Optimization of Extraction Methods and Dyeing Standardization of Nila Leaves (*Indigoferatinctoria* Linn.) as Natural Dyes

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**ABSTRACT.** Indigosol is commonly used as a synthetic dye to generate blue color. However, synthetic dyes are detrimental to health and environment because they contain heavy metals. Nila plant (*Indigoferatinctoria* Linn.) contains indican glucoside which produces indoxyl exotic blue gold color and known as indigo. Indigo produce the dull-colored, when applied to cotton material. This study aims to get the optimize extraction method and standard dyeing of nila as cotton material natural dye, in order to generate sharper blue color. Nila leaves were extracted by variation time of maceration at 12, 24, 36, 48 hours and variation boiling temperature at 70, 80, 90 °C in aquadest, with pH 11 and 13. The indigo level was measured using spectrophotometer at λ 611 nm. The optimal result were applied to prima and primissima cotton material and then tested in fade resistance to leaching with staining scale (SNI-08-0285-1998) and color depth with spectrophotometer method with λ 380 - 780 nm. This study showed that in maceration and boiling method, pH significantly affected the indigo level. The treatment of 12-hour maceration at pH 11 and boiling at 80-90°C at pH 11 were the most optimal treatment to produce sharp blue color, both in prima and primissima cotton material. The best scale of fade resistance was 3-4, which was indigo extract with 12-hour at pH 11 in the maceration method and applied in primissima cotton. It can be concluded that maceration method generates sharper dye than the boiling method in primissima cotton material.

**Keywords:** cotton material, indigo, maceration, natural dye

**INTRODUCTION**

The use of synthetic dyes in the batik industry in Indonesia has been increasing since the 20th century, causing environmental and health problems [1]. One of the blue dye was commonly used in the batik industry was indigosol. It composes of a benzene ring which was very stable and difficult to be degraded [2]. The used of natural dyes was needed to reduce the negative effects of using synthetic dyes.

*Indigoferatinctoria* Linn. was one of the plants that can be used as a natural dye batik because it was contain indican which will be hydrolyzed into indigo and be able to give blue color on the fabric [3]. The
quality of indigo natural dye obtained from *Indigofera* species[4]. *Indigofera* plant contains the indole precursor, there are isatan and indican[5], [6] and [7]. According to [8] that isatan not found in the *I. tinctoria* leaves which have been stored at room temperature and the temperature 40°C, but the indican level increased in the leaves that are stored at room temperature and the temperature 40°C. Indican was hydrolyzed by water and indoxyl β-glucosidase enzyme. Two molecules of indoxyl were oxidized with air to form oxindole [9]. Oxindole will be able to form indigo blue [10]. The bond between indigo blue with Ca then forms a precipitate pasta. The process of changing indoxyl into indigo greatly influenced by pH [9]. According to [3] at pH 11 indigos formed more than 80% and the formation of indigo in proportional to the increase in pH. The process of coloring fabrics using natural dye indigo was also affected by pH [11]. In this process, indigo can be reduced to leuco-indigo in order to bind the cellulose [12]. Reduction intention to change unsoluble-indigo become soluble-indigo, there are changes of leuco-indigo into bifenate ions and monofenolate ions[13]. Reduction of indigo into leuco-indigo occurs at pH <5.5 then forms monofenolate ion at pH 5.5 up to 11, further at pH>11 bifenate ion is established then will have returned to form indigo [11]. Monofenolate ion has high water solubility as well as has high the affinity and the highest absorption speed to the cellulose fiber while bifenate ion has less affinity towards the cellulose fiber of fabric [16]. The making of natural dye indigo is usually done by fermentation (submergence) process but indigo precursor are released from the leaf tissue into aquadest on heating [8 and 14]. However the result of indigo pasta with fermentation method was a less sharp color when applied on the fabric so was needed modification to the conditions of method.

This study aimed to extract indigo leaves as batik natural dye and to generate sharper blue color by applicative methods. This research was carried out to: (1) evaluate the effect of variation time and pH in the maceration method toward levels of indigo (2) evaluate the effect of temperature and pH in the boiling method on levels of indigo (3) evaluate color of indigo pasta produced by maceration and boiling method that is applied as a natural dye batik on prima and primisimafabric.

**MATERIALS AND METHODS**

**Materials**

The materials were used in this study include leaves of indigo (*Indigoferatinctoria* Linn.) obtained from KulonProgo, Yogyakarta-Indonesian. The leaves were used are the young leaves. Other required materials were indigo carmine, distilled water, NaOH, HCl, CH₃COOH, H₂SO₄ (all of these chemical compounds were the pure analytical), CaO, brown sugar, alum, prima and primisimafabric. The tools were used in this research include scale semi-analytical, visible spectrophotometer, pH meter, crock meter, UV-PC, electric stoves, glassware, dark tube, filter, and jars.

**Sample Collection**

Nila plant (*Indigoferatinctoria*) that has been aged 1.5-2 months were collected in the morning at around 6:00 to 08:00 pm. The leaves were used are the young leaves at the ends of plant's stems. Approximately 15-20 cm rod ends were cut with a knife then were separated the leaves with stems branch.

**Maceration**

A total of 150 gr of fresh indigo leaves were been fermentation (submergence) in 1500 ml aquadest a jar covered with aluminum foil on the outside and allowed to stand for 12, 24, 36, 48 hours at room temperature. The fermentation solution is filtered to separate the pulp of the leaves. Its solution was taken 10 ml and the absorbance was recorded at 611 nm. The fermentation solution had been filtered was added 4.5 gr of quicklime then stirred periodically for 10-15 minutes so there was contact with oxygen until the color of the solution changing to blue. The solution was added NaOH or HCl in order to make pH of the solution were 11 or 13. When the pH is reached, the indigo solution was incubated at room temperature for 20 hours and was filtered to obtain pasta (sediment) indigo.
Boiling
A total of 150 gr of fresh indigo leaves were boiled in 1500 ml aquadest at 60-70°C, 70-80°C, and 80-90°C temperature. Leaves were inserted into aquadest was being heated when temperature reached the limit of temperature range. It was boiled for 10 minutes. The boiling solution was filtered and cooled to 30°C than was taken 10 ml to recorded absorbance at λ 611 nm. The fermentation solution had been filtered was added 4.5 gr of quicklime then stirred periodically for 10-15 minutes so there was contact with oxygen until the color of the solution changing to blue. The solution was added NaOH or HCl in order to make pH of the solution were 11 or 13. When the pH is reached, the indigo solution was incubated at room temperature for 20 hours and was filtered to obtain pasta (sediment) indigo.

Quantitative Analysis Indigo
Quantitative analysis indigo was conducted by the visible spectrophotometer method with a standard calibration indigo. A total of 8 mg standard indigo carmine dissolved in 20 ml of H2SO4 then added 500 ml of boiling water and tested spectrophotometer with λ 611 nm. Indigo paste dissolved in 20 ml of H2SO4 then added 500 ml of boiling water and tested spectrophotometer with λ 611 nm. The best results of the test spectrophotometer characterized by the highest absorbance are used for batik cloth dyeing stage prima and primisima further tested the fade endurance.

Reduction Step
The pasta (10 gr) had resulted from maceration and boiling method were dissolved in 100 ml water then was added 10 gr of brown sugar as a reductant. The mixture was incubated at room temperature for 20 hours.

Coloring Step
Indigo solution has been reduced then ready to be used for dyeing. The primaand primisimafabric (30x30 cm) were boiled with alum to removed compound attached the fabric. The fabric was dried then dipped in the indigosolution for 1 minute. The fabric was aerated (avoid direct contact with sunlight) for 10 minutes. The fabric was dipped in indigo solution again then aerated back. This process was repeated 5 times. After the fabric dyed were dried then washed with CH3COOH to remove lime and remnants of a dye was not fixed.

Fade Resistance Test to Leaching
The fabric has been dyed (10x4 cm) was placed between two pieces of white fabric then was sewn on all four sides next. The fabric was included in the soap solution at 40-50°C temperature and stirred for 30 minutes. Furthermore, the fabric was rinsed twice with water and rinsed with cold running water for 10 minutes. The fabric was squeezed and the seam was released on three sides. The color changed was assessed with gray scale and with a staining scale (SNI 08-0285-1998).

Color Depth Test (Reflectance)
The fabric has been dyed and white fabric (5x5 cm) was measured reflectance using a Spectrophotometer (UV-PC) at a wavelength of 380 nm to 780 nm.

Data Analysis
The measured parameter was solution absorbance of maceration and boiling method, pasta weight was resulted, scoring of fade resistance test and color depth test. All variable were analyzed by ANOVA using a two-way randomized block design with three replications. Means were separated on the basis of least significant difference (LSD) only when the ANOVA F-test showed significant at 0.05 probability levels [17].

RESULTS AND DISCUSSION
Maceration Method
The highest absorbance in maceration method was 48 hour treatment and the lowest was 12 hours treatment while the 24 hours treatment was not significantly different from the 36 hour treatment. It indicated that the duration of maceration caused the absorbance of indigo solution moves up due to the
content indoxyl high. Indoxyl was formed by hydrolysis process from the indican [10]. In maceration process, the concentration of solvent (distilled water) was lower than the concentration of the liquid inside cell consequently the solvent penetrated the cell wall and entered the cavity of the cell. Inside the cell cavity, indican was hydrolyzed by the water and β-glucosidase converted into indoxyl [14]. This process was repeated consequently the solution concentration was balance between outside and inside the cell.

Figure 1a. shows that the weight of pasta at pH 13 is always higher than pH 11. This is possible because more ion bifenolat formed at pH 13. Ca of calcium oxide binds to the O- group of bifenolations then precipitated pasta. Therefore, the formation of bifenolat ion directly proportional with precipitation of pasta. This was supported research by [15] that the amount of indigo pasta increased when added calcium oxide (CaO), while levels of indigo in the paste decreased when added CaO. In the process of maceration, after soaking process the indigo solution was filtered to separate the pulp leaves, then the solution was aerated and added CaO. The aeration process caused two molecules of indoxyl were oxidized and formed oxindole. Oxindole was oxidized to indigo [5]. Indigo was reduced by brown sugarto formed leuco-indigo which soluble in water. At pH <11 leuco indigo was oxidized to monofenolat ion and at a pH of>11 will have formed ion bifenolat [16]. The addition of calcium oxide in order to shift the pH toward alkaline and reduced indigo in precipitated pasta, also take a part in the formation of pasta. CaO was a basic oxide that can reduce leuco indigo into indigo when formation of pasta.

The best effect of pH can view of the average each treatment, Figure 1b. showed that the highest indigo levels obtained at 48 hours of pH 11 while the highest pasta weight at 36 hours of pH 13. At pH 13 pasta indigo formed more than at pH 11 because it is influenced by the formation of ion bifenolat. When the amount of indigo extracted at pH 11 and 13 does not vary much but dissolved into a paste which is more consequently the indigo levels in the pasta at pH 13 is always lower than the pH 11.

![Graph](image1.png)

**FIGURE 1.** The weight of indigo pasta produced from maceration method with a variation of time (a), levels of indigo in the pasta in maceration with temperature variations (b).

**Boiling Method**

The highest absorbance in boiling method was obtained at 80-90°C temperature treatment with an average of absorbances was 0.756 while at 80-90°C temperature treatment was not significantly different from the 70-80°C treatment. This result explained that boiling temperature directly proportional with the absorbance of the solution. The high temperatures caused the cell walls lysis so the mixture in the cell out, including the indican [9 and 19]. Indican was hydrolyzed by H₂O and β-glucosidase enzyme to formed indoxyl [3]. The high temperature and heating for too long can damage the indoxyl consequently at a
temperature > 90°C the indigo solution was a brown color. Figure 2a. shows that the pasta weight at pH of 13 is always higher than pH 11. The temperature rise also led to weight pasta higher but indigo levels were not affected by temperature boiling. Figure 2b. shows that the highest indigo level at 80-90°C pH 11. Similarly, with the maceration method, the results boiling method showed that the indigo level at pH 11 is always higher than the treatment pH 13.

![Figure 2a. Weight of indigo pasta produced from boiling method with variation of time](image1)

![Figure 2b. Levels of indigo in the pasta in boiling with temperature variations](image2)

**FIGURE 2.** Weight of indigo pasta produced from boiling method with variation of time (a), Levels of indigo in the pasta in boiling with temperature variations (b)

**Fade Resistance Test and Reflectance**

In coloring process, sugar was used for reduce the negative effect of sodium dithionate [18]. Table 1. shows that application of indigo pasta in prima and primisima fabric from maceration method give the sharper blue color than the indigo pasta boiling method results. In addition, the reflectance test results also supported it. The reflectance values of indigo pasta from maceration method in fabric applications resulted lower than the boiling pasta indigo. Lowest reflectance values of maceration method that was 14.9% at 12 hours pH 11 treatment prima fabric, while the highest reflectance value was 38.81% at 12 hours pH 13 treatment prima fabric. In the boiling method, the lowest reflectance values was 26.2% at pH 13 80-90°C treatment primisima fabric and the highest reflectance value was 43.56% at 80-90°C pH 13 treatment prima fabric. Low reflectance value was the color that was getting older because the reflectance test principle is the reflection of light. Primisima fabric contains cellulose which is higher than the prima so that the dye affinity is higher. Affinity is the ability of the fabric fibers to bind color [20]. Primisimalastetal yarn fabric (fabric density) is higher so that the cellulose fibers are held higher than the primafabric. The higher the content of cellulose fibers, the more OH ions that will bind to substances indigo [20 and 21]. This is what causes staining primisimafabric sharper, and more resistant to fade.
TABLE 1. Staining pasta indigo concentration of 10% and a fade resistance on the fabric and reflectancetest Prima and Primisima

| Treatment          | Coloring | Fade value | Reflectance (%) |
|--------------------|----------|------------|-----------------|
|                    | Prima    | Primisima  | Prima           | Primisima |
| Boiling 80-90°C pH 11 | 2-3      | 3          | 30,88           | 35,17     |
| Boiling 80-90°C pH 13 | 3-4      | 3          | 43,56           | 26,2      |
| Maceration 80-90°C pH 11 | 2-3      | 3-4        | 14,9            | 18,31     |
| Maceration 80-90°C pH 13 | 3        | 3          | 38,81           | 22,98     |

CONCLUSION
1. In the maceration method, time treatment had no effect on levels of indigo but treatment of pH significantly affect the levels of indigo. At pH 11 indigo levels produced higher than pH 13. The treatment of 12 hours pH 11 in maceration method is the most optimal treatment to produce a sharp blue color.
2. In the boiling method, temperature and pH treatment have a significant effect on levels of indigo. Treatment 80-90°C pH 11 in the boiling method is a most optimal treatment to produce a sharp blue color.
3. Pasta was resulted by maceration method produces a sharper blue color and better the reflectance values than the pasta was resulted by boiling method when applied to the prima and primisimafabric.

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