Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Optimizing the extraction parameters of epigallocatechin gallate using conventional hot water and ultrasound assisted methods from green tea

Sena Saklar Ayyildiz[a,*], Bulent Karadeniz[a], Nihan Sagcan[b], Banu Bahar[a], Ahmet Abdullah Us[c], Cesarettin Alasalvar[a]

[a] TÜBİTAK Marmara Research Center, Food Institute, P.O. Box 21, 41470, Gebze, Kocaeli, Turkey
[b] Çorlu Vocational School, Namik Kemal University, TR 59860, Çorlu, Tekirdağ, Turkey
[c] Department of Food Engineering, Yıldız Technical University, TR 34210, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 25 December 2017
Received in revised form 24 May 2018
Accepted 4 June 2018
Available online 18 June 2018

Keywords:
Green tea
Catechins
Epigallocatechin gallate
Ultrasound extraction
Hot water extraction
Response surface methodology

ABSTRACT

Epigallocatechin gallate (EGCG) is the main polyphenol present in green tea and is also known to possess various beneficial health effects. Extractions of EGCG, epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) from green tea were investigated by both conventional hot water extraction and ultrasound assisted extraction (UAE) with water and ethanol. Response Surface Methodology was used in order to optimize the extraction conditions for obtaining maximum amount of EGCG in the extracts. Pilot scale conventional hot water extraction and UAE with water in continuous flow mode were also conducted for industrial use. UAE with ethanol was significantly ($p < 0.05$) efficient to extract more EGCG, EGC, ECG, and EC than conventional hot water extraction and UAE with water. At optimum conditions, almost 100 and 50% more EGCG content was obtained in UAE with ethanol than conventional hot water extraction and UAE with water, respectively. The optimal conditions for UAE of EGCG with ethanol were 66.53 °C, 43.75 min and, 67.81% ethanol. Conventional hot water extraction can easily be scaled up to a pilot scale and can be used for green tea beverage production. UAE should also be scaled up to a pilot scale by an efficient design either using an ultrasound probe in a larger volume continuously or ultrasound probes on each side of the extractor tank with an agitation system.

1. Introduction

The green tea catechins are among the strongest natural antioxidants. They are the most biological active groups of the tea components, which are thought to contribute to the health benefits of green tea. They can also account for 30–40% of the extractable solids of dried green tea leaves. Major catechins in green tea are: (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG), among which EGCG is the major catechin with most bioactive and cancer preventive properties (Singh et al., 2011; Ju et al., 2007). Epicatechins can change to their epimers that are non-epicatechins, which are known as: (−)-gallocatechin gallate (GCG), (−)-catechin gallate (CG), (−)-gallocatechin (GC), and (+)-catechin (C) (Banerjee and Chatterjee, 2015).

Extraction is the major step in obtaining catechins from green tea. Different techniques have been used to extract the bioactive compounds from green tea, including maceration, infusion, microwave assisted extraction (Li and Jiang, 2010), ultrasonic assisted extraction (UAE) (Lee et al., 2013), supercritical carbon dioxide (Chang et al., 2000), soxhlet extraction and ultrahigh pressure extraction (Jun et al.,

* *Corresponding author.*
E-mail address: sena.saklar@tubitak.gov.tr (S. Saklar Ayyildiz).
https://doi.org/10.1016/j.fbp.2018.06.003

Table 1 – Coded[a] and actual values of the independent variables, experimental data[b] (g/100 g) and regression equation[c,d] coefficients and regression analysis of catechin contents for conventional hot water extraction.

	Coded and actual values			EGCG	EGC	ECG	EC	Coefficient	EGCG	EGC	ECG	EC
	A (T, °C)	B (t, min)	C (R, ml/g)									
1	−1(75)	−1(15)	−1(15)	2.98	1.89	0.50	0.35	b_0	−7.19	−3.75	−0.15	−4.31
2	−1(75)	+1(45)	+1(25)	3.38	2.12	0.55	0.40	b_1	0.17*	0.08	0.04*	0.10*
3	0(80)	0(30)	0(20)	3.56	2.21	0.59	0.42	b_2	0.15*	0.13*	0.01*	$9.8 \times 10^{-3*}$
4	+1(85)	+1(45)	−1(15)	3.72	2.12	0.67	0.42	b_3	−0.04	0.08	-9.1×10^{-3}	0.03
5	0(80)	0(30)	0(20)	3.57	2.22	0.59	0.42	b_{11}	-7.3×10^{-4}	-3.7×10^{-4}	-2.2×10^{-4}	$-6.1 \times 10^{-4*}$
6	+1(85)	−1(15)	+1(25)	3.76	2.26	0.64	0.40	b_{22}	$-7.1 \times 10^{-4*}$	$-2.7 \times 10^{-4*}$	-6.3×10^{-5}	$-5.5 \times 10^{-5*}$
7	−1(75)	+1(45)	−1(15)	3.25	2.16	0.54	0.39	b_{33}	6.8×10^{-4}	$-2.2 \times 10^{-3*}$	-2.2×10^{-4}	-3.0×10^{-4}
8	+1(85)	+1(45)	+1(25)	3.41	1.81	0.62	0.39	b_{12}	-9.0×10^{-4}	$-1.1 \times 10^{-3*}$	-4.7×10^{-18}	-3.3×10^{-5}
9	−1(75)	−1(15)	+1(25)	2.95	1.91	0.48	0.37	b_{13}	7.0×10^{-4}	6.5×10^{-4}	3.0×10^{-4}	-1.0×10^{-4}
10	+1(85)	−1(15)	−1(15)	3.21	1.84	0.54	0.36	b_{23}	-1.2×10^{-3}	$-1.3 \times 10^{-3*}$	-2.0×10^{-4}	-1.3×10^{-4}
11	0(80)	0(30)	0(20)	3.45	2.03	0.58	0.41	R^2	0.92	0.98	0.91	0.88
12	0(80)	0(30)	0(20)	3.43	2.06	0.57	0.43	F	10.43	39.01	8.74	6.79
13	−1.682(72)	0(30)	0(20)	2.98	1.91	0.45	0.31	P	<0.05	<0.05	<0.05	<0.05
14	0(80)	−1.682(5)	0(20)	2.48	1.62	0.41	0.32					
15	0(80)	0(30)	0(20)	3.29	1.74	0.55	0.35					
16	0(80)	+1.682(55)	0(20)	3.40	1.83	0.62	0.39					
17	+1.682(88)	0(30)	0(20)	3.70	1.83	0.63	0.39					
18	0(80)	0(30)	0(20)	3.45	1.94	0.59	0.40					
19	0(80)	0(30)	+1.682(28)	3.57	1.76	0.53	0.39					
20	0(80)	0(30)	−1.682(12)	3.29	1.89	0.56	0.38					

Abbreviations: EGCG: Epigallocatechin gallate, EGC: Epigallocatechin, ECG: Epicatechin gallate, and EC: Epicatechin.

[a] A: temperature, T (°C), B: time, t (min), and C: tea to water ratio, R (ml/g).

[b] Data are expressed as mean of three measurement on a dry weight basis.

[c] $Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$ where A: temperature (°C), B: time (min), and C: tea to water ratio (ml/g).

[d] Significance level = *$p < 0.05$.

2011), among others. UAE is preferred mode of tea catechins due to the increased efficacy of extraction process at lower temperature by retaining their antioxidant activity. Extraction parameters such as solvent, temperature, time, ratio of material to solvent and variety of tea influence efficiency of an extraction process. Emergence of novel techniques eases the extraction process, minimize extraction cost, time, and maximize the yield. Simultaneously, it is tried to retain maximum antioxidant potential and sensory attributes namely colour, flavour, sweetness reducing proteins and pectin contents (Banerjee and Chatterjee, 2015).

The catechins are polar compounds and solubilize in polar organic solvents such as ethanol, methanol, water, and acetone. During extraction using polar organic solvents, other components such as caffeine and chlorophyll also get extracted (Gadkari and Balaraman, 2015). Perva-Uzunalic et al. (2006) reported that the highest extraction efficiency of catechins was obtained by extraction with 50% aqueous acetone as 99.3%, with 50% acetonitrile as 99.8% and with 80% ethanol as 89.1%, respectively. There are numerous studies showing that hot water and ethanol are effective in extracting catechins from green tea. Vuong et al. (2011) studied the extraction of catechins from green tea using hot water, they showed that the best temperature and time combination for catechin extraction was at 80 °C for 30 min. Liang et al. (2007) also reported that the concentrations of EGCG increased with extraction temperature up to 80 °C and then decreased at 100 °C due to epimerisation reactions. They found that EGCG concentration was 55.8 mg/g tea at 80 °C extraction. They also studied the ethanol extraction and found that, the concentration of EGCG extracted by 50% ethanol, was 26.3% higher than those extracted in water at 80 °C. Moreover, Choung et al. (2014) determined 40% ethanol solution with UAE for optimal extraction of catechins and caffeine.

Due to the growing environmental problems and the adverse health effects of organic solvents, water is preferred as a cheap and safe solvent, and is widely used for producing green tea beverages. Ethanol is generally preferred to produce green tea extract powder. To obtain the maximum efficiency from conventional hot water extraction and UAE, extraction conditions of catechins need to be optimized and easily scaled-up. Therefore, the aim of this study was to optimize the parameters of conventional hot water and UAE with water and ethanol for green tea to maximize the EGCG concentration by using Response Surface Methodology (RSM) and to scale up a pilot scale extraction for industrial use.

2. Material and methods

2.1. *Chemicals*

Epigallocatechin gallate (95%), epigallocatechin (95%), epicatechin gallate (98%), epicatechin (98%), methanol (99.9%), ethanol (99.8%), acetonitrile Chromasolv® for HPLC (≥99.9%), and ethylenediaminetetraaceticacid (EDTA) were obtained from Sigma Aldrich (Bo&Ga, Kaplan Metal, Prolab, and Interlab Co., Istanbul, Turkey.) Ascorbic acid and acetic acid were purchased from VWR Chemicals (Prolab, Istanbul, Turkey). Ethanol (96%) was obtained from TEKKIM Chemicals (Bursa, Turkey). Standard reference material (SRM 3255), *Camellia sinensis* Extract – Green Tea – was obtained from Sigma Aldrich Co. (National Institute of Standards & Technology-NIST, MD, USA).

2.2. *Samples*

Green tea was procured from the ÇAYKUR Green Tea Processing Plant (Rize, Turkey), at the beginning of the first harvest season of June 2014. Green tea having a particle size between 1–1.6 mm and moisture content 2.7% was used for the extractions.

2.3. *Experimental design*

RSM was used to optimize the extraction parameters of EGCG and other catechins present in green tea. A 3-factor, 5-level Rotatable Central Composite Design (CCD) was used for conventional hot water extraction and UAE with ethanol. The