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Properties and Stability of Oil-in-Water Emulsions Stabilized by Coconut Skim Milk Proteins

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Protein fractions were isolated from coconut: coconut skim milk protein isolate (CSPI) and coconut skim milk protein concentrate (CSPC). The ability of these proteins to form and stabilize oil-in-water emulsions was compared with that of whey protein isolate (WPI). The solubility of the proteins in CSPI, CSPC, and WPI was determined in aqueous solutions containing 0, 100, and 200 mM NaCI from pH 3 to 8. In the absence of salt, the minimum protein solubility occurred between pH 4 and 5 for CSPI and CSPC and around pH 5 for WPI. In the presence of salt (100 and 200 mM NaCI), all proteins had a higher solubility than in distilled water. Corn oil-in-water emulsions (10 wt %) with relatively small droplet diameters (d₃₂ ~ 0.46, 1.0, and 0.5 µm for CSPI, CSPC, and WPI, respectively) could be produced using 0.2 wt % protein fraction. Emulsions were prepared with different pH values (3-8), salt concentrations (0-500 mM NaCl), and thermal treatments (30-90 °C for 30 min), and the mean particle diameter, particle size distribution, ζ -potential, and creaming stability were measured. Considerable droplet flocculation occurred in the emulsions near the isoelectric point of the proteins: CSPI, pH ~4.0; CSPC, pH ~4.5; WPI, pH ~4.8. Emulsions with monomodal particle size distributions, small mean droplet diameters, and good creaming stability could be produced at pH 7 for CSPI and WPI, whereas CSPC produced bimodal distributions. The CSPI and WPI emulsions remained relatively stable to droplet aggregation and creaming at NaCl concentrations of \leq 50 and \leq 100 mM, respectively. In the absence salt, the CSPI and WPI emulsions were also stable to thermal treatments at ≤80 and ≤90 °C for 30 min, respectively. These results suggest that CSPI may be suitable for use as an emulsifier in the food industry.

KEYWORDS: Oil-in-water emulsion; coconut protein; coconut skim milk protein; protein isolate; protein concentrate; ultrafiltration; isoelectric precipitation

INTRODUCTION

Coconut (*Cocos nucitera* Linn.) is widely cultivated as an agricultural product in South and Southeast Asia, that is, India, The Philippines, Indonesia, and Thailand (*1*). Aside from being an important source of vegetable oil, the endosperm of fully matured coconut fruit is extracted to obtain coconut milk, which is an essential ingredient for traditional Asian foods and desserts.

In the coconut oil industry, dried coconut meat ("copra") is commonly used as the raw material for oil extraction either by organic solvent or by mechanical pressing (2). Efforts have been undertaken to utilize the protein that remains in the coconut meal after oil extraction for human consumption. Despite its

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fibrous, and the protein quality is severely lowered by the condition of oil extraction (3). Several methods of protein extraction from coconut meal have been reported, including use of chemicals such as NaOH, HCl, CaCl₂, and NaCl (4, 5) and enzymatic treatment using cellulase or protease (6, 7). However, the quality and functionalities of these protein products are adversely affected by the oil and protein extraction conditions. The oil extraction process from fresh coconut meat, the so-called "wet coconut process", was developed to gain high-quality coconut oil and to reduce losses from microbial spoilage, insects, and rodents during drying, storage, and transportation of copra (3). There are three main steps involved in the wet process: (i) extraction of coconut milk (emulsion); (ii) separation of coconut milk, cream, and skim milk; and (iii) breaking of the cream emulsion to recover the oil. Coconut protein is an important byproduct obtained from skim milk separation in the second step. Many methods can be used to separate and concentrate

relatively high protein content (18-25%), coconut meal is too

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these protein fractions including heat coagulation, isoelectric precipitation, salt precipitation, centrifugation, and ultrafiltration (8, 9). Currently, this protein is either discarded or used as animal feed. It would be highly beneficial to convert this underutilized protein species into a value-added food ingredient (9-12). Nevertheless, information about the type, concentration, and functional properties of the major protein fractions within coconut is required to achieve this goal.

If a coconut protein ingredient is going to find widespread application as an emulsifier in the food industry, it is important to establish the range of environmental conditions under which it can successfully exhibit its functional properties (e.g., pH, ionic strength, and thermal processing). The objective of this study was to investigate the influence of solution conditions on the stability of oil-in-water (O/W) emulsions stabilized by protein fractions extracted from coconut: coconut skim milk protein isolate (CSPI) and coconut skim milk protein concentrate (CSPC).

MATERIALS AND METHODS

Materials. The mature coconut used as a source of coconut meat throughout this research was a native coconut palm variety grown in the Thanam district, Pattani province, Thailand. Whey protein isolate (WPI) (BiPRO JE 361-1-450) was obtained from Davisco Foods International Inc. (Le Sueur, MN). As stated by the manufacturer, the powdered WPI had a composition of 97.6 wt % protein, 2.0 wt % ash, and 0.3 wt % fat (dry weight basis) and 4.7 wt % moisture (wet weight basis). All other reagents were of analytical grade or purer.

Preparation of Coconut Skim Milk Proteins. The mature coconuts were dehusked and cracked to remove coconut water. The fresh coconut kernels were finely comminuted through an electric grater. Coconut milk was obtained by mixing one part of coconut meat with one part of water and pressing using a hydraulic press (Thai Sakaya-A2) in a double-layer cloth bag. Coconut milk was centrifuged at room temperature to separate it into two phases: cream and skim milk. In this study, only the coconut skim milk fraction was used to isolate the protein fractions. The coconut skim milk was stored at 4 °C overnight, which led to the separation of the system into a soluble supernatant phase and an insoluble precipitate phase. The soluble supernatant phase was used to extract the two different protein fractions used in this study.

Coconut Skim Milk Protein Concentrate. A portion of the soluble supernatant phase was concentrated at a concentration factor of 4 using an ultrafiltration unit (Pellicon2 Cassette, Millipore Corp., Bedford, MA) equipped with a cellulose membrane [PLCGC 10K regenerated cellulose, molecular weight cutoff (MWCO) of 10000 Da, membrane area = 0.5 m^2]. The soluble supernatant phase was delivered to the membrane at a feed pressure of 30 psi. The temperature of the retentate was controlled at 20-25 °C using a temperature-controlled water bath. The concentrated solution that was retained in the ultrafiltration unit contained the CSPC fraction.

Coconut Skim Milk Protein Isolate. Another portion of the soluble supernatant phase was adjusted to pH 3.9 with 0.1 or 1.0 M HCl, which led to the formation of a precipitate. This system was centrifuged at 5500g for 30 min to separate the precipitate from the supernatant, after which the supernatant was poured away and the precipitate was collected. The precipitate was then redispersed into an aqueous solution adjusted to pH 7.0 using 0.1 or 1.0 M NaOH. This aqueous solution contained the CSPI fraction.

The CSPC and CSPI fractions were then freeze-dried and analyzed for the content of protein, fat, moisture, and ash using official methods (13).

Protein Solubility (PS). Aqueous solutions of the protein fractions (0.1% w/v) were prepared by dispersing the powders in distilled water containing 0, 100, or 200 mM NaCl adjusted to pH values ranging from 3 to 8. The protein solutions were stirred with a magnetic stirrer at 4 °C overnight, centrifuged at 13200g for 30 min, and filtered through filter paper (Whatman no. 1). The protein content of the filtered supernatant was determined according to the Lowry method using whey

protein isolate as a standard. The percent PS was calculated using the following expression: $PS = 100(P_S/P_T)$, where P_S is the protein concentration remaining in the supernatant after centrifugation and filtration and P_T is the total protein concentration present in the original solution.

Emulsion Preparation. The buffer solution used to prepare the emulsions was a 5 mM phosphate buffer (containing 0.02 wt % sodium azide as an antimicrobial agent) adjusted to pH 6.2. This pH was selected because it is close to the pH of the aqueous phase of many coconut milk-based products. The protein solutions were prepared by dispersing the desired amount (0.1-1.0 wt %) of protein into buffer solution and stirring overnight at 4 °C.

O/W emulsions were prepared by blending 10 wt % corn oil and 90 wt % protein solutions using a high-speed blender for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland). This emulsion was passed two times through a high-pressure valve homogenizer (APV-Gaulin, model Mini-Lab 8.30H, Wilimington, MA) at 3000 psi.

Particle Size Determination. The particle size distribution of the emulsions was measured using a laser light scattering instrument (Malvern Mastersizer, Malvern Instruments, Worcestershire, U.K.). This instrument measures the angular dependence of the intensity of laser light ($\lambda = 632.8$ nm) scattered by dilute emulsion and then finds the particle size distribution that gives the best agreement between theoretical predictions and experimental measurements. To prevent multiple scattering effects, the emulsions were diluted to a droplet concentration of ~0.005 wt % using buffer solution at the pH and NaCl content of the sample. A refractive index ratio of 1.08 was used in the calculations of the particle size distribution. The particle size was reported as surface-volume mean particle diameter, $d_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$, and as weight-average mean particle diameter, $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$, where d is the diameter and n is the number of particles. All measurements were made on at least two freshly prepared samples, and the results were reported as the mean and standard deviation.

ζ-Potential Measurements. The electrical charge (*ζ*-potential) of oil droplets in the emulsions was determined using a particle electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments). The *ζ*-potential is determined by measuring the direction and velocity of droplet movement in the applied electric field. Emulsions were diluted to a droplet concentration of ~0.001 wt % using buffer solutions of the appropriate pH and NaCl concentration to avoid multiple scattering effects. The diluted emulsions were mixed thoroughly and then injected into the measurement chamber of the instrument. The *ζ*-potential of each individual sample was calculated from the average of five measurements on the diluted emulsion, and the results were reported as the mean and standard deviation.

Creaming Stability Measurements. Ten grams of emulsion sample was transferred into a test tube (with 15 mm internal diameter and 125 mm height), which was tightly sealed with a plastic cap and then stored at room temperature. After storage, a number of emulsions separated into an opaque (cream) layer at the top and a turbid or transparent (serum) layer at the bottom. The total height of the emulsions in the tubes (H_E) and the height of the serum layer (H_S) were measured. The extent of creaming index provided indirect information about the extent of droplet aggregation in an emulsion: the higher the creaming index, the faster the droplets move and, therefore, the more droplet aggregation has occurred. All experiments were carried out in at least duplicate using freshly prepared samples, and results are reported as the mean and standard derivation of these measurements.

RESULTS AND DISCUSSION

Composition of Coconut Skim Milk Proteins. The protein, moisture, fat, and ash contents of the CSPI and CSPC used in this study are shown in **Table 1**. These measurements indicate that the extracted protein fractions were highly contaminated with nonprotein components, that is, fat, carbohydrate, and minerals. The protein and fat contents were higher in CSPI than in CSPC, whereas the ash and carbohydrate contents were higher in content in CSPI. Differences in the total protein content

 Table 1. Proximate Composition^a (Percent) of Coconut Skim Milk

 Protein Isolate (CSPI) and Coconut Skim Milk Protein Concentrate (CSPC)

constituent	CSPI	CSPC
moisture	2.69 ± 0.02	6.5 ± 0.3
protein ^b	59.6 ± 0.6	45.6 ± 0.1
fat	28.5 ± 0.5	0.79 ± 0.08
ash	5.25 ± 0.02	8.84 ± 0.02
carbohydrate ^c	3.9 ± 1.0	36.6 ± 0.6

^a Values are for triplicate determination. ^b 6.25 was use as the nitrogen conversion factor. ^c Estimated by difference.



Figure 1. Protein solubility curve of coconut skim milk protein isolate (CSPI), coconut skim milk protein concentrate (CSPC), and whey protein isolate (WPI) in distilled water as related to a pH range of 3–8.



Figure 2. Protein solubility curve of coconut skim milk protein isolate (CSPI), coconut skim milk protein concentrate (CSPC), and whey protein isolate (WPI) in 100 mM NaCl as related to a pH range of 3–8.

and in the types and amounts of impurities present could certainly affect the emulsifying and stabilizing properties of these protein fractions. In addition, the type, denaturation, and aggregation of the proteins present may also play an important role in determining their functional properties. It should therefore be recognized that this study is only a first step toward establishing whether protein fractions extracted from coconuts can be used as emulsifiers in the food industry and that more work is certainly required to isolate purer protein fractions and to establish the influence of denaturation and aggregation on their functionality. We are currently preparing a manuscript that will provide a more detailed molecular characterization of the coconut protein fractions used in the present study.

Solubility Characteristics of Coconut Skim Milk Proteins. The pH dependence of the protein solubility (PS) of CSPI, CSPC, and WPI in 0, 100, and 200 mM NaCl is shown in Figures 1–3. In the absence of salt (0 mM NaCl), all samples had U-shaped solubility profiles (Figure 1). The minimum PS



Figure 3. Protein solubility curve of coconut skim milk protein isolate (CSPI), coconut skim milk protein concentrate (CSPC), and whey protein isolate (WPI) in 200 mM NaCI as related to a pH range of 3–8.

for both CSPI and CSPC occurred around pH 4 and 5, which is close to the reported isoelectric points (pI) of these proteins (14). A similar effect of pH on the solubility profiles of coconut proteins has been reported by previous workers (4, 9, 14). For WPI, the minimum protein solubility was around pH 5, which is close to the reported isoelectric point of this protein, pI \sim 4.8 (15, 16). The solubility of CSPI, CSPC, and WPI increased when the pH was altered either below or above the pI. The low solubility of proteins near their pI can be attributed to the fact that they have zero net charge at this pH, so there is no electrostatic repulsion between the molecules or even a slight electrostatic attraction between oppositely charged patches on different molecules. At pH values above or below the pI, the protein has a net negative or positive charge, and so there is a strong electrostatic repulsion between the molecules, which prevents them from aggregating and leads to a greater protein solubility (17).

The pH solubility profiles of CSPI, CSPC, and WPI measured in the presence of salt were quite different from those measured in distilled water (**Figures 1–3**). The solubility of the proteins at their pI values increased when the NaCl concentration was increased from 0 to 200 mM. Protein solubility was also strongly dependent on protein type: WPI > CSPC > CSPI. The relatively low solubility of CSPI may have been because of some denaturation during its precipitation. There are a number of reasons that account for the increase in protein solubility at the pI when salt concentration increases. The salt ions interact with oppositely charged groups, which decreases any electrostatic attraction between protein molecules and causes more protein solvation (17).

Influence of Initial Protein Concentration on Emulsion Formation and Stability. In this section, we examine the influence of initial protein concentration on the mean diameter of the droplets produced by a high-pressure valve homogenizer. An experiment was carried out to determine the minimum amount of coconut skim milk protein required to form stable emulsions. Corn oil-in-water emulsions (10 wt % oil, pH 6.2) were prepared with different initial protein fraction concentrations (0.1–1.0%) and protein types (CSPI, CSPC, and WPI). The mean particle diameter of the emulsions was measured 24 h after homogenization (**Figure 4**).

For all three proteins there was a steep decrease in mean particle diameter when the protein concentration was increased from 0.1 to 0.2 wt %, after which the mean particle diameter reached a relatively constant value ($d_{3,2} \sim 0.4-0.5 \,\mu$ m for CSPI and WPI and $d_{3,2} \sim 1.0-1.5 \,\mu$ m for CSPC). This result indicated that emulsions with smaller droplet sizes could be produced using CSPI and WPI than by using CSPC. In all subsequent



Figure 4. Influence of protein concentration on mean particle size.



Figure 5. Influence of pH on the ζ -potential of oil-in-water emulsions stabilized by coconut skim milk protein isolate (CSPI), coconut skim milk protein concentrate (CSPC), and whey protein isolate (WPI).

experiments 0.2 wt % protein was used to prepare the emulsions, because this protein concentration enabled us to produce emulsions containing relatively small droplets. It should be noted that the actual protein concentrations are somewhat lower than this value, because the protein fractions consisted of about 46, 60, and 95% for CSPC, CSPI, and WPI, respectively.

There are a number of possible reasons to account for the observed decrease in droplet size with increasing protein concentration. The amount of droplet surface area that can be stabilized by the protein increases and the rate at which the protein adsorbs to the droplet surfaces increases, thereby facilitating droplet disruption and retarding droplet coalescence by forming protective membranes around the droplets. The ability of proteins to generate repulsive interactions (e.g., steric and electrostatic) between the oil droplets and to form an interfacial membrane that is resistant to rupture also plays an important role in stabilizing the droplets against flocculation and coalescence during long-term storage (18). The differences of emulsifying ability between different proteins may have been because they have different surface loads, surface activities, or adsorption rates (19).

Influence of pH on Emulsion Stability. To investigate the influence of pH on emulsion stability, we prepared 10 wt % corn oil-in-water emulsions stabilized by CSPI, CSPC, and WPI at pH 3–8 as described above. The dependence of the droplet charge (ζ -potential), mean particle diameter, particle size distribution, and creaming stability of these emulsions was measured after they had been stored at room temperature for 1 day.

The pH dependence of the ζ -potential of the droplets in the CSPI and CSPC emulsions was compared with that of the WPI emulsions (**Figure 5**). In general, the pH dependence of the ζ -potential was fairly similar for CSPI, CSPC, and WPI. The ζ -potential of the droplets in the CSPI and CSPC emulsions

was highly positive ($\sim +30$ mV) at pH 3, became less positive with increasing pH until it reached zero (pH \sim 4.0 for CSPI and pH \sim 4.5 of CSPC), and then became increasingly negative as the pH was further increased, until it reached a value of ~ -39 mV at pH 8. These measurements suggested that the isoelectric points of the coconut proteins were around pH 4 and 5, which is close to previously reported values (14). The ζ -potential of the droplets in the WPI stabilized emulsion went from positive ($\sim +60 \text{ mV}$) to negative ($\sim -70 \text{ mV}$) when the pH was increased from 3 to 8 and had a value close to zero around pH 4.8, which is close to the value expected for WPI (15), and then became increasingly negative as the pH was increased further. The charge density of the droplets in the WPI stabilized emulsions was more positive at low pH and more negative at high pH than either CSPI or CSPC. These differences may have been due to differences in the type and number of ionizable amino acids in the different proteins or because of different levels of multivalent mineral ion impurities that could bind to the droplet surfaces and alter the droplet charge.

The particle size distributions and mean particle diameters of a series of 10% corn oil-in-water emulsion stabilized by different protein fractions were determined as a function of pH (Figures 6 and 7). For CSPI, the particle size distributions of the emulsions at pH 3 and 7 were fairly similar, showing evidence of considerable droplet aggregation leading to the formation of a bimodal distribution (Figure 6a). The mean particle diameter was relatively high ($d_{43} > 30 \,\mu\text{m}$) from pH 3 to 5, with a maximum value around pH 4, and was somewhat smaller ($d_{43} \approx 10 \ \mu \text{m}$) at pH 6–8 (**Figure 7**). For CSPC, the particle size distribution was either bimodal or multimodal at all pH values, indicating that the droplets were aggregated (Figure 6b). The mean particle diameter was relatively high $(d_{43} > 20 \,\mu\text{m})$ at all pH values from 3 to 8, with a maximum value around pH 5 (Figure 7). These measurements indicated that there was substantial droplet aggregation in the coconut protein emulsions at all pH values. For WPI, the particle size distribution measurements showed that there was a single population of relatively small droplets at pH 3 and 7, indicating that the droplets were stable to aggregation. On the other hand, there was a large population of big particles at pH 5, indicating that considerable droplet aggregation occurred near the pI of the protein (Figure 6c). Measurements of the mean particle diameter of the WPI stabilized emulsions indicated that there was significant droplet aggregation at pH 4 and 5 (Figure 7) but that the emulsions were relatively stable at higher and lower pH values.

The creaming stability of the emulsions stabilized by CSPI and CSPC was much worse than those stabilized by WPI (**Figure 8**). The CSPI and CSPC emulsions were highly unstable to creaming at pH 3-6 and were still fairly unstable to creaming at pH 7 and 8. On the other hand, the WPI emulsions were stable to creaming at all pH values, except pH 5, which was close to the pI of the proteins. The rapid creaming observed in some of the emulsions can be attributed to droplet aggregation.

The observed differences in the stability of the various emulsions to droplet aggregation and creaming can be attributed to the influence of the proteins on the colloidal interactions between the droplets. In the WPI emulsion, it seems that the electrical charge on the emulsion droplets is sufficiently large to prevent droplet flocculation when the pH is ≥ 1 unit away for the p*I*. On the other hand, the emulsions stabilized by coconut proteins are not stable to droplet aggregation at most pH values, which may be attributed to the fact that there is a



Figure 6. Droplet size distribution of 10 wt % corn oil emulsions containing 0.2 wt % (a) CSPI, (b) CSPC, and (c) WPI at pH 3, 5, and 7 with storage at room temperature for 24 h. The graphs show volume percentage of droplets in each diameter size class.

relatively low electrical charge on the droplets compared to WPI (**Figure 5**).

Influence of Salt on Emulsion Stability. In general, all emulsions exhibited similar behavior, with the extent of droplet aggregation and creaming increasing with increasing salt concentration (Figures 9 and 10). In the CSPI and CSPC emulsions, there was some droplet aggregation and creaming at relatively low salt concentrations (0-50 mM NaCl), which became more extensive at higher salt concentrations (>50 mM NaCl). On the other hand, the WPI emulsions were relatively stable to droplet aggregation and creaming at low ionic strengths (<200 mM), but became strongly aggregated at higher ionic strengths.

These results were interpreted in terms of the electrostatic interactions between the emulsion droplets. At low salt concentrations there is a relatively strong electrostatic repulsion between the droplets, which helps prevent them coming into close contact; however, once a critical salt concentration is exceeded, the electrostatic repulsion is no longer strong enough



Figure 7. Influence of pH on mean particle size of emulsion: (a) particle sizes were reported as volume-weighted mean diameters, d_{43} (μ m); (b) particle sizes were reported as surface-volume mean diameters, d_{32} (μ m).



Figure 8. Influence of pH on creaming stability of emulsion.

to overcome the attractive forces (e.g., van der Waals and hydrophobic) acting between the droplets, and so they aggregate.

Influence of Thermal Processing on Emulsion Stability. Particle size and creaming stability of 10 wt % corn oil-in-water emulsions stabilized by 0.2 wt % CSPI, CSPC, and WPI were measured after samples had been heated from 30 to 90 °C for 30 min and stored overnight at room temperature. For CSPI and CSPC, the mean particle size and creaming instability of the emulsions were relatively high at all holding temperatures from 30 to 90 °C (Figures 11 and 12). There was a slight increase in droplet aggregation at the highest holding temperatures, suggesting that there was some additional thermally induced droplet aggregation. The emulsion stabilized by CSPI appeared to be somewhat more stable than the one stabilized by CSPC, because it had a smaller mean particle size and exhibited less creaming. The emulsions stabilized by WPI had relatively small particle diameters and good creaming stability at all holding temperatures, indicating little evidence of droplet



Figure 9. Influence of NaCl on mean particle size of emulsion: (a) particle sizes were reported as volume-weighted mean diameters, d_{43} (μ m); (b) particle sizes were reported as surface-volume mean diameters, d_{32} (μ m).



Figure 10. Influence of NaCl on creaming stability of emulsion.



Figure 11. Influence of heat treatment on mean particle size of emulsion (d_{32}) .

aggregation. This can be attributed to the fact that the electrostatic repulsion between the WPI-stabilized droplets was sufficiently large to overcome the attractive interactions (e.g.





Figure 12. Influence of heat treatment on creaming stability of emulsion.

van der Waals and hydrophobic attraction), even when the proteins unfolded and increased the surface hydrophobicity of the droplets (16).

Conclusions. This work has shown that coconut protein fractions can be used to prepare oil-in-water emulsions using high-pressure valve homogenization. CSPI appears to be more effective as an emulsifier than CSPC because it produces smaller droplets during homogenization and because it is better at protecting the droplets against aggregation and creaming when exposed to environmental stresses (pH, NaCl, and heating). On the other hand, neither of the coconut proteins was able to create emulsions as stable as those created by WPI. Coconut proteins may therefore be most effective at stabilizing emulsions that are fairly viscous, when droplet flocculation and creaming are not major problems, for example, desserts, yogurts, sauces, and dressings. Nevertheless, we recognize that the coconut protein fractions used in this study were highly contaminated with nonprotein material (such as minerals, lipids, and carbohydrates) and that they may have been subject to denaturation and aggregation during the extraction and purification procedures. We therefore recommend that future studies should identify more efficient extraction techniques to isolate and purify the proteins and then test the functionality of these purer protein fractions.

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