Indonesian Journal of Pharmaceutics
IdJP
ISSN : 2597-8748

Vol. 6, Issue 1, 2024 (01-16)
http://journal.unpad.ac.id/IdJP

Formulation of Black Garlic Tea with The Addition of Ginger (Zingiber officinale), Rosella (Hibiscus sabdariffa L), and Packaging Design

Efri Mardawati[1], Cahyani Rizki Sendi[1], Devi Maulida Rahmah[1], Totok Pujianto[1], Herlina Marta[1], Norisca Aliza Putriana[*2]

[1]Faculty of Agricultural Industrial Technology, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang KM 21, Jatinangor 45363, Indonesia

[2]Department of Pharmacy, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang KM 21, Jatinangor 45363, Indonesia

Submitted: 18/01/2024, Revised: 25/01/2024, Accepted: 28/01/2024, Published: 29/04/2024

ABSTRACT

Indonesia is globally recognized for its abundant biodiversity and is considered one of the most biodiverse countries in the world. Over centuries, local communities have explored the benefits of natural ingredients such as garlic to be used as herbal medicine. Although garlic has numerous health benefits, raw consumption is still uncommon due to its strong aroma and taste. To address this issue, black garlic, a derived product from garlic, has been developed to minimize its strong characteristics. Therefore, this research aimed to investigate formulation of black garlic tea with the addition of ginger and rosella flowers in terms of bioactive components, including total phenolic content, total flavonoid content, antioxidant activity, and organoleptic characteristics of taste and aroma. The method used involved the extraction of 12 samples. The results indicated that the highest phenolic content was 16.708 ± 0.0356 mg GAE/g with formulation of black garlic, rosella, and ginger at a ratio of 1 g: 3 g: 2 g. The highest flavonoid content was observed in formulation of black garlic, rosella, and ginger at a ratio of 2 g: 3 g: 2 g, which amounted to 13.560 ± 0.536 mg QE/g. Furthermore, formulation with black garlic, rosella, and ginger at a ratio of 2 g: 3 g: 2 g exhibited the highest antioxidant activity of 63.21%. These results showed that formulation of black garlic tea with the addition of ginger and rosella flowers could enhance the levels of bioactive components, including total phenolic content, total flavonoid content, and antioxidant activity.

Keywords: Antioxidant Activity; Black Garlic; Ginger; Rosella; Total Flavonoid Content; Total Phenolic Content.

*Corresponding author,
e-mail: norisca@unpad.ac.id N. A. Putriana)

https://doi.org/10.24198/idjp.v6i1.52667

1. Introduction

Herbal beverages are commonly made from various natural parts of the plant, including leaves, stems, roots, fruits, buds, and flowers. These beverages are a rich source of natural bioactive compounds such as flavonoid, alkaloids, terpenoids, saponins, coumarins, phenolic acids, and carotenoids (Prisdiany et al, 2021).

Garlic (Allium sativum) is a type of tuber that has been widely used as a seasoning in food and an ingredient in herbal medicine. One of the derivatives product from garlic is Black Garlic, which is produced through spontaneous heating at specific temperatures and humidity. Garlic passes through the Maillard or Browning process, changing its color from white to brown and finally black due to the incubation process at a sufficiently high temperature and humidity. This transformation increases certain content, particularly antioxidant when compared to fresh garlic (Kimura et al, 2015).

Ginger (Zingiber officinale) is another plant that is widely used as an ingredient in herbal medicine with numerous benefits. Furthermore, ginger is a popular spice and medicinal plant, with several varieties commonly used for different purposes, such as red, elephant, and java ginger. The compounds found in ginger have anti-carcinogenic, non-toxic, and non-mutagenic properties in high concentrations. Some of these compounds, including gingerol, shogaol, and zingerone, exhibit pharmacological and physiological activity, namely antioxidant, anti-inflammatory, analgesic, anti-carcinogenic, and cardiotonic effects. A specific group of antioxidant compounds found in ginger is phenolic compounds, specifically flavonoid, which act as polar scavengers of free radicals (Fakhrudin et al, 2015)

In 2021, ginger production had a significant increase of approximately 40%, reaching 307 thousand tons compared to 183 thousand tons in 2020 (Badan Pusat Statistik, 2021). This is due to the naturally high content of pharmacological antioxidant compounds that effectively and efficiently inhibit superoxide and hydroxyl free radicals produced by cancer cells. Furthermore, it has been widely used in Traditional Chinese Medicine since at least the ninth century for treating nausea, diarrhea, and digestive complaints (Romm et al, 2010). The biological activity of ginger include antioxidant, anti-inflammatory, anticancer, antidiabetic, and antitumor effects (Azeez.& Lunghar, 2021)

Rosella (Hibiscus sabdariffa L.) is a plant of the Malvaceae family that grows abundantly in tropical regions such as Java and Kalimantan. The flowers of the rosella plant contain several beneficial substances for the human body and are distinguished by their acidic taste, providing a refreshing sensation and a pinkish-red color. This sour taste is due to the presence of vitamin C (0.002-0.005%), citric acid, malic acid, with total acidity of 13%, and glycolic acid (Utari et al, 2017). Therefore, the flowers are widely used by the Indonesian community to make infused beverages, considering the benefits of taste and color that are well-received by the people.

Among the several constituents, rosella contains anthocyanins, which act as natural antioxidant capable of neutralizing free radicals. Antioxidant are compounds that can donate electrons to oxidizing agents, thereby inhibiting their activity. Additionally, rosella is a well-known plant because its flower calyx can be used to make health drinks believed to be beneficial in treating various conditions such as hypertension and diabetes, as well as acting as a diuretic (Mardiah & Sulaeman, 2010).

The active compounds that play a significant role in the calyx of rosella flowers include gossypetin, anthocyanins, and hibiscus glucosides (Utari et al, 2017). The red color of the flowers is due to the presence of anthocyanins, which act as antioxidant, contributing to the treatment of degenerative diseases. Anthocyanins have a conjugated double-bond system that enables the function of antioxidant with radical scavenging mechanisms (Mardiah & Sulaeman, 2010).

Previous research has shown an increase in antioxidant and anti-inflammatory levels in the combination of garlic and ginger (Imo, 2019). This results align with earlier investigations that explored the combination of garlic and ginger, indicating higher anti-inflammatory properties and antioxidant levels compared to only garlic and ginger (Okoh, 2014). A significant increase in antioxidant levels of ginger, rosella, and lemongrass has also been reported (Suseno et al, 2022). Another research showed changes in physicochemical properties, including increased antioxidant levels, when fresh garlic was fermented to become black garlic (Kimura et al, 2017).

Considering the potential of high antioxidant content in black garlic, ginger, and rosella flowers, efforts are needed to enhance the acceptability of the taste and aroma characteristics of black garlic. To achieve this, further research and testing are necessary to determine the appropriate formulation for black garlic herbal beverages by adding ginger and rosella flowers to determine antioxidant levels and organoleptic characteristics. Therefore, this research aimed to identify the proper formulation for combining black garlic with ginger and rosella flowers to produce a product with high antioxidant content and desirable organoleptic characteristics in terms of taste and aroma. The analysis was carried out to identify a suitable packaging model for black garlic herbal beverages with added ginger and rosella flowers from a consumer perspective.

2. Method

2.1. Research Material

This research was conducted at the Pedca Utara and the Pilot Plan Laboratory of the Faculty of Agricultural Technology, Universitas Padjadjaran. Data collection was conducted from December 2022 to February 2023. The materials used consisted of single garlic, ginger, and rosella flowers. Other materials employed for the analysis process included distilled water, methanol, ethanol, 10% (w/v) AlCl3, 10% Folin-Ciocalteau, 7.5% (w/v) Sodium Carbonate (NaCO3), 5% (w/v) Sodium Nitrite (NaNO2), 1 N Sodium Hydroxide (NaOH), DPPH (2,2-diphenyl-1-picrylhydrazyl), Quercetin, and Gallic Acid.

2.2. Ratio Formulation

The analysis of ratio formulation was conducted using an experimental method with a two-factor completely randomized factorial design. The first factor was the ratio formulation of the base materials mixture, which included black garlic, ginger, and rosella flowers. Formulation ratios to be tested were: 1:1:1 (A), 1:0:1 (B), 1:1:0 (C), 1:3:1 (D), 2:3:2 (E), and 1:3:2 (F).

The second factor was the size factor of each base material, including black garlic, ginger, and rosella flowers. The sizes were prepared as powder by grinding the samples using a blender and in the form of thin dried slices.

2.3. Packaging Analysis

The organoleptic test was conducted with 15 panelists using the hedonic quality test method or preference. The panelists were asked to provide their responses about their likes or dislikes and degrees of preference. These degrees of preference were referred to as the hedonic scale. For example, in terms of "like," the hedonic scale included categories such as "like very much," "like," "like slightly," "neither like nor dislike", "dislike,“ "dislike very much," and "dislike extremely." The hedonic scale can be expanded or narrowed (Unpad, 2008) and the test discussed the secondary and primary packaging (tea bag) based on the existing hedonic scale, as indicated in Table 1.

Table 1. Table of Hedonic Quality Test Design

Test Variables	
Preference	
Hedonic Scale	Numeric Scale
Like very much	4
Like	3
Like slightly	2
Dislike	1

2.4. Data Analysis

The data obtained were processed using Microsoft Excel 2019 software, and subjected to ANOVA (Analysis of Variance) through IBM SPSS Statistics 25 software following the outlined procedures (Harsojuwono et al, 2021). When the results indicated a significant effect of the experimental factors, the analysis was continued with Duncan Multiple Range Test (DMRT) at a 5% confidence level to determine significant differences between the groups.

The data obtained from the hedonic test were analyzed using non-parametric tests, namely the Kruskal-Wallis and Mann-Whitney post hoc tests, to determine significant differences between groups. The rank test results were analyzed descriptively using Microsoft Excel 2019, and the Kruskal-Wallis and Mann-Whitney tests were conducted with SPSS 25 software.

3. Result

3.1. Total Phenolic Content

Based on the total phenolic content test using the ANOVA method, the following results were obtained:

Table 2. ANOVA variance test result on total Phenolic Content

Source	SS	df	Mean Square	f-count	f-table	p-value
Corrected Model	3.851a	11	0.350	332.166	2.22	0.000044
Intercept	16.017	1	16.017	15195.514	4.26	0.000026
Ratio	3.104	5	0.621	588.953	2.62	0.000068
Size	0.378	1	0.378	358.236	4.26	0.00014
Ratio * Size	0.370	5	0.074	70.166	2.62	0.00039
Error	0.025	24	0.001			
Total	19.894	36				
Corrected Total	3,877	35				
a. R Squared = 0.993 (Adjusted R Squared = 0.990)						

Source: Experimental Data, 2023

a. Total Flavonoid Content

Based on the total flavonoid content test using the ANOVA method, the following results were obtained:

Table 3. ANOVA Variance Test Results on Total Flavonoid Content

Source	SS	df	Mean Square	f-count	f-table	p-value
Corrected Model	0.377[a]	11	0.034	67.640	2.22	0.000033
Intercept	3.463	1	3.463	6826.520	4.26	0.000059
Ratio	0.296	5	0.059	116.491	2.62	0.00038
Size	0.072	1	0.072	141.924	4.26	0.00029
Ratio * Size	0.010	5	0.002	3.932	2.62	0.01009
Error	0.012	24	0.001			
Total	3.853	36				
Corrected Model	0.390	35				
a. R Squared = 0.969 (Adjusted R Squared = 0,954)						

Source: Experimental Data, 2023.

b. Antioxidant Activity

Based on the antioxidant activity test using the ANOVA method, the following results were obtained:

Table 4. The ANOVA Multiple Test Result on Total Flavonoid Content

Source	SS	df	Mean Square	f-count	f-table	p-value
Corrected Model	0.637a	11	0.058	34.765	2.22	0.000
Intercept	10.484	1	10.484	6291.712	4.26	0.000
Ratio	0.426	5	0.085	51.106	2.62	0.000
Size	0.081	1	0.081	48.690	4.26	0.000
Ratio * Size	0.130	5	0.026	15.638	2.62	0.000
Error	0.040	24	0.002			
Total	11.161	36				
Corrected Total	0.677	35				
a. R Squared = 0.941 (Adjusted R Squared = 0.914)						

Source: Experimental Data, 2023

c. Organoleptic Test

Based on the organoleptic test, the following results were obtained:

Table 5. The Results of the Organoleptic Testing for All Samples

Parameter	The mean values of the Hedonic Testing for each sample					
	D1	D2	E1	E2	F1	F2
Black garlic taste	3.20±0.775abc	2.80±0.941ac	3.47±0.640b	2.67±0.816ac	3.20±0.676a	2.60±0.828c
Ginger taste	3.27±0.704ab	2.67±1.113abc	3.40±0.737b	2.53±0.834c	3.07±0.884abc	2.80±1.014abc
Sour taste	3.53±0.516a	2.73±1.033ab	3.40±0.507a	2.73±0.884b	3.47±0.640a	2.87±0.913b
Black garlic aroma	3.27±0.594a	2.80±0.862b	3.33±0.816ab	2.73±1.033ab	3.33±0.617ab	2.60±0.737bc
Ginger aroma	2.93±0.799a	2.27±0.799b	2.87±0.834ab	2.33±0.816ab	3.53±0.640c	2.73±0.704ab

Source: Experimental Data, 2023

Table 6. The Overall Hedonic Test Results for Black Garlic Tea

Sample	Raw Material	Ingredient Form			Score
		Black Garlic (g)	Rosella Flowers (g)	Ginger (g)	
D1	Powder	1	3	1	36
D2	Slices	1	3	1	39
E1	Powder	2	3	2	48
E2	Slices	2	3	2	40
F1	Powder	1	3	2	47
F2	Slices	**1**	**3**	**2**	**52**

d. Packaging Design

Based on the packaging design test, the following results were obtained:

Table 7. Packaging Selection Test Results

Packaging Form	Average
Secondary Packaging	
Triangle Zip Lock	3.400
Aluminum Tube	**3.667**
Rectangular Box	3.400
Primary Packaging	
Triangular Pyramid	3.467
Drawstring Bag	**3.600**
Circle	3.400

4. Discussion

4.1. Total Phenolic Content

Based on testing total phenolic content in each used ingredient, black garlic exhibited the highest content, followed by rosella, and ginger. Total phenolic content in black garlic, in the form of powder and thin slices, was 4.198 mg GAE/g and 3.938 mg GAE/g. Total phenolic content in rosella, in the form of powder and thin slices, was 3.753 mg GAE/g and 3.494 mg GAE/g. For ginger, total phenolic content in powder and thin slices was 3.543 mg GAE/g and 2.679 mg GAE/g, respectively.

Total phenolic content obtained from individual ingredients played a significant role in determining the overall total phenolic content in each tested formulation. Formulation with the highest value was found in sample F1, with a composition of black garlic, rosella, and ginger in powder form at the ratio of 1g:3g:2g.

Total phenolic content from each ingredient in formulation F1 showed that the composition of rosella was the highest compared to black garlic and ginger. Although black garlic and ginger had the same composition of 1 g, black garlic content was in the first position, influencing the high total phenolic content. The combination of black garlic with rosella considerably affected phenolic content in formulation F1. This aligned with formulation B1, where the composition of black garlic with rosella resulted in total phenolic content of 6.255 ± 1.886 mg GAE/g, higher than formulation C1, which had the composition of black garlic and ginger with a value of 3.531 ± 1.960 mg GAE/g. Based on the quantitative results obtained, further testing was conducted using Microsoft Excel 2019 software. The processed data were subjected to ANOVA (Analysis of Variance) to determine the effect of the specifications on the ratio and size parameters used. The data was presented in the form of variance analysis

The ANOVA variance test result on the effects of the ratio and size treatments of black garlic, ginger, and rosella flowers, as well as their interaction on total phenolic content, showed that the overall model was highly significant, as indicated by the p-value (0.00039) > alpha (0.05). The treatment ratios showed an f-count of 588.953, while the f-table was 2.62. Since the f-count was greater than the f-table and the p-value (0.000068) > alpha (0.05), it was concluded that there is at least one treatment level of the ratios had a significant effect on total phenolic content. To determine which ratio of black garlic, ginger, and rosella flowers most significantly affected total phenolic content, further testing using Duncan's test was conducted.

For the size treatment of black garlic, ginger, and rosella flowers, the f-count was 358.236, and the f-table was 4.26. Since the f-count > f-table and the p-value (0.00039) > alpha (0.05), it was concluded that there was a highly significant difference in the effect of the size treatment of black garlic, ginger, and rosella flowers on total phenolic content.

The results of the Duncan test on the ratio treatments of black garlic, ginger, and rosella flowers, divided the six treatment levels into five subsets. Ratios that showed no significant difference were placed in the same subset, while those with significant variation were put in separate subsets. In subset one, there was 1:1:0, subset two had 1:1:1 and 1:0:1, subset three consisted of 1:3:1, subset four had 2:3:2, and subset five comprised 1:3:2. From the calculations in the Duncan test, it was discovered that the highest total phenolic content value was in the ratio of 1:3:2 of black garlic, ginger, and rosella flowers, while the lowest exhibited a ratio of 1:1:0.

Total phenolic content test was conducted to determine the difference in phenolic compound levels between the powdered mixture of black garlic-rosella-ginger (odd-numbered) and the thinly sliced mixture of black garlic-rosella-ginger (even-numbered), which indicated the potential antioxidant activity. The method employed was based on the reduction strength of the hydroxyl groups of phenolic compounds using the Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent was reduced to form a blue-colored molybdenum complex, which was measured using a UV-Vis spectrophotometer. At the same time, phenolic compounds were oxidized to phenolate ions. Subsequently, Na2CO3 was added to create an alkaline environment to facilitate the dissociation of the proton in phenolic compounds, forming phenolate ions. Gallic acid is a standard solution because it is a heteropoly acid with three hydroxyphenolic groups that will be oxidized by Folin-Ciocalteu (Januarti et al., 2019). The chemical structure of plants polyphenolic compounds that exhibited heat resistance affected total phenolic content. The increase or decrease in total phenolic content with increasing temperature was also affected by the type of food material.

The use of raw materials in dry form affected the resulting total phenolic content. This was because drying damaged cell walls and released phenolic compounds from insoluble components such as proteins, thereby causing an increase during extraction (Nyangena et al, 2019). Similarly, a previous research stated that total phenolic content of oven-dried mangoes was higher than fresh ones

(Gümüşay et al, 2015).

3.2. Total Flavonoid Content

Based on testin g total flavonoid content in each used ingredient, black garlic had the highest value, followed by rosella and ginger. Total flavonoid content in black garlic, in the form of powder and thin slices, was 37.567 mg GE/g and 34.056 mg GE/g, while rosella had values of 19.149 mg GE/g and 18.544 mg GE/g, respectively. As for ginger, total flavonoid content in powder and thin slices is 12.428 mg GE/g and 9.335 mg GE/g, respectively.

Total flavonoid content obtained from each used ingredient determined total content in each tested formulation. Based on formulation with the highest total flavonoid content was found in sample E1, with a composition of black garlic, rosella, and ginger in powder form with the respective ratio of 2g:3g:2g. In formulation E1, the composition of rosella was the highest compared to others, which significantly contributed to the high flavonoid content. Although black garlic and ginger had the same composition of 2g, black garlic content was in the first position, influencing the high total content. The combination of black garlic with rosella considerably also affected content in formulation E1. This aligned with the testing in formulation B1, where the composition of black garlic with rosella resulted in total flavonoid content of 8.242 ± 0.670 mg GE/g. This value was higher than formulation C1, which had the composition of black garlic and ginger with total of 36.281 ± 0.479 mg GE/g/.

The ANOVA variance test showed the effects of the ratio and size treatments of black garlic, ginger, and rosella flowers, as well as their interaction on total flavonoid content. The results showed that the overall model was significant, as indicated by the p-value (0.00033) < alpha (0.05). Moreover, it was found that for the ratio treatment of black garlic, ginger, and rosella, the calculated f-count was 116.491, and the f-table was 2.62. Since the f-count > f-table and the p-value (0.00038) > alpha (0.05), it can be concluded that there was at least one treatment level of the ratio of black garlic, ginger, and rosella with a highly significant effect on total flavonoid content. Further investigation was carried out using the Duncan test to determine the ratio most affecting total flavonoid content

For the size treatment of black garlic, ginger, and rosella flowers, the calculated f-count was 141.924, and the f-table was 4.26. Since the f-count > f-table, and the p-value (0.00029) > alpha (0.05), there was a highly significant difference in the effect of the size treatment on total flavonoid content. Based on the interaction between the ratio and size treatments of black garlic, ginger, and rosella flowers, the f-count was 3.932, and the f-table was 2.62. The f-count > f-table and the p-value (0.01009) > alpha (0.05) suggested that at least one interaction level of the ratio and size treatments of black garlic, ginger, and rosella flowers with a highly significantly affected total flavonoid content.

The results of the Duncan test on the ratio treatments of black garlic, ginger, and rosella flowers, divided the six treatment levels into four subsets. Ratios that showed no difference were placed in the same subset, while significantly different ratios were put separately. In subset one, there was only 1:1:0, and subset two had ratios 1:3:2, 1:1:1, and 1:3:1. In subset three, there were ratios 1:3:1 and 1:0:1, while subset four had only ratio 2:3:2. From the calculations in the Duncan test, it was discovered that the highest total flavonoid content was in the ratio 2:3:2 of black garlic, ginger, as well as rosella flowers, and the lowest was in the ratio 1:1:0.

In this research, the quantitative analysis of flavonoid compounds in black garlic extract was carried out using the method of complex formation with aluminum chloride. A quercetin standard curve was used as a reference in determining total flavonoid content. This method was based on the formation of a complex between aluminum chloride and the ketone group at the C-4 atom and the hydroxyl group at the C-3 or C-5 atom, as part of the flavone and flavonol groups. Quercetin was used as the standard because it was found to be a strong flavonoid compound in the flavonol group. Flavonols were also known for their characteristic presence in flavonoid compounds that were widely distributed in plants. Furthermore, several medicinal plants exhibited high levels of quercetin content.

3.3. Antioxidant Activity

Antioxidant activity in black garlic tea was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method following a modified procedure (Choi et al, 2014). The wavelength used was 517 nm and the incubation time was set to 30 minutes in a dark place. Antioxidant activity in black garlic tea was tested to determine the percentage of DPPH free radical inhibition by antioxidant compounds present in black garlic tea.

Based on antioxidant activity testing of each ingredient used, black garlic showed the highest activity, followed by rosella, and ginger. Antioxidant activity of black garlic in the form of powder and thin slices was 73.39% and 73.04%, rosella had values of 72.50% and 67.23%, while ginger showed 66.25% and 64.02%, respectively.

Antioxidant activity obtained from each ingredient played a significant role in determining the overall total antioxidant activity in the tested formulation. Formulation with the highest antioxidant activity was found in sample E1 with a composition of black garlic, rosella, and ginger in powder form at a ratio of 2 g: 3 g: 2 g, respectively. Based on total flavonoid content of each ingredient, in formulation E1, rosella had the highest content compared to black garlic and ginger. This contributed significantly to the high antioxidant activity in formulation E1 compared to black garlic and ginger. Although black garlic and ginger have the same composition of 2 g, black garlic content was at level 1, which affected the high antioxidant activity. The combination of black garlic with rosella significantly affected antioxidant activity in formulation E1. Similarly, formulation B1, with a composition of black garlic and rosella, had antioxidant activity of 58.84%, higher than formulation C1, with a composition of black garlic and ginger, at 51.0%.

The ANOVA analysis showed the effect of treatment factors, namely the ratio and size on the three plants as well as their interaction on antioxidant activity. The results showed that the overall model was significantly high because of the obtained p-value (0.000) < alpha (0.05). Furthermore, for the treatment factor of black garlic, ginger, and rosella ratio, the f-count was 51.106, and f-table was 2.62. Since the f-count > f-table and the p-value (0.000) > alpha (0.05), there was at least one treatment level of the ratios that significantly affected antioxidant activity. Further analysis using the Duncan test was conducted to identify which specific black garlic, ginger, and rosella ratios had the most significant effect on antioxidant activity.

For the treatment factor of black garlic, ginger, and rosella size, the f-count was 48.690, while the f-table is 4.26. The f-count > the f-count and the p-value (0.000) > alpha (0.000 < 0.05) indicated a significantly high difference in the effect of the treatment level sizes.

The results of Duncan test on the treatment factor of black garlic, ginger, and rosella ratio, divided the six treatment levels into four subsets. Ratios that showed no significant difference were placed in the same subset, while those with a specific variation were put in different subsets. In subset one, there were 1:0:1, 2:3:2, and 1:1:1, subset two had 1:3:2, subset three had 1:3:1; and in subset four, there was a ratio of 1:1:0.

The increase in the percentage of inhibition in black garlic sample was due to the rise in antioxidant compounds present in black garlic. This was observed in the testing of total phenolic and flavonoid, where there was an increase in content of the compounds due to their antioxidant properties. Based on previous research, another compound that contributed to the increased antioxidant activity in black garlic was S-allyl-cysteine (SAC) (Bae et al, 2014).

3.4. Organoleptic Test

The tests used in this research were discrimination and hedonic quality testing. The discrimination testing was conducted to determine whether there were sensory or organoleptic differences between two or more samples. This test was carried out to assess the effects of different treatments given to the samples or identify similarities or differences between the tested samples. Meanwhile, hedonic quality testing was performed to determine the panelists impressions of the tested samples. Hedonic quality can be general, such as liking-disliking, good-bad, or specific, in terms of taste and aroma.

Antioxidant activity of black garlic tea was assessed through a hedonic test. The taste results showed that the highest preference was obtained in formulation E1 with black garlic, ginger, and rosella in the ratio of 2 g: 3 g: 2 g in powder form, with an average hedonic score of 3.47±0.640b. This indicated a strong black garlic taste. The hedonic results for the taste in the next formulation were D1 with a composition of 1 g: 3 g: 1 g in powder form and E1 with a composition of 2 g: 3 g: 2 g, with values of 3.20±0.775abc and 3.20±0.676a, respectively. This was due to the significant effect of black garlic content in each formulation when combined with other ingredients (Intania, 2019).

Based on the results above, a Kruskal-Wallis test was performed on the parameter of black garlic taste, which showed a P-value of < α (0.05). This indicated that there was a significant difference between the effects of treatments (D1, D2, E1, E2, F1, F2) on black garlic taste. To identify significantly different groups, a

Mann-Whitney test was conducted, and found that the preference level for black garlic taste did not differ significantly because the P-value > α (0.05) for D2 and E2. Furthermore, it was observed that there was a significant difference (P < α, 0.05) between D2 and F1, E1 and F2, E2, and F1, as well as F1 and F2.

The Kruskal-Wallis test result on the parameter of ginger taste showed that the P-value > α (0.05) or H0 was accepted. This indicated that there was no significant difference between the effects of treatments (D1, D2, E1, E2, F1, F2) on ginger taste. To identify significantly different groups, a Mann-Whitney test was conducted.

The hedonic test result for ginger taste was most significant in formulation E1 with black garlic, ginger, and rosella in the sequence of 2 g : 3 g: 2 g in powder form, with an average score of 3.40±0.737b. This indicated that ginger taste was significantly strong compared to other formulation. The next hedonic result for ginger taste was formulation F1 with a composition of 1 g: 3 g: 2 g in powder form. Ginger in powder form produced a more pronounced taste due to its smaller surface area. A smaller surface area facilitated the extraction process (Maksum & Purbowati, 2017).

Based on the results above, the Mann-Whitney test was conducted as a follow-up test to show that the preference level for ginger taste did not differ significantly with P>α (0.05) in D2, F1, and F2. Furthermore, it was discovered that there was a significant difference with P<α (0.05) between D1 and E2, as well as E1 and E2.

The Kruskal Wallis test result on the sour taste parameter showed the P value<α (0.05). This indicated that there was a significant difference between the effects of treatments (D1, D2, E1, E2, F1, F2) on the sour taste. The hedonic test result also showed that the most significant taste was in formulation E1 with black garlic, ginger, and rosella in a sequence of 1 g: 3 g: 1 g in powder form, with an average score of 3.53±0.516a. This showed that the sour taste was mainly derived from rosella due to its relatively high vitamin C content (Dianasari & Fajrin, 2015).

To determine significant differences between groups, the Mann-Whitney test was conducted. The result showed that the preference level for black garlic (BG) aroma was not significantly different, as the P value>α (0.05) for D1, E1, and F1, as well as for E2 and F2. However, a significant difference was observed with a P value<α (0.05) between D1 and E2, D1 and F2, D2 and F1, E1 and E2, as well as E2 and F1.

The Kruskal Wallis test result on black garlic aroma showed the P value<α (0.05), indicating a significant difference between the effects of the treatments (D1, D2, E1, E2, F1, F2) on black garlic taste (Abdel-Gawad et al, 2018). The hedonic test for black garlic aroma showed that the most preferred aroma was found in formulation E1 with a composition of black garlic, ginger, and rosella in the ratio of 2 g: 3 g: 2 g in powder form, with an average score of 3.33 ± 0.816ab. Formulation F1 with a composition of 1 g: 3 g: 2 g also obtained a high hedonic score of 3.33 ± 0.617ab. Moreover, the aroma of garlic was produced due to the decrease in pH, which affected enzyme activity and provided the characteristic taste (Lu et al, 2017).

To observe significant differences between groups, the Mann-Whitney test was performed. The results show that the preference level for black garlic aroma did not significantly differ because the P value>α (0.05) for E1, E2, and F1. However, there was a significant difference (P < α (0.05)) between D1 and D2, D1 and F2, E1 and F2, as well as F1 and F2.

The hedonic test for ginger aroma was most prominent in formulation F1 with black garlic, ginger, and rosella in the ratio of 2 g: 3 g: 2 g, in powder form, with an average score of 3.53±0.516a. The characteristic aroma of ginger was obtained from essential oils (volatile compounds) such as cineol, borneol, geraniol, linalool, and pharmsen (Irfan et al, 2015). The Kruskal-Wallis test on ginger aroma parameter showed that the P-value was less than α (0.05), indicating a significant difference in the effect of treatments (D1, D2, E1, E2, F1, F2) on ginger aroma. Subsequently, the Mann-Whitney test was performed to observe the differences between groups. The results showed that the preference level for black garlic aroma did not differ significantly with P>α (0.05) in E1, E2, and F2. This indicated a significant difference with P>α (0.05) between D1 and D2, D1 and F1, D2 and F1, E1 and F1, E2 and F1, as well as F1 and F2.

The Kruskal-Wallis test was followed by the Mann-Whitney test to determine the hedonic value or preference level by examining the highest average hedonic scores for each parameter. The hedonic test scores from each parameter were used as supporting data to produce formulation with taste and aroma adjusted.

The hedonic quality test result using the available scale showed the highest preference score for sample C2 with formulation of black garlic, ginger, and rosella in a ratio of 1:3:2 in the form of thin slices. This was used as supporting data for further development and commercialization of the product. The preference scores were presented in Table 6.

3.5. Packaging Design

The selection of packaging design was carried out using the hedonic quality or preference test to determine the secondary and primary packaging forms (tea bags). The technical aspect of this testing involved presenting 6 design to the panelists, consisting of 3 design for the secondary and 3 for the primary packaging (tea bag). Subsequently, the results obtained were presented in Table 7.

The most preferred secondary packaging was design of the aluminum tube, with an average score of 3.667, described as like. Compared to others, the points obtained were not significantly different. The triangular zip lock packaging design obtained an average score of 3.40, with a similar description of liking as the rectangular box packaging, which received an average score of 3.400. This indicated that the interest of panelists mostly favored the aluminum tube design as the preferred secondary packaging for black garlic tea.

The selection of appropriate secondary packaging that needed to be analyzed, involved safety and protection, efficiency, as well as marketing and branding (Anisa, 2017). The evaluation of safety and protection was used to determine whether packaging provided adequate protection against physical damage, moisture, light, or other environmental elements during the production process until the product reached the consumers (Siagian et al, 2020). The evaluation of efficiency in secondary packaging involved design that minimized material waste, space, or labor during packaging and distribution process. Secondary packaging, in terms of branding and marketing, was carried out to strengthen product branding. An attractive design attracted consumers' attention, delivered product information clearly, and distinguished from other products (Gunawan et al, 2015).

The testing for selecting design of the primary packaging, specifically, the shape of tea bag was carried out. The results showed that the highest average score was 3.60, with a description of "like" for designing tea bag as a drawstring pouch. Compared to other packaging design, the average scores were not significantly different, with 3.40 and a description of "like" for the drawstring pouch design and 3.47 with a description of "like" for the circle design and the triangular pyramid design. This indicated that the preferred primary packaging (tea bag) was the drawstring pouch design to be used as the primary packaging for black garlic tea.

Packaging was designed to enclose dried tea leaves, preventing quick deterioration and scattering of the dried tea leaves quality as well as cleanliness. Tea bags were packaged in rectangular cardboard boxes and further wrapped in plastic for protection from external damage, while loose tea was packaged in dried tea leaves wrapped in paper (Rahastine, 2018). The presentation of loose tea had a larger quantity and a more aromatic fragrance. However, the presentation required a longer process to release its aroma compared to instant tea bags that were steeped briefly and lifted for single servings with the aroma dissipating quickly.

Based on the Regulation of the Head of the Indonesian Food and Drug Authority (BPOM) Number HK.03.1.23.07.11.6664 of 2011 regarding the Supervision of Food Packaging, tea bags were made from paper, typically of kraft type, coated with polyethylene plastic that served for heat sealing. The paper packaging industry for food no longer uses chlorine compounds as bleaching agents. Therefore, this requirement should be included when applying for product safety assessment. The polyethylene used for sealing did not melt at the boiling point of water, as observed when tea bag was unable to be opened during steeping with hot water. In addition to paper bags, tea bags can also be made from plastic materials such as nylon, polyethylene terephthalate (PET), or polylactic acid (PLA) (Saragih et al, 2021). Tea packaging development from steeping to tea bags retains certain visual elements while losing others. These visual elements include specific signs or symbols, with preserved features and new packaging innovations.

5. Conclusion

In conclusion, this research showed that the combination of black garlic, ginger, and rosella increased the highest total phenolic content obtained by formulation with a ratio of 1:3:2 in powder form, at 16.708±0.0356 mg GAE/g. There was also an increase in the highest total flavonoid content, achieved by formulation with a ratio of 2:3:2 in powder form, at 13.560 ± 0.536 mg QE/g. Furthermore, the combination enhanced the highest antioxidant activity, obtained by formulation with a ratio of 2:3:2 in powder form, with an inhibition of 63.21%. According to the organoleptic testing results, the most preferred formulation was achieved by the ratio of 2:3:2 in thin slice form. The aluminum tube secondary packaging design was mainly favored, while the drawstring bag form was preferred for the primary packaging.

6. Acknowledgements

The authors are grateful to individuals/institutions/agencies involved in supporting the implementation of this research, including fund providers, analysis, and testing services.

7. References

[1] Abdel-Gawad M, Abdel-Aziz M, ElSayed M. El-Wakil E, Abdel-Lateef E. in Vitro Antioxidant, Total Phenolic and Flavonoid Contents of Six Allium Species Growing in Egypt. J Microbiol Biotechnol Food Sci. 2018;8(2):343–346.

[2] Azeez TB, Lunghar J. Anti-inflammatory effects of turmeric (Curcuma longa) and ginger (Zingiber officinale). Inflamm Nat Prod, 2021.

[3] Badan Pusat Statistik. Produksi Tanaman Biofarmaka (Obat) 2018-2020. Hortikultura, 2021. Available at: https://www.bps.go.id/indicator/55/63/1/produksi-tanaman-biofarmaka-obat-.html.

[4] Bae SE, Cho SY, Won YD, Lee SH, Park HJ. Changes in S-allyl cysteine contents and physicochemical properties of black garlic during heat treatment. Lwt, 2014;55(1):397–402. https://doi.org/10.1016/j.lwt.2013.05.006

[5] Choi IS, Cha HS, Lee YS. Physicochemical and antioxidant properties of black garlic. Molecules, 2014;19(10):16811–16823. https://doi.org/10.3390/molecules191016811

[6] Dianasari D, Fajrin A. Uji Aktivitas Antidiabetes Ekstrak Air Kelopak Bunga Rosella (Hibiscus sabdariffa L.) pada Tikus dengan Metode Induksi Aloksan. J Farm Sains Dan Terap. 2015;2(1):54–58.

[7] Fakhrudin MI, Anam C, Andriani M. Karakteristik oleoresin jahe berdasarkan ukuran dan lama perendaman serbuk jahe dalam etanol Characteristics of ginger oleoresin based on powder size and submerging duration in ethanol. 2015;13(1):25–33. https://doi.org/10.13057/biofar/f130104.

[8] Gümüşay ÖA, Borazan AA, Ercal N, Demirkol O. Drying effects on the antioxidant properties of tomatoes and ginger. Food Chem. 2015;173:156–162. https://doi.org/10.1016/j.foodchem.2014.09.162

[9] Gunawan S, Damayanti MN, Cahyadi J. Perancangan Desain Kemasan Almond Crispy Cheese Merek “Gosyen” Sebagai Oleh-oleh Khas Surabaya. Jurnal DKV Adiwarna, 2015:1–17. http://publication.petra.ac.id/index.php/dkv/article/viewFile/3252/2941

[10] Harsojuwono BA, Arnata IW, Puspawati. Rancangan Percobaan: teori, aplikasi SPP dan Excel. In Angewandte Chemie International Edition, 2021;6(11):951–952.

[11] Imo C. Medicinal Properties of Ginger and Garlic: A Review. Curr Trends Biomed Eng Biosci. 2019;18(2). https://doi.org/10.19080/ctbeb.2019.18.555985

[12] Irfan M, Fakhrudin, Anam C, Andriani M. Karakteristik oleoresin jahe berdasarkan ukuran dan lama perendaman serbuk jahe dalam etanol Characteristics of ginger oleoresin based on powder size and submerging duration in ethanol. 2015;13(1):25–33. doi: 13057/biofar/f130104.

[13] Kimura S, Tung YC, Pan MH, Su NW, Lai YJ, Cheng KC. Black garlic: A critical review of its production, bioactivity, and application. JFDA. 2017;25(1):62–70. https://doi.org/10.1016/j.jfda.2016.11.003.

[14] Lu X, Li N, Qiao X, Qiu Z, Liu P. Composition analysis and antioxidant properties of black garlic extract. J Food Drug Anal. 2017;25(2):340–349. doi: 10.1016/j.jfda.2016.05.011.

[15] Maksum A, Purbowati ISM. Optimasi Ekstraksi Senyawa Fenolik Dari Kelopak Bunga Rosella (Hibiscus Sabdariffa) Berbantu Gelombang Mikro Optimization Phenolic Microwave Extraction ofRoselle Calyx (Hibiscus Sabdariffa). Agrin, 2017;21(2):91–104.

[16] Mardiah ALD, Sulaeman A. Ekstraksi Kulit Batang Rosella (Hibiscus sabdariffa L .) Sebagai Pewarna Merah Alami. Jurnal Pertanian, 2010;1(1):1–8.

[17] Nyangena IO, Owino WO, Imathiu S, Ambuko J. Effect of pretreatments prior to drying on antioxidant properties of dried mango slices. Sci Afr. 2019;6:e00148. https://doi.org/10.1016/j.sciaf.2019.e00148

[18] Okoh MP. Anti-Oxidative Properties of Garlic, Onion and Ginger Extracts. 2016;October 2014.

[19] Prisdiany Y, Puspitasari IM, Putriana NA, and Syamsunarno MRAA. Potensi Tanaman Herbal Antidiabetes untuk Minuman Obat: Sebuah Literatur Review. Indones J Clin Pharm. 2021;10(2):144. doi: 10.15416/ijcp.2021.10.2.144.

[20] Rahastine MP. Analisa Makna Desain Kemasan Pada Produk Teh Di Indonesia. Jurnal Komunikasi, 2018;9(1):72–78. http://ejournal.bsi.ac.id/ejurnal/index.php/jkom

[21] Romm A, Hardy ML, Mills S. Ginger. Botanical Medicine for Women's Health; 2010, 536–538.

[22] Saragih SFJ, Suter IK, and Yusasrini NLA. .Aktivitas Antioksidan Dan Sifat Sensoris Teh Herbal Celup Kulit Anggur (Vitis vinifera L.) Pada Suhu Dan Waktu Pengeringan. J Ilmu dan Teknol Pangan. 2021;10(3):424. doi: 10.24843/itepa.2021.v10.i03.p10.

[23] Siagian IDN, Bintoro VP, Nurwantoro. Karakteristik Fisik, Kimia dan Organoleptik Teh Celup Daun Tin dengan Penambahan Daun Stevia (Stevia Rbaudiana Bertoni) sebagai Pemanis. Jurnal Teknologi Pangan, 2020;4(1):23–29.

[24] Suseno R, Surhaini, Rahmi SL, Yanti F. Characteristics and sensory properties of lemongrass, roselle, and ginger formulation herbal tea. IOP Conf Ser Earth Environ Sci. 2022:951(1). doi: 10.1088/1755-1315/951/1/012096.

[25] Unpad. Bahan Ajar Penilaian Indera. Fakultas Teknologi Industri Pertanian; 2008.

[26] Utari DF, Djaeni M, Ariani N, Hidayat R. Ekstraksi Antosianin dari Kelopak Bunga Rosella (Hibiscus sabdariffa L.) Berbantu Ultrasonik: Tinjauan Aktivitas Antioksidan Ultrasonic Aided Anthocyanin Extraction of Hibiscus sabdariffa L. Flower Petal: Antioxidant Activity. J Apl Teknol Pangan. 2017;6(3):71.