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Evaluation on the effect of butternut pumpkin (*Cucurbita moschata*) maturity stage on the bioactive components and antioxidant activity of pumpkin flour

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ABSTRACT

Pumpkin flour is a processed product high in carotenoids and a good source of nutrients. This nutritional content of pumpkin flour was considered to be influenced by the fruit maturity stage. Therefore, this research aimed to evaluate pumpkin flour's chemical properties, bioactive components, and antioxidant activity at different maturity stages. This also used honey pumpkins with a maturity stage of 15, 20,25, and 30 days after fruit set(DAFS). The results showed that the maturity stage of butternut pumpkin affects the moisture content and bioactive components such as phenols, flavonoids, and carotenoids of flour produced. Furthermore, the highest antioxidant activity was found in pumpkin flour with a maturity stage of 25 DAFS with IC50 values of 85.31 µg/mL (DPPH) and 64.39 g/mL (ABTS). Therefore, butternut pumpkins with a maturity stage of 25 DAFS can be processed into flour with antioxidant properties.

Keywords: antioxidant activity, bioactive components, maturity stage, pumpkin flour.

Introduction

Butternut pumpkin also called honey pumpkin (*Cucurbita moschata*) is categorized in the Cucurbitaceae family (Oloyede et al., 2012). Pumpkin is widely grown in Indonesia, including Lampung Province. In addition to being used as a raw material in food production, these vegetables are high in carotene, therefore, they can potentially be a source of functional foods. However, as an agricultural product, pumpkin has several disadvantages such as seasonal, non-uniform size, and being bulky requires a large storage space.

Processing pumpkin into flour is an alternative to fresh fruit, used as a main ingredient in a particular food industry. This is because flour is more secure in its availability, extends shelf life, is available all year around, reduces storage space requirements, facilitates transportation, and can be mixed with other ingredients more easily (Pereira et al., 2020).

The quality of pumpkin flour can be affected by the degree of maturity. Pumpkin plants normally exhibit the highest rate of fruit set between 35- 45 days after transplanting (Stapleton et al., 2000). Then about three weeks later, the pumpkin fruit size develops rapidly and is accompanied by accumulation of metabolites until it reaches maximum volume ten days later. In this stage, the fruit is regarded as being fully developed or fully mature, and the ripening of fruits normally is achieved 50 days after flowering. During this ripening stage, tremendous biochemical changes take place in the maturing fruit. These changes can be observed as changes in color, sweetness, texture, and chemical contents including bioactive compounds of the fruits.

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Bioactive compounds mainly carotenoids and phenolics are important constituents of pumpkins. Carotenoids and phenolics have been reported to have many health benefits such as anti-aging, anti-cancer, and antioxidant. The carotenoid content of fresh *Cucurbita maxima* pumpkins harvested at 60 days (2.83 mg/100 g) was higher than the 50 and 40 days (Muenmanee et al., 2016). Similarly, the total phenolic content of young pumpkins (14 days) was higher than mature pumpkins (Oloyede et al., 2012). In addition to their changes in content throughout the maturity stages, the bioactive compound content of pumpkin is also affected by heat processing. Ouyang et al. (2022) reported the levels of carotenoids and carotenoid esters in pumpkin (*C. maxima*) slices as affected by hot air drying (60–100 °C, 6–17 h). Other researchers exposed phenolic compounds are the most labile to heat, because already at the stage of vegetable pretreatment such as by cutting, shredding, and removing the peel, enzymatic processes are induced, which reduce the antioxidant content (Piłat & Zadernowski, 2017). However, previous studies reported that the bioactive compounds contained in pumpkin flour mainly produced from different stages of maturity are still very sparce . Therefore, this paper reveals the total phenols, flavonoids, carotenoids, and antioxidant activity of pumpkin flour at different maturity stages.

Material and methods

Materials and tools

The main research materials and equipment were butternut pumpkin (*Cucurbita moschata*) harvested from Horticultural Park, Sabah Balau, Tanjung Bintang, South Lampung, DMSO, 96% ethanol, methanol, acetone, beta carotene, and other chemicals to test the characteristics of products purchased from chemical suppliers such as PT Elo Karsa Pratama, Jakarta. The essential tools used include a boiling pot, a Memmert dryer/oven, a disk mill type flouring device (Ramesia, FCT-Z300), a standard Tyler 80 mesh sieve, as well as tools for analysis such as the G-10S Thermo Scientific UV-VIS spectrophotometer, Brabender Visco amylograph, and scanning electron microscopy (SEM/ ZEISS EVO MA 10 brand).

Research method

This research used a completely randomized block design with 4 treatments of pumpkin maturity stage (15, 20, 25, and 30 days after fruit set/ DAFS) with 6 replications. The data were subjected to analysis of variance (ANOVA) and further tested using the Least Significant Difference (LSD) at a level of 5%.

Pumpkin flour preparation

Pumpkins with maturity stages of 15, 20, 25, and 30 DAFS, counted from the ovule began to appear (days after fruit set/DAFS), were peeled, washed under running water, sliced into 1-2mm size, blanched with a ratio of water and sample (2:1) and a temperature of 70 - 80°C for 1 minute, drained and then dried in an electric oven (Memmert) at temperature of 60°C for 10 hours, ground and sieved using a 60 mesh sieve, then packed in plastic bags and stored at 20°C until analysis was performed.

Parameters analyzed

The flour samples were analyzed for moisture, total phenolics, total flavonoids, total carotenoid contents, antioxidant activity, and granular morphology using the following methods:

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1) Moisture content analysis

The analysis was based on the method of AOAC No.925.10, 2005. A total of 2 g samples were dried in an oven at 105°C for 3-5 hours. The difference in weight obtained from before and after drying is the moisture content in the pumpkin.

2) Sample extraction for analysis of total phenols, flavonoids, and antioxidant capacity

A total of 5 g samples were macerated in 20 mL 96% ethanol for 24 hours at 4°C in an unlighted room and then filtered using filter paper. The extract obtained was placed in opaque bottles and stored in an unlighted room at 4°C (Nurdjanah et al., 2017).

3) Total phenolic content analysis

The total phenolic content was determined according to the method used by Nurdjanah et al. (2017) First, a total of 1 ml extract was placed in a tube, and added 1 ml of 50% Folin-Ciocalteu reagent, homogenized, and left for 3 minutes. Furthermore, 1 ml of 1 N Na2CO3 was added and left for 10 minutes. A total of 7 ml of ionized water was also added and incubated for 2 hours at 20-25°C in an unlighted room. The absorbance was then read with a UV-Vis spectrophotometer at a wavelength of 725 nm. The absorbance values were plotted on a pre-established standard gallic acid curve using the linear regression equation.

4) Total flavonoid analysis

The method of Sultana et al. (2009) was used to analyze total flavonoids. One ml of extract was mixed with 4 ml of distilled water, 0.3 ml of 5% NaNO2 solution, and left for 5 min. Furthermore, 0.3 ml of 10% AlCl3 was added and left for 6 min. Two ml of 1 M NaOH and 2.4 ml of distilled water were then added and homogenized. The absorbance of the solution was read at 380 nm. The results were plotted against a pre-established quercetin standard curve using the linear regression equation.

5) Total carotenoid analysis

A total of 5 g of pumpkin flour is dissolved in 10 mL of petroleum ether and homogenized for 30 seconds. The sample was centrifuged at 3000 rpm for 10 minutes. The filtrate was taken up, then 10 ml of acetone was added to the residue, and the mixture was homogenized for 30 seconds. In addition, the samples were centrifuged at 3000 rpm for 10 minutes. The filtrate was taken, then the residue was added with 5 mL of petroleum ether and 5 mL of acetone and homogenized for 30 seconds. The sample was then centrifuged for 10 minutes at 3000 rpm, and the filtrate was taken. The filtrate obtained was collected and washed with 10 ml of 0.1 M NaCl, and the organic phase was taken. The washing was carried out twice. First, the obtained organic solution was read for absorbance using a UV-VIS 10S Thermo Scientific spectrophotometer at 450 nm. These absorbance values were then plotted on a pure beta-carotene curve standard for quantitative calculations(Jaramillo et al., 2018).

6) Antioxidant activity analysis

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The antioxidant activity test was carried out using the DPPH and the ABTS methods. The DPPH (2,2-diphenyl-2-picryl-hydroxyl) was conducted according to Nurdjanah et al. (2017)) as follows: A total of 0.0078 g of DPPH was dissolved in 100 mL of 96% ethanol. Furthermore, 1 mL of pumpkin flour extract solution was added with 2 mL of DPPH solution, then incubated at 37°C for 30 minutes in an unlighted room. The absorbance was read at 517 nm using UV-VIS spectrophotometer 10S Thermo Scientific. Finally, the calculation of the percentage of antioxidant activity against DPPH radicals was carried out using the formula below:

% Antioxidant=(Ak-As)/Ak, where:

Ak = Control Absorbance (DPPH without sample)

As = Sample Absorbance

The ABTS method was conducted as described by Brand-Williams et al. (1995) as follows:

The pumpkin flour extract (100 µL) was added with 2.9 mL of 7 mM ABTS solution and then incubated at 37oC for 30 minutes in a dark room. Furthermore, the absorbance was read at a wavelength of 734 nm using a 10S thermoscientific UV-VIS spectrophotometer. The antioxidant activity against ABTS radicals was calculated using the formula below Brand-Williams et al. (1995): % Antioxidant=(Ak-As)/Ak

Description: Ak = Control Absorbance, As = Sample Absorbance

Determination of IC_{50} for DPPH and ABTS methods

Determination of IC_{50} (the concentration of an antioxidant that can cause 50% of DPPH or ABTS to lose its radical properties).

DPPH method

A total of 1 g pumpkin flour sample with a maturity stage of 25 DAFS (highest antioxidant activity) was added jointly with 10 mL of 96% ethanol and then left for 24 hours at room temperature. Furthermore, the extract solution was taken up to 25, 50, 75, 100, and 125 µL, then 1 mL of 0.2 mM DPPH solution and 96% ethanol were added until the volume reached 10 mL. This method was adapted from (Xiang *et al*., 2018) with slight modifications. The sample was incubated for 30 minutes in an unlighted room. The absorbance of the solution was read at a wavelength of 517 nm using a 10S Thermo Scientific UV-VIS spectrophotometer, and the results were plotted on a curve to determine the linear regression equation.

ABTS method

A total of 1 g pumpkin flour sample with a maturity of 25 DAFS (highest antioxidant activity) was added jointly with 10 mL of 96% ethanol and then left for 24 hours at room temperature. Furthermore, 75, 150, 225, and 300 µL of the extract solution were taken and added with 1 mL of ABTS solution and 96% ethanol until the volume reached 3 mL. The sample was incubated for 30 minutes in an unlighted room. The absorbance was read at a wavelength of 734 nm, and the results were plotted on a curve to determine the linear regression equation (Almeida et al., 2011).

Flour granule morphology

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Micrograms of flour granules are obtained using a ZEISS Scanning Electron Microscope (SEM) type EVO MA 10. The sample was placed in a double-sided carbon taped holder and then coated with Au-Pd using a sputter coater (QUORUM). The micrographs were obtained with an acceleration voltage of 10.00 kV with magnifications of 1000 and 2500 times (Pratiwi et al., 2020)

Results and discussion

Moisture content

The moisture content of pumpkin flour ranges from 7.23 – 9.01% (Figure 1). The age at which the pumpkin was harvested had a significant influence (P <5%). The moisture content of pumpkin flour at the maturity stage of 15 DAFS (7.23%) is lower than at maturity of 20 DAFS (8.13%), 25 DAFS (8.15%), and 30 DAFS (9.01%).

This was probably due to the changes in the structure of the cell wall toward the increasing maturity stage (Goulao & Oliveira, 2008) During fruit development, the cell wall deposition rate decreases and cell wall swelling increases, and consequently, this affects water holding capacity and water retention capacity (Schumann et al., 2020) According to Lapčíková et al. (2021) there are water molecules between different phases, such as the interphase region between starch grains, proteins, and other phases. The migration of water molecules into the evaporating phase depends on the microstructure of the dispersion matrix, such as the stability and tendency of the phase separation. Therefore, the commodity with the stable network matrix can withstand the water vapor transfer rate than the less stable network matrix.

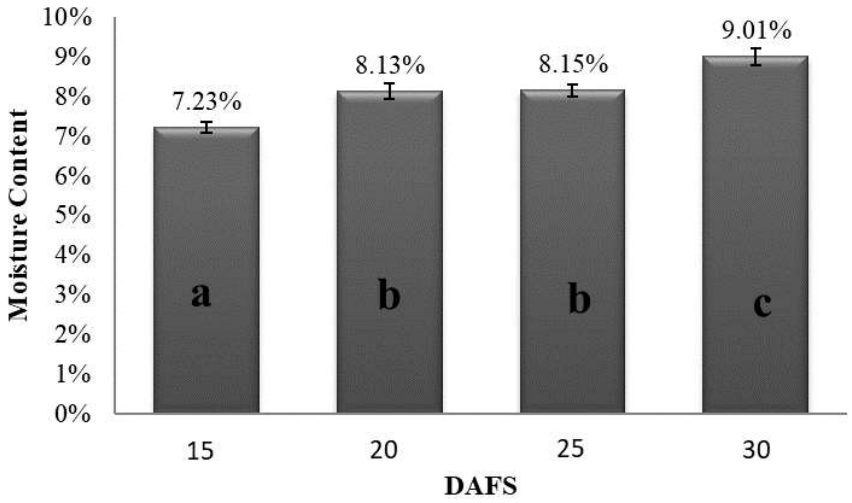

Figure 1. Effect of maturity stage on the moisture content of pumpkin flour

Total phenolic content

The maturity stage of the pumpkin has a very significant influence (P <5%) on the total phenol content. The total phenol content in pumpkin flour decreases with the degree of ripeness of the pumpkin (Figure 2). For example, pumpkin flour with a maturity stage of 15 DAFS (30.37 mgGAE/g)

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has a higher total phenol content than those with a maturity stage of 20 DAFS (25.71 mgGAE/g), 25 DAFS (20.30 mgGAE/g), and 30 DAFS (11.22 mgGAE/g).

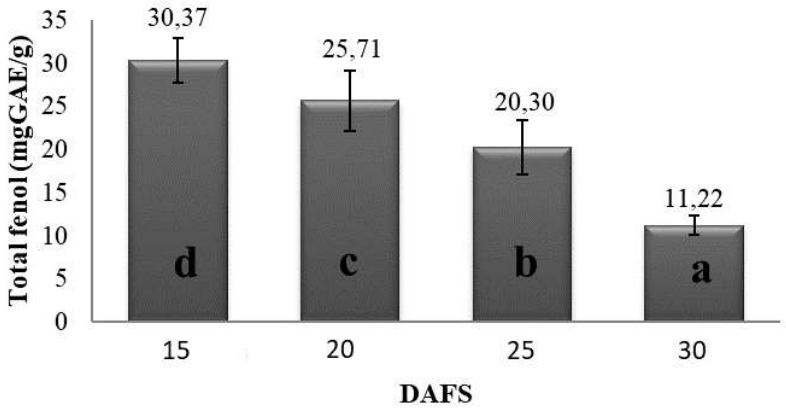

Figure 2. Effect of maturity stage on total phenol of pumpkin flour

This is in accordance with (Sharma & Rao, 2013), which stated that the total phenol content of fresh flesh pumpkin (*Cucurbita maxima*) decreased with the maturity level of pumpkin fruit around 0.56 – 0.42 mg/g. In contrast, the results of Oloyede et al. (2012) stated that the mature flesh (*Cucurbita pepo*) pumpkin had a high phenol content of 0.33 mg/g compared to the young pumpkin of 0.1 mg/g. The phenol component is a secondary metabolite produced by plants. The secondary metabolites synthesized by plants have a protective function against pathogens. Therefore, the decrease in total phenol occurs probably because it is used as a mechanism to protect plants from diseases and pests (Jamiołkowska, 2020).

Total flavonoids

Flavonoids belong to the bioactive components of the group of polyphenols. They can resist free radicals as antioxidants. The maturity stage or the age of the pumpkin harvested has a very significant influence (P <5%) on the total flavonoids in the pumpkin flour. Total flavonoids tend to decrease with the maturity level of the pumpkin (Figure 3).

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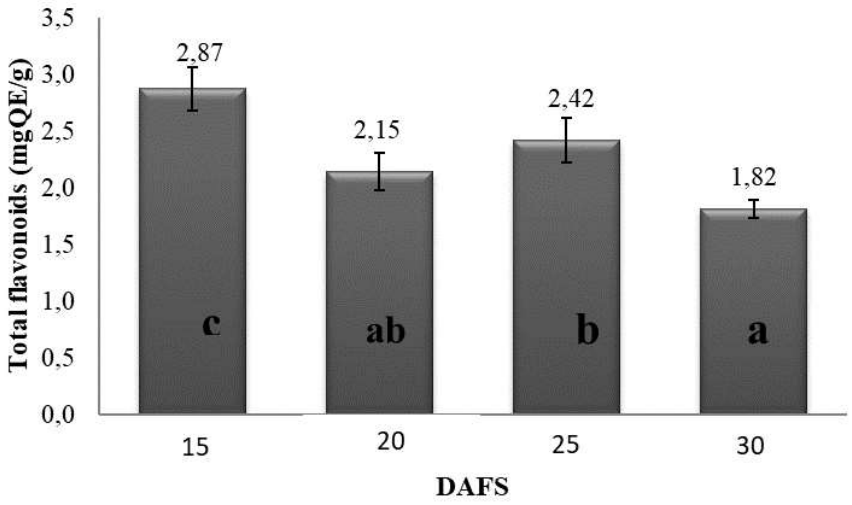

Figure 3. Effect of maturity stage on total flavonoids of pumpkin flour

The total flavonoids of pumpkin flour with a maturity stage of 15, 20, 25, and 30 DAFS are 2.87, 2.14, 2.42, and 1.82 mgQ/g, respectively. The decrease in flavonoids can also be associated with a reduction in total phenol content (Figure 2) since flavonoids are phenolic components. According to Arena et al. (2012), the accumulation of flavonoids in berries decreased with the maturity level. This decrease occurs due to the degradation of flavonoids during the biosynthesis of other compounds. According to Jamiołkowska (2020), the flavonoids produced by plants are also present in secretions in plant parts such as roots, fruits, and leaves that act on pathogenic microorganisms.

Total carotenoids

The pumpkin maturity stage has a very significant effect (P<5%) on the total carotenoids of pumpkin flour. The total carotenoids of pumpkin flour tend to increase with the maturity level of the pumpkin (Figure 4). The total carotenoids of pumpkin flour with a maturity stage of 25 and 30 DAFS are 14.45 and 14.18 mg/100 g, respectively, higher than pumpkin flour with a maturity stage of 15 DAFS at 7.28 mg/100 g and 20 DAFS at 10.92 mg/100 g. This is in line with Sharma & Rao (2013), which stated that the total carotenoids of pumpkin fruit increased from 0.67-7.47 mg/100 g along with the maturity level of the pumpkin. The highest accumulation of carotenoid compounds is found during the ripening phase. The variety can influence the biosynthesis and metabolism of carotenoids in vegetables and fruits, temperature, availability of nutrients in the soil, DAFS light intensity, maturity level, and post-harvest (Kulczynski & Gramza-Michałowska, 2019) . Carotenoids function as a contributor of yellow color to pumpkin at an early stage which then turns to orange during ripening (Norshazila et al., 2012). The dominant types of carotenoids in pumpkin species are β-carotene, α-carotene, lutein, zeaxanthin, and violaxanthin (Provesi et al., 2011).

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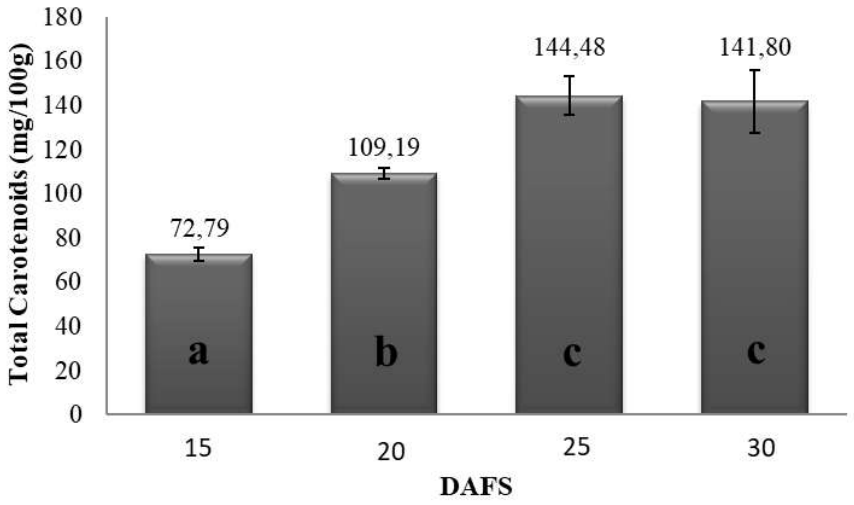

Figure 4. Effect of maturity stage on total carotenoids of pumpkin flour

Antioxidant activity

Antioxidant activity is an activity that can counteract or reduce free radicals. The maturity stage of the pumpkins has a very significant influence on the antioxidant activity of the flour in both the DPPH and ABTS methods (Figure 5).

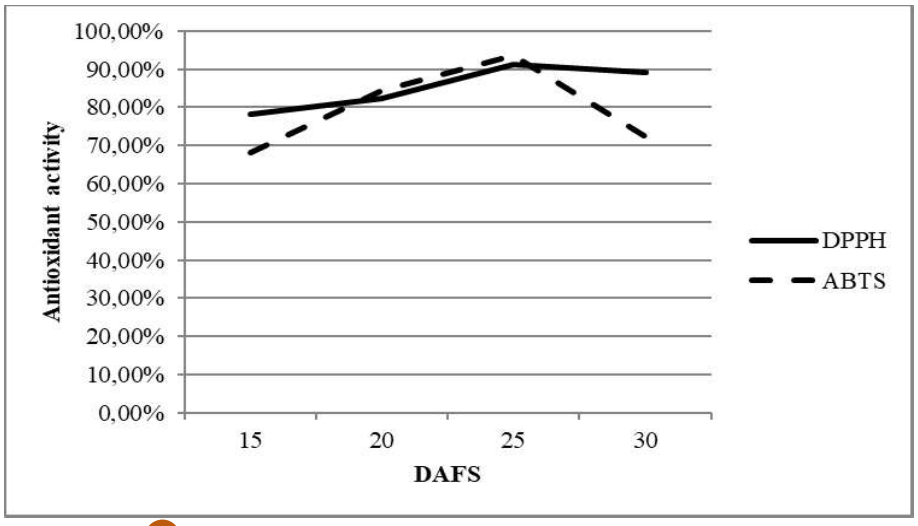

Figure 5. Effect of maturity stage on antioxidant activity of pumpkin flour

Pumpkin flour can reduce free radicals DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azinobis (3-ethylbenzthiazolin-6-sulfonic acid)). The antioxidant activity of pumpkin flour in scavenging DPPH radicals is 78.33% (15 DAFS), 82.44% (20 DAFS), 91.26% (25 DAFS) and 89.30% (30 DAFS). Meanwhile, the antioxidant activity in counteracting ABTS free radicals is 68.20% (15 DAFS), 83.01% (20 DAFS), 91.14% (25 DAFS), and 72.24% (30 DAFS). According to (Oloyede et al., 2012) the antioxidant activity of pumpkin increases with the maturity level. This is because there is an increase in bioactive compounds that can counteract free radicals. In this research, the antioxidant activity of pumpkin flour is more strongly influenced by total carotenoids (Figure 4), which tend to increase

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compared to total phenols content (Figure 2), and flavonoids (Figure 3) which tend to decrease with increasing maturity levels. According to Abbas et al. (2020), an increase in antioxidant activity is significantly correlated with an increase in the content of neoxanthin, violaxanthin, lutein, and β-carotene. In addition to the decrease of bioactive compounds as maturity degree increased, Piepiórka-Stepuk et al. (2023) reported that the concentration of bioactive compounds of pumpkin decreased during heat treatment.

IC_{50}

The IC50 test was carried out on pumpkin flour with the highest antioxidant activity at the maturity stage of 25 DAFS. IC50 is the concentration of the sample that can counteract free radicals up to 50% obtained by the regression equation. The IC50 values of pumpkin flour with a maturity stage of 25 DAFS are 85.31 µg/mL (DPPH) and 64.39 g/mL (ABTS). This indicates that the antioxidant activity of pumpkin flour is moderate. According to Amarowicz and Pegg, (2019) the smaller the IC50 value obtained from a test compound, the more effective the compound is as a radical scavenger, but the higher the IC50 value, the more weakly the activity of the antioxidant. Therefore, compounds are classified as very strong, strong, moderate, and weak antioxidants when the IC values are < 50, 50-100, 100-150, and 151-200 ppm, respectively.

Flour granule morphology

The SEM revealed that the granule structure of pumpkin flour with a maturity stage of 25 DAFS (Figures 6 and 7) was very compact, irregular in shape with a rough surface, and size of over 10 µm. This compact granule structure and rough surface may be due to the complex between fat and protein (Singh et al., 2007; Nakhon et al., 2017). In the micrograph obtained, the starch granules were not visible because the starch was not extracted, which was still covered in its natural position with cell walls in the form of cellulose, hemicellulose, and pectin. The starch granules of Cucurbita pepo and Cucurbita maxima were reported to have a spherical and dome shape with a size of 1-10 µm (Singh et al., 2007; Nakhon et al., 2017).

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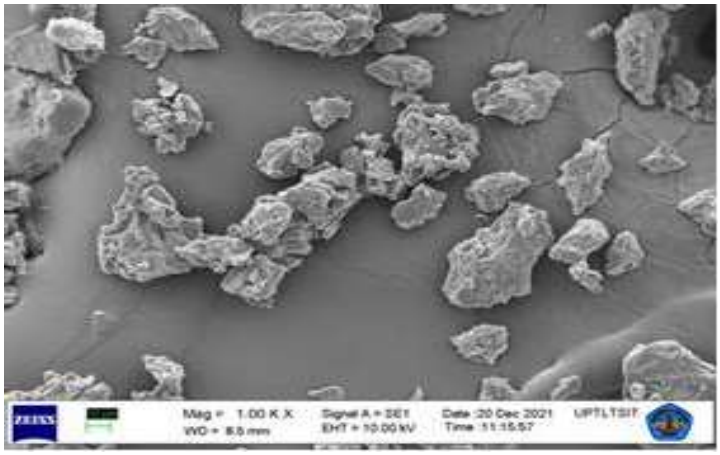

Figure 6. Morphology of pumpkin flour granules with 1000 times magnification

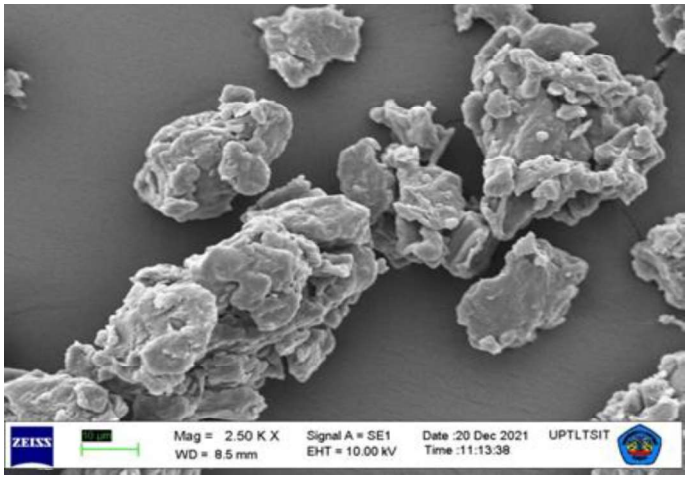

Figure 7. Morphology of pumpkin flour granules with 2500 times magnification

Conclusions

This study proves the maturity stage significantly affected the water content and bioactive components such as phenols, flavonoids, and carotenoids of pumpkin flour. The highest antioxidant activity was found in pumpkin flour with a maturity stage of 25 DAFS with IC 50 values of 85.31µg/mL (DPPH) and 64.39 g/mL (ABTS). This opens the possibility of developing butternut pumpkin flour-based functional food products.

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