Subcritical Water Extraction of Phenolic Compounds from Black Tea Processing Waste

Neslihan KAYRA, Gizem ÖZAN, Ezgi ÖZCAN, Sevcan ERŞAN F. Yeşim EKİNCİ, Özlem GÜÇLÜ-ÜSTÜNDAĞ*

Yeditepe University, Department of Food Engineering, 34755, Istanbul, Turkey
ozlemg.ustundag@yeditepe.edu.tr & Fax: +90 216 578 0004

ABSTRACT

Black tea processing waste (BTPW) can be used as a raw material for the recovery of bioactive phenolic compounds. High temperatures used in subcritical water (SCW) extraction, a green alternative to traditional methods, results in higher extraction efficiency but might result in modification of extract composition due to degradation reactions. Therefore, extraction temperature should be optimized considering yield and composition/bioactivity of extracts. In this study, SCW extraction of BTPW was investigated at 90-180°C to determine the effect of temperature on the phenolic profile and antioxidant activity of extracts. BTPW samples (2g), obtained at a black tea factory in the Black Sea region of Turkey in July 2012, were extracted using a built in-house SCW extractor (extraction column: 1/2'' o.d × 20 cm) at 90, 120, 150, and 180ºC for 30 min using a flow rate of 2 mL/min. Caffeine and phenolic (gallic acid (GA), catechins and theaflavin) content of SCW extracts were analyzed using HPLC-PDA. Antioxidant activity of SCW extracts was also investigated by ABTS radical cationdecolorization assay. Extraction yield (%w/w) increased from 22.06±2.58 to 36.35±3.2 with a temperature increase from 90 to 180°C. Caffeine, GA, catechin (C), epicatechin (EC), epicatechingallate (ECG), epigallocatechingallate (EGCG), and gallocatechingallate (GCG) were detected in the SCW extracts. While caffeine content of SCW extracts decreased with increasing temperature, GA content increased from 6.47±1.16 mg/g extract at 90°C to 17.39±1.14 mg/g extract at 180°C due to degradation of phenolic compounds such as theaflavins. The increase in GCG content at 120-150°C, which was accompanied by a decrease in EGCG content of the extracts can be attributed to epimerization reactions. Total catechin content increased with temperature up to 150°C whereas a sharp decrease was observed at 180°C. SCW extracts at 180°C were mainly composed of GA (17.39±1.14 mg/g extract) and caffeine (39.14±0.03 mg/g extract). Antioxidant activity of SCW extracts, which ranged between 2.75±0.5 and 3.61±0.3 mg Trolox Equivalent (TE)/mL extract, showed an increasing trend with temperature due to the increase of extraction yield. In conclusion, SCW extraction of BTPW at high temperatures may provide opportunity to obtain extracts with different compositions and bioactivities.

INTRODUCTION

Generally, by-products of food industry are not reused as raw material for other products, in many cases; they are used as animal feed or diverted to landfill. Recently, there has been increasing interest on the utilization of these streams as a source of bioactive compounds for the development of added value products such as food ingredients and nutraceuticals [1, 2]. Black tea is one of the most popular beverages worldwide and Turkey is one of the biggest black tea producers in the world. Black tea processing waste, which does not have commercial value and is usually burned or used as compost, can be used as a source of bioactive phenolic compounds [3].

Subcritical water (SCW) extraction involves the use of water as a solvent at temperatures higher than its normal boiling point (100°C) by the use of pressure. The polarity of SCW decreases with increasing temperature giving it a similar solvent power to that of organic solvents such as ethanol and methanol. The modified solvent power in addition to enhanced mass transfer properties obtained at higher temperatures makes SCW a green sustainable alternative to organic solvents for the extraction of a wide range of natural compounds [4]. SCW extraction of bioactive compounds, mainly phenolics, from food and agricultural by-products such as onion skin [5], potato peel [6], winery waste [7], citrus pomaces [8] and peel [9], and mango leaves [10] has been widely investigated. There are limited studies on the processing of black tea processing waste using SCW [11], supercritical carbon dioxide [12,13], and traditional extraction methods [14], which mainly focused on recovery of caffeine.

Although high temperatures used in SCW extraction improves extraction efficiency, stability of the extracted compounds needs to be considered as thermal degradation of existing compounds may occur affecting bioactivity [15].

In this study, SCW extraction of antioxidant phenolic compounds from black tea processing waste and the effect of extraction temperature on the phenolic profile and antioxidant activity of the extracts were investigated.

MATERIALS AND METHODS

Materials

Black tea processing waste (BTPW) samples were obtained from the grading step at a black tea factory in the Black Sea region of Turkey at the second harvest season of 2012. Samples were kept in sealed bags at -20°C until utilized. ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt) (≥98%), Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylicacid) (97%), potassium persulfate (ACS grade, ≥99%), EDTA (Ethylenediaminetetraaceticacid disodium salt, dihydrate) (ACS grade, ≥99%), L-ascorbic acid (98%), (+)-catechin (C) (≥99.0%), gallic acid monohydrate (GA) (≥ 98.0%), caffeine (≥99.0%), (-)-epicatechin (EC) (≥98%), (-)-epicatechin gallate (ECG) (≥98%), (-)-epigallocatechin (EGC) (≥95%), (-)-epigallocatechin gallate (EGCG) (≥95%), (-)-catechin gallate (CG) (≥98%),(-)-gallocatechin gallate (GCG) (≥98), tea extract from black tea (≥80% theaflavin (TF)) were purchased from Sigma-Aldrich (USA) and used for antioxidant and HPLC analysis. HPLC grade acetonitrile and methanol were purchased from JT Bakers (USA) and VWR (Belgium), respectively. Milli-Q-water from a Millipore Water Purification System (Milli-Q A10, France) was used for all experiments.

Subcritical Water Extraction

SCW extractions were carried out using an extractor built in-house as shown in Fig. 1. The main components of the extractor include a column (1/2’’ o.d×20 cm) where extraction takes place, an HPLC pump (Dionex-Ultimate 3000, Thermo Scientific, USA) to introduce water, an oven (Venticell, Germany) for heating, heating and cooling coils, thermocouples, pressure gages, and a back pressure regulator. SCW extraction of BTPW samples (2 g) was carried out at four temperatures (90, 120, 150 and 180ºC) for 30 min at 1000 psi using 2 ml/min water flow rate. SCW extracts were freeze dried (Christ, Alpha 2-4LD Plus, Germany) and stored at

-20°C until further analysis. SCW extractions were performed in duplicate and extraction yields were determined gravimetrically**.**

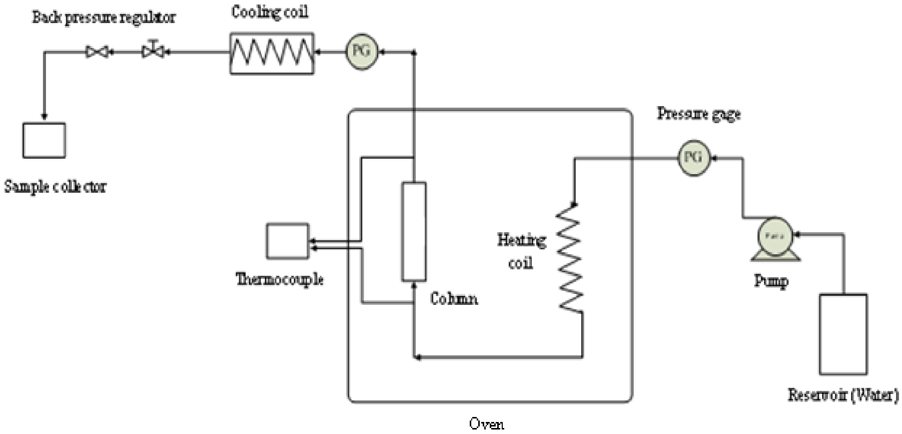

Figure 1: Subcritical water extractor

Antioxidant Activity

Antioxidant activities of the SCW extracts were determined using ABTS radical cation decolorization assay [16] using a microplate reader (Thermo Scientific, Multiskan Go, Finland) with modifications as described in Güçlü Üstündağ and Mazza [17] in duplicates. Trolox was used as a standard and results were expressed as mg of Trolox equivalent (TE) per mg freeze-dried extract.

HPLC Analysis

Caffeine and phenolic content (gallic acid, catechins and theaflavins) of SCW extracts were analyzed using an HPLC-PDA (Thermo Scientific, USA) using a Phenomenex Luna phenyl-hexyl column (4.6 mm x 250 mm, 5µm) according to ISO 14502-2:2005 [18]. Identification of caffeine and phenolics was done by comparing the retention times of the sample peaks with those of standards. Quantification of catechins, gallic acid and caffeine was carried out using standard calibration curves. While catechin, gallic acid and caffeine amounts were expressed as mg/g freeze dried extract, theaflavin content was expressed as area %. Standard extraction procedure using %70 methanol as outlined in the ISO method was used for comparison purposes [18].

RESULTS AND DISCUSSION

Phenolic Profile of Subcritical Water Extracts

The phenolic profile of the BTPW samples (determined using 70% methanol extraction according to the standard ISO method), and of the subcritical water extracts of BTPW are given in Table 1. BTPW samples contained GA, caffeine, EC, EGCG, ECG and TF. In addition to these compounds, C and GCG were also detected in the SCW extracts (Table 1). Total catechins, total TF, GA and caffeine contents of the SCW extracts were in the range of 4.97-17.48 mg/g, 1.25-2.93 (% Area), 6.47-17.39 mg/g and 39.14-68.87 mg/g, respectively.

Table 1: Composition of SCW black tea processing waste extracts obtained at 90-180°C**.**

	Extracts[b]				
	SCW90	**SCW120**	**SCW150**	**SCW180**	**ISO**
Catechins (mg/g)[a]					
GA	6.47±1.16	10.66±1.23	13.44.±0.31	17.39±1.14	2.76±0.11
Caffeine	68.87±0.98	61.35±3.63	49.79±0.16	39.14±0.03	70.46±2.11
GC	ND[c]	ND	ND	ND	ND
EGC	ND	ND	ND	ND	ND
C	3.10±0.72	3.25±0.89	3.20±0.33	2.71±0.72	ND
EC	9.11±0.69	5.09±1.53	2.68±0.03	0.28±0.40	19.92±0.36
EGCG	4.35±0.61	4.92±0.54	2.68±0.08	0.29±0.40	4.46±0.23
GCG	ND	2.34±0.09	4.00±0.12	1.28±0.43	ND
ECG	0.92±0.04	0.91±0.00	1.02±0.15	0.41±0.22	1.12±0.08
Total Catechins	17.48±1.98	16.51±2.87	13.58±0.41	4.97±1.38	25.51±0.59
Total Theaflavins (% Area)	2.93±0.14	2.26±0.29	1.25±0.21	ND	19.12±0.12

[a]GA: Gallic acid, GC: Gallocatechin, EGC: Epigallocatechin, C: Catechin, EC: Epicatechin, EGCG: Epigallocatechin gallate, GCG: Gallocatechin gallate, ECG: Epicatechin gallate [b]SCW90-180: Subcritical water extracts obtained at 90-180 ºC, ISO: 70% methanol extracts obtained according to the ISO method. [c]ND: not determined. Data were expressed as mean ± SD (n = 2) on a dry extract basis.

While caffeine, total catechins and TF content of SCW extracts decreased with increasing temperature, GA content increased from 6.47±1.16 mg/g extract at 90°C to 17.39±1.14 mg/g extract at 180°C due to degradation of gallated catechins and theaflavins (Figure 2). SCW extracts at 180°C were mainly composed of GA (17.39±1.14 mg/g extract) and caffeine (39.14±0.03 mg/g extract), while TF were not detected.

Total catechin and TF content of the extracts, which were 17.48±1.98 and 2.93±0.14 at 90ºC, respectively, decreased by 6 % and 23 % at 120°C, and by 22 % and 57 % at 150°C. No TF was observed at 180°C, while catechins decreased by 72%. These results are in agreement with the previous findings which showed lower thermal stability for TF than catechins [19]. For example, heating at 100°C for 3 h led to 25% catechin loss in a catechin-theaflavin mixture, whereas TF was completely destroyed [19].

While GCG was not detected in the BTPW samples and in the SCW extract obtained at 90°C, 120°C SCW extract contained 2.34 mg/g GCG. The GCG content increased when temperature was increased up to 150°C accompanied by a drop in content of EGCG, however a sharp decrease of GCG was observed at 180°C (Figure 3). These results indicate that epimerization of EGCG occurred at temperatures > 90°C resulting in the formation of its epimer, GCG. Epimerization of catechins has been observed by many researchers at temperatures higher than 80-100°C in water, buffer solutions and tea infusions [20, 21, and 22]. Seto et al 1997 [20] reported that epimerization of EGCG was hardly observed in McIlvaine buffer solution at 80-100°C, whereas the percentage epimerization of EGCG to GCG reached 55% at higher temperatures (120 and 130°C) after 30 min.

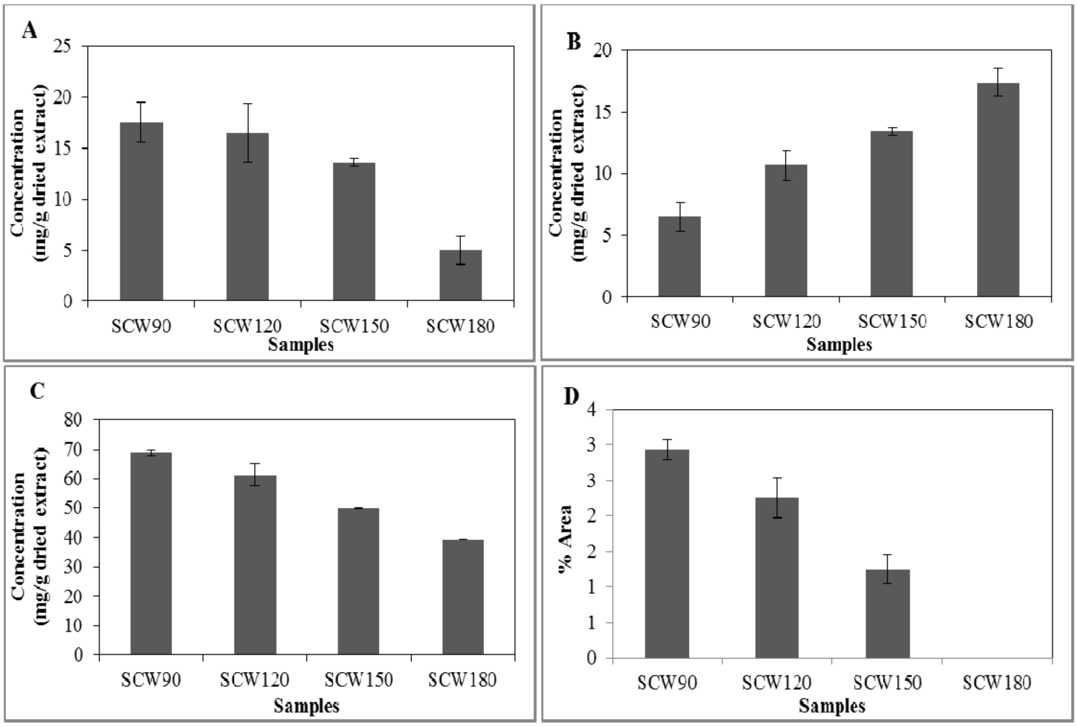

Figure 2: Effect of SCW extraction temperature on the composition of BTPW extracts obtained at 90, 120, 150 and 180 ºC (SCW90-SCW180). A; total catechins, B; gallic acid, C; caffeine, D; theaflavins. Data were expressed as mean ± SD on dry basis.

An increase in epimerization with temperature has also been widely reported [21, 22]. When temperature was increased from 25 to 130°C (for 20 min), EGC and EGCG in distilled water decreased from 42 to 11 mg/l and 160 to 40 mg/l, respectively while their epimers GC and GCG increased from 6 to 33 mg/l and 5 to 50 mg/l [21].

The decrease in the GCG concentration observed in this study at 180°C can be attributed to thermal degradation reactions. Degradation reactions were shown to take place simultaneously with epimerization reactions, resulting in a decrease at the concentration of the produced epimers at higher temperatures and times [22]. In their study on the kinetics of epimerization and degradation reactions of EGCG, Wang et al [22] observed a maximum in the concentration of the GCG at all temperatures investigated (120-165°C) which was more pronounced and occurred earlier at the higher temperatures indicating increasing degradation with time. For example, GCG concentration first increased to a maximum (50%) at 3 min, and then decreased due to thermal degradation at 165°C, a similar maximum in GCG concentration (30-40%) was observed at around 20-40 min at 120°C [22].

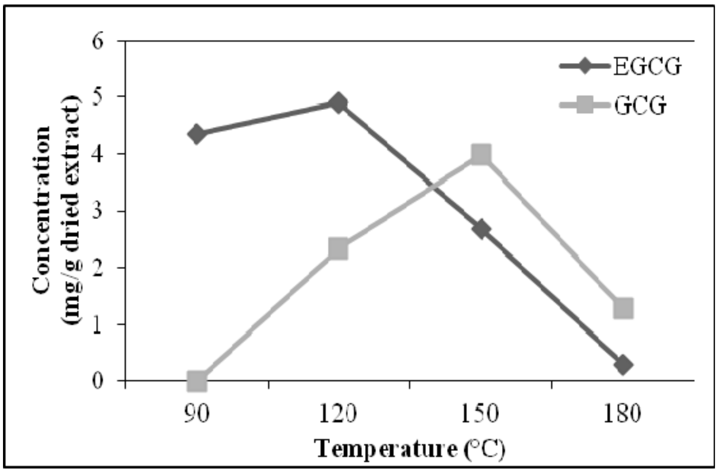

Figure 3: Effect of temperature on the concentration of epigallocatechin gallate (EGCG) and gallocatechin gallate (GCG) in BTPW extracts.

Antioxidant Activity

Antioxidant activities of SCW extracts of BTPW ranged between 0.30-0.38 mg TE/mg extract with the lowest value obtained at 180 °C (Table 2). However, when the antioxidant activity values were expressed as mg TE/ml extract, the highest antioxidant activity was observed at 180°C (3.61 mg TE/mL extract). These results indicate that the decrease in the TF and catechin contents of the extracts, which are the main antioxidant compounds in black tea, with temperature (Figure 2), did not affect the antioxidant activity of the freeze dried extracts up to 180°C due to the antioxidant activity of the breakdown products. The increasing trend with temperature observed in antioxidant values expressed as mg TE/mL extract reflects the increase in the extraction yield with temperature.

Table 2: Antioxidant activity of SCW extracts of BTPW samples

Extracts	Antioxidant Activity	
	(mg TE/mg extract)	*(mg TE/ml extract)
SCW90	0.37± 0.031	2.75± 0.549
SCW120	0.35± 0.015	2.65± 0.350
SCW150	0.38± 0.010	3.26± 0.437
SCW180	0.30± 0.004	3.61± 0.273

*Calculated based on extraction yields of BTPW samples (22.06, 22.69, 25.62 and 36.35 % (w/w) at 90, 120, 150 and 180 ºC, respectively).

CONCLUSION

Subcritical water extraction can be used to obtain phenolic extracts with antioxidant properties from BTPW. With increasing extraction temperature epimerization and degradation reactions take place resulting in extracts with different compositions and bioactivities.

Therefore the effect of temperature on the stability of tea phenolics and the modifications in the bioactivity of the extracts should be considered for process design.

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