The Effect of Citric Acid Concentration on Physico-Chemical Properties of Porang Flour

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Abstract:
The research was conducted to determine the effect of the citric acid concentration on the physical and chemical properties of porang flour (Amorphophallus oncophyllus). This study used a completely randomized design (CRD) with several levels of citric acid concentration: 0%, 2.5%, 5%, 7.5% and 10% and 4 replications. The treatment of soaking porang tubers in various concentrations of citric acid had a significant effect on calcium oxalate, water, and protein content, as well as yield, color L*, and b*, water and oil absorption capacity, and pH but had no significant effect on a*. Citric acid concentration of 10% is considered the most appropriate treatment which produce porang flour with calcium oxalate content of 35.70 mg/100g, water content of 8.92%, and protein content of 3.24%, L* value (88.75), a* value (5.75), b* value (33.25) with a very soft orange description, water absorption capacity of 1,686.26%, oil absorption capacity of 107.56%, pH 3.28, and yield 21.89%.

Keywords: Porang Tuber, Porang Flour, Citric Acid, Calcium Oxalate

1. Introduction
Porang plants (Amorphophallus oncophyllus) in particular has been widely recognized widespread on the island of Java but still very limited or there has not been much effort to develop it (Pasaribu et al. 2019). Porang plants have a chance large for export, so it is necessary to develop. According to the Coordinating Ministry for Economic Affairs in 2020, the export value of people in Indonesia reached USD 19.65 million with volume of 8,570 tonnes. Porang tubers contain carbohydrates, proteins, fats, minerals, vitamins and dietary fiber and a relatively high glucomannan. Porang contains up to approx. 36% mannan on a dry basis (porang flour) (Dwiyono et al. 2014). Glucomannan is a substance in the form of complex sugars and fiber soluble which is the highest source in Indonesia itself, said to come from the Porang plant. In use in the food sector, glucomannan has very good water absorption as well is one of the most dietary fiber thick, and gives a gel effect, until recently used for binding, thickening, substituting preservatives, and fat substitutes (Handayani & Herlinasari, 2020).

Porang tuber contains considerably amount of calcium oxalate which limits its use for food. Rasmito & Widari (2018) reported that every 100 grams of fresh porang tubers contains 0.25% calcium oxalate, while on a dry basis in the form of flour porang, existing calcium oxalate compounds reached 0.81% (Dwiyono et al. 2014). Calcium oxalate in food may cause skin irritation, itching & cause crystals formation in the kidneys (calcium oxalate stones). The threshold levels of calcium oxalate in food is worth 71 mg/100g (Kumoro et al., 2014).

Oxalate content in porang tubers can be removed by physical, chemical or mechanical treatment. Washing tubers with water is usually ineffective in reducing calcium oxalate which is insoluble in water. Washing is only able to reduce oxalic acid compounds. Sutrisno (2011) has carried out the process of porang tubers into flour using the Hammer Mill method and separated calcium oxalate from glucomannan using cyclones. The method then was found to be ineffective in dissolving calcium oxalate and caused destruction in glucomannan due to the heat generated from the flour processing. Purwaningsih & Kuswiyanto (2016) report that soaking slices of taro tuber with citric acid reduced calcium oxalate content. The soaking in 5% citric acid for 15 minutes was able to reduce by 41.74% calcium oxalate.
Citric acid is an acidic solution which releases H\(^+\) ions. H\(^+\) ions from citric acid may bind to the oxalate ion from calcium oxalate compounds to form acidic compounds oxalate, while calcium ions will bind with citric acid to form calcium citrate. Both of these compounds are water soluble so it will dissolve and be wasted together with soaking solution. Besides being able to reduce calcium, citric acid is also able to inhibit browning reaction. The objective of this study is to determine the effect of citric acid concentration on physico-chemical properties of porang flour.

2. Research Methods

Material
Porang was harvested in Karang Dapo Village, North Musi Rawas Regency, South Sumatra, Indonesia. The tubers have diameter 8-10 cm, length 5-8 cm and weight 500-600 grams with a planting age of 1.5 years. The chemicals such as citric acid and alcohol were technical grades. NaOH, NH\(_4\)OH, CaCl\(_2\), KMnO\(_4\), H\(_2\)SO\(_4\), Methyl Red, Bromocresol Green, and HCl were analytical grades. For color analysis, a color box and digital camera Nikon Coolpix P1000 were used.

Methods
This research was conducted using Completely Randomized Design (CRD) with treatment concentration of citric acid solution (0%, 2.5%, 5%, 7.5%, 10%) and 4 repetitions. Porang flour preparation was carried out following method describe previously (Melidia 2021). The porang tubers were cleaned, peeled and sliced ±5 mm thick. 800 g of porang tuber slices were washed thoroughly and immersed in a certain citric acid solution (0%; 2.5%; 5%; 7.5% and 10%) into 1500 mL distilled water. Soaking was carried out for 30 minutes, drained, rinsed and dried in an oven at 50°C for 24 hours. Chips of porang tuber chips were ground using a blender until smooth and sieved using a 60-mesh sieve. The resulting flour was packed in sealed plastic bag and stored at room temperature until further analysis.

Research Procedures
Water Content
Water content was measured using gravimetry.

Protein Content
Protein content was determined using micro Kjeldahl and calculated with conversion factor 6.25.

Calcium Oxalate (AOAC, 1990)
Determination of calcium oxalate levels in Porang flour is done by permanganometry titration which consist of 3 steps as follows:

\textit{Destruction process}
Two grams of porang flour was dissolved in 190 mL of distilled water. 10 mL of 6 M HCl was added to the flour solution and heated at 100°C for 1 hour. The mixture was allowed to cool and become object of dilution up to 250 mL using distilled water.

\textit{Oxalate deposition}
125 mL of the sample solution was transferred into beaker and four drops of methyl red indicator was added. To this solution, concentrated NH\(_4\)OH was added drop by drop until the solution paled yellow (pH 4-4.5). This mixture was then heated to 90°C, cooled and filtered to remove precipitate containing iron ions. Then reheated to 90°C and 10 mL of 5% CaCl\(_2\) solution was added with stirring using a magnetic stirrer. The solution was stored overnight (±12 hours) at ±5°C.

\textit{Permanganate titration}
The solution was centrifuged at 2500 rpm for 5 minutes. The precipitate is dissolved in 10 mL 20% H\(_2\)SO\(_4\). Shortly after that, diluted with distilled water up to 300 mL. 125 mL of the filtrate was heated until nearly boiled. Then the filtrate was titrated with standardized KMnO\(_4\) solution until the color changes to pink that lasts for 30 seconds.

\[
\text{Calcium Oxalate (mg/100g)} = \frac{T \times V_{me} \times DF \times 10^{4}}{ME \times mf}
\]
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Note:
T: Volume of KMnO₄ (mL)
V_me: Volume-mass equivalent (1 mL of solution KMnO₄ 0.05 M, equivalent to 0.00225 g anhydrous oxalic acid)
DF: Dilution Factor (2,4)
ME: KMnO₄ equivalent (5)
mf: Mass of flour used (gram)

Standardization of 0.05 M KMnO₄ solution

0.05 M KMnO₄, oxalic acid dihydrate (C₂H₂O₄·2H₂O) 0.05 M (BM=126 gram/mol) and 1 M sulfuric acid were prepared. KMnO₄ solution to be standardized was placed in a dark (brown) burette, while 10 mL oxalic acid dihydrate and 5 mL aqueous sulfuric acid were placed into Erlenmeyer and was heated to 70°C. Titrate the mixture immediately with KMnO₄ solution under hot conditions. The titration was stopped when the solution turned pink. The concentration of KMnO₄ was calculated using the following equation:

\[ V_1 \times M_1 = V_2 \times M_2 \]

Note:
V₁: Volume of KMnO₄ used (mL)
M₁: Concentration of KMnO₄ solution (M)
V₂: Volume of oxalic acid dihydrate (mL)
M₂: Concentration of dihydrate oxalic acid solution (M)

Color

Color was analyzed using a simple digital imaging method. Sample was placed in a uniform container and in the color box as described previously. Samples were photographed using a Nikon Coolpix P1000 digital camera with closed plank box position and distance camera to sample ±40 cm. Photos were analyzed using Adobe Photoshop CS5 software to obtain the values of L*, a*, and b* and descriptions color was obtained in www.colorhexa.com.

Water Absorption Capacity (Falade & Christopher, 2015)

1 gram of porang flour was mixed with 10 mL distilled water in a centrifuge tube of known weight. The mixture was then vortexed 3 times with 10 minutes pause in between. The mixture was then centrifuged at 2000 rpm for 30 minutes. The supernatant was discarded, the precipitate in the tube was air-dried and weighed. The difference in the weight of the tube is the amount of water contained absorbed by flour. Water absorption capacity is presented as the percentage of water absorbed by 100 grams material.

\[ \text{Water Absorption Capacity (%) } = \frac{(c-a)-b}{b} \times 100\% \]

Note:
a = empty tube weight
b = sample weight
c = weight of tube and precipitate

Oil Absorption Capacity (Falade & Christopher, 2015)

Oil absorption capacity of porang flour was analyzed using similar procedures as water absorption capacity as explained above, except for 10 ml of distilled water which was replaced with 10 mL of deep corn oil.

Acidity (pH)

The acidity was measured using a calibrated pH meter with buffer pH 4 and 7. The sample was prepared by dissolving 1 g of porang flour with 20 mL of distilled water.
3. Results and Discussion

Calsium Oxalate

Calcium oxalate (CaC₂O₄) is an undesired compound in porang flour. Calcium oxalate differs from oxalic acid in terms of their solubility in water. Calcium oxalate is difficult to dissolve in water so washing tuber with water cannot reduce its content of calcium oxalate. Washing and soaking process of porang tubers using acid solutions can reduce the content of calcium oxalate (Wardani and Handrianto, 2019). Calcium oxalate can be dissolved in water if it is converted to oxalic acid (H₂C₂O₄). The average value of calcium oxalate flour content porang can be seen in Table 1.

Table 1. Calcium oxalate, water and protein content of porang flour processed using different concentrations of citric acid solution.

<table>
<thead>
<tr>
<th>Citric acid concentration (%)</th>
<th>Calcium Oxalate (mg/100g)</th>
<th>Water Content (%)</th>
<th>Protein Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>469.96 ± 54.27a</td>
<td>5.25 ± 0.54a</td>
<td>6.58 ± 2.38b</td>
</tr>
<tr>
<td>2.5</td>
<td>189.34 ± 23.49c</td>
<td>6.73 ± 0.83b</td>
<td>5.92 ± 2.90ab</td>
</tr>
<tr>
<td>5</td>
<td>140.75 ± 28.77a</td>
<td>7.55 ± 1.05bc</td>
<td>4.60 ± 1.80ab</td>
</tr>
<tr>
<td>7.5</td>
<td>61.28 ± 5.60a</td>
<td>8.34 ± 1.12c</td>
<td>3.29 ± 1.10a</td>
</tr>
<tr>
<td>10</td>
<td>35.70 ± 7.07a</td>
<td>8.92 ± 1.06d</td>
<td>3.24 ± 1.73a</td>
</tr>
</tbody>
</table>

Note: The numbers followed by uppercase letters same in the same column are not significantly different at the 5% level according to the DnMRT.

Lukitaningsih et al (2012) explained that citric acid has a good ability to penetrate the idioblast cell wall where calcium oxalate is stored. Hence, more calcium oxalate crystals will be released and dissolved in an acidic medium and can further be washed with water. Citric acid in solution form citrate tri-anion which binds calcium from calcium oxalate. In acid condition, divalent oxalate ion (C₂O₄²⁻) is deprotonated thereby reducing the binding potential with Ca²⁺ to form un-dissolved calcium oxalate. It causes an increase in dissolved oxalate that is drained along with the soaking solution. In addition, Wulf-Johansson et al (2010) also explains that the bond potential between ions oxalate with calcium will be reduced on acidic conditions. This leads to formation of oxalic acid which is more water soluble. The following is a chemical reaction that takes place during soaking of porang tubers in citric acid solution:

\[ \text{CaC}_2\text{O}_4 + 2\text{C}_6\text{H}_8\text{O}_7 \rightarrow \text{Ca}_3(\text{C}_4\text{H}_2\text{O}_7)_2 + 3\text{H}_2\text{C}_2\text{O}_4 \]

Calcium oxalate content in porang flour decreased with increasing concentration citric acid solution. In line with previous report that calcium oxalate content of porang tubers soaked with belimbing wuluh’s juice (Wardani & Handrianto, 2019). According to Kumoro (Kumoro et al., 2014) the threshold for calcium oxalates in foodstuffs is 71 mg/100g. Based on this, the soaking treatment of porang tubers on 7.5% and 10% citric acid solutions are eligible to produce flour within the acceptable calcium oxalate content.

Water content

Water content plays a major role in the stability of product quality. Water content that exceeds the standard will make the product susceptible to the growth of microorganisms (Yuniar 2016). The percentage of water content of porang flour is directly proportional to the increase in concentration citric acid solution. The average value of moisture content of porang flour can be seen in Table 1.

During soaking, porang slices which contain glucomannan absorb water. Glucomannan consists mainly of 33% D-glucose and 67% D mannose, with a large number of hydroxyl groups (OH) leading to its ability to absorb water. When the slices are dried, the tuber chips are covered by a thin membrane like film. There are more films in the surface of the chips with the increase concentration of citric acid solution. The presence of film in the surface of porang chips cause water being hindered during drying leading to higher water content (Table 1). The percentage of water content of porang flour produced in all treatments complies with SNI 3751-2009 requirements (wheat flour quality requirements), determined by the maximum water content 14.5%.
Protein Content

The average value of protein content of porang flour can be seen in Table 1. The protein content of porang flour ranges from 3.24 – 6.58% (Table 1). The average value of the highest protein content (6.58%) is found in porang flour treated with 0% citric acid. This value is in line with other report (Pasariibu et al. 2019). A protein has a certain pH which is called isoelectric pH (generally ranges from 4.8 to 6.3), in which proteins have zero charge (balanced positive and negative charges). At this point level protein solubility is very low, this is because stronger protein-to-protein interactions compared to protein-water interactions. However, protein solubility will increase if given excess acid treatment, this happens because positive ions on acids that cause proteins which is originally charged neutral becomes positively charged leading to an increase in solubility. The average value of protein content is decreasing with increasing concentration of citric acid solution. The increase in acid concentration increases the solubility of protein in immersion water and therefore more protein is leached during soaking. Similar result in pandan mat fruit powder is also reported (Paiki, et al. 2018).

Color

Color is an important parameter because it is the first thing seen by consumer. The color of porang flour is characterized by value L* (brightness level), a* value (level redness), and b* value (level of yellowness). Visually the color of the resulting flour is visible difference in color from the control treatment (solution citric acid 0%) which is darker, whereas the highest citric acid immersion treatment was seen the color is getting brighter. Average value of L*, a*, and b* porang flour can be seen in Table 2.

Table 2. Average value of L* a* b* of porang flour processed using various concentrations of citric acid solution

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Colour Description</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80.75 ± 5.74a</td>
<td>3.75 ± 0.50</td>
<td>27.00 ± 2.94a</td>
<td>Very Soft Orange</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>88.50 ± 3.70b</td>
<td>4.00 ± 0.82</td>
<td>28.50 ± 2.38a</td>
<td>Very Soft Orange</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>91.00 ± 2.83b</td>
<td>4.75 ± 2.36</td>
<td>29.75 ± 1.50ab</td>
<td>Very Soft Orange</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>90.00 ± 2.94b</td>
<td>5.25 ± 2.06</td>
<td>33.50 ± 3.79b</td>
<td>Very Soft Orange</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>88.75 ± 3.95b</td>
<td>5.75 ± 3.77</td>
<td>33.25 ± 2.75b</td>
<td>Very Soft Orange</td>
<td></td>
</tr>
</tbody>
</table>

Note: Numbers followed by the same uppercase letter in the same column are not significantly different (p>5%) according to DnMRT

The L* value indicates the brightness level, where the greater the value of L*, the level the higher the brightness of the product, and so does conversely a small L* value then the product will getting darker (Pardede & Ridwansyah, 2017). Soaking porang tubers in various concentrations of solutions citric acid significantly affected the L* value. Citric acid is a chelating agent which binds copper ions (cofactor PPO enzyme) and prevents browning (Anggraini & Fitria, 2021).

In addition, citric acid decreases the pH so that the enzyme polyphenol oxidase (PPO) becomes inactive. Along with the decline in pH, the enzymes are protonated, thus loses their impactful negative charge on decreased enzyme activity.

The b* values of porang flour are increased along with the increase in concentration of citric acid solution. The b* value ranges from 27.00 to 33.25 so the color tends to be yellowish. Porang tubers contain high levels of carotene up to 40 mg/kg but need further investigation to ensure whether the carotene in porang flour is retained during soaking with higher concentration of acid solution.
Water Absorption Capacity

Water absorption capacity is one of several factors that affect flour quality. Water absorption is the ability of internal food ingredients to absorb and retain water in the material molecules (Diniyah et al., 2018). The values of porang flour’s water absorption capacity can be seen in Table 3.

Table 3. Average values of water and oil absorption capacity, pH and yield of porang flour treated with various concentration of citric acid solution

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Water Absorbance Capacity (%)</th>
<th>Oil Absorbance Capacity (%)</th>
<th>pH</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1876.30 ± 53.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127.57 ± 7.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.21 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.55 ± 1.36&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5</td>
<td>1842.19 ± 31.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.97 ± 4.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.07 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.13 ± 0.95&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>1732.43 ± 34.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>109.52 ± 5.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.82 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.33 ± 2.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>7.5</td>
<td>1712.72 ± 68.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.68 ± 5.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.44 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.26 ± 1.87&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>1686.26 ± 34.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.56 ± 5.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.28 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.89 ± 2.70&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Numbers followed by the same lowercase letter in the same column are not significantly different (p>5%) according to DnMRT

The percentage of water absorption capacity of porang flour is related to the availability of starch and glucomannan content in flour. Starch and glucomannan content in porang flour according to (Dwiyono et al., 2014) are 31.36% and 36.07% respectively. The role of glucomannan in porang flour is thought to be greater in terms of absorbing water when compared to the starch. This is due to the ability of starch to absorb water only by 30%, while glucomannan reaches 138 – 200%. Glucomannan ability in absorbing water is due to the content of hydroxyl groups (OH) in the structure. It is known that the absorption of water is due to formation of strong hydrogen bond between the hydroxyl groups (OH) of glucomannan and water. In addition to the role of starch and glucomannan, the capacity of water absorption is also affected by protein content and water content in flour.

Oil Absorption Capacity

Oil absorption capacity is a characteristic of flour which shows the ability of flour to physically bind the oil. Absorption oil is affected by the content of starch, fat as well as proteins that have non-polar groups in flour (Ali et al., 2016). The absorption capacity of oil is important flour quality characteristics maintain flavor (taste) and as well improved mouthfeel in food products (Diniyah et al., 2018). The concentration of citric acid solution has a very significant effect on the percentage of oil absorption capacity of porang flour. The average value of flour oil absorption porang can be seen in Table 3.

Similar to water absorption capacity, the oil absorption capacity decreased concomitantly with increasing concentration of the citric acid solution. Proteins that have non-polar chains are capable of bonding with oil. Ability to absorb the oil from porang flour indicates the presence of lipophilic components. Falade & Christopher (2015) describes chemical components major factor influencing adsorption capacity oil is a protein, which consists of hydrophilic and hydrophobic parts. Acid side in non-polar amino acids chain can form hydrophobic interactions with a lipid hydrocarbon chain. So the percentage of oil absorption may directly proportional to the percentage of protein content on porang flour. Other than protein, starch also have the ability to bond with oil (lipid) (Muchlisiyah et al. 2016). With the ability to absorb oil in starch indicates that the starch has parts lipophilic in its constituent components (Falade & Christopher, 2015). The higher the amylose content, the ability to absorb oil will be higher (Qin et al. 2016). Amylose can form complex with oil (lipid) in the form of amylose-lipid. As can be seen in Table 3, the oil absorption capacity of porang flour decreased concomitantly with increasing concentration of citric acid. This may relate to the leaching of some amylose during soaking leaving less availability of amylose which can form complex with oil (lipid).

Degree of Acidity (pH)

The pH value is closely related to product quality. Products with high degree of acidity (pH<7) will last longer due to microbes inactivity. The average pH of porang flour in various citric acid concentrations can be seen in Table 3. Based on ANOVA, concentration of citric acid has significant effect on the pH of
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