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Research Article

Comparison the chemical components and functional properties of fish protein isolate from *Pangasius hypophthalmus* byproducts to other protein isolates

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Abstract

Research was undertaken on the chemical composition and foaming, emulsifying abilities of fish protein isolate (FPI) which was obtained by the hydrolysis of *Pangasius hypophthalmus* byproducts. These were then compared to commercial soy protein isolate (SPI) and commercial whey protein isolate (WPI). The results showed that the foaming ability of FPI was equivalent to WPI and higher than SPI. The highest foaming abilities of FPI, WPI, SPI were 94.61±1.03% (at pH=7); 96.42±1.12% (at pH=7); 80.54 ± 0.89% (at pH=8) respectively. Emulsifying abilities of FPI and SPI were equal and both reached the highest values at pH=7. The maximum emulsifying ability of FPI was 21.03±1.01 mL oil/g FPI while the highest one of SPI was 21.56±0.91 mL oil/g SPI. Emulsifying ability of WPI was lower than FPI, SPI. The protein component in FPI, SPI and WPI was higher than 90%. The very low lipid contents in FPI, WPI, SPI were 0.94±0.18%; 0.81±0.05%; 0.39±0.08% respectively. Moisture and ash contents of the FPI, WPI, SPI were 2.86±0.90% and 4.94±0.16%; 3.01±0.02% and 5.17±0.06%; 4.18±0.42 and 4.45±0.24% respectively.

Keywords: FPI, SPI, WPI, hydrolysis, foaming ability, emulsifying ability, striped catfish, Vietnam.

Introduction

Improving the functional properties of protein products (protein hydrolysates (PH); protein concentrates (PC); protein isolates (PI), including: solubility, water holding, oil holding, emulsifying, and foaming characteristics are a major challenge for food science [1]. The protein products may be used as food ingredients or food additives due to their functional properties [2]. The use of fish waste has been increasing interests in years. It is considered to be a safe, high-protein material with many nutritional

benefits [3]. FPI contains proteins with small molecular weight and can be called peptone from fish [4]. FPI functional properties, as well as biological activities depend on its origin and production methods [5]. *Pangasius hypophthalmus* Sauvage is a popular species in Vietnam for capture-based aquaculture as it is a prolific spawner and finds a ready market. For *Pangasius hypophthalmus* byproducts, under controlled conditions, enzymatic hydrolysis influences the molecular weight, hydrophobicity and polar groups of the proteins in final products [6]. The characteristics of the proteins in FPI directly affect its functional properties, such as emulsifying and foaming abilities [7, 8]. Both whey and soy proteins are by-products of the industry. The functional properties of WPI and SPI are mainly solubility, emulsifying, gelling and foaming abilities [9, 10]. Commercial SPI has been manufactured from defatted soy flakes by separation of the soy proteins from both the soluble and the insoluble carbohydrate fractions of the soybean. WPI is obtained by removing sufficient non-protein constituents from whey so that the finished dry product contains no less than 90% protein [11]. Fat from the whey is first removed by micro-filtration (MF) and then ultra-filtration (UF) or nano-filtration (NF). In addition to concentrating protein and fractionating whey into individual proteins, WPI can be subjected to controlled enzyme hydrolysis in order to yield smaller protein fragments [12].

There are numerous studies on the foaming, emulsifying properties of WPI [13, 14, 15]; SPI [16, 17, 18] and some studies on FPI [19, 20]. However, there are no comparative studies among these. Therefore, the objective of this study is to conduct a comparative examination for the foaming and emulsifying abilities, as well as chemical components of FPI to those of commercial WPI and SPI.

Materials and Methods

Materials

By-products (the spine and head) of *Pangasius hypophthalmus* were received from Can Tho Fish Joint Stock Company (CAFICO) in the Mekong River Delta, Vietnam. These were refrigerated, transported to the laboratory, divided into small units for each experiment and stored at - 20^0C until used.

Enzymes Alcalase 2.4L was purchased from EAC Co., Ltd. (sole-exclusive agent for Novozyme in Ho Chi Minh City, Vietnam).

Commercial SPI (SPI_4) was purchased from Prestige L.O. Limited (France). According to brochure Prestige L.O. Limited: SPI was obtained by removing soluble carbohydrates, defatted soy meal by using aqueous or alkali extraction of proteins at a pH range of 7-10; dispersion of the precipitate on alkaline medium (pH 8.0), further processing by ultra-filtration and freeze-dried to get SPI powder.

Commercial WPI was purchased from Labrada Nutrition (Toronto, Canada). According to brochure of Labrada Nutrition: WPI was obtained by removing sufficient non-protein constituents from whey; fat was first removed by cooling, then microfiltration and ultra-filtration or nano-filtration; free-dried to get WPI powder.

All chemical reagents used for the experiments were in analytical grade.

Methods

Hydrolysis process and collection FPI

Pangasius hypophthalmus by-products were hydrolyzed by protease (Alcalaze 2.4L) under controlled condition.

After hydrolysis, filtering was done to separate the solid and liquid, inactivating enzyme Alcalaze 2.4L by heat treatment at 90^0C/10 minutes, as per recommendation of Novozymes. Hydrolyzed solution was then cooled to 4^0C for a preliminary de-fatting, vacuum filtered through non-ash paper and then centrifuged to de-fat at the speed of 15,000 rpm for 20 minutes.

The solution obtained after centrifugation was brought to freeze-dry to get FPI powder. FPI powder is used to study the foaming and emulsifying abilities.

Chemical analysis of PI

The moisture and ash content were determined according to the AOAC standard methods 930.15 and 942.05 respectively**.** Total nitrogen content of FPI was determined by using the Kjeldahl method. Lipids were determined gravimetrically after Soxhlet extraction of dried samples with hexane. All measurements were performed in triplicate.

Determination of PI foaming ability

Pangasius hypophthalmus by-products were hydrolyzed by protease (Alcalaze 2.4L) under controlled conditions to get FPI with highest foaming ability as follows: enzyme/substrate (E/S) ratio of 0.2% (v/w); hydrolysis temperature is 64^0C; hydrolysis time is 92 minutes. After collecting the FPI powder, comparing foaming ability of FPI to the ones of WPI, SPI.

Foaming ability of PI was determined by the method of Tsumura [21]: 0.25 g FPI would be dissolved in 25 ml of distilled water. The mixture was adjusted to pH 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 by 0.5N NaOH or HCl. Then it was stirred by electric mixer to create foam system at room temperature. The sample after stirring was poured into the instrument (flash) for measuring both the total volume in foaming phase and the volume of separated water after 30 seconds. Foaming ability was calculated as follows:

$$FA(\%)= \frac{V_f - V_w}{V_i} * 100$$

Where: V_f: total volume in foaming phase; V_w: volume of separated water; V_i: volume of initial mixture.

Determination of PI emulsifying ability

Pangasius hypophthalmus by-products were hydrolyzed by protease (Alcalaze 2.4L) under controlled conditions to get FPI with highest emulsifying ability as follows: pH 7.4; E/S ratio is 0.19% (w/v), temperature at 62^0C, hydrolysis time is 80 minutes. After collecting the FPI powder, emulsifying ability of FPI was compared to that of WPI and SPI.

Emulsifying capacity of PI was measured as described by Rakesh and Metz [22], with some modification. One gram of each freeze-dried sample was transferred into a 250 mL beaker and dissolved in 50 mL of 0.5 N NaCl and then 50 mL of soybean pure oil was added. The solution was adjusted to pH of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 by 0.5N NaOH or HCl. Homogenizing the solution for 120 sec. at 10.000 rpm to make an emulsion. The mixture was transferred into centrifuge tubes, kept under a water-bath at 90^0C for 10 min and then centrifuged at 3000 rpm for 20 min. Emulsifying capacity was calculated using the equation:

$$EC \text{ (mL oil/g FPI)} = (V_A - V_R)/W_S$$

Where: V_A is the volume of oil added to form an emulsion; V_R is the volume of oil released after centrifugation; W_S is the weight of the sample.

Statistical analysis
All analytical determinations were carried out in triplicate and mean values with standard deviation (SD) are presented. Results were analyzed statistically by ANOVA using SPSS 15.0 to ascertain whether differences were significant at $p<0.05$.

Results and Discussion

Chemical composition of PI

The chemical composition of FPI from *Pangasius hypophthalmus*, commercial WPI and commercial SPI were determined. The results are shown in Table 1.

Table 1. Chemical composition(*) of the PI.

Source	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
FPI	90.14 ± 1.60^a	0.94 ± 0.18^a	2.86 ± 0.90^a	4.94 ± 0.16^a
Commercial WPI	90.74 ± 0.84^a	0.81 ± 0.05^a	3.01 ± 0.02^b	5.17 ± 0.06^b
Commercial SPI	90.08 ± 1.43^a	0.39 ± 0.08^b	4.18 ± 0.42^c	4.45 ± 0.24^c

Note. (*) Results reported are means of triplicate samples ± standard deviation. Values in the same column with different superscripts are significant different at $p<0.05$

Based on the results in Table 1, the protein content of all three PIs was higher than 90% and have no significant difference ($p <0.05$). Protein content in the aforementioned PIs is similar. High protein content reflected the quality of the PIs. Moisture and ash contents in WPI, FPI and SPI were different ($p <0.05$). The FPI moisture is lowest while SPI is highest. Ash content of WPI is highest and the lowest one belongs to SPI.

Regarding FPI from *Pangasius hypophthalmus*, the study results were found to be similar to the findings of other investigators who reported protein content ranging from 78% to 93% for lyophilized hydrolysate or FPI samples from *Pollachius virens* [23]; *Catla catla* [24]; Salmon [7] and Pacific whiting muscle [25]. Ash and moisture contents in FPI from *Pangasius hypophthalmus* byproducts were equal to ones of the FPI from Silver catfish (3.99% ÷ 5.61% and 3.33% ÷ 4.45% respectively) [26].

The fat content in all 3 types of PI was very low (all less than 1%). The SPI fat content was lowest in comparison with WPI, FPI. The fat content in WPI and FPI was similar and had no significant difference ($p <0.05$). Lipid content in FPI and WPI was higher than SPI because both WPI and FPI were derived from animals, while SPI is derived from vegetables [12]. The lipid in FPI was highest due to *Pangasius hypophthalmus* belonging to the fat catfish group. The lipid content in *Pangasius hypophthalmus* by-products was 32.21±1.89% [6]. In general, PI obtained from different production methods, the chemical compositions were almost similar but functional properties could be very different (these will be examined in the following section).

Emulsifying ability of PI

Based on the results presented in Figure 1, WPI emulsifying ability is lowest and there is significant difference ($p <0.05$) compared with emulsifying ability of SPI and FPI. Emulsifying abilities of FPI and SPI are similar and have no significant differences ($p <0.05$). Emulsifying ability of all WPI, SPI, FPI have reached the lowest value at lightly acid pH (pH=5). The highest emulsifying capabilities of SPI, FPI and WPI were obtained at pH=7.0. The maximum emulsifying ability of WPI compared with the

maximum of FPI, SPI was only 83.95% and 86.07% respectively. At pH values which were lower than 7.0, the emulsifying ability of PI was low. In contrast, at the pH values of 7.0 or higher, emulsifying ability of Ps achieved maximum values and then declined slightly.

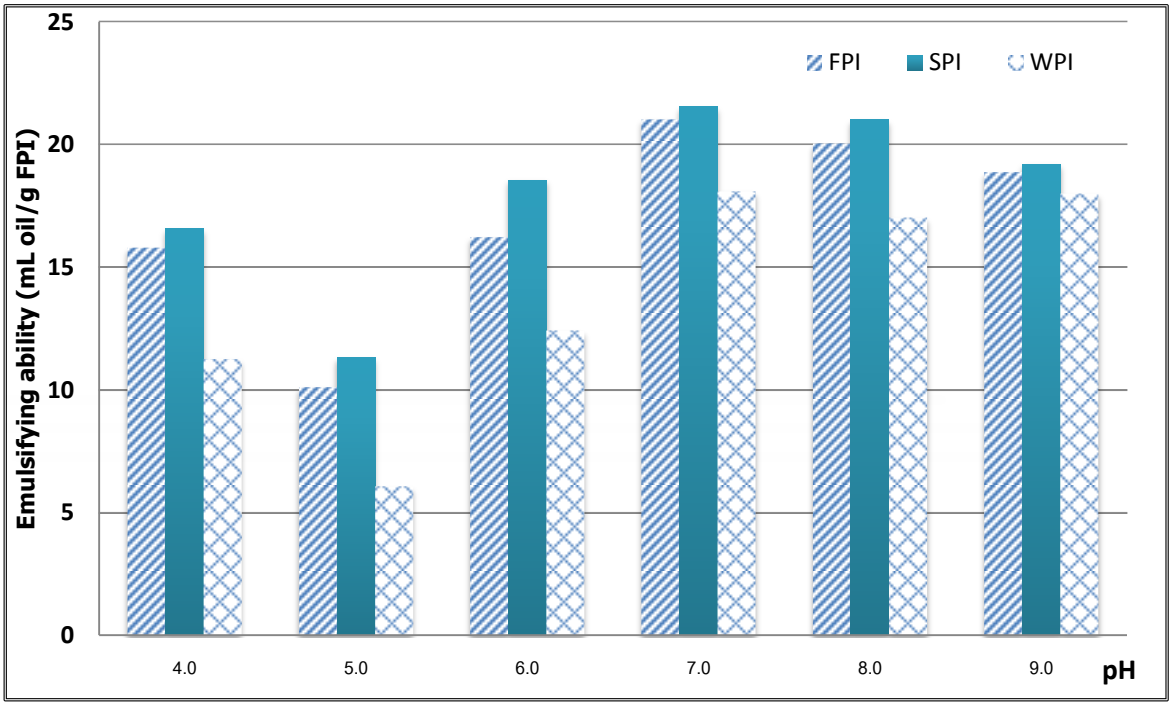

Figure 1. The emulsifying ability of WPI, FPI, SPI.

This is explained as follows: a significant increase in emulsifying capacity of PI at pH=7.0 may be due to higher quantities of soluble proteins in PI [27]. The pH also affects emulsifying properties by changing the solubility and surface hydrophobicity of proteins, as well as the charge of the protective layer surrounding the lipid globules. Ions alter the electrostatic interactions, conformation, solubility of the proteins and hydrophilic - lipophilic balance [28]. WPI emulsifying ability was lower than FPI and SPI because as the number of medium and small size proteins (7-20 kDa) in FPI and SPI are similar and accounted for 65 - 75% of their protein content, while this WPI size of protein group was only 35.8%. This group of proteins has an important role in forming the emulsifying ability of PIs [1, 29, 30]. On the other hand, environmental pH also affects emulsifying properties by changing the solubility and surface hydrophobicity of proteins, as well as the charge of the protective layer surrounding the lipid globules. Ions alter the electrostatic interactions, conformation, solubility of the proteins and hydrophilic-lipophilic balance [28]. At high pH values, the emulsifying ability of PI increased because at these pH values, small and medium polypeptides (from 7÷20 kDa) can be unfolded due to negative charges. Repulsion could result from this change, allowing for better orientation at the interface [25]. This could result in a more efficient exposure of hydrophilic and hydrophobic residues in these peptides, promoting a major interaction at the oil-water (O:W) interface. Since the lowest solubility occurred at pH 4.0, 5.0 and 6.0, peptides could not move rapidly to the interface. Additionally, the net charge of the peptide will be minimized at these pH values. So the emulsifying ability of PI decreased.

The results from this study are similar to what has been reported from Taheri [31] regarding emulsifying ability of FPI from rainbow trout (*Onchorhynchus mykiss*) viscera; Klompong *et al*., [32] about emulsifying ability of FPI from yellow-striped trevally; Foh, *et al* [33] about emulsifying ability of FPI from Tilapia *(Oreochromis niloticus)*; Alexandra, R.T. [12] about emulsifying ability of WPI, SPI.

Foaming capability of PI

The foaming properties of the three PIs were determined at pH values of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The foaming capacity is shown in Figure 2.

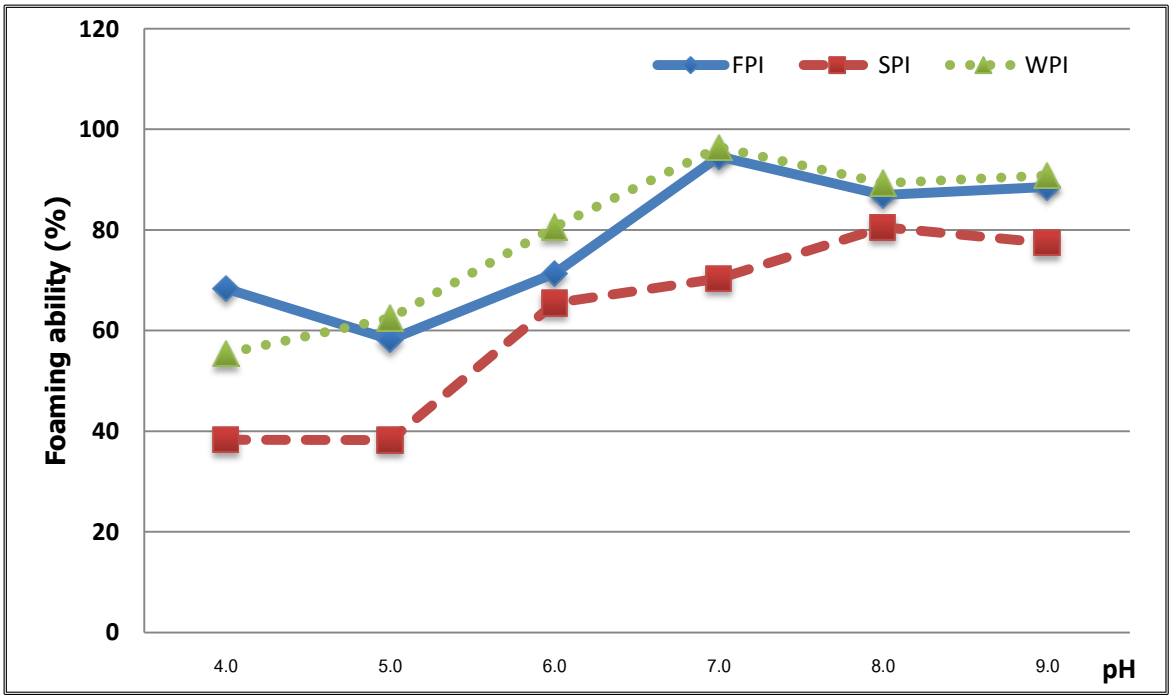

Figure 2. The foaming ability of WPI, FPI, SPI.

The foaming capacity of FPI, WPI and SPI ranged between 58.21±0.78% ÷ 94.61 ± 1.03%; 55.46%±0.09 ÷ 96.42±1.12%; and 38.26±0.47% ÷ 80.54±0.98%, respectively. The foaming capacities of all three types of PI have reached the lowest value at pH of 4.0 ÷ 5.0; when pH increases, the foaming ability of WPI, SPI, FPI tends to increase up to a maximum at pH of 7.0 ÷ 8.0. At neutral to alkaline pH, the foaming ability of FPI, SPI and WPI was higher than that at light acid pH. Foaming ability of WPI and FPI was similar and much higher than SPI. The highest foaming ability of WPI, FPI reached at pH=7.0 and had no statistical significance ($p < 0.05$). The highest foaming ability of SPI was only 83.50% and 85.13% compared with WPI and FPI respectively.

This is explained as follows: Foaming ability is related to decreasing rate of the surface tension of the air/water interface caused by absorption of protein molecules [34]. Good foaming ability was linked with flexible protein molecules, which reduces surface tension. Low foaming ability on the other hand can be related to highly ordered globular proteins, which resists surface denaturation. The basic requirements of proteins as good foaming agents are the ability to: (1) absorb the proteins rapidly at air water interface during bubbling, (2) undergo rapidly conformational change and rearrangement at the interface and (3) form a cohesive viscoelastic film via intermolecular interactions. The first two factors are essential for better foaming ability whereas the third is important for the stability of the foam [12, 34, 35, 36]. Although the total protein in WPI, FPI and SPI is similar; the ratio of proteins in the same molecular weight groups is different. This will affect the foaming ability of each PI [27, 33]. It should be noted that the adsorption rate to the air-water interface may be influenced by the molecular size, protein structure and hydrophobicity of the hydrolysates [37]. These are highly dependent on both producing methods of PIs and the parent protein from which they are obtained, as well as the hydrolysis procedure. The hydrolysis of protein produces a range of peptides that possess altered hydrophobicity, net charge and conformation in comparison to the native molecule. Their reduced molecular weight

makes them more flexible, form a stable interfacial layer and increase the rate of diffusion to the interface, which in turn improves foaming ability [38]. The foaming ability of all WPI, FPI and SPI are low at low pH due to the lowest foaming ability being attributed to the protein behaviour around its isoelectric point. At high pH, it was likely due to the increased net charges on the protein, which weakened the hydrophobic interactions but increased the flexibility of the protein. This allowed the protein to diffuse more rapidly to the air-water interface to encapsulate air particles and then enhance the foam formation [39].

The results of this study were equivalent to the previous ones that have been reported. SPI foaming ability is less than foaming ability of WPI and SPI [1, 29]. Foaming ability of FPI from Tilapia *(Oreochromis niloticus)* could reach 89.63% while SPI is only 71.52% at pH 7.0 ÷ 8.0 [33]. The highest foaming ability of SPI from defatted soy is about 80% [30]. The highest foaming ability of FPI from Sardine *(Sardinella aurita)* reached 92.8% at pH=7 [40].

Conclusion

Protein isolate obtained from enzymatic hydrolysis of *Pangasius hypophthalmus* byproducts has relatively good abilities of foaming and emulsifying. FPI foaming ability was equivalent to WPI and higher than that of SPI. Emulsifying ability of FPI from *Pangasius hypophthalmus* byproducts was equivalent to SPI one and higher than WPI. Highest foaming ability of FPI from *Pangasius hypophthalmus* byproducts and WPI were 94.61±1.03% and 96.42±1.12% (at pH=7.0) respectively. The lowest FPI foaming ability was 58.21±0.78% (at pH=5.0) while WPI was 55.46±0.09 (at pH=4.0). The highest emulsifying ability of FPI was 21.03±1.01mL oil/g FPI (at pH=7) and SPI was 21.56±0.91mL oil/g SPI (at pH 7). The lowest emulsifying ability of FPI and SPI (both at pH=5) are 10.11±0.26 mL oil/g FPI and 11.32±0.62 mL oil/g SPI respectively. The protein content in FPI, SPI and WPI was similar and over 90%. Fat content in FPI, WPI and SPI was 0.94±0.18%; 0.81±0.05% and 0.39±0.08% respectively. This was a very low amount of fat (less than 1%). Other chemical compositions, such as moisture and ash content of the FPI, WPI, SPI were 2.86±0.90% and 4.94±0.16%; 3.01±0.02% and 5.17±0.06%; 4.18±0.42 and 4.45±0.24% respectively.

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