Optimization of the Different Formulations for the *Eucalyptus* Blended Tea Based on Response Surface Method

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Abstract. *Eucalyptus* tea is an effective herbal drink because it reduces cholesterol and blood sugar levels. However, the overall acceptability of *Eucalyptus* tea was not good, so it needs to be blended with black tea and cinnamon to improve the sensory characteristics. This study aims to obtain an optimum blended *Eucalyptus* tea formula that meets customer requirements. This study used the response Surface Method (RSM) with Central Composite Design (CCD) using two factors, namely the percentage of black tea powder and cinnamon powder on *Eucalyptus* leaves powder. The parameters studied to obtain the optimum formula were moisture content, extract content, color, and sensory scoring including color, aroma, taste, and overall preference of blended *Eucalyptus* tea. The results showed that the optimum formula for blended tea of *Eucalyptus* was 48.5% black tea and 29.8% cinnamon.

1. Introduction

Indonesia has many types of herbs, which are used as the main ingredient in herbal drinks. One type of herbal drink that is most commonly consumed is herbal tea. Herbal tea has many health benefits. It is the most popular non-alcoholic beverage in the world [1]. Herbal teas are made from spices, flowers, vegetables, leaves, nuts, barks, and roots of medicinal plants that are green and dry and sold loosely or wrapped in bags [2]. Herbal tea is fragrant, has a calming effect on the mind, and high antioxidant contents [3]. Herbal teas are increasing because they provide good sensory properties (taste and aroma) and have health benefits [4]. One of the herbs, which is of Indonesian heritage and has health benefits, is *Eucalyptus* leaves. *Eucalyptus* leaves can also be processed into herbal teas. The *Eucalyptus* tea has been studied and patented by Shimabukuro [Shimabukuro] that started by steaming, rumpling-twisting, and drying *Eucalyptus* leaves. *Eucalyptus* tea can reduce blood sugar and cholesterol levels [Shimabukuro]. *Eucalyptus* leaf extract contains 1,8-cineole [5], terpenoids [6], flavonoids [7], tannins [8], and others. In the preliminary study, we have developed herbal teas from 100% *Eucalyptus* in teabag packaging due to its practicality, familiarity, and durability [9]. Consumer acceptability of *Eucalyptus tea* is very poor since the aroma and taste do not meet the consumer requirements. Therefore, it is recommended to increase consumer acceptance with blended *Eucalyptus* leaves with other herbs. Gogoi [10] stated that blending a tea with other ingredients makes excellent blended tea that improves its taste and aroma. Blended teas can be made from tea with other tea, tea with fruits, and tea with spices or herbs [10]. The tea that blended with different herbs brings nice flavors, aromas, and colors [11]. Each has its flavor and taste, but blending with other ingredients can affect the tea flavor [12]. The consumer of blended tea can expect potency from the ingredients and the synergistic effects of both the taste and the aroma.
[13]. The determination of supporting herbal ingredients to be blended is based on health benefits and their effect on improving taste and aroma.

In the preliminary research, several supporting herbal ingredients have been blended with powdered eucalyptus leaves, including ginger, rosella, cinnamon, black tea, and secang, and packed in a teabag. The panelists' sensory test results, black tea, and cinnamon were the selected herbal ingredients. The polyphenolic compounds present in black tea are associated with beneficial effects in preventing coronary heart disease (CHD) and cancer and protecting against dental caries and bone loss [14]. Besides, anti-aging, anti-diabetic, and many other health benefits associated with black tea consumption are described [15]. Cinnamon (Cinnamomum burmannii) effectively improves glycemia [16] both in healthy and diabetic subjects. The cinnamon bioactive compounds revealed high antioxidant properties in human and animal models on oxidative stress through inhibiting lipid peroxidation [17]. However, studies on determining the optimum formulation in the manufacture of blended tea from Eucalyptus leaves, black tea, and cinnamon have never been carried out.

This study aims to determine the optimum formulation in the manufacture of blended tea from Eucalyptus leaves, black tea, and cinnamon using Response Surface Methods (RSM) based on each formula's physical and sensory characteristics. Revealed from this research the best blended tea composition which is acceptable to consumers.

2. Materials and methods

2.1. Materials

The four primary blended tea materials are fresh Eucalyptus leaves, black tea, cinnamon, and tea bags. The Eucalyptus leaves were obtained from the Eucalyptus tree in Kamal District, Bangkalan Regency, Indonesia. The plucking was carried out on Eucalyptus leaves, which have a homogeneous level of greenness. After that, the withering and drying process was carried out with a sun dryer until the moisture content is 5-6%. The dried leaves were then grinded to a powder form 80 mesh. Black tea "Kertowono" purchased in the local market in size of 80 mesh. Cinnamon is purchased in the local market in dried form, then grinded to a powder with 80 mesh size.

2.2. The process of making blended tea in teabag packaging

The process of making blended tea begins with mixing eucalyptus leaf powder, black tea powder, and cinnamon powder according to the percentage specified in the research design. The mixing process must be homogeneous so that the blended tea is perfectly blended. After that, put the blended tea in a tea bag with a net weight of 2.5 grams, then give the teabag string and label. Sealing the top of the teabag and make sure there were no leaks in the teabag.

2.3. Experimental design using Response Surface Method (RSM)

This study's experimental design includes two independent variables: a percentage of black tea powder and percentage of cinnamon powder and nine response variables as water content, extract content, color (L*, a*, b*), the score of color, taste, aroma and overall preferences. The central composite design (CCD) was used to examine the effects of independent variables and their interaction on the response variables in brewed Eucalyptus blended tea. The percentages of black tea powder and cinnamon powder are based on the amount of Eucalyptus leaves powder used.

This study's general model was set the independent variables at five levels: $-1.414$, $-1$, $0$, $+1$, and $+1.414$. Based on the preliminary research, each level's values were assigned as follows: the percentage of black tea powder was ranged from 40-70%, and the percentage of cinnamon powder was ranged from 20-40%. By using the Design Expert 7.0 stat-ease software, adjust the range for each independent variable so that the experimental conditions for the percentage of black tea powder were [33.79% ($-1.414$), 40% ($-1$), 55% (0), 70% ($+1$), and 76.21% ($+1.414$)] and the percentage of cinnamon powder were [15.86% ($-1.414$), 20% ($-1$), 30% (0), 40% ($+1$), and 44.14% ($+1.414$)]. In the CCD design, the center points (0,0) were carried out with five repetitions. The total treatment was 13 treatments (8 non-center points and five center points) were generated to predict the optimal formula of Eucalyptus blended tea for consumer acceptability. The experimental design was presented in Table 1.
Table 1. The experimental design using CCD

| Treatment | Coded | Uncoded |
|-----------|-------|---------|
|           | The percentage of black tea powder (%) | The percentage of cinnamon powder (%) | The percentage of black tea powder (%) | The percentage of cinnamon powder (%) |
| 1         | +1    | 70      | 10         | 40       |
| 2         | +1    | 70      | 20         | 20       |
| 3         | 0     | 1.414   | 55         | 44.14    |
| 4         | -1    | 40      | 20         | 20       |
| 5         | -1    | 40      | 40         | 40       |
| 6         | +1.414| 0       | 76.21      | 30       |
| 7         | 0     | 0       | 55         | 30       |
| 8         | 0     | 0       | 55         | 30       |
| 9         | 0     | 0       | 55         | 30       |
| 10        | 0     | 0       | 55         | 30       |
| 11        | -1.414| 0       | 33.79      | 30       |
| 12        | 0     | -1.414  | 55         | 15.86    |
| 13        | 0     | 0       | 55         | 30       |

2.4. The procedure of physical characteristics analysis

2.4.1. Measurement of moisture content
Samples were measured for water content using the Ohaus MB25 moisture analyzer to obtain the blended tea's water content. Place the 2 grams of sample in the sample holder on the moisture analyzer, close the sample area, and run the moisture analyzer until the sample's water content is obtained.

2.4.2. Measurement of soluble ethanol extract content
The measurement procedure for ethanol-soluble extract content was based on Herbal Pharmacopoeia [2008]. First, weighed 5 g of blended tea powder, then added 100 ml of 96% ethanol in a clogged flask, shake periodically for the first 6 hours, leave for 18 hours. Thus, filtered and evaporated 20 ml of the filtrate at a temperature of less than 78 °C until fixed weight. Calculated the percentage of ethanol-soluble extract content using the following formula [18]:

\[
\text{Etanol soluble extract content} = \frac{w_1 - w_2}{w_1 - w_0} \times 100\% \quad (1)
\]

where \( w_0 \) is the weight of the empty dish, \( w_1 \) is the weight of the dish and used sample, and \( w_2 \) is the weight of the plate and dried sample.

2.4.3. Color
Color analysis of brewed blended tea was obtained by the color reader (Konika Minolta CR-10). The color assay was conducted with the Hunter system to determine L* (lightness-darkness), a* (redness-greenness), and b* (yellowness-blueness) values. Higher L* values indicate a lighter tea infusion color, whereas lower L* values indicate a darker tea infusion color. A negative a* value indicates greenness, whereas a positive a* value indicates redness. A lower negative b* value indicates more blueness, whereas a higher positive b* value indicates more yellowness.

2.5. Sensory Evaluation
The sensory evaluation was performed by 30 semi-trained panelists consisting of students and lecturers from the Department of Science and Agricultural Technology, University of Trunojoyo Madura, Indonesia. The panelists were instructed to evaluate each sensory characteristic, including color, aroma, taste, and overall acceptability of brewed blended tea samples. This evaluation using a scoring scale of 1-5 for color, aroma, and taste evaluation. The evaluation of overall acceptability using hedonic scale 1-5.
Blended tea in teabag packaging was brewed using the same water volume, water temperature, and brewing time. All samples were served in 100 ml cups under warm conditions, and the same concentration of sugar was added for each sample. Most of the people (61%) consumed tea with added sugar [19]. The product information has been blinded and labeled as samples A, B, ..., M. Samples were served in random order. The water was served among the sample for mouth washing to reduce residual effects between samples.

2.6. Analysis of statistics and response surfaces
Statistical comparisons were analyzed using a one-way analysis of variance (ANOVA). The difference was considered significant at $p$-value < 0.05. T-test and Duncan’s test analyzed the significant differences between experimental values and predicted values at 95% confidence level. The responses were modeled by using RSM as a function of formulation condition. The regression model equation is composed of two factors to estimate the linear or quadratic and interactive coefficients.

3. Results and Discussion
3.1. Physical and sensory characteristics of blended tea
There were 13 treatments performed using CCD in this study. The data for each treatment are shown in Table 2.

| The experimental conditions | The physical characteristics | The sensory characteristics |
|-----------------------------|------------------------------|-----------------------------|
| black tea powder (%) | cinnamon powder (%) | Water content (%) | Extract content (%) | L* | a* | b* | Aroma | Color | Taste | Overall acceptability |
| 40 | 20 | 6.94 | 4.95 | 22.93 | 3.78 | 26.28 | 3 | 3.17 | 3.8 | 3.97 |
| 70 | 20 | 6.55 | 5.86 | 23.1 | 4.23 | 26.4 | 2.96 | 3.33 | 3.86 | 3.98 |
| 40 | 40 | 7.04 | 5.53 | 24.65 | 3.38 | 24.58 | 3.2 | 2.93 | 4.12 | 3.93 |
| 70 | 40 | 6.45 | 6.12 | 25.8 | 2.95 | 25.1 | 2.97 | 3.33 | 4.02 | 3.86 |
| 33.79 | 30 | 7.45 | 5.94 | 23.93 | 3.15 | 23.98 | 2.8 | 2.87 | 3.73 | 3.76 |
| 76.21 | 30 | 7.13 | 5.62 | 23.25 | 3.05 | 24.98 | 3.27 | 3.63 | 3.96 | 3.9 |
| 55 | 15.86 | 6.89 | 5.52 | 23.05 | 3.25 | 24.88 | 3.8 | 3.3 | 3.25 | 3.73 |
| 55 | 44.14 | 6.65 | 6.38 | 23.9 | 3.78 | 25.35 | 3.56 | 3.03 | 3.8 | 4.03 |
| 55 | 30 | 7.17 | 6.24 | 23.15 | 3.7 | 25.7 | 3.86 | 3.3 | 3.93 | 4.2 |
| 55 | 30 | 7.06 | 6.12 | 25.15 | 3.15 | 24.35 | 3.66 | 3.4 | 4.07 | 4.17 |
| 55 | 30 | 7.32 | 6.23 | 25.13 | 2.95 | 23.58 | 3.86 | 3.5 | 4.15 | 4.37 |
| 55 | 30 | 7.09 | 6.18 | 24.58 | 2.88 | 23.78 | 3.47 | 3.6 | 4.2 | 4.23 |
| 55 | 30 | 6.84 | 6.15 | 24.43 | 3.25 | 24.68 | 3.96 | 3.53 | 4.07 | 4.4 |

The physical and sensory analysis (Table 1) were then analyzed by RSM to determine the appropriate regression model to predict the response variable's value from the percentage value of black tea and cinnamon powder. This analysis can also be seen as the effect of black tea and cinnamon and their interactions on each response variable. The appropriate regression model is determined on a high R square value model and an adjusted R square value.
The values of water content ranged from 6.45 to 7.45%. These values have met Indonesian National Standard that the maximum moisture content of dry tea is 10% [20]. The results of the RSM analysis show that the quadratic regression model is the recommended one with an R square value of 0.6722. The regression equation for water content is:

$$\text{Water content} = 5.57 - 0.015A + 0.15B - 3.333E - 0.004 AB + 1.144E - 0.004 A^2 - 2.343E003 B^2$$

The percentage of cinnamon powder has a significant effect on the water content of blended tea. This study's results are appropriate [21] who reported that the higher the addition of cinnamon powder to noni blended tea, the more water content of the blended tea will increase to a maximum point and decrease again. Cinnamon powder is a powder from cinnamon woods which has hygroscopic properties or easily absorbs and releases water. Therefore, the higher the percentage of cinnamon powder, the water content of blended tea tends to increase (Fig. 1A). Meanwhile, the percentage of black tea powder and the interaction between the percentage of black tea powder and cinnamon percentage in blended tea affect the water content of blended tea (Fig. 1B).

**Figure 1.** Response surface plot showing the effect of tea percentage and cinnamon percentage on the physical and sensory characteristics of blended tea *Eucalyptus*, black tea, and cinnamon.
cinnamon powder had no significant effect on blended tea's water content. In this study, the water content of cinnamon powder was 8.77±0.46%, which was higher than the water content of black tea powder that only 6.3±0.84%.

The extract content values range from 4.95-6.38%. These values have not fulfilled the SNI (Indonesian National Standard) for Black tea in teabag packaging because the minimum extract content is 11%. The RSM analysis results show that the quadratic regression model is the best, with an R square value of 0.6782. The regression equation for water content = -1.12 -0.15 A + 0.16 B - 5.33E-004 AB - 1.176E-003 A^2 - 1.795E-003 B^2. The percentage of black tea powder has a significant effect on the extracted content of blended tea. Extract content from black tea from plantations on the island of Bali, according to Paramita et al. [22], is 16.64 ± 0.414%, and the extracted water-soluble content is 31.84 ± 0.6446. Thus, if the tea powder added is higher, the extracted content will increase (Fig. 1B). Meanwhile, the percentage of cinnamon powder and the interaction between the percentage of black tea powder and cinnamon powder had no significant effect on blended tea extract content.

The blended tea color parameter is measured using the Hunter method, including L*, a*, and b* values. The variance analysis showed that the percentage of black tea powder, cinnamon powder, and their interaction did not significantly affect the L *, a *, and b * values of blended tea. The color of the blended tea is from light brown to dark brown. The more tea powder or cinnamon powder is added, the color will be darker due to the contents of theaflavin and thearubigin, which give the copper-red and brown color on brewed tea [23,24]. The theaflavin and thearubigin contents of black tea are 0.5621% and 5.9414%, respectively. Meanwhile, black tea's theaflavin and thearubigin contents are 0.5179% and 5.2777% [25].

The score for the aroma parameter ranges from 2.8-3.96. The quadratic regression model is the best, with an R square value of 0.7370. The regression equation for aroma score = -3.98 + 0.23 A + 0.089 B - 3.222E-004 AB - 1.979E-003 A^2 - 1.219E-003 B^2. The percentage of black tea powder in the linear and quadratic terms had a significant effect on the blended tea's aroma score. The higher the percentage of black tea powder, the more preferred (Fig. 1C). However, if you add too much black tea powder, consumer acceptance will also decrease (Fig. 1C). Ahmad et al. [25] stated that black tea's aroma is more dominant than cinnamon aroma due to its theaflavin and thearubigin contents. The characteristics of various kinds of tea consist of a balance of very complicated mixtures of aroma compounds in tea due to the total phenolic content and compound that affect the aroma properties [26]. The percentage of cinnamon powder had no significant effect on the aroma value of blended tea. However, Fig. 1C shows that the more cinnamon powder is added, the more preferred the blended tea aroma is. The aroma score will decrease if the addition is excessive because cinnamon also contains a high total phenolic content and compound that affect the aroma properties.

The sensory scores for color parameters ranged from 2.87-3.63. The hedonic scale used is 1 (dislike extremely) to 5 (like extremely). The RSM analysis results show that the quadratic regression is the suggested model with an R square value of 0.8717. The regression equation for the color score = 0.68 + 0.057 A + 0.063 B + 3.889E-004 AB - 5.001E-004 A^2 - 1.542E-003 B^2. The percentage of black tea powder in the linear and quadratic terms significantly affects the color preference score of the blended tea. The color of the blended tea is from brown to dark brown. The more tea powder added, the more preferred the consumer but too much, the lower the score (Fig. 1D). These results were consistent with the research of Ahmad et al. [25] that the greater the proportion of black tea, the darker the resulting color and the less preferred by consumers. The percentage of cinnamon powder had a significant effect on the blended tea color score's quadratic term. Cinnamon powder also gives the tea its brown color because of its theaflavin and thearubigin contents [25]. The more cinnamon powder is added, the brown the blended tea will be (Fig. 1D).

The sensory scores for the taste parameter ranged from 3.25-4.2. The quadratic regression is the suggested model with an R square value of 0.7180. The regression equation for the taste score = 0.41 + 0.036 A + 0.16 B – 2.667E-004 AB – 2.365E-004 A^2 – 2.133E-003 B^2. The percentage of cinnamon powder in the linear and quadratic terms significantly affects the taste score. Cinnamon powder has high
The optimal formula of blended tea is Blended Eucalyptus powder with black tea powder 53.95% and cinnamon powder 29.8% that is predicted to produce color, aroma, taste, and overall acceptability values are 3.433, 3.720, 4.109, and 4.156, respectively. Meanwhile, the predicted value of water content and extract contents are 6.947% and 5.867%.

4. Conclusion
The optimal formula of blended tea is Blended Eucalyptus powder with black tea powder 53.95% and cinnamon powder 29.8% that is predicted to produce color, aroma, taste, and overall acceptability values are 3.433, 3.720, 4.109, and 4.156, respectively. Meanwhile, the predicted value of water content and extract contents are 6.947% and 5.867%. Further research analyzed the chemical characteristics, including phenol, the content of 1,8 cineol, antioxidant and antimicrobial content of the Eucalyptus blended tea in the optimum formula.

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References
[1] Guimarães R, Barros L, Carvalho A M and Ferreira I C F R 2011 Infusions and decoctions of mixed herbs used in folk medicine: Synergism in antioxidant potential Phyther. Res. 25 1209–14
[2] Farr S 2016 Healing herbal teas—learn to blend 101 specially formulated teas for stress management, common ailments, seasonal health, and immune support (Storey Publishing)
[3] Aoshima H, Hirata S and Ayabe S 2007 Antioxidative and anti-hydrogen peroxide activities of various herbal teas Food Chem. 103 617–22
[4] Joubert E, de Beer D and Malherbe C J 2017 Herbal teas—Exploring untapped potential andstrengthening commercialisation South African J. Bot. 110 1–3
[5] Inouye S, Yamaguchi H and Takizawa T 2001 Screening of the antibacterial effects of a variety
of essential oils on respiratory tract pathogens, using a modified dilution assay method. J. Infect. Chemother. 7 251–4

[6] Lee C K 1998 Ursane triterpenoids from leaves of Melaleuca leucadendron Phytochemistry 49 1119–22

[7] Wollenweber E, Wehde R, Dörr M, Lang G and Stevens J F 2000 C-Methyl-flavonoids from the leaf waxes of some Myrtaceae Phytochemistry 55 965–70

[8] Yoshida T, Maruyama T, Nitta A and Okuda T 1996 An hydrolysable tannin and accompanying polyphenols from Melaleuca leucadendron Phytochemistry 42 1171–3

[9] Mu’Tamar M F F, Ulya M and Hidayat K 2019 Product development of black Piper retrofractum Vahl tea (black PrV tea) IOP Conf. Ser. Earth Environ. Sci. 230

[10] Gogoi R C 2016 Blending of tea – the development Two a Bud 61 53–6

[11] Zaman S, Alam M K, Ahmed S S, Uddin M N and Bari M L 2014 The Prevalence of E. coli O157: H7 in the Production of Organic Herbs and a Case Study of Organic Lemongrass Intended for Use in Blended Tea Agric. Food Anal. Bacteriol. 4 164–76

[12] Kim J H, Lee J H, Choi Y K and Chun S S 2018 A lexicon for descriptive sensory evaluation of blended tea Prev. Nutr. Food Sci. 23 364–73

[13] Yang J E and Lee J 2020 Consumer perception and liking, and sensory characteristics of blended teas Food Sci. Biotechnol. 29 63–74

[14] Ruxton C H S 2008 Black tea and health Nutr. Bull. 33 91–101

[15] Khan N and Mukhtar H 2013 Tea and Health: Studies in Humans Curr. Pharm. Des. 19 6141–7

[16] Allen R W, Schwartzman E, Baker W L, Coleman C I and Phung O J 2013 Cinnamon use in type 2 diabetes: An updated systematic review and meta-analysis Ann. Fam. Med. 11 452–9

[17] Amin K A and Abd El-Twab T M 2009 Oxidative markers, nitric oxide and homocysteine alteration in hypercholesterolicimic rats: Role of atorvastatine and cinnamon Int. J. Clin. Exp. Med. 2 254–65

[18] Kemenkes RI 2011 Farmakope Herbal Indonesia Edisi I 2011 Kementerian Kesehatan Republik Indonesia (Jakarta: Kementerian Kesehatan Republik Indonesia)

[19] De Godoy R C B, Deliza R, Gheno L B, Licodiedoff S, Frizon C N T, Ribani R H and dos Santos G G 2013 Consumer perceptions, attitudes and acceptance of new and traditional mate tea products Food Res. Int. 53 801–7

[20] Badan Standarisisasi Nasional Indonesia 2014 Teh hitam celup (Jakarta: Badan Standarisisasi Nasional)

[21] Nurminabari I S 2019 Pengaruh perbandingan serbuk kayu manis (Cinnamomum burmannii) dengan cengkeh (Syzygium aromaticum L.) dan konsentrasi gula stevia (Stevia rebaudiana B.) terhadap karakteristik teh mengkudu J. Ilmu dan Teknol. Pangan 8 419–29