

THE POTENTIAL OF MATURE PANDAN LEAVES AS A SOURCE OF CHLOROPHYLL FOR NATURAL FOOD COLORANTS

[Potensi Daun Pandan Tua sebagai Sumber Klorofil untuk Pewarna Makanan Alami]

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ABSTRACT

Plant leaves are the primary source of natural colorants for food, mainly due to their chlorophyll content. However, the plant types and the degree of leaf maturity determine the quality and quantity of the chlorophyll. This study aimed to determine the best maturity level of pandan (*Pandanus amaryllifolius* Roxb.) leaves that serves as potential source of chlorophyll for natural food colorants. Eighty three pandan plants obtained from six different farming locations in Bantul Regency, Yogyakarta, Indonesia were used as samples. The leaves were grouped into four levels of maturity using descriptive statistics based on their morphology, anatomy, color, and chlorophyll contents. The results showed that the average number of leaves ranged from 20-24 leaves per plant (at 95% confidence interval), and 96.4% of the plant had a maximum of 24 leaves. The leaf maturity was grouped into (1) young, (2) medium, (3) mature, and (4) over mature, corresponding to leaf number 1-6, 7-12, 13-18, and 19-24, respectively. The higher the leaf maturity, the higher the chlorophyll content. However, the over mature leaves were only slightly different from the mature ones. In addition, pandan leaves have specific flavor and contain carotenoid, phenolic, and flavonoid substances. Anatomically, the mesophyll's size was greatest in the mature leaves, while the size of chloroplast was not significantly different from medium to over mature leaves. Based on the chlorophyll content and mesophyll size, it was concluded that mature pandan leaves were the best source of chlorophyll, containing chlorophyll of 623.08 mg/100 g dry weight (DW).

Keywords: chlorophyll, food colorants, leaf maturity, *Pandanus amaryllifolius* Roxb., photomicrography

ABSTRAK

Daun merupakan sumber utama pewarna alami untuk makanan terutama karena kandungan klorofilnya. Namun, jenis tanaman dan tingkat ketuaan daun menentukan kualitas dan kadar klorofil. Penelitian ini bertujuan untuk mengetahui tingkat ketuaan terbaik daun pandan (*Pandanus amaryllifolius* Roxb.) sebagai sumber klorofil potensial untuk pewarna alami pangan. Sebanyak 83 tanaman pandan digunakan sebagai sampel, yang diperoleh dari enam lokasi pertanian berbeda di Kabupaten Bantul, Yogyakarta, Indonesia. Daun dikelompokkan dalam empat kelompok berdasarkan tingkat ketuaan daun menggunakan statistik deskriptif dan dianalisis morfologi, anatomi, warna, dan kadar klorofilnya. Hasil penelitian menunjukkan bahwa rata-rata jumlah daun antara 20-24 ruas (pada tingkat kepercayaan 95%) dan 96,4% mempunyai jumlah daun maksimal 24 ruas. Tingkat ketuaan daun dikelompokkan menjadi (1) daun muda, (2) medium, (3) tua, dan (4) sangat tua sesuai dengan ruas daun ke 1-6, 7-12, 13-18, dan 19-24 setelah tunas, secara berurutan. Semakin tinggi kematangan daun maka semakin tinggi pula kandungan klorofilnya. Namun demikian, pada daun sangat tua hanya berbeda sedikit dengan daun tua. Selain itu, daun pandan memiliki aroma yang khas dan mengandung zat karotenoid, fenolik, dan flavonoid. Secara anatomis, ukuran mesofil paling besar ada pada daun tua, sedangkan ukuran kloroplas tidak berbeda nyata antara daun medium hingga daun tua. Berdasarkan kadar klorofil dan ukuran mesofil dapat disimpulkan bahwa daun pandan yang paling baik sebagai sumber klorofil adalah daun tua dengan kadar klorofil 623,08 mg/100 g berat kering (bk).

Kata kunci: klorofil, *Pandanus amaryllifolius* Roxb., pewarna makanan, photomicrography, tingkat ketuaan

INTRODUCTION

Consumers consider food color as the primary attribute of food before purchasing because it represents the quality and authenticity of the food and indicates successful food processing (Simon *et al.*, 2017; Schifferstein *et al.*, 2019). Recently, food colorants have played a significant role in the food industry with an upward trend in the market and are expected to grow steadily from 10 to 15% per year (Institute of Food Technology, 2016).

The primary type of colorants that is widely used is synthetic colorants. Synthetic colorants have many advantages over natural colorants, but several studies have shown their adverse effects on human health. Many studies suggested that artificial colorants might cause hyperactivity and learning problems in children (Arnold *et al.*, 2012) and could induce DNA damages in rat colon (Shimada *et al.*, 2010). Therefore, many consumers prefer to choose food with natural colorants as it is considered safe. There are Indonesian indigenous plants that are usually used as a food coloring agent, namely pandan (*Pandanus amaryllifolius* Roxb.) (Wakte *et al.*, 2012) and suji (*Dracaena angustifolia*) (Indrasti *et al.*, 2019). Pandan and suji leaves are used as a source of green colorants.

The major substance in pandan leaves that is used as the green coloring agent is chlorophyll. Traditionally, the use of pandan leaves, as natural colorants, is done by mixing pandan leaf extract with food materials during food preparation and processing. However, the color intensity usually decreases during processing. Therefore, the optimization of processing methods to stabilize the color intensity is needed, for example, by the encapsulation process (Porrarud and Pranee, 2010). In the commercial processing of chlorophyll, the feasibility of production must be considered, and the main factor of this feasibility is the chlorophyll content in leaves (Miazek and Ledakowicz, 2013).

The chlorophyll content in leaves is mainly influenced by the type of plants and leaf maturity. Plants as the primary source of commercial chlorophyll production are alfalfa, nettle, spinach, and other types of grasses (Viera *et al.*, 2019). Devenci and Uzun (2011) observed the chlorophyll content in spinach from the initial stage (cotyledon) to the mature stage of plant growth. The results showed that the longer the spinach grew, the higher the chlorophyll content. Kamble *et al.* (2015) stated that the chlorophyll content of old leaves was higher than that of young leaves in several plants. Besides, Prawira-Atmaja *et al.* (2018) found that the chlorophyll content in *Camellia sinensis* var. *sinensis* leaves was positively correlated with the degree of leaf maturity. However, other researchers argued that during plant growth there is a degradation

process of chlorophyll (Hörtensteiner and Kräutler, 2011), which occurs enzymatically by peroxidase, Mg-dechelataze, pheophorbide a oxygenase (PaO), red-chlorophyll catabolite reductase, and chlorophyllase enzymes (Indrasti *et al.*, 2018) resulting in decreased chlorophyll levels. Hu *et al.* (2013) found that the degree of leaf maturity of *Arabidopsis thaliana* affected its chlorophyll content. The four-month aged leaves contained higher chlorophyll than the eight-month aged leaves because chlorophyll might degrade during the over mature period. In addition to chlorophyll degradation, the older the pandan leaves, the higher the fiber content, thus complicating the extraction process leading to low extracted chlorophyll. Interestingly, Limantara *et al.* (2015) explained that some factors affect the chlorophyll content in commercial green vegetables, namely leaf size, thickness, and variation in chloroplast distribution. Pandan leaf extract had a total chlorophyll content of 17.82 mg/g fresh weight (FW) (Senklang and Anprung, 2010), while the chlorophyll content in different degrees of leaf maturity has not been studied yet. This study aimed to evaluate the effects of leaf maturity on the chlorophyll content, color, and chemical characteristics of pandan leaves and to determine the best section of pandan leaves as the most potential source of chlorophyll for natural food colorants.

MATERIALS AND METHODS

Materials

Pandan leaves were collected from Bantul Regency, Yogyakarta, Indonesia. Sampling was done in September and October 2018 from six different farming locations (A, B, C, D, E, and F) covering 100 plants. However, only 83 plant samples were used for further studies after field selection. The field selection was conducted to gather complete pandan plants whose leaves have not been harvested. Plant samples were wrapped with black polyethylene (PE) plastics and stored in a cooling box while being transported and analyzed.

Leaf maturity classifications

The morphology of the plant samples was visually observed, and the number of leaves was counted. After that, the leaves were classified into four groups based on the degree of leaf maturity, namely (1) young, (2) medium, (3) mature, and (4) over mature by descriptive statistics as computed using SPSS (IBM, 2011). The number of leaves was determined based on mean estimation by Z distribution at a 95%-significance level (Mendenhall and Sincich, 2016).

After grouping, the most potential farming location was selected from the six areas. A total of

12 plants from each garden were analyzed in three replications for their color and chlorophyll content. The data were then analyzed statistically using the one-way analysis of variance (ANOVA) (IBM, 2011). The selected garden was used as the primary source of raw materials for further studies.

Lastly, to determine which group is the best chlorophyll source, each leaf of different maturity levels was taken from two pandan plants obtained from the selected garden location. All the leaf samples were analyzed in three replications for their color and morphology and their proximate, carotenoid, phenolic, flavonoid, and chlorophyll contents. The data were analyzed statistically using ANOVA (IBM, 2011).

Determination of chlorophyll contents (Nikolaeva *et al.*, 2010)

Briefly, fresh pandan leaves were cut crosswise at a size of 1-2 cm, dry-milled for three min, weighed accurately (5.0 g), extracted with 20 mL of acetone (Merck, Germany), mixed, and centrifuged at 8,000 rpm for 15 min. The supernatant was then filtered with the Whatman filter paper grade 1 and 42 with pore sizes of 11 and 2.5 μm , respectively, and adjusted to 25 mL in a volumetric flask. The absorbance value of the extract was measured by a UV-Vis spectrophotometer (Shimadzu, Japan) at 663 and 645 nm. Total chlorophyll, chlorophyll a, and chlorophyll b contents were calculated by the equation (1), (2), and (3), in which fw= fresh weight, DF= dilution factor, and W= sample weight.

Total Chlorophyll (mg/g fw) =

$$(20.24 A_{645} + 8.02 A_{663}) \times \frac{DF}{(W \times 1000)} \dots\dots\dots (1)$$

Chlorophyll a (mg/g fw) =

$$(12.70 A_{663} - 2.69 A_{645}) \times \frac{DF}{(W \times 1000)} \dots\dots\dots (2)$$

Chlorophyll b (mg/g fw) =

$$(22.90 A_{645} - 4.69 A_{663}) \times \frac{DF}{(W \times 1000)} \dots\dots\dots (3)$$

Determination of color values (Pathare *et al.*, 2013)

The color values of pandan leaves were determined using Chromameter (CR-400, Konica Minolta Optic Inc., Japan) with L, a, and b color system in which L is for lightness, +a= redness and -a= greenness (-), and +b= yellowness, and -b= blueness. Chroma (C) and hue (h°) were computed using equation (4) and (5).

$$C = \sqrt{(a^2 + b^2)} \dots\dots\dots (4)$$

$$\text{hue } (h^\circ) = \arctan \frac{b}{a} \dots\dots\dots (5)$$

Determination of total carotenoid content (Khandaker *et al.*, 2012)

Total carotenoid was determined by the spectrophotometric method. Briefly, fresh pandan leaves were cut crosswise at a size of 1-2 cm, dry-milled for three min, and weighed accurately (1.0 g). The slurry sample was extracted with 100 mL of acetone, stirring until the carotenoids dissolved. The extract was filtered with the Whatman filter paper grade 42 (pore size= 2.5 μm). The absorbance value of the supernatant was measured by a UV-Vis spectrophotometer at wavelengths of 480, 646 and 663 nm. The carotenoid content was calculated based on equation (6), in which V= sample volume (mL) and W= sample weight (g). Conversion into mg/g units was carried out with an average molecular weight of 545 g/mol carotenoids.

Total Carotenoid ($\mu\text{mol/g}$)=

$$\frac{((A_{480} + (0.114 \times A_{663}) - (0.638 \times A_{645})) \times (V \times 10^3))}{112.5 \times W} \dots\dots\dots (6)$$

Determination of total phenolic and flavonoid contents (Pujimulyani *et al.*, 2010)

Total phenolics were determined by the Folin-Ciocalteu method. Briefly, 50 μL of extract solution was mixed with 250 μL of the Folin-Ciocalteu solution (Sigma Chemical Co., St. Louis) and allowed to stand for one min. Then, 750 μL of 20% Na_2CO_3 (Merck, Germany) was added and swirled until homogeneous. The solution was then added with distilled water until 5 mL. After incubation for 2 hours at room temperature, the absorbance was measured with a UV-Vis spectrophotometer at 760 nm. Total phenolics were calculated using a gallic acid standard (Sigma Chemical Co., St. Louis) and expressed as milligram equivalent gallic acid per gram dry weight (mg EAG/g DW).

Total flavonoids were determined by a colorimetric method. Briefly, 50 μL of extract, 4 mL of distilled water, and 0.3 mL of 10% NaNO_2 solution were mixed in a test tube. After six min, 0.3 mL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (Merck, Germany) solution was added and allowed to stand for another five min. After that, 4 mL of 10% NaOH solution was added, and the volume was adjusted to 10 mL with distilled water and swirled. After incubation for 15 min at room temperature, the absorbance was measured by a UV-Vis spectrophotometer at 510 nm. The extract was replaced with distilled water as blank. Total flavonoids were calculated using a quercetin stan-

dard (Sigma Chemical Co., St. Louis) and expressed as milligram equivalent quercetin per gram dry weight (mg EQ/g DW).

Proximate analysis (AOAC, 1990)

The proximate analysis of pandan leaves was also carried out. The proximate composition includes the moisture, ash, lipid, protein, and carbohydrate contents (by difference) of pandan leaves.

Leaf anatomy measurements (Rahayu *et al.*, 2011)

The leaf anatomy was observed based on photomicrography of the transverse section of the pandan leaves. Mesophyll width, parenchyma length, and chloroplast size were measured using a binocular microscope (BOECO BM 180-SP, Germany) and a digital microscope camera (Optilab, Upgrade Edition).

Statistical analysis

IBM SPSS version 20 software (2011) was used to statistically analyze the data with the interpretation based on the One-way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) with a 95% confidence interval ($P < 0.05$).

RESULTS AND DISCUSSION

Plant morphology

Cultivated pandan plants in Bantul Regency grew in clumps (5-15 stems in each clump), creeping on land until a height of 0.5-1.2 m (Figure 1). However, Wakte *et al.* (2009) mentioned that pandan plants could grow as high as 4.5 m. The stem was cylindrical, 0.5-1.5 cm in diameter, with a small taproot.

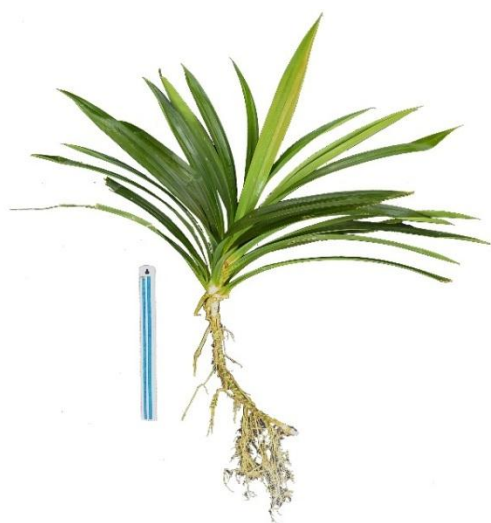


Figure 1. *Pandanus amaryllifolius* Roxb. plant

The leaves formed a sword-like shape with 19-55 cm in length and 1.5-3.2 cm in width (Figure 1). The upper surface of the leaves was bright with prickly tips. In Bantul Regency, pandan plants were cultivated in paddy fields as well as in some private gardens. Two out of the six studied locations were in paddy fields located in Kalipakis, Tirtonirmolo, Kasihan (A) and in Nulis, Argomulyo, Sedayu (F), and the rest were in private gardens (Siyangan, Triharjo, Pandak (B); Gedongsari, Wijirejo, Pandak (C); Dagen, Pendowoharjo, Sewon (D); and Panggang, Argomulyo, Sedayu (E)). Naturally, pandan plants grow in clumps and can consist of many plants per clump without a pre-cutting treatment. Wakte *et al.* (2009) classified the plant density as (1) low (<5 plants per 50 m²), (2) medium (5-50 plants per 50 m²), and (3) high (>50 plants per 50 m²). Our survey showed no location with a plant density of fewer than five plants per 50 m². The plant density in all paddy fields was high or exceeded 50 plants per 50 m². In contrast, the plant density in the private gardens was categorized as a medium, consisting of 10-20 plants per 50 m² (Table 1).

Table 1. Characteristics of the farming areas in Bantul Regency

Farming Locations	Types of Land	Plants Density (plants/ 50 m ²)
A	Paddy field	>50, high
B	Private garden	10-20, medium
C	Private garden	>50, high
D	Private garden	10-20, medium
E	Private garden	10-20, medium
F	Paddy field	>50, high

Characteristics of pandan leaves from different locations

The total chlorophyll content of pandan leaves from different farming locations is presented in Figure 2. The total chlorophyll content showed the total amount of chlorophyll in the leaves, consisting of chlorophyll a and b, where it varied from 77.75 to 125.39 mg/100 g FW.

The results showed that high chlorophyll contents were obtained from the pandan leaves grown in A, C, and D locations, and hence served as the primary source of materials. The pandan leaves from those locations had high chlorophyll contents because the gardens were shaded by other trees with a sunlight intensity ranging from 3605±1.83 Lux. Zhang *et al.* (2015) stated that a decrease in light intensity would significantly increase the chlorophyll content of the oriental lily. The chlorophyll content of pandan leaves was similar to commercial chlorophyll from different plants. For instance, alfalfa and spinach leaves contained chlorophyll of 39-460 mg/100 g FW (Petisco *et al.*, 2009) and 288.02-1032.95 mg/g DW (Deveci and Uzun, 2011), respectively.

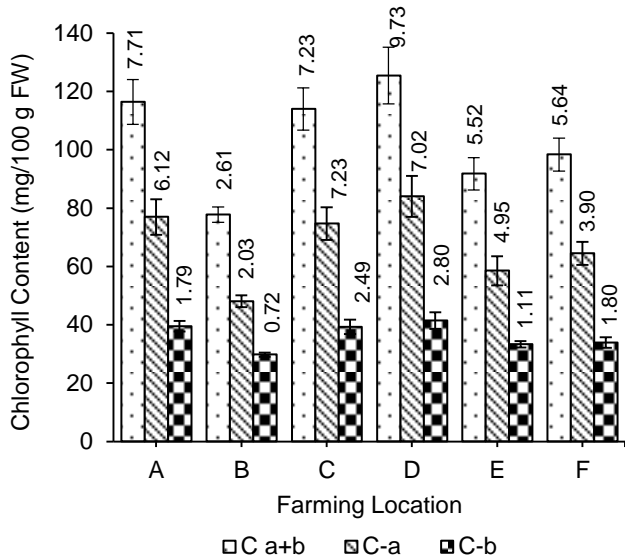


Figure 2. Chlorophyll total (C a+b), chlorophyll a (C-a) and chlorophyll b (C-b) of pandan leaves from different farming locations in Bantul Regency

The leaves with high chlorophyll contents had greenness (-a value) ranging from -14.56 to -15.75, yellowness (b value) ranging from 21.29-23.57, saturation ranging from 39.89 to 46.14, and hue angle ranging from 124.20 to 125.59 (Figure 3).

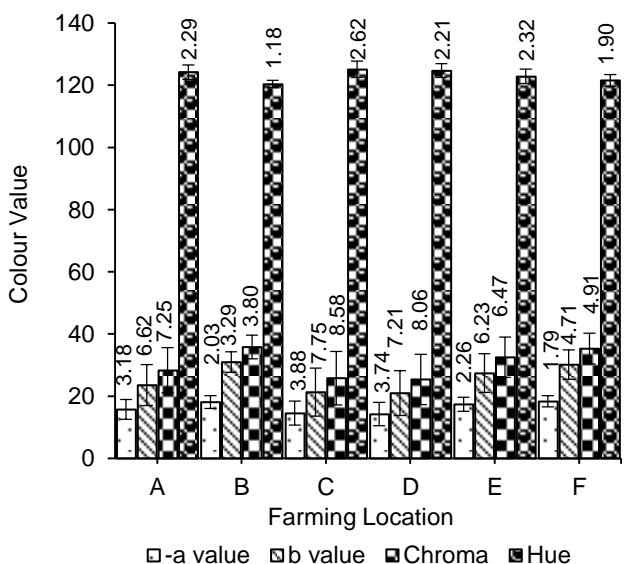


Figure 3. Color values of pandan leaves from different farming locations in Bantul Regency; greenness (-a value), yellowness (b value), color saturation (chroma) and hue angle (hue)

These results were similar to Sowndhararajan *et al.* (2015). They found that pandan leaves with -a value of between -7 to -15, b value of 15-45, and L value of 30-50, are a good source of chlorophyll. Hue values in the range of 120° in the HSL color system show green, while more than 120° to 160° indicate bluish-green to blue. The bluish-green color is the color of chlorophyll a. Conversely, the HSL color system with less than 120° to 60° shows a yellowish-green to yellow. The yellowish-green color is the color of chlorophyll b. The results of the color study of pandan leaves from A, C, and D locations were identical to each other.

Leaf maturity classifications

The chlorophyll content depends on the plant types, growing condition, climate, and soils (Li *et al.*, 2018). Furthermore, it also depends on leaf maturity (Kamble *et al.*, 2015). Figure 4 depicts leaf numbering based on leaf maturity. The numbering starts after shoots, in which the remaining leaves bundling the stem totally (Wakte *et al.*, 2010; Liew, 2012). Wakte *et al.* (2010) divided pandan leaves into six groups with the number of leaves per group ranging from three to five internodes. The grouping was carried out to select the potential leaf segment with high total phenolic contents. Gil *et al.* (2012) divided the leaf maturity of vegetables into three groups, namely immature, mature, and overmature. In this study, the immature was divided into young and medium. Therefore, this study used four groups of leaf maturity. The average number of leaves was 20.40-23.55, rounded to 20-24. The leaves in section 1-6 were classified as young, 7-12 as medium, 13-18 as mature, and 19-24 as over mature (Table 2). The results showed that 96.4% of the plant samples had no more than 24 leaves (Figure 5).

Chemical and color properties of different aged leaves

Harvesting pandan leaves as a whole is incorrect because it will inhibit plant growth. Mataliana *et al.* (2015) suggested 30-35 days of cyclical harvesting. As a chlorophyll source, therefore, it is necessary to determine the optimal leaf maturity. The chlorophyll contents of pandan leaves from different degrees of maturity are shown in Figure 6. The older the leaves, the higher the chlorophyll a and the total chlorophyll content. However, in the over mature group, the increased chlorophyll content was not significantly different from the mature one. Similarly, the older the leaves, the higher the chlorophyll b. Based on the duncan's multiple range test (DMRT), the highest content of chlorophyll a and total chlorophyll was found in mature and over mature leaves.

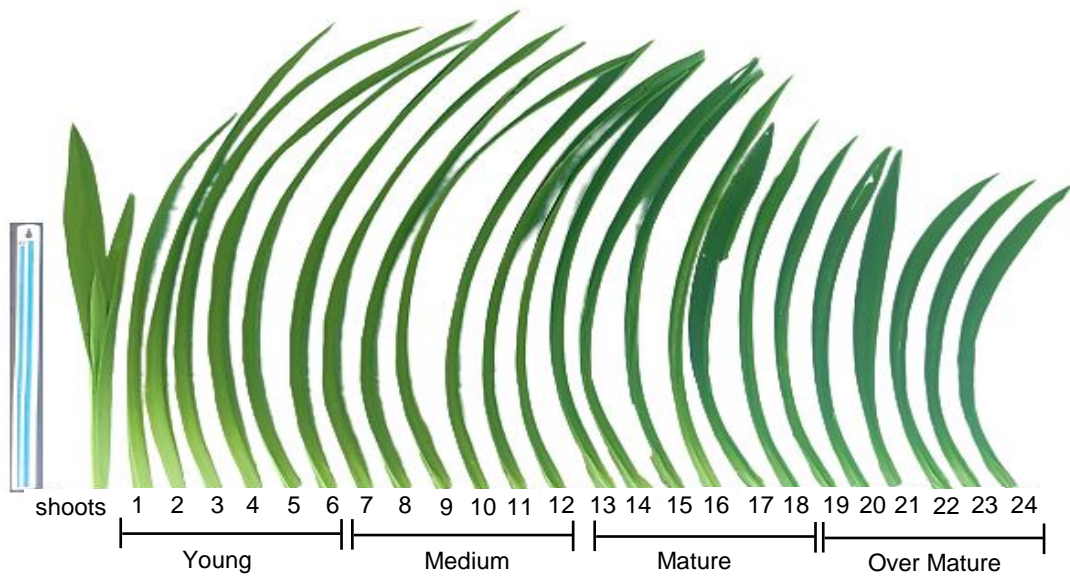


Figure 4. Leaf section numbering

Table 2. Different categories of pandan leaves based on leaf maturity

Categories	Leaf Section Numbers	Leaf Maturity Groups
1	1-6	Young
2	7-12	Medium
3	13-18	Mature
4	19-24	Over mature

the leaves, the higher the hue angle value, while the color saturation, yellowness (b value), and greenness (-a value) were smaller, but the mature and over mature leaves were not differed significantly (Figure 7). During the aging process, chlorophyll a will degrade into chlorophyllide a, pheophorbide a, and finally into non-fluorescent chlorophyll catabolite (NFCC), which is colorless (Rios *et al.*, 2014).

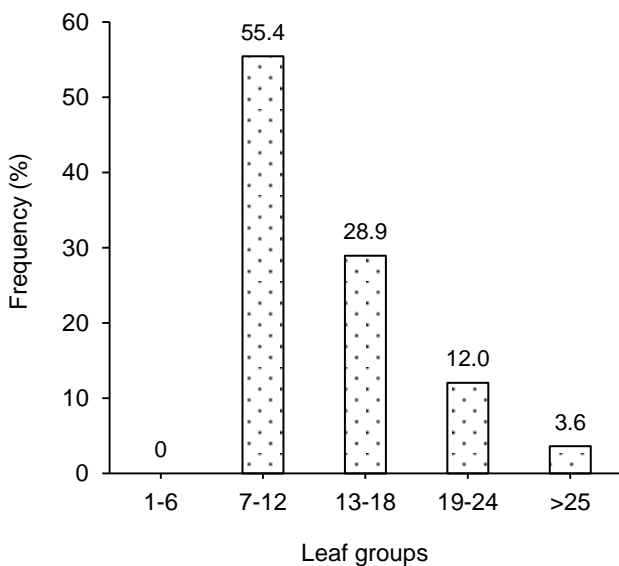
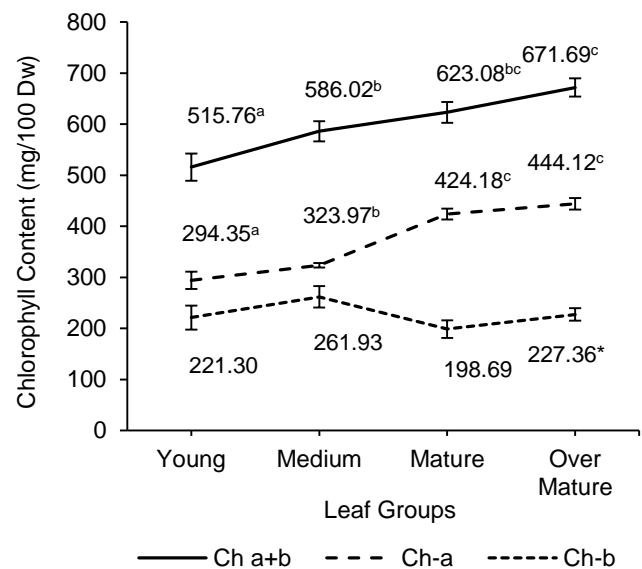


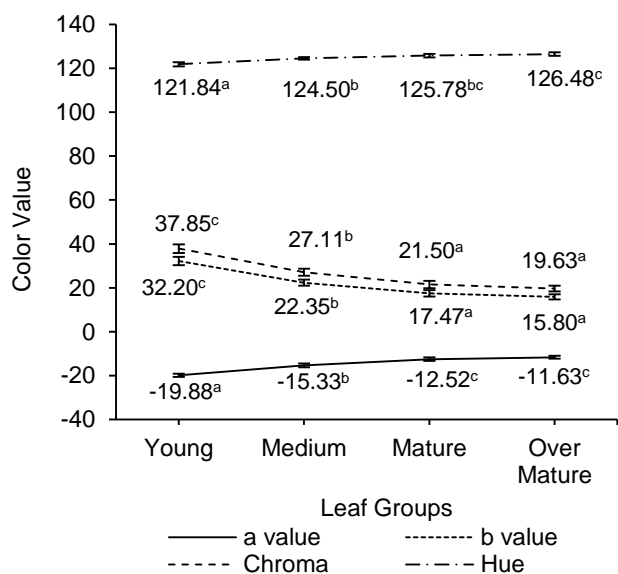
Figure 5. Frequency of pandan plants population in various leaf groups

When mature leaves were getting older, their chlorophyll a and total chlorophyll were still getting higher, and their chlorophyll b was also getting higher, affecting the a, b, L and C values. The older



Note: Different notations (a, b, bc, and c) indicate a significant difference among groups (young, medium, mature, and over mature) ($P < 0.05$). * = Means are not significantly different ($P > 0.05$)

Figure 6. Chlorophyll total (Ch a+b), chlorophyll a (Ch-a) and chlorophyll b (Ch-b) from different degrees of maturity



Note: Different notations (a, b, bc, and c) indicate a significant difference among groups (young, medium, mature, and over mature) or on each color parameter analyzed ($P < 0.05$)

Figure 7. Color values of pandan leaves from different degrees of maturity

Table 3 shows that, as increased leaf maturity, the moisture and carbohydrate contents tended to decrease, while the protein, total carotenoid, and total flavonoid contents tended to increase steadily; and the ash, lipid, and total phenolic contents tended to increase obviously.

The increased total carotenoid was observed until the medium maturity and then remained constant afterward. Based on the results of the correlation analysis, it was known that total chlorophyll contents were significantly correlated ($P < 0.05$) with the chlorophyll a ($R = 0.437$), total carotenoid ($R = 0.542$), and total phenolic contents ($R = 0.468$), but not correlated ($P > 0.05$) with total flavonoid and chlorophyll b contents. These results were different from the results of Prawira-Atmaja *et al.* (2018), showing that the chlorophyll contents of *Camellia sinensis var. sinensis* leaves were negatively correlated with the total phenolic contents. This might be

because, in Prawira-Atmaja's study, the leaf segment was from 1 to 4, while in this study, the leaf segment was wider (from 1 to 24). The total carotenoid, phenolic, and flavonoid contents provided an added value to the pandan leaves as a natural colorant besides having a specific aroma. It is known that carotenoids have antioxidant activity (Young and Lowe, 2018). The phenolic and flavonoid substances from pandan leaves also have the potency for being antioxidant (Suryani *et al.*, 2017) and anti-hyperglycemic agents (Suryani *et al.*, 2018).

The anatomy of pandan leaves and their potential section

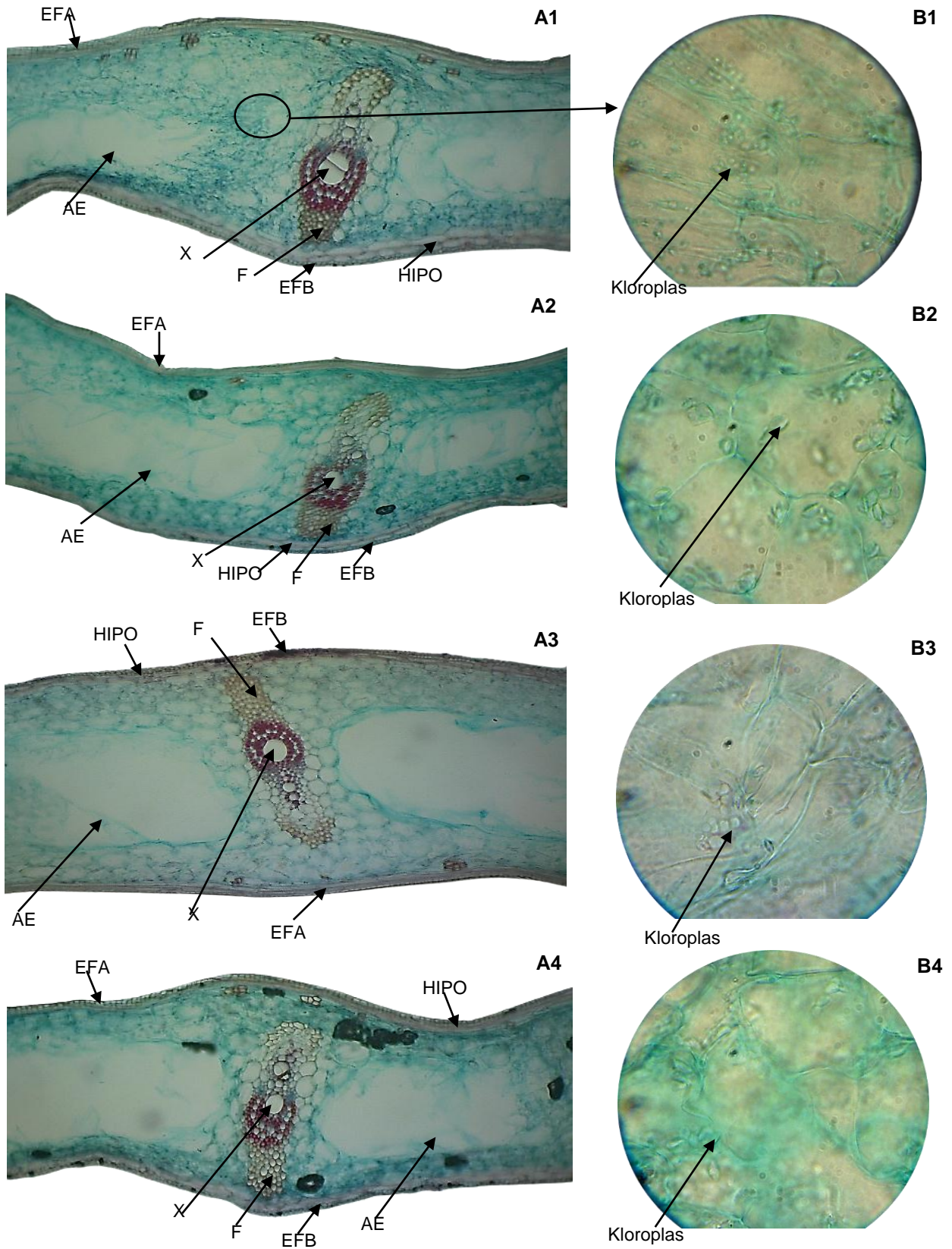
According to the results of chlorophyll a and total chlorophyll contents, pandan leaves on sections greater than 12 were a potential source of chlorophyll. In Figure 8A, the mesophyll cells are shown by a bluish-green area, where the chloroplasts are located (Figure 8B). The photomicrograph (Figure 8) clearly shows that leaves were getting thicker as increased maturity. However, the leaves became thinner when over mature. When leaves are getting older, the colorless cells occupied greater space (AE). This indicated that the disintegration process occurred at a higher rate leading to a hollow formation. This was consistent with the results of the analysis of carbohydrate contents in over mature leaves, which tended to increase as increased fibers. The increased fibers make the grinding process more difficult (Liu *et al.*, 2016) so that the result of chlorophyll extraction is low.

In addition, Table 4 shows that the mesophyll size was getting bigger as increased leaf maturity, but it decreased when the leaves were over mature. The chloroplast size was also getting more prominent as increased maturity, but it remained constant after the leaves reached medium maturity. According to Wild and Wolf (1980), the size of chloroplasts is very important as a source of chlorophyll as large chloroplasts have much higher chlorophyll contents than small chloroplasts. In contrast, the parenchyma length was not affected by leaf maturity. Based on the mesophyll and chloroplast size, mature pandan leaves are more appropriate as the chlorophyll source.

Table 3. Chemical properties of pandan leaves from different degrees of maturity

Leaf Groups	Moisture Contents (% FW)	Proteins (% DW)	Lipids (% DW)	Ash (% DW)	Carbohydrate (% DW)	Total Carotenoids (mg/g DW)	Total Phenolics (mg EAG/100 g DW)	Total Flavonoids (mg EQ/100 g DW)
Young	82.90 ^c	16.53 ^a	1.39 ^a	8.55 ^a	73.53 ^c	1.04 ^a	316.26 ^a	17.11 ^a
Medium	80.87 ^b	18.09 ^b	1.69 ^b	8.60 ^a	71.63 ^b	1.47 ^b	334.57 ^b	18.49 ^a
Mature	80.65 ^b	18.96 ^b	2.40 ^c	8.87 ^{ab}	69.77 ^a	1.49 ^b	343.64 ^b	18.47 ^a
Over mature	80.03 ^a	17.92 ^b	2.25 ^c	9.22 ^b	70.61 ^{ab}	1.50 ^b	386.21 ^c	24.48 ^b

Note: Different letters within a column are significantly different ($P < 0.05$)



Note: Groups 1= young, 2= medium, 3= mature, and 4= over mature leaves. EPB= Lower Epidermis, EPA= Upper Epidermis, HIPO= Hypodermis, F= Phloem, X= Xylem, and AE= Aerenchyma. Scale Bar: 100 µm

Figure 8. Photomicrography of transverse sections of pandan leaves (A) and chloroplasts (B)

Table 4. Size of mesophyll, parenchyma, and chloroplast of pandan leaves from different degrees of maturity

Leaf Groups	Size of Mesophyll (μm)	Length of Parenchyma (μm)*	Size of Chloroplast (μm^2)
Young	322.80 ^a	690.41	5.35 ^a
Medium	382.49 ^b	681.07	12.31 ^b
Mature	428.20 ^c	731.97	12.60 ^b
Over mature	378.12 ^b	739.21	13.05 ^b

Note: Different letters within a column are significantly different ($P < 0.05$); *= Means are not significantly different ($P > 0.05$)

CONCLUSION

It can be concluded that pandan plants in Bantul Regency, Yogyakarta, Indonesia were estimated to have 20-24 leaves with 96,4% of them had no more than 24 leaves. Based on leaf section numbering, the leaf maturity could be classified as (1) young, (2) medium, (3) mature, and (4) over mature, corresponding to leaf number 1-6, 7-12, 13-18, and 19-24 after shoots, respectively. The leaf maturity defined the chlorophyll content. The higher the leaf maturity, the higher the chlorophyll content. However, the over mature leaves were not significantly different from the mature ones. Anatomically, the greatest mesophyll size was found in mature leaves; however, the chloroplast size remained constant for leaves on sections greater than six. Based on the chlorophyll content and mesophyll size, mature pandan leaves are a good source of chlorophyll for natural food colorants.

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