

Evaluation of drying-grinding and wetting-grinding mediated fabrication of pork skin functional protein powders: the underlying mechanism responsible for superior properties and functionalities

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Abstract: The concept of healthiness and sustainability have triggered consumers into choosing healthier food, especially “clean-label” products. Porcine skin is a natural “clean-label” raw material, and direct usage of whole pork skin as an additive has been proven as an efficient way to deal with the by-product issues and also satisfies the “clean label” demands of consumers. However, efficient approaches to convert bulk skin into handy powders in a green-fabrication manner while maintaining its unique properties have yet to be fully investigated. Herein, we provided two green approaches, drying-grinding, and wetting-grinding, to prepare pork skin functional protein powder (FPP), and their chemical composition, structure, properties, stability, and functionality are systematically investigated. Specifically, FPP prepared by two methods exhibit similar chemical composition and great thermal stability. Notably, FPP prepared by drying-grinding method is superior in flow ability, water dispersion, and texture properties of FPP gels as compared to wetting-grinding method. Structural analyses revealed that the superior properties of FPP prepared by drying-grinding method depend on the intrinsic natural triple helix structure of collagen. Overall, this work revealed the underlying key factors for the preparation of FPP with excellent properties, and highlighted that the FPP prepared by drying-grinding method is more suitable for practical application as “clean-label” additives in the food industry.

Keywords: pork skin; functional protein powder; drying-grinding; wetting-grinding; underlying structure

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1 Introduction

In recent decades, lifestyle changes have increased the demand of consumers for novel and healthier food products, and gradually enhanced the awareness of consumers of health risks by special food components or food additives^[1]. A valuable survey revealed that consumers tend to believe that most of food additives have a negative effect on their health and even possess potential hazards, thus preferring food products without additives and with natural ingredients^[2]. Therefore, with the increasing requirement for healthier food products, researchers and industries are shifting their focus on the fabrication of natural food additives or ingredients and aiming to develop novel clean-label food products^[3–4]. For instance, Diantom et al.^[5] investigated the potential of potato fiber as a texturing agent to substitute xanthan in the tomato industry and proved its capability to be used as a “clean label” substitute. Cămară et al.^[6] evaluated the functional properties of Chia mucilage powder as a phosphate replacer in low-fat Bologna sausages and suggested that 2% Chia mucilage gel can be applied to substitute 50% phosphate in low-fat Bologna sausages. Therefore, the fabrication and preparation of clean-label ingredients from edible animals and

plants and evaluated their properties and functionalities for the innovation of healthy food products is becoming a hotspot in the food industry recently^[3,7–8].

In the meat industry, the production of meat products such as sausages would produce a large amount of pork skin by-product, which would bring a disposal cost and ecological issues. It is known that the composition of pork skin is mainly composed of protein molecules and over 87% account for collagen^[9]. The typical structure of collagen has been proved to be a right-handed triple helix generated by the supercoiling of the three parallel α -chains, and each α -chains contains a lot of Gly-X-Y repeating amino acid motifs^[9–11]. Currently, massive studies revealed that the unique structure and sequence features endow collagen and its derivative product (gelatin) with great functionalities, and they have been widely used as an additive in food products, medicine, and cosmetics^[12–15]. However, the extraction or preparation of collagen or gelatin from animal skins is usually accompanied by the usage of acidic or alkali agents as well as the loss of nutritional components^[16], which is not coincident with the concept of “clean-label”. In fact, pork skin is known to be edible and has been

processed into many snacks. For example, the main source of skin jelly products is pork skin, and a standard (DB 2104/T 0001.4–2019) for skin jelly has been disclosed in China. Besides, it can also be used directly as additives in the food industry. For example, Alves et al.^[17] used pork skin and green banana flour as additives to prepare Bologna-type sausages, which can efficiently reduce fat while maintaining its texture properties. Santos et al.^[18] prepared two pork skin-based emulsion gels and investigated their capability in replacing animal fat in hot-dog style sausages. The results illustrated that the replacement of 50% pork fat by the emulsion gel has little effect on the sensory parameters of sausages. Therefore, those investigations indicated that direct usage of pork skin as additives might be an efficient way to deal with the by-product issues and also satisfies the “clean label” demands of consumers. However, the pork skin usually exists in form of large bulk pieces, which are not suitable for direct application. Thus, efficient methods to process pork skin into handy powder products in a green-fabrication manner while maintaining its functionality are of great importance for industrialization supply in the food industry when used as a natural ingredient or additive.

Herein, we provided two green-fabrication methods, drying-grinding method (DGM) and wetting-grinding method (WGM), to prepare porcine skin functional protein powder (FPP), and focused on providing a systematic comparison of two different methods in the chemical composition, properties, stability, and functionality of FPP. Specifically, the effect of fabrication methods on the chemical composition, texture properties, water and oil holding capacity, and powder dispersity of FPP have been evaluated. Subsequently, the primary structure and triple helix structure as well as the micromorphology of FPP prepared by DGM and WGM have been further characterized by multiple analytic techniques. Finally, the thermal stability of PFF has also been evaluated. This work provided an efficient method for the fabrication of porcine skin FPP and may facilitate the application of porcine skin as natural “clean label” additives in food industry.

2 Materials and methods

2.1 Materials

The porcine skin was purchased from local market and the skin was obtained from adult boar. Sucrose fatty acid esters were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Hydrochloric acid, sulfuric acid, anhydrous ethanol, petroleum ether and other reagents were purchased from Chongqing Wansheng Chuandong Chemical Co., Ltd. All other chemicals used in this study were of analytical grade.

2.2 Preparation of FPP through DGM and WGM mediated green fabrication

The porcine skin FPP were prepared by using two different methods: DGM and WGM mediated green fabrication method. The detailed procedures for DGM are as follows: The porcine skin was preliminarily washed and cleaned with water, followed by removing the fat layers. Then, the skin samples were heated in boiling water for 6 min, and the grease layer was further scraped off. The resulting skin was cut into pieces (3 mm × 3 mm) by using a JZM-JR351 meat grinder (Zhongshan Jinzheng Household Electric Appliance Co., Ltd., Guangdong, China). Subsequently, the porcine skin was added to a degreasing buffer (0.03% sucrose fatty acid ester solution) with a ratio of solid to liquid 1:2 (*m/V*), and the mixture

was suffered ultrasonic treatment (20 min, 60 °C, 120 W) by using a KQ3200DV ultrasonic equipment to promote the degreasing events. During degreasing, the buffer was replaced with a clean solution once. The porcine skin pieces were washed with water and suffer to drying treatment by using a DGG-9140A oven (Shanghai Qixin Scientific Instrument Co., Ltd., Shanghai, China). The drying conditions were as follows: 40, 55, and 70 °C for 24, 12, and 8 h, respectively. Finally, the dried porcine skin was smashed with an SYFM-8 II ultramicro pulverizer (Jinan Songyue Machinery Co., Ltd., Shandong, China) to obtain FPP, and the resulting samples were named as FPP40, FPP55, and FPP70. In the case of the WGM, the pre-treatment of the skin is identical to the DGM. Differently, after degreasing, the resulting samples were processed by using a JM-LB80-1D colloid grinder (Wenzhou Xiaojie Machinery Company, Zhejiang, China) to obtain skin slurry. Then, the resulting samples are further dried by using a YC-510 spray dryer (Shanghai Pilotech Instrument Equipment Co., Ltd., Shanghai, China) with hot air temperatures of 160 and 190 °C respectively, and the two samples are termed as FPP160 and FPP190.

2.3 Chemical composition analysis

The moisture, ash, protein, fat, and collagen contents of porcine skin FPP prepared by using DGM and WGM approaches were determined by referring to the previously reported method^[19]. The collagen content was determined by measuring the hydroxyproline concentration, which was conducted according to the PRC National Standard of GB/T 9695.23–2008. The collagen content was calculated through the multiplication of the hydroxyproline concentration with a coefficient of 9.75.

2.4 Texture properties analysis

To explore the texture properties of FPP, the FPP gels are prepared. Specifically, FPP samples (1.5 g) are mixed with 30 mL distilled water and followed by the homogenization at 9 000 r/min for 2 min with a XHF-DY high-speed disperser (Ningbo Xinzhi Biotechnology Co., Ltd., Zhejiang, China). The resulting samples were heated in a water bath at 80 °C for 1 h, and subsequently stored at 4 °C for 16 h. Texture properties analyses were carried out by using a TA TOUCH texture analyzer (Baosheng Industrial Co., Ltd., Shanghai, China). The test parameters were set as follows: the pre-test speed was 1.0 mm/s, the test speed was 1.0 mm/s, the post-test speed was 1.0 mm/s, the deformation was 40%, and the probe was TA 10.5.

2.5 Water and oil holding capacity analysis

The water holding capacity (WHC) and oil holding capacity (OHC) are determined according to the previous methods with some modifications^[19]. Specifically, in the case of WHC, 0.5 g sample was mixed with 10 mL of distilled water in a 50 mL centrifuge tube, and the resulting mixture was dispersed using a QL-901 vortex oscillator (Haimen Qilin Bell Instrument Manufacturing Co., Ltd., Jiangsu, China). After storing at 4 °C for 1 h, the resulting samples were centrifuged at 10 000 × *g* for 15 min by using a MULTIFUGE X3R centrifugal machine (Thermo Fisher Technology China Co., Ltd., Shanghai, China). The supernatants were decanted. During the process, the weights of the centrifuge tubes, samples, and moisture losses were all recorded. The WHC was calculated by using the following equation:

$$\text{WHC}(\%) = \frac{m_2 - m_1 - m}{m_1} \times 100 \quad (1)$$

Where m was the weight of the centrifuge tube (g); m_1 was the weight of the sample (g); m_2 was the weight of the samples after centrifugation (g).

As for the OHC, the determination method and calculation are similar to the WHC while the distilled water was substituted with 10 mL soybean oil.

2.6 Dispersity analysis

FPP samples (0.25 g) were soaked in 5 mL of distilled water (pre-stored at 4 °C for a period) and stored at 4 °C. The images of the samples were recorded every 5 min. After 10 min, the mixtures were magnetically stirred for 5 min and followed by image recording. Finally, the sample was stirred with a glass bar, and the dispersion of the sample in water was observed and photographed.

2.7 Bulk density and tap density measurement

The bulk density (ρ_{Bulk}) and tap density (ρ_{Tap}) were measured according to the previous method^[30]. Specifically, samples were added into a measuring cylinder and the volume of the powders (V) was measured. The ρ_{Bulk} , Hausner' ratio (HR) and Carr' index (CI) of the sample are calculated by following equations:

$$\rho_{\text{Bulk(Tap)}} = \frac{m}{V} \quad (2)$$

$$\text{HR} = \frac{\rho_{\text{Tap}}}{\rho_{\text{Bulk}}} \quad (3)$$

$$\text{CI}(\%) = \frac{\rho_{\text{Tap}} - \rho_{\text{Bulk}}}{\rho_{\text{Tap}}} \times 100 \quad (4)$$

Where m was the weight of powder (g); V was the bulk (tapped) powered volume (cm^3).

2.8 Scanning electron microscopy (SEM) observation

The microstructure of all the samples was observed by using a SEM (Phenom Pro, Phenom World, Netherlands). The samples were fixed on a bronze stub, sputter-coated with gold and subject to scanning electron microscopy analysis with an acceleration voltage of 10 kV.

2.9 Fourier transform infrared (FTIR) spectroscopy analysis

The FTIR spectroscopy analysis was carried out by employing a Spectrum100 FTIR spectrometer (Perkin Elmer company, USA). The spectra of all the samples were recorded in the range of 4 000–600 cm^{-1} with a resolution of 4 cm^{-1} .

2.10 Differential scanning calorimetry (DSC) analysis

The DSC experiments were conducted by using a DSC4000 instrument (Perkin Elmer company, USA). Sample (2.5 g) was

added to the crucible and the DSC data were collected in the range of 10–200 °C with a heating rate of 10 °C/min and nitrogen flow rate of 50 mL/min.

2.11 Sodium-dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE experiment was carried out by using an 8% separating gel and 5% stacking gel. Sample (8 mg/mL) was prepared with 10% (m/V) SDS as solvent, and the resulting samples were kept in water bath at 80 °C for 2 h. After being mixed with the loading buffer, the samples are subject to water-boiling for 5 min. After electrophoresis, the resulting gel was stained with Coomassie Brilliant Blue.

2.12 Statistical analysis

All the measurements were repeated three times. The data and figures were processed and analyzed with SPSS 18.0 and Origin 2019. Least significant difference (LSD) method was used to analyze the significance of the data. A value of $P < 0.05$ indicates to be statistically significant.

3 Results and discussion

3.1 Chemical composition and appearance of FPP

To explore the chemical composition of porcine skin functional protein powders prepared by DGM and WGM, the moisture, ash, protein, fat, and collagen contents have been determined. As can be seen in Table 1, the chemical composition of all prepared porcine skin FPP was identical with content varying and protein was the main component, giving the content of 91%–95%, and most of the proteins are determined as collagen (over 79%). This was not surprising because the skin is mainly composed of collagen proteins^[9–10]. In addition, it was obvious that the collagen content of protein powder prepared by DGM was a little lower than that of FPP prepared by WGM. It might be ascribed to the loss of degraded collagen during the initial drying stage in the oven. Besides, it can be observed that the fat content of the FPP can be reduced to less than 3%. This is because the sucrose fatty acid ester solution was employed during the degreasing stage, which can remove most of the fat in pork skin. Besides, the appearance of protein powders was further compared. According to Figures 1A and 1B, protein powders prepared by DGM and WGM exhibit distinct differences. In the case of pork skin FPP prepared by DGM, it was beige and not easy to clump. Inversely, pork skin FPP prepared by WGM was white and easy to aggregate. Moreover, as compared to the protein powders prepared by DGM, protein powders prepared by WGM exhibited a smaller particle size. Therefore, all those results suggested that porcine skin FPP prepared by two methods exhibits a similar chemical composition,

Table 1 Chemical composition of porcine skin FPP (%).

Samples	Moisture content	Fat content	Ash content	Collagen content	Protein content
FPP40	9.63 ± 0.57 ^a	2.61 ± 0.09 ^a	0.64 ± 0.05 ^b	79.15 ± 2.43 ^b	91.38 ± 0.21 ^c
FPP55	8.26 ± 0.26 ^b	2.67 ± 0.07 ^a	0.79 ± 0.06 ^a	80.06 ± 1.19 ^b	94.58 ± 0.42 ^a
FPP70	7.70 ± 0.14 ^{bc}	2.77 ± 0.16 ^a	0.61 ± 0.10 ^b	81.98 ± 1.92 ^b	94.30 ± 0.43 ^{ab}
FPP160	7.56 ± 0.27 ^c	1.46 ± 0.04 ^c	0.62 ± 0.03 ^b	90.95 ± 0.86 ^a	93.19 ± 1.56 ^b
FPP190	7.10 ± 0.23 ^c	1.95 ± 0.19 ^b	0.72 ± 0.06 ^{ab}	90.33 ± 0.61 ^a	93.74 ± 1.08 ^{ab}

Note: Different letters for each column are statistically different ($P < 0.05$).

but the FPP prepared by drying-grinding seems to exhibit greater stability against powder aggregation.

3.2 Evaluation of dispersion property of FPP

The dispersion property of porcine skin FPP in cold water (4–10 °C) is one of the most important prerequisites for their practical application when used as additives in the processing of food products, such as the meat industry. It can be observed from Figure 1C that the swelling degree of all porcine skin FPP samples increased with the extension of soaking time, illustrating the great water absorption capability. It should be notable that FPP samples (FPP160 and FPP190) prepared by WGM showed obvious clumps after swelling, and the FPP samples can be not dispersed in water. Inversely, the FPP40, FPP55, and FPP160 prepared by DGM were gradually dispersed in water. Remarkably, after magnetic stirring, FPP samples prepared by DGM were almost homogeneous-dispersed in cold water, while the clumps of FPP samples prepared by WGM were still tightly clustered and could not be dispersed in water. Further, after undergoing the stirring by glass rod, the clumps of FPP160 and FPP190 became smaller, but they still could not be completely dispersed in water. Therefore, all those results

demonstrated that porcine skin FPPs prepared by DGM possess good dispersion property in cold water and the dispersion is better than the FPP samples prepared by WGM. In addition, in the case of temperature variation, it seems that there was no significant difference in the dispersion properties of FPP samples prepared by DGM or WGM.

It is known that the dispersing time and agglomeration rate of protein powder is closely related to the powder flowability, and the flowability is highly dependent on the adhesion, shape, and particle size distribution of protein powder^[21]. In order to evaluate the flowability of the pork skin FPPs, the bulk density and the tap density of prepared FPP samples were measured, and the HR and CI were calculated. Figure 2A showed that the bulk density of FPP samples prepared by DGM was significantly higher than that of FPP samples prepared by WGM, indicating that the gap volume between the protein powders prepared by DGM was much smaller. Moreover, the data of tap density also showed the similar trends. It may be that the smaller size of FPP prepared by WGM leads to stronger adhesion and larger contact areas between the protein powder particles, thus it is easy to gather into clumps and generate large gaps between the powder blocks, which is not easy to

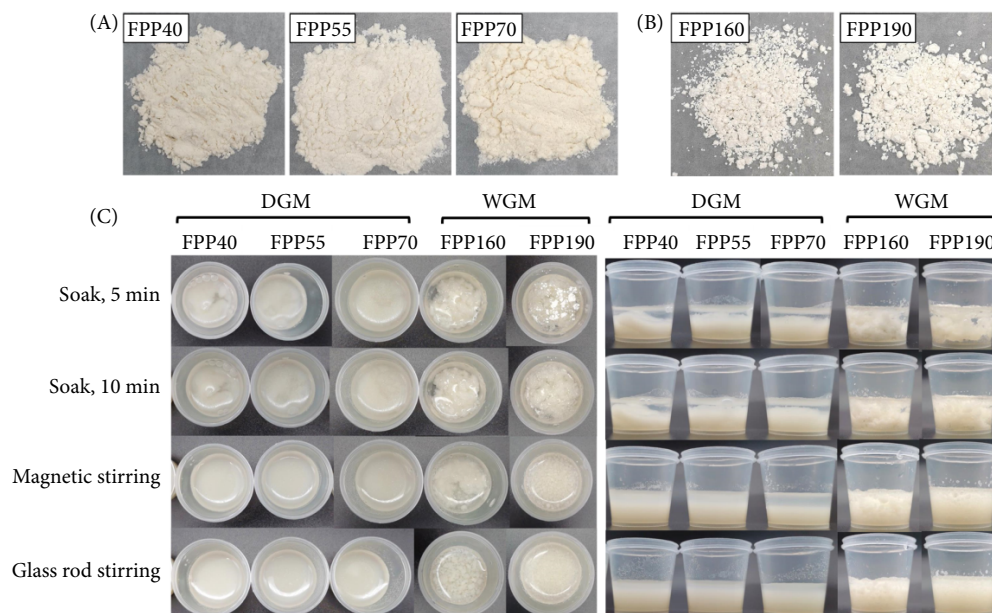


Figure 1 Appearance of FPP prepared by (A) DGM and (B) WGM. (C) The dispersion of FPP in water at different stages.

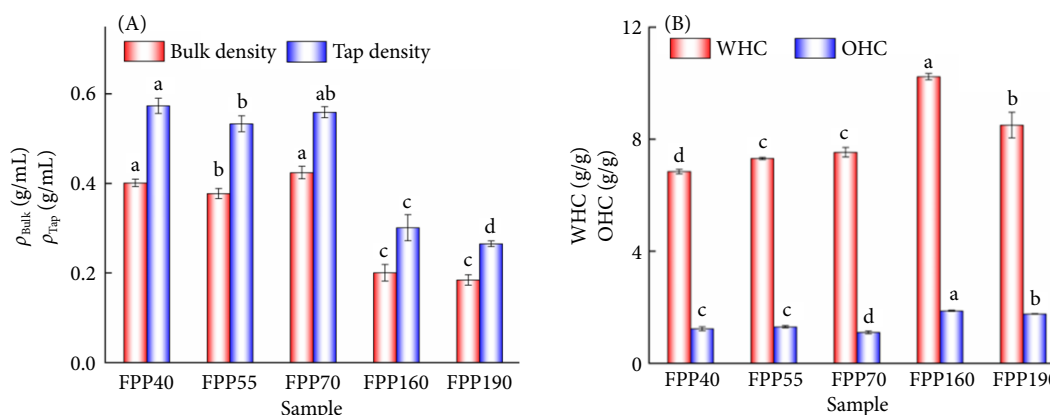


Figure 2 (A) Bulk density and tap density of FPP. (B) Water holding capacity (WHC) and oil holding capacity (OHC) of functional protein powder. Different letters indicate significant differences between groups of the same indicator ($P < 0.05$).

be removed by tapping. Such results were consistent with the dispersion properties of FPP in water. Besides, according to Table 2, the HR and CI of FPP55 and FPP70 were slightly smaller than that of other samples, indicating that they possess good flowability and weak adhesion forces between particles. Therefore, the results revealed that the dispersion performance of FPP prepared by DGM was better than that of FPP prepared by WGM. Moreover, FPP55 and FPP70 seem to be more convenient when used as additives in meat processing.

Table 2 Hausner' ratio (HR) and Carr' index (CI) of FPP.

Sample	FPP40	FPP55	FPP70	FPP160	FPP190
HR	1.43 ± 0.04 ^a	1.41 ± 0.06 ^a	1.32 ± 0.03 ^b	1.50 ± 0.03 ^a	1.44 ± 0.07 ^a
CI (%)	29.99 ± 2.21 ^a	28.21 ± 3.00 ^a	24.21 ± 1.53 ^b	33.41 ± 1.57 ^a	30.41 ± 3.50 ^a

Note: Different letters in the same line indicate significant differences ($P < 0.05$).

3.3 Water and oil holding capacity of functional protein powder

Meat products are prone to lose water and nutritional components during processing which leads to the decline of food quality. Therefore, to avoid those problems, some of the additives such as thickeners, and emulsifiers, are usually added to the products to enhance their water and oil retention ability and aim to improve the texture of products. As we all know, the main component of skin, collagen or gelatin, has been extensively used in the food industry. Thus, the pork skin FPP is also a great potential additive to improve food quality. The WHC indicates the ability to retain water

molecules and is one of the most important characteristics for the practical application of porcine skin FPP. According to Figure 2B, it can be observed that the WHC of FPP prepared by DGM was significantly lower than that of WGM ($P < 0.05$). This phenomenon might be that the particle size of the FPP prepared by WGM was much smaller. Since the smaller particle size would endow the FPP particles a larger specific surface area, which gives a larger area to absorb water molecules. In addition, for the FPP prepared by DGM, with the increase of temperatures, the WHC was enhanced. This phenomenon might be associated with the exposure of additional hydrophilic groups that can facilitate the binding of water molecules. Inversely, in the case of FPP prepared by WGM, the WHC of FPP160 was higher than FPP190. It might be that higher temperature would lead to the degradation of protein, thus showing a lower WHC. In addition, the OHC is also an important indicator of FPP as it can evaluate the binding ability of protein molecules to oil molecules, which can reflect the capability of flavor retention. On the basis of Figure 2B, the effect of preparation method and processing parameters on the OHC was similar to that of WHC. Thus, all those results demonstrated that all the FPP showed great water and oil holding capacity, and FPP prepared by DGM was a little lower than that of FPP prepared by WGM.

3.4 Texture properties analysis

FPP is one of the important raw materials for the preparation of gel products, thus to evaluate the effect of preparation methods on the gel quality of FPP samples, the FPP gels were fabricated and their texture properties were measured (Figure 3). It can be observed that the values of hardness, chewiness, and cohesion of FPP prepared by DGM were higher than those of WGM ($P < 0.05$), implying that the texture properties of FPP40, FPP55, and FPP160 were

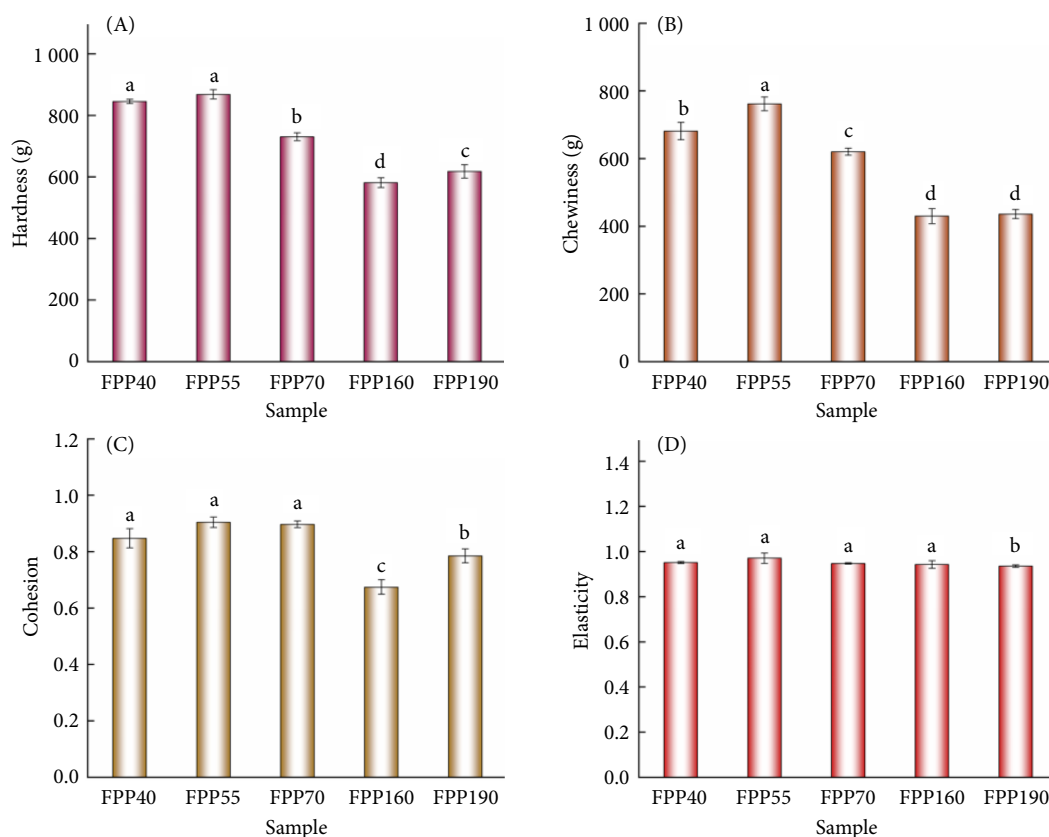


Figure 3 Texture properties analysis of FPP gels. (A) Hardness; (B) Chewiness; (C) Cohesion; (D) Elasticity. Different letters indicate significant differences ($P < 0.05$).

significantly better than that of FPP160 or FPP190. On the basis of the chemical composition analyses, the collagen content of FPP prepared by DGM was little lower than that of FPP prepared by WGM. However, the texture properties of FPP40, FPP55, and FPP160 are reversely better. In general, the texture properties are associated with collagen content and intrinsic structure. Thus, the reason might be that the natural structure of collagen in FPP40, FPP55, and FPP160 was not completely destroyed, while the intrinsic structure of collagen in FPP160 or FPP190 was damaged during the preparation process. In addition, all samples showed excellent elasticity, indicating a high degree of gel recovery after initial compression. Overall, it suggested that preparation of FPP through DGM would endow the protein powder with better texture properties.

3.5 Micromorphology of FPP

To further investigate the micromorphology of pork skin FPPs, the SEM observation was carried out. According to Figure 4, it can be clearly found that the particle shape of FPP prepared by DGM exhibit an irregular and fragmented morphology. Moreover, as compared to FPP prepared by WGM, the particle size of FPP prepared by DGM was significantly larger than that of FPP prepared by WGM. This phenomenon might be attributed to the different preparation procedures of the two types of protein powders. In the case of particle morphology of FPP160 and FPP190, they both exhibit a spherical shape with surface depression, and adjacent FPP particles tend to gather with each other to generate larger clusters. Such result was in agreement with the optical observation, and also explained the differences in dispersion properties of two types of FPP. More specifically, due to the smooth surface of flaky protein powder prepared by DGM, it was more likely to disperse after swelling in water. Inversely, the inter-molecular conjugation of between adjacent spherical FPP160 or FPP190 made them to form large aggregates, which might suppress the water absorption and the swell events, thus leading to poor dispersion.

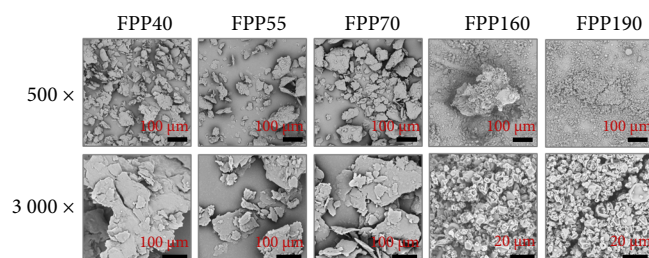


Figure 4 SEM image of FPP prepared by (A) DGM and (B) WGM.

3.6 SDS-PAGE result

To further explore the effect of fabrication method on the primary structure of porcine skin FPPs and investigate the underlying mechanism of such superior properties and functionalities, SDS-PAGE experiment was conducted and the results were illustrated in Figure 5. It is obvious that FPP samples prepared by two different methods exhibited distinct differences in the composition of protein bands in SDS-PAGE, suggesting that fabrication method can significantly affect the primary structure of porcine skin FPP. For the FPP prepared by DGM, three protein bands with molecular weight near 200 and 120 kDa can be observed. According to the previous results, collagen proteins

consist of two α chains (α_1 and α_2) and a β chain, forming a triple helix structure^[23]. The band with molecular weight of 200 kDa were termed as β chains, and the bands near 120 kDa were α_1 and α_2 chains, respectively. Thus, the results indicated that the FPP samples are mainly composed of the α_1 chain, α_2 chain and β chain of collagen, implying that a large proportion of the α and β chains of collagen and its triple helix structures were retained during the process and preparation. In the case of FPP prepared by WGD, protein band with molecular weight of 200 kDa disappeared and a fraction of protein bands with molecular weight lower than 100 kDa were observed. These results indicated that most of the collagen β chain is decomposed into small molecular components. Moreover, a small fraction of α chain also will undergo degradation upon increasing the temperature. Thus, utilization of wetting-grinding mediated fabrication method to prepare FPP can significantly lead to the degradation of α chain and β chain of collagen, and even may lead to the destruction of the triple helix structure of collagen. Meanwhile, this result also gives directed explanation why the texture properties of FPP prepared by drying-grinding method are much better.

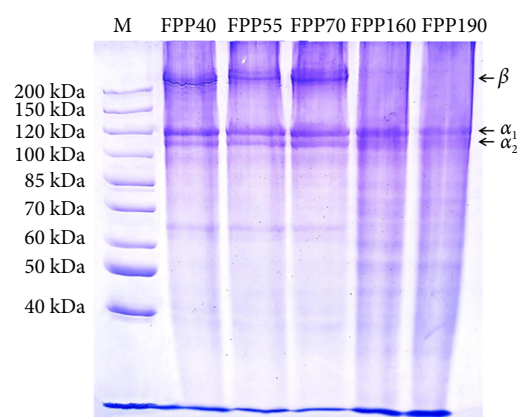


Figure 5 SDS-PAGE analysis of FPP prepared by DGM and WGM. Lane M, protein Markers and their corresponding molecular masses. The α chains and β chain are marked with arrows.

3.7 FTIR analysis of functional protein powder

To further explore the effect of preparation methods on the intrinsic triple helix structure of porcine skin FPPs, the FTIR data were collected. As can be seen in Figure 6 and Table 3, in the amide A band, the characteristic peaks of FPP40, FPP55, and FPP70 appeared at 3 281 cm^{-1} , and the characteristic peaks of FPP160 and FPP190 appeared at 3 286, 3 285 cm^{-1} , respectively. In general, the amide A band represents the N-H stretching vibration and hydrogen bond bonding. Moreover, it has been reported that the position of the characteristic peak usually moves to a lower wave number when the N-H group participates in the formation of hydrogen bonds^[23]. Thus, it means that more N-H groups were involved in the generation of hydrogen bonds for FPP40, FPP55, and FPP70 samples. Moreover, it inferred that the triple helix structures of collagen in FPP prepared by DGM seem to be more stable^[24]. Amide B bands are associated with asymmetric stretching of CH_2 ^[25]. The characteristic peaks of FPP40, FPP55, and FPP70 in the amide B band appeared at 2 924, 2 925, and 2 924 cm^{-1} , close to the reported 2 924 cm^{-1} , while the characteristic peaks of FPP160 and FPP190 appeared at 2 928 cm^{-1} with low intensity. It implied that the triple helix structure of collagen in FPP160 and FPP190 might be destroyed.

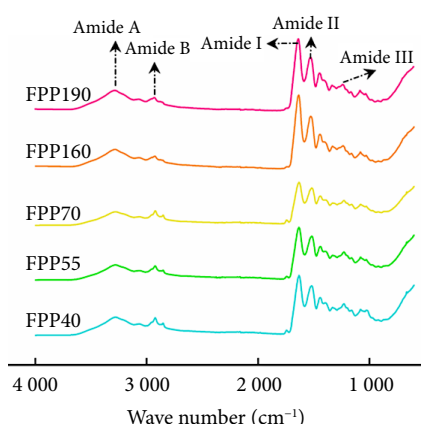


Figure 6 FTIR spectrum of FPP prepared by DGM and WGM.

Table 3 The amide stripping peak position in FTIR of FPP prepared by DGM and WGM (cm⁻¹).

Amide stripping peak	FPP40	FPP55	FPP70	FPP160	FPP190
Amide A	3 281	3 281	3 281	3 286	3 285
Amide B	2 924	2 925	2 924	2 928	2 928
Amide I	1 633	1 633	1 633	1 635	1 635
Amide II	1 520	1 520	1 520	1 528	1 526
Amide III	1 231	1 231	1 229	1 238	1 237

The amide II band is mainly related to C–N stretching and N–H bending vibration. The amide III band is mainly related to C–N stretching, N–H bending vibration, C=C stretching, and C–H bending^[26]. In the amide II band, the characteristic peak positions of FPP160 and FPP190 appeared at 1 528 and 1 526 cm⁻¹, while the characteristic peak positions of FPP40, FPP55, and FPP70 moved to a lower wave number of 1 520 cm⁻¹. In amide III band, the characteristic peak wave number of FPP40 and FPP55 was 1 231 cm⁻¹, the characteristic peak number of FPP70 was 1 229 cm⁻¹, and the characteristic peak number of FPP160 and FPP190 was 1 238 and 1 237 cm⁻¹, respectively. It has been reported that slight deviation of the characteristic peak of the amide III band towards the low wave number is related to the formation of collagen fibrils, and intramolecular and intermolecular hydrogen bonds are responsible for the stability of the triple helix structure of collagen macromolecules^[27]. The movement of the characteristic peaks of the amide II and III bands towards the low wave number indicated that the intermolecular hydrogen bond in collagen was enhanced. Thus, all those suggested that WGD might break the hydrogen bonds in collagen. On the contrary, FPP samples prepared by DGM preserved most domain of the intrinsic triple helix structure of collagen, resulting in stronger hydrogen bond interaction in the collagen of FPP40, FPP55, and FPP70 samples.

Besides, it has been reported that the ratio of absorbance value between amide III band and the peak at 1 450 cm⁻¹ can reflect the integrity of the triple helix structure of collagen. When the ratio is close to 1.0, the triple helix structure of collagen is well-kept^[28]. After calculation, the ratio of FPP40, FPP55, and FPP70 were determined as 0.841 9, 0.832 9, and 0.846 1, respectively. The ratio of FPP160 and FPP190 were determined as 0.686 9 and 0.097 4, respectively. It is clear that the ratio of the FPP samples prepared by DGM was all higher than 0.83, while the ratio of the FPP samples prepared by WGM was lower than 0.7. Thus, it implied that the triple helix structure of the FPP samples prepared by DGM can be well preserved as compared to the WGM.

3.8 DSC analysis

It is known that good thermal stability is quite important and may be beneficial for the utilization of FPP during food processing. Thus, to evaluate the thermal stability of prepared FPP, all the samples were subjected to the DSC analysis (Figure 7). For FPP prepared by DGM, the melting temperature (T_m) value was gradually increased upon the drying temperature increased. This result implied that a higher drying temperature would endow protein powder a better thermal stability. Since a lower drying temperature needs longer time to obtain the protein powder, thus it is probable that the long drying duration may result in a comparatively looser structure of collagen, thus giving a lower thermal stability. As for FPP prepared by WGM, the T_m of spray drying samples at 190 °C was lower than that of drying samples at 160 °C. According to the results of FTIR spectrum analysis, it might be that the intermolecular forces within the triple helical structure were largely destructed although the triple helical structure of collagen in FPP190 was more complete than that of drying samples at 160 °C, thus resulting in the reduction of its thermal stability. On the basis of the above results, it seemed that FPP55, FPP70, and FPP160 possess better thermal stability.

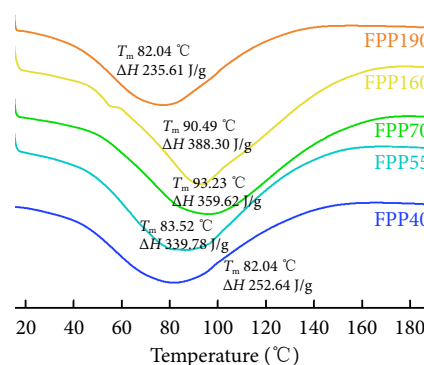


Figure 7 DSC diagram of functional protein powder prepared by DGM and WGM.

4 Conclusion

In this study, we provided two efficient green-fabrication approaches to prepare porcine skin FPP through DGM and WGM, and a systematic comparison of preparation methods in their properties, stability, and functionality were investigated, and the underlying mechanism responsible for superior properties and functionalities has been illustrated. The results demonstrated that the fabrication method of FPP has little effect on their chemical composition, but the FPP prepared by DGM exhibited better flowability and water dispersion property, and the texture properties of FPP gel was superior to that of FPP prepared by WGM. The structural analysis revealed that the excellent properties of FPP prepared by DGM were highly associated with the reservation of the α and β chains of collagen and its triple helix structure. Thus, the FPP fabricated by DGM seemed to be more suitable for the application as “clean-label” additives in the food industry. Besides, for DGM, longer drying time at lower temperatures might lead to loss of the ordered structure of collagen. Overall, this work highlighted that the drying-grinding mediated green-fabrication method might be an efficient approach to prepare porcine skin functional protein powders and facilitate their application as natural “clean-label” additives in the food industry.

Conflict of interest

The authors declare that they have no known competing financial interests.

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References

- [1] A. T. Noguerol, M. J. Pagán, P. García-Segovia, et al., Green or clean? Perception of clean label plant-based products by omnivorous, vegan, vegetarian and flexitarian consumers, *Food Res. Int.* 149 (2021) 110652. <https://doi.org/10.1016/j.foodres.2021.110652>.
- [2] S. Shim, S. H. Seo, Y. Lee, et al., Consumers' knowledge and safety perceptions of food additives: evaluation on the effectiveness of transmitting information on preservatives, *Food Control* 22 (2011) 1054–1060. <https://doi.org/10.1016/j.foodcont.2011.01.001>.
- [3] J. Aschemann-Witzel, P. Varela, A. Peschel, Consumers' categorization of food ingredients: Do consumers perceive them as 'clean label' producers expect? An exploration with projective mapping, *Food Qual. Prefer.* 71 (2019) 117–128. <https://doi.org/10.1016/j.foodqual.2018.06.003>.
- [4] B. Jeganathan, T. Vasanathan, F. Temelli, Isolation of clean-label faba bean (*Vicia faba* L.) proteins: a comparative study of mild fractionation methods against traditional technologies, *Innov. Food Sci. Emerg. Technol.* 84 (2023) 103285. <https://doi.org/10.1016/j.ifset.2023.103285>.
- [5] A. Diantom, F. Boukid, E. Carini, et al., Can potato fiber efficiently substitute xanthan gum in modulating chemical properties of tomato products?, *Food Hydrocoll.* 101 (2019) 105508. <https://doi.org/10.1016/j.foodhyd.2019.105508>.
- [6] A. K. F. I. Câmara, V. A. S. Vidal, M. Santos, et al., Reducing phosphate in emulsified meat products by adding chia (*Salvia hispanica* L.) mucilage in powder or gel format: a clean label technological strategy, *Meat Sci.* 163 (2020) 108085. <https://doi.org/10.1016/j.meatsci.2020.108085>.
- [7] R. H. Belmiro, L. D. C. Oliveira, A. A. L. Tribst, et al., Techno-functional properties of coffee by-products are modified by dynamic high pressure: a case study of clean label ingredient in cookies, *LWT-Food Sci. Technol.* 154 (2022) 112601. <https://doi.org/10.1016/j.lwt.2021.112601>.
- [8] B. Jeganathan, J. Gao, F. Temelli, et al., Potential of air-currents assisted particle separation (ACAPS) technology for hybrid fractionation of clean-label faba bean (*Vicia faba* L.) protein, *J. Food Eng.* 339 (2023) 111265. <https://doi.org/10.1016/j.jfoodeng.2022.111265>.
- [9] S. Min, Y. Jo, S. Park, Potential application of static hydrothermal processing to produce the protein hydrolysates from porcine skin by-products, *LWT-Food Sci. Technol.* 83 (2017) 18–25. <https://doi.org/10.1016/j.lwt.2017.04.073>.
- [10] C. Tang, K. Zhou, Y. Zhu, et al., Collagen and its derivatives: from structure and properties to their applications in food industry, *Food Hydrocoll.* 131 (2022) 107748. <https://doi.org/10.1016/j.foodhyd.2022.107748>.
- [11] A. M. Puzkarska, D. Frenkel, L. J. Colwell, et al., Using sequence data to predict the self-assembly of supramolecular collagen structures, *Biophys. J.* 121 (2023) 3023–3033. <https://doi.org/10.1016/j.bpj.2022.07.019>.
- [12] Z. Li, C. Ruan, X. Niu, Collagen-based bioinks for regenerative medicine: Fabrication, application and prospective, *Medicine in Novel Technology and Devices.* 17 (2023) 100211. <https://doi.org/10.1016/j.medntd.2023.100211>.
- [13] W. Peng, D. Li, K. Dai, et al., Recent progress of collagen, chitosan, alginate and other hydrogels in skin repair and wound dressing applications, *Int. J. Biol. Macromol.* 208 (2022) 400–408. <https://doi.org/10.1016/j.ijbiomac.2022.03.002>.
- [14] M. Wang, Z. Yin, M. Zeng, Construction of 3D printable Pickering emulsion gels using complexes of fiber polysaccharide-protein extracted from *Haematococcus pluvialis* residues and gelatin for fat replacer, *Food Hydrocoll.* 137 (2023) 108350. <https://doi.org/10.1016/j.foodhyd.2022.108350>.
- [15] X. Feng, H. Dai, Y. Yu, et al., Adjusting the interfacial property and emulsifying property of cellulose nanofibrils by ultrasonic treatment combined with gelatin addition, *Food Hydrocoll.* 133 (2022) 107905. <https://doi.org/10.1016/j.foodhyd.2022.107905>.
- [16] S. Park, H. Kim, Effect of wet- and dry-salting with various salt concentrations on pork skin for extraction of gelatin, *Food Hydrocoll.* 131 (2022) 107772. <https://doi.org/10.1016/j.foodhyd.2022.107772>.
- [17] L. A. A. dos Santos Alves, J. M. Lorenzo, C. A. A. Gonçalves, et al., Production of healthier bologna type sausages using pork skin and green banana flour as a fat replacers, *Meat Sci.* 121 (2016) 73–78. <https://doi.org/10.1016/j.meatsci.2016.06.001>.
- [18] M. D. Santos, P. E. S. Munekata, M. Pateiro, et al., Pork skin-based emulsion gels as animal fat replacers in hot-dog style sausages, *LWT-Food Sci. Technol.* 132 (2020) 109845. <https://doi.org/10.1016/j.lwt.2020.109845>.
- [19] A. Abbou, N. Kadri, F. Dahmoune, et al., Optimising functional properties and chemical composition of *Pinus halepensis* Mill. seeds protein concentrates, *Food Hydrocoll.* 100 (2019) 105416. <https://doi.org/10.1016/j.foodhyd.2019.105416>.
- [20] D. Arepally, T. K. Goswami, Effect of inlet air temperature and gum Arabic concentration on encapsulation of probiotics by spray drying, *LWT-Food Sci. Technol.* 99 (2018) 583–593. <https://doi.org/10.1016/j.lwt.2018.10.022>.
- [21] X. Qi, L. Cheng, X. Li, et al., Effect of cooking methods on solubility and nutrition quality of brown rice powder, *Food Chem.* 274 (2018) 444–451. <https://doi.org/10.1016/j.foodchem.2018.07.164>.
- [22] C. Shi, C. Bi, M. Ding, et al., Polymorphism and stability of nanostructures of three types of collagens from bovine flexor tendon, rat tail, and tilapia skin, *Food Hydrocoll.* 93 (2019) 253–260. <https://doi.org/10.1016/j.foodhyd.2019.02.035>.
- [23] A. Sionkowska, J. Kozłowska, M. Skorupska, et al., Isolation and characterization of collagen from the skin of *Brama australis*, *Int. J. Biol. Macromol.* 80 (2015) 605–609. <https://doi.org/10.1016/j.ijbiomac.2015.07.032>.
- [24] M. Andonegi, K. D. L. Caba, P. Guerrero, Effect of citric acid on collagen sheets processed by compression, *Food Hydrocoll.* 100 (2020) 105427. <https://doi.org/10.1016/j.foodhyd.2019.105427>.
- [25] J. H. Muyonga, C. G. B. Cole, K. G. Duodu, Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*), *Food Chem.* 85 (2004) 81–89. <https://doi.org/10.1016/j.foodchem.2003.06.006>.
- [26] C. Stani, L. Vaccari, E. Mitri, et al., FTIR investigation of the secondary structure of type I collagen: new insight into the amide III band, *Spectrochim Acta Part A* 229 (2020) 118006. <https://doi.org/10.1016/j.saa.2019.118006>.
- [27] K. Pietrucha, Changes in denaturation and rheological properties of collagen-hyaluronic acid scaffolds as a result of temperature dependencies, *Int. J. Biol. Macromol.* 36 (2005) 299–304. <https://doi.org/10.1016/j.ijbiomac.2005.07.004>.
- [28] Y. Zhang, Z. Chen, X. Liu, et al., SEM, FTIR and DSC investigation of collagen hydrolysate treated degraded leather, *J. Cult. Herit.* 48 (2021) 205–210. <https://doi.org/10.1016/j.culher.2020.11.007>.