Research Article

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# Protein–peptide nutritional material prepared from surimi wash-water using immobilized chymotrypsin–trypsin

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## Abstract

**BACKGROUND: In the production process of surimi, large quantities of wastewater are produced. Thus it would be interesting to develop an efficient protocol for the recovery of protein from hairtail surimi wash-water.**

**RESULTS: A technique involving the use of immobilized chymotrypsin–trypsin (I-CT) was developed, providing a practical method for the preparation of protein–peptide nutritional material (PPNM). Under optimized reaction conditions, the recovery rate of nitrogen of surimi wash-water was measured as 98.3 ± 2.9%. Nutritional evaluation of the protein–peptide fraction demonstrated that it contained all essential amino acids (EAA) for humans, accounting for 44.1% of the total amino acid (TAA) content, which was determined to be 78.2 g per 100 g dry matter. The essential amino acid index (EAAI) and biological value (BV) were 101.7 (>95) and 76.7 respectively. A wide range of volatile flavor compounds (>50), including aldehydes, ketones, alcohols, hydrocarbons and heterocyclic compounds, were identified in PPNM by gas chromatography/mass spectrometry (GC/MS) analysis.**

**CONCLUSION: An efficient and practical protocol for the recovery of protein from hairtail surimi wash-water has been developed. The PPNM prepared in this work could be used as a nutraceutical and as an ingredient of functional foods.**


**Keywords:** protein–peptide nutritional material; surimi wash-water; immobilized chymotrypsin–trypsin; nutritional evaluation; volatile flavor compounds

## INTRODUCTION

Surimi serves as a potential raw material for a variety of food products, which have become increasingly popular owing to their unique textural properties as well as high nutritional value.[1] In the eastern coastal cities of China, hairtail is one of the most popular commercial marine fish. The production of hairtail surimi has attracted the interest of seafood manufacturers owing to its abundant stock and relatively low price.

Surimi is concentrated myofibrillar protein obtained from fish flesh. In the production process of surimi, a large amount of freshwater is required for the cleaning, mincing and washing operations. This process produces large quantities of wastewater.[2,3] Surimi wash-water typically contains 15–30% of the initial protein in fish,[4] resulting in considerable waste of protein resources.[5–7] The annual output of surimi amounts to 200 000 tons, generating more than 6000 tons of protein resource waste. Consequently, there is a demand to find an efficient way to recover fish proteins from surimi wash-water, which would not only reduce the negative environmental impact and the cost of waste disposal but also generate additional profits. Using conventional methods such as ohmic heating,[8] centrifugation,[9] filtration[10] and isoelectric point precipitation,[11] protein recovery is relatively low.

Recently, several attempts have been made to recover proteins from surimi wash-water using a variety of techniques and to transform them to higher-value products. For instance, ultrafiltration was employed to recover myofibrillar protein from surimi wash-water,[6,12] but there are problems centered on severe fouling of membranes using this method.[13] Methods based on precipitation triggered by shifting the pH and using organic solvents have been employed to recover water-soluble proteins from surimi wash-water, achieving a recovery rate of protein of up to 65%.[1] However, the volatility of organic solvents leads to challenges to industrial safety issues and to an increase in the processing cost. Protein has also been recovered from surimi wash-water by use of chitosan–alginate complex

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as a protein adsorption agent,[14,15] but protein recovery was relatively low.

In the present study, immobilized chymotrypsin–trypsin (I-CT) was employed for the preparation of protein–peptide nutritional material (PPNM) from hairtail surimi wash-water. The effect of the hydrolysis conditions (pH, temperature, reaction time and amount of enzyme) on the properties of the resulting PPNM was investigated using nitrogen recovery (NR), trichloroacetic acid nitrogen solubility index (TCA-NSI) and residual I-CT activity as the evaluation indices. Finally, the amino acid composition and main flavor components of the PPNM were analyzed by automatic amino acid analysis and gas chromatography/mass spectrometry (GC/MS).

## MATERIALS AND METHODS

### Materials and reagents

The experimental hairtail (*Trichiurus lepturus* Linnaeus) with an average weight of 200 g per fish, purchased in Hangzhou market, were caught in March and authenticated by Zhejiang Research Institute of Marine Fisheries (Zhejiang, China). I-CT with an activity of $1340 \pm 50$ U $g^{-1}$ was prepared by covalently conjugating a trypsin–chymotrypsin mixture onto modified polyvinyl chloride (PVC) microspheres according to a previous report.[16] Kjeldahl catalysts were purchased from Büchi (Flawil, Switzerland). Acrylamide, *N*,*N*′-methylenebisacrylamide, glycine, sodium dodecyl sulfate, ammonium persulfate, peptide standards and tetramethylethylenediamine were purchased from Bio-Rad (Hercules, CA, USA). Tricine was purchased from Amresco (Solon, OH, USA). Ready-to-use protein molecular weight standards (high and low) were purchased from Takara Bio Inc. (Dalian, China). Amino acid mixture standard (containing 17 amino acids) was purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals and solvents used in this study were of analytical or high-performance liquid chromatography (HPLC) grade.

### Sample preparation

The fresh hairtail were scaled, gutted and their heads and fins removed before being minced by a flesh separator with an aperture of 3 cm (Dingcheng Machine Co., Guangdong, China). A 100 g portion of mince was mixed with 500 mL of distilled water in a beaker with stirring for 10 min. The supernatant was separated from the solid particles by centrifugation, then filtered with a 200-mesh sieve to further remove the residual solids (fat and minced meat). The filtrate was stored in a clean bottle with a tight-fitting lid at −18 °C before use.

### Chemical analysis

The contents of dry matter, crude fat and crude protein were determined according to national standards (China) GB/T 8858–1988 (vacuum drying method),[17] GB/T 5009.6-2003 (Soxhlet method)[18] and GB/T 5009.5-2010 (Kjeldahl method)[19] respectively. The protein fractions of surimi wash-water were divided into three components as follows. First, 40 mL of surimi wash-water was centrifuged at $15\,000 \times g$ for 20 min. This resulted in a lower sediment comprising suspended minced fish and an upper clear liquid containing water-soluble protein (WSP) and non-protein nitrogen (NPN)-containing compounds. WSP was precipitated by adding 10 mL of 500 g $L^{-1}$ TCA solution to the mixture and stirring for 30 min at 30 °C. The WSP was separated by centrifugation at $15\,000 \times g$ for 10 min, leaving NPN in solution. The protein and nitrogen contents of these three components were measured according to GB/T 5009.5-2010 (Kjeldahl method).[19]

### Hydrolysis of surimi wash-water

The pH of surimi wash-water was adjusted to 7.0–9.0 by the addition of 0.1 mol $L^{-1}$ NaOH solution. The hydrolysis of surimi wash-water was carried out in the presence of I-CT at a suitable pH value and temperature. After hydrolysis, the I-CT was separated from the reaction solution by centrifugation at $15\,000 \times g$ for 5 min. The supernatant was stored at −20 °C before further analysis.

### Molecular weight determination of hydrolysate of surimi wash-water

The molecular weight distribution of the enzymatic hydrolysate derived from hairtail surimi wash-water was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[20] and tricine sodium dodecyl sulfate polyacrylamide gel electrophoresis (tricine-SDS-PAGE).[21]

The hydrolysate (15 $\mu$L) and molecular weight markers were loaded into separate wells. Electrophoresis was performed at room temperature. Gels were fixed in 500 g $L^{-1}$ methanol and 100 g $L^{-1}$ acetic acid for 30 min, stained with 0.25 g $L^{-1}$ Coomassie Brilliant Blue R-250 overnight and de-stained in 100 g $L^{-1}$ acetic acid.[20] The relationship between electrophoretic mobility of protein–SDS complexes and protein molecular weight was regressed to be logarithmic. The molecular weights of the hydrolysate proteins were calculated from a standard curve derived from the relative migration rate and logarithmic relative molecular mass of the protein markers.

### NR and TCA-NSI of hydrolysis

The NR of hydrolysis, defined as the percentage of nitrogen content of hydrolysate in the total nitrogen content of surimi wash-water, was calculated based on the consumption of base by the Kjeldahl method according to the following equation:

$$\text{NR}\ (\%) = [\text{nitrogen content in hydrolysate}\ (\text{mg}) / \text{total nitrogen content in surimi wash-water}\ (\text{mg})] \times 100 \quad (1)$$

**Table 1.** Results of orthogonal test of enzymolysis

| Run | pH | Time (min) | Temperature (°C) | Enzyme addition (g per 100 mL) | NR (%) |
|---|---|---|---|---|---|
| 1 | 7.8 | 15 | 50 | 2 | $66.19 \pm 1.75$ |
| 2 | 7.8 | 30 | 55 | 4 | $95.12 \pm 2.04$ |
| 3 | 7.8 | 45 | 60 | 6 | $100.00 \pm 0.00$ |
| 4 | 8.2 | 15 | 55 | 6 | $98.94 \pm 1.63$ |
| 5 | 8.2 | 30 | 60 | 2 | $89.52 \pm 1.04$ |
| 6 | 8.2 | 45 | 50 | 4 | $100.00 \pm 0.00$ |
| 7 | 8.6 | 15 | 60 | 4 | $88.45 \pm 1.33$ |
| 8 | 8.6 | 30 | 50 | 6 | $100.00 \pm 0.00$ |
| 9 | 8.6 | 45 | 55 | 2 | $87.73 \pm 2.20$ |
| $K_1$ | 261.31 | 253.58 | 266.19 | 243.44 | |
| $K_2$ | 288.46 | 284.64 | 281.79 | 283.57 | |
| $K_3$ | 276.18 | 287.73 | 277.97 | 298.94 | |
| $k_1$ | 87.10 | 84.53 | 88.73 | 81.15 | |
| $k_2$ | 96.15 | 94.88 | 93.93 | 94.52 | |
| $k_3$ | 92.06 | 95.91 | 92.66 | 99.65 | |
| $R$ | 9.05 | 11.38 | 5.20 | 18.50 | |

**Table 2.** Protein components (mg mL$^{-1}$) in surimi wash-water and its hydrolysate

| Index | Dry matter | Crude protein | WSP | NPN |
|---|---|---|---|---|
| Surimi wash-water | 8.194 ± 0.097 | 6.776 ± 0.043 | 2.257 ± 0.009 | 1.923 ± 0.010 |
| Hydrolysate | 8.253 ± 0.110 | 6.735 ± 0.061 | 2.805 ± 0.051 | 3.937 ± 0.064 |

The TCA-NSI, used for evaluating the proportion of low-molecular-weight peptides and free amino acids in hydrolysate, was measured by the AOAC method.[22] In brief, 5 mL of 500 g L$^{-1}$ TCA solution was added to 20 mL of hydrolysate and the resulting mixture was centrifuged at 15 000 × *g* for 5 min. The supernatant was analyzed for nitrogen content in TCA-soluble peptides and free amino acids, namely NPN compounds, using the Kjeldahl method. The TCA-NSI was calculated as follows:

$$\text{TCA-NSI}\ (\%) = [\text{TCA-soluble nitrogen content in hydrolysate (mg)} / \text{total nitrogen content in surimi wash-water (mg)}] \times 100 \quad (2)$$

### Optimization of hydrolysis conditions

In order to optimize the hydrolysis conditions of surimi wash-water in the presence of I-CT, to obtain the highest NR, a three-level/four-factor orthogonal experimental design using IBM SPSS statistics software was applied. Nine tests were required as shown in Table 1. Based on the results obtained in single-factor tests, the three levels for the variables were selected as pH 7.8, 8.2 and 8.6, hydrolysis time 15, 30 and 45 min, temperature 50, 55 and 60 °C and enzyme/substrate (E/S) ratio 2, 4 and 6 g per 100 mL, and the measured variable response was NR (%). Each enzymatic hydrolysis experiment was performed in triplicate.

### Preparation of PPNM

Concentration of the hydrolysate was undertaken by low-temperature vacuum drying (50 °C) followed by spray drying (air capacity 800 m$^3$ h$^{-1}$, inlet air temperature 250 °C, air exhaust temperature 120 °C, spray revolutions 5000 rpm, collecting pot temperature 50 °C) to yield PPNM as a dry powder. The dried PPNM was stored in a bottle with a tight-fitting lid at −18 °C for future use. The contents of dry matter, crude fat and crude protein in PPNM were determined according to the methods mentioned above. The molecular weight distribution of the enzymatic hydrolysate was estimated by SDS-PAGE.[21]

### Evaluation of nutritional value and analysis of volatile flavor compounds in PPNM

The amino acid composition of PPNM was analyzed by an automatic amino acid analyzer (L-8800, Hitachi Ltd., Tokyo, Japan). Amino acid score (AAS), chemical score (CS), essential amino acid index (EAAI) and biological value (BV) were monitored in order to evaluate the overall nutritional value of PPNM.[23,24] The volatile compounds in PPNM were analyzed by GC/MS using headspace solid phase microextraction (HS-SPME).[25] Xcalibur software[26] was used to analyze the data, retrieving the unknown compounds by matching with a standard NIST 2.0 spectral library. Unknown compounds were authenticated when the pros and cons of matching were greater than 800. After deducting the peaks of siloxane group-containing compounds and non-olfactory compounds from the chromatogram, the total peak area of volatile constituents was statistically analyzed and relative peak areas of each component were calculated by area normalization.

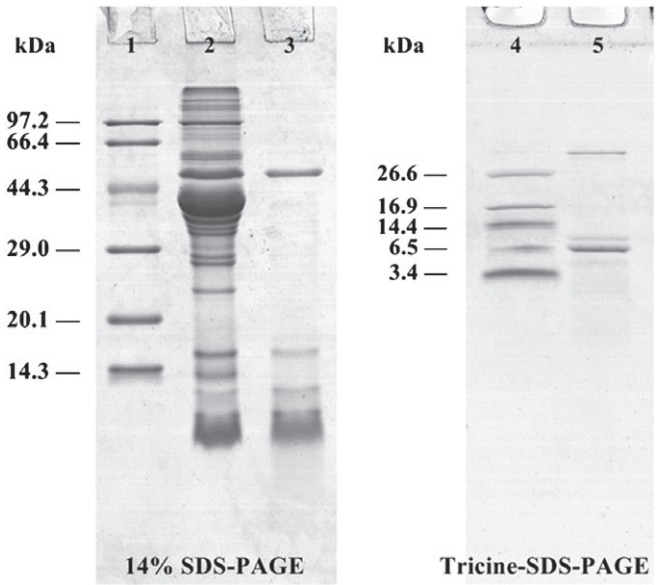


**Figure 1.** Molecular weight distribution of enzymatic hydrolysates of hairtail surimi wash-water measured by SDS-PAGE and tricine-SDS-PAGE: lane 1, protein molecular weight marker; lane 2, hairtail surimi wash-water; lanes 3 and 5, enzymatic hydrolysates of hairtail surimi wash-water; lane 4, peptide molecular weight marker.

The contribution of volatile components to the overall flavor of PPNM was evaluated by relative odor activity value (ROAV).[27] Compounds with ROAV ≥ 1 are defined as key flavor components, while those with 0.1 ≤ ROAV < 1 are defined as main flavor components.

## RESULTS AND DISCUSSION

### Proximate composition

The proximate composition of hairtail surimi wash-water in this study was determined to be 8.19 ± 0.097 mg mL$^{-1}$ dry matter, 0.54 ± 0.014 mg mL$^{-1}$ crude fat and 6.78 ± 0.043 mg mL$^{-1}$ crude protein. The pH of hairtail surimi wash-water was 6.79 ± 0.01. It was found that 38.4% of the protein in surimi wash-water was from suspended fish particles (2.605 ± 0.024 mg mL$^{-1}$), which are small-sized particles from which protein recovery is relatively difficult. WSP (2.257 ± 0.009 mg mL$^{-1}$) mainly consisted of sarcoplasmic protein in hairtail surimi wash-water, and NPN (1.923 ± 0.010 mg mL$^{-1}$) mainly contained oligopeptides and free amino acids, which are flavoring compounds and flavoring precursors for food. Other workers have produced surimi wash-waters with protein concentrations falling in the range 0.8 – 16 mg mL$^{-1}$,[4,28,29] which involved multiple washing steps. The value reported in this work (6.78 mg mL$^{-1}$) was within this range.

**Table 3.** Amino acid profile of PPNM

| Essential amino acid (EAA) | Content(g per 100 g DM) | Non-essential amino acid (NEAA) | Content(g per 100 g DM) |
|---|---|---|---|
| Leucine | 7.260 ± 0.235 | Glutamic acid | 9.175 ± 0.264 |
| Lysine | 6.829 ± 0.147 | Aspartic acid | 8.734 ± 0.302 |
| Valine | 4.784 ± 0.111 | Lactamine | 5.863 ± 0.142 |
| Phenylalanine | 4.259 ± 0.152 | Glycine | 5.768 ± 0.153 |
| Isoleucine | 3.817 ± 0.089 | Arginine | 3.285 ± 0.098 |
| Threonine | 3.410 ± 0.124 | Serine | 3.161 ± 0.105 |
| Tryptophan | 2.163 ± 0.096 | Proline | 2.466 ± 0.067 |
| Methionine | 1.965 ± 0.073 | Histidine | 2.183 ± 0.069 |
| EAA total | 34.487 | Tyrosine | 2.174 ± 0.082 |
| | | Cysteine | 0.938 ± 0.030 |
| | | NEAA total | 43.747 |

**Table 4.** Nutritional evaluation of PPNM

| Amino acid | FAO/WHO essential amino acid model (mg $g^{-1}$ protein) | Whole egg protein model (mg $g^{-1}$ protein) | PPNM | | | |
|---|---|---|---|---|---|---|
| | | | AAS | CS | EAAI | BV |
| Thr | 40 | 47 | 109** | 93 | 101.67 | 76.70 |
| Val | 50 | 66 | 122 | 93 | | |
| Met + Cys | 35 | 57 | 106* | 65* | | |
| Phe + Tyr | 60 | 93 | 137 | 88** | | |
| Try | 10 | 17 | 277 | 163 | | |
| Ile | 40 | 54 | 122 | 90 | | |
| Leu | 70 | 86 | 133 | 108 | | |
| Lys | 55 | 70 | 159 | 125 | | |

*First limiting amino acid;
**second limiting amino acid.

### Optimization of enzymolysis conditions

Based on the results of single-factor tests, an orthogonal test was employed to further optimize the enzymolysis conditions (Table 1). It was demonstrated that the amount of added I-CT had the most significant effect on enzymolysis ($R = 18.50$), with the enzymolysis time being the second most important factor ($R = 11.38$). However, the enzymolysis temperature and pH also produced some impact on performance. The NR obtained in runs 3, 4, 6 and 8 was close to 100% in each case. Taking economic factors into account, namely temperature and reaction time, the reaction conditions of run 4 were finally selected for the enzymolysis of surimi wash-water. The optimized hydrolysis conditions were found to be: pH 8.2, enzyme 6 g per 100 mL, reaction time 15 min and temperature 55 °C. Using this system, the NR of surimi wash-water was predicted to be 98.9% (Table 1). A validation assay was conducted using the above optimal conditions to confirm the recovery rate of nitrogen, being determined as 98.3 ± 2.9%. The NR achieved using the present method is higher than those obtained by traditional methods such as fractional isoelectric precipitation (NR 74.4%)[28] and flocculation (NR 86.7%).[29]

### Composition of protein in PPNM

In order to compare the composition of protein in PPNM with that in surimi wash-water, the composition of the hydrolysate of surimi wash-water was determined (Table 2). The results indicated no major differences in the contents of dry matter and crude protein. The contents of WSP and NPN in PPNM were increased by 24.3 and 104.7% respectively, which was attributed to the hydrolytic degradation of 'fish particles'.

### Molecular weight of PPNM

The distribution of the molecular weights of PPNM obtained under the selected optimal conditions was analyzed by SDS-PAGE and tricine-SDS-PAGE. As shown in Fig. 1, the molecular weights of most proteins in surimi wash-water were higher than 20.1 kDa (lane 2), and these main proteins were not found in PPNM (lane 3), except for the protein with molecular weight 55.3 kDa. These data support the fact that most proteins were partially degraded into low-molecular-weight peptides and amino acids. The tricine-SDS-PAGE result (lane 5 in Fig. 1) demonstrated that the molecular weights of PPNM mainly ranged between 2 and 6.5 kDa. In lane 5, the blue color of the band with molecular weight 6.5 k Da was intense. A number of lighter bands with molecular weights ranging between 2 and 4.8 kDa were also detected.

### Nutritional evaluation of PPNM

The amino acid composition and contents of PPNM are presented in Table 3. PPNM contained all essential amino acids (EAA) for humans, and the total amino acid (TAA) content was 78.2 g per 100 g dry matter (DM). The contents of non-essential amino acids (NEAA) in PPNM were also high, especially those of glutamic acid and aspartic acid. The EAA accounted for 44.1% of the TAA content.

The nutritional value of PPNM was evaluated by the FAO/WHO essential amino acid model[11] and the whole egg protein model[30]

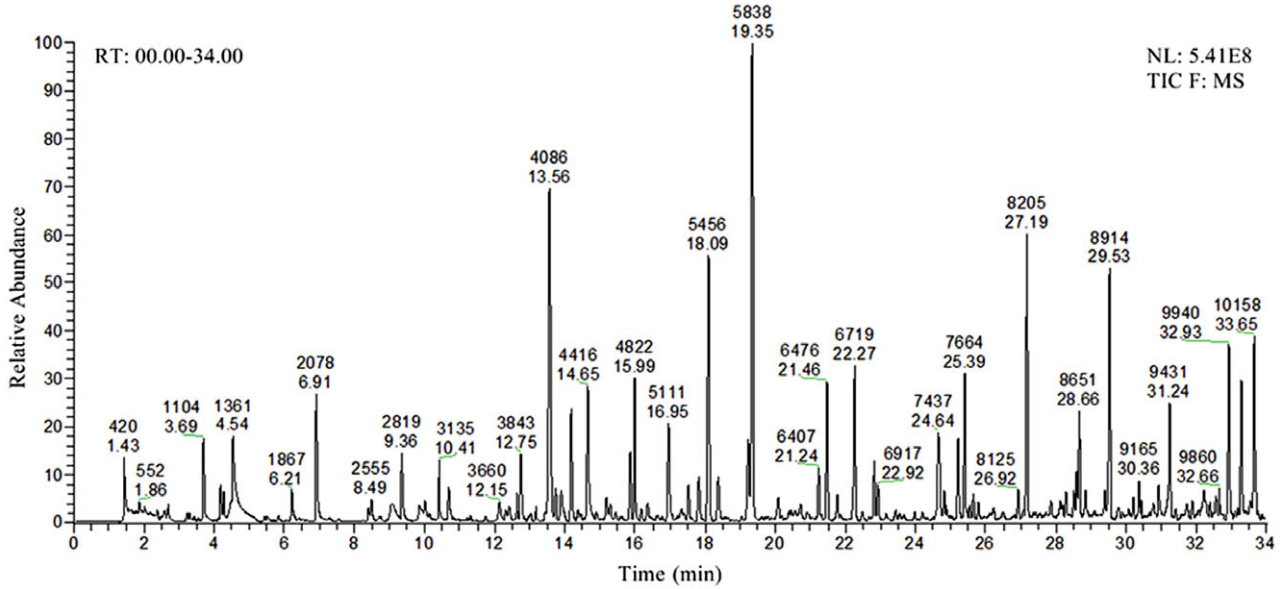


**Figure 2.** Total ion chromatogram of volatile components in PPNM.

**Table 5.** Relative content of volatile compounds in PPNM

| Compound | Retentiontime (min) | Relative peak area (%) | Perception threshold (μg kg$^{-1}$) | Compound | Retention time (min) | Relative peak area (%) | Perception threshold (μg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|
| *Aldehydes* | | | | *Ketones* | | | |
| 3-Methylbutyraldehyde | 3.31 | 0.18 ± 0.01 | 0.2 | 2,3-Butanedione | 2.58 | 0.34 ± 0.06 | 2.3–6.5 |
| 2-Methylbutyraldehyde | 3.44 | 0.09 ± 0.00 | 1 | 2,3-Pentanedione | 4.27 | 0.54 ± 0.03 | 5.13 |
| Pentanal | 4.18 | 0.58 ± 0.03 | 3 | 2-Nonanone | 17.52 | 0.78 ± 0.01 | 38.9 |
| *trans*-2-Pentenal | 6.21 | 0.65 ± 0.03 | 1500 | Acetophenone | 19.35 | 12.8 ± 0.30 | 65 |
| Caproaldehyde | 6.92 | 2.56 ± 0.08 | 4.5 | 2-Decanone | 21.77 | 0.54 ± 0.02 | 7.94 |
| *trans*-2-Hexenal | 9.36 | 1.36 ± 0.05 | 17 | Geranylacetone | 30.36 | 0.76 ± 0.04 | 60 |
| Heptaldehyde | 10.41 | 1.19 ± 0.02 | 3 | *Hydrocarbons* | | | |
| *cis*-4-Heptenal | 10.69 | 0.75 ± 0.00 | 0.8 | Trichloromethane | 2.71 | 0.31 ± 0.00 | 100000 |
| *trans,trans*-2,4-Hexadienal | 12.31 | 0.19 ± 0.00 | 10 | Methylbenzene | 5.45 | 0.10 ± 0.00 | 200 |
| *trans*-2-Heptenal | 13.17 | 0.31 ± 0.00 | 13 | Phenethylene | 10.14 | 0.08 ± 0.00 | 730 |
| Octyl aldehyde | 14.18 | 2.17 ± 0.05 | 0.5 | 3-Ethyltoluene | 12.15 | 0.46 ± 0.14 | – |
| Benzaldehyde | 14.65 | 3.01 ± 0.09 | 3 | 2-Ethylmethylbenzene | 13.03 | 0.18 ± 0.09 | – |
| *trans,trans*-2,4-Heptadienal | 15.99 | 2.89 ± 0.11 | 10 | D-Limonene | 13.73 | 0.58 ± 0.04 | 10 |
| *trans*-2-Octenal | 16.95 | 2.44 ± 0.01 | 3 | Hendecane | 14.46 | 0.10 ± 0.01 | – |
| Nonanoic aldehyde | 18.09 | 6.13 ± 0.09 | 1 | 1,4-Dichlorobenzene | 15.29 | 0.33 ± 0.01 | 48 |
| Benzeneacetaldehyde | 18.38 | 1.15 ± 0.01 | 4 | Tetradecane | 25.78 | 0.33 ± 0.03 | – |
| *trans*-2-Nonenal | 21.24 | 1.10 ± 0.04 | 0.08 | Pentadecane | 28.66 | 1.85 ± 0.17 | – |
| *trans,cis*-2,6-Nonadienal | 21.46 | 2.70 ± 0.07 | 0.01 | Heptadecane | 31.25 | 2.20 ± 0.40 | – |
| Decanal | 22.27 | 3.15 ± 0.01 | 0.1 | 2,6,10,14-Tetramethylpentadecane | 33.30 | 2.53 ± 0.25 | – |
| 4-Ethylbenzaldehyde | 22.92 | 0.70 ± 0.01 | – | Nonadecane | 33.65 | 3.34 ± 0.54 | – |
| *trans,trans*-2,4-Nonadienal | 23.98 | 0.15 ± 0.01 | 0.09 | *Alcohols* | | | |
| *trans*-2-Decenal | 24.81 | 0.44 ± 0.03 | 0.3 | *trans*-2-Octen-1-ol | 5.83 | 0.13 ± 0.01 | 40 |
| Undecanal | 25.65 | 0.44 ± 0.00 | 5 | 1-Octen-3-ol | 12.63 | 0.51 ± 0.00 | 1 |
| 2-Undecenal | 27.86 | 0.40 ± 0.07 | – | 2-Decen-1-ol | 16.36 | 0.50 ± 0.06 | – |
| Dodecenal | 28.59 | 0.59 ± 0.02 | 2 | Nonanol | 20.45 | 0.15 ± 0.00 | 50 |
| *Heterocyclic compounds* | | | | 2,7-Octadien-1-ol | 22.83 | 1.09 ± 0.02 | – |
| 2-Methylfuran | 2.38 | 0.17 ± 0.01 | 9 | *Esters* | | | |
| 2-Ethylfuran | 3.70 | 1.54 ± 0.10 | 2.3 | Methyl salicylate | 23.66 | 0.09 ± 0.00 | 40 |
| 2-Pentylfuran | 12.75 | 1.45 ± 0.04 | 5 | *Acids* | | | |
| Benzothiazole | 26.23 | 0.30 ± 0.03 | 80 | Acetic acid | 3.25 | 0.12 ± 0.00 | – |

Relative peak area was expressed as mean ± standard deviation; —, perception threshold of compound was undetected.

**Table 6.** ROAV of volatile compounds of PPNM

| Compound | ROAV | Sensory description |
|---|---|---|
| *Key flavor components (ROAV ≥ 1)* | | |
| Decanal | 100.00 | Sweet and wax fragrance, floral and orange aromas |
| *trans*-2-Nonenal | 43.65 | Green, wax and cucumber fragrance |
| Nonanoic aldehyde | 19.46 | Grease, floral, wax and orange aromas |
| Octyl aldehyde | 13.78 | Fat, orange and honey aromas |
| *trans*,*cis*-2,6-Nonadienal | 10.71 | Sweet, green and vegetable aromas |
| *trans*,*trans*-2,4-Nonadienal | 5.29 | Ester and floral aromas |
| *trans*-2-Decenal | 4.66 | Fat and meat aromas, mushroom scents |
| Benzaldehyde | 3.19 | Nutty and cheery aromas, almond bitterness |
| *cis*-4-Heptenal | 2.98 | Vegetable and linseed oil aromas |
| 3-Methylbutyraldehyde | 2.86 | Almond and nutty aromas |
| *trans*-2-Octenal | 2.58 | Green and fat aromas |
| 2-Ethylfuran | 2.13 | Bean, malty and malty aromas |
| Caproaldehyde | 1.81 | Green and leaf aromas, fishiness, fruity |
| 1-Octen-3-ol | 1.62 | Mushroom aromas, grease smell, fishiness |
| 1-Octen-3-ol | 1.26 | Nutty aromas, sweet almond smell, fishiness |
| *Main flavor components (0.1 ≤ ROAV < 1)* | | |
| Dodecenal | 0.94 | Soap, aldehydic and wax fragrance, orange aromas |
| 2-Pentylfuran | 0.92 | Bean, vegetable and fruity aromas, earth smells |
| *trans*,*trans*-2,4-Heptadienal | 0.92 | Green, aldehydic and chicken aromas |
| Benzeneacetaldehyde | 0.91 | Hyacinth fragrance |
| Acetophenone | 0.63 | Almond smells |
| Pentanal | 0.61 | Fruit and bread aromas |
| 2,3-Pentanedione | 0.33 | Caramel and cream aromas |
| 2-Methylbutyraldehyde | 0.29 | Green and nutty aromas, fruity |
| Undecanal | 0.28 | Wax, citrus and floral aromas |
| *trans*-2-Hexenal | 0.25 | Green and aldehydic aromas, fruity, spicy flavors |
| 2-Decanone | 0.22 | Fruity, musty |
| D-Limonene | 0.18 | Sweet, citrus and lemon aromas |

Only flavor components with ROAV ≥ 0.1 are presented.

(Table 4). The protein nutritional value of PPNM was estimated by AAS and CS from a different perspective. Based on the analysis of AAS and CS, methionine plus cystine were found to be the first limiting amino acids, while threonine and phenylalanine plus tyrosine were the second limiting amino acids (Table 4). The AAS of all EAA are higher than those in the FAO/WHO essential amino acid model. Tryptophan and lysine have AAS 2.77 and 1.6 times the FAO/WHO standards respectively. Thus PPNM could supplement the lack of tryptophan in corn food and could also be a desirable ingredient of rice-based food which is relatively low in lysine. The results also demonstrated that the EAAI was 101.7 (>95), which means that the EAA composition of PPNM was close to that of standard whole egg protein, suggesting that PPNM is a high-quality protein source. The BV reflects the degree of systemic bioavailability after digestion of proteins. The BV of PPNM was 76.7, being similar to that of beef (76) and higher than those of wheat (67), peanuts (59) and pork (74). Therefore the composition of amino acids in PPNM was extremely good and in accordance with the protein reference pattern provided by the WHO/FAO.

### Volatile constituents and key flavor components of PPNM

The volatile constituents in PPNM were extracted using SPME and analyzed and identified by GC/MS. The total ion chromatogram of the volatile constituents is presented in Fig. 2. By comparison with a standard NIST 2.0 spectral library, 55 different volatile flavor compounds were identified in PPNM, including aldehydes, ketones, alcohols, hydrocarbons and heterocyclic compounds (Table 5). Among them, some volatile components were found to possess large relative peak areas, such as acetophenone (12.8%), nonanoic aldehyde (6.1%), nonadecane (3.3%), decanal (3.2%) and benzaldehyde (3.0%). The perception threshold of some volatile constituents is given in Table 5.[31,32] Aldehydes are known to make a significant contribution to the whole food flavor profile. They possess low perception thresholds and can overlap with other volatile compounds, exhibiting an accumulated effect on flavor.[31] The characteristics of flavor change from green aromas, fruity aromas, nutty aromas to cheese aromas with increasing concentration of aldehydes.[31]

Owing to the large differences in perception threshold between some flavor compounds, the overall contribution of each flavor compound to PPNM should be evaluated by considering both their relative content and their perception threshold. According to the results presented in Table 5, decanal has low perception threshold and high relative content, thereby making the greatest contribution to whole flavor. Thus the ROAV of decanal was defined as 100 and the ROAVs of other volatile flavor components were calculated based on comparison with decanal.[31] The ROAVs of volatile flavor components and their sensory descriptions are presented in Table 6. PPNM was found to contain 15 key flavor components (ROAV ≥ 1) and 12 main flavor components (0.1 ≤ ROAV < 1).

## CONCLUSIONS

An efficient novel approach to recycle protein sources from surimi wash-water using I-CT is described here. This method has the advantages of high nitrogen recovery (98%) and simplicity of manipulation. The PPNM prepared in this work possesses high nutritional value. The results showed that the EAAI was 101.7 (>95), indicating that the EAA composition of PPNM was close to that of standard whole egg protein. Fifty-five volatile flavor compounds have been identified in PPNM by GC/MS, mainly including aldehydes, ketones, alcohols, hydrocarbons and heterocyclic compounds. The PPNM prepared as described in this work could be used as a nutraceutical and as an ingredient of functional foods.

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## REFERENCES

1 Bourtoom T, Chinnan MS, Jantawat P and Sanguandeekul R, Recovery and characterization of proteins precipitated from surimi wash-water. *LWT – Food Sci Technol* **42**:599–605 (2009).
2 Afonso MD and Bórquez R, Review of the treatment of sea food processing wastewaters and recover of proteins therein by membrane separation processes – prospects of the ultrafiltration of wastewaters from the fish meal industry. *Desalination* **142**:29–45 (2002).
3 Lin S, Chen L and Chen H, The change of thermal gelation properties of horse mackerel mince led by protein denaturation occurring in frozen storage and consequential air floatation wash. *Food Res Int* **38**:19–27 (2005).
4 H-Kittikun A, Bourneow C and Benjakul S, Hydrolysis of surimi wastewater for production of transglutaminase by *Enterobacter* sp. C2361 and *Providencia* sp. C1112. *Food Chem* **135**:1183–1191 (2012).
5 Zhang Z, Yang B and Huang X, Recovery of proteins from washings of surimi by ferric chloride. *Fish Sci Technol Info* **24**:207–210 (1997).
6 Lin TM, Park JW and Morrissey MT, Recovered protein and reconditioned water from surimi processing waste. *J Food Sci* **60**:4–9 (1995).
7 Thongraung C and Kangsanan S, Influence of pH, *NaCl and pre-incubation on utilisation of surimi wash water in generation of antioxidative material by using the Maillard reaction. Int J Food Sci Technol* **45**:1696–1702 (2010).
8 Huang L, Chen Y and Morrissey MT, Coagulation of fish proteins from frozen fish mince wash water by ohmic heating. *J Food Process Eng* **20**:285–300 (1997).
9 Ramírez JA, Velazquez G, Echevarría GL and Torres JA, Effect of adding insoluble solids from surimi wash water on the functional and mechanical properties of Pacific whiting grade A surimi. *Bioresour Technol* **98**:2148–2153 (2007).
10 Sanmartin E, Arboleya JC, Villamiel M and Moreno FJ, Recent advances in the recovery and improvement of functional proteins from fish processing by-products: use of protein glycation as an alternative method. *Compr Rev Food Sci Food Saf* **8**:332–344 (2009).
11 Suh JS, Cho SY, Son KT, Lee JS and Lee EH, Recovery and utilization of proteins and lipids from the washing wastewater in marine manufacture by isoelectric point shifting precipitation method: 3. *Utilization of the recovered lipids as the material for a processed food. J Kor Fish Soc* **28**:157–162 (1995).
12 Khatprathum A, Siriwongpaisaan P and Youravong W, Concentration of protein in fish mince wash water discharged from Surimi processing plant by ultrafiltration. *Desalin Water Treat* **21**:1–7 (2010).
13 Morr CV, Whey protein concentrate: an update. *Food Technol* **30**:18–19 (1976).
14 Savant VD and Torres JA, Fourier transform infrared analysis of chitosan based coagulating agents for treatment of surimi waste water. *J Food Technol* **1**:23–28 (2003).
15 Wibowo S, Velazquez G, Savant V and Torres JA, Surimi wash water treatment for protein recovery: effect of chitosan–alginate complex concentration and treatment time on protein adsorption. *Bioresour Technol* **96**:665–671 (2005).
16 Li DF, Ding HC and Zhou T, Covalent immobilization of mixed proteases, trypsin and chymotrypsin, onto modified PVC microspheres. *J Agric Food Chem* **61**:10447–10453 (2013).
17 China National Food Safety Standards, *Method for Determination of Dry Matter and Water Content in Fruit and Vegetable Products*. GB/T 8858–1988 (1988).
18 China National Food Safety Standards, *Determination of Fat in Foods*. GB/T 5009.6-2003 (2003).
19 China National Food Safety Standards, *Determination of Protein in Foods*. GB/T 5009.5-2010 (2010).
20 Schägger H, Tricine–SDS-PAGE. *Nat Protocols* **1**:16–22 (2006).
21 Schägger H and von Jagow G, Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* **166**:368–379 (1987).
22 AOAC, *Official Methods of Analysis*. Association of Official Analytical Chemists, Arlington, VA (1984).
23 FAO/WHO Ad Hoc Expert Committee, Energy and protein requirements. *FAO Nutr Meet Rep Ser* **52**:40–73 (1973).
24 Yuan Y, Li XY, Yang XS and Huang HL, Analysis and comparison on the nutritional components of different Antarctic krill meals. *Mar Fish* **34**:457–463 (2012).
25 Bu JZ, Dai ZY, Zhou T, Lu YB and Jiang QQ, Chemical composition and flavour characteristics of a liquid extract occurring as waste in crab (*Ovalipes punctatus*) processing. *J Sci Food Agric* **93**:2267–2275 (2013).
26 Zhou SD, Chen XX, Xiao SY, Lei Y, Wang LL, Zhou LP, *et al.*, Studies on the chemical components and antibacterial activity of essential oil from the leaves of *Rubus corchorifolius*. *J Chin Med Mater* **32**:1547–1550 (2009).
27 Liu D, Zhou G and Xu X, 'ROAV' method: a new method for determining key odor compounds of Rugao ham. *Food Sci* **29**:370–374 (2008).
28 Lu SJ, Mei SW, Liu XH, Jin TY and Qi XM, Research of protein recovery from surimi wastewater by isoelectric precipitation. *Sci Technol Food Ind* **33**(18):93–95 (2012).
29 Wu YX, Jin T, Wang JP, Le Y and Lin GF, Research on the recovery of protein from surimi wash water of hairtail *Trichiurus haumela* by natural algae flocculant sodium alginate. *Oceanol Limnol Sin* **43**:335–339 (2012).
30 Mitchell HH and Block RJ, Some relationships between the amino acid content of proteins and their nutritive values for the rat. *J Biol Chem* **163**:599–620 (1946).
31 Sun B, *The Edible flavouring Techniques*. Chemical Industry Press, Beijing, pp. 23–350 (2003).
32 Gu SQ, Tao NP, Wu N, Zhang JJ, Wang XC and Zhang W, A new method based on ROAV value to identify the characteristic key volatile compounds of crab flavor. *Sci Technol Food Ind* **33**(13):410–416 (2012).

  