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FULL PAPER

Quality of purula (rice seasoning for anemia from soy protein hydrolysate and seaweed) during pilot plant scale development using drum drying process

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Abstract

Purula is a new rice seasoning product made from soy protein hydrolysate and seaweed. It contains low molecular weight (<20 kDa) biopeptide from soybean that can enhance iron absorption and fortified with vitamin and mineral to prevent anemia. This research aimed to monitor the quality of purula and its main ingredient during pilot plant scale process. The two main ingredients of purula are soy protein hydrolysate flour and seaweed slurry. Steam flash explosion and enzymatic hydrolysis treatment were applied to the soybean, followed by drum drying to produce soy protein hydrolysate flour. Brown and green algae were blanched and grinded to make seaweed slurry. Then, they were mixed with flour mix, drum dried, and added with vitamin and mineral to produce purula. Proximate, microbiological, and heavy chemical analysis were conducted on samples taken during stages of purula processing. Moisture content and thickness of Purula flakes were examined during pilot plant process. Molecular weight of protein analysis was also conducted on raw soybean, soy protein hydrolysate, and purula. Microbiological and chemical quality of soy protein hydrolysate flour, seaweed slurry, and purula conform with the food safety requirement. This indicates that Purula is safe for human consumption. Purula flakes produced by drum drying process also share consistent results of moisture content and thickness of Purula flake within 5 days of production. Low molecular weight of protein (<20 kDa) was produced on soy protein hydrolysate and still intact on purula finished product. This study concluded that purula is able to be properly produced on pilot plant scale.

1. Introduction

National Research and Innovation Agency of Indonesia has developed a new food product called purula. Purula is classified as functional food in the form of rice seasoning designed for preventing and overcoming anemia. Anemia is a major health problem in Indonesia. Approximately, 23% of female adolescents in Indonesia and 12% of male adolescents suffer from anemia. Moreover, almost half of pregnant women (48.9%) in Indonesia have anemia (Indonesia Ministry of Health, 2018). This number is quite huge and need to be addressed immediately. People with anemia often feel fatigue, weakness, dizziness, reduced immune system, and anemia in pregnant woman increases the risk of giving birth to malnourished and stunted children (Tampy *et al*., 2020). Therefore, anemia prevention is a major concern for the Indonesian government.

The Indonesian government has launched a program for providing iron supplementation for adolescent females and pregnant women. This iron tablet is given once a week for school children totalling 90 tablets prior to the maternity for pregnant woman. However, there are still some concerns regarding iron tablet supplementation. It is reported that oral iron supplementation caused up to 60% of patients with gastrointestinal side effects such as constipation, nausea, and bloating (Bloor *et al*., 2021)

Thereby, purula is designed as an alternative of the iron tablet supplement. The bioactive peptide is derived from the soy protein hydrolysate which contains low molecular weight protein (<20 kDa). This protein exhibits the characteristic to enhance the absorption of iron intake (Giarni *et al*., 2020; Susanti *et al*., 2020).

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Meanwhile, the seaweed provides additional mineral for purula. Moreover, purula also fortified with vitamin and mineraI mix to meet the nutritional requirement for anemia supplementation.

The production process consists of producing the main ingredients, soy protein hydrolysate flour and seaweed mix slurry, and making purula using drum dryer process. The soy protein hydrolysate is obtained using mechanical hydrolysis through steam flash explosion followed by enzymatical hydrolysis utilizing protease from bromelain (Laily, 2018). Drum drier is then used to make soy protein hydrolysate flour. Meanwhile, the seaweed slurry is made from red and green algae. These soy protein hydrolysate flour and seaweed slurry are then mixed with flour mix to make purula dough and then drum dried (Laily, 2019). Initially, this process is done in the laboratory within small scale production. Currently, it is increased to pilot plant scale process in order to enlarge the production output. However, the quality of such process needs to be controlled, regarding its main ingredients and finished product. The quality of the sample should conform with the product requirement, mainly microbiological and heavy metal characteristics for food safety, and should show consistency among the batches. Thereby, this paper aims to monitor the quality of Purula and its main ingredient during pilot plant scale process.

2. Materials and methods

2.1 Experimental setup and sample preparation

The production of purula consisted of three steps, they were production of soy protein hydrolysate flour, production of seaweed slurry, and production of purula. In this research, 100 kg of soy protein hydrolysate flour and 500 kg of seaweed slurry were made as main ingredients in order to produce 500 kg of purula. For characterisation, 1 kg of soy protein hydrolysate flour, 1 kg of seaweed slurry, and 1 kg of purula were taken as sample.

2.1.1 Production of soy protein hydrolysate

A total of fifteen kg of soybean was soaked for 4 hrs and rinsed. Then, the rinsed soybean was treated with steam flash explosion using steam blaster for 15 mins at 3 bar pressure and 130°C temperature. After that, the soybean was mixed with water (1:2) (w/w) and grinded until it became slurry. The soybean slurry was then hydrolysed using protease from bromelain enzyme for 2 hrs at 60°C. This process produced 90 kg of soy protein hydrolysate.

This process was repeated six times to make 450 kg of soy protein hydrolysate. The soy protein hydrolysate then was mixed with corn flour (20%) (w/w) and sugar (6%) (w/w) to make the soy protein hydrolysate dough. The dough was drum dried (175°C) to make soy protein hydrolysate flour.

2.1.2 Production of seaweed slurry

A total of nine kg of dried red and brown algae mix was soaked overnight. Then, the soaked algae mix was rinsed, and resoaked using 1% $Ca(OH)_2$ at pH 4.5 for 1 hr. After this deodorization process, the algae mix was blanched at 90°C for 5 mins and was grinded using Universal Fritter and Colloid Mill to make seaweed slurry. This process was repeated for seven times to produce 540 kg of seaweed slurry

2.1.2 Production of purula flake

One kg of soy protein hydrolysate flour and 5 kg of seaweed slurry were mixed with flour mix and water to make 25 kg purula dough. Then, this Purula dough was drum dried at 10 bar pressure, 175°C temperature, and 0.8 rpm rotary speed. This process lasted for 1 hr and produced 10 kg of purula. This process was repeated 40 times within 5 days, 8 times for each day, to make 400 kg of raw purula. This 400 kg of raw purula was mixed with 100 kg of vitamin, mineral, and flavor premix to make 500 kg of purula.

2.2 Analysis

2.2.1 Proximate, microbiological, and heavy metal quality of soy protein hydrolysate flour

For proximate analysis, standard AOAC (Association of Official Analytical Chemists) methods was used. For moisture content, gravimetry method was used. Total fat analysis was conducted using Soxhlet method according to AOAC 2003.005. Ash content analysis was conducted using gravimetry method according to AOAC 923.03. Protein content was conducted using kjeldahl with 6.25 conversion factor (Maehre *et al*., 2018). For lead analysis, SNI (Indonesia National Standard) 19-2896-1998 method about analysis of metal contaminant on food product was used (SNI, 1998a). SNI 01-4866-1998 method about analysis of arsenic contaminant method on food product was used for determining arsenic content on the soy protein hydrolysate flour (SNI, 1998b). ISO standard no 7251 was used for *E. coli* measurement (ISO, 2012a). For Total Plate Count, ISO (International Organization for Standardization) standard no 4833-1 horizontal method of microbiological enumeration was used (ISO, 2015).

2.2.2 Proximate, microbiological, and heavy metal quality of seaweed slurry

Similar with soy protein hydrolysate flour, standard

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AOAC method was used for proximate analysis. Moisture content was conducted using gravimetry method, total fat analysis was conducted (AOAC 2003.005) using soxhlet, and protein content analysis was conducted using kjeldahl method with 4.59 conversion factor (Maehre *et al*., 2018). ISO standard no 21527 (ISO, 2012b) was used for mold and yeast measurement, ISO standard no 4833-1 was used for Total Plate Count of microorganism (ISO, 2015). ISO standard no 21528 (ISO, 2016) was used for detection and enumeration of *Enterobacteriaceae*. For lead (Pb) analysis, SNI 19-2896-1998 method about analysis of metal contaminant method on food product was used (SNI, 1998a). SNI 01-4866-1998 about analysis of arsenic contaminant method on food product was used for determining arsenic content on the soy protein hydrolysate flour (SNI, 1998b).

2.2.3 Moisture content and thickness of purula flake

Moisture analyzer HE53 Mettler Toledo was used for quick moisture content analysis of purula flake. First, the heating module was opened and the empty sample pan was positioned in the sample pan handler. The sample pan handler was placed on the draft shield. After that, the heating module was closed and the balance was set to zero. Then, the heating module was reopened and 2 g of purula flakes sample was loaded onto the module. The sample was distributed properly to obtain great result. The heating module was then closed and the start button was pressed in order to conduct the measurement. Within minutes, the final result was presented on the LCD display. The room temperature during measurement was 24°C.

The thickness measurement of purula flake was conducted using monotaro mini digital outer micrometer. The measurement of moisture content and thickness of purula flake sample were conducted for each batch of drum drying process

2.2.4 Electrophoresis of raw soybean, soy protein hydrolysate and purula

Molecular weight of the protein was estimated using modified Laemmli (1970) method. Prior to electrophoresis, raw soybean, soy protein hydrolysate, and Purula sample were grinded smoothly and 1 g of each sample were diluted into 1 M buffer tris-HCl pH 8.5, The diluted samples were then shaken for 1 hr at room temperature using shaker incubator, and were centrifuged at 10000 rpm for 20 mins. The supernatants were kept and stored at -20°C as sample stocks.

For electrophoresis, the glycine-SDS-PAGE (12.5% gel) was used as separation gel. As much as 10 µL of sample stocks were mixed with 15 µL buffer (0.01 M Tris-HCl pH 6.8; 0.1% SDS; 0.1% 2-mercaptoethanol) and kept in water bath (100°C) for 5 mins. For standard ladder, Precision Plus Protein 10-250 kDa (All Blue Standards, BioRad, Hercules, California) was used as markers for glycine-SDS-PAGE. Coomassie brilliant blue R250 (1 g) in acetic acid (10 mL), methanol (40 mL), and distilled water (40 mL) solution was used as staining solution. For destaining solution, acetic acid (10 mL), methanol (40 mL), and aquadest (40 mL) solution were used.

2.2.5 Microbiological and heavy metal quality of purula

For lead (Pb), mercury (Hg), and cadmium (Cd) analysis, SNI 19-2896-1998 method about analysis of metal contaminant method on food product was used (SNI, 1998b). For total plate count (TPC) analysis, ISO 4833-1:2015 about horizontal method of microbiological enumeration was used. For *Salmonella* analysis, ISO 6579:2015 about horizontal method of salmonella enumeration was used. For *Bacillus cereus* analysis, ISO 7931:2012 method about horizontal method of *B. cereus* enumeration was used (ISO, 2004a). For *Clostridium perfringens* analysis, ISO 7937:2012 about horizontal method of clostridium perfringens enumeration was used (ISO, 2004b).

3. Results and discussion

3.1 Proximate, microbiological, and heavy metal quality of soy protein hydrolysate

Table 1 shows the quality of soy protein hydrolysate flour which can be classified into three categories, proximate, chemical (heavy metal) quality, and microbiological quality. The moisture content of soy protein hydrolysate is 6.2%. Moisture content is important in determining the quality of a food. There is a correlation between moisture content of the food and its

Table 1. The quality of soy protein hydrolysate

Parameter	Unit	Result	Standard*
Moisture content	%	6.2	-
Protein	%	16.64	-
Fat	%	7.68	-
Ash	%	1.86	-
Lead (Pb)	mg/kg	<0.041	< 0.3
Arsenic (As)	mg/kg	<0.001	<0.5
Microbiology:			
Total Plate Count	CFU/g	1.1×10^3	$<10^5$
Escherichia coli	MPN/g	<3	<7.4

*Heavy metal standard refers to SNI (2009) heavy metal – 7837:2009, food category 06.0, Cerealia including legumes; microbiological standard refers to BPOM (National Agency of Drug and Food Control of Indonesia, 2019) standard for microbiological contaminant on processed food, food category: 06.0 Cerealia including legumes (flour and starch)

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shelf life and perishability. In general, higher moisture content gives higher water activity and thus lowers shelf life of the food. The free water on the food is a good medium for microbes to spoil it. Therefore, it is important to maintain the moisture content of the food based to the standard of certain food type. According to Famurewa *et al*. (2008), soy flour with moisture content lower than 6.99% and kept at environment with relative humidity less than 50% is ideal for prolonged shelf life.

The protein content in soy protein hydrolysate is 16.64%. This number was lower than the Initial protein content of grobogan soybean (43.9%) according to Balitkabi (Research Center for Various Legumes and Tubers, 2016). Furthermore, it was also lower than protein content of soybean flour from other researchers. Farzana and Mohajan (2015) reported that the protein content of dehulled soybean was 49.3%. Omah *et al*. (2022) reported that the protein content of soybean flour ranges from 29.05 to 49.63%. Suryana *et al*. (2022) reported that the protein content of sun dried and oven dried soy flour ranged between 37.56% to 38.09%.

However, it should be noted that this soy protein hydrolysate was mixed with fillers during drum drying process, which are corn flour and sugar. It is hypothesized that the addition of such fillers, decreased the protein content of soy protein hydrolysate. Moreover, the soy protein hydrolysate not intended to be main source of protein. Its role is to increase the absorption of mineral during digestion process (Giarni *et al*., 2020; Susanti *et al*., 2020).

Initially, the fat content in grobogan soybean is 18.64% (Research Center for Various Legumes and Tubers, 2016). However, the fat content in soy protein hydrolysate is 7.68%. This fat content was lower than fat content of soybean flours reported by Farzana and Mohajan (2015) (31.8%), Omah *et al*. (2022) (ranged from 15.09% to 18.65%), and Suryana *et al*. (2022) (ranged from 19.21% to 19.59%). Again, it is postulated that the low-fat content was caused by the corn flour and sugar flour mixed during processing. Fillers safeguard the material to be dried by acting as a protective layer and an exterior wall, preventing volatile component loss and denaturation. They also prevent the browning process and protect the food product from the loss of aroma, vitamin, and other heat sensitive substances (Setyadjit and Sukasih, 2015). The ash content of soy protein hydrolysate flour is 1.86%. This indicates that the soy protein hydrolysate contains some minerals.

In terms of heavy metal contamination, the two categories of heavy metals utilized as food safety criteria for flour product are lead (Pb) and arsenic (As). Arsenic is naturally found on contaminated soil and water that have been contacted with arsenic rich rock. It can also be found on the soil and ground water through the introduction of herbicides and pesticides that contain arsenic during agricultural practices. Arsenic poisoning can give some serious health problems such as heart disease, cancer, diabetes mellitus, and melanosis (Bhattacharya *et al*., 2010; Khan *et al*., 2010). Meanwhile, lead is commonly found as an industrially heavy metal and widespread in air, water, soil, and food (Raikwar *et al*., 2008). Chronic and acute lead poisoning can cause miscarriages, low birth weight, hypertension, and cardiovascular disease (Flora *et al*., 2012).

Soy protein hydrolysate flour contains <0.041 mg/kg of lead and <0.001 mg/kg of arsenic. These numbers were in accordance with the heavy metal food safety standard for lead (<0.3 mg/kg) and arsenic (<0.5 mg/kg) (Indonesia National Standard, 2009). During processing, food grade products without any chemical contamination were used as ingredients to ensure the quality of soy protein hydrolysate. This value of heavy metal contamination which has met the standard indicates that this product is safe for consumption

Microbiological parameter of soy protein hydrolysate flour conforms with the standard of BPOM (National Agency of Drug and Food Control of Indonesia, 2019). The TPC and *E. coli* of soy protein hydrolysed flour is 1.1×10^3 and <3 MPN. This number is acceptable since they are lower than maximum standard, which are $<10^5$ CFU/g for TPC and <7.4 MPN/g for *E. coli*. The production of soy protein hydrolysate utilized heat treatment using steam flash explosion and drum drying. During these process, high temperature was required to produce high quality flake. The temperature of steam flash explosion reached 130°C and the temperature of drum drying reached 175°C. It is hypothesized that the heat treatment kills the microbes and contributes to the low number of TPC and *E. coli* on soy protein hydrolysate.

3.2 Proximate, microbiological, and heavy metal quality of seaweed slurry

The proximate quality of seaweed slurry is shown on Table 2. Based on Table 2, The protein and fat content of seaweed slurry is only 0.78% and 0.75%. These numbers are very low compared to the initial protein and fat content of red and brown algae, the ingredient of seaweed slurry. According to Echave *et al*. (2021), red algae have a protein level ranging from 20 to 47% of their dry weight whereas brown algae have a protein content of 3–15%. The lipid content of seaweed is typically between 1 and 4% dry weight, but are high in polyunsaturated fatty acids (PUFA) (Paiva *et al*., 2016).

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The low fat and protein content of seaweed slurry was caused by the addition of water during the process of seaweed slurry. The weight of seaweed slurry itself is 10 times higher than the initial weight of dried seaweed. This weight is mostly from the water. Therefore, the highest composition of seaweed slurry is its moisture content (95.32%). However, this seaweed slurry is not intended as main source of protein and fat. Seaweed slurry is used as flavour enhancer ingredient in Purula and also to add some minerals.

Table 2. The quality of seaweed slurry

Parameter	Unit	Result	Standard*
Moisture content	%	95.32	-
Protein	%	0.78	-
Fat	%	0.75	-
Arsenic (As)	mg/kg	0.31	<1.0
Lead (Pb)	mg/kg	<0.041	< 0.5
Mercury (Hg)	mg/kg	<0.0002	< 0.03
Microbiology:			
Total Plate Count	CFU/g	$3.8x10^8$	-
Yeast and Mold	CFU/g	$7.5x10^5$	-
Enterobacteriaceae	MPN/g	<3.0	<3.0

*Heavy metal standard refers to SNI (2009) heavy metal – 7837:2009, food category 04.0, fruit, vegetable, seaweed, legume; microbiological standard refers to BPOM (National Agency of Drug and Food Control of Indonesia, 2019) standard for microbiological contaminant on processed food, food category: 04.2.2.7 food ingredient form vegetable and seaweed pulp

As for the heavy chemical safety of seaweed slurry, it should be noted that rapid urbanization and large-scale industrial activity around the world have greatly led to increased metals contamination in the aquatic system in recent years (Saraee *et al*., 2013). Toxic metals are quickly collected in multicellular marine macroalgae after they are introduced into aquatic systems where seaweeds are cultivated or grow naturally (Khandaker *et al*., 2021). Therefore, it is important to measure the heavy metal contamination of seaweed products.

Arsenic (As), lead (Pb), and mercury (Hg) are three standards of heavy chemical contamination for the safety of seaweed slurry. Based on Table 2, seaweed slurry contained 0.31 mg/kg of arsenic, <0.041 mg/kg of lead, and <0.0002 mg/kg of mercury. These numbers are lower than the standard of arsenic (<1.0 mg/kg), lead (<0.5 mg/kg), and mercury (<0.03 mg/kg) (SNI, 2009). Consequently, the seaweed slurry produced on this research is safe for ingredient of Purula and human consumption.

Seaweed slurry contained total plate count of 3.8×10^8 CFU/g and *Enterobacteriaceae* concentration of 3.0 MPN/g. The number of *Enterobacteriaceae* is still within the acceptable range of 3 MPN/g according to the BPOM standard (National Agency of Drug and Food Control of Indonesia, 2019). The standard, however, does not specify a maximum number of total plate count. This is owing to the fact that seaweed slurry is classified as an ingredient vegetable that requires further processing. As a result, the total plate count of the end product, Purula, should be less than the maximum number based on the standard.

3.3 Moisture content and thickness of purula flake

The moisture content and thickness of purula flake are shown on Table 3, comprising the production day and batch. Each batch consists of 25 kg of dough that takes approximately 1 hr of processing time. Each day consists of 8 batches that takes approximately 8 hrs of processing time. From Table 3 it is shown that average moisture content per day for Purula flake in day 1 is 4.66%, day 2 is 6.46%, day 3 is 4.36%, day 4 is 5.09%, and day 5 is 3.96%. The result shows that day to day changes of moisture content is relatively stable. One of the advantages of the drum drying process is the uniformity of moisture content on the dried product (Leilayi *et al*. 2019)

The result also shows that none of the average of moisture content per day of Purula flake exceeds 7%. According to Cordova *et al*. (2020), if the dried product is intended to be a dehydrated food ingredient, similar to a flour, the maximum moisture content should not exceed 15%. However, lower moisture content, which is correlated to the lower water activity, gives prolonged shelf life of the product and better quality of crispness (Lewicki *et al*. 2007)

The correlation between moisture content and water activity can be explained through sorption isotherm theory. Generally, lower moisture content means lower water activity property of the food matrixes. Low water activity, between 0.2-0.3, can inhibit the growth of microorganism, slow the rate of fat oxidation, and increase the durability of the material (Lindriati and Maryanto, 2016).

Several factors contribute to the final moisture content of drum dryer product. Two of them are steam pressure and rotation speed of the drum dryer. Drum surface temperature is increased by higher steam pressure. High surface temperature reduces feed viscosity and the flow of liquid on the drum dryer. Consequently, the final product moisture is decreased (Suriyajunhom and Phongpipatpong, 2018). High rotation speed decreases feed residence time on the drum surface. Thereby, the shorter residence time increases the final moisture product (Chia and Chong, 2015). In this

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Table 3. Moisture content and thickness of purula flakes

Day	Batch	Inlet dough (kg)	Finished Good (kg)	Moisture (%)		Thickness (mm)				
				Per batch	Average per day	1	2	3	Average per batch	Average per day
1	1	25	10	4.58	4.66±1.10	0.62	0.65	0.52	0.60	0.55±0.06
	2	25	10	4.33		0.59	0.61	0.67	0.62	
	3	25	10	2.91		0.56	0.50	0.45	0.50	
	4	25	10	5.23		0.56	0.60	0.60	0.59	
	5	25	10	5.48		0.50	0.41	0.50	0.47	
	6	25	10	6.35		0.55	0.55	0.59	0.56	
	7	25	10	3.49		0.32	0.60	0.60	0.51	
	8	25	10	4.89		0.60	0.52	0.59	0.57	
2	1	25	10	5.60	6.46±1.10	0.39	0.61	0.64	0.55	0.61±0.08
	2	25	10	6.50		0.56	0.47	0.65	0.56	
	3	25	10	4.21		0.63	0.67	0.41	0.57	
	4	25	10	7.01		0.31	0.79	0.64	0.58	
	5	25	10	6.94		0.74	0.48	0.58	0.60	
	6	25	10	7.70		0.63	0.72	0.60	0.65	
	7	25	10	6.47		0.75	0.81	0.75	0.77	
	8	25	10	7.24		0.59	0.69	0.42	0.57	
3	1	25	10	4.40	4.36±1.34	0.79	0.65	0.63	0.69	0.58±0.06
	2	25	10	5.23		0.61	0.65	0.69	0.65	
	3	25	10	4.76		0.64	0.50	0.68	0.61	
	4	25	10	5.03		0.52	0.59	0.50	0.54	
	5	25	10	3.02		0.48	0.44	0.59	0.50	
	6	25	10	3.09		0.53	0.57	0.61	0.57	
	7	25	10	2.73		0.51	0.58	0.60	0.56	
	8	25	10	6.64		0.57	0.49	0.50	0.52	
4	1	25	10	3.71	5.09±1.44	0.49	0.53	0.65	0.56	0.57±0.06
	2	25	10	7.00		0.47	0.48	0.55	0.50	
	3	25	10	4.83		0.46	0.50	0.55	0.50	
	4	25	10	2.93		0.53	0.56	0.44	0.51	
	5	25	10	6.16		0.61	0.49	0.67	0.59	
	6	25	10	4.21		0.77	0.63	0.57	0.66	
	7	25	10	6.62		0.74	0.64	0.41	0.60	
	8	25	10	5.22		0.57	0.76	0.61	0.65	
5	1	25	10	3.86	3.96±1.49	0.60	0.56	0.78	0.65	0.61±0.10
	2	25	10	3.33		0.76	0.62	0.83	0.74	
	3	25	10	2.31		0.65	0.77	0.60	0.67	
	4	25	10	3.61		0.58	0.49	0.51	0.53	
	5	25	10	3.50		0.72	0.79	0.61	0.71	
	6	25	10	3.45		0.57	0.38	0.44	0.46	
	7	25	10	4.19		0.63	0.65	0.57	0.62	
	8	25	10	7.40		0.39	0.50	0.66	0.52	

research, 10 bar of steam pressure and 0.8 rpm of rotary speed was used.

Table 3 also shows the thickness of Purula, as listed on batch and day to day basis. The average of thickness per day of purula day 1 is 0.55 mm, day 2 is 0.61 mm, day 3 is 0.58 mm, day 4 is 0.57 mm, and day 3 is 0.61 mm. It can be inferred that the thickness of Purula is relatively uniform around 0.6 mm. The main factor that contributes to the thickness of drum drying product is the load of the dry mass. It correlates to the quantity of mass processed on the drum dryer. Feed slurries with higher solid content result in thicker final product (Kalogianni *et al*., 2002; Vallous *et al*. 2002).

3.4 Electrophoresis of raw soybean, soy protein hydrolysate and purula

Figure 1 shows the SDS-PAGE profile of raw soybean, soy protein hydrolysate, and Purula. It can be seen that the protein band characteristics are different for each sample. The raw soybean protein consists of different subunits based on its molecular weight. Generally, two main fractions of soybean protein are 7S (β -conglycinin) and 11S (glycinin). Both factions compose more than 70% of soybean soluble protein.

The 7S, β-conglycinin fraction, is composed of proteins with molecular weight ranged between 150 – 180 kDa. This molecule has three subunits: α', α, and β. The main protein in 7S is glycoprotein. This

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glycoprotein contains carbohydrate group which is attached on aspartic acid residue and N end of the molecule. The 11s, glycinin fraction, has a molecular weight of 360 kDa. This fraction is a protein molecule with stable quartenary structure formed through disulfide and electrostatic bond, as well as hydrophobic interaction.

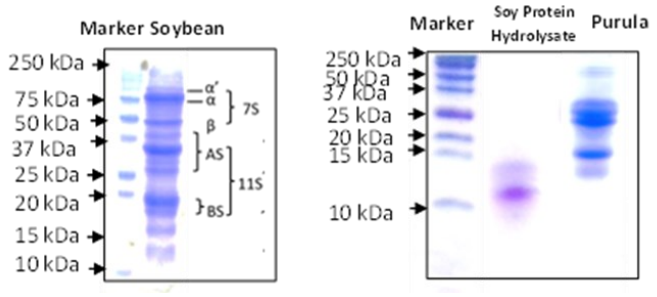

Figure 1. Electrophoresis profile of soybean, soy protein hydrolysate and purula

The glycinin comprises subunit A (acid), and subunit B (base). Between subunits, there is disulfide bond connection. Therefore, one glycinin molecule consists of 6 A-SS-B subunits on the form of double layer hexagonal structure. According to Lakemond (2000), the base polypeptide is located within the interior molecule of glycinin.

Table 4 shows the identification of protein subunit based on the molecular weight. The subunits are: β-conglycinin which consists of α (80 kDa), α' (75 kDa), and β (50 kDa), and glycinin which comprises subunit A (31-45 kDa) and B (17-20 kDa). The identifications of other researchers are also expressed on Table 4. It shows the difference of molecular weight. The molecular weight of α', α, dan β of this research is similar to those reported by Mujoo and Trinh (2003), and the molecular weight of A and B is similar to those reported by Denavi *et al*. (2009) and Pena-Ramos and Xiong (2002).

Table 5 shows the molecular weight of soy hydrolysate from different source and different hydrolysis method. Cintya *et al*. (2020) examined commercial soy hydrolysate and found that they have molecular weight ranged between 15-75 kDa. Nguyen *et al*. (2014) soy protein hydrolysate using flavourzyme and produced protein factions with molecular weight 12-31 kDa. Mao *et al*. (2013) used papain and alcalase on soy protein isolate and produced hydrolysate protein with molecular weight 5-30 kDa. Xie *et al*. (2012) hyrdrolysed defatted soybean meal using protamex enzyme and produced <1 kDa protein factions. Bao *et al*. (2011) used different enzymes, alcalase and pancreatin, to produce protein hydrolysate. The result shows that alcalase produced 1-10 kDa protein factions and pancreatin produced >50 kDa protein factions. From these data, it is clear that those reported by Xie *et al*. (2012) were the lowest molecular weight of protein hydrolysate. It is possible that the utilization of defatted soybean meal enhances the effectivity of hydrolysis since the fat has been omitted so did not interfere the hydrolysis process.

This research utilized double stage hydrolysis which is thermal hydrolysis via steam flash explosion method and enzymatic hydrolysis using bromelain enzyme. These processes produced 10-15 kDa soy protein

Table 4. Molecular weight of soybean subunits from different researchers

Subunit	Result from this research	Denavi *et al*. (2009)	Manjaya *et al*. (2007), Deak *et al*. (2007), Ruiz-Henestrosa *et al*. (2007)	Sadeghi *et al*. (2006)	Natarajan *et al*. (2004)	Tsumura *et al*. (2004)	Mujoo and Trinh (2003)	Pena-Ramos and Xiong (2002)
1) β-conglycinin								
α'	80	72	71	90.5	-	45-91	80	45-116
α'	75	68	67	71.5	68	45-91	75	
B	50	52	50	55.2	48	45-91	50	
2) glycinin								
Acid	31-45	35	-	37.6	-	14.4-42	34	31-45
Base	17-20	20	-	19.8	-	14.4-42	15	14-21

Table 5. Molecular weight of soy protein hydrolysate on different protein sources and different hydrolysis methods

Researcher	Source of Protein	Hydrolysis Method	Soy protein hydrolysate molecular weight
This research	Soy bean	Thermal hydrolysis: steam flash explosion followed by enzymatic hydrolysis: bromelain	10-15 kDa
Cintya *et al*. (2020)	Commercial soy hydrolysate	-	15-75 kDa
Nguyen *et al*. (2014)	Soybean	Enzymatic: flavourzyme	12-31 kDa
Maomao *et al*. (2013)	Soy protein isolate	Papain and alcalase	5 - 30 kDa
Bao *et al*. (2011)	Soy sauce lees	Enzymatic: alcalase	1-10kDa
		Enzymatic: Pancreatin	>50 kDa
Xie *et al*. (2012)	Defatted soybean meal	Enzymatic: Protamex	<1 kDa

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hydrolysate as can be seen in Figure 1. According to Giarni *et al*. (2020), the low molecular weight soy exhibits the characteristic as iron binding protein. The molecular bands are shifted again in purula finished good, which comprises larger protein on 50 kDa, around 25 kDa, and smaller protein which is under 20 kDa. This can be attributed by the addition of other ingredients during the making of purula. However, the smaller protein band around 15 kDa is still intact.

3.5 Heavy metal and microbiological quality of purula

As a final food product, the heavy metal contaminations are an important parameter for determining the food safety of purula product. It is a matter of fact that the utilization of such heavy metals plays an important role in sustaining modern human activity. It can be found in human activity ranging from agricultural practices, urban activity, to industrial development (Jan *et al*., 2015). Thus, the risk of heavy metals exposing human has been increased, including from food sources. The heavy metal intake on human body via contaminated food leads to a series of adverse effects. Children disability, dementia, nervous system disorder, insomnia, kidney and liver diseases are the symptoms of heavy metal poisoning (Jan *et al.*, 2011). Therefore, the Indonesia authority has issued the standard regarding safe level of heavy metals in food for human consumption.

Three main parameters of heavy metals in food product are lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) concentration. Table 6 shows the lead concentration (<0.013 mg/kg), mercury concentration (<0.0002 mg/kg), cadmium concentration (<0.0013 mg/kg), and arsenic concentration (<0.0002 mg/kg) of Purula. Fortunately, all of these concentrations are still below the standard, which is 0.3 mg/kg for lead, 0.05 mg/kg for mercury, 0.1 mg/kg for cadmium, and 0.5 mg/kg for arsenic (Indonesia National Standard, 2009).

These results are in accordance with the heavy metal concentration of purula ingredients, soy protein hydrolysate and seaweed slurry, which are within the safe range of heavy metal contamination standard. Furthermore, during processing of purula, all of the flour mixes are selected food grade ingredient that contain no harmful substances for human consumption. Consequently, purula is safe for human consumption regarding heavy metal quality.

In terms of microbiological safety, four important parameters that should be measured in final food product are *Salmonella*, *B. cereus*, *C. perfringens*, and TPC. *Salmonella*, *B. cereus*, and *C. perfringens* are pathogen bacteria that can cause severe diseases when consumed by human. In some cases, they can even resist the food processing technology and survive in the final food product without altering its physical characteristics (Setlow *et al*., 2006; De Knegt *et al*., 2015; Hailegebreal, 2017). Therefore, it is important to measure these microbes that can cause food borne diseases if consumed undetected.

Table 6. Quality of Purula flakes

Parameter	Unit	Result	Standard*
Lead (Pb)	mg/kg	<0.0134	<0.3
Mercury (Hg)	mg/kg	<0.0002	<0.05
Cadmium (Cd)	mg/kg	<0.0013	<0.1
Arsenic (As)	mg/kg	<0.0002	<0.5
Microbiology			
Salmonella	CFU/25 g	Negative	Negative
B. cereus	CFU/g	<10	$<10^4$
C. perfringens	CFU/g	Negative	$<10^3$
Total Plate Count	CFU/g	1.4×10^3	$<3\times10^5$
Yeast and Mold	CFU/g	7.5×10^5	-
Enterobacteriaceae	MPN/g	<3.0	<3.0

*Heavy metal standard refers to SNI (2009) heavy metal – 7837:2009, food category 06.0, Cerealia including legumes; microbiological standard refers to BPOM (National Agency of Drug and Food Control of Indonesia, 2019) standard for microbiological contaminant on processed food, food category: 06.0 Cerealia including legume (flour and starch)

Table 6 also shows the microbiological properties of purula. Three of them are *Salmonella* (negative colony/25 g), *B. cereus* (<10 CFU/g), *C. perfringens* (negative CFU/g), and TPC (1.4×10^3 CFU/g). All of these three parameters were below the acceptable standard for *Salmonella* (negative colony/25 g), *B. cereus* ($<10^4$ CFU/g), *C. perfringens* ($<10^3$ CFU/g), and Total Plate Count (3×10^5 CFU/g). Despite one of the Purula ingredients (seaweed slurry) had a high TPC (3.8×10^8 CFU/g), Table 6 shows a low purula TPC (1.4×10^3 CFU/g). This result is below the acceptable standard (3×10^5 CFU/g). This microbiological properties data of purula shows that purula production using high-temperature drum drying technique (at 175°C) was able to eliminate germs within acceptable level and produce a safe purula product for human consumption.

4. Conclusion

This research shows that purula finished good and primary ingredients (soy protein hydrolysate and seaweed slurry) can be produced at a pilot plant size to manufacture high-quality products. In terms of heavy chemical and microbiological contaminant level, soy protein hydrolysate, seaweed slurry, and purula conform with the National Agency of Drug and Food Control of standards. Purula is therefore safe for human consumption. Purula flakes have a consistent moisture level and thickness over the course of 5 days of

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production. The soy protein hydrolysate contains low molecular weight of protein (<20 kDa) which is still intact on purula finished good.

Conflict of interest

The authors declare no conflict of interest.

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