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Modeling and optimization of green tea precipitation for the recovery of catechins

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ABSTRACT

Green tea catechins are claimed to have several health benefits (e.g. antioxidant, antimutagenic and antiviral) with increasing applications in the food and pharmaceutical markets. By using the tea creaming effect and by enhancing it, as a phase separation via precipitation, it is possible to recover a large amount of polyphenols from the cream phase without using toxic solvents.

A design of experiments (DoE) together with statistical analysis allows a description of the system with polynomial models and enables the determination of the optimal conditions that maximize the catechins recovery, while minimizing the amount of caffeine, which is considered a contaminant. A total of four influence factors are studied in this DoE: hydroxypropylmethylcellulose and polyvinylpyrrolidone (used as precipitation agents), temperature and pH, of which only pH is found not to be significant.

With the optimal combination of factors it is possible to separate and recover up to 67% of the catechins present in the green tea extract solution, while increasing the ratio of catechins/caffeine by 60%.

1. Introduction

Tea polyphenols, especially the flavanoids like catechins, their dimeric and oligomeric oxidized forms (theaflavins and thearubigins) but also flavonolglycosides, are mainly responsible for the organoleptic properties of tea, including color and taste [1]. Green tea is the type of tea with the highest content of catechins, including the four main catechins: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) (see Fig. 1).

Green tea catechins have been associated to several health benefits due to the *in vitro* antioxidant, antimutagenic and antiviral effects [2–5]. They are, therefore, regarded as desired components with several applications in a variety of areas such as foods, cosmetics and pharmaceuticals. The increased interest in green tea polyphenols for use as food ingredients or health supplements has led to more research into separation and purification of these components from the tea solution. There are, however, other components present in a tea extract (such as proteins, carbohydrates, and caffeine) which need to be separated from the catechins. The separation of some of the tea components is not a straightforward process, due to the similarities in molecular size and solubility.

Most of the research about polyphenols separation focuses on the extraction steps [6–8] and although there is previous research about the tea cream effect as a separation route for the tea catechins [9,10], there is no research using multiple precipitation mechanisms and that can generate models for the separation and isolation of catechins.

Tea cream formation occurs after the extraction process upon cooling, as some components differ considerably in solubility, both in hot and cold water. This natural occurring precipitation phenomenon is considered to be caused by interaction of extracted natural polymeric molecules, like proteins and pectins, and complex formation of these with small molecules like catechins, other polyphenols (e.g. flavonol glycosides) and caffeine [11]. Above a certain solids concentration, spontaneous demixing is the predominant mechanism of cream formation, showing the substantial insolubility of polyphenols at low temperature [12].

Several factors have been identified in literature that influence the amounts of catechins and caffeine in the cream and clear phases when the precipitation takes place. The following separation inducing factors are tested:

- Addition of a salt.

The addition of aluminum chloride to a green tea aqueous solution, causes the precipitation of green tea catechins and could be used for their isolation [13]. Zinc chloride is also mentioned in

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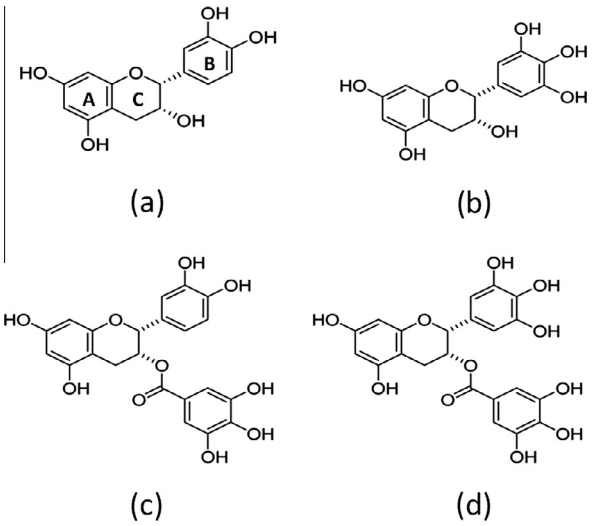

Fig. 1. Catechins chemical structure; (a) epicatechin (EC), (b) epigallocatechin (EGC), (c) epicatechin gallate (ECG) and (d) epigallocatechin gallate (EGCG).

literature for the formation of aggregates of Zn^{2+} and phenolic hydroxyl groups in the basic pH range [14]. An inorganic salt (ammonium sulfate) can also be used for polyphenol precipitation, since it is one of the best known salts for precipitation by salting out [15].

- Addition of a precipitation agent.

  Polyvinylpyrrolidone (PVP) is a commonly used as precipitating agent for specific polyphenolic compounds (including catechins) [16], due to the hydrophobic interactions and the formation of intermolecular hydrogen bonds between the hydroxyl group (—OH) of the polyphenol and the carbonyl group (—C=O) of PVP [17]. Methylcellulose (MCL) is used for precipitation of tannins and for several precipitation-based assays, with the additional advantage of being non-toxic and food-grade [18,19]. Tang and co-workers found that the polyphenol interactions with cellulose are positively correlated to the molecular size, the number of galloyl groups and the hydrophobicity of the polyphenols [20].

- Temperature: green tea cream formation is characterized by the separation into immiscible phases below an upper critical solution temperature and is dependent on the amount of solids in the tea solution and on the temperature [12,21].
- pH can also influence the amount of cream formation and the amount of polyphenols in each phase [11]. However, due to the low stability of green tea catechins in neutral or alkaline pH conditions, only the acidic pH conditions are tested [22].

Due to the high complexity of a green tea aqueous solution and tea cream formation, in terms of the amount and diversity of components, together with their multiple interactions, it is very difficult to fully characterize and model such a system. In our opinion, the use of design of experiments (DoE) coupled with statistical analysis is a very efficient way to design and model a procedure to separate the key components. The DoE also produces a higher knowledge (including interaction effects), when compared to the more usual approach of studying one or two factors at a time. In combination with statistical analysis allows the determination of the parameters and/or combinations of parameters, which significantly influence the selected responses [23].

A design of experiments (DoE) based on a response surface methodology (RSM) and using a Box–Behnken design, is selected to describe the operational window (design space) with polynomial models, setting the objective of maximizing the amount of catechins and minimizing the amount of caffeine in the cream phase.

Screening experiments have been performed to select the stronger effect (addition of a salt or addition of a precipitation agent) that influences the objective. Two responses are defined for the outcome of the DoE: yield of catechins (Y_{cat}) and yield of caffeine (Y_{caff}).

2. Experimental

2.1. Reagents and equipments

Acetonitrile, EDTA and citric acid are analytical grade and obtained from Sigma–Aldrich. Zinc chloride and aluminum chloride (⩾99% purity) are from Fluka analytical. Glacial acetic acid (HPLC grade), ethanol absolute and NaOH solutions (0.5 N) are purchased from Merck KGaA. Polyvinylpyrrolidone (PVP) with an average molecular weight of the polymer of 10,000 (PVP-10), Methylcellulose (MCL) with a viscosity of 0.4 Pa s, Hydroxypropylmethylcellulose (HPMCL) with a viscosity of 0.08–0.1 Pa s, ammonium sulfate (⩾99% purity) and Polyvinylalcohol (PVA) are also obtained from Sigma–Aldrich. The individual polyphenol standards and the freeze-dried dry green tea powder are supplied by Unilever R&D. All the water used in the experiments is Milli-Q gradient (Millipore). The ultracentrifuge is from Beckman Coulter (Optima L-90K) and the pH meter is from Inolab (WTW series-pH 730).

2.2. Experimental method

Freeze-dry green tea powder is dissolved in 30 ml water at 85 °C in an Erlenmeyer, with stirring for 10 min, to prepare an aqueous green tea solution with a solids content of 4% (w/w). The salt(s) or the precipitation agent(s) are then added to the tea solution, with stirring, followed by the pH adjustment (with a NaOH solution or with citric acid).

The tea mixture is then cooled at a constant rate (average rate: 1.5 °C/min) until it reaches the target temperature of the experiment. Afterwards the tea is centrifuged for 25 min at 20,000 rpm at the target temperature. The clear phase is then decanted from the cream fraction and both fractions are weighted. The cream phase is then extracted with 20 ml of ethanol at 20 °C for 2 h (with stirring). The tea extract, clear phase as well as the ethanol extracts of the cream phase are afterwards analyzed separately via HPLC, for catechins and caffeine content, and the values presented as yield of catechins in the cream (Y_{cat}) and yield of caffeine in the cream (Y_{caff}).

$$Y_{caff}(\%) = \frac{mass\ caffeine\ in\ cream\ phase}{mass\ caffeine\ in\ extract\ phase} * 100 \quad (1)$$

$$Y_{cat}(\%) = \frac{mass\ catechins\ in\ cream\ phase}{mass\ catechins\ in\ extract\ phase} * 100 \quad (2)$$

2.3. Analytical methods: HPLC analysis

Caffeine and individual catechins concentrations are determined by HPLC analysis on an Agilent 1220 Infinity LC gradient system equipped with a variable wavelength detector, which is set at a wavelength of 278 nm. The analysis is performed in a phenyl hexyl column (Luna 5 μm Phenyl-Hexyl, 100 Å, LC Column 250 × 4.6 mm, from Phenomenex), by gradient elution at 30 °C, using 2% acetic acid in water (v/v) (eluent A) and 2% acetic acid