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Master's Thesis of Agriculture

**Effects of various plant-based proteins
on the quality characteristics of high
moisture texturized vegetable protein
through an extrusion process**

**다양한 식물성 단백질이 고수분 압출성형 식물성
조직 단백질의 품질 특성에 미치는 영향**

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Effects of various plant-based proteins on the quality characteristics of high moisture texturized vegetable protein through an extrusion process

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이 논문을 농학석사 학위논문으로 제출함

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ABSTRACT

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According to increasing interest in the meat analogue market, research on the fibrous structure of the meat analogues that mimics the muscle fiber structure of the meat is actively conducted. Soy protein isolate is widely used because of its good gelation ability, but it has a too hard texture. Therefore, the objective of this study is to produce meat analogues with a fibrous structure and a softer texture by mixing different protein sources including pea protein isolate, fababean protein concentrate, and pumpkin seed protein concentrate. The properties of the texturized vegetable protein (TVP) were characterized with measurements of rheological properties, appearance, color, moisture content, texturization index, and microstructure imaging. The moisture content was all maintained at similar levels by process design. The storage modulus of protein slurry tended to decrease as the content of soy protein isolate with the highest gelling ability decreased. It was confirmed that the texturized structure of high moisture TVPs was well formed from the appearance of the fibrous structure connected without breaking. The tendency of texture properties,

hardness, springiness, cohesiveness and chewiness were decreased when the proportion of soy protein isolate was decreased, similar to the tendency of storage modulus of protein slurry. The texturization index and the microstructure image showed the proportion of soy protein isolate effects on the fibrous structure of TVP. This study showed that the high moisture TVP can be produced using various plant-based protein materials, and setting the mixing ratio of plant-based materials to make TVP with fibrous texture is important. In addition, it showed that pea protein isolate was material with the potential for making the high moisture TVPs due to its quality characteristics such as appearance and color.

Keyword : High moisture extrusion, Meat analogue, Texturized vegetable protein

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I . INTRODUCTION

Worldwide interest in plant-based protein alternatives is rising in relation to animal ethics, environment, and health issues (Singh et al., 2021). In particular, consumers have a high interest in meat analogues that can replace meat, so research on plant-based meat analogues are actively conducted. People prefer meat analogues with a texture comparable to actual meat, so texture improvement is one of the main issues in meat analogue research (Fiorentini et al., 2020). Because the structure of analogues replicates the fiber structure of meat, consumers can feel the texture of meat in meat analogues.

The extrusion using high temperature and high pressure is a widely used when making meat analogues, and it is divided into low moisture extrusion and high moisture extrusion according to the moisture content. Low moisture meat analogue (LMMA) expand when extruded, causing moisture to vaporize (Lee, Oh, et al., 2022), so it has many large air cells and sponge-like structures, and before use, it had to be rehydrated (Guy, 2001), (Guo et al., 2020). However, when producing high moisture meat analogue (HMMA), the cooling die prevents sudden pressure changes and prevents internal moisture evaporate, so makes the texture similar to actual meat (J. Zhang

et al., 2022), (Z. Zhang et al., 2022). Currently, LMMA is already widely used, but due to the textural potentiality of HMMA, many studies are being conducted on high moisture extrusion. Especially, the studies of improving the texture of HMMA by adjusting the parameters of extrusion such as feeding rate, screw rate, barrel temperature, and material sources are performed (Maung et al., 2020), (Kaleda et al., 2021), (Xiao et al., 2022).

Soy protein is most commonly used as a component of meat analogues because it has good gelling properties. However, soy protein is associated with allergy and GMO issues (Webb et al., 2022) and the texture of soy-based meat analogues is too hard. So, many other proteins are being used as a way to solve these problems. Especially, pea protein is widely used because of its softer texture and less allergy risk (Osen et al., 2014), (Kantanen et al., 2022). But, gelling properties of pea protein are lesser than soy protein, so it is still hard to make proper texture with pea protein (Shand et al., 2007).

Fababean protein is a pulse that is often used to make TVP as pea protein. Fababean protein has attention due to its low fat and fiber-rich nutrients (do Carmo et al., 2021). And it is widely used in the research on TVPs because it has similar functions to soy protein, such as water holding capacity, oil

holding capacity, and foam stability (Kantanen et al., 2022). Seed protein such as pumpkin seed protein is also used to produce TVPs because of its potential to replace soy in plant-based foods. Additionally, the study of using pumpkin seed protein to make TVP is still in the initial phase (Kyriakopoulou et al., 2021).

In this study, I tried to produce high moisture TVPs having a soft texture and texturized fibrous structure by mixing pea protein, fababean protein, pumpkin seed protein and soy protein. And texture characteristics of the produced TVPs were analyzed to determine whether the interaction of the two proteins affected the formation and texture of TVPs.

II. MATERIALS AND METHODS

2.1. The extrusion condition setup

2.1.1. Materials

To determine extrusion conditions to produce high moisture TVPs, preliminary experiments were conducted by changing extrusion conditions such as raw materials, mixing ratio of raw materials, moisture content, screw speed, feed rate, and barrel temperature.

Two types of soy protein isolate (SPI), SPI-S from Solbar Ningbo Protein Technology Co., Ltd (Ningbo, China), SPI-Y from Shandong Yuwang Ecological Food Industry Co., Ltd (Yucheng, China), and pea protein isolate (PPI) from Roquette Frères (Lestrem, France) were used to setting the extrusion condition.

Corn starch (CS) and wheat gluten (WG) were obtained from Solbar Ningbo Protein Technology Co., Ltd and Roquette Frères, respectively.

2.1.2. Extrusion

The twin-screw extruder (FX-40, Milling Ind., Seoul, Korea) with a cooling die (50 × 10 × 500 mm (W × H × L)) was used to extrusion step. Then, the mixture was cooled down by a cooling die. The extrusion parameters are shown in Table 1.

Table 1. The extrusion parameters (raw materials and its ratio, moisture content, screw rate, feed rate, and barrel temperature) to setup the extrusion conditions

Materials	Sample	Ratio	Moisture content (%)	Screw rate (rpm)	Feed rate (rpm)	Barrel temperature
SPI-Y + WG	YW1	9:1	60	200	350	60-80-120-90
	YW2	9:1	69	100	350	
	YW3	9:1	76	100	150	
	YW4	9:1	76	200	100	
	YW5	9:1	76	300	100	70-90-120-90
	YW6	8:2	64	400	200	
	YW7	8:2	76	300	100	
	YW8	8:2	76	400	100	
SPI-Y + CS	YC1	9:1	57	600	400	90-120-130-120
	YC2	9:1	57	1000	300	90-120-150-120
SPI-Y + WG + CS	YWC1	7:2:1	57	500	300	90-120-150-120
	YWC2	7:2:1	57	800	300	
	YWC3	7:2:1	57	1000	300	
	YWC4	7:2:1	57	1000	400	
SPI-S + CS	SC1	9:1	57	600	400	90-120-130-120
	SC2	9:1	57	700	100	
	SC3	9:1	57	800	200	
	SC4	9:1	57	800	400	
	SC5	9:1	57	1000	400	
PPI + WG	PW1	8:2	64	200	200	70-90-120-90
	PW2	8:2	64	300	200	
	PW3	8:2	76	300	100	

2.2. High moisture TVPs made of plant-based protein mixture

2.2.1. Materials

Soy protein isolate (SPI), pea protein isolate (PPI), fababean protein concentrate (FPC), pumpkin seed protein concentrate (PSPC), and corn starch (CS) were obtained from Solbar Ningbo Protein Technology Co., Ltd, Roquette Frères, AGT Foods (Regina, SK, Canada), Nutraceuticals Group (Little Falls, NJ, USA), and Samyang Corporation (Seoul, Korea) respectively.

2.2.2. Rheological properties

To confirm the gelling properties of the mixture of soy protein and pea protein, discovery hybrid rheometer (AR 1500 ex, TA instruments, New Castle, Delaware, USA) was used with a diameter of 40 mm steel plate. The slurry consisted of SPI, PPI and CS with 20% w/w, SPI, FPC and CS with 30% w/w, and SPI, PSPC and CS with 30% w/w and the detail proportion is according to Table 2, respectively. The protein mixture was hydrated overnight. For the temperature sweep, the sample got the temperature change with a constant 0.02 strain level. The sample was heated from 25 °C to 95 °C, and maintained at 95 °C. The temperature was then reduced until it reached 25 °C. The results were represented as storage modulus (G') and loss modulus (G'')

Table 2. The proportion of raw materials (SPI, PPI, FPC, SPC, and CS) for the slurry and TVP mixture

Materials	Sample	Proportion (wt%)		
		SPI	PPI	CS
SPI + PPI	S9P0	9	0	1
	S8P1	8	1	1
	S7P2	7	2	1
	S6P3	6	3	1
	S5P4	5	4	1
	S4P5	4	5	1
	S3P6	3	6	1
	S2P7	2	7	1
	S1P8	1	8	1
	S0P9	0	9	1

Materials	Sample	Proportion (wt%)		
		SPI	FPC	CS
SPI + FPC	S9F0	9	0	1
	S8F1	8	1	1
	S7F2	7	2	1
	S6F3	6	3	1

Materials	Sample	Proportion (wt%)		
		SPI	PSPC	CS
SPI + PSPC	S9PS0	9	0	1
	S8PS1	8	1	1
	S7PS2	7	2	1
	S6PS3	6	3	1
	S5PS4	5	4	1

2.2.3. Extrusion

The mixture of SPI, CS and PPI or FPC or PSPC, was used to produce high moisture TVPs and the proportion of the mixture is shown in Table 2, respectively. Then the PPI mixture and water were blended for an hour and a half in the 5:6 ratio (approximately 58%), and the FPC and PSPC mixture and water also blended for same time with PPI mixture in the approximately 64% of moisture contents (5:8), with the kneader (HDP-020S, Hunwoo, Seoul, Korea) and hydrated overnight. The high moisture extrusion of TVPs was performed using a twin-screw extruder with a cooling die. The blended sample was fed through the feeder at the rate of 7.5 kg/h and extrusion with the screw speed of 800 rpm. The temperatures of each barrel were 60, 80, 100, and 120 °C (for PPI mixture), and 90, 120, 130, and 120 °C (for FPC and PSPC mixture) respectively in order near the feeder. The denaturation temperature of pea protein is about 75~85 °C (J.L. Messian, 2015), and it is lower than the protein denaturation temperature of other protein (soy protein: about 90 °C (S.R. Euston, 2009) fababeam protein: 82~95.4 °C (D.Martineau-Côté, 2022) and pumpkin seed protein: 93.4~96.6 °C (L Rezig, 2013)). Then, the mixture was cooled down through 20 °C for PPI, and 15 °C for FPC and PSPC at cooling die section.

2.2.4. Moisture content

To determine the moisture content, AACC 44-19.01 method was used. TVPs were dried in an oven at 105 °C for 16 h.

2.2.5. Appearance

TVPs were cut into 2×5 cm and were torn in the flow direction to check the internal fibrous structure. The appearance images of TVPs were taken using a fixed camera (450D, Canon, Tokyo, Japan) within a black photo box with a light source.

2.2.6. Color

The color of TVPs was followed Hunter' s color system (L^* , a^* , and b^*) with a chromameter (CR-400, Konica Minolta Sensing Inc., Tokyo, Japan).

2.2.7. Textural properties

The textural properties of TVPs were measured by a texture analyzer (TA.XTplusC, Stable Micro Systems, Surrey, UK). Hardness, springiness, cohesiveness, and chewiness data were determined using a 100 mm diameter compression plate probe. SPI and PPI mixing TVPs were cut into $2 \times 2 \times 1$ cm and compressed with 60% strain at a test speed of 2 mm/s. TVPs made of SPI and FPC mixture and SPI and PSPC mixture were cut into $3 \times 3 \times 1$ cm, and were compressed at a test speed of 2 mm/s with 50% and 60%, respectively.

The texturization index was calculated with slight modifications to the method by (Mohamad Mazlan et al., 2020). Samples ($3 \times 3 \times 1$ cm) were cut using a Warner-Bratzler shear probe in longitudinal which is parallel to TVP flow direction, and transversal which is vertical to TVP flow direction at a speed of 2 mm/s to 100% of its

original height. Figure 1 showed the cutting direction of TVPs. The texturization index was calculated as the equation below.

$$\text{Texturization index} = \frac{\text{Transversal cutting strength (N)}}{\text{Longitudinal cutting strength (N)}}$$

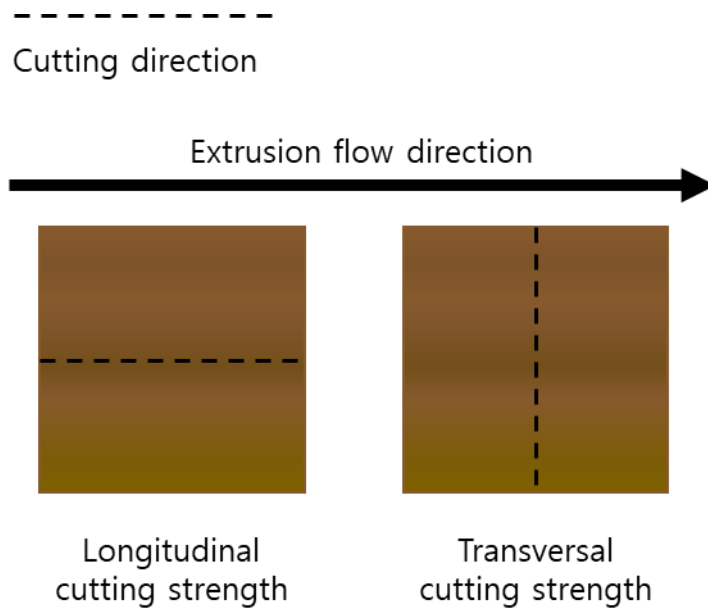


Figure 1. Longitudinal and transversal cutting direction of high moisture TVPs for TI index measurement.

2.2.8. Scanning electron microscopy

To take the microstructure image of TVPs, Field–Emission Scanning Electron Microscope (SIGMA, Carl Zeiss, Oberkochen, Germany) was used. TVPs were cut into small pieces and freeze–dried. Then, samples were coated with platinum at 30 mA for 120 s. Samples were scanned with a 5 kV acceleration voltage and 100× magnification in a vacuum.

2.3. Statistical analysis

Statistical analysis was performed using SPSS software (version 26.0, SPSS Inc, Chicago, IL, USA) by the one–way analysis of variance and Tukey’ s honestly significant difference (HSD). Statistical significance on each sample was considered at $p < 0.05$. All tests were conducted at least three times. The mean \pm standard deviation (SD) was used to represent the results.

III. RESULTS AND DISCUSSION

3.1. The extrusion condition setup

Figure 2 showed the appearance of TVPs made with the condition of Table 1. The samples YW1 and YW6, the samples were made with SPI-Y and WG, and the moisture content was under 64%, the crack was shown in the TVP. There were grain-like structure and nonuniform TVPs produced when the feed rate was 100 and 150 rpm. However, over 350 rpm of feed rate, the surface of TVPs was uniform. The sample that was blended with SPI-Y and CS did not form TVPs when screw rate of 600 rpm and the feed rate of 400 rpm. When using SPI-S and CS mixture, the TVPs were not produced neither at the condition of 700 rpm of screw rate and 100 rpm of feed rate (SC2), nor 800 rpm of screw rate and 200 rpm of feed rate (SC3). TVPs consisted of PPI and WG, were too watery and didn't form well.

Considering the result, screw rate at 800 rpm and feed rate at 300 rpm were used for the extrusion of TVP.



Figure 2. The appearance of TVPs for setting the extrusion condition.

3.2. High moisture TVPs made of plant-based protein mixture

3.2.1. Rheological properties

The rheological properties of the protein slurry were shown in Figure 3. The G' of the protein slurry decreased rapidly during the heating process, and then increased during the cooling period. And the G' of the final gel was higher when using FPC than when using PSPC. The G' was reduced as the elastic behavior of the protein decreased due to the destruction of ionic and hydrogen bonds, and the denaturation of protein molecules during the heating period (Wang et al., 2017). Also, the increase of G' for the cooling process was a property of a protein called gel reinforcement, generally due to strengthening of attractive forces such as hydrogen bonds and ionic interactions between proteins (Clark & Lee-Tuffnell, 1998; Zhang et al., 2019). After completing the cooling process, the G' was declined as the content of SPI decreased, which might be related that the gelling property of SPI being higher than that of PPI, FPC, and PSPC.

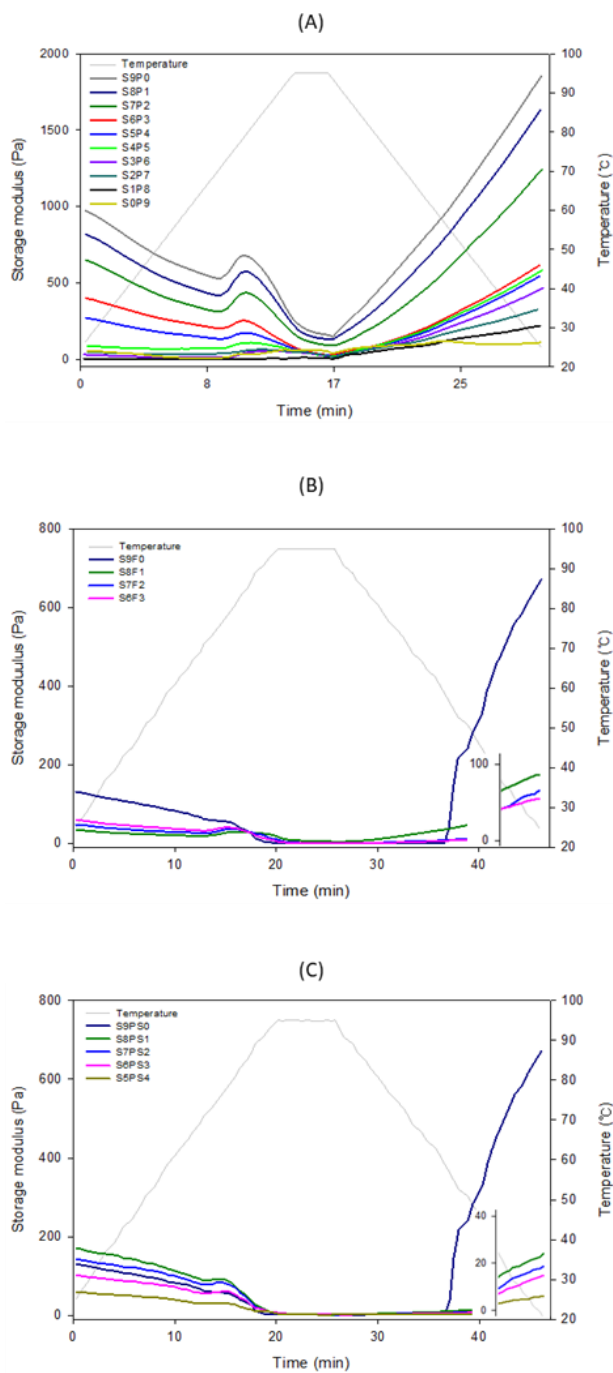


Figure 3. Storage modulus (G') of protein slurries: (A) Pea protein isolate, (B) Fababean protein concentrate, and (C) Pumpkin seed protein concentrate.

3.2.2. Moisture content

The moisture contents of SPI and PPI blending TVPs ranged between 55.06 ± 0.24 and $57.43 \pm 0.23\%$ (Table 3), which were little lesser than the expected moisture content of 58%. The moisture contents of TVPs made of SPI and FPC blending ($61.19 \pm 0.21 \sim 62.86 \pm 0.08$) and SPI and PSPC blending ($62.43 \pm 0.10 \sim 64.30 \pm 0.01$) were close to the moisture content of 64%, which was the expected moisture content. According to (Zahari et al., 2020), a slight difference in moisture content could be caused by an irregular feed rate of the blended samples through the extrusion process. It was determined that the effect of moisture content was restricted in the properties of TVPs because the moisture contents of TVPs were controlled.

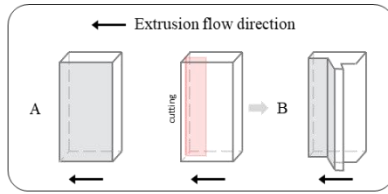
Table 3. Moisture content of high moisture TVPs

Materials	Sample	Moisture content (%)
SPI + PPI	S9P0	56.79 ± 0.26 ^a
	S8P1	55.06 ± 0.24 ^c
	S7P2	55.79 ± 0.09 ^b
	S6P3	56.98 ± 0.19 ^a
	S5P4	57.16 ± 0.30 ^a
	S4P5	56.74 ± 0.13 ^a
	S3P6	57.03 ± 0.22 ^a
	S2P7	57.43 ± 0.23 ^a
	S1P8	57.04 ± 0.04 ^a
	S0P9	57.02 ± 0.17 ^a
SPI + FPC	S9F0	62.38 ± 0.14 ^b
	S8F1	61.19 ± 0.21 ^c
	S7F2	62.86 ± 0.08 ^a
	S6F3	62.15 ± 0.08 ^b
SPI + PSPC	S9PS0	64.30 ± 0.01 ^a
	S8PS1	64.16 ± 0.09 ^a
	S7PS2	63.31 ± 0.07 ^b
	S6PS3	62.43 ± 0.10 ^c
	S5PS4	62.59 ± 0.07 ^c

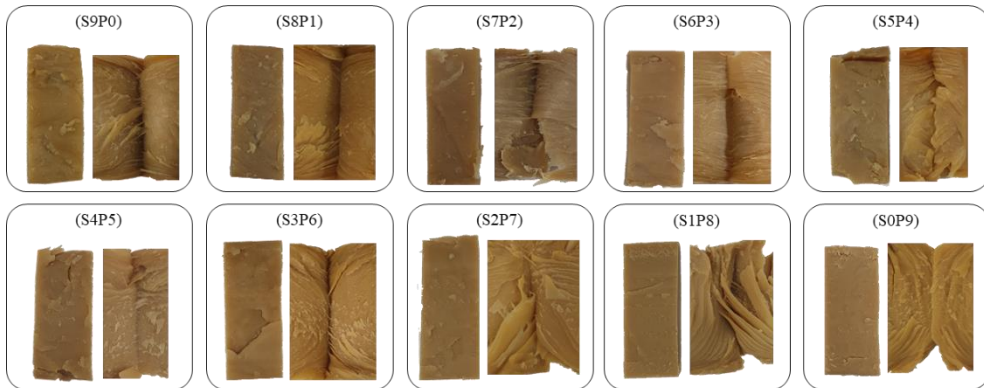
All data represent the mean of triplicates. Different letters in same materials represent the significant differences ($p < 0.05$).

3.2.3. Appearance

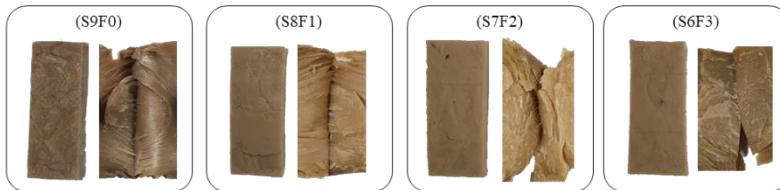
The external surface and internal fibrous structure of high moisture TVPs are shown in Figure 4. TVPs were molded by a cooling die shape. Most of samples reveal the characteristics of the high moisture TVPs using cooling die, layered and fibrous structures which is the feature of high moisture TVP as demonstrated in (Samard et al., 2019) and (Ferawati et al., 2021). However, when the samples were torn to check the internal structure, the weaker gel was broken as the SPI content was lower. Therefore, the fibrous structure was not shown in the S6F3 sample, especially.



SPI + PPI



SPI + FPC



SPI + PSPC



Figure 4. The images of high moisture TVPs. The left image is an outer surface of high moisture TVPs, and the right image is an internal fibrous structure of high moisture TVPs.

3.2.4. Color

During the extrusion process, the color of TVPs changed because of various reactions, such as the Maillard reaction and caramelization (Zahari et al., 2020). Table 4 presents the color of TVPs. The L^* (lightness) of the TVP made of PPI mixture increased slightly with decreasing SPI levels. The a^* (redness) and b^* (yellowness) also tended to increase with the ratio of SPI reducing. In total, the lower the SPI content, the brighter and closer to brown color. The color of the TVP made of FPC mixture showed similar tendency with TVP made of PPI mixture. As the proportion of PSPC increased, L^* , b^* tended to increase and a^* tended to decrease.

According to (Zahari et al., 2022), the color of TVPs is related to barrel temperature during extrusion, the moisture content of TVPs, and the color of raw materials. In this study, it suggests the color and proportion of raw materials had the most considerable effect on color difference of TVPs because the moisture content and barrel temperature were set at the same level. The color of TVPs is important because consumers recognized it the first appearance characteristic (Bakhsh et al., 2022). Therefore, the greenness color when PSPC ratio increased, can be a property that customers avoid.

Table 4. Lightness (L^*), redness (a^*), and yellowness (b^*) of high moisture TVPs

Materials	Sample	L^*	a^*	b^*
SPI + PPI	S9P0	51.05 ± 1.60 ^d	0.79 ± 0.23 ^d	11.78 ± 2.27 ^b
	S8P1	52.49 ± 1.81 ^{cd}	1.15 ± 0.21 ^d	13.72 ± 0.54 ^b
	S7P2	54.81 ± 1.22 ^{bcd}	0.98 ± 0.07 ^d	13.13 ± 0.33 ^b
	S6P3	53.32 ± 2.75 ^{cd}	1.03 ± 0.04 ^d	13.72 ± 1.02 ^b
	S5P4	59.66 ± 0.58 ^a	1.80 ± 0.35 ^c	19.71 ± 1.08 ^a
	S4P5	54.80 ± 1.02 ^{bcd}	1.93 ± 0.09 ^c	17.58 ± 0.79 ^b
	S3P6	55.55 ± 0.43 ^{bc}	2.87 ± 0.12 ^a	18.78 ± 0.90 ^a
	S2P7	56.02 ± 1.25 ^{abc}	2.28 ± 0.11 ^{bc}	18.69 ± 0.54 ^a
	S1P8	55.03 ± 0.63 ^{bc}	2.53 ± 0.07 ^{ab}	19.26 ± 0.56 ^a
	S0P9	57.71 ± 0.72 ^{ab}	2.81 ± 0.12 ^a	19.68 ± 0.48 ^a
SPI + FPC	S9F0	55.95 ± 0.25 ^c	2.79 ± 0.13 ^a	12.91 ± 0.06 ^c
	S8F1	55.71 ± 0.29 ^c	1.77 ± 0.01 ^c	11.64 ± 0.13 ^d
	S7F2	58.77 ± 0.07 ^b	2.29 ± 0.01 ^b	16.59 ± 0.11 ^b
	S6F3	59.49 ± 0.01 ^a	2.48 ± 0.10 ^b	20.14 ± 0.34 ^a
SPI + PSPC	S9PS0	55.95 ± 0.25 ^c	2.79 ± 0.13 ^a	12.91 ± 0.06 ^c
	S8PS1	54.11 ± 0.48 ^c	1.66 ± 0.74 ^b	13.69 ± 1.37 ^c
	S7PS2	56.15 ± 0.67 ^c	-0.73 ± 0.13 ^c	16.73 ± 0.29 ^b
	S6PS3	63.89 ± 1.16 ^a	-1.90 ± 0.39 ^d	19.24 ± 0.33 ^a
	S5PS4	60.10 ± 2.11 ^b	-3.19 ± 0.06 ^c	18.23 ± 0.92 ^{ab}

All data represent the mean of triplicates. Different letters in the same column in the same materials represent the significant differences ($p < 0.05$).

3.2.5. Textural properties

The texture properties of high moisture TVPs are shown in Figure 5. The hardness tended to decrease significantly as the content of SPI decreased from S9P0 (443.09 ± 42.49 N) to S0P9 (242.33 ± 9.33 N), from S9F0 (367.95 ± 6.42 N) to S6F3 (326.89 ± 11.01 N) and from S9PS0 (430.71 ± 12.92 N) to S5PS4 (328.41 ± 1.87 N), respectively. According to Table 5, the springiness (PPI mixture: $0.91 \pm 0.01 \sim 0.79 \pm 0.04$, FPC mixture: $0.90 \pm 0.03 \sim 0.80 \pm 0.02$, PSPC mixture: $0.91 \pm 0.01 \sim 0.83 \pm 0.01$), cohesiveness (PPI mixture: $0.79 \pm 0.01 \sim 0.50 \pm 0.06$, FPC mixture: $0.84 \pm 0.01 \sim 0.78 \pm 0.01$, PSPC mixture: $0.89 \pm 0.01 \sim 0.73 \pm 0.01$), chewiness (PPI mixture: $318.214 \pm 31.884 \sim 96.273 \pm 18.395$, FPC mixture: $280.15 \pm 3.58 \sim 203.28 \pm 7.54$, PSPC mixture: $348.70 \pm 3.12 \sim 198.05 \pm 0.57$) of also showed a tendency to decrease.

The hardness affects a major role in the texture characteristics of food. The hardness of the TVPs made of PPI, FPC, and PSPC mixture tended to reduce as the content of SPI decrease, similar tendency with the G' of protein slurry. The springiness, cohesiveness, and chewiness also had same trend. Therefore, the texture properties of TVPs were estimated that related to the gelling properties of raw protein materials.

According to (Chiang et al., 2019), disulfide bonds have influence on the formation of the binding structure of TVP. SPI consisted with high ratio of glycinin, a protein high in sulfur, and sulfur content of legumin, vicilin and cucurbitin is less than glycinin. Therefore, it is assumed that the differences of sulfur content in protein raw materials affected the formation of hard disulfide bonds. And it is suggested that other plant-based proteins interfered with structuring the protein bonds of SPI, resulting in texture characteristics differences.

The TI is an important indicator of the degree of texture formation of TVP. Because of differences in viscosity caused by cooling from the surface and flow rate caused by friction, U-shaped structure are formed (Lee, Choi, et al., 2022). $TI > 1$ represents transversal cutting strength is higher than longitudinal cutting strength. Therefore, fiber structures were formed in a direction parallel to the TVP flow, and it determined that the anisotropy structure, like real meat, is constructed well (Lee et al., 2023). Figure 6 shows the TI of high moisture TVPs. The TI of S9P0 (1.04 ± 0.01) was higher than S0P9 (0.89 ± 0.00) and tended to reduce as the content of SPI decreased. The TVP samples with the content of SPI above 50% (S9P0, S8P1, S7P2, S6P3, and S5P4) indicated that the fibrous structure was formed well because the TI was higher than 1. And the TI of TVP made of FPC mixture also decreased (1.06

± 0.00 to 0.99 ± 0.02) with decreasing SPI ratio and S6F3, TI is less than 1, suggested that meat-like structure is not formed well. When the PSPC mixture TVPs, the TI showed the tendency to decrease with reducing SPI content (1.19 ± 0.01 to 0.98 ± 0.01).

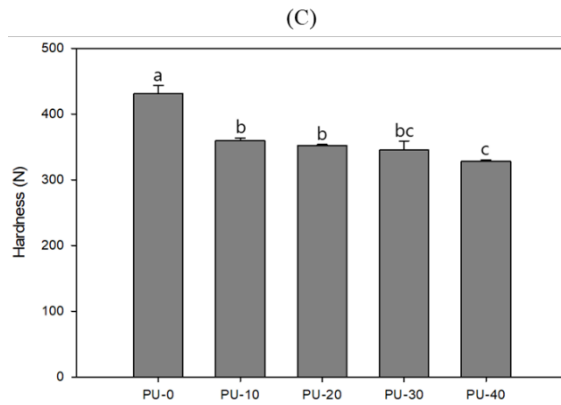
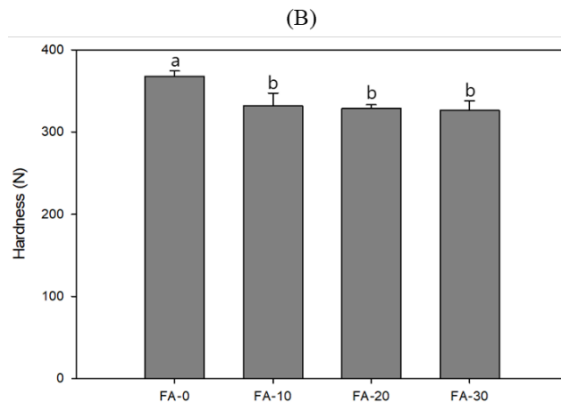
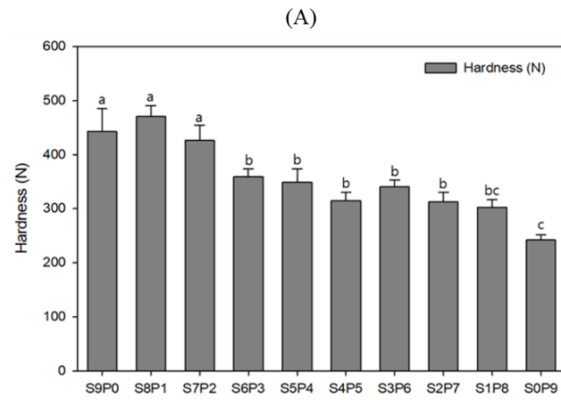


Figure 5. Hardness of high moisture TVPs: (A) PPI mixture TVPs, (B) FPC mixture TVPs, and (C) PSPC mixture TVPs. All data represent the mean of triplicates. Different letters represent the significant differences ($p < 0.05$).

Table 5. Texture properties (springiness, cohesiveness, chewiness) of high moisture TVPs

Materials	Sample	Springiness	Cohesiveness	Chewiness
SPI + PPI	S9P0	0.91 ± 0.01 ^{ab}	0.79 ± 0.01 ^{ab}	318.21 ± 31.88 ^a
	S8P1	0.92 ± 0.00 ^{ab}	0.81 ± 0.01 ^a	348.05 ± 11.49 ^a
	S7P2	0.93 ± 0.03 ^a	0.78 ± 0.01 ^{abc}	310.30 ± 10.25 ^a
	S6P3	0.88 ± 0.02 ^{ab}	0.78 ± 0.01 ^{abc}	245.69 ± 6.34 ^b
	S5P4	0.90 ± 0.01 ^{ab}	0.76 ± 0.02 ^{abc}	238.74 ± 16.21 ^b
	S4P5	0.88 ± 0.01 ^{ab}	0.74 ± 0.01 ^{abc}	204.58 ± 9.29 ^{bcd}
	S3P6	0.89 ± 0.00 ^{ab}	0.71 ± 0.04 ^{bc}	213.76 ± 17.33 ^{bc}
	S2P7	0.85 ± 0.05 ^{bc}	0.70 ± 0.06 ^{cd}	185.44 ± 22.39 ^{cd}
	S1P8	0.86 ± 0.03 ^{abc}	0.62 ± 0.02 ^d	161.24 ± 11.44 ^d
	S0P9	0.79 ± 0.04 ^c	0.50 ± 0.06 ^e	96.27 ± 18.40 ^e
SPI + FPC	S9F0	0.90 ± 0.03 ^a	0.84 ± 0.01 ^a	280.15 ± 3.58 ^a
	S8F1	0.84 ± 0.04 ^{ab}	0.80 ± 0.01 ^b	223.55 ± 12.15 ^b
	S7F2	0.87 ± 0.01 ^{ab}	0.76 ± 0.01 ^c	216.83 ± 7.47 ^b
	S6F3	0.80 ± 0.02 ^b	0.78 ± 0.01 ^{bc}	203.28 ± 7.54 ^b
SPI + PSPC	S9PS0	0.91 ± 0.01 ^a	0.89 ± 0.01 ^a	348.70 ± 3.12 ^a
	S8PS1	0.88 ± 0.01 ^b	0.84 ± 0.02 ^b	266.16 ± 5.27 ^b
	S7PS2	0.87 ± 0.00 ^b	0.79 ± 0.01 ^c	240.46 ± 2.65 ^c
	S6PS3	0.84 ± 0.01 ^c	0.76 ± 0.02 ^{cd}	220.21 ± 6.21 ^d
	S5PS4	0.83 ± 0.01 ^c	0.73 ± 0.01 ^d	198.05 ± 0.57 ^e

All data represent the mean of triplicates. Different letters in the same column in the same materials represent the significant differences ($p < 0.05$).

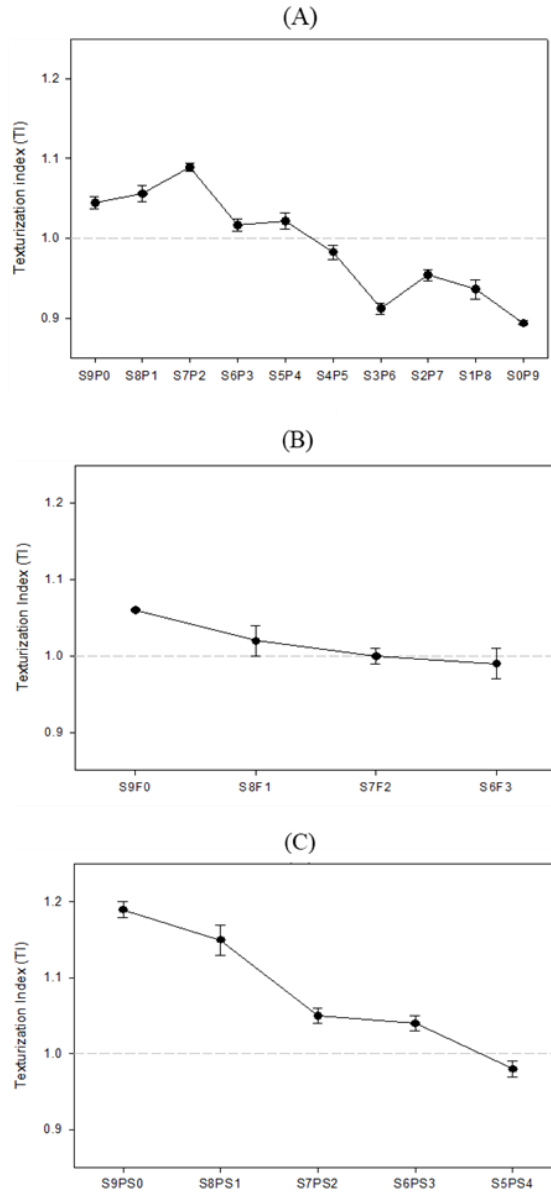


Figure 6. Texturization index of high moisture TVPs. (A) PPI mixture TVPs, (B) FPC mixture TVPs, (C) PSPC mixture TVPs. All data represent the mean of five replicates. Different letters represent the significant differences ($p < 0.05$) among the TI.

3.2.6. Scanning electron microscopy

Figure 7 represents the microstructure of TVP through SEM. The parallel fibrous structure was shown through the image of the sample that TI above 1, such as S7P2, S9F0, and S8PS1. The microstructure of TVPs forms directional and fibrous structures like real meat, chicken, beef, and pork according to (Samard & Ryu, 2019). But the texture of TVPs and real meat could be different because the numbers amount and forms of air cells are different. However, the fibrous structure was not generated well in the S0P9, S6F3, and S5PS4 (TI < 1). The SEM image of S5PS4 showed a network-like structure without breaking. However, the microstructure of S0P9 showed a dense structure, and at S6F3, the network seemed to be cut off. So, low SPI would inhibit the formation of a fibrous structure that is similar to real meat, and this result agreed with the texture properties and the TI.

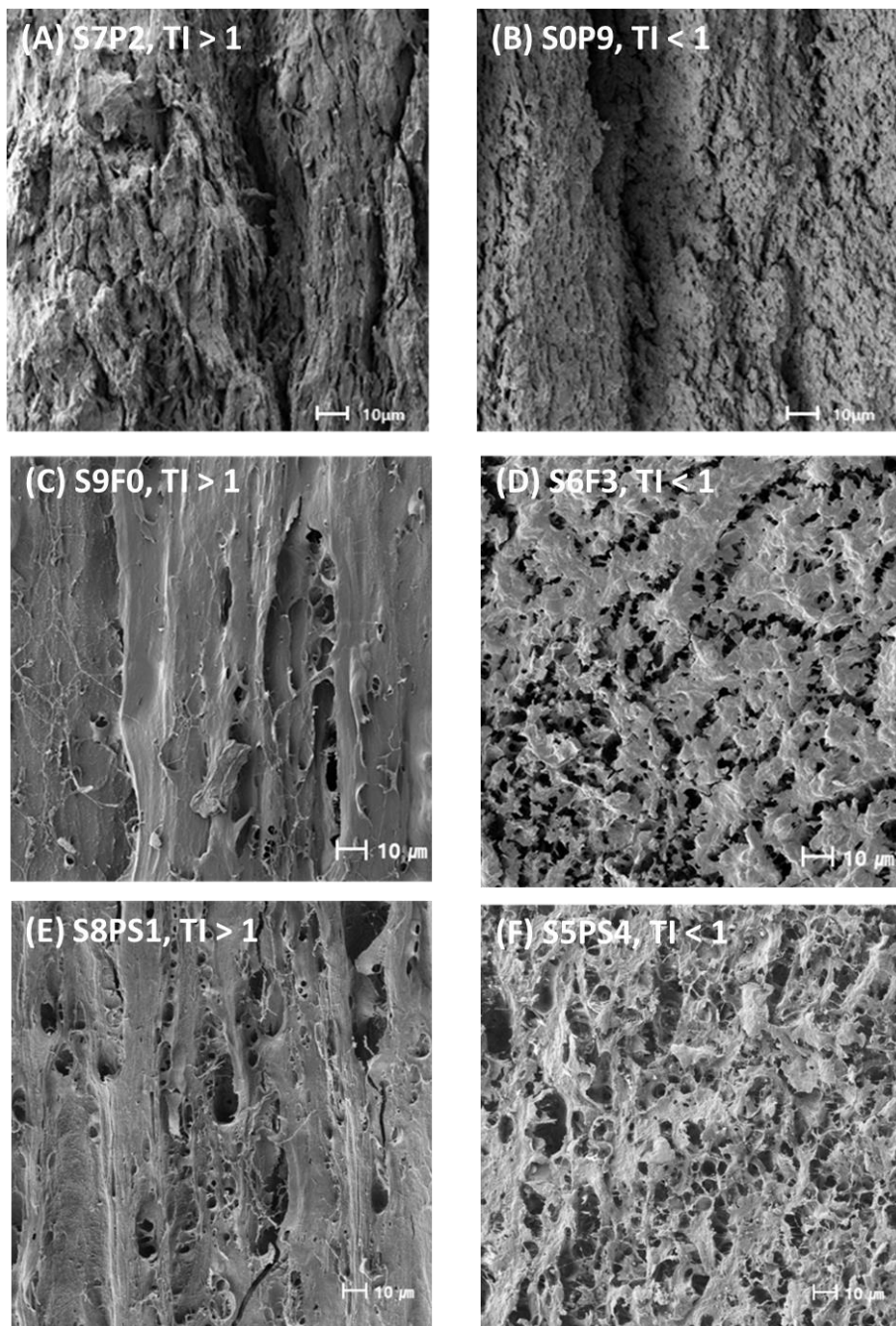


Figure 7. Microscopic morphological images of high moisture TVPs made of SPI and PPI mixture through SEM: (A) S7P2, (B) S0P9, (C) S9F0, (D) S6F3, (E) S8PS1, (F) S5PS4.

IV. CONCLUSION

In this study, the effect of blending different plant-based proteins in SPI on the quality characteristics of high moisture TVP was investigated. Since SPI has a higher gelling property than PPI, FPC, and PSPC, rheology properties such as G' of protein slurry tended to decline as the content of SPI decreased. The texture properties such as hardness, springiness, cohesiveness and chewiness of TVPs also tended to reduce as the SPI content decreased. The TI also showed a tendency to decrease as SPI content was reduced. However, the TI exceeded 1 only when the content of SPI was 50% or more, 70% or more, and 60% or more in the PPI, FPC, and PSPC mixture TVP, respectively. And in this case, it was confirmed that the fibrous structure was well formed. Likewise, through the microscopic image, it was determined that when the TI is less than 1, a clear fibrous structure was not formed.

Therefore, it was suggested that TVP with desired texture characteristics and fibrous structure could be created by controlling the blending ratio of other plant-based protein materials added to SPI. Especially, this study suggesting that pea protein isolate is the most potential source because the green color of pumpkin seed protein concentrate may seem to be a bad characteristic for consumers, and the limited content

to blend of faba bean protein concentrate. Further studies are needed to create a TVP with desired texture properties by mixing various other proteins in SPI and applying it to the high moisture extrusion method. Also, by performing a sensory analysis on the texture characteristics of TVPs, the quality properties that consumers actually feel can be analyzed.

V. 국문초록

대체육 시장에 대한 관심이 증가함에 따라 육류의 근섬유 구조를 모방한 식물성 조직화 단백질의 섬유 구조에 대한 연구가 활발히 진행되고 있다. 분리대두단백은 겔화 능력이 좋아 연구에 널리 사용되지만, 실제 육류보다 단단한 질감을 가진다는 한계점이 있다. 따라서 본 연구에서는 분리대두단백에 분리완두단백, 농축잠두단백, 농축호박씨단백 등의 다른 단백질 공급원을 각각 혼합하여 부드러운 질감을 가지고, 섬유같은 겔 구조를 형성하는 조직화단백을 생산하고자 하였다. 조직화단백의 특성 분석을 위해 단백질 슬러리의 물성 특성, 외관, 색도, 수분함량, 질감 특성 분석, 조직화 지수, 주사전자현미경을 통한 내부 구조 분석이 진행되었다. 단백질 슬러리의 저장 탄성률은 겔화 능력이 가장 높은 분리대두단백의 함량이 감소함에 따라 감소하는 경향이 나타났다. 만들어진 고수분 압출성형 조직화단백은 끊어지지 않고 연결된 실 같은 구조가 육안으로 보이는 것에서 겔이 잘 형성되었음을 확인할 수 있었다. 분리대두단백의 비율이 감소하였을 때의 경도, 탄력성, 응집성, 씹힘성의 경향은 단백질 슬러리의 저장 탄성률과 유사한 감소하는 경향을 보였다. 조직화지수와 전자주사현미경을 통한 내부 구조 분석을 통해 특정 정도 이상의 분리대두단백 함량이 식물성 조직화 단백질의 섬유 구조 형성에 영향을 끼치는 것을 확인할 수 있었다. 따라서 본 연구는 다양한 식물성 단백질 소재를 이용하여 질감이 개선된 고수분 식물성 조직화 단백질을 제조할 수 있음과, 겔 구조 형성을

위해서는 원료별 배합비 설정이 중요하다는 것을 보여주었다. 또한 분리완두단백을 혼합한 조직화 단백질 외관 등의 품질 특성이 가장 우수하였으며, 조직화 단백을 만드는 것에 잠재력을 가진 소재임을 보여주었다.

주요어: 고수분 압출성형, 대체육, 식물성조직화단백

학번: 2021-24011

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