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Optimization of soft tofu and ginger drink formula as components of soft tofu dessert using response surface methodology (RSM)

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Abstract

The objective of this research was to optimize formula composition of soft tofu and ginger drink as components of soft tofu dessert using response surface methods. Two factors (independent variables) and three responses (dependent variables) were determined to obtain optimum formula for each component. As many as 16 formulas were delivered from Design-Expert based on the studied factors range. These formulas then were tested one by one by measuring the determined responses. The optimum formula for soft tofu was obtained by combining two factors: 1) soy concentration (0.33 g/mL) and 2) $CaSO_4$ concentration (0.01 g/mL). The factors for ginger drink were 1) ginger concentration (0.23 g/mL) and 2) sugar concentration (0.37 g/mL). These optimum formulas were chosen according to the desirability value of model for soft tofu and ginger drink formulas which were 0.847 and 0.896 respectively. Verification was done by remaking soft tofu and ginger drink using the optimum formulas and remeasuring the responses as well. Responses for soft tofu with optimum formula were hardness (116.80 gf), solid content (11.21%), and protein content (4.78%). Different responses for ginger drink with optimum formula were total phenolic content (0.61 mg GAE/g), reducing sugar content (10.99 mg/g), and total soluble solid (26.73°Brix). These values were closed to the prediction values resulted by the Design-Expert. In conclusion, the model of formula optimization was suitable to produce soft tofu and ginger drink with optimum responses in the studied range.

Introduction

Soft tofu dessert is made from two main components, soft tofu and ginger drink which are offered together as a warm dish. Soft tofu (douhua) is produced by coagulating soymilk using coagulants and the process does not involve any pressing (Shurtleff and Aoyagi, 2013). The coagulation of soft tofu is resulted by gelling formation as a result of aggregation of soy protein. Gelling formation of protein is related to solid content of soy milk and concentration of coagulant (Chang *et al*., 2009). Many types of coagulants usually used for soft tofu preparation, including $CaSO_4$, $CaCl_2$, Glucono-d-lactone (GDL), etc. In this research, $CaSO_4$ was used as coagulant, and it is allowed as food additives with requisition of Good Manufacturing Practises (CAC, 1995, 2008). Shih *et al.* (1997) used Response Surface Methodology (RSM) to optimize soft tofu with soy concentration, coagulant concentration, temperature, and stirring time as the variables. The observed responses was protein content, hardness, brittleness, and elasticity. Ju Chen *et al.* (2005) determined some optimization factors, including the concentration of soy milk and coagulant, and observed hardness as one of the responses. The hardness itself increased with increasing of protein and solid content in the soymilk which reinforced the interactions amongst protein molecules during gelation then forming a stronger network of the gels (Ho Chang *et al*., 2011). Wadikar and Premavalli (2012) also used RSM to optimize formula of ginger drink. The factors were the concentration of ginger, lemon, and sugar whereas the responses were acidity, total solid content, and sensory acceptance. Kumar *et al.* (2014) also determined ginger amount as one of the factors and measured antioxidant acitivity as one of the responses.

RSM is a tool that combines statistical and mathematical technics and widely used in development, improvement, optimization of process, design, and formulation of products (Myers *et al.,* 2009). Mixture design is a part in the RSM that is assigned for designing new formulation. This design

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make possible to determine ideal composition of each variable (factor) of a mixture in order to obtain a product with the best or optimum properties (response) (Granato and Calado, 2014). However, Design-Expert also provides possibilites to not use mixture design in formulation as long as the proportional criteria can not be applied in the experiment. If the components (factors) do not depend on each other, then users can experiment using response surface design rather than mixture design.

According to Yolmeh and Jafari (2017), the application of RSM in various food industries processes involve some steps which influence significantly to its successfull application. Those steps are including appropriate selection of RSM design, independent variables (screening), levels of the factors, and also validity evaluation of the optimum condition that is predicted through RSM.

RSM allows users to optimize process, content (formula), or mixture of both process and content parameter. Khazaei *et al.* (2016) studied optimization of anthocyanin extraction process of saffron petals with RSM. They observed several parameters such as extraction time, extraction temperature, % ethanol used, and solvent ratio. Some studies associated with development food products for example; Tan *et al*. (2015) used central composite response surface design to optimize encapsulation of bitter melon extract and determined concentration and ratio to stock solution as the factors, Kumar *et al.* (2014) developed rich antioxidant-ready to eat food containing Coleus aromaticus using response surface design, Diamante *et al.* (2013) studied the effect of apple juice, blackcurrant, and pectin levels on selected qualities of Apple-Blackcurrant fruit leather, Melo *et al*. (2013) used RSM to optimize peach nectar acceptability and implemented concentration of aspartame and acesulfame-K as the factors, Nadeem *et al*. (2012) developed, characterized, and optimized protein level in date bars using response surface design, formulation and optimization of muffin using composite flour (Purnomo *et al.*, 2012), process optimization of sorghum noodle (Muhandri *et al.,* 2013), formulation of sago noodle substituted with mungbean flour (Yuliani *et al.*, 2015), , optimization of sensory evaluation of soy-based dessert (Granato *et al*., 2010), formula optimization of egg tofu (Murad *et al.*, 2015), optimization of prebiotic and probiotic concentration in the making process of synbiotic yoghurt (Pandey and Mishra, 2015), development of ginger-based appetizer (Wadikar *et al*., 2010), and optimization of ginger tea extract (Makanjuola *et al*., 2015).

Product optimization usually involves multple responses and in RSM there is popular technique regarding optimization of multiple responses by using simultaneous optimization technique (Derringer and Suich, 1980 in Myers *et al.*, 2009). The procedure uses desirability functions by converting each response (yi) into an individual desirability function (di) that varies over the range ($0 \leq di \leq 1$). The value di = 1 means that the response is as its goal or target and when the value di = 0 means the response is outside an acceptable region. Those individual desirability then were made into overall desirability functions ($D = d_1 d_2 ... d_m)^{1/m}$ where there are m responses. Design variables are chosen to maximize the overall desirability in the design optimization.

Studies regarding the optimization of chemical and physical properties for the two components are still unknown. According to Jin He and Qiang Chen (2013), standardization of formula will make an easier way to promote existing or new food products to people. Besides, there are still no basic standard formula for the making of soft tofu dessert. Therefore, the aim of this study was to obtain optimum formula of soft tofu and ginger drink as components of soft tofu dessert and using D-optimal response surface design as the optimization tool.

Materials and methods

The materials used as soft tofu formula were: Genetic Modified Organism (GMO) soybean (Bola brand) obtained from Gerbang Cahaya Utama-Rumah Tempe Indonesia, $CaSO_4$ obtained from SGP-Setia Guna, and drinking water (Aqua, Danone). Ginger drink was made using red ginger rhizome (*Zingiber officinale* var. Rubrum or JAHIRA 2 variety) obtained from Balittro, Bogor, red palm sugar (Alfamidi), white cane sugar (Gulaku), and drinking water (Aqua, Danone).

Making process of soft tofu and ginger drink

Soft tofu was made by soaking the soybean (500 g) in water for 6 hours then were washed and peeled to remove the skin. Afterwards, it was ground for 1 minute by adding 1.5 L of drinking water and filtered using white cloth to obtain soymilk. The soymilk was boiled (90°C, 10 minutes) and removed its foams. Later, it was poured into a container containing $CaSO_4$ with concentration of 1.5 g/L of soymilk. Furthermore, this mixture was placed at room temperature until the soft tofu was formed.

Ginger rhizome was washed to make it clean from soil and other wastes. The rhizome was grated without any peeling of the skin. As many as 750 g of grated ginger was added with 5 L of drinking water,

Table 1. Design matrix of soft tofu and ginger drink formula

Formula	Soft tofu: Soy concentration (g/mL)	Soft tofu: $CaSO_4$ concentration (g/mL)	Ginger drink: Ginger concentration (g/mL)	Ginger drink: Sugar concentration (g/mL)
1	0.2000	0.0100	0.3000	0.3900
2	0.1400	0.0099	0.0500	1.0000
3	0.3300	0.0069	0.0500	1.0000
4	0.2600	0.0093	0.1300	0.2700
5	0.1400	0.0079	0.3000	0.3900
6	0.3300	0.0100	0.0500	0.1700
7	0.2400	0.0035	0.2100	0.1700
8	0.1800	0.0059	0.3000	1.0000
9	0.1400	0.0035	0.2100	0.4800
10	0.2500	0.0063	0.0500	0.1700
11	0.1400	0.0099	0.1700	1.0000
12	0.3300	0.0035	0.2500	0.6900
13	0.3300	0.0100	0.1600	0.6300
14	0.3300	0.0035	0.2100	0.1700
15	0.1400	0.0035	0.3000	1.0000
16	0.3300	0.0069	0.0500	0.5800

Table 3. Goal criteria and importance for each optimization variables of soft tofu and ginger drink

Samples	Variables	Goal	Lower	Upper	Importance
Soft tofu	Soybean concentration	In range	0.143	0.33	+++
	$CaSO_4$ concentration	In range	0.0035	0.010	+++
	Hardness	Maximize	21.13	118.50	+++
	Solid content	Maximize	0.06	0.11	+++
	Protein content	Maximize	2.50	6.10	+++
Ginger drink	Ginger concentration	In range	0.05	0.30	+++
	Sugar concentration	In range	0.17	1.00	+++
	Total phenolic content	Maximize	0.22	0.39	+++
	Reducing sugar content	Target = 10	5.23	19.68	+++
	Total soluble solid	Minimize	13.70	52.67	+++

red palm sugar (750 g), and white cane sugar (1750 g). This blended formula then was boiled (90°C, 10 minutes) and filtered using white cloth to obtain ginger drink.

Design and optimization of soft tofu and ginger drink formula

Formula optimization was started by determining lower and upper limit value for the variables (factors) which were the concentration of soybean and $CaSO_4$ for soft tofu formula and the concentration of ginger and sugar for ginger drink formula. The sugar concentration was a combination of red palm sugar and white cane sugar in ratio of 3:7. The limit values were obtained by trial and error process. Based on the trial and error process, we found that the chosen limit value for soybean concentration was ranged from 0.10 – 0.50g/mL, whereas for $CaSO_4$ concentration was 0.001 – 0.016 g/mL. For ginger drink formula, the limit value for ginger concentration was ranged from 0.05 – 0.75 g/mL, whereas for sugar concentration was ranged from 0.17 – 2.50 g/mL. These ranges then was input into the Design-Expert to be analysed for further steps.

Design optimization was conducted by using Design-Expert® ver. 8.0.1 software (trial version). The range of soybean concentration and $CaSO_4$ concentration was entered as the optimization factor for soft tofu formula whereas the range of ginger concentration and sugar concentration was the factor for ginger drink formula. The ranges can be referred to Table 3. Those values of the factor concentration in Table 1 or 3 were a result of constraint optimization provided by Design-Expert. After inputting the values, Design-Expert issued as many as 16 design formulas for each component. All of the formulas were made appropriately according to the output concentrations and run in order. The soft tofu formulas were measured for three functional properties as the responses, including hardness, solid content, and protein content. While total phenolic content, reducing sugar content, and total soluble solid were measured as the response for ginger drink formulas. The design matrix for soft tofu and ginger drink formula were presented in Table 1.

Textural analyses of soft tofu hardness

Soft tofu hardness was measured according to Ho Chang *et al.* (2011) by using texture analyser TA-XT2 instrument (Stable Micro Systems). Hardness point was the peak value of the graphic resulted from measurement when the probe's instrument pressed the soft tofu. Soft tofu was made in the container with its dimension of 15 x 10 x 5 cm. Soft tofu was prepared until the height reached 3 cm from the bottom of the container. The setting of textural analyses was pre-test speed (2 mm/s), test speed (2 mm/s), post-test speed (5 mm/s), rupture test distance (1%), distance (50%), force (205 g), time (5 s), count (5), trigger type (auto 5 g), probe type (5 mm), and probe height (45 mm).

Solid content of soft tofu

The solid content was obtained by measuring water content of each soft tofu formula using gravimetric method (AOAC, 2012). Solid content is a difference between 100% value with the obtained water content value.

Protein content of soft tofu

Protein content was analysed using Kjeldahl method (AOAC, 2012) based on destruction, distillation, and titration of samples. Protein content is a multiplication of % nitrogen and a conversion factor (6.25).

Total phenolic content of ginger drink

The phenolic content was measured according to Maizura *et al.* (2011) by using Folin-Ciocalteau reagent. As many as 75 µL was reacted with 5 mL Folin-Ciocalteau reagent. After 5 minutes, 4 mL Na_2CO_3 (7.5%) was mixed with sample. The mixture was placed at room temperature for 2 hours. A series of standard solution were made using gallic acid with different concentrations (100, 200, 400, 600, 800, and 1000 mg/g) to obtain a standard curve. Absorbance value was measured at 765 nm using spectrophotometer (UV-Vis, Thermo Scientific, Genesys 20). Total phenolic content was stated as mg GAE/g of sample.

Reducing sugar content of ginger drink

Reducing sugar was measured according to Gusakov *et al*. (2011). A DNS solution was prepared to measure the content of reducing sugar. A series of glucose solution was made with different concentration (100, 200, 400, 600, 800, and 1000 mg/g). Sample (600 µL) was reacted with DNS (400 µL) then boiled at 99.9°C for 10 minutes. A total of 5mL mixture solution was made by adding 4mL of distilled water. The solution was stirred with vortex and measured the absorbance value at 540nm using spectrophotometer (UV-Vis, Thermo Scientific, Genesys 20). Reducing sugar content was stated as mg glucose/g of sample.

Total soluble solid of ginger drink

Total soluble solid was measured according to Wadikar and Premavalli (2012). A table refractometer (Spectronic Instruments) was used to measure the total soluble solid of ginger drink. As many as three drops of ginger drink were put into the objective glass of the instrument. Total soluble solid was stated as degree of Brix (°Brix).

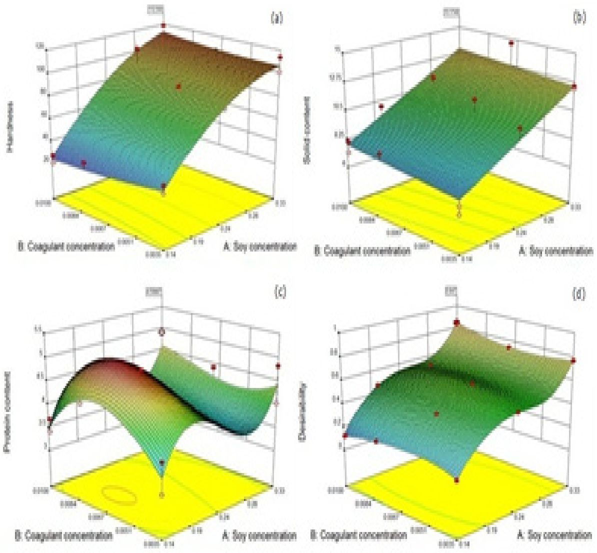

Figure 1. Optimization Response of soft tofu formula; (a) hardness, (b) solid content, (c) protein content, and (d) desirability of soft tofu optimum formula

Proximate analysis of both formulas

The optimum products were analysed to obtain the moisture content (gravimetric method), ash (gravimetric method), fat (soxhlet), protein (kjeldahl method), and carbohydrate content (by difference) (AOAC 2012).

Results

Optimum formula of soft tofu

The optimum model is presented at Table 2. Figure 1 (a, b, c) shows three dimensional views of the three observed responses. Desirability value of the optimum formula was 0.847. It means that the higher the desirability value, the more suitable the model to predict formula which results in the acquired optimum response. Figure 1(d) represent a three dimensional graphic for desirability value of soft tofu optimum formula.

A combination between 0.33 g/mL of soybean concentration and 0.010 g/mL of $CaSO_4$ concentration was proved to be an optimum formula to make soft tofu in this study. A verification process was conducted to confirm that the predicted value resulted from Design-Expert was fitted with the later verification value. Hardness value and protein content were in the range of 95% CI, whereas solid content was in the range of 95% PI. These values approached to the predicted value that resulted from Design-Expert. This means that the model was suitable to determine soft tofu formula in the studied ranges.

Table 2. Responses of soft tofu and ginger drink formula produced by using response surface design (Design-Expert)

Samples	Parameter	p-value Model	p-value Lack of Fit	Adjusted R^2	Predicted R^2	Adequate Precision	Equation
Soft tofu	Hardness	<0.0001 (sig)	0.2854 (n sig)	0.9482	0.9165	17.631 (>4,0)	Hardness = +82.54 + 39.60A - 1.56B + 4.47AB - 14.44A^2 + 1.97B^2
	Solid content	<0.0001 (sig)	0.7210 (n sig)	0.8518	0.8096	14.368 (>4,0)	Solid Content = +10.16 + 2.66A + 0.36B
	Protein content	0.0061 (sig)	0.9766 (n sig)	0.6465	0.4309	7.992 (>4,0)	Protein Content = +4.35 - 1.57A + 0.083B - 0.35B^2 + 0.66AB^2 + 1.44A^4
Ginger drink	Total phenolic content	0.0002 (sig)	0.2710 (n sig)	0.8218	0.7042	11.171 (>4.0)	Total Phenolic Content = +0.60 + 0.071C + 0.17D + 0.053CD - 0.11C^2 -0.048D^2
	Reducing sugar content	<0.0001 (sig)	0.1593 (n sig)	0.9506	0.9212	19.267 (>4.0)	Reducing Sugar Content = +13.79 - 0.052C + 6.33D + 0.17CD - 1.06C^2 - 0.95D^2
	Total soluble solid	<0.0001 (sig)	0.3514 (n sig)	0.9826	0.9706	32.057 (>4.0)	Total Soluble Solid = +35.87 - 2.05C + 17.56D + 0.48CD - 0.85C^2 - 3.76D^2

Note: A = soybean concentration (g/mL), B = $CaSO_4$ concentration (g/L), C = ginger concentration (g/mL), D = sugar concentration (g/mL), sig = significant (α=0.05), n sig = not significant (α=0.05)

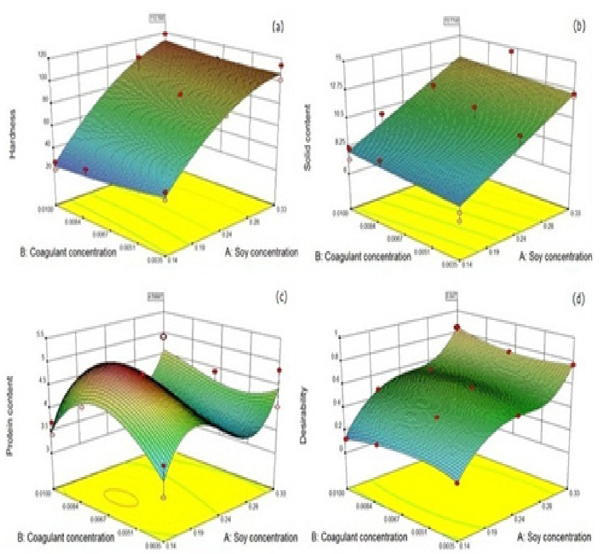

Figure 1. Optimization Response of soft tofu formula; (a) hardness, (b) solid content, (c) protein content, and (d) desirability of soft tofu optimum formula

Optimum formula of ginger drink

The optimum formula of ginger drink was made by combining ginger concentration (0.23 g/mL) and sugar concentration (0.37 g/mL). A ratio of 3:7 was determined as the composition of red palm sugar and white cane sugar used in this study. The composition of these two sugars was 0.11g/mL and 0.26 g/mL for red palm sugar and white cane sugar respectively. The model of optimum formula to produce optimum response was presented at Table 2. Figure 2 (a, b, c) was three dimensional graphic which shows the three observed responses in the optimization of ginger drink formula. The desirability value of the optimum formula was 0.896 (Figure 2(d)).

Comparison between prediction and verification value of ginger drink optimum formula had been done as well. Total phenolic content and total soluble solid were in the range of 95% PI, whereas reducing sugar content was in the range of 95% CI. The near value between predicted and verified value was like the previous soft tofu formula. This means the model of ginger drink formula was suitable to determine the optimum response in the studied ranges.

Nutrition content of both optimum formulas

Soft tofu and ginger drink which were produced using the optimum formula had nutritive values supported by conducting analysis for moisture, ash, protein, fat, and carbohydrate content.

Discussion

Optimum formula of soft tofu

Different models were obtained for each parameters or responses in this study (Table 2). According to the p-value, all models were significant ($p<0.05$). The lack of fits were not significant, the adequate precisions were more than 4.0, and the coefficient of determinations for hardness and solid content responses closed to 1.00. However, protein content had low R square values which were 0.6465 (adjusted R^2) and 0.4309 (predicted R^2). The same result had been observed by Kumar *et al.* (2014) who found the adjusted and predicted R square values of vitamin C response were 0.5547 and 0.4206 respectively, for optimization of ready to eat appetizer. Another study (Purnomo *et al.*, 2012) also found low R square values for taste (0.3612 and 0.1342), texture (0.4035 and 0.2405), and for overall responses (0.6848 and 0.5669). Based on the observed values the models could describe the measured responses. All responses were then optimized by determining desired goal and importance level of the variable as indicated in Table 3. The importance level was determined as default (+++) for all responses. Optimum formula obtained from optimization of hardness, solid content, and protein content of soft tofu was 0.33 g/mL (soy concentration) and 0.01 g/mL ($CaSO_4$ concentration). Desirability value of optimum formula was 0.847. The higher desirability value indicated the high suitability of formula to achieve the desired responses. In this study, 84.70% of the desired responses could be achieve by the optimum formula.

Hardness is one of textural properties of soft tofu that had been observed in this study. Soft tofu hardness can be affected by total soluble solid of the soymilk. A higher value of total soluble solids has positive correlation with the hardness of tofu. A study performed by Rekha and Vijayalakshmi (2013) found that to produce a softer texture of tofu, the soymilk needed total soluble solid about 7°Brix, whereas for harder texture it needed about 9°Brix. This study used soymilk which contained about 7.67°Brix. So, the soluble solid content of the soymilk would result in soft texture to the tofu. Textural properties of tofu are affected by its gelling formation. Gelling formation are influenced by soy protein interaction with coagulant and other components, such as phytic acid. During the formation, intermolecular interaction of the soy protein can be decelerated by the role of coagulant. Consequently, it will result in a more homogenous structure of the tofu. This homogenous structure has an impact for the tofu's strength so that it will not easily perform syneresis (Seog *et al.*, 2008). Increasing of gelation rate also correlated with increasing of coagulants concentration, but decreased with increasing of solid content of soymilk. The hardness value increased with increasing solids content and coagulant concentration as well (Ho Chang *et al.*, 2011). There were three categories of tofu's hardness reported by Midayanto and Yuwono (2014) which were soft (3 – 5N), elastic (5 – 7N), and hard (7 – 9N).

The optimum soft tofu had a hardness value of 116.80gf which was still in the range of 95% CI. Design-Expert predicted a near value which was 112.57 gf, so that the hardness properties had been rightly verified. The hardness value was equal to 1.15N so that it was still below the category of soft hardness according to Midayanto and Yuwono (2014). In addition, Yuan and Chang (2010) also found that hardness was significantly influenced by distance or probe penetration of texture analyser instrument (50 and 75%). In this study, the distance was set into 50%.

Hardness was affected by soy concentration significantly compared to $CaSO_4$ concentration (Figure 1(a)). In other words, $CaSO_4$ concentration did not give significant influence to the hardness in this study ($p>0.05$). The equation in Table 2 showed that the coefficient of soy concentration variable (A) was higher than the coefficient of $CaSO_4$ concentration variable (B). The using of $CaSO_4$ with different concentration (0.3 – 0.5% w/v) did not show any significant differences between the observed hardness value of tofu (Jayasena *et al.*, 2014). Ho-Chang *et al.* (2011) found that the solid content of soymilk produced a steeper slope then the coagulant concentration in the graphic explaining its relation to hardness. Another study showed that addition of $CaSO_4$ did not produce tofu with increasing hardness as well. The role of phytic acid content was also responsible for the tofu hardness. If tofu was produced from soymilk that did not contain or had less phytic acid, the texture of tofu would be very soft and brittle. This condition resulted to the non significant texture altough the $CaSO_4$ concentration was increasing (Setyono, 1994).

Solid content and coagulant concentration were known to affect yield and protein content of tofu. Solid content would influence tofu hardness as well. In this study, solid content was mainly controlled by soy concentration and only increased slightly by influence of $CaSO_4$ concentration in this study (Figure 1(b)). A higher coefficient of soy concentration was also found in model equation (Table 2) for solid content. Benassi *et al.* (2011) measured hardness, yield, solid content, and protein content as quality parameters of tofu. The using of $CaSO_4$ (2%) resulted in hardness (4%), protein content (12%), and solid content (22%). Another study found that 1% of $CaSO_4$ resulted to the best tofu appearance which had soft, compact, and not very hard texture (Kanlayakrit and Phromsak, 2014). Prabhakaran *et al.* (2006) used 0.4% $CaSO_4$ which performed not only stronger but also softer texture compared to other coagulants. Other study that used another coagulant such as $CaCl_2$ (0.3 – 0.5%) found an increasing in hardness parameter as well (Leiva *et al.*, 2011). However, soy concentration (0.33 g/mL) acted to have more influence in case of solid content in this study. Soy concentration or water-to-soybean ratio generally was adjusted to maintain total solids content in tofu production. In other words, the amount of water added to produce soymilk would affect the solids content of soft tofu, including its dry matter (protein, fat, ash and carbohydrate) content. This condition would show further consequences to physical and chemical properties of soft tofu.

Layer (skin) sometime resulted on the top of tofu during the making process. Yuan and Chang (2007) discovered that the skin appeared as an effect of high pressure applied during the making of tofu. It has reported that tofu without skin had protein content ranged from 4.40 – 7.50%, whereas tofu produced with skin had 7.20 – 12.10%. Another study stated that protein content of tofu varied from 3.33 – 4.96% (Midayanto and Yuwono, 2014). The optimum soft tofu contained 4.78% protein which was slightly difference with the predicted value (4.54%). This value was in the range of 95% CI and proved to be rightly verified. The differences of protein content could be influenced by soybean variety, soaking time, pressure condition, and coagulant type. In general, soybean which had higher protein contained lower fat and sugar content. However, higher protein content did not show any positive correlation with a better value of hardness (Yuan and Chang, 2010). Because protein content is related with gelling formation of soft tofu, it will have an impact of hardness result.

Protein content was influenced by soy concentration in a type of cubic curve, wheras coagulant concentration show a parabolic effect to protein content (Figure 1(c)). When it was referred to its perturbation plot in the Design-Expert software, the changes of soy concentration would result in the wide range of protein content changes, but no such effect for the changes of $CaSO_4$ concentration. A steep slope or curvature in a factor showed that the response was sensitive to that factor. Therefore, in this model, protein content was sensitive to soy concentration.

Optimum formula of ginger drink

All parameters showed quadratic model with p-value less than 0.05 (significant). Optimization was conducted by applying desired goal and importance level as indicated in Table 3. The importance level was determined as default (+++) for all responses. Optimum formula obtained from optimization of total phenolic content, reducing sugar content, and total soluble solids was 0.23 g/mL (ginger concentration) and 0.37 g/mL (sugar concentration). In this study, desirability value of ginger drink optimum formula was 0.896. It indicated that the optimum formula could achieve 89.60% of the desired responses in this study.

Destandau *et al*. (2013) in Makanjuola *et al*. (2015) described that total phenolic content was not increasing always along with the increasing of ginger concentration. An increasing of total phenolic content appeared at 0.17 – 0.24 mg/mL of ginger concentration. However, it tent to decrease if more than those level. This decline was caused by a rising of solid content compared to its extraction solvent which later resulted a fewer of available surface for the solvent to enter the substrate and solve the target molecule. The phenolic content of ginger rhizome itself was known to be about 8.38 – 8.40 mg/g ginger. In addition, an extraction process using hot water (100°C) resulted in higher polyphenol content compared to normal water (30°C) and other solvents (Shirin and Jamuna, 2010). Kishk and Sheshetawy (2010) obtained optimum formula and process by using 0.72% ginger and 56.12°C as extraction temperature. The use of ginger more than 0.72% was known to potentially have prooxidant effect. Heating process such as roasting (± 320°C) and boiling (± 100°C) showed total phenolic content up to 29.94 and 30.87 mg/g respectively during 2 – 6 minutes of heating (Purnomo *et al.*, 2010). Heating process helped to release antioxidative substances from plant cells by degrading the cell wall. Some of ginger glycoside substances could be hydrolised to form aglycon substances by heating process. Aglycon was known to have higher antioxidant activity compared to its glycoside form. The more glycosidic bond which could be degraded will also result in the higher reducing sugar content (Rahmawati and Yunianta, 2015). An addition of sugar and ginger into a traditional drink of Nigeria (Zobo) could increase its total phenolic, flavonoid, and vitamin C content significantly (Oboh and Okhai, 2012).

Sugar concentration has more influence to total phenolic content compared to ginger concentration (Figure 2(a)). This condition might be caused by the higher concentration of sugar (0.37 g/mL) compared to ginger concentration (0.23 g/mL). Addition of sucrose increased total phenolic content in Secang drink, a traditional beverage from Indonesia, as well. It was expected that Folin Ciocalteau method could be interfered by the presence of sugar molecule, in this case sucrose, which could be reduced by tungsten and molybdenum in the formation of methal oxide. Therefore, it affected the absorbance value (Zulfahmi and Nirmagustina, 2012). Other research also showed the phenolic content of cane sugar (Nayaka *et al*., 2009), glucose and mannose (Haghparast *et al*., 2013).

Reducing sugar was mainly controlled by sugar concentration rather than ginger concentration (Figure 2(b)). A targeted reducing sugar content was determined in this study as 10 mg/g. It was based on the reducing sugar content of palm sugar according to Indonesian National Standard (SNI, 1995) about quality requisition of palm sugar. Reducing sugar content was measured because it showed influence to antioxidant content and activity of the samples.

Reducing sugar was known to have an effect of anthocyanine degradation rate during heating, as a result of phurphural ring and 5-(hydroximethyl) phurphural formation (Cisse *et al*., 2009 in Krishna *et al.*, 2014). In other study, reducing sugar with six carbon (hexose) such as D-glucose and D-mannose had higher antioxidant activity compared to reducing sugar with five carbon atoms (pentose) such as D-arabinose (Haghparast *et al*., 2013). A correlation between sugar and phenolic content had been studied as well. Two types of cane sugar which was jaggery (traditional cane sugar) and brown sugar showed different content of phenolic substance. Jaggery showed a ten fold higher phenolic content than brown sugar which was 3837 and 372 µg GAE/g respectively. The least phenolic content was found in white cane sugar (sucrose) and refined sugar which was 31.5 and 26.5 µg GAE/g respectively (Nayaka *et al.*, 2009). The combination of palm sugar and white cane sugar was giving benefit in this optimization study. White cane sugar (sucrose) was not a reducing sugar and known to not have any antioxidative effect. However, it had been proved to increase consumer acceptance trough altering sensory attributes of food products. The use of sucrose significantly affected consumer acceptance, especially for sweetness, richness, and smoothness attribute (Wangcharoen, 2012). Meanwhile, palm sugar contain reducing sugar so it had an antioxidative effect. This properties was a consequence of Maillard reaction product, caramelization product, and phenolic acid inside (Naknean and Meenune, 2011).

Total soluble solids was also affected by sugar significantly compared to ginger (Figure 2(c)). Some studies showed that total soluble solid of ginger drink was affected by the sugar and ginger concentration respectively (Wadikar and Premavalli, 2012). An addition of ginger extract into bit drink decreased the total soluble solid but on the contrary with addition of sucrose (Chasparinda *et al*., 2014). There was a positive correlation between oleoresin content with the total soluble solids and reducing sugar content (Eleazu *et al*., 2012).

Nutrition content of the two optimum formulas

The nutritional content was obtained by implementing proximate analysis of the two optimum products. Soft tofu had 88.82% moisture content which was obtained from soybean:water ratio of 1:3 or 0.33 g/mL. The protein content was about 4.84% which approached the predicted and verification value of protein content. According to Midayanto and Yuwono (2014), various tofu products contained 78.82 – 85.27% of moisture content, whereas the protein content could be about 3.33 – 4.96%. Fat, carbohydrate, and ash content were 3.60, 1.48, and 1.26% respectively.

Ginger drink composition contain two types of sugars, thereby resulting in higher carbohydrate content (19.72%). This beverage also contained at about 27.62% of total solids, since it had 72.38% of moisture content. This high content of total solids came from the use of two kinds of sugars (palm and cane) and from the ginger as well. Smaller content of total solids was observed by Mayani *et al.* (2014), since it used only sucrose and lower ginger concentration (0.1%). Fat content reached 7.05% which was considered as a result from fat content of ginger rhizome, its oleoresin substance, and also from the sugar especially palm sugar. It has been reported that ginger rhizome could contain about 5.62% of crude fat (Odebunmi *et al*., 2009) and could reach 7%. It also contained 5-10% of oleoresin (Eze and Agbo, 2011). Meanwhile, the addition of palm sugar could increase the fat content of ginger drink. Palm sugar had 0.20-0.34% of fat reported by Radam *et al.* (2014), another research reported 0.09% by Yulianingsih and Yuwono (2015), and study performed by Kurniasari and Yuwono (2015) showed the fat content of coconut sugar reached 1.30%. Red ginger consisted mainly oleoresin substances such as gingerol and shogaol which gave the pungent properties. The structure of gingerol and shogaol has a phenyl group and a long chain structure, which resulted in their hydrophobic characteristic.

Conclusion

This study resulted two optimum formulas for soft tofu and ginger drink as the components of soft tofu dessert. A combination of soy (0.33 g/mL) and $CaSO_4$ (0.01 g/mL) concentration resulted an optimum formula of soft tofu. Consequently, this formula would produced soft tofu with optimum functional properties including hardness (116.80 gf), solid content (11.21%), and protein content (4.78%). Three functional properties of ginger drink were measured as results from a combination of ginger (0.23 g/mL) and sugar (0.37 g/mL) concentration. These properties included total phenolic content (0.61 mg GAE/g), reducing sugar content (10.99 mg glucose/g), and total soluble solid (26.73°Brix). Both of the optimum formulas had desirability values 0.847 and 0.896 respectively for soft tofu and ginger drink. This value was near the highest value (more than 0.800) which were means that the suitability of the model was more desirable.

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