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Research Article

Thermal Processing Effects on the Chemical Constituent and Antioxidant Activity of Okara Extracts Using Subcritical Water Extraction

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Subcritical water extraction (SWE) has been employed for the extraction of bioactive compounds from plant materials with cost-effectiveness, less consuming time, and environmental sustainability. To explore the effects of thermal processing during SWE, total organic content (TOC), total sugar, polysaccharides, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (ABTS and DPPH assays) of eight aqueous extracts have been quantitatively investigated. The results indicated that elevated temperatures indeed resulted in significant changes in the constituents and antioxidant activities of okara extracts. Among them, the extract obtained at 220°C exhibited the highest total phenolic, flavonoid content, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity, and ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)] radical-scavenging activity. However, phenolic compounds were destroyed after the treatment above 230°C, suggesting that any polymer processing is improper to undertake at higher than this value to achieve the high antioxidant activity. Moreover, a significant positive correlation between TPC or TFC and antioxidant capacity (DPPH and ABTS) values was detected.

1. Introduction

Okara is rich in bioactive compounds including dietary fiber, protein, polysaccharides, and phenolic compounds, many of which have been claimed to contribute to the antioxidant activity due to their redox properties derived from various possible mechanisms: free radical-scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity [1]. For instance, phenolic compounds play an important role in stabilizing lipid peroxidation and inhibiting various types of oxidizing enzymes [2]. Nevertheless, the differences in the structures and substitutions may influence the phenoxyl radical stability and impair the antioxidant properties.

Subcritical water extraction (SWE) is defined as hot water at temperatures ranging between 100°C and 374°C under high pressure to maintain water in the liquid state [3]. As one of an environmentally friendly and efficient technique, it has been a wide range of applications, such as extraction, hydrolysis, and wet oxidation of organic compounds [4, 5]. Elevated temperatures can modify the dielectric constant and ionization constant of water, resulting in the possibility of tuning its polarity and accelerating hydrolysis, thereby obtaining organic compounds instead of using organic solvents during food industry processing [6].

It is well known that heating extraction is an essential processing procedure, especially during SWE processing, which is attributed to the oxidation, thermal degradation, and leaching of bioactive compounds from fresh vegetables [7]. Depending upon the morphology and nutritional properties of raw materials, different heating conditions, such as heating duration and temperatures, have either positive or negative effects on the antioxidant properties of vegetables [8].

However, no information is available on the effects of thermal treatments on bioactive compounds and antioxidant activity in okara under subcritical water conditions. Therefore, the objective of this study was to investigate the

effects of different temperatures under subcritical water extraction conditions on a range of potentially health-related chemical constituents and antioxidant capacities, in order to evaluate the potential of SWE for the production of high bioactive extracts from okara.

2. Materials and Methods

2.1. Chemicals and Standard Solutions. Ascorbic acid, Folin–Ciocalteu reagent, ethanol, sodium carbonate, gallic acid, sodium nitrite, aluminium nitrate, sodium hydroxide, disodium hydrogen phosphate, potassium persulphate, sodium dihydrogen phosphate, phenol, and D-glucose were purchased from Wako Pure Chemical (Osaka, Japan). 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich, Inc. (Saint Louis, MO, USA). All the other chemical reagents were of analytical grade.

2.2. Sample Preparation. Okara (78.8% moisture content) was purchased from Inamoto Co., Ltd. (Tsukuba, Japan). Residual water was removed by drying at 60°C for 5 h, ground in a high-speed disintegrator (IFM-800, IWATANI, JAPAN) to obtain a fine powder (250 μm), and then refluxed with 85% ethanol at 70°C for 4 h to defat, deactivate enzymes, and remove interference components. The residue was thermally dried at 50°C and vacuum packed to decrease lipid oxidation.

2.3. Extraction Procedure. A schematic diagram of the semicontinuous SWE unit is shown in Figure 1. Subcritical water extraction was performed in a 200 mL SUS-316 stainless steel tube reactor (MMS-200, OMLABO, JAPAN) with the maximum operating pressure of 20 MPa and temperature of 300°C. The extraction system consists of an extraction vessel (i.d. 38.5 mm × 197 mm) covered with a heating jacket, a Bourdon tube pressure gauge, a pressure release valve, and a temperature sensor connected with a temperature controller by the thermocouple to monitor the real-time temperature. In a typical run, 1.0 g of pretreated okara powder was loaded inside the extraction reactor. After adding a fixed liquid-solid ratio of 30 mL/g, the vessel was electrically heated to each specific temperature, which generally took less than 8 min. The extraction process then lasted 10 min at the required temperature (160–230°C). The extracts were centrifuged at 3600 rpm for 10 min and separated from the soluble liquid portion through a filter paper (Whatman number 1). All of the supernatant were stored at 4°C in the dark before use.

2.4. Determination of Total Organic Carbon (TOC). TOC was measured by a TOC analyzer (TOC-VCSN, Shimadzu, Japan) after the samples being centrifuged and filtrated through 0.45 μm membrane. More details on the determination of TOC have previously been reported [9].

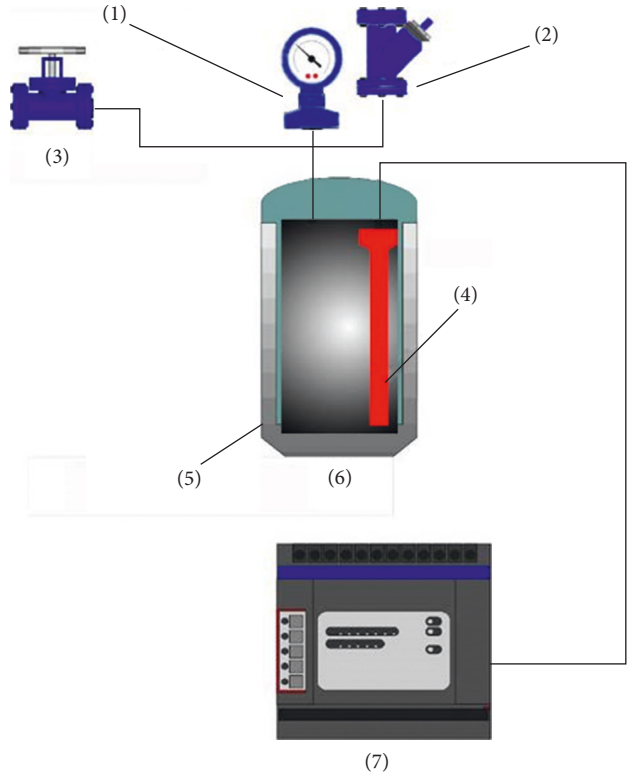

Figure 1: Experimental schematic diagram of the subcritical water system for crude polysaccharide production from okara. (1) Pressure gauge, (2) safety head, (3) pressure release valve, (4) temperature sensor, (5) insulation jacket, (6) SWT reactor, and (7) temperature controller and thermocouple.

2.5. Determination of Total Sugar. The total sugar was determined by the phenol-sulfuric acid method with certain modifications [10]. The color reaction was initiated by mixing 1 mL of solution with 0.5 mL of a 5% phenol solution and 2.5 mL of concentrated sulfuric acid, and the reaction mixture was incubated in a boiling water bath for 15 min. After cooling to room temperature, the optical density (O. D.) of the mixture was determined at 490 nm and the total carbohydrate content was calculated with D-glucose as a standard. The results were expressed as milligram of glucose equivalent per gram of okara [11].

2.6. Determination of Polysaccharides. The filtrate was precipitated by adding dehydrated ethanol to a final concentration of 80% (v/v) and stored at 4°C for 12 h. The precipitate was harvested as crude polysaccharides after centrifugation at 7500 rpm for 15 min and washed triple times using dehydrated ethanol. After being redissolved in ultrapure water, the aqueous solution was subjected to remove proteins by using the Sevag reagent [12], dialyzed with deionized water for 72 h, and concentrated under reduced pressure. Finally, the polysaccharide product was collected after lyophilization. Polysaccharide content was assayed using the phenol-sulfuric acid method [13]. All the results were expressed as mg of glucose equivalent per g of the pretreated okara.

2.7. Analysis of Total Phenolic Compounds. Total phenolic content (TPC) was determined on the basis of the Folin–Ciocalteu colorimetric method [14]. The sample (0.5 mL) and 2 mL of sodium carbonate (75 g/L) were added to 2.5 mL of 10% (w/v) Folin-Ciocalteu reagent. After 30 min of reaction at room temperature (intermittent shaking for color development), the absorbance was measured at 765 nm. The TPC was determined from the linear equation of a standard curve prepared with gallic acid.

2.8. Analysis of Total Flavonoid Compounds. The total flavonoid content (TFC) in extract was measured by a colorimetric assay [15]. The extract (5 mL) was added to a 10 mL flask, and then 5% $NaNO_2$ solution (0.3 mL) was added. After mixed well, the solution was allowed to stand for 6 min at room temperature; 5% $Al(NO_3)_3$ solution (0.3 mL) was added to the flask, mixed well, and kept for 6 min at room temperature. At last 4% NaOH solution (4.4 mL) was added, mixed well, and kept for 12 min at room temperature. Absorbance was read on a UV spectrophotometer at 510 nm, and the total flavonoid contents (%) were estimated using calibration curves. Total flavonoid content in samples was calculated from a calibration curve ($R^2 = 0.999$) using rutin and expressed as mg of rutin equivalent (RE) per g of dry weight.

2.9. Antioxidant Activities

2.9.1. DPPH Radical-Scavenging Activity Assay. DPPH radical-scavenging activity of extractions was evaluated according to a literature procedure with a slight modification [13]. Aliquots (0.5 mL) of various concentrations (0.3125–10.00 mg/mL) of extracts were mixed with 3 mL (25 μg/mL) of a MeOH solution of DPPH and then were shaken vigorously and allowed to stand in the dark for 30 min. The absorbance was measured with a spectrophotometer at 517 nm against a blank. Decrease of the DPPH solution absorbance indicated an increase of the DPPH radical-scavenging activity. Ascorbic acid was used as positive controls. DPPH radical-scavenging activity was calculated according to the following equation:

$$\text{scavenging activity } (\%) = \left(1 - \frac{A_1}{A_0}\right) \times 100\%, \quad (1)$$

where A_0 is the absorbance without samples and A_1 is the absorbance containing the samples.

Results were expressed in millimole (mM) ascorbic acid equivalent (AAE) per 1 g of a sample on a dry weight basis.

2.9.2. ABTS Radical-Scavenging Activity Assay. ABTS radical-scavenging activity was measured using the methods with some modifications [16]. ABTS was dissolved in distilled water at a final concentration of 7.0 mM and mixed with a potassium persulphate solution at a final concentration of 2.45 mM. The mixture was conserved in the dark at ambient temperature for 12–16 h before use. For individual sample experiment, prepared $ABTS^{\cdot+}$ solution was diluted with 0.01 M phosphate buffer saline (PBS, pH 7.4) to achieve its absorbance within 0.70 ± 0.02 at 734 nm wavelength. And then a constant volume (0.15 mL) of various concentrations of samples (0.3125–10.00 mg/mL) was reacted with 2.85 mL of $ABTS^{\cdot+}$ solution by mixing vigorously. Eventually, the absorbance was measured at 734 nm after incubation at ambient temperature for 10 min. Ascorbic acid was used as positive controls. The scavenging activity of the ABTS free radicals was calculated using the following equation:

$$\text{scavenging activity } (\%) = \left[1 - \frac{(A_1 - A_2)}{(A_3 - A_4)}\right] \times 100\%, \quad (2)$$

where A_1 is the absorbance of ABTS solution + sample, A_2 is the absorbance of potassium persulphate + sample, A_3 is the absorbance of ABTS solution + distilled water, and A_4 is the absorbance of potassium persulphate + distilled water.

Results were expressed in millimole (mM) ascorbic acid equivalent (AAE) per 1 g of a sample on a dry weight basis.

2.10. Statistical Analysis. The data obtained in this study were expressed as the mean of three replicate determinations plus or minus the standard deviation (SD). The content of total phenolic content was calculated as mean ± SD ($n = 3$) and expressed as mg gallic acid equivalents (GAEs)/g dry weight. The Pearson correlation coefficient (R) and $p$value were used to show correlations and their significances (SPSS 19.0 for Windows, SPSS Inc. IL, USA). Probability values of $p < 0.05$ and $p < 0.01$ were considered to be statistically significant and extremely significant, respectively.

3. Results and Discussions

3.1. Extraction Yields

3.1.1. General. As other processing steps like milling, grinding, and homogenization, extraction can recover and isolate phytochemicals from plant materials and is important for obtaining extracts with acceptable yields and strong antioxidant activity [17]. Generally, extraction efficiency is associated with the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances [18]. Besides, the yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time, and composition of the sample. Among these influential factors, extraction temperature is one of the crucial parameters, especially under the subcritical condition.

In the current study, a reaction time was 10 min and a liquid-solid ratio was 30 mL/g based on previous optimal extraction conditions using response surface methodology [19]. The various extractions from okara at different temperatures were presented in Table 1. The results revealed a considerable diversity in the chemical constituents in the extracts investigated in the present study. For TOC, it was kept relatively stable until 180°C, which produced the highest value of $30.00 \pm 0.73\%$ and then sharply decreased to

Table 1: Chemical constituents and antioxidant activities of different okara extracts by using SWE.

Temperature (°C)	TOC (%)	Total sugar (mg·GE/g)	Polysaccharides (mg·GE/g)	TPC (mg·GAE/g)	TFC (mg·RE/g)	DPPH (mM·AAE/g)	ABTS (mM·AAE/g)
160	29.83 ± 0.75	210.36 ± 4.35	145.61 ± 3.09	23.32 ± 0.14	15.60 ± 0.87	0.85 ± 0.14	31.54 ± 0.73
170	28.27 ± 0.79	174.02 ± 2.41	92.41 ± 0.23	27.12 ± 0.37	18.27 ± 0.19	3.39 ± 0.27	45.00 ± 2.30
180	30.00 ± 0.73	163.77 ± 1.42	61.86 ± 2.98	39.05 ± 0.46	20.31 ± 0.40	7.31 ± 0.35	73.77 ± 1.15
190	20.64 ± 0.73	112.56 ± 6.14	48.86 ± 1.40	50.50 ± 0.54	28.12 ± 0.88	18.73 ± 1.15	103.28 ± 2.41
200	21.73 ± 0.35	89.16 ± 1.81	19.01 ± 1.14	67.25 ± 0.73	49.00 ± 1.40	23.75 ± 0.36	126.37 ± 0.88
210	22.17 ± 0.25	61.08 ± 2.09	3.13 ± 0.045	71.14 ± 0.54	58.12 ± 0.64	38.67 ± 1.14	156.19 ± 3.47
220	20.86 ± 0.20	39.09 ± 0.93	0.54 ± 0.0091	76.18 ± 0.45	66.12 ± 0.69	43.43 ± 0.58	177.12 ± 8.77
230	20.30 ± 0.23	22.83 ± 0.44	0.30 ± 0.014	70.82 ± 0.65	65.91 ± 0.66	41.83 ± 1.53	169.61 ± 9.70

20.64 ± 0.73%, possibly owing to a weak hydrolysis reaction, pyrolysis, and gasification of the organic compounds [3]. The trend of total sugar was similar to that of TOC. With the increasing temperatures, total sugar reduced continuously from 210.36 ± 4.35 mg·GE/g to 22.83 ± 0.44 mg·GE/g. There was a wide range of variations in total polysaccharides, from 0.30 mg·GA/g to 145.61 mg·GA/g. The results were consistent with the fact that total sugars of the aqueous phase comprise mixtures of poly-, oligo-, di-, and monosaccharides [20].

3.1.2. Total Phenolic Content. Phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables [21]. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [22]. The total phenolic content of diverse extracts in this study was determined based on the aforementioned reagent method. TPC values were calculated from the gallic acid standard calibration curve $y = 0.0056x - 0.0003$ with $R^2 = 0.9995$, where x is the absorbance and y is the concentration of gallic acid equivalents expressed as mg GAE/g. Significant differences were found for TPC among the extracts at various subcritical temperatures. The TPC of various aqueous extracts was in the range of 23.32 ± 0.14 mg·GAE/g to 76.18 ± 0.45 mg·GAE/g (increased by 3.27 folds) under subcritical conditions (Table 1). The cleaving of the esterified and glycosylated bond or the formation of the Maillard reaction during the heating process may be responsible for the increase in total phenolics after heating [8, 23]. However, when the heating temperature rose to 230°C, a slight decrease (70.82 ± 0.65 mg·GAE/g) occurred, mainly because of accelerated interaction decomposition of target products under the elevated temperature. And the results are in good agreement with the published results related to the total phenolic content of canola meal achieved by subcritical water technology [24].

3.1.3. Total Flavonoid Content. Flavonoids are the naturally occurring polyphenols representing one of the most prevalent classes of compounds in vegetables, nuts, fruits, and beverages such as coffee, tea, and red wine [25]. TFC values were calculated from rutin standard calibration curve $y = 0.0012x + 0.0027$ with $R^2 = 0.992$, where x is the absorbance and y is the concentration of rutin equivalents expressed as mg·RE/g. The TFC of okara extracted at different temperatures in this study is shown in Table 1. An increasing trend of TFC in first seven extracts was found as the temperature elevated, whereas there was a moderate decrease in the TFC when the temperature reached to 230°C. The total flavonoid contents varied from 15.60 ± 0.87 (160°C) to 66.12 ± 0.69 mg·RE/g (220°C) with the difference of 4.24-fold in the initial flavonoid contents. The decrease in the total flavonoid at a higher temperature could be attributed to the degradation of flavonoids, which possibly depends on the structure of particular flavonoids [8]. Our results are in agreement with the findings of Ioku et al. [26] who found that total flavonoid content increased after heating at a certain temperature and magnitude of time, whereas too much exposure to severe conditions reduced the content of total flavonoid content. Moreover, in most fruits and vegetables, flavonoids contain glycosidic bonds and exist as dimers and oligomers, and the industrial processing such as heating or boiling results in the formation of monomers by the hydrolysis of glycosidic bonds [27].

3.2. Antioxidant Activities

3.2.1. DPPH Radical-Scavenging Activity. Since different trends have been found in numerous antioxidant activity assays, it is of necessity to evaluate and compare the antioxidant capacity of extracts by means of multiple assays rather than one single test. Hence, the total antioxidant activity of extracts in this study needed to be measured using the DPPH assay and the ABTS assay, respectively.

DPPH radical is a stable free radical with a characteristic absorption maximum at 517 nm [28]. The DPPH radical-scavenging activity assay is sensitive enough to accommodate samples in a short period of time and detect active ingredients at low concentrations [29]. The DPPH scavenging radical effects of the eight different aqueous extracts from okara and standard antioxidant (ascorbic acid) were compared and shown in Figure 2. In this study, ascorbic acid showed DPPH radical-scavenging activity of 97.30 ± 0.12% at the initial concentration of 0.3125 mg/mL, exhibiting excellent scavenging ability on DPPH radicals. And altered concentrations of all the SWE extracts (0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/mL) showed DPPH scavenging activities in a dose-dependent manner. As shown in Table 1, all aqueous extracts on the DPPH assay followed an

ascending the order of 160°C < 170°C < 180°C < 190°C < 200°C < 210°C < 230°C < 220°C. And it was obvious that DPPH assays varied to a great extent, ranging from 0.85 to 43.43 mM AAE/g among the extract accessions when the temperature rose from 160°C to 220°C. In other words, DPPH radical-scavenging activities of extracts significantly increased ($p < 0.05$) as the temperature elevated. The results indicated that SWE plays a positive role on promoting the quantity of antioxidant metabolites in the obtained extracts and advancing radical-scavenging activity. Moreover, it could be attributed to the hydrolysis of glycoside bonds of phenolic compounds, thus increasing the number of phenolic hydroxyl groups and consequently the antioxidant activity of the extracts, taking into account TPC and TFC content in the extracts.

3.2.2. Total Antioxidant Activity Using ABTS. Since the characteristic absorption spectrum of ABTS cation radical can be determined at 414, 645, 734, and 815 nm [30, 31] because of the presence of antioxidants [32], common organic radical cation ($ABTS^+$) assay is one of the most widely applied methods to assess antioxidant activity [33]. In addition, other reports indicated that ABTS radical-scavenging activity is more of accuracy, sensitivity, and robustness for screening single ingredient and other complex antioxidant mixtures such as plant extracts, beverages, and biological fluids [34].

Eight extracts at various temperature levels were measured and compared for their free radical-scavenging activities against ABTS radicals, and the results of the scavenging ability of SWE extracts on ABTS free radicals are shown in Figure 3. Apparently, all the extracts reduced the absorbance at 734 nm, and the concentration of the extracts was directly proportional to the reduction. In the ABTS radical-scavenging assay, noteworthy differences among values of all the extracts were found ($p < 0.05$). As the control group, ascorbic acid at a concentration of 0.3125–10 mg/mL was also available to produce excellent inhibition of ABTS radicals. To be more specific, levels of ABTS radical-scavenging activities followed a descending order of 220°C > 230°C > 210°C > 200°C > 190°C > 180°C > 170°C and 160°C with values of 77.06%, 72.94%, 72.13%, 61.83%, 51.57%, 37.27%, 19.10%, and 15.01% at the initial concentration of 0.3125 mg/mL, respectively. When the concentration rose to 2.5 mg/mL, almost all the samples were equal to the effect produced by ascorbic acid at the same concentration, except for the sample processed at 160°C. Another result should be pointed out that the effect of scavenging capacity on ABTS radicals was as significant as that of ascorbic acid when the concentration of test solution was greater than 5 mg/mL, which indicated that there was no difference on ABTS radical-scavenging activity between aqueous extracts and ascorbic acid at a relatively higher concentration.

Also, it should be noted that DPPH and ABTS assays were conducted in ethanol and water media, respectively [35]. From a mechanistic perspective, the DPPH radical-scavenging assay emphasizes the capacity of the extract

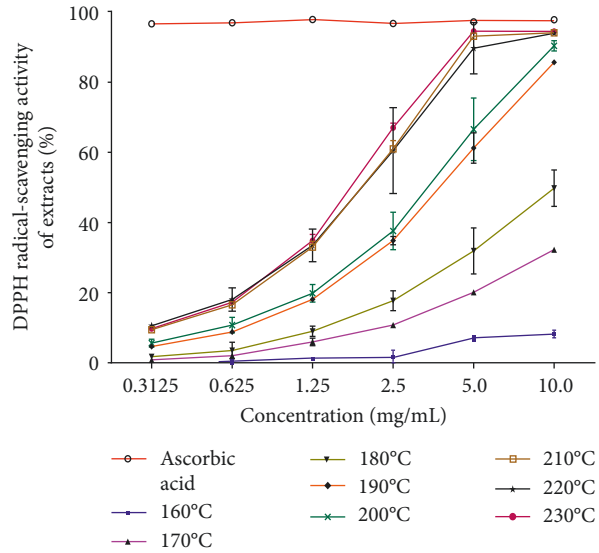

Figure 2: DPPH radical-scavenging assays of different aqueous extracts from okara by SWE and standard antioxidant (ascorbic acid). Data were expressed as mean ± standard deviation of triplicate samples.

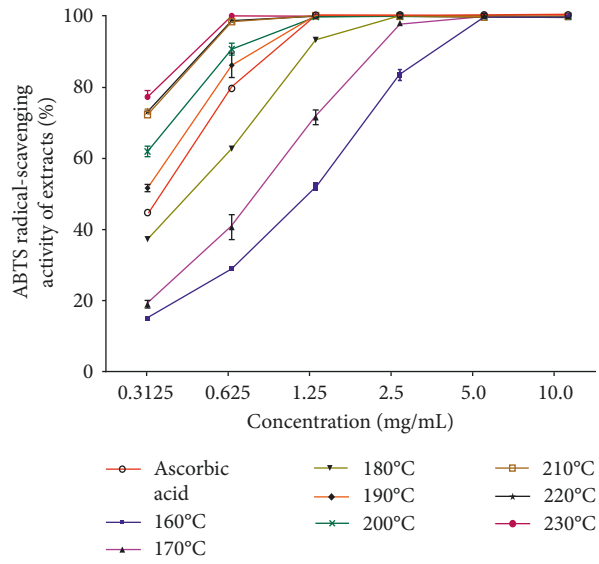

Figure 3: ABTS radical-scavenging assays of different aqueous extracts from okara by SWE and standard antioxidant (ascorbic acid). Data were expressed as mean ± standard deviation of triplicate samples.

transferring electrons or hydrogen atoms, while the ABTS radical-scavenging activity could reflect the hydrogen donating and the chain-breaking capacity of the extract [36]. The order of ABTS radical-scavenging activity was consistent with that of the DPPH radical, suggesting thermal treatment had decisive effects on scavenging radicals dissolved in organic or aqueous solvent, to some extent.

3.3. Correlations among Constituents and Antioxidant Activities. In order to evaluate the suitability and reliability

Table 2: Correlation coefficients between constituents and antioxidant activities of okara extracts by SWE.

R (R^2)	TOC	Total sugar	Polysaccharides	TPC	TFC	DPPH	ABTS
TOC	1	—	—	—	—	—	—
Total sugar	0.899 (0.808)**	1	—	—	—	—	—
Polysaccharides	0.815 (0.664)*	0.952 (0.906)**	1	—	—	—	—
TPC	−0.878 (0.771)**	−0.967 (0.935)**	−0.959 (0.920)**	1	—	—	—
TFC	−0.821 (0.674)*	−0.968 (0.937)**	−0.897 (0.805)**	0.959 (0.920)**	1	—	—
DPPH	−0.857 (0.734)**	−0.981 (0.962)**	−0.914 (0.835)**	0.962 (0.925)**	0.982 (0.945)**	1	—
ABTS	−0.872 (0.760)**	−0.993 (0.986)**	−0.954 (0.910)**	0.980 (0.960)**	0.973 (0.947)**	0.988 (0.976)**	1

R: correlation coefficient. R^2: coefficient of correlation. *Significant at $p < 0.05$. **Significant at $p < 0.01$.

of the antioxidant assay for measurement of total antioxidant activity of extracts from okara by SWE, Pearson correlation coefficients between the antioxidant capacities (DPPH and ABTS), total organic carbon, total sugar, polysaccharides, total phenolic content, and total flavonoid content for all extracts prepared under different extraction temperatures were calculated and the results are shown in Table 2. According to the results of the present study, significant correlations ($p < 0.05$) were detected in all cases, which revealed that values of antioxidant activity determined by two different methods were comparable and reliable.

TOC showed strongly linear relationship with total sugar ($R = 0.899$; $R^2 = 0.808$, $p < 0.01$), and polysaccharides ($R = 0.815$; $R^2 = 0.664$, $p < 0.05$), which is in accordance with the fact that carbon element is essential in these two compounds. It should be noted that TOC, total sugar, and polysaccharides were negatively correlated with two selected *in vitro* assays, suggesting there was no direct relation between the three aforementioned carbohydrates and antioxidant abilities under these subcritical water conditions. Too much exposure to elevated temperatures contributed to their degradation and inactivation. The correlation coefficient for DPPH and ABTS assay ($R = 0.988$; $R^2 = 0.976$, $p < 0.01$) indicated that the values of antioxidant activities assayed by these two different methods were significantly correlated, associating with spectrophotometry-based assay methods and elimination ability of the radical cation [37].

Phenolic compounds in extracts, like other antioxidative compounds, are believed to account for a major portion of the antioxidant activity in many plants due to interaction with free radicals by acting as electron donor of hydrogen atoms. Measurements of the scavenging effects of DPPH and ABTS radicals showed that radical-scavenging capacity increased with increase in TPC. Apparently, TPC exhibited a strong correlation with both DPPH ($R = 0.962$; $R^2 = 0.925$, $p < 0.01$) and ABTS ($R = 0.980$; $R^2 = 0.960$, $p < 0.01$). Consistently, as main ingredients of TPC ($R = 0.959$; $R^2 = 0.920$, $p < 0.01$), TFC also has closed connections with both DPPH ($R = 0.982$; $R^2 = 0.945$, $p < 0.01$) and ABTS ($R = 0.973$; $R^2 = 0.947$, $p < 0.01$). The high correlations obtained in this work could suggest that the antioxidant activities of these extracts are resulted from phenolic compounds which contribute to the DPPH and ABTS scavenging activities. All these results differ from previous studies on *Amaranthus mantegazzianus* [38]. Three factors may bring about the differences between the results of this study and other studies: (1) the difference in plant matrix; (2) the method and conditions of extraction (temperature, time, and solvents) led to differences in compositions and antioxidant activities; (3) the difference of phenolic structures like possessing a higher number of hydroxyl groups [39]. It is worth noting that there must be some other soluble compounds existing in the extracts, like proteins, peptides, and pigments, might be also responsible for the antioxidant activity partly [40].

4. Conclusions

Thermal processing had significant effects on the compositions and antioxidant activities on okara extracts in this study. Specifically, TOC, total sugar, and polysaccharides in extracts decreased under elevated temperature due to some reactions like pyrolysis and gasification. The highest yields of TPC and TFC were observed in the extract obtained as extraction temperature was 220°C with the other two fixed parameters (residence time of 10 min and liquid-solid ratio of 30 mL/g). Among the various aqueous extracts, all aqueous extracts showed high antioxidant activity, which indicated SWE played important roles in the increase of the antioxidant capacities of okara extracts on DPPH scavenging activity and ABTS assay. According to correlation comparisons, the total phenolic contents of the extracts were coherent with the antioxidant activities of the extracts, pending further analysis of their specific composition to explain the underlying mechanism. This finding could be useful to the disposal processing of okara by-products in the food industry.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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