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Nutritional quality analysis of high-moisture extrudates containing mixed proteins from soy and surimi

Anna Hu,†[a] Yujie Zhang,†[a] Jinchuang Zhang, *[a] Tongqing Li,[a] Zhaojun Wang [b] and Qiang Wang *[a]

High-moisture extrusion technology emerges as a prime choice for preparing alternative protein products with a meat-like texture. However, the nutritional aspects of these products, prepared from a blend of plant and animal proteins, remain unclear. This study investigated the nutritional qualities of extrudates derived from soy protein isolate (SPI) and surimi, exploring ratios ranging from 90 : 10 to 50 : 50, with varied extrusion temperature (125 °C, 135 °C and 145 °C) and moisture content (65%, 70% and 75%). Results revealed the significant role played by surimi in enhancing both amino acid and fatty acid contents in high-moisture extrudates originating from SPI and surimi. Notably, the first limiting amino acid score (AAS/MET + CYS) increased significantly from 88.82 to 109.50 as the surimi content increased from 10% to 50%. Moreover, the levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the extrudates significantly increased, concurrently reducing the n-6/n-3 fatty acid ratio. At a higher moisture content (70–75%), increasing extrusion temperature bolstered the fatty acid content in the extrudates. When the SPI–surimi ratio was 90 : 10, the gastric digestibility of the extrudates was the highest (60.20%). Meanwhile, the highest small intestinal digestibility was 93.07% at a SPI–surimi ratio of 70 : 30. At lower extrusion temperatures (125–135 °C), increasing moisture content led to a notable increase in the small intestinal digestibility of the extrudates. SPI–surimi ratios and hydro-thermal combined parameters have significant effects on the *in vitro* digestibility of high-moisture extrudates. This study could contribute to the improvement of nutritional qualities of alternative protein products based on mixed proteins from soy and surimi.

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Sustainability spotlight

According to the UN's Sustainable Development Goals (SDGs), alternative protein products containing mixed proteins from soy and surimi can contribute to sustainability in several aspects. Firstly, this study focuses on the nutrition analysis of high-moisture extruded mixed proteins from soy and surimi, including amino acid and fatty acid contents, *etc.*, which corresponds to SDG 2, improving nutrition and promoting sustainable agriculture. Secondly, partially replacing animal protein with plant protein has the potential to mitigate chronic diseases like heart disease and diabetes, thereby contributing to the achievement of SDG 3 of healthier food and diets. Thirdly, this study is also beneficial for achieving SDG 13 for fewer greenhouse gas emissions through alternative protein product development. In conclusion, this study has a positive impact on the UN SDGs.

Introduction

The global population is projected to reach about 10 billion individuals by 2050,[1] resulting in a rapid rise in the global demand for protein sources. The global demand for animal-derived meat products is expected to reach 455 million tons.[2] To meet this demand, it is necessary to explore new protein sources to complement traditional ones. Alternative protein sources[3] such as plant proteins (grains, legumes, tubers, and oilseeds), insect proteins, microorganisms (fungi and bacteria) and aquatic proteins (algae) are gaining attention. These sources are grown and processed in ways that reduce greenhouse gas emissions,[4,5] land, and water resource wastage.[6] They are already used in food, cosmetics and pharmaceuticals.[1,7] Various meat-like alternative protein products have been developed from sustainable sources.[8,9] Soy protein is known for its excellent gelation properties and fibrous structure formation.[10–12] Surimi, derived from animals, contains unsaturated fatty acids (*e.g.*, DHA and EPA). Mixing soy protein with surimi can create alternative protein products with comprehensive nutrition quality.[13,14] These products have

[a]*Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Key Laboratory of Agro-Products Processing, Ministry of Agriculture and Rural Affairs, Beijing 100193, China. E-mail: zhangjinchuang1002@163.com; wangqiang06@caas.cn*

[b]*State Key Laboratory of Food Science and Resource, Jiangnan University, Wuxi, 214122, China*

† Equal contribution.

garnered attention, particularly in terms of their nutrition profile,[14,15] including amino acids, fatty acids and digestibility.

Combining soy protein and surimi results in products with superior nutritional properties.[13,16] Researchers have explored different processing methods,[7,8] such as ultra-high pressure, microwave heating, 3D printing and ultrasonic technology, to enhance the quality of surimi-based products. Food extrusion technology has also been used to improve the digestibility and texture of soy protein and surimi blends.[17] Kaur *et al.*[18] showed that adjusting the ratios of surimi and wheat protein could enhance the digestibility of extrudates. Adding soy protein can increase the content of essential amino acids,[19] but a higher extrusion temperature and a lower moisture content may lead to amino acid loss.[20]

High-moisture extrusion is a promising method for creating alternative protein products with a meat-like texture.[21] One of the advantages of high-moisture extrusion is that the extrudates produced are ready-to-eat and have an improved fibrous structure.[22] It is energy-efficient and environmentally sustainable,[9,23] improving the digestibility of both plant and animal proteins while reducing anti-nutritional factors.[24] At present, raw materials mainly consist of plant proteins such as soy protein, pea protein, and wheat gluten. Gradually, animal proteins have been added, enriching the products with a variety of nutrients, including proteins, lipids, carbohydrates, minerals, vitamins and dietary fiber.[13,16,17] Extrusion can be used to imitate the texture of marine products, such as by adding surimi during extrusion.[25] Altering the raw material ratio and extrusion process parameters during the high-moisture extrusion can further enhance nutritional properties.[17,26] Kaur *et al.*[18] showed that the content of essential amino acids and fatty acids can be increased as the surimi content increased. Lin *et al.*[27] showed that the dietary fiber content of surimi and the antioxidant capacity were enhanced with the addition of wheat. Pudtikajorn *et al.*[28] reported that the addition of surimi increased the nutritional quality of fish tofu. Sorensen *et al.*[29] reported that a low extrusion temperature improved the digestibility of extruded feeds. Delgado *et al.*[30] found that different extrusion temperatures, screw speeds and moisture contents changed the nutritional content of extrudates. However, the nutritional qualities of mixed proteins from soy and surimi under high-moisture extrusion conditions (moisture content ranging from 40% to 80%) remain uncertain.[31]

This study aims to analyze nutritional changes in extrudates through high-moisture extrusion, varying SPI–surimi ratios and extrusion parameters. It also seeks to explore the effect of SPI–surimi ratios on amino acids and fatty acids in extrudates and examine how hydrothermal parameters affect these nutritional aspects. Additionally, the digestibility of the mixed proteins from soy and surimi was investigated. These findings reveal the nutritional potential of alternative protein products with a mixture of plant and animal proteins prepared using high-moisture extrusion.

Materials and methods

Materials

Soy protein isolate (SPI) was supplied by Yihai Kerry Co., Ltd. (Shanghai, China), containing 90.81% protein (dry basis), 5.55% moisture, 0.36% fat (dry basis) and 4.67% ash content (dry basis). Surimi was purchased from Shengteng Seafood Co., Ltd. (Qingdao, China), containing 52.78% protein (dry basis), 67.97% moisture, 8.38% fat (dry basis) and 1.73% ash content (dry basis).

High-moisture extrusion experiments

Before extruding, the SPI and surimi were mixed using a mixer (JHF-20L, Zhengzhou Jinhe Machinery Manufacture Co., Ltd, China). The extrusion experiments of the SPI–surimi mixtures were carried out using a co-rotating twin-screw food extruder (FMHE36-24, FUMACH, China) with a screw diameter of 36 mm and a length/diameter ratio of 24 : 1. The extruder barrel was segmented into a feeding zone and five temperature-controlled zones. At the exit of the barrel, a long cylindrical cooling die with a diameter of 22 mm was attached. The extrusion conditions of different SPI–surimi ratios and hydro-thermal combined parameters were set according to Tables 1 and 2, respectively. The cooling die was kept at 50 °C controlled by the running moisture.[8]

Determination of amino acids

The amino acid score (AAS) was estimated from the amount of protein required to provide the minimal essential amino acid (EAA) pattern for adults, using the FAO/WHO (2007) reference pattern and according to the equation:[32]

$$\mathrm{AAS} = \frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in requirement pattern}} \quad (1)$$

The chemical score (CS) and the essential amino acid index (EAAI) were calculated by the method of the equations:[33]

$$\mathrm{CS} = \frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in 1 g egg protein}} \quad (2)$$

$$\mathrm{EAAI} = \sqrt[n]{\frac{(\mathrm{EAA}_1 \times 100)(\mathrm{EAA}_2 \times 100)(\ldots)(\mathrm{EAA}_n \times 100)[\text{sample}]}{(\mathrm{EAA}_1 \times 100)(\mathrm{EAA}_2 \times 100)(\ldots)(\mathrm{EAA}_n \times 100)[\text{reference}]}} \quad (3)$$

The biological value (BV) is the ratio of the amount of nitrogen used by the human body and the amount of nitrogen absorbed by the body after protein was digested and absorbed. BV was calculated using eqn (4):[34]

$$\mathrm{BV} = (1.09 \times \mathrm{EAAI}) - 11.70 \quad (4)$$

The nutritional index (NI) was used to comprehensively describe the protein content and amino acid composition patterns, which was calculated using eqn (5):[35]

$$\mathrm{NI} = \mathrm{EAA} \times \text{protein (g/100 g)}/100 \quad (5)$$

Table 1 High-moisture extrusion conditions with different SPI : surimi ratios

Number	SPI : surimi	Extrusion temperature (°C)	Moisture content (%)	Screw speed (rpm)	Feed rate (kg h^{-1})
1	90 : 10	135	70	210	7
2	80 : 20	135	70	210	7
3	70 : 30	135	70	210	7
4	60 : 40	135	70	210	7
5	50 : 50	135	70	210	7

Table 2 High-moisture extrusion conditions with different hydro-thermal combined parameters

Number	SPI : surimi	Extrusion temperature (°C)	Moisture content (%)	Screw speed (rpm)	Feed rate (kg h^{-1})
1	80 : 20	125	65	210	7
2	80 : 20	125	70	210	7
3	80 : 20	125	75	210	7
4	80 : 20	135	65	210	7
5	80 : 20	135	70	210	7
6	80 : 20	135	75	210	7
7	80 : 20	145	65	210	7
8	80 : 20	145	70	210	7
9	80 : 20	145	75	210	7

Determination of fatty acids

The fatty acid profiles were analyzed in a previous study.[33] The atherosclerosis index (IA) and thrombosis index (IT), used to assess the effect of fatty acids in extrudates on human cardiovascular diseases, were calculated according to eqn (6) and (7),[36] respectively.

$$\mathrm{IA} = \frac{C_{12:0} + 4 \times C_{14:0} + C_{16:0}}{\sum(\mathrm{MUFA} + \mathrm{PUFA})} \quad (6)$$

$$\mathrm{IT} = \frac{C_{14:0} + C_{16:0} + C_{18:0}}{0.5 \times \sum \mathrm{MUFA} + 0.5 \times \sum \text{n-6 PUFA} + 3 \times \sum \text{n-3 PUFA} + \frac{\text{n-6}}{\text{n-3}}} \quad (7)$$

The monounsaturated fatty acid and the polyunsaturated fatty acid were denoted as MUFA and PUFA, respectively.

In vitro protein digestibility

The *in vitro* protein digestibility (IVPD) of the extrudates was determined according to a previous study.[8] With some modifications, 0.1 g triturated extrudates were diluted with 15 mL of 0.1 M HCL and preheated at 37.5 °C for 10 min. 2 mg pepsin (Sigma-Aldrich Ltd., St Louis, USA) was added into the preheated solution and kept at 37.5 °C for 3 h. The pepsin hydrolysis was ended by adding 7.5 mL of 0.2 M NaOH. The solution was collected to analyze the gastric IVPD. The simulated intestinal digestibility started with adding 7.5 mL of 0.2 M phosphate buffer (pH 8.0) containing 4 mg trypsin (Sigma-Aldrich Ltd., St Louis, USA) into the solution of the ended pepsin hydrolysis, and then the solution was heated at 37 °C for 4 h. The trypsin hydrolysis was ended by boiling for 10 min. The final solution was collected. All of the collected solution was precipitated with isopycnic 10% trichloroacetic acid for 1 h and then centrifuged at 1000 *g* for 30 min.[37] The liquid supernatant was collected to determine the protein content. The blank sample was prepared by treatments under the described conditions without the extrudate sample. The IVPD of the extrudates was calculated using the equation:[34]

$$\mathrm{IVPD}\ (\%) = (P_s - P_0)/P_e \times 100\% \quad (8)$$

The P_s, P_0 and P_e represent the protein content of the liquid supernatant, the blank and the triturated extrudates, respectively.

Statistical analysis

Analysis of variance (ANOVA) was used to analyze all data through Statistical Product and Service Solutions software (version 26.0, SPSS Inc., Chicago, USA). Duncan's test was used to evaluate the comparisons between treatments. The statistical significance level was set at 0.05. Principal component analysis (PCA) was performed using The Unscrambler X 10.4.

Results and discussion

Amino acid evaluation

Effect of SPI–surimi ratios. As can be seen in Table 3, the amino acid content increased as the surimi content increased from 10% to 40%, which was consistent with the study of others.[38] It was also found that the most abundant amino acids were Glu (190.05–222.38 mg per g protein), Asp (110.72–120.92 mg per g protein) and Leu (75.10–89.69 mg per g protein). However, the scarcest amino acids were Cys (7.76–9.00 mg per g protein), Trp (9.77–11.20 mg per g protein) and Met (11.49–16.33 mg per g protein). Aberoumand and Baesi[39] and Hughes *et al.*[40] also found that Glu, Asp and Leu took up the highest proportion of amino acids in the surimi and SPI and Cys and Met were the scarcest amino acids of SPI, suggesting that the high-moisture extrusion had no effect on the general composition of amino acids in SPI and surimi blends. Fig. 1 displays the amino acid scores (AASs) and chemical scores (CSs) of the extrudates at different SPI–surimi ratios. It indicated that Met + Cys was the first limiting amino acid, and the corresponding scores of AAS and CS were 88.82–109.50 and 55.83–68.83, respectively, which were increased as the surimi content increased from 10% to 50%. The corresponding values for Tyr + Phe were the highest in the range of 191.65–218.28 and 121.38–138.25, respectively. Hughes *et al.*[40] found that the first limiting amino acid was Met + Cys in SPI, and Phe + Tyr got the highest AAS values. These results indicated that SPI played a role in the AAS evaluation of extrudates with different SPI–surimi ratios by high-moisture extrusion processing. At the SPI–surimi ratios of 80 : 20, 60 : 40 and 50 : 50, the AAS of the extrudates was more than 100, suggesting that the amino acid contents of the extrudates was much higher than those of the FAO/WHO and the extrudates at these ratios could meet the requirements of adults' body.[41]

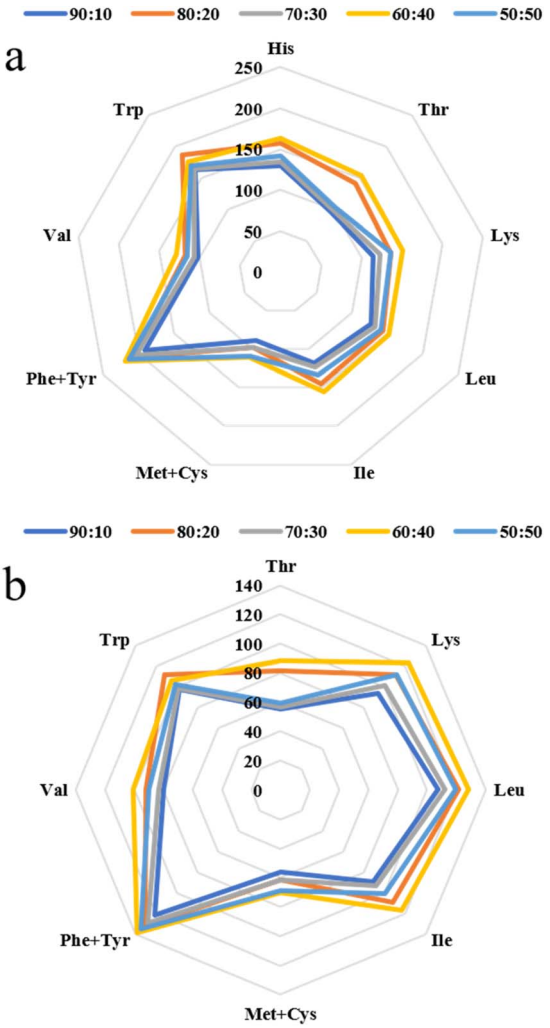

Fig. 1 Amino acid scores (AASs) (a) and chemical scores (CSs) (b) of the extrudates with different SPI–surimi ratios.

Table 3 Amino acid content of the extrudates with different SPI–surimi ratios[a]

Amino acids (mg per g protein)		SPI–surimi ratios				
		90 : 10	80 : 20	70 : 30	60 : 40	50 : 50
Essential amino acids (EAAs)	THR	22.05 ± 0.51c	32.55 ± 0.62b	22.71 ± 0.53c	35.23 ± 0.11a	23.78 ± 1.57c
	VAL	39.80 ± 0.11e	46.15 ± 0.64b	41.76 ± 0.11d	50.36 ± 0.68a	44.96 ± 0.25c
	MET	11.49 ± 0.13c	13.00 ± 0.01b	13.10 ± 0.28b	15.50 ± 0.52a	16.33 ± 0.48a
	ILE	35.54 ± 0.27e	43.43 ± 0.45b	37.02 ± 0.16d	46.73 ± 0.30a	40.25 ± 0.61c
	LEU	75.10 ± 0.30d	85.07 ± 2.02b	78.41 ± 0.26c	89.69 ± 0.66a	83.59 ± 0.93b
	TRP	9.77 ± 0.25b	11.20 ± 0.36a	9.85 ± 0.13b	10.57 ± 0.52ab	10.17 ± 0.11b
	PHE	43.62 ± 0.16d	45.77 ± 0.25c	46.17 ± 0.52c	48.18 ± 0.94a	47.12 ± 0.39ab
	LYS	51.63 ± 0.45d	61.21 ± 1.12b	55.40 ± 0.47c	67.69 ± 0.81a	61.61 ± 0.28b
Non-essential amino acids (NEAAs)	ASP	110.72 ± 0.16c	113.85 ± 1.85bc	115.81 ± 0.70b	120.92 ± 1.45a	119.93 ± 1.46a
	HIS	19.55 ± 0.08d	23.60 ± 0.42b	20.25 ± 0.13d	24.49 ± 0.54a	21.23 ± 0.24c
	ARG	59.54 ± 0.80c	71.98 ± 1.10a	62.50 ± 0.42bc	75.65 ± 0.81a	64.45 ± 3.37b
	PRO	51.02 ± 4.12a	40.94 ± 2.45b	51.75 ± 1.24a	41.94 ± 1.03b	46.64 ± 2.65b
	CYS	8.05 ± 0.05b	8.48 ± 0.66ab	8.53 ± 0.03ab	9.00 ± 0.25a	7.76 ± 0.27b
	TYR	29.21 ± 0.23c	32.90 ± 0.26b	31.00 ± 0.74c	34.77 ± 0.28a	33.79 ± 1.38ab
	SER	40.60 ± 1.01c	43.58 ± 0.83b	41.45 ± 0.47c	47.58 ± 0.26a	41.89 ± 0.51c
	GLU	190.05 ± 0.14d	207.33 ± 1.34b	198.52 ± 1.84c	222.38 ± 2.50a	208.33 ± 1.51b
	GLY	32.38 ± 0.01c	37.68 ± 0.89b	32.91 ± 0.40c	39.49 ± 0.48a	33.67 ± 0.64c
	ALA	34.97 ± 0.36d	41.40 ± 1.83ab	37.07 ± 0.52cd	43.51 ± 0.37a	39.54 ± 1.46bc
Total amino acids (TAAs)		865.01 ± 46.32c	960.06 ± 14.23b	904.17 ± 4.93bc	1023.60 ± 12.01a	945.01 ± 12.05b

[a] Different letters in the same row mean significant differences ($p < 0.05$).

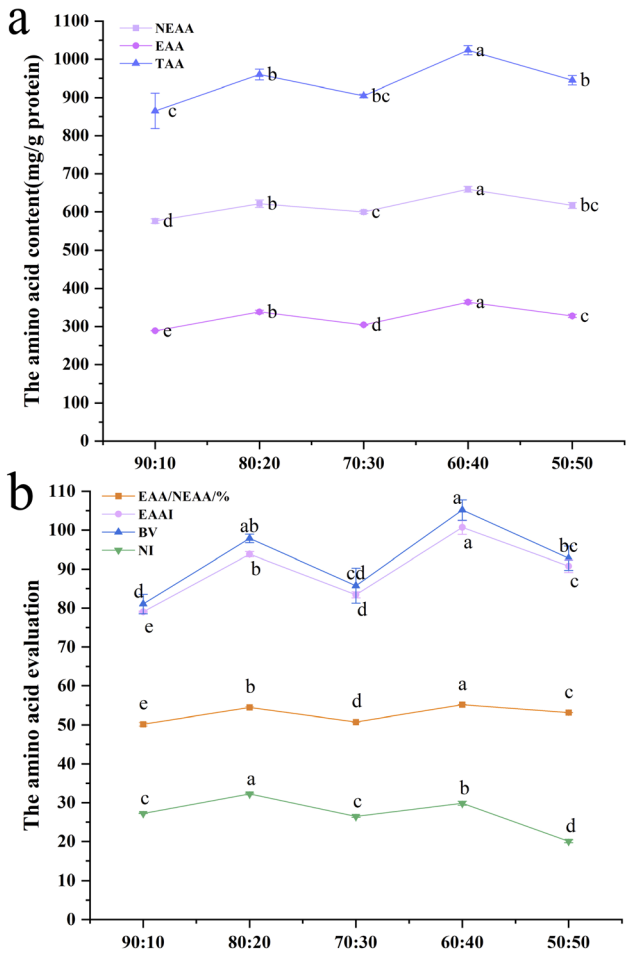

Fig. 2 The amino acid content with NEAA, EAA and TAA (a) and the amino acid evaluation with EAA/NEAA, EAAI, BV and NI (b) of the extrudates with different SPI–surimi ratios, and different letters indicate significant differences ($p < 0.05$).

In Fig. 2, the EAA/EAAI values were between 50.17% and 55.16%, which could almost reach the reference values of 60% recommended by the FAO/WHO. At a SPI–surimi ratio of 60 : 40, the TAA, EAA, NEAA, EAA/NEAA, EAAI and BV of the extrudates were significantly higher than those of others. In Fig. 2b, at a SPI–surimi of 80 : 20, the NI of the extrudates was 32.24, which was significantly higher than that of all the others. Results showed that as the surimi content increased from 10% to 50%, the extrudates were rich in various amino acids and the amino acid pattern was more balanced, especially at a SPI–surimi ratio of 60 : 40. Ai *et al.*[19] also reported that fish meal from surimi can improve the balance of the amino acid pattern. When the surimi content was excessive (50%), the interactions between soy protein and surimi protein molecules became weaker, while the protein–protein interactions of surimi were enhanced, which might not be conducive to the retention of amino acids.

The principal component analysis (PCA) soring plot and factor loading plot can make it easier to discriminate the differences of the samples visually and help to determine the degree of contribution of the variances (PC1-75% and PC2-24%). According to Fig. 3, EAA, NEAA and TAA were significantly related to the ratio of 60 : 40. And the NI was critically related to the ratio of 80 : 20.

Effect of hydro-thermal combined parameters. Table 4 shows the amino acid contents of the extrudates with different hydro-thermal parameters. All the extrudates were rich in Glu (186.66–217.33 mg per g protein), Asp (100.32–116.70 mg per g protein) and Leu (73.88–82.10 mg per g protein), but lack of Cys (7.78–9.45 mg per g protein). Meanwhile, at a moisture content of 75%, as the extrusion temperature increased from 125 °C to 145 °C, the TAA content decreased from 968.68 mg g^{-1} to 876.47 mg g^{-1}. This might be due to the degradation of amino acids by the Maillard reaction, which was consistent with Iwe *et al.*[42] who also found the loss of Arg (21%) and Asp (14%) as extrusion temperature increased from 135 °C to 160 °C due to the Maillard reaction.[43] Csapó *et al.*[44] also found the loss of Lys (21%) in soy protein as extrusion temperature increased from 101 °C to 220 °C. Furthermore, the TAA content was the highest (968.68 mg per g protein) at a moisture content of 75% and extrusion temperature of 125 °C, while it was the lowest (828.36 mg per g protein) at a moisture content of 70% and extrusion temperature of 135 °C, suggesting that the increasing

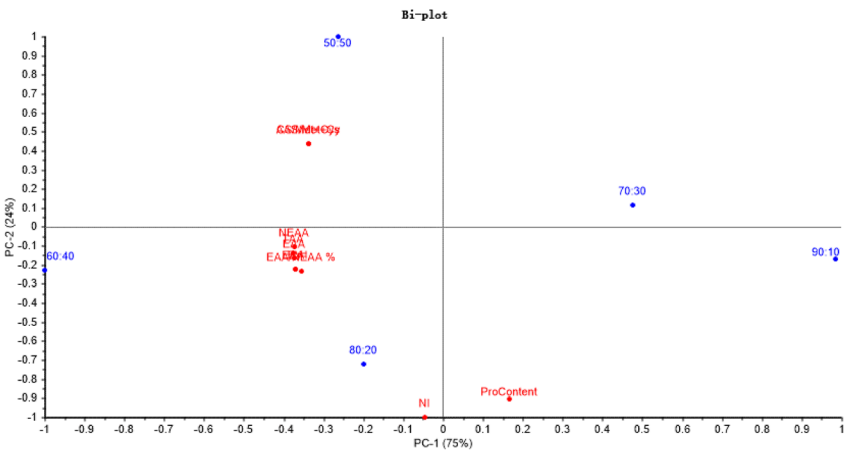

Fig. 3 The PCA Bi-plots of the extrudates with different SPI–surimi ratios.

Table 4 Amino acid contents of the extrudates with different hydro-thermal parameters[a]

Amino acid contents (mg per g protein)		Hydro-thermal parameters								
		125 °C-65%	135 °C-65%	145 °C-65%	125 °C-70%	135 °C-70%	145 °C-70%	125 °C-75%	135 °C-75%	145 °C-75%
EAA	THR	26.70 ± 1.82bcd	28.96 ± 0.47abc	26.62 ± 1.22cd	29.14 ± 0.06abc	25.73 ± 0.76d	29.25 ± 0.18abc	31.67 ± 2.06a	29.80 ± 0.59ab	29.32 ± 2.02abc
	VAL	45.55 ± 3.31ab	48.31 ± 0.12a	44.68 ± 2.23ab	46.50 ± 0.79ab	42.18 ± 1.27b	47.60 ± 0.13a	47.83 ± 0.91a	44.75 ± 0.23ab	42.93 ± 2.85b
	MET	12.59 ± 0.78abc	13.41 ± 0.38a	12.41 ± 0.38abc	12.61 ± 0.07abc	11.45 ± 0.29c	12.94 ± 0.49ab	13.05 ± 0.21a	12.45 ± 0.26abc	11.8 ± 0.77bc
	ILE	39.32 ± 2.63ab	41.67 ± 0.01a	38.83 ± 1.99ab	41.36 ± 0.64a	37.04 ± 1.14b	41.91 ± 0.66a	42.96 ± 1.17a	40.47 ± 0.10ab	39.50 ± 2.86ab
	LEU	75.18 ± 4.54abc	79.53 ± 0.06ab	73.88 ± 3.47bc	78.84 ± 1.28ab	69.87 ± 2.42c	79.00 ± 0.49ab	82.10 ± 3.28a	78.45 ± 0.22ab	75.38 ± 5.61abc
	TRP	12.06 ± 0.52ab	12.37 ± 0.69a	10.57 ± 0.40cd	11.09 ± 0.35bc	10.26 ± 0.35cd	10.92 ± 0.42bcd	11.18 ± 0.29abc	11.05 ± 0.82bcd	9.84 ± 0.30a
	PHE	43.64 ± 2.79ab	46.96 ± 0.06a	43.83 ± 1.56ab	45.00 ± 0.78ab	41.55 ± 0.95b	46.76 ± 0.31a	46.52 ± 1.73ab	44.27 ± 0.82ab	42.86 ± 3.85ab
	LYS	59.41 ± 4.07abc	62.89 ± 0.39a	58.31 ± 2.40abc	61.09 ± 0.95ab	54.70 ± 1.41c	62.29 ± 0.54a	63.16 ± 1.48a	59.59 ± 0.35abc	55.93 ± 4.03bc
NEAA	ASP	107.57 ± 7.38abc	115.41 ± 0.94ab	105.89 ± 4.55abc	111.51 ± 1.34ab	100.32 ± 2.67c	113.71 ± 0.91ab	116.70- ± 4.18a	109.97 ± 0.47abc	104.8 ± 7.74bc
	HIS	21.41 ± 1.37abc	22.47 ± 0.69ab	20.97 ± 0.82bc	22.33 ± 0.06ab	19.79 ± 0.42c	22.53 ± 0.24ab	23.19 ± 0.87a	22.17 ± 0.15ab	21.41 ± 1.51abc
	ARG	68.40 ± 4.94abc	71.96 ± 1.09ab	66.79 ± 2.86abc	70.41 ± 0.48ab	63.13 ± 1.50ab	71.34 ± 0.44ab	72.59 ± 2.36a	68.86 ± 0.72abc	65.33 ± 4.43bc
	PRO	30.77 ± 0.26d	33.9 ± 0.63bcd	31.11 ± 2.89cd	35.08 ± 0.3abc	30.09 ± 1.24d	34.96 ± 0.77abc	38.21 ± 2.79a	36.52 ± 0.10ab	36.52 ± 2.45ab
	CYS	8.32 ± 0.30abc	8.79 ± 0.66abc	9.04 ± 0.69ab	8.90 ± 0.40abc	7.78 ± 0.18c	8.76 ± 0.16abc	9.45 ± 0.69a	8.93 ± 0.34abc	8.00 ± 0.21bc
	TYR	27.72 ± 1.70bc	29.43 ± 0.17ab	27.85 ± 1.04bc	29.74 ± 0.35ab	26.47 ± 0.84c	29.79 ± 0.25ab	31.37 ± 1.36a	30.28 ± 0.23ab	29.13 ± 2.03abc
	SER	39.40 ± 2.86bc	42.88 ± 0.75abc	39.20 ± 2.17bc	42.95 ± 0.18abc	37.73 ± 1.15c	43.18 ± 0.30ab	46.78 ± 2.95a	43.29 ± 2.18ab	42.15 ± 3.12abc
	GLU	200.27 ± 14.25abc	214.26 ± 1.55a	197.65 ± 8.58abc	208.47 ± 2.50ab	186.66 ± 5.47c	211.89 ± 1.48ab	217.33 ± 6.60a	205.66 ± 1.90abc	192.45 ± 14.31bc
	GLY	31.54 ± 2.27bc	33.85 ± 0.19ab	31.58 ± 1.07bc	34.19 ± 0.19ab	30.49 ± 1.07c	34.15 ± 0.10ab	36.06 ± 1.40a	34.12 ± 0.30ab	33.56 ± 2.21abc
	ALA	34.42 ± 2.15bc	36.86 ± 0.24ab	34.46 ± 1.29bc	36.09 ± 0.31abc	33.12 ± 0.74c	37.47 ± 0.02ab	38.53 ± 1.77a	36.20 ± 0.35abc	35.55 ± 1.82abc
TAA		884.27 ± 56.83ab	943.91 ± 8357a	873.67 ± 38.8ab	925.30 ± 11.02ab	828.36 ± 20.30b	938.45 ± 5.40a	968.68 ± 35.52a	916.83 ± 8.10ab	876.47 ± 62.12ab

[a] Different letters in the same row mean significant differences ($p < 0.05$).

Table 5 Amino acid scores (AASs) and chemical scores (CSs) of the extrudates with different hydro-thermal parameters[a]

Amino acid evaluation (scores)		Hydro-thermal parameters								
		125 °C-65%	135 °C-65%	145 °C-65%	125 °C-70%	135 °C-70%	145 °C-70%	125 °C-75%	135 °C-75%	145 °C-75%
AAS	HIS	142.72 ± 9.14abc	149.82 ± 4.61ab	139.78 ± 5.47bc	148.85 ± 0.40ab	131.95 ± 2.83c	150.20 ± 1.63ab	154.58 ± 5.82a	147.75 ± 0.99ab	142.72 ± 10.08abc
	THR	116.08 ± 7.93bcd	125.89 ± 2.04abc	115.71 ± 5.32cd	126.71 ± 0.25abc	111.85 ± 3.27d	127.18 ± 0.81abc	137.68 ± 8.95a	129.55 ± 2.57ab	127.46 ± 8.74abc
	LYS	132.02 ± 9.06abc	139.75 ± 0.86a	129.57 ± 5.32abc	135.75 ± 2.12ab	121.56 ± 3.15c	138.42 ± 1.19a	140.35 ± 3.30a	132.41 ± 0.76abc	124.28 ± 8.97bc
	LEU	131.07 ± 8.79ab	138.90 ± 0.01a	129.43 ± 6.66ab	137.85 ± 2.16a	123.45 ± 3.78b	139.68 ± 2.21a	143.19 ± 3.87a	134.89 ± 0.33ab	131.67 ± 9.5ab
	ILE	127.43 ± 7.70abc	134.8 ± 0.10ab	125.21 ± 5.88bc	133.62 ± 2.17ab	118.43 ± 4.10c	133.89 ± 0.83ab	139.15 ± 5.57a	132.97 ± 0.37ab	127.77 ± 9.52abc
	MET + CYS	95.05 ± 3.54abc	100.91 ± 1.75a	97.50 ± 1.74abc	97.77 ± 0.30abc	87.41 ± 1.30c	98.64 ± 2.22ab	102.27 ± 0.95a	97.18 ± 1.21abc	90.00 ± 3.49bc
	PHE + TYR	187.79 ± 11.83ab	201.03 ± 0.59a	188.63 ± 6.83ab	196.68 ± 2.95ab	179.00 ± 4.72b	201.45 ± 1.47a	204.97 ± 8.13a	196.18 ± 2.76ab	189.45 ± 15.48ab
	VAL	119.80 ± 8.48ab	127.13 ± 0.32a	117.58 ± 5.73ab	122.37 ± 2.02ab	111.00 ± 3.24b	125.26 ± 0.34a	125.87 ± 2.33a	117.76 ± 0.57ab	112.97 ± 7.31b
	TRP	200.93 ± 8.70ab	206.12 ± 11.38a	176.10 ± 6.72cd	184.85 ± 5.85bc	171.04 ± 5.84cd	181.97 ± 7.09bcd	186.29 ± 4.88bc	184.13 ± 13.7bcd	163.89 ± 5.02d
CS	THR	66.75 ± 4.56bcd	72.38 ± 1.17abc	66.54 ± 3.06cd	72.86 ± 0.14abc	64.32 ± 1.87d	73.13 ± 0.47abc	79.17 ± 5.14a	74.50 ± 1.48ab	73.29 ± 5.02abc
	LYS	108.02 ± 7.42abc	114.34 ± 0.70a	106.02 ± 4.35abc	111.07 ± 1.73ab	99.46 ± 2.57c	113.26 ± 0.97a	114.84 ± 2.69a	108.34 ± 0.63abc	101.69 ± 7.33bc
	LEU	107.40 ± 6.49ab	113.62 ± 0.08a	105.54 ± 4.96cd	112.62 ± 1.82bc	99.82 ± 3.46cd	112.85 ± 0.70bcd	117.29 ± 4.69abc	112.07 ± 0.32bcd	107.69 ± 8.03d
	ILE	98.30 ± 6.59abc	104.18 ± 0.01ab	97.08 ± 5.00bc	103.39 ± 1.62ab	92.59 ± 2.84c	104.76 ± 1.65ab	107.40 ± 2.91a	101.17 ± 0.24ab	98.75 ± 7.13abc
	MET + CYS	59.74 ± 2.23abc	63.43 ± 1.10a	61.29 ± 1.10abc	61.46 ± 0.18abc	54.94 ± 0.82c	62.00 ± 1.40ab	64.29 ± 0.59a	61.09 ± 0.76abc	56.57 ± 2.19bc
	PHE + TYR	118.93 ± 4.37ab	127.32 ± 0.65ab	119.47 ± 5.54ab	124.57 ± 2.42ab	113.37 ± 5.58b	127.58 ± 0.86ab	129.82 ± 3.81a	124.25 ± 3.02ab	119.98 ± 10.85ab
	VAL	91.10 ± 6.60ab	96.61 ± 0.25a	89.37 ± 4.46ab	93.00 ± 1.58ab	84.35 ± 2.53b	95.19 ± 0.26a	95.66 ± 1.82a	89.51 ± 0.45ab	85.86 ± 5.71b
	TRP	120.56 ± 5.23ab	123.67 ± 6.83a	105.66 ± 4.03cd	110.91 ± 3.51bc	102.63 ± 3.50cd	109.18 ± 4.26bcd	111.77 ± 2.93abc	110.47 ± 8.22bcd	98.34 ± 3.01d

[a] Different letters in the same row mean significant differences ($p < 0.05$).

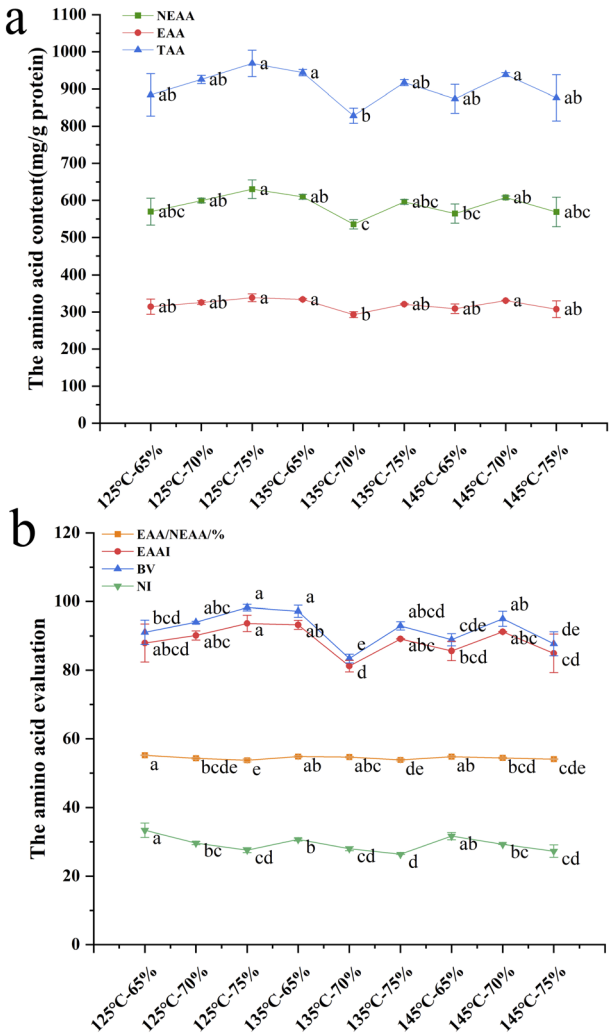

Fig. 4 The amino acid content with TAA, EAA and NEAA (a) and the amino acid evaluation with EAA/NEAA, EAAI, BV and NI (b) of the extrudates with different hydro-thermal parameters, and different letters indicate significant differences ($p < 0.05$).

extrusion temperature would significantly disrupt the content of amino acids in the extrudates at a higher moisture content (70–75%).

Table 5 shows the AAS and CS of the extrudates under different hydro-thermal parameters and the PHE + TYR of the extrudates showed the highest scores, which were 179.00–204.97 and 113.37–129.82, respectively. The AAS and CS of the amino acids were more than 100 except Met + Cys, which can be seen as the first limiting amino acid with the corresponding scores of 90.00–102.27 and 54.94–64.29, respectively. The result indicated that the extrusion parameters had no large effect on the first limiting amino acid of the SPI–surimi extrudates. At the same time, it showed that the amino acid composition of the extrudates could meet the recommended intake.[41]

In Fig. 4, at a moisture content of 70% and extrusion temperature of 135 °C, the TAA, EAA, NEAA, EAAI and BV of the extrudates were significantly lower, and the EAA/NEAA values were between 53.71% and 55.18%, which could reach the reference values of 60% recommended by the FAO/WHO. At a certain temperature (125–145 °C), the EAA/NEAA and NI decreased dramatically as the moisture content increased from 65% to 75%. It indicated that at a certain temperature (125–145 °C), increasing moisture content could decrease the EAA/NEAA values slightly, and the amino acid pattern of the extrudates was also changed. Zahari *et al.*[45] found that the amino acid pattern of the extrudates was more balanced at a moisture content of 65%. In this study, when the extrusion temperature was 125 °C, as the moisture content increased from 65% to 75%, the NEAA, EAA, TAA, EAAI and BV increased remarkably. At a moisture content of 75%, when the extrusion temperature increased from 125 °C to 145 °C, NEAA, EAA, TAA, EAAI and BV decreased dramatically, indicating that higher extrusion temperature would destroy the extrudates' amino acid pattern.[46] It was further shown that the amino acid content and amino acid balance of the SPI–surimi extrudates could be improved by changing the extrusion parameters.

Fig. 5 shows that PC1 and PC2 could explain 72% and 25% of the total variance, respectively. Moreover, the NEAA and TAA

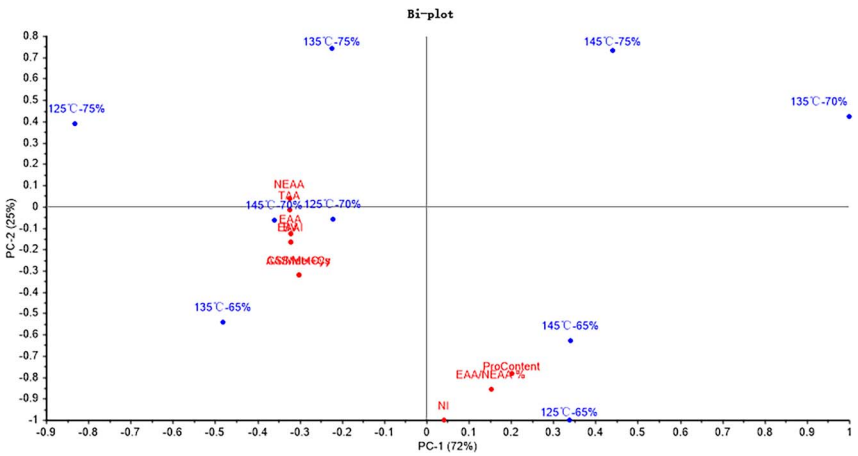

Fig. 5 The PCA Bi-plots of the amino acid evaluation of the extrudates with different hydro-thermal parameters.

Table 6 Fatty acid contents of the extrudates with different SPI–surimi ratios[a]

Fatty acid contents (mg per g fat)			SPI–surimi ratios				
			90 : 10	80 : 20	70 : 30	60 : 40	50 : 50
C4:0	SFA	Butyric acid	0.00 ± 0.00c	1.76 ± 0.31a	1.20 ± 0.09b	1.21 ± 0.07b	0.00 ± 0.00c
C12:0	SFA	Lauric acid	3.69 ± 0a	0.00 ± 0.00c	0.00 ± 0.00c	1.04 ± 0.03b	1.01 ± 0.11b
C14:0	SFA	Myristic acid	3.57 ± 0.06d	6.47 ± 0.53bc	6.09 ± 0.71c	7.79 ± 0.03ab	9.18 ± 1.09a
C15:0	SFA	Pentadecanoic acid	1.04 ± 0.06c	1.83 ± 0.24b	2.11 ± 0.25b	2.52 ± 0.02a	2.76 ± 0.04a
C16:0	SFA	Palmitic acid	184.91 ± 3.22d	251.37 ± 4.24a	225.55 ± 2.74b	202.32 ± 0.59c	191.83 ± 11.24cd
C16:1n7	MUFA	Palmitoleic acid	2.08 ± 0.02d	5.57 ± 0.57c	6.89 ± 0.62b	9.07 ± 0.37a	10.38 ± 0.74a
C17:0	SFA	Pearlescent fatty acid	2.23 ± 0.06c	3.68 ± 0.29b	3.71 ± 0.05b	4.41 ± 0.17a	4.75 ± 0.28a
C18:0	SFA	Stearic acid	45.61 ± 0.35c	67.08 ± 2.72a	60.58 ± 1.01b	58.13 ± 0.74b	55.79 ± 3.64b
C18:1n9c	MUFA	Oleic acid	64.35 ± 0.56ab	67.55 ± 4.25a	61.94 ± 1.27ab	58.05 ± 0.74b	60.26 ± 5.51ab
C18:2n6c	PUFA n-6	Linoleic acid	253.4 ± 5.56ab	259.25 ± 2.36a	246.28 ± 0.95b	184.92 ± 0.67c	157.18 ± 6.87d
C20:0	SFA	Arachidonic acid	1.34 ± 0.10b	1.49 ± 0.28ab	1.67 ± 0.53ab	2.10 ± 0.27ab	2.23 ± 0.25a
C18:3n3	PUFA n-3	Alpha-linolenic acid	24.56 ± 0.80a	21.73 ± 0.10b	22.70 ± 0.06b	16.02 ± 0.01c	14.66 ± 0.67d
C22:0	SFA	Behenic acid	3.38 ± 0.25b	5.29 ± 0.08a	4.47 ± 0.42a	4.31 ± 0.17ab	3.24 ± 0.77b
C20:4n6	PUFA n-6	Arachidonic acid	1.17 ± 0.24d	2.58 ± 0.65c	4.61 ± 0.97b	6.85 ± 0.24a	8.02 ± 0.31a
C24:0	SFA	Lignocarboxylic acid	3.52 ± 0.04bc	4.55 ± 0.71a	3.70 ± 0.15ab	3.21 ± 0.10bc	2.78 ± 0.29c
C20:5n3	PUFA n-3	EPA	1.44 ± 0.37e	4.62 ± 0.10d	6.17 ± 0.14c	9.24 ± 0.38b	10.30 ± 0.45a
C22:6n3	PUFA n-3	DHA	6.44 ± 0.07e	18.47 ± 0.57d	26.51 ± 2.04c	37.40 ± 1.26b	41.12 ± 1.62a

[a] Different letters in the same row mean significant differences ($p < 0.05$). SFA denotes saturated fatty acids, UFA denotes unsaturated fatty acids, MUFA denotes monounsaturated fatty acids and PUFA denotes polyunsaturated fatty acids. n-3 and n-6 denote PUFA types.

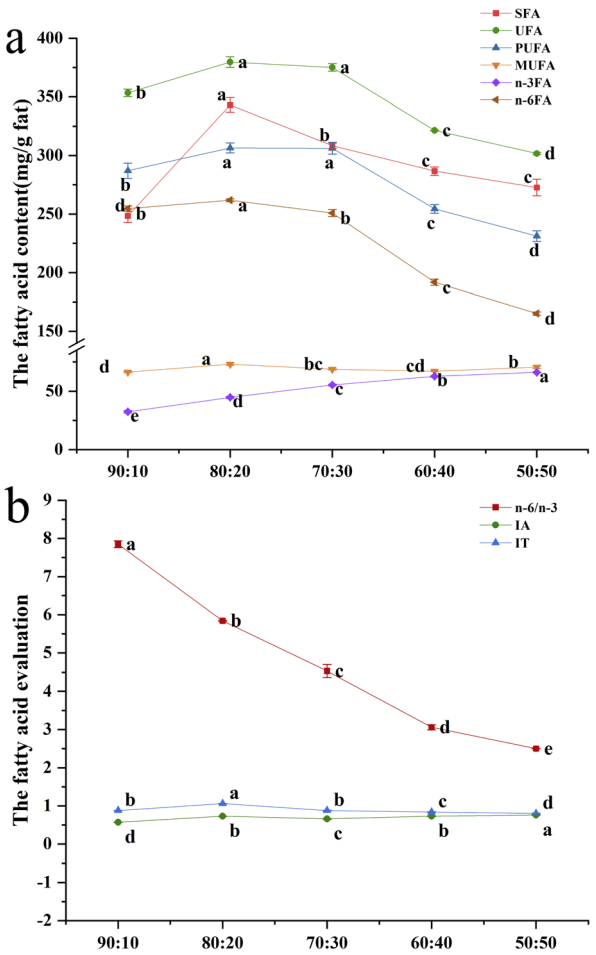

Fig. 6 The fatty acid content with the SFA, UFA, PUFA, MUFA, n-3FA and n-6FA (a) and the fatty acid evaluation with n-6/n-3, IA and IT (b) of the extrudates with different SPI–surimi ratios, and different letters indicate significant differences ($p < 0.05$).

were significantly related to the hydro-thermal combination parameters of 125 °C-75%. And the NI was significantly related to the hydro-thermal combined parameters of 125 °C-65%.

Fatty acid evaluation

Effect of SPI–surimi ratios. Table 6 shows the contents of 17 fatty acids of the extrudates with different SPI–surimi ratios. The contents of palmitic acid (184.91–251.37 mg per g fat) and linoleic acid (157.18–259.25 mg per g fat) were much higher especially at a SPI–surimi ratio of 80 : 20, and the lowest fatty acid was butyric acid (0.00–1.76 mg per g fat). It showed that the high-moisture extrusion processing had no significant effect on the most abundant fatty acids in the SPI–surimi extrudates. As the ratio of surimi increased from 10% to 50%, the eicosapentaenoic acid (EPA) of the extrudates increased significantly from 1.44 mg per g to 10.30 mg per g and the docosahexaenoic acid (DHA) content increased prominently from 6.44 mg g^{-1} to 41.12 mg g^{-1}. This result was consistent with Jannat *et al.*[47] who also found that the addition of surimi resulted in the increase of DPA and EHA, which further confirmed that the surimi enhanced the unsaturated fatty acids (UFA) of the alternative protein foods.[48]

As can be seen in Fig. 6, at a SPI–surimi ratio of 80 : 20, the extrudate showed the highest saturated fatty acid (SFA), unsaturated fatty acid (UFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), n-6 fatty acid (n-6FA) contents and the highest IT values. Meanwhile, at a SPI–surimi ratio of 50 : 50, the n-6FA, UFA and PUFA contents were the lowest and the IA values were the highest. Moreover, the n-6 fatty acid content/n-3 fatty acid content (n-6/n-3) values gradually decreased as the surimi content increased from 10% to 50%, and it might be related to the increasing n-3 fatty acid

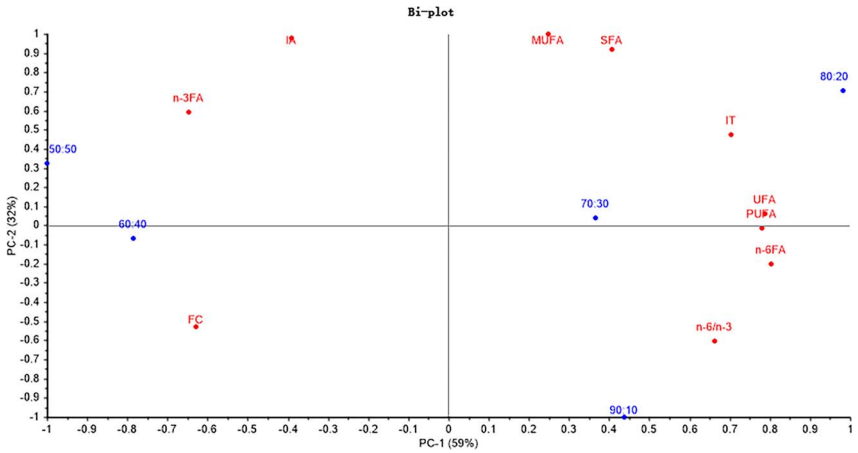

Fig. 7 The PCA Bi-plots of the fatty acid evaluation of the extrudates with different SPI–surimi ratios.

content from 32.44 mg g^{-1} to 66.08 mg g^{-1}, indicating the enhanced ability of extrudates to prevent chronic diseases. The above results indicated that the fatty acid levels were the highest and the antioxidant properties of extrudates increased significantly when at a SPI–surimi ratio of 80 : 20 during the high-moisture extrusion processing.[49]

Fig. 7 shows that PC1 and PC2 could explain 59% and 32% of the total variance, respectively. The SFA, UFA, PUFA, MUFA and IT were significantly related to the ratio of 80 : 20. Additionally, the n-6/n-3 was positively related to the ratio of 90 : 10.

Effect of hydro-thermal combined parameters. As shown in Table 7, palmitic acid (157.20–284.01 mg per g fat) and linoleic acid (176.89–308.46 mg per g fat) accounted for the highest portion of all the extrudates; however, the butyric acid (0.00–1.50 mg per g fat) content was the lowest. At a certain extrusion temperature (125–145 °C), the EPA and DHA contents of the extrudates decreased dramatically as the moisture content increased from 65% to 75%. Čolović *et al.*[50] also found that increasing the moisture content could lead to less fatty acid contents during the high-moisture extrusion processing because of the inactivated lipase. However, when the moisture content was at 70%, as the extrusion temperature increased from 125 °C to 145 °C, the EPA and DHA contents increased significantly.

As shown in Fig. 8, when the extrusion temperature was constant, the six different fatty acid contents (SFA, UFA, MUFA, PUFA, n-3FA, and n-6FA) and two indicators (n-6/n-3 and IT values) both decreased dramatically as the moisture content increased from 65% to 75%. Azam *et al.*[51] reported the effect of low moisture on the nutritional properties of the extrudates, which was positive for increasing the various fatty acids. When the moisture content was 65%, the increasing extrusion temperature could lead to less fatty acid contents. It might be caused by lipid oxidation and thermal decomposition according to a study.[50] Wang *et al.*[52] also reported that fatty acids were broken down due to the action of high temperature, high pressure and high shear. It is generally believed that fatty acids can form complexes with carbohydrates and proteins in the extrusion process.[53] Interestingly, at higher moisture contents (70–75%), increasing extrusion temperature (from 125 °C to 145 °C) enhanced the fatty acid contents due to inactivation of fatty acid hydrolases.[31]

Fig. 9 shows that PC1 and PC2 could explain 77% and 17% of the total variance, respectively. The SFA, UFA, PUFA n-3FA and n-6FA were significantly irrelated to the hydro-thermal combined parameters of 125 °C-70%. Moreover, the n-6/n-3, IA and IT were dramatically irrelated to the hydro-thermal combined parameters of 125 °C-75%.

In vitro digestibility

Effect of SPI–surimi ratios. As can be seen in Fig. 10, as the surimi content increased from 10% to 50%, the gastric digestibility (GD) decreased from 60.20% to 24.63% firstly but then increased significantly to 53.02%. At a SPI–surimi ratio of 70 : 30, the GD value was the lowest, which should be considered as the turning point of the mixed protein ratios for gastric digestion. This might be related to higher gel strength at this ratio according to our previous study.[8] Furthermore, the vegetable ingredients of plant-based meat significantly reduced the number of gastric parietal cells and pepsin activity.[54] An enzyme activity test also confirmed that the plant-based meat significantly decreased pepsin activity but increased trypsin activity.[55] Moreover, the increased surimi content could lead to an increase in chain proteins, which promoted the contact between the pepsin and binding points. In terms of small intestinal digestibility (SD), the highest SD was 93.07% at a SPI–surimi ratio of 70 : 30. It was perhaps related to the increase of the surimi content and the increase of the intestinal pepsin activity according to a previous study.[54] The lowest SD was only 12.16% with 40% surimi addition and further research should be necessary.

Effect of hydro-thermal combined parameters. Fig. 10 shows the GD and SD of the extrudates with different hydro-thermal parameters. When the extrusion temperature was set at 125 °C, the GD increased from 12.65% to 30.18% and the SD

Table 7 Fatty acid contents of the extrudates with different hydro-thermal parameters[a]

Fatty acid contents (mg per g fat)			Hydro-thermal parameters								
			125 °C-65%	135 °C-65%	145 °C-65%	125 °C-70%	135 °C-70%	145 °C-70%	125 °C-75%	135 °C-75%	145 °C-75%
C4:0	SFA	Butyric acid	1.19 ± 0.08b	1.2 ± 0.05b	1.15 ± 0.20b	0.95 ± 0.10b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	1.50 ± 0.25a
C12:0	SFA	Lauric acid	1.64 ± 0.03a	1.44 ± 0.58a	1.42 ± 0.49a	0.94 ± 0.14a	1.12 ± 0.42a	0.88 ± 0.04a	0.00 ± 0.00b	1.26 ± 0.14a	1.39 ± 0.29a
C14:0	SFA	Myristic acid	10.33 ± 0.20a	9.80 ± 3.99ab	7.98 ± 1.51abc	5.72 ± 0.29c	6.83 ± 0.79abc	7.22 ± 1.19abc	4.64 ± 0.17c	6.29 ± 0.48bc	7.29 ± 0.59abc
C15:0	SFA	Pentadecanoic acid	1.78 ± 0.22a	1.57 ± 0.23ab	1.39 ± 0.41abc	1.00 ± 0.04c	1.18 ± 0.06bc	1.09 ± 0.08bc	1.10 ± 0.10bc	1.05 ± 0.29bc	1.40 ± 0.08abc
C16:0	SFA	Palmitic acid	284.01 ± 4.68a	280.80 ± 4.32a	253.63 ± 2.36b	157.20 ± 3.47f	203.92 ± 0.07d	215.78 ± 8.86c	175.73 ± 0.49e	177.10 ± 4.96e	221.47 ± 0.91c
C16:1n7	MUFA	Palmitoleic acid	3.54 ± 0.16ab	3.72 ± 0.92a	3.39 ± 0.29ab	2.21 ± 0.26cd	2.69 ± 0.11bcd	2.69 ± 0.15bcd	2.16 ± 0.11d	2.37 ± 0.17cd	3.05 ± 0.10abc
C17:0	SFA	Pearlescent fatty acid	3.32 ± 0.15a	3.23 ± 0.08ab	3.02 ± 0.08b	1.74 ± 0.12d	2.30 ± 0.09c	2.31 ± 0.14c	1.90 ± 0.15d	1.90 ± 0.14d	2.51 ± 0.02c
C18:0	SFA	Stearic acid	75.26 ± 0.61a	72.31 ± 1.25a	67.65 ± 0.91b	39.82 ± 1.02f	51.58 ± 1.17d	53.54 ± 2.24d	43.36 ± 0.14e	44.82 ± 1.48e	57.23 ± 1.64c
C18:1n9c	MUFA	Oleic acid	102.09 ± 1.24a	96.35 ± 1.83b	88.85 ± 1.04c	60.37 ± 1.80f	76.52 ± 1.30e	77.16 ± 2.97de	58.43 ± 0.44f	62.00 ± 2.72f	81.34 ± 1.38d
C18:2n6c	PUFA n-6	Linoleic acid	305.84 ± 4.45a	308.46 ± 9.05a	276.19 ± 8.92b	176.89 ± 0.45e	227.69 ± 4.79c	238.82 ± 3.83c	206.75 ± 0.02d	199.34 ± 10.28d	241.15 ± 3.22c
C20:0	SFA	Arachidonic acid	2.14 ± 0.03a	1.81 ± 0.43abc	2.08 ± 0.44ab	0.00 ± 0.00e	1.14 ± 0.11d	1.53 ± 0.20cd	1.35 ± 0.15cd	0.00 ± 0.00e	1.59 ± 0.03bcd
C18:3n3	PUFA n-3	Alpha-linolenic acid	27.09 ± 0.28a	26.95 ± 0.87a	24.61 ± 0.98b	15.96 ± 0.17f	19.95 ± 0.51cd	20.90 ± 0.76c	18.39 ± 0.28de	17.39 ± 1.11ef	21.01 ± 0.24c
C22:0	SFA	Behenic acid	5.79 ± 0.18a	5.86 ± 0.55a	4.75 ± 0.53ab	2.11 ± 0.10e	3.47 ± 0.33cd	3.89 ± 0.51bc	2.40 ± 0.03de	2.61 ± 1.07de	3.91 ± 0.06bc
C20:4n6	PUFA n-6	Arachidonic acid	2.58 ± 0.42abc	3.45 ± 0.10a	2.88 ± 0.67ab	1.90 ± 0.16bc	2.02 ± 0.33bc	2.21 ± 0.38bc	1.82 ± 0.72bc	1.59 ± 0.34c	2.47 ± 0.16abc
C24:0	SFA	Lignocarboxylic acid	5.30 ± 0.45a	5.25 ± 0.12ab	4.72 ± 0.05b	2.46 ± 0.00e	3.32 ± 0.16cd	3.71 ± 0.26c	2.95 ± 0.12de	2.63 ± 0.22e	3.40 ± 0.23cd
C20:5n3	PUFA n-3	EPA	2.06 ± 0.05ab	2.21 ± 0.42a	1.82 ± 0.03ab	1.17 ± 0.29c	1.52 ± 0.05bc	1.62 ± 0.24abc	1.82 ± 0.07ab	1.51 ± 0.27bc	1.71 ± 0.28abc
C22:6n3	PUFA n-3	DHA	16.47 ± 0.06ab	16.74 ± 1.22a	15.11 ± 0.48bc	9.61 ± 0.18g	12.32 ± 0.74ef	13.48 ± 0.12de	13.17 ± 0.87def	11.67 ± 0.46f	14.37 ± 0.58cd

[a] Different letters in the same row mean significant differences ($p < 0.05$). SFA denotes saturated fatty acids, UFA denotes unsaturated fatty acids, MUFA denotes monounsaturated fatty acids and PUFA denotes polyunsaturated fatty acids. n-3 and n-6 denote PUFA types.

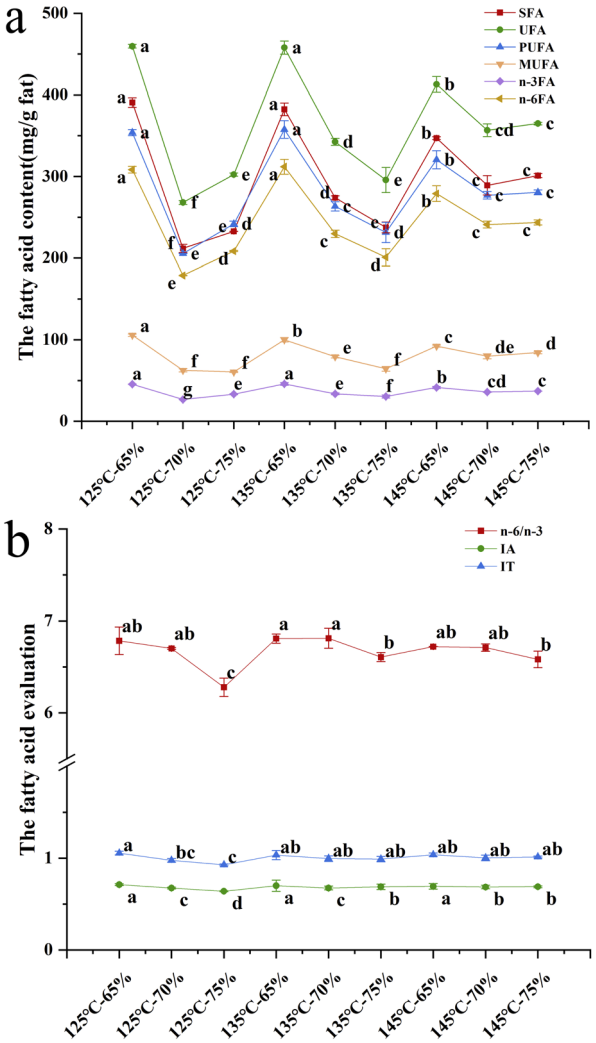

Fig. 8 The fatty acid content with the SFA, UFA, PUFA, MUFA, n-3FA and n-6FA (a) and the fatty acid evaluation with n-6/n-3, IA and IT (b) of the extrudates with different hydrothermal parameters, and different letters indicate significant differences ($p < 0.05$).

increased from 13.67% to 35.64% as the moisture content increased from 65% to 75%. While the extrusion temperature was set at 145 °C, the GD decreased from 31.52% to 19.24% firstly and then increased to 30.41%, and the SD increased from 17.81% to 20.03% firstly and then decreased to 3.25%. It might be because most protein substances are decomposed in the stomach, and the low concentration of substrate leads to a sharp decline in the digestibility of the small intestine.[55,56] At high extrusion temperature, the denaturation of protein molecules aggravated, the protein spatial structure was destroyed, the peptide chain was expanded, and the amino acid was dissociated.

Comprehensive nutritional evaluation of SPI–surimi extrudates

Effect of SPI–surimi ratios. Fig. 11 shows that PC1 and PC2 could explain 65% and 16% of the total variance, respectively. The protein content, UFA, PUFA, n-6FA, n-6/n-3 and SD were positively correlated with PC1, while others were negatively correlated with PC1. The moisture content of the extrudates (MC), FC, SD, GD and n-6/n-3 was negatively correlated with PC2, while others were positively correlated with PC2. These nutritional indicators have a large impact on the evaluation of the comprehensive nutritional quality of the SPI–surimi extrudates at different ratios. The analysis revealed that the ratio of 80 : 20 was in the first quadrant, mainly influenced by AC, PUFA, UFA, and n-6FA on comprehensive nutritional quality evaluation. The ratio of 60 : 40 was distributed in the second quadrant, mainly influenced by EAA, EAAI, AAS (Met + Cys), CS (Met + Cys), and n-3FA. The ratio of 50 : 50 was in the third quadrant, mainly influenced by MC, GD and FC, and the ratios of 90 : 10 and 70 : 30 were in the fourth quadrant, mainly influenced by SD and n-6/n-3.

Effect of hydro-thermal combined parameters. Fig. 12 shows that PC1 and PC2 could explain 44% and 28% of the total variance, respectively. The SD, GD, MC, EAA, EAAI, AAS (Met + Cys) and CS (Met + Cys) were negatively correlated with PC1

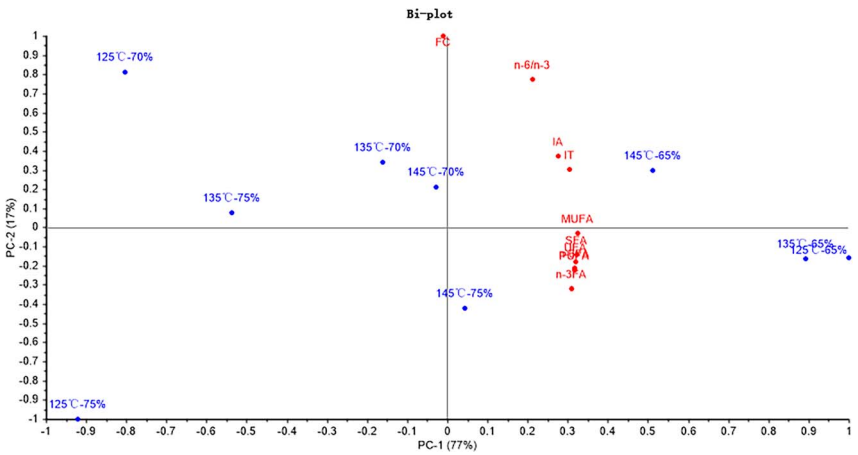

Fig. 9 The PCA Bi-plots of the fatty acid evaluation of the extrudates with different hydro-thermal parameters.

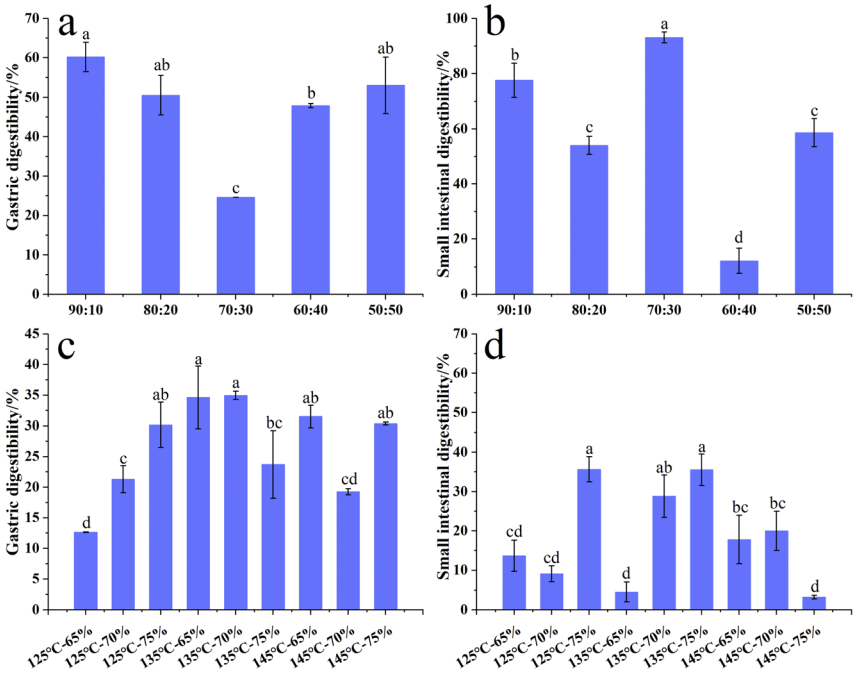

Fig. 10 Simulated gastric digestibility and small intestinal digestibility of the extrudates with different SPI–surimi ratios (a and b) and different hydrothermal parameters (c and d), and different letters indicate significant differences ($p < 0.05$).

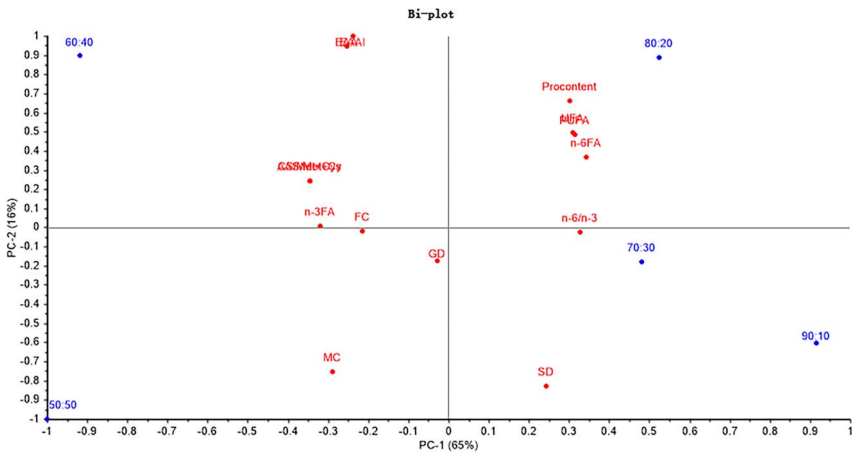

Fig. 11 The PCA Bi-plots of the extrudates with different SPI–surimi ratios for comprehensive nutritional evaluation.

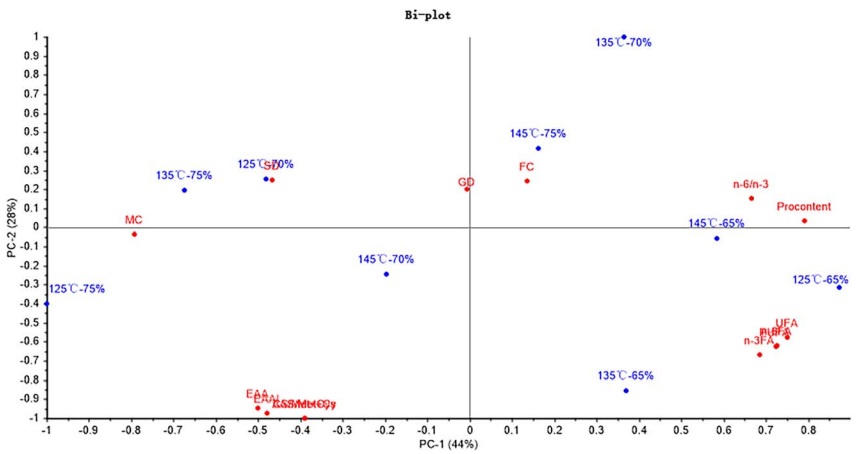

Fig. 12 The PCA Bi-plots of the extrudates with different hydro-thermal parameters for comprehensive nutritional evaluation.

while others were positively correlated with PC1. The protein content, n-6/n-3, FC, GD and SD were positively correlated with PC2, while others were negatively correlated with PC2. These nutritional indicators have a large impact on the evaluation of the comprehensive nutritional quality of the SPI–surimi extrudates at different ratios. The PCA showed that 135 °C-70% and 145 °C-75% were in the first quadrant and mainly influenced by the protein content and n-6/n-3. 125 °C-70% and 135 °C-75% were distributed in the second quadrant and were mainly influenced by SD and GD. 145 °C-70% and 125 °C-75% were in the third quadrant and mainly influenced by EAA, EAAI, AAS (Met + Cys) and CS (Met + Cys). 125 °C-65% and 135 °C-65% were in the fourth quadrant and mainly influenced by PUFA, UFA, n-6FA and n-3FA.

Conclusions

When the surimi content increased from 10% to 50%, the AAS significantly increased from 88.82 to 109.50. Furthermore, the EPA and DHA levels in the extrudates increased notably, going from 1.44 mg g^{-1} to 10.30 mg g^{-1} and from 6.44 mg g^{-1} to 41.22 mg g^{-1}, respectively. These findings suggest that surimi plays a crucial role in improving both amino acid and fatty acid contents in high-moisture extrudates derived from SPI and surimi. Additionally, when the moisture content reached 75%, elevating the extrusion temperature from 125 °C to 145 °C resulted in a significant decrease in the essential amino acid content. In a certain extrusion temperature range (125–145 °C), the EPA and DHA contents of the extrudates decreased substantially as the moisture content increased from 65% to 75%. It was found that higher extrusion temperature and increased moisture content disrupted the amino acid patterns in the extrudates, while simultaneously enhancing certain fatty acid levels. Conversely, a lower extrusion temperature (125 °C) and lower moisture content (65%) contributed to higher EPA and DHA levels. During the high-moisture processing, with an SPI–surimi ratio of 70 : 30, the lowest GD was 24.63%, while the highest SD reached 93.07%. Higher moisture levels (70% and 75%) were associated with greater SD, and increasing the temperature at a lower moisture content (60%) or increasing moisture content at a lower temperature (125 °C) leads to an obvious increase in GD during high-moisture extrusion processing.

Author contributions

Anna Hu: investigation, validation, formal analysis, and writing – original draft. Yujie Zhang: methodology, investigation, data curation, and writing – original draft. Jinchuang Zhang: conceptualization, methodology, formal analysis, writing – review & editing, and supervision. Tongqing Li: visualization and validation. Zhaojun Wang: writing – review & editing and supervision. Qiang Wang: funding acquisition and supervision.

Conflicts of interest

There are no conflicts to declare.

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