Research article

Physicochemical, functional properties and antioxidant activity of protein extract from spent coffee grounds using ultrasonic-assisted extraction

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Abstract: Spent coffee grounds, the residue from coffee brewing, are still underutilized even though they contain several useful organic compounds including proteins. To valorize the spent coffee grounds, the spent coffee ground protein was investigated using ultrasonic-assisted extraction as a pretreatment to conventional extraction. The pretreatments involved different ultrasound amplitudes (40%, 60% and 80%) and extraction times (10, 20 and 30 min) and their effects on the physicochemical and functional properties including antioxidant activity of protein extract. It was found that the protein content extracted was increased approximately 2 times, compared to the initial spent coffee grounds. Furthermore, the ultrasonic-assisted extraction affected the physicochemical properties, functional properties and antioxidant activity of the protein extract. The 80% amplitude for 10 min extraction time improved the foaming capacity, foaming stability, emulsifying activity index and the emulsifying stability index of protein extract. The pretreatment at 20 min extraction time provided the highest antioxidant activity (933.92–976.03 mM Trolox eq/g protein extract) and the highest total phenolic content (267.66–304.81 mg GAE/g protein extract). Nonetheless, protein extract using ultrasonic-assisted extraction resulted in higher total phenolic content and antioxidant activity without changes in the protein structure as confirmed by changes in FT-IR spectra and SDS-PAGE profiles. Thus, the spent coffee ground protein can be an interesting and alternative plant protein with functional properties for food application. Moreover, this work showed the feasibility to reduce waste and the food waste valorization.
Keywords: spent coffee grounds; protein extract; ultrasonic-assisted extraction; antioxidant activity; functional properties

1. Introduction

Coffee is a popular beverage worldwide. Spent coffee grounds (SCG) are the residual material obtained from coffee brewing. SCG have been studied as a source of polysaccharides [1] and bioactive compounds [2]. However, SCG are still underutilized even though they contain several useful compounds for the food industry. One of these interesting compounds is protein. It has been reported that SCG have 13–17% protein content [2,3]. Thus, there is a need to transform coffee by-products into a value-added compound for reuse in the food system. Generally, alkali treatment is the conventional method used to extract protein from rice bran [4], coconut by-products [5], walnut [6] and legumes [7]. Tao et al. [8] reported that an extreme alkaline treatment could reduce cell wall rigidity and improve the functional properties of okara protein.

At present, ultrasonic techniques, especially high intensity ultrasound, are widely used in the food industry for extraction, emulsification, crystallization, depolymerization, fermentation and microbial deactivation [6]. The sound waves produce high shear forces to disrupt the cell walls and allow solvent penetration into the material cells resulting in the release of compounds [9]. Application of ultrasonic-assisted extraction (UAE) is simple and effective technique compared to conventional extractions (CE). Moreover, several studies showed that UAE enhanced protein extraction combined with conventional solvent extraction [10]. For example, a high extraction yield of protein was obtained from defatted soy flakes at 46% ultrasound amplitude, compared to the control sample without ultrasound [11]. Preece et al. [12] reported that the protein extraction yield from okara using pilot-scale UAE increased by 4.2%. Furthermore, UAE improved the water and oil absorption and protein yield of rice bran protein concentrate compared with CE [4] and ultrasonic treatment increased the water solubility and improved the emulsifying properties of walnut proteins [6]. In addition, UAE was used as a pretreatment to enhance the aggregates of okara protein which improved the foaming stability index and emulsion stability [8]. Wen et al. [13] found that protein extraction yield from coffee silverskin using alkaline extraction by UAE was increased by 2.8 times, compared to CE. Nonetheless, Connolly et al. [14] reported that the alkaline extracted brewers’ spent grain had a protein content ranging from 38.96 to 46.16%. Most works have been done on the extraction of polysaccharides, caffeine and polyphenols from SCG. However, there is scarce on the study of the extraction and characterization of protein from SCG. Due to the remarkable amount of SCG disposal, UAE might be a technique to extract a relatively valuable protein from SCG with the improvement of functional properties and antioxidant activity to further use for pharmaceutical and food application. Therefore, the objective of this study was to determine the effect of UAE as a pretreatment to CE on the properties of SCG protein (SCGP).

2. Materials and methods

2.1. Materials

Spent coffee grounds (SCG) as a coffee by-product were collected from a coffee shop in
Nakhon Pathom province, Thailand, being derived from a mixture of the Robusta (90%) and Arabica (10%) coffee varieties. Folin-Ciocalteu reagent was purchased from Merck KGaA (Darmstadt, Germany). Gallic acid monohydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox ((±)-6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate (Na₂CO₃) was purchased from Ajax Fine-chem Pty Ltd (Taren Point, New South Wales, Australia). All chemicals and reagents used in this study were analytical grade.

2.2. Methods

2.2.1. Preparation of spent coffee grounds

The SCG were collected and dried at 60 °C for 24 h in a hot-air dryer (RedLINE RF 115, Tuttlingen, Germany) until the moisture content was less than 5% (wet basis). The SCG were defatted using petroleum ether as solvent using a solid:liquid ratio of 50:500 g/mL per bottle (1000 mL) in a shaking water bath (Memmert WNB 7–45, Schwabach, Germany) at 28 °C for 24 h. Then, the defatted SCG were dried overnight under a hood at room temperature (28 ± 2 °C) until constant weight was reached. The samples were kept in a polyethylene zip-lock bag and stored at room temperature for further analyses.

2.2.2. Protein extraction of spent coffee grounds

2.2.2.1. Conventional extraction

Protein extraction of SCG was done according to the modified method of Rodsamran and Sothornvit [5]. The SCG (60 g) were mixed with distilled water (720 mL) as solvent and the mixture was adjusted to pH 11 using 0.7 M tri-sodium orthophosphate (Na₃PO₄). The mixture was continually stirred at 50 °C for 1 h in a water bath. After that, the mixture was centrifuged at 10,000 × g and 0 °C for 10 min using a refrigerated centrifuge (Eppendorf centrifuge 5804R, Hamburg, Germany) to obtain the supernatant. To increase the protein content, the supernatant was precipitated by adjusting to pH 4 using 3M HCl. Precipitated proteins were washed with distilled water, centrifuged and then stored at −50 °C for 48 h in a freezer (Elcold DK-9500, Hobro, Denmark) and lyophilized using a freeze dryer (Scanvac Coolsafe 100-4 Pro, Lyng, Denmark) for 24 h. The dried SCGP samples were kept in polyethylene zip-lock bags at room temperature (28 °C) prior to further analyses.

2.2.2.2. Ultrasonic-assisted extraction as a pretreatment compared to conventional extraction

UAE of SCG was used as a pretreatment prior to continuing with CE. Briefly, the SCG-solvent sample (pH 11) was extracted using different ultrasound amplitudes (40, 60 and 80%) and extraction times (10, 20 and 30 min) with the pulse duration mode (20 s on and 20 s off) of an ultrasound processor (VCX 750, Sonics & Materials, Inc., Newtown, CT, USA) and a 25 mm diameter stainless probe. After the pretreatment with UAE, the sample was extracted by heating in a water bath (CE) as described in section 2.2.2.1.
2.2.3. Physicochemical properties

Protein content

The total protein contents of samples were evaluated using the Kjeldahl method with a conversion factor of 6.25 based on the Association of Official Analytical Chemists (AOAC) methods [15]. A protein sample (1.0 g) was digested in the presence of a Kjeldahl catalyst (5 g) and 20 mL of concentrated H₂SO₄ in a digestion flask by boiling until the solution was clear. Then, the solution was cooled at room temperature and then cautiously added 60 mL of distilled water and connected for distillation. The 50 mL of 40% NaOH solution was added to the solution to form the ammonia gas. The ammonia was trapped in a 50 mL of 4% H₃BO₃ solution. Then approximately 150 mL of distillate was collected and titrated with 0.2 N HCl and 6–7 drops of methyl red as an indicator until the solution changes from green to pinkish. The blank was also done the same way without sample. Protein content was calculated using Eq. (1):

\[
\text{Protein content (\%)} = \frac{(A - B) \times N \times 1.4007 \times 6.25}{W}
\]  

where: \(A\) = volume (mL) of 0.2 N HCl used for sample; \(B\) = volume (mL) of 0.2 N HCl used in blank; \(N\) = Normality of HCl; \(W\) = weight (g) of sample.

2.2.4. Functional properties

2.2.4.1. Water and oil absorption capacity

The water absorption capacity (WAC) and oil absorption capacity (OAC) were evaluated according to the method of Rodsamran and Sothornvit [5]. A protein sample (20 mg) and 1.5 mL of distilled water or soybean oil were mixed in a vortex for 20 s in a 2 mL centrifuge tube and then allowed to stand at 30 °C for 30 min. The tubes were centrifuged at 10,000 × g for 20 min at room temperature. The free water or oil was removed using a pipette and the water or oil-absorbed sample was weighed. The WAC and OAC were expressed as grams of water or oil absorbed per gram of protein sample.

2.2.4.2. Foaming capacity and stability

The foaming capacity (FC) and foaming stability (FS) method were determined using the method of Rodsamran and Sothornvit [5]. A protein sample (200 mg) was prepared in 20 mL distilled water (V), adjusted to pH 11 with 0.7 M Na₂PO₄ solutions and stirred at 30 °C for 30 min. Then, the protein solution was homogenized at 919 × g for 1 min in a high speed homogenizer (Polytron® PT-MR 3100D, Kinematica AG, Luzern, Switzerland). The whipped protein sample was transferred into a 50 mL graduated cylinder and the volume was recorded at 0 min (\(V_0\)) and 60 min (\(V_1\)). The FC and FS were calculated using Eqs. (2) and (3), respectively.

\[
\text{FC} = \frac{V_0 - V}{V} \times 100
\]  

\[
\text{FS} = \frac{V_1}{V_0} \times 100
\]
\[ FS = \frac{V_f - V}{V} \times 100 \]  

(3)

2.2.4.3. Emulsifying properties

The emulsifying activity index (EAI) and the emulsifying stability index (ESI) were calculated according to the modified method of Pearce and Kinsella [16]. A sample of protein solution (10 mg/mL, WE) was adjusted to pH 11 with 0.7 M Na\(_3\)PO\(_4\) solutions and stirred at 30 °C for 30 min. A sample of 18 mL of protein solution was mixed with soybean oil (2 mL) and then homogenized at 13,500 rpm for 1 min. A sample of 50 μL of the emulsion was pipetted at 0 and 10 min from the bottom of the tube and diluted with 5 mL of 0.1% sodium dodecyl sulfate (SDS) solution. After homogenization, the absorbance of the emulsion at 0 min (A\(_0\)) and 10 min (A\(_{10}\)) was measured at 500 nm using a spectrophotometer (Shimadzu UV–Visible 1800, Tokyo, Japan). The EAI and ESI were calculated using Eqs. (4) and (5), respectively.

\[
EAI (m^2/g) = \frac{2 \times 2.303 \times A_0}{0.1 \times WE} 
\]

(4)

\[
EAI (\text{min}) = \frac{A_0 \times 10}{A_0 - A_{10}} 
\]

(5)

2.2.4.4. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was run according to the method of Samsalee and Sothornvit [17] in a Mini Protein II electrophoresis unit (Bio-Rad Laboratories Inc., Richmond, CA, USA). The dried precipitate proteins were dissolved in distilled water (5 mg/mL), adjusted to pH 11, mixed for 1 min using a vortex and centrifuged at 12,000 \(\times\) g for 10 min. The supernatant protein solutions (20 μL) were mixed with 20 μL of sample buffer (containing 950 μL Laemmli buffer and 50 μL β-mercaptoethanol) and then heated at 90 °C for 10 min. Ten μL of each sample and marker (Precision Plus Protein All Blue standard, Bio-Rad Laboratories Inc., Richmond, CA, USA) were loaded onto 4–20% precast polyacrylamide gel (Mini-Protein® TGXTM Precast Gels). Electrophoresis was performed in an electrode buffer (containing 25 mM Tris-HCl, pH 8.3, 0.19 M glycine and 0.1% SDS) at 120 V for approximately 40 min. Protein was stained with 0.125% Coomassie brilliant blue G 250 and destained with 30% methanol and 10% acetic acid.

2.2.4.5. Total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu assay. The protein solution (1 mg/mL) was prepared in distilled water and stirred at 30 °C for 30 min. The solution was centrifuged at 126 \(\times\) g for 10 min. The 0.8 mL supernatant samples were mixed with 4 mL of 10% Folin-Ciocalteu reagent and then 3.2 mL of 10% sodium carbonate were added to each mixture and vortexed for 20 s. The mixtures were incubated at room temperature for 2 h. The absorbance of each
sample was measured at 750 nm against a blank using a spectrophotometer. Gallic acid was used as a standard and the TPC was expressed as milligrams of gallic acid equivalent (GAE) per gram of sample.

2.2.4.6. Antioxidant activity based on DPPH assay

The antioxidant activity was determined according to the modified method of Geremu et al. [18]. The protein solution (1 mg/mL) was prepared in distilled water and stirred at 30 °C for 30 min. The solution was centrifuged at 126 × g for 10 min. Extracts (2 mL) were mixed with 4 mL of 0.4 mM methanolic solution of DPPH. The mixtures were stored at room temperature in the dark for 30 min and the absorbance was measured at 517 nm against a blank using a spectrophotometer. The percentage of radical-scavenging ability was calculated based on Eq. (6) and the results were expressed as millimolar of Trolox equivalent per gram of sample.

\[
\text{Scavenging ability (\%) } = \left[ 1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right] \times 100
\]  
(6)

2.2.4.7. Fourier transform infrared (FT-IR) spectra

The FT-IR spectra of protein samples were determined using a Perkin Elmer Spectrum 100 instrument (PerkinElmer Inc., Waltham, MA, USA) for the wavenumber range 4000–650 cm\(^{-1}\), with the attenuated total reflectance technique. Spectra were recorded in absorbance mode based on 16 scans per spectrum at a resolution of 4 cm\(^{-1}\). The interference of water and CO\(_2\) from air was deducted during scanning.

2.2.4.8. X-Ray Diffraction Analysis

X-ray diffraction (XRD) patterns of SCGP samples were performed using a X-ray diffraction (Aeris 600W, PANalytical, Netherlands) operating at a CuKα wavelength of 0.154 nm. The samples were exposed to the X-ray beam with the X-ray generator running at 40 kV and 15 mA. Distribution patterns were obtained at 2θ angles, 10 to 70 °C at room temperature (25 °C) and step size of 0.02°.

2.3. Statistical analyses

A completely randomized design was used in this experiment. Three replications were used to determine each property. Data were subjected to analysis of variance and Duncan’s multiple range test was used to determine significant differences at the 95% confidence interval. Analysis was performed using the SPSS package (SPSS 11.0 for Windows; SPSS Inc.; Chicago, IL, USA).

3. Results and discussion

3.1. Physicochemical properties

The total protein content of SCG was 15.97% (Table 1) similar to the values reported by
Mussatto et al. [2] and Ballesteros et al. [3] in the same material (13.6 and 17.44\%, respectively). Variations in the protein content corresponded to the variety of the coffee beans and the brewing conditions used [3]. There was no effect of UAE on the total protein content of SCGP, compared with that using CE alone. The total protein content of all SCGP varied from 29.48 to 33.95\% (Table 1). According to previous studies, the extraction of protein from other related by-products were reported such as brewers' spent grain (38.96–46.16\%) [14] and defatted rice bran (76.09\%) [4]. The higher protein content compared to our results might be due to the effects of different extraction conditions (such as extraction method, extraction temperature and extraction time) including types of raw materials. Although the protein content of SCGP might not be as high, the large amount of SCG disposal is significant and the waste valorization opens an opportunity and challenge for the future researchers. Nevertheless, different UAE extraction times (10, 20 and 30 min) did not result in any significant differences in the protein contents at the same amplitude (Table 1). We hypothesized that the protein content from SCG was extracted and completely released within the first 10 min of UAE extraction.

Table 1. Physicochemical properties, antioxidant activity and total phenolic content of spent coffee grounds and spent coffee ground protein using conventional extraction and ultrasonic-assisted extraction.

| Extraction condition | Ultrasonic extraction time (min) | Protein content (%) | Total phenolic content (mg GAE/g SCGP) | DPPH (mM Trolox eq/g SCGP) |
|---------------------|----------------------------------|---------------------|---------------------------------------|-----------------------------|
| SCG                 | -                                | 15.97 ± 1.10\a       | -                                     | -                           |
| CE                  | -                                | 32.11 ± 0.52\bcd     | 159.83 ± 13.80\a                     | 576.17 ± 0.48\a            |
| UAE 40\%            | 10                               | 32.42 ± 2.08\bcd     | 157.26 ± 4.63\a                      | 591.63 ± 75.03\ab          |
| UAE 40\%            | 20                               | 33.95 ± 1.62\cd      | 304.81 ± 3.94\bc                     | 933.92 ± 49.90\c           |
| UAE 40\%            | 30                               | 31.55 ± 1.36\bcd     | 297.73 ± 31.23\bc                    | 784.85 ± 16.90\cd          |
| UAE 60\%            | 10                               | 31.20 ± 1.05\bc      | 317.73 ± 50.38\bc                    | 937.11 ± 27.13\c           |
| UAE 60\%            | 20                               | 32.23 ± 2.39\bcd     | 288.65 ± 6.94\b                      | 976.07 ± 22.53\c           |
| UAE 60\%            | 30                               | 32.52 ± 1.24\bcd     | 344.82 ± 1.39\c                      | 859.28 ± 82.38\d           |
| UAE 80\%            | 10                               | 29.90 ± 2.07\bc      | 164.76 ± 1.19\a                      | 694.59 ± 60.05\bc          |
| UAE 80\%            | 20                               | 29.48 ± 1.85\b       | 267.66 ± 35.33\b                     | 961.17 ± 24.37\c           |
| UAE 80\%            | 30                               | 31.78 ± 1.89\bcd     | 139.29 ± 9.31\a                      | 712.41 ± 27.61\c           |

Data are mean ± standard deviation. Different superscripts (a, b, c, d, e) in each column indicate significant (p< 0.05) differences due to protein extraction method.

3.2. Functional properties

3.2.1. Water and oil absorption capacity

The WAC of SCGP using CE alone was 2.98 g/g protein sample (Figure 1). The extraction time of UAE did not affect the WAC but different amplitude levels resulted in a significant difference in the WAC of SCGP. The SCGP using UAE at 40\% amplitude for 10 and 30 min had the highest WAC value compared to the other SCGP samples. This might have been due to the effect of
cavitation in UAE breaking the covalent bonds of biopolymeric chains and increasing the mobility of molecules to absorb water compared to that using CE alone [19]. Similarly, Chittapalo and Noomhorm [4] reported that rice bran protein concentrates using UAE had higher WAC values compared to the CE method.

![Figure 1](image_url)

**Figure 1.** Water absorption capacity (WAC) and oil absorption capacity (OAC) of spent coffee ground protein using conventional extraction and ultrasonic-assisted extraction at different amplitudes and times of extraction. Different letters (a, b, c) indicate significant (p < 0.05) differences in each property. Error bars show standard deviation.

The UAE at 40% and 60% amplitudes had higher OAC values of SCGP than from using 80% amplitude (Figure 1). Changing the extraction time of UAE had no significant effect on the OAC values. Again, the cavitation effect of UAE was possibly a reason for the increase in the OAC of SCGP. The OAC of SCGP using CE alone was 2.92 g/g protein sample, being similar to the results for all SCGP samples using UAE at 80% amplitude. The higher values of WAC and OAC of the protein made it a suitable ingredient for food products such as breads, cakes and muffins where both hydration and shortening are desirable parameters [7].

### 3.2.2. Foaming capacity and stability

The foaming capacity and stability of SCGP using CE alone were 72.50% and 51.25%, respectively (Figure 2). Using UAE did not help to improve the foaming capacity and stability of SCGP compared to using CE alone. In contrast, the foaming properties significantly increased in the SCGP using UAE at 80% amplitude for 10 min. This might have been due to the higher amplitude of UAE given the uniform dispersion of the protein and fat particles that finally improved the foaming property [20]. Similarly, a significantly large increase was reported in the foam capacity of
ultrasound-treated wheat gluten with increasing ultrasound power levels (60%, 80% and 100% amplitudes for 10 min), which might have resulted from denaturation of the wheat protein due to the exposure of more hydrophobic regions [21]. The hydrophobic regions are taken into account in adsorption on the air-water interface molecules [20,21]. Therefore, UAE might be considered to improve the protein properties.

![Figure 2](image.png)

**Figure 2.** Foaming capacity and foaming stability of spent coffee ground protein using conventional extraction alone and ultrasonic-assisted extraction at different amplitudes and times of extraction. Different letters (a, b, c, d) indicate significant (p < 0.05) differences in each property. Error bars show standard deviation.

3.2.3. Emulsifying properties

The EAI evaluates the area of interface stabilized per unit weight of protein, whereas ESI evaluates the ability of the emulsion to resist changes to its structure over a certain period of time [22]. UAE at the different amplitude levels and extraction times did not improve the ESI compared to the CE treatment except at 80% amplitude for 10 min of UAE treatment (Figure 3). The emulsion activity of protein is altered by the interfacial interaction of water-oil interfaces which are controlled by several factors such as the ability of protein molecules to absorb at the water-oil interface, the strength of the membrane around the oil droplets and the ability of protein molecules to organize the hydrophobic and hydrophilic groups at the water-oil interface [23].
**Figure 3.** Emulsifying activity index (EAI) and emulsion stability index (ESI) of spent coffee ground protein using conventional extraction alone and ultrasonic-assisted extraction at different amplitudes and times of extraction. Different letters (a, b, c) indicate significant ($p < 0.05$) differences in each property. Error bars show standard deviation.

**Figure 4.** SDS-PAGE profiles of spent coffee ground protein using conventional extraction (CE) alone and ultrasonic-assisted extraction at different amplitudes and times of extraction.
3.2.4. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The ultrasound amplitude and time of extraction did not affect the protein pattern of SCGP (Figure 4). All SCGP samples following the CE and UAE had the highest protein band intensity (at around 15–20 kDa) compared to SCG. There was a clear, lower protein band intensity in the initial SCG, while the protein band intensity increased in SCGP after the extraction process of SCG using CE or UAE corresponding to the total protein content of samples. Bau et al. [24] reported that seeds of Coffea arabica contain the main reserved protein, consisting of two main bands at approximately 35 and 20 kDa. Moreover, SCGP using UAE did not modify the protein patterns in SDS-PAGE corresponding to the results in wheat gluten [21] and walnut protein isolate [6]. This indicated that the ultrasonic treatment did not alter the primary structure of the proteins [25]. Furthermore, this result confirmed that the molecular weight of the SCGP was not changed when using ultrasound treatment.

3.2.5. Total phenolic content

The UAE at 60% amplitude for 30 min produced a significantly higher (by 2 times) TPC compared with CE alone (Table 1). Nevertheless, it did not differ with SCGP extraction at either 60% amplitude for 10 min or at 40% amplitude for 20 and 30 min. The ultrasonic waves accelerate heat and mass transfer during extraction processes; thus, they eventually disrupt the plant cell walls via cavitation effects and release the bioactive compounds [26]. However, the TPC decreased with 80% amplitude and a longer extraction time over 20 min. This was probably due to the longer extraction time resulting in the degradation of bioactive compounds [27]. It is implied that not only the conditions of UAE but also the different structures of samples are important factors affecting the TPC of extract obtained. Nonetheless, the TPC values of SCGP in this study (139.29–344.82 mg GAE/g protein extract) were in a range of TPC reported in the enzymatically hydrolyzed SCG (291.86 mg GAE/g sample) [28]. The difference might be due to the different raw materials. We hypothesize that the higher TPC values of SCGP were come from the other compositions left in the protein extract such as polysaccharide which also provided TPC as well.

3.2.6. Antioxidant activity based on DPPH assay

The antioxidant activity of SCGP tended to increase following UAE (Table 1). The extraction time caused no significant difference in antioxidant activity at 60% amplitude. The highest antioxidant activity was obtained at 40% amplitude for 20 min, 60% amplitude for 10, 20, 30 min and 80% amplitude for 20 min ($p > 0.05$). This might have been due to UAE helping to accelerate solvent penetration and to release more active compounds than from using CE alone, resulting in higher antioxidant activity. As seen, the antioxidant activity of SCGP increased with extraction using UAE compared to using CE alone. This suggested the possibility of reusing SCG as an antioxidant compound. Antioxidant compounds have numerous applications in food, cosmetic, and pharmaceutical areas because they can protect against chronic and degenerative diseases and decrease the risk factors of cardiovascular diseases [3].
3.2.7. Fourier transform infrared spectra

The FT-IR spectra of all SCGP using the CE and UAE resulted in similar peaks (Figure 5). This is implied that there were no changes in the structure of the protein extract using UAE compared to using CE alone. The different amplitude levels and extraction times did not produce any differences in protein structure. Amide-I was found in the range between 1600 and 1700 cm\(^{-1}\), which corresponded to four conformation types of secondary structure of proteins: α-helix (1650–1660 cm\(^{-1}\)), β-sheet (1610–1640 cm\(^{-1}\), 1670–1690 cm\(^{-1}\)), β-turn (1660–1670 cm\(^{-1}\)) and random coil (1640–1650 cm\(^{-1}\)) [8]. These regions were associated with chlorogenic acid and caffeine found in SCG [3]. The major bands of the SCGP were prominent at approximately 3279, 2925, 1645, 1515 and 1232 cm\(^{-1}\), which were assigned to amide A (NH-stretching coupled with hydrogen bonding), amide B (CH stretching and –NH\(^3+\)), amide I (C=O stretching/ hydrogen bonding coupled with COO) and amide II (bending vibration of the N-H groups and stretching vibration of the C-N groups), amide-III (vibration in plan of C-N and N-H groups of bound amide), respectively [17].

![Figure 5. FT-IR spectra of spent coffee ground protein using conventional extraction alone and ultrasonic-assisted extraction at different amplitudes and times of extraction.](image)

3.2.8. X-Ray Diffraction

Figure 6 shows the X-ray diffractograms of SCGP using UAE at 40%, 60% and 80% amplitude
for 30 min. Diffractograms of other samples have not been presented due to the similar effects of different times on the structural properties of the samples. All SCGP samples had similar crystalline sharp peaks at 16.4°, 20.1°, 31.6°, 45.5° and 56.6° (2Θ). The presence of a peak (2Θ) at approximately 20° in all SCGP samples indicated the presence of β-sheet structure of the proteins [29], which were in agreements with the results on FT-IR. As shown, the peak intensity at 16.4° decreased with increasing ultrasound amplitude levels. This result could be related to the crystalline structure of SCGP was partially destroyed by ultrasonic treatment at high amplitude levels. Likewise, the crystallinity values of soluble dietary fiber from coffee peel by ultrasound-assisted enzymatic extraction were slightly lower than those of soluble dietary fiber from coffee peel by enzymatic extraction alone [30]. Moreover, the peak intensity at 31.6° of SCGP was related to diffraction of sample as a semi-crystalline polymer [30].

![XRD diffractograms of spent coffee ground protein using ultrasonic-assisted extraction at](image)

**Figure 6.** XRD diffractograms of spent coffee ground protein using ultrasonic-assisted extraction at: (A) 40 %, (B) 60% and (C) 80% amplitude for 30 min.

4. Conclusions

The UAE at different amplitude levels had little effect on the WAC and OAC values of SCGP. However, UAE at 80% amplitude for 10 min improved the foaming capacity, foaming stability, EAI and ESI of SCGP. Nevertheless, UAE produced high TPC and antioxidant activity of SCGP. Furthermore, UAE did not impact the structure of protein. Thus, the protein extract from SCG can be used as an alternative as food supplement or as functional food in a food system. This work shows the alternative way of waste valorization of SCG due to the large amount of SCG disposal at present.
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Conflict of interest

We have no conflicts of interest to declare.

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