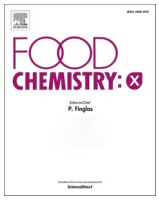
Food Chemistry: X 21 (2024) 101179

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Tilapia-soybean protein co-precipitates: Focus on physicochemical properties, nutritional quality, and proteomics profile

Qingguan Liu [a,b,c,d,e], Li Tan [a,b,c,d,e], Pengzhi Hong [a,b,c,d,e], Huanming Liu [a,b,c,d,e], Chunxia Zhou [a,b,c,d,e,*]

[a] College of Food Science and Technology, Guangdong Ocean University, Zhanjiang 524088, China
[b] Guangdong Provincial Key Laboratory of Aquatic Product Processing and Safety, Zhanjiang 524088, China
[c] Guangdong Provincial Engineering Technology Research Center of Marine Food, Zhanjiang 524088, China
[d] Guangdong Provincial Modern Agricultural Science and Technology Innovation Center, Zhanjiang 524088, China
[e] Southern Marine Science and Engineering Guangdong Laboratory (Zhanjiang), Zhanjiang 524088, China

ARTICLE INFO

Keywords:
Tilapia protein
Soybean protein
Protein co-precipitates
Physicochemical properties
Nutritional quality
Proteomic analysis

ABSTRACT

The development of binary protein systems featuring superior nutritional properties and applied range is an interesting and challenging task in the food industry. In this study, the tilapia-soybean protein co-precipitates (TSPCs) with different mass ratios of tilapia meat and soybean meal were constructed. Results of physicochemical properties showed that the highest solubility and thermal stability values of TSPCs were 81.90 % and 90.30 °C, respectively. TSPCs have the full complement of amino acids and enhanced nutritional quality compared to tilapia protein isolate (TPI) and soybean protein isolate (SPI). $TSPC_{2:1}$ and $TSPC_{1:1}$ contained the highest levels of tryptophan, aspartic acid, glycine, histidine, and arginine relative to TPI and SPI. The *in vitro* protein digestibility and protein digestibility corrected amino acid scores of TSPCs were also higher than that of SPI. SDS-PAGE revealed that TSPCs contained protein subunits from TPI and SPI. Moreover, the lysine-to-arginine ratio and β subunit were greatly correlated with protein digestibility with correlation coefficients of −0.962 ($P < 0.01$) and −0.971 ($P < 0.01$), respectively. Compared to SPI, TSPCs displayed a lower lysine-to-arginine ratio and β-conglycinin content, which improved its digestibility. Proteomic analysis indicated that $TSPC_{1:1}$ had 989 unique proteins, which gives TSPCs enhanced biological properties compared to TPI and SPI, allowing them to participate in a broad range of biochemical metabolic and signal transduction pathways. The study would advance the utilization of mixed proteins toward exceptional food industry applications.

1. Introduction

Proteins are crucial macronutrients and a vital structural component in many foods. They provide the necessary amino acids for human growth, development, and protein synthesis, including hormones, enzymes, and antibodies (Lv, Zhao, & Ning, 2017). Protein-based ingredients play various technological roles in formulated foods and contribute to food texture, flavor, and other consumer-related properties (Loveday, 2019). However, proteins are heterogeneous in composition, structure, and other characteristics. The application value of food proteins is dependent on their source and processing.

Mixed protein systems from different sources have gained attention due to their improved nutrition and functional properties. The combination of animal and plant proteins has proven effective in improving individual protein properties and addressing environmental and resource scarcity issues (Alves & Tavares, 2019). Tilapia is a widely-cultured fish in Africa and Asia, while soybean is the largest source of plant proteins. Tilapia and soybean proteins are used in several food products due to their high nutritional value, low price, and potential for human consumption (Shaheen et al., 2016; Kang et al., 2022). Mixing tilapia and soybean proteins could be a nutritionally favorable combination due to their different levels of sulfur amino acids and aromatic amino acids (Shaheen et al., 2016; Tan, Tan, & Tan, 2023). The formulation of products containing tilapia and soybean proteins can take advantage of the differences in their essential and non-essential amino acids to design products with unique nutrition and molecular structure. As consumer demand for aquatic foods increasing, mixed aquatic-plant proteins have caught the attention of functional food manufacturers

* Corresponding author at: College of Food Science and Technology, Guangdong Ocean University, Zhanjiang 524088, China.
E-mail address: chunxia.zhou@163.com (C. Zhou).

https://doi.org/10.1016/j.fochx.2024.101179
Received 5 September 2023; Received in revised form 8 January 2024; Accepted 1 February 2024
Available online 5 February 2024

(Tahergorabi et al., 2015; Lin et al., 2017). Therefore, the mixing of tilapia and soybean proteins warrants further investigation.

Co-precipitation is one of the most common methods for mixed proteins (Alu'datt et al., 2013), which can be an effective strategy for blending tilapia fish protein and soybean protein. Previous studies have confirmed that whey-soy protein co-precipitates contain protein components of whey protein and soybean protein (Alu'datt, Alli, & Nagadi, 2012). Protein co-precipitates with the potential to improve the nutrition and functional properties of original raw proteins. Comparative studies have found that the nutritional value of protein co-precipitates is higher than individual protein isolates due to the altering of amino acid compositions (Thompson, 1977; Youssef, Abu-Foul, & Moharram, 1995). The soybean-wheat co-precipitated protein (SWCP) with improved gelation properties than pure proteins (Tian et al., 2022a). In another report, the emulsification, foaming, and nutritional properties of soy-wheat co-precipitated protein were enhanced compared to pure protein (Tian et al., 2022b). Incorporation of protein co-precipitates can also enhance the sensory acceptability of many food products. Beef patties extended with milk co-precipitates have the best appearance, flavor, and texture and are generally the most acceptable (Thomas, Mcbride, Turner, & Abad, 1978). The low-fat ground pork patties complexed with 7 % milk co-precipitate resulted enhanced microbiological properties and refrigerated storage stability (Kumar & Sharma, 2003). Overall, a wide range of nutrition, function, and sensory properties allows protein co-precipitates to be used as an ingredient in many food systems by contributing necessary processing properties.

The nutritional quality of protein is often a key factor for evaluating food quality, especially during the production of a formulated product (Swieca et al., 2019; Harshani et al., 2021). Usually, the nutritional quality of food protein is primarily based on protein content, amino acid composition, and digestibility (Loveday, 2019; Kowalczewski et al., 2019). The amino acid score and protein digestibility corrected amino acid score are important factors for assessing protein quality, which can directly reflect the utilization of protein by the body (University & Organization, 2007). In addition, studying the molecular structure of food proteins leads to understanding the components that make up a whole protein, which is often vital to understanding its nutritional value (Yu, Mckinnon, Christensen, & Christensen, 2004). In a mixed protein system, structural diversity and amphiphilicity of food proteins allow them to interact with other proteins in food products under certain conditions (Alves & Tavares, 2019). Thus, the extent of structural changes in these proteins during food processing may modulate these interactions and dramatically impact the nutritional quality of food.

In recent years, there have been increasing numbers of studies on the correlation between protein nutrition and protein structure. The effect of protein structure on nutritional value may be due to differences in molecular structure, resulting in different sites of action with digestive enzymes, thereby releasing differing kinds and quantities of oligopeptides and amino acids (Yu, 2007; Gomaa et al., 2018). However, although changes in molecular structure will affect the nutritional quality of proteins, the role of amino acids in this process remains unclear. Existing studies have confirmed that amino acid composition may be the main modulator responsible for the physiological function of proteins, which may affect their digestibility (Yang et al., 2012). At the same time, protein co-precipitates formed by the interaction of different proteins may lead to the generation of new proteins, which may have potential biological functions. Usually, these unique proteins can be identified using proteomics techniques. The biological functions of unique proteins can be analyzed through Gene Ontology (GO), and the metabolic and signal transduction pathways can be analyzed through the Encyclopedia of Genes and Genomes (KEGG) (Zhao, Sun, Liu, Cheng, Wang & Guo, 2022).

Based on our previous study, TSPCs were developed, and the structure–function relationship of TSPCs has been discussed (Tan et al., 2019). However, there is a lack of in-depth research on the physicochemical properties, nutritional quality and correlation with protein structure, and proteomics profile. Therefore, this study aims to prepare TSPCs with different mass ratios of tilapia-soybean protein and further research its physicochemical properties, nutritional quality, and proteomics profile. This study will provide a theoretical basis for promoting the application of TSPCs in the food industry.

2. Materials and methods

2.1. Materials

Fresh Nile tilapias (Oreochromis niloticus) were slaughtered and eviscerated in room temperature at the local market (Zhanjiang, Guangdong province, China) by market personnel to collect fish muscle. Fresh fish muscle with a protein content of 17.73 % (nitrogen × 6.25, wet) was placed in an incubator with ice packs and promptly sent to the laboratory for immediate use or frozen at −20 °C for further use. Soybean meal, with a protein content of 53.31 % (nitrogen × 6.25, wet), was purchased from Shandong Wandefu Biotechnology Co., Ltd. (Dongying, China). Pepsin (1:15000 USP units/mg of protease) and pancreatin (300 USP units/mg of protease) were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Other chemicals and reagents used were of analytical grade.

2.2. Preparation of tilapia-soybean protein co-precipitates (TSPCs)

TSPCs were prepared according to our previous study (Tan et al., 2019). Briefly, tilapia meat was mixed with soybean meal in different mass ratios (3:1, 2:1, 1:1, 1:2, and 1:3, w/w) and then mixed with cold deionized water (1:9, w/v). The pH of the mixtures was adjusted to 11.0 using 2.0 M NaOH, followed by stirring at 600 rpm for 30 min using a magnetic stirrer (IKA R05, USA). The supernatant was collected after centrifuging (10,000*g*, 4 °C) for 20 min. The pH of the supernatant was adjusted to 4.5 by 2.0 M HCl and subjected to centrifugation (10,000*g*, 4 °C, 20 min) for precipitate collection. The precipitate was adjusted to pH 7.0 and dialyzed in cold deionized water for 48 h. The dialysate was Freeze-dried to obtain TSPCs with different mass ratios of tilapia meat and soybean meal ($TSPC_{3:1}$, $TSPC_{2:1}$, $TSPC_{1:1}$, $TSPC_{1:2}$, and $TSPC_{1:3}$). The tilapia protein isolate (TPI) from tilapia meat was prepared as previous study (Foh, Xia, Amadou, & Jiang, 2011) with slight modification, which is that the pH of alkaline dissolution and acidic precipitation was 11.0 and 5.5, respectively. The soybean protein isolate (SPI) from soybean meal was prepared as described by Yang et al. (2014) with some modification, which is that the pH of alkaline dissolution and acidic precipitation was 8.0 and 4.5, respectively. All protein samples were stored at −20 °C for further use. In addition, the protein content of all protein samples was analyzed by the fully automatic Kjeldahl nitrogen analyzer (VADODEST 450, Gerhardt, Germany). The batch replication has been conducted during all steps of TSPCs preparation.

2.3. Physicochemical properties of tilapia-soybean protein co-precipitates

2.3.1. Analysis of basic components

The basic components of tilapia-soybean protein co-precipitates, including crude protein, crude fat, moisture, and ash content, were determined by referring to the method of Ban et al. (2020) with some modifications. The content of crude protein was determined via the Kjeldahl method, using a conversion factor of 6.38. The content of crude fat was analyzed using the Soxhlet method. The contentof moisture was determined by an rapid moisture analyzer (HC103, METTLER TOLEDO, Switzerland). The content of ash was determined by dry-ashing using a muffle furnace.

2.3.2. Determination of solubility

Protein solubility was determined using a modified version of previously described methods (Foh et al., 2011). Protein samples (300 mg) were dispersed in 30 mL of distilled water and stirred for 30 min at room

temperature. The pH of the mixture was adjusted to 7.0 and centrifuged at 10,000*g* for 20 min. The protein content of the clear supernatant was determined by the Kjeldahl method, using a conversion factor of 6.25. Solubility was calculated using the following equation:

$$\text{Protein solubility}(\%) = \frac{Supernatantproteincontent}{Proteincontentbeforecentrifugation} \times 100 \quad (1)$$

2.3.3. Determination of thermal stability

The denaturation temperature of the protein co-precipitates was evaluated by a synchronous thermal analyzer (STA449F3, Netz, Germany). Samples (8 mg) were sealed in an aluminum pan and heated from 30 °C to 150 °C with a rate of 10 °C/min and a nitrogen purge rate of 50 mL/min. The peak temperature value represents thermal stability.

2.4. Nutritional properties of tilapia-soybean protein co-precipitates

2.4.1. Determination of amino acid

Sixteen amino acids and tryptophan were quantified according to the method of Horwitz and Latimer (2005). Protein samples underwent acid digestion with 6 M HCl at 110 °C for 24 h. Each hydrolysate was transferred into a 50 mL volumetric flask and diluted with distilled water. Sixteen amino acid content was analyzed using a fully Automatic Amino Acid Analyzer (S-443D, Sykam, Germany) after precolumn derivatization with o-phthaldialdehyde (OPA). Furthermore, the sample was subjected to alkaline hydrolysis with 5 M NaOH at 110 °C for 22h, and tryptophan was analyzed using Liquid Chromatography (1260, Agilent, USA). The amino acid content was expressed as g of amino acid/100 g of protein.

2.4.2. Determination of in vitro protein digestibility (IVPD)

The IVPD was determined using a modified version of previously described methods (Vilela, Lands, Chan, Azadi, & Kubow, 2006; Kaspchak, Mafra, & Mafra, 2018). Briefly, gastric digestion was completed using pepsin (3.33 mg/mL) and protein (3.0 mg/mL) at pH 2.0 (adjusted with 1.0 M HCl). The system was stirred for 1.5h at 37 °C using a thermostatic bath oscillation (HHS, Shanghai Boxun Industrial Co., Ltd., Shanghai, China) and then neutralized to pH 7.0 using 1.0 M NaOH. For intestinal conditions, a pancreatin solution (pH 7.0, 0.1 M phosphate buffer) was added to the gastric digestion solution to obtain a final concentration of 0.5 mg/mL. The mixture was stirred for 3.0 h at 37 °C. Sodium carbonate was added to the mixture at 5.0 mg/mL to obtain pH 10.0 for terminating reaction. Protein quantification during the gastrointestinal digestion process was based on the Bradford method (Bradford, 1976). The IVPD values were calculated using the following equation:

$$\text{IVPD}(\%) = \frac{Digestedproteincontent}{Totalproteincontent(3.0mg/mL)} \times 100 \quad (2)$$

2.4.3. Calculation of amino acid score (AAS) and protein digestibility corrected amino acid score (PDCAAS)

The AAS was calculated by dividing the amount of essential amino acid by the reference pattern of amino acid requirements for non-athletic individuals, as established by the University and Organization (2007). The amino acid with the lowest score was identified as the limiting amino acid (LAA). The PDCAAS was calculated using the amino acid score and *in vitro* protein digestibility of protein samples. AAS and PDCAAS were calculated using Eqs. (3) and (4):

$$\text{AAS} = \frac{\text{Content of essential amino acid in sample}}{\text{Content of essential amino acid in reference protein}} \quad (3)$$

$$\text{PDCAAS}(\%) = \text{AAS of LAA} \times \text{in vitro protein digestibility} \quad (4)$$

Where the reference protein was the WHO/FAO/UNN adult amino acid requirements.

2.4.4. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE was performed using a modified version of previously described methods (Alu'datt et al., 2012). Freeze-dried protein samples were formulated into the same concentration of protein solution and heated (95 °C, 5-10 min) in sample buffer (10% SDS, 0.5 M β-mercaptoethanol, 0.5 M Tris-HCl, pH 6.8, 2% glycerol, and 0.1% bromophenol blue). The acrylamide content for the stacking and resolving gels was 5 % and 12 %, respectively. A series of standard protein markers (16.0-270 kDa, Beyotime Biotechnology, Shanghai, China) was used in the electrophoresis to aid in the identification of protein bands in the samples. Electrophoresis was carried out on a vertical gel electrophoresis unit (Mini-Protean II; Bio-Rad Laboratories, Richmond, CA) at 15 mA, and bands were stained with Coomassie Brilliant Blue R-250. Destaining was performed using 40% methanol and 10% acetic acid. Densitometric analysis was performed on all bands on the SDS-PAGE gels using the Gel Imaging System (GelDoc XR+, Bio-Rad, USA).

2.5. Proteomic analysis of tilapia-soybean protein co-precipitates

2.5.1. Proteolysis of samples

Briefly, 100 μg of protein was added to a 1.5 mL centrifuge tube. Then, add the enzyme solution with a ratio of Trypsin (μg) to substrate protein of 1:20 (w/w). After vortexing, the mixture was incubated at 37 °C for 2 h. Next, the digested peptide solution was subjected to desalting. Freeze-dry the desalted peptide solution and store it until analysis.

2.5.2. Separation by liquid chromatographic

The peptide samples were dissolved in mobile phase A (2 % ACN, 0.1 % FA) and centrifuged at 20,000*g* for 10 min. The resulting supernatant was injected into the sample and separated by liquid chromatography. The sample was first enriched and desalted by a trap column, then connected in series with a C18 column (75 μm inner diameter, 3 μm particle size, 25 μm column length) at a flow rate of 300 nL/min for liquid chromatography separation. The following gradient was used: 0–5 min, 5 % mobile phase B (98 % ACN, 0.1 % FA); 5–45 min, mobile phase B linearly increased from 5 % to 25 %; 45–50 min, mobile phase B increased from 25 % to 35 %; 50–52 min, mobile phase B increased from 35 % to 80 %; 52–54 min, 80 % mobile phase B; 54–60 min, 5 % mobile phase B. Finally, the liquid phase was connected directly to the mass spectrometer for subsequent experiments.

2.5.3. Mass spectrometry analysis

After separation by liquid chromatography, the peptides were ionized by the nanoESI source and enter the Q-Exactive HF tandem mass spectrometer for DDA (data-dependent acquisition) mode to detect the full spectrum of proteins. The main parameter settings were as follows: the ion source voltage was set to 1.9 kV; the primary mass spectrometer scans at the range of 350–1600 *m/z* with a resolution of 60,000; the secondary mass spectrometer with an initial *m/z* fixed at 100 and a resolution of 15,000. Parent ions for secondary fragmentation were screened based on charge (2+ to 6+), peak intensity (>10,000), and ranking in the top 30. Ion fragmentation was performed using HCD, and fragment ions were detected by Orbitrap. The dynamic exclusion time was set to 30 s. The AGC settings were 3E6 for the first level and 1E5 for the second level.

2.5.4. Bioinformatics analysis

Proteomics analysis involves converting raw mass spectrum data into mass spectrum peak files, which are then searched for matches with sequences in the database. In this study, all original mass spectrometry data were first converted into MGF format using Proteome Discoverer in the Thermo Scientific tool. The converted MGF file was then analyzed using Mascot 2.3.02 (Matrix Science, https://www.matrixscience.com/) to search the UniProt (https://www.uniprot.org/) protein sequence

database and obtain the final protein identification results. The results generated by the search engine were preprocessed with Percolator and re-scored to improve the accuracy of correct matching and random matching. Finally, GO and KEGG annotation analyses were performed based on the credible protein identification results.

2.6. Statistical analysis

All experimental results were measured in triplicate or more. Statistical differences were analyzed using SPSS (V19.0, SPSS Inc., Chicago, USA) following a one-way analysis of variance (ANOVA) and Duncan's Multiple Range tests ($P < 0.05$). The Pearson correlation method was used for correlation analysis based on the normality test results. The experimental results were expressed as mean ± standard deviation (Mean ± SD) unless otherwise specified.

3. Results and discussion

3.1. Physicochemical properties of tilapia-soybean protein co-precipitates

3.1.1. Basic composition

The basic components of TPI, TSPC with different mass ratios of TPI and SPI, and SPI were showed in Table S1. The crude protein content of TPI, TSPC, and SPI was more than 90 %, indicating high purity. The crude protein content of TSPC was found to be between that of TPI and SPI, which is consistent with previous research on protein co-precipitats (Youssef, Abu-Foul, & Moharram, 1995). Foods with high protein content can better meet the human body's amino acid requirements, and the high protein content of TSPC suggests its potential as a base material for food. TPI had a slightly higher crude fat content than SPI, while TSPC was between TPI and SPI in terms of ash content. The moisture content of all protein samples was less than 1 %, indicating that the storage conditions were suitable for research purposes. Moreover, TSPC can be easily prepared through a simple alkali dissolution-isoelectric precipitation process, which suggests its potential for widespread use in the food industry.

3.1.2. The solubility of protein co-precipitates

Protein solubility is a critical index that can affect many functional properties of proteins for use in beverages, formula and as an indicator of protein changes during food processing (Zhu et al., 2010). Therefore, the solubility of TSPCs obtained through alkaline extraction and isoelectric precipitation at pH 7.0 was studied. As shown in Fig. 1A, the solubility of TSPCs was 40 % higher than that of TPI. The highest and lowest solubility of TSPCs were 81.90 % of $TSPC_{1:3}$ and 52.25 % of $TSPC_{3:1}$, respectively. These results suggest that the extraction method of protein co-precipitates altered the solubility of the raw material proteins. Furthermore, protein solubility is correlated with amino acid composition (Niu et al., 2012). There was no significant increase in solubility between $TSPC_{1:1}$ and $TSPC_{2:1}$, which may be due to the similar amino acid content of aspartic acid and glutamic acid. Similarly, $TSPC_{1:3}$ and $TSPC_{1:2}$ did not show significant increases in solubility, which may be due to the similar amino acid content of lysine and histidine. These results are consistent with our previous research, which found no significant difference in solubility between $TSPC_{1:1}$ and $TSPC_{2:1}$ at pH 7.0 due to the influence of protein molecule structure (Tan et al., 2019). Overall, the higher solubility of TSPCs suggests their potential for use in the food and beverage industry.

3.1.3. The thermal stability of protein co-precipitates

Thermal properties can reflect the chemical composition and structure of protein polymers. Fig. 1B shows the change in the thermal denaturation temperature of TSPCs. The thermal denaturation temperature of TPI and SPI is 76.63 °C and 91.63 °C, respectively. TSPCs exhibit higher thermal stability than TPI, with the thermal denaturation temperature significantly increasing from 84.63 °C for $TSPC_{3:1}$ to 90.30 °C for $TSPC_{1:3}$. These results suggested that the thermal stability of TSPCs was improved due to the introduction of soybean protein. Some researchers have reported that the thermal stability of proteins can be influenced by their amino acid composition, with methionine having a disadvantageous effect (Yokot et al., 2006). The reduced content of methionine in TSPCs compared to TPI may have contributed to their improved thermal stability. Moreover, studying the solid-state behavior of TSPCs can help us better understand their stability during storage and predict their thermal behavior in various food processing systems, such as pasteurization, for potential applications in pasta (Goes et al., 2016) and other food products.

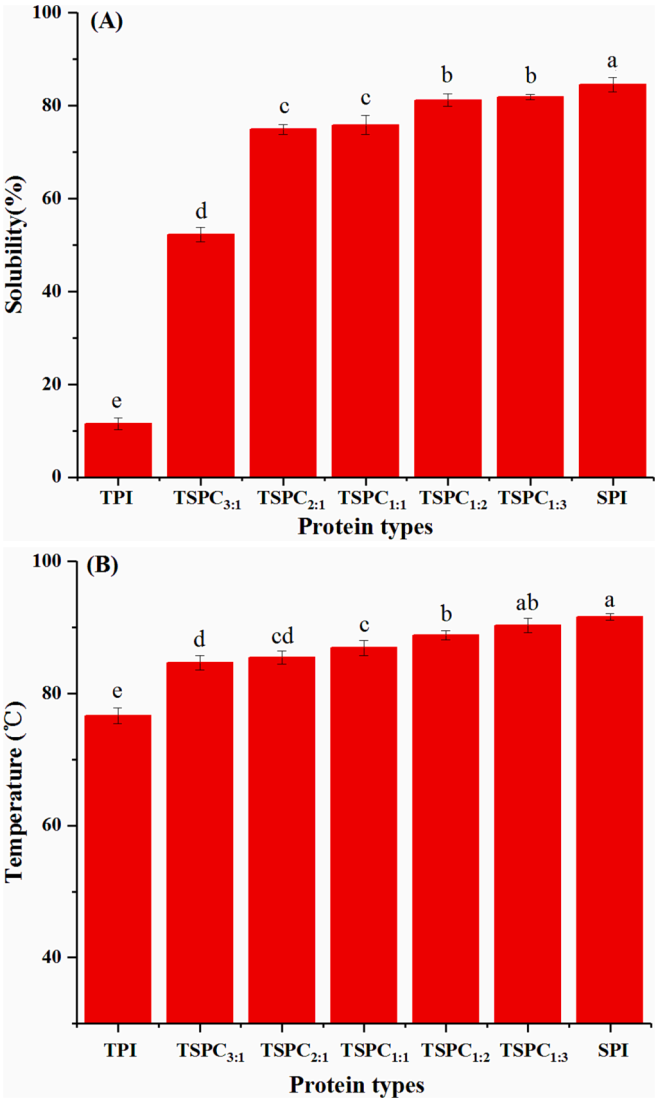


Fig. 1. The solubility (A) and thermal stability (B) of tilapia protein isolate (TPI), tilapia-soybean protein co-precipitates (TSPCs), and soybean protein isolate (SPI). Different letters within the same figure indicate significant differences ($P < 0.05$).

3.2. Nutritional properties of protein co-precipitates

3.2.1. The amino acid composition of protein co-precipitates

Table S2 showed that TPI and SPI had significantly different levels of amino acids, including sulfur amino acids and aromatic amino acids ($P < 0.05$), which is consistent with previous studies indicating that TPI has high levels of essential amino acids, while SPI has higher levels of non-essential amino acids (Shaheen et al., 2016; Chamba et al., 2015). In contrast, TSPCs maintained a balanced amino acid composition similar

to that of TPI and SPI. Furthermore, TSPCs had higher contents of phenylalanine, tryptophan, serine, proline compared to TPI and threonine, methionine, lysine, alanine compared to SPI. Notably, $TSPC_{2:1}$ and $TSPC_{1:1}$ had the highest levels of tryptophan, aspartic acid, glycine, and histidine relative to TPI and SPI. These findings are consistent with a previous study on double protein components, which reported higher amino acid compositions in the protein components than in the raw materials (Wang, Xu, Chen, Zhou, & Wang, 2018). Moreover, $TSPC_{2:1}$ and $TSPC_{1:1}$ had the highest total amino acid levels among all the TSPC proportions tested. These results suggest that $TSPC_{2:1}$ and $TSPC_{1:1}$ have a superior amino acid profile and could serve as a daily protein substitute. The high amino acid contents of TSPCs is crucial for the Asian population to achieve dietary balance and supplement limited amino acids. Furthermore, studies have shown that pasta mixed with fish powder and durum wheat semolina can improve nutraceutical and nutritional potential (Desai, Brennan, & Brennan, 2018). Therefore, TSPCs containing tilapia and soybean proteins could yield products with exceptional nutritional value.

The amino acid composition was analyzed to determine the ratios of amino acids. As shown in Table S2, the amino acid ratios of TSPCs exhibited irregular patterns. $TSPC_{2:1}$ and $TSPC_{1:1}$ had a higher amount of essential amino acids, with 30.24 and 29.71, respectively, compared to SPI. The amount of non-essential amino acids in $TSPC_{2:1}$ and $TSPC_{1:1}$ was also higher than that of TPI, with 49.27 and 49.62, respectively. These results suggest that $TSPC_{2:1}$ and $TSPC_{1:1}$ have a higher nutritional value compared to other TSPC proportions. Furthermore, the ratios of essential amino acids to total amino acids and essential amino acids to non-essential amino acids were higher in TSPCs than in SPI. This is consistent with previous research showing that the amino acid composition of protein co-precipitates was higher than raw protein isolates (Youssef et al., 1995). Overall, TSPCs prepared by mixing tilapia and soybean can compensate for the lack of most non-essential amino acids in TPI and the lack of most essential amino acids in SPI, which may have a positive effect on the body's nitrogen balance.

3.2.2. The AAS of protein co-precipitates

The AAS is used to determine the effectiveness of dietary nitrogen absorption in meeting essential amino acid requirements at a safe level of protein intake. According to Table 1, the first limiting amino acids of TPI, TSPCs, and SPI were valine, valine, and methionine, respectively. This finding is consistent with previous research that identified valine or methionine as the limiting amino acid in tilapia and soybean protein. Additionally, $TSPC_{1:1}$ had the best amino acid profile among other TSPCs and can meet human nutritional requirements. The AAS of threonine, valine, methionine, isoleucine, leucine, lysine, tryptophan, and histidine in $TSPC_{1:1}$ was higher than that of SPI, with an AAS exceeding 100. Interestingly, $TSPC_{1:1}$ showed the highest AAS of tryptophan and histidine compared to TPI and SPI. This result is similar to previous research that found higher protein scores in soybean-whey and cottonseed-whey co-precipitates compared to individual protein precipitates. Overall, TSPCs can result in a full complement of essential amino acids and enhance overall nutritional value compared to tilapia and soybean.

3.2.3. The IVPD of protein co-precipitates

In this experiment, *in vitro* simulated gastrointestinal digestion experiments were used to calculate the IVPD of TSPCs, which was found to be higher than that of SPI (80.89 %), as shown in Table 1. The increase in tilapia proportion in raw materials led to a gradual increase in IVPD of TSPC ($P < 0.05$), which is due to the greater digestibility of fish protein compared to soy protein (University & Organization, 2007). Protein with high digestibility can result in higher amino acid levels after digestion, enhancing nutritional value. However, higher protein digestibility has been linked to lifestyle diseases (Yang et al., 2012). The IVPD of TSPCs was between that of TPI and SPI, which may be more beneficial to health. Furthermore, digestibility is closely related to the amino acid composition of the protein. The ratio of arginine to lysine has been shown to affect the digestibility of rice protein (Yang et al., 2012). According to the data in Table S2, the content of ratio arginine to lysine of TPI, $TSPC_{3:1}$, $TSPC_{2:1}$, $TSPC_{1:1}$, $TSPC_{1:2}$, $TSPC_{1:3}$, and SPI was 0.61, 0.82, 0.93, 0.96, 1.03, 1.08, and 1.17, respectively. Therefore, the higher IVPD of TSPC compared to SPI may be due to the decreased number of peptide bonds between arginine and lysine required for digestion in TSPCs.

3.2.4. The PDCAAS of protein co-precipitates

Assuming that the amino acid profile can be used to determine the efficiency of absorbed dietary nitrogen, the PDCAAS should be used to predict the biological value of a protein. The calculated PDCAAS, based on food protein digestibility and amino acid score, has been widely used to evaluate the protein quality of food products (University & Organization, 2007). As shown in Table 1, TSPCs consistently had a higher PDCAAS than SPI (0.70), which is similar to previous reports where the PDCAAS of SPI was 0.77 and 0.63 (Chamba et al., 2015). Among the different TSPC proportions, $TSPC_{1:1}$ had the highest nutritional value with a PDCAAS of up to 0.86. This value is comparable to the nutritional quality of beef protein, which has a PDCAAS of 0.92. In contrast, kidney beans have a PDCAAS of 0.68, and egg whites have a PDCAAS of 1.0 when used for food proteins. Therefore, TSPCs have a high nutritional quality and could be a suitable nutritional supplement in the food industry.

3.2.5. SDS-PAGE of protein co-precipitates

The protein subunits' contributions to format protein co-precipitates were analyzed using SDS-PAGE. Fig. 2 depicts the SDS-PAGE pattern of TPI, TSPCs, and SPI. TPI exhibited bands of MHC, AC, TM α_1, TnT, and

Table 1
Comparison of AAS, IVPD and PDCAAS of tilapia protein isolate (TPI), tilapia-soybean protein co-precipitates (TSPCs), and soybean protein isolate (SPI). AAS was amino acid score; IVPD represented *in vitro* protein digestibility; PDCAAS indicated protein digestibility corrected amino acid score.

Items		TPI	$TSPC_{3:1}$	$TSPC_{2:1}$	$TSPC_{1:1}$	$TSPC_{1:2}$	$TSPC_{1:3}$	SPI
Amino acid	Reference [a] (mg/g)	AAS (%)						
Thr	23	163	132	137	132	119	123	116
Val	39	113	98	99	101	94	98	96
Met	16	214	135	106	111	96	98	87
Ile	30	149	127	130	128	118	126	124
Leu	59	130	108	110	107	98	104	103
Phe + Tyr	30	234	236	226	225	225	240	254
Lys	45	192	138	142	130	116	118	112
Trp	6	137	155	152	185	162	172	172
His	15	152	141	128	161	154	156	160
IVPD (%)		96.62 ± 0.71[a]	86.60 ± 1.72[b]	85.61 ± 0.53[b]	85.48 ± 0.64[b]	83.55 ± 0.64[b]	82.69 ± 0.64[c]	80.89 ± 0.75[c]
PDCAAS		1.09	0.85	0.85	0.86	0.78	0.81	0.70

Different letters in the same amino acid indicate significant differences between the data ($P < 0.05$).
[a] WHO/FAO/UNU amino acid requirements of adult.

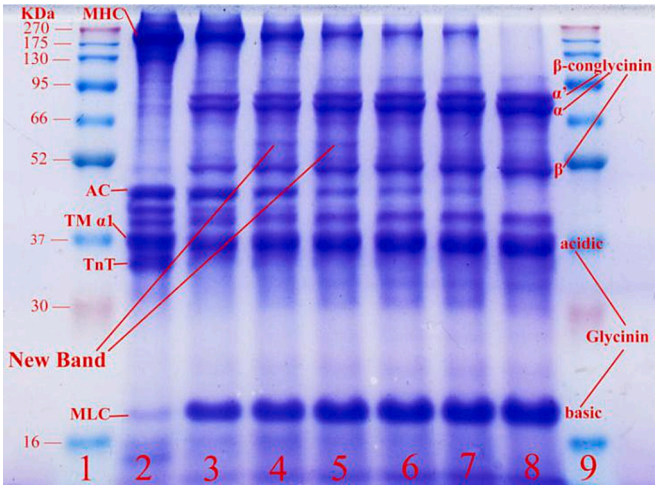


Fig. 2. SDS-PAGE of TPI, TSPCs, and SPI. Lanes 1 and 9 represent standard samples; Lane 2 represents TPI; Lane 8 represents SPI; Lanes 3, 4, 5, 6, 7, represent different proportions of proteins co-precipitates ($TSPC_{3:1}$, $TSPC_{2:1}$, $TSPC_{1:1}$, $TSPC_{1:2}$, $TSPC_{1:3}$); MHC indicates myosin heavy chain; AC indicates actin; TM α_1 indicates tropomyosin fragment; TnT indicates troponin T; MLC indicates myosin light chain.

MLC, which are common tilapia proteins (Yongsawatdigul & Piyadhammaviboon, 2007). SPI had bands of β-conglycinin and glycinin, which are common in soybean proteins (Li et al., 2018). TSPCs showed protein bands observed in both TPI and SPI, indicating that most subunits from both proteins were included in the protein co-precipitates. Similar results were reported for whey-soy protein co-precipitates (Alu'datt et al., 2012). With an increase in soybean proportion in raw materials, the contents of myosin heavy chain and actin in TSPCs gradually decreased, and β-conglycinin and glycinin increased (Table S3). Soybean protein has excellent solubility and thermal stability, making it suitable for food heat processing (Ricci et al., 2018). The SDS-PAGE assay confirmed that TSPCs had higher solubility and thermal stability than TPI (Fig. 1). However, soybean globulin's β-conglycinin was not conducive to protein digestion (Yang et al., 2016). The changes in protein subunit suggested that the IVPD of TSPCs was between TPI and SPI. Overall, the protein co-precipitates contained subunits from both original protein sources, indicating that TSPCs' preparation effectively modified tilapia and soybean proteins to enhance their potential applications. These results agree with previous observations that two protein components from different sources can be integrated into a novel protein with improved nutritional and functional properties (Wang et al., 2018).

3.2.6. Correlation analysis between nutritional quality and molecular structure

The IVPD of TSPCs was found to be between TPI and SPI, which may due to the combination effect of amino acid compositions and protein subunits (the proportion of protein subunits occupied by SDS-PAGE gel was shown in Table S3). Table S4 illustrates the correlation coefficients of the effects of amino acids and protein subunits on protein digestibility. The digestive qualities of proteins are affected by their amino acid compositions, as trypsin specifically hydrolyzes peptide bonds between lysine and arginine. The correlation analysis revealed that the lysine to arginine ratio had a significant effect on protein digestibility ($R = -0.962$, $P < 0.01$). This result confirmed that the IVPD of TSPCs was higher than that of SPI. Our findings agreed with previous research, which found that the digestibility of rice protein was closely related to the ratio of arginine to lysine (Yang et al., 2012). Moreover, the β-conglycinin of SPI was negatively correlated with protein digestibility, with the β subunit showing the greatest correlation ($R = -0.971$, $P < 0.01$). Researchers have reported that the β subunit of β-conglycinin required a longer time for complete digestion than the α' subunit and α subunit (Sadeghi, Nikkhah, Shawrang, & Shahrebabakb, 2006). Therefore, the digestibility of the five proportions of TSPCs was significantly higher than that of SPI.

3.3. Proteomics of protein co-precipitates

3.3.1. Proteins identification

It was found that $TSPC_{1:1}$ had the highest nutritional quality among the five different ratios of tilapia-soybean protein co-precipitates. Therefore, follow-up omics studies will focus on $TSPC_{1:1}$. The protein spectrum of TPI, $TSPC_{1:1}$, and SPI was analyzed using proteomics technology. A comparison was made between the protein types detected in the three proteins. As depicted in Fig. 3, TPI had a total of 2222 proteins identified, SPI had a total of 5878 proteins identified, while $TSPC_{1:1}$ had 5092 proteins identified, which was between TPI and SPI. $TSPC_{1:1}$ shared 859 and 3244 proteins with TPI and SPI, respectively, while 989 proteins were only detected in $TSPC_{1:1}$. These findings indicate that the protein composition of $TSPC_{1:1}$ differed significantly from that of TPI and SPI. Furthermore, the protein types in $TSPC_{1:1}$ were much more diverse than those in TPI.

3.3.2. Gene Ontology (GO) functional annotation

All identified proteins were subjected to GO annotation based on the three categories of biological process (BP), cellular component (CC), and molecular function (MF). As shown in Fig. 4, TPI, $TSPC_{1:1}$, and SPI were found to participate in 11, 12, and 12 molecular functions, respectively. Among them, TPI, $TSPC_{1:1}$, and SPI shared 10 molecular functions. Additionally, translation regulator activity was detected in both TPI and $TSPC_{1:1}$ but not in SPI, while protein tag was identified in both $TSPC_{1:1}$ and SPI but not in TPI. These findings suggest that TSPC indeed integrates the molecular functions of TPI and SPI. Further analysis of the molecular functions revealed that compared to TPI, TSPC had more protein tags. Compared to SPI, TSPC had more translation regulator activity but less nutrient reservoir activity.

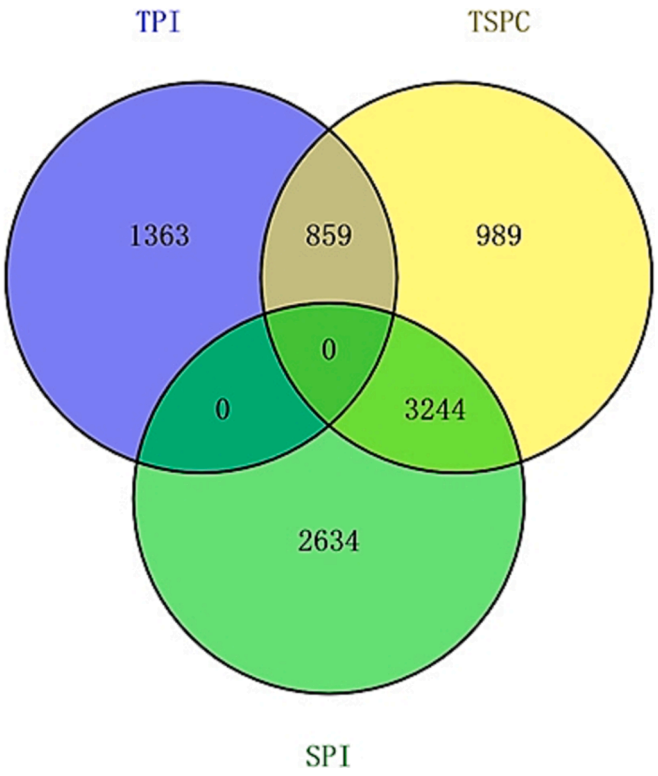


Fig. 3. Venn diagram of identified proteins in TPI, $TSPC_{1:1}$, and SPI.

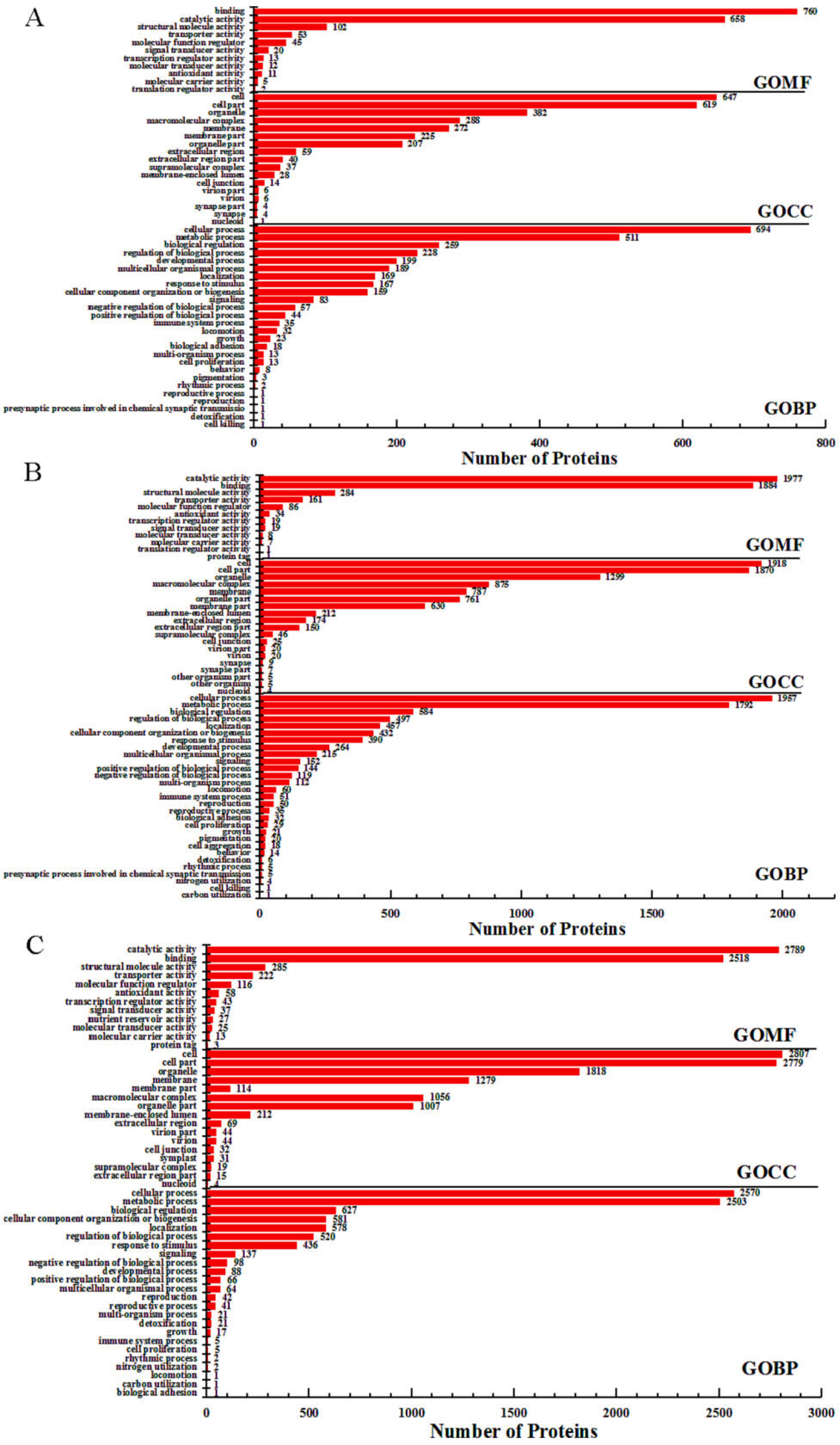


Fig. 4. Gene Ontology analysis of TPI (A), $TSPC_{1:1}$ (B), and SPI (C). GOMF represents molecular function; GOCC represents cellular composition; GOBP represents biological process.

Regarding cellular components, TPI, $TSPC_{1:1}$, and SPI were found to participate in 17, 19, and 16 cellular components, respectively. Among them, TPI, $TSPC_{1:1}$, and SPI shared 15 cellular components. Furthermore, synapse and synapse part were annotated in both TPI and $TSPC_{1:1}$ but not in SPI. Compared to TPI, $TSPC_{1:1}$ participated in other organism part and other organism cellular components. Compared to SPI, $TSPC_{1:1}$ had more synapse, synapse part, other organism part, and other organism cellular components but less symplast. In addition, $TSPC_{1:1}$ uniquely participated in other organism part and other organism cellular components.

In terms of biological processes, TPI, $TSPC_{1:1}$, and SPI were found to participate in 26, 29, and 24 biological processes, respectively. Among them, TPI, $TSPC_{1:1}$, and SPI shared 22 biological processes. $TSPC_{1:1}$ and TPI participated in behavior, cell killing, pigmentation, and presynaptic process involved in chemical synaptic transmission that were not found in SPI. On the other hand, carbon utilization and nitrogen utilization were identified in both $TSPC_{1:1}$ and SPI but not in TPI. Compared to TPI, $TSPC_{1:1}$ participated in cell aggregation, carbon utilization, and nitrogen utilization. Compared to SPI, $TSPC_{1:1}$ participated in cell aggregation, behavior, cell killing, pigmentation, and presynaptic process involved in chemical synaptic transmission. Additionally, $TSPC_{1:1}$ uniquely participated in cell aggregation. These results indicate that the preparation of TSPC indeed enhanced the biological properties of both TPI and SPI.

3.3.3. *Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis*

The KEGG pathway analysis was conducted to determine the metabolic and signal transduction processes involved in the identified proteins. As shown in Fig. 5, TPI, $TSPC_{1:1}$, and SPI participated in 328, 401, and 134 KEGG pathways, respectively. Due to the large number of KEGG pathways involved in co-precipitated proteins, the top ten of KEGG pathways were selected for further analysis. The proportions of proteins involved in TPI, $TSPC_{1:1}$, and SPI accounted for 50.81 %, 89.84 %, and 75.26 % of all pathways, respectively, with metabolic pathways being the most important pathway for all three proteins. However, $TSPC_{1:1}$ showed significant differences between TPI and SPI in the number of KEGG pathways involved, with 80 more pathways than TPI and 267 more pathways than SPI. These findings suggest that $TSPC_{1:1}$ can participate in more biochemical metabolic and signal transduction pathways, making it superior to TPI and SPI. Further analysis of the 45 KEGG pathways unique to $TSPC_{1:1}$ revealed that microbial biosynthesis and microbial metabolism in different environments were the most important metabolic pathways unique to $TSPC_{1:1}$, with the proportions of participating proteins being 33.48 % and 30.76 %, respectively. The unique antibiotic biosynthesis pathway suggests that $TSPC_{1:1}$ differs significantly from TPI and SPI in terms of disease resistance, drug component generation, accumulation, and metabolism.

Various types of *N*-glycan biosynthesis (Figure S2) are an important pathway for polysaccharide biosynthesis and belong to the biosynthesis and metabolism of sugar in the KEGG metabolic pathway. This pathway is related to the biosynthesis of resveratrol (Che, Shi, Gao, & Zhang, 2016) and the pharmacological effects of Cordyceps sinensis (Xin et al., 2019). Therefore, It's an important focus of functional food processing and biomedicine research. Table S5 shows the types of $TSPC_{1:1}$ proteins involved in the biosynthesis of various types of *N*-glycans, with Glyco_s_transf_1 domain-containing protein and α-1,2-mannosidase (alpha-1,2-Mannosidase) being among the 989 protein types unique to $TSPC_{1:1}$ (Fig. 3). This uniqueness may explain why $TSPC_{1:1}$ exhibits specificity in the biosynthesis of various types of *N*-glycans. These results suggest that the differences in protein composition of $TSPC_{1:1}$ lead to its participation in unique biological pathways not found in TPI and SPI.

4. Conclusions

TSPCs were prepared effectively by the alkali dissolution-isoelectric point precipitation method. The improved solubility and thermal stability confirmed that TSPCs can be widely used in food processing. $TSPC_{1:1}$ had the highest nutritional value, as proved by the results of amino acid composition, AAS, and PDCAAS. Interestingly, It has been confirmed that the lysine-to-arginine ratio and β-conglycinin presented substantial effects on protein digestibility. Proteomic analysis indicated that $TSPC_{1:1}$ possessed enhanced biological properties compared to TPI and SPI due to the 989 unique proteins identified. The improved physicochemical properties, high nutritional quality, and excellent biological characteristics make $TSPC_{1:1}$ a promising candidate for application as a functional high-protein nutritional supplement in the food industry.

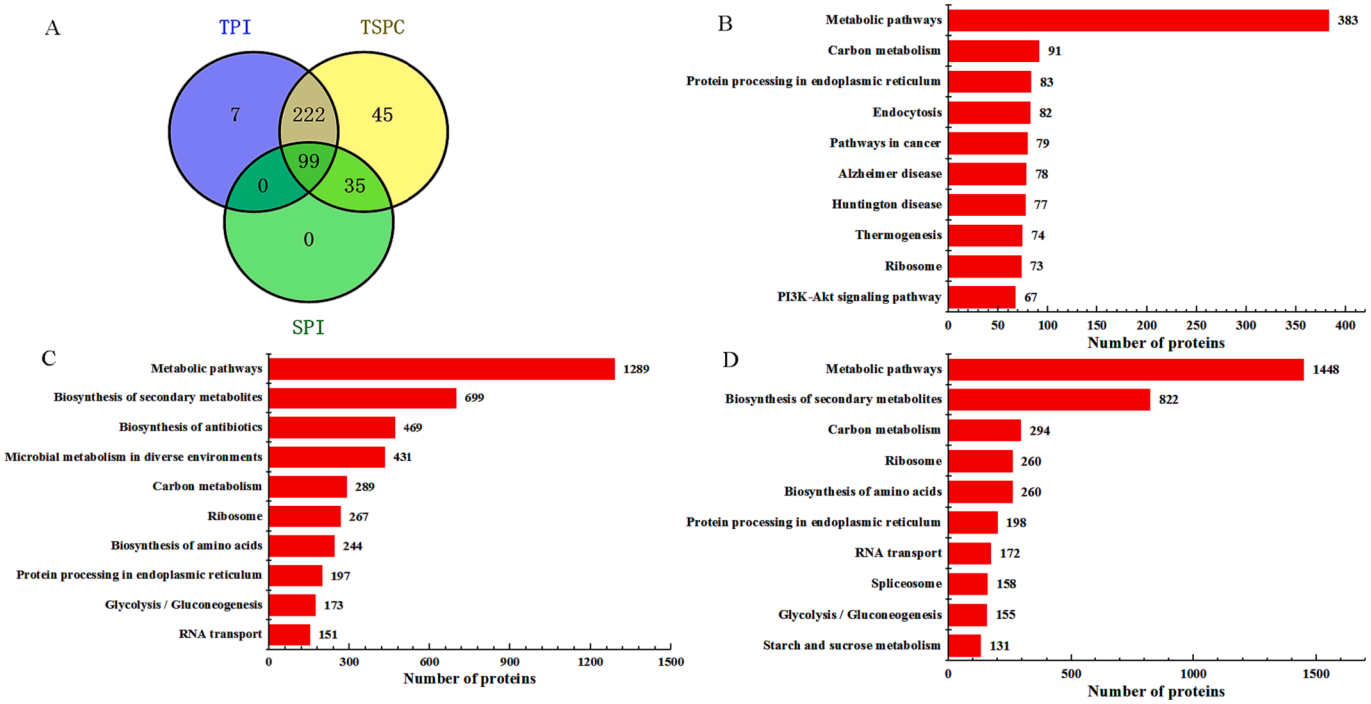


Fig. 5. Venn diagram of all KEGG pathways of different samples (A) and top ten KEGG pathway analysis of TPI (B), $TSPC_{1:1}$ (C), and SPI (D).

Author contributions

Qingguan Liu designed the study and drafted the original manuscript. Li Tan, Pengzhi Hong, and Huanming Liu collected test data and carried out the formal analysis. Chunxia Zhou contributed materials and funding.

CRediT authorship contribution statement

Qingguan Liu: Writing – review & editing, Writing – original draft, Investigation. **Li Tan:** Investigation, Formal analysis, Data curation. **Pengzhi Hong:** Formal analysis. **Huanming Liu:** Formal analysis. **Chunxia Zhou:** Writing – review & editing, Supervision, Resources, 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.

Data availability

Data will be made available on request.

Acknowledgements

This work is supported by Science and Technology Planning Project of Guangdong Province (2015A020209168, 2017A020208067), Innovation and Development Project about Marine Economy Demonstration of Zhanjiang City (XM-202008-01B1). The authors sincerely thank the anonymous reviewers for their valuable comments that have led to the present improved version of the original manuscript. In addition, the authors thank Jiali Chen for her technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101179.

References

Alves, A. C., & Tavares, G. M. (2019). Mixing animal and plant proteins: Is this a way to improve protein techno-functionalities? *Food Hydrocolloids, 97*, Article 105171.

Alu'datt, M. H., Al-Rabadi, G. J., Alli, I., Ereifej, K., Rababah, T., Alhamad, M. N., & Torley, P. J. (2013). Protein co-precipitates: A review of their preparation and functional properties. *Food and Bioproducts Processing, 91*(4), 327–335.

Alu'datt, M. H., Alli, I., & Nagadi, M. (2012). Preparation, characterization and properties of whey-soy proteins co-precipitates. *Food Chemistry, 134*(1), 294–300.

Ban, Q., Liu, Z., Yu, C., Sun, X., Jiang, Y., Cheng, J., & Guo, M. (2020). Physiochemical, rheological, microstructural, and antioxidant properties of yogurt using monk fruit extract as a sweetener. *Journal of Dairy Science, 103*(11), 10006–10014.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Chamba, M. V., Hua, Y., Murekatete, N., & Chen, Y. (2015). Effects of synthetic and natural extraction chemicals on yield, composition and protein quality of soy protein isolates extracted from full-fat and defatted flours. *Journal of Food Science and Technology, 52*(2), 1016–1023.

Che, J., Shi, J., Gao, Z., & Zhang, Y. (2016). Transcriptome analysis reveals the genetic basis of the resveratrol biosynthesis pathway in an endophytic fungus (Alternaria sp. MG1) isolated from Vitis vinifera. *Frontiers in Microbiology, 7*, 1257.

Desai, A. S., Brennan, M. A., & Brennan, C. S. (2018). Effect of fortification with fish (Pseudophycis bachus) powder on nutritional quality of durum Wheat Pasta. *Foods, 7*, 62.

Foh, M. B. K., Xia, W. S., Amadou, I., & Jiang, Q. X. (2011). Influence of pH shift on functional properties of protein isolated of tilapia (Oreochromis niloticus) muscles and of soy protein isolate. *Food and Bioprocess Technology, 5*(6), 2192–2200.

Gomaa, W. M. S., Mosaad, G. M., & Yu, P. (2018). On a Molecular basis, investigate association of molecular structure with bioactive compounds, anti-nutritional factors and chemical and nutrient profiles of canola seeds and co-products from canola processing: comparison crusher plants within Canada and within china as well as between Canada and China. *Nutrients, 10*(4), 519.

Goes, E. S. D. R., Souza, M. L. R. d., Michka, J. M. G., Kimura, K. S., Lara, J. A. F. d., Delbem, A. C. B., & Gasparino, E. (2016). Fresh pasta enrichment with protein concentrate of tilapia: nutritional and sensory characteristics. *Food Science and Technology, 36 (1)*, 76-82.

Harshani, N., Gamini, S., Swarna, W., Renuka, S., Danny, H., & Terrence, M. (2021). Comparative analysis of the nutritional profiles of selected solanum species grown in Sri Lanka. *Journal of Food Composition and Analysis, 103847*.

Horwitz, W., & Latimer, G. W. (2005). *Official methods of analysis of AOAC international*. Gaithersburg, MD: AOAC International.

Kang, Z. L., Bai, R., Lu, F., Zhang, T., Gao, Z.-S., Zhao, S. M., Zhu, M. M., & Ma, H. J. (2022). Effects of high pressure homogenization on the solubility, foaming, and gel properties of soy 11S globulin. *Food Hydrocolloids, 124*, Article 107261.

Kaspchak, E., Mafra, L. I., & Mafra, M. R. (2018). Effect of heating and ionic strength on the interaction of bovine serum albumin and the antinutrients tannic and phytic acids, and its influence on in vitro protein digestibility. *Food Chemistry, 252*, 1–8.

Kowalczewski, P. L., Olejnik, A., Bialas, W., Rybicka, I., Zielinska-Dawidziak, M., Siger, A., Kubiak, P., & Lewandowicz, G. (2019). The nutritional value and biological activity of concentrated protein fraction of potato juice. *Nutrients, 11*(7), 1523.

Kumar, M., & Sharma, B. D. (2003). Quality characteristics of low-fat ground pork patties containing milk co-precipitate. *Asian Australasian Journal of Animal Sciences, 16*(4), 588–595.

Li, J., Zhou, R., Ren, Z., Fan, Y., Hu, S., Zhuo, C., & Deng, Z. (2018). Improvement of protein quality and degradation of allergen in soybean meal fermented by Neurospora crassa. *LWT-Food Science and Technology, 101*, 220–228.

Lin, D., Lu, W., Kelly, A. L., Zhang, L., Zheng, B., & Miao, S. (2017). Interactions of vegetable proteins with other polymers: Structure-function relationships and applications in the food industry. *Trends in Food Science & Technology, 68*, 130–144.

Loveday, S. M. (2019). Food proteins: Technological, nutritional, and sustainability attributes of traditional and emerging proteins. *Annual Review of Food Science and Technology, 10*, 311–339.

Lv, C., Zhao, G., & Ning, Y. (2017). Interactions between plant proteins/enzymes and other food components, and their effects on food quality. *Critical Reviews in Food Science and Nutrition, 57*(8), 1718–1728.

Niu, X., Li, N., Chen, D., & Wang, Z. (2012). Interconnection between the protein solubility and amino acid and dipeptide compositions. *Protein and Peptide Letters, 20*(1), 88–95.

Ricci, L., Umilta, E., Righetti, M. C., Messina, T., Zurlini, C., Montanari, A., Bronco, S., & Bertoldo, M. (2018). On the thermal behavior of protein isolated from different legumes investigated by DSC and TGA. *Journal of the Science of Food and Agriculture, 98*(14), 5368–5377.

Sadeghi, A. A., Nikkhah, A., Shawrang, P., & Shahrebabakb, M. M. (2006). Protein degradation kinetics of untreated and treated soybean meal using SDS-PAGE. *Animal Feed Science and Technology, 126*(1–2), 121–133.

Shaheen, N., Islam, S., Munmun, S., Mohiduzzaman, M., & Longvah, T. (2016). Amino acid profiles and digestible indispensable amino acid scores of proteins from the prioritized key foods in Bangladesh. *Food Chemistry, 213*, 83–89.

Swieca, M., Gawlik-Dziki, U., Jakubczyk, A., Bochnak, J., Sikora, M., & Sulibarska, J. (2019). Nutritional quality of fresh and stored legumes sprouts - Effect of Lactobacillus plantarum 299v enrichment. *Food Chemistry, 288*, 325–332.

Tahergorabi, R., Matak, K. E., & Jaczynski, J. (2015). Fish protein isolate: Development of functional foods with nutraceutical ingredients. *Journal of Functional Foods, 18*, 746–756.

Tan, L., Hong, P. Z., Yang, P., Zhou, C. X., Xiao, D. H., & Zhong, T. J. (2019). Correlation between the water solubility and secondary structure of tilapia-soybean protein co-precipitates. *Molecules, 24*(23), 4337.

Tan, S. T., Tan, S. S., & Tan, C. X. (2023). Soy protein, bioactive peptides, and isoflavones: A review of their safety and health benefits. *PharmaNutrition, 25*, Article 100352.

Tian, T., Tong, X., Ren, K., Cao, J., Yuan, Y., Yang, J., Zhu, J., Miao, L., Yang, S., Yu, A., Wang, H., & Jiang, L. (2022a). Influence of protein ratios on the structure and gel properties of soybean-wheat co-precipitated proteins. *LWT-Food Science and Technology, 170*, Article 114045.

Tian, T., Ren, K., Tong, X., Peng, X., Lian, Z., Lyu, B., Du, M., Wang, H., & Jiang, L. (2022b). Co-precipitates proteins prepared by soy and wheat: Structural characterisation and functional properties. *International Journal of Biological Macromolecules, 212*, 536–546.

Thompson, L. U. (1977). Co-precipitation of rapeseed and cheese whey proteins using acid and heat treatment. *Canadian Institute of Food Science and Technology Journal, 10*(1), 43–48.

Thomas, M. A., Mcbride, R., Turner, A. D., & Abad, G. H. (1978). Effect of extending beef patties with milk co-precipitates. *Journal of Food Science, 43*, 162–164.

University, U. N., & Organization, W. H. (2007). *Protein and amino acid requirements in human nutrition: Report of a Joint FAO/WHO/UNU expert consultation*. World Health Organization.

Vilela, R. M., Lands, L. C., Chan, H. M., Azadi, B., & Kubow, S. (2006). High hydrostatic pressure enhances whey protein digestibility to generate whey peptides that improve glutathione status in CFTR-deficient lung epithelial cells. *Molecular Nutrition & Food Research, 50*(11), 1013–1029.

Wang, T., Xu, P., Chen, Z., Zhou, X., & Wang, R. (2018). Alteration of the structure of rice proteins by their interaction with soy protein isolates to design novel protein composites. *Food & Function, 9*, 4282–4291.

Xin, X., Yin, J., Zhang, B., Li, Z., Zhao, S., & Gui, Z. (2019). Genome-wide analysis of DNA methylation in subcultured Cordyceps militaris. *Archives of Microbiology, 201*(3), 369–375.

Yang, J., Guo, J., Yang, X. Q., Wu, N. N., Zhang, J. B., Hou, J. J., Zhang, Y. Y., & Xiao, W. K. (2014). A novel soy protein isolate prepared from soy protein

concentrate using jet-cooking combined with enzyme-assisted ultra-filtration. *Journal of Food Engineering, 143*, 25–32.
Yang, L., Chen, J.-H., Zhang, H., Qiu, W., Liu, Q.-H., Peng, X., Li, Y.-N., & Yang, H.-K. (2012). Alkali treatment affects in vitro digestibility and bile acid binding activity of rice protein due to varying its ratio of arginine to lysine. *Food Chemistry, 132*(2), 925–930.
Yang, Y., Wang, Z., Wang, R., Sui, X., Qi, B., Han, F., Li, Y., & Jiang, L. (2016). Secondary structure and subunit composition of soy protein in vitro digested by Pepsin and its relation with digestibility. *BioMed Research International, 2016*, 1–11.
Yokot, K., Satou, K., & Ohki, S.-Y. (2006). Comparative analysis of protein thermostability: Differences in amino acid content and substitution at the surfaces and in the core regions of thermophilic and mesophilic proteins. *Science and Technology of Advanced Materials, 7*(3), 255–262.
Yongsawatdigul, J., & Piyadhammaviboon, P. (2007). Gel-enhancing effect and protein cross-linking ability of tilapia sarcoplasmic proteins. *Journal of the Science of Food and Agriculture, 87*(15), 2810–2816.
Youssef, A. M., Abu-Foul, N. S., & Moharram, Y. G. (1995). Preparation and characteristics of co-precipitate proteins from oilseeds and legumes seeds. *Food / Nahrung, 5*(6), 475–482.
Yu, P., Mckinnon, J. J., Christensen, C. R., & Christensen, D. A. (2004). Using synchrotron-based FTIR microspectroscopy to reveal chemical features of feather protein secondary structure: Comparison with other feed protein sources. *Journal of Agricultural and Food Chemistry, 52*, 7353–7361.
Yu, P. (2007). Protein secondary structures (α-helix and β-sheet) at a cellular level and protein fractions in relation to rumen degradation behaviours of protein: A new approach. *British Journal of Nutrition, 94*(5), 655.
Zhao, Z., Sun, X., Liu, N., Cheng, J., Wang, C., & Guo, M. (2022). Comparative analysis of caseins in Saanen goat milk from 3 different regions of China using quantitative proteomics. *Journal of Dairy Science, 105*(7), 5587–5599.
Zhu, K. X., Sun, X. H., Chen, Z. C., Peng, W., Qian, H. F., & Zhou, H. M. (2010). Comparison of functional properties and secondary structures of defatted wheat germ proteins separated by reverse micelles and alkaline extraction and isoelectric precipitation. *Food Chemistry, 123*(4), 1163–1169.