



High catechins/low caffeine powder from green tea leaves by pressurized liquid extraction and supercritical antisolvent precipitation



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ARTICLE INFO

Article history:

Received 2 March 2015

Received in revised form 20 April 2015

Accepted 23 April 2015

Available online 1 May 2015

Keywords:

Green tea

Decaffeination

Ethyl lactate

Pressurized liquid extraction

Supercritical antisolvent process

ABSTRACT

This paper reports a method to produce a solvent-free extract from green tea leaves with high content of catechins and low content of caffeine, in two steps and using only “green” solvents. The method is based on the pressurized liquid extraction (PLE) of the green tea leaves using ethyl lactate as solvent, followed by a selective precipitation procedure using the supercritical carbon dioxide (SCCO₂) antisolvent (SAS) technique.

PLE was accomplished at 100 °C and 10 MPa, on the basis of a previous work. The influence of pressure (15–30 MPa) and temperature (50 °C and 70 °C) on the SAS precipitation process was experimentally studied in terms of precipitation yield, concentration of key bioactive compounds (caffeine and monomeric catechins) and total content of phenols of the precipitates. Additionally, a comparison using a different organic solvent (ethanol) for the extraction and precipitation steps is reported.

The precipitates obtained from the ethyl lactate PLE extracts were decaffeinated (giving less than 1% mass caffeine in the dry matter) and the concentration of catechins was close to 23% mass. The total phenolic content of the precipitates was up to 590 mg of gallic acid equivalents per g of precipitate, which represents an increase of up to 25% with respect to the PLE extracts.

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1. Introduction

Tea is obtained from the leaves of the plant *Camellia sinensis* being green tea one of the most consumed types of tea. Historically, green tea has been consumed in Oriental countries like China and Japan, but its consumption in Europe and United States has increased in recent years due to its potential health benefits. Moreover, green tea extracts are also used as ingredients in other beverages (e.g. ready-to-drink beverages), in ice-creams, etc. Green tea leaves contain several bioactive compounds, such as methylxanthine alkaloids and phenolic compounds. Caffeine is the most abundant alkaloid in green tea, being the main responsible for the stimulating effects of tea. Caffeine content in tea leaves is usually around 2–5% mass of the dry weight [1–3]. Some adverse well-known effects derived from caffeine consumption include sleep deprivation, rise in blood pressure, tachycardia, abortion and miscarriages, depending on the intake concentration [4–6].

Regarding phenolic compounds, green tea is a very rich source of polyphenols (up to 30% mass of tea solids) and the major phenolic compounds are catechins [7–9]. Moreover, the main green tea catechins (flavan-3-ols) are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG), usually being EGCG the principal catechin present in green tea leaves (50–80% of total catechins in tea) [10].

Besides the contribution of catechins to tea taste, important pharmacological properties have been associated to their consumption, including antioxidant [11], anticancer [10,12], anti-inflammatory [13], antiaging [14], anti-hypercholesterolemic activity [15], antibiotic and antiviral effects [16,17].

Due to these beneficial properties of catechins and the adverse effects of caffeine, several methods have been studied to produce green tea extracts with high catechin concentration but free of caffeine. Chlorinated solvents, such as chloroform, have been used to isolate catechin compounds free from caffeine by sequential fractionation of aqueous tea extracts [18]. Chlorinated solvents are effective solvents to extract caffeine but their use has been severely reduced owing to its toxic solvent residues.

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Alternatively, Dong et al. [19] used ethyl acetate (a so-called “green” solvent, of low environmental impact [20]) to isolate catechins from an aqueous green tea extract and the extract was subsequently treated with citric acid solution to remove the caffeine.

Other methods involve fractionation of a green tea extract by selective precipitation of catechins with a precipitation agent [21] and different adsorption separation processes. Several adsorbents have been tested, such as lignocellulose [22,23] and lignocellulose copolymerized with N-vinylpyrrolidone [24], activated carbon [25], polyvinylpyrrolidone [26,27], polyamide [28], poly(acrylamide-co-ethylene glycol dimethacrylate) [29] and different macroporous polymeric resins [30,31].

Despite the fact that substantial amounts of caffeine may be removed, many steps are needed starting from the tea leaves, large quantities of solvents are used and the loss of catechins in some cases is appreciable. Moreover, a final additional step is needed in all cases to remove the solvent and produce a dry product ready to use.

On the other hand, supercritical carbon dioxide (SCCO₂) has been used as antisolvent to precipitate food ingredients that are initially dissolved in organic solvents [32–35]. The solute is usually a single compound and the SAS technique is applied with the aim to produce particles of controlled morphology. In the supercritical antisolvent (SAS) technique, the liquid solvent and the antisolvent are miscible, while the solute is not soluble in the supercritical mixture. Upon contact, CO₂ removes the organic solvent from the liquid mixture, leaving a dry solute behind. Precipitation is dictated among other things by the phase behavior of the system, which is a reflection of solute–solvent–antisolvent interactions. Thus, if the initial solution is not of a single compound but a mixture, it is possible that not all solutes precipitate at the same process conditions. In this case, controlling particle morphology is less relevant, while the principle of selective precipitation can be exploited as a way to enrich the precipitate in the compounds of interest. This is a powerful idea that leads to reduced number of steps in purification and drying procedures. The concept has been applied by several authors in the fractionation of natural products [36–40], although it has not been reported for the decaffeination of tea extracts.

In the case of green tea, Sosa et al. [41] used SCCO₂ as antisolvent to precipitate catechins from an extract in the presence of an encapsulating agent. The extract had been previously obtained by microwave-assisted extraction using acetone as the solvent. The aim of the work was not to enrich the extracts in a particular compound but to evaluate the encapsulation efficiency. Nevertheless, the authors mentioned that around 13% of the extracted caffeine was present in the precipitate while more than 90% of the EC and ECG was recovered for one of the experiments, which evidences that the precipitate had been enriched in catechins. However, not enough data was presented to evaluate the selectivity of the extraction, precipitation and the quality of the final product.

In a similar line of research, we have recently reported the use of ethyl lactate for the selective decaffeination of green tea leaves [42] by pressurized liquid extraction (PLE). Ethyl lactate was selected as extractive solvent since it is a bio-renewable agrochemical solvent, environmental benign and permitted by the U.S. Food and Drug Administration (FDA) as pharmaceutical and food additive. The extracts obtained by this method contained both catechins and caffeine, but the extraction was proven to be selective toward caffeine indicating that caffeine is more soluble than catechins in ethyl lactate. Based on these results we speculated that a mixture ethyl lactate/CO₂ would dissolve caffeine more than catechins and lead to selective precipitation. The aim of this work was to evaluate the use of the SAS technique employing SCCO₂ as antisolvent to precipitate catechins from an ethyl lactate extract while removing caffeine and the organic solvent. For comparison, data

using ethanol instead of ethyl lactate is also reported. The selectivity of the process is discussed in terms of precipitation yield and catechins/caffeine mass ratio of the precipitates.

2. Material and methods

2.1. Samples and reagents

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

Ultrapure CO₂ was provided by Air Products (Bochum, Germany). Ethyl lactate (99% purity) was obtained from Alfa-Aesar (Ward Hill, MA, USA). Acetonitrile (HPLC grade) and phosphoric acid (≥98% purity) were obtained from Merck (Darmstadt, Germany) and ethanol (99.7% purity) from Solveco AB (Stockholm, Sweden). Folin–Ciocalteu reagent was purchased from Sigma–Aldrich (St. Louis, MO, USA).

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

The total content of caffeine and catechins in the vegetal material was measured using an exhaustive method as reference [2]. For this purpose, 200 mg of green tea leaves were extracted at 70 °C with 20 mL of an aqueous ethanol solution (30% v/v) 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 until the extraction yield in the corresponding cycle was lower than 2% of total yield. The content of caffeine and catechins determined by HPLC was, respectively, 22.4 mg and 82.5 mg per g of green tea leaves.

Even though this extraction method has very likely induced degradation of the catechins to a certain extent [43], it was chosen as it is similar to the ISO standard procedure for the determination of total content of catechins in green tea leaves [44] and to other exhaustive methods reported in literature [1,45,46]. Furthermore, we carried out extractions at the conditions described in the present paper using different extraction times (0.5 h, 1 h, 2 h y 4 h) and we observed the highest caffeine and catechins extraction yield after 4 h.

2.2. Pressurized liquid extraction

Extractions were carried out in an Accelerated Solvent Extraction system ASE 200 from Dionex Corporation (Sunnyvale, CA, USA).

Based on previous results [42], PLE assays with ethyl lactate and ethanol were carried out at 10 MPa and 100 °C, considering that this temperature ensures minimal thermal degradation of catechins while providing high concentration of catechins in the extract. The solid vegetal sample was dispersed with sea sand (vegetal material/sand ratio = 1 g/g).

The experimental procedure was as follows: the cells employed (11 mL capacity) were filled with the corresponding amount of sample and dispersant and automatically placed in an oven. Each cell was filled with the solvent up to the set pressure and was heated-up to the desired temperature. Then, a batch extraction was carried out for 20 min and afterwards the cell was washed with fresh solvent. The solvent was subsequently purged using nitrogen gas until the complete depressurization of the system. The extracts produced were stored under refrigeration until use.

The extracted amount of solutes is expressed as mg of dry extract per g of dry tea leaves. In Table 1, the concentration values

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