

Journal of Chemistry and Technologies

pISSN 2663-2934 (Print), ISSN 2663-2942 (Online).

journal homepage: http://chemistry.dnu.dp.ua



UDC 637.146.344

FUNCTIONAL PROPERTIES OF FOUR KINDS OF OILSEED PROTEIN ISOLATES

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 Received 26 October 2020; accepted 20 April 2021; available online 1 May 2021

Abstract

Plant proteins can be applied in food processing as alternatives to animal protein. Recently, popular additives of plant proteins such as soybean protein and peanut protein are widely applied in food products, like meat products, beverages, and breads. Besides, new plant proteins such as pumpkin seed protein and sunflower seed protein also get more and more attention in food industry. These plant proteins need to satisfy some special functional properties such as oil-absorbing ability and emulsifying property for developing new food products by industry. It is necessary to analyze these functional properties and make a comparison for better utilization. Since the alkaline solution and acid precipitation is still an effective method to satisfy the demand of industrial large-scale production to protein isolates. In the present study, using alkali solution and acid precipitation, four kinds of oilseed protein isolates such as peanut protein isolate (PEPI), pumpkin seed protein isolate (PUPI), sunflower seed protein isolate (SUPI), and soybean protein isolate (SOPI) were prepared from defatted peanut meal, defatted pumpkin seed meal, defatted sunflower seed meal and defatted soybean meal. The functional properties including water-absorbing ability (WA), oil-absorbing ability (OA), wetting time (WT), emulsifying ability (EA), emulsifying stability (ES), foaming ability (FA), and foaming stability (FS) of these four kinds of protein isolates were investigated. The results showed that different protein isolates exhibited different functional properties. Compared to the FA of PUPI (3.88 ± 1.69 %), SOPI (7.76 ± 2.04%), and SUPI (9.33 $\pm 1.39\%$), the FA of PEPI showed the highest value of $18.18 \pm 0.97\%$. The PUPI exhibited the shortest WT of 11.02 ± 3.13 s and the highest OA of 1.34 ± 0.004 mL/g. Besides, SOPI showed the highest EA value of 100.84 ± 9.69 m²/g. Notably, although not all functional properties of PUPI showed the best value, PUPI exhibited the shortest WT and the highest OA value, which makes it a candidate additive in meat products and might improve the OA and palatability of meat products. Moreover, PUPI does not contain chlorogenic acid, which is contained in SUPI, and therefore does not influence the organoleptic and nutritional quality of food products.

Keywords: protein isolate; alkali solution and acid precipitation; functional properties

ФУНКЦІОНАЛЬНІ ВЛАСТИВОСТІ ЧОТИРИХ ВИДІВ БІЛКОВИХ ІЗОЛЯТІВ НАСІННЯ ОЛІЙНИХ КУЛЬТУР

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Анотація

Рослинні білки можуть застосовуватися в харчовій промисловості як альтернатива тваринного білка. Останнім часом такі популярні добавки рослинних білків, як соєвий білок та арахісовий білок, широко застосовуються в харчових продуктах, таких як м'ясні продукти, напої та хліб. Крім того, рослинним білкам, таким як білок насіння гарбуза та білок насіння соняшнику, також приділяють дедалі більше уваги у харчовій промисловості. Ці рослинні білки повинні задовольняти функціональні властивості, такі як жироутримуюча здатність та емульгуюча здатність. Проаналізовано ці функціональні властивості та проведено їх порівняння. У цьому дослідженні для виділення чотирьох видів білкових ізолятів олійних культур, використовували метод лужного розчинення з подальшим кислотним осадженням. Ізолят білка арахісу (ІБА), ізолят білка насіння гарбуза (ІБНГ), ізолят білка насіння соняшнику (ІБНС) та ізолят білка сої (ІБС) готувались із знежиреного борошна відповідного виду. Були досліджені та проаналізовані функціональні властивості, такі як вологоутримуюча здатність (ВУЗ), жиропоглинаюча здатність (ЖПЗ), час змочування (ЧЗ), емульгуюча здатність (ЕЗ), стійкість емульсії (СЕ), піноутворююча здатність (ПЗ) та стійкість піни (СП) всіх чотирьох видів ізолятів білка. Результати показали, що різні ізоляти білка виявляли різні функціональні властивості. ПЗ І БА показала найвище значення 18.18±0.97 %, порівняно з ПЗ ІБНГ (3.88±1.69 %), ІБС (7.76±2.04 %) та ІБНС (9.33 ±1.39 %). ІБНГ демонстрував найкоротший ЧЗ 11.02±3.13 і найвищий показник ЖПЗ 1.34±0.004 мл/г.

*Corresponding author: e-mail: a.helikh.snau@gmail.com © 2021 Oles Honchar Dnipro National University doi: 10.15421/082116 Крім того, ІБС показав найвище значення СЕ 100.84 ± 9.69 м² / г. Зазначено, що, хоча не всі функціональні властивості ІБНГ демонстрували найвищі показники, проте, ІБНГ демонстрував найкоротший ЧЗ та найвище значення показника ЖПЗ, що робить його перспективним в якості добавки в м'ясні продукти та може покращити ЖПЗ та смакові якості м'ясних продуктів. Більше того, ІБНГ не містить хлорогенової кислоти, яка міститься в ІБНС, а отже, не впливає на органолептичні та харчові якості харчових продуктів.

Ключові слова: білковий ізолят; лужне розчинення та кислотне осадження; функціональні властивості

ФУНКЦИОНАЛЬНЫЕ СВОЙСТВА ЧЕТЫРЕХ ВИДОВ БЕЛКОВЫХ ИЗОЛЯТОВ СЕМЯН МАСЛИЧНЫХ КУЛЬТУР

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Аннотация

Растительные белки могут применяться в пищевой промышленности в качестве альтернативы животного белка. В последнее время такие популярные добавки растительных белков, как соевый белок и арахисовый белок, широко применяются в пищевых продуктах, таких как мясные продукты, напитки и хлеб. Кроме того, растительным белкам, таким как белок семян тыквы и белок семян подсолнечника, также уделяют все больше внимания в пищевой промышленности. Эти растительные белки должны удовлетворять функциональные свойства, такие как жиро поглощающая способность и эмульгирующая способность. Проанализированы эти функциональные свойства и проведено их сравнение. В этом исследовании для выделения четырех видов белковых изолятов масличных культур, использовали метод щелочного растворения с последующим кислотным осаждением. Изолят белка арахиса (ИБА), изолят белка семян тыквы (ИБСТ), изолят белка семян подсолнечника (ИБСП) и изолят белка сои (ИБС) готовились с обезжиренной муки соответствующего вида. Были исследованы и проанализированы функциональные свойства, такие как влагоудерживающая способность (ВУЗ), жиропоглощающая способность (ЖПС), время смачивания (ВС), эмульгирующая способность (ЭС), стабильность эмульсии (СЭ), пенообразующая способность (ПО) и устойчивость пены (УП) всех четырех видов изолятов белка. Результаты показали, что различные изоляты белка проявляли различные функциональные свойства. ПО ИБА показала высокое значение 18.18 ± 0.97 % по сравнению с ПО ИБСТ (3.88 ± 1.69 %), ИБС (7.76 ± 2.04 %) и ИБСП (9.33 ± 1.39 %). ИБСТ демонстрировал кратчайший BC 11.02 ± 3.13 и самый высокий показатель ЖПЗ 1.34 ± 0.004 мл/ч. Кроме того, ИБС показал высокое значение СЭ $100.84 \pm 9.69 \text{ m}^2/\text{ч}$. Отмечено, что, хотя не все функциональные свойства ИБСТ демонстрировали высокие показатели, однако, ИБСТ демонстрировал кратчайшее ВС и высокое значение показателя ЖПС, что делает его перспективным в качестве добавки в мясные продукты и может улучшить ЖПС и вкусовые качества мясных продуктов. Более того, ИБСТ не содержит хлорогеновой кислоты, которая содержится в ИБСП, а, следовательно, не влияет на органолептические и пищевые качества пищевых продуктов.

Ключевые слова: белковый изолят; щелочное растворение и кислотное осаждение; функциональные свойства

Introduction

Protein can not only provide important nutrients for human body, but also have an important impact on the basis and physiochemical properties of food. Since the production of animal protein sources gives a great pressure on the environment, plant proteins have been paid more and more attention recently. Compared to animal proteins, plant proteins have an advantage of high vield, low cost and large variety of species. The most popular vegetable proteins applied are oilseeds, cereals, and legumes [1]. Nowadays, soybean protein and peanut protein have been widely used in meat products and beverage industries. The meat products include meat patties, sausages, meatballs and so on [2]. Adding plant proteins into these meat products can not only increase the protein content, decrease the fat content of the products, but also decrease the production costs because plant proteins have a lower price than muscle proteins. The commonly used plant proteins in meat products is sovbean protein, such as soybean protein isolate and

soybean protein concentrate [3]. Except soybean protein and other popular plant proteins mentioned above, new plant proteins, such as flaxseed protein, cranberry seed protein, chia seed protein, pumpkin seed protein, hemp protein, and sunflower seed protein get more and more attention [4].

As an ingredient or additive in food processing, plant proteins need to satisfy some special functional properties. The functional properties, such as oil-absorbing ability, waterabsorbing ability, emulsifying ability, and foaming ability are the intrinsic physicochemical characteristics which have impact on the performance of protein in complex systems during manufacturing, storage, processing, preparation [5]. They are influenced by the composition, structure, and conformation of ingredient proteins. The typical functional properties will determine the applications of such proteins for developing new food products by industry. For example, plant proteins for sausagetype processed meats require good emulsification, plant proteins for doughs require good hydration and water-absorbing ability, plant proteins for whipped toppings require good foaming ability and color control is vital in many products, especially in breads. In many circumstances, several functional properties might be needed in a particular product at the same time [6]. For instance, meat products not only need good emulsification, but also need good waterabsorbing ability and good oil-absorbing ability [7]. Because different sources of proteins showed different functional properties, it is necessary to make a comparison of different kinds of protein isolates. In this study, the difference of the functional properties of PUPI, PEPI, SUPI, and SOPI, including oil-absorbing ability, waterabsorbing ability, emulsifying ability, emulsifying stability, foaming ability, and foaming stability was investigated. Traditional method of alkaline solution and acid precipitation was used to get PUPI, PEPI, SUPI, and SOPI from pumpkin seed meal, peanut meal, sunflower seed meal, and soybean meal. Although there are other methods, such as ethanol-water extraction, reverse micelle method, the method combining alkaline extraction with membrane separation and so on. However, the process of ethanol-water extraction consumes a great deal of organic solvent [8]. The process of preparation of reverse micelle is complicated and also involves the utilization of surfactants. And the equipment of the ultrafiltration and diafiltration membrane is hard to clean and expensive. Until now, the alkaline solution and acid precipitation is still effective to satisfy the demand of industrial large-scale production to achieve high yielding and purified protein like protein isolates [9]. Using the method of alkaline solution and acid precipitation, protein constituents are separated from non-protein constituents (sugar, fiber and anti-nutritional chemicals) by dissolving proteins in alkaline solution and then precipitating at isoelectric points. This paper describes the functional and technological properties of PUPI, PEPI, SUPI, and SOPI and their potential applications.

The aim of the study. The functional properties are very important for the applications of protein isolates to develop new food products by industry. The functional properties including waterabsorbing ability (WA), oil-absorbing ability (OA), wetting time (WT), emulsifying ability (EA), emulsifying stability (ES), foaming ability (FA), and foaming stability (FS) of peanut protein isolate (PEPI), pumpkin seed protein isolate (PUPI), sunflower seed protein isolate (SUPI), and soybean protein isolate (SOPI) were investigated

in order to have a better understanding of their potential applications in food processing.

Statement of the main material. Pumpkin seed meal is a by-product during the production of pumpkin seed oil. The protein content of pumpkin seed meal is more than 60 % and can be used in food industry. Pumpkin seed protein is mainly composed of four components: water-soluble albumin, salt-soluble globulin, alkali-soluble glutenin and alcohol-soluble proline [10]. It contains all essential amino acids for human body and can also provide children essential amino acid histidine. The amino acid pattern of pumpkin seed protein is similar to the essential amino acid pattern of human body [11] (Table 1). The pumpkin seed protein isolate obtained by Vinayashree et al is 92.59 % [11]. As a high-quality plant protein, pumpkin seed protein has great development potential and wide application prospect. However, pumpkin seeds are mainly used to produce healthy oil. The remaining pumpkin seed meal or cake has not been effectively used. Therefore, the development of pumpkin seed protein by pumpkin seed meal is benefit and necessary [12].

Soy product consumption has been associated with many potential health benefits in decreasing the risk of chronic diseases like obesity, cardiovascular disease, insulin-resistance/type II diabetes, certain type of cancers, and immune disorders. These physiological functions are due to soy proteins either as intact soy protein or more commonly as functional or bioactive peptides degraded from soybean processing [13]. Soybean protein is widely used in food processing and feed industry. The proportion of protein in soybean is about 40 %. The soybean protein isolate obtained by Hettiarachchy et al is 92% [14]. High lysine content and low methionine made soybean protein an ideal amino acid complementary food for cereal protein [15] (Table 1). The main storage proteins in soybean are 7 S globulin (βconglycinin) and 11 S globulin (glycinin), which account for 70 % of the total protein [16].

Peanut is one of the four most important oil crops in the world. Peanut has comprehensive nutrients composition and equilibrium content. The protein in peanut is about 25.6 %. Peanut protein consists of two globulins (arachin and conarachin) and a certain number of other proteins [17]. Peanut protein contains eight essential amino acid [18] (Table 1) and is easily digested and absorbed by human body, and its digestion coefficient can reach more than 90 %. The peanut protein obtained by Zheng et al is 89 % [18]. Compared with soybean protein, peanut

protein has the advantages of less flatulence factor and anti-nutrition factor [19]. Besides, the nitrogen solubility index of peanut protein is relatively high, and it can be easily added to various foods to improve quality and strengthen nutrition [20].

It is well known that the nutritional quality of edible oil of sunflower seeds ranks among the best vegetable oils in cultivation [21]. The sunflower

seed also contains many kinds of vitamins and minerals, including Ca, P, Fe, Zn, K and other beneficial mineral elements. The protein content of it is about 20 % \sim 28 %. It has complete amino acid and reasonable proportion [22]. Besides, sunflower seed protein is rich in aromatic amino acids (Table 1). It has been demonstrated that sunflower seed protein can significantly lower the depression symptoms [23].

Table 1.

Amino acid composition (mg/100 mg protein) of SOPI, PEPI, PUPI and SUPI. "-" Tryp was not analyzed. alkali solution and acid precipitation $=2TA0\times100c\times\Phi\times10000$ rpm/min). 50µL of emulsifying was taken from the 2 Foaming ability (%)= (volume after whopping-volume before whipping) ml Foaming ability (%)= $\frac{\text{(volume before whipping) ml}}{\text{(volume after standing for 30 min - volume before whipping)ml}}$ Foaming stability (%) = $\frac{\text{(volume after standing for 30 min - volume before whipping)ml}}{\text{(volume after whipping for 30 min - volume before whipping)ml}}$

The data were averages of triplicate observations and were subjected to one-way analysis of various (ANOVA), followed by Duncan's multiple range

Results and Discussion

In this study, four kinds of plant protein isolates, namely peanut protein isolate (PEPI),

pumpkin seed protein isolate (PUPI), soybean protein isolate (SOPI), and sunflower seed protein isolate (SUPI) were prepared from defatted peanut meal, defatted pumpkin seed meal, defatted soybean meal, and defatted sunflower seed meal by the method of alkali solution and acid precipitation, respectively. The functional properties including oil-absorbing ability (OA), water-absorbing ability (WA), wetting time (WT), emulsifying ability (EA), emulsifying stability (ES), foaming ability (FA), and foaming stability (FS) of

these four kinds of protein isolates were investigated and showed in Fig. 1–7.

Oil-absorbing ability indicates the amount of oil which per unit weight of the protein can be absorbed [27]. It can improve the absorption and retention ability of the food to fat, reduce the loss of fat in manufacturing operations, and then improve the palatability and flavor of food. In this study, the oil-absorbing ability of PEPI, PUPI, SOPI, and SUPI was determined. As shown in Fig. 1, the oil-absorbing ability (OA) of PUPI showed the highest value $(1.34 \pm 0.004 \,\mathrm{mL/g})$, which was 1.34 times more than PEPI $(1.00 \pm 0.04 \,\mathrm{mL/g})$ and SUPI $(1.00 \pm 0.03 \,\mathrm{mL/g})$, and 1.56 times more than SOPI $(0.86 \pm 0.05 \text{ mL/g})$. Although the OA of PUPI was significantly (p < 0.05) higher than other three protein isolates, there was no significant (p > 0.05)difference between the OA value of PEPI and SUPI (Fig. 1). The OA of PEPI and SUPI was significantly (p < 0.05) higher than that of SOPI (Fig. 1). Protein with high oil-holding ability could be applied in food industries for ground meat formulation, meat substitutes and extenders. According to these obtained data, it might be suggested that PUPI have more advantages to reduce the loss of fat in meat processing, reduce the fat intake in frying process and improve the taste of the products.

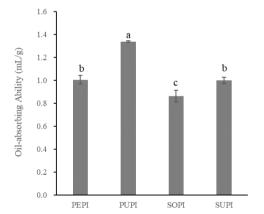


Fig. 1. The oil-absorbing ability of PEPI, PUPI, SOPI, and SUPI.

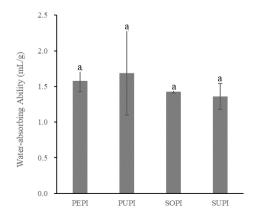


Fig. 2. The water-absorbing ability of PEPI, PUPI, SOPI, and SUPI.

Water-absorbing ability is an indication of the quantity of water, which a unit weight of the protein can absorb [27]. The high water-absorbing ability not only reduces the costs of the and helps on emulsification. As shown in Fig. 2, the water-absorbing ability (WA) of PEPI, PUPI, SOPI, and SUPI was 1.58 ± 0.15 mL/g, 1.69 ± 0.59 mL/g, 1.42 ± 0.02 mL/g, and 1.36 ± 0.18 mL/g, respectively. No significant difference (p > 0.05) was found in these four isolates, which might indicate that the water-absorbing ability of these four isolates has no significant (p > 0.05) difference during food processing.

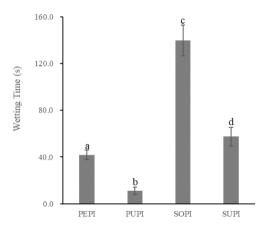


Fig. 3. The wetting time of PEPI, PUPI, SOPI, and SUPI.

Wetting time (WT) can describe the interaction between plant proteins and water. Wetting time was visually examined and corresponded to the time necessary to wet all the particles. Short wetting time indicates that the plant proteins can contact with water quickly and wetting quickly. As shown in Fig. 3, the wetting time (WT) of PUPI showed the lowest value ($11.02 \pm 3.13 \, \text{s}$), which was about 26.36% of PEPI ($41.80 \pm 4.05 \, \text{s}$), 19.19% of SUPI ($57.44 \pm 8.06 \, \text{s}$), and 7.87% of SOPI ($139.94 \pm 13.20 \, \text{s}$). The shortest wetting time of PUPI might indicate that when PUPI is used as a functional ingredient in food products, the wetting stage might take less time during food processing, when it is compared to PEPI, SOPI, and SUPI.

Foaming ability (FA) of protein not only depends on its own interfacial properties, such as the ability to reduce surface tension, the flexibility of molecular structure, amphiphilicity, charge distribution. and other physicochemical properties, but also on the ionic strength, pH, temperature, evaporation, the system, competitive adsorption of other proteins and so on [28]. In food industry, protein isolates are often used to help in the formation and stabilization of food foam. Some of the food systems where foam formation is crucial include beverages, cakes, and whipped toppings [29]. In this study, from Fig. 4, the highest foaming ability (FA) was PEPI (18.18 \pm 0.97 %), which was about 4.69 higher than PUPI (3.88 \pm 1.69 %), 2.34 higher (3.88 \pm 1.69 %) than SOPI (7.76 \pm 2.04 %), and 1.95 higher (3.88 \pm 1.69 %) than SUPI (9.33 \pm 1.39 %). However, there was no significant difference (p > 0.05) among the FA of PUPI, SOPI, and SUPI (Fig. 4). It might be suggested that PEPI have more potential to be added into desserts, such as cakes when it is compared to other three protein isolates.

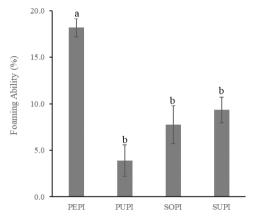


Fig. 4. The foaming ability of PEPI, PUPI, SOPI, and SUPI.

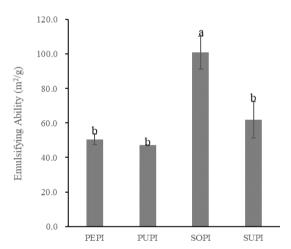


Fig. 6. The emulsifying ability of PEPI, PUPI, SOPI, and SUPI.

Emulsions are considered to be a dispersion in which small droplets are in a surrounding liquid phase. In the food industry, there are two types of emulsions. One is oil-in-water (O/W) mixtures, like milk and creams. The another is water- in-oil (W/O) mixtures, like margarine and butter [30]. Emulsions are formed in the presence of an emulsifier. Protein is a kind of emulsifier, which contains both of polar and non-polar amino acid residues. Emulsifying ability (EA) and emulsifying stability (ES) have been used widely to determine

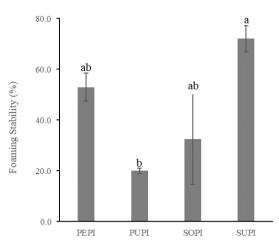


Fig. 5. The foaming stability of PEPI, PUPI, SOPI, and

Foaming stability is also an important property for cake and ice cream. As presented in Fig. 5, SUPI showed the highest foaming stability (FS) of 72.04 \pm 5.17 %, which was 1.36 higher than PEPI (52.96 \pm 5.50 %), 3.60 higher than PUPI (20.00 \pm 1.00 %), and 2.21 higher than SOPI (32.54 \pm 17.87 %). It might be suggested that the foaming stability of SUPI is better than PUPI when they are introduced in the production of the dessert or ice creams.

emulsifying properties. EA is defined as the quantity of oil emulsified by a certain unit of emulsifier at the emulsifying collapse point [31]. As shown in Fig. 6, the emulsifying ability of SOPI $(100.84 \pm 9.69 \, \text{m}^2/\text{g})$ was 1.99 higher than PEPI $(50.57 \pm 3.19 \, \text{m}^2/\text{g})$, 2.14 higher than PUPI $(47.08 \pm 0.01 \, \text{m}^2/\text{g})$, and 1.63 higher than SUPI $(61.82 \pm 10.59 \, \text{m}^2/\text{g})$. There was no significant (p > 0.05) difference among other three protein isolates (Fig. 6). In this study, SOPI showed the highest emulsifying ability among these four isolates. The emulsifying properties of soy protein have long been utilized as processing aids in the comminuted meat field.

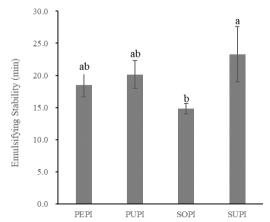


Fig. 7. The emulsifying stability of PEPI, PUPI, SOPI, and SUPI.

Emulsification is one of the important functional properties of protein, which plays a role in maintaining the stability of dispersed and continuous phases in food [32]. ES is the ability of the emulsifier to stabilize an oil-water mixture. Otherwise, it might result in phase separation. As we can see from Fig. 7, the emulsifying stability (ES) of SUPI showed the highest value (23.30 \pm 4.31 min), which was 1.26 times more than PEPI $(18.47 \pm 1.80 \text{ min})$, 1.16 times more than PUPI $(20.13 \pm 2.18 \, \text{min})$, and $1.57 \, \text{times more than SOPI}$ $(14.83 \pm 0.84 \text{ min})$. There was no significant (p > 0.05) difference among the ES of SOPI, PEPI, and PUPI. Although the emulsifying ability of SOPI was higher than that of SUPI, the ES of SUPI showed significant (p < 0.05) higher value than that of SOPI (Fig. 7). It might be suggested that SUPI have better property than SOPI to keep the stability of an oil-water mixture in the food processing such as ice creams.

Conclusions

In the present study, PEPI showed the highest foaming ability (FA) of 18.18 ± 0.97 %, while the foaming stability (FS) of PEPI (52.96 ± 5.50 %) was not significant (p > 0.05) compared to that of PUPI (20.00 \pm 1.00 %), SOPI (32.54 \pm 17.87 %), and SUPI (72.04 \pm 5.17 %). The PUPI showed the shortest wetting time (WT) of 11.02 ± 3.13 s and the highest oil-absorbing ability (OA) of 1.34 ± 0.004 mL/g. The SOPI showed the highest emulsifying ability (EA) of $50.57 \pm 3.19 \,\mathrm{m}^2/\mathrm{gwhile}$ its emulsifying stability (ES, 18.47 ± 1.80 min) was significantly (p < 0.05) lower than ES of SUPI $(23.30 \pm 4.31 \text{ min})$. These results will give a deeper understanding of the functional properties of PEPI, PUPI, SUPI, and SOPI and provides a theoretical basis for their applications. It is noteworthy that although not all functional properties of PUPI were the best. The shortest WT and highest OA of PUPI make it a potential additive in meat products. Because the price of plant proteins is lower than that of muscle proteins. Consequently, the application of PUPI can also reduce the cost of the meat products and increase the profit. Besides, the substances like chlorogenic and caffeic in SUPI significantly change the color of the isolates [33]. And these substances decrease the nutritional value of the isolates because their interaction with some amino acids such as lysine and methionine [33]. While PUPI does not contain chlorogenic acid, and therefore does not affect the organoleptic and nutritional quality when it is introduced into meat products.

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