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Optimization of roselle tea bag brewing by variation of particle weight and type of tea bags

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ABSTRACT

Roselle flower petals contain bioactive compounds such as anthocyanin and vitamin C which function as primary antioxidants and can clinically improve the body's immune system. To improve the body's immune system, you can consume roselle in the form of herbal tea. There are several important aspects that must be considered when making herbal tea bags, such as the weight of the tea particles and the type of tea bag used. This research aims to determine the optimization of the properties of brewing roselle tea bags with variations in particle weight and type of tea bag and to determine the physicochemical and sensory characteristics of brewing roselle tea bags under optimum conditions. The research was carried out using the Response Surface Methodology (RSM) optimization method using Design Expert version 13 software with an Optimal Custom Design experimental design. The results of the research showed that the optimal brewing of roselle tea bags was by varying the particle weight treatment of 3.82 grams using nylon tea bags. The physicochemical characteristics of brewing roselle tea bags under optimum conditions are anthocyanin content (7.464±0.355 mg/L), antioxidant activity (92.47%), vitamin C content (2.534 mg/g), pH (2.47) and color. lightness (15.38) redness (-1.95) and yellowness (3.46). The sensory characteristics of brewing roselle tea bags under optimum conditions are that they have a distinctive aroma of roselle, the taste tends to be very sour, the color is slightly dark red, produces an astringent aftertaste and the panelists' preference for the product is neutral.

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INTRODUCTION

Since March 2023, WHO (World Health Organization) has officially revoked the status of PHEIC (Public Health Emergency of International Concern) for the Covid-19 disease outbreak. However, the corona virus still exists and people are still advised to maintain cleanliness, a healthy lifestyle and body immunity to ward off the virus. Viruses are disease agents that can threaten health at any time, especially when the body's immune system is weakened (Hafid Muliana, Syachriyani 2022) Therefore, maintaining the body's immune system is the most important thing at this time. One way that can be done is to consume nutritious food and additional nutrients that have health benefits, especially increasing the body's immunity. These additional nutrients can be obtained by consuming herbal tea. Herbal tea is defined as a drink made not from the tea plant (Camellia sinensis), but from one or a mixture of several ingredients, a combination of dry leaves, seeds, wood, fruit or flowers of other plants that have benefits (Yusasrini 2021).

One herbal tea that is widely known is herbal tea from the flowers of the roselle plant. Roselle (Hibiscus sabdariffa L.) is a herbal plant where all parts of the plant can be used for health, especially the flower petals. Roselle plants have three varieties, there are red roselle, purple roselle and white roselle. White roselle has lower antioxidant activity than red roselle and purple roselle. However, red roselle is more commonly consumed than purple roselle (Hartutik et al. 2023). Red roselle flower petals contain bioactive compounds such as anthocyanin and vitamin C. Anthocyanin is a flavonoid compound which functions as a primary antioxidant and can clinically improve the body's immune system (Kusnadi et al. 2021). Apart from acting as an antioxidant, anthocyanin compounds are believed to also be able to cure various diseases such as diabetes, hypertension, cancer and liver disorders (Yani et al. 2021).

Herbal tea is usually made in the form of tea bags, which is known to be easy and practical to use. When making tea bags, especially herbal tea bags, there are several important aspects that must be considered, such as the weight of the tea particles and the type of tea bag used. Particle weight is known to influence the absorption of bioactive compounds contained in tea. If the weight of the tea particles in the bag is small, the bioactive compounds infused in the tea will be low. Meanwhile, the greater the weight of the tea particles in the bag, the narrower the space for absorption of tea particles by water in the brewing process (Yadav et al. 2017). Mu'tamar et al. (2021) also examined the effect of the particle weight of eucalyptus herbal tea bags between 2-3 grams. The research results showed that the greater the weight of the particles being brewed, the higher the extract content obtained.

The effect of using bag packaging on the brewing of tea products, especially roselle tea bags, needs to be identified. Currently, tea bag packaging has various shapes and materials used, such as tea bags made from paper, nylon and non-woven fabric. The bag used to wrap tea products can affect the appearance and taste characteristics of the brewed tea. This is because tea bags with particle weight and type of tea bag are considered to be related to the brewing characteristics of the tea product (Jha et al. 2020). However, until now it has not been identified how particle weight and the type of tea bag used affect the characteristics of roselle tea bag brewing.

This research will discuss further how these two treatment variations affect the physical, chemical and sensory characteristics of roselle herbal tea bags using optimization methods. The optimization method chosen in this research is Response Surface Methodology (RSM). Response Surface Methodology is a statistical method that can develop, improve and optimize processes with several factors that can influence response variables. The aim of this research is to determine the optimum conditions for roselle tea bags with variations in particle weight and type of tea bag and to determine the physicochemical and sensory characteristics of roselle tea bags at optimum conditions.

METHODE

Materials

The materials used in this research were roselle flowers, potassium chloride, sodium acetate, hydrochloric acid (Merck), acetic acid (Merck), amylum indicator, iodine, methanol, DPPH (diphenyl-2-pycrilhidrazil) (Sigma Aldrich), distilled water and mineral water.

The main equipment used in this research were tea bags made of paper, nylon and non-woven fabric, sieves (20 mesh), grinder, analytical scales, test tubes (Iwaki), beakers glass (Herma),

erlenmeyer (Iwaki), measuring glass (Pyrex), volumetring flask (Pyrex), burette (Pyrex), clamp, stand, cuvette, vortex (VM-300), pH meter (pH-009(I)A), thermometer, color reader, cabinet dryer (code 16090670), UV-1800 spectrophotometer (Shimadzu), plastic cups, plastic gloves, filter paper, stove, gas, pan, and spoons.

Experimental Design

This research refers to a Completely Randomized Design (CRD). The method used is optimization using Response Surface Methodology (RSM) with an experimental design using Design Expert version 13 software and an optimization method using Optimal Custom Design. The factors chosen for optimization were the weight of tea particles (grams) with a lower and upper limit range of 1-4 grams as a numerical factor and the type of tea bag as a categorical factor. The response that will be the focus of the research is anthocyanin levels (mg/L). The experimental design recommended by the Design Expert version 13 software is shown in Table 1.

Analysis Procedure

Roselle flower petals that had been dried using a cabinet dryer (T = 55°C, t = 30 minutes) and ground using a grinder were weighed and put into tea bags according to each treatment (Table 1). The tea was brewed under the same conditions with hot water at 85°C for 5 minutes and stirred twice. The dipping frequency was 15 dipiing in 1 minute with a resting time of 45 seconds (Yadav et al. 2017). The brewed roselle tea bags then analyzed for anthocyanin content as an optimization variable. Analysis of anthocyanin levels was measured using the pH differential method using potassium chloride buffer pH 1 and sodium acetate buffer pH 4.5. The absorbance of the sample is then measured with a wavelength of 510-700 nm (Unawahi et al. 2022).

Optimization, Verification and Validation Procedure

The data obtained were analyzed using ANOVA using Design Expert software version 13 with the RSM (Response Surface Methodology) method. The test result data must be significant and the lack of fit is not significant. Optimization is carried out using the Optimal Custom Design method. This method will perform optimization based on the factor data and response measurements entered. Optimization will show the most optimal treatment recommendations according to the software. The most optimal treatment is indicated by the maximum desirability value (closer to 1.0) (Ramadhani et al. 2017).

Table 1 Experimental design recommended by the Design Expert version 13 software

Run	Space Type	Factor 1 Particle weight (gram)	Factor 2 Type of tea bag
1	Edge	2,155	Non-woven
2	Edge	1,39	Non-woven
3	Center	2,5	Paper
4	Edge	2,14	Nylon
5	Edge	1,42	Nylon
6	Edge	3,58045	Non-woven
7	Vertex	4	Paper
8	Edge	2,89	Non-woven
9	Edge	3,625	Nylon
10	Edge	2,89	Nylon
11	Vertex	1	Paper
12	Edge	3,625	Nylon
13	Edge	3,58045	Non-woven
14	Edge	1,39	Non-woven
15	AxialCB	1,75	Paper
16	Center	2,5	Paper
17	Edge	1,42	Nylon

The aim of verification and validation is to proving predictions of response values (anthocyanin levels) resulting from optimization using Design Expert software. Validation is carried out to test the optimum treatment results recommended by the software in reality. The test was carried out by repeating 3 times on the best treatment until the sample was completely valid.

Characterization Procedure

Physicochemical analysis (such as antioxidant activity, vitamin C, pH, color) and sensory analysis were also measured as characterization variables. Antioxidant activity was tested using the DPPH method with a concentration of 0.16 mM DPPH. Antioxidant activity, in this case the ability to scavenge free radicals, is calculated as percent inhibition (Sheikh et al. 2009). Vitamin C levels were measured with the iodometric titration method using 1% amylum indicator and titrated with 0.01 iodine (Sudarmadji 2010). pH is measured using a pH meter. The pH meter is calibrated using pH 7 and 4 buffers and then cleaned with distilled water. Next, the sample pH was measured (Widagdha and Nisa 2015). Color testing is measured using a color reader by taking a 5 mL sample, then placing it in a cuvette. A color reader is a tool that can measure light by reflecting and converting it to a set of coordinates such as L, a*, and b*. These coordinates will show a three-dimensional system that contains all colors (Deskawi et al. 2015).

Sensory analysis was measured using a scoring test, involving 25 semi-trained panelists who were asked to give their impressions of the aroma, taste, color, aftertaste and preference of roselle tea bag samples with variations in the treatment of tea particle size and the most optimal type of tea bag. The intensity of sensory testing was modified from the research of (Fatima et al. 2020) for aroma parameters (1 = very atypical to 5 = very typical), taste (1 = very not sour to 5 = very sour), color (1 = pink to 5 = dark red), aftertaste (1 = not astringent at all to 5 = very astringent), and liking (1 = strongly dislike to 5 = very like). The results are presented as average values and discussed.

RESULTS AND DISCUSSION

The effect of particle weight of tea bag on anthocyanin levels

The effect of particle weight on anthocyanin levels as a response variable for brewing roselle tea bags has been identified in this study. Particle weight has a significant influence on the anthocyanin content of the resulting roselle tea bag.

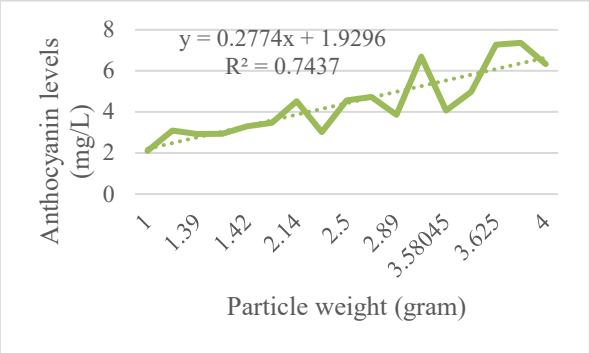


Figure 1 Graph of the effect of particle weight on anthocyanin levels

Based on Figure 1, the anthocyanin content as a response variable for brewing roselle tea bags shows different results depending on the weight of the particles used. In general, the greater the weight of the tea particles being brewed, the higher anthocyanin levels resulted. The same volume of solvent with a greater amount of particle weight will produce greater anthocyanin levels. This is because the surface contact area of the particles with the solvent increases (Mastuti et al. 2021).

The effect of tea bag type on anthocyanin levels

The use of different types of tea bags has a significant effect on the average anthocyanin content of the roselle tea bags produced.

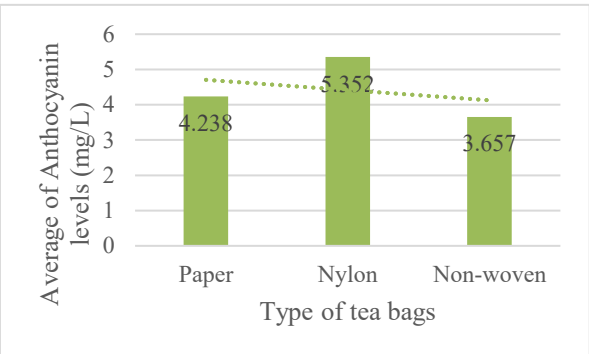


Figure 2 Average anthocyanin levels in various types of tea bags

Based on Figure 2, of the three types of tea bags, the highest average anthocyanin levels were produced from brewing tea using nylon bags. This higher anthocyanin content is due to a more uniform pore structure, the permeability value and percent porosity of nylon tea bags is higher compared to the other two types of tea bags. This increases the absorption of anthocyanins from brewing roselle tea bags (Jha et al. 2020).

Optimization of roselle tea bag brewing

Anthocyanin levels as a response variable for each roselle tea bag brewing treatment were measured. The results of anthocyanin levels for each treatment are shown in Table 2.

Table 2 shows that each treatment of the particle weight factor and type of tea bag produces anthocyanin levels as a response value with different levels. The lowest anthocyanin content of 2.104 mg/L was produced from treatment with a particle weight of 1 gram using a paper tea bag. The highest anthocyanin content of 7.264 mg/L was produced from treatment with a particle weight of 3.625 grams using a nylon tea bag. Both factors show an interaction between treatments as shown in Figure 3.

Table 2 Results of response to anthocyanin levels

Run	*Space Type*	Faktor 1 Particle weight (gram)	Faktor 2 Type of tea bag	Respon Anthocyanin levels (mg/L)
1	Edge	2,155	Non-woven	3,022
2	Edge	1,39	Non-woven	3,089
3	Center	2,5	Paper	4,559
4	Edge	2,14	Nylon	4,525
5	Edge	1,42	Nylon	2,939
6	Edge	3,58045	Non-woven	4,074
7	Vertex	4	Paper	6,329
8	Edge	2,89	Non-woven	3,857
9	Edge	3,625	Nylon	7,280
10	Edge	2,89	Nylon	6,696
11	Vertex	1	Paper	2,104
12	Edge	3,625	Nylon	7,364
13	Edge	3,58045	Non-woven	4,976
14	Edge	1,39	Non-woven	2,922
15	AxialCB	1,75	Paper	3,473
16	Center	2,5	Paper	4,726
17	Edge	1,42	Nylon	3,306

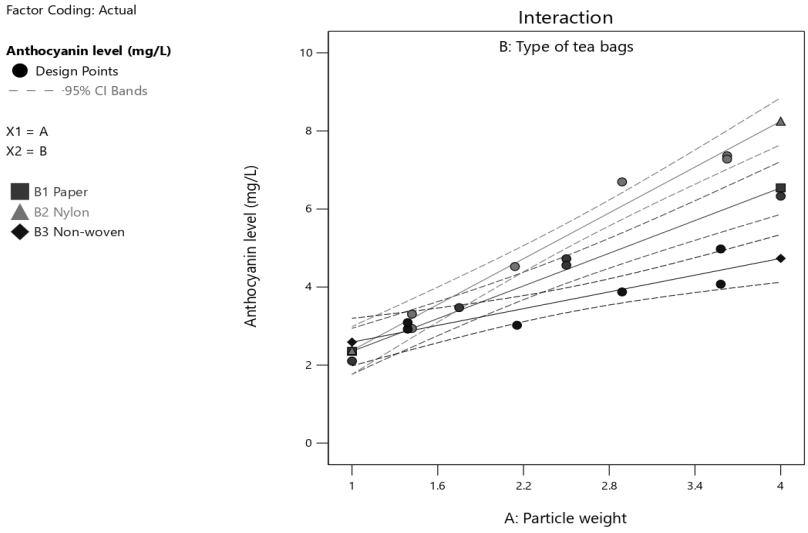


Figure 3 Graph of interaction between the two factors and response

Table 3 Results of ANOVA analysis factors of responses

Analysis	Model (P-value)	Lack of Fit (P-value)	R^2	Adjusted R^2	Adequate Precision
Anthocyanin levels	<0,0001	0,3494	0,9671	0,9522	24,4685

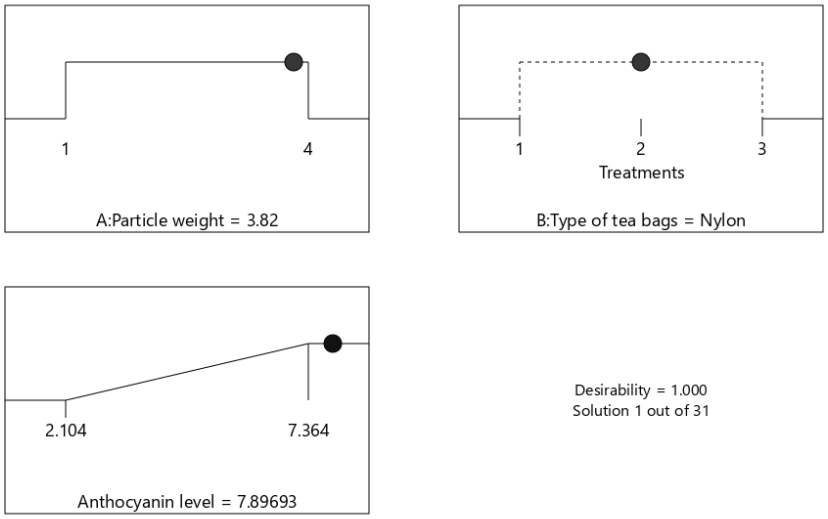


Figure 4 Prediction of the most optimal treatment according to the software

Table 4 Results of Verification and Validation

Actual Anthocyanin levels*	Standard Deviation	Predicted Anthocyanin levels**	95% Interval Prediction**	
			Low	High
7,464	0,355	7,897	7,185	8,608

Notes: *Processed data, 2023. **Design Expert version 13.

Table 5 Physicochemical characteristics of brewing roselle tea bags under optimum conditions

No.	Analysis	Result
1.	Anthocyanin levels (mg/L)	7,464±0,355
2.	Antioxidant activity (%)	92,47
3.	Vitamin C (mg/g)	2,534
4.	pH	2,47
5.	Color	
	Lightness (L*)	15,38
	Redness (a*)	-1.95
	Yellowness (b*)	3.46

The highest anthocyanin levels resulted from the interaction between the highest particle weight treatment using nylon tea bags. The lowest anthocyanin levels resulted from the interaction between the lowest weight treatment using paper tea bags.

Anthocyanin levels as a response variable have been analyzed using Design Expert software Version 13. The next step is the response data analysis process for optimization using ANOVA analysis. The results of the ANOVA analysis are shown in Table 3.

Based on Table 3, it can be seen that the significant model indicates that the two variations in particle weight treatment and type of tea bag have a P-value of less than 0.05 or are significant, which means that these two variables have a real influence on the anthocyanin content in roselle tea

bags. Anova analysis shows appropriate results supported by a high adjusted R-squared value (0.9522) and adequate precision of more than 4 (Mawi et al. 2018). After the ANOVA analysis requirements are met, optimization can be continued and the most optimal treatment predictions can be obtained.

The prediction results show that the most optimal treatment solution is roselle tea bags with a particle weight of 3.82 grams in nylon tea bags. This prediction was determined to be the most optimum treatment because it showed a desirability value close to or equal to 1. This prediction of the most optimum treatment was then carried out for verification and validation.

The average anthocyanin levels from the verification results was 7.464 ± 0.355 mg/L as shown in Table 5. This response value was close to the predicted value and was between the lowest prediction interval and the highest prediction interval (7.185 < 7.464 < 8.608). Based on the verification results, the treatment predictions shown by the Design Expert program, tea bags with a particle weight of 3.82 grams and nylon type tea bags, are valid as the best and most optimum treatment for roselle tea bag drink products.

Characterization of Brewing Roselle Tea Bags under Optimum Conditions

The physicochemical characteristics of brewing Rosella tea bags under optimum conditions are shown in Table 5.

The anthocyanin level of roselle tea bag brewing under optimum conditions was 7.464 ± 0.355 mg/L. (Purbowati et al. 2016) who extracted rosella with microwaves at 250 Watt power, using ethanol with a concentration of 78.36 for 4.91 minutes produced anthocyanin levels of 14.80 ± 0.08 mg/L. Differences in solvents and extraction or brewing conditions can cause differences in the anthocyanin levels of roselle tea bags. Ethanol is the extractant that is considered the most effective for extracting anthocyanin compounds (Amogne et al. 2020).

The antioxidant activity of brewing roselle tea bags under optimum conditions is 92.47%. (Juniarka et al. 2011) identified the antioxidant activity and anthocyanin content of roselle flower petals. The research results showed that roselle flowers extracted using the multilevel maceration method with methanol:formic acid (97:3) had antioxidant activity of 38.22% with anthocyanin amounts of 3.65 mg/L. The antioxidant activity of brewing roselle tea bags under optimum conditions was higher compared to the study because the amount of anthocyanin compounds contained was also greater. This proves that antioxidant activity is influenced by antioxidant compounds, in this case especially the amount of anthocyanin compounds.

The analysis results showed that the level of vitamin C contained in roselle tea bags at optimum conditions was 2.534 mg/g. (Purbowati et al. 2016) stated that roselle extract resulting from optimization of the microwave-assisted extraction method with 250 Watt power, methanol concentration of 78.36 for 4.91 minutes had a vitamin C content of 10.74 ± 0.14 mg/g, which was more large compared to the vitamin C levels of roselle tea bag brewing in this study. (Purbowati et al. 2016) stated that each extraction method has a different level of effectiveness, causing the extracted bioactive compounds to have different levels.

The test results showed that the pH value of roselle tea bags at optimum conditions was 2.47. The pH value of brewing roselle tea bags tends to be low due to the acid content in the raw material, namely roselle flowers. Roselle flowers contain acidic compounds such as ascorbic acid or what is commonly called vitamin C, so they have a sour taste and low pH (Ramadhani et al. 2017).

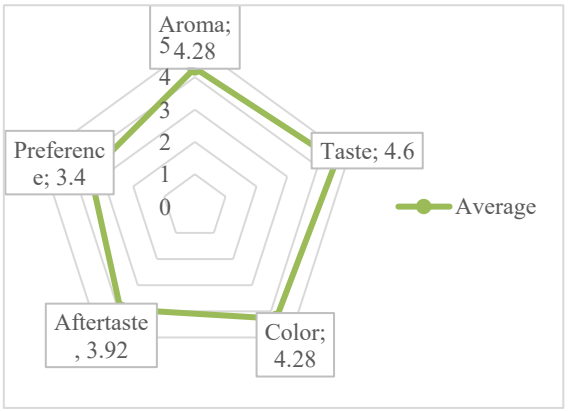


Figure 5 Sensory analysis graph for brewing rosella tea bags

Brewing roselle tea bags under optimum conditions has a brightness level (L*) of 15.38. This value shows that brewing roselle tea bags under optimum conditions has a fairly dark color. The redness value (a*) of brewing roselle tea bags under optimum conditions is -1.95, indicating that the sample has a color that tends to be bluish red

because of the concentration of the sample. The yellowness value (b*) is 3.46. Roselle tea bags have a deep red color. This red color is produced from the anthocyanin compound content in roselle flowers. According to (Mulyani et al. 2024), the dark color of the brew can be caused by the temperature and brewing time because the compounds contained can be extracted more completely. The darker color of the brew can also be caused by the lower pH value.

The sensory characteristics of brewing roselle tea bags under optimum conditions are that they have a typical aroma of roselle (4.28), the taste tends to be very sour (4.6), the color is slightly dark red (4,28), produces an astringent aftertaste (3,92) and the panelists' preference for the product is neutral (3,4). Brewed roselle tea bags have a distinctive aroma due to the volatile compounds contained in them, causing the brewed roselle tea to have a distinctive sour aroma (Hastuti and Kusnadi 2016). The taste of brewing roselle tea bags under optimum conditions tends to be very sour because it contains quite a high amount of ascorbic acid and malic acid, giving a sour taste with a fresh sensation to roselle herbal tea drinks (Nurilawaty et al. 2013). This astringent taste is obtained from the flavonoid compounds contained in roselle flowers, namely tannins (Djailolo et al. 2022).

The color of this brew is influenced by the dye contained in roselle flowers, namely anthocyanin. Apart from that, the pH level also affects the color produced from brewing roselle tea bags. A decrease in the pH value can change the anthocyanin compound to become more red in color (Hastuti and Kusnadi 2016). The overall preference of the panelists for brewing roselle tea bags under optimum conditions is neutral. The taste of roselle tea bags which is classified as very sour is the reason why the panelists gave a neutral assessment of this roselle tea bag product. Therefore, further research needs to be carried out to find out the right formulation for making roselle tea bags so that they are more preferred.

CONCLUSION

The results of the research showed that the most optimal brewing of roselle tea bags was by varying the particle weight treatment by 3,82 grams using nylon tea bags. The physicochemical characteristics of brewing roselle tea bags under optimum conditions are anthocyanin content (7,464±0.355 mg/L), antioxidant activity (92,47%), vitamin C content (2,534 mg/g), pH (2,47) and color. lightness (15,38) redness (-1,95) and yellowness (3,46). The sensory characteristics of brewing roselle tea bags under optimum conditions are that they have a distinctive aroma of roselle, the taste tends to be very sour, the color is slightly dark red, produces an astringent aftertaste and the panelists' preference for the product is neutral. Based on these results, it can be concluded that the particle weight and type of tea bag have an influence on the results of brewing roselle tea bag.

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