

The Investigation of Influenced Factor on Antioxidant Level of Tea Bags from Pelawan Leaves (*Tristaniopsis merguensis* Griff) by Using Taguchi Method

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ABSTRACT: Pelawan (*Tristaniopsis merguensis* Griff) possesses a plethora of health-promoting constituents. The local populace on Bangka Island asserts that Pelawan leaves exhibit therapeutic properties that may combat cancer. This study was undertaken to examine the antioxidant properties of tea bag products derived from Pelawan leaves. The methodology employed in this experiment was the Taguchi approach, incorporating three factors and two levels, specifically leaf type (shoots and non-shoots), drying technique (solar and oven), and tea formulation (original and spice-infused). The orthogonal array applied is $L_4(2^3)$, adhering to the principle of "the smaller the better," wherein a diminished IC_{50} value indicates enhanced quality (elevated antioxidant content). The predominant factor influencing the antioxidant capacity identified is the original tea composition, contributing a remarkable 86% to the overall variance. It is projected that the Pelawan leaf tea bag product may yield an IC_{50} value of $14.7 \pm 278.207 \mu\text{g/ml}$ when prepared with the original composition. The preferred drying technique is the use of an oven or drying apparatus, as it produces superior antioxidant outcomes compared to solar drying methods.

KEYWORDS: Antioxidant, Pelawan leaves, Tea bag, Taguchi.

1. INTRODUCTION

As one of the foremost tropical nations, Indonesia encompasses a comprehensive forest area measuring 120,495,702.96 hectares ^[1]. The extensive forested regions of Indonesia are distributed throughout its 38 provinces, one of which includes the Bangka Belitung Islands. According to data provided by the Bangka Belitung Islands Provincial Forestry Service in 2014, it was reported that the province of Bangka Belitung Islands possessed a total forest area of 4,619,107.6 hectares, of which 1,388.0 hectares constituted the Pelawan Namang Forest area, located in Namang Village, Central Bangka Regency ^[2]. The Pelawan Namang Forest is characterized by a considerable diversity of natural resources, wherein the prominent forest products derived from this region include bitter pelawan honey, sweet pelawan honey, pelawan toadstool, pepper, tomatoes, and Pelawan tea ^[3].

The composition of this original Pelawan teabag variant consists of only dried Pelawan leaves. The distinctive taste of the original Pelawan tea bag is the astringent taste that comes from pelawan leaves, as shown in Fig. 1. It was stated in the previous study that different compositions with cinnamon, star anise and cloves addition in teabag will increase consumer preference and make it daily consumption ^[4]. In this study, it also asserted that the modified Pelawan leaf tea bags had several beneficial ingredient for health, including antioxidants and bioactive compounds.

Previous studies related to Pelawan leaves Revealed that Pelawan leaves contain several bioactive compounds including antioxidants, ethanol, flavonoids, alkaloids and tannins ^[5]. The antioxidant content contained in Pelawan leaves is $14.02 \pm 1.08 \text{ ppm}$ ^[6]. The anti-cholesterol content of Pelawan leaves can reduce cholesterol levels by 42% ^[7]. For the content of flavonoids, alkaloids, and tannins, it is known to have positive test results in Pelawan leaves ^[8, 10]. It also revealed in another study that Pelawan leaves can inhibit the bacterial activity such as *Escherichia Coli* ^[11]. Another function of Pelawan leaves is as antidiabetic ^[12]. Antioxidants can donate hydrogen atoms, to inhibit the rate of oxidation of low-density lipoproteins and chelate metal ions so that oxidative stress can be overcome ^[13]. The free radicals can trigger the pathogenesis of diseases including the visual system, neurobehavioral system, cardiovascular system, immune system, excretory and urinary systems, diseases related to premature babies, and AIDS ^[14]. By consuming food or drinks that are rich in antioxidants, you will increase the protection of cell DNA from free radical damage.



Fig. 1 Pelawan Tree (*Tristania sp.*)

Pelawan tea is purported by local inhabitants to possess the capability to neutralize free radicals, attributable to the presence of antioxidants within pelawan leaves. Nonetheless, there exists a dearth of empirical research specifically addressing pelawan leaf tea bags, thereby precluding a definitive understanding of whether the antioxidant properties endure post-production and the extent of antioxidant concentration in this tea. To ascertain a formulation of pelawan tea bags characterized by elevated antioxidant levels, it is imperative to identify an optimal composition. This research endeavor is designed to establish the appropriate formulation of pelawan tea bags with superior antioxidant levels through experimental methodologies employing the Taguchi method, incorporating various factors and levels.

The Taguchi method aim to attain this objective by rendering the product or process robust and less susceptible to various influencing factors [15, 16, 17]. The application of the Taguchi method as a Design of Experiment (DOE) is predicated upon the premise that the execution of this design is comparatively more straightforward than that of a factorial design, which necessitates conducting experiments in their entirety. This is due to the fact that the Taguchi design does not require as extensive a number of trials as a full factorial combination of factors, thereby facilitating a reduction in both time and costs associated with the testing process.

2. RESEARCH METHODS

This investigation constitutes an empirical research endeavor aimed at identifying a causal relationship between two variables as delineated by the researcher, while systematically controlling for extraneous variables [18]. The study was executed through the examination of multiple factors that potentially affect the antioxidant content in Pelawan tea bags, including the leaf type, drying methodology, and tea composition, as illustrated in Table 1. All factors and their respective levels were employed to generate several variants of tea bags. Subsequently, the tea bags with distinct variants underwent testing for their antioxidant content, which was analyzed through hypothetical testing utilizing Analysis of Variance (ANOVA).

Table 1. Factor and level of experiment

Code	Faktor	Level 1	Level 2
A	Type of leaf	leaf shoots	Non leaf shoots
B	Drying method	Sun	Oven
C	Tea composition	Original	Mixed

2.1. Calculation of the Level Effect of Factors on the Average Response Variable

At this stage, the calculation of the influence of factors on the average response variable were carried out. Then an Analysis of Variance (ANOVA) was performed on the average IC50 value for each factor. Pooling up was employed on the factor that has the least squared sum value. The calculation of the percentage contribution value was conducted to determine the effect of each factor. Calculation of confidence intervals for the predictive value of the influence of factors on the average response variable.

2.2. Calculation of the Level Effect of Factors on Variability (S/N) of Response Variables

After calculation of the influence of factors on the average response variable were done, the Analysis of Variance (ANOVA) was performed on the average value of the S/N ratio for each factor. Pooling up was employed on the factor that has the least squared sum value. The calculation of the percentage contribution value were carried out to determine the effect of each factor. Confidence interval calculation for the predicted value of the influence of the factor on the S/N ratio on the response variable.

3. RESULT AND ANALYSIS

3.1 Planning the Taguchi Experiment Design

Factors and experimental levels determined from previous studies are shown in table 2.

Table 2. Factor classification

Control factor	Noise factor	Signal factor
Leaves type	raw material storage time	Water temperature
Drying method	Raw material storage temperature	Tea steeping time
Tea composition	Fineness of materials	

The orthogonal matrix was determined based on the number of factors, number of levels, and degree of freedom values as a reference for the minimum experiment that needs to be carried out. The value of the degrees of freedom in the orthogonal array must be greater than the value of the degrees of freedom of the research being carried out. The degrees of freedom in this study are 3, so the orthogonal array which has a greater degree of freedom value equal to 3, the closest is $L_4(2^3)$ with a degree of freedom value of 4. By using the $L_4(2^3)$ matrix, the experiment will be carried out 4 times with a combination factors as in Table. 3.

Table 3. Ortogonal Matrix $L_4(2^3)$

$L_4(2^3)$			
Experiment	Coloumn /Factor		
	A	B	C
1	1	1	1
2	1	2	2
3	2	1	2
4	2	2	1

The experiment was carried out according to the predetermined treatment combinations. In general, the experiment in making samples for the four variants of pelawan leaf tea bags is shown in figure 2.

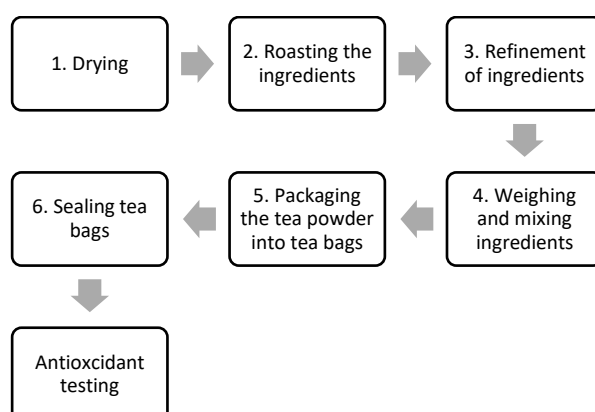


Fig 2. Experiment steps

The result of tea bags that were made for antioxidant test is shown in figure 3.



Fig. 3. Teabags from Pelawan Leaves (*Tristanopsis merguensis* Griff)

The next stage after finishing making the four variants of pelawan leaf tea bags is the process of measuring the antioxidant levels. To carry out previous measurements, each sample must be brewed with the same treatment as one another. Here's how to make a pelawan leaf tea bag solution.

1. To make the tea solution, the first thing to do is put the Pelawan teabag into the cup,
2. Heat the mineral water to a temperature of 100°C, for each cup of Pelawan dip tea you need 150 ml of water.
3. Pour the heated water into the glass containing the pelawan teabag, let the tea that has been poured with hot water for 5 minutes.
4. After 5 minutes remove the tea bag and stir the solution using a spoon.
5. The contrarian tea is ready.

The antioxidant testing was carried out on the four samples with 2 replications for each variant. The antioxidant test used was the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) method. The antioxidant obtained from the calculation of antioxidant concentration (ppm) which can reduce 50% of free radicals, namely IC₅₀ value. The IC₅₀ (inhibition concentration 50) categories is shown in table 4 ^[19] & [20].

Table 4. The IC₅₀ categories

Category	IC50 value
Very Strong	< 50 ppm
Strong	50 – 100 ppm
Medium	100- 150 ppm
Weak	150 - 200 ppm

The results of laboratory tests on the four variants of pelawan leaf tea bags is shown in table 4 and Figure 4. The antioxidant efficacy of the tea bag extracts was assessed utilizing the DPPH radical scavenging assay, with results articulated as IC₅₀ values, as delineated in Table 4. The IC₅₀ value is quantitatively defined as the concentration requisite to achieve a 50% inhibition of DPPH free radicals. Diminished IC₅₀ values are indicative of heightened antioxidant efficacy.

Table 5 delineates IC₅₀ values that span from 32.02 µg/mL to 683.94 µg/mL across four distinct experimental conditions, each replicated twice. Among the evaluated samples, Experiment 4 demonstrated the most pronounced antioxidant activity, exhibiting an average IC₅₀ of 32.58 µg/mL, while Experiment 3 manifested the least robust activity, characterized by an average IC₅₀ of 668.28 µg/mL.

Table 5. Antioxidant level, IC₅₀ dpph (µg/ml)

Experiment	Replication 1 (A)	Replication 2 (B)
1	175,83	170,23
2	451,35	451,29
3	683,94	652,62
4	33,14	32,02

3.2 Calculation of the effect of factor level on the average level of antioxidant content

To determine the influence of the factors level on the average level of antioxidant content of Pelawan teabags, data processing on the results of testing the level of antioxidant content was carried out. Table 6 give the information of antioxidant level of teabag Pelawan.

Table 6. Antioxidant level of teabag Pelawan

Experiment	Factor			Replication		Average
	A	B	C	1	2	
1	1	1	1	175,83	170,23	173,03
2	1	2	2	451,35	451,29	451,32
3	2	1	2	683,94	652,62	663,28
4	2	2	1	33,14	32,02	32,58

Table 7. Calculation Results of the Influence of Factors on the Average Level of Antioxidant Content of Pelawan Tea Bags

Level	Factor		
	A	B	C
Level 1	312,175	418,155	102,805
Level 2	347,93	241,95	557,3
Difference	38,255	178,705	456,995
Ranking	3	2	1

According to table 7, it can be seen that the leaf type factor (factor A) is indicated to have the smallest influence value on the average level of antioxidant content of Pelawan teabags. The variance analysis of the average level of antioxidant content of Pelawan teabags is shown in table 8.

Table 8. ANOVA of the average level of antioxidant content of Pelawan teabags

Source of Variation	DoF	Sum of Square	Mean Square	F-Count
A	1	1.278,42	1.278,42	-
B	1	31.048,2	31.048,2	-
C	1	206.565,7	206.565,7	-
Error	-	-	-	-
Total	3			

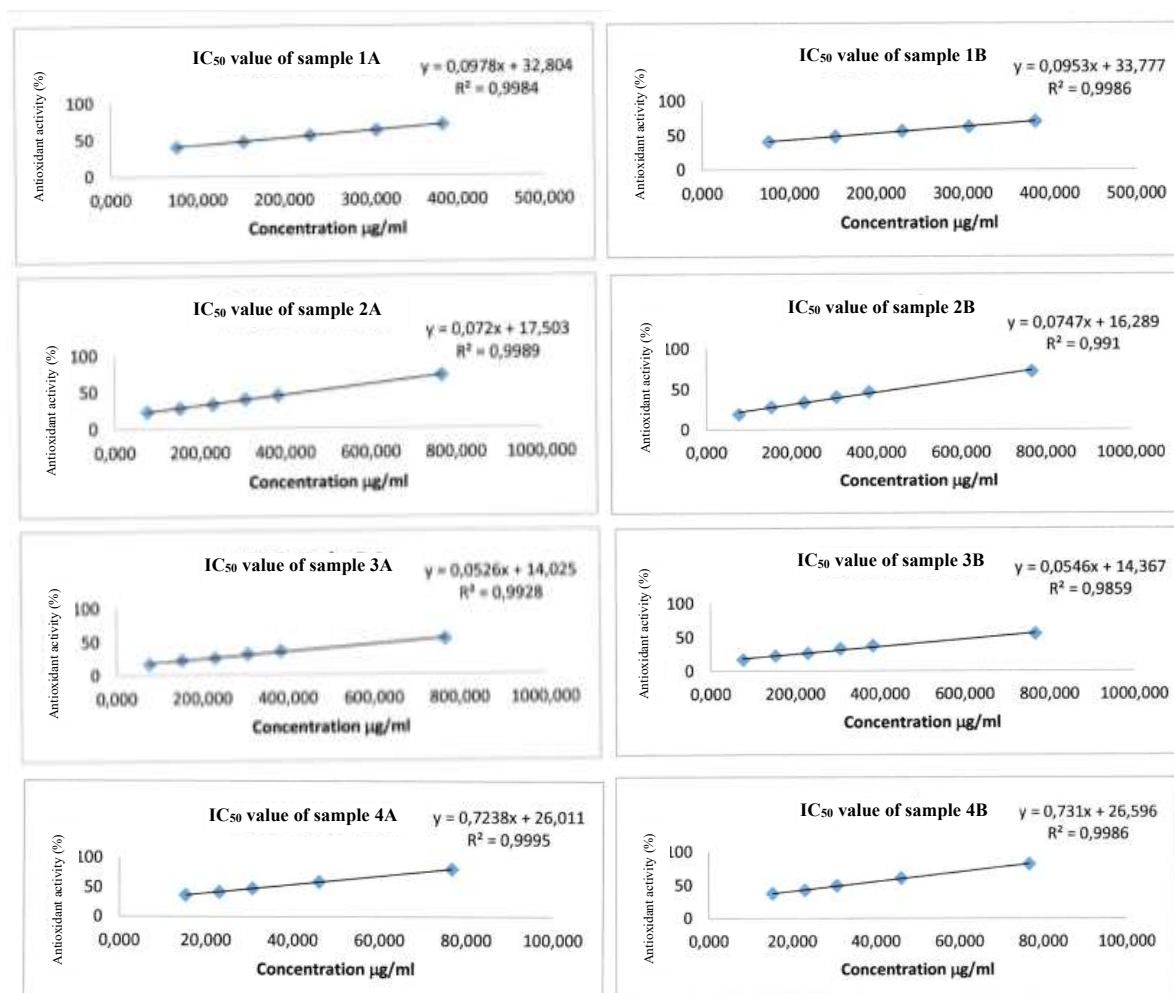


Fig 4. Antioxidant activity from IC₅₀ value

The degrees of freedom of error is 0 (zero). Accordingly, it is not possible to calculate the value of squared sum errors (SSerror) and the mean squared error (MSerror). Without an MS error value, it is not possible to calculate the F-count value for each factor. Therefore, to perform ANOVA calculations, pooling up (merging) factors into errors was carried out. Pooling up factors were carried out for factors that did not significantly influence the level of antioxidant content. Factor pooling was done by combining the factors with the least squared sum value of an insignificant factor which will later be combined into the total squared error (SSerror) value [21]. The results of the ANOVA calculation after pooling up the factor for the leaf type factor (factor A) are shown in table 9.

Table 9. ANOVA of the average level of antioxidant content of Pelawan teabags after *Pooling Up*

Source of variation	DoF	Sum of square	F-Count	F (0,05:1:1)
A	<i>POOLED</i>			161,45
B	1	31.048,2	24,29	
C	1	206.565,7	161,58	
Error	1	1.278,42	1	
Total	3	238.892,33		

It was determined from ANOVA that only factor C had a significant effect on the level of antioxidant activity of Pelawan tea bags. Meanwhile, factor B had no influence on the level of antioxidant activity of Pelawan tea bags. The percent contribution value of each factor, namely:

$$\begin{aligned}\rho_B &= \frac{29.769,78}{238.892,33} \times 100\% = 12\% \\ \rho_C &= \frac{205.287,29}{238.892,33} \times 100\% = 86\% \\ \rho_{\text{error}} &= \frac{3.835,26}{238.892,33} \times 100\% = 2\%\end{aligned}$$

It was obtained that the percent error contribution for calculating the S/N ratio was 2%, this is still below the provisions of the Taguchi method where the value of the maximum error contribution percentage is $\leq 50\%$ so that the percent contribution is accepted and said to be reliable [22]. The predicted value of the average level of antioxidant content in pelawan tea bags namely:

$$S/N_{\text{prediksi}} = -34,848 \mu\text{g/ml}$$

The confidence interval value for the average S/N ratio of the level of antioxidant content in pelawan leaf tea bags is:

$$-78,067 \mu\text{g/ml} \leq E(S/N) \leq 8,37 \mu\text{g/ml}$$

3.3 Calculation of the Influence of Factor Levels on the Variability (S/N) of Antioxidant Content Levels

The level of antioxidant activity has the characteristic of smaller is better, where the lower the IC₅₀ value, the better.

Table 10. S/N ratio of smaller the better

Experiment	Factor			Replication		S/N Ratio
	A	B	C	1	2	
1	1	1	1	175,83	170,23	-46,57
2	1	2	2	451,35	451,29	-54,85
3	2	1	2	683,94	652,62	-58,24
4	2	2	1	33,14	32,02	-32,07

Table 11. The influence of factors on the S/N ratio of antioxidant levels Teh Celup Pelawan

Level	Factor		
	A	B	C
Level 1	-50,711	-52,407	-39,321
Level 2	-45,156	-43,461	-56,547
Difference	5,554	8,946	17,226
Ranking	3	2	1

It is shown that the leaf type factor (factor A) was indicated to have the smallest influence value on the average level of antioxidant content of Pelawan teabags. The analysis of variance (ANOVA) calculation is shown in table 12.

Table 12. ANOVA influence of factors on the S/N ratio of antioxidant content of Pelawan teabag

Source of Variation	of DoF	Sum of Square	Mean square	F-Count
A	1	1.278,42	1.278,42	-
B	1	31.048,2	31.048,2	-

C	1	206.565,7	206.565,7	-
Error	-	-	-	-
Total	3			

Pooling up factors were employed for factors that did not significantly influence the antioxidant content. Pooling up factors was done by combining factors with the least squares sum value of the insignificant factor which will later be combined into a sum of squared error (SSerror) value.

Table 13. ANOVA influence of factors on the S/N ratio of antioxidant content of Pelawan teabag after Pooling Up

Source of Variation	DoF	Mean Square	F-Count	F (0,05:1:1)
A	<i>POOLED</i>			161,45
B	1	80,029	80,029	
C	1	296,723	296,723	
Error	1	30,852	30,852	
Total	3	407,604		

The ANOVA calculation revealed that both factor B and factor C had no influence on the level of antioxidant activity of pelawan teabags. The percent contribution value of each factor is:

$$\rho_B = \frac{49,18}{407,604} \times 100\% = 12\%$$

$$\rho_C = \frac{265,87}{407,604} \times 100\% = 65\%$$

$$\rho_{\text{error}} = \frac{92,56}{407,604} \times 100\% = 23\%$$

It is determined that the percentage error contribution for the S/N ratio is 23%, below the provisions of the Taguchi method where the maximum error contribution percentage is $\leq 50\%$. Therefore, the contribution percentage is accepted. The estimated value for the average S/N ratio to the level of antioxidant content in pelawan leaf tea bags is: $S/N_{\text{prediksi}} = -34,848 \mu\text{g/ml}$

The confidence interval value for the average S/N ratio of the level of antioxidant content in pelawan leaf tea bags is: $-78,067 \mu\text{g/ml} \leq E(S/N) \leq 8,37 \mu\text{g/ml}$

4. DISCUSSION

This investigation assessed the antioxidant potential of Pelawan leaf tea bags employing the Taguchi method, with IC_{50} values serving as the principal metric of effectiveness. The research data illustrates a wide assortment of IC_{50} values, from $32.58 \mu\text{g/mL}$ to $668,28 \mu\text{g/mL}$, influenced by the different combinations of parameters that were carefully examined. According to established classifications of antioxidant activity [23], the most effective sample (Experiment 4, $IC_{50} = 32.58 \mu\text{g/mL}$) is categorized as “very strong” ($<50 \mu\text{g/mL}$), in contrast to the least effective sample (Experiment 3, $IC_{50} = 663.28 \mu\text{g/mL}$), which exhibited minimal antioxidant activity. The diversity that found in this study underscores the necessity of choosing the most effective processing parameters for bioactive compounds in Pelawan Tea.

Among the three factors evaluated, tea composition exhibited the most pronounced influence, accounting for 86% of the variance in antioxidant levels. The original (pure Pelawan) tea consistently showcased superior antioxidant activity compared to formulations infused with spices. This observation indicates that the incorporation of cinnamon, cloves, or star anise could potentially dilute or chemically engage with the phytochemicals inherent in Pelawan, thereby compromising the overall antioxidant effectiveness. This result shows that Pelawan leaves are abundant in flavonoids, alkaloids, and tannins, which are the bioactive compounds essential for the neutralization of free radicals, and this finding align with the previous studies. Therefore, preserving the original composition is imperative for optimizing antioxidant effectiveness.



The drying method emerged as the second most significant factor influencing antioxidant activity. Oven drying demonstrated enhanced antioxidant efficacy relative to solar drying, that indicating the regulated thermal conditions can more effectively conserve heat-sensitive phenolic compounds. It is revealed from the previous researches that unregulated sunlight exposure increases the deterioration of antioxidants present in herbal and agricultural goods. Consequently, prioritizing oven or mechanical drying methods is advisable for the commercial-scale production of Pelawan tea.

The impact of leaf type (shoots versus non-shoots) was minimal relative to other factors. Both young and mature leaves demonstrated substantial antioxidant activity, indicating that producers possess leeway in the selection of raw materials without significantly compromising quality. This flexibility is advantageous for large-scale harvesting and sustainable resource management.

The robust antioxidant performance of Pelawan tea ($IC_{50} = 32.58 \mu\text{g/mL}$ for the most effective treatment) is favorably comparable to other recognized natural antioxidants, such as mangosteen rind and green tea, which also exhibit similarly low IC_{50} values [24]. This outcome reinforces local beliefs regarding the therapeutic potential of Pelawan tea in mitigating diseases associated with oxidative stress, including cardiovascular and metabolic disorders [25].

The findings yield explicit recommendations for the development of Pelawan tea products that the original composition should be preserved to attain maximum antioxidant advantages for tea composition, and oven drying or controlled mechanical drying is preferred over solar drying.

5. CONCLUSION

The results of the analysis using the Taguchi method stated that there was one factor that had a significant influence on the antioxidant level of Pelawan tea bags, namely the composition of the tea with the original variant, which provided a percent contribution of 86%. Thus, to get pelawan leaf tea bags that contain strong antioxidants, it is recommend to use the original variant of the tea composition. It is estimated that the Pelawan leaf tea bag product can have an IC_{50} value of $14.7 \pm 278.207 \mu\text{g/ml}$ from original composition. The recommend drying method is by using oven/drying machine to get better antioxidant results compared to drying method by sun light.

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