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Sensory evaluation of flavoring agent addition in soy-based beverage

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Abstract: Soy-based beverages offer numerous health benefits, but the presence of lipoxygenases causes an unpleasant off-flavor sensation that hinders acceptability. The addition of a suitable flavoring agent has been shown to enhance the acceptability of soy-based beverages. This study aimed to investigate preferences towards two flavored variants of soy-based beverages: original chocolate made from cocoa powder and vanilla made from vanilla essence. Before comparing the sensory parameters, which included appearance, taste, odor, texture, and overall assessment, the ratio of basic components such as soy and cow milk powder, sugar, and maltodextrin was studied. Fifty-seven untrained panelists evaluated the three flavored variants of soy-based beverages. The chocolate-flavored soy-based beverage received a significantly higher score in taste, odor, texture, and overall assessment ($\alpha = 0.05$). Hence, adding cocoa powder as a flavoring agent in soy-based beverages effectively improved the sensory attributes.

Keywords: soy based-beverage, flavoring agent, sensory evaluation

Introduction

Soy-based beverages have gained widespread popularity over the past two decades due to their nutritional content and health benefits [1]. Soy milk, a soy-based beverage, was the fastest-growing market, reaching a 62% market share in the US in 2010 [2]. The main raw material of soy-based beverages, soybean (*Glycine max* (L.) Merr), contains various phytochemical compounds that depend on its genotype. Indonesian soybeans contain the highest phenolic compound contributing to antioxidant activity among twenty-four genotypes selected from North and South America, Australia, Egypt, Syria, India, and China [3]. Isoflavone is the most abundant compound found in soybean and offers some pharmacological benefits to humans [4]. Three types of isoflavones - genistein, daidzein, and glycitein - are present in soybean, but genistein has been identified as the most potent and has been the subject of numerous studies [1]. In addition, soybean also contains lunasin, a plant peptide that has been indicated for cancer prevention [5].

In addition to its beneficial compounds, soybean is also rich in lipoxygenases (LOX-1, LOX-2, and LOX-3), responsible for soy-based beverages' unpleasant taste and odor [6]. These enzymes play a role in the peroxidation of polyunsaturated fatty acids (linolenic, linoleic, and arachidonic acids) and in the formation of volatile alcohols, ketones, aldehydes, and methanethiol, which result in grassy and beany flavors. Meanwhile, non-volatile compounds produced by the activity of LOXs cause astringency and bitterness [7]. LOX-1 has been reported to play a role in the bleaching process of beans, fruits, and vegetables, indicating freshness by turning green to yellowish [7,8]. Additionally, LOX-2 and LOX-3 work as pigment bleachers of linoleic acid. Among these three enzymes, LOX-2 is primarily responsible for the unfavorable odor in soybean [7]. Therefore, soy-based beverages with deactivated LOXs are generally more acceptable [9]. LOXs can be deactivated by high temperature, pressure, and pulsed light [10,11].

The acceptability of soy-based beverages can be improved by adding flavoring agents or sugar, as well as by mixing soy milk or soy powder with cow milk [12]. The addition of vanilla- and fruit-based flavoring agents has been shown to increase the acceptability of soy milk and soy yogurt [12]. In the US, the top flavored soy milk variants are chocolate, vanilla, and strawberry [2]. Therefore, in this study, chocolate and vanilla were chosen as the flavoring agents. The ratio of cow milk (skim milk and full cream), sugar, and soy powder has been investigated. The effect of adding maltodextrin to the beverage was also examined. This study aimed to determine the most favorable combination of these components and identify the flavoring agent that would yield the highest sensory scores, indicating the highest level of acceptability.

Methods

Design, location, and time

The study comprised two preliminary and single main study. The first preliminary study aimed to assess the effect of the ratio of soy and cow milk powder as well as the source of cow milk powder on the preferences of panelists. Eight untrained panelists at the National Research and Innovation Agency in Cibinong, Bogor evaluated eight formulas (Table 1). The second preliminary study involved a semi-trained panelist at the Post-Harvest Research and Development Center of the Ministry of Agriculture in Bogor who evaluated one selected formula from the first study and two modified formulas (Table 2). In the main study, fifty-seven panelists were recruited for the hedonic test. This sample size was deemed sufficient to achieve a statistical power (P) of 1.0 and an alpha (α) of 0.05 or 0.10 [13]. The main hedonic test was conducted in 2018 at the Laboratory of Pharmaceutics, Faculty of Pharmacy, University of Jember, East Java, under the permission of the Ethical Clearance Committee at Faculty of Medicine, University of Jember with reference number 1.179/H25.1.11/KE/2018.

Sampling, materials and tools

The panelists were recruited through simple random sampling among the students at the Faculty of Pharmacy, University of Jember Indonesia. The panelists had to meet the requirements of The Guideline for Organoleptic or Sensory Evaluation – National Standard of Indonesia SNI 01-2346-2006. Furthermore, they were required to be free from high risk of metabolic disorders such as diabetes mellitus and were also asked to sign an informed consent.

The formulations used in the preliminary studies were composed of soy powder (Mahkota, Malang Indonesia), skim milk A (Tropicana Slim, Jakarta Indonesia), skim milk B (Prolac, Butterworth Malaysia), full cream (Indoprima, Semarang Indonesia), sugar (Gulaku, Lampung Indonesia), and food grade maltodextrin (Hingmao, Shandong China). From these formulations, one was selected and combined with cocoa powder (van Houten, Jakarta Indonesia) or vanilla essence (Larome, Tangerang Indonesia) for further evaluation by a larger number of panelists.

All the ingredients were mixed using a mixer with a flat paddle according to the formula shown in Table 1. Then, the resulting products were packed in 250 aluminum foil pouches with a zipper. The products were prepared in a clean room laboratory at Research Center for Biotechnology, Indonesian Institute of Sciences (National Research and Innovation Agency). Afterward, they were sent to the Faculty of Pharmacy, University of Jember, using an expedited courier service. The products were stored in a dry place at room temperature for no longer than two weeks and were opened immediately before conducting the sensory evaluation. During the presentation day, each formula was poured into a cup, mixed with 100 ml of warm water (40 °C), and stirred completely with a plastic stirrer.

Data collection

The experiment was conducted after lunchtime between 2:00 PM to 4:00 PM, or at least 90 minutes after the meal [14]. Each panelist tasted each sample once and recorded their feedback on a given questionnaire. The panelists were provided with mineral water to cleanse their palates between samplings. The panelists were then given at least 60 seconds before completing the questionnaire.

In the first preliminary study, a simple hedonic scaling was chosen to reduce bias interpretation because a small population of untrained panelists was involved. The hedonic scale ranged from 1 to 4 and included: color (1 = white; 2 = yellowish white; 3 = yellow; 4 = brownish yellow), taste (1 = very unpleasant; 2 = slightly pleasant; 3 = pleasant; 4 = very pleasant), texture (1 = very aqueous; 2 = aqueous; 3 = viscous; 4 = very viscous), odor (1 = very unpleasant; 2 = slightly pleasant; 3 = pleasant; 4 = very pleasant), acceptability (1 = very unacceptable; 2 = unacceptable; 3 = acceptable; 4 = very acceptable), and solubility (1

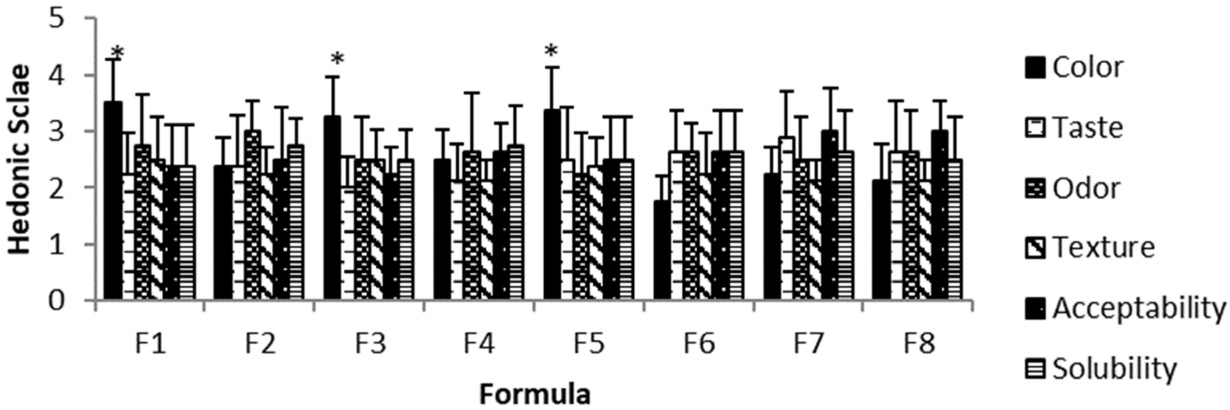

Figure 1. Hedonic scores for eight formulas in the first preliminary study. Analysis of variance was performed with α = 0.05, *n* = 8

Table 1. Formulas of soy based beverage; variations of cow milk sources and ratio of soy-cow milk powder in formulas for the first preliminary study

Composition	F1 (g)	F2 (g)	F3 (g)	F4 (g)	F5 (g)	F6 (g)	F7 (g)	F8 (g)
Sugar	8	8	8	8	8	8	8	8
Skim milk A	5	7	0	0	0	0	2	3
Soy powder	7	5	7	5	7	5	5	5
Full cream milk	0	0	5	7	0	0	4	2
Skim milk B	0	0	0	0	5	7	2	3

*A: Tropicana Slim; ** B: Prolac; F: code of formula (Formula 1 – Formula 8), g: gram

= very insoluble; 2 = insoluble; 3 = soluble; 4 = very soluble).

In the second preliminary study and the main study, a 9-point hedonic scale was used. The scale ranged from 1 to 9 and included categories: 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely [15].

Data analysis

Statistical analysis was performed using Stata SE 15.0. Normality distribution was analyzed using Shapiro – Wilk W'test and Shapiro – Francia W'test. The significance of the formula to the parameter was analyzed using the variance method. All groups with normally distributed data were compared to others using multivariate variance analysis (MANOVA) and continued with pairwise comparison Tukey method. The effect of various treatments in the group with abnormally distributed data was compared using the Kruskal-Wallis method. All of the statistical analysis was performed with α = 0.05. Correlation among parameters was measured with Pearson correlation coefficient with α = 0.05. Principal component analysis (PCA) was performed to rank the mean values of the parameters.

Results

Preliminary study to determine the basic formula of the soy-based beverage

The variations in the ratio of soy-cow milk powder and the sources of skim milk did not significantly affect the parameters of the soy-based beverage. However, adding 2 grams of soy powder in formulas F1, F3, and F5 significantly affected the hedonic scale of color between brownish yellow and yellow (Table 1, Figure 1). Unfortunately, an excess of 2 grams of soy powder negatively correlated with other parameters (correlation coefficient = -0.1021 for taste; -0.2011 for odor; -0.2671* for acceptability; and -0.1338 for solubility), except for texture or viscosity (correlation coefficient = 0.0897). The soy-based beverage has a pseudoplastic flow and thixotropic characteristics;

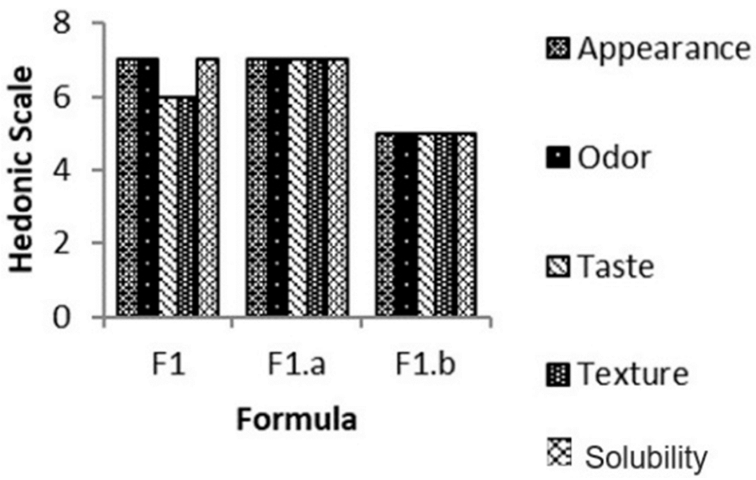

Figure 2. Hedonic scores for three formulas with modification of soy powder and sugar amount, and maltodextrin addition in the second preliminary study

Table 2. Modification of F1 by adding maltodextrin and adjusting sugar-soy powder ratio (formulas in the second preliminary study)

Composition	F1 (g)	F1.a (g)	F1.b (g)
Sugar	8	3	3
Skim milk A*	5	5	7
Soy powder	7	10	12
Maltodextrin	-	0.07	0.07

*A: Tropicana Slim

therefore, an increase in soy powder amount leads to higher viscosity [16]. The additional soy powder, which closely correlated with the increase of color and texture scale, significantly lowered acceptability. The high hedonic scale of taste was positively correlated with the scale of acceptability (correlation coefficient = 0.7263).

Formula F1 was chosen randomly for the second preliminary study because all of the formulas were considered similar in general. Maltodextrin was added to enhance the formula and alleviate any negative effects from a higher quantity of soy powder. Maltodextrin is a product of starch hydrolysis commonly used to improve particle size, hygroscopicity, and solubility [17]. This compound is non-toxic and non-irritating and has been widely used in food and pharmaceutical products [18].

In the second preliminary study, three formulas (Table 2) were evaluated by semi-trained panelists. The addition of 0.07 g maltodextrin in F1.a indicated effectiveness in improving solubility. The solubility score of F1.a was higher than F1, which had more soy powder, indicating the positive impact of maltodextrin. However, the ability of maltodextrin to increase solubility appeared limited. F1.b, which had the highest amount of soy powder, showed the lowest solubility score (Figure 2).

Maltodextrin, besides improving solubility, is also recognized as a sweetening agent with high dextrose equivalency [19,20]. Maltodextrin is usually devoid of taste and aroma [18]. This study utilized maltodextrin with high dextrose equivalency, which reduced sucrose from 8 g to 3 g without affecting the beverage's sweetness (Table 2). The modification of F1 by adding maltodextrin and adjusting the sugar and soy powder amount led to increased hedonic scales (Figure 2). Consequently, F1.a was deemed superior to other formulas in all parameters and was used in the primary hedonic test as the default or original variant of soy-based beverages.

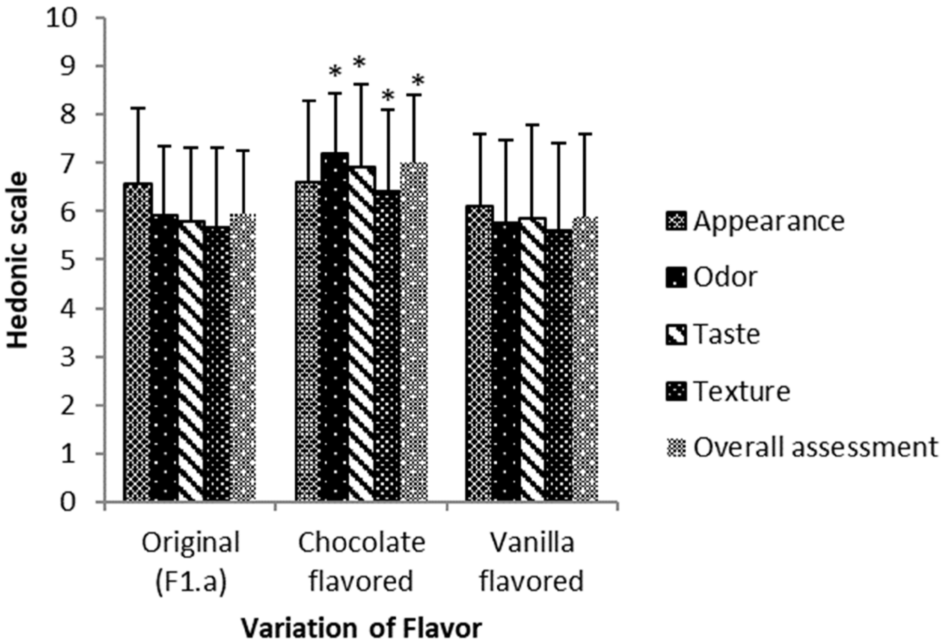

Figure 3. Hedonic scores for three formulas with flavor variation in the main study. Analysis of variance was performed with α = 0.05, *n* = 57. *) indicates a significant difference of respective sensorial parameter among the three groups

Hedonic test to evaluate preference of flavoring agent in the soy-based beverage

In the main hedonic test, formula F1.a was supplemented with flavoring agents, specifically cocoa powder (1.5 g) and vanilla essence (0.05 g). The soy-based beverage with chocolate flavor was scored significantly higher in taste, odor, texture, and overall assessment parameters, except for appearance (Figure 3). The appearance showed a moderate to low correlation with other parameters (correlation coefficient = 0.4735 for appearance vs. odor; 0.3880 for appearance vs. taste; 0.2852 for appearance vs. texture; and 0.4538 for appearance vs. overall assessment). Taste and overall assessment had the strongest correlation among the correlations of each two parameters (correlation coefficient = 0.8060), followed by odor vs. overall assessment (correlation coefficient = 0.6870), odor vs. taste (correlation coefficient = 0.6592), and texture vs. overall assessment (correlation coefficient = 0.6454).

Principal component analysis (PCA) demonstrated that component 1 (PC 1) had the highest variance among the five components with an eigenvalue greater than 1 (3.20) and explained the highest proportion of variance (64.11%), followed by PC 2 (15.44%), PC 3 (10.65%), PC 4 (6.18%), and PC 5 (3.62%). The component loadings from PC1 revealed that overall assessment and taste had the strongest correlation, followed by odor and texture (Figure 4).

The excess soy powder decreased the preference of the panelists for the beverage, but maltodextrin and an appropriate flavoring agent effectively overcame the unpleasant mouthfeel. Soy-milk beverages with chocolate flavor were preferred in all key parameters, as determined by PCA. Cocoa powder was found to be more effective in improving the characteristics of soy-milk beverages compared to vanilla essence or the original/without flavoring agent beverage.

Discussion

In a previous study, Deshpande and Chinnan compared the effect of chocolate and vanilla flavor in soy-peanut-based beverages [21]. They reported that chocolate flavor increased the acceptability of the beverage, yet vanilla flavor lowered the acceptability and caused an unpleasant sensation. The vanilla flavor is a sensation taste from vanillin, either naturally extracted from *Vanilla sp* beans or chemically synthesized. The vanillin offers a pleasing odor as the primary sensation; however, because the vanilla flavoring agent has been frequently applied in sweet food and beverage such as ice cream, flavor-modified milk, and desserts, it reflects a 'sweet' taste as a secondary sensation. Nevertheless, at a molecular level, vanillin activates bitter taste receptors, namely TAS2R14; TAS2R20; and TAS2R39 [22].

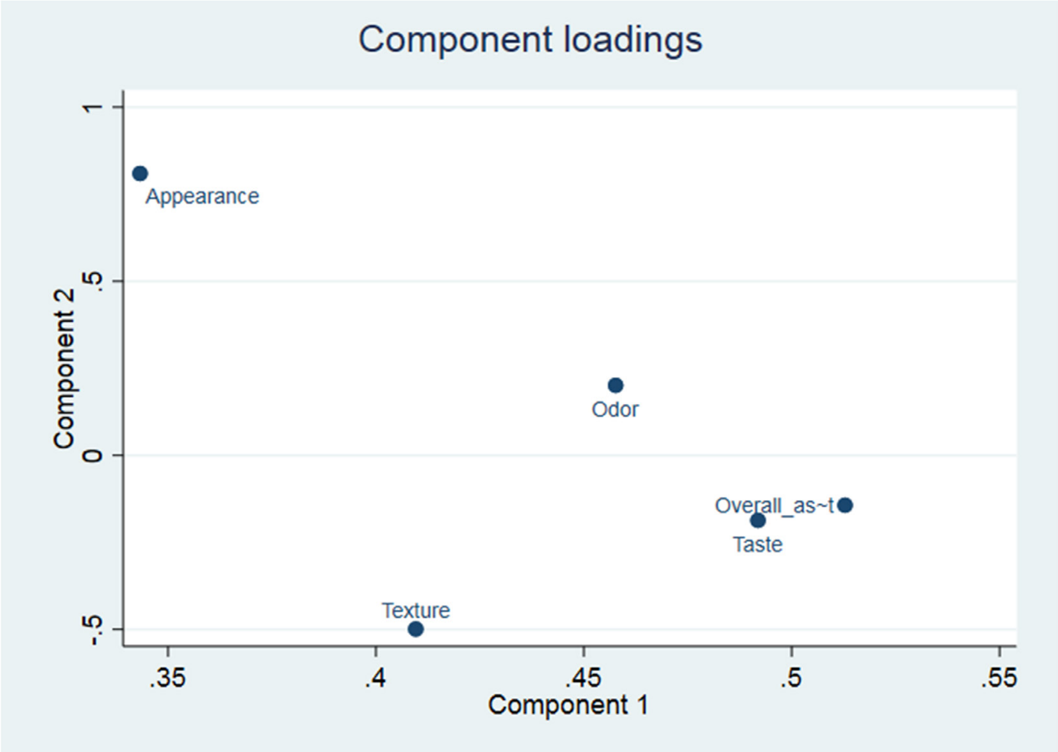

Figure 4. Component loading of principal component analysis performed in parameters tested in the main study. Only components from PC1 were considered and interpretated

Selamat et al. investigated the effect of cocoa powder on the taste and solubility of lecithin soy beverages. Their findings revealed a positive correlation between the increased amount of cocoa powder in the beverage and the elimination of the unpleasant taste of soy lecithin [23]. The soluble fat in cocoa powder can mask an unpleasant taste; however, inappropriate use may worsen the product's taste [24]. Moreover, cocoa powder has been used to alleviate the extremely bitter taste of several active pharmaceutical ingredients [25-27]. Cocoa fat, which accounts for approximately 10-12% of cocoa powder, is responsible for enhancing the solubility of the beverage through a polar cosolvency mechanism. Triglyceride and fatty acid, the primary components of cocoa fat, play an essential role in this process [28].

The soy milk beverage with chocolate flavor also received the highest score in odor preference. Cocoa powder was proven to alleviate and mask the unpleasant odors of beverages, such as spirulina [29]. These previous studies support the use of chocolate flavoring agents in soy milk beverages, particularly by utilizing cocoa powder, as they received significantly higher scores in the evaluated parameters compared to those with vanilla flavor or the original version.

Conclusion

The study results indicate that the flavoring agent is the most significant ingredient in determining the sensory acceptability of soy-based beverages. Specifically, soy-based beverages added with cocoa powder as the flavoring agent were found to be significantly more pleasing to the senses across a range of parameters compared to vanilla-flavored soy-based beverages and the original beverage without any added flavoring.

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Declaration of conflict interest

The authors declare no conflict of interest.

Author contributions

RDP was the project coordinator, conducted the experiment and data analysis, wrote the manuscript, VAR was the hedonic coordinator, SZ and KSD conducted the experiment, RN and DD were consultants for data analysis, HH was consultant for the formulation.

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