Formula Optimization of Traditional Functional Beverage *Minasarua* from Bima West Nusa Tenggara Indonesia

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Abstract

Minasarua is a traditional beverage from Bima, West Nusa Tenggara Indonesia which consists of spices, fermented black glutinous rice (FBGR), *tai mina* (dried *blondo*) and palm sugar. The product is consumed by local people with various formulas, especially in terms of types and quantities of spices and FBGR. This study aimed to optimize the formula of *Minasarua* which has optimum antioxidant activity. The method used was D-Optimal Mixture Design using Design Expert 13 software with independent variables, namely ginger, turmeric, Javanese chili pepper (JCP), pepper and FBGR. There were 21 formulas of *Minasarua* with different percentages of each component observed in this research. The antioxidant activity (DPPH method), total phenolic content (Folin Ciocalteu method), total flavonoid content (aluminum chloride colorimetric assay) and total anthocyanin (pH differential method) were tested using a spectrophotometer in response to all the formulas. The results of the optimization showed that *Minasarua* with 16.59 % ginger, 0.30 % turmeric, 4.50 % JCP, 3.83 % pepper and 74.78 % FBGR were able to respond to the antioxidant activity of 51.23 mg TE/100 g, with total phenolic, flavonoid and anthocyanin were 112.03 mg GAE/100 g, 278.31 mg QE/100 g and 1.14 mg/100 g, respectively, and the desirability of 0.98. There was no significant difference between the predicted and verification values of the 4 responses. Therefore, this formula is recommended as the best formula which has an optimum antioxidant activity to produce *Minasarua* as a functional beverage.

Keywords: Antioxidant, Formula, *Minasarua*, Optimization

Introduction

Minasarua is a traditional beverage from Bima, West Nusa Tenggara, Indonesia, which is usually consumed in warm conditions and intended to warm the body [1]. The product has authentic characteristics that are different from other traditional drinks. The uniqueness of *Minasarua* can be seen from its yellowish-brown color and its sweet-spicy-slightly sour taste. Those sensory properties come from its constituent components, namely spices, FBGR, *tai mina* (dried *blondo*) and brown sugar. Several spices are used in *Minasarua*, such as ginger, turmeric, pepper and JCP. Those spices can potentially reduce free radicals.

Spices are part of plants that are rich in bioactive compounds and have a distinctive taste [2,3]. For instance, ginger (*Zingiber officinale* Rosc.) contains powerful antioxidants that can eliminate free radicals. This role comes from bioactive compounds, especially gingerol, shogaols, paradols and zingerone [4]. The antioxidant role of turmeric (*Curcuma longa* Linn) is estimated to come from the content of bioactive compounds of the phenol group, curcuminoids. There are 3 main curcuminoid contents in turmeric, namely curcumine (60 - 70 %), demethoxycurcumin (20 - 27 %) and bisdemethoxicurcumin (10 - 15 %) [5]. Apart from curcuminoids, several minor bioactive compounds are also found in turmeric, such as caffeic acid, catechin, epicatechin, chlorogenic acid, cinnamic acid, coumarin, ferulic acid, gallic acid, genistein, myricetin and quercetin [6]. Curcumin becomes the main constituent of the turmeric extract, which has important properties for therapeutic development. Pepper (*Piper nigrum*) and JCP (*Piper retrofractum* Vahl *syn. Piper chaba* Hunter) belong to the *Piperaceae* family, which contains natural compounds that are beneficial for the body [7]. The *Piperaceae* group has been reported to have antioxidant, antibacterial, antitumor, antimutagenic, antidiabetic and antiinflammatory properties [8]. Therefore, the gingerol compound in ginger [9], curcumin and quercetin in turmeric [5,6], and piperin in pepper and JCP [10] are predicted to have the capability of being antioxidants for the body.

Minasarua not only consists of various types of spices but also contains FBGR. The FBGR contains lactic acid bacteria (LAB), which act as probiotic bacteria [11]. Consuming foods containing LAB has a positive impact as a booster of the body's immune system [12]. Anthocyanins in black glutinous rice (BGR) are also a flavonoid group [13] that will support the antioxidant activity of the product. So, the constituent inside of *Minasarua* reasonably becomes the agent of antioxidants in the body. Those facts are also in line with empirical studies in the local community. The local community empirically believes that consuming *Minasarua* can increase body stamina and prevent fatigue after a day's activities [14].

The benefits of *Minasarua* consumption can be reached by formulating a great recipe for *Minasarua*. A great recipe will affect the quality of the product, especially in terms of functional properties. Previous research showed that combining several types of spices, such as ginger, cardamom, lemongrass and cloves, in traditional drinks demonstrated higher amounts of phenolic compounds and the ability to scavenge free radicals compared to traditional drinks with fewer spices [15,16]. The formulation of various types of spices to make a drink or food product has been widely researched; for example, *wedang uwuh*, which consists of red ginger, cloves, sappan wood, black grass jelly leaves and soursop leaves [17]. However, research combining various types of spices and fermented products in traditional beverages is potentially rich in bioactive compounds. Therefore, it is necessary to optimize the formula of *Minasarua* products in order to obtain the best functional properties.

No one has made an optimization regarding the *Minasarua* formula. In Bima, local producers make *Minasarua* with different recipes (including different types of spices, the number of spices and the number of FBGR). This condition causes the diverse quality of *Minasarua* in terms of sensory and functional properties. A high concentration of spices contributes to high antioxidant activity but increases the pungency taste. Formula optimization is needed to combine all materials in a great formulation so functional properties can be reached. The optimization process is scientifically approached by using a D-optimal mixture design. D-optimal mixture design can be used to determine the optimum amounts of compositions in a food or pharmacy product toward the responses [18]. This design is applied in experiments when more substances are mixed to generate a new experimental region [19]. Consequently, the study aimed to optimize the formula of the *Minasarua* beverage, which has high antioxidant activity, total phenolics, total flavonoids and total anthocyanin.

Materials and methods

Materials

The spices used for *Minasarua* beverage were ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* Linn) and pepper (*Piper nigrum*) which were purchased from the local market in Malang, East Java, Indonesia, while dried JCP (*Piper retrofractum* Vahl syn. *Piper chaba* Hunter) was obtained from local market in Bima, West Nusa Tenggara, Indonesia. *Tai mina* (a residue that is left over after coconut oil extraction) and coconut oil were also purchased from a local producer in Bima. Furthermore, BGR, *ragi tape* (a local dried starter containing yeast), palm sugar and mineral water were purchased from the local market in Malang. The chemicals used in the test were DPPH (2,2-diphenyl picrylhydrazyl), quercetin, trolox, gallic acid, $AlCl_3$, $NaNO_2$, Na_2CO_3, NaOH, KCl and $CH_3CO_2Na.3H_2O$ (Merck); methanol and ethanol (Smart Lab); Follin ciocalteau (Merck); and HCL 2N.

Experimental methods

The research design in this experiment used D-Optimal Mixture Design with Design Expert 13 software. The aim was to obtain a model that could explain the interaction between the spices ratio variables and FBGR used for the responses in this study, namely antioxidant activity, total phenolics, total flavonoids and total anthocyanins. Optimization begins with determining the limitations that will be used. Determination of the limit is obtained based on the percentage of each component from 3 *Minasarua* products sold by local producers. Based on the references, the lowest and highest values for each component served as the minimum and maximum limits in the design, as presented in **Table 1** (reducing the scale to 1:100). Furthermore, the data from **Tabel 1** was processed and gained 21 new formulations like in **Table 2**.

Table 1 Minimum limits (low) and maximum limits (high) of each component.

Composition	Low (%)	High (%)
Ginger	10	30
Turmeric	0.1	0.3
Javanese chili pepper (JCP)	1.5	4.5
Pepper	1.5	4.5
Fermented black glutinous rice (FBGR)	60	80

Table 2 Formulation results of optimization *Minasarua* with a design expert.

Run	Ginger (%)	Turmeric (%)	JCP (%)	Pepper (%)	FBGR (%)
1	10.77	0.23	4.50	4.50	80.00
2	23.57	0.30	4.50	4.50	67.13
3	30.00	0.10	2.50	1.50	65.90
4	13.90	0.10	2.50	3.50	80.00
5	13.70	0.30	1.50	4.50	80.00
6	13.90	0.10	4.50	1.50	80.00
7	30.00	0.23	4.50	1.50	63.77
8	30.00	0.30	1.50	1.50	66.70
9	16.77	0.23	1.50	1.50	80.00
10	13.70	0.30	1.50	4.50	80.00
11	10.77	0.23	4.50	4.50	80.00
12	30.00	0.10	4.50	4.50	60.90
13	30.00	0.10	1.50	4.50	63.90
14	21.27	0.10	1.50	1.50	75.63
15	21.15	0.20	3.00	4.50	71.15
16	18.13	0.30	4.50	3.50	73.57
17	25.63	0.10	1.50	1.50	71.27
18	15.20	0.30	3.00	1.50	80.00
19	30.00	0.23	1.50	3.50	64.77
20	30.00	0.30	4.50	4.50	60.70
21	13.90	0.10	4.50	1.50	80.00

Minasarua beverage preparation
Fresh spices of ginger and turmeric were washed, drained, and peeled to remove the skin and physical contaminants. Dried spices of pepper and JCP were sorted and cleaned from any dust or dirt. Cleaned ginger, turmeric, pepper and JCP were ready to weigh based on the formulation of every run.

Preparation of FBGR
BGR was washed with tap water and soaked for 3 h [16]. After draining the water, BGR was steamed for 40 min. Subsequently, the steamed BGR was cooled at room temperature for about 40 min. After that, cold steamed BGR was inoculated with 1.5 % (w/w) of ragi tape and ready to be yeasted and fermented for 2 days in a closed container. The FBGR also weighed based on the formula.

Making *Minasarua*

The process of making *Minasarua* began with grinding all spices (ginger, turmeric, pepper and JCP) in 20 mL of mineral water using a blender. This process was carried out for 1 min until the spices became puree. A 500 mL beaker glass was placed on a 300-watt electric stove which had been turned on. Ten mL of coconut oil was put into the beaker glass and waited for a while until it was hot enough. The pureed spices were put into the beaker glass containing hot coconut oil to saute for 2 min. The remaining pureed spices in the blender were rinsed using 10 mL of mineral water and then added to the sauteed spices. Next, 50 mL brown sugar water and 20 mL mineral water were added to the spice mixture, and cooked for 5 min at 80 - 95 °C. After that, FBGR was added to the mixture and cooked for 2 min. The 5 g of *tai mina* (dried *blondo*) were entered into the mixture and cooked for 1 min. The *Minasarua* is ready.

Sample preparation

Samples were prepared by weighing 0.5 g of sample and dissolving in 5 mL of aquades (solvent). The sample was homogenized and centrifuged at 6,000 rpm for 20 min [20]. After the centrifugation process, the supernatant (liquid) was separated from the pellets (solid). The part of the supernatant was used to test antioxidant activity, total phenolics, total flavonoids and total anthocyanins.

Antioxidant activity

The antioxidant activity of the *Minasarua* beverage was measured using DPPH radical scavenging capacity method [21] with certain modifications. Zero-point 5 mL of the sample was mixed with 2.85 mL of 30 µg/mL of DPPH radical solution (in methanol). The mixture was incubated for 30 min in the dark at 25 ± 2 °C. After incubation, the absorbance of the mixture was measured using a spectrophotometer at 517 nm. Trolox (concentrations 0, 50, 100, 150, 200 and 250 µg/mL) was used as the standard curve. Each treatment was performed in triplicate. The result was expressed as mg trolox equivalent (TE)/100 g dry weight.

Total phenolic content

The total phenolic content of *Minasarua* beverage was determined by the Folin-Ciocalteu method [22,23] with some modifications. First, 0.5 mL of the sample solution was mixed with 2.5 mL of 10 % Folin Ciocalteau reagent, and incubated for 5 min at 25 ± 2 °C. Second, 2 mL of 7.5 % Na_2CO_3 was added to the mixture and then incubated for 30 min. At the end, the absorbance was measured at 756 nm using a spectrophotometer. Gallic acid (concentrations 0, 20, 40, 60, 80 and 100 µg/mL) was used as a standard curve. Every sample was measured in triplicate and the results are expressed as mg gallic acid equivalent (GAE)/100 g dry weight.

Total flavonoid content

The total flavonoid content of the *Minasarua* beverage was measured using aluminum chloride colometric assay [24] with modification. One mL of the sample solution was diluted with 4 mL of distilled water, then added with 0.3 mL of 5 % $NaNO_2$. The mixture was incubated for 5 min. Subsequently, 0.3 mL of 10 % $AlCl_3$ was added into the mixture, then vortexed and incubated for 6 min. After that, 2 mL of 1 M NaOH and 2.4 mL of distilled water were added, and incubated again at 25 ± 2 °C for 15 min. After incubation, the absorbance was measured using a spectrophotometer at 322 nm with each sample measured in triplicate. Quercetin (concentrations 0, 20, 40, 60, 80 and 100 µg/mL) was used as a standard curve. The result was expressed as mg quercetin equivalent (QE)/100 g dry weight.

Total anthocyanin

The total anthocyanin was determined by using the pH differential method [25]. The anthocyanin analysis procedure used 2 types of buffer solutions, namely potassium chloride (KCL) buffer solution (0.025 M, pH 1) and sodium acetate trihydrate ($CH_3CO_2Na \cdot 3\ H_2O$) buffer solution (0.4 M, pH 4.5). pH adjustment in making potassium chloride and sodium acetate buffers using concentrated HCL 2N. Two test tubes were prepared. Each test tube was filled with 1 mL of sample. Next, 4 mL of potassium chloride buffer solution (0.025 M) pH 1 was added to the 1st test tube and 4 mL of sodium acetate buffer solution (0.4 M) pH 4.5 in the 2nd test tube. The solution was left for 15 min. After incubation for 15 min, the absorbance of both pH treatments was measured using a spectrophotometer at 510 and 700 nm. The absorption value was expressed as mg/100 g dry weight.

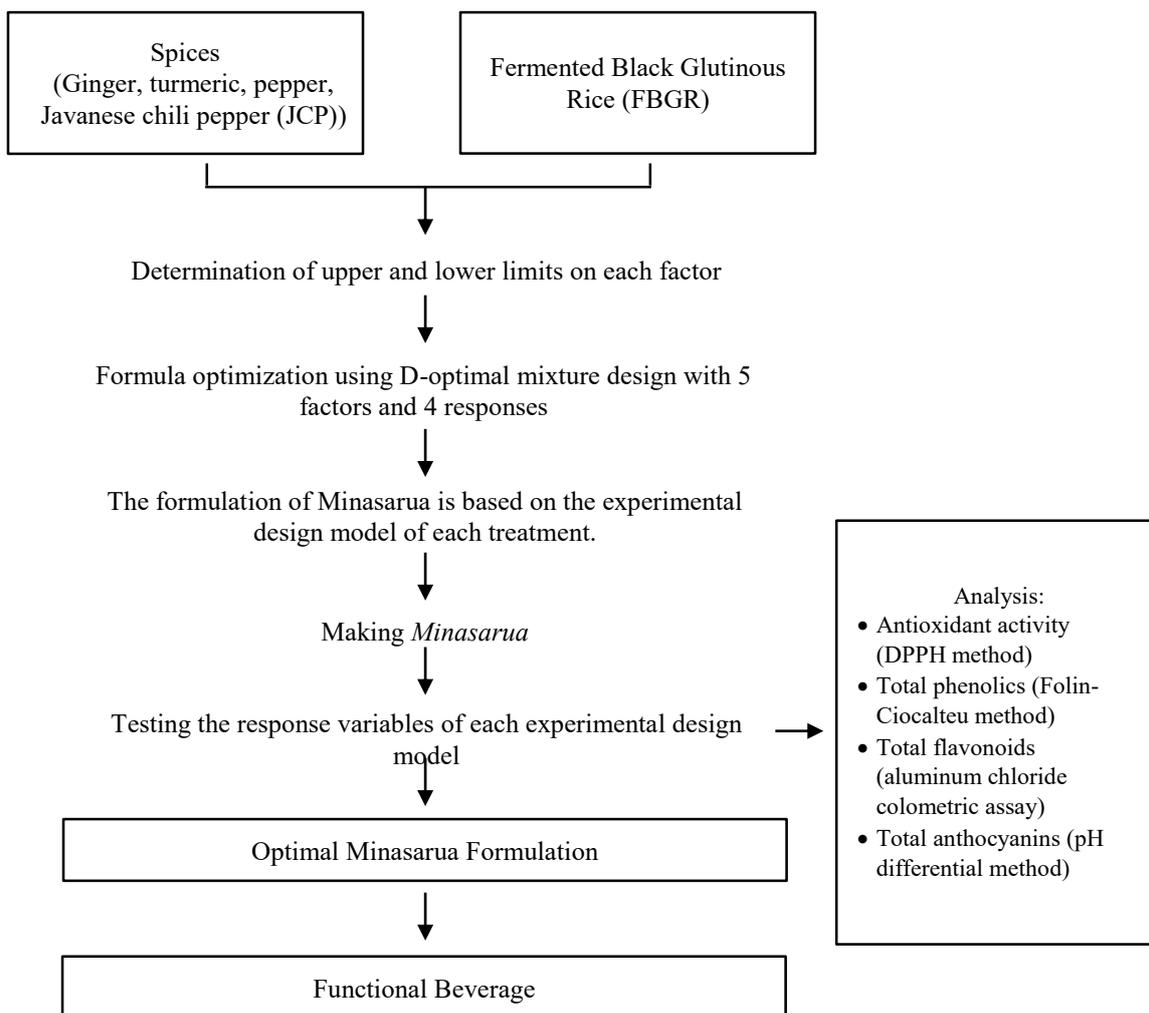The overview flow chart of this study can be seen in **Figure 1**.

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┌─────────────────────────────┐     ┌─────────────────────────────┐
│          Spices             │     │  Fermented Black Glutinous  │
│ (Ginger, turmeric, pepper,  │     │        Rice (FBGR)          │
│ Javanese chili pepper (JCP))│     │                             │
└─────────────────────────────┘     └─────────────────────────────┘
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Determination of upper and lower limits on each factor

Formula optimization using D-optimal mixture design with 5 factors and 4 responses

The formulation of Minasarua is based on the experimental design model of each treatment.

Making *Minasarua*

Testing the response variables of each experimental design model

Analysis:
- Antioxidant activity (DPPH method)
- Total phenolics (Folin-Ciocalteu method)
- Total flavonoids (aluminum chloride colometric assay)
- Total anthocyanins (pH differential method)

Optimal Minasarua Formulation

Functional Beverage

Figure 1 The overview flow chart of formula optimization *Minasarua*.

Results and discussion

The results of the reformulation *Minasarua* based on the Design Expert Program with the D-optimal Mixture method in the response model are listed in **Table 3**. In addition, every parameter is analyzed statistically so that the analysis of variance in terms of the response model is presented in **Table 4**. The response models are the antioxidant activity, total phenolic content, total flavonoid content and total anthocyanin. The quadratic model is suggested by the Design Expert for every single response.

Table 3 Results of antioxidant activity, total phenolic, total flavonoid and total anthocyanin of each *Minasarua* formula.

Run	Antioxidant activity (mg TE/100 g)	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg QE/100 g)	Total anthocyanin (mg/100 g)
1	43.35	102.31	178.74	0.88
2	46.25	104.92	212.07	0.86
3	33.60	79.94	89.19	0.48

Run	Antioxidant activity (mg TE/100 g)	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg QE/100 g)	Total anthocyanin (mg/100 g)
4	30.25	64.41	93.33	0.30
5	39.35	91.62	115.05	0.71
6	34.64	84.71	105.59	0.56
7	30.50	69.29	94.05	0.33
8	28.78	53.92	72.97	0.18
9	33.67	81.24	88.56	0.56
10	41.03	95.44	123.51	0.76
11	42.89	98.74	175.77	0.86
12	40.00	93.62	120.09	0.23
13	39.92	92.76	117.93	0.71
14	39.85	91.86	126.67	0.86
15	36.25	85.26	106.13	0.61
16	52.67	108.15	282.61	1.11
17	41.96	98.22	142.25	0.86
18	31.71	75.40	105.77	0.56
19	32.67	77.33	100.00	0.43
20	33.64	80.14	102.79	0.35
21	38.46	90.25	108.65	0.58
Lower limit	28.78	53.92	72.97	0.18
Upper limit	52.67	108.15	282.61	1.11

Table 4 The analysis of variance of the response quadratic model.

Responses	*p*-value of model	*p*-value lack of fit	R^2	Adjusted R^2	Predicted R^2	Adequate precision
Antioxidant Activity	< 0.0001	0.5844	0.9696	0.9241	0.7504	16.4216
Total Phenolic Content	< 0.0001	0.8682	0.9905	0.9682	0.8535	26.4579
Total Flavonoid Content	< 0.0001	0.3471	0.9975	0.9918	0.8264	55.6588
Total Anthocyanin	< 0.0001	0.3538	0.9960	0.9867	0.8279	37.9062

Antioxidant activity analysis

The mixture design analysis recommended a quadratic model to be used for the antioxidant activity response. This quadratic model was significant with a *p*-value < 0.05, which was < 0.0001, as shown in **Table 4**. The significant result indicated that there was a significant effect of the components (ginger, turmeric, JCP, pepper and FBGR) on the response of antioxidant activity in the *Minasarua* formula. The significance model was supported by an insignificant Lack of Fit. This indicates that the model presented was appropriate to the results of the ANOVA model. If the model influences the response, the data mismatch should not have an effect. Therefore, the lack of fit result obtained in this analysis was very good and supported the results of the ANOVA model. Other data that strengthen the model's accuracy were R^2, adjusted R^2, predicted R^2 and adequate precision. The R^2 value was 0.9696. This value indicates that the factors (X) were able to influence the response (Y) by 96.96 %. Only 3.04 % of the response was influenced

by other factors. The high R^2 value could also be an indicator that the data was good enough, meaning that it was not spread out (has been homogeneous). Not only that, the difference between adjusted R^2 and predicted R^2 was less than 0.20, which means that the predicted R^2 of 0.7504 was in reasonable agreement with the adjusted R^2 of 0.9241 and it was fit to the model. For adequate precision (signal-to-noise ratio measurements) offered adequate signal because the result (16.4216) was more than 4 and this model could be used to navigate the design space. Furthermore, **Figure 2** displayed a normal plot of residuals. This normal plot was figuring a good data distribution because all points (runs) approached the linear line.
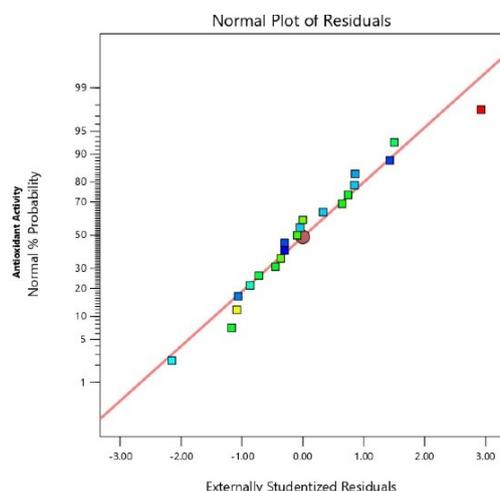
Figure 2 Normal graph plot of residual response antioxidant activity.

The synergistic and antagonistic interaction between all components in the mixture formula is displayed in the equation below.

$$Y = - 4.38\ A + 25766.40\ B + 300.25\ C + 2.87\ D - 0.16\ E - 262.49\ AB$$
$$- 3.24\ AC + 0.10\ AE - 256.09\ BC - 255.11\ BD - 257.96\ BE - 3.07\ CD - 3.14\ CE \qquad (1)$$

Notes: A = ginger, B = turmeric, C = JCP, D = pepper and E = FBGR.

A positive value in the regression equation indicates a beneficial effect on optimization because it is synergistic, while a negative value indicates an opposite or antagonistic effect between the factor and response. Based on that equation, it showed that the linear coefficients (turmeric, JCP and pepper) and quadratic coefficients (ginger and FBGR or AE) have a positive effect on the response of antioxidant activity. This positive effect means each component, which gave positive value, could work synergistically to provide high antioxidants in the product. This condition is also found in **Figure 3**.
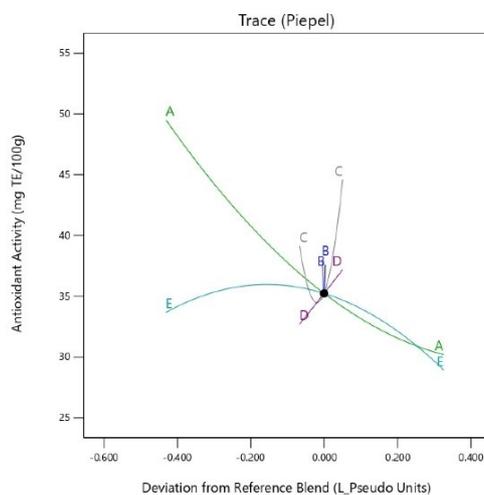

Figure 3 Trace blend factors of response antioxidant activity.

Figure 3 demonstrates the relationship between all components in the product. Based on the equation and **Figure 3**, ginger and FBGR showed a decreasing graph, which means adding ginger and FBGR individually could decline the value of antioxidant activity in the *Minasarua* product because the percentages of those components in the formula were high enough and it will give impact on percentages of other components. On the other hand, adding turmeric, JCP and pepper to the formula potentially increases the antioxidant activity of *Minasarua* product. The antioxidant activity of turmeric comes from the presence of electron donor groups, particularly the phenolic hydroxyl groups. This group is an important component to increase the antioxidant activity of turmeric [26]. In addition, the presence of carbon-carbon double bonds, β-diketo groups and phenyl ring with hydroxyl groups and o-methoxy also contribute to the antioxidative properties of turmeric [27]. Pepper and JCP belong to the *Piperaceae* family, the *Piper* genus which contains natural compounds that are beneficial to the body [7]. The *Piperaceae* group has been reported to have antioxidant, antibacterial, antitumor, antimutagenic, antidiabetic and anti-inflammatory properties [8]. Overall, the effect of blending all components on antioxidant activity is plotted in **Figure 4**.
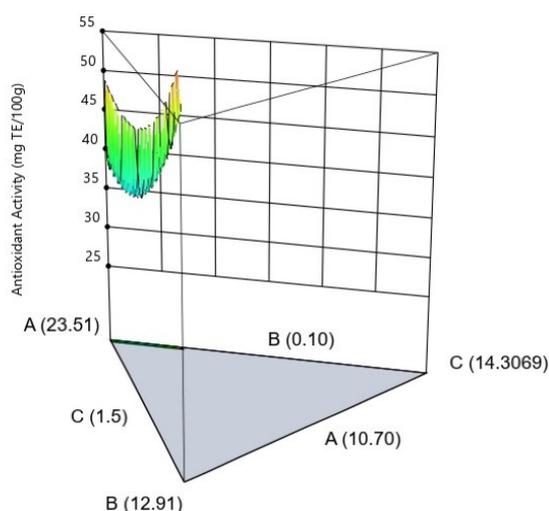
Figure 4 3D surface plot of response antioxidant activity.

Total phenolic content analysis

The range of total phenolic content in 21 runs was 53.9244 - 108.151 mg GAE/100 g, as shown in **Table 3**, and they have a normal distribution, as figured in **Figure 5**. Those data statistically were significant, because the *p*-value was < 0.0001, displayed in **Table 4**. Moreover, the program analysis suggested a quadratic model for the total phenolic content response. The quadratic model will give the optimum peak when all components are combined. This quadratic model has been tested for model accuracy through lack of fits, R^2, adjusted R^2, predicted R^2 and adequate precision data. The lack of fits (0.8682) exhibited insignificant data, which means that the divergence of data was minimal and did not affect the model. The R^2 (0.9905), adjusted R^2 (0.9682), predicted R^2 (0.8535) and adequate precision (26.4579) also gave a good value that supports the model. Thus, a quadratic model was recommended for this response to find an optimum formula that will provide high total phenolic content.
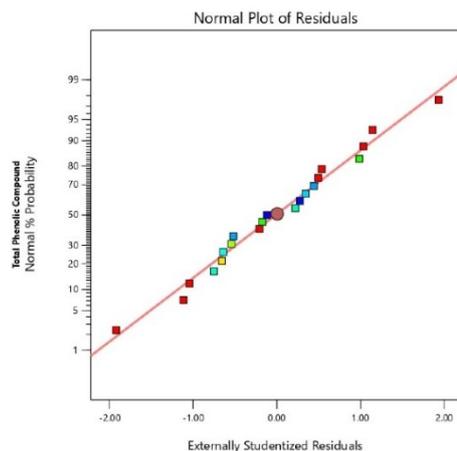
Figure 5 Normal graph plot of residual response total phenolic content.

The high total phenolic content product could be produced by combining all components (ginger, turmeric, JCP, pepper and FBGR). The interaction between all components in the mixture formula may give a synergistic or antagonistic effect on the total phenolic content of the product. This reaction could be predicted from the equation below.

$$Y = -5.99\,A - 3707.79\,B + 577.76\,C - 34.88\,D + 0.04\,E + 25.23\,AB - 6.23\,AC$$
$$+ 0.55\,AD + 0.16\,AE + 42.15\,BC + 72.99\,BD + 38.71\,BE - 5.46\,CD - 6.06\,CE + 0.29\,DE \qquad (2)$$

Notes: A = ginger, B = turmeric, C = JCP, D = pepper and E = FBGR.

Based on the equation, the linear coefficient (JCP and FBGR) and quadratic coefficients (ginger and turmeric or AB; ginger and pepper or AD; ginger and FBGR or AE; turmeric and JCP or BC; turmeric and pepper or BD; turmeric and FBGR or BE; pepper and FBGR or DE) have a positive value on the response of total phenolic content. It means the interaction between them possible to produce high total phenolic content synergistically. Furthermore, the effect of each component on the total phenolic content is figured out in **Figure 6**.
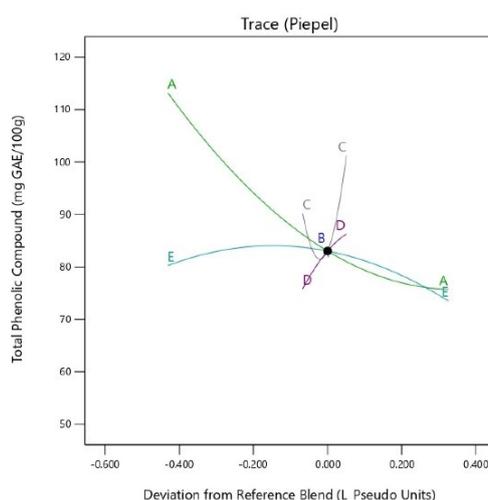
Figure 6 Trace blend factors of response total phenolic content.

The trace blend factors of response total phenolic content in **Figure 6**. presented a similar diagnostic with antioxidant activity results. The difference between them is just in factor B (turmeric), which was stagnant at that point, and did not give any increase. This condition happened because of the phenolic compound inside of components. According to the results from the equation and **Figure 6**, JCP was

becoming an element that promoted positive and high values for total phenolic content. JCP contains phenolic compounds such as 5-O-caffeoylquinic acid, 4-p-coumaroylquinic acid, 5-p-coumaroylquinic acid, chavibetol, hydroxychavicol and phenolic acid (vanillic, caffeic, ferulic, protocatechuic and rosmarinic acid) [28]. Not only containing various types of phenolic compounds, JCP also contains piperine, which can increase the bioavailability of other polyphenolic compounds. This capability was presented by [29], who found that piperine in *Piper* species is able to increase the bioavailability of curcumin. Panahi *et al.* [30] clarified that the increase in curcumin absorption by piperine was caused by piperine's ability to reduce the activity of the glucuronidase enzyme. The glucuronidase enzyme plays a role in the glucuronidation process, which causes curcumin to not be optimally absorbed by the body because it is considered a foreign substance (xenobiotic). So that, when piperine could reduce the enzyme, curcumin or other polyphenolic compounds can absorb optimally. Thus, it can be seen that the total phenol in the *Minasarua* reformulation product is influenced by its constituent components, especially JCP. The 3D surface plot of each component to the response of total phenolic content can be seen in **Figure 7**.
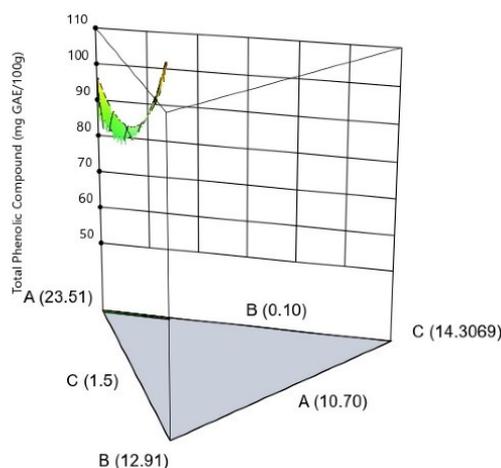

Figure 7 3D surface plot of response total phenolic content.

Total flavonoid content analysis

For the total flavonoid content response, a quadratic model was suggested by the program analysis. As indicated in **Table 4**, this quadratic model had a *p*-value < 0.0001. The notable outcome showed that the ingredients (ginger, turmeric, JCP, pepper and FBGR) had a substantial impact on the response in the *Minasarua* formula. An insignificant Lack of Fit (0.3471) supported the significance model. This suggests that the provided model was suitable for the ANOVA model's outcomes. Consequently, the lack of fit results from this study was excellent and corroborated the findings. R^2, adjusted R^2, predicted R^2 and adequate precision are additional data that increase the model's accuracy. R^2 came in at 0.9975. This value shows that the factors (X) were able to have a 99.75 % influence on the response (Y). Only 0.25 % of the responses were impacted by extraneous variables. Additionally, the adjusted R^2 (0.9918) and the predicted R^2 (0.8264) just only have differences of less than 0.2, indicating that the model was fit and the predicted R^2 of 0.750 was in an acceptable amount of agreement with the adjusted R^2. Moreover, the result of adequate precision (55.6588) was more than 4, this model could be utilized to travel the design space. Furthermore, a normal plot of the residuals is shown in **Figure 8**. Since all points (or runs) were close to a linear line, this normal plot accurately represented the data distribution.

The interaction between all components in the mixture formula presented in the equation below.

$$Y = -33.74\,A + 2.22\,B + 2004.34\,C - 1989.69\,D - 4.58\,E - 2264.41\,AB$$
$$-21.85\,AC + 20.96\,AD + 0.75\,AE - 2173.38\,BC - 2222.26\,BD - 2231.82\,BE \qquad (3)$$
$$+1.95\,CD - 21.01\,CE + 21.34\,DE.$$

Notes: A = ginger, B = turmeric, C = JCP, D = pepper and E = FBGR.

Based on the equation, the linear coefficient (turmeric and JCP) and quadratic coefficients (ginger and pepper or AD; ginger and FBGR or AE; JCP and pepper or CD; pepper and FBGR or DE) have a positive value on the response of total flavonoid content. It means the interaction between them possible to

produce high total phenolic content synergistically. Furthermore, the effect of each component on the total flavonoid content is figured out in **Figure 9**.
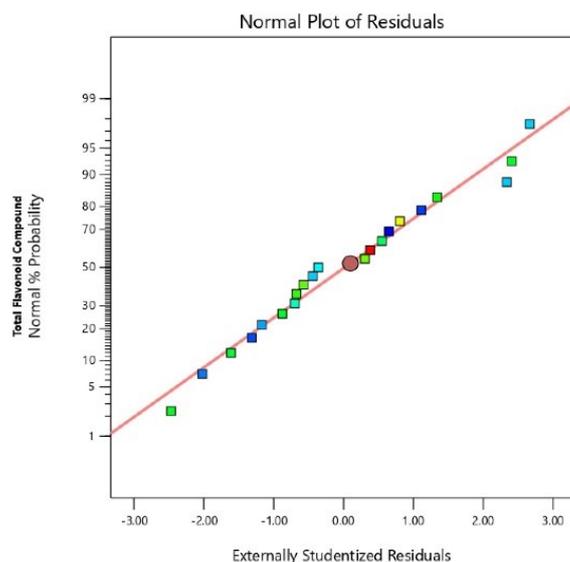

Figure 8 Normal graph plot of residual response total flavonoid content.
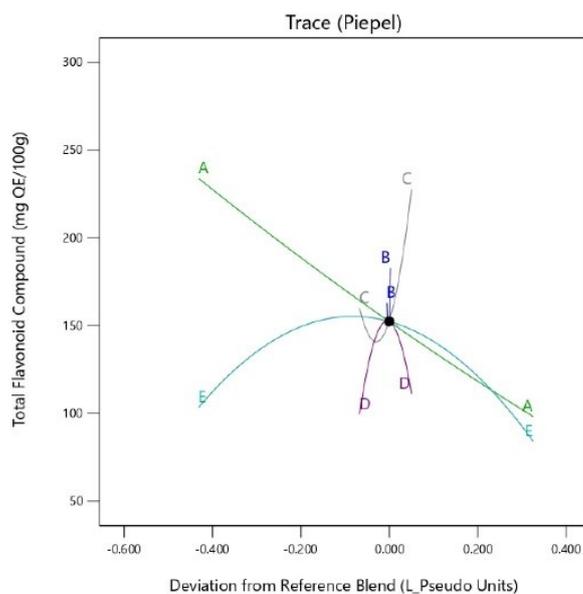

Figure 9 Trace blend factors of response total flavonoid content.

The equation and **Figure 9** figured that only turmeric and JCP have positive regression. This positive regression was shown from the rising amount of factors B and C after reaching the optimum point. Although other factors presented a declining graph while increasing percentages of components, the mixture of all components could produce high flavonoids. This high value of total flavonoid content in *Minasarua* was predicted to come from turmeric, JCP, pepper and FBGR. FBGR is forecasted to become part of the formula that produces high flavonoids because its interaction with pepper created positive regression. Anthocyanin in the FBGR included the flavonoid group. This bioactive compound supported the high value of flavonoids in *Minasarua*. Overall, the effect of blending all components on antioxidant activity is plotted in **Figure 10**.
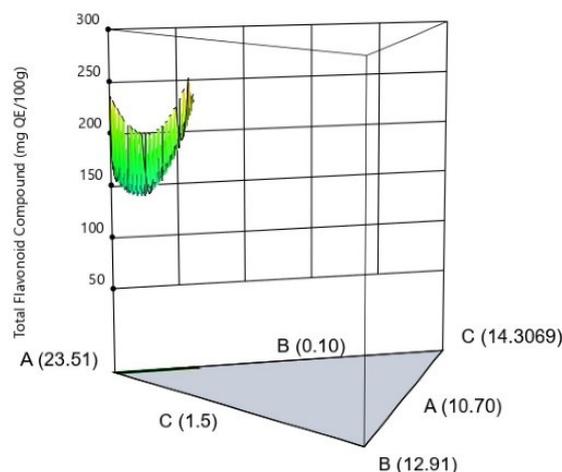
Figure 10 3D surface plot of response total flavonoid content.

Total anthocyanin analysis

The quadratic model was recommended by mixture design analysis for the total anthocyanin response. That quadratic model was significant with a *p*-value < 0.05, which was < 0.0001, as shown in **Table 4**. This significant result defined that the *Minasarua* formula's components (ginger, turmeric, JCP, pepper and FBGR) had a considerable impact on the total anthocyanin response. Additionally, an insignificant Lack of Fit (0.3538) supported the significance model. This reveals that the model was appropriate to the results of the ANOVA model. The R^2, adjusted R^2, predicted R^2 and adequate precision also strengthened the model's accuracy. The R^2 value was 0.9960. This value implied that the factors (X) were able to influence the response (Y) by 99.60 %. Only 0.40 % of the response was influenced by other factors. The high R^2 value could also be an indicator that the data was good enough, meaning that it was not spread out (has been homogeneous), as shown in **Figure 11**. In addition, the difference between adjusted R^2 and predicted R^2 was less than 0.2, which means that the predicted R² of 0.8279 was in reasonable agreement with the adjusted R² of 0.9867 and it was fit to the model. For adequate precision (signal-to-noise ratio measurements) offered adequate signal because the result (37.9062) was more than 4 and this model could be used to navigate the design space.
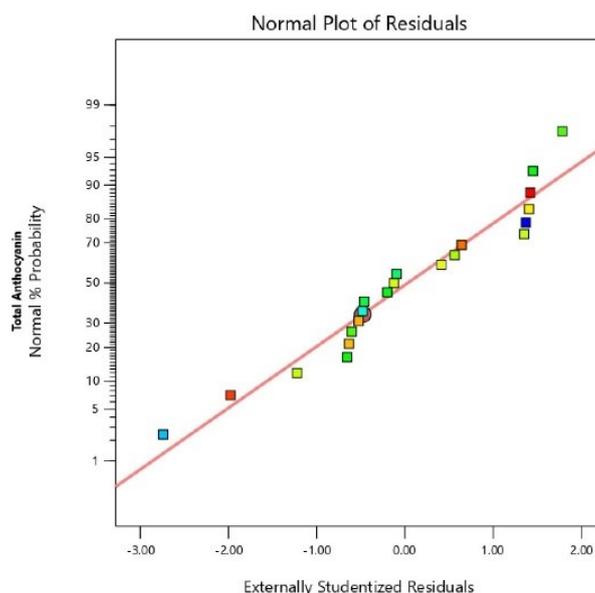

Figure 11 Normal graph plot of residual response total anthocyanin.

$$Y = -0.15\ A + 43.00\ B + 8.68\ C - 0.03\ D - 0.01\ E - 0.61\ AB - 0.097\ AC + 0.001\ AD \quad (4)$$
$$+ 0.003\ AE + 0.27\ BC - 0.003\ BD - 0.43\ BE - 0.10\ CD - 0.09\ CE + 0.0004\ DE.$$

Notes: A = ginger, B = turmeric, C = JCP, D = pepper and E = FBGR.

The synergistic and antagonistic interaction between all components in the mixture formula can be seen in the equation above. Based on the equation, turmeric, JCP, ginger and pepper or AD, ginger and FBGR or AE, turmeric and JCP or BC, and also pepper and FBGR or DE offered positive value. This positive effect means each component could work synergistically to provide high total anthocyanin in the product. Turmeric could contribute to the high value of anthocyanin because in the turmeric there anthocyanin which has a positive correlation with the flavonoid and antioxidant activity in the turmeric [31]. Actually, FBGR consists of high anthocyanin, but the fermentation process and cooking process while making *Minasarua* makes the degradation of anthocyanin inside of product [32]. Nevertheless, *Minasarua* products still have anthocyanin content inside of the product. **Figures 12** and **13** figure out the interaction between all components in the mixture formula. Based on **Figure 12**, the increasing quantity of FBGR can raise the total anthocyanin of the product, but after reaching the optimum point it will decline gradually.
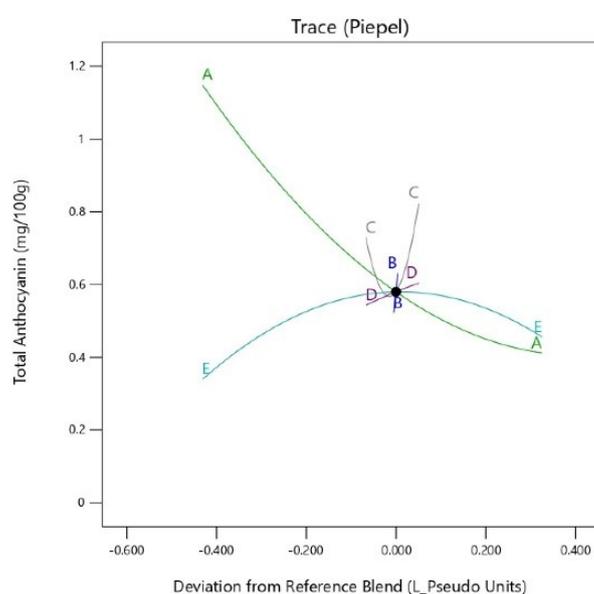

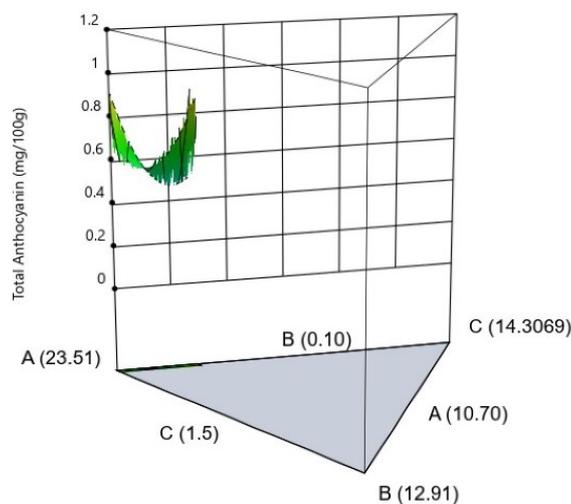**Figure 12** Trace blend factors of response total anthocyanin.

Figure 13 3D surface plot of response total anthocyanin.

Based on the analysis and explanation of the *Minasarua* formulation, there is a relationship between antioxidant activity, total phenolic content, total flavonoid content and total anthocyanin content. The relationship between all parameters is related to the chemical structure of the compound. The antioxidant activity of the product primarily comes from its constituents, which are rich in phenolic, flavonoid and anthocyanin compounds. Flavonoids and anthocyanin belong to the phenolic compounds group that have strong antioxidant activity [33,34]. The antioxidant activity of flavonoids arises from their high ability to scavenge most oxidizing molecules, including singlet oxygen and various free radicals [34]. The high flavonoid content of a product can increase the value of phenolic compounds and the antioxidant activity of the product. This condition is in line with the previous study that observed *Zingiberaceae* plants. Muflihah *et al.* [35] presented information that the antioxidant activity of *Zingiberaceae* plants emerged from their chemical composition because they were rich in total phenolic content, total flavonoid content, terpenoids, alkaloids and organic acids. The total phenolic and flavonoid content have been considered as contributors to antioxidant activity, mainly because of their special redox characteristics. This is also associated with the fact that the techniques used for assessing the antioxidant activities and the phenolic content are the same, namely using an electron transfer mechanism [33]. Therefore, it is known that the antioxidant activity increases proportionately with the quantity of phenolics.

Optimization formula of *Minasarua* product

The optimization formula was processed using Design Expert 13. All components were set up "in range", and all parameters were "maximize", as presented in **Table 5**. Those criteria were set to optimize functional properties in the product. The high value of antioxidant activity, total phenol, total flavonoid and total anthocyanin could be interpreted as the success of optimizing the formula. Therefore, the best solution for this optimization is presented in **Table 6**. That solution has a desirability of 0.98. This condition means that the predicted factors can give the maximum score of predicted responses, and around 98 % were influenced by suggested factors. Furthermore, the predicted value of the formula was verified using T-test analysis in Minitab, and the result showed an insignificant difference between the predicted and verified data. This T-test analysis provided information that the optimum data has been successfully verified.

Tabel 5 Materials and responses criteria.

Criteria	Goal	Lower Limit	Upper Limit
Ginger	in range	10.70	30.00
Turmeric	in range	0.10	0.30
Javanese chili pepper (JCP)	in range	1.50	4.50
Pepper	in range	1.50	4.50
Fermented Black Glutinous Rice	in range	60.70	80.00
Antioxidant Activity	maximize	28.78	52.67
Total Phenolic Content	maximize	53.92	108.15
Total Flavonoid Content	maximize	72.97	282.61
Total Anthocyanin Content	maximize	0.18	1.11

Table 6 The solution formula of *Minasarua* for all responses.

Ginger (%)	Turmeric (%)	Javanese chili pepper (%)	Pepper (%)	Fermented black glutinous rice (%)	Antioxidant activity (mgTE/100 g)	Total phenolic content (mgGAE/100 g)	Total flavonoid content (mgQE/100 g)	Total anthocyanin (mg/100 g)	Desirability
16.59	0.30	4.50	3.83	74.78	51.23	112.03	278.31	1.14	0.98

Conclusions

Optimizing the formula of *Minasarua* using the scientific method, specifically the D-optimal mixture design, can produce *Minasarua* with the best functional properties. The optimal formulation makes *Minasarua* as a functional beverage because of the existence of several functional parameters, such as antioxidants, phenolics, flavonoids and anthocyanins. The best formula to produce functional beverage *Minasarua* is 16.59 % ginger, 0.30 % turmeric, 4.50 % JCP, 3.83 % pepper and 74.78 % FBGR. That formulation can get the antioxidant activity around 51.23 mg TE/100 g, total phenolic content 112.03 mg GAE/100 g, total flavonoid content 278.31 mg QE/100 g, and total anthocyanin 1.14 mg/100 g with the desirability of 0.98. Thus, the formula mentioned is the most optimum for producing the best antioxidant activity, total phenolics, total flavonoids and total anthocyanins. Furthermore, this formulation can be tested *in vivo* to check the efficacy of functional properties to the body's health.

Acknowledgments

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