea leaves was also accomplished. Table 2 shows the extraction yield, caffeine concentration and caffeine recovery obtained using ethyl lactate and ethanol solvents. Values reported for extraction yield and caffeine content are the average values obtained from duplicate experiments; the *ARSD* (see eq. 2) were, respectively, 4.3% and 3.6%.

Both solvents employed exhibit similar behavior regarding the extraction of caffeine. As expected, extraction yield increased considerably with temperature, with a remarkable increase at 200°C in the case of ethyl lactate particularly in the extraction of green coffee beans. As can be observed in Figure 3, the increases observed for entire and ground beans samples are, respectively, 4.8% and 1.5% when temperature increase from 100°C to 150°C, while these values are 8.5% and 3.7% when temperature increase from 150°C to 200°C. That is, a significant increase of the solvent power of ethyl lactate is observed when the extraction temperature became higher than normal boiling point of the solvent (154°C).

The concentration of caffeine in the green tea extracts decreased with increasing temperature, demonstrating that the removal of caffeine from the green tea leaves is much selective at the lower temperature investigated (100°C). Further, according to the reported values for the content of caffeine in green tea leaves (20-40 mg/g) (Park et al., 2007; Perva-Uzunalic et al.,

2006) it appears that the ASE using ethyl lactate may provide high removal of caffeine also from green tea leaves.

3.2 Analysis of the co-extraction of other bioactive substances from green coffee beans

As mentioned before, coffee oil and phenolic compounds (mainly chlorogenic acids) are also present in coffee beans and are valuable components of coffee due to their positive biological activity. Further, these substances have an important role during coffee roasting since the high temperatures provoke their transformation into key compounds of coffee flavor and aroma (Farah et al., 2006). Thus, it is desirable to reduce the removal of phenolic compounds and coffee oil during decaffeination.

The co-extraction of these compounds is described in Tables 3 and 4, where is reported the content (mass %) of total phenolic compounds (TPC) and lipid-type compounds (LTC) determined in the extracts. By increasing the extraction temperature, increasing concentrations of TPC were found in the samples (particularly in the case of ground coffee beans) while decreasing concentrations of LTC were determined. Further, considerably higher concentrations of LTC were extracted in the case of ground beans in comparison with entire beans. The main fatty acids identified in the samples were palmitic, linoleic and stearic acids (see Figure 4) in accordance with the literature (Dussert et al., 2008).

Taking into consideration the mean values reported in the literature for the content of phenolic acids and coffee oil in green coffee beans, that is, respectively, 46 mg (Alonso-Salces et al., 2009) and 108 mg (Oliveira et al., 2007) per gram of beans, the co-extraction of phenolic and lipid compounds was assessed and compared. Table 5 shows the recovery of TPC and LTC calculated for the different extracts obtained. For all solvents studied, including ethyl lactate, the co-extraction of phenolic compounds and coffee oil represent, respectively, less than 28% and 21% of the corresponding amounts present in the raw material. Particularly, ethyl lactate behavior is quite similar to that of ethanol. Further, ethyl lactate extracted similar amounts of coffee oil but almost twice amounts of phenolic compounds in comparison with ethyl acetate. These results may be attributed to the higher polarity of ethyl lactate in comparison with ethyl acetate, due to the hydroxyl group present in its chemical structure.

Conclusions

The potential use of ethyl lactate in the extraction of caffeine from natural matter, namely green coffee beans and green tea leaves, was presented in this work. Accelerated Solvent Extraction (ASE) of green coffee beans at 200°C provided higher caffeine recovery using ethyl lactate than when using ethyl acetate. Further, also high caffeine recoveries were obtained in the ASE of green tea leaves. Thus, ethyl lactate seems to be a good agrochemical solvent for the extraction of caffeine from vegetal sources.

In the case of the extraction of green coffee beans, preliminary study of the co-extraction of other important substances present in the natural matter also derived in encouraging the potential use of ethyl lactate. The co-extraction of phenolic compounds and coffee oil was, respectively, lower than 28% and 10%. Additional studies are under development in order to determine optimal conditions to selectively extract caffeine from tea leaves, minimizing the co-extraction of catechin-type bioactive substances.

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Table 1. Extraction yield (g of extract / g of beans \times 100), caffeine content (g caffeine / g extract \times 100) and caffeine recovery (mg of caffeine / g of beans) obtained in the ASE of green coffee beans using ethyl lactate, ethanol and ethyl acetate.

		extraction temperature		
		100°C	150°C	200°C
Solvent: ethyl lactate				
entire beans	extraction yield	0.31 ± 0.06	1.48 ± 0.02	13 ± 3
	caffeine content	2.6 ± 0.6	5.3 ± 0.5	4.3 ± 0.3
	caffeine recovery	0.08	0.78	5.36
ground beans	extraction yield	1.79 ± 0.03	2.75 ± 0.05	10.1 ± 0.1
	caffeine content	3.86 ± 0.03	6.2 ± 0.2	5.8 ± 0.2
	caffeine recovery	0.69	1.71	5.87
Solvent: ethanol				
entire beans	extraction yield	0.4 ± 0.2	1.41 ± 0.04	5.7 ± 0.6
	caffeine content	5 ± 2	7.5 ± 0.3	7.8 ± 0.5
	caffeine recovery	0.21	1.06	4.40
ground beans	extraction yield	2.4 ± 0.1	3.6 ± 0.1	8.7 ± 0.3
	caffeine content	4.8 ± 0.4	7.29 ± 0.01	6.8 ± 0.2
	caffeine recovery	1.16	2.59	5.93
Solvent: ethyl acetate				
entire beans	extraction yield	0.23 ± 0.03	0.38 ± 0.07	3.3 ± 0.3
	caffeine content	6.8 ± 0.2	16 ± 2	11.8 ± 0.2
	caffeine recovery	0.16	0.59	3.83
ground beans	extraction yield	1.57 ± 0.08	1.97 ± 0.07	4.5 ± 0.2
	caffeine content	6.0 ± 0.4	10.25 ± 0.07	10.2 ± 0.2
	caffeine recovery	0.94	2.02	4.54

Table 2. Extraction yield (g of extract / g of tea leaves \times 100), caffeine content (g caffeine / g extract \times 100) and caffeine recovery (mg of caffeine / g of tea leaves) obtained in the ASE of green tea leaves using ethyl lactate and ethanol.

		extraction temperature		
		100°C	150°C	200°C
Solvent: ethyl lactate				
	extraction yield	14.3 \pm 0.6	26 \pm 3	50 \pm 1
	caffeine content	8.4 \pm 0.3	7.2 \pm 0.5	4.5 \pm 0.2
	caffeine recovery	11.98	18.92	22.58
Solvent: ethanol				
	extraction yield	19.0 \pm 0.5	29.0 \pm 0.2	40 \pm 2
	caffeine content	10.3 \pm 0.3	8.1 \pm 0.1	5.9 \pm 0.2
	caffeine recovery	19.62	23.45	23.91

Table 3. Total phenolic compounds (TPC) in green coffee beans extracts measured using the Folin & Ciocalteu reagent (g of gallic acid equivalents / g of extract \times 100). *ARSD* (average relative standard deviation) = 4.12 %.

	extraction temperature		
	100°C	150°C	200°C
ethyl lactate			
entire beans	2.51	5.31	8.67
ground beans	10.64	11.77	12.67
ethanol			
entire beans	5.84	5.87	13.94
ground beans	8.97	9.84	13.91
ethyl acetate			
entire beans	5.32	8.94	17.44
ground beans	3.07	5.71	15.74

Table 4. Content of lipid-type compounds (LTC) (g oil / g extract \times 100) obtained in the green coffee beans extracts using ethyl lactate, ethanol and ethyl acetate. *ARSD* (average relative standard deviation) = 7.71 %.

	extraction temperature		
	100°C	150°C	200°C
ethyl lactate			
entire beans	12.9	3.2	4.7
ground beans	48.4	36.9	10.4
ethanol			
entire beans	17.4	8.8	6.6
ground beans	41.8	31.2	25.6
ethyl acetate			
entire beans	16.0	17.9	13.6
ground beans	79.7	60.6	29.0

Table 5. Recovery^a of total phenolic compounds (TPC) and lipid-type compounds (LTC) (g extracted / g of beans x 100) in the ASE of green coffee beans using ethyl lactate, ethanol and ethyl acetate.

			extraction temperature		
			100°C	150°C	200°C
Solvent: ethyl lactate					
entire beans	TPC		0.2	1.7	23.7
	LTC		0.4	0.4	5.5
ground beans	TPC		4.1	7.0	27.8
	LTC		8.0	9.4	9.7
Solvent: ethanol					
entire beans	TPC		0.6	1.8	17.1
	LTC		0.7	1.1	3.5
ground beans	TPC		4.8	7.6	26.2
	LTC		9.4	10.3	20.6
Solvent: ethyl acetate					
entire beans	TPC		0.3	0.7	12.3
	LTC		0.3	0.6	4.1
ground beans	TPC		1.0	2.4	15.3
	LTC		11.6	11.1	12.0

^a TPC and LTC present in raw material were taken from the literature (Alonso-Salces et al., 2009; Oliveira et al., 2007)

Figure captions

Figure 1. Scheme of ASE device employed in the extraction of caffeine from green coffee beans and green tea leaves.

Figure 2. Total caffeine recovered in 3 g of green coffee beans: kinetic behavior of caffeine extraction using ethanol at 60°C (ambient pressure) in a Stuart Orbital shaker.

Figure 3. Extraction yield obtained in the ASE extraction of green coffee beans and green tea leaves using ethyl lactate. (■) entire coffee beans; (●) ground coffee beans and (▲) tea leaves.

Figure 4. Fatty acid profile obtained by GC analysis of green coffee beans (entire samples) ASE extracts obtained at 100°C, 10 MPa and using ethyl acetate.

Figure 1.

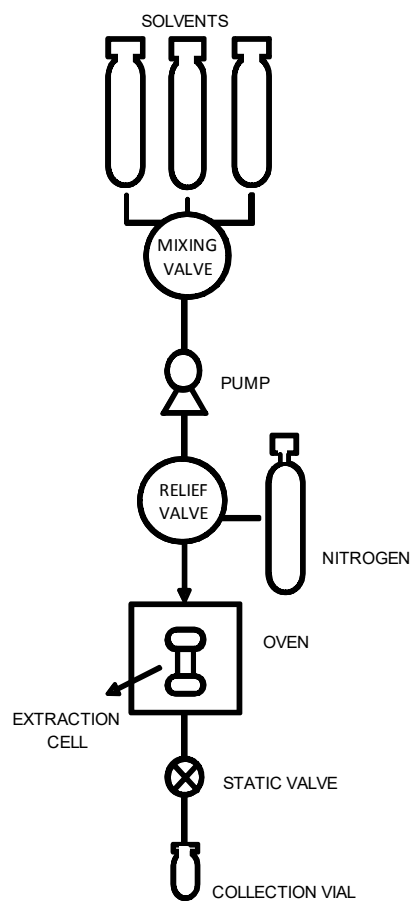


Figure 2.

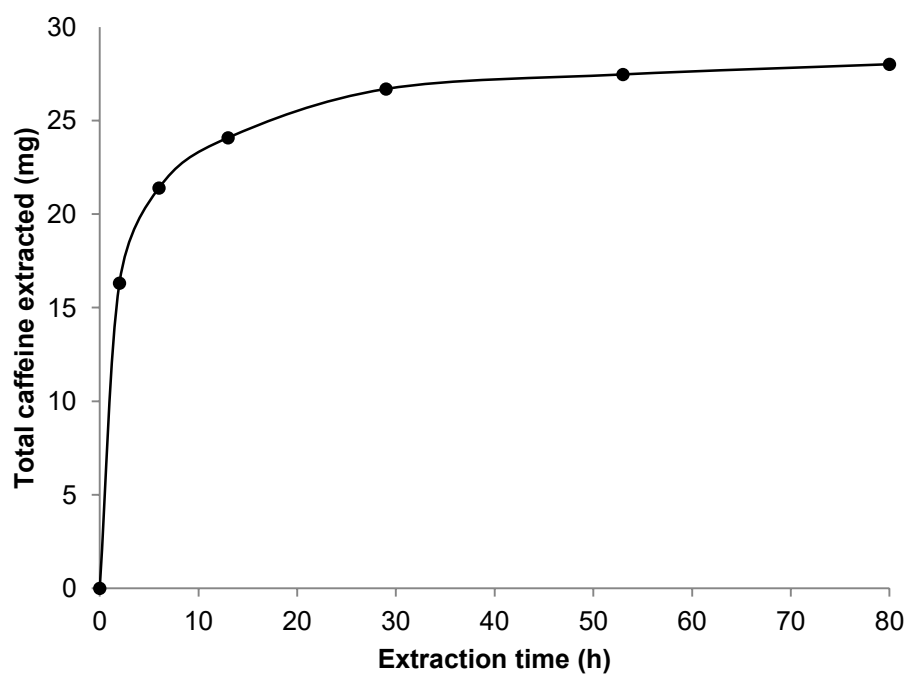


Figure 3.

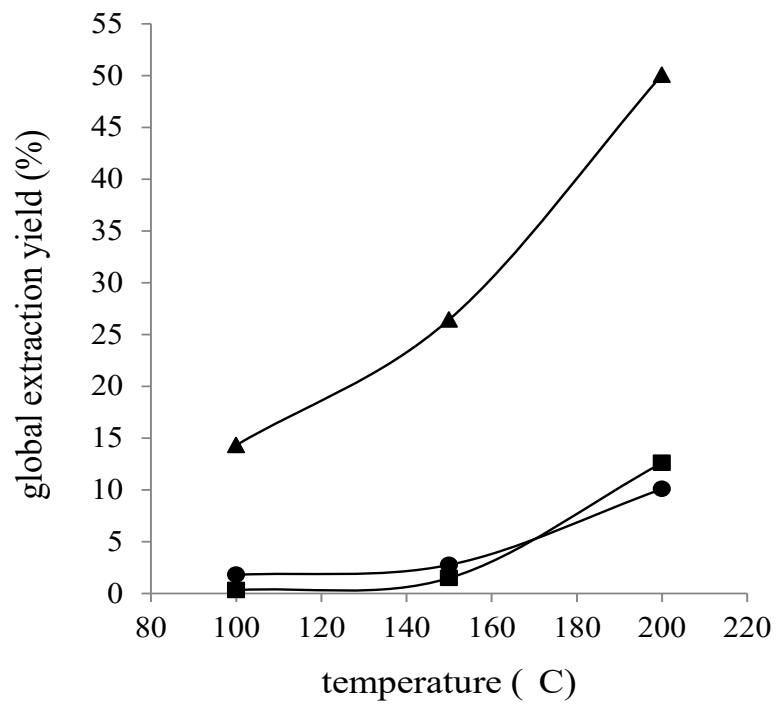


Figure 4.

