

1.1. Ultrasound-assisted extraction with methyl alcohol

The SBTP and FR were grounded in conical beaker. Methanol was added at solid to solvent ratio of 1:5 (w/v). The equipment was kept overnight at 25 °C in a Ultrasonic Cleaner (KQ-300-DE, China, Kunshan). The suspension was obtained by the centrifugation at 5000 rpm for 5min. The extraction was repeated twice. The supernatants were mixed together and filtered. The solution was evaporated by a rotary evaporator (Shenshun, R501, China), followed by nitrogen gas. The extracts obtained were dissolved in methanol and kept at -20 °C for further evaluation.

1.2. SBTP and FR waste composition

The moisture content of materials was confirmed by a moisture meter (MB45, Ohaus). The chemical constitution (protein, lignin, crude lipids, crude fiber, and ash) of SBTP and FR was confirmed according to AOAC method.¹ Total carbohydrates were evaluated by subtracting protein, fat, ash, and TDF. All processes were conducted in triplicate, and values were expressed as mg/g dry sample.

1.3. Total polyphenol, carotenoid and flavonoid content,

Folin-Ciocalteu method was selected to conduct total polyphenol assay of the wastes.² Briefly, the extracts (3mL) were mixed with 0.5 mL of Folin-Ciocalteu reagent and 10mL of sodium carbonate solution (10%). The absorbance was recorded at 765 nm after 2-hour reaction in darkness. Total polyphenol assay was presented by comparing the curve of gallic acid as a reference standard.

Total carotenoid content was confirmed according to the method of Aprodu et al 2017 with some modifications.³ In brief, 1 mL of extract was added to 0.5 mL of 0.05 g/L NaCl, shaking for 30 s, followed by centrifugation at 4000 rpm for 10 min. After properly diluted, the absorbance of the solution was evaluated at 450 nm. The final results were conducted through plotting a calibration curve with β -carotene as a reference standard.

The flavonoid content was performed by the aluminum chloride colorimetric method with some modifications.⁴ A volume of 1.25 mL of the extract was mixed with 3% NaNO₂ solution (1 mL) in a 10 mL brown volumetric flask. The reagent was preserved for 6 min, followed by the addition of AlCl₃ (10%, 2mL). The solution stood for 5 min, and 3 mL of NaOH (1 M) was added. The container was filled to the volume with methanol. The mixture was kept in darkness for 20 min, and the final results were conducted through plotting a calibration curve with quercetin as a reference standard.

1.4. Tocopherol and tocotrienol

Extracts (1mL) were added to a 10 mL brown volumetric flask, followed by n-hexane dissolved to the curve. The mixture was filtered through a nylon syringe filter (0.22 μ m) before injected into a Waters 2695 HPLC for evaluation. The condition was as follows: LC separation (Waters Spherisorb® NH₂ analytical column, 4.6 mm \times 250 mm, 5 μ m), mobile phase (n-hexane/isopropanol, 98:2 v/v), flow rate (0.8 mL/min), column temperature (40 °C), injection volume (10 μ L), excitation wavelength (298 nm), emissions wavelength (330 nm).

1.5. Antioxidant activity *in vitro*

The DPPH radical scavenging ability of extracts was measured by the method of Mi et al⁵. One mL of extract and 1 mL of DPPH solution were mixed together. The mixture was shaken rigorously and stored in darkness for 2 h at room temperature. The results were confirmed at 517 nm using Microplate Reader (Thermo, 1510) and expressed as inhibition percentage. The calculation was as follows:

$$\text{Inhibition percentage (\%)} = [1 - (\text{A}_{517} \text{ of extracts}) / (\text{A}_{517} \text{ of blank})] \times 100\%.$$

The ABTS radical scavenging ability of extracts was confirmed according to Mi et al⁵. ABTS working solution was composed of ABTS reagent (7 mmol/L, 25mL), potassium persulfate solution (2.45 mmol/L, 440 μ L). The working solution was prepared in advance (12-16h), and diluted to the same absorbance (0.7 ± 0.01) with methanol before used. Methanolic extracts (10 μ L) was added to ABTS working solution (1 mL) and the mixture stood in darkness for 20 min after shaken. The absorbance was recorded at 734 nm by Microplate Reader. The final results were evaluated as inhibition percentage by the following formula:

$$\text{Inhibition percentage (\%)} = [1 - (\text{A}_{517} \text{ of extracts}) / (\text{A}_{517} \text{ of blank})] \times 100\%.$$

The FRAP assay was estimated in accordance with the method of Shi et al⁶. FRAP solution, containing acetate buffer (25 mL, 0.1 mol/L, pH 3.6), TPTZ solution (2.5 mL, 10 mmol/L) and FeCl₃ solution (2.5mL, 20 mmol/L), was prepared and maintained at 37 °C. The diluted extract (0.1 mL) and FRAP reagent (1mL) were added to a 5 mL graded tube, and distilled water was added to the volume. The tubes were persevered at room temperature for 10 min after shaken completely. The solution was centrifuged at 10,000 rpm for 3 min, and the supernatant was moved to 96-well plate for measurement. The absorbance was confirmed at 593 nm by Microplate Reader, and the distinction between sample absorbance and blank absorbance was evaluated as the result of the FRAP radical scavenging ability.

References

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