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Drying methods affect physicochemical and functional properties of quinoa protein isolate

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ABSTRACT

Quinoa protein possesses great amino acid profiles and can be a potential food ingredient with broad applications. The objective of this study was to investigate the effect of different drying methods, namely freeze drying, spray drying, and vacuum drying on the functional and physicochemical properties of quinoa protein isolate, e.g., morphology, amino acid composition, SDS-PAGE profile, sulfhydryl/disulfide content, secondary structure, surface hydrophobicity, and thermal stability. The freeze-dried protein exhibited the highest emulsification capacity and stability and oil binding capacity, which was contributed to its higher surface hydrophobicity, while the spray-dried sample had the highest solubility and water absorption capacity at pH 7. Gels (8%) prepared with the freeze-dried protein had higher elastic and viscous modulus than that from others. The freeze-dried protein had the highest maximal denaturation temperature but lowest enthalpy, which may be attributed to its higher amount of random coil but lower percent of regular α-helix and β-sheet structures. Overall, quinoa protein isolate from different processing methods demonstrated distinct functional properties. This information will be useful to optimize quinoa protein production and benefit its applications.

1. Introduction

The demand for proteins in human diet has been steadily increasing in recent years due to the increased awareness of their nutritional value and functional properties. Compared with animal proteins, production of plant protein is more sustainable and requires much less water, land, and fossil energy resources. Currently, wheat gluten, soy protein, and pea protein are the most available plant proteins. In order to meet the future needs of more diverse, affordable, and superior plant protein ingredients, additional protein sources should be vigorously explored and investigated. Quinoa (*Chenopodium quinoa* Wild.), known as a pseudocereal and ancient grain, is consumed mostly by people in the Andean region (Abugoch et al., 2009). Quinoa possesses good resistance to drought, frost, soil salinity environmental conditions and can be tolerate to a wide range of soil pH (Abugoch et al., 2009; Steffolani et al., 2016). Because of the relatively high protein content as well as balanced amino acid compositions, quinoa is receiving increased popularity as a new food and protein source (Abugoch et al., 2009). Quinoa contains higher content of lysine (5.1–6.4%), methionine (0.4–1.0%), and cysteine than common cereal grains (Elsohaimy et al., 2015). It is also a good source of fiber, polyunsaturated fat, minerals, vitamins and phytochemicals such as polyphenols and flavonoids (Abugoch et al., 2009; Hager, Wolter, Jacob, Zannini, & Arendt, 2012). These functional nutrients could help lower the risk of chronic disease and potentially promote human health.

The major proteins in quinoa are 11S globulin and 2S albumin, which account for about 37% and 35% of the total grain protein, respectively (Abugoch James, 2009; Kaspchak et al., 2017). Quinoa proteins possess good functional properties, for example, emulsification, foaming, gelation, water and oil binding properties, and solubility (Abugoch James, 2009; Abugoch et al., 2008; Steffolani et al., 2016). Kaspchak et al. (2017) indicated that quinoa protein formed strong and stable gels at pH 3.5 with heating to 70–90 °C, and the gel formation potential was affected by pH through altering the secondary structure as well as protein solubility. Steffolani et al. (2016) found that quinoa protein had better water and oil binding capacity compared with some legume proteins, although the properties differed among different quinoa varieties. The functional properties of proteins are dependent on many factors, such as hydrophilic/hydrophobic ratio, water activity, ionic force, pH, temperature, charge density and changes in environment (Abugoch James, 2009).

Protein functional properties are dependent on processing conditions. A few studies have been conducted on the functionality of quinoa proteins with different extraction or processing methods. Lilian et al.

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(2008) indicated that quinoa protein extracted at pH 9 had higher solubility at isoelectric point than that extracted at pH 11, although the proteins from both extractions had similar amino acid composition and water holding capacity. Mir et al. (2019) and Vera et al. (2019) reported that ultrasound treatment at high intensity improved functional properties of quinoa protein, especially gelling behavior. Ruiz et al. (2016) investigated the effect of extraction pH of quinoa protein on heat-induced aggregation and gelation properties. They found that with extraction at pH 8 or 9, protein aggregation was enhanced in the formation of a semi-solid gel, while at pH 10 or 11, the protein had less aggregation, lost particle arrangements, and could not form gels. Therefore, protein processing methods and conditions are critical in determining their functionalities. Previous studies reported that different drying methods affected the functional properties of cowpea and bambara proteins (Mune & Sogi, 2016), peanut protein (Liu, Li, Jiang, Yang, & Zhang, 2019), and whitecheek shark protein hydrolysates (Alinejad, Motamedzadegan, Rezaei, & Regenstein, 2017). Freeze drying prevents most of protein deterioration and minimizes microbiological reaction, but it is a more expensive and time-consuming drying process. Spray drying is more time efficient and one of the most popular processes used in the food industry, although it may cause some quality deterioration. Vacuum drying is considered as a simple and popular technique; however, it can be expensive for the large scale production and some degradation of heat sensitive products (Alinejad et al., 2017; Pratap Singh et al., 2020). To our knowledge, there is little information available about the effect of dehydration methods of quinoa proteins on their functional and physicochemical properties. Therefore, the objective of this study was to investigate the effect of different drying methods, namely freeze drying, spray drying, and vacuum drying on physicochemical and functional properties of quinoa protein isolates. Critical protein functional properties including water/oil absorption capacity, emulsification and foaming properties, solubility, and gel properties were analyzed. Protein physicochemical characteristics including amino acid composition, surface hydrophobicity, sulfhydryl/disulfide content, SDS-PAGE profile, secondary structure, thermal stability, and morphology were also evaluated. Selecting appropriate processing methods will benefit industry in optimizing protein production and functional properties for targeted food applications.

2. Materials and methods

2.1. Materials

Commercial white quinoa (*Chenopodium quinoa* Wild.) flour (11.4% moisture, 12.6% protein, 2.4% ash) was provided by Ardent Mills (Denver, CO, USA). Sodium dodecyl sulfate (SDS), 8-anilinonaphthalene-1-sulfonic acid (ANS), Tris-HCl, β-mercaptoethanol, urea, ethylenediaminetetraacetic acid (EDTA), sodium sulfite, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Amino acid standards and analysis kit (EZ: faast kit) were purchased from Phenomenex (Torrance, CA, USA).

2.2. Quinoa protein extraction

Quinoa flour was defatted with hexane at a flour/ hexane ratio of 1: 4 (w/v) for 1 h and 3 times prior to protein extraction. The defatted quinoa flour was placed in a fume hood for at least 24 h to evaporate residue hexane. Quinoa protein isolate was extracted based on a literature method (Ruiz et al., 2016) with some modifications. Briefly, 100 g defatted quinoa flour was dispersed into 1000 mL deionized water (DI), and the suspension was stirred for 1 h at room temperature with pH maintained at 10 using 1 M NaOH, followed by centrifugation at 8000 g for 20 min at 4 °C using Avanti J-E centrifuge (Beckman Coulter, Indianapolis, USA). The supernatant was collected, pH was adjusted to 4 with 1 M HCl, and the sample was stored at 4 °C for 2 h to maximize protein precipitation, followed by another centrifugation to recover the protein precipitate. The precipitated protein was washed with DI water twice and then adjusted to neutral pH. The re-dispersed quinoa protein suspension (10%) was dried respectively by using a freeze-dryer (Freezone 4.5L, Labconco Corporation, Kansas City, MO, USA) (-40 °C, 3 days), vacuum dryer (Fisher Scientific, Hampton, NH, USA) (40 °C, 2 days at –28 inHg), and Buchi Mini spray dryer B-290 (BUCHI Corporation, New Castle, DE, USA) (inlet temperature 180 °C, outlet temperature 60 °C, 90% of aspirator, and 10% of pump speed). The protein suspension was further homogenized before feeding to the spray drier. All dried protein powders were ground after drying and kept at 4 °C for further analysis. The quinoa protein content and moisture were measured following AACC Method 46–30.01 and 44–19.01, respectively.

2.3. Measurement of particle size

Particle size of quinoa protein samples was measured using a laser diffraction particle size distribution analyzer LA-910 (Horiba, Ltd., Kyoto, Japan). Each protein sample was dispersed in DI water at 0.1% concentration, and the sample was transferred into the reservoir tank of the instrument filled with water. The sample was further mixed inside the instrument with a set of agitating blades and ultrasonic vibration to achieve uniform dispersion of the protein particles in the water, which was then analyzed based on the diffraction of laser scattered by the particles. Both particle size distribution and average particle size were collected from the software.

2.4. Color analysis

The color of quinoa proteins from different drying methods was measured using a Minolta Chroma Meter CR-300 colorimeter (Minolta Camera Co., Osaka, Japan). Three color components were collected from the measurement, including L* (- black to + white), a* (- green to + red), and b* (-blue to + yellow).

2.5. Atomic force microscopy (AFM)

Atomic force microscope (AFM) imaging of the quinoa proteins was analyzed following our previous method (Wang et al., 2020) and carried out using a Bruker Innova AFM instrument (Billerica, MA, USA) operated in a contact mode.

2.6. Amino acid analysis

Amino acids composition of the proteins was analyzed following the method of Steffolani (Steffolani et al., 2016) with some modifications. One hundred milligram protein was transferred to 8 mL 6 M HCl, mixed well and vortexed for 5 s, with nitrogen gas purging for 5 min. The sample tube was sealed and transferred to an oven set at 110 °C for protein hydrolysis for 24 h. Tryptophan was analyzed using 5 M NaOH alkaline hydrolysis under the same condition. Amino acid extraction and derivatization were carried out using EZ: faast kit from Phenomenex (Torrance, CA, USA). Two microliter derivatized sample was injected to a GC–MS QP2010-SE system (Kyoto, Kyoto Prefecture, Japan) equipped with a ZB-AAA capillary column (10 m × 0.25 mm i.d., Phenomenex, Torrance, CA, USA). The parameters were set as the following: injection temperature at 300 °C, flow rate of the carrier gas (helium) at 0.6 mL/min, oven temperature at 110 °C with heating at 20 °C/ min to 320 °C, split ratio at 10, and the ion source at 240 °C. Aspartic acid, methionine, glutamic acid, and phenylalanine were separated using SIM mode based on their major ions. Each amino acid concentration was calculated based on the calibration curve established with respective amino acid standard. The amino acids were determined based on the standard solution chromatogram provided by Phenomenex (Torrance, CA) based on their elution time and mass spectra.

2.7. Functional properties

2.7.1. Solubility

Solubility of quinoa proteins in water was measured at pH 3 to 11 with 4% solid content. After pH was adjusted to the desired level using 1 M NaOH or HCl, the suspension was further stirred for 30 min at room temperature, followed by centrifugation at 4000 g for 30 min. Protein concentration in the solution was determined according to the Biuret method and analyzed using a double beam spectrophotometer (VWR UV-6300PC) at 540 nm absorbance (Elsohaimy et al., 2015).

2.7.2. Oil/water absorption capacity

Oil/ water absorption capacities were analyzed following the method of Elsohaimy (Elsohaimy et al., 2015) with small modifications. For oil absorption capacity (OAC), approximately 1 g protein was accurately weighted (O_0) and thoroughly mixed with 10 mL soybean oil in a 15 mL centrifuge tube (O_2), then allowed to stand for 30 min at room temperature, and followed by centrifugation at 3000 g for 30 min (Z 366 K centrifuge, Hermle Benchmark, USA). The supernatant was discarded, and the test tube was inverted for 2 min to drain the excess oil and weighed (O_1). The OAC was calculated as: $OAC = \frac{O1 - O2 - O0}{O0}$. For water absorption capacity (WAC) test, protein was accurately weighted (W_0, approximately 0.6 g) and thoroughly mixed with 10 mL DI water in a 15 mL centrifuge tube (W_2), followed by centrifugation at 3000 g for 30 min. The supernatant was discarded, and the tube containing the protein was weighted (W_1). The WAC was calculated as: $WAC = \frac{W1 - W2 - W0}{W0}$, and determined at pH 5–8. The OAC and WAC results were expressed as g oil/ g protein and g H_2O/ g protein, respectively.

2.7.3. Emulsion capacity and stability

Emulsion capacity and stability were measured according to a literature method (Yasumatsu et al., 2011) with small modifications. Quinoa protein (1.75 g) was homogenized with 25 mL DI water for 30 s using a Waring blender. Soybean oil (25 mL) was then added to the suspension and homogenized for another 30 s. The emulsion was then centrifuged at 1100 g for 5 min. Emulsion capacity (EC) was calculated as: $EC = \frac{H1}{H0} \times 100$, where H_0 is the height of the total emulsion in the tube, and H_1 is the height of emulsified layer in the tube. For emulsion stability, the emulsion was heated at 80 °C for 30 min and then centrifuged similarly. The emulsion stability (ES) was calculated similarly as for EC. Both ES and EC were measured at pH 5, 6, 7 and 8, respectively.

2.7.4. Foaming capacity and stability

Foaming capacity and stability were measured according to a literature method (Kaushik et al., 2016) with some modification. Briefly, 0.5 g protein was dispersed into 50 mL DI water in a beaker. The suspension was homogenized with a high-performance disperser (Ingenieurbure CAT, Germany) for 2 min at 20,000 rpm, immediately transferred to a graduated cylinder, and volume was recorded (V_1). Foam capacity (FC) was calculated as $FC = \frac{V1 - V2}{V0} \times 100$, where V_0 is the total volume of protein suspension, and V_2 is the total volume of protein suspension solution after homogenization at 0 min. The total volume was recorded at 0, 15, 30, 45, 60, 75 and 90 min, respectively. Foam stability (FS) was calculated by the equation: $FS = \frac{vt}{v0} \times 100$, where Vt is the volume of foam at a certain time after homogenization. The FC and FS were measured at pH 5, 6, 7 and 8, respectively.

2.7.5. Gel rheology

Gel rheological properties were measured using a Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA, USA) with a PP 20 parallel plate with a gap size of 500 μm. Protein was dispersed in DI water with mixing to a final concentration of 8%, which was heated in boiling water for 1 h, cooled, and kept in a refrigerator at 4 °C for 2 h. Frequency sweep was conducted in the range of 0.1 to 10 Hz at 25 °C with 1% strain. Rheological properties in terms of shear storage modulus ($\acute{G}$) and loss modulus ($\acute{G}$) were recorded.

2.8. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The protein sample was dispersed into 1% SDS/NaPhos buffer (pH 7.0, 5 mg/mL) and vigorously mixed overnight followed by centrifugation at 10,000 g for 5 min at room temperature (Chen et al., 2019) before running the gel. Twenty microliter supernatant was mixed with 10 μL Laemmli buffer (0.01 M Tris-HCl, 10% (w/v) SDS, , 0.1% (w/v) bromophenol blue, and 10% (v/v) glycerol) with (reducing condition) or without (non-reducing condition) 2% (v/v) β-mercaptoethanol. The sample was then boiled for 10 min, and 15 μL solution was loaded on the gel slab which consists of 12% resolving gel (pH 8.8) and 4% stacking gel (pH 6.8). The electrophoresis was run using a PowerPac 1000 (Bio-Rad, USA) with running buffer prepared by diluting 100 mL 10 X Tris/Glycine/SDS buffer with DI water at the constant voltage 220 V, and the gel was stained with Coomassie Brilliant Blue for 1 h, followed by de-staining with DI water overnight.

2.9. Sulfhydryl/ disulfide content

Free sulfhydryl groups were determined according to a previous method (Rombouts, Jansens, Lagrain, Delcour, & Zhu, 2014) with some modifications. Briefly, 30 mg protein was added to 3 mL reaction buffer A (8 M urea, 0.2 M Tris-HCl, 3 mM EDTA, 1% SDS, pH 8.0), and then vortexed for 30 s and mixed vigorously for 1 h. After that, 0.3 mL of buffer B (10 mM DTNB in 0.2 M Tris-HCl, pH 8.0) was added, mixed vigorously for another 1 h, followed by centrifugation at 13,600 g for 15 min at room temperature. Total sulfhydryl content was measured using our previous method (Chen et al., 2019). Ten milligram protein was added into 1 mL reaction buffer which included 3 mM EDTA, 1% SDS, 0.2 M Tris-HCl, 0.1 M sodium sulfite, at pH 9.5 and 0.5 mM 2-nitro-5-thiosulfobenzoate (NTSB), vortexed and mixed vigorously in dark for 1 h, followed by centrifugation at 13,600 g for 15 min. The supernatant (0.3 mL) was diluted with 2.7 mL of the reaction buffer without NTSB. The absorbance was measured at 412 nm using a double beam spectrophotometer (VWR UV-6300PC, Radnor, PA, USA). The free or total SH content was calculated by the equation: $C(SH) = \frac{A}{\varepsilon b}$, where A is the absorbance, ε is the extinction coefficient of 13,600 $M^{-1}cm^{-1}$, b is the cell path length. The disulfide (SS) was calculated by the equation: $C(SS) = \frac{C_{tSH} - C_{fSH}}{2}$, where C_{tSH} is the total SH content, C_{fSH} is the free SH content.

2.10. FTIR and protein secondary structure

FTIR spectra of quinoa protein were collected with a PerkinElmer Spectrum 400 FT-IR/FT-NIR Spectrometer (PerkinElmer, Inc., Waltham, MA, USA) equipped with an attenuated total reflectance cell (ATR) according to our previous method (Chen et al., 2019). A total of 64 scans was run for each sample at an interval of 4 cm^{-1} in the range of 400–4000 cm^{-1}, and protein secondary structures were determined from the amide I region (1600–1700 cm^{-1}). The data was quantified using OriginPro 2016 software (OriginLab, Inc., Northampton, MA, USA) to fit the peaks, and a second derivative method was used to find the anchor points. The curve was then auto baseline-subtracted and rescaled to smooth the derivative by using Savitzky-Golay method. The protein secondary structure was then determined based on the peak wavenumber and peak area.

2.11. Surface hydrophobicity

Surface hydrophobicity of the extracted proteins was measured according to a previous method (Timilsena, Adhikari, Barrow, & Adhikari,

2016) with some modifications. Protein solutions in DI water with concentrations of 0.0125–0.1% (w/v) were prepared. Each prepared sample (4 mL) was mixed with 20 μL of 8 mM 8-anilinno-1-napthalenesulfonic acid (ANS) in a 15 mL test tube, vortexed for 30 s, and incubated in dark for 15 min at room temperature. The mixture (250 μL) was then added to a 96-well microplate, and fluorescent intensity was measured using a microplate spectrophotometer (BioTek, Synergy H1 Hybrid, Highland Park, Winooski, VT) at 390 nm for excitation and 460 nm for emission. Fluorescent intensity of each diluted protein samples without ANS was also measured. Protein surface hydrophobicity was calculated based on the absorbance difference between the sample with and without ANS plotted against protein concentration, and the linear slope was considered as the relative surface hydrophobicity.

2.12. Differential scanning calorimetry (DSC)

Thermal properties of quinoa proteins were determined by differential scanning calorimeter (DSC) (Q200, TA instrument, Schaumburg, IL). Approximate 5 mg protein powder was accurately weighed and sealed in a high-volume stainless-steel pan. The sample was heated from 20 to 250 °C at a heating rate of 10 °C /min in an inert environment using nitrogen with the gas flow at 50 mL/min. Protein denaturation temperature and enthalpy were calculated using Universal Analysis 2000 software.

2.13. Statistical analysis

All the tests were conducted in at least duplicate. Data were analyzed using SAS statistical software, version 9.4 (SAS Institute, Cary, NC, USA). Results were evaluated by one-way ANOVA. Tukey's post-hoc test was used to assess the significant differences among individual data set. The results were presented as means ± standard deviation (SD), and $p < 0.05$ was considered as the significant level.

3. Results and discussions

3.1. Protein physical and compositional characteristics

Moisture content of the protein powders obtained through different drying methods was not significantly different (13 – 14%) (Table 1). Protein content ranged from 83.2 to 86.2%. The freeze- and vacuum-dried quinoa proteins had a slightly higher protein content than that from the spray drying, which was caused by the loss of some protein fractions during the later process. Spray-dried proteins had significantly smaller and finer particle sizes (10.43 μm) than that from freeze-dried (44.24 μm) and vacuum-dried proteins (38.25 μm). The freeze- and vacuum-dried proteins required further grinding after drying to reduce the particle size, which determined the average powder particle size and size distribution (Yu, Ahmedna, & Goktepe, 2007). Protein particle size distribution is shown in Fig. 1, and AFM images are presented in Figure S1 (Supplementary Document). The spray-dried protein powders exhibited narrower and more uniform distribution, while the freeze-dried and vacuum-dried proteins showed similar distribution patterns (Fig. 1).

Color characteristics of the protein powders were significantly different ($p < 0.05$) (Table 1). Freeze- and vacuum-dried protein powders showed brownish color, while spray-dried proteins showed creamy white color (Figure S2, Supplementary Document). Based on the lightness (L*) values, spray-dried protein was the lightest, while the vacuum-dried protein was the darkest. This result was in agreement with a previous study of chia seed protein isolates (Timilsena et al., 2016). The color properties were determined by the intrinsic characteristics of proteins, protein purity and pigment contamination, particle sizes, etc. Shaviklo et al. (2010) reported that high speed homogenization before spray drying may disrupted the protein tissue and can lead to removal of some pigments.

Table 1
Physical and functional properties of quinoa proteins from different drying methods. *Means with different letters within each attribute denote significant differences ($p < 0.05$).

Physical property	Freeze dry	Spray dry	Vacuum dry
L*	52.44 ± 1.11^{b}	77.60 ± 0.98^{a}	48.86 ± 0.65^{c}
a*	2.70 ± 0.17^{a}	0.93 ± 0.09^{b}	2.78 ± 0.22^{a}
b*	11.72 ± 0.37^{a}	8.41 ± 0.18^{b}	12.20 ± 0.17^{a}
Moisture content (%)	13.32 ± 0.45^{a}	14.11 ± 0.54^{a}	13.58 ± 0.09^{a}
Protein content (%)	86.19 ± 0.18^{a}	83.22 ± 0.13^{b}	86.13 ± 0.44^{a}
Mean particle size (μm)	44.24 ± 3.64^{a}	10.43 ± 0.23^{b}	38.25 ± 6.00^{a}
Functional properties			
Oil absorption capacity (g oil/g protein) pH 7	3.19 ± 0.01^{a}	1.19 ± 0.05^{b}	0.94 ± 0.04^{c}
Water absorption capacity (g H_2O/g protein)			
pH 5	1.43 ± 0.13^{de}	1.43 ± 0.06^{de}	1.38 ± 0.11^{de}
pH 6	1.52 ± 0.21^{cde}	2.03 ± 0.25^{b}	1.29 ± 0.00^{e}
pH 7	1.84 ± 0.07^{bcd}	2.76 ± 0.12^{a}	1.46 ± 0.03^{cde}
pH 8	1.26 ± 0.41^{e}	1.94 ± 0.10^{bc}	1.55 ± 0.05^{bcde}
Emulsion capacity (%)			
pH 5	40.60 ± 1.15^{cd}	39.46 ± 1.00^{cd}	14.27 ± 1.44^{e}
pH 6	44.72 ± 1.04^{c}	40.68 ± 0.83^{cd}	35.00 ± 1.10^{d}
pH 7	56.63 ± 5.01^{ab}	51.55 ± 2.89^{b}	41.46 ± 1.44^{c}
pH 8	61.01 ± 4.44^{a}	59.32 ± 3.93^{a}	44.39 ± 1.51^{c}
Emulsion stability (%)			
pH 5	30.18 ± 3.30^{ef}	17.28 ± 0.95^{h}	5.95 ± 0.73^{i}
pH 6	42.04 ± 1.15^{cd}	37.72 ± 1.78^{de}	19.80 ± 2.16^{gh}
pH 7	51.90 ± 5.02^{ab}	47.99 ± 5.79^{bc}	25.35 ± 3.29^{fg}
pH 8	57.98 ± 3.46^{a}	55.35 ± 3.34^{ab}	24.41 ± 2.02^{fgh}

Aspartic acid, glutamic acid and leucine are the most abundant amino acids in quinoa proteins (Table S1, Supplementary Document). Compared with most cereal proteins, such as wheat, barley or sorghum, quinoa protein possesses higher amount of lysine. Amino acid compositions of the quinoa proteins from different drying methods were mostly similar except for alanine and glycine, which were higher in freeze-dried proteins compared with the other two proteins (Table S1). This could be attributed to the partial thermal degradation of these amino acids during the drying involving intensive heating (Abugoch James, 2009). Feyzi et al. (2018) and Uribe et al. (2019) also found that amino acid profiles of green seaweed and fenugreek proteins were significantly different from different drying methods. The freeze- dried green seaweed had significantly higher glycine and alanine content than vacuum- and spray-dried samples, which indicated that the amino acids were more susceptible to drying technologies and could be lost, changed or destroyed during processing (Uribe et al., 2019). In addition, extraction methods (Abugoch et al., 2008) and cultivate varieties (Steffolani et al., 2016) could also affect the amino acid compositions of proteins.

3.2. Functional properties

3.2.1. Solubility

Protein solubility depends on its hydrophilic-lipophilic balance and the thermodynamics of its interaction with water (Ghribi et al., 2015). As expected, minimal solubility was observed for quinoa proteins from all the drying methods at the isoelectric point around pH 4.5, and solubility increased when the pH was further increased or decreased beyond the isoelectric point (Fig. 2A) (Tang, 2007; Zhao et al., 2013). The freeze- and spray-dried quinoa proteins possessed similar solubility trend from pH 3 to 11. The vacuum-dried protein showed much lower solubility when the pH was above 6, but higher solubility when the pH was below 6. The maximal solubility values (pH 11) of the proteins from freeze drying, spray drying, and vacuum drying were 93.7, 95.3 and 61.3%, respectively. The lower solubility of vacuum-dried protein could be due to more severe protein denaturation during the drying

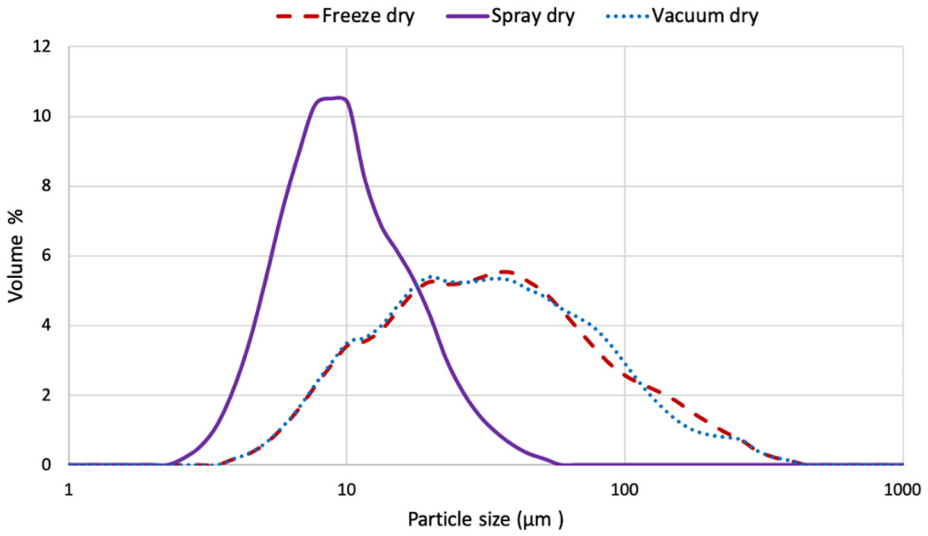

Fig. 1. Particle size distribution of quinoa proteins from different drying methods.

process (Ghribi et al., 2015). Vacuum drying may promote hydrophobic interchange reaction among the protein molecules and film formation on the solution surface, resulted in protein aggregation (Ghribi et al., 2015). Zidani et al. (2012) reported that the vacuum drying allows the water vaporization at low temperature (below 25 °C) and heat transfer occurred by conduction and radiation. It is not advantageous for vacuum drying to work at higher temperature, since the solubility would be decreased. Higher solubility of freeze-dried protein was attributed to the less protein denaturation during the process. Approximately 90% of water was removed as a vapor causing minimum salts or carbohydrates migration to the drying surface, thus the interactions were reduced between components and solubility was minimally affected. The spray drying process had less extent of denaturation than vacuum-dried protein, since the outlet temperature (60 °C) was lower than denaturation temperature (191.4 °C), and the process of spray drying was fast and the residence time of protein inside the drying chamber was very short (Timilsena et al., 2016).

3.2.2. *Oil/water absorption capacity*

Oil absorption capacity (OAC) indicates the ability of protein to absorb and retain fat, and water absorption capacity (WAC) is a critical attribute in determining water retention functionality, swelling, solubility and gelation properties of proteins, both of which affect food texture and quality (Foegeding & Davis, 2011). The freeze-dried protein had significantly higher OAC than spray- and vacuum-dried proteins (Table 1). Oil absorption capacity is related to the amount of exposed hydrophobic amino acid residues in the protein and hydrophobic amino acid content. Freeze-dried protein possessed significantly higher surface hydrophobicity than the proteins from the other two drying methods (Table 2), which contributes to the highest oil absorption capacity of

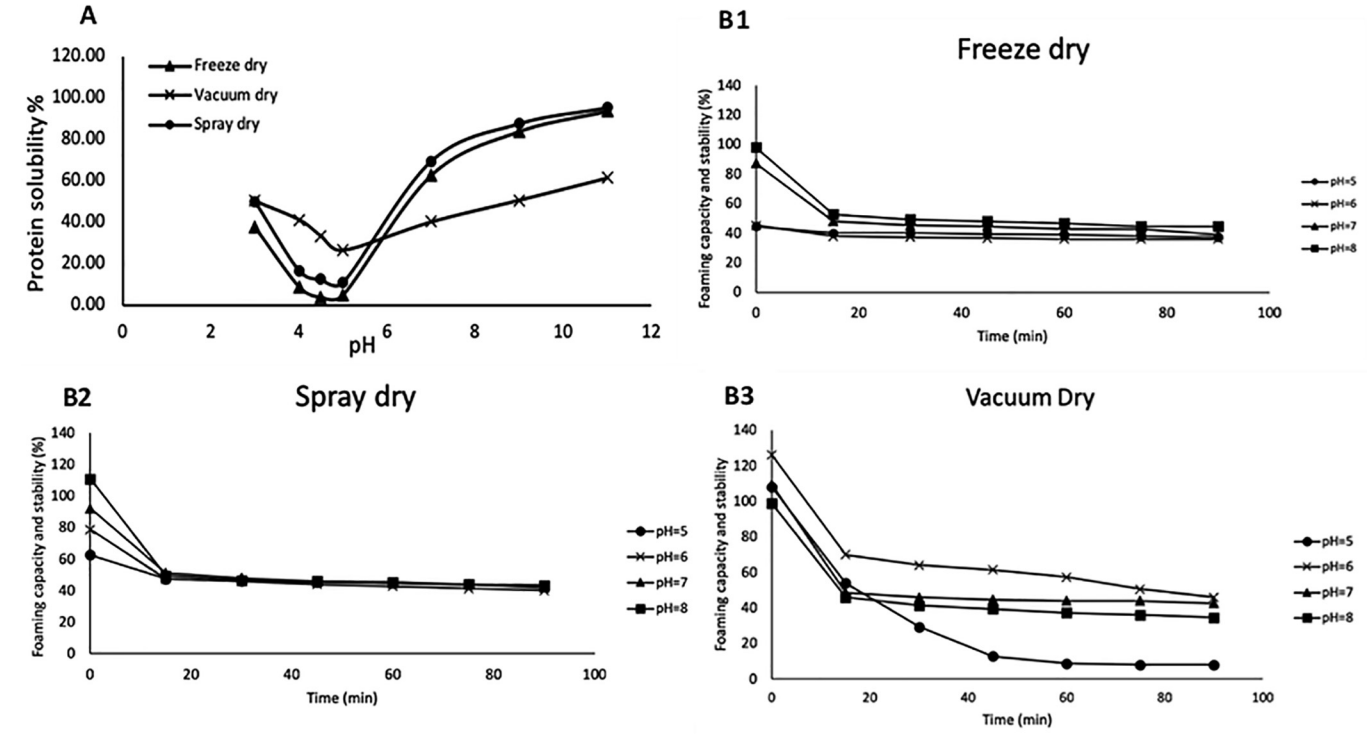

Fig. 2. Solubility (A) and foaming properties (B1-B3) of quinoa proteins from different drying methods.

Table 2
Structural and thermal properties of quinoa proteins from different drying methods. *Means with different letters within each property denote significant differences ($p < 0.05$).

Property	Freeze dry	Spray dry	Vacuum dry
Secondary structure			
β-sheet (%)	16.13 ± 1.16[b]	34.05 ± 2.86[a]	28.87 ± 7.75[a]
random coil (%)	51.27 ± 3.18[a]	0	31.08 ± 3.18[b]
α-helix (%)	15.99 ± 1.22[c]	52.47 ± 2.93[a]	26.24 ± 5.15[b]
β-turn (%)	12.32 ± 3.11[a]	13.51 ± 0.32[a]	11.85 ± 5.26[a]
Relative surface hydrophobicity (H_0)	360,937 ± 11,426[a]	293,106 ± 3,721[b]	32,915 ± 1,538[c]
Free SH (nmol/mg)	11.30 ± 0.85[a]	13.05 ± 0.01[a]	7.93 ± 0.14[b]
Total SH (nmol/mg)	48.97 ± 7.38[a]	44.45 ± 0.26[a]	40.10 ± 2.45[a]
S−S (nmol/mg)	18.44 ± 3.07[a]	15.76 ± 0.04[a]	16.10 ± 1.28[a]
T_d (°C)	131.22 ± 0.09	/	/
	183.23 ± 0.30	/	/
	220.43 ± 0.03[a]	191.42 ± 2.62[c]	208.29 ± 0.21[b]
ΔH_d (J/g)	3.41 ± 0.10	/	/
	1.08 ± 0.13	/	/
	8.49 ± 0.48[c]	38.79 ± 1.27[a]	25.05 ± 0.08[b]
T_a (°C)	/	147.65 ± 1.93[b]	172.00 ± 1.17[a]
ΔH_a (J/g)	/	17.94 ± 0.18[a]	18.71 ± 1.48[a]

freeze-dried proteins.

The WAC of quinoa protein was dependent on protein drying methods and pH values (Table 1). Relatively lower WAC values were observed when the pH was close to the isoelectric point of the protein. When pH values increased from 5 to 7, the WAC gradually increased as well, which was related to the alteration of the electrical charge distribution and net charge values with the pH. At pH 8, freeze-dried quinoa proteins had the lowest WAC, while vacuum-dried protein had the highest WAC compared to that at other pH values. Overall, spray-dried protein exhibited relatively better WAC than the proteins from the other two methods, which may be attributed to its finer particle size and larger specific surface area (Ragab, Babiker, & Eltinay, 2004; Yu et al., 2007). Water absorption capacity is related to the swelling phenomenon of hydrated protein matrix. Thus, changes of protein conformation with increasing binding sites under high temperature could lead to better WAC functionality (Zayas, 1997). Our result was in agreement with Yu et al. (2007), but different from Timilsena et al. (2016), who indicated that spray-dried chia seed protein isolate had no significant differences of WAC from vacuum-dried proteins. This is because the water absorption behavior of protein can be affected by protein sources and structures, testing pH, and other constituents in the materials, such as residue polysaccharides (Ragab et al., 2004; Steffolani et al., 2016).

3.2.3. Emulsifying activity and stability

Emulsifying properties of proteins, such as emulsifying capacity (EC) and emulsifying stability (ES), are useful functional properties and play a critical role in food applications. The EC is the ability to absorb oil and water at the interfacial area to form emulsion, which depends on the size, shape, charge, composition, and hydrophobicity of protein molecules (Ragab et al., 2004). The ES is related to the stability of emulsion over a certain time under specific conditions, and it depends on the magnitude of these interactions (Karaca, Low, & Nickerson, 2011; Ma et al., 2011). The EC of quinoa proteins ranged from 14.3 to 61.0% (Table 1), and the vacuum-dried protein had significantly lower EC compared to the proteins from other drying methods. The oil–water interface is dominated by hydrophobic interactions, and exposure of the non-polar hydrophobic residues at the interface greatly affects the emulsifying properties. Relatively higher surface hydrophobicity can lead to stronger binding between emulsifier and oil droplet and better emulsifying properties of the protein (Gong et al., 2016). The lower emulsifying properties of vacuum-dried protein could also be attributed to the lower solubility. The EC significantly increased with pH for all the proteins. When the pH is close to the isoelectric point (pH = 5), electrostatic repulsion among the molecules is the lowest, which resulted in protein aggregation, and lower solubility and emulsifying properties. When the pH value is higher than the isoelectric point, the protein molecules had a net negative charge which greatly enhanced protein-water interaction and resulted higher solubility, therefore, the EC was increased. The ES ranged from 6.0 to 58.0% (Table 1). The freeze-dried quinoa protein had relatively higher ES than spray-dried protein, and the vacuum-dried protein had the lowest ES at all pH values. The better emulsifying properties of the freeze-dried protein may be attributed to its higher surface hydrophobicity and favorable dissociation at oil and water interfaces (Ghribi et al., 2015). Zhao et al. (2013) and Liu et al. (2019) reported that spray-dried rice dreg and peanut proteins had higher emulsion capacity than freeze-dried proteins due to the smaller particle size and higher solubility, but freeze-dried protein had higher emulsion stability than spray-dried protein.

3.2.4. Foaming capacity and stability

The foaming properties including foam capacity (FC) and foam stability (FS) are important functionality that is utilized for aeration and whipping purpose in food industry. Foam formation is dependent on the interfacial film that is formed by the proteins and its ability to keep the air bubble in the suspension and slow down the coalescence rate (Ghribi et al., 2015; Ma et al., 2011). The foaming properties of quinoa proteins made from different drying methods at pH 5–8 are shown in Fig. 2B. As pH increasing, the FC (i.e., defined as the value at 0 min) increased for both freeze- and spray-dried proteins. In contrast, the vacuum-dried protein showed the highest FC at pH 6 and the lowest FC at pH 8. The spray- and freeze-dried proteins had similar foaming stability (20 to 90 min). The vacuum-dried protein had similar FS as the freeze- and spray-dried proteins at pH 7–8, but much lower stability at pH 5. The lower FC observed around isoelectric point is attributed to the low protein solubility. The increased FC at pH value higher than 5 could be explained by the increased protein solubility due to the increase in the net charge of protein in the aqueous dispersion. The increased repulsive force among the charged molecules decreased protein aggregation and reduced the coalescence of air bubbles (Timilsena et al., 2016). Protein with smaller particle size, such as that from spray drying, could be more rapidly absorbed during whipping to generate more foams (Zhao et al., 2013). Protein needs to be adequately unfolded and molecularly flexible in order to form interfacial membranes around the air bubbles (Aluko & Monu, 2003). Aluko & Monu (2003) reported that enzymatically hydrolyzed quinoa protein possessed better foaming capacity, because hydrolysis reduced the molecular size and increased the flexibility of the protein to form interfacial membranes.

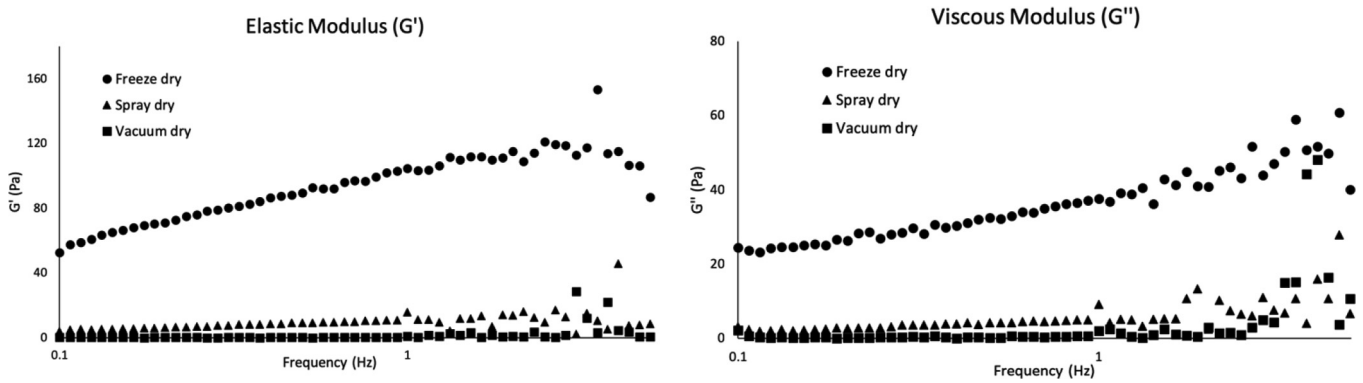

Fig. 3. Rheological properties (Ǵ and Ǵ) of quinoa protein gels.

3.2.5. Gel rheology

Shear storage modulus (G') and loss modulus (G") of heat-induced quinoa protein gels are shown in Fig. 3. The freeze-dried quinoa protein exhibited much higher G' and G" compared with that from spray and vacuum drying. The G' of the freeze-dried protein was higher than G", implying the formation of stronger gels with better resistance to stress-induced rupture. The spray- and vacuum-dried protein gels had similar range of G' and G", indicating weaker gelation properties of the proteins from these two drying methods. The freeze-dried protein was less denatured during processing compared with the other two proteins, and most of the intrinsic properties were retained with higher solubility, which favored protein interaction and aggregation during the heating process to form gel networks (Ruiz et al., 2016). On the other side, the vacuum-dried protein could hardly form gels, and the G' and G" values were low. This result was in agreement with Joshi et al. (2011), and they found that vacuum-dried lentil protein could not form gels as rapidly as freeze-dried lentil protein because of the poor protein solubility. Therefore, higher concentration or longer heating time were required for the vacuum-dried proteins to form a gel.

3.3. SDS-PAGE

SDS-PAGE profiles (non-reducing and reducing) of the quinoa proteins are shown in Fig. 4. Quinoa proteins are comprised of albumin and globular chenopedin proteins, which exhibited a complex band profile.

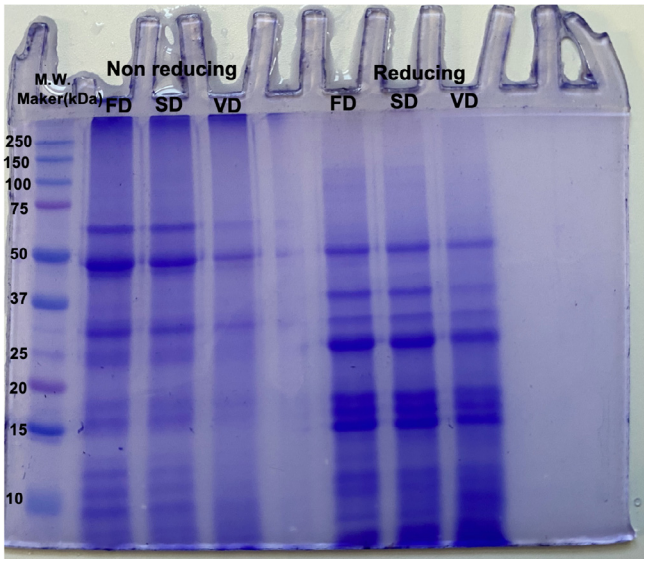

Fig. 4. SDS-PAGE of quinoa proteins from different drying methods under non-reducing and reducing conditions: Lane 1-molecular weight marker; FD-freeze dry, SD- spray dry, VD- vacuum dry.

The globular chenopedin has a hexamer structure and consists of six pairs of basic and acidic polypeptides with molecular weight of 22–23 kDa and 32–39 kDa, respectively, linked by single disulfide bond (Dakhili, Abdolalizadeh, Hosseini, Shojaee-Aliabadi, & Mirmoghtadaie, 2019; Ruiz et al., 2016). Under non-reducing condition, more intensive bands were observed for the freeze-dried protein, while much weaker bands were noticed for the vacuum-dried protein, and band intensity for spray-dried protein was in between. This result is expected because the freeze drying is the mildest drying process with the lowest temperature among the three methods evaluated. The vacuum drying process requires much longer time, though at a lower temperature, than the spray drying, which still caused severe protein denaturation, aggregation, and crosslinking. This reduced protein solubility, leading to very weak bands in the SDS-PAGE profile. This observation was in consistent with the solubility results, which showed that the vacuum-dried quinoa protein had the lowest solubility than freeze- and spray-dried proteins. With breakdown of intramolecular disulfide bonds and unfolding of protein molecules by the reducing agent, the band of higher molecular weight disappeared and several new bands at lower molecular weight range appeared in the reduced SDS-PAGE profile (Timilsena et al. 2016).

3.4. Sulfhydryl/ disulfide content

The content of free sulfhydryl group (SH) as well as disulfide bond (SS) of the quinoa proteins are presented in Table 2. The free SH content of freeze- and spray-dried proteins was significantly higher than that of vacuum-dried protein, indicating more intensive oxidation of free SH to form disulfide bonds during vacuum drying (Visschers and De Jongh, 2005). The total SH and SS were not significantly different among all the drying methods, ranging from 40.1 to 49.0 nmol/mg and 15.8 to 18.4 nmol/mg, respectively. Zhao et al. (2013) noticed that spray drying led to higher protein denaturation than freeze drying, although there was no significant differences of free SH content for the freeze- and spray-dried rice dreg protein isolate. Gong et al. (2016) indicated that the free SH content of freeze- and spray-dried peanut protein was not significantly different, but the freeze-dried peanut protein had relatively higher SS content. This is in accordance with changes in protein hydrophobicity, which suggested that the higher amount of exposed hydrophobic groups would increase the formation of disulfide bond from adjacent free SH groups, and this interchange reaction may lead to the different extent of aggregation (Gong et al., 2016).

3.5. Secondary structure

The secondary structure composition of quinoa proteins derived from the amide I band (1600–1700 cm^{-1}) is summarized in Table 2. The quinoa proteins prepared from different dry methods exhibited

significant differences in secondary structures. The freeze-dried protein possessed higher amount of random coil than β-sheet and β-turn, while no random coil structure was observed in the spray-dried protein. The spray drying process altered the protein secondary structure and promoted protein assembly into more regular β-sheet and α-helix structures. Zhao et al. (2013) reported that the spray-dried rice dreg protein had higher β-sheet than the freeze-dried protein, which is similar to our result. This is probably because more β-sheet structure could be formed in aggregated protein molecules. However, Gong et al. (2016) reported that the freeze-dried peanut protein had higher β-sheet than the spray-dried protein, while the spray-dried peanut protein exhibited higher α-helix, and they explained that this was attributed to the shrinkage of droplet during spray drying and related to the concentration of solute during freeze drying process (Gong et al., 2016). Overall, it seems that protein secondary structure composition is influenced by both protein types and drying methods.

3.6. Surface hydrophobicity

Protein surface hydrophobicity is related to the amount and type of hydrophobic amino acid residues exposed at the surface of the protein and affected by protein unfolding and denaturation (Timilsena et al., 2016). The surface hydrophobicity could also influence intermolecular protein–protein and protein-lipid interactions and determines protein surface activities that are important to functional properties such as solubility, emulsification, and foaming. The differences in surface hydrophobicity of all the quinoa proteins could be attributed to the degree of denaturation of the proteins during different drying processes. The freeze-dried protein exhibited the highest surface hydrophobicity, while that of the vacuum-dried protein was the lowest (Table 2). This could be attributed to a certain extent of denaturation occurred in the freeze-dried quinoa protein by exposing hydrophobic regions; while the vacuum-dried protein had more intensive denaturation due to the hydrophobic interchange reaction among the protein molecules, and it was also related to the film formation on the protein surface, and resulted in protein aggregation (Hu et al., 2010). This observation agreed with the higher oil absorption capacity for the freeze-dried protein compared to the spray- and vacuum-dried proteins (Table 1). Gong et al. (2016) and Mune & Sogi (2016) also found that freeze-dried peanut protein, cowpea and bambara bean proteins exhibited higher hydrophobicity than the spray- and vacuum-dried proteins. However, other study showed that vacuum-dried fenugreek protein had the highest surface hydrophobicity, followed by spray-dried and freeze-dried proteins (Feyzi et al., 2018).

3.7. Thermal properties

DSC thermograms are presented in Figure S3, and denaturation temperatures (T_d), aggregation temperature (T_a), and phase transition enthalpy (ΔH_a and ΔH_d) are summarized in Table 2. During the first scan from 20 to 250 °C, one major endothermic peak was observed for all the three proteins with peak temperatures around 190 to 220 °C, which was attributed to protein denaturation. Freeze-dried protein exhibited two additional endothermic denaturation peaks at 131 and 183 °C. These multiple denaturation peaks were caused by the complex composition of quinoa albumin and globular chenopedin proteins (Dakhili et al., 2019; Ruiz et al., 2016). The two denaturation peaks at lower temperatures were not shown for spray- and vacuum-dried proteins, which is probably because the proteins were partially denatured during drying. However, there was a significant exothermic peak for the spray- and vacuum-dried proteins at 148 and 172 °C, respectively, and this is because of protein aggregation during heating (Goyal, Chaudhuri, & Kuwajima, 2014). Ruiz et al. (2016) reported that extraction methods could affect the denaturation temperature. They found that there was one endothermic peak for the protein extracted at pH 8 to 10; however, no endothermic peak was found when the protein was extracted at 11, due to protein denaturation during extraction. No endothermic or exothermic peaks were observed during the second DSC heating scan of all the three proteins (Figure S3), indicating that the protein denaturation and aggregation transitions are non-reversable.

4. Conclusion

In this study, quinoa proteins were prepared using freeze drying, spray drying and vacuum drying methods and systematically characterized side by side. The color, protein content, and particle size of freeze- and vacuum-dried proteins were similar, while the spray-dried protein had significantly finer particles, lighter color, and lower protein content. The freeze-dried protein was less denatured during processing and exhibited better functional properties than the spray- and vacuum-dried proteins. The protein from freeze drying method had the highest emulsification capacity and stability as well as oil absorption capacity due to its higher surface hydrophobicity. Gels prepared from the freeze-dried protein had higher elastic and viscous modulus than that from spray- and vacuum-dried proteins. Conclusions from functional properties were well supported by protein structural features from SDS-PAGE, sulfhydryl and disulfide analysis, secondary structure, surface hydrophobicity, and thermal characterization. Overall, quinoa protein demonstrated good functional properties. This study provides useful guidance for the industry to optimize protein production and will benefit their applications as a new protein ingredient.

CRediT authorship contribution statement

Yanting Shen: Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Xiao Tang:** Investigation, Writing - review & editing. **Yonghui Li:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127823.

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