The effect of leaf age and drying method on physico-chemical characteristics of pandan (*Pandanus amaryllifolius* Roxb.) leaves powder

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Abstract. *Pandanus amaryllifolius* leaves contain chlorophyll as green pigment and volatile compounds in the form of 2-acetyl-1-pyrroline which can give color and aroma to food products. Following the zero waste concept, the general purpose of this research to convert pandan leaves into a powder so that it can be fully utilized. The specific purpose to investigate the effect of age of leaves and drying methods on the physicochemical of pandan leaves powder. Pandan leaves powder was made from young and old with 3 drying methods that were cabinet, vacuum, and freeze drying. The result showed the age of leaves and drying method had a significant effect on moisture content, ash content, density, water absorption capacity, oil absorption capacity, pH, color, total chlorophyll, total phenolic content, and antioxidant IC$_{50}$. Pandan leaves powder from old leaves and vacuum drying had the highest total chlorophyll, total phenolic content, and antioxidant activity than others.

1. Introduction

*Pandanus amaryllifolius*, commonly known as pandan is a tropical plant that is widely used as a natural coloring and flavor in food and beverage processing. Pandan leaves contain chlorophyll as a green pigment [1] and volatile compounds in the form of 2-acetyl-1-pyrroline [2] which can give distinctive color and aroma to food products. Pandan leaves also contain many functional compounds such as phytochemicals like steroids, carbohydrates, phenols, isoflavones, coumestrol, lignans, alkaloids, glycosides, amino acids and vitamins, terpenoids, flavonoids, saponins [3]. Pandan leaves can be used to refresh the body, reduce fever, and relieve indigestion [4]. Besides that, pandan leaves are traditionally used as a medicinal plant as antimicrobial, anti-diabetic, anti-viral, anti-neoplastic, antioxidant, anti-diuretic, analgesic and neuroprotective. Pandan leaves also contain fibers that can help overcome constipation [5].

Pandan leaves based on their age are divided into young and old leaves. Young leaves tend to have a relatively higher water content compared to older leaves. Leaves with older age will experience an increase in metabolism and genetics which causes an increase in chlorophyll production in leaf [6]. Pandan leaves with the highest content of 2-acetyl-1-pyrroline are leaf number 12-14 from apex [2]. However, older leaves have higher fibers content than younger ones as a result of their metabolism.
Besides that, older leaves also have higher chlorophyll and phenolic compounds. Chlorophyll was synthesized during leaf growth and development [6] along with phenol components [7].

Natural colorants from plant-based materials have gained increasing popularity due to the health consciousness of consumers. The use of pandan leaves as a natural coloring and aroma has been done, but mostly done by pounding pandan leaves and then extracted using water. In this study, we make natural color and aroma from pandan leaves in powder form with the aim that all parts of pandan leaves can be used with zero waste concept. Pandan leaves powder can be made by reducing water through the drying process. The drying process not only reduces water in the leaves but also makes some changes in its properties. The most common changes that occur due to heat from the drying process are the loss of chlorophyll and aroma in pandan leaves [8]. The selection of drying methods is needed to minimize the damage quality of the product. Low temperature and low RH in the drying process can improve the product quality [9].

Many drying methods have been developed in this modern era, including cabinet, vacuum, and freeze drying. The cabinet drying method is one of type of drying most often used in industry or lab scale. It has an air re-circulation that can be adjusted to keep the RH and temperature constant [10]. Negi and Roy reported that cabinet drying can keep the chlorophyll higher than solar drying in dried beet and amaranth green leaves [11]. The vacuum drying method has a difference from the other drying method. During the vacuum drying process, sub-atmospheric pressure is reached so the water vapor may decrease with lower temperatures [12]. Shravya et al. reported that the vacuum drying method can keep the highest chlorophyll and fiber than the other drying method on guava leaves powder making [13]. Freeze drying is a drying method by sublimation through freezing [10]. Roshanak et al. reported the effect of the freeze drying method in antioxidant activity, phenolic, and chlorophyll content of green tea leaves [14]. Freeze drying will produce dry green tea leaves with higher chlorophyll but lower antioxidant activity and phenols content than the oven drying method. Therefore, the objectives of this study were to examine the effect of pandan leaves age and drying method on the pandan leaves powder quality as a natural colorant and aroma.

2. Materials and Methods

2.1. Materials

Fresh pandan leaves were collected from Malang, East Java, and sorted based on the leaves’ age which is divided into young and old leaves. Young pandan leaves were taken from shoots 1-7 from shoots, while old leaves were taken from shoots 8 down. Young leaves had greener color, old leaves had a darker color and harder tissue. Pandan leaves powder was dried in Food Processing Technologies Laboratory, Agriculture Technology Faculty, Universitas Brawijaya, Malang, Indonesia.

2.2. Preparation of dried pandan leaves powder

Pandan leaves are sorted by age according to shoot position when picking (young and old). Young pandan leaves were taken from shoots 1-7 from shoots, while old leaves were taken from shoots 8 down. Pandan leaves were cleaned and washed. Pandan leaves were cut into small pieces with a size of about 2x0.5 cm uniformly, then dried using a cabinet dryer at 40°C for 6 hours, vacuum dryer at 40°C for 6 hours, and freeze dryer at -75°C for 16 hours. Dried pandan leaves were crushed using a blender until smooth around 10 minutes and ball mill with a speed of 115-118 rpm for 30 minutes. Pandan leaf powder was sieved with a 100 mesh and keep in a closed food container.

2.3. Physical and chemical analysis

The moisture content, ash, and fiber of fresh pandan leaves and pandan leaves powder were determined using standard methods by AACC [15]. Bulk density was determined by measure the volume and weight of powder. The color of fresh pandan leaves and pandan leaves powder was analyzed using CR-300 chroma-meter (Minolta, Japan). Data were reported in the 3 parameters, namely L*, a*, and b*. L* represents the color lightness of the sample being tested. Positive a* value
will symbolize redness, while a * negative will symbolize increasingly greenish color. A positive b* value will symbolize the more yellowish color, while a negative b * value will symbolize the color bluer.

2.4. Water and oil absorption capacity

Water absorption capacity was determined with weighed pandan leaves powder 1 gram and mixed with 10 ml distilled water in a centrifuge tube. Samples were allowed to stand at room temperature (30 ± 20°C) for 1 hour. Then the sample and water in a centrifuge tube were put into a centrifuge with a speed of 2000 rpm for 30 minutes. The volume of the supernatant was measured. Water absorption capacity was measured in percentage of the weight of the panda leaves powder used.

Oil absorption capacity was determined with weighed pandan leaves powder 1 gram then mixed with 10 ml corn oil in a centrifuge tube with a little mixing. The suspension was centrifuged at 3000 rpm for 15 minutes, then the supernatant was discarded and the tube content re-weighed. Oil absorption capacity was measured in percentage of the weight of the pandan leaves powder used.

2.5. Total chlorophyll, total phenolic content, and antioxidant IC50 analysis

Total Chlorophyll was calculated using spectrophotometry. A total of 1 gram pandan leaves powder was weighed and placed into an erlenmeyer (for fresh samples mashed using a mortar). Samples were added 50 ml 96% ethanol and the container was allowed to stand in a light-tight condition. Containers containing samples and solvents were placed into a beaker glass filled with water which was placed on a water bath shaker at 60°C for 45 minutes. The extraction results are filtered using fine filter paper. The extracted filtrate was analyzed using UV-Vis spectrophotometry at wavelengths of 645 and 663 nm. Total chlorophyll is obtained by calculating the absorbance results using the formula:

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\text{Total Chlorophyll content concentration (mg / L)} = 8.02 \times A_{663} + 20.2 \times A_{645}
\]  

(1)

The total phenolics content in the pandan leaves powder was determined by spectrophotometry. Pandan leaves powder 10 mg were dissolved in 50 ml of ethanol. A 0.5 ml result of the solution was added 2.5 ml 10% Folin Ciocalteau, 2 ml 7.5% Na2CO3 was added, and then incubation for 30 minutes. Absorbance was measured at 756 nm using a spectrophotometer. The concentration of total phenolic content was calculated using gallic acid as a standard.

The free radical-scavenging activity was assayed, based on the reduction of DPPH radicals in ethanol, which causes an absorbance drop at 517 nm. 4 ml of DPPH in ethanol (0,1 mM) was added 1 ml 0, 40, 80, 120, 160, and 200 ppm of extract pandan leaves powder. After 20 minutes of incubation, absorbance was measured, and antioxidant activity was found based on the concentration levels. The graphic was created on excel to find the concentration of samples needed to have 50% antioxidant activity.

2.6. Statistical analysis

All the tests were independent with four replicates. Mean values of experimental data were carried out by analysis of variance (ANOVA) using Minitab 17 software. Tukey HSD was used at α < 0.05.

3. Results and Discussion

3.1. Characteristics of fresh pandan leaves

The characteristics of fresh pandan leaves can be seen in Table 1. The moisture content of young leaves was 80.91% higher than the old leaves with a moisture content of 75.18% (P <0.05). But, the total chlorophyll of young leaves was 1.20 mg/g which is lower than old leaves by 2.55%. The total phenolics content of young leaves was 30.04 mg/g also lower than old leaves by 48.96 mg/g. Chlorophyll was synthesized during leaf growth and development [6] along with phenol components.
This also caused differences in the IC₅₀ antioxidant levels of the two samples. Where young leaves had an IC 50 antioxidant of 396.61 ppm was no more effective than the old leaves of 351.29 ppm. The antioxidant activity of a sample was strongly influenced by its functional components such as phenol and chlorophyll which can act as antioxidants in counteracting free radicals. Chlorophyll and its derivatives were able to act as antioxidants in counteracting free radicals.

The color of pandan leaves was strongly influenced by the total chlorophyll contained as a green pigment. Young leaves had a relatively no greener and darker color than old leaves. Young leaves had a higher level of lightness that was 53.07 compared to old leaves which was 44.42. The redness level of young leaves was higher, which was -8.97 compared to old leaves with -12.20. The a* value shows that old leaves more greener than young leaves. The yellowness of young leaves was higher by 23.17 than old leaves which was 12.70. This color was strongly influenced by the chlorophyll content of the pandan leaves produced. Higher chlorophyll will give lower the lightness, lower the redness, and lower the yellowness.

### Table 1. Characteristics of fresh pandan leaves.

| Parameter                  | Young      | Old        | P-Value |
|----------------------------|------------|------------|---------|
| Moisture content (%)       | 80.91 ± 0.80 | 75.18 ± 1.37 | 0.027   |
| Total chlorophyll (mg/g)   | 1.20 ± 0.08  | 2.55 ± 0.11  | 0.000   |
| Total phenolics content (mg/g) | 30.04 ± 1.33 | 48.96 ± 2.42 | 0.001   |
| Antioxidant IC 50 (ppm)    | 396.61 ± 3.73 | 351.29 ± 2.46 | 0.001   |
| Color                      |            |            |         |
| L*                         | 53.07 ± 1.04 | 44.42 ± 1.25 | 0.017   |
| a*                         | -8.97 ± 0.39 | -12.20 ± 0.46 | 0.017   |
| b*                         | 23.17 ± 1.64 | 12.70 ± 1.03 | 0.019   |

Notes: Means of 3 replicates ± standard error. The same symbol shows the insignificant different on Tukey’s HSD test α=0.05

### 3.2. Chemical and physical characteristics of dried pandan leaves powder

The chemical and physical characteristics of pandan leaves powder can be seen in Table 2. The different age of leaves and drying method had a significant effect (p> 0.05) on the moisture content, ash content, density, water and oil absorption capacity of pandan leaves powder. The moisture content of pandan leaves powder respectively ranged from 6.30 to 10.10%. Young pandan leaves powder with freeze dryer had the highest moisture content than others, while the old pandan leaves with vacuum dryer had the lowest moisture content. It is caused by the different age of leaves and characteristics of the dryer. Young leaves had higher moisture content than old leaves, while the vacuum dryer was more effective than the cabinet and freeze dryer. During maturation, the leaves will lose water which causes the water content of young leaves to be higher than old leaves. The result of vacuum drying has the lowest moisture content due to this drying process which is most effective compared to another drying with the help of a vacuum pump that is owned by a vacuum dryer [12]. The higher the drying temperature, the lower the water content [16].

The ash content of pandan leaves powder respectively ranged from 8.54 to 11.55%. Young pandan leaves powder from freeze dryer had the lowest ash content than others, while the old pandan leaves from the vacuum dryer had the highest ash content. Ash content refers to mineral content will be influenced by soil conditions, fertilization, and irrigation during plant growth [17]. This indicates that old leaves absorb more minerals during growth compared to young leaves, so the ash content was higher.

The density of pandan leaves powder ranged from 0.16to 0.22 g/cm³. Young pandan leaves powder had lower density than old pandan leaves powder. The vacuum dryer had the lowest density than the cabinet and freeze dryer. The density of pandan leaves powder very depends on the moisture content.
of the powder. The density of a material is strongly influenced by its water content. The higher the water content, the higher the density [18]. Vacuum drying had the lowest moisture content so that it had the lowest density compared to the others.

The water absorption capacity (WAC) of pandan leaves powder was ranged 663.20 to 898.51%. Young pandan leaves powder from freeze dryer had the lowest WAC, while old pandan leaves powder from vacuum dryer had the highest WAC than the others. Besides that, the oil absorption capacity (OAC) of pandan leaves powder was ranged 363.60 to 421.27%. Young pandan leaves powder from freeze dryer had the lowest OAC, while old leaves powder had the highest OAC than other samples. The presence of food fibers in a material can increase the absorption of water and oil [19]. Some soluble fibers such as hemicellulose and pectin have a good ability to absorb water, whereas lignin and cellulose have a lower ability to absorb water [20]. Also, several non-polar side chains can bind to the oil hydrocarbon chain, thereby increasing the oil binding capacity [21]. The fibers in the leaves themselves are formed during growth. Pandan leaves with older age will have a higher fiber content compared to young leaves as a result of metabolism [22]. WAC ability of pandan leaves powder dependent on the amount and nature of the water / hydrophilic binding component [23].

In this study, vacuum drying had the highest WAC than the others. This is thought to be due to vacuum drying being able to maintain the water-binding component in the pandan leaves powder and having a low moisture content in the powder. The high water content will cause saturation of water bonds in the powder which causes a decrease in water absorption.

### Table 2. Chemical and physical characteristics of pandan leaves powder.

| Sample | Moisture content (%) | Ash content (%) | Density (g/cm³) | WAC (%) | OAC (%) |
|--------|----------------------|-----------------|-----------------|---------|---------|
| Young  |                      |                 |                 |         |         |
| CD     | 8.47 ± 0.25 c        | 9.72 ± 0.26 c   | 0.20 ± 0.00     | 741.89 ± 33.21 b | 385.93 ± 6.87 bc |
| VD     | 7.87 ± 0.18 d        | 10.81 ± 0.51 b  | 0.17 ± 0.00 d   | 842.38 ± 10.37 a | 398.89 ± 10.15 ab |
| FD     | 10.10 ± 0.17 a       | 8.54 ± 0.29 d   | 0.22 ± 0.01 a   | 663.20 ± 22.65 b | 363.60 ± 9.73 c  |
| Old    |                      |                 |                 |         |         |
| CD     | 7.64 ± 0.27 d        | 10.42 ± 0.24 b  | 0.19 ± 0.00 c   | 777.67 ± 29.15 c | ab         |
| VD     | 6.30 ± 0.17 e        | 11.55 ± 0.19 a  | 0.16 ± 0.00 e   | 898.51 ± 30.01 a | 421.27 ± 19.63 a |
| FD     | 9.38 ± 0.43 b        | 9.55 ± 0.29 c   | 0.20 ± 0.00 b   | 737.98 ± 17.73 b | 377.49 ± 13.57 bc |

Notes: Means of 3 replicates ± standard error. The same symbol shows the insignificant different on Tukey’s HSD test α=0.05. CD cabinet drying, VD vacuum drying, FD freeze drying, WAC water absorption capacity, OAC oil absorption capacity.

The color and pH of pandan leaves powder can be seen in Table 3. The different age of leaves and drying method had a significant effect (p<0.05) on the lightness (L*), redness (a*), yellowness (b*), and pH of pandan leaves powder. L* of pandan leaves powder respectively ranged 61.60-65.03. Young pandan leaves powder with cabinet dryer had the highest L*, while old pandan leaves powder with vacuum dryer had the lowest L*. The decrease of L* value is caused by the presence of chlorophyll content in pandan leaves powder. Older leaves had the highest chlorophyll content than younger ones. High chlorophyll gives a darker green color on pandan leaves powder. The decrease of L* value was also caused by the degradation of chlorophyll during the drying phase. Vacuum drying was able to maintain the color pigment of pandan leaf powder than other drying [14].

Redness (a*) of pandan leaves powder was ranged from -3.31 to 1.04. Young pandan leaves powder from the cabinet had the highest a*, while old leaves powder with vacuum dryer had the lowest a*. More negatives a* value that indicates the color of pandan leaves powder was more green. More positives that indicate the redness of the pandan leaves powder increased. Increased a* values in dried pandan leaves powder may be due to epimerization reactions that occur when chlorophyll is exposed to heat during drying. The reaction causing chlorophyll changed to pheophytin and can continued to forms pyropheophytin which has a green to olive-brown color [24].
Yellowness (b*) of pandan leaves powder respectively ranged from 14.79 to 17.37. Young pandan leaves powder from cabinet dryer had the highest b* value, while old pandan leaves powder from vacuum dryer had the lowest b* value than others. Chlorophyll can be degraded by heat into its derivative compounds, such as pheophytin caused by the loss of Mg^{2+} nuclei in the porphyrin ring during the drying process. The impact of chlorophyll degradation is a decrease in the intensity of the chlorophyll green color which slowly changes to a brownish yellow color which is the color of pheophytin [25].

The pH of pandan leaves powder respectively ranged from 5.33 to 5.71. Young pandan leaves powder from the cabinet dryer had the highest pH, while old pandan leaves powder from the vacuum dryer had the lowest pH than others. The pH of pandan leaves powder from the vacuum drying of old leaves had the lowest pH due to the highest phenolic content than others. The presence of phenol compounds which undergo oxidation can reduce the pH.

**Table 3.** Color and pH of pandan leaves powder.

| Sample | L*      | a*     | b*      | pH     |
|--------|---------|--------|---------|--------|
| Young  | 65.03 ± 0.09 a | 1.04 ± 0.04 a | 17.37 ± 0.24 a | 5.71 ± 0.02 a |
| VD     | 62.96 ± 0.63 c | -2.08 ± 0.07 d | 15.66 ± 0.20 bc | 5.49 ± 0.02 c |
| FD     | 63.88 ± 0.20 b | -1.01 ± 0.03 c | 16.10 ± 0.16 b | 5.64 ± 0.05 ab |
| Old    | 63.81 ± 0.44 b | 0.33 ± 0.01 b | 16.24 ± 0.43 b | 5.53 ± 0.10 bc |
| VD     | 61.60 ± 0.05 d | -3.31 ± 0.03 f | 14.79 ± 0.20 d | 5.33 ± 0.05 d |
| FD     | 62.81 ± 0.19 c | -2.26 ± 0.05 e | 15.36 ± 0.28 cd | 5.48 ± 0.05 c |

Notes: Means of 3 replicates ± standard error. The same symbol shows the insignificant different on Tukey’s HSD test α=0.05. CD cabinet drying, VD vacuum drying, FD freeze drying.

3.3. **Total chlorophyll, total phenolic content, antioxidant IC_{50} of dried pandan leaves powder**

Total chlorophyll content, total phenolics content, and antioxidant IC_{50} of pandan leaves powder can be seen in Table 4. The different age of leaves and drying methods had a significant effect (p<0.05) on total chlorophyll content, total phenolics content, and antioxidant IC_{50} of pandan leaves powder. The total chlorophyll content of pandan leaves powder respectively ranged from 6.88 to 13.82 mg/g. Young pandan leaves powder had total chlorophyll lower than old pandan leaves powder. It was caused by old leaves contain more chlorophyll than young leaves. The vacuum dryer had the highest chlorophyll content than the cabinet and freeze dryer. A vacuum dryer can keep the chlorophyll from damage during the drying process. The reduction in total chlorophyll during the heating process can occur because chlorophyll loses the core group Mg^{2+} and the degradation of the chlorophyll pigment into the pigment pheophytin and pyropheophytin which form a brown color [25]. During the heating process, the organic acids in the tissue are released. These organic acids trigger Mg^{2+} ions released from chlorophyll, causing chlorophyll degradation more quickly.

The total phenolics content of pandan leaves powder respectively ranged from 48.87 to 96.34 mg/g. Young pandan leaves powder had total phenols lower than old pandan leaves powder. It was caused by old leaves contain more phenolic content than young leaves. The vacuum dryer had the highest phenolic content than the cabinet and freeze dryer. A vacuum dryer can keep the total phenolic content from damage during the drying process. In most previous studies, freeze-drying was considered as one of the appropriate methods to preserve the quality of plants during processing. But in this study, the vacuum had higher phenol and chlorophyll content than freeze. Drying with temperatures over 50°C causes a decrease in the yield of essential oils and the total phenolic content of different plant species [26].

Antioxidant IC_{50} of pandan leaves powder was ranged from 181.51 to 264.97 ppm. Young pandan leaves powder had antioxidant no more effective than old pandan leaves powder. The antioxidant activity was strongly influenced by its functional components such as phenol and chlorophyll which can act as antioxidants in counteracting free radicals. Pandan leaves powder from vacuum drying had...
the highest antioxidant activity than the cabinet and freeze. It is believed that the high total phenolic content might contribute to the high antioxidant activity. The higher temperature used, the potential damage to some antioxidant components was higher, so that components such as phenol and chlorophyll will be damaged and have decreased antioxidant activity.

Table 4. Functional properties of pandan leaves powder.

| Sample | Total Chlorophyll (mg/g) | Total Phenolics Content (mg/g) | Antioxidant IC₅₀ (ppm) |
|--------|-------------------------|-------------------------------|------------------------|
|        |                         |                               |                        |
| Young  |                         |                               |                        |
| CD     | 6.88 ± 0.56 d           | 48.87 ± 1.10 f                | 264.97 ± 0.62 a        |
| FD     | 10.76 ± 0.55 b          | 87.47 ± 1.89 b                | 184.70 ± 3.19 d        |
| Old    |                         |                               |                        |
| CD     | 8.07 ± 0.59 c           | 58.12 ± 1.72 e                | 260.50 ± 0.34 b        |
| FD     | 13.82 ± 0.77a           | 96.34 ± 1.96 a                | 142.19 ± 1.32 e        |
|         | 10.33 ± 0.13 b          | 77.74 ± 3.67 c                | 181.51 ± 1.20 d        |

Notes: Means of 3 replicates ± standard error. The same symbol shows the insignificant different on Tukey’s HSD test α=0.05. CD cabinet drying, VD vacuum drying, FD freeze drying.

4. Conclusions
Fresh pandan leaves of different age of leaves had different physical and chemical characteristics. The age of leaves and drying method has a significant effect on moisture content, ash content, density, WAC, OAC, pH, lightness (L*), redness (a*), yellowness (b*), total chlorophyll, total phenolics content, and antioxidant IC₅₀ of pandan leaves powder. Pandan leaves powder with old leaves will contain higher chlorophyll and phenol that was caused the powder had darker color and more effective as an antioxidant than young leaves powder. Older leaves had a higher metabolic and genetic rate, so chlorophyll production in the leaves is higher. The phenols component was synthesized by plants during growth and development. Pandan leaves with older age also had higher fiber content than young leaves as a result of metabolism. Vacuum drying was the best method for this study. Vacuum drying can keep the color, chlorophyll, phenol, and antioxidant during drying than the cabinet and freeze drying.

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