FOODSCITECH
Food Science and Technology Journal

Saponin Content Reduction in Maja Leaf Tea (*Aegle marmelos* L. Corr.) A Study of Different Blanching Methods and Duration

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ABSTRACT

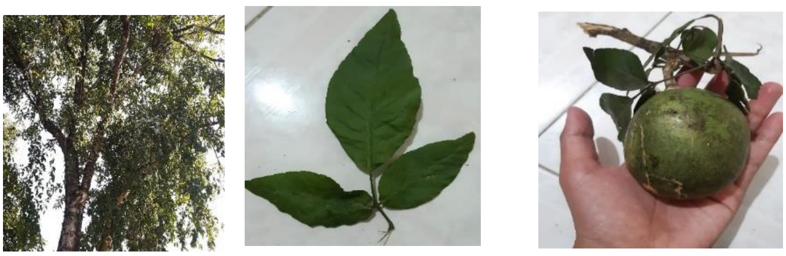
The potential of maja plant leaves (Aegle marmelos L. Corr.*) in Indonesia is important to research, because it can be a new alternative herbal tea. It is known that maja leaves contain saponin. Saponin levels can decrease with blanching treatments such as boiling and steaming. This research uses an experimental method, with a completely randomized design (CRD) consisting of two factors, the first factor (C) is the method of blanching maja leaf tea with two levels, namely C1: Boiling and C2: Steaming, and the second factor (L) is blanching time with three levels, namely L1: 5 minutes, L2: 7 minutes and L3: 9 minutes which is repeated three times. The research data were analyzed using ANOVA (Analysis of Variance) in SPSS version 23. If the results of analysis showed that there were significant differences between treatments (p<0.05), then a further test was carried out using the LSD and Tukey HSD test at the level confidence α=5%. Organoleptic test analysis was analyzed using the liking (hedonic) test, followed by the Kruskal Wallis test. The best determination of all research parameters is carried out using the Effectiveness Test. The consclucion of the experiment reseach, it shows that the interaction between different blanching methods and duration have a significant effect on saponin levels, a very significant effect on water content and the C2L2 treatment is the best treatment with the highest yield value is 0.82 with the research variables is saponin 15.14 mg/ml, water content 3.47%, average color 5.52, aroma 4.79 and taste 4.88.*

Keywords*: Aegle marmelos; blanching; maja leaf; saponin*

INTRODUCTION

Indonesia is a tropical country rich in natural resources, with diverse flora that offers untapped potential for food and non-food applications. One such plant is *Aegle marmelos* L. Corr., commonly known as the maja plant. Originating from India, this plant is often mistaken for *Crescentia cujete* (berenuk), although the two species differ significantly in morphology. Maja leaves are particularly notable for their bioactive compounds, including tannins, flavonoids, phenols, alkaloids, and saponins. (Nigam and Nambiar, 2015).

The morphology of the maja plant can be seen in Figure 1.



(a) Maja Tree (b) Maja Leaves (c) Maja Fruit

Figure 1. The morphology of the maja plant

FOODSCITECH, 7 No. 2 December 2024
ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.9195

Saponins are secondary metabolites with unique properties, such as their ability to produce foam in aqueous solutions. They offer numerous health benefits, including anti-inflammatory, antioxidant, and cholesterol-lowering effects. However, saponins can also have antinutritional properties, such as hemolytic activity on red blood cells, and may impart a bitter taste if present in high concentrations. Therefore, reducing saponin levels is essential to enhance the sensory quality and safety of food products derived from maja leaves. (Sahidin, 2006).

Saponins are a group of triterpenoid compounds. These compounds are capable of producing foam in aqueous solutions due to their soap-like and surfactant properties (Sahidin, 2006). Saponins are classified into two types: steroid saponins and triterpenoid saponins. Upon hydrolysis, they yield sapogenin and sapogenin, respectively (Hanani, 2015). Pharmacologically, steroid saponins can be used to treat rheumatism, anemia, diabetes, syphilis, impotence, and fungal infections, while triterpenoid saponins possess antibacterial, antifungal, anti-inflammatory, and expectorant properties (Evans, 2002 in Darma and Marpaung, 2020). Saponins can also hemolyze red blood cells, thus the saponin content in maja leaves must be kept below a certain limit. The reduction of saponin content can be treated through boiling and steaming (Gunawan, 2018).

Blanching is one of the processing methods commonly used to reduce certain compounds in food materials, including saponins. The blanching process can be carried out using two primary methods: water blanching and steam blanching. Additionally, the duration of blanching is an important factor that influences the effectiveness of reducing bioactive compounds such as saponins. However, improper processing may lead to the loss of other beneficial compounds or compromise the physical quality of the material.

Given this background, the present study aims to investigate the effects of different blanching methods and durations on reducing saponin content in maja leaf tea. The goal is to identify the optimal blanching conditions for producing high-quality tea with reduced bitterness and enhanced consumer acceptance.

Research on maja plants is still limited. This information is needed in the field of education as a source of scientific information, therefore in this study, a study will be conducted on the different ways and duration of blanching to reduce the saponin content of maja leaf tea (*Aegle marmelos* L. Corr.). Preliminary research results show that boiling at a temperature of 80±2℃ for 3 minutes reduces the saponin content in maja leaves by 13 mg/ml. Based on the background, the researcher is interested in conducting research with the title “Reduction of Saponin Content in Maja Leaf Tea (*Aegle marmelos* L. Corr.) A Study of Different Blanching Methods and Duration".

METHODS

Materials and Equipment

The primary material used in this research was maja leaves, which were sourced from Bejijong Village, Trowulan District, Mojokerto Regency. The maja tree, with a height of up to 762 cm and sharp thorny branches, required the leaves to be harvested by cutting the branches using a knife or machete. Only young branches and shoots were collected. After collection, the branches were wrapped in sacks to ensure adequate aeration, protect them from direct sunlight, and prevent mechanical damage (Samsuri and Fitriani, 2013)

Young leaves were selected due to their lower saponin content compared to mature leaves. The characteristics of maja leaves (Aegle marmelos L. Corr) closely resemble those of kaffir lime leaves (Citrus hystrix), as both belong to the same family, Rutaceae. Hence,

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.9195

the choice of maja leaves as a green tea ingredient was based on these similarities. The harvested leaves were young shoots, specifically from the second to the fifth leaf nodes (Ali et al., 2015).

Production Process

The picked leaves were then sorted to select good leaves that were not damaged, broken, or rotten. After that, the leaves were cleaned by rinsing them with running water. The clean leaves were then cut into approximately 1 cm wide pieces using a knife. In the processing of maja leaf tea, the leaves were blanched with two treatments: boiling and steaming for 5, 7, and 9 minutes. The leaves were then drained and air-dried at room temperature of 32 ± 2℃ for 10 minutes, followed by drying using a food dehydrator at a temperature of 40 ± 2℃ for 6 hours (Modified from Gunawan, 2018).

Chemicals Analysis

Saponin

Based on the research conducted by (Ngginak, 2021) on saponin content analysis, 200 grams of the ground sample were soaked in 400 mL of methanol (p.a) solvent in an Erlenmeyer flask for 2x24 hours.

The initial foam indicator test was done by mixing 10 mL of distilled water with 0.5 grams of sample filtrate in a test tube. The solution was shaken for 2 minutes, then 1 drop of 2N HCl solution was added. The test tube containing the acid solution was allowed to stand to observe the foam reaction. The foam formed had a diameter of one to three centimeters for approximately 30 seconds.

The initial color indicator test was done by adding 0.5 grams of sample filtrate containing 10 mL of chloroform solution. The test tube was placed in a water bath for approximately 5 minutes. Lieberman Burchard (LB) reagent was added until a color formed. Triterpene saponins were indicated by a brown color, while steroid saponins were indicated by a blue or green color.

Furthermore, the saponin content was measured using spectrophotometry. 30 grams of dried maja leaves were ground, then the sample was extracted with 1 mL of methanol until all compounds were dissolved. The extracted sample was placed in a cuvette. The cuvette was inserted into a UV-Vis spectrophotometer with a wavelength of 260-370 nm.

Moisture Content

A 5-gram sample of ground maja leaf tea was weighed in a pre-weighed weighing bottle. The sample was dried in an oven at 105℃ ± 1℃ for 4 hours, then cooled in a desiccator and weighed. The sample was then heated in the oven for 30 minutes, cooled in a desiccator, and weighed. This treatment was repeated until a constant weight was achieved (the difference between successive weighings was less than 0.2 mg). The weight loss represents the amount of water in the sample (Andarwulan et al., 2011).

The formula for calculating moisture content is as follows:

$$\text{Moisture content (\%)} = \frac{(\text{initial weight} - \text{final weight})}{\text{initial weight}} \, x \, 100\% \qquad (1)$$

Statistical Analysis

Parametric data, including saponin content and moisture content, were analyzed using Analysis of Variance (ANOVA) in SPSS version 23. If the analysis results showed a

FOODSCITECH, 7 No. 2 December 2024
ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.9195

significant difference between treatments (p<0.05), then a post-hoc test using LSD/Tukey HSD/Duncan test was conducted at a confidence level of α=5%, depending on the value of the Coefficient of Variation (CV). If the CV value was below 5%, the LSD test was used, if the CV value was 5-10%, the Tukey HSD test was used, and if the CV value was above 10%, the Duncan test was used (Syahri, 2003).

Non-parametric data, namely organoleptic values including colour, taste, and aroma, were analyzed using the mean hedonic score, followed by the Kruskall Wallis test to determine the difference between treatments (Ayustaningwarno, 2014). The organoleptic assessment is tested by 75 panelists.

Determination of the best treatment from all research parameters, including parametric data (saponin content and moisture content) and non-parametric data (color, taste, aroma), was done using the Effectiveness Test (Susanto, 2000).

RESULT AND DISCUSSIONS

Table 1 Average saponin content of maja leaf tea

Treatment Code	Treatment	Average saponin content (mg/ml)
C1L1	Boiling 80℃: 5 minutes	20,97±1
C1L2	Boiling 80℃: 7 minutes	20,09±1
C1L3	Boiling 80℃: 9 minutes	17,35±1
C2L1	Steaming 80℃: 5 minutes	15,77±1
C2L2	Steaming 80℃: 7 minutes	15,14±1
C2L3	Steaming 80℃: 9 minutes	14,03±1

The table above shows that different treatments have different effects on the saponin content of maja leaf tea. Treatment C2L3 showed a different effect compared to other treatments on the saponin content of maja leaf tea with the lowest saponin content of 14.03 mg/ml, while treatments C2L1 and C2L2, which were steaming at 80℃ for 5 and 7 minutes, respectively, had the same effect on the saponin content of maja leaf tea, which was 15.77 mg/ml and 15.14 mg/ml, respectively. Treatment C1L3 showed a different effect compared to other treatments with a content of 17.35 mg/ml, and treatments C1L1 and C1L2 had the same effect on saponin content, which was 20.97 mg/ml and 20.09 mg/ml, respectively.

Treatment C2L3 resulted in the lowest saponin content of 14.03 mg/ml, while treatments C1L1 and C1L2 had the same effect with the highest saponin content of 20.97 mg/ml and 20.93 mg/ml, respectively. Saponin has the physical property of being soluble in water (Gunawan, 2018) and is damaged when heated at high temperatures (Muflihah, 2015 in Puspitasari, 2018), so the longer the blanching time, the lower the saponin content in maja leaf tea (*Aegle marmelos* L. Corr). The steaming blanching process can quickly reduce saponin content. This is because steaming uses water vapor as a medium. The steam produced by boiling water can bind to saponin compounds and evaporate them. In the process, steaming is done openly, meaning the steamer is not closed. The combination of treatment C2L3, which is steaming for 9 minutes, can reduce saponin content to 14.03 mg/ml from the control of 40.83 mg/ml. While in the combination of treatment C1L1, which is boiling for 5 minutes, can reduce saponin content to 20.97 mg/ml from the control. Boiling reduces saponin content less, because boiling uses water as a medium. In the process, maja leaves are boiled together with water, so that bioactive compounds can dissolve with

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.9195

water (Hastuti, 2012).

There is no specific threshold for saponin content in green tea, but the WHO states a maximum threshold of 0.12% for saponin in quinoa (a type of cereal). According to (Shi et al, 2004 in Ehsen et al, 2016), saponin at a concentration of 10-12 mg/ml can lower cholesterol in experimental rabbits. Treatment C2L3 with a saponin content of 14.03 mg/ml indicates that steaming for 9 minutes is still insufficient in reducing saponin content to 10-12 mg/ml.

Based on the quality standards for green tea stated in SNI No. 3945:2016, there is no information regarding the minimum or maximum limits of saponin content in green tea that is declared safe for consumption, but there is no toxicity at a consumption of 26 mg/ml of saponin, however, levels above 30 mg/ml are detrimental (Sharma et al, 2023). Therefore, the saponin content in all treatments is still below the consumption threshold.

Table 2 Average moisture content of maja leaf tea

Treatment Code	Treatment	Average moisture content (%)
C1L1	Boiling 80℃: 5 min	3,76 ±1
C1L2	Boiling 80℃: 7 min	4,26 ±1
C1L3	Boiling 80℃: 9 min	5,05 ±1
C2L1	Steaming 80℃: 5 min	4,07 ±1
C2L2	Steaming 80℃: 7 min	3,47 ±1
C2L3	Steaming 80℃: 9 min	3,4 ±1

The table above shows that the interaction between different blanching methods and blanching durations of maja leaf tea has a very significant effect on the moisture content of maja leaf tea. Treatment C1L3, which is steaming for 9 minutes, resulted in the highest moisture content of maja leaf tea at 5.05%, while treatment C2L3 resulted in the lowest moisture content at 3.41%.

Treatments C1L1, C2L2, and C2L3 had the same effect. Similarly, treatments C1L1, C2L1, and C2L2 had the same effect. Treatments C1L1, C1L2, and C2L1 also had the same effect, while treatment C1L3 had a different effect from the other treatments.

The similarity in the effects of treatments C1L1, C1L2, C2L1, C2L2, and C2L3 can be attributed to the similar effects of boiling and steaming at specific time intervals. Treatments C1L1 and C2L2 showed similar effects, likely due to the fact that boiling for 5 minutes (C1L1) reduced moisture content by 3.76% from the control, while steaming for 7 minutes (C2L2) reduced it by 3.47%. Similarly, treatments C2L2 and C2L3 showed no significant difference, indicating that steaming for 7 or 9 minutes had a similar effect on moisture reduction.

Treatments C1L1 and C2L1 had similar effects. Boiling for 5 minutes reduced the moisture content to 3.76% compared to the control. This is because boiling for 5 minutes reduces the compound content in the maja leaves due to blanching, while treatment C2L1, which is steaming for 5 minutes, reduced the moisture content to 4.07% from the control. Steaming for 5 minutes did not show a significant reduction in moisture content. Treatments C2L1 and C2L2, which are steaming for 5 and 7 minutes, respectively, did not show significant differences. Treatments C1L1 and C1L2 had similar effects, with reductions of 3.76% and 4.26% from the control, respectively.

In boiling treatments, a longer boiling time resulted in an increase in moisture content. This is because longer boiling times can increase water absorption (Gregory, 2010). On the

FOODSCITECH, 7 No. 2 December 2024
ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.9195

other hand, steaming treatments resulted in a decrease in moisture content as the steaming time increased. This is because the moisture content in the food material decreases after the cooking process (Hassaballa et al., 2009 in Zulisyanto et al., 2016). Consistent with the statement of (Adawyah, 2008), the hot steam flowing over the surface of the material increases the water vapor pressure, causing water to move by diffusion from the material to the surface. Eventually, after the water content of the material decreases, the water vapor pressure will decrease until it reaches equilibrium with the surrounding air.

Based on the Indonesian National Standard (SNI) No. 3945:2016, the quality requirement for moisture content in green tea is a maximum of 8%. Therefore, it can be concluded that the moisture content of maja leaf tea under different blanching methods and durations meets the quality requirements for green tea.

Table 3 Average organoleptic color of maja leaf tea

Treatment Code	Treatment	Average Color
C1L1	Boiling 80℃: 5 min	4,12 ±1
C1L2	Boiling 80℃: 7 min	3,69 ±1
C1L3	Boiling 80℃: 9 min	3,55 ±1
C2L1	Steaming 80℃: 5 min	5,93 ±1
C2L2	Steaming 80℃: 7 min	5,52 ±1
C2L3	Steaming 80℃: 9 min	4,15 ±1

The table above shows that treatment C2L1, which is steaming for 5 minutes, resulted in the highest tea color of 5.93, meaning that the color of the maja leaf tea was considered pleasant by the panelists. On the other hand, treatment C1L3, which is boiling for 9 minutes, resulted in the lowest tea color of 3.55, meaning that the tea color was considered neutral by the panelists.

The color of the leaves is influenced by flavonoid compounds (Gunawan, 2018). These compounds have the characteristic of being water-soluble and heat-sensitive, so the longer the heating time, the lower the color value of the maja leaf tea.

The difference in the evaluation results between boiling and steaming treatments is due to the flavonoid content, which is easily soluble in water. Therefore, the evaluation results of treatments C1L1 to C1L3 were lower than those of treatments C2L1 to C2L3.

Based on the Indonesian National Standard (SNI) No. 3945:2016, the quality requirement for the color of green tea is a very bright to dull yellowish green. The color of the maja leaf tea produced with a value of 3.55-5.93, which is considered neutral to pleasant, indicates that the color of the maja leaf tea meets the specified quality standards.

The results of the Kruskal-Wallis test for the color of maja leaf tea, as shown in Appendix 10, indicate that there is a significant difference between all treatments ($p = 0.014 < \alpha = 0.05$), which means that different blanching methods and durations have a very significant effect on the color of the maja leaf tea.

Table 4 Average organoleptic aroma of maja leaf tea

Code Treatment	Treatment	Average aroma
C1L1	Boiling 80℃: 5 min	4,43 ±1
C1L2	Boiling 80℃: 7 min	4,35 ±1
C1L3	Boiling 80℃: 9 min	4,03 ±1
C2L1	Steaming 80℃: 5 min	5,16 ±1
C2L2	Steaming 80℃: 7 min	4,79 ±1
C2L3	Steaming 80℃: 9 min	4,27 ±1

The table above shows that steaming at 80℃ for 5 minutes resulted in the highest tea aroma of 5.16, meaning that the aroma of the maja leaf tea was considered somewhat pleasant by the panelists. On the other hand, boiling at 80℃ for 9 minutes resulted in the lowest tea aroma of 4.03, meaning that the tea aroma was considered neutral by the panelists.

From the table above, it can be seen that the longer the blanching process, the lower the panelists' preference for the tea aroma. This is evident in the 9-minute blanching time in treatments C1L3 and C2L3, which have an average aroma rating of 4.03-4.27, considered low. This is because the leaves contain tannin compounds, which are water-soluble, so the longer the blanching time, the lower the aroma rating of the tea.

Boiling and steaming treatments have different aroma evaluation ratings. This is because boiling uses water as a medium, so the aromatic compounds in the leaves are reduced as they are carried away by the water, while steaming uses water vapor (Zulisyanto *et al.,* 2016*)*.

Maja leaf tea has a characteristic green tea aroma with an organoleptic value of 4.03-5.16, indicating that the green tea aroma was rated neutral to somewhat pleasant by the panelists, so the aroma of maja leaf tea is in accordance with the Indonesian National Standard (SNI) No. 3945:2016.

Based on the results of the Kruskal-Wallis test for the aroma of maja leaf tea, as shown in Appendix 12, it is known that there is a significant difference between all treatments ($p = 0.014 < \alpha = 0.05$), which means that different blanching methods and durations have a very significant effect on the aroma of maja leaf tea.

Table 5 Average organoleptic taste of maja leaf tea

Treatment Code	Treatment	Average taste
C1L1	Boiling 80℃: 5 min	3,87 ±1
C1L2	Boiling 80℃: 7 min	4,07 ±1
C1L3	Boiling 80℃: 9 min	4,27 ±1
C2L1	Steaming 80℃: 5 min	4,84 ±1
C2L2	Steaming 80℃: 7 min	4,88 ±1
C2L3	Steaming 80℃: 9 min	5,29 ±1

The table above shows that steaming at 80℃ for 9 minutes resulted in the highest tea taste of 5.29, meaning that the taste of the maja leaf tea was considered somewhat pleasant by the panelists. On the other hand, boiling at 80℃ for 5 minutes resulted in the lowest tea taste of 3.87, meaning that the taste of the maja leaf tea was considered neutral by the panelists.

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.9195

This taste evaluation is due to the maja leaf containing saponin and tannins compounds. Saponin compounds are water-soluble but heat-sensitive, so the longer the blanching time, the lower the saponin content. Saponins have a characteristic bitter taste, so the higher the saponin content, the lower the taste rating (Ngginak *et al.,* 2021). This can be seen from the evaluation results of treatments C1L1, C1L2, and C1L3, which show an average tea taste of 3.87-4.27, meaning that the taste produced was considered somewhat unpleasant to neutral by the panelists.

Based on the Indonesian National Standard (SNI) No. 3945:2016, the quality requirement for the taste of green tea is from very good, characteristic of green tea, to unpleasant. The taste of maja leaf tea produced with a value of 3.87-5.29, which is considered neutral to somewhat pleasant, indicates that the taste of the maja leaf tea is in accordance with the specified quality standards.

The results of the Kruskal-Wallis test for the taste of maja leaf tea, as shown in Appendix 14, indicate that there is a significant difference between all treatments ($p = 0.011 < \alpha = 0.05$), which means that different blanching methods and durations have a very significant effect on the taste of the maja leaf tea.

Table 6 Result Value (NH) research variables

Parameters	**Treatment**					
	C1L1	**C1L2**	**C1L3**	**C2L1**	**C2L2**	**C2L3**
Saponin	0	0,001	0,115	0,165	0,19	0,22
Moisture content	0,173	0,106	0	0,132	0,212	0,22
Taste	0	0,028	0,056	0,141	0,142	0,2
Aroma	0,07	0,057	0	0,2	0,135	0,042
Color	0,04	0,006	0	0,17	0,141	0,043
Total	0,283	0,198	0,171	0,808	0,82	0,725

The table above shows that treatment C2L2, which is boiling at 80℃ for 7 minutes, is the best treatment as it has the highest overall score of 0.82 with the following criteria: saponin content of 15.12 mg/ml, water content of 3.47%, taste of 4.88 (somewhat pleasant), aroma of 4.79 (somewhat pleasant), and color of 5.52 (pleasant).

CONCLUSION

The results of the study on the reduction of saponin content in maja leaf tea: A study of different blanching methods and durations can be concluded that different blanching methods have a very significant effect on the saponin content and moisture content of maja leaf tea, where steaming is better than boiling. Different blanching durations have a very significant effect on saponin content and a significant effect on the moisture content of maja leaf tea. The interaction between different blanching methods and durations has a significant effect on saponin content and a very significant effect on the moisture content of maja leaf tea. Treatment C2L2, which is boiling at 80℃ for 7 minutes, is the best treatment with the highest Score Value (NH) of 0.82 with the following research variable criteria: saponin=15.12 mg/ml, moisture content=3.47%, taste=4.88 (slightly like), aroma=4.79 (slightly like), colour=5.52 (like).

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