ISSN: 2347-467X, Vol. 12, No. (2) 2024, Pg. 699-707

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Optimization of Volumetric Ratio of Soy Milk and Concentration of Porang Gel on the Properties of Soy Milk Fortified with Ginger Extract

AHMAD ZAKI MUBAROK[1,2]* and OCHA SALMA SAMESTA[1]

[1]Department of Food Science and Biotechnology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia.
[2]Porang Research Center, Universitas Brawijaya, Malang, Indonesia.

Abstract

Soy milk is a typical soy protein product and a good source of essential nutrients, however "beany" off-flavor makes soy milk unpleasant or unacceptable to many consumers. The addition of ginger extract in this research is intended to mask the "beany" off-flavor in soy milk. Soy milk is a beverage with an emulsion system that exhibits unstable characteristics during storage. Creaming and sedimentation in protein-rich beverages can be addressed by enhancing system stability using polysaccharides such as glucomannan from the porang plant. Glucomannan has the ability to bind water effectively, restricting molecular movement and thereby improving the stability of soy milk. The objective of this research is to determine the optimal values for volumetric ration of soy milk and concentration of porang gel that yield the desired responses in terms of physical stability, viscosity, and antioxidant activity of soy milk using response surface methodology. The models predicted that the optimal formula was soy milk volumetric ratio of 69.3 (v/v) and porang gel concentration of 1.3%. The verification experiments were conducted in three replications. The results showed that physical stability, viscosity and IC_{50} value of soy milk were 0.90±0.05, 132.0±5.5 cP and 187.5±35.2 ppm under optimal formulation. The optimization results were validated and found no statistically significant differences at a 5% significance level. Soy milk with good physical stability, viscosity, and antioxidant activity can be prepared with this formulation.

Article History
Received: 14 March 2024
Accepted: 18 August 2024

Keywords
Beverage;
Ginger;
Glucomannan;
Soybean;
Soy Milk.

CONTACT Ahmad Zaki Mubarok ahmadzaki@ub.ac.id Department of Food Science and Biotechnology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia.

Doi: https://dx.doi.org/10.12944/CRNFSJ.12.2.17

Introduction

Soy milk is a plant-based milk beverage derived from soybeans. Soy milk has a good nutritional content due to its high protein content. Soy milk has played a crucial role as a valuable source of high-quality protein and its absence of lactose ensures its suitability for consumption by those with lactose intolerance.[1] Numerous research studies have shown the associated advantages of the use of soy products to help prevent conditions such as obesity, diabetes, heart disease, blood cholesterol, cancer, kidney disease, osteoporosis, and blood pressure regulation.[2] Despite its considerable nutritional worth and other positive health attributes, soy milk encounters resistance from consumers due to an unpleasant beany flavor taste. A number of studies demonstrated that the strange flavour arises from the activity of enzyme lipoxygenase on the lipid component during the production of soy milk.[3]

Ginger is characterised by its pungent taste and contains bioactive compounds that provide advantageous effects, including vitamins C and E, phenolic compounds, flavonoids, carotenoids, tannins, and flavones.[4] These compounds are responsible for diverse biological functions of ginger including its antimicrobial, antioxidant, immunomodulatory, anti-inflammatory effects, and other beneficial properties.[5] Ginger serves as a flavor enhancer in carbonated beverages, pickles, confectionery, sauces, and baked goods.[6] The addition of ginger has been proven to enhance the nutritional quality and sensory attributes of soy milk.[7] Therefore, in this study, soy milk was developed with the addition of ginger extract to enhance flavor and increase antioxidant content.

Soy milk is an emulsion of oil, water, and protein. This emulsion is susceptible to creaming, flocculation, sedimentation, and coalescence, leading to the instability of the product which affects its taste and quality.[8] Several studies were conducted to overcome the physical separation of emulsions by adding polysaccharides. The presence of alginate increased the viscosity and improved pH stability of the emulsion-based gelled systems.[9] Incorporating gum arabic into flaxseed protein concentrate and soybean protein concentrate stabilized emulsion would strengthen their stability.[10] Inulin has been used to enhance the stability of emulsions containing β-lactoglobulin, where inulin at low concentrations plays a role in protein adsorption at the boundary between oil and water, whereas higher concentrations of inulin exhibit thickening properties.[11] Xanthan gum is frequently added to emulsions to improve stability by increasing the viscosity of the continuous phase and retarding the creaming process.[12]

Glucomannan is a hydrocolloidal polysaccharide typically extracted from tubers of *Amorphophallus konjac* and *Amorphophallus muelleri* Blume.[13] *Amorphophallus muelleri* Blume, known as porang in Indonesia, contains a high level of water-soluble glucomannan. Glucomannan was employed as a stabilizer and also as a gelling or viscosifying agent due to its impressive capabilities in thickening and retaining water.[14] It is believed that glucomannan offer numerous health benefits, such as positively modulating gut microflora, reducing blood cholesterol and sugar levels, improving immune function, and promoting weight loss.[15,16] There are few studies on using glucomannan for improving the stability of soy milk emulsions. Therefore, the objective of this study was to produce stabilized soy milk fortified with ginger extract with enhanced stability and antioxidant activity. This research was conducted to determine the values for volumetric ration of soy milk and concentration of porang gel that yield the optimal responses in terms of physical stability, viscosity, and antioxidant activity of soy milk using response surface methodology. Porang gel is obtained by mixing porang flour with water at a specific concentration. To the best of our knowledge, there has been no work done on the addition of porang gel to soy milk fortified with ginger extract. The addition of porang gel to soy milk, besides those contributed to the stabilization of emulsions, may also enhance health benefits.

Materials and Methods

Materials

The materials used in this research are soybeans, ginger (*Zingiber officinale* var. *amarum*), porang flour, 0.1 mM DPPH reagent, and methanol. Porang flour was prepared in our laboratory using a centrifugal grinder.[17]

Experimental Design

Response Surface Methodology (RSM) was carried out using Design Expert Version 13.0 with two independent factors, those are volumetric ratio of soy milk and concentration of porang gel (Table 1). Three responses: stability, viscosity, and IC_{50} value, were selected as dependent variables. The Central Composite Design, with 4 factorial points, 4 axial points and 5 repilcates at the center point were used to build a model. The model's adequacy was assessed using Lack of fit value, R^2 value, and Adjusted R^2 value with its statistical significance determined through an F-test.

Table 1: Independent variables and factor levels in the central composite design

Independent variables	Factor level				
	- α	-1	0	1	α
Volumetric ratio of soy milk (v/v, X_1)	55.9	60.0	70.0	80.0	84.2
Concentration of porang gel (%, X_2)	0.8	1	1.5	2	2.2

Experimental Procedures

Porang Gel Preparation

The process of making porang gel begins with weighing the porang flour as required and adding water according to the necessary concentration (w/v) for the treatment. Then, stirring is carried out to homogenize the porang flour solution with a magnetic stirrer for 2 hours at room temperature.

Ginger Extract Preparation

Dried ginger powder is dissolved in hot water at a temperature of 80°C at a concentration of 3%(w/v), and stirred with a magnetic stirrer for 5 minutes. After that, filtration is performed using filter paper to obtain ginger extract.

Soy Milk Preparation

The process of making soy milk with the addition of ginger extract begins with washing soybeans using clean water. Then, the soybeans are soaked for 6 hours using water at a ratio of soybeans to water of 1:3 (w/v). The soybeans are soaked again in hot water to soften the tissue and deactivate the lipoxygenase enzyme for 30 minutes. Afterward, the soybeans are peeled, and water is added at a ratio of 1:6 (w/v) before blending them using a blender. Then, the mixture is filtered to obtain soy milk and separate the remaining residue. Soy milk is mixed with a ginger extract solution (3% (w/v) concentration) at a ratio of 90:10 (soy milk to ginger). The soy milk is then heated to a temperature of 90°C for 15 minutes. The porang gel with each concentration is added to soy milk according to the specified treatment in the Table 2. Then, the samples are stirred with a hotplate stirrer for a duration of 1 hour.

Experimental Analysis

Physical Stability Analysis

10 mL of soy milk were transferred into cylindrical plastic tubes and centrifuged at 4,500 rpm for 5 min. Physical stability of soy milk was calculated by the following equation:

$$\text{Stability} = V_1 / V_0$$

Where V_1 is the liquid volume of stable soy milk remained in the tube after centrifugation and V0 is the initial volume of soy milk transferred into the tube.

Viscosity Analysis

Viscosity analysis is performed to obtain the viscosity value of a liquid substance using viscometer (Elcometer 2300 RV). A 100 ml sample is tested for its viscosity using a viscometer by immersing the spindle into the sample. The spindle rotates for a period of 20 to 30 seconds. The viscosity measurement results are displayed on the viscometer screen and recorded as the measurement outcome.

Radical scavenger activity (IC_{50}) analysis

The preparation of methanol extract of soy milk was carried out in a 100 mL volumetric flask. At a concentration of 100 ppm, 10 mg of soy milk samples was prepared and methanol was added to a total volume of 100 mL. At a concentration of 200 ppm, 20 mg of soy milk samples was prepared and methanol was added to a total volume of 100 mL. At a concentration of 300 ppm, 30 mg of soy milk samples was prepared and methanol was added to a total volume of 100 mL. And at a concentration of 400 ppm, 40 mg of soy milk sample was prepared and methanol was added to a total volume of 100 mL. 1 mL the methanol extracts of soy milk samples (100, 200, 300, and 400 ppm) was combined with 3 mL of methanolic solution containing DPPH radicals, where the final concentration of the DPPH is 0.2 mM. The mixture was thoroughly shaken and left to stand for 30 minutes in the dark, after which the absorbance was measured at 517 nm. The capability of the test material to scavenge DPPH radicals was calculated as (%) = [absorbance of the control - (absorbance of the sample)/(absorbance of the control] × 100%. The radical scavenger activity was expressed in terms of the quantity of antioxidants required to reduce the initial DPPH absorbance by 50% (IC_{50}). The IC_{50} value for each sample was calculated graphically by plotting the percentage reduction of DPPH as a function of the sample concentration.

Results and Discussion

The addition of porang gel to various volumes of soy milk affects the stability, viscosity, and antioxidant activity of soy milk, optimization is necessary to produce soy milk with the best characteristics. The first factor in this study is the volume of soy milk, with an upper limit of 80 (v/v) and a lower limit of 60 (v/v). The second factor is the concentration of porang gel, with an upper limit of 2% and a lower limit of 1%. The responses obtained include physical stability, viscosity (cP), and antioxidant activity (ppm). The response analysis results are presented in Table 2.

Table 2: Response obtained for each experimental running

Run	Actual		Response 1	Response 2	Response 3
	Volumetric ratio of soy milk (v/v)	Concentration of Porang gel (%)	Stability	Viscosity (cP)	IC_{50} (ppm)
1	60.00	1.00	0.7	130	179.2
2	55.86	1.50	1.0	270	284.9
3	70.00	1.50	1.0	200	232.3
4	70.00	1.50	1.0	182	212.1
5	84.14	1.50	0.6	155	204.0
6	80.00	2.00	0.9	410	372.5
7	80.00	1.00	0.5	105	153.8
8	70.00	1.50	1.0	178	209.2
9	60.00	2.00	1.0	540	377.1
10	70.00	1.50	1.0	183	227.6
11	70.00	2.21	1.0	620	412.4
12	70.00	0.79	0.2	75	127.5
13	70.00	1.50	1.0	190	227.7

Physical Stability

The physical stability response is measured using the creaming level, where the closer it is to 1, the more stable the emulsion will be, and no creaming is formed. The data from the response analysis indicate that the highest stability response is achieved when the soy milk ratio is low, and the porang gel concentration is high, with a stability value of 1. The lowest stability response is observed when the soy milk ratio is high, and the porang gel concentration is low, with a stability value of 0.2. The analysis of variance (ANOVA) results shows that the soy milk ratio has a p-value of 0.0060, and the porang gel concentration (B) has a p-value of <0.0001, indicating that both the soy milk ratio and the porang gel concentration significantly affect the physical stability response. The interaction value AB has a p-value of 0.5453, suggesting that the interaction does not significantly affect the physical stability response. The soy milk ratio squared variable (A^2) has a p-value of 0.0296, and the porang gel concentration squared variable (B^2) has a p-value of 0.0005, which means that these variables have a significant impact on the physical stability response (Table 3).

The ratio of soy milk significantly affects stability response (Figure 1A). The higher the soy milk ratio, the lower the physical stability value, making the emulsion increasingly unstable. Soy milk can be considered a colloidal system, where small particles of soy protein are dispersed in water. This colloidal systems are inherently unstable because the protein particles have a tendency to aggregate over time.[18] The concentration of porang gel significantly influences stability response. The higher the concentration of porang gel, the increased stability value. This is because porang gel has a high content of glucomannan, which has the ability to bind water effectively, thus can be used as an emulsion stabilizer and lengthening the physical stability. When glucomannan added to soy milk, they can absorb and bind water molecules, forming a gel-like structure.[14] This helps in preventing the separation of water from the soy protein particles, contributing to the overall stability of the system. The presence of glucomannan can increase the viscosity of the soy milk. This enhanced viscosity helps in maintaining a uniform suspension of soy protein particles, preventing settling or aggregation.[15]

The interaction mechanism between the soy milk ratio and porang gel is that when the soy milk ratio is low and the porang gel concentration is high, the emulsion system becomes more stable. This is because a lower soy milk ratio means reduced protein content in the soy milk, which decreases sedimentation, and the glucomannan in the porang gel can bind water molecules in the soy milk, preventing phase separation. However, as the volumetric ratio of soy milk decreases, the nutritional content of the soy milk product will also decrease, although the presence of glucomannan from porang gel provides benefits as a source of dietary fiber.

Table 3: ANOVA results for physical stability of soy milk

Source	Sum of square	df	Mean square	f-value	p-value	Significance
Model	0.7674	5	0.1535	24.79	0.0003	Significant
A - Volumetric ratio of soy milk	0.0937	1	0.0937	15.13	0.0060	Significant
B - Concentration of porang gel	0.4192	1	0.4192	67.72	<0.0001	Significant
AB	0.0025	1	0.0025	0.4038	0.5453	Not significant
A^2	0.0459	1	0.0459	7.42	0.0296	Significant
B^2	0.2285	1	0.2285	36.92	0.0005	Significant
Residual	0.0433	7	0.0062			
Lack of Fit	0.0433	3	0.0144			
Pure Error	0.0000	4	0.0000			
Cor total	0.8108	12				

Table 4: ANOVA results for viscosity of soy milk

Source	Sum of square	df	Mean square	f-value	p-value	Significance
Model	3.412E+05	5	68247.57	390.60	<0.0001	Significant
A - Volumetric ratio of soy milk	12611.46	1	12611.46	72.18	<0.0001	Significant
B - Concentration of porang gel	2.759E+05	1	2.759E+05	1579.23	<0.0001	Significant
AB	2756.25	1	2756.25	15.77	0.0054	Significant
A^2	2013.39	1	2013.39	11.52	0.0115	Significant
B^2	49686.00	1	49686.00	284.37	<0.0001	Significant
Residual	1223.07	7	174.72			
Lack of Fit	923.87	3	307.96	4.12	0.1026	Not Significant
Pure Error	299.20	4	74.80			
Cor total	3.425E+05	12				

Viscosity

The highest viscosity response is achieved with the treatment of relatively low soy milk ratio and high porang gel concentration, measuring 620 cP. The lowest viscosity response is observed with the treatment of high soy milk ratio and low porang gel concentration, measuring 75 cP. The ANOVA results shows that the soy milk ratio has a p-value of <0.0001, and the porang gel concentration has a p-value of <0.0001, indicating that both the soy milk ratio and porang gel concentration significantly influence the viscosity response. The interaction AB has a p-value of 0.0054, indicating that the interaction significantly affects the viscosity response. The soy milk ratio squared variable (A2) has a p-value of 0.0115, and the porang gel concentration squared variable (B2) has a p-value of <0.0001, meaning that these variables also have a significant impact on the viscosity response (Table 4).

The ratio of soy milk ratio factor significantly influences viscosity response (Figure 1B). The higher the soy milk ratio, the more the viscosity will decrease or become more liquid. When the soy milk ratio is increased, the proportion of porang gel will decrease, resulting in a reduction of glucomannan content in the soy milk as well. The concentration of porang gel significantly influences viscosity response. The higher the concentration of porang gel, the more the viscosity will increase or become thicker. Glucomannan is a water-soluble dietary fiber that has thickening and gelling properties.[19] When added to soy milk, it increases viscosity, giving the milk a thicker consistency. If you decrease the concentration of glucomannan in soy milk, there will be fewer thickening agents present, resulting in a lower viscosity.

The interaction between the volumetric ratio of soy milk and porang gel concentration has a significant effect on the viscosity response. A lower soy milk ratio and a higher porang gel concentration will increase viscosity. The viscosity level of the soy milk product needs to be considered, as excessively high viscosity can make the product more difficult to flow and may reduce consumer preference.

Radical Scavenger Activity (Ic_{50})

The IC_{50}, which stands for half-maximal inhibitory concentration, quantifies the level of a substance, like an antioxidant, required to reduce a specific biological or chemical response by 50%. A lower IC_{50} value indicates higher antioxidant activity because it means that a lower concentration of the antioxidant is needed to effectively neutralize or reduce the oxidative activity by 50%. The results of the ANOVA show that the soy milk ratio (A) has a p-value <0.0001 and the concentration of porang gel (B) has a p-value of 0.0137, indicating that the soy milk ratio and the concentration of porang gel significantly affect the antioxidant activity response. The interaction value AB has a p-value of 0.5265, indicating that the interaction does not significantly affect the antioxidant activity response. The squared soy milk ratio variable (A2) has a p-value of 0.0421, and the squared porang gel concentration (B2) has a p-value of 0.0024, meaning that these variables significantly influence the antioxidant activity response (Table 5).

Table 5: ANOVA results for IC50 of soy milk

Source	Sum of square	df	Mean square	f-value	p-value	Significance
Model	92779.89	5	18555.98	76.08	<0.0001	Significant
A - Volumetric ratio of soy milk	2606.78	1	2606.78	10.69	0.0137	Significant
B - Concentration of porang gel	83949.47	1	83949.47	344.17	<0.0001	Significant
AB	108.16	1	108.16	0.4434	0.5265	Not Significant
A^2	1502.72	1	1502.72	6.6	0.0421	Significant
B^2	5240.80	1	5240.80	21.49	0.0024	Significant
Residual	1707.41	7	243.92			
Lack of Fit	1275.86	3	425.29	3.94	0.1092	Not Significant
Pure Error	431.55	4	107.89			
Cor total	94487.30	12				

The ratio of soy milk significantly affects the antioxidant activity response (Figure 1C). The higher the soy milk ratio, the stronger the antioxidant activity. This is because this soy milk product contains ginger extract in this research, where ginger is known to have antioxidant activity. The antioxidant compound in ginger such as gingerol, zingerone, and shogaol were found to be highly active.[20] The concentration of porang gel significantly affects the antioxidant activity response. The higher the concentration of porang gel, the weaker the antioxidant activity. This is because porang gel does not contain compounds that are suspected to function as antioxidants. The antioxidant value decreases as the concentration of porang gel increases because as more porang gel is used, the ratio of soy milk decreases, and antioxidant activity relies solely on soy milk with the addition of ginger extract. Therefore, the determination of the porang gel addition level needs to be considered carefully, ensuring it is at the right concentration to provide product stability while maintaining optimal antioxidant content.

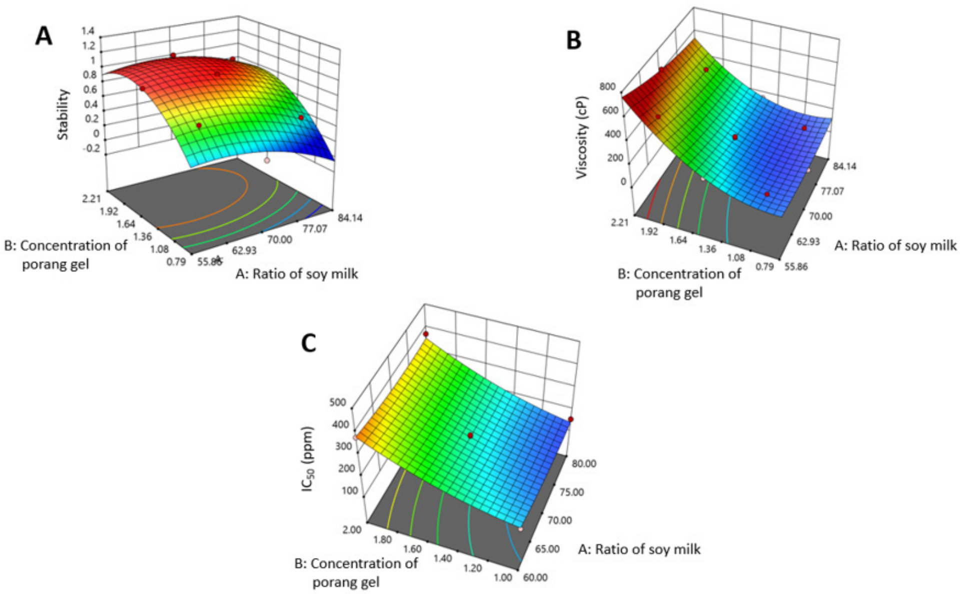

Fig. 1: Response surface plots for stability (A), viscosity (B) and IC_{50} (C) of soy milk as a function of ratio of soy milk and concentration of porang gel

Predicted Optimization and Verification

The determination of the optimum point is based on the highest values of physical stability and the lowest values of viscosity and antioxidant activity responses. The results showed that the optimum condition was as follow, the predicted optimum soy milk volumetric ratio was 69.3 (v/v) and porang gel concentration was 1.3%. These results produce soy milk with the physical stability of 0.87, viscosity of 123.8 cP, and it has an antioxidant activity with an IC_{50} value of 183.8 ppm with the desirability value of 0.85. The verification experiments were conducted in three replications. The results showed that physical stability, viscosity and IC_{50} value of soy milk were 0.90±0.05, 132.0±5.5 cP and 187.5±35.2 ppm, respectively. The values closely aligned with the model's predictions, showing a difference of less than 5%. The consistency of responses, with less than a 5% variance between predicted and experimentally obtained results, affirmed the model's accuracy.

Conclusion

Physical stability, viscosity, and IC_{50} value of the soy milk fortified with ginger extract were affected by the volumetric ratio of soy milk and concentration of porang gel. The experimental results show that as the volumetric ratio of soy milk increases, the physical stability value of the soy milk decreases, leading to a more unstable emulsion. Conversely, a higher concentration of porang gel enhances the stability of the soy milk. As the volumetric ratio of soy milk increases, the viscosity decreases, making the mixture more liquid. Conversely, as the concentration of porang gel increases, the viscosity rises, making it thicker. The antioxidant activity becomes stronger if the volumetric ratio of soy milk increases. In contrast, as the concentration of porang gel increases, the antioxidant activity becomes weaker. The findings indicated that the optimal conditions were as follows: the predicted optimal volumetric ratio for soy milk was 69.3 (v/v), and the porang gel concentration was 1.3%. As a continuation of this research, sensory testing can be conducted to determine how well this soy milk product is accepted by consumers in terms of taste and preference.

Acknowledgment
The authors would like to thank to all lecturers and laboratory staff of the Department of Food Science and Biotechnology, Faculty of Agricultural Technology, Universitas Brawijaya who helped in the implementation of this research.

Funding Sources
The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest
The authors declares no conflict of interest.

Authors' Contribution
Ahmad Zaki Mubarok - Conceptualization and design of the study, laboratory supervision, interpretation of data, and writing the article. Ocha Salma Samesta – Conceptualization and design of the study, laboratory experiments, data curation and interpretation of data.

Data Availability Statement
The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement
The document accurately and thoroughly presents the authors' original research and analysis.

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