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Optimization of meat analog production from concentrated soy protein and yam (*Xanthosoma sagittifolium*) powder using pasta machine

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Abstract

The objectives of this study were to determine the effect of *Xanthosoma sagitifolium* powder (XSP), mixing time and water on meat analog characters and to optimize these parameters in meat analog production using Response Surface Methodology with Box-Behnken design. Meat analog is produced from mixing XSP and concentrated soy protein using a household pasta machine. The observation was made at the range of XSP proportion 0 - 80%, mixing time 6 - 18 mins and water addition 70 - 150%. Fourier Transform Infra-red (FTIR) was employed to determine the alteration of the functional bond. The results of FTIR observation showed that the highest intensity obtained when XSP proportion, mixing time and water addition are 20%, 9 mins and 140% respectively. The experimental data obtained from the Box-Behnken design was in accordance with the second order polynomial equation. Moreover, the 3D response surface derived from mathematical models was used to optimize the production condition. The optimum production conditions are XSP proportion of 30%, water addition of 140% and mixing time of 12 mins which produced meat analog having WHC of 145.3542±5.7384%, OHC of 43.7869±7.8725% and texture of 90.32±0.37 gr/mm.

1. Introduction

There has been growing food demand including protein-based diet driven by the increasing world population. In several developing countries, there are problems related to animal protein malnutrition. In addition, the awareness of healthy food has shifted the consumption trend from animal protein towards plant protein. Meat analog is a vegetable protein-based food texturized to imitate meat products (Sheard *et al.,* 1984). This diet becomes suitable for vegetarian diet and is considered as healthy food. Meat analog becomes more superior compared with animal meat based on the ability to reduce the risks of heart disease and cancer. Meat analog usually is made from soy protein as a major component and is fabricated with extrusion cooking to produce fiber-like structure (Lin *et al.*, 2000; Yao *et al.*, 2004).

The fiber-like structure in meat analog depends on the material of its components, extrusion conditions and moisture content. The combination of temperature enhancement and shear rates during screw rotation results in protein and carbohydrate melting (Krintiras, 2015). Extrusion machines are commonly large-scale machines and cannot be utilized in daily cooking. However, household pasta machine is a simple extrusion machine which has been used in daily cooking activities for making pasta at a common household kitchen. The application of this machine in meat analog production facilitates the meat analog provision and ease of consumption.

Furthermore, the addition of carbohydrate into a meat analog matrix probably increases texture and fibrous characteristic. *Xanthosoma sagittifolimum* yam is a good source of carbohydrate which can be cultivated under less favorable land conditions. Yam can be normally consumed boiled, fried, steamed or roasted. Besides its high starch content of 74.66±0.66 to 80.46±0.62%, yam has various types of phytochemicals (Senanayake *et al.,* 2012). The most predominant phytochemicals are diosgenin, saponin, dioscorine, alkaloid and flavonoid which make yam offer diverse health benefits. The addition of *Xanthosoma sagittifolimum* powder (XSP) in meat analog production is expected to increase the physical properties and to improve functional properties of health.

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The chemical bonds performed in meat analog matrix are disulfide bonds, hydrogen bonds and hydrophobic interaction (Hager, 1984). The bond is performed in protein-protein interaction to form matrix structure in meat analog. Protein content that increases in meat analog results in fine structure (Maurice and Stanley, 1978). The carbohydrate content supports the structure and improves the texture of meat analog (Sheard *et al.*, 1984; Rareunrom *et al.,* 2008). The percentage of soy protein isolate increases from 20-80% of expansion ratio but reduces cutting force and fibrous characteristic (Rareunrom *et al.,* 2008). There are considerable discrepancies in the appearance of the resulting product, between low moisture (up to 35%) and high moisture (over 50%) extrusion. Although the extrudates have differed in appearance, protein-protein interaction which is contributed in meat analog structure remains unchanged (Chiang, 2007). Consequently, water addition probably affects its texture and appearance.

The first objective of this study is to examine the alteration of chemical bonds in meat analog matrix driven by the variation on XSP proportion, mixing time and water addition. The observations are executed using Fourier Transform Infrared (FTIR). The second objective is to determine the optimum parameter from XSP proportion, mixing time and water addition based WHC, OHC and texture data. The optimization is implemented with RSM (Response Surface Methodology). RSM is an effective statistical technique which can reduce the period and number of experiments. The meat analog in this research is developed using a household pasta machine.

2. Materials and methods

2.1 Materials

The raw materials used were Concentrated Soy Protein (CSP) is obtained from "NURA" (local shop) containing 66.14% protein, 0.16% lipid, 5.75% water, 4.43% ash and 27.94% carbohydrate. *Xanthosoma sagittifolium* powder (XSP) was made from *Xanthosoma sagittifolimum* tubers with modification on dipping solution wherein sodium chloride 200 ppm was used rather than sodium metabisulphite of 20 ppm (Owuamanam, 2010). The XSP contains 2.37% protein, 0.33% lipid, 8.27% water, 4.42% ash and 84.61% carbohydrate. Moreover, mineral water ("Aqua") is added to adjust moisture contain.

2.2 Design experiment

The research was divided into two stages. The objective of the first stage was to determine the alteration of chemical bonds using FTIR. Observations were made on the variations of XSP proportion (0, 20, 40, 60 and 80%), mixing time (6, 9, 12, 15 and 18 mins) and water addition (80, 100, 120 and 140% w/w). The aim of the second stage was to determine the optimum condition in the production of meat analog by using RSM method; furthermore, Box-Behnken design was chosen. There were three independent variables consisting of XSP proportion (X1), mixing time (X2) and water addition (X3). After randomization using Box-Behnken design, fifteen experimental conditions were tested and optimized. The randomization result was shown in Table 1. The response variables were WHC, OHC and texture. The RSM was run using Minitab V 17.0 for Windows.

Table 1. Box-Behnken design with coded levels and actual levels

No	Coded Level			Actual Level		
	x_1	x_2	x_3	x_1 (%)	x_2 (Min)	x_3 (%)
1	-1	1	0	0	18	110
2	0	1	1	40	18	140
3	0	0	0	40	12	110
4	1	1	0	80	18	110
5	-1	0	-1	0	12	80
6	-1	0	1	0	12	140
7	1	0	1	80	12	140
8	1	0	-1	80	12	80
9	0	1	-1	40	18	80
10	0	-1	-1	40	6	80
11	0	0	0	40	12	110
12	0	0	0	40	12	110
13	-1	-1	0	0	6	110
14	1	-1	0	80	6	110
15	1	-1	1	80	6	140

The meat was made wherein, XSP was blended with CSP to obtain varied proportions. Water (80°C) then was added in the extruder according to the research design. Afterwards, the dough was mixed and blended for a certain duration using household pasta machine (Healthy Power Noodle, *Fastworld*) then extruded through the die which has been modified. The extrusion process was classified as cool extrusion. Variations of XSP concentration, water addition and mixing time are applied according to the research design. After the extrusion process, the meat was cooked with 100°C steam for 30 mins and then it was dried in a regulating oven at 50°C for 20 hrs.

2.4 FTIR analysis

Samples for FTIR spectroscopy were prepared by drying meat analog at 50°C for 20 hrs to later was pulverized into a fine powder. Two milligrams of powder were mounted in the sample holder of the FTIR machine (Alpha-Bruker) and spectra were observed in the range 4000–450 cm^{-1}.

2.5 Texture analysis

The dry meat analog was rehydrated by cooking in boiling water for 15 mins. The texture of meat analog was determined by using Rheotex Type of SD-700 (Sun Scientific Co-LTD); moreover, the penetrations of head was set at 0.15 mm, ten measurements were conducted for each sample. The texture was reported as gram/mm.

2.6 Water and oil - holding capacity (WHC and OHC).

This method was used with slight modifications (Chau, 1997). One gram of dry meat analog was stirred in 10 mL of distilled water or coconut oil (using "Dorang" local product). Vortex was used in 2 mins. The dispersion was then centrifuged at 3000 rpm for 30 mins. WHC or OHC was expressed as the weight of water or oil held by 1 g of the sample.

3. Results and discussion

3.1 The effect of XSP proportion on FTIR spectra

Figure 1 shows that 20% XSP meat analog has the highest intensity in the range of 3500 - 3200 cm^{-1} and 1750 – 1250 cm^{-1} which is almost as high as 0% and 40% of XSP, and the lowest intensity is obtained from meat analog with 80% of XSP proportion. These areas represent amide A and B, OH stretch, NH bend, NH_2 bend, ring aromatic stretch, C=C stretch, and C=O stretch. These are the characteristics of FTIR protein spectra (Kong and Yu, 2007). After XSP proportion is varied from 0% - 40%, the interaction in the meat analog matrix will be controlled by protein-protein interaction. When XSP is added to 20%, the highest interaction was obtained.

In the range of 1158 – 1080 cm^{-1}, the highest intensity is obtained from meat analog with 80% of XSP proportion, followed with 20% and 60% of XSP (Figure 1). The lowest intensity is obtained from meat analog with 0% of XSP proportion. These areas represent C-OH stretching in carbohydrate molecule (Huang *et al.*, 2006). The 20% of XSP addition had intensity band almost as high as 60% of XSP.

According to Figure 1, it can be presumed that the interaction between carbohydrate and protein is not a permanent bond, but it is a repulsive interaction wherein the expansion of the protein-protein matrix is supported by the existence of carbohydrate. Starch is distributed into the protein and fibrous matrix is not incorporated into the protein fiber (Noguchi, 1989). The role of carbohydrate is stabilized by the labile hydrophobic interactions in a protein-protein matrix whereas the microscopic work shows that carbohydrate gel acts as the cement in the protein matrix (Coomaraswamy and Flint, 1973). The optimum interaction is obtained when XSP is added to 20% in which the FTIR spectrum has the highest intensity.

There is a shift to the right of the peak in the area of 1158 – 1080 cm^{-1} from the XSP addition of 0% to 40%. Once the XSP addition increased from 40% to 80% peaks, it does not shift. The shifts of absorption bands are probably related to interactions between the functional groups (Bodirlau and Teaca, 2009). The addition of XSP into meat analog matrix is probably shifted the protein-carbohydrate interaction especially in the area of C-OH stretching. It is presumable that the

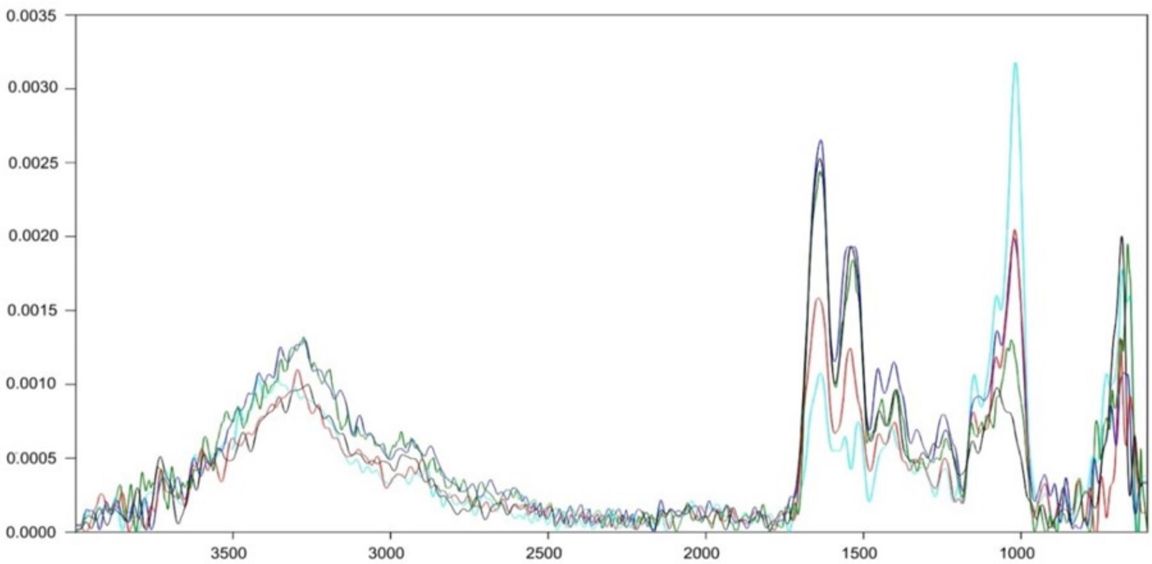

Figure 1. Spectral comparation of meat analog with 120% water addition and a 12-minute mixing time from different levels of XSP proportion (Note: the y axis is absorbance units; the x axis is wavenumber cm^{-1}; --- = 0%; --- = 20%; --- = 40%; --- = 60%; --- = 80%).

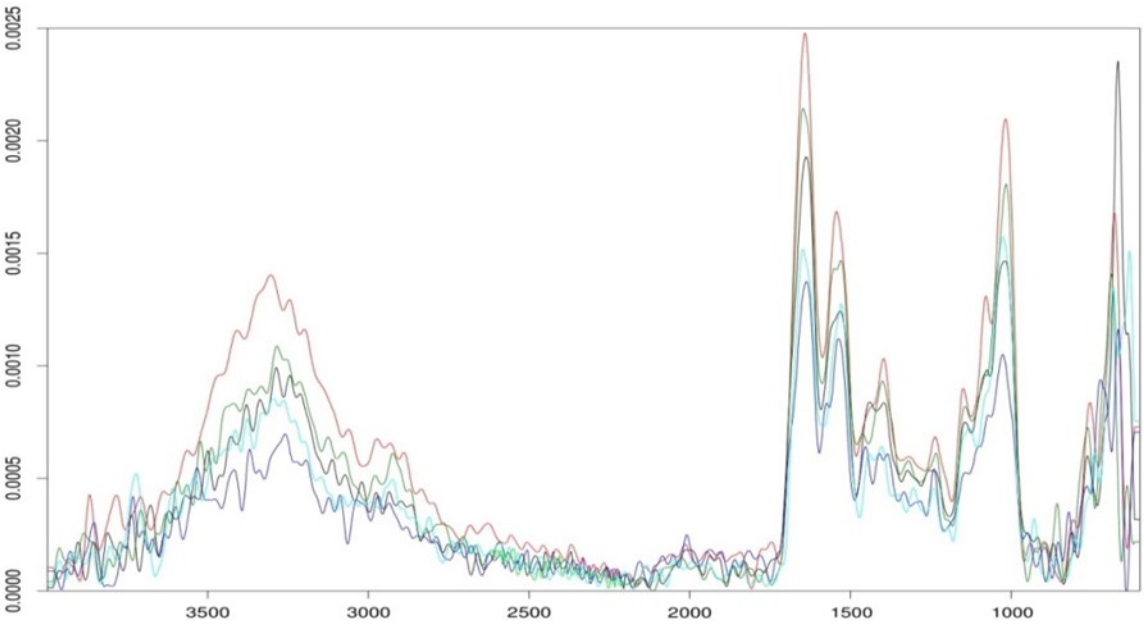

Figure 2. Spectral comparation of meat analog from 30% XSP and 120% water addition with different levels of mixing time (Note: the y axis is absorbance units; the x axis is wavenumber cm-1; --- = 0%; --- = 20%; --- = 40%; --- = 60%; --- = 6 mins; --- = 9 mins; --- = 12 mins; --- = 15 mins; --- = 18 mins).

interaction of carbohydrate-protein effects on C-OH stretch in the carbohydrate molecule.

3.2 The effect of mixing time on FTIR spectra

The increase of mixing time from 6 – 9 mins, enhanced intensity of FTIR spectra as it is clearly shown in the range of 3500 – 3000 cm^{-1} (Figure 2), in the area of OH stretch. Prolonging mixing time from 6 – 9 mins enhances the distribution of water molecules causing an increased level of hydrogen bonding formation. The spectra in the area of 3750 – 2400 cm^{-1} is related to the OH stretching which is responsible for the hydrogen bonding; moreover, the intensity enhancement in these regions is probably related to hydrogen bond formation (Henrique, 2007).

Figure 2 shows that prolonging mixing time from 9 – 18 mins results in the decrease of intensity, probably because there are existing damaged structures of the meat analog matrix when mixing time increased from 9 – 18 mins. The decrease in absorption band intensity is probably related to the reduction of macromolecule interaction. The research in line with this research in which prolonged shear time has resulted in the torque curve trend as the sharp torque presented an increasing and decreasing trend of stress and strain (Manski, 2008; Krintiras, 2015).

3.3 The effect of water addition on FTIR spectra

The FTIR spectra (Figure 3) shows that generally there is increased pattern in the intensity in all areas when water addition levels up. Probably, water does not only promote the interaction of protein-protein, but it also promotes protein-carbohydrate and carbohydrate-carbohydrate interactions. There is no different interaction in the meat analog matrix between different levels of water added as it is presented in Figure 3, where there is no different shape of FTIR spectra with different levels of water additions.

Studying protein-protein interaction of isolated soy protein from extrusion processing points out that although there are different appearances between meat analog produced with low and high-water content, there are no differences in protein-protein interaction contributing to the texturization (Chiang, 2007). An important role of water in meat analog development is in the separation of proteins, which promotes the formation of protein fibrous structure. During extrusion, when protein undergoes a plastic melt, water combined with carbohydrate would act as phase separation which enhances protein interaction (Noguchi, 1989).

3.4 The result of response surface methodology (RSM)

RSM is an important tool to optimize process condition for product improvement. RSM consists of a group of mathematical and statistical procedure which can reduce the number of trials and provide an approach to achieve optimization. The average values of WHC, OHC and texture of meat analog are shown in Table 2.

Multiple regression analysis is applied to the experimental data and the second order of polynomial equation for WHC value as a parameter is as follows (only coefficient with p<0.05 (significant effect) model term are shown; R^2 = 99.3%):

$$Y = 476.003 - 3.950x_1 - 5.29x_2 - 3.056x_3 + 0.033x_1^2 + 0.354x_2^2 + 0.011x_3^2 - 0.053x_1x_2 \quad (1)$$

$$p-\text{values}: 0.000(\text{intercept}); 0.000(x_1); 0.120(x_2); 0.001(x_3); 0.000(x_1^2); 0.021(x_2^2); 0.004(x_3^2); 0.018(x_1x_2)$$

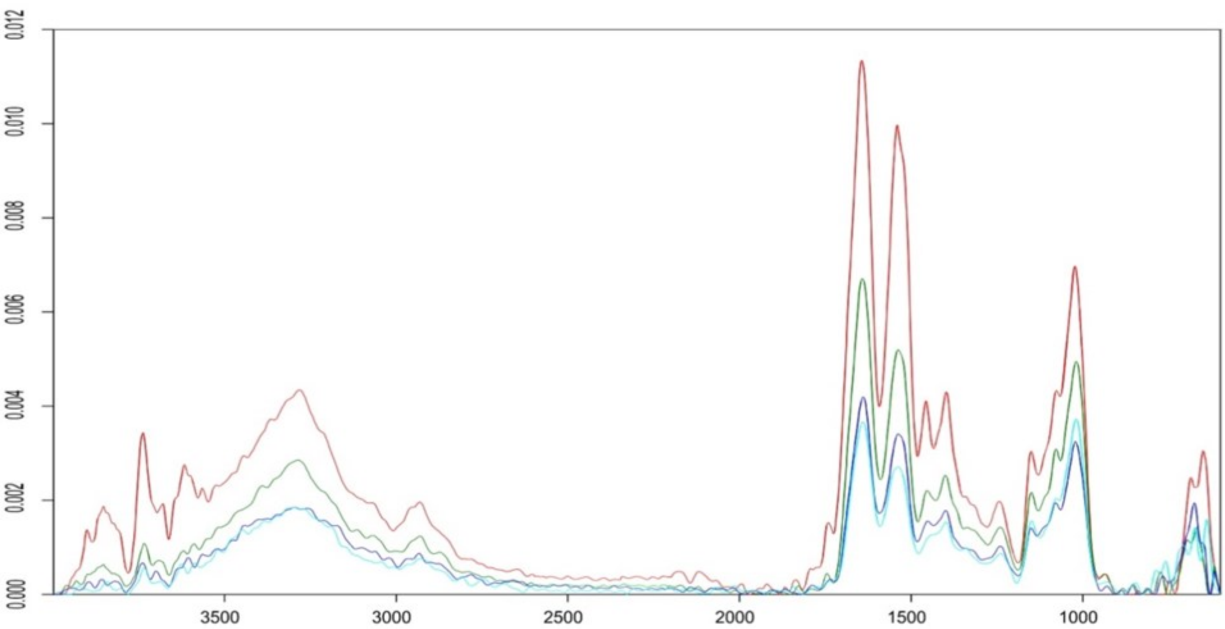

Figure 3. Spectral comparation of meat analog from 30% XSP and 12 mins mixing time with different levels of water addition (Note: the y axis is absorbance units; the x axis is wavenumber cm-1--- 140% ; --- 120% ; --- 100% ; --- 80%).

Where Y is WHC value, x_1 is the variable of XSP proportion, x_2 is the variable of mixing time and, x_3 is variable of water addition.

Table 2. The result of response variables

Samples	WHC (%)	OHC (%)	Texture (gr/mm)
1	289.08±7.36	63.89±1.88	28.9±0.9
2	154.70±6.53	55.00±2.57	50.1±0.3
3	137.89±5.57	41.09±0.88	107.9±1.1
4	115.32±3.42	35.01±0.72	136.9±1.1
5	301.21±9.23	43.49±0.83	12.8±0.6
6	264.17±2.73	50.01±0.99	45.7±0.4
7	92.99±3.47	31.28±1.69	108.8±1.4
8	155.74±7.53	21.04±1.36	134.8±1.1
9	189.67±8.63	43.69±0.66	64.7±1.1
10	182.58±4.83	28.79±0.86	52.1±0.7
11	132.16±6.73	40.63±1.32	99.7±0.9
12	126.76±8.75	41.40±0.76	98.9±0.9
13	256.08±5.78	34.11±1.01	49.9±1.2
14	133.34±5.89	26.09±1.22	162.0±1.2
15	124.49±4.89	32.22±0.66	119.2±0.9

Equation 1 shows that the effect of mixing time on decreasing WHC is greater than XSP proportion and water addition in the linear model terms. Although p-value for x_2 >0.05, it is not eliminated from the model because it is required to support the hierarchy for x_2^2 and x_1x_2 ($p<0.05$). A better understanding of the influence of XSP proportion, mixing time and water addition on WHC value would be described by applying descriptive technique through response surface diagram.

Figure 4a shows that when water is added at constant value of 110%, the effect of XSP proportion is greater than the mixing time, in which an increase of XSP proportion decreases WHC. If the mixing time is set at constant level (12 mins), the effect of XSP proportion is greater than the water addition (Figure 4b). The effect of water addition is greater than the mixing time, it occurs when meat analog is produced at constant XSP proportion (40%) (Figure 4c). The conclusion of the second order of polynomial data and descriptive technique is XSP proportion having the greatest effect on WHC value.

WHC is an important character of fresh meat since it affects the quality of the end product. Polysaccharides are extensively used as ingredients in a number of meat products to obtain desirable binding characteristics, texture and appearance (Perez-Mateos and Montero, 2000). The effect of polysaccharides on WHC has been widely studied. The addition of sodium alginate into pork muscle gel enhances WHC (Cong-Gui *et al.*, 2006). The effect of soy protein concentrate on WHC is better than xanthan gum when added to silver carp surimi (Hasanpour *et al.*, 2012). WHC of proteins is affected by their interaction with carbohydrate (Tolstoguzov, 1997). The result of this research showed that the addition of XSP as a carbohydrate source in meat analog matrix decreased WHC significantly.

The second order of polynomial equation for OHC value as an individual parameter is as follows (only coefficients with $p<0.05$ (significant effect) model term are shown, and $R^2 = 99.7\%$):

$$Y = 5.695 + 0.140x_1 + 0.981x_2 + 0.308x_3 - 0.001x_1^2 - 0.001x_3^2 - 0.022x_1x_2 + 0.009x_2x_3 \quad (2)$$

$$p - \text{values}: 0.274(\text{intercept}); 0.006(x_1); 0.042(x_2); 0.004(x_3); 0.002(x_1^2); 0.003(x_3^2); 0.000(x_1x_3); 0.005(x_2x_3)$$

Where Y is OHC value, x_1 is the variable of XSP proportion, x_2 is the variable of mixing time and, x_3 is variable of water addition.

Equation 2 shows that the effect of mixing time on increasing OHC is greater than water addition and XSP

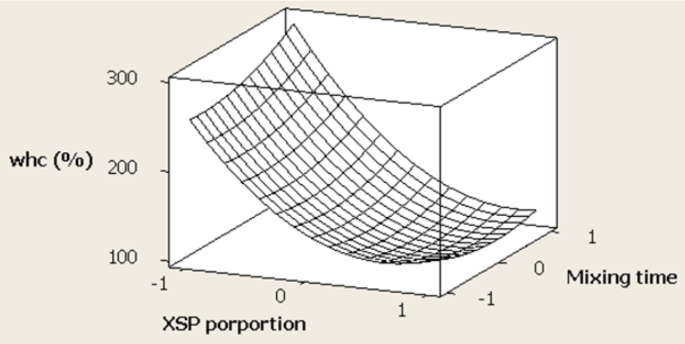

Figure 4a. Surface plot of WHC vs. mixing time; XSP proportion.

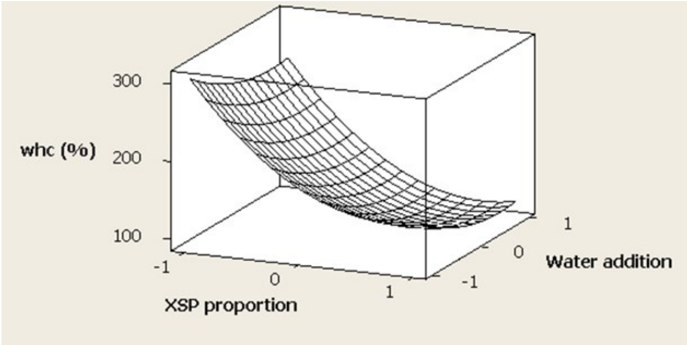

Figure 4b. Surface plot of WHC vs. water addition; XSP proportion.

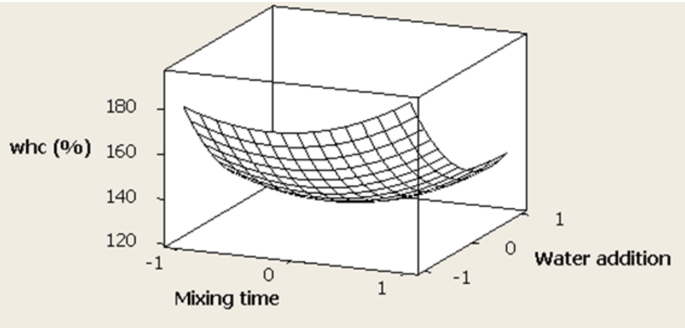

Figure 4c. Surface plot of WHC vs. water addition; mixing time.

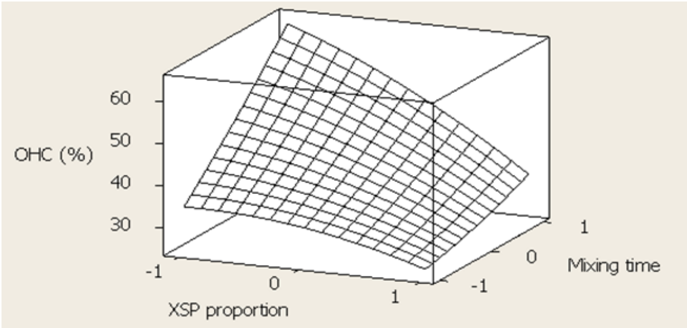

Figure 5a. Surface plot of OHC vs. mixing time; XSP proportion.

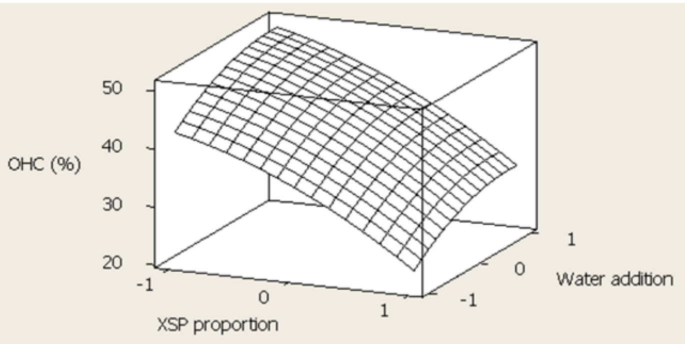

Figure 5b. Surface plot of OHC vs. water addition; XSP proportion.

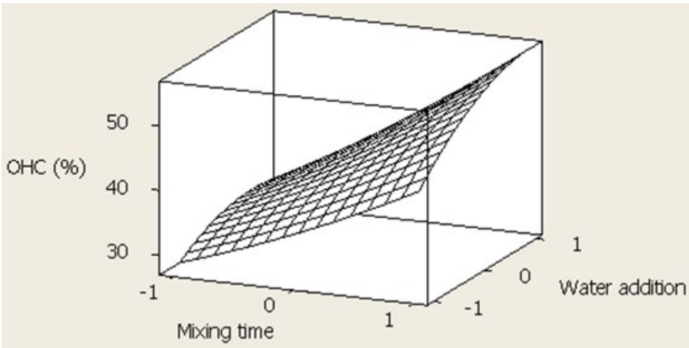

Figure 5c. Surface plot of OHC vs. water addition; mixing time.

proportion in the linear model terms and is also greater than those of other factors. The effect of XSP proportion, mixing time and water addition on OHC is clearly described by applying a descriptive technique through response surface diagram. Figure 5a shows that at constant water addition, prolonging mixing time increases the effect of XSP proportion on leveling down OHC. The enhancement of XSP proportion decreases the effect of mixing time on increasing OHC. If mixing time is set at constant level, the effect of XSP proportion decreases the OHC, but water addition increases the OHC, in which the effect of XSP proportion is greater (Figure 5b). Figure 5c shows that the effects of mixing time and water addition were almost linear in increasing OHC when XSP proportion is constant (40%). This result can be confirmed with Equation 2, in which the term of X_2^2 has been deleted from the equation.

The WHC and OHC are functional properties of the meat which depends on pore size and the charges of molecules. High OHC indicates the enhanced hydrophobic character of proteins, causing fat physically bind with protein by capillary attraction. The protein exposes non-polar amino acids and enhances hydrophobicity as a result in increasing oil absorption (Akinyede and Amoo, 2009). OHC is important properties in meat products like sausages and effects on shelf life (Chen *et al*., 2006). Therefore, meat analog with highest OHC is favorable in sausage formulations where high OHC is required.

Multiple regression analysis is applied to the experimental data and the second order of polynomial equation for texture value as an individual parameter is as follows (only coefficients with $p<0.05$ (significant effect) model term are shown; R^2=97.8%):

$$Y = 283.655 + 2.279x_1 + 7.224x_2 + 4.905x_3 - 0.015x_3^2 - 0.009x_1x_3 - 0.085x_2x_3 \quad (3)$$

$$p-\text{values}: 0.000(\text{intercept}); 0.000(x_1); 0.008(x_2); 0.000(x_3); 0.001(x_3^2); 0.010(x_1x_3);\ 0.002(x_2x_3)$$

Where Y is texture value, x_1 is the variable of XSP proportion, x_2 is the variable of mixing time and, x_3 is variable of water addition.

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Equation 3 shows that the effect of mixing time on increasing texture is greater than water addition and XSP proportion in the linear model terms, but the p-value for X_2 (mixing time) coefficient is greater than 0.05. Therefore, water addition has the greatest influence on texture. The effect of XSP proportion, mixing time and water addition on texture is clearly described by applying descriptive technique through response surface diagram.

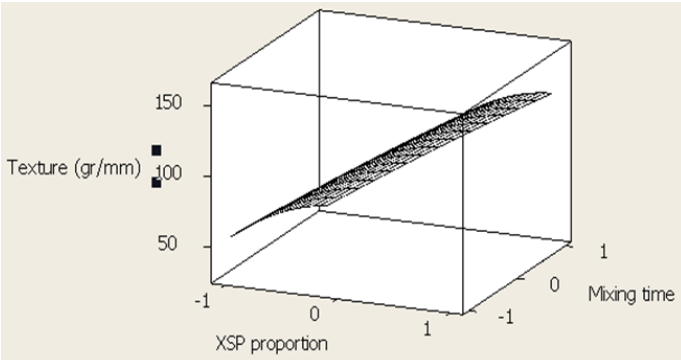

Figure 6a. Surface plot of texture vs. mixing time; XSP proportion.

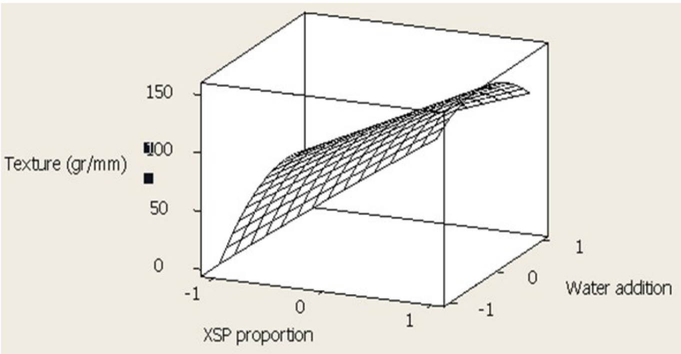

Figure 6b. Surface plot of texture vs. water addition; XSP proportion.

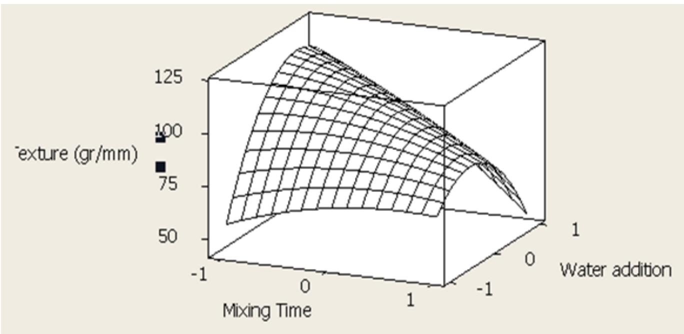

Figure 6c. Surface plot of Texture vs. water addition; mixing time.

Figure 6a and Figure 6b show that the effect of XSP proportion is linear at both constant water addition and mixing time. Equation 3 confirms with the term X_1^2 deletion. Figure 6a and Figure 6c show the same tendency for mixing time when both water addition and XSP proportion are set at constant level**.** Equation 3 confirmed with the term X_2^2 deletion. The effect of water addition was quadratic. It is presented in Equation 3 that there is the term of X_3^2. Figure 6c shows that there is a different effect of mixing time on texture when water is added at different levels. At low water addition, prolonging mixing time slightly increases texture, but at high water addition, the result is in contrast, in which prolonging mixing time decreases texture.

The optimum conditions and predicted responses are presented in Table 3. For convenience for the production process, the optimum conditions are modified to XSP proportion of 30%, water addition of 140% and mixing time of 12 mins. The WHC value of the meat analog produced from this research is highly similar to pork muscle gel from another research conducted by Chen *et al*. (2006) and is lower than Isolated Soy Protein from the research conducted by Kempka *et al.* (2014).

4. Conclusion

Meat analog can be produced from the mixing of concentrated soy protein and *Xanthosoma sagittifolium* powder with the application of household pasta machine. The FTIR spectra show that 20% of XSP proportion, 9 minutes of mixing time and 140% of water addition have the highest absorption intensity. This result is probably related to the interaction of macromolecule-macromolecule and macromolecule-water. XSP proportion, mixing time and water addition become the significant factors affecting on water holding capacity, oil holding capacity and meat analog texture. The XSP proportion has the greatest effect on those three parameters. The optimal operation condition in meat analog production is XSP proportion of 30%, water addition of 140% and mixing time of 12 mins. The characters of the meat analog produced are 145.3542±5.7384% of WHC, 43.7869±7.8725% of OHC and 90.32±0.37 gr/mm of texture.

Conflict of Interest

The authors declare no conflict of interest.

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