

Effect of Rice Flour and Cassava Flour Blended with Soy Protein Isolate on Low Moisture Extrusion for Development to Textured Vegetable Protein

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Abstract

Soy protein isolate (SPI) was blended with rice or cassava flour for extruded to texture vegetable protein (TVP). The physicochemical properties of all samples were investigated. The feeding rate at 50 rpm of extrusion was the suitable condition for this experiment due to a better %CV of expansion degree, higher (water absorption index) WAI, and lower (water solubility index) WSI than that of other feeding rates. The SPI-TVP provided lower expansion degree, moisture content, WAI, and WSI while hardness and cohesiveness were higher than that of SPI+Rice and SPI+Cassava TVP. The SPI-TVP blended with a binder to be TVP-minced meat showed the higher hardness, gumminess, and chewiness but lower in cohesiveness than SPI+Rice and SPI+Cassava of TVP-minced meat. The properties of TVP-minced meat were related to the binder types and TVP extrudate, which attributed to the extrusion condition and raw materials properties.

Keywords: Soy protein isolate, Texture vegetable protein, Extrusion

Introduction

Soy protein isolates and concentrates were the most commonly used ingredients for structure of meat analogues products such as sausage-, burger- and meat muscle-like (Kyriakopoulou, et al., 2021). Soy-based products accounted for the largest market share of



more than 48% in 2019 due to their protein-rich contents. Moreover, the rising popularity of the vegan diet, particularly in European countries, has pushed the demand for plant-based meat products in the region. Plant Based Food (especially, vegetable meat) is considered as an alternative protein food or future food. Textured vegetable proteins (TVP) are products that have been transformed from a flour-type material mixed with other ingredients, into one which has a meat-like texture by using extrusion technique. Extrusion is a process in which a mixture of ingredients is forced through an opening in a die, and finally cut to a specific size or shape. This method is normally used for produce TVP (MacDonald & Reitmeier, 2017). The soy proteins are the most commonly used base materials for TVP and the resulting TVP product provides chewiness and fibrous character which are guite similar to the real-meat texture (Featherstone, 2015). Several food industries have tried to produce plant-based meat with high nutritional value. The opportunity of plant-based meat product is that plant-based meat alternative products and plant protein ingredients need to offer optimized nutrition, taste and functionality compared to the real meat. Additionally, taste is also the main reason for consumer's choice. Delivering good taste has traditionally been a challenge in the plantbased meat category. Hence, designed of plant-based meat nutrition to equal the animal alternatives in taste and texture, among them convenience products are the key driven for plant-based meat development (Osen & Schweiggert-Weisz, 2016).

Purpose/Objective

The objective of this research was to investigate effect of rice flour or cassava flour blended with soy protein isolate on low moisture extrusion process and developed the extrudate to textured vegetable protein.

Research Scope

Scope of this study was focus on the effect of rice flour or cassava flour blended with soy protein isolate on low moisture extrusion process. The physicochemical properties such as expansion degree, color value, WAI, WSI and texture profiles of all samples were investigated. Then, the TVP extrudate were developed to TVP-minced meat.



Methodology

1. Raw materials

Soy protein isolate (protein 90.3%, moisture 6.0%, ash 4.8%) was purchased from Krungthepchemi (F014SP, Krungthepchemi, Thailand). Rice flour (Choheng rice vermicelli factory Co., Ltd.) and cassava flour (Somsaha Thaveewatana Co., Ltd.) were used in this study.

2. Extrusion process

All extrusion experiments were performed by a co-rotating twin-screw food CTE-D22L32 Twin- screw extruder (CHAREON TUT Co., LTD., Thailand). The sample powders were prepared and mixed in 3 mixtures as SPI: rice flour in ratio 70: 30 (SPI+Rice), SPI: cassava flour in ratio 70: 30 (SPI+Cassava), and pure SPI. The moisture content of the sample powder was adjusted to 25% (wet basis) and the screw speed was 450 rpm. The sample powder was fed to the extruder at varying speeds as 30 to 55 rpm of feeding paddle. A short expansion die was attached to barrels with a diameter of 4 mm. The extruder barrel temperatures were set for six heating zones as 60°C, 90°C, 110°C, 120°C, 140°C, and 120°C, respectively. The die temperature was set at 90°C and the cutting knife was set at 120 rpm. Extrudates were collected when the operation condition was at a steady state. The texture vegetable protein (TVP) extrudates were continued drying at 60°C for 24 h to decrease moisture content before being kept in polyethylene until measurement (Wu, et al., 2018).

3. Protein content of the extrudate product

The total protein content in the extrudate samples was calculated based on the nitrogen content (N) according to the Dumas combustion using a nitrogen analyzer FP 528 (Leco Corporation, St. Joseph, MI, USA) with a conversion factor of 6.25 (Osen, et al., 2015).

4. Expansion degree

The expansion degree (ED) of the extrudate samples were measured as follows:

ED= Diameter of product/Diameter of die

(1)

The diameter of extrudate product was measured for 20 replications in each sample for determine ED and coefficient of variation (%CV) (Noguchi, et al., 1982).

295



5. Moisture content

The moisture content of extrudate product was measured. The 2 g of sample was dried at 105 $^{\circ}$ C in an oven (SLW 35 STD; POL-EKD APARATURA SP.J., Poland) until constant weight. The moisture content of the sample was calculated using the following equation: *Moisture content (%) = (m1-m2) x 100/m1* (2)

When m1 was sample weight before dried, and m2 was sample powder weight after dried.

6. Water absorption index (WAI) and water solubility index (WSI)

Water absorption index (WAI) and water solubility index (WSI) of extrudate product were determined. The extrudate sample was ground to approximate size as 180 μ m. The extrudate powder 2.5 g was dispersed in 25 g of distilled water, then stirred for 30 min. The dispersion was rinsed into centrifuge tubes and adjust weight to 32.5 g. The samples were centrifuged at 5000 rpm for 10 min. The supernatant was decanted for determination of its solid content and the sediment was weight. WAI and WSI were calculated using equation,

WAI (g/g) = weight of sediment/ weight of dry solids	(3)
WSI (g/g) = weight of dissolved solids in supernatant/ weight of dry solids	(4)

(Singh, et al., 2007)

7. Color

Color of the extrudate product was measurement with Minolta colorimeter (CR-400/CR-410, Japan). Color in terms of luminosity, light versus dark (L*), red versus green (a*) and yellow versus blue (b*) were measured.

8. Texture analysis of texture vegetable protein

Texture analysis was measured with LLOYD TA plus Material Tester (Lloyds, England). The hydrated extrudate sample (2.6 parts $H_2O/1$ part TVP) 10 g were placed in plastic cylinder (diameter = 45 mm, depth 20 mm) and pressed to make a smooth surface. A cylinder

296



25 mm plastic probe was used to determine 70% compression with 5 mm/s. The samples were measured hardness, adhesiveness, cohesiveness, and springiness (Crowe & Johnson, 2001).

9. Texture profile analysis of TVP- minced meat

The TVP 60 g from extrudate product were mixed with a blended paste (modified starch, coconut oil, canola oil, and beet root powder) 40 g to form ground meat patties. The 60 g of ground meat patties were placed into a cylindrical mold size 16 X 67 mm and hand patting. The patties were kept at -20°C until cooked. The frozen patties were thawed at 4°C for 24 h and then were cooked to an internal temperature of 70°C. The patties were allowed to cool to room temperature, and a 13X25 mm core sample was taken from the center of each patty for textural analysis. Texture analysis of cooked TVP- minced meat. Texture profile analysis was performed using a LLOYD TA plus Material Tester (Lloyds, England). A 45 mm anvil was used to determine 70% compression at a rate of 1mm/s. Samples were evaluated for hardness, springiness, adhesiveness, cohesiveness, gumminess, and chewiness. Eight texture analysis were performed on each sample (Crowe & Johnson, 2001).

10. Statistical analysis

All the samples were analyzed statistically using SPSS version 22. Different means were investigated by ANOVA and Duncan's multiple range tests at a level of significance of 0.05.

Findings/ Results

1. Protein content of the extrudate product

All types of materials after extrusion were measured protein content which showed protein content of pure protein extrudate higher than SPI+Rice and SPI+Cassava extrudate (Table 1). The results could explain by the protein content decreased with the blending of rice flour and cassava flour, and rice flour could have protein content (6%) more than cassava flour (2%).



Table 1 Protein content of extrudate product

Type of material	Protein (%)
SPI+Rice	59.89±0.37b
SPI+Cassava	57.10±0.41a
Pure SPI	82.36±1.58c

* Different letters significant difference at $p \le 0.05$.

** SPI+Rice was mixing of soy protein isolate and rice flour in ratio 70: 30, SPI+cassava was mixing of soy protein isolate and cassava flour in ratio 70: 30, and Pure SPI was using only soy protein isolate.

2. Expansion degree of TVP extrudate sample

The expansion degree of the extrudate sample showed in Table 2. In various feeding rates, the trend of expansion degree might be increased with increasing feeding rate. For compared the expansion degree and %CV between feed rate in each raw material, the feeding rate at 50 rpm might be a suitable condition for the extruder in this experiment that due to the feeding rate at 50 rpm could possess the lowest %CV of the expansion degree for three types of the raw material extrudate. Moreover, the expansion degree tended to increase with the protein isolate mixed with flour especially mixed with rice flour. The expansion degree of extrudate might be related to the type of starch that was mixed with soy protein isolate, and also could be related to the phase transition effect of soy protein on the expansion of extrudates (Seker, 2005). The increase in expansion ratio with blended flour was similar to de Mesa et al. (2009), Seker (2005), and Zasypkin & Lee, (1998). The expansion during extrusion related to elastic and expansion properties of starch and protein damaged, starch could form a continuous matrix that permitted water vapor to expand due to melting viscosity of starch lower than that of protein (de Mesa, et al., 2009; Seker, 2005). The high protein ratio extrudates would be lower expansion degree compared to high starch ratio extrudates could depend on protein properties, extrusion conditions and feed composition (Beck, et al., 2018).



21 March, 2022

Table 2 The expansion degree of extrudate sample

Food	Expansion degree			%CV		
Feed	SPI+Rice	SPI+Cassava	Pure SPI	SPI+Rice	SPI+Cassava	Pure SPI
30	2.15±0.30bcde	2.02±0.57bc	1.57±0.40a	13.80	28.21	25.80
35	2.07±0.57bcd	1.59±0.51a	1.43±0.58a	27.47	31.82	40.54
40	2.28±0.53def	2.04±0.34bc	1.52±0.55a	23.21	16.43	36.21
45	2.32±0.33ef	1.96±0.61bc	1.91±0.36b	14.35	31.15	18.81
50	2.76±0.34g	2.42±0.39f	2.02±0.31bc	12.21	16.30	15.51
55	2.31±0.78ef	2.17±0.48cde	2.14±0.34bcde	33.88	22.29	15.99

* Different letters significant difference at $p \le 0.05$.

3. Color and appearance of TVP extrudate sample

The color differences of extrudate samples were identified using CIE L*a*b* coordinates. The color parameters were defined in the terms of light versus dark (L*), red versus green (a*), and yellow versus blue (b*). The color and appearance of the extrudate sample were shown in Fig.1. The TVP extrude sample of pure SPI appeared in darkness-brown more than SPI+Rice and SPI+Cassava as showed the L* value of pure SPI lower than that of SPI+Rice and SPI+Cassava, and a* and b* value of pure SPI higher than that of SPI+Rice and SPI+Cassava. The results might relate to the blending of flour that had color in whiteness.

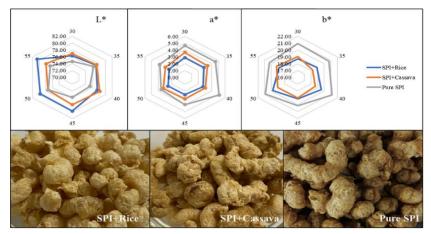


Figure 1 Color and appearance of TVP extrudate sample

299



4. Moisture content and Aw of TVP extrudate sample

The moisture content and water activity or Aw were the important properties of products because the sample with possessed the high moisture content and Aw could reduce the quality of a dried product during storage due to the multiplication of the microorganisms would require Aw of the sample more than the minimum value (0.6) (Yu & Lv, 2019). The moisture content and Aw of the extrudate sample were in the range of 7.27-9.50% and 0.45-0.55, respectively (Table 3).

Table 3 Moisture content and Aw of extrudate sample

Moisture (%)			Aw			
reeu	SPI+Rice	SPI+Cassava	Pure SPI	SPI+Rice	SPI+Cassava	Pure SPI
30	8.76±1.05ab	8.77±0.81ab	8.14±0.43ab	0.54±0.00b	0.55±0.02b	0.46±0.03a
35	8.16±1.97ab	9.02±1.13ab	8.32±0.13ab	0.54±0.01b	0.54±0.00b	0.45±0.03a
40	9.18±1.52ab	9.15±0.94ab	8.22±0.21ab	0.54±0.01b	0.54±0.01b	0.45±0.05a
45	8.24±1.21ab	9.50±0.94b	7.27±0.32a	0.54±0.01b	0.54±0.01b	0.46±0.04a
50	8.93±1.25ab	8.83±1.10ab	7.83±0.57ab	0.53±0.02b	0.54±0.01b	0.48±0.05a
55	8.21±1.54ab	9.12±1.20ab	7.63±0.18ab	0.53±0.02b	0.54±0.01b	0.47±0.02a

* Different letters significant difference at $p \le 0.05$.

The trend of moisture content and Aw of pure SPI extrudate was lower than SPI+Rice and SPI+Cassava that might be due to moisture content of rice flour (13.17%) and cassava flour (13.60%) were more than that of SPI (6%).

5. Water absorption index (WAI) and water solubility index (WSI)

The WAI of the TVP extrudes sample tended to increase with increasing feeding rate in all of SPI+Rice, SPI+Cassava, and pure SPI (Table 4). The results were similarly trending with the expansion degree of the samples that the trend increased with increasing feeding rate.



Even though, the results of WSI showed in a conversed trend that WSI decreased with increasing feeding rate.

Table 4 WAI and WSI of TVP extrudate sample	
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Food	WAI			WSI		
Feed	SPI+Rice	SPI+Cassava	Pure SPI	SPI+Rice	SPI+Cassava	Pure SPI
30	2.11±0.21abcd	2.11±0.08abcd	2.07±0.16abcd	0.042±0.006ab	0.067±0.009def	0.054±0.005bcd
35	2.07±0.25ab	2.06±0.05ab	2.04±0.17abcd	0.041±0.004ab	0.071±0.008f	0.063±0.001cde
40	2.19±0.34abcdef	2.27±0.12bcdef	1.78±0.17a	0.041±0.015ab	0.062±0.008def	0.071±0.028def
45	2.18±0.21bcdef	2.35±0.04def	2.15±0.06abcde	0.038±0.004a	0.066±0.008ef	0.048±0.004ab
50	2.38±0.27cdef	2.37±0.17ef	2.16±0.03abcde	0.034±0.007a	0.060±0.006def	0.041±0.002ab
55	2.23±0.11bcdef	2.37±0.15f	2.10±0.23abc	0.043±0.007a	0.063±0.009def	0.043±0.002abc

* Different letters significant difference at $p \le 0.05$.

In high feeding rate, The WAI and WSI of SPI+Cassava extrudate were higher than that of pure SPI extrudate. The high screw speed of extrusion would be leading to starch degradation which WAI could decrease with increasing SPI ratio due to a reduction in the starch content (de Mesa, et al., 2009). The WAI of extrudate would related with polymeric chains that WAI increased with increasing the length of polymeric chains while WSI was correlated with molecular degradation (Seker, 2005). Moreover, water absorption would decrease with an increase in the ratio of SPI according to water absorption was also afforded by gelatinized starch and an insoluble component that protein could only depress water absorption slightly (Noguchi, et al., 1982).

6. Texture profile analysis of texture vegetable protein and TVP- minced meat and

The results from expansion degree, %CV (Table 2) and WAI (Table 4) of TVP extrudate sample showed the result of feeding rate at 50 rpm better than that of other feeding rates. Thus, the TVP extrudate at 50 rpm feeding rate was chosen for measurement texture



properties. The hardness and springiness of pure SPI extrudate were higher than SPI+Rice and SPI+Cassava extrudate. However, adhesiveness and cohesiveness of pure SPI extrudate tended lower than that of SPI+Rice and SPI+Cassava extrudate.

Table 5 Texture profile analysis of texture vegetable protein

Texture properties	SPI+Rice 50	SPI+Cassava 50	Pure SPI 50
Hardness (N)	1.78±0.20a	2.20±0.52a	4.18±0.70b
Adhesiveness (Nmm)	0.28±0.12a	0.76±0.14b	0.24±0.05a
Cohesiveness	2.28±0.24b	2.01±0.17b	1.94±0.17a
Springiness	0.55±0.04b	0.50±0.02a	0.59±0.07c

* Different letters in each row significantly difference at $p \le 0.05$.

For the TVP-minced meat, The TVP extrudate was mixed with a binder before cooking and investigated the texture properties. The hardness, adhesiveness, and cohesiveness of TVP-minced meat could be similar trends in the texture properties of TVP extrudate (Table 6). However, the springiness could not significantly different when TVP extrudate mixed with binder. Moreover, the texture of pure SPI extrudate mixed with binder could have a higher value in gumminess and chewiness more than that of SPI+Rice and SPI+Cassava extrudate mixed with binder. The TVP with high protein content would have high hardness and chewiness due to the protein available might contribute to the gelation of protein (Yeater, et al., 2017).



Table 6 Texture profile analysis of TVP- minced meat

Texture properties	SPI+Rice	SPI+Cassava	Pure SPI
Hardness (N)	15.34±2.47a	14.03±2.54a	18.45±1.75b
Adhesiveness (Nmm)	4.62±1.13a	5.42±0.98b	4.78±0.41a
Cohesiveness	5.35±0.42b	5.47±0.50b	4.62±0.38a
Springiness	0.58±0.00a	0.57±0.01a	0.59±0.01a
Gumminess	2.88±0.45a	2.58±0.48a	4.02±0.53b
Chewiness	1.66±0.27a	1.48±0.28a	2.37±0.33b

* Different letters in each row significantly difference at $p \le 0.05$.

Starch could affect the extrudate structure formation due to gelatinization and degradation as water absorbed and expanded in a mixing zone; hydrogen bond of starch molecule disrupted, starch gelatinized, protein- starch interacted, protein aggregated, and fibrous structure formed in a melting zone; water evaporated, starch granule puffed, and air cell form in TVP at the die (Zhang, et al., 2019). Texture properties of TVP-minced meat would be affected additives such as lipid, carbohydrate, salt, and moisture to form the gel matrix, and enhanced hardness and chewiness during cooking; and the combination of protein and polysaccharide could influentially modify the texture values (Gao, et al., 2015).

Conclusion

In the extrusion process, the material feeding rate affected the working condition of the extruder and extrudate properties. The blending of rice flour and cassava flour could change the protein content of matrix extrudate and affect the structure formation which was related to the extrudate properties both in TVP and TVP mixed with a binder to be minced meat. Thus, the properties of TVP-minced meat were related to the binder and TVP extrudate, which attribute to the condition of raw material for extrusion and condition for adjusting the extrusion.



Reference

- Beck, S. M., Knoerzer, K., Foerster, M., Mayo, S., Philipp, C., & Arcot, J. (2018). Low moisture extrusion of pea protein and pea fibre fortified rice starch blends. Journal of Food Engineering, 231, 61–71. https://doi.org/10.1016/j.jfoodeng.2018.03.004.
- Crowe, T. W., & Johnson, L. A. (2001). Twin-screw extrusion texturization of extrudedexpelled soybean flour. Journal of the American Oil Chemists' Society, 78(8), 781– 786. https://doi.org/10.1007/s11746-001-0342-8.
- de Mesa, N. J. E., Alavi, S., Singh, N., Shi, Y.-C., Dogan, H., & Sang, Y. (2009). Soy proteinfortified expanded extrudates: Baseline study using normal corn starch. Journal of Food Engineering, 90(2), 262–270. https://doi.org/10.1016/j.jfoodeng.2008.06.032.
- Featherstone, S. (2015). 8 Ingredients used in the preparation of canned foods. In S. Featherstone (Ed.), A Complete Course in Canning and Related Processes (Fourteenth edition, pp. 147-211). Oxford: Woodhead Publishing.
- Gao, X., Zhang, W., & Zhou, G. (2015). Emulsion stability, thermo-rheology and quality characteristics of ground pork patties prepared with soy protein isolate and carrageenan. Journal of the Science of Food and Agriculture, 95(14), 2832–2837. https://doi.org/10.1002/jsfa.7023
- Kyriakopoulou, K., Keppler, J. K., & van der Goot, A. J. (2021). Functionality of Ingredients and Additives in Plant-Based Meat Analogues. **Foods, 10**(3), 600. https://doi.org/10.3390/foods10030600.
- MacDonald, R., & Reitmeier, C. (2017). Chapter 6 Food Processing. In R. MacDonald & C. Reitmeier (Eds.), **Understanding Food Systems** (pp. 179-225). Academic Press.
- Noguchi, A., Kugimiya, W., Haque, Z., & Saio, K. (1982). Physical and Chemical Characteristics of Extruded Rice Flour and Rice Flour fortified with Soybean Protein Isolate. Journal of Food Science, 47(1), 240–245. https://doi.org/10.1111/j.1365-2621.1982.tb11069.x.
- Osen, R., Toelstede, S., Eisner, P., & Schweiggert-Weisz, U. (2015). Effect of high moisture extrusion cooking on protein–protein interactions of pea (Pisum sativum L.) protein isolates. International Journal of Food Science & Technology, 50(6), 1390–1396. https://doi.org/10.1111/ijfs.12783.



- Osen, R., & Schweiggert-Weisz, U. (2016). **High-Moisture Extrusion: Meat Analogues**. In Reference Module in Food Science): Elsevier.
- Seker, M. (2005). Selected properties of native or modified maize starch/soy protein mixtures extruded at varying screw speed. Journal of the Science of Food and Agriculture, 85(7), 1161–1165. https://doi.org/10.1002/jsfa.2086.
- Singh, B., Sekhon, K. S., & Singh, N. (2007). Effects of moisture, temperature and level of pea grits on extrusion behaviour and product characteristics of rice. Food Chemistry, 100(1), 198–202. https://doi.org/10.1016/j.foodchem.2005.09.042.
- Wu, M., Sun, Y., Bi, C., Ji, F., Li, B., & Xing, J. (2018). Effects of extrusion conditions on the physicochemical properties of soy protein/gluten composite. International Journal of Agricultural and Biological Engineering, 11, 205–210. https://doi.org/10.25165/j.ijabe.20181103.4162.
- Yeater, M., Casco, G., Miller, R. K., & Alvarado, C. Z. (2017). Comparative evaluation of texture wheat ingredients and soy proteins in the quality and acceptability of emulsified chicken nuggets. **Poultry Science, 96**(12), 4430–4438. https://doi.org/10.3382/ps/pex250.
- Yu, Y., & Lv, Y. (2019). Degradation kinetic of anthocyanins from rose (*Rosa rugosa*) as prepared by microencapsulation in freeze-drying and spray-drying. *International* Journal of Food Properties, 22(1), 2009–2021.

https://doi.org/10.1080/10942912.2019.1701011.

- Zasypkin, D. V., & Lee, T.-C. (1998). Extrusion of Soybean and Wheat Flour as Affected by Moisture Content. Journal of Food Science, 63(6), 1058–1061. https://doi.org/10.1111/j.1365-2621.1998.tb15854.x.
- Zhang, J., Liu, L., Liu, H., Yoon, A., Rizvi, S. S. H., & Wang, Q. (2019). Changes in conformation and quality of vegetable protein during texturization process by extrusion. Critical Reviews in Food Science and Nutrition, 59(20), 3267–3280.
 https://doi.org/10.1080/10408398.2018.1487383.