Journal of Engineering Science and Technology
Vol. 14, No. 1 (2019) 122 - 137

OPTIMISING PROTEIN AND TOTAL DISSOLVED SOLID TO SYNTHESIZE SOY SAUCE FROM SOYBEAN RESIDUE USING BOX-BEHNKEN DESIGN

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Abstract

Soybean residues are the residue of milk processing with high protein content. It is often benefited as cattle meal; however, it is processed to produce soy sauce in this research. It aims to optimise the protein and totally dissolve solid content in the soy sauce synthesis with optimum water/soybean mass ratio, sodium chloride and time. Soybean is immersed for 10 hours in distilled water, blended at water/soybean mass ratio 4:1, 5:1, and 6:1, and filtered to get the residue. The maximum protein in the residue is 12.48% at water/soybean mass ratio 5:1; therefore, the synthesis uses the ratio to benefit the protein. The residue is fermented for four days until the mould grows in its surface. It is fermented in sodium chloride of 20, 25 and 30%. The synthesis is optimum at water/soybean mass ratio 5:1, fermentation time of four weeks and the sodium chloride concentration 25%. Optimising the soy sauce protein follows the second order polynomial with protein 1.01% and total dissolved solid 40.13%. These results are compared to SNI 3543.2:2013. The solid meets the quality requirements, but protein is still below the standard. In brief, the condition can be implemented to synthesize soy sauce from the soybean residue.

Keywords: Fermentation, Protein, Residue, Soy sauce, Water content.

1. Introduction

Soybeans are the legume crop used as oilseed [1]. They are the ordinary material in Asia processed into foods such as soy sauce. Soy sauce is a brown liquid used as flavouring agents for fish, meat, etc. [2]. Soybean and soy sauce are two common terms used in the industry. Soybean with high protein influences the synthesis and quality of soybean milk. It has been popular for years and resulted in a byproduct during its process. It is soaked for eight hours and boiled at 90-100 ºC for 15 minutes [3]. Soybean residues are the excessive soybean product from the process considered as waste. Soybean 1 kg processed into milk results in the residue 1.4-1.8 kg [4]. Currently, the residue is useful as cattle woof, fertilizer or thrown into a landfill. There has been an increase of the residue amount being discarded.

The bean quality depends on agricultural practices, organic or conventional. It makes the soybean residue composition is different from the soybean. Li et al. [5] commented it contains protein 15.2-33.4%, fat 8.3-10.9%, fiber 42.4-58.1%, carbohydrate 3.8-5.3% and ash 3.0-4.5%. The moisture of the residue is 70-80%, too high for good preservation [2]. Consequently, the residue will decompose rapidly. It is extensively used in research in flavour production by moulds or yeast. However, it is usually in the form of soybean curd residue from the fermentation process.

Vong et al. [6] stated that the fermented residue contains carbohydrate, 62.57%, protein 23.48%, fat 8.73% and ash 3.77%. There is no report about the residue use from direct processing of soybean for making soy sauce consumed by the community. Soybean residue can be fermented into soy sauce without reducing its nutrient by a simple process. The fermentation is influenced by the microbe, temperature, oxygen, medium water content, medium pH, the nutritional content of raw material and salt content. A nutritional value at the fermentation is much influenced by the process. The soy sauce fermentation could be a solid fermentation and liquid fermentation (moromi). Solid fermentation takes time about 3-5 days [7]. The microbe used is Aspergillus oryzae, Penicillium sp and Fusarium sp.

Fungus (called koji) is dried and soaked in salt water 20-30% (w/v) [8]. In this study, soybean residue as the nutrient is investigated to ferment the protein with koji. Koji submersion in the salt solution is called moromi fermentation. The moromi fermentation takes time for 2-4 weeks [9]. The caramel is also added in soy sauce as a sweetener to affect the soy sauce taste. A free amino acid is an added element to give soy sauce an aroma. In Indonesian standard, salty soy sauce minimally contains protein 4% w/w, total dissolved solid 10% w/w and sodium chloride 3% w/w [10]. Soy sauce generally contains total nitrogen 1.00-1.65%, sodium chloride 17-19%, reducing sugar 1.5-5.0% and organic acid 1-2% w/v [11]. The saltless than 20% can cause putrefaction, as it is higher it inhibits the protease enzyme.

This research focusses on the soybean residue use before discarding without passing the initial process. It aims to optimise the fermentation condition and the protein content in the soy sauce. The optimising of other compounds will be published in another publication. Some novelties of the research are as follows. Firstly, the residue is made through a direct process of soybean with Anjosmoro variety (not soybean curd residue). The residue protein is fermented by Rhizopus oryzae and tempeh moulds. Secondly, the proteins before and after fermentation are measured. Finally, the condition to get the optimum protein and total dissolved solids content are determined by Response Surface Method (RSM). It is a method for optimising the process by analysing the effect of some parameters affecting the efficiency. According to Charunnisak et al. [12], it eases in determining the optimum value and process data by Box-Behnken Design (BBD) to get the optimum condition and relevant process variable. It is carried out to eliminate error systematically. Optimising usually uses many response surface designs such as Central composite, Doehlert and BBD. The BBD is appropriate because it uses formulas with a simple combination. The BBD result can effectively figure out the number of researches carried out. The RSM uses to optimise the protein and total dissolved solids in the soy sauce have not been investigated so far. Therefore, it is urgent to find a time-saving way to produce soy sauce with efficient salt concentration.

2. Experimental Procedure

The materials used were soybean from a distributor in Banda Aceh, galangal, lime leaves, bay leaves and pokak tea. The soybean with Anjasmoro variety was soaked for 10 hours to outgrow its size and be soft. Selen mixture was made by mixing SeO_2 (99.5%, Merck), K_2SO_4 (commercial grade, 3B Scientific (Wuhan Corp), and $CuSO_4{\cdot}5H_2O$ (99.999%, Merck). Other compounds were H_2SO_4 (analytical standard grade, Merck) and HCl 0.01 *N*. The NaOH 30% was made by dissolving 150 grams NaOH into 350 mL water and kept in a bottle. The H_3BO_3 2% was made by dissolving 10 grams H_3BO_3 in 500 mL distilled water. After it was cold, it was removed into a glass bottle. It was mixed with 5 mL indicator.

2.1. Synthesis of soybean residue

The method used was synthesis the soybean residue as a raw material of soy sauce. For each production, soybeans were soaked (1:5 w/v) for half hour, filtered, dropped into hot water (1:10 bean/water mass), and boiled for 10 minutes with 0.2% salt. It was flushed and kneaded to peel off the hull for around five minutes. This residue 200 grams were blended for 3 minutes in a blender (RIGCHINA MD-326S) with three mass ratios of water/soybean as in Table 1 and filtered using a muslin. The protein was analyzed as Indonesian standard (SNI 01-2891-1992). The water and ash of the residue with the highest protein were also analysed because they were related to higher calcium in cells.

Table 1. Code and input variable of RSM.

Variable	Code	-1	0	+1
Water/soybean mass ratio	*A*	4	5	6
Sodium chloride (% w/v)	*B*	20	25	30
Fermentation time (week)	*C*	3	4	5

2.2. Test method for protein analysis

The sample 0.51 grams were weighed and put into Kjeldahl 100 ml. Then, it was added by 2 grams of selen mixture and 25 mL of concentrated H_2SO_4. It was boiled on a heater until the solution becomes greenish clear (about two hours). It was entered into a flask 100 ml and diluted appropriately until the line mark. The solution 5 mL was added by 5 mL sodium hydroxide 30% and phenolphthalein and distilled. The distillation took time around 10 minutes and used 10 ml borate acid 2% mixed with an indicator for the collector. Then, the tip of the cooler was rinsed with distilled water. It was titrated with a hydrochloric acid solution 0.01 *N*. The blank determination was also carried out. Each sample was analyzed three times, and the result was averaged. The protein calculation followed Eq. (1).

$$Protein\ content = \frac{(V_1 - V_2) \times N \times 0.014 \times fk \times fp}{w} \times 100\% \quad (1)$$

where w is the sample mass, V_1 is the volume of HCl 0.01 N for sample titration, V_2 is the volume of HCl for blank titration, N is the normality of HCl, fk is the conversion factor for protein from the residue (6.25), and fp is the dilution factor.

2.3. Test method for water analysis

The residue 1-2 grams were dried in an oven at 105 ºC for 3 hours, cooled in an exicator, weighed, and repeated to get a constant mass. The water content was calculated as in Eq. (2).

$$Water\ content = \frac{m}{w} \times 100\% \quad (2)$$

where m is the mass loss after drying (gram) and w is sample mass before drying (gram).

2.4. Test method of ash analysis

The residue 2-3 grams were weighed in a porcelain cup whose mass was known. It was burnt, ashed in a furnace at 550 ºC until the ashing is complete, cooled in an exicator; and weighed until the mass was constant. The ash followed Eq. (3).

$$Ash\ content = \frac{w_1 - w_2}{w} \times 100\% \quad (3)$$

where w is the mass of the residue before ashing (gram), w_1 is the mass of the sample and cup after ashing (gram), w_2 is the mass of empty cup (gram).

2.5. Synthesis of soy sauce

The soybean residue was submerged in water for 10 hours and dried at 30 ºC for four hours. After cooling, it was inoculated with a thickness of two centimetres on a winnowing tray. It used yeast 0.3% and was incubated for four days. This step was the first step of fermentation. It used Rhizopus oryzae that is a brownish fine powder, and tempeh mould. In the second day, it was reversed, joggled, and incubated until the fourth day. The result was marked by the growth of white mould and green, yellowish spore. The fermentation room was equipped with a blower to set the humidity 85%. After four days, the soybean residue and the mould above the tray were scrapped. It was put into a tank and added with salt. It was immersed in sodium chloride as in Table 1. During the immersion, the material was agitated twice. It was left at room temperature until the soy sauce was synthesized. Accordingly, the material was kept in an open area to get sunlight.

The solution was filtered to take the concentrate of the soybean. The filtration was carried out twice using filter cloth to get the free impurity-soybean concentrate. It was boiled with caramel sugar until its aroma and taste were formed. Galangal, lime leaves, bay leaves, pokak tea were added into the mixture. As the soy sauce was relatively viscous, it was entered into a container tank. It was filtered to get pure and clean soy sauce to channel into a container vessel. The protein and total dissolved solid content of the soy sauce were then analysed.

2.6. Research design

The relationship between the independent variables and the result was determined using RSM. The variables were water/soybean mass ratio (*A*), sodium chloride concentration (*B*) and fermentation time (*C*). The process variable was shown in Table 1.

Optimising by the Design-Expert 10.0.1 software with BBD was carried out randomly in 17 times of treatment in Table 2. The responding variables were the residue, the soy sauce protein, and the dissolved solid content.

Table 2. Box-behnken design.

Run	Standard	*A*	*B*	*C*
1	17	5	25	4
2	7	4	25	5
3	4	6	30	4
4	12	5	30	5
5	16	5	25	4
6	1	4	20	4
7	5	4	25	3
8	3	4	30	4
9	13	5	25	4
10	9	5	20	3
11	14	5	25	4
12	8	6	25	5
13	15	5	25	4
14	11	5	20	5
15	10	5	30	3
16	2	6	20	4
17	6	6	25	3

2.7. Test method of total dissolved solid

The soy sauce was weighed, added by distilled water, filtered using filter paper number 41 into a flask 100 ml. The paper was washed; the solution was added by distilled water and was stirred. The solution 25 mL was entered into a cup, vaporized in a bath until dry, left in an oven at 105 ºC for 2-3 hours, cooled in an exicator, and weighed until its mass was constant. The TDS was as in Eq. (4).

$$\%\, total\ dissolved\ solid = \frac{\frac{100}{25} \times (w_1 - w_2) \times 100\%}{w} \times 100\% \quad (4)$$

where w_1 is the cup and sample mass after vaporising (gram), w_2 is the cup mass (gram), w is the sample mass (gram).

3. Results and Discussion

3.1. The effect of the water/soybean mass ratio on the protein content of soybean residue

Table 3 shows qualified soybean with larger mass and higher protein. Thakur and Hurburgh [13] commented that the protein in other varieties is much lower (34.8-36%) than that in this research. Here, 3-mono-chloro propane-1,2-diol is not analysed because it is not required by Indonesian salty sauce standard and no proper test could measure it.

Table 3. Characteristics of the qualified soybean and soy sauce.

Characteristic	Soybean	Soy sauce
Name	Anjosmoro	Salty soy sauce
Colour	Yellow	Blackish brown
Mass of 100 grains (g)	14.8	-
Protein content (%)	41.8-42.1	0.44-1.01
Water content in the residue (%)	16.09	-
Ash content in the residue (%)	1.08	-
Total dissolved solid	-	36.55-41.80

Soybean variety in this research has relatively high protein content. As a result, the protein content of the soybean residue resulted in this research is also higher than that in the earlier research [5]. As the protein in the soybean increases, it needs more water to extract the protein. The water content in the residue is also higher because this research uses qualified soybean. An effect of the water/soybean mass ratio on the protein content in the residue follows the second order polynomial. By the model, the protein can be predicted for other water/soybean mass ratios. The protein content is analyzed using the procedure in the method section. The protein content is analyzed using three water/soybean mass ratios as in Table 1. Figure 1 shows the water/soybean mass ratio effect on the average percentage of the residue protein. It shows that protein content increases until 12.48% in water/soybean mass ratio 5:1, then it declined. The residue composition is higher than that of previous research with the moisture content of 68.03%, ash 1% and proteins 8.08% [14]. It may be because the earlier research used more water to prepare the residue with water/soybean mass ratio 6:1.

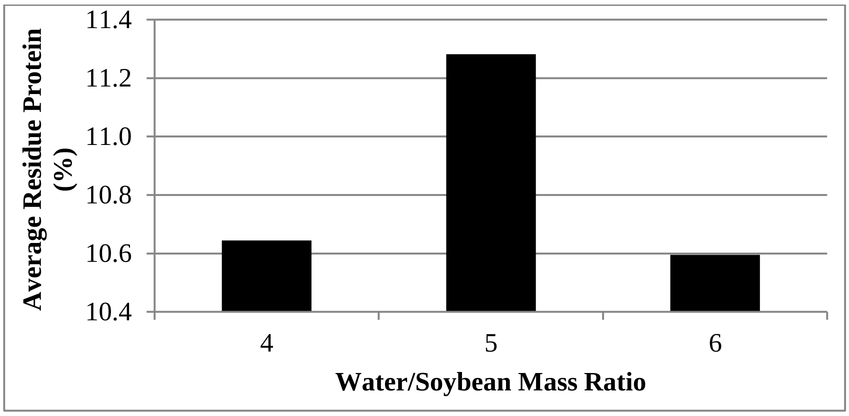

Fig. 1. Effect of water/soybean mass ratio on protein of the residue.

It is noted that the low water content in the residue enables its preservation for a longer time. If the residue is properly dried, all of its component increases, so its ratio influences its properties. The percentage shows that protein composition in the residue at water/soybean mass ratio 5:1 has been relatively saturated as soy sauce material. In contrast, the lowest protein content is 9.42% in water/soybean mass ratio 6:1 as shown in Table 4. It is relatively higher than the previous research with 85% moisture and 3.6% protein [15], probably because the residue was made of black soybean. It seems that the protein content in soybean residue is mainly influenced by the water or the moisture in the residue.

Table 4. Composition of soybean residue and soy sauce by BBD.

Run	Water/ soybean mass residue	NaCl (%)	Fermentation time (weeks)	Residue protein (%)		Soy sauce protein (%)		Dissolved solid (%)	
				Actual	Prediction	Actual	Prediction	Actual	Prediction
1	**5**	**25**	**4**	**12.48**	**12.48**	**1.01**	**1.01**	**40.16**	**40.13**
2	**4**	**25**	**5**	**10.89**	**10.76**	**0.53**	**0.60**	**41.80**	**41.79**
3	6	30	4	10.51	10.74	0.84	0.91	37.91	38.12
4	5	30	5	11.78	11.78	0.83	0.81	41.36	41.22
5	5	25	4	12.48	12.48	1.01	1.01	40.16	40.13
6	4	20	4	10.34	10.11	0.74	0.67	40.72	40.68
7	4	25	3	10.22	10.45	0.88	0.94	40.67	40.85
8	4	30	4	11.03	11.16	0.68	0.63	41.76	41.95
9	5	25	4	12.48	12.48	1.01	1.01	40.16	40.13
10	5	20	3	9.95	9.95	0.79	0.81	38.81	38.71
11	5	25	4	12.48	12.48	1.01	1.01	40.16	40.13
12	6	25	5	11.81	11.58	0.90	0.85	41.20	41.17
13	5	25	4	12.48	12.48	1.01	1.01	40.16	40.13
14	**5**	**20**	**5**	**11.02**	**11.38**	**0.44**	**0.44**	**41.65**	**41.74**
15	5	30	3	11.23	10.87	0.77	0.77	39.19	38.86
16	6	20	4	10.59	10.59	0.47	0.52	39.78	39.76
17	**6**	**25**	**3**	**9.42**	**9.42**	**0.90**	**0.83**	**36.55**	**36.71**

As the water in the residue increases, there is a tendency that the protein content will decrease. To optimise the residue protein, the mathematical model in Eq. (5) can be used:

Soybean residue protein:

$$12.48 - 0.019\,A + 0.33\,B + 0.59\,\mathrm{C} - 0.19\,AB$$

$$+ 0.43\,\mathrm{AC} - 0.13\,\mathrm{BC} - 1.14\,\mathrm{A}^2 - 0.73\,\mathrm{B}^2 - 0.76\,\mathrm{C}^2 \quad (5)$$

The equation in terms of code factors can be used to predict the soybean residue protein for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The equation is useful for identifying the impact of the factors by comparing the factor coefficients.

3.2. Effect of independent variables on the soy sauce protein

The constraint of the variable can be written mathematically using the result data:

4 $\leq$ Water/soybean mass ratio $\leq$ 6
20% $\leq$ Sodium chloride concentration $\leq$ 30%
Three weeks $\leq$ Fermentation time $\leq$ Five weeks

Analysis of Variance (ANOVA) distinguishes the average value of at least three data by comparing its variance and deciding the response. Table 5 shows the interaction significance between the dependent and response variables. The R^2 of the protein of the residue and soy sauce is closer to the model of more than 90%. The result appears correct and significant with the polynomial model. Table 5 also shows the effect of interaction among variables. The probability value (p-value) larger than F that equals to 0.1 shows the significant or insignificant variable interaction. The value higher than 0.1 shows that the model is insignificant.

Table 5. Anova for the probability value of protein and dissolved solid composition of soy sauce.

Source	Residue protein (%)		Soy sauce protein (%)		Dissolved solid (%)	
	P-value	Remark	P-value	Remark	P-value	Remark
Model	0.0002	Significant	0.0013	Significant	< 0.0001	Significant
A	0.8532		0.1875		**< 0.0001**	
B	0.0116		**0.0094**		0.1607	
C	**0.0005**		0.0124		**< 0.0001**	
AB	**0.2059**		**0.0157**		**< 0.0001**	
AC	0.0170		0.0364		**< 0.0001**	
BC	0.3778		0.0193		**0.0811**	
A^2	**< 0.0010**		**0.0097**			
B^2	**0.0010**		**0.0004**			
C^2	**0.0008**		0.0280			
R^2	**96.73%**		**94.43%**		**99.05%**	
Adj-R^2	92.51%		87.26%		98.49%	

The water/soybean mass ratio and fermentation time interaction (AC) has the p-value of 0.0364. However, it has the largest p-value for soy sauce protein. It means that the fermentation time interaction with water/soybean mass ratio is less influential than the sodium chloride interaction at the ratio. As the fermentation time increases until a particular time, the soy sauce protein will be higher as shown in Fig. 2. The water/soybean mass ratio and sodium chloride content have the lowest interaction significance (0.0157) in the protein synthesis of soy sauce. The lowest p-value shows that the ratio and content interaction (AB) has a higher effect on the soy sauce protein. Figures 2 and 3 display the result of the optimising of soy sauce protein.

The increasing of the ratio (*A*) provides a positive effect on the protein of soy sauce. All compounds in soybean will be fewer as the mass ratio increases. The more influential variables in this research are water/soybean mass ratio as in Fig. 3. As the mass ratio and sodium chloride increase to a particular point, the protein content in soybean residue becomes higher. The soy sauce results in the lowest protein content 0.44% with water/soybean mass ratio of 5:1 and sodium chloride content 20%. Box-Behnken shows that the optimum content of soy sauce protein is 1.01% as in Table 3 with water/soybean mass ratio 5:1 and sodium chloride content 25%. It is also the highest protein content.

Sodium chloride content (B) also enriches the protein content of the soy sauce with p-values 0.0094, 0.0157, and 0.0004. Therefore, both *A* and *B* variables are selected as significant interaction. The protein of soy sauce tends to increase as the increase of the sodium chloride until a particular limit as shown in Fig. 3. High protein content may be because the soybean protein is one of the parts to form the soy sauce. Its increase is influenced by the water/soybean mass ratio. Therefore, both factors tend to influence the protein content in the soy sauce. The sodium chloride content during fermentation will change because the liquid in net cells leaves the cell. However, when it becomes higher, the protein in the soy sauce will be lower. The total nitrogen resulted may achieve maximal and the peptidase activities are low because of enzyme denaturation. The content adjustment can be carried out periodically; however, it was set as variable determined. This soy sauce has less protein content than the protein of others with the value of 2.05-3.88% [2]. It is because the soybean residue is used as the material, not soybean whose protein content is higher. Besides, this research uses Rhizopus oryzae and tempeh mould, whereas the previous research uses Aspergillus oryzae and Aspergillus flavus. The protein content of soy sauce also relates to the nitrogen in the residue using soybean with a mass of more than 10 g/100 soybeans. The total nitrogen can show the amount of protein used in the soy sauce and increase the flavour.

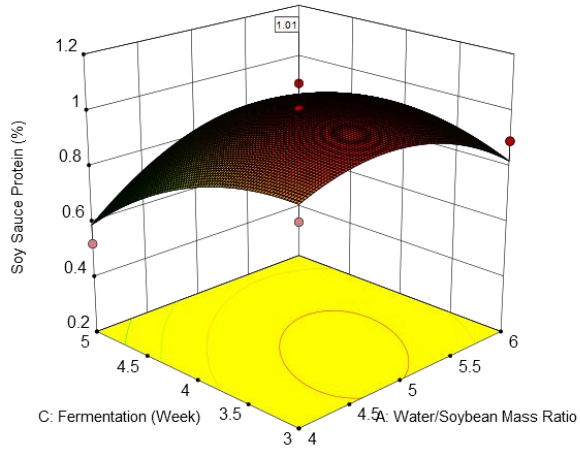

Fig. 2. Effects of water/soybean mass ratio and fermentation duration on the soy sauce protein.

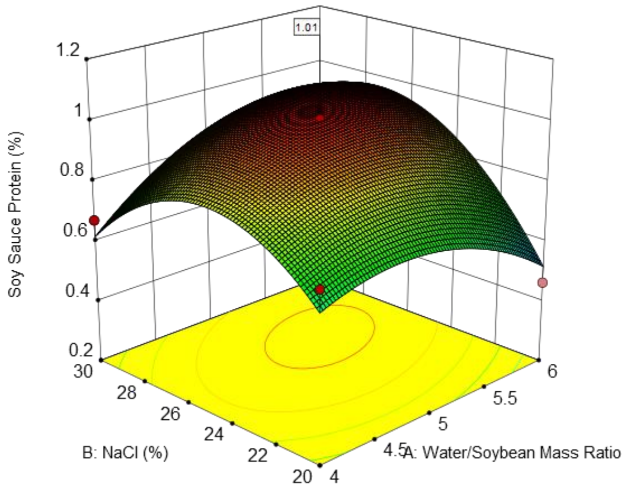

Fig. 3. The effects of water/soybean mass ratio and sodium chloride on the soy sauce protein.

The soybean has the relatively narrow surface area of pores to spread sodium chloride evenly. Maximum total nitrogen can be obtained when sodium chloride is absent. It decreases with increasing of sodium chloride concentration. The salty soy sauce has higher sodium chloride, thus its total nitrogen is less and more protein is hydrolyzed. It makes the protein content remains in the soy sauce become relatively less. In this research, it is not measured because it is not required in SNI 3543.2:2013. Nitrogen implies a protein digestibility during fermentation. An excessive dilution during brine fermentation may affect the nitrogen content used in dark soy sauce. The high content of nitrogen is a sign of the acid-hydrolysis process. It differentiates soy sauce synthesized by hydrolysis from that made by fermentation. The total nitrogen increases with high yields. The higher level of nitrogen implies greater protein hydrolysis into small peptides and amino acids.

During the protein content analysis, the soy sauce fermentation takes place well. Its protein is like soy sauce of tofu residue with a protein content of 2.61% at four-week fermentation and increases to 6.83% in six weeks [16]. An equation can predict it by modelling a relationship between the variables and the protein. Design Expert software provides Eq. (6) to calculate the protein of the soy sauce.

$$\text{Protein (\%)} = 1.01 + 0.035\,A + 0.085\,B - 0.08\,C + 0.11\,AB + 0.088\,AC + 0.1\,BC - 0.12\,A^2 - 0.21\,C^2 - 0.091\,C^2 \quad (6)$$

The water/soybean mass ratio (*A*) and sodium chloride concentration (B) variability equal the protein content shown by a positive constant. It suggests that the holes of the residue network are too big to hold the protein by hydrophobic interaction because of the ratio effect. The phenomenon is like that of hydrophobic interaction of the protein with isoflavones in tofu production [17]. As the ratio increases, the protein of soy sauce increases in the range of 0.44-1.01%. SNI 3543.2:2013 determines that the protein of the soy sauce is minimally 4%. The protein made of the three mass ratios of water/soybean investigated has not fulfilled the standard. This tendency is useful while synthesising the soy sauce from a large-scale soybean residue. For quality and technical easiness, the ratio should be 5:1 with the sodium chloride 25% to make the protein content close to the standard.

The soybean content and types, fermentation time, and yeast also influence the soy sauce protein. The yeast produces a protease enzyme to extract protein actively into soy sauce. During the immersion, this enzyme breaks the protein, fat, and carbohydrate into amino acids, peptides, sugar, and fatty acid to give a unique aroma. Sugar has resulted from the enzyme action on the starch and protein of the residue during brine fermentation. It results in a sweet taste in the salty soy sauce because of the minimum sugar. The bacteria expected to grow are the Rhizopus oryzae producing acid. Glucose is degraded and converted to organic acids by the bacteria [17].

An industry usually adds sugar, molasses, and caramel to improve its taste during the heating. The reaction between sugar and peptides influences molecular weights of peptides. The reddish colour of the soy sauce may be from the reaction of sugars, an amino group on a protein or amino acid, and peptidase. It is the Maillard reaction. The reaction of protein with carbonyl-containing compounds such as sugar produces modified protein.

This protein reactivity is primarily because of the amino group of lysine. The amino group reacts to convert its L-lysine into N-fructofuranosyl-lysine to the protein. The protease promoting hydrolysis increases the protein in the 4 days then, the sugar and other ingredient accumulation decline its activity. This increase slows down, some proteins are degraded, the others need more time to be degraded. The sugar and all compounds formed probably causes the protein less. The sugar is not analysed because it is not required in SNI standard of salty soy sauce.

3.3. The effect of independent variables on the total dissolved solids

Total Dissolved Solid (TDS) is one of the significant parameters in industrial soy sauce. It is total solid that is resisted on a filter with a particle size of 20 - 25 µm. Its categories are bacteria and dissolved crystal, organic compounds and dissolved gases. The impact of TDS is its contribution to the dark colour of soy sauce. The R^2 value of the TDS is closer to the model more than 90% as in Table 5. The interaction of water/soybean mass ratio and sodium chloride concentration (AB) has an important effect on the total dissolved solids of soy sauce. Both variables show that the significant variation is dominated by the interaction. It is in accordance with p-value of AB less than 0.0001 for total dissolved solids. The contour plot shows the effect of the ratio versus the total dissolved solids as in Fig. 4. The increasing of the ratio gives a positive impact on the TDS of soy sauce. The TDS are in the range of 36.55-41.80% in the soy sauce with the highest content increases in the water/soybean mass ratio 4:1 and sodium chloride 25%. The increasing of the ratio results in the predicted total dissolved solids of 36.71-41.79% in the range of 4:1-6:1. The contact between particles may be increased more densely, and the solid is hard to enter among particles at the condition. It fulfils SNI 3543.2:2013, that the soy sauce has TDS minimally 10%. All water/soybean mass ratios result in the total dissolved solid higher than 10%.

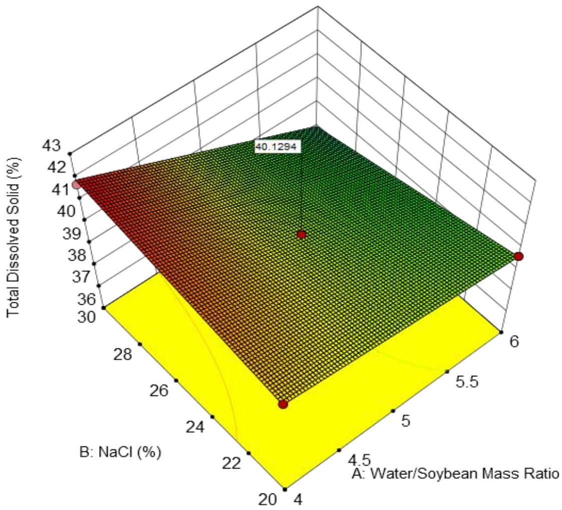

Fig. 4. Effect of the water/soybean mass ratio and sodium chloride on the total dissolved solids.

Another important research variable is sodium chloride content. The setting of the sodium chloride content could give the same impact. It could influence the mixture stability in the soybean residue. The residue can decay as a result of the decrease of the preservation effect of the salt. The sodium chloride causes microbe contained in the residue is hard to ferment. The less microbe is a signal that the soy sauce contains only a few dissolved solids. This salt must be used at least 20% because the soy sauce will smell bad if it is too low. However, the sodium chloride in the fermentation process is useful to regulate the water supply for a microorganism. It functions to draw out net-cell liquid containing saccharides as a nutrient for microorganism growth and influence the dissolved solids in the soy sauce. The salt solution extracts the nitrogen compounds from hydrolysis results of enzymes in soybean residue [18]. As a result, the total content of solids increases as occurs in commercial soy sauce whose total solids 15.67%. Some solids are soluble and some are insoluble. The total dissolved solids decline at a ratio 6:1 and sodium chloride content 25%. The increasing of sodium chloride shows that the

TDS slightly increases. This shows that as the sodium chloride content increases, the TDS also increases up to a particular point.

Figure 5 shows the change of TDS during the fermentation in a tank. The longer fermentation results in the increase of TDS. The highest TDS is 41.74% achieved by the sodium chloride of 20% in five weeks. When it is relatively high, hydrolysis to become simpler substances by an anaerobic bacteria needs a longer time of hydraulic in a tank. This time of three weeks is not enough for degradation and hydrolysis biologically of dissolved solids. In this batch process, the bacteria have enough time to degrade solids in soy sauce. Therefore, the TDS can be higher. It means they need more than three weeks for good fermentation. The optimum fermentation time is four weeks for the reason of time efficiency as in Fig. 5. Intermediate may limit the degradation process and there is an inhibition possibility because of the rise of ammonia. Soy sauce can be used as a flavoring or is added by more water to diminish the TDS content if necessary.

There is no comparable research about the total dissolved solids in soy sauce so far. Based on studies by Feng et al. [19], therefore, it was difficult to decide on which, the condition of soy sauce is better. Consequently, it is necessary to use a suitable evaluation method using BBD. The optimum value of the total dissolved solid seems 40.13% as shown in Table 2 based on BBD. Equation (7) is the BBD result in getting the optimum value of TDS.

Total dissolved solid:

$$40.13 - 1.19\,A - 0.092\,B + 1.35\,C - 0.73\,AB + 0.88\,AC - 0.17\,BC \tag{7}$$

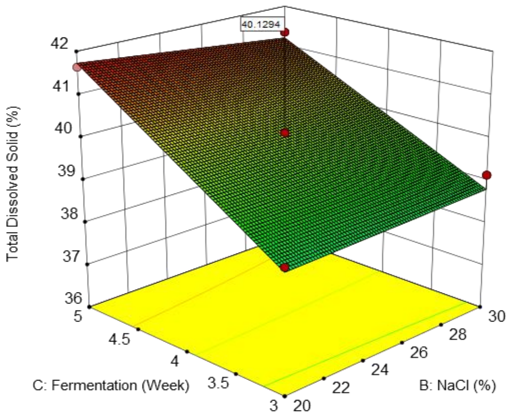

Fig. 5. Effect of sodium chloride and fermentation week on the total dissolved solids.

3.4. Optimising with box-Behnken design

Figures 2 to 5 show the relationships between three variables and the response. Every plot shows the effect of two variables in a range where the others are in zero conditions. The response surface describes every influential variable interaction on the protein composition of the residue and soy sauce, and on the dissolved solids. The contour shows the characteristic and the largeness of the effect of interaction among response variables. An elliptical contour plot shows the interaction among variables influencing each other. The spherical contour shows that the variable interaction is less influential on the protein composition of the residue and the dissolved solid than that of the elliptical plot as in Fig. 3.

The soybean residue and soy sauce protein, and dissolved solid content at sodium chloride content 25% increases significantly in the range of 9.42-12.48%; 0.44-1.01%; and 36.71-41.79%, respectively. This optimum protein content of soybean residue is in the water/soybean mass ratio 5:1, sodium chloride content 25% and fermentation time in four weeks. The ratio and the sodium chloride content cause the increase in protein until a particular limit. The four weeks of fermentation time will ease the reaction, and more microbes can result in a change required maximally. This reaction causes an increase in protein and dissolved solid content in soy sauce. As the water/soybean mass ratio is higher than 5:1; thus, it results in the dissolved protein, peptide, and amino acid in great quantities. The mass ratio higher than 5:1 causes the protein of soy sauce is less. By ignoring the effect of the ratio on the protein content of soy sauce, it is clear that the protein of soy sauce increases in the range of 0.44-1.01%. The protein composition declines while the water/soybean mass ratio is higher than 5:1. This is because the microbe does not carry out fermentation anymore at a particular ratio and sodium chloride concentration. The high sodium chloride makes the changing of the dissolved protein into peptides does not occur in the soy sauce. It was reported that appropriate salt concentration could influence the enzyme hydrolysis. However, when the salt concentration was higher than 13%, microbe and enzymes that were not resistant were suppressed [20]. This relates to the effect of sodium chloride in protein fermentation. As sodium chloride enters the fermented system, it blocks the growth of the decomposing microbe. The peptide formed experiences hydrolysis to result in glycine and alanine as shown in Fig. 6.

$$^{+}NH_3\text{-}CH_2\text{-}\overset{\overset{O}{||}}{C}\text{-}NH\text{-}\underset{\underset{CH_3}{|}}{CH}\text{-}\overset{\overset{O}{||}}{C}\text{-}O\text{-} + H_2O \rightleftharpoons {}^{+}NH_3\text{-}CH_2\text{-}\overset{\overset{O}{||}}{C}\text{-}O^{-} + {}^{+}NH_3\text{-}\underset{\underset{CH_3}{|}}{CH}\text{-}\overset{\overset{O}{||}}{C}\text{-}O\text{-}$$

Fig. 6. Hydrolysis of a peptide into glycine and alanine.

Table 6 shows the optimum condition with BBD from every variable. Optimising takes place to reach a target expected from every response. The target is determined in the form of a particular value range. Desirability is in the value 0-1. As they equal to one; thus, the desired condition is more appropriate. Table 6 shows five options to gain the most optimum water/soybean mass ratio in producing soy sauce protein. The ratio is optimum at a ratio 5:1 in four-week fermentation. The protein content declines while the sodium chloride content is above 25%. There is an optimum concentration of sodium chloride, while fermentation to form the peptide. Hoang et al. [21] explained that the high brine content cause enzyme denaturation; therefore, the activities of peptidase are low. The decrease of salt causes the increase in water activity; thus, rapid fermentation at optimum sodium chloride concentration is expected (four weeks). The fermentation synthesizes the soy sauce protein optimally at sodium chloride concentration 25%.

The optimum condition selected is the third condition, namely at four-week fermentation and sodium chloride 25%. It exhibits the residue protein, 12.48%, the protein of soy sauce, 1.01%, the TDS 40.13% and the desirability 1.000. Industry requires soybean with high protein; thus, the second, the third and the fourth options are selected for the highest protein. However, the second option needs more sodium chloride concentration, whereas the fourth option needs a longer fermentation time. Therefore, the third option is as the selected optimum operation to achieve lower sodium chloride concentration and lower fermentation time. It is important to result in the most efficient process condition.

The optimising to get the optimum condition for fermentation of soybean residue results in the water/soybean mass ratio of 5:1. To confirm the predicted optimum response; thus, three tests are carried out at the optimum condition. Table 7 shows the average value of the percentage of soybean residue protein is 12.48%, the soy sauce protein 1.00% and the dissolved solid 40.06%. The BBD design implementation is appropriate for processing the soybean residue to synthesize the soy sauce [4]. It is the most popular surface design to optimise a process. The results in this research cannot be compared to other designs such as Doehlert design to confirm the results because it requires more variations.

In Doehler designs the level (variation), quantity is different for each variable. In a three-variable Doehlert design, one variable is investigated at five levels, another is studied at seven levels, and the other is studied at three levels. Therefore, the matrix and the dimensions depend on the structured involved.

Table 6. Optimising value of RSM with soybean residue sample.

Variable	1	2	3	4	5
Water/soybean mass ratio	5.3	5.1	**5.0**	4.9	4.6
NaCl concentration	26.22	27.16	**25.00**	24.68	25.38
Fermentation time (week)	3.4	3.6	**4.0**	4.1	3.7
Residue protein (%)	11.68	12.48	**12.48**	12.48	12.14
Soy sauce protein (%)	1.01	1.01	**1.01**	1.00	1.00
Dissolved solid of soy sauce (%)	38.759	39.405	**40.130**	40.290	40.398
Desirability	1.000	1.000	**1.000**	1.000	1.000

Table 7. Experimental value of the response with soybean residue sample.

Response	1	2	3	Average	Standard of deviation
Residue protein (%)	12.46	12.48	12.50	**12.48**	**0.02**
Soy sauce protein (%)	0.99	1.01	1.00	**1.00**	**0.01**
Dissolved solid of soy sauce (%)	40.45	40.13	39.6	**40.06**	**0.43**

4. Conclusions

The water/soybean mass ratio and sodium chloride concentration are more influential than the fermentation time in the soybean residue fermentation. As the ratio is higher by a 5:1 point; thus, the percentage of the soy sauce protein increases. The protein percentage tendency is different from the dissolved solid tendency. As the ratio increases; thus, the dissolved solid percentage of soy sauce decreases. This fermentation is useful to get the high protein and less dissolved solid of soy sauce than the soybean residue obtained from various industries of soybean milk. With water/soybean mass ratio 5:1, sodium chloride content 25% and four-week fermentation, thus the protein content of soybean residue 12.48%, the soy sauce protein 1.00% and the dissolved solid 40.06% can be obtained. The solid has fulfilled minimal standard of 10% as mentioned in SNI 3543.2:2013 in Indonesia. This solid percentage proves that the residue is suitably used for soy sauce synthesis. Such condition can be implemented for soybean residue from other soybean industries.

Acknowledgements

Thanks to Putri Sri Wahyuni and Ratih Yunitasari for their help to analyse data and financial support in this research.

Nomenclatures

A	Water/soybean mass ratio
B	Sodium chloride content, %
C	Fermentation Time, hour
fk	Conversion factor for protein from the residue
fp	Dilution factor
m	Mass loss of the sample after drying, kg
N	Normality of standard solution. N
V_1	Volume of standard solution for sample titration, m^3
V_2	Volume of standard solution for blank solution, m^3
w	Mass of the sample before drying or ashing or vaporising, kg
w_1	Mass of the sample and cup after ashing or vaporising, kg

Abbreviations

BBD	Box-Behnken Design
RSM	Response Surface Methodology
SNI	Standar Nasional Indonesia

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