The Stability of Extract *Indigofera tinctoria* for Color Indicator

PG Putri\(^1\), E Warsiki\(^2\), and Sugiarto\(^3\)

\(^1\)Post Graduate program of Agroindustrial Technology, Bogor Agricultural University, Bogor, Indonesia
\(^2\)Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor, Indonesia
Email: Pridata.ginaputri@apps.apb.ac.id

**Abstract.** Color indicator was one of intelligent packaging that give information to the consumer based on color change. In the manufacture of color indicator, *Indigofera tinctoria* was used as a source of natural coloring agent. *Indigofera tinctoria* leaves contain *indicanglucoside* compound which indigo color can be hydrolyzed with water to indoxyl which produce blue color. Natural dyes used as color indicator must have a certain stability that is influenced by certain factors such as temperature and pH. In the research extraction of indigo leaves was fermented for 24 hours. The extract was stored at different temperature levels at refrigerator (2 ± 2 °C), room temperature (25 ± 2 °C) and high temperature (40 °C). The effect of pH on the stability of indigo dye extract was carried out by measuring the absorbance value at pH 1 to 14. The stability of the extract on temperature decreases with the coefficient of determination of \(R^2 = 0.99\) by linear regression of \(y = 0.026x + 170\) and the stability of extract to pH \(R^2 = 0.94\) by the equation of \(y = 0.05x + 0.016\).

1. **Introduction**

Packaging plays an important role in protecting food products from damage either physical or chemical damage. One of the packaging development is the smart label indicator. Indicator label serves to provide information to consumers regarding product quality degradation or monitor the fault of the storage temperature of a product. In making the indicator label, a color indicator from a natural dye source is used.

One plant that is potentially used as a natural dye is *Indigofera tinctoria*. The taxonomy of indigofera plants included in Kingdom Plantae, Magnoliophyta Division, Class Magnoliopsida, Order Fabales, Family Fabaceae, Genus *Indigofera*, Species *Indigofera tinctoria* Linn [1]. Leaves contain tannins, flavonoids, alkaloids, glycosides, and phenols [2].

*Indigofera tinctoria* plants contain indigo pigments, that is indoxyl compounds that dissolve in water and are easily oxidized to blue indigo. The content of dyes contained in them has a blue-green color, depending on the fixator. Extraction of the *Indigofera tinctoria* Linn plant will produce color green and blue color when fermented. Fresh indigo green leaves there is glucose that forms *indicanglucoside* when indican fermentation will be hydrolyzed by glucosidase enzymes in the leaves so as to produce indoxyl which will give blue color [3].

To get the dye from the plant *Indigofera tinctoria* Linn is done by fermentation. In addition, the color resistance of indigo dyes is not good for the bright light of the day so that when exposed to bright light indigo dyes will fade [4]. With indigo color resistance that is not good for high temperatures or...
bright light, indigo dyestuff has the potential as a dye on the indicator label for the product to
be susceptible to temperature [4].

Until now the research on the measurement of indigo dyestuff extracts is still limited, so it is
necessary to measure stability at several temperatures. The aim of the study was to determine the level
of stability of the color of indigoferatinctoria against several temperatures during storage and various
pH. The results of this study are needed in making the indicator label.

2. Materials and Method
The material that will be used in this research is Indigoferatinctoria leaves, water, aquades, HCl, NaOH.
The tools used are measuring cups, cup glasses, pH meters, and spectrophotometers. This study
includes (i) extraction of indigoferatinctoria leaves; and (ii) temperature stability test and pH of indigo
extract.

2.1. Indigoferatinchoria Leaf Extraction
Extraction of indigo leaves is done by fermentation, indigo leaves are weighed and added water
according to treatment and left for 24 hours. The leaves were separated by filtering then added 4 g / L
water solution and the mixture was left for 12 hours. The yellow top layer is removed and the blue
bottom layer is taken as an indigo natural dye. The extract was then analyzed for pH. All processes are
carried out in conditions that are protected from light.

2.2. Stability of Indigofera tinctoria
Stability of indigo dyes extracts for factors, namely: the effect of pH and storage temperature. Absorption values show the color density of extracts of indigo dyes.

2.2.1 Stability of pH
The effect of pH on the stability of indigo dyes extract was done by measuring the absorbance value at
pH 1 and 7. The indigo dye extract was diluted with 10% concentration and the extract solution was
adjusted by using a solution of NaOH or HCl to obtain the extract with a pH between 1 and 7, if a
precipitate is formed, the sediment must be filtered. The extract solution whose pH has been measured
is measured at the maximum absorbance value at a predetermined wavelength.

2.2.2 Stability of Temperature
The effect of temperature on the stability of the extract of indigo dyes was observed by measuring
the absorbance value. In this study used storage conditions with different temperature levels, namely at
room temperature (25 ± 2 °C), refrigerator (2 ± 2 °C), high temperature (40 °C). The way it works is
that the indigodye extract is diluted with water to a 10% concentration then the extract solution is
stored according to the temperature level used. Each extract value was measured using UV-Vis
spectrophotometer.

3. Results and Discussion

3.1. Indigoferatinchoria Leaf Extraction
The dye extraction produced in this study has a pH of 5, the pH measurements obtained are not much
different from indigofera extraction research [5]. Which is blue tilapia paste at pH 4.8. Extraction has
a dark blue color with ohue 316.6.

3.2. pH Stability of Indigofera tinctoria
The effect of pH on the stability of indigo is done by observing and measuring the absorbance value
at wavelength of 611 nm at pH 1 to 14. Indigo groups with different pH conditions shown in Figure
1.
The effect of pH is the most important factor to indigocolor. Indigo dyes have four different colors depending on pH conditions. Indigotin and reduced non-ionic forms are at a pH below 9-9.5, pH> 10 on reduced non-ionic structures to form mono-phenolics. Almost all indigo molecules will form mono-phenolics at a pH of around 11.5 [6]. In addition, the pH also affects the different substantivity for the fiber, at different pH values will affect the form of indigo in solution [7]. Indigo at pH 1 and pH 14 shown in Figure 2.

The more alkaline pH of indigo paste produced the more concentrated blue. Adjusting the pH of the solution can maintain the condition of the process to remain stable because the pH of the solution that is suitable will produce the right indigo color aging. The more pH of the indigo paste is getting blue, the acid pH produces a transparent purple color. The value of indigo absorbance ranges from 0.02 - 0.1032. The highest absorbance value is at pH 14 which is 0.1032. Absorption pH value in Figure 3.
The value of indigo absorbance ranges from 0.02 - 0.1032 with linear regression \( y = 0.05x + 0.016 \). The highest absorbance (0.1032) is on pH 14. The increase pH value resulted in the higher absorbance value. But meanwhile, in the fermentation process of indigo plants, more acidic solutions resulted in more indigo extract, this is due to strong acids which release protons (H\(^+\)) will completely affect the breaking of the glycoside bonds, the glucosides will then break down into indoxyl and glucose, the indoxyl produced will be oxidized to indigo. The more indigo glucoside which decomposes into indoxyl and glucose, the more indigo will be produced [8].

### 3.3. Temperature Stability of Indigofera tinctoria

The effect of pH on the stability of indigo substances was carried out by storing indigo extract at a temperature in the room (25 ± 2 °C), refrigerator (2 ± 2 °C), high temperature (40 °C). Storage at refrigerator temperature (2 ± 2 °C) has a better level of stability than at high temperatures (40 °C). This is in accordance with the nature of indigo dyes that are susceptible to bright light. The color resistance of indigo dyes is not good for the bright light of the day so that when exposed to bright light indigo dyes will fade [4]. The color change that occurs in bright light storage is very significant. The longer the storage, the color that is caused to fade. Value of Temperature Absorbance in Figure 4.

At low temperatures the absorbance value is 0.145 while at high temperatures is 0.093 with linear regression \( y = -0.026x + 0.170 \). The higher the storage temperature, the absorbance value will decrease. The pattern of reduction in absorbance value is the same as the research measuring the stability of chlorophyll dyestuff [9]. This means that the resulting concentration of dyes decreases. The decrease in absorbance value is caused by the influence of the given temperature so that it changes the indigo structure. According to [10]. Beside that, certain temperatures can result in the breaking of the
bond chain between molecules in a dye solution. In addition, a decrease in the length of the absorbance value can also be caused by the damage to the structure of dyes due to the enzymatic oxidation reaction during storage.

4. Conclusion
The storage of indigo extract at temperature (40 ° C) makes the color of indigo extract become lighter. While the effect of pH on indigo extract is important in creating bluecolor. The blue color will produce on alkaline indigo paste, but in acidic condition, the color will get a transparent purple color. The effect of pH to indigo color production is needed to inform as colorant of label indicator in intelligent packaging.

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