Optimization of functional beverage formula made from turmeric, tamarind, and ginger by D-optimal mixture design

Reggie Surya¹*, Andreas Romulo¹ and Eliana Susilo²
¹Department of Food Technology, Faculty of Engineering, Bina Nusantara University, Jakarta 11480, Indonesia
²Department of Food Science and Technology, Faculty of Agricultural Engineering, IPB University, Bogor, Indonesia
*Corresponding author: reggie.surya@binus.edu

Abstract. Turmeric, tamarind, and ginger are widely used as ingredients of Indonesian traditional beverage known as jamu. This latter is known for its beneficial properties for human health, such as high antioxidant capacity, which make it potential to be developed as functional beverage. This study aimed at optimizing functional beverage formula made from turmeric, tamarind and ginger with regard to antioxidant capacity and sensory qualities using D-optimal mixture design. It was revealed that the optimum formula was the mixture containing 5.0%(v/v) turmeric extract, 9.9%(v/v) tamarind extract, 15.1%(v/v) ginger extract, 40%(v/v) sugar solution, and 30%(v/v) water. Such a formula exhibited an antioxidant capacity of (0.184 ± 0.002) mgAEq/mL and an overall hedonic score of 4.27 (out of 7).

1. Introduction

Herbs and spices are potential ingredients for functional food owing to their beneficial properties for human health. Such benefits are mainly related to the presence of a wide array of bioactive compounds exerting integrated antioxidant and anti-inflammatory activities [1]. Numerous in vitro and in vivo studies have demonstrated the potential of herbs and spices in stimulating digestive system, reducing lipid levels in blood and showing antibacterial, antiviral and anticarcinogenic activities [2].

In Indonesia, herbs and spices are commonly used in not only in cuisine but also as main ingredients of traditional beverage with pharmacological properties known as jamu. From a cultural point of view, consuming jamu to prevent or cure illnesses has been done by Indonesians for hundreds of years, from generation to generation [3]. Nowadays, even in the era of allopathic medicine, jamu remains popular and is still the first choice of some Indonesian people who prefer implementing natural means for their health.

Several spices that are frequently used as ingredients for jamu are turmeric (Curcuma longa), tamarind (Tamarindus indica) and ginger (Zingiber officinale). These spices exert biological properties that are linked to maintaining good health and treating diseases [4-6]. Turmeric is rich in curcumin, a polyphenolic compound with strong antioxidant activities that has been shown to aid in the management of multiple health problems: metabolic syndrome, arthritis, hyperlipidemia, anxiety, neurodegenerative disorders, liver and pulmonary disorders, cardiovascular disease, inflammation, and cancer [7]. Tamarind has been reported to possess antimicrobial, antidiabetic, antivenomic, antioxidant, antimalarial, cardioprotective, hepatoprotective, laxative, antiatherosclerotic and anti-hyperlipidemic activities [5]. Health-related properties of ginger that have been evidenced include
antioxidant, anti-inflammatory, antimicrobial, anticancer, cardiovascular protective, neuroprotective, respiratory protective, antiobesity, antidiabetic, antinausea and antiemetic [8].

Sensorial quality and functional characteristics are important parameters to take into account to produce functional foods that possess beneficial effects for health and are accepted by consumers [9]. The major problem of jamu is that despite its advantageous health-related benefits, people are often reluctant to drink it due to its unpleasant or strong taste and aroma. Thus, this experiment aimed at determining the optimal formulation of a functional beverage made from turmeric, tamarind and ginger in terms of antioxidant capacity and consumer’s sensorial acceptability. The tool used to find the optimal formulation was D-optimal mixture design, an experimental design that enables food formula optimization with small number of runs and, therefore, allows low cost of analysis relatively to other available experimental designs for formula optimization [10]. In this present work, the combination of turmeric, tamarind and ginger was optimized using D-optimal mixture design in order to meet desirable characteristics of our functional beverage including high antioxidant capacity and satisfying consumer’s hedonic acceptability.

2. Materials and Methods

2.1. Spice extraction and sugar stock preparation

The spices (turmeric, tamarind and ginger harvested at age of 11-12 months) were purchased at a local market in Bogor, Indonesia. They were peeled, washed, cut into small pieces and blended with commercialized mineral water (Aqua®) in 1:1 (v/v) ratio (except in 4:1 (v/v) ratio for tamarind). The spice purée was then filtered and the supernatant was kept in the refrigerator for 12 hours to let decantation process before being used in the formulation.

Sugar stock solution was prepared by diluting coarse granulated sugar with boiling commercialized mineral water (Aqua®) in 1:1 (v/v) ratio. Total dissolved solids in the sugar stock solution was 39-40° Brix.

2.2. Formulation design using Design-Expert 7.0®

Following the obtention of spice extract and sugar stock solution that would be used in the beverage formulation, the experiment was continued towards formulation and response design by using statistical software Design-Expert 7.0® from Stat-Ease Inc. This software is dedicated to performing experimental designs, including mixture design and optimization (D-optimal method design) used in this experiment to obtain the optimal formula of functional beverage from combination of spices (turmeric, tamarind and ginger).

The variables used in the formulation design were determined as follows : (1) water (30%(v/v)) and sugar stock solution (40%(v/v)) as control variables, (2) combinations of spice extract solution (turmeric, tamarind and ginger) with a total concentration of 30%(v/v) as independent variables and (3) dependent variables consisting of antioxidant capacity and hedonic rating score on product’s color, odor, taste and overall properties. The design of experiment for our functional beverage formulae as proposed by Design-Expert 7.0® is shown in Table 1.

2.3. Antioxidant capacity (DPPH radical scavenging) analysis

Antioxidant capacity of the tested formulae was analyzed as previously described [11]. Briefly, 1 mL of sample solution was mixed with 7 mL of methanol (Sigma Aldrich) and 2 mL of DPPH (2,2-diphenyl-1-picrylhydrazil) 1 mM. The absorbance of the sample solution was then measured by UV-Vis spectrophotometer (λ=520 nm). The standard curve was determined by using a series of ascorbic acid solutions (0-500 ppm) plotted with (A blank–A sample) values. The antioxidant capacity of the samples was expressed as AEAC (ascorbic acid equivalent antioxidant capacity, mgAEq/mL), a value denoting the amount of ascorbic acid (mg) equivalent to 1 mL of sample needed to reduce the similar amount of DPPH. All data were generated from 3 independent experiments (n=3) and were analyzed by using SPSS software (ANOVA test followed by Tukey’s test, p≤0.05).
Table 1. Design of experiment for functional beverage formulae proposed by software Design-Expert 7.0®

| Formula | Water (°/v) | Sugar stock (°/v) | Turmeric (°/v) | Tamarind (°/v) | Ginger (°/v) |
|---------|------------|------------------|----------------|----------------|-------------|
| 1       | 30.0       | 40.0             | 0.0            | 0.0            | 30.0        |
| 2       | 30.0       | 40.0             | 19.9           | 5.0            | 5.1         |
| 3       | 30.0       | 40.0             | 10.0           | 9.9            | 10.1        |
| 4       | 30.0       | 40.0             | 10.0           | 10.0           | 10.0        |
| 5       | 30.0       | 40.0             | 5.1            | 19.9           | 5.0         |
| 6       | 30.0       | 40.0             | 0.0            | 15.0           | 15.0        |
| 7       | 30.0       | 40.0             | 0.0            | 30.0           | 0.0         |
| 8       | 30.0       | 40.0             | 5.0            | 5.1            | 19.9        |
| 9       | 30.0       | 40.0             | 30.0           | 0.0            | 0.0         |
| 10      | 30.0       | 40.0             | 10.0           | 10.0           | 10.0        |
| 11      | 30.0       | 40.0             | 0.0            | 0.0            | 30.0        |
| 12      | 30.0       | 40.0             | 15.0           | 0.0            | 15.0        |
| 13      | 30.0       | 40.0             | 0.0            | 30.0           | 0.0         |
| 14      | 30.0       | 40.0             | 15.0           | 0.0            | 15.0        |
| 15      | 30.0       | 40.0             | 24.9           | 5.1            | 0.0         |
| 16      | 30.0       | 40.0             | 15.0           | 15.0           | 0.0         |
| 17      | 30.0       | 40.0             | 0.0            | 15.0           | 15.0        |
| 18      | 30.0       | 40.0             | 30.0           | 0.0            | 0.0         |

2.4. Hedonic rating analysis
Hedonic rating analysis was done as previously described [12] by using 70 untrained panelists who were used to drinking jamu at daily basis. In this analysis, the panelists were asked to rate their preferences (scale 1-7 from the lowest to the highest preference) towards four sensorial attributes of the provided jamu samples: color, aroma, taste and overall properties. Data analysis was done by using SPSS software (ANOVA test followed by Tukey’s test, p≤0.05).

2.5. Response analysis, optimization and formula verification
The values of all dependent variables obtained from all proposed beverage formulae were analyzed by Design-Expert 7.0® that provided an optimal polynomial function for each dependent variable with probability value ≤0.05 and maximum R-squared value. For optimization, the software provides a degree of importance (scale 1-5 from the lowest to the highest) to be attributed to each dependent variable. The parameter chosen to be maximum (degree of importance of 5) was antioxidant capacity while other dependent variables were given a degree of importance of 3. Afterwards, the software provided a formula expected to exhibit maximum antioxidant capacity that needed verifying by a series of analyses as mentioned above. Predicted and real values of the optimal formula were analyzed by using t-test (α=0.05).

3. Results and Discussion
In this experiment, we used three sorts of spices that are generally used in Indonesia as ingredients for jamu: turmeric, tamarind and ginger. As a functional food, jamu is expected to provide beneficial properties to human health, one of which is its potential as source of antioxidants. Many human
diseases have been demonstrated to be linked to oxidative stress and consumption of food containing potent antioxidants may protect human cells against such a detrimental phenomenon [13]. Turmeric contains curcumin which itself is a potent antioxidant that breaks free radical chains; and its consumption upregulated serum activities of cellular antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase [14-15]. Polyphenols, including caffeic acid and tannins in turmeric seed are bestowed with protective properties towards human cells against oxidative stress [16-17]. Tamarind seed coat extract has been reported to restore hepatic antioxidant enzyme levels in CCl4-treated rats [18]. Ginger contains bioactive compounds acting as antioxidants such as gingerol, shogaol and paradol that make it potential to be developed into functional food [6,8]. 6-shogaol can improve cellular ability to eliminate oxidative stress through the activation of Nrf2 (nuclear factor erythroid 2-related factor 2), a transcription factor of cellular antioxidant enzymes [19]. Based on this information, we decided to highlight antioxidant capacity as an important parameter determining the functionality of our beverage.

**Figure 1.** A) Antioxidant capacity and B) hedonic scores on color (i), aroma (ii), taste (iii) and overall (iv) properties of the 18 tested formulae of functional beverage. Data were obtained from three independent experiments and were expressed as mean ± standard deviation. Means with the same letter are not significantly different at $\alpha=0.05$. 
Figure 1 displays antioxidant capacity and hedonic scores of the 18 tested formulae proposed by software Design-Expert 7.0®. Formulae 7 and 13 containing 30%(v/v) tamarind extract showed the least antioxidant capacity compared to the other formulae (Figure 1A). This phenomenon might be explained by the fact that the tamarind extract was diluted in water 4 times as high as the other spices (curcuma and ginger). Tamarind seed extract has been demonstrated to exert potent antioxidative properties and ability to restore antioxidant activities of radical-damaged murine liver cells [18]. Among all formulae containing single spice, turmeric-based beverage formulae demonstrated the highest antioxidant capacity. In accordance with experiments conducted by Saranya et al. [20], methanol extract of turmeric exhibited antioxidant activities twice as high as ginger. Interestingly, formula 8 containing 5%(v/v) turmeric extract, 5.1%(v/v) tamarind extract and 19.9%(v/v) ginger extract turned out to exhibit the highest antioxidant capacity among all tested formulae, indicating a synergistic interaction among the three spices. The synergistic antioxidative activities in turmeric and ginger have been previously reported by Maizura et al. [21]. Such a synergism in a mixture of natural products may improve its effectiveness at lower doses and thus, reduce the side effects caused by the excessive use of antioxidants [22].

In terms of hedonic score on color, no significant difference was observed among all the 18 tested formulae at $\alpha=0.05$ (Figure 1B i). Our panelists clearly showed a particular preference for formulae 1 and 11 containing 30%(v/v) ginger extract in terms of aroma, taste and overall properties (Figure 1B ii–iv). Formula 8, despite exhibiting the highest antioxidant capacity, obtained relatively low overall hedonic score compared to the other formulae. On the other hand, formulae 1 and 11 obtained the highest scores in our hedonic rating test but their antioxidant capacity was relatively low. For this reason, we were interested in finding a formula that would obtain maximum antioxidant capacity and overall hedonic score using D-optimal mixture design provided by software Design-Expert 7.0®. Firstly, the software generated graphs representing polynomial equations for antioxidant capacity and overall hedonic score as function of the combinations of the three spices (Figure 2).

![Figure 2](image_url)

**Figure 2.** 3-D graphs representing polynomial equations for A) antioxidant capacity and B) overall hedonic rating as function of spice combination (A : turmeric extract, B : tamarind extract, C : ginger extract ; all expressed in %(v/v)). Color gradation goes from red representing high values of dependent variables to blue representing low values of dependent variables.
Secondly, based on these equations and graphs, we set the software to generate the optimal formula of our functional beverage by putting the importance of antioxidant capacity to the maximum (degree of importance 5/5). We assumed that antioxidant capacity is the most important parameter that should define the beneficial properties of our beverage as functional food. Without neglecting the importance of consumer’s sensorial acceptance towards our product, we set the software to optimize all hedonic rating parameters to 4.5/7 by putting the degree of importance of 3/5. Following this setting, the software provided us a formula expected to possess optimal antioxidant capacity and overall hedonic rating consisting of 5%(v/v) turmeric extract, 9.9%(v/v) tamarind extract and 15.1%(v/v) ginger extract. Afterwards, we verified the expected antioxidant capacity and overall hedonic rating as predicted by the software by performing similar experiments as precedently done. The comparison between expected and real values of antioxidant capacity and overall hedonic rating of the optimized formula is shown in Table 2.

Table 2. Predicted and verified values of functional beverage optimized formula. No significant difference between predicted and verified values observed at α=0.05.

| Response          | Optimum formula: | Predicted value | Verified value | 95% CI | 95% CI | 95% PI | 95% PI |
|-------------------|------------------|-----------------|----------------|--------|--------|--------|--------|
|                   | turmeric 5% + tamarind 9.9% + ginger 15.1% |                 |                |        |        |        |
| Antioxidant capacity (mgAEq/mL) | 0.1767 | 0.1841 | 0.1700 | 0.1800 | 0.1600 | 0.1900 |
| Hedonic rating    |                  |                 |                |        |        |        |
| Color             | 4.50             | 4.40            | 4.18           | 4.82   | 3.72   | 5.28   |
| Odor              | 4.06             | 4.20            | 3.83           | 4.28   | 3.09   | 5.03   |
| Taste             | 4.40             | 4.20            | 4.17           | 4.63   | 3.92   | 4.88   |
| Overall           | 4.44             | 4.27            | 4.14           | 4.74   | 3.80   | 5.09   |

As presented in Table 2, the predicted and verified values of the analyzed responses did not differ statistically as the verified values were included in 95% CI and PI range. The antioxidant capacity of the optimized formula (0.1841 mgAEq/mL) might be lower compared to the highest antioxidant capacity previously obtained in formula 8 (0.1925 mgAEq/mL, Figure 1A). However, the optimized formula had better overall hedonic rating than formula 8 (4.27 vs. 3.31). Anggarwal et al. reported that among four key indicators in choosing food (taste, nutrition, cost and convenience), US adults put taste as the most important determinant [9]. Verbeke stated that taste is a critical parameter in determining people’s willingness to consume functional food since less and less consumers accepted to compromise on taste for health in the case of functional food [23]. Furthermore, Petit suggested that promoting the taste of healthy food may lead to healthy food behaviors of consumers. Therefore, health benefits and sensorial acceptance are two crucial factors that should be taken into account equally in developing functional food [24].

In this experiment, we used D-optimal mixture design that has been considered as an efficient tool to determine the best ingredient combination in formulation [25]. This method has been widely used to find optimal formulation in various food products by considering different food characteristics, including bread, probiotic beverage, chewing gums, energy drink and hot chocolate beverage [26-30]. Through this study, we demonstrated for the first time that D-optimal mixture design can be used to
optimize jamu formulation by taking into account its antioxidant capacity and consumer’s sensorial acceptability, thus developing the potential of jamu as functional beverage.

4. Conclusions
Optimization in functional food formulation using D-optimal mixture design can be performed to find the best combination of ingredients in a formula with maximum desirable characteristics. In functional food development, the formulation should be designed to obtain maximum sensorial qualities and beneficial properties in order to create a healthy food product that is accepted by the consumers. In our study, the optimization through D-optimal mixture design using software Design-Expert 7.0® suggested the optimal functional beverage (jumu) formula consisting of 5.0%(v/v) turmeric extract, 9.9%(v/v) tamarind extract, 15.1%(v/v) ginger extract, 40%(v/v) sugar solution, and 30%(v/v) water. This formula exhibited antioxidant capacity of (0.184 ± 0.002) mgAEq/mL and overall hedonic score of 4.27 (out of 7).

References
[1] Serafini M and Peluso I 2016 Curr. Pharm. Des. 22 6701-15
[2] Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J and Perz-Alvarez JA 2010 Crit. Rev. Food Sci. Nutr. 51 13-28
[3] Elfahmi, Woerdenberg HJ and Kayser O 2014 J. Herb. Med. 4 51-73
[4] Hewlings SJ and Kalman DS 2017 Foods 6 92
[5] Kuru P 2014 J. Trop. Biomed. 4 676-813
[6] Mashhadi NS, Ghiasvand R, Askari G, Hariri M, Darvishi L and Mofid MR 2013 Int. J. Prev. Med. 4 S36
[7] Pulido-Moran M, Moreno-Fernandez J, Ramirez-Tortosa C and Ramirez-Tortosa M 2016 Molecules 21 264
[8] Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T and Li HB 2019 Foods 8 6
[9] Aggarwal A, Rehm CD, Monsivais P and Drewnowski A 2016 J. Trop. Biomed. 4 676-813
[10] Eriksson L 2008 Design of Experiments : Principles and Applications (Malmö : MKS Umetrics AB)
[11] Sharma OP and Bhat TK 2008 Food Chem. 113 1202-5
[12] Lim J 2011 Food Qual. Prefer. 22 733-47
[13] Tan BL, Norhaizan ME, Liew W and Rahman HS 2018 Front Pharmacol. 9 1162
[14] Xu X, Meng X, Li S, Gan R, Li Y and Li H 2018 Nutrients 10 1553
[15] Sahebkar A, Serbanc MC, Ursoniuc S and Banach M 2015 J. Funct. Foods 18 898-909
[16] Razali N, Junit SM, Ariffin A, Ramli NS and Abdul Aziz A 2015 BMC Complement Altern. Med. 15 438
[17] Sinchayakit P, Ezure Y, Srirang S, Pongbangpho S, Povichit N and Suttajit M 2011 Nat. Prod. Commun. 6 829
[18] Sandesh P, Velu V and Singh RP 2014 J. Food Sci. Technol. 51 1965-73
[19] Chen H, Fu J, Chen H, Hu Y, Soroka DN, Prigge JR, Schmidt EE, Yan F, Major MB and Chen X 2014 Chem. Res. Toxicol. 27 1575-85
[20] Saranya B, Sulikarali T, Chindhu S, Muneeb AM, Leela NK and Zachariah TJ 2017 Journal of Spices and Aromatic Crops 26 27-32
[21] Maizura M, Aminah A and Wan Aida WM 2011 Int. Food. Res. J. 18 526-31
[22] Chouhan S, Sharma K, Zha J, Guleria S and Koffas MA 2017 Frontl. Microbiol. 8 2259
[23] Verbeke W 2006 Food Qual. Prefer. 17 126-31
[24] Petit O, Merunka D, Anton J-L, Nazarian B, Spence C, Cheok AD, Raccah D and Oullier O 2016 PLoS One 1 e0156333.
[25] Bono A, Sarbatly R, Kaluvan S, Rajin M and Krishnaiah D 2008 Intern. J. Phys. Sci. 3 45-9
[26] Azarbad HR, Tehrani MM and Rashidi H 2019 J. Agr. Sci. Tech. 21 101-15
[27] Gao YF, Hamid N, Gutierrez-Maddox N, Kanton K, Kitundu E 2019 Foods 8 214
[28] Al Hagbani T, Alomare C, Salawi A and Nazzal S 2018 *Int. J. Pharmaceutics* **553** 210-9
[29] Shibly VK, Radhakrishna K and Bawa AS 2013 *Int. J. Food Sci. Tech.* **48** 742-8
[30] Dogan M, Toker OS, Aktar T and Goksel M 2013 *Food Bioprocess Tech.* **6** 783-94