



Subcritical water extraction of polyphenolic compounds from sorghum (*Sorghum bicolor* L.) bran and their biological activities

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ABSTRACT

Subcritical water extraction (SWE), an environment-friendly technique, was applied to extract polyphenolics from sorghum bran. Extraction temperatures (°C), time (min), and solid-liquid ratio (mL/g) were investigated and optimized by Box-Behnken design. The optimized conditions for SWE was 144.5 °C of temperature, 21 min of time, and 35 mL/g of solid-liquid ratio, with a polyphenolics yield of 47.253 ± 0.375 mg GAE/g dw, which was in good agree with the predicted value. Comparing with hot water extraction (HWE), SWE resulted in a higher yield of polyphenolics, higher radical scavenging activities, and more efficient antiproliferative activity. Furthermore, major polyphenolic compositions of the extracts were identified and quantified by HPLC-ESI-MS/MS. Taxifolin, taxifolin hexoside, oligomeric procyanidins, and epicatechin were the most abundant polyphenolic compounds in the extracts. Taken together, SWE can be used as a effective extraction method for polyphenolics from sorghum bran, which could be used as a potential source of natural antioxidants.

1. Introduction

Sorghum (*Sorghum bicolor* L.) is a gluten-free grain and ranked as the fifth most planted crop in the world and mainly consumed as staple food in Asia and Africa. Sorghum is considered as a source of nutrients and has prominent potential in health promotion (de Moraes Cardoso, Pinheiro, Martino, & Pinheiro-Sant'Ana, 2017). Epidemiological studies have demonstrated that consumption of sorghum grain and its products reduced the incidence of esophageal cancer (van Rensburg, 1981) and other chronic disease (Slavin, Jacobs, & Marquart, 1997). Modern pharmacological researches have also reported the effects of sorghum and its extracts on cancer cells inhibition, glycemic control, cardiovascular disease prevention, and so on (de Moraes Cardoso, et al., 2017; Stefoska-Needham, Beck, Johnson, & Tapsell, 2015). Those benefits could be attributed to abundant phytochemicals (such as polysaccharides, lipids, proteins, and phenolic) in sorghum (de Moraes Cardoso et al., 2017). Among those phytochemicals, phenolics are believed to play a critical role in various properties of sorghum (Yang, Allred, Geera, Allred, & Awika, 2012). Almost all kinds of phenolics are found in sorghum and most of them are existed in the outer layers (bran). However, sorghum bran is often stripped and discarded during the refining process of sorghum. Therefore, sorghum bran as a potential source of phenolics have attracted a great deal of attention.

In general, extract method is the first stage affecting the research and utilization of phenolics from sorghum bran. The conventional

methods (including refluxing, hot water, maceration and soxhlet extraction) for extracting of phenolics are commonly time consuming, high organic solvents (generally ethanol, methanol, and acetone) using and low phenolics yield extracting. In recent years, several emerging technologies, including enzymatic ultrasonic, pulsed-electric field, accelerated solvent, supercritical fluid, microwave, and subcritical water (SW) have been used to extract phenolics from plant materials (Pasrija & Anandharamakrishnan, 2015). Among these technologies, accelerated solvent extraction (ASE) (Barros, Dykes, Awika, & Rooney, 2013) and ultrasonic assisted extraction (UAE) (Luo et al., 2018) for phenolics from sorghum bran were already investigated, with the desired total phenolic contents (TPC) of 45 mg GAE/g dw (dry weight) and 49.74 mg GAE/g dw, respectively. However, both ASE and UAE have consumed a lot of ethanol, similarly as conventional methods. Therefore, developing a more environmentally extraction methods for extraction of sorghum bran phenolics is still necessary.

Subcritical water extraction (SWE), an environment-friendly technique, also named pressurized hot water extraction, is based on using water with modified physical properties as the extraction solvent. It is performed in liquid state at various temperatures (100–374 °C) under sufficient pressures (1–22.1 MPa), which are the critical temperature and pressure, respectively. SWE is recognized as a rapid and efficient extraction tool for extraction of active compounds from plant materials (Duan et al., 2014; Luo et al., 2017; Wang, Chen, & Lü, 2014). In recent years, SWE has been successfully used to extract phenolics from spent

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coffee grounds (Xu, Wang, Liu, Yuan, & Gao, 2015) and *Crocus sativus* petals (Ahmadian-Kouchaksaraie, Niazmand, & Najafi, 2016). To our best of our knowledge, there has been no research report on extraction of polyphenolics from sorghum bran with subcritical water.

The aim of this study was to investigate the SWE variables (time, temperature, and solid-liquid ratio), and optimize the extraction conditions for the yield of polyphenolics from sorghum bran. In addition, the antioxidant activities, and anti-proliferative activity of the SWE extracts were investigated and compared with the hot water extraction (HWE) extracts. Besides, the polyphenolic compositions of SWE extracts were identified by HPLC-ESI-MS/MS.

2. Material and methods

2.1. Chemicals and reagents

Folin-Ciocalteu reagent, sodium carbonate, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), and 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (Shanghai, China). Gallic acid and (+)-catechin were obtained from Chengdu Herbpurify Co., Ltd. (China). Non-essential amino acid (NEAA), Dulbecco's modified Eagle's medium (DMEM), HBSS buffer (pH 7.2), and trypsinization (0.25% trypsin with EDTA) were purchased from HyClone (Logan, USA), and heat-inactivated fetal bovine serum (FBS) was purchased from Gibco Life Technologies (Carlsbad, USA). HPLC grade acetonitrile was purchased from Tedia Company, Inc. (USA). All other chemicals and reagents were of analytical reagent grade.

2.2. Sorghum bran samples

Sorghum wholegrain was collected from Liaoning Province, China. The grain was decorticated using a dehuller to obtain a bran yield of approximately 10%. Bran was ground into powder using a pulverizer (XS-10B, Longxin, China) to pass through a 60 mesh sieve, then stored at -20°C until extraction.

2.3. Subcritical water extraction (SWE)

The SWE experiment was carried out with a laboratory-built apparatus in accordance with recommended method described by He et al. (2011). In the typical experimental instance, the sample was loaded onto a polytetrafluoroethylene (PTFE) hydrothermal reactor (inner volume, 20 mL), with a stainless steel jacket. The temperature was monitored and controlled by an air dry oven. In each extraction experiment, 10 mL of distilled water was added into PTFE reactor, while the addition weight of sorghum bran sample was changed according to the solid-liquid ratio. The extraction time began to record after 1 min heating of the reactor in air dry oven. After extraction, extracts were immediately centrifuged (5000 rpm/min, 5 min) and placed in a volumetric flask (10 mL) and the volume was completed with distilled water. The extract solutions were collected and stored at -20°C until further analysis.

2.4. Hot water extraction (HWE)

HWE was performed in a beaker heated with a water bath at 95°C . The extraction time and solid-liquid ratio were conducted in the same with SWE. After extraction, the extracts were centrifuged (5000 rpm/min, 5 min) and placed in a volumetric flask (10 mL) and the volume was completed with distilled water. The extracts were stored at -20°C prior to analysis.

2.5. Experimental design

2.5.1. Single factor experiments

In this part, the effects of the following parameters were investigated: temperature (110 – 190°C), time (5 – 40 min), and solid-liquid ratio ($10:1$ – $50:1$ mL/g). According to the single-factor experimental results, the levels of each factors were obtained.

2.5.2. Response surface methodology (RSM) experiments

On the basis of the single-factor test result, the optimal values of 3 factors were secondly studied. Box-Behnken design (BBD) (Ahmadian-Kouchaksaraie et al., 2016) for SWE was employed to determine the best combination of extraction variables for the maximization of polyphenolics. Extraction temperature (X_1 , $^{\circ}\text{C}$), time (X_2 , min), and solid-liquid ratio (X_3 , mL/g) were the independent variables, and their coded and uncoded levels were presented in Table S1. Total polyphenolic contents (TPC, Y) taken as the response for the design experiment was given in Table S2. The optimization and regression analysis of RSM results were analyzed using Design Expert 9.0 software (Stat-Ease Inc., Minneapolis, USA). Finally, the lack of fit, coefficient of determination (R^2), adjusted coefficient of determination (R_{adj}^2), coefficient of variation (CV), and F -value were used for evaluating the adequacy of the obtained models for SWE.

2.6. Determination of TPC

TPC was measured using the Folin-Ciocalteu method (Singleton & Rossi, 1965) with little modification. Briefly, a $20\ \mu\text{L}$ extract solutions (both SWE and HWE) properly diluted with distilled water were mixed with $80\ \mu\text{L}$ of Folin-Ciocalteu reagent (10% , v/v). After 4 min, $100\ \mu\text{L}$ of Na_2CO_3 (7.5% , w/v) was added. After reacted for 2 h at room temperature, the absorbance was measured at $765\ \text{nm}$ using a microplate reader (Infinite M200Pro, Tecan, Austria). The estimation of TPC was calculated by a calibration curve obtained with gallic acid and expressed as gallic acid equivalents (mg GAE/g dw).

2.7. Purification of crude extracts

Both SWE and HWE extraction solutions were purified based on previously published method (Garcia-Castello, et al., 2015): after extraction, the crude extraction solutions were loaded onto an AB-8 macroporous resins column and then washed using distilled water (4 times of column volume) to remove sugars and organic acids. Finally, 60% (v/v, 4 times of column volume) ethanol was used to elute polyphenolics and then collected the elution fraction. After removal of ethanol, the purified extracts were lyophilized and stored at -20°C until further use.

2.8. Identification and quantification of polyphenolics

The purified extracts were analyzed by a Shimadzu LC-20AT HPLC system (Shimadzu, Kyoto, Japan) comprised of a diode array detector (DAD; SPD-M20A). The separation of extracts were performed on an Agilent Zorbax C18 chromatography column ($250\ \text{mm} \times 4.6\ \text{mm}$, $5\ \mu\text{m}$). The mobile phase consisted of water (0.1% formic acid, solvent A) and acetonitrile (solvent B), using a gradient elution of 0 – 5 min, 5 – 10% B; 5 – 10 min, 10 – 20% B; 10 – 25 min, 20 – 35% B; 25 – 35 min, 35 – 50% B; 35 – 50 min, 50 – 70% B; 50 – 57 min, 70 – 5% B; 57 – 60 min, 5% B. The flow rate was kept at $1\ \text{mL/min}$ and monitored at $280\ \text{nm}$ at room temperature. Polyphenolic compositions of extracts were determined using an Ion Trap-Mass Spectrometry (Thermo LXQ, USA) with an ESI source. Data acquisition were performed with Xcalibur 2.0 software (Thermo Finnigan, USA) and recorded across the range m/z 50 – 1500 in both positive and negative ion modes.

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