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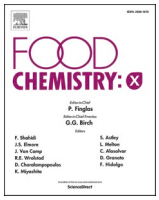

Pilot-scale extraction of polyphenols from spent black tea by semi-continuous subcritical solvent extraction

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ABSTRACT

Spent black tea (SBT) is a residue from tea beverage production and considered as a potential source of active polyphenols. This study aimed to develop a pilot-scale process on semi-continuous subcritical solvent extraction (SSE) of polyphenols from SBT by exploiting the lab-scale knowledge. Treatment of SBT with ethanol–water (50% w/w) as solvent at 125 °C and 0.3 MPa achieved a significantly higher yield of polyphenols (80.82 g gallic acid equivalents/kg black tea) with antioxidant activity (64.20 g gallic acid equivalents/kg black tea), compared to hot water extraction (HWE). SSE increased the soluble matter content in extracts than HWE. Based on the results of LC-MS, theaflavin-3,3′-digallate was the most abundant polyphenol from a total of 12 compounds to be extracted by SBT with 50% ethanol. The results suggested that SSE can be used as a scale-up extraction method to recover polyphenols from SBT.

1. Introduction

Black tea [*Camellia sinensis* (L.) O. Kuntze (family: *Theaceae*)] is a popular herbal beverage and contains numerous types of polyphenols that are beneficial to human health. The crucial step in the manufacture of black tea which is called fermentation leads to the formation of these polyphenols. Therefore, the hot water infusion produced from tea leaves can be considered as a therapeutic beverage for consumers interested in health and wellbeing, in addition to its role as a thirst quencher. Numerous studies have analysed black teas for the presence of antioxidant polyphenols, which offer health benefits such as anti-ageing, anti-diabetic, and anti-cancer effects, and preventative effects against cardiovascular and gastrointestinal diseases (Zhang, Qi, & Mine, 2019). As a result of the perceived benefits of tea consumption, world tea production has increased to 5.73 million tons in 2016, of which black tea accounts for 78% (Falla, Demasi, Caser, & Scariot, 2021).

Black tea infusion is prepared by steeping dried tea leaves in hot water for 5–10 min. After brewing, spent tea leaves become a waste product that requires disposal. However, the mild conditions of brewing are not sufficient to extract all the available polyphenols in tea and appreciable amounts of polyphenols remain in spent black tea leaves. These remaining polyphenols exist as non-extractable polyphenols (NEPPs), which may complex with protein and cell wall polysaccharides (Durazzo, 2018). In particular, the ready-to-drink (RTD) tea industry disposes of huge amounts of SBT, estimated to be more than 0.12 million tons annually all over the world (Mukhtar, Mushtaq, Akram, & Adnan, 2018). RTD iced tea is ready prepared tea generally consumed cold and available as a powder format or as RTD tea bottles. Because of the convenience of its consumption, the global bottled tea market is projected to show a compound annual growth rate of nearly 4% through to 2027 (Tea and Coffee Trade Journal, 2019).

In the system of circular economy, valorisation of food by-products can be used as a source for bioactive compounds, particularly antioxidant polyphenols for further application as functional ingredients in the food industry (Rajapaksha & Shimizu, 2021). Also, the potential and feasible utilization of black tea waste is of high research endeavour. In this context, the recovery of phenolic compounds from SBT requires an efficient and scalable extraction method. Our previous study evaluated the potential of hot pressurised liquid extraction under subcritical conditions (subcritical solvent extraction; SSE) for laboratory-scale recovery of polyphenols from SBT and optimised the processing conditions (Rajapaksha & Shimizu, 2020). SSE is an environmentally friendly method (Shimizu, Ushiyama, & Itoh, 2019), which uses a pressurised liquid kept below its critical point (374 °C for water) and above its boiling point (100 °C for water). The SSE technique promotes the extraction of active compounds without changing their chemical

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integrity, with reduced solvent volume and extraction times (Liang, Nielsen & Christensen, 2020). For scale-up of this extraction methodology into the design of an industrial plant, a pilot-scale extraction system must be tested. To the best of our knowledge, pilot-plant-scale data for the extraction of polyphenols from SBT using SSE or scale-up studies have not been reported to date. Hence, the aim of the work reported here was to assess the potential of recovering antioxidant polyphenols by SSE at pilot-plant scale to propose a suitable scale-up method. Furthermore, non-extracted polyphenols that remained in SBT after hot water extraction were identified.

2. Materials and methods

2.1. Materials

Black tea (expiration date: 09/09/2021) was supplied by Mitsui Norin (Tokyo, Japan). Gallic acid was supplied by Sigma-Aldrich (Shanghai, China), 2,2-diphenyl-1-picrylhydrazyl (DPPH) by Sigma-Aldrich (Taufkirchen, Germany), Folin–Ciocalteu phenol reagent by Sigma-Aldrich (St Louis, MO, USA), and sodium carbonate, ethanol, and methanol by Fujifilm Wako Pure Chemical (Osaka, Japan). Distilled water was used in all the experiments. All other chemicals and solvents used for liquid chromatography–mass spectrometry (LC–MS) were HPLC grade.

2.2. Extraction procedure

The prior knowledge on laboratory-scale use of SSE was considered in the development of a pilot-scale process (Rajapaksha & Shimizu, 2020). In addition, maximum pressure limit of the pilot-scale reactor and the safety matters also determined the process temperature and ethanol concentration of SSE in pilot-scale experiment. Pilot-scale semi-continuous SSE was conducted in a 200-L extractor (TEX0513) as shown in Fig. 1. As given in Table 1, three main extraction trials (T1–T3) were conducted. The first extraction was performed with raw black tea (5 kg) in 1:1 ethanol–water mixture (100 kg, 200 kg/h) at 125 °C (SSE: subcritical solvent extraction). Before extraction, the reactor was purged with N_2 gas to pressurise the inside up to 0.3 MPa (0.25–0.35 MPa). To achieve a semi-continuous process, extracts were expelled in 15 kg fractions while aqueous ethanol was supplied continuously. The second and third experimental runs were carried out to extract the phenolic compounds from SBT. First, raw black tea (5 kg) was extracted with hot water at 90 °C (125 kg, 300 kg/h) and hot water extracts (HWE) were collected for analysis. After removal of HWEs, the remaining SBT leaves were treated by SSE, which using an ethanol–water mixture (T2, 50% ethanol; T3, 40% ethanol) with a solid-to-solvent ratio of 1:20 (100 kg, 200 kg/h) at 125 °C and 0.3 MPa. The extracts were then expelled as 15 kg fractions while the extraction solvent was supplied continuously. To cool the extractor, an extra 20 kg of water was supplied at the end of the ethanolic extraction and the residual liquid was drained. Temperature and pressure in the reactor were recorded during the extraction. The collected extracts were stored at − 20 °C until further analysis.

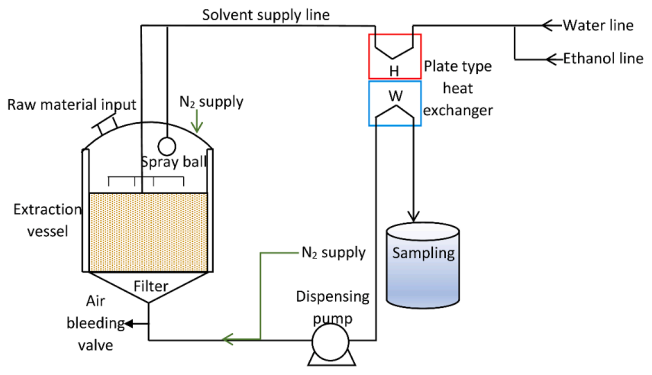

Fig. 1. Schematic diagram of the hot pressurised extraction equipment at pilot-plant scale.

Table 1
Experimental trials and their conditions.

Trial	Source	Extraction conditions	Symbol
T1	BT	125 °C, 50% aqueous ethanol, 0.3 MPa	T1
T2	BT	Hot water, 90 °C	HWE
	SBT	125 °C, 50% aqueous ethanol, 0.3 MPa	T2 et
T3	BT	Hot water, 90 °C	HWE
	SBT	125 °C, 40% aqueous ethanol, 0.3 MPa	T3 et

Abbreviations: BT, black tea; SBT, spent black tea.

2.3. Determination of total solids content and brix

Extract (1 mL) was evaporated to dryness in a laboratory oven at 105 °C for 24 h until constant weight was obtained. The dry weight was recorded to give the total solids (TS) content (g/mL). The total soluble solids in homogenized extracts were determined by a digital refractometer (RX–5000α–Plus, Atago, Japan) with an accuracy of ± 0.010% at temperature of 25 °C and were expressed in brix values (0–100 %).

2.4. Analysis of total phenolic content

The total phenolic content (TPC) of the liquid extract was measured using the Folin–Ciocalteu (FC) method. Briefly, the obtained extract was diluted (1:100) with solvent, and then a 1.0-mL aliquot of the extract (performed in triplicate) was transferred into a test tube and mixed thoroughly with 5.0 mL of FC reagent diluted (1:10) with distilled water. After 3 min, 5.0 mL of sodium carbonate solution (7.5%, w/v) was added and mixed. The mixtures were then allowed to stand for 1 h in darkness before measuring the absorbance using a UV–Vis spectrophotometer (V-560, JASCO, Tokyo, Japan) at 756 nm against a blank. Gallic acid was used as the standard for preparation of the standard curve (7.812–250 μg/mL, $R^2 = 0.998$). The TPC values were expressed as grams of gallic acid equivalent per kilogram (dry weight) of material (g GAE/kg).

2.5. Analysis of antioxidant activity

Antioxidant activity (AA) was determined by measuring the DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging activity using the method of Brand-Williams, Cuvelier, & Berset (1995) with slight modification. Fresh DPPH reagent (2.9 mL, 0.1 mM) and diluted (1:70) liquid extract (0.1 mL) were mixed and incubated at room temperature for 20 min before the absorbance was measured at 517 nm (V-560, JASCO). DPPH reagent in solvent without the sample were considered as the control. Gallic acid was used as the standard for preparation of the standard curve (3.90–62.5 μg/mL, $R^2 = 0.997$). DPPH scavenging capacity was expressed as grams of gallic acid equivalent per kilogram (dry weight) of material (g GAE/kg).

2.6. Liquid chromatography–mass spectrometry (LC–MS) analysis

LC–MS analysis of extracts was performed using a Nexera-XR liquid chromatograph (Shimadzu, Kyoto, Japan) coupled to a mass spectrometer (LTQ-Orbitrap XL, Thermo Fisher Scientific, Waltham, MA, USA). Extract (5 μL) was injected into an Inert Sustain AQ-C18 column (1.9 μm, 2.1 × 100 mm, GL Sciences, Tokyo, Japan) maintained at 35 °C. Aqueous formic acid (0.1%, solvent A) and 0.1% formic acid in acetonitrile (solvent B) were used as mobile phase. Gradient elution was performed at 150 μL/min using the following program: 0% B 0 min, 0% B 2 min, 95% B 20 min, 95% B 24 min, 20% B 25 min, 20% B 30 min.

Electrospray ionisation (ESI) was used in negative mode. Other instrument settings: source voltage, 4.0 kV; nitrogen sheath gas flow rate,

30 L/ min; auxiliary gas flow rate, 10 L/min; sweep gas flow rate, 0 L/ min; capillary temperature, 300 °C; capillary voltage, 30 V; tube lens voltage, 80 V. All extracts were evaluated in FTMS mode, and the mass range was acquired by full range acquisition covering m/z 100–1800. Samples were typically diluted (1:50) with solvent A. Data analysis was achieved using XCalibur software v2.0.7 (Thermo Fisher Scientific).

2.7. Statistical analysis

Statistical analyses were carried out using Minitab 19.1.1. (Minitab, State College, PA, USA) to determine significance of difference. One-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to compare and identify significant differences ($p < 0.05$) between group means. The results were reported as the mean value of three repeated experimental trials.

3. Results and discussion

3.1. Study of temperature and pressure during extraction

The preheated water at 90 °C was supplied to the reactor for HWE. However, the temperature inside the reactor during HWE showed some fluctuation between 70 °C and 83 °C (Fig. 2a), which could be due to the heat transfer into the biomass and reactor wall through conduction. Upon the removal of extract, the outlet temperature increased, although that level was maintained at around 80 °C. During SSE, four phases were observed. The first phase started with the supply of water–ethanol mixture and the heating and pressurisation of solvent (Fig. 2b). The second phase began when the system reached maximum temperature and pressure, and with the removal of extracts as 15 kg fractions. At the beginning of the second phase, the pressure was maintained around 0.3 MPa but it decreased gradually, possibly because of a slight reduction in temperature. However, this semi-continuous process can minimise the degradation of antioxidant phenolic compounds by shortening the overall extraction time (Asofiei, Calinescu, Trifan, & Gavrila, 2019). During the third phase, the temperature and pressure decreased as the solvent supply was turned off and the extractor was cooled by water showering. The fourth phase included draining of all extracts after the water shower.

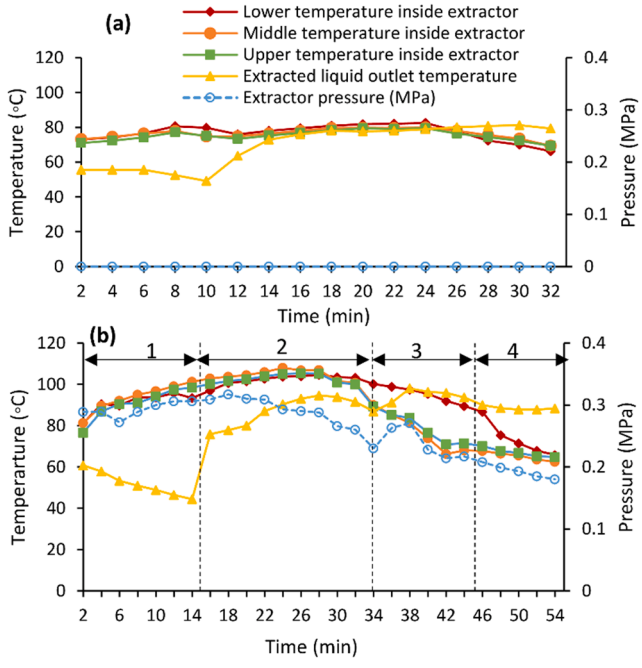

Fig. 2. Temperature and pressure variation during the HWE: hot water extraction (a), and SSE: subcritical solvent extraction (b).

3.2. Total solids content and brix value

TS content and the brix value of all fractions for the three main extractions are shown in Fig. 3. The TS content of extracts represents both soluble and insoluble matter, while brix is used to analyse the percentage of soluble solids in the solution. The TS values were lower in all fractions of the T1 extract and were higher in the T2 and T3 samples. In contrast to the trend in TS content, the highest brix value was observed in T1 sample, while T3 showed the lowest value. These results reflect the trend of higher soluble solids in T1 extract than insoluble material, and T3 contained higher content of insoluble material than T1 and T2. Moreover, the HWEs showed lower brix and higher TS content than other hydroethanolic fractions, which indicates a higher content of insoluble particles in HWEs. From T2 and T3, the ethanolic fractions of T2 (T2 et) contained higher levels of soluble solids. Soluble matters would be expected to include sugars, polyphenols, alkaloids, and soluble amino acids, which have more affinity to hydroethanolic mixtures than pure water. Thus, it is noteworthy that the SSE technique could facilitate the mass transfer from SBT, and that the ethanol–water mixture enhanced the solubility of compounds in SBT. This finding is consistent with the prior result showing that increased solubility and extraction efficiency was obtained by pressurized liquid extraction of phenolic compounds from jabuticaba skins (Santos, Veggi & Meireles, 2012).

3.3. Analysis of homogenized fractions of the extract

3.3.1. Total phenolic content and antioxidant activity

The quantitative analyses of TPC and AA of the HWEs and homogenous samples from T1, T2 et, and T3 et were performed (Fig. 4a,b). The extract of T1 from raw black tea had the highest values of TPC and AA, owing to the presence of polyphenols not extracted previously. HWE removed less than half of the antioxidant polyphenols from T1 and a large amount remained in the SBT. SSE carried out as the second extraction using 50% hydroethanolic solvent at 125 °C and 0.3 MPa (T2 et) extracted significantly higher amounts of polyphenols with

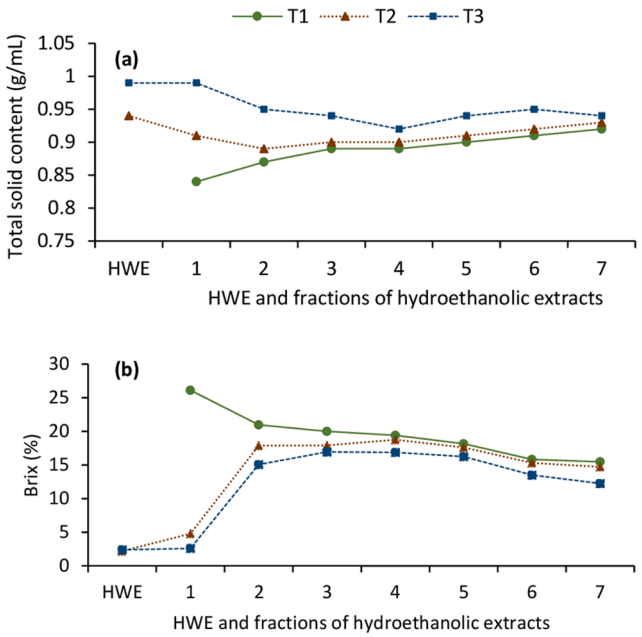

Fig 3. Total solids content (a), and brix value (b) of HWE: hot water extract and all fractions of hydroethanolic extract in T1: SSE of black tea with 50% ethanol, T2: HWE + SSE of spent black tea with 50% ethanol, and T3: HWE + SSE of spent black tea with 40% ethanol. SSE: subcritical solvent extraction.

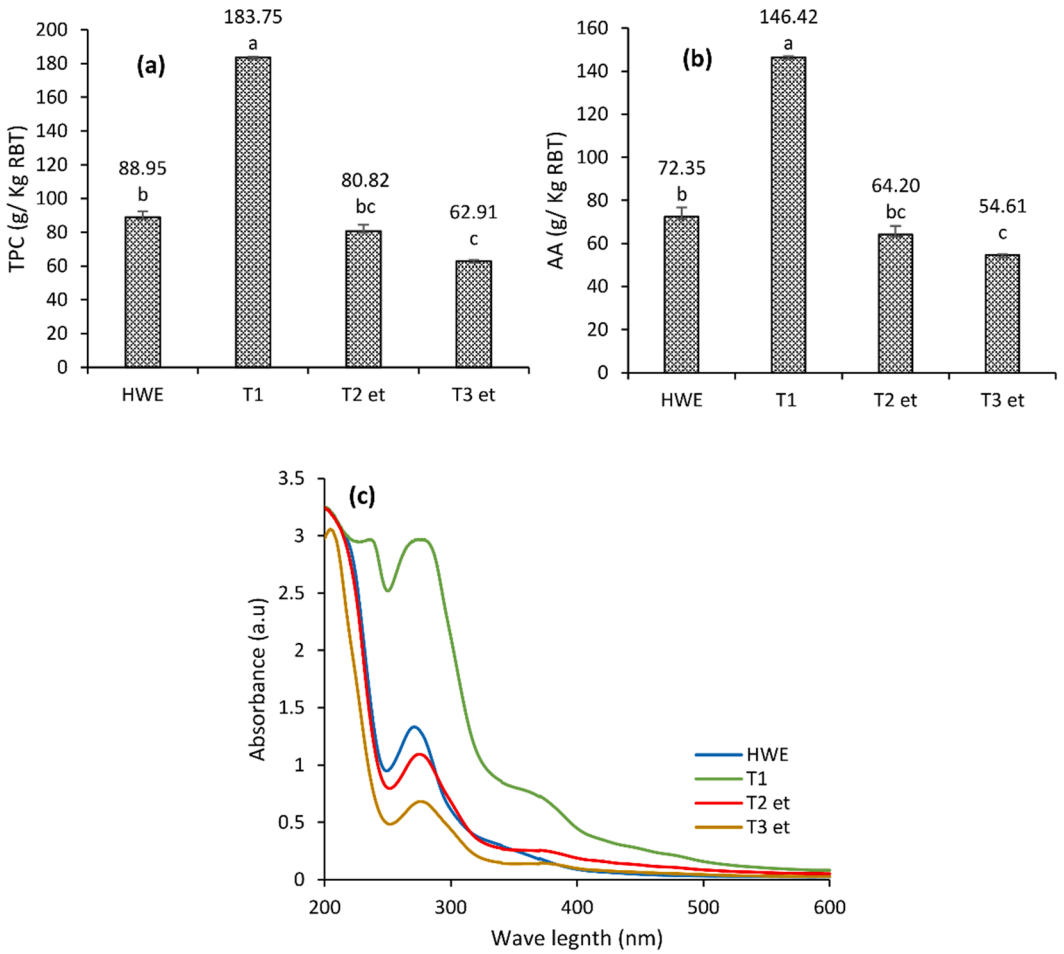

Fig. 4. Total phenolic content (TPC) (a), antioxidant activity (AA) (b), and UV–visible absorption spectra (c) of HWE: hot water extraction, T1: SSE of black tea with 50% ethanol, T2 et: SSE of spent black tea with 50% ethanol, and T3 et: SSE of spent black tea with 40% ethanol. SSE: subcritical solvent extraction. Bars marked with different letters (a–c) are significantly different ($p < 0.05$).

antioxidant activity (80.82 and 64.20 g GAE/kg black tea, respectively) ($p < 0.05$). Lowering the ethanol concentration from 50% to 40% caused decreased polyphenol yields with AA. This result was consistent with our previous work, which showed that increased temperature and increased ethanol concentration favoured greater recovery of polyphenols. This was attributed to the creation of a moderately polar medium and a greater ability of ethanol to establish intermolecular interactions with polyphenols (Rajapaksha & Shimizu, 2020). Moreover, a solvent of ethanol–water can enhance the extraction efficiency by reducing the polarity even at the temperature as 125 °C, thus increasing the solubility and diffusivity of phenolics at high temperatures (Allcca-Alca et al., 2021).

3.3.2. *UV–visible absorption spectrum*

UV–vis spectra of the extracts were recorded from 200 to 600 nm and are shown in Fig. 4c. All extracts showed a maximum absorption at 280 nm, which likely corresponds to the polyphenols in black tea, in particular theaflavin and catechin derivatives, phenolic acids, and alkaloids. In addition, extracts from SSE showed another absorption peak at 360 nm. This result is confirmed by Fig. S1, which shows a reddish-brown colour in the extract, likely caused by thearubigins in black tea (Uchida, Ogawa, & Yanase, 2016).

3.4. *Total phenolic content and antioxidant activity of T2 extracts*

The HWE and each fraction (15 kg) of the T2 et were analysed for TPC and AA. As presented in Table S1, the content varied significantly from one fraction to the next. The maximum amount of phenolic content was obtained from the second fraction of the ethanol–water extract and the TPC values were in accordance with the AA values in same fractions. Rupturing of cell walls facilitates solvent access and leads to extraction of more polyphenols in the initial fractions.

The TPC and AA decreased in later fractions because of the removal of most of the polyphenols in the initial fractions. However, the first hydroethanolic fraction had low levels of TPC and AA, probably because of mixing of solvent with the remaining in HWE. The first four T2 et fractions (60 kg of extracts) extracted almost 75% of the phenolic content. Thus, it is noteworthy that 60 kg of aqueous ethanol would be sufficient to extract more than 75% of misspent polyphenols from 5 kg of black tea; in other words, the solvent-to-solid ratio of 12:1 is better than 20:1. Use of this result will lead to efficient extraction in terms of cost and environmental considerations.

3.5. *Phenolic composition in HWE, T2 et, and T1*

The overview of the relative abundance of the major polyphenols available in the extracts is presented in Fig. 5 and their fragmented ions, retention times, and tentative identifications are summarised in Table S2. While HWE reflects normal tea infusion, T2 et extract reflects the residues that remain in SBT leaves. Qualitative analysis showed that HWE could extract relatively few compounds, mainly theasinensin C, theasinensin-gallate, theaflavin and apigenin and kaempferol linked with carbohydrate moieties. After SSE treatment of SBT leaves, the collected T2 et extracts contained some moderately polar polyphenols,

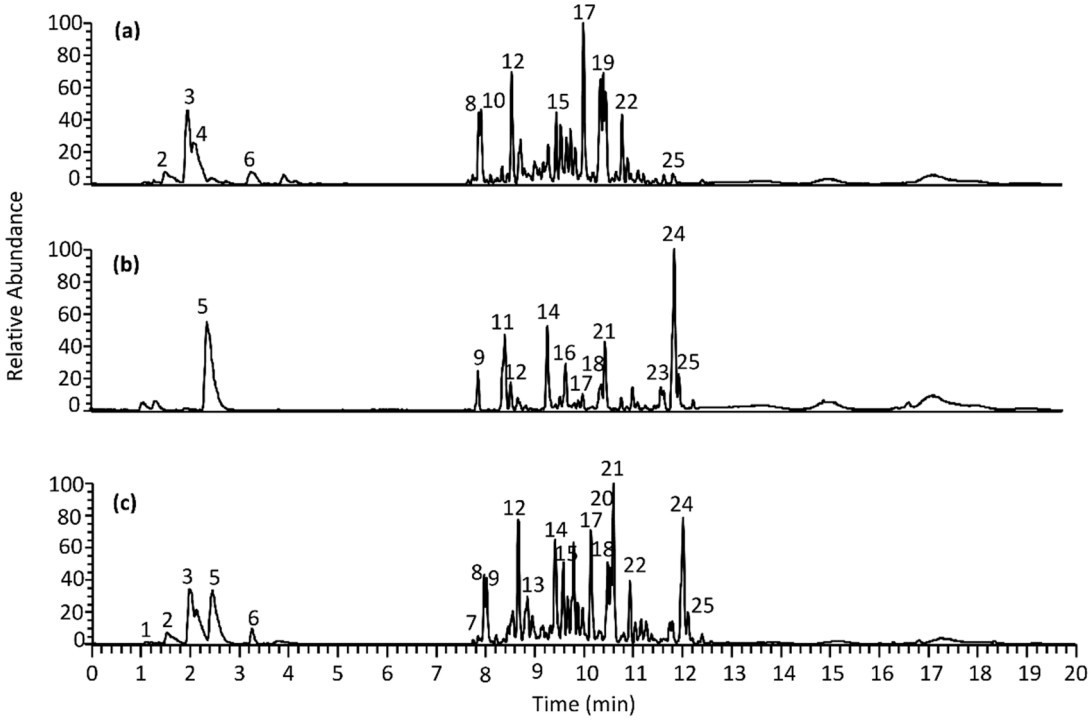

Fig. 5. Total ion chromatograms obtained at negative MS ionisation for HWE: hot water extraction (a), T2 et: SSE of spent black tea with 50% ethanol (b), and T1: SSE of black tea with 50% ethanol (c). SSE: subcritical solvent extraction. Peaks identification: 1. Caffeic acid 2. Caffeic acid derivative 3. Quinic acid 4. Unknown 5. Malic acid 6. Unknown 7. Gallic acid hexoside 8. Unknown 9. Gallic acid 10. 5-O-Galloylquinic acid 11. Unknown 12. Theasinensin-gallate 13. Galloyl-HHDP-hexoside 14. Theasinensin A 15. Theaflavin 16. Epigallocatechin-gallate 17. Theasinensin C 18. Quercetin-3-O-rutinoside 19. Apigenin-6, 8-C- dihexoside 20. *P*2 (EGCG digallate dimer) 21. Epicatechin-gallate 22. Kaempferol-3-O-glucoside 23. Epitheaflagalline-3-gallate 24. theaflavin-3 3′-digallate 25. Theaflavin-3-gallate.

such as galloyl esters of theaflavin and hydrophobic fractions of high molecular weight theasinensin. The remaining polyphenols in T2 et can be considered to be NEPPs, which are an understudied fraction of polyphenols. NEPPs mostly occur as conjugates with macromolecules such as polysaccharides and proteins. Because of their presence in food by-products, they are mostly neglected as waste (Pérez-Jiménez & Saura-Calixto, 2018). Theaflavin-3,3′-digallate (TF3) was identified as the most abundant polyphenol in T2 et extract but was not detected in HWE. TF3 is the major theaflavin found in black tea and is formed from the co-oxidation of (−)-epicatechin gallate and (−)-epigallocatechin gallate (EGCG) during black tea production (Pan, Li, Rankin, Rojanasakul, Tu & Chen, 2018). This result indicates that TF3 has a greater ability to solubilize in the hydroethanolic solvent at high temperature and pressure than in hot water. A similar observation was reported by Friedman, Levin, Choi, Kozukue & Kozukue, (2006). In addition to TF3, theasinensin A was relatively abundant in SBT leaves. Theasinensin and theaflavin have been known as the catechin dimers that serve as precursors of thearubigins (TR). Recent studies have proposed that TRs are generated by oxidative cascade-type reactions of catechin (Drynan, Clifford, Obuchowicz, & Kuhnert, 2012; Kuhnert, Drynan, Obuchowicz, Clifford, & Witt, 2010). In addition, malic acid, gallic acid, epigallocatechin-gallate, quercetin-3-*O*-rutinoside, epicatechin-gallate, and epitheaflagalline-3-gallate were tentatively identified as abundant polyphenols in T2 et and T1 extracts.

In the total ion spectrum of T1 extract, 18 phenolic compounds were identified including the polyphenols identified in HWE and T2 et. These results confirmed that the extractable polyphenol fraction from black tea using hot water was significantly less than the fraction obtained by SSE. As mentioned above, most of the NEPPs in SBT were inaccessible to the solvent during the HWE process, probably because of hydrophobic interactions between NEPPs and matrix compounds. These hydrophobic interactions can be weakened by increasing the temperature and pressure in SSE because they are impeded by the decrease of ionic strength (Domínguez-Rodríguez, Marina, & Plaza, 2017). In addition, decreased viscosity and surface tension in solvents facilitates the solubility and diffusivity of phenolics, resulting in higher extraction yields for a variety of NEPPs.

4. Conclusions

In the present study, applicability on pilot-scale extraction of polyphenols from spent black tea by semi-continuous subcritical solvent extraction (SSE) was evaluated. SSE led to increased diffusivity and solubility of phenolics. The use of 1:1 ethanol–water solvent at 125 °C and 0.3 MPa extracted significantly higher phenolic content from spent black tea compared to hot water extraction (HWE). A variety of non-extractable polyphenols in spent black tea leaves, which remained after HWE, were extracted by SSE. Further studies in quantitative analysis of phenolic compounds can be proposed for better identification of all extracts.

CRediT authorship contribution statement

Surakshi Rajapaksha: Investigation, Formal analysis, Writing – original draft. **Naoto Shimizu:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2021.100200.

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