



# Study on the relationship between raw material characteristics of soybean protein concentrate and textured vegetable protein quality

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## Abstract

To investigate which protein properties affect the quality of high moisture textured vegetable protein (HTVP), we compared 12 commercial Soy Protein Concentrate (SPC). The comparison revealed that characteristics of different SPCs vary greatly, significant impact on the quality of extrusion products. There were significant differences in the degree of texturization, textural properties, and the sensory score of products. Statistical correlation analysis between SPC raw materials and products showed that the quality of products was significantly affected by the nitrogen solubility index (NSI), water holding capacity (WHC), emulsifying properties (EAI), foaming capacity (FC), gel strength, and sulfhydryl content of SPC raw materials. Our research provides a way to stabilize extrusion product quality.

**Keywords:** soy protein concentrate; raw material characteristics; textured vegetable protein; quality properties.

**Practical Application:** The phenomenon of product quality instability often arises in the process of producing high moisture textured vegetable protein, which is closely related to the raw materials of soy protein concentrate used. It is useful to explore the relationship between the raw material characteristics of soy protein concentrate and the quality of high moisture textured vegetable protein products to address product instability in industry.

## 1 Introduction

High moisture extrusion cooking (moisture content  $\geq 40\%$ ) started in the 1990s and was gradually developed based on low moisture extrusion technology. It is considered an emerging and promising technology to obtain fibrous structure similar to animal meat from plant protein (Kumar et al., 2017; Zhang et al., 2022). High moisture textured vegetable protein (HTVP) is an ideal substitute for animal meat due to its high protein, low saturated fat, no cholesterol, zero hormones, good digestibility, rich in essential amino acids, and direct edible advantages (Samard & Ryu, 2019).

Soy ingredients are the most commonly used in meat analogues because of their characteristic functional properties, such as water-holding, fat-absorbing, gelling, and emulsifying capacities (Kyriakopoulou et al., 2019). Soy protein concentrate (SPC) is a further purification of low-temperature defatted soybean meal. After removing the water-soluble or alcohol-soluble non-protein part, the product contains more than 65% protein. SPC-based meat analogues have been reported to be easier to extrude and more pronounced anisotropic than formulations based on soy protein isolate (SPI) under similar conditions (Chiang et al., 2019). The result may be that the polysaccharides in SPC contribute to the formation of the dispersed phase (Wittek et al., 2021a). Furthermore, SPC, at low cost, is used as raw material to produce textured vegetable protein, which has good economic benefits (Wittek et al., 2021b; Chajuss, 2001).

Protein as the skeleton material of textured vegetable protein, protein intrinsic physicochemical properties, such as

particle size, nitrogen solubility index (NSI), 11S and 7S content, and functional properties (hydration ability and active surface properties) by influencing the heat transfer rate, specific mechanical energy, and water or other protein interaction and so on, further affect the quality of extrusion products (Beniwal et al., 2021; Zhang et al., 2022). During extrusion cooking, larger particles typically require a large input of mechanical energy (Singh & Koksel, 2021; Onwulata & Konstance, 2006). Wang et al. (2012) concluded that the extruded products with higher NSI, smaller particle size, and higher 11S globulin content had better fibrous structure. Furthermore, Liu et al. (2022) added 11S and 7S to the raw materials and concluded that a higher 11S protein content resulted in a brighter color, better texture properties, and a higher degree of texturization. Existing studies primarily explore products extruded from types of protein raw material (Schreuders et al., 2019; Palanisamy et al., 2019), extrusion parameters (Ferawati et al., 2021; Pietsch et al., 2019), system parameters (Chen et al., 2010; Zhang et al., 2015) and additives (Chen et al., 2021; Dou et al., 2022) etc. However, the relationship between the characteristics of protein raw materials and the quality of extruded products has not been studied deeply. So far, it is not clear which properties of protein raw materials are critical to the production of products.

Therefore, this study aims to establish possible connections between SPC raw material characteristics and the quality of HTVP, to solve the phenomenon of unstable product quality. A wide range of tests (physicochemical and functional properties, etc.)

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were performed to characterize SPC. Furthermore, extruded product indexes were measured. The relationship between SPC raw material characteristics and extruded product quality was explored by cluster analysis and correlation analysis, which provided a theoretical basis for the selection of raw material and control of product quality in the processing of plant protein texturization.

## 2 Materials

Twelve commercial SPC were used, obtained from different batches from six manufacturers (Yihai Kerry, Yuwang, Vanderfor, Yuxin, Jiahua, and DuPont) in China, marked SPC 1-12. SPI contains, on a dry basis, 94.24% protein, 5.99% moisture, 4.64% ash, and 0.39% fat. Wheat gluten (WG) contains about 87.66% protein, 5.32% moisture, 2.00% ash, and 2.11% fat. SPI and WG were purchased from Yihai Kerry Food Industry Co., LTD. (Henan, China).

### 2.1 Raw material characteristics of SPC

#### Chemical composition of SPC

The moisture, ash, and fat in the samples were determined according to the method of AACC (American Association of Cereal Chemists, 1999a, b, c). The crude protein content of the samples was determined by the Kjeldahl method ( $N \times 6.25$ ) (National Health and Family Planning Commission of the People Republic of China, 2016).

#### Nitrogen solubility index

Nitrogen solubility index (NSI) reference to AACC - 46 - 23 (American Association of Cereal Chemists, 1999d).

#### pH

The SPC was prepared into 8% slurries with distilled water pH 7.0 as a dispersant. The pH of the solution was determined using a pH meter (HI2210, HANNA, Italy).

#### Water and oil holding capacity (WHC and OHC)

The determination of WHC or OHC was performed according to Zahari et al. (2021). 3 g SPC was accurately weighed into a centrifuge tube, 17 mL distilled water or soybean oil was added, shaken for 1 min, then stood for 15 min, centrifuged at 5 000 g for 10 min, and the supernatant was poured out and weighed. The WHC and OHC were calculated by the following formula (Equation 1).

$$WHC \text{ or } OHC (\%) = \frac{M_3 - M_2 - M_1}{M_1} \times 100\% \quad (1)$$

Where  $M_1$  = the mass of the SPC,  $M_2$  = the mass of the empty centrifuge tube, and  $M_3$  = the total weight of SPC and centrifuge tube after the supernatant was decanted.

#### Emulsifying activity index (EAI) and emulsifying stability index (ESI)

EAI and ESI were determined according to the method of Ahmed et al. (2018) and Wani et al. (2015). The SPC was

prepared into 1 g/100 mL of slurries, and the solution was stirred for 30 minutes, centrifuged at 3,000 g for 15 min, 5 mL soybean oil was added to 15 mL supernatant, and homogenized for 1 min at 10,000 rpm. At 0 min and 10 min, the absorbance was measured at 500 nm. The EAI and ESI were calculated by the following formula (Equations 2-3).

$$EAI(m^2/g) = \frac{2 \times 2.303 \times A_0 \times N}{\theta \times L \times C \times 10000} \quad (2)$$

$$ESI(\text{min}^{-1}) = \frac{A_0 \times 10}{A_0 - A_{10}} \quad (3)$$

Where  $A_0$  and  $A_{10}$  are the absorbance values measured at 0 min and 10 min;  $N$  = the dilution ratio (100);  $\theta$  = the oil volume fraction of the emulsion (0.25);  $L$  = the optical path (0.01);  $C$  = the initial protein concentration (g/mL).

#### Foaming capacity (FC) and foaming stability (FS)

FC and FS were determined according to the procedure used by Yust et al. (2010). Slurries of 1 g/100 mL were prepared with distilled water, and the sample was stirred for 30 min to ensure complete dissolution. After centrifugation at 3,000 g for 10 min, 25 mL protein solution was homogenized at 10,000 rpm for 1 min. The volume of the foam was measured after homogenization and standing for 25 min. The FC and FS were calculated by the following formula (Equations 4-5).

$$FC(\%) = \frac{V_1}{V_0} \times 100\% \quad (4)$$

$$FS(\%) = \frac{V_t}{V_1} \times 100\% \quad (5)$$

Where  $V_0$  = the initial volume of protein solution,  $V_1$  = the foam volume after homogenization for 1 min, and  $V_t$  = the foam volume after standing for 25 min.

#### RVA viscosity

The viscosity of the proteins was measured on a Rapid Visco Analyzer (RVA-TM, Perten, Sweden). SPC was prepared in 10% slurries, and rapidly stirred at 960 rpm for 15 s and 160 rpm for 10 min. The temperature was constant at 25 °C, and the value was recorded at 5 min.

#### Gel strength

The determination was performed according to Jin et al. (2013). Preparation of gel: The SPC was made into 10% slurries, homogenized at 10,000 rpm with a high speed homogenizer for 5 s, and 20 mL was placed in a beaker, covered with plastic wrap, and heated in a water bath at 90 °C for 35 min. After removal, it was cooled for 20 min and placed in a refrigerator at 4 °C for 24 h. Gel strength was measured on a physical property tester (Ta. XT Plus, Stable Micro-systems, UK) with a P/0.5 probe at a speed of 1 mm/s.

### Sulfhydryl content

Ellman's reagent was used to determine the content of free sulfhydryl groups in the samples. Take 100 mg of SPC, add 4.7 g guanidine hydrochloride, and then dissolve in 10 mL of buffer. Take 1 mL of the prepared solution, add 4 mL of urea-guanidine hydrochloride solution and 0.05 mL of Ellman's reagent, stand still for 30 min, then measure the absorbance value at the wavelength of 412 nm (Equation 6).

$$SH(\mu\text{mol/g}) = \frac{73.53 \times A_{412} \times D}{C} \quad (6)$$

Where  $A_{412}$  = the absorbance value of the sample at 412 nm; D = the dilution factor; C = the sample concentration (mg(dry base)/mL).

### 11S/7S

SDS-PAGE was performed according to the method of Yadav et al. (2010).

## 2.2 High moisture extrusion cooking

A CLETRAL Ev025 (Firminy, France) type twin screw extruder was adopted with a screw diameter of 25.5 mm and an aspect ratio of 24. The barrel contains six temperature control areas, which are heated by an electric heating cylinder and cooled by circulating water.

The formula of extruder ingredients includes SPI, SPC, and WG in a ratio of 3: 4: 3 and 5% corn starch. The mixtures were fed into the extruder at a constant speed of 3.4 kg/h. Based on preliminary experiments and operational stability of the extruder, water entered the extruder at a constant speed of 3.0 L/h, the screw speed was 280 rpm, and the temperatures of extruder barrel were kept at 30, 90, 120, 140, 150, and 160 °C from the first zone to the sixth zone, respectively. After the extrusion material was stable, it was collected, packed with a vacuum seal, and stored at -18 °C for further experiments.

## 2.3 Color

The color difference meter (CR-400, Konica Minolta, Japan) was used for determination, and  $L^*$  and  $\Delta E^*$  values were recorded.

## 2.4 Texture properties

The texture properties of the extrudates were evaluated using a TA.XT plus Texture Analyzer (Stable Micro Systems, UK).

The shape samples (2 cm × 2 cm × 0.5 cm) were compressed to 75% of the original thickness with a P/36R probe at a speed of 2mm /s, and the hardness, springiness, and chewiness were recorded. Samples of the same shape were cut using a knife blade to 75% of their original thickness at the same speed, and the cutting strength was recorded.

## 2.5 Sensory evaluation

Ten professional sensory evaluators were invited to evaluate 12 HTVPs. The sensory evaluation criteria are shown in Table 1.

## 2.6 Statistical analysis

All experiments were performed at least three times, and the results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) and Duncan's multiple interval test were used to compare the results. Origin 2021 was used for mapping.

# 3 Results and discussion

## 3.1 Analysis of SPC raw material characteristics

The chemical composition of SPC 1-12 is summarized in Table 2. The moisture and ash content of the 12 SPCs follows the General Standard for Soy Protein Products CXS 175-1989 (Food and Agriculture Organization, 2019) for SPC moisture content ( $\leq 10.0\%$ ) and ash content ( $\leq 8.0$ ). With the exception of SPC 5, crude protein content is just under 65%, and all others met the crude protein content standard (65%~90%).

The raw material characteristics of protein are the basis and guarantee of producing extruded plant protein. The characteristics of SPC 1-12 raw materials are shown in Table 3. There are significant differences in the characteristics of SPC from different manufacturers and different batches of the same manufacturer, which may be related to the soybean varieties (Min et al., 2005), production process (Preece et al., 2017), and storage conditions (Ziegler et al., 2018) used by the manufacturer. All SPCs used in this study were prepared using the alcohol method. The NSI of SPC produced by the alcohol method is generally lower (<15%). Still, the NSI difference in the measurement results is very significant, which is mainly caused by the different degrees of protein denaturation caused by process conditions, such as leaching concentration (Peng et al., 2021), drying methods (Ghribi et al., 2015) and different modification methods (Sui et al., 2021; Moreno et al., 2020).

**Table 1.** Sensory evaluation criteria of HTVP.

Apparent state (0.1)	Color (0.2)	Taste and flavor (0.3)	Structure state (0.4)	Score
A rough, uneven surface with numerous burrs	Dark color, obvious gelatinization	Bad taste, poor chewing	No fiber structure, no complete forming state	1~3
Slightly rough surface, less burr	Slightly dark yellow, a little gelatinization	No bad taste, slightly better chewing	Less fibrous structure, slightly formed state	4~6
Smooth surface, fewer burrs	Yellow, no gelatinization	No abnormal taste, good chewiness	Apparent fiber structure, well-formed state	7~9
Smooth surface, no burrs	Bright yellow, no gelatinization	Clear aroma, full of chewy	Rich fiber structure, perfectly formed state	10

**Table 2.** Chemical composition of SPC.

	Moisture (%)	Ash (%)	Crude protein (%)	Fat (%)
SPC 1	7.64 ± 0.01	6.06 ± 0.01	69.32 ± 0.01	0.10 ± 0.01
SPC 2	6.79 ± 0.01	4.54 ± 0.01	67.99 ± 0.01	0.34 ± 0.01
SPC 3	7.37 ± 0.01	6.56 ± 0.01	68.68 ± 0.01	0.35 ± 0.01
SPC 4	6.36 ± 0.01	4.72 ± 0.01	69.30 ± 0.01	0.30 ± 0.01
SPC 5	6.04 ± 0.01	4.73 ± 0.01	64.88 ± 0.01	0.13 ± 0.01
SPC 6	6.49 ± 0.01	7.18 ± 0.01	66.60 ± 0.01	0.08 ± 0.01
SPC 7	6.54 ± 0.01	4.32 ± 0.01	69.49 ± 0.01	0.20 ± 0.01
SPC 8	6.99 ± 0.01	4.62 ± 0.01	65.85 ± 0.01	0.10 ± 0.01
SPC 9	7.58 ± 0.01	4.61 ± 0.01	66.23 ± 0.01	0.37 ± 0.01
SPC 10	6.92 ± 0.01	6.79 ± 0.01	69.73 ± 0.02	0.31 ± 0.01
SPC 11	7.48 ± 0.01	4.40 ± 0.01	70.48 ± 0.01	0.07 ± 0.01
SPC 12	7.32 ± 0.01	6.73 ± 0.01	67.94 ± 0.01	0.06 ± 0.01

Studies had found that the protein solubility of ethanol-treated lentil protein isolate was below 10% when 35~55% ethanol was applied and increased to approximately 30% when the water/ethanol ratio increased from 55 to 75% (Chang et al., 2019). There are significant differences in WHC, OHC, and EAI among different SPCs. The higher WHC may be due to the lower loss of soluble protein and the higher polar amino acids, while OHC is attributed to protein hydrophobicity and the binding performance of non-polar amino acid side chains to fat (Ghribi et al., 2015). EAI refers to the ability of protein to quickly adsorb on the oil-water interface, and ESI is to evaluate the stability of the protein staying at the water-oil interface for a period of time (Yao et al., 2022). There is no significant difference in ESI among SPCs. There are significant differences in FC, divided into two levels. SPC 2, 4, 5, 7, 8, 9, and 11 are the ones with higher FC, and SPC 1, 6, and 10 are the ones with lower FC. Differences in FS are also very significant. SPC 2, 5, 8, and 9 are free of foam, and SPC 6 and 10 are relatively stable at 40%. The foaming ability of protein mainly depends on its soluble part. Generally, there is a strong positive correlation between FC and NSI. At the same time, it can be seen that the data of FC and FS data show opposite trends. The viscosity can be divided into three apparent levels. 19.00~22.00 cP is the lowest batch, 164.50~397.50 cP is the middle batch, and 1 093.50~2 188.50 cP is the highest batch. The three clusters of raw materials have a very significant difference. There are significant differences in sulfhydryl content, composition, and gel strength among SPCs. Sulfhydryl oxidation generates new disulfide bonds to organize protein molecules in a more orderly manner and form a more stable network structure (Bruneel et al., 2011). Therefore, free sulfhydryl groups have a great impact on the formation of gel and protein cross-linking. The ratio of 7S and 11S is different in different soybean varieties, which has an important impact on the emulsification (Cheng et al., 2006) and gel ability (Shi et al., 2005) of soybean protein. 11S globulin contains more disulfide bonds and sulfhydryl groups, so when the proportion of 11S globulin increases, high gel strength. In conclusion, SPC raw material characteristics of different manufacturers and different batches produced by the same manufacturer are significantly different.

### 3.2 Color and functional properties analysis

Color L\* is lightness value. The larger L\*, the brighter the sample.  $\Delta E^*$  indicates the degree of difference between each value and the standard whiteboard. Table 4 shows that there are significant differences in color between products. L\*,  $\Delta E^*$  coefficient of variation is 1%, 2%, and the coefficient of variation is small (<15%). The color data for 12 HTVP products did not vary much, suggesting that SPC raw materials have little impact on the color of the products. Therefore, the color index of product quality can be considered a secondary factor in selecting raw materials.

WHC and OHC are related to the taste and flavor of products, reflecting the adsorption and retention capacity of HTVP products for water or oil, which depends on protein composition, protein denaturation, and the degree of interaction with water and oil (Samard & Ryu, 2019). Table 3 shows significant differences in WHC and OHC among products, with the maximum WHC being 289.89% and the minimum WHC being 222.61%. The maximum OHC was 109.94%, and the minimum OHC was 90.72%. After the extrusion of high temperature, high pressure, and high shear, the molecular structure of proteins from different raw materials is destroyed and assembled to form different structures. However, the variation coefficient of WHC and OHC of the product was small (<15%), indicating that different SPC had little influence on the WHC and OHC of the product.

### 3.3 Product texture properties and sensory analysis

The texture properties of HTVP play an important role in people's acceptability and repeat purchases. In Figure 1, the hardness, springiness, and chewiness range from 18 145.40 to 29 551.09, 0.85 to 0.97, and 13 706.35 to 24 497.62, and the coefficient of variation is 16.10%, 3.72%, and 22.39%, respectively. Through the significance analysis and coefficient of variation of HTVP texture characteristics, it can be concluded that there are significant differences in the hardness and chewiness of HTVP. Combined with the sensory score, people's acceptance is relatively high when the hardness is kept at about 20 000 g, and the chewiness is maintained at 15 000 g. If the hardness and



**Table 3.** Raw material characteristics of SPC.

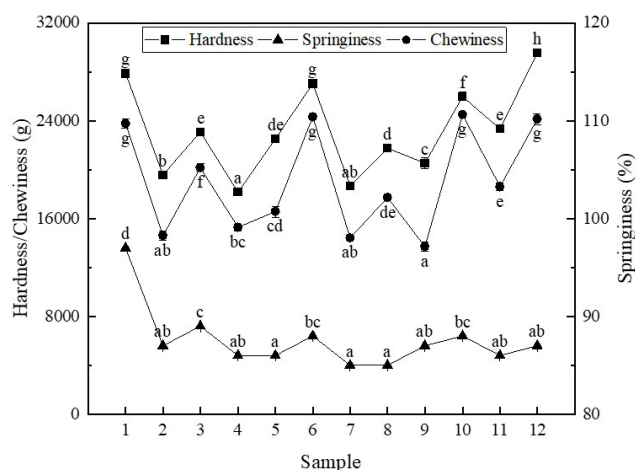
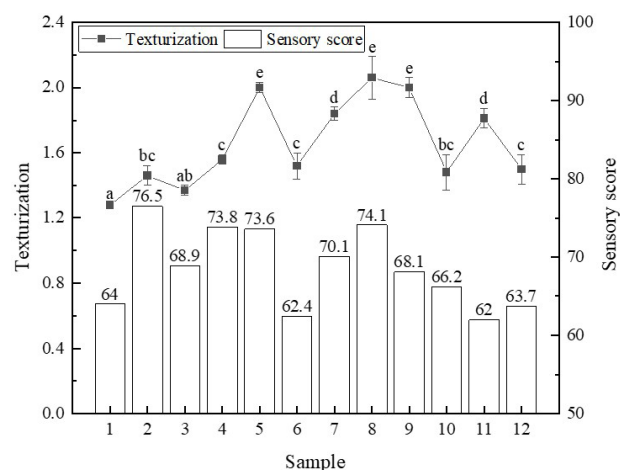
Sample	NSI (%)	pH	WHC (%)	OHC (%)	EAI (m <sup>2</sup> /g)	ESI (min <sup>-1</sup> )	FC (%)	FS (%)	Viscosity (cP)	Gel strength (g)	Sulphydryl content (μmol/g)	11S/7S
SPC1	6.24 ± 0.01 <sup>a</sup>	7.10 ± 0.04 <sup>b</sup>	285.06 ± 0.07 <sup>a</sup>	84.00 ± 0.01 <sup>ab</sup>	8.36 ± 0.12 <sup>bcd</sup>	20.28 ± 2.10 <sup>ef</sup>	41.87 ± 0.01 <sup>a</sup>	20 ± 0.01 <sup>b</sup>	22.00 ± 0.01 <sup>a</sup>	39.42 ± 1.36 <sup>c</sup>	4.69 ± 0.19 <sup>e</sup>	1.07 ± 0.03 <sup>a</sup>
SPC2	81.40 ± 0.01 <sup>c</sup>	7.49 ± 0.08 <sup>c</sup>	483.34 ± 0.07 <sup>ef</sup>	118.00 ± 0.04 <sup>f</sup>	9.83 ± 0.30 <sup>f</sup>	14.71 ± 0.64 <sup>g</sup>	146.53 ± 0.02 <sup>d</sup>	0 ± 0.03 <sup>a</sup>	397.50 ± 10.50 <sup>c</sup>	128.26 ± 4.84 <sup>e</sup>	5.31 ± 0.15 <sup>f</sup>	1.56 ± 0.28 <sup>bc</sup>
SPC3	6.62 ± 0.01 <sup>a</sup>	7.01 ± 0.03 <sup>a</sup>	288.08 ± 0.06 <sup>a</sup>	80.00 ± 0.01 <sup>a</sup>	7.85 ± 0.46 <sup>e</sup>	18.92 ± 2.32 <sup>bcdef</sup>	46.05 ± 0.01 <sup>b</sup>	36.57 ± 0.01 <sup>g</sup>	21.50 ± 0.50 <sup>a</sup>	20.62 ± 1.75 <sup>a</sup>	5.28 ± 0.37 <sup>f</sup>	1.17 ± 0.09 <sup>ab</sup>
SPC4	92.18 ± 0.01 <sup>f</sup>	7.31 ± 0.01 <sup>f</sup>	443.78 ± 0.08 <sup>d</sup>	150.00 ± 0.01 <sup>g</sup>	10.27 ± 0.63 <sup>g</sup>	14.7 ± 1.44 <sup>ab</sup>	146.53 ± 0.04 <sup>d</sup>	34.29 ± 0.01 <sup>f</sup>	164.50 ± 25.5 <sup>b</sup>	82.89 ± 5.63 <sup>d</sup>	4.70 ± 0.1 <sup>e</sup>	1.81 ± 0.01 <sup>bcd</sup>
SPC5	37.41 ± 0.03 <sup>c</sup>	7.55 ± 0.01 <sup>f</sup>	539.95 ± 0.08 <sup>g</sup>	131.00 ± 0.02 <sup>f</sup>	8.95 ± 0.36 <sup>de</sup>	19.38 ± 3.70 <sup>def</sup>	146.53 ± 0.01 <sup>d</sup>	0 ± 0.01 <sup>a</sup>	1819.50 ± 38.50 <sup>c</sup>	136.92 ± 0.44 <sup>f</sup>	2.95 ± 0.06 <sup>a</sup>	1.49 ± 0.29 <sup>abc</sup>
SPC6	5.78 ± 0.01 <sup>a</sup>	7.01 ± 0.04 <sup>a</sup>	295.94 ± 0.08 <sup>a</sup>	89.00 ± 0.01 <sup>bc</sup>	7.94 ± 0.47 <sup>ab</sup>	19.73 ± 4.40 <sup>def</sup>	41.87 ± 0.01 <sup>a</sup>	40.00 ± 0.01 <sup>h</sup>	20.50 ± 0.50 <sup>a</sup>	22.13 ± 2.33 <sup>a</sup>	3.30 ± 0.12 <sup>ab</sup>	1.02 ± 0.01 <sup>a</sup>
SPC7	37.00 ± 0.01 <sup>c</sup>	6.99 ± 0.01 <sup>a</sup>	425.62 ± 0.07 <sup>c</sup>	119.00 ± 0.08 <sup>e</sup>	8.82 ± 0.15 <sup>cde</sup>	21.47 ± 2.85 <sup>f</sup>	146.53 ± 0.01 <sup>d</sup>	31.90 ± 0.01 <sup>e</sup>	250.50 ± 32.50 <sup>b</sup>	124.77 ± 9.25 <sup>e</sup>	4.05 ± 0.08 <sup>cd</sup>	1.52 ± 0.26 <sup>bc</sup>
SPC8	46.60 ± 0.01 <sup>d</sup>	7.81 ± 0.01 <sup>b</sup>	474.26 ± 0.08 <sup>c</sup>	131.00 ± 0.02 <sup>f</sup>	10.64 ± 0.63 <sup>g</sup>	17.21 ± 2.11 <sup>abcde</sup>	146.53 ± 0.01 <sup>d</sup>	0 ± 0.01 <sup>a</sup>	1093.50 ± 7.50 <sup>d</sup>	141.81 ± 5.2 <sup>fg</sup>	3.51 ± 0.11 <sup>b</sup>	2.37 ± 0.14 <sup>de</sup>
SPC9	38.45 ± 0.01 <sup>c</sup>	7.84 ± 0.01 <sup>b</sup>	492.90 ± 0.16 <sup>f</sup>	125.00 ± 0.05 <sup>f</sup>	9.98 ± 0.52 <sup>f</sup>	15.94 ± 0.78 <sup>abcd</sup>	146.53 ± 0.01 <sup>d</sup>	0 ± 0.01 <sup>a</sup>	2188.50 ± 76.50 <sup>f</sup>	144.76 ± 3.94 <sup>g</sup>	3.62 ± 0.34 <sup>bc</sup>	2.49 ± 0.39 <sup>f</sup>
SPC10	4.33 ± 0.01 <sup>a</sup>	7.00 ± 0.03 <sup>a</sup>	287.12 ± 0.05 <sup>a</sup>	92.00 ± 0.02 <sup>c</sup>	8.31 ± 0.40 <sup>bc</sup>	15.42 ± 1.69 <sup>abc</sup>	41.87 ± 0.01 <sup>a</sup>	40.00 ± 0.01 <sup>h</sup>	21.00 ± 0.01 <sup>a</sup>	41.39 ± 0.72 <sup>c</sup>	4.27 ± 0.27 <sup>de</sup>	1.55 ± 0.06 <sup>bc</sup>
SPC11	32.76 ± 0.01 <sup>b</sup>	7.15 ± 0.01 <sup>b</sup>	381.16 ± 0.04 <sup>b</sup>	107.00 ± 0.03 <sup>d</sup>	9.03 ± 0.18 <sup>e</sup>	15.89 ± 1.92 <sup>abcd</sup>	146.53 ± 0.01 <sup>d</sup>	28.57 ± 0.01 <sup>d</sup>	189.00 ± 24.10 <sup>b</sup>	156.05 ± 5.43 <sup>b</sup>	3.51 ± 0.03 <sup>b</sup>	2.06 ± 0.5 <sup>de</sup>
SPC12	4.83 ± 0.01 <sup>a</sup>	7.00 ± 0.05 <sup>a</sup>	282.20 ± 0.05 <sup>a</sup>	92.00 ± 0.01 <sup>c</sup>	7.95 ± 0.33 <sup>ab</sup>	18.43 ± 2.41 <sup>bcdef</sup>	50.24 ± 0.01 <sup>c</sup>	25.00 ± 0.01 <sup>c</sup>	19.00 ± 0.01 <sup>a</sup>	30.62 ± 2.47 <sup>b</sup>	4.75 ± 0.5 <sup>e</sup>	1.32 ± 0.29 <sup>ab</sup>
Range	4.33–92.18	6.99–7.84	282.20–539.95	80.00–150.00	7.58–10.64	14.70–21.47	41.87–146.53	0–40	19–2188.50	20.62–156.05	2.95–5.31	1.02–2.49
CV	91.08%	4.44%	24.76%	20.02%	18.98%	13.17%	50.63%	78.54%	147.13%	61.29%	18.98%	29.68%

Average values with different superscripts within the same column are significantly different at  $p < 0.05$ . CV is short for the coefficient of variation.

**Table 4.** color and functional properties of HTVP.

Sample	L*	$\Delta E^*$	WHC (%)	OHC (%)
1	77.23 ± 0.33 <sup>bc</sup>	25.92 ± 0.49 <sup>bcd</sup>	247.22 ± 0.05 <sup>bc</sup>	96.64 ± 0.03 <sup>bc</sup>
2	77.09 ± 0.42 <sup>bc</sup>	26.12 ± 0.42 <sup>cde</sup>	279.11 ± 0.04 <sup>g</sup>	93.47 ± 0.02 <sup>ab</sup>
3	77.36 ± 0.04 <sup>cd</sup>	25.82 ± 0.03 <sup>bcd</sup>	238.38 ± 0.03 <sup>b</sup>	90.72 ± 0.02 <sup>a</sup>
4	78.40 ± 0.09 <sup>e</sup>	24.79 ± 0.14 <sup>a</sup>	285.19 ± 0.06 <sup>fg</sup>	103.01 ± 0.01 <sup>de</sup>
5	76.99 ± 0.09 <sup>bc</sup>	26.11 ± 0.26 <sup>cde</sup>	250.08 ± 0.04 <sup>c</sup>	104.37 ± 0.03 <sup>e</sup>
6	76.73 ± 0.37 <sup>b</sup>	26.12 ± 0.45 <sup>cde</sup>	222.61 ± 0.04 <sup>a</sup>	95.52 ± 0.01 <sup>b</sup>
7	76.82 ± 0.24 <sup>bc</sup>	26.32 ± 0.11 <sup>de</sup>	252.58 ± 0.07 <sup>cd</sup>	103.38 ± 0.03 <sup>de</sup>
8	77.24 ± 0.23 <sup>bc</sup>	25.99 ± 0.51 <sup>bcd</sup>	268.69 ± 0.01 <sup>ef</sup>	109.94 ± 0.01 <sup>f</sup>
9	76.02 ± 0.23 <sup>a</sup>	26.74 ± 0.35 <sup>ef</sup>	289.89 ± 0.07 <sup>h</sup>	104.38 ± 0.03 <sup>e</sup>
10	77.84 ± 0.18 <sup>de</sup>	25.31 ± 0.04 <sup>ab</sup>	238.54 ± 0.10 <sup>b</sup>	97.09 ± 0.01 <sup>bc</sup>
11	76.09 ± 0.27 <sup>a</sup>	27.09 ± 0.13 <sup>f</sup>	260.10 ± 0.02 <sup>de</sup>	100.28 ± 0.02 <sup>cd</sup>
12	77.91 ± 0.19 <sup>de</sup>	25.46 ± 0.3 <sup>abc</sup>	237.99 ± 0.03 <sup>b</sup>	94.71 ± 0.02 <sup>b</sup>
Range	76.02~78.40	24.79~27.09	222.61~289.89	90.72~109.94
CV	1%	2%	1.79%	1.92%

Average values with different superscripts within the same column are significantly different at  $p < 0.05$ . CV is short for the coefficient of variation.


**Figure 1.** Textural properties of HTVP.

**Figure 2.** Texturization degree and sensory of HTVP.

chewiness are too high or too low, it will reduce the taste and decrease the approval of people.

The degree of texturization is an important index used to evaluate the quality of HTVP products. For example, Zhang et al. (2018a) used it to describe the degree of fibrosis of products. The higher the value of texturization degree, the better the fiber structure. In Figure 2, The texturization degree of the 12 HTVPs ranges from 1.28 to 2.06, and the coefficient of variation is 16.32%. The range of a batch with a reasonable degree of texturization is about 2.00, mainly No.5, No.8, and No.9. However, Osen et al. (2014) concluded that when the cooking temperature exceeded the denaturation temperature of the protein, the functional characteristics of the protein played a small role in the process of fiber formation. This is inconsistent with the experimental results, possibly due to the different extrusion materials used and the significant differences in the quality of SPC raw materials measured in this experiment.

Sensory evaluation has been used as a valid tool for assessing the quality of food products, bringing predictive results on the

acceptance of a product by consumers (Priulli et al., 2021). For TVP to replace meat in the daily diet, these products need to be accepted by the public in terms of their overall preferences. In addition, sensory assessment combined with instrumental measures (e.g. texture and colour) can also better guide product processing and recipe optimization, helping to improve the end product (Fiorentini et al., 2020). Sensory scores for the 12 HTVPs are shown in Figure 2. The sensory evaluation of HTVP shows that No.2 has the highest score. No.2 HTVP, showing a light yellow color, has a flat surface without burrs, good taste, flavor, and fiber structure, so the overall score is the highest. The lowest score is No.1, which was low because of its poor taste, smell and fiber structure, dark color, hard taste and lack of acceptance.

### 3.4 Cluster analysis of HTVP quality

To find the optimal HTVP and the most suitable SPC raw material characteristics, the quality of HTVP is clustered. The results are shown in Figure 3, Table 5, and Table 6.

**Table 5.** Quality characteristics of HTVP after clustering.

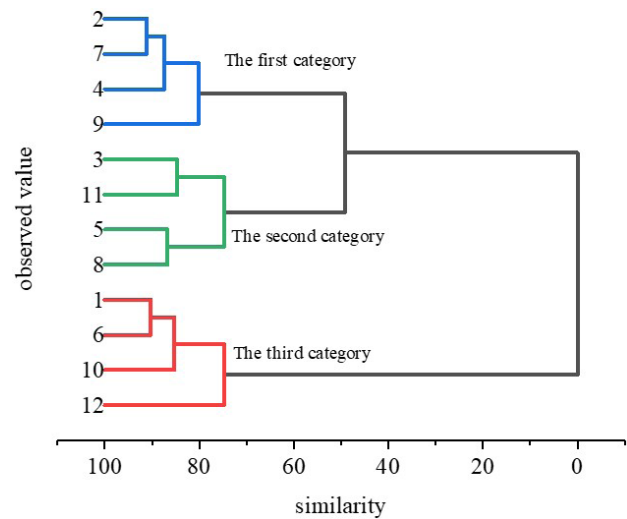
	The first category	The second category	The third category
WHC (%)	276.69	254.31	236.59
OHC (%)	101.06	101.33	95.99
Texturization degree	1.72	1.81	1.44
Sensory score	72.13	69.65	64.08
Hardiness (g)	19 239.63	22 678.52	27 610.77
Springiness (%)	86.00	87.00	90.00
Chewiness (g)	14 506.14	18 270.11	24 181.82
L*	77.08	76.92	77.43
$\Delta E^*$	25.99	26.25	25.70

**Table 6.** Characteristics of SPC raw materials of different classes after clustering.

	The first category	The second category	The third category
NSI (%)	62.26	30.85	5.30
pH	7.41	7.38	7.03
WHC (%)	461.41	420.86	287.58
OHC (%)	128.00	112.25	89.25
EAI (m <sup>2</sup> /g)	9.73	9.12	8.14
ESI (min <sup>-1</sup> )	16.71	17.85	18.47
FC (%)	146.53	121.41	43.96
FS (%)	16.55	16.29	31.25
Viscosity (cP)	750.25	780.88	20.63
Gel strength (g)	119.95	113.85	33.39
Sulfhydryl content ( $\mu\text{mol/g}$ )	4.42	3.81	4.25
11S/7S	1.85	1.77	1.24

The 12 products were divided into three categories and the clustered HTTPP quality characteristics are shown in Table 5. The WHC, OHC, and sensory scores of the first category are the highest among the three types. The second category has the highest degree of texturization, followed by the first. The hardness and chewiness of the third group are the highest. According to the data presented above, springiness and color are not significantly different among the three groups, and the hardness and chewiness keep at the appropriate level. Comprehensive analysis showed that the first type of product had an excellent WHC, OHC, and the highest sensory score, indicating the best acceptance. Although the degree of texturization of the first type is inferior to the second type of products, the difference is slight. So the first type of sample can be used as the optimal product. According to products 2, 7, 4, and 9 included in the first category, the optimal range of the HTVP protein quality index can be selected as follows: WHC is 252.58%~289.89%, OHC is 93.47%~104.38%, texturization degree is 1.46~2.00, hardness is 18 145.40 g~20 536.49 g, springiness is 85.00%~87.00%, and chewiness is 13 706.35 g~15 277.18 g.

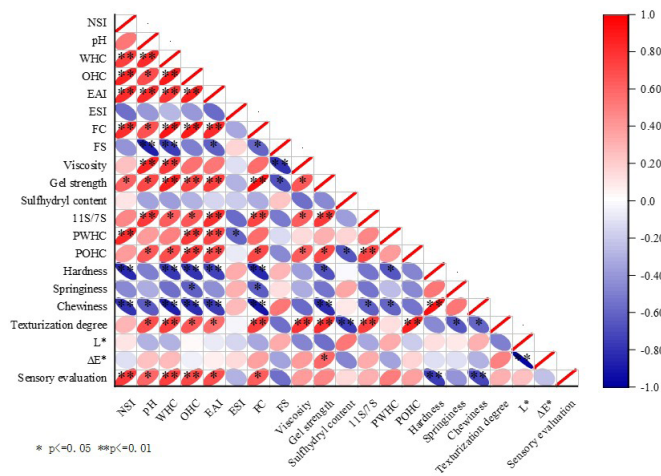
The characteristics of raw materials corresponding to the three categories of HTVP products obtained by cluster analysis are shown in Table 6. The functional characteristics of SPC raw

**Figure 3.** Pedigree of HTVP quality characteristics cluster analysis.

materials corresponding to the first category of products with the best product quality performance are the highest, such as NSI, WHC, OHC, EAI, FC, and gel strength. Therefore, it can be concluded that the functional characteristics of SPC play an essential role in promoting the quality of HTVP. In addition, moisture, ash, crude protein, fat content, and color of the three kinds of HTVP related raw materials do not have noticeable differences. To sum up, when the quality of HTVP is better, the required range of SPC raw material characteristics is as follows: NSI is 50.44%~70.56%, pH is 6.99~7.84, WHC is 425.62%~492.90%, OHC is 118.00%~150.00%, EAI is 8.82~10.27%, ESI is 14.70%~21.47%. FC is 140.54%~150.82%, FS is 10.34%~23.55%, viscosity is 397.50 CP~856.29 cP, gel strength is 82.00 g~144.76 g, sulfhydryl content is 3.62  $\mu\text{mol/g}$ ~5.31  $\mu\text{mol/g}$ , the ratio of 11S to 7S is 1.52~2.49.

### 3.5 Correlation analysis

The correlation analysis results between SPC raw materials and HTVP quality indexes are shown in Figure 4. The influence of raw materials on products is mainly discussed in this part. The WHC of the product is positively correlated with NSI, OHC, EAI, and negatively correlated with ESI of the SPC raw material. The water holding capacity of proteins is directly proportional to their water binding capacity, so WHC is positively correlated with NSI. The NSI, WHC, OHC, and EAI of SPC raw materials have significant correlation. The OHC of the product is positively correlated with pH, WHC, OHC, EAI, FC, viscosity, gel strength, 11S/7S, and negatively correlated with the sulfhydryl content of raw materials. The hardness and chewiness of HTVP are negatively correlated with NSI, WHC, OHC, EAI, FC, and gel strength of raw materials. The chewiness is also negatively correlated with pH and 11s/7s of raw materials. The springiness is negatively correlated with the OHC and FC of raw materials. The higher the NSI value, the lower the degree of protein denaturation and the better the texture properties of extruded products (Zhang et al., 2017). In addition, the stronger the binding capacity of the protein raw material with water or oil, the softer the product.



**Figure 4.** Correlation analysis between SPC raw material and HTVP quality indexes.

The texture degree of HTVP is positively correlated with pH, WHC, OHC, EAI, FC, viscosity, gel strength, and 11S/7S of raw materials. It is negatively correlated with the content of the sulfhydryl group. Increasing the pH value of raw materials appropriately can improve the organizational quality of products. Alkaline conditions promoted the formation of gluten network and induced a more fibrous cross section and the regular surface of gluten extrudate (Li et al., 2018). The viscosity is the internal friction force when the material flows. The greater the viscosity value, the slower the flow speed of the material in the barrel, and the more orderly fiber structure may be formed in the cooling mold (Chen et al., 2010). Proteins with poor gel ability, such as peanut protein, are difficult to form rich fibrous structure during high moisture extrusion (Zhang et al., 2018b). Soybean 11S protein has a higher content of sulfur amino acids, and the tensile and shear strength of heat-induced gels are higher than 7S protein. It may be easier to promote the formation of fiber structure through covalently cross-linking during extrusion, thus improving the texture degree of HTVP (Liu et al., 2022). The oxidation reaction that occurs during extrusion reduces the content of sulfhydryl group, which is conducive to the formation of disulfide bonds, and the protein rearranged to form new chemical cross-linking in the die (Peng et al., 2022). The sensory evaluation of HTVP is positively correlated with the NSI, pH, WHC, OHC, EAI, and FC of SPC. According to the above, we can try to produce better quality extrusion products by changing some characteristics of SPC raw materials.

## 4 Conclusion

In conclusion, we conclude that the characteristics of SPC raw materials produced by different manufacturers and different batches of the same manufacturer vary significantly. SPC was extruded with other raw materials to prepare HTVP, and it was found that there were significant differences in the quality of the various products, in which the texture properties, degree of texturization, and sensory score were significantly different. At the same time, WHC, OHC, and color coefficient of variation were small, and the differences between the data were slight.

According to the correlation analysis results of SPC and HTVP quality, the SPC raw material characteristics that have a very significant impact on product quality are NSI, WHC, EAI, FC, gel strength, and sulfhydryl content.

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