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Formula Optimization and Physicochemical Characterization of Tempe Drink Powder

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Abstract

Tempe is chosen as the main ingredient of tempe drink powder (TDP) due to its protein digestibility, phytochemical compounds, as well as vitamins and minerals. Previous studies had been conducted to develop TDP formula. The commercial TDP formula showed that improvement of quality aspects needs to be done so the product has better physical and chemical characteristics. In order to optimize the TDP formula, the viscosity, water solubility index (WSI), water absorption index (WAI), sedimentation index (SI), proximate, antioxidant activity, isoflavone content, GABA content, and physicochemical properties were analyzed. The optimized formula was done using the mixture experiment optimization method with optimization d-optimal to obtain the best formula. The optimization result showed that the best formula proportion consisted of 70% (w/w)Tempe flour, 18.23% (w/w) maltodextrin and 1.77% (w/w)guar gum. The best formula was chosen due to having better chemical characteristics compared with the commercial TDP and commercial soy drink powder (SDP), with protein content of 42.61%, antioxidant activity of 58.36 mgAEAC/100g, daidzein and genistein isoflavones of each 48.18 and 140.06 mg/100g and GABA of 21.24 mg/g. Based on the physical characteristics, the optimum formula had a lower viscosity value (18.67 cP) and WAI (2.58g/g) as well as a higher SI value (10.18%) and WSI (9.70%) compared with the commercial TDP. The optimum TDP formula has fulfilled the quality requirements based on the Indonesian National Standard (SNI 7612:2011) regarding soy drink powder.

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Introduction

Tempe is one of the Indonesian food products that is made from synergetic fermentation of soybean with the help of mold (*Rhizopus* spp) and lactic acid bacteria.[1] Compared with soybean, Tempe has a higher protein and carbohydrate digestibility. During the fermentation process, the occurrence of enzymatic hydrolysis helps in breaking down the complex compounds into simpler, easily digested and absorbed compounds.[2,3] During the fermentation process, the phytic acid that exists in the soybean is reduced by the phytase enzyme secreted by the mold.[4] The protein is hydrolyzed into short-chain peptides and free amino acids by protease enzyme, while the carbohydrate is hydrolyzed onto shorter saccharide compounds by amylase enzyme and the fat is hydrolyzed into free fatty acids[5] In addition, there are several vitamin contents, specifically higher vitamin B in Tempe compared with soybean. The vitamins which are commonly found in Tempe are Vitamin B1, Vitamin B2, pantothenic acid, niacin, vitamin B6 and vitamin B12.[5–7]

One of the most potential processed Tempe products is Tempe drink powder (TDP). Processing Tempe into TDP can provide an alternative way to consume Tempe,[8] since Tempe is commonly consumed as food. TDP can be one of the innovations in soy-based drinks that are being heavily promoted as a health beverage. Several studies have shown that consuming Tempe drink powder three glasses/day for 4 weeks consecutively will reduce blood LDL levels significantly in hypercholesterolemia subjects,[8] reduce blood pressure in hypertension patients[9] and reduce blood glucose in diabetes patients.[10,11] Tempe flour consumption is also shown to increase estrogen serum[12] and prevent osteoporosis.[13,14]

Several studies had successfully processed Tempe juice and fresh Tempe into TDP product.[15–17] The Formula of fresh Tempe-based TDP has been commercially traded and patented.[18] However, commercial TDP from the patented formula has a large serving size to achieve the desired protein content and does not have good physical characteristics, thus, it is least preferred by the consumers. Consequently, commercial TDP formula still needs to be improved in terms of its physical and chemical characteristic aspects.

In this study, the TDP was made with Tempe flour as the key ingredient. Tempe flour had high calcium bioavailability and was even similar to casein, a protein found in milk, suggesting it has the potential to be used as an alternative calcium source aside from animal products like as dairy products.[13]. Tempe flour is also able to increase estrogen serum better than tofu flour, casein and estradiol, so it has the potential to reduce the risk of cardiovascular disease.[12] Therefore, Tempe flour can be further processed into a functional beverage.

Mixture design or mixture experiment (ME) is commonly used to design a formula optimization experiment since it saves time, cost and energy. ME is an experiment design method that is used to combine several ingredients composition to produce a final product with desired characteristics.[19] The utilization of ME for optimization of TDP formula was expected to obtain the most optimum TDP formula with improved physical and chemical characteristics compared with the commercial TDP and other similar commercial products

Materials and Methods
Material

The main ingredient used for this study was fresh Tempe made from soybean obtained from Rumah Tempe Indonesia (RTI), located in Cilendek, Bogor. Other ingredients consisted of guar gum, maltodextrin (DE value of 10 – 12), low-calorie sweetener (combination of sorbitol, erythritol and steviol glycoside) and vanilla identical flavor which were purchased from online marketplace at Zeelandia official store.

Tempe Flour production

Tempe was initially sliced to a thickness less than 0.5 cm using a slicer (Geprufte Sicherheit, Alexanderwerk Inc., Mongomeryville, Pennsylvannia). Then, the Tempe was steam blanched using steamer (Armfield® UOP 10) at 100°C for 10 minutes. Then, the Tempe was dried using a fluidized bed dryer at 43 ± 1oC for 4 hours. The dried Tempe was milled using pin disc mill (Alecanderwek Inc., Germany) and sieved using 100-mesh sieve.[20]

Optimum TDP formula production

The additive ingredients such as sugar, maltodextrin, guar gum and flavor were mixed together using mixer.

The mixture was added with Tempe flour gradually until all components were mixed homogenously.[16] The compositional formula based on design of experiment (DOE) is presented in Table 1. The optimum formula was estimated by the highest desirability graph (Figure 2). The optimization process resulted in optimum TDP formula which was compared with the commercial TDP.[18] In addition, the optimum formula would also be compared with soy drink powder product with observed chemical parameters namely antioxidants, isoflavones and GABA.

Table 1: Compositional formula based on experimental design

Run	Formula		
	Tempe flour (%) (w/w)	Maltodextrin (%) (w/w)	Guar gum (%) (w/w)
1	75	15	0
2	75	15	0
3	77	10	3
4	77	10	3
5	70	20	0
6	78.5	10	1.5
7	70	18.5	1.5
8	70	20	0
9	70	17	3
10	80	10	0
11	73.5	13.5	3
12	72.125	17.125	0.75
13	76.375	12.125	1.5
14	72.125	15.625	2.25
15	80	10	0
16	70	17	3

Viscosity

The viscosity analysis was done using Viscometer instrument (Brookfield, Model LVT, Brookfield Engineering Laboratory, Middleborough, Massachusetts). Sample of one-time serving (20 g) was added with distilled water at room temperature in accordance with the serving suggestion (200 mL). The sample was poured in a glass and then spindle size (No. 1) was chosen with rotation speed of 60 rpm at 25°C to obtain the sample viscosity which stated in cP (centipoise) unit.[21]

Water Solubility Index (Wsi)

Powder sample of 1 g was weighed and added with 25 mL of distilled water at room temperature. The mixture was homogenized using a homogenizer (Armfield L4R) for 5 minutes. Then, the solution was centrifuged at 760 x g speed for 10 minutes. Then, 20 mL of supernatant was poured into a plate and dried to determine its weight. The supernatant was dried on a plate in an oven at 105°C for 5 hours and weighed every 2 hours to ensure a constant weight of the supernatant. WSI is calculated as g/100 g percentage.[22]

Sedimentation Index (Si)

The determination of SI value was done by diluting 10 g of powder sample into 100 mL distilled water in 100 mL measuring glass, then the sedimentation height declining was observed for 30 minutes. SI (%) is calculated based on the ratio between transparent volume against total volume.[23]

Water Absorption Index (Wai)

One g of powdered sample was weighed and put into centrifuge tube filled with 15 mL of distilled water and vortexed until homogenized. The sample was centrifuged at 3000 rpm for 10 minutes(BECKMAN, J2-MC, Minnesota, rotor JA-14)at 21°C. The supernatant liquid was separated from the mixture and the residue was weighed. WAI is calculated as g/g.[22]

Proximate, Dietary Fiber and Crude Fiber

The proximate, dietary fiber and crude fiber analysis referred to AOAC procedure,[24] which consisted of water content, ash content with gravimetry method, fat content with Soxhlet method, protein content with Kjeldahl method and carbohydrate content was determined using by difference method. The dietary fiber was analyzed using enzymatic method while crude fiber was analyzed using acid-base digestion method

Antioxidant Activity, Isoflavone Aglycones Contentand GABA

The tested sample of 0.1 mL was added with 3.9 mL DPPH (0.075 mM) in methanol then waited for 30 minutes for the reaction to occur. The absorbance was measured at 517 nm wavelength using UV-Vis spectrophotometer GenesysTM 150 (Thermo Fisher Scientific, Waltham, MA, USA). The standard curve

was prepared in respective concentrations of 10, 25, 50, 75 and 100 µg/mL. The obtained antioxidant activity is equivalent to ascorbic acid activity as DPPH radical scavenger.[25]

The extraction of isoflavone sample was started by adding 6 mL of HCL 1 M and 24 mL acetonitrile into 2 g sample. The sample was centrifuged at 3500 rpm speed for 10 minutes. Supernatant of 20 µL was injected into C-18 15 cm × 4.6 mm i.d., 5 µm (LiChrospher, Merck Millipore, USA) column. The utilized mobile phase was methanol: ammonium acetate 1mM with the ratio of 6:4 and rate of 1 mL/minute. The detector used for the analysis was UV Detector at 265 nm wavelength[26]. Data was collected using a HPLC Agilent UV-Vis VWD.

Sample of 1 g was inserted into centrifuge tube, added with 5 mL of distilled water and vortexed. The sample was put into a shaker at 200 rpm speed for 1 hour. Next, the sample was centrifuged for 30 minutes at 3000 rpm speed at 4°C. The supernatant was strained using 0.45 mikron nylon filter paper. Sample filtrate of 0.5 mL was reacted with 0.2 mL borate buffer (pH 9.0) and 1 mL of phenol 6% (b/v). The mixture was vortexed and cooled for 5 minutes before being heated in a water bath at 95 ± 1°C for 10 minutes. Then, 0.5 mL of 60% (v/v) ethanol was added into the mixture and vortexed. The absorbance was measured at 645 nm wavelength using UV-Vis spectrophotometer Genesys TM 150 (Thermo Fisher Scientific, Waltham, MA, USA). The GABA standard curve was made at concentration range of 0 – 300 ppm.[27]

Heavy Metals Contaminant Analysis
The analysis of heavy metal Cadmium (Cd), lead (Pb), Tin (Sn), Mercury (Hg) and Arsen (As) were done with inductively coupled plasma-mass spectrometry (ICP-MS) (Thermoscientific iCAP RQ).[28] One g of sample was reacted with 0.5 mL hydrogen peroxide 30% (v/v) and 4 mL nitric acid 65% (v/v) inside the PTFE vessels. The vessels were put in microwave for 15 minutes when the temperature was increased to 120°C and then left at constant temperature for 10 minutes. Then, the temperature was raised to 160°C for 20 minutes and then rested for 15 minutes. Then, the vessels were cooled at 22°C for 30 minutes. The mixture was diluted with 50 mL of deionized water and ready to be injected.

Colour and Degree of Whiteness Analysis
The equipment used for the analysis was chromameter (Konica Minolta CR-310). The obtained data from the color measurement consisted of L*, a* and b* value.[29] The measurement of whiteness degree was done using whiteness meter device (Kett C-130).[30]

Total Dissolved Solid, Bulk Density and Water Activity(a_w)
The total dissolved solid analysis procedure was performed by using hand refractometer(ATAGO Model N-3E, Japan) and stated in °Brix.[31] The bulk density was calculated based on comparison between sample weight against its volume in g/mL.[32] Water activity (aw) analysis was done by using Shibaura aw meter WA-360 (Fukushima Shibaura Electronics co., Ltd.).[31]

Microbiology Analysis
Total plate count analysis was performed in accordance with Indonesian National Standard(SNI 4833-1:2015), which calls for sample homogenization with buffered peptone water solution. The sample was then diluted and cultured on acidified potato dextrose agar media in a petri dish. After three days of incubation at 25-26°C, the mold colonies formed mycelia-filled rings.
Total molds analysis was performed in accordance with Indonesian National Standard(SNI 4833-1:2015). Potato dextrose agar (PDA) media was weighed up and diluted in distilled water before being heated until dissolved. For 15 min, the media solution was sterilized. Acidified potatoe dextrose agar (APDA) medium was made by adding 10% of tartaric acid to the media and homogenized with buffered peptone water (BPW). The solution was serially diluted in BPW at 1:9 (b:v) ratio before being grown in a petri dish with 1 mL of each solution. The plates were incubated for 2-3 days at 25-26°C

Data Analysis
The TDP formula was optimized using the Mixture Design Method and d-optimal optimized design in Stat-Ease Design Expert 12 (DX12) software. The response results were inputted into DX12 software and analyzed using ANOVA and a mathematical model. DX 12 software will offer a variety of mathematical models based on the conditions of the response test results. The physical, chemical, and microbiology properties were analyzed by

ANOVA and differences between means by Duncan test using SPSS (Ver 25, Chicago, IL). Significance was considered at the level of 5%.

Results and Discussion
Analysis of Response Optimization

The upper and lower limit is used in optimization process based on the results of literature studies and trials DX 12 software is designed to do experiments that obtained results of 16 runs. The measurement result of the TDP formula optimization response (Table 2) showed that the range of viscosity response of the TDP product ranged between 6 to 46.5 cP, SI value ranged between 4.96 to 14.9%, WAI value between 2.29 to 4.01 g/g and WSI value ranged between 6.56 – 10.78%. The summary of ANOVA analysis result for each optimization response can be seen in Table 3 and 3D optimization response curve is presented in Figure 1.

Table 2: The results of the response measurement of various optimization treatments of Tempe flour, maltodextrin and guar gum

Run	Results			
	Viscosity (cP)	SI (%)	WAI (g/g)	WSI (%)
1	7.5	14.20	2.49	8.81
2	7.5	13.59	2.50	8.62
3	46.5	6.60	4.01	6.73
4	46	6.75	3.81	6.56
5	6	12.90	2.29	10.78
6	27	10.50	2.96	7.32
7	25	8.88	2.64	9.93
8	9	13.16	2.35	10.67
9	42	5.37	3.45	9.32
10	12	14.70	2.60	6.72
11	40	6.52	3.40	8.52
12	19	11.92	2.43	9.54
13	30	10.30	2.61	8.27
14	36	7.59	2.75	9.78
15	8	14.90	2.56	6.72
16	42	4.96	3.45	9.32

Notes: SI = Sedimentation index, WAI = Water adsorption index, WSI = Water solubility index

Based on the analysis of variance, the chosen model for viscosity and SI responses were linear, WSI was cubic and WAI was special cubic (Figure 1). The chosen model for overall responses had significant value lower than 0.05, which means that all models were significant to the chosen response and model, as well as had an error probability less than 5%. All responses also had no insignificant Lack of fit values (p>0.05). The non-significant Lack of fit value showed that the chosen model is a model which possesses the suitability of good response data and is not significant to model inaccuracy.

Water Solubility Index (WSI) Response Analysis

WSI parameter in drink powder product is a crucial indicator because it affects the consumer perception, especially when dissolving the drink powder. There are several factors that affects WSI value, namely the composition of easily soluble ingredients, the concentration of food additives and processing condition.[33–35] The criteria of an acceptable powder product are easy to absorb water, drown and dissolved in water, compared to floating and prone to coagulate drink powder product.[36]

The chosen model for WSI analysis response was cubic model. The model had Lack of fit value of 0.5297 and R2 value of 0.9986 (Table 3). The equation for interpretation WSI response is as follows.

WSI (%)=6.72 A+10.72 B-162.7 C+0.0126 AB+291.52 AC+298.44 BC-252.61 ABC-1.27 AB(A-B)-124.67 AC(A-C)-143.51 BC(B-C)

The product WSI value for TDP ranged from 6.56 to 10.78% (Table 2). The value indicates that the TDP was easily soluble in water. The value was lower compared with TDP product (fresh Tempe based formula),[16] yacon juice powder[37] and pomegranate juice powder.[34] The equation shows that WSI response was affected by the concentration of Tempe flour (A), maltodextrin (B), guar gum (C) and the interaction of all three factors (ABC). The increase in WSI value was affected by Tempe flour concentration and maltodextrin due to the positive coefficient value. On the contrary, the decline of WSI value was affected by guar gum concentration due to the negative coefficient value. Based on the

equation, it is shown that TDP made with higher Tempe flour and maltodextrin had higher WSI value. The WSI value was affected by the high carbohydrate and protein content as well as the low content of hydrophobic compounds in the product.[37,38] The macromolecule components interact with each other and form complexes such as amylose-lipid and protein-starch which will affect the WSI value.[35] The addition of too many hydrocolloid ingredients (such as guar gum) can result in lower product solubility since it will interfere the interaction between water and carbohydrate polymer chain.[39]

Table 3: Analysis of variance (ANOVA) result on the experiment responses

Responses	Model	Significant (p<0.05)	Lack of fit (p>0,05)	R^2	Adj. R^2	Pred. R^2	Adeq. Precision
Viscosity (cP)	Linear	<0.0001	0.2376	0.9854	0.9832	0.9783	43.5658
SI (%)	Linear	<0.0001	0.4102	0.9945	0.9937	0.9921	77.9710
WSI (%)	Cubic	<0.0001	0.5297	0.9986	0.9966	0.9984	61.7044
WAI (g/g)	Special cubic	<0.0001	0.4463	0.9901	0.9835	0.9651	33.6111

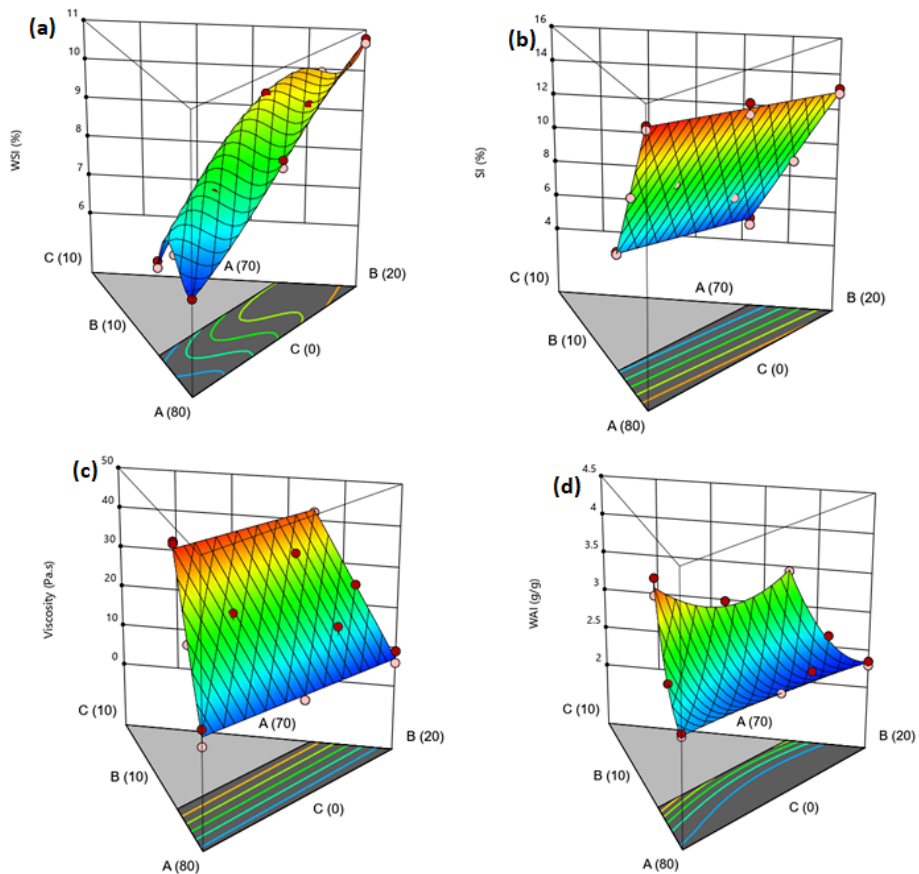Notes: SI = Sedimentation index, WAI = Water adsorption index, WSI = Water solubility index

Fig. 1: 3D response curve of (a) WSI, (b) SI, (c) viscosity and (d) WAI

The SI value indicates the stability of dispersion system of a certain mixture. The SI value would affect consumer acceptance since SI value affect the appearance of the beverage product.[40] The product is considered stable when it has low SI value, because it cannot easily form sedimentation after being diluted in water.[41,42] Several factors which affect the SI value of a product are particle size, stabilizer concentration and ratio of particle and its solvent.[40,43]

The chosen model for SI response analysis was linear model. The model had Lack of fit value of 0.4102 and R^2 value of 0.9945 (Table 3). The equation used to interpret SI response is as follows.

SI (%)=14.83 A+12.99 B-12.48 C

The SI value estimation in TDP product ranged from 4.96 to 14.9% (Table 2). The SI value was considered as sufficiently high compared to chocolate beverage with hydrocolloid.[40,41] The obtained equation shows that SI response was affected by Tempe flour (A), maltodextrin (B) and guar gum (C). The increase in SI value is heavily affected by several factors, namely Tempe flour concentration and maltodextrin due to the positive coefficient. Meanwhile, SI value decline is affected by guar gum concentration due to the negative coefficient. Based on the equation, it is shown that TDP which contains lower Tempe flour and maltodextrin as well as higher guar gum will lower the SI value, which makes the product more stable. Guar gum is considered to be able in preventing sedimentation and maintaining the texture of the beverage.[44,45] The presence of stabilizer such as guar gum can form stable structure that can retain the dispersion system due to its capability in ensnaring particles into the matrix.[46]

Viscosity Response Analysis
Viscosity is an important parameter because it indicates texture or mouthfeel of a certain beverage product, which in the end affects consumer acceptance.[47,48] Viscosity occurs due to the interaction between the dispersing phase and the dispersed solids within the product. Viscosity has a relationship with SI value, where the higher the viscosity value, the more stable the dispersed system within the product. Several factors that affect the viscosity of a product, such as the concentration of the stabilizer and the ratio between the particles and its solvent.[40,43]

The chosen model to be analyzed for viscosity response was linear model. The model had lack of fit value of 0.2376 and R^2 value of 0.9854 (Table 3). The equation used to interpret the viscosity response is as follows.

Viscosity (cP)=10.52 A+7.20 B+125.51 C

The estimation of TDP viscosity value ranged between 6 to 46.5 cP (Table 2). The value showed that the TDP was a viscous beverage. The result is similar to protein-based beverage added with carrageenan as a thickener[49] and chocolate beverage which was added with xanthan gum.[41] Based on the equation, it is shown that viscosity response is influenced by Tempe flour (A) concentration variation, maltodextrin (B) and guar gum (C). The increase in viscosity was also influenced by factors such as Tempe flour concentration, maltodextrin and guar gum since it had positive coefficient. Guar gum has pseudoplastic properties which can increase the product viscosity when added in low concentration.[50] Several hydrocolloids have a synergistic effect, such as interaction between guar gum and other polysaccharides which could increase viscosity and gel power.[51,52] Guar gum is able to increase the viscosity by forming a strong hydrogen bond with its polar solvent.[53] Galactomannan in guar gum binds water, which results in gel formation and viscosity increase. The viscosity decreases when the galactomannan is decomposed by being heated up to 90oC or the pH lowered to below 3.5.[53]

Water Absorption Index (WAI) Response Analysis
WAI parameter shows the capability of a material to absorb water. Materials with high WAI tend to be easily dispersed in water. WAI parameter can also be used to observe hydrophilicity and gelation capacity from macromolecules, such as starch and protein in flour as well as determining the volume capacity of starch that can be occupied by water that can show the hydration ability of certain materials[35,54]

The chosen model for WAI response analysis was special cubic model. The model had lack of fit value of 0.4463 and R2 value of 0.9901 (Table 3). The equation used to interpret the WAI response is as follows.

WAI (g∕g)=2.57 A+2.34 B+15.99 C+0.1735 AB-12.8 AC-14.13 BC-10.04 ABC

The estimation of WAI value for TDP was from 2.29 to 4.01 g/g(Table 2) which indicates good rehydration capability. The value was higher compared to TDP (fresh Tempe based formula)[16] similar with the WAI value of goldenberry powder.[55] Based on the equation, WAI response was affected by the Tempe flour (A) concentration, maltodextrin (B), guar gum (C) and interaction between all of the factors (ABC) which was shown by the positive coefficient value. It can be said that TDP made from higher percentage of Tempe flour, maltodextrin and guar gum will increase the WAI value. The ability of Tempe flour, maltodextrin and guar gum in increasing the WAI value was caused by the presence of hydrophilic molecules and the high content of soluble fiber that is able to strongly bind water.[55] Guar gum contains a large polysaccharide in the form of galactomannan, at least 75%. Guar gum has a high content of galactomannan, which is at least 75%. The galactomannan has roles in binding water due to having many hydrophilic groups. Protein is one of the most important molecules which can increase WAI value do the hydrophilic unit it possesses.[56,57].

Tdp Formula Optimization
The optimization step was done to obtain the best variables of TDP formula by optimizing the analyzed responses. Desirability value is a function to achieve the desired result based on the existing variable criteria. The desirability value shows the degree of accuracy of the optimum solution58. Response is

considered to be optimum when the desirability value is close to 1. The variable goal criteria of Tempe flour, maltodextrin and guar gum were in range with interest scale of 3 (+++). The criteria goal for viscosity, SI, WSI and WAI each were in respective targeted (20 cP), targeted (9.93%), in range and in range. The interest scale for each response variable was 3 (+++).

The optimization result was one optimum formula with the highest desirability value of 0.943. The 3D desirability curve can be seen in Figure 2. The obtained optimum formula was 70% Tempe flour, 18.23% maltodextrin and 1.77% guar gum. The optimum formula was verified to observe the model capability in predicting the optimum value. The study is considered to be verified if the response testing results are in Confident Interval (CI) value of 95% and Prediction Interval (PI) value of 95%.
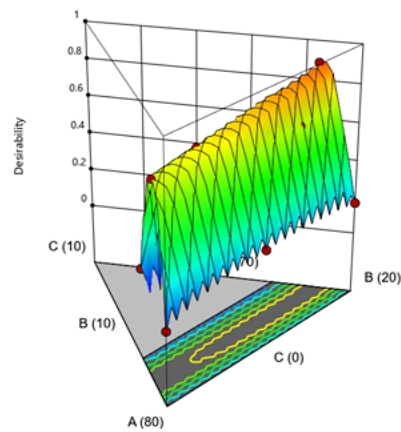

Fig. 2: 3D graph of desirability

Table 4: Verification of the optimum formula

Variable	Prediction value	Verification value	95% CI		95% PI	
			Low	High	Low	High
WSI (g/100g)	9.90	9.70	9.70	10.11	9.67	10.15
SI (%)	9.93	10.18	9.69	10.17	9.51	10.36
Viscosity (cP)	21.42	19.83	19.71	23.13	18.39	24.45
WAI (g/g)	2.49	2.58	2.39	2.59	2.35	2.62

Notes: SI = Sedimentation index, WAI = Water adsorption index, WSI = Water solubility index

The verification result (Table 4) showed that all formula responses were in range with 95% CI value and 95% PI value. The result showed that the formula had the suitable response testing result with the recommended prediction by the verified software and formula. The optimum formula of Tempe flour, maltodextrin and guar gum is able to produce TDP with sufficiently consistent viscosity, SI, WAI and WSI values.

Physical, Chemical and Microbiology Analysis of TDP
Proximate, Dietary Fiber, Crude Fiber and Total Sugar
The proximate, dietary fiber, crude fiber and total sugar composition are presented in Table 5. The TDP with an optimum formula which fulfilled the SNI 7612:2011 criteria test based on water, ash, fat, protein and crude fiber content parameters. The analysis of water, crude fiber and total sugar did not show significant differences (p>0.05) between the optimum formula and the commercial TDP formula. The protein, ash and fat in the optimum formula were higher compared with the commercial TDP formula, respectively 42.61, 1.75 and 28.85%. The results were higher compared to TDP(fresh Tempe based formula)[16] and Tempe flour.[17] However, the protein content of the optimum formula was lower than the Tempe beverage.[59] This was due to the difference in Tempe composition used in the product

formulation. Reformulation was able to increase TDP protein content up to 12.33%. These result show that the optimum formula showed "higher protein" due to the optimum formula containing 40.8% higher protein than the commercial TDP formula (Table 5). "Higher protein" claims can be attained when the products have a higher protein content of at least 25% based on Indonesian FDA Regulation No. 01 the Year 2022 on regarding Supervision of Claims on Processed Food Labels and Advertisement.

The carbohydrate and dietary fiber content in the optimized formula was lower than the commercial TDP formula, with each 26.79 and 14.32%. This was due to the commercial TDP formula utilized other ingredients in higher amount such as wheat flour and maltodextrin in higher amount which increases the carbohydrate content. The result was also higher compared with other soy-based beverages such as soymilk[60] and extract Tempe drink.[15] The dietary fiber content of the commercial TDP formula has fulfilled the high fiber claim requirements based on Indonesian FDA Regulation No. 01 the Year 2022 regarding Supervision of Claims on Processed Food Labels and Advertisement. The dietary fiber maintains the digestive system function and prevents diabetes, obesity, cardiovascular diseases and colon-related diseases.[61,62] Based on the result, it can be concluded that TDP contains protein and dietary fiber sources that are beneficial for health.

Table 5: Chemical, heavy metal contamination and microbiology characteristics

Parameter	Tempe flour	Tempe drink powder		Standard*
		Commercial formula	**Optimum formula**	
Proximate (%)				
Moisture	6.70 ± 0.04[a]	6.49 ± 0.41[a]	6.20 ± 0.03[a]	max. 10.0
Ash	1.63 ± 0.08[a]	1.25 ± 0.05[b]	1.75 ± 0.00[a]	max. 6.0
Protein	47.56 ± 0.07[a]	30.22 ± 3.01[b]	42.61 ± 0.13[a]	min. 30.0
Fat	33.53 ± 0.21[a]	13.50 ± 0.49[c]	28.85 ± 0.11[b]	min. 17.0
Carbohydrate	17.27 ± 0.26[c]	55.02 ± 2.82[a]	26.79 ± 0.05[b]	-
Dietary fiber	15.34 ± 0.14[b]	19.57 ± 0.71[a]	14.32 ± 0.15[b]	-
Crude fiber	1.13 ± 0.16[b]	3.04 ± 0.15[a]	1.60 ± 0.13[a]	max. 3.0
Total sugars	ND	8.70 ± 0.11[a]	7.83 ± 2.48[a]	-
Minerals (mg/kg)				
Cd	ND	ND	ND	max. 0.2
Pb	ND	ND	ND	max. 0.25

Sn	ND	ND	ND	max. 40
Hg	ND	ND	ND	max. 0.03
As	ND	ND	ND	max. 0.25
Microbiology (CFU/g)				
Total mold count	$1.6 \times 10^3 \pm 0.07$	< 10	< 10	max. 5×10^1
Total plate count	$1.45 \times 10^5 \pm 0.07$	$4.80 \times 10^3 \pm 0.42$	$6.90 \times 10^3 \pm 0.14$	max. 5×10^4

Mean on the same row followed by different letters show significant differences (p<0.05)
Note: ND = Not detected, * = Indonesian National Standard (SNI 7612:2011) regarding soy drink powder

Mineral Characteristic

Several minerals are harmful to health due to their toxicity, such as Pb, As, Hg, Sn and Cd. In Table 5, it is shown that the commercial TDP formula and optimum formula, there were no heavy metals contamination detected in the product, which fulfills the SNI 7612:2011 testing criteria requirements. The SNI requires heavy meatal threshold of soy drink powder are respectively 0.2, 0.25, 40, 0.03 and 0.25 mg/kg. The heavy metal contamination is included into the critical testing criteria due to its toxicity and can be accumulated in the human body through the bioaccumulation mechanism.[63] In addition, several heavy metals such as Cd, Pb and Hg are highly toxic even in extremely low concentration.[64–66] TDP also fulfills the heavy metal contamination requirements by CODEX STAN 175-1989 for protein product produced from soybean. Therefore, TDP is a safe product for consumption.

Microbiology Characteristics

The optimum formula had total plate count and mold total count of each <10 and 6.90 x 103 colony/g. The result was below the threshold of microbiology characteristics based on SNI 7612:2011, thus fulfills the requirements. The Indonesian National Standard set a limit for total plate count and total mold count, respectively 5.0×10^4 dan 5.0×10^1 colony/g. The growth of mold on TDP was caused by many factors, including Tempe fermentation which involves *Rhizopus* spp. mold. The TDP has fulfilled the microbiology contamination based on CODEX STAN 175-1989, namely free from microorganism that are harmful for health. Therefore, TDP is a product that is safe for consumption.

Physical Properties

The physical properties comparison between the optimum formula and the commercial TDP formula can be seen in Table 6. Water activity (aw) of the commercial TDP formula and optimum formula were on 0.23-0.24 range. The value is considered to be within the safe zone for drink powder product in preventing the growth of microorganisms. The water activity level that limits the growth of all microorganisms is 0.60 a_w.[67]

On the total solublesolid parameter, the commercial TDP formula and optimum formula each had 5.25 and 7.02 °Brix. The result was higher compared to TDP (fresh Tempe based formula).[16] The differences were affected by the variation of filler concentration, stabilizer and sugar which were added into the drink formula. Product with higher total soluble solids is better due to the high concentration of water-soluble solids.[68]

In Table 6, the optimum TDP formula had lower bulk density (0.43) compared with the commercial TDP formula (0.59). The lower bulk density in optimum TDP formula was due to the addition of maltodextrin. Maltodextrin is considered a skin-forming material, and employing it as a food additive might cause air collection and trapping inside the particles, causing it to become less dense and porous.[36] The drying method utilized in this study also contributes to the bulk density difference, namely fludized bed drying method. The low temperature used in fluidized bed drying method made the particles less compact and stiff, otherwise, the high temperature used in commercial TDP, namely oven drying method, may have caused collapse which resulted in more compact and rigid products. These characteristics resulted in higher bulk density.[68] The high bulk density is highly favorable in powdered product due to the narrow space between molecules, which could increase the cohesivity between particles. Low bulk density causes the water vapor to easily enter the product and accelerate the coagulation and damage on the product. The result was higher compared

with TDP (fresh Tempe based formula)[16] and lower compared to spray-dried soy beverage.[32]

The commercial TDP formula had a higher viscosity value (32.17 cP) compared with the optimum formula (18.67 cP). Higher viscosity value will increase the SI. In Table 6, the commercial TDP formula had lower SI value (5.56%) compared with the optimum formula (10.18%). This is in accordance with study before which mentioned that product with high viscosity tends to have a more stable dispersion system.[40,43]. There are several factors that affects viscosity and SI value, namely stabilizer concentration and size of dispersed particles in suspension.

The commercial TDP formula had a higher WAI value (2.82 g/g) compared with the optimum formula (2.58 g/g). On the contrary, the commercial TDP formula with lower WSI value (7.06%) than the optimum formula (9.70%). The commercial TDP formula had higher WAI compared with the optimum formula due to the higher hydrocolloid concentration. The hydrocolloid is a hydrophilic molecule and considered as a water-soluble fiber which has the ability to bind water.[55] However, excessive amount of hydrocolloid component will result in lower solubility in water because it interferes the interaction between water and carbohydrate polymer chain.[39]

Table 6: Physical properties

Parameter	Tempe drink powder	
	Commercial formula	**Optimum formula**
aw	0.23 ± 0.00[a]	0.24 ± 0.00[a]
Total soluble solid (oBrix)	5.25 ± 0.28[b]	7.02 ± 0.05[a]
Bulk density (g/mL)	0.59 ± 0.01[a]	0.43 ± 0.00[b]
Viscosity (cP)	32.17 ± 0.76[a]	18.67 ± 0.76[b]
SI (%)	5.56 ± 0.29[b]	10.18 ± 0.55[a]
WAI (g/g)	2.82 ± 0.31[a]	2.58 ± 0.36[b]
WSI (%)	7.06 ± 0.53[b]	9.70 ± 0.18[a]

Mean on the same row followed by different letters show significant differences (p<0.05)

Table 7: Color characteristics

Parameter	Tempe drink powder	
	Commercial formula	**Optimum formula**
L*	79.71 ± 0.10[b]	82.69 ± 0.01[a]
a*	-1.60 ± 0.23[a]	-1.56 ± 0.12[a]
b*	19.17 ± 0.35[a]	19.84 ± 0.74[a]
Degree of whiteness	52.30 ± 0.21[b]	55.95 ± 0.14[a]

Mean on the same row followed by different letters show significant differences (p<0.05)

The colour parameter is an important parameter in a product since it determines the purchasing decision as well as consumer acceptance. In Table 7, it is shown that there are differences in L* value and degree of whiteness between the optimum formula and the commercial TDP formula. Otherwise, the a* and b* value did not show significant differences (p>0.05) between the optimum formula and the commercial TDP formula. The optimum formula had higher L* value and degree of whiteness,

respectively 82.69 and 55.95. The result was comparably higher than soy drink powder.[68] The optimum TDP formula was dried at 43°C to prevent browning on the flour. The addition of maltodextrin into the formula also resulted in brightness increase of the product.[38]

Potential of TDP for Health
According to epidemiological study, consuming Tempe drink powder three glasses every day for four weeks consecutively will improve health and reduce disease risk, such as hypercholesterol,[8] hypertension[9] and diabetes.[10,11] TDP contains various bioactive compounds which have the ability to maintain health, such as isoflavones, GABA and its antioxidant activity. In Table 8, it is shown that the fermentation process, formulation difference and TDP production method affect significantly on isoflavones content, GABA and antioxidant activity. The optimum formula has total isoflavones (daidzein and genistein), GABA content and antioxidant activity respectively 188.24 mg/100g, 21.24 mg/g and 58.36 mg AEAC/100g. The obtained total isoflavones were higher if compared to the commercial TDP formula, soy drink powder and Tempe powder[11,69,70]. There is an effect of fermentation of soybean to the increase of isoflavone aglycon on the Tempe drink. The soybean fermentation is able to increase the total isoflavones aglycon as well as to decrease the anti-nutrition compounds, which can increase the isoflavones bioavailability.[71] The fermentation process can hydrolyze the glycoside bond of the isoflavone glucoside(daidzine, glycitine and genistine) into its aglycon form (daidzein, glycytein

and genistein) due to the activity of β-glycosidase.[72] Isoflavone in its aglycon form is easily absorbed by the human body and also has bioactive properties, such as being capable of reducing the development of cancer cells and averting free radicals.[73,74]

The combination of GABA in the optimum TDP formula is higher compared with the commercial TDP formula and soybean drink powder. The result was higher compared to fresh Tempe product.[75,76] The type of culture used for the fermentation process affects the resulted GABA content, therefore each fermented product has different GABA content.[77] GABA content in fermented product has been studied to have antioxidant activity and inhibition of angiotensin-converting enzyme (ACE).[78–80]

The increase of antioxidant activity in TDP was also influenced by the high isoflavone compound (phenol) and GABA since it has the ability in averting free radicals. The antioxidant activity of optimum TDP formula was higher compared with the commercial TDP formula. The higher the Tempe composition in the drink formula, the higher the antioxidant activity, since Tempe contains various phytochemical compounds which are able to tackle free radicals, such as bioactive peptides, phenolic (isoflavones), saponins and vitamin E.[11,70,81,82] The antioxidant activity in Tempe drink is also higher compared with soy drink due to the fermentation process from Tempe production that could release the phenolic compound and produce peptides fraction with low molecular weight (less than 1 kDa), which has high antioxidant activity.[83,84]

Table 8: Antioxidant activity, isoflavones and GABA content

Parameter	Tempe flour	Tempe drink powder		Soy drink powder
		Commercial formula	Optimum formula	
Antioxidant activity (mg AEAC/100 g)	65.50 ± 0.51[a]	28.60 ± 1.18[c]	58.36 ± 0.51[b]	20.50 ± 1.85[d]
Daidzein isoflavone (mg/100g)	52.46 ± 0.08[a]	29.24 ± 0.05[ab]	48.18 ± 0.00[a]	5.11 ± 0.00[c]
Genistein isoflavone (mg/100g)	140.21 ± 0.08[a]	77.98 ± 0.05[ab]	140.06 ± 0.00[a]	11.92 ± 0.00[c]
GABA (mg/g)	24.63 ± 0.29[a]	10.84 ± 0.16[c]	21.24 ± 0.56[b]	2.12 ± 0.07[d]

Mean on the same row followed by different letters show significant differences (p<0.05)

Conclusion

The formula optimization on tempe drink powder using mixture experiment (ME) established the effect of tempe flour, maltodextrin and guar gum concentration on viscosity, SI, WSI and WAI value. The optimum TDP formula was 70% Tempe flour, 18.23% maltodextrin and 1.77% guar gum, as determined by the ME method. The optimum formula was demonstrated to be capable of improve the physical properties of the commercial TDP formula, specifically for the viscosity and WSI value. The optimum formula had a higher protein content, antioxidant capacity, total isoflavones and GABA content, and it has the potential to be further developed as a functional drink. The optimum TDP formula also fulfilled the quality requirements in accordance with the Indonesian National Standard (SNI 7612:2011) regarding soy drink powder on chemical, heavy metal contamination and microbiology parameters.

Conflict of Interest

The authors declare no conflict of interest.

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