The brewing conditions optimization of Eucalyptus blended tea bags

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Abstract. Blended tea is a mixed herbal drink made from herbal plants' leaves, roots, flowers, or stems. The blended tea that formulation has been reported in previous studies is composed of Eucalyptus leaves, cinnamon, and black tea. However, the optimum brewing conditions of Eucalyptus blended tea have not been studied. This study aims to determine the optimum brewing conditions of Eucalyptus blended tea using Response Surface Methodology (RSM) with Central Composite Design (CCD). This study used two factors, the loading per tea bag (2 g, 2.5 g, 3 g) and brewing time (2, 4, and 6 minutes). The parameters used were water-soluble extract content, color (L, a, b), and overall acceptability. The results showed that a weight of 2.48 g with a brewing time of 3.45 minutes was the optimum condition for brewing blended tea of Eucalyptus, cinnamon, and black tea.

1. Introduction
The health advantages of Eucalyptus tea are numerous. It can reduce blood sugar levels as well as cholesterol levels [1]. Eucalyptus leaf extract contains 1,8-cineole [2], terpenoids [3], flavonoids [4], tannins [5], and others. According to the preliminary study, Eucalyptus tea’s customer acceptability is quite low because the taste and flavor do not fulfill customer expectations. As a result, blending Eucalyptus leaves with other herbs is suggested to improve customer acceptance.

Blended teas were a tea blend with other tea, tea with fruits, and tea with spices or herbs [6]. When tea is blended with other herbs, it produces more pleasant aromas, colors, and flavors [7]. Each blended tea ingredient has its taste and aroma, however, combining one ingredient can change the blended tea flavor [8]. The blended tea customers can expect efficacy from the ingredients and synergistic effects from the taste and flavor [9]. The health advantages and effects on increasing taste and flavor are used to decide which supporting herbal ingredients should be blended. Teabags can be used to combine herbs, spices, and other plant ingredients [10].

Teabags have broad applications in the beverage industry, as they are convenient, easy to use, and dispose-off with a higher preference because they are easy to prepare and handle [11]. Teabag packaging was chosen due to its familiarity, practicality, and durability [12]. It was previously reported that the optimum blended tea bag formula was composed of Eucalyptus leaf powder mixed with 53.95% of black tea powder and 29.8% cinnamon powder [13]. In addition to the proper formulation, consumer acceptance of herbal tea products is also influenced by the design of teabags. Acceptability, steeping power, and extraction efficiency are all influenced by the tea bag’s design. Selecting raw material for paper, pore size, bag size and shape, and loading capacity are some of the parameters that might affect
the quality of tea bags. [11]. At the same time, other important parameters which also affect the brewing of tea bag includes loading in the tea bag [14], particle size [15,11], the temperature of the brewing water [16,11,17], the volume of the brewing water [11] and the brewing time [16,11,17].

The brewing process conditions affect the extraction yield, total phenolic compounds and antioxidant activity [16,17], and tea’s sensory characteristics [15-17]. The tea packaging must include serving suggestions, including the volume of the brewing water, brewing time, and brewing temperature. Meanwhile, the loading per bag is predetermined by the manufacturer. Because Eucalyptus blended tea is a new product, it is necessary to investigate the optimum brewing conditions to maximize consumer acceptability of Eucalyptus blended tea.

The purpose of this study is to determine the optimum brewing conditions of Eucalyptus blended tea using Response Surface Methods (RSM) based on its sensory characteristics. This study revealed the optimum brewing conditionS of Eucalyptus blended tea that is acceptable to consumers.

2. Material and methods

2.1. Material
Fresh Eucalyptus leaves, cinnamon, black tea, and tea bags are the four primary blended tea ingredients. The Eucalyptus leaves were collected from a tree in Bangkalan Regency, Indonesia. The greenness of the Eucalyptus leaves is uniform. Eucalyptus leaves were withered and sun dried until the moisture content was 5-6 percent. The powder was then ground to an 80 mesh fine powder. Black tea "Kertosowo" purchased in the local market in size of 80 mesh. Cinnamon was purchased dried in the local market and ground to an 80 mesh powder.

2.2. The procedure of producing blended tea
Producing blended tea bag starts with blending powdered Eucalyptus leaves, 53.95% powdered black tea, and 29.8% powdered cinnamon. The percentages of powdered black tea and powdered cinnamon are calculated using the amount of powdered Eucalyptus leaves. The blended tea ingredients must be mixed and shifted to be perfectly blended. Fill a tea bag with the blended tea and a net weight based on the experimental design Table 1. Sealing the teabag's top and ensuring there were no leaks in the teabag.

| Treatment |
|-----------|
| Code 1   |
| Code 2   |
| Code 3   |
| Code 4   |
| Code 5   |
| Code 6   |
| Code 7   |
| Code 8   |
| Code 9   |
| Code 10  |
| Code 11  |
| Code 12  |
| Code 13  |

2.3. RSM Experimental design
This study has two independent variables: loading per tea bag and brewing time, and five dependent variables: water-soluble extracts, color (a*, L*, b*), and overall acceptability. The central composite
design (CCD) ‘face centered’ was used to investigate the impacts of independent variables and their interactions on dependent variables in brewed Eucalyptus blended tea.

The independent variables in this study were set to three levels: -1, 0, +1, so that the experimental conditions for the loading of teabag were (2, 2.5, 3 g) and the brewing time was (2, 4, 6 minutes). The center points (0,0) were repeated five times in the CCD experimental design. The total treatment was 13 (8 non-center points and five center points) created to estimate Eucalyptus blended tea’s optimal brewing condition for consumer acceptability. Table 1 summarizes the experimental design.

2.4. The physical characteristics analysis procedure

2.4.1. Water-soluble extracts measurement. Herbal Pharmacopoeia [18] was used to guide the measurement of water-soluble extract content. First, weigh 5 g of blended tea powder, then add 100 ml of water and chloroform in a clogged flask, shake periodically for the first 6 hours, then leave for 18 hours. Thus, 20 ml of the filtrate was filtered and evaporated until fixed weight at a temperature less than 78 °C. The following formula [18] was used to calculate the percentage of water-soluble extract content:

\[
\text{Water-soluble extract content (\%) = \left( \frac{w_1 - w_2}{w_1 - w_0} \right) \times 100 \times 100\%}
\]

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 samples.

2.4.2. Color analysis. The color reader (Konika Minolta CR-10) was used to analyze the brewed blended tea color. This analysis use the Hunter system to determine \( L^* \) (lightness-darkness), \( a^* \) (redness-greenness), and \( b^* \) (yellowness-blueness) values. Higher \( L^* \) values indicate a lighter color of tea infusion, and vice versa. Greenness is indicated by a negative \( a^* \) value, while redness is indicated by a positive \( a^* \) value. A lower negative \( b^* \) value indicates greater blueness, while a higher positive \( b^* \) value indicates greater yellowness.

2.5. Sensory analysis
The sensory analysis was carried out by 30 untrained panelists from the Department of Agroindustrial Technology at the University of Trunojoyo Madura in Indonesia. The panelists were instructed to use a hedonic scale of 1-7 to rate the overall acceptability of brewed Eucalyptus blended tea samples.

The same water volume and temperature (70 °C) were used to brew blended tea in teabag packaging because most herbs have active compounds that are unstable at high temperatures. All samples were served in 100 ml cups (70°C) at room temperature. Because most people (61%) consumed tea with added sugar [19], so in this study was added the same sugar concentration for each sample. Samples were served in random order The 13 samples has been blinded and labeled as samples using the three-digit number code (316, 171, …, 321). The water was served between samples for cleansing to neutralize and reduce the residual effects of the previous samples.

2.6. Statistical and response surface analysis
The statistical comparisons in this study used Analysis of variance. A p-value of 0.05 was considered significant, and the T-test and Duncan's test were used to examine the significant differences between experimental and predicted values at a 95 percent confidence level. RSM was used to model the responses as a function of the brewing condition. To estimate the linear or quadratic and interactive coefficients, the regression model equation is composed of 2 factors. Several parameters' goals, weights, and importance are determined during the optimization process. The solution chosen has a desirability value close to 1 [20].
3. Results and discussion

3.1. Physical characteristics and overall acceptability of Eucalyptus blended tea

The data for 13 treatments are shown in Table 2.

| The Experimental conditions | Physical characteristics | Sensory evaluation (Overall acceptability) |
|-----------------------------|--------------------------|-------------------------------------------|
| Loading per tea bag (gram)  | The brewing time (minutes) | Extract content (%) | a* | L* | b* | |
| 2                           | 2                        | 6.28                      | 3.95           | 25.275  | 26.15  | 5.2 |
| 2.5                         | 4                        | 6.58                      | 5.525           | 26.05  | 28.1  | 5.7 |
| 2.5                         | 4                        | 6.65                      | 4.45           | 25.25  | 26.25  | 5.6 |
| 2.5                         | 4                        | 6.62                      | 5.125           | 25.55  | 26.375  | 5.5 |
| 3                           | 2                        | 6.54                      | 4.425           | 24.7  | 27.175  | 5 |
| 3                           | 6                        | 6.97                      | 4.5            | 26.025  | 29 | 4.8 |
| 2                           | 4                        | 6.56                      | 4.625           | 29.9  | 27.825  | 5 |
| 2                           | 6                        | 6.35                      | 4.525           | 28.2  | 28.35  | 4.8 |
| 3                           | 4                        | 6.94                      | 4.35           | 25.525  | 26.675  | 4.7 |
| 2.5                         | 4                        | 6.68                      | 4.9            | 26  | 28.3  | 5.6 |
| 2.5                         | 6                        | 6.82                      | 4.7            | 24  | 28.175  | 5.1 |
| 2.5                         | 4                        | 6.56                      | 4.375           | 25.85  | 28.15  | 5.4 |
| 2.5                         | 2                        | 5.82                      | 4.5            | 24  | 26.575  | 5.1 |

The physical and sensory evaluations Table 2 were then evaluated with RSM to identify the best regression model to estimate the value of the dependent variable based on the loading per tea bag and brewing time. A high value of R square and an adjusted R square are used to select the most appropriate regression model.

The extract content values range between 5.82 and 6.97 percent. The best regression model based on RSM analysis is a quadratic model with an R square value of 0.8988. The regression equation for water-soluble extract content is 

\[ y = +6.8 - 2.11A + 0.74 B - 5.00E-003 AB + 0.52 A^2 - 0.075 B^2 \]

The brewing time has a significant impact on the water-soluble extract content of Eucalyptus blended tea. The black tea extract content of Bali plantation is 16.64 ± 0.414% [21]. Thus, the water-soluble extract content will increase if the loading per tea bag is higher (Fig. 1A).

Meanwhile, the loading per tea bag and the interaction of the loading per tea bag and the brewing time had no significant effect on the water-soluble extract content of blended tea. The water-soluble extracts are estimated from the tannin group [22]. Black tea and eucalyptus leaves also contain tannins [5], so that the higher the loading per tea bag, the higher the water-soluble extracts.

The color analysis on a* parameter values ranges from 3.95-5.525. The recommended regression model is quadratic with an R square value of 0.4482. The regression equation for a* is

\[ y = -5.69 +6.98 A + 0.80 B - 0.125 AB - 1.28 A^2 - 0.052 B^2 \]

The loading per tea bag, the brewing time, and interaction between the two had no significant effect on the a* parameter of blended tea. It is presumably because the distance from one level to another is very close, so there is no significant effect. However, based on Fig.1B, the higher the loading per tea bag, the higher the a* value. However, the higher the net weight, the lower the value of a* at a certain point. Water with a fixed volume will have the maximum ability to absorb extracts from blended tea. At a certain point, it will decrease or become stationary by increasing the ingredients' net weight. Fig. 1B shows that the higher the brewing time, the higher the a* value. More active compounds are extracted, especially theaflavin and thearubigin compounds in tea, which cause color changes [23,24].
Figure 1. The response surface plot demonstrating the impact of loading per tea bag and brewing time on the physical characteristics and overall acceptability of *Eucalyptus* blended tea
A. The Surface Plot of Extract content; B. The Surface Plot of a*; C. The Surface Plot of L*; D. The Surface Plot of b*; E. The Surface Plot of Overall acceptability

The color analysis on L* parameter values ranges from 24-29.9. The best regression model based on RSM analysis is quadratic model with an R square value of 0.8403. The regression equation for \( L^* = +69.69 -40.97 A + 4.76 B - 0.49 AB - 0.43 B^2 \). The loading per tea bag and the brewing time significantly affected the L* parameter, but the interaction between the two had no significant effect.
The more loading per tea bag and brewing time is added, the darker (Fig. 1C) because of theaflavin and thearubigin, which give brewed tea a brown color and copper-red [23,24]. Black tea contains 0.5621 percent theaflavin and 5.9414 percent thearubigin, respectively.

The color analysis on b* parameter values ranges from 26.15-29. The recommended regression model based on RSM analysis is a quadratic model with an R square value of 0.4734. The regression equation for b* = +25.16 +0.17 A + 0.47 B. The brewing time significantly affects the b* parameter, but the loading per teabag has no significant effect. The higher the brewing time, the higher the b* value and vice versa (Fig. 1D) because more active compounds were extracted, resulting in a yellow color change. The color will be changed due to the theaflavin and thearubigin content, which can give brewed tea a brown color and copper-red [23,24].

The score of overall acceptability ranges between 4.7 and 5.7. The recommended regression model based on RSM analysis is quadratic model with an R square value of 0.7724. The regression equation for overall acceptability = -5.15 + 8.43 A + 0.20 B +0.05 AB –1.76 A^2 – 0.047 B^2. The loading per tea bag in the linear and quadratic terms significantly affected the blended tea's overall acceptability score. The greater the loading per tea bag, the greater the overall acceptability (Fig. 1E). However, if the loading per teabag is excessive, the overall acceptability will suffer (Fig. 1E). According to Savitri et al. [25], the greater the proportion of black tea, the greater the panelists' acceptability. Blending tea with a few herbs or spices, on the other hand, has a greater consumer acceptance than black tea [25,26]. Ahmad et al. [26] stated that cinnamon-spiced tea had the greatest overall preference for improving overall acceptability of Eucalyptus blended tea. The greater the loading per teabag means the black tea and cinnamon are extracted to increase overall acceptability.

### 3.2 Optimization process

Optimization was done to determine the optimal brewing conditions that maximize consumer acceptance and water-soluble extracts value. The optimization process takes using five criteria, including all dependent variables according to Table 3.

| Parameters   | Goal          | Lower | Target | Upper | Weight | Importance |
|--------------|---------------|-------|--------|-------|--------|------------|
| Extract content | Maximum       | 6.2   | 6.65   | 6.65  | 1      | 2          |
| a*           | Target        | 3.9   | 4.60   | 5.50  | 1      | 1          |
| L*           | Minimum       | 25    | 25     | 29.90 | 2      | 3          |
| b*           | Target        | 26.0  | 27.0   | 29.00 | 1      | 1          |
| Overall acceptability | Maximum | 5.2   | 5.50   | 5.50  | 3      | 5          |

The results show that the optimum brewing condition is at a loading per teabag of 2.48 grams, and brewing time is 3.45 minutes with a desirability value of 0.861. This optimum point was chosen because it has the highest desirability value or close to 1 [20]. Under optimal conditions, it is predicted to produce Eucalyptus blended tea with water-soluble extracts of 6.5045%, a* 4.7997, L* 25.4440, b* 27.2109, and an overall acceptability score of 5.4985. These optimal parameters are verified under optimum conditions and produce Eucalyptus blended tea with water-soluble extracts of 6.21%, a* 4.823, L* 25.227, b* 26.865, and an overall acceptability score of 5.564.

### 4. Conclusions

The optimal brewing condition of Eucalyptus blended tea is the loading per tea bag is 2.48 grams and the brewing time is 3.45 minutes that is predicted to produce water-soluble extracts, a*, L*, b* and overall acceptability values are 6.5045%, 4.7997, 25.4440, 27.2109, and 5.4985, respectively. These optimal parameters are verified under optimum conditions and produce Eucalyptus blended tea with
water-soluble extracts of 6.21%, a* 4.823, L* 25.227, b* 26.865, and an overall acceptability score of 5.564. Further research looked into the chemical characteristics, such as total phenolic compound and the antioxidant activity in the optimum formula and brewing conditions of blended tea.

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References
[1] Shimabukuro K 1996 Process for production of Eucalyptus tea. United States Patent 005578338
[2] 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
[3] Lee C K 1998 Ursane triterpenoids from leaves of Melaleuca leucadendron Phytochemistry 49 1119–22
[4] 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
[5] Yoshida T, Maruyama T, Nitta A and Okuda T 1996 An hydrolysable tannin and accompanying polyphenols from Melaleuca leucadendron Phytochemistry 42 1171–3
[6] Gogoi R C 2016 Blending of tea – the development Two a Bud 61 53–6
[7] 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
[8] 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
[9] Yang J E and Lee J 2020 Consumer perception and liking, and sensory characteristics of blended teas Food Sci. Biotechnol. 29 63–74
[10] Alakali J S, Ismaila A R, Alaka I C, Faasema J and Yaji T A 2016 Quality evaluation of herbal tea blends from ginger and pavetta cassipes European J. of Medicinal Plants 12 (4) 1–8
[11] Bassi P, Kumar V, Kumar S, Kaur S, Gat Y and Majid I 2019 Importance and prior considerations for development and utilization of tea bags; a critical review J. of Food Process Eng. e13069
[12] 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
[13] Mu’tamar M F F, Fakhry M and Ulya M 2021 IOP Optimization of the different formulations for the Eucalyptus blended tea based on response surface method IOP Conf. Ser. Earth Environ. Sci. 757
[14] Dheke P P and Patwardhan A W 2019 Mathematical modeling of tea bag infusion kinetics J. of Food Eng. 274 109847
[15] Castiglioni S, Damiani E, Astolfi P and Carloni P 2015 Influence of steeping conditions (time, temperature, and particle size) on antioxidant properties and sensory attributes of some white and green teas Int. J. Food Sci Nutr. Early Online 1–7
[16] Pérez-Burillo S, Giménez R, Ruffián-Henares J A, Pastoriza S 2017 Effect of brewing time and temperature on antioxidant capacity and phenols of white tea: relationship with sensory properties Food Chem. 218 111-118
[17] Ploenkuthan R, Sripromma P, Amornraks A, Yasurin P, Sripromma P, Asavasanti S, Soontrunnarudrungsri, A 2019 Effected brewing time and temperature of centella asiatica tea on antioxidant activity and consumer acceptance Proceed. of the 2019 9th Int. Conf. on Bioscience, Biochem and Bioinformatics 82-85
[18] Ministry of Health RI 2011 Indonesian Herbal Pharmacopoeia Edition I 2011 Ministry of Health of the Republic of Indonesia (Jakarta: Ministry of Health of the Republic of 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] Montgomery D C 2001 Design and analysis of experiments. New York: John Wiley & Sons
[21] Paramita N L P V, Andani N M D, Putri I A P Y, Indriyani N K S and Susanti N M P 2019 Characteristics of black tea simplicia from Camelia sinensis Var. assamica from the Bali Cahaya Amerta tea plantation, Angseri Village, Baturiti District, Tabanan Regency, Bali J. Kim. 13 58
[22] Voigt, R 1994 Pharmaceutical technology textbook. (Translation). Yogyakarta: Gajah Mada University Press
[23] Halim M O, Widyawati P S and Budianta D W 2015 Effect of proportion of beluntas leaf flour (Pluchea indica Less) and black tea on physicochemical properties, organoleptic properties, and antioxidant activity of beverage products J. Teknol. Pangan dan Gizi 14 10–6
[24] Rohdiana D 1999 Evaluation of theaflavin and thearubigin content in packaged dry tea JKTI 9 29–32
[25] Savitri K A M, Widarta I W R and Jambe A A G N 2019 Effect of comparison black tea (Camellia sinensis ) and red ginger (Zingiber officinale var . Rubrum) on the characteristics of teabag J. Ilmu dan Teknol. Pangan 8 419–29
[26] Ahmad I, Das T T, Yasin M and Hossain M A 2016 Study on biochemical compounds, antioxidant activity and organoleptic taste of some spice tea Agric. Food Sci. Res. 3 53–8