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Antioxidant Activity and Total Phenol Content of Steamed Pumpkin (Cucurbita Moschata) Flour with Skin Colour Variations

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Abstract

The aim of this study was to analyze the antioxidant activity and total phenolic content of three steamed pumpkin flour: the steamed yellow-yellow, brown-orange, and the steamed green-white pumpkin which derived from Cucurbita moschata, one of the multipurpose pumpkin plant in Indonesia. Steamed pumpkin flour was developed by steaming, sun-drying and milling. The DPPH method was carried out to evaluate the antioxidant activity of the steamed pumpkin flour, while the Folin–Ciocalteu method was performed to measure the total phenolic content and expressed as Gallic Acid Equivalent (GAE). Purple steamed flour pumpkin had the highest antioxidant activity (4.87%) anticoagulant activity and phenolic content (0.268 mg GAE/g) followed by brown-orange and green-white steamed flour pumpkin (0.23% and 0%) and phenolic content (0.256 mg GAE/g and 0.166 mg GAE/g) respectively. These differences were because of genetic variation and processing conditions, with bright colored pumpkins yielded higher carotenoid and phenolic content. The steamed pumpkin flour possess great potentiality for use in different food products since they can essentially be considered as a functional food ingredient and the processing conditions and variety selection should be optimized for maximizing their bioactivity.

***Keywords**: Cucurbita moschata, Antioxidant activity, Phenol.*

I. INTRODUCTION

These properties have a lot of potential for developing local food products due to the cultural richness, diversity of food, and abundant natural resources that Indonesia has. The variety of vegetables, fruits, tubers, and grains produced from various regions of Indonesia gives the opportunity to develop food products from local foods that are underutilized and can add diversity in the diet to improve community nutrition. Pumpkin (*Cucurbita moschata*) is a leading crop belonging to the cucurbitaceae family, which is well adaptive to Indonesia's tropical climate (Ranonto et al., 2015).

Pumpkin is a seasonal vine that is typically harvested 50 to 60 days after planting. Pumpkin is a type of yellow to orange fleshy, and hard-shelled fruit with an interior layer containing seeds (Rismaya et al., 2018). Proper storage condition with a long storage time, strong adaptability towards environmental condition, and unique flavor of pumpkin stimulate its popularity with scar market, etc. Pumpkin is not only useful agronomically but also abundant in nutritional values which makes it an important source of food (Zufahmi et al., 2015).

While the 12 global pumpkin species exist, cucurbita moschata is the most widely cultivated types of pumpkin species in Indonesia (Rismaya et al., 2018). Pumpkin used in a variety of cuisines, including the global and

Indonesian cuisine, is found in several dishes, including soups, desserts, juices, chips, flour, and others because of its versality. The downside is that pumpkin-based products are perishable, so these are hard to keep fresh. Pumpkin flour would prolong its freshness, make it easier to eat and yield a much more durable, multi-purpose product. Converting pumpkin into flour extends its shelf life and allows it to have diverse applications, including in bread and pancakes, is developed (Men et al., 2021).

Pumpkin contains antioxidant activity and supports immunity, metabolism and eye health due to the high content of beta-carotene, lutein and vitamins A, B1, C and E (Ranonto et al., 2015; Mardiah et al., 2021; Men et al., 2021). According to Murkovic et al. (2002), health benefits of pumpkin, for example, glucose regulator, reduced cholesterol, as well as cardiovascular risk factors, were also attributed to their antioxidant compounds. Pumpkin extract possesses strong antioxidant activity, reflected in the low values of IC50 obtained from studies.

Moreover, pumpkin is rich in carotenoids, phenols, flavonoids and polysaccharides that provide antioxidant, anti-inflammatory and digestive health benefits (Yulianawati & Isworo, 2012; Nawirska-Olszanska et al., 2014; Aukkanit & Sirichokworrakit, 2017). According to Lismawati et al. (2021) and Mardiah et al. (2021), the yellow to orange flesh contains plenty of Beta Carotene, which marks its exception proven in nutrition which helps in fighting free radicals which are associated with degenerative diseases like cancer, diabetes, heart diseases.

Pumpkin is a potent source of phenolic compounds with antioxidant and antimicrobial activity, and can neutralize free radicals by both donating electrons and serving as a metal chelator. Pumpkin's (Christodoulou et al., 2022) antioxidant activity has also been noted, as the Folin-Ciocalteu method is a widely used method to determine total phenolic content. There are, however, still limited studies on antioxidant activity and phenolic content of steamed pumpkin flour based on difference skin color.

These factors play a role in the elucidating of such information, so this study takes this urge to break down these factors for better understanding of the search for functional food and the benefits of it, and helps in understanding how to utilize local food resources in Indonesia. The results of antioxidant activity and total phenolic content of these steamed pumpkin flours in this study can improve public health by providing an alternative source of antioxidant-rich and beneficial substitutes for consumers, and provide a rationale for farmers to produce more economically valuable pumpkin species.

II. MATERIALS AND METHODS

This study was carried out from June to August 2024 at UPT Laboratorium Terpadu (Integrated Laboratory), Universitas Nusa Cendana, Kupang. Equipment used for experimentation included analytical balance, a rack of test tubes, dropper pipettes, graduated pipettes, beakers, spat chula, erlenmeyer flask, volumetric flask, spray bottle, knife, steamer, strainer, blender, airtight container, oven, vial bottle, rotary vacuum evaporator, and UV-Vis spectrophotometer. Pumpkin pumpkin type, filter paper, tissue, distilled water, ethanol (70%), methanol, DPPH, gallic acid, Folin-Ciocalteu reagent, sodium carbonate ($Na2CO3$), aluminum foil, and plastic wrap were the materials used.

The study was carried out in five steps, such as Sample collection and preparation, Sample extraction, Antioxidant activity testing, Preparation of Gallic acid standard curve, Determination of total phenolic content.

➢ *Sample Collection and Preparation*

The type of pumpkin used was parang pumpkin sourced from Baun, Amarasi Barat District. Some pumpkins are selected for their bright yellow, orange and yellow-green colors. The pumpkins were thoroughly washed, halved and separated into skin, seeds and flesh. The flesh was then steamed until cooked and separately processed for each type before being crushed and pressed to extract water. The pulp was dried under sunlight for 1-3 days, then ground into powder and sieved with an 80-mesh sieve. Steamed pumpkin flour was then kept in sealed bags, and vacuumed-sealed bags stored for an additional analysis as illustrated in Fig. 1.

➢ *Sample Extraction*

The process consisted of successive maceration of 10 g of steamed pumpkin flour with 100 mL of 70% ethanol, for 24 hours at room temperature, totaling three times. The mixture was filtered and the filtrate was concentrated with rotary vacuum evaporator at 50^{O}C to obtain a more concentrated extract.

➢ *Antioxidant Activity Testing*

The stock solution of the DPPH was prepared as follows; 0.01 g of DPPH was dissolved in 100 mL methanol. The blank solution was prepared by adding 2 mL of DPPH to 2 mL of 70% ethanol, vortexing it, and then after dark incubation for 30 minutes, the respective maximum absorbance was measured between 700-400 nm using UV-Vis spectroscopy. The antioxidant activity was expressed as the percentage of radical scavenging (% inhibition). A 1000 ppm stock solution of each sample was prepared by dissolving 0.050 g of extract in 50 mL of 70% ethanol. Two milliliters (mL) of the stock solution was mixed with 2 mL of DPPH, vortexed, and incubated for 30 min, in the dark, and then the maximum absorbance at the blank's wavelength was measured.

➢ *Gallic Acid Standard Curve Preparation*

Stock solution of gallic acid (1000 ppm) was prepared by dissolving 0.025 g of gallic acid in 25 mL of methanol. Dilutions were performed to obtain concentrations from 0.25 to 6 ppm. Thus, in each diluted solution, 1 mL was reacted with 5 mL of Folin-Ciocalteu region and allowed to stand for 3 min, then mixed with 4 mL of 7.5% $Na2CO3$ and incubated at room temperature for 60 min. The absorbance was recorded at 740 nm to obtain a standard curve.

➢ *Determination of Total Phenolic Content*

Each steamed pumpkin flour type was mixed with 0.1 mL methanol and diluted to 10 mL in methanol. To perform, 5 ml of the solution were reacted with 5 mL of Folin–Ciocalteu reagent and 4 mL of 7.5 % Na2CO3 and incubated for 60 min at room temperature. The absorbance at 740 nm was measured using a UV-Vis spectrophotometer. Tests were conducted in duplicates, and total phenolic content was determined with the equation from the dilution calibration curve, using gallic acid as a standard. All standard reagents and solutions, such as Folin-Ciocalteu reagent and 7.5% Na2CO3, were prepared according to standard procedures to ensure accuracy in clinical chemical analyses.

III. RESULTS AND DISCUSSION

The research was started with the selection of raw materials and divided into three parts: skin, pulp, and seeds of fruits harvested; washed to ensure they were free of contaminants and they were peeled. The flesh was steamed to retain nutrients and inhibit enzyme activity, then crushed, pressed, and sun-dried for 1–3 days. Ultimately, the desiccated meat was ground to a fine flour to perform this analysis (Fig.1). The maceration method of extraction of secondary metabolites with 70% ethanol was selected in order to extract polar and nonpolar compounds such as phenolics and flavonoids. The macerate was filtered, and concentrated (using a rotary vacuum evaporator, 50OC). The extract was used to test for antioxidant activity and total phenol content. The antioxidant activity was determined by DPPH method at 516 nm. DPPH is a purple solution that disappears in the presence of antioxidants, and these reactions are measured through UV-Vis spectrophotometry. Inhibition percentage of each type of steamed pumpkin flour is shown in Table 1.

Fig 1 Steamed Pumpkin Flours

Table 1 Results of % Inhibition

Sample	Blank Absorbance	Sample Absorbance	% Inhibition
Green-White Pumpkin	1,271	1,327	0
Orange-Brown Pumpkin	1,271	1,268	0,23
Yellow-Yellow Pumpkin	1,271	1,209	4,87

As per the % inhibition calculation for the three different types of steamed pumpkin flour samples, steamed yellow pumpkin flour sample showed the highest antioxidant activity compared to others. Nonetheless, the antioxidant activity of this sample was still very weak (It inhibition value was only 4.87%). Results demonstrate that steam yellow pumpkin flour's antioxidant potential is only modest in comparison to previous studies that produce more potent levels.

According to Purwanto et al. (2017), The IC50 for the concentration of antioxidant fractions (x-axis) against the percentage of inhibition (y-axis) is derived from measurements made by a number of replications. The corresponding percentage of inhibition was calculated and then it was used to determine the IC50 which gives an idea of the concentration of extract that would inhibit 50% of free radical activity. A smaller IC50 value indicates a greater antioxidant capacity of a sample, as a lower concentration is required to inhibit 50% of free radicals. In this study, however, the antioxidant activity test on steamed pumpkin flour was conducted without varying the concentration of the extract, making it impossible to calculate the IC50 value. Thus, despite the higher antioxidant activity of the steamed pumpkin flour sample compared with the other samples, its antioxidant potential remained relatively low. The nonextraction process applied can also be considered, or even the lack of antioxidant compounds concentration in the sample.

Total Phenolic Content was measured by the Folin-Ciocalteu method and reported as Gallic Acid Equivalent (GAE). Total phenol content was given in the unit of GAE (Gallic Acid Equivalents), which is mg of gallic acid in 1 g of sample. Table 2 showed that the total phenol content of each sample using gallic acid calibration curve equation ($y = 0.0168x + 0.0356$).

Table 2 Total Phenolic Content of Methanol Extract of Steamed Pumpkin Flour (*Cucurbita moschata*)

Sample	Absorbance	Average	Concentration (ppm)	TPC (mg GAE/g)	TPC (%)
Green-White Pumpkin	0,049				
	0,05	0,050	0,831	0,166	16,618
Orange-Brown Pumpkin	0,057				
	0,057	0,057	1,278	0,256	25,561
Yellow-Yellow Pumpkin	0,058				
	0,058	0,058	1,338	0,268	26,754

Data from steamed pumpkin flour samples for total phenol content analysis revealed a significant variation. Steamed yellow-yellow pumpkin flour had the highest phenol content with 0.268 mg GAE/g and steamed green-white pumpkin flour had a lower phenol content (0.166 mg GAE/g) and to a variety of factors including variety and pumpkin and drying temperature. The phenol amount can be significantly influenced by the type of pumpkin, as the genetic variation of the plant influences the biosynthesis and accumulation of phenolic compounds in the tissue of the plant. Besides, pumpkins with brighter colors, such as bright yellow or orange, generally contain higher levels of carotenoids and phenols. This concurs with the results reported by Evahelda et al. (2017), which indicated that lighter bluish-pink plant materials correlate with increased antioxidant activity and phenolic content.

IV. CONCLUSION

Steamed yellow-yellow pumpkin flour samples exhibited the strongest antioxidant activity and total phenolic content compared to the other two pumpkin varieties with an antioxidant activity of 4.87% and 0.268 mg GAE/g total phenolic content. But, the antioxidant activities of steamed brown-orange (0.23%) and green-white pumpkin flours (0%) were less potent compared to dried yellow-orange pumpkin flour, which corresponded with 0.256 mg GAE/g and 0.166 mg GAE/g in terms of phenolic content. Variations of pumpkin made by different varieties and under different processing conditions like drying temperature, affected the content of the antioxidants. Pumpkins with bright colors (e.g. yellow or orange) featured higher levels of carotenoids and phenolic compounds, making them more nutritionally valuable and with higher antioxidant capacity. Optimized variety selection and processing methods, therefore, cement the functional attributes of the pumpkin ingredient in the end product.

RECOMMENDATIONS FOR FUTURE RESEARCH

The study concluded that it is essential to conduct additional research to improve the understanding and use of pumpkin-based products. Conduct researches like comparative analysis over regions to assess differences in the bioactive compound content and antioxidant activity, and as finding the effect of a location on a nutritional quality. This research can further be enhanced for an effect of different processing technology like freeze-drying or vacuum drying method so that a morphological and chemical characterization of phenolic compounds and its antioxidant activity in steamed pumpkin flour can be recognized. Research could be further expanded to include the preparation of functional food products such as baked goods, beverages, or nutraceuticals while maintaining the bioactive properties of steamed pumpkin flour during production. Lastly, human health impact studies in the form of clinical trials can confirm the health-protective effects of phenolic compound-rich pumpkin-based products and promote the role in the prevention and treatment of diseases.

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