

**Effect of Tween Addition 80 On Natural Dye Extraction into Suji Leaf (*Pleomele angustifolia* N.E. Brown) and Pandan Leaves (*Pandanus amaryllifolius* Roxb)**Darti Yulianti<sup>1,\*</sup>, Sunardi<sup>1</sup>, Widayanti Wibowo<sup>1</sup><sup>1</sup>Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Indonesia, Kampus Baru UI Depok, Jawa Barat-16424

\*email: darti.yulianti@ui.ac.id

**Abstract**

Today, natural dyes are beginning to be abandoned and people tend to use synthetic dyes as food dyes. However, the use of synthetic dyes as food coloring can have negative effects such as toxic and carcinogenic. Therefore, it is necessary to find a safe and cheap source of natural dyes. One source of natural dyes that can be utilized are suji leaves and pandan leaves. This study aims to obtain the best chlorophyll extracts physically and chemically with the addition of surfactants Tween 80. Surfactant Tween 80 is used because it is not toxic so safe for food. In this research, extraction of chlorophyll from suji leaves and pandan leaves with variation of stirring time and variation ratio of Tween 80/aquades then extraction result with time and ratio of Tween 80/aquades optimum in freeze dryer. With 7 hours and Tween 80/aquades ratio (5:10), total chlorophyll content of 30,221 mg/L was obtained for suji leaf while 18,573 mg/L for pandan leaves. The extraction results were characterized by UV-Vis spectrophotometer. The results show that the wavelength of suji leaves is 663 and the pandan leaves is 433 nm.

**Keywords:** leaf suji, pandan leaves, chlorophyll, surfactant Tween 80, extraction**Introduction**

Today, natural dyestuffs are becoming obsolete and people tend to use synthetic dyes. Synthetic color is used more often because it has advantages, such as varied colors, very efficient because it only needs a small amount, the color is relatively more homogeneous, brighter and cheaper. However, the use of synthetic dyes as food or beverage dyes can have a negative impact that is toxic and carcinogenic. Thus, many people want to reuse natural dyes for food coloring, since natural pigments are still considered safer to date. Suji leaves and pandan leaves are a source of green dyes that begin to be abandoned due to the existence of synthetic dyes. Since ancient leaves suji and pandan leaves have been widely used to dye food and how to use it is also easy, ie leaves pounded, plus water, then filtered. However, this method is impractical and has a weakness that is the green color resulting from dye leaves suji and pandan leaves less than the maximum. The

green color produced from suji leaf extract and pandan leaves is easily transformed into brownish or brownish green due to acid treatment, heat and enzymatic browning reaction during processing (Putri *et al.*, 2000). Green chlorophyll can turn brownish green and turn brown due to substitution of magnesium by hydrogen to form feofitin (Hutajalu *et al.*, 2008). To obtain a maximum green color it is necessary to use an extracting solution suitable to the nature of chlorophyll where chlorophyll is soluble in it. The process of dissolving chlorophyll is facilitated with the help of surfactant. The use of Tween 80 which includes non ionic surfactants, in the process of dissolving chlorophyll may suppress the formation of feofitin.

### **Materials and Methods**

10 grams of leaves Leaves suji and pandan leaves crushed then added solution Tween 80 and aquades with a ratio of 1:10 and stirred at a speed of 480 rpm. The next process is the filtering of suji leaf extract and pandanus leaves that are still rough using a filter cloth to obtain the extract of suji leaves and pandan leaves. . The extraction was done by variation of stirring time 1, 2, 3, 4, 5, 6, and 7 hours. The extraction process is also carried out with Tween 80 / aquades variations of 1:10, 2:10, 3:10, 4:10 and 5:10 with optimum agitation time. The extract of chlorophyll obtained from the extraction process was then analyzed chlorophyll content. The measurement of total chlorophyll content in the extract follows the Gross principle (1991). For the total chlorophyll content analysis, the extraction results (1.5 ml) were mixed with 80% acetone (8.5 ml) then left for one night in the refrigerator. The mixture is centrifuged for 10 minutes. Analysis of total chlorophyll content was measured at 645 nm and 663 nm using a UV-Vis spectrophotometer. Calculation of chlorophyll content is done by using the following formula:

$$\text{Total chlorophyll content (mg/L)} = 20.2 A_{645.0 \text{ nm}} + 8.02 A_{663.0 \text{ nm}}$$

$$\text{Chlorophyll content a (mg/L)} = 12.72 A_{663.0 \text{ nm}} - 2.59 A_{645.0 \text{ nm}}$$

$$\text{Chlorophyll content b (mg/L)} = 22.9 A_{645.0 \text{ nm}} - 4.67 A_{663.0 \text{ nm}}$$

Chlorophyll extract which has the largest chlorophyll content used as powder form with freeze drying method.

**Results and Discussion****Chlorophyll Extraction with Variation of Stirring Time**

Chlorophyll extraction is easier by using Tween 80. Tween 80 is included in the polysorbate emulsifier class feed additives. For the aqueous emulsion oil (o/w) the recommended balance of hydrophilic-lipophil (HLB) is in the range 8-18. The value of HLB Tween 80 is 15. Chlorophyll in the thylakoid membrane chloroplast. In the membrane, the chlorophyll is bound to an integral protein and is located between the lipid bilayer. Use of Tween 80, which includes non ionic surfactants, in chlorophyll extraction may help to form anionic surfactants. Anionic surfactants, such as SDS, increase the negative charge on the surface of the chloroplast membrane then produce H<sup>+</sup> ions and increase to form feofitin (Vargaz dan Lopez, 2003). Chlorophyll content of extraction from suji leaves and pandan leaves with variation of stirring time is shown in Fig. 1 and 2.

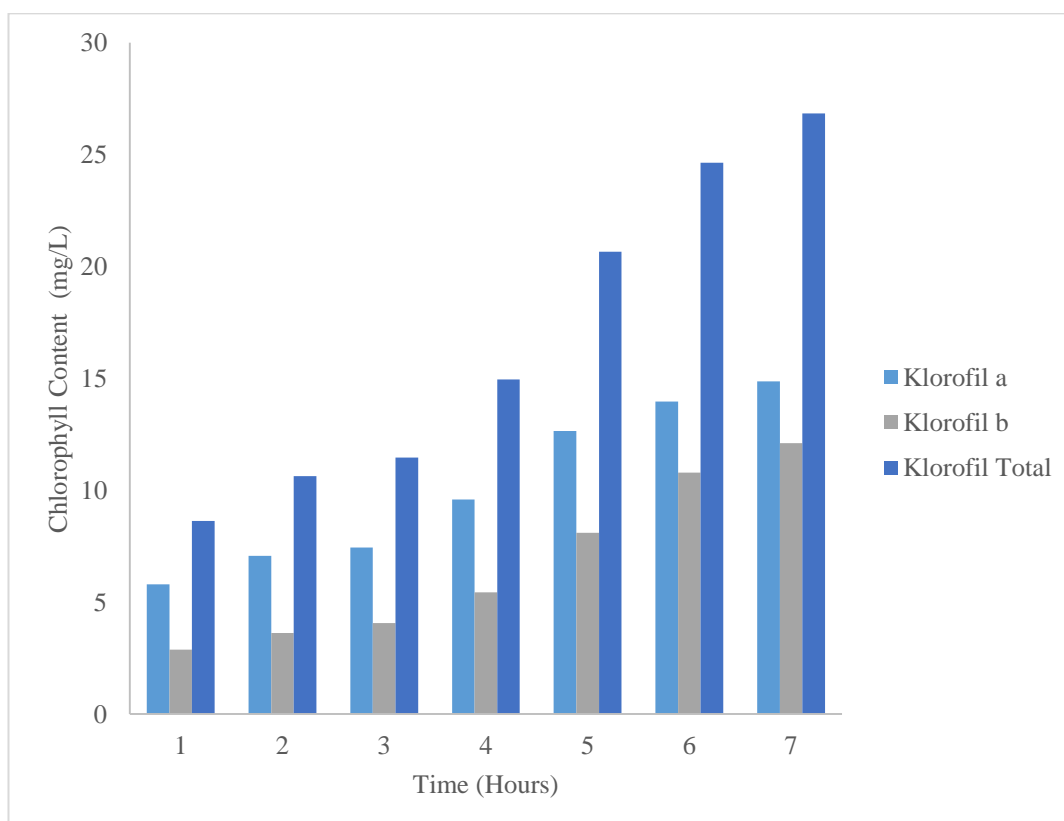


Fig. 1. Effect of Stirring Time on Value of Chlorophyll a, Chlorophyll b and Total Chlorophyll from Suji Leaf Extraction Result with Tween 80 Addition

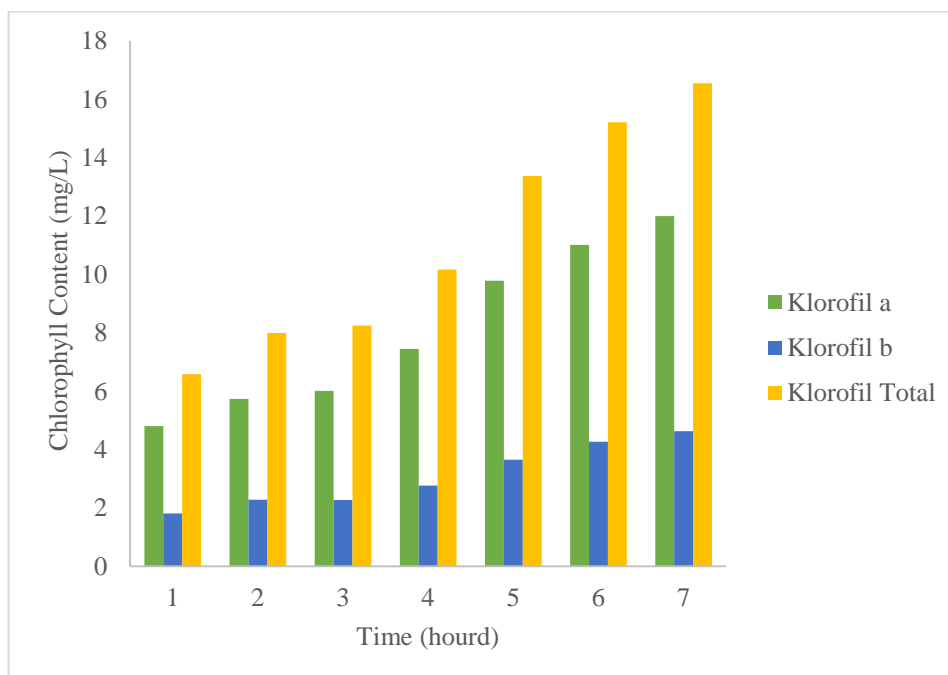


Fig. 2. Effect of Stirring Time on Value of Chlorophyll a, Chlorophyll b and Total Chlorophyll from Pandan Leaf Extraction Result with Tween 80 Addition

The extraction results from both suji and pandan leaves showed that the longer the stirring time, the higher chlorophyll content extracted both chlorophyll a, chlorophyll b and total chlorophyll content (Fig. 1 and Fig. 2). This happens because the length of stirring time causes the contact between the material and the solvent to last longer so that the solubility of chlorophyll increases. The use of Tween 80 also has an effect on the increase in chlorophyll content where Tween 80 can help chlorophyll which is lipophilic emulsified in water (o/w emulsion), besides facilitating contact between chlorophyll with chlorophyllase enzyme. Chlorophyllase works to hydrolyze the phytyl groups thereby converting them into water-soluble chlorophyllids.

**Chlorophyll Extraction with Tween Variation 80: Aquadest**

After the variation of stirring time, proceed to search for Tween 80: optimum aquades. The way of extraction was the same as in extraction with variation of stirring time. For extraction of suji leaves, the stirring time for 7 hours with variations of Tween 80:aquades of 1:10, 2:10, 3:10, 4:10, and 5:10. As for pandan leaf extraction done time of stirring for 7 hours with variations Tween 80:aquades

of 1:10, 2:10, 3:10, 4:10, and 5:10. Chlorophyll content of extraction of suji leaves and pandan leaves with variations of Tween 80: aquades are presented in Fig. 3 and 4.

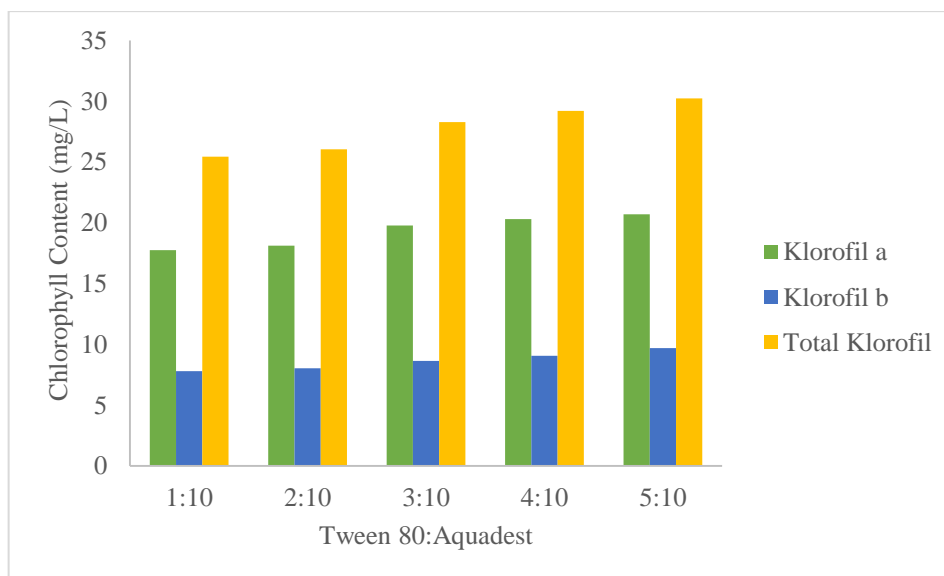


Fig. 3. Effect of Tween 80: aquades on the Value of Chlorophyll a, Chlorophyll b content, and Total Chlorophyll Content from the Result of Suji Leaf Extraction

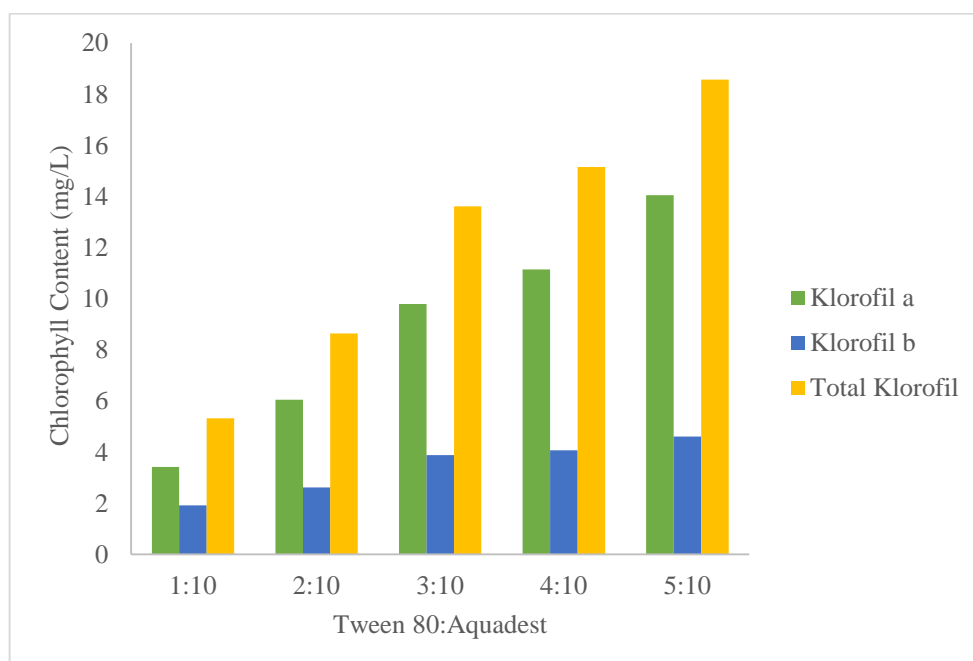


Fig. 4. Effect of Tween 80: aquades on the Value of Chlorophyll a, Chlorophyll b content, and Total Chlorophyll Content from Pandan Leaf Extraction Result

According to Figures 3 and 4 it is stated that the more Tween 80s added the chlorophyll content of both chlorophyll a, chlorophyll b and total chlorophyll will increase. This happens because of the nature of Tween 80 which is hydrophilic, ie easily soluble in water. So that more Tween 80 is added it will bring more chlorophyll to dissolve. In addition Tween 80 can reduce the interface voltage between chlorophyll and medium as well as forming micelles so that chlorophyll will be carried away by micelles dissolved into the medium (Martin *et al.*, 1993). Good extraction results from suji leaves and pandan leaves with 7 hours stirring time and comparison Tween 80: aquadest (5:10), each in freeze dryer to obtain the result of powder.

#### Characterization Result with UV-Vis

Graph showing the absorbance of suji leaves and pandan leaves is presented in Fig. 5.

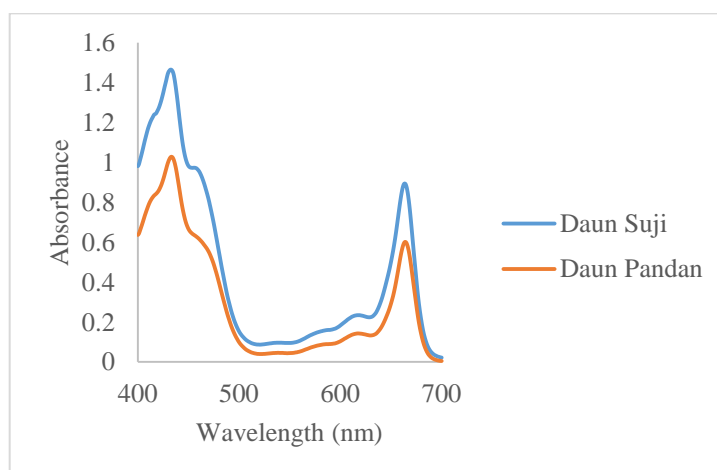


Fig. 5. Graph of Relation Between Wavelength and Absorbance of Suji Leaf Extraction Result and Pandanus Leaves

Fig. 5 showed that the highest peak value is the absorbance of the suji leaf sample and the second graph for the absorbance of the pandan leaves. The absorption curve of suji leaf sample has 7 peak wavelength and pandan leaf sample has 7 peak wavelength. These peaks indicate a particular type of chlorophyll that plays a role in the photon absorption process with the various wavelengths that are involved in the sample. The molecules of chlorophyll leaf composition include: chlorophyll a, chlorophyll b and carotenoid. Chlorophyll a is the most important

pigment, chlorophyll b and carotenoid as complementary pigments. Each pigment is distinguished by its absorbing spectrum of absorbed wavelength. The graph above is a mixture of various components of pigment chlorophyll, which still contains chlorophyll a, chlorophyll b and beta-carotene. Chlorophyll a has a maximum absorbance at wavelengths of 662 nm, 430 nm and 410 nm. From Figure 5 it can be seen that the absorbance spectrum of chlorophyll a achieved the sample at the same wavelength of 663 nm and 430 nm region. The spectrum of chlorophyll absorbbs a sample of suji leaves and pandan leaves respectively at a wavelength of 663nm and at a wavelength of about 430 nm. Thus, the suji leaf sample has the ability to absorb photons better than pandan leaves. Chlorophyll b has a maximum absorbance at 642 nm and 453 nm wavelengths. The spectrum of chlorophyll b absorbbs coincides with chlorophyll a so that the peaks are not visible whereas for the presence of carotenoids in the leaves is not apparent due to chlorophyll pigment. The spectrum of chlorophyll a a absorption at 663 nm indicates that at that wavelength chlorophyll a absorbs the maximum of red color while its absorbance at 430 nm indicates that chlorophyll a absorbs the maximum blue color. Chlorophyll a green-blue color will reflect green and absorb the red color, because the red color spectrum has a long wavelength and blue spectrum has a great energy then the two color spectrum is able to penetrate the Earth's atmosphere layer and absorbed maximally by chlorophyll molecules leaf (Arrohmah, 2007). Fig. 5 has shown that the suji leaf sample has a higher absorbance than the pandan leaf sample.

### **Conclusion**

Surfactant Tween 80 can help dissolve the chlorophyll organic compounds in aquades used as extracting fluids to increase the amount of chlorophyll extracted from suji leaves and pandan leaves. The longer the stirring time the amount of the dye extracted will increase. The greater the ratio of Tween 80 / aquades the amount of extracted dye will increase. The result of UV-Vis analysis shows wave peak at 663 nm and 433 nm for chlorophyll a.

**References**

- Arrohmah. (2007). Studi Karakteristik Klorofil pada Daun sebagai Material Photodetector Organic. *Skripsi*. Surakarta: Jurusan Fisika, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sebelas Maret.
- Clydesdale F.M. dan F.J. Francis. (1976). *Pigments*. In O.R. Fennema. Principles of Food Science. Marcel Dekker, Inc., New York.
- Counsell, J.N. (1981). *Natural Colour for Food and Other Uses*. Applied Science Publishers Ltd, London.
- Eskin N.A.M. (1979). *Plant Pigment Flavors and Textures: The Chemistry and Biochemistry of Selected Compounds*. Academic Press, New York.
- Gross J. (1991). *Pigments in Vegetables: Chlorophylls and Carotenoids*. Van Nostrand Reinhold, New York.
- Heaton, James., dan Alejandro G. Marangoni. (1996). Chlorophyll Degradation in Processed Foods and Senescent Plant Tissue. *Journal of Trends in Food Science and Technologi*, VII, 8-15.
- Hutajalu, T.F., Hartanto, Subagia. (2008). Proses Ekstraksi Zat Warna Hijau Klorofil Alami untuk Pangan dan Karakteristiknya. *J Riset Industri* Vol. 2 (1): 44-45. Bogor: Balai Besar Industri Agro.
- Joslyn, M.A dan G. Mackinney. (1940). The conversion of chlorophyll to pheophytin. *J. Am. Chem. Soc.* 62:231.
- King, A.E. et al. (2001). Chlorophyll Stability in Spinach Dehydrated by Freeze-Drying and Controlled Low-Temperature Vacuum Dehydration. *Food Research International*, 34, pp. 167-175.
- Martin, A., Bustamante, P., and Chun, A.H.C. (1993). *Physical pharmacy*, 4<sup>th</sup> Ed., 324-361, Lea and Febiger, Philadelphia, London.
- Muchtadi, D. (1992). *Fisiologi Pasca Panen Sayuran dan Buah-Buahan*. PAU Pangan dan Gizi IPB. Bogor.
- Perez-Gregorio, M.R. et al., (2011). Changes in antioxidant flavonoids during freeze-drying of red onions and subsequent storage. *Journal of Food Control*, 22(7), pp. 1108-1113.



- Prangdimurti, E. (2007). Kapasitas Antioksidan dan Daya Hipokolesterolemik Ekstrak Daun Suji (*Pleomele angustifolia* N.E.Brown). *Disertasi*. Bogor: Sekolah Pasca Sarjana, Institut Pertanian Bogor.
- Putri, W.D.R., E. Zubaidah, N. Sholahudin. (2000). Ekstraksi Pewarna Alami Daun Suji, Kajian Pengaruh Blanching dan Jenis Bahan Pengektrak. *J Tek Pert.* Vol 4 (1): 13-24. Jurusan Teknologi Pertanian, Fakultas Teknologi Pertanian, Universitas Brawijaya.
- Rowe, R.C., J.S. Paul, J.W. Paul. (2009). *Handbook of Pharmaceutical Exipients*. London: Pharmaceutical Press. Hal. 1-974.
- Schwartz S.J dan J.H von Elbe. (1983). Kinetics of Chlorophyll Degradation to Pyropheophytin in Vegetables. *J.Food Sci.* 48:1303-1306.
- Vargas F.D dan O.P. Lopez. (2003). *Natural Colorant For Food and Nutraceutical Uses*. CRC Press, London. P 221-232.
- Yilmaz, C., and V. Gokmen. (2016). *Chlorophyll in Encyclopedia of Food and Health*, Volume 2, editor B. Caballero, P. M. Finglas and F. Toldra, 1st ed. Academic Press, Oxford, 37-41.