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Effects of the Variety and Harvesting Age on Physicochemical Characteristics and Nutritional Composition of Hybrid Pumpkin *(Cucurbita moschata)* in Vietnam

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This study aimed to evaluate the effects of variety and harvesting age on the physicochemical characteristics and nutritional composition of the fruits from three hybrid pumpkin varieties grown in Tra Vinh province of Vietnam. An increment in nutrient accumulation between 60 and 80 days old was observed in all three studied varieties. Adequate accumulation of dry matter, fat, fiber and ash in the fruit was achieved at 70 days old for the F1 pumpkin variety - long fruit (A1) while its maximum accumulation of protein and total phenolic content (TPC) was at 65 days old. A1 variety reached the highest total soluble solid (TSS) content at 75 days old. The F1 pea pumpkin variety (A2) had the highest accumulation of ash and fiber content at 60 days while the contents of fat, protein, carbohydrate and dry matter were maximal values at 65, 75, 70 and 70 days. The highest accumulation of fat, fiber, and carbohydrate was reached at 70 days old for the F1 hybrid pumpkin variety - round fruit (A3). The research results can be used to determine the appropriate harvesting age of the fruits to increase the value of the pumpkin varieties.

1. Introduction

Pumpkin is widely grown and consumed worldwide. According to FAO (2022), the global production of pumpkin fruits in 2021 was more than 23.7 million t. Although pumpkin production has been distributed on all continents, Asia led the world in production (accounting for 50.3 %). Currently, pumpkin varieties are very diverse and abundant including hybrids, and local varieties (FAO, 2022). The fruits of the indigenous pumpkin varieties have a variety of shapes, lengths (13.21 - 91.99 cm), diameters (9.46 - 55.4 cm), and weights (0.59 - 8.75 kg). The pumpkin flesh is rich in nutritional compositions including moisture (79.0 - 93.0 %), protein (0.76-19.61 %), fat (0.04 - 3.81 %), ash (0.57 - 13.45 %), carbohydrates (4.38 - 53.32 %), total fiber (0.51 - 2.97 %) (Men et al., 2021). The soluble solids contents varied from 4.97 to 17.20 °Brix (Carvalho et al., 2015). The pumpkin flesh also contains healthy compounds such as vitamin C content (10.84 - 83.05 mg/100 g) (Men et al., 2021), TPC (13.3 - 369 mg/100 g) (Hagos et al., 2023). These compounds have a significant correlation with antioxidant activity (Piepiórka-Stepuk et al., 2023), which has a positive impact on human health. It is increasingly used as a functional food (Yang et al., 2022). The cultivation period of the indigenous pumpkin varieties is relatively long (75 to 180 days after sowing) (Ramachandran et al., 2022). Nowadays, hybrid pumpkin varieties are widely grown in the world because of their high yield and short harvest time (FAOSTAT, 2022). The fruits of the hybrid pumpkin varieties are a good source of nutrients such as carbohydrates, protein, fat, fiber, ash, and healthy ingredients such as carotenoids, vitamin C, amino acids and unsaturated fatty acids (Amin et al., 2019). In Vietnam, farmers are interested in growing hybrid varieties due to the high yield and the low cost of cultivation. The information on the change of nutritional compositions and the physicochemical characteristics according to

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the harvesting age of the hybrid pumpkin varieties is still incomplete. This study aimed to evaluate the effect of the hybrid pumpkin varieties and harvesting age on the changes in TPC, vitamin C content, nutritional compositions, and physicochemical properties of hybrid pumpkin varieties during the maturation stage from 60 to 80 days old.

2. Materials and Methods

Chemicals: Foline-Ciocalteu, Na_2CO_3, C_2H_5OH, ascorbic acid test, NaOH and H_2SO_4 were supplied by Merck, Germany; Acid gallic was supplied by Sigma-Aldrich, USA. Other chemicals originated from China.
Pumpkin varieties: A1 (F1 hybrid pumpkin variety, long fruit) and A3 (F1 hybrid pumpkin variety, round fruit) were provided by Tan Loc Phat Seed Co., Ltd, A2 (F1 pumpkin variety, pea fruit) was provided by Agricultural Co., Ltd, Vietnam. The pumpkin varieties were grown at Hanh My Cooperative, Tra Vinh province, Vietnam.
Experiment procedures: The length and diameter of the pumpkin fruits by the days old from 60 to 80 were measured on the same marked fruits on the pumpkin plant. Three other fruits of each pumpkin variety (A1, A2, A3) were directly harvested from the pumpkin plants at the days old of 60, 65, 70, 75 and 80 (Figure 1). They were then transported to the laboratory for analysis on the same day. They were then peeled and seeded, and 100 g of the flesh of each pumpkin fruit was directly used to analyze the physicochemical properties and biological compounds in this study. All experiments were performed in triplicates.

Figure 1: Flowchart to evaluate the quality change of pumpkin according to the variety and harvesting age

2.1 Conventional physicochemical analysis

The length and diameter of the fruits were determined by the vernier caliper (Fu et al., 2016). The density of the pumpkin fruits was determined based on the ratio between the weight and volume of the fruit (Clark et al., 2007), and the volume of the fruit was measured by the water displacement method (Fu et al., 2016). TSS in the juice samples from pumpkin flesh was determined by a hand refractometer (ATAGO Master-α), the results are expressed as degrees Brix (°Bx). pH of the juice samples was measured by the pH meter (HANNA HI99161). The moisture content was determined gravimetrically after drying 2 g of fresh pumpkin sample for 10 h at 105 °C until constant weight by the No. 925.10 method of AOAC; The total ash content was determined by the No. 942.05 method of AOAC by burning samples at 650 °C to a constant mass; The total crude fiber content was analyzed by the No. 985.29 method of AOAC; The crude fat content was determined by Soxhlet extraction with ether solvent in 8 h, the solvent was then evaporated and the fat was determined gravimetrically by the No. 920.39 method of the AOAC. The crude protein content was calculated from nitrogen content determined by the Kjeldahl method according to the No. 920.87 method of AOAC, and protein was calculated using 6.25 as the Nitrogen conversion factor (Helrich, 1990); Total carbohydrates content was calculated by difference: the carbohydrates content = 100 - (moisture + proteins + fat + ash) (Barros et al., 2010).

2.2 Bioactive compounds analysis

TPC was determined by the Folin-Ciocalteu method (Darsini et al., 2013) using gallic acid as a standard curve. 10 g of the pumpkin flesh sample and 30 g of 85 % ethanol were pureed for 2 min in a blender. The mixture was then homogenized at 13000 rpm for 2 min. Next, the mixture was filtered through filter paper. The filtrate was diluted with distilled water to get the absorbance within the range of the standard curve. 3 mL of the sample and 0.5 mL of Foline Ciocalteu reagent were then added into the test tube. The test tube was placed in the dark. After

3 min, 2 mL of 20 % sodium carbonate was added and incubated for exactly 1 min in a boiling water bath (100 °C). It was then cooled and measured the absorbance at 650 nm using a spectrophotometer (Shimadzu, UV 1800). The results are expressed in mg gallic acid equivalents (GAE) per 100 g of pumpkin flesh.

Vitamin C content was measured by a reflectometric method. 100 g of the pumpkin flesh and 100 g of distilled water were pureed for 2 min. The mixture was then homogenized at 13000 rpm for 2 min. Next, the mixture was filtered through filter paper. The filtrate was used to measure vitamin C content using a Handheld Reflectometer (RQflex® Plus 10, Merck). Ascorbic acid in the sample reduced yellow molybdophosphoric acid in the Reflectoquant® Ascorbic acid test strips to phosphormolybdenum blue (EMD Millipore, 2015).

2.3 Statistical analysis

The significance difference ($p < 0.05$) of experiments was evaluated by ANOVA using the Statgraphics®centurion XV software. The results are shown in the average ± standard deviation of triplicates.

3. Results and Discussion

3.1 Accumulation of nutritional compositions of the pumpkin by the harvesting age and varieties

The content of nutritional compositions in the fruit flesh of pumpkin varieties such as moisture, fat, protein, fiber, carbohydrate and ash had a statistically significant change in the period from 60 to 80 days old (Table 1).

Table 1: Effect of the variety and harvesting age on the nutritional compositions content in the pumpkin flesh

Pumpkin varieties	Harvesting ages	Moisture (%)	Fat (%)	Protein (%)	Fiber (%)	Carbohydrate (%)	Ash (%)
A1	60	85.1 ± 0.4^{ab}	0.60 ± 0.03^{bc}	0.59 ± 0.13^{def}	2.5 ± 0.2^{i}	12.9 ± 0.5^{fg}	0.77 ± 0.08^{f}
A1	65	83.6 ± 0.4^{def}	0.61 ± 0.08^{bc}	1.05 ± 0.05^{ab}	3.5 ± 0.2^{cdef}	13.7 ± 0.3^{cde}	0.97 ± 0.04^{de}
A1	70	82.7 ± 0.4^{gh}	0.72 ± 0.07^{ab}	1.11 ± 0.16^{a}	3.9 ± 0.1^{ab}	14.4 ± 0.2^{bc}	1.11 ± 0.05^{ab}
A1	75	83.1 ± 0.3^{efg}	0.73 ± 0.03^{a}	1.08 ± 0.11^{a}	3.4 ± 0.3^{cdefg}	14.0 ± 0.4^{cd}	1.02 ± 0.05^{cde}
A1	80	83.0 ± 0.4^{fg}	0.72 ± 0.02^{ab}	1.14 ± 0.05^{a}	3.3 ± 0.1^{defg}	14.0 ± 0.4^{cd}	1.08 ± 0.04^{bcd}
A2	60	83.8 ± 0.7^{de}	0.30 ± 0.08^{f}	0.42 ± 0.09^{fg}	3.8 ± 0.3^{abc}	14.4 ± 0.6^{bc}	1.14 ± 0.06^{ab}
A2	65	83.7 ± 0.3^{de}	0.35 ± 0.05^{f}	0.65 ± 0.10^{cde}	4.2 ± 0.3^{a}	14.0 ± 0.4^{cd}	1.22 ± 0.02^{a}
A2	70	82.5 ± 0.1^{gh}	0.58 ± 0.12^{de}	0.74 ± 0.09^{cde}	3.8 ± 0.2^{abc}	15.0 ± 0.2^{ab}	1.21 ± 0.08^{a}
A2	75	83.2 ± 0.1^{efg}	0.71 ± 0.06^{ab}	0.67 ± 0.14^{cde}	3.7 ± 0.1^{bcd}	14.3 ± 0.0^{c}	1.14 ± 0.04^{ab}
A2	80	82.1 ± 0.9^{h}	0.70 ± 0.07^{ab}	0.81 ± 0.28^{cd}	3.6 ± 0.4^{bcde}	15.2 ± 0.8^{a}	1.20 ± 0.11^{a}
A3	60	85.4 ± 0.1^{a}	0.41 ± 0.02^{ef}	0.32 ± 0.08^{g}	2.8 ± 0.1^{hi}	13.0 ± 0.0^{fg}	0.93 ± 0.05^{e}
A3	65	84.6 ± 0.0^{bc}	0.45 ± 0.04^{de}	0.55 ± 0.14^{ef}	3.1 ± 0.4^{gh}	13.2 ± 0.2^{efg}	1.12 ± 0.08^{abc}
A3	70	84.5 ± 0.6^{bc}	0.56 ± 0.01^{cd}	0.85 ± 0.15^{bc}	3.4 ± 0.4^{cdefg}	12.9 ± 0.6^{g}	1.17 ± 0.04^{ab}
A3	75	84.1 ± 0.3^{cd}	0.61 ± 0.05^{cd}	0.77 ± 0.04^{cde}	3.1 ± 0.2^{fgh}	13.4 ± 0.2^{defg}	1.14 ± 0.09^{ab}
A3	80	84.1 ± 0.2^{cd}	0.55 ± 0.01^{cd}	0.69 ± 0.20^{cde}	3.2 ± 0.1^{efg}	13.5 ± 0.2^{def}	1.08 ± 0.08^{bcd}

Note: Values in the same column which have different superscripts are significantly different ($p < 0.05$) by Duncan's multiple range test

For A1, the moisture content in the fruit flesh reached the lowest value ($p < 0.05$) of 82.7 ± 0.4 % from 70 days old onwards. Moisture content is an important parameter to evaluate the quality of pumpkin fruits during the maturation stage because when the moisture content in the fruit decreases, the accumulated dry matter content in the fruit increases (Muenmanee et al., 2016). The content of fat (0.72 ± 0.07 %), fiber (3.9 ± 0.1 %), carbohydrates (14.4 ± 0.2 %) and ash (1.11 ± 0.05 %) reached the highest values at 70 days old while the highest value of protein content (1.11 ± 0.16 %) was at 65 days old and maintained until 80 days old. In general, these compositions tended to get the highest peak at 70 days old, except for moisture content. The retention of these nutrients during the harvest stage of 75 and 80 days old could be explained by the nutrient accumulation in the fully mature fruit have reached a maximum value for nurturing seeds (Sonu et al., 2013).

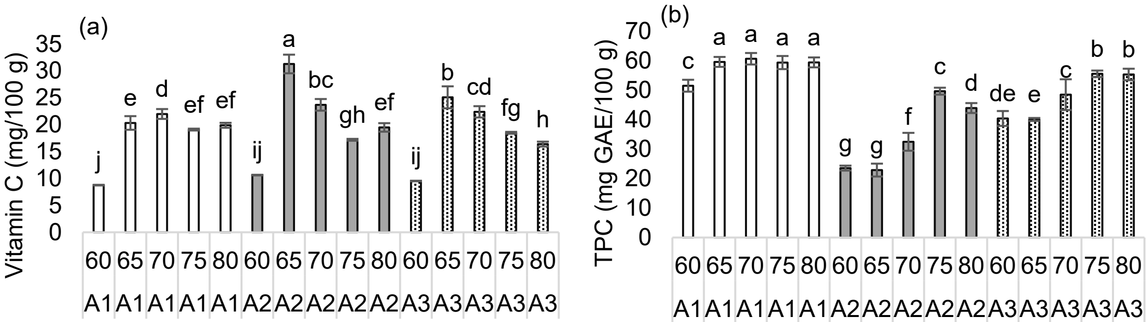
For A2, the change of nutritional compositions in the fruit also has a significant change from 60 to 80 days old. The lowest moisture content (82.1 ± 0.9) observed at 80 days old, indicated the highest accumulated dry matter in the fruits during this period. Besides, the highest values of fat, protein, fiber, carbohydrate and ash content were obtained at 0.71 ± 0.06 % (at 75 days old), 0.65 ± 0.10 % (at 65 days old), 3.8 ± 0.3 % (at 60 days old), 15.0 ± 0.2 % (at 70 days old) and 1.14 ± 0.06 % (at 60 days old).

For A3, the moisture content of the fruit reached the lowest value from 75 days old onwards. The fat, protein, fiber, carbohydrate and ash contents reached the highest values ($p < 0.05$) of 0.56 ± 0.01 % (at 70 days old), 0.85 ± 0.15 % (at 70 days old), 3.4 ± 0.4 % (at 70 days old), 13.4 ± 0.2 % (at 75 days old) and 1.12 ± 0.08 % (at 65 days old).

Overall, A1 reached the highest values of the dry matter, fat, and protein content at an earlier harvesting age than A2 and A3. A previous study reported that the dry matter content in the pumpkin flesh was lower than in this study (Amin et al., 2019). The highest fat content of A1 was less than that reported by the previous study (0.89 %) (Kim et al., 2012). The protein content in the pumpkin flesh of this study was in good agreement with that reported by the previous study (0.98 %) (See et al., 2007). These differences could be explained by genetic differences in pumpkin varieties (Amin et al., 2019).

3.2 Accumulation of bioactive compounds of the pumpkin by the harvesting age and varieties

Similar to the change in the nutritional compositions, the vitamin C content and TPC in the flesh of pumpkin fruits also changed significantly ($p < 0.05$) according to the harvesting age and the pumpkin variety.
For A1, the vitamin C content reached the highest value (22 ± 0.9 mg/100 g) at 70 days old ($p < 0.05$). The highest value of TPC (59.6 ± 1.7 mg GAE/100 g) was at 65 days old.
For A2, the vitamin C content reached the highest (31.3 ± 1.7 mg/100 g) at 65 days old ($p < 0.05$), and TPC reached the highest value (9.7 ± 1.2 mg GAE/100 g) at 75 days old.
For A3, the greatest content of vitamin C (25.1 ± 2.0 mg/100 g) at 65 days old, and the highest TPC was 55.5 ± 1.1 mg GAE/100 g at 75 days old (Figure 2).



Note: *Means ± standard deviations of triplicate analysis. Values which have different superscripts are significantly different ($p < 0.05$) by Duncan's multiple range test.

Figure 2: Effect of the pumpkin varieties (A1, A2, A3) and the harvesting ages (60, 65, 70, 75, and 80) on (a) vitamin C content, (b) TPC in the pumpkin flesh

The increase in vitamin C content during fruit ripening may be related to the conversion of starch to glucose, which is a substrate for the pathway of ascorbate biosynthesis (Bulley and Laing, 2016). The reduction of Vitamin C during the post-ripening of the pumpkin fruits may be caused by the conversion of vitamin C to sugar due to ascorbic acid dehydrogenase (Ramachandran et al., 2022).
The highest content of vitamin C and TPC in three pumpkin varieties of this study was higher the content of vitamin C (19.38 mg/100 g) and TPC (13.296 mg GAE/100 g) as previously reported in the *Cucurbita maxima* pumpkin variety (Piepiórka-Stepuk et al., 2023). According to Yang et al., (2022), the vitamin C content (35 mg/100 g) of *Cucurbita moschata* pumpkin was higher than in this study whereas TPC (39 mg GAE/100 g) was lower than this study. The differences in bioactive compounds might be due to different pumpkin varieties, care conditions and natural environmental factors. The increase in TPC could be attributed to complex biochemical reactions occurring during fruit ripening which led to the production of phenolic compounds and volatile compounds (Yang et al., 2022). There are also a few other factors that might be responsible for the differences in the quality and quantity of the phytochemicals listed above such as sunlight, soil, season, growing area, cultivar, and maturity stage (Dragovic-Uzelac et al., 2007). During the fruit ripening stage, the decrease in TPC resulted from polyphenol oxidation caused by polyphenol-oxidase (Fawole and Opara, 2013). The cultivation efficiency of crops could be reduced due to excessive prolongation of the time to harvest agricultural products. It is highly recommended to harvest at the period when the fruits had fully accumulated the nutritional compositions.

3.3 Physical properties of the pumpkin influenced by the harvesting age and varieties

The fruit density was often correlated with the chemical composition, dry matter content, cell size and intercellular distance in the fruits (McGlone et al., 2007). In this study, the fruit density of A1 and A3 was higher than A2. A1 and A3 had a slight decrease in fruit density from 75 days old (Table 2). This could be due to the process of respiration and water evaporation under the high-temperature conditions of the cultivation environment (Paliyath et al., 2009).
For the fruit length and diameter values of the A1, A2 and A3 fruits, there were no statistically significant differences from 60 to 80 days old as shown in Table 2. A1 had the highest fruit length (18.4 - 18.6 cm) and the

lowest diameter (8.2 - 8.4 cm) compared to A2 (8.8 - 12.5 cm of length and 9.8 - 14.2 cm of diameter) and A3 (8.8 - 9.0 cm of length and 14.4 - 14.5 cm of diameter).

Table 2: Effect of the variety and harvesting age on the physical properties of the pumpkin fruits

Pumpkin varieties	Harvesting ages	Length of fruit (cm)	Diameter of fruit (cm)	Density (g / cm^3)	pH	TSS (^{o}Bx)
A1	60	18.6 ± 1^a	8.4 ± 0.1^c	0.96 ± 0.02^{ab}	6.59 ± 0.02^{ij}	5.8 ± 0.3^d
A1	65	18.6 ± 1^a	8.4 ± 0.1^c	0.99 ± 0.07^a	6.85 ± 0.04^a	8.8 ± 0.3^b
A1	70	18.5 ± 0.9^a	8.3 ± 0.1^c	0.97 ± 0.01^{ab}	6.72 ± 0.03^{de}	8.8 ± 0.3^b
A1	75	18.4 ± 0.9^a	8.3 ± 0.1^c	0.97 ± 0.01^b	6.74 ± 0.01^{cd}	9.5 ± 0.0^a
A1	80	18.4 ± 0.9^a	8.2 ± 0.1^c	0.97 ± 0.02^{ab}	6.63 ± 0.01^{gh}	9.0 ± 0.0^b
A2	60	12.5 ± 0.4^b	9.8 ± 1.0^b	0.93 ± 0.01^b	6.63 ± 0.02^{hi}	6.2 ± 0.3^{cd}
A2	65	12.5 ± 0.4^b	10.0 ± 1.1^b	0.94 ± 0.04^b	6.80 ± 0.05^b	6.3 ± 0.3^c
A2	70	12.4 ± 0.4^b	10.0 ± 1.1^b	0.94 ± 0.01^b	6.69 ± 0.03^{ef}	6.2 ± 0.3^{cd}
A2	75	12.5 ± 0.4^b	10.0 ± 1.1^b	0.94 ± 0.02^b	6.67 ± 0.01^{fg}	6.2 ± 0.3^{cd}
A2	80	12.5 ± 0.4^b	10.0 ± 1.1^b	0.94 ± 0.02^b	6.62 ± 0.01^{hi}	6.0 ± 0.0^{cd}
A3	60	8.8 ± 0.1^c	14.2 ± 0.6^a	0.93 ± 0.02^b	6.59 ± 0.01^{ij}	5.2 ± 0.3^e
A3	65	9.0 ± 0.2^c	14.4 ± 0.9^a	0.97 ± 0.01^{ab}	6.77 ± 0.02^{bc}	5.8 ± 0.3^d
A3	70	8.9 ± 0.4^c	14.5 ± 0.9^a	0.97 ± 0.02^{ab}	6.62 ± 0.05^{hi}	6.2 ± 0.3^{cd}
A3	75	8.8 ± 0.3^c	14.5 ± 1.1^a	0.93 ± 0.01^b	6.67 ± 0.01^{fg}	6.0 ± 0.0^{cd}
A3	80	8.8 ± 0.3^c	14.4 ± 1.0^a	0.86 ± 0.03^c	6.55 ± 0.02^j	6.0 ± 0.0^{cd}

Note: Values in the same column which have different superscripts are significantly different ($p < 0.05$) by Duncan's multiple range test

The pH value in the fruit flesh of three pumpkin varieties tended to increase slightly in the days old from 60 to 65. They then decreased slightly in the period from 70 to 80 days old (Table 2). This pH change could be attributed to the conversion of starch into soluble sugar in the initial ripening stage of the fruits (Paliyath et al., 2009), leading to an increase in the pH value. Organic acids such as citric acid, malic acid, and fumaric acid in the pumpkin fruit might then increase in the post-ripening period leading to a slight decrease in the pH value (Nawirska-Olszańska et al., 2014). These results were in agreement with a previous study that reported the pH of pumpkin ranged in the range of 6.6 - 6.9 (Renquist et al., 2005). Among the three pumpkin varieties studied, the highest TSS of A1 (9.5 ^{o}Bx) was observed higher than A2 (6.3 ^{o}Bx) and A3 (6.8 ^{o}Bx). The days old which reached the highest value of TSS ($p < 0.05$) were 75 for A1, 65 for A2, and 70 for A3. The increment in TSS during the ripening stage of pumpkin may be caused by the conversion of starch to sugar (Paliyath et al., 2009).

4. Conclusion

The nutrient accumulation and physicochemical characteristics in the pumpkin depended on variety and harvest age. The content of fat, protein, and carbohydrates increased to the highest value; it was then maintained according to the harvesting age. while vitamin C and TPC, pH tended to rise to the peak value and slightly decrease with increasing in the harvesting age. In contrast, moisture content tended to decrease with increasing in the harvesting age. TSS of A1 was the highest. Fruit length and diameter of pumpkin varieties were stable from 60 to 80 days old. The results provide vital information to determine the proper harvesting age and pumpkin variety for the processing purposes of the pumpkin fruits to increase the value of pumpkins.

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