ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Technol. Aliment. 23(1) 2024, 15–27
www.food.actapol.net pISSN 1644-0730 eISSN 1898-9594 https://doi.org/10.17306/J.AFS.001180
ORIGINAL PAPER
Received: 01.10.2023
Accepted: 08.11.2023

EFFECT OF HARVEST STAGES ON PHYSICOCHEMICAL PROPERTIES, BIOACTIVE COMPOUNDS, AND ANTIOXIDANT ACTIVITY OF OKRA (*ABELMOSCHUS ESCULENTUS* L.) FOR PROCESSING APPLICATIONS

Ho Thi Ngan Ha[1,3]✉, Nguyen Thi Ngoc Giang[2,3], Phan Uyen Nguyen[1,3], Diep Kim Quyen[1,3], Tran Nguyen Tuong Vy[1,3], Le Hoang Bao Ngoc[1,3]

[1]Faculty of Agriculture and Natural Resources, An Giang University
No. 18, Ung Van Khiem Street, Dong Xuyen Ward, Long Xuyen City, An Giang Province, **Vietnam**
[2]Experimental-practical Area, An Giang University
No. 18, Ung Van Khiem Street, Dong Xuyen Ward, Long Xuyen City, An Giang Province, **Vietnam**
[3]Vietnam National University Ho Chi Minh City
Ho Chi Minh City, **Vietnam**

ABSTRACT

Background. Okra (*Abelmoschus esculentus* L.) is regarded as a good source of carbohydrates, protein, lipids, fiber, vitamins, and minerals. It also presents significant antioxidant capabilities, mostly due to its substantial amounts of vitamin C, chlorophyll, β-carotene, and phenolic. However, a high variation in the characteristics and composition of okra fruit has been reported among different harvest stages.
Material and methods. Data on physical parameters, nutritional values, content of bioactive compounds, and antioxidant activity of the "Mai Vang" okra variety cultivated in Vietnam were recorded from 5 to 17 days after flowering at an interval of 2 days.
Results. The results demonstrated that quick fruit development occurred between 11 and 17 days, and fruits at this stage were of highest fresh weight, length, diameter, and firmness. However, the okra at the stage of 15 days old had the optimum value of nutritional components (protein and lipid) and bioactive substances (β-carotene and phenolic), as well as antioxidant activity, which is very suitable for processing food products.
Conclusion. The findings are useful for understanding the change in physicochemical properties and the accumulation of nutrients and bioactive compounds in okra fruits during maturation. They can also help in identifying the best stage of maturation for the production of healthy products.

Keywords: *Abelmoschus esculentus*, antioxidant, bioactive, day after flowering, physicochemical

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is a member of the Malvaceae family that is derived from the tropical, subtropical, and warm temperate areas of the world (Petropoulos et al., 2018). Okra has a prominent position among vegetable fruits due to its high nutritional and therapeutic value, ease of cultivation, wider adaptation to varied weather conditions, year-round cultivation, high yield, resistance to numerous diseases and pests, and export potential (Reddy et al., 2012; Meena et al., 2017).

Okra contains a lot of mucilage, is low in calories but rich in nutrients, and is a very good source of fiber.

✉htnha@agu.edu.vn, https://orcid.org/0000-0002-5675-0922

© Copyright by Wydawnictwo Uniwersytetu Przyrodniczego w Poznaniu
CC BY

It also contains bioactive compounds such as phenolic, vitamin C, carotenoids, thiamin, folic acid, riboflavin, oxalic acid, niacin, and amino acids. Moreover, okra is a good source of minerals (K, Ca, P, Mg) and is very low in cholesterol and saturated fat (Habtemariam, 2019). In addition to direct consumption, okra fruit is also exploited by the pharmaceutical industry due to its high content of functional compounds such as polysaccharides and flavonoids (Romdhane et al., 2020). Currently, plant proteins are mainly derived from soybeans. Meanwhile, okra seeds have a high protein content and unique flavor that can be used as an alternative and sustainable source of vegetable protein. Many *in-vitro* and *in-vivo* studies have demonstrated that okra fruit possess a variety of bioactivities, including cardiovascular, renal, gastric, and neuroprotection (Habtemariam, 2019), antioxidant, anti-diabetic, and anti-hyperlipidemic (Durazzo et al., 2018), anti-fatigue, anti-bacterial (Petropoulos et al., 2018), anti-inflammatory, and analgesic (Alves et al., 2018).

Although okra fruit is rich in nutrients and has health benefits, so far, the research on the processing and application of okra benefits in food products has not received much attention. In Vietnam, okra is primarily consumed for its edible immature fruit in fresh, boiled, fried, or grilled form. In addition, some companies have also signed off-take contracts with farmers to purchase for export to Japan and Cambodia in the form of freezing. Besides, another type of product has also been produced, which is freeze-dried okra in whole or sliced form. In the world, only a few studies have also been carried out to create powdered products (Adelakun et al., 2009; Chen et al., 2015; Samakradhamrongthai et al., 2022) or dried tea from okra fruit (Minh et al., 2019). Powder products have been added to a number of products such as biscuits (Akoja and Coker, 2018), chicken meatballs (Liu et al., 2021), gluten-free bread (Tufaro et al., 2022), and soup products (Omoniyi et al., 2021).

Several studies have been carried out to investigate the effect of harvest age on the quality of okra fruit intended for fresh consumption, that is, young fruit with a soft texture and sweet taste (Petropoulos et al., 2018). However, there have been no studies investigating the overall quality change of okra fruit applied at later stages in the processing process, with priority given to the content of nutritional components and bioactive compounds besides the physicochemical properties. Therefore, the present study was carried out to investigate the impact of picking time on the quality of the "Mai Vang" cultivar of okra, which is a variety widely grown by farmers in Vietnam, with the aim of obtaining materials for processing purposes.

MATERIAL AND METHODS

Material

Okra seeds of the "Mai Vang" cultivar were provided by Song Bao Store (Ho Chi Minh City, Vietnam). The planting process was carried out at Sau Ton Farm (Provincial Road 943, Vinh Trung Hamlet, Vinh Trach Commune, Phu Hoa Town, Thoai Son District, An Giang Province, Vietnam). The fieldwork was undertaken from March 2022 to June 2023.

Experimental design

Okra seeds were incubated as seedlings in the soil trays first. After about 2–3 days, the seeds germinated. When plants reached a height of 20 cm, they were transferred to the garden for planting (0.5 m inside each row and 0.8 m between rows). The process of incubation and planting was carried out at Sau Ton Farm. At the time of flowering, 200 flowers were directly tagged. Later, twenty tagged fruits were picked after flowering from 5 to 17 days at an interval of 2 days. Harvest time was in the morning (before 8 a.m.). After harvesting, the fruits were put into cartons (perforated) and transported to the Food Technology Laboratory of An Giang University within 1 h. In the laboratory, the fruits were washed with distilled water, then dried with absorbent paper, and removed the peduncle and calyx. All the okra fruits were divided into two groups. The first group was used to measure the physicochemical characteristics, including fruit weight and size, firmness, color, and moisture content. The second group was freezing dried and then ground to pass through a 60-mesh sieve. Subsequently, the samples were stored at –20°C for further analysis of the remaining indicators (content of mucilage, nutritional components, bioactive compounds, and DPPH free radical scavenging ability). Three times, in three distinct seasons (March 2022, July 2022, and March 2023), the cultivation process was repeated. About 40–50 days after planting, the tree began to flower.

Analytical methods

Fruit weight and size parameters

An analytical balance with an accuracy of 0.0001 g (PR-series, Ohaus, USA) was used to determine the weight of the fruit. A digital caliper with a sensibility of 0.01 mm (MC 01120028, Gaogen, China) was used to measure the fruit length and polar diameter.

Firmness

Fruit firmness was measured using a RheoTex (SD 700, Sun Science, Japan). A cylindrical probe with a flat end and a diameter of 1 cm was used. The force required to press into the middle of fruits at the equator for a distance of 4 mm was recorded and expressed in g.

Color (L^*, a^*, b^*)

Fruit color was determined using a Colorimeter (CR400, Konica-Minolta, Japan) based on the CIE (Commission Internationale de L'Eclairage) three-dimensional color space expressed by L^*, a^*, and b^* values.

Mucilage content

Mucilage content was determined by extraction method with ethanol (de Alvarenga Pinto Cotrim et al., 2016). The sample (5 g) was added to 100 mL of distilled water and kept for 24 h. The mixture was filtered through a muslin cloth. The filtrate was given 50 mL of ethanol and slowly stirred until mucilage precipitated. A pre-weight Whatman No.4 paper was used to filter the mixture. The filter paper containing residue was dried at 105°C to constant weight, from which mucilage content (%) was calculated.

Moisture, ash, protein, and lipid content

Moisture content was determined by drying the samples in an oven at 105°C until a constant weight was obtained (AOAC, 2005; Method No. 925.40). Ash content was determined by heating at 550–600°C until the samples burned completely (AOAC, 2005; Method No. 942.05). Nitrogen content was determined using the Kjehldal method (AOAC, 2005; Method No. 984.13), and crude protein content was calculated by multiplying the nitrogen content by 6.25. Lipid content was determined using the conventional Soxhlet extraction method (AOAC, 2005; Method No. 920.39).

Carbohydrate content

Carbohydrate content was determined using the phenol-sulfuric acid method (Jian et al., 2017). For complete hydrolysis, 5 mL of 2.5N HCl were added to the sample (1 g), which was then boiled in a water bath for 3 h. After being cooled to room temperature, the mixture was totally neutralized by adding solid Na_2CO_3. The mixture was filtered and made up to 100 mL. The filtrate (0.5 mL) was combined with 2.5 mL of 96% H_2SO_4 and 0.5 mL of phenol solution. The absorbance was measured at 490 nm after 30 min. The carbohydrate content was calculated using Equation 1, where X was the content of glucose derived from the standard curve (0.05–0.5 mg/mL), k was the dilution factor, V was the extract volume (mL), and m was the sample weight (g).

$$\text{Carbohydrate (\%)} = \frac{X \times k \times V}{10 \times m} \tag{1}$$

Cellulose content

Cellulose content was determined based on the stability of cellulose to acids (Mui, 2007). The sample, which was weighed (1 g) after drying to a constant weight at 105°C, was then put in a flask along with 15 mL of concentrated HNO_3 and 1.5 mL of concentrated H_2SO_4. The mixture was boiled for 30 min after inserting the reflux refrigeration tube. The mixture was diluted with hot water and filtered through a piece of Whatman No. 4 paper. Next, the precipitate was washed with hot distilled water, then with 96% ethanol, and finally with ethyl ether. The filter paper containing cellulose was dried at 105°C to a constant weight, from which the cellulose content (%) was calculated.

Pectin content

Pectin content was determined by the pectate calcium method (Girma and Worku, 2016). The sample (1 g) was filled to 100 mL with distilled water and filtered through a piece of Whatman No. 4 paper. The filtrate (20 mL) was mixed with 100 mL of 0.1 N NaOH solution. After 7h, the mixture was added to 50 mL of 0.1 N CH_3COOH, left for 5 min, and then added to 50 mL of 1 N $CaCl_2$. After 1 h, the sample was boiled for 5 min and then filtered through filter paper. The filter paper containing the precipitate was dried at 105°C to a constant weight. The pectin content was calculated by Equation 2, where P was the precipitate weight

(g), 0.92 was the conversion coefficient from calcium pectate to pectin, and *m* was the sample weight (g).

$$\text{Pectin (\%)} = \frac{P \times 0.92}{20} \times \frac{100}{m} \times 100 \quad (2)$$

Chlorophyll content

The chlorophyll content was determined by the colorimetric method of extract (Trang et al., 2021). The sample (2 g) was filled to a volume of 100 mL with acetone and NH_4OH (9/1, v/v) solvent. After being kept in a dark glass bottle for 24 h, the mixture was centrifuged at 7000 × g for 10 min. The absorbance of the extract was measured at 663 nm and 645 nm by a UV-Vis spectrophotometer (V-730, Jasco, Japan) with an 80% acetone blank. The chlorophyll content was calculated using Equations 3–5, where *A* was the absorbance, *V* was the extract volume (mL), and *m* was the sample weight (g).

$$\text{Chlorophyll a (\%)} = [12{,}7 \times (A_{663}) - 2{,}69 \times (A_{645})] \times \frac{V}{10^6 \times W} \times 100 \quad (3)$$

$$\text{Chlorophyll b (\%)} = [22{,}9 \times (A_{645}) - 4{,}68 \times (A_{663})] \times \frac{V}{10^6 \times W} \times 100 \quad (4)$$

$$\text{Chlorophyll a + b (\%)} = \text{Chlorophyll a (\%)} + \text{Chlorophyll b (\%)} \quad (5)$$

β-carotene content

β-carotene content was determined by the colorimetric method of extraction (Biswas et al., 2011). The sample (0.1 g) was put in a test tube along with 5 mL of cooled acetone and kept for 15 min at 4°C, then vortexed at high speed for 10 min, and finally centrifuged at 7000 × g for 10 min. The supernatant was collected, and the sample was re-extracted as above. Both supernatants were combined and made up to 50 mL. The absorbance was measured at 449 nm. The β-carotene content was calculated using Equation 6, where *X* was the content of β-carotene derived from the standard curve (0.03–30 µg/mL), *k* was the dilution factor, *V* was the extract volume (mL), and *m* was the sample weight (g).

$$\text{Carotene (µg/g)} = \frac{X \times k \times V}{m} \quad (6)$$

Vitamin C content

Vitamin C content was determined using the colorimetric method (Abeysuriya et al., 2020). The sample (0.2 g) was extracted with 8 mL of a solution containing 3% (w/v) meta-phosphoric acid and 8% (v/v) glacial acetic acid by shaking at 150 rpm for 1 h. The mixture was filtered through a piece of Whatman No. 4 paper. The extract (0.8 mL) was added to 50 µL of 3% bromine water, 25 µL of 10% thiourea, and 200 µL of 2,4-dinitrophenylhydrazine and kept at 37°C for 3 h. After cooling in an ice bath for 30 min, the mixture was added to 1 mL of cooled 85% H_2SO_4 and measured for absorbance at 520 nm. The vitamin C content was calculated using Equation 7, where *X* was the content of L-ascorbic acid derived from the standard curve (0.01–0.1 mg/mL), *k* was the dilution factor, *V* was the extract volume (mL), and *m* was the sample weight (g).

$$\text{Vitamin C (mg/100 g)} = \frac{X \times k \times V \times 100}{m} \quad (7)$$

Total phenolic content and antioxidant activity

Total phenolic content was determined using the Folin-Ciocalteu reagent, and antioxidant activity was estimated by the DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging assay (Teixeira et al., 2013). The sample (5 g) was filled to a volume of 50 mL with 95% ethanol and extracted for 60 min. The mixture was then separated by a centrifuge at 7000 × g for 10 min.

The supernatant (0.2 mL) was added to 1.0 mL of 10% Folin-Ciocalteu reagent, left for 5 min, and then added to 1.2 mL of 5% Na_2CO_3. After 2 h, the absorbance was recorded at 750 nm. The total phenolic content was calculated as the gallic acid equivalent (Equation 8). Where C was the content of gallic acid derived from the standard curve (0.01–0.1 mg/mL), *k* was the dilution factor, *V* was the extract volume (mL), and *m* was the sample weight (g).

$$\text{Phenolic (mgGAE/100 g)} = \frac{X \times k \times V \times 100}{m} \quad (8)$$

The supernatant (0.1 mL) was added to 2 mL of DPPH (0.21 mM in 95% ethanol) and kept for 1 h before absorbance reading at 517 nm. For the control, the sample extract was replaced with 95% ethanol. The percentage of DPPH free radical scavenging was calculated by Equation 9, where $A_{control}$ and A_{sample} were the absorbances of the control and sample solutions, respectively.

$$DPPH\ (\%) = (A_{control}\ A_{sample}) / A_{control} \times 100 \quad (9)$$

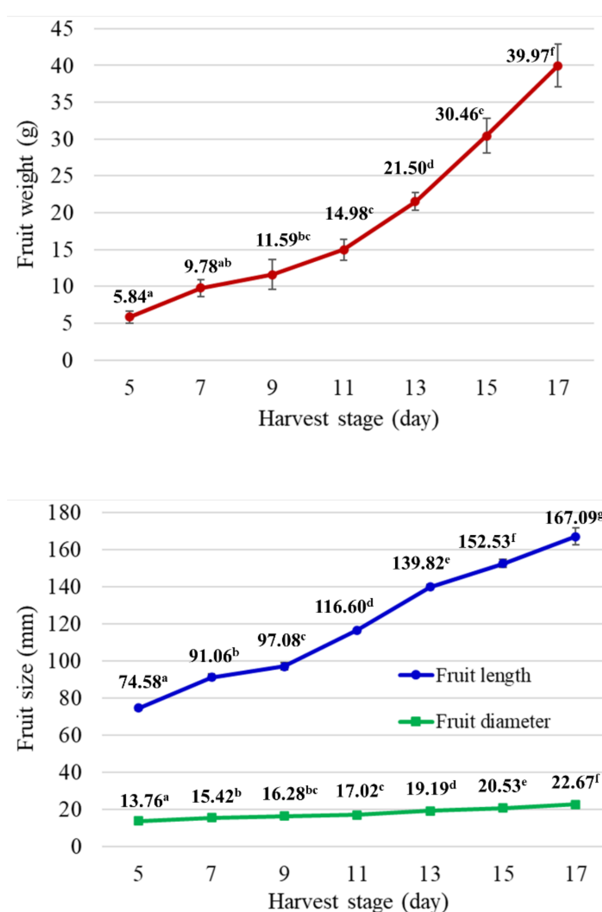
Data analysis

The experiment was set up using a randomized complete design with three replications. The obtained results were expressed as mean values and standard deviations (SD). The data was graphed using Microsoft Excel software and statistically analyzed by Portable Statgraphics Centurion software (version 15.2.11.0, Statpoint Technologies, Inc., VA, USA) using one-way analysis of variance (ANOVA) with the test of significant difference of the treatment means through Least Significance Difference (LSD) at 95% confidence ($p = 0.05$).

RESULTS AND DISCUSSION

Okra fruits of “Mai Vang” variety at different harvest stages (from 5 to 17 days after flowering) (Fig. 1) were analyzed for physicochemical characteristics, bioactive compound content, and antioxidant activity.

Fig. 1. Morphological properties of “Mai Vang” okra fruit at different harvest stages



Notes: values with different superscripts within a line are significantly different at 5% significance level ($p < 0.05$).

Fig. 2. Effect of harvest stage on the fruit weight (a) and fruit size (b)

Fruit weight and size

The weight and size of each fresh fruit are considered the primary harvest indicators for okra fruit (Olivera et al., 2012). The fresh weight, length, and polar diameter all rose significantly ($p < 0.05$) over the course of maturation and reached the maximum values on the 17th day after flowering (Fig. 2). During this time, the fruit grew from 5.84 g to 39.97 g in weight, 74.58 mm to 167.09 mm in length, and 13.76 mm to 22.67 mm in diameter. The weight and size only rose slightly in the initial phases (from 5 to 11 days) but then increased strongly during the later stages. Fruit development in the majority of plants can be divided into three stages. The earliest phase includes the growth of the ovary and the decision to stop or continue with additional cell division and fruit development, known as fruit formation. Cell division drives the majority of fruit growth during the second stage. The third phase begins once cell division is finished. Fruit continues to expand at this time, mostly through cell expansion, until the fruit reaches its full size. This stage of development is the most pronounced and physiologically significant due to the strong sink activity generated by the expanding cells (Chen et al., 2022). These findings are entirely consistent with those of Barnwal et al. (2017)

who observed that the maximum fresh weight of fruit accumulated after 16 days of flowering in the "Kashi Pregati" okra and 18 days after flowering in the "Kashi Kranti" okra. These scientists also noticed a tendency toward a decline in the fresh weight of fruit after that. Dehydration, maturity drying and conversion of fruit reserves, and assimilation of seed reserves could all contribute to this change.

Firmness and color

It was discovered that the fruit firmness increased from 5 days (1530 g force) to 11 days (3467 g force) after flowering before accelerating up to 17 days (6722 g force) (Table 1). The difference in firmness between the okra fruits harvested at 15 and 17 days was statistically significant. The firmness of okra is attributed to the pericarp thickness and fiber development in the pericarp. Barnwal et al. (2017) found that the pericarp thickness gradually grew from 0.66 mm to 1.47 mm as the harvest period was extended from 4 to 14 days after flowering.

Similar findings were reported by Ngangbam and Jahangir (2011), who observed an initial increase in pericarp thickness with maturity.

All three values L^*, a^*, and b^* were used to evaluate the color of the fruit. Darkness to brightness is represented by the "L" values, which rise from 0 to 100. Both remaining values, "a" standing for green to redness and "b" for blue to yellow, go from negative to positive (Castro et al., 2019). As shown in Table 2, the L^* value increased as the fruit advanced towards maturity and reached the maximum at 17 days after flowering. The two remaining colorimetric values (a^* and b^*) also changed in a similar manner. This indicates that the brightness and yellow steadily grow while the green gradually fades as the fruits mature. Cachero and Belonias (2017) also discovered that the young, immature okra fruits can be dark green, pale green, or emerald in color, depending on the variety, and at maturity, the fruits turn brown and split into segments.

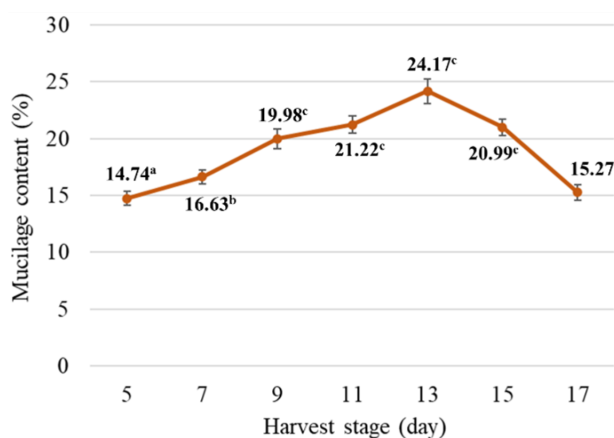
Mucilage content

Okra mucilage is a mixture of natural polysaccharides, consisting of the monosaccharides D-galactose, L-rhamnose, and galacturonic acid associated with proteins and minerals (Gajadhar, 2023). Through in vitro and in vivo research, okra mucilage in particular has shown functional health properties, including anticancer, antioxidant, antibacterial, hypoglycemic, and antiulcerogenic activities. It is also able to bind cholesterol and bile acids, which removes toxins from the liver (Dantas et al., 2021). Mucilage content was expressed in the percentage of fresh fruit weight and presented in Figure 3. The mucilage content of okra fruits increased considerably from 14.74% to 24.17% during maturation stages, from 5 to 13 days after flowering, but then decreased strongly to 20.29% and 15.27% on the 15th and 17th days, respectively. This

Table 1. Effect of harvest stage on fruit firmness and colorimetric value

Harvest stage day	Firmness g force	Colorimetric value		
		L^*	a^*	b^*
5	1 530 ±113^{a}	55.95 ±1.19^{a}	–16.29 ±1.04^{a}	18.58 ±0.31^{a}
7	2 133 ±106^{ab}	58.23 ±1.53^{b}	–15.83 ±1.13^{ab}	19.47 ±0.37^{ab}
9	2 707 ±187^{bc}	59.63 ±1.79^{bc}	–15.39 ±1.02^{abc}	19.84 ±0.65^{abc}
11	3 467 ±127^{cd}	60.57 ±1.40^{cd}	–15.02 ±1.03^{abc}	20.23 ±1.01^{abc}
13	4 213 ±146de	61.37 ±1.13^{cd}	–14.83 ±1.07^{abc}	20.67 ±1.20^{bc}
15	5 103 ±124^{e}	62.67 ±0.99^{de}	–14.42 ±0.97^{bc}	21.47 ±1.80^{cd}
17	6 722 ±354^{f}	63.87 ±0.61^{e}	–13.63 ±0.89^{c}	22.66 ±1.57^{e}

Notes: each value represented the mean ±standard deviation. Values with different superscripts within a column were significantly different at 5% significance level ($p < 0.05$).



Notes: values with different superscripts are significantly different at 5% significance level ($p < 0.05$).

Fig. 3. Effect of harvest stage on the mucilage content

finding was similar to the results of Bini (2003), who discovered that the mucilage content of 20 cultivars of okra increased during three stages of harvest (from 5 to 9 days after flowering).

Content of nutritional composition

The nutritional content of okra fruits varies depending on the stage of harvest, as seen in Table 2. Carbohydrates were the macronutrients with the highest concentration, while ash, fat, and protein were present in lower quantities.

The average fruit moisture content decreased with the advancement of maturity from 5 days (90.59%) to 17 days (84.43%) after flowering. This result is relatively similar to that of Ngangbam and Jahangir (2011), who found that the okra moisture content increased up to the 6th day of fruit flowering; then, it marginally decreased. According to Samnotra (2002), the development of seeds and fiber in the fruit may be the cause of the decline in fruit moisture content during maturation.

The protein content of okra fruit is very high, compared to other fresh vegetables with a contribution from 2.45% to 4.05%. Consequently, this material could serve as a good source of protein in the human diet. This result is higher than the data analyzed by Petropoulos et al. (2018) for various okra cultivars in two fruit sizes (3–5 cm and > 7 cm). This could be probably attributed to differences in the tested genotypes. Protein content was high in immature okra fruit (it reached 3.17% after 5 days of flowering) and tended to decrease during the early growth. Similar findings were made by Gemede et al. (2015), who also found that protein concentration of mature fruit (2.51%) was lower than that of young fruit (2.57%). However, the percentage of protein rose gradually at stages after 9 days and reached the highest level (4.05%) when the okra got to the 17th day; however, there was no significant difference with the 15th day (3.91%). The reason may be that after the maturation stage, the okra fruit gradually ages, and the main components in the seed are gradually synthesized, including protein.

In addition to protein, carbohydrates play a significant role in okra fruits. The carbohydrate level was relatively low on the 5th day (5.34%) and climbed gradually during fruit development to peak at a value of 9.19% after 17 days of flowering. This is the result of cell growth and expansion, increased synthesis of energy and cellular components, and in particular the formation of more fiber during the aging period of the fruit (Heller et al., 2004). Cellulose and pectin are two major carbohydrate components in okra, in which cellulose is an insoluble fiber and pectin is a soluble fiber (Sengkhamparn et al., 2009). When a piece of fruit is young, the structure is soft, and the fiber content is low. During maturation, the amount of moisture gradually decreases simultaneously with the formation of dry matter components, especially cellulose. The conversion of insoluble protopectin to soluble pectin under the action of the protopectinase enzyme rapidly increases the pectin content. In the aging process, the fruit continues to synthesize the cellulose, making the structure become stiffer. Contrastingly, the amount of pectin decreases due to the increasing pectinase enzyme activity (Trong et al., 2023). As a result, cellulose production rose as the harvest age increased, whereas pectin production only increased in the early stages (5–13 days) before declining. Pectin is the primary polysaccharide present in okra mucilage and has been found to be responsible for the viscous characteristic of okra extracts (Kpodo et al., 2017).

Okra fruit has a relatively low lipid content – only 0.17% in 5-day-old fruits. Lipid is concentrated primarily in the seed (Jarret et al., 2011). During the maturation process, the seed synthesizes more lipid; therefore, the lipid content of fruits gradually increased

Table 2. Effect of harvest stage on the content of nutritional components of okra fruits

Harvest stage, day	Moisture %	Protein %	Lipid %	Carbohydrate %	Cellulose %	Pectin %	Ash %
5	90.59 ±0.47^{e}	3.17 ±0.09^{c}	0.175 ±0.012^{a}	4.35 ±0.30^{a}	1.09 ±0.04^{a}	1.26 ±0.04^{a}	1.259 ±0.065^{e}
7	89.98 ±0.53^{de}	2.87 ±0.15^{b}	0.270 ±0.009^{b}	5.51 ±0.32^{b}	1.26 ±0.05^{b}	1.43 ±0.03^{b}	0.830 ±0.071^{d}
9	89.52 ±0.61^{cd}	2.45 ±0.08^{a}	0.326 ±0.017^{b}	6.28 ±0.11^{c}	1.51 ±0.03^{c}	1.71 ±0.06^{c}	0.543 ±0.047^{ab}
11	88.95 ±0.38^{c}	3.03 ±0.10^{bc}	0.501 ±0.031^{c}	6.46 ±0.19^{c}	1.73 ±0.06^{d}	1.82 ±0.05^{d}	0.416 ±0.038^{a}
13	87.47 ±0.72^{b}	3.62 ±0.13^{d}	0.608 ±0.043^{d}	7.07 ±0.38^{d}	2.17 ±0.05^{e}	2.07 ±0.02^{e}	0.584 ±0.051^{bc}
15	85.35 ±0.29^{a}	3.91 ±0.18^{e}	0.745 ±0.030^{e}	8.36 ±0.33^{e}	2.70 ±0.07^{f}	1.80 ±0.04^{d}	0.746 ±0.062^{cd}
17	84.43 ±0.80^{a}	4.05 ±0.27^{e}	0.789 ±0.033^{e}	9.19 ±0.24^{f}	2.92 ±0.04^{g}	1.39 ±0.03^{b}	0.875 ±0.079^{d}

Notes: each value represented the mean ± standard deviation. Values with different superscripts within a column were significantly different at 5% significance level ($p < 0.05$).

but at a slower rate than that of carbohydrates, reaching 0.78% on the 17th day with no significant difference between the 15th and 17th days. A lower content of lipid was detected by Petropoulos et al. (2018) for various okra accessions pods (0.139% to 0.321%) in both sizes of fruits. This variation can be explained by the fact that the age of harvest is different, since okra fruits in the study of these authors were gathered when the size was fairly small (3–5 cm and > 7cm), whereas okra fruit in our study was harvested at later stages (7.46 to 16.71 cm in size).

Moreover, a fluctuation in ash content during the fruit development of okra was also observed.

It steadily dropped from 1.26% to 0.58% between days 5 and 13 after flowering, before rising to 0.87% on the 17th day. This demonstrated that young fruits had a higher ash concentration. Olivera et al. (2012) observed similar outcomes in terms of ash content and noted a tendency for it to decrease as okra fruit size increased.

Content of bioactive compounds and DPPH free radical scavenging ability

The harvest stage had a substantial impact on bioactive component levels. The chlorophyll and vitamin C content decreased when fruits were harvested at

Table 3. Effect of harvest stage on content of bioactive compounds

Harvest stage, day	Chlorophyll, mg/100 g	β-carotene, mg/100 g	Vitamin C, mg/100 g	Phenolic, mgGAE/100 g
5	9.05 ±0.55^{d}	0.563 ±0.051^{a}	23.51 ±2.60^{d}	25.81 ±3.92^{a}
7	8.58 ±0.62^{cd}	0.668 ±0.021^{b}	24.84 ±3.57^{d}	29.70 ±5.46^{ab}
9	7.28 ±0.19^{c}	0.731 ±0.017^{bc}	19.89 ±2.01^{cd}	32.40 ±4.65^{ab}
11	5.79 ±0.46^{b}	0.802 ±0.024^{c}	16.88 ±2.25^{bc}	37.40 ±4.18^{bc}
13	5.00 ±0.28^{ab}	0.916 ±0.064^{d}	15.36 ±2.30^{bc}	43.04 ±5.41^{cd}
15	4.73 ±0.31^{ab}	1.032 ±0.068^{e}	12.73 ±2.13^{ab}	50.35 ±3.86^{d}
17	3.72 ±0.27^{a}	1.085 ±0.033^{e}	9.57 ±1.28^{a}	48.32 ±1.46^{d}

Notes: each value represented the mean ±standard deviation. Values with different superscripts within a column were significantly different at 5% significance level ($p < 0.05$).

a later stage, while an increase was observed for both β-carotene and total phenolic (Table 3). Consequently, compared to younger fruit, older fruits exhibited a better capacity to scavenge the DPPH free radical (Fig. 3).

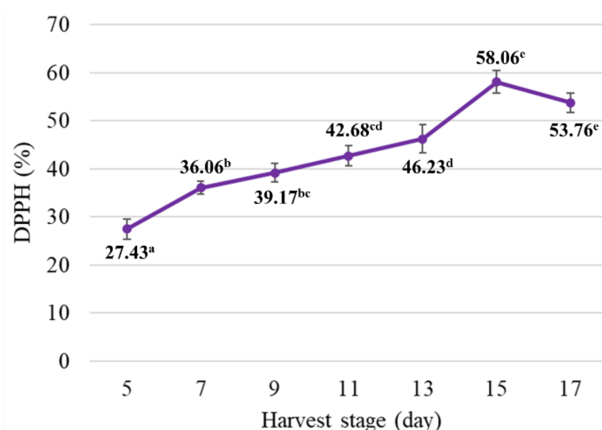
Specifically, there were two opposing trends in the content of the pigments chlorophyll and β-carotene. While β-carotene climbed steadily from 0.563 mg/100 g to 1.085 mg/100 g, chlorophyll gradually declined from 9.05 mg/100 g to 3.72 mg/100 g. The reported levels of β-carotene were higher than those reported for okra pods by Gemede et al. (2015) (185 μg/100 g), whereas Rai and Balasubramanian (2009) showed significantly higher values for both β-carotene and chlorophyll content (10 and 60 mg/100 g, respectively). This is likely because of variations in genotype and/or pod size at harvest stage. Regarding the influence of harvest stage on pigments content, Petropoulos et al. (2018) also reported a decrease of chlorophyll concentration with okra pod development. Chandra et al. (2021) proposed that increased acidity may have significant effects on chlorophyll pigments. The breakdown of chlorophyll and the synthesis of carotenoids are what cause the color change in the fruit during maturation (Bramley, 2013).

Similar to chlorophyll, the level of vitamin C decreased from 23.51 mg/100 g to 9.57 mg/100 g.

The loss of vitamin C may be due to the ascorbate oxidase enzyme, whose activity is greatly dependent on the pH of the vegetable. Ascorbic acid is converted into dehydroascorbic acid by this enzyme (Adetuyi et al., 2008).

Besides polysaccharides in mucilage, okra fruits should be noted for their contribution of phenolic components (Olivera et al., 2012). According to Shen et al. (2019), the primary bioactive compounds in okra fruits – those responsible for all their different bioactivities – are phenolic compounds. Therefore, the total phenolic content at various maturation stages was investigated in order to better understand how phenolic compounds accumulate in okra fruits during maturation and to obtain okra fruits with high amounts of health-beneficial phenolic compounds. In contrast to vitamin C, the total phenolic content increased from 25.81 to 50.35 mgGAE/100 g at the stages from 5 to 15 days and then showed signs of decreasing slowly to 48.32 mgGAE/100 g at 17 days after flowering. A possible reason for this phenomenon could be the cessation or slow rate of biosynthesis of new phenolic components during fruit maturation along with a dilution effect when fruits become larger (Kulkarni and Aradhya, 2005). Wu et al. (2020) discovered the phenolic substances including catechin, isoquercitrin, protocatechuic acid, quercetin, quercetin-3-O-gentiobioside, and rutin in the fruit and its extracts. Nampuak and Tongkhao (2020) found catechin, epigallocatechin gallate, and quercetin compounds in the mucilage of okra.

Antioxidant activity is an important indicator to evaluate the potential beneficial health effects of foods (Moharram and Youssef, 2014). The bioactive compounds present in okra fruits contribute to their antioxidant capacity, which is demonstrated by the ability to scavenge the DPPH free radical. The fruits had low DPPH readings while they were at the immature stage (days 5 to 11). This value then gradually increased until it reached its peak on the fifteenth day (58.06%) before beginning to decline (Fig. 3). The phenolic compounds may be the main contributor toward the antioxidant capacity of okra fruits. In fact, many previous studies have also found good correlations between the antioxidant activity and phenolic contents of okra fruits (Chao et al, 2014; Ahmed and Kumar, 2016; Shen et al., 2019).



Notes: values with different superscripts are significantly different at 5% significance level ($p < 0.05$).

Fig. 4. Effect of harvest stage on DPPH free radical scavenging ability

In the current study, noticeable variations in okra fruit physicochemical characteristics and content of bioactive compounds were observed at different

maturation stages. It could be seen that the maximum β-carotene, total phenolic, and DPPH values were found in 15-day-old okra fruit. In addition, at this harvest stage, the content of two nutritious components, namely protein and fat, reached its peak. The amount of these ingredients did not considerably increase when the harvest period was extended to 17 days; contrastingly, pectin, vitamin C, and chlorophyll content decreased. This is undesirable for the processor even though there was a slight increase in carbohydrate amount due to the contribution of cellulose. Therefore, it can be concluded that in the cultivar “Mai Vang” under study fruits should be picked at 15 days after flowering for processing purposes.

CONCLUSION

The findings of our study indicated that, in addition to the conventional approach of picking immature fruit for fresh consumption, the option of harvesting larger fruit should be also considered to be used for processing purposes such as powder, dried, pickled, or canned products with increased nutritional value and antioxidant properties. Further evidence that the aged okra fruits should be investigated as functional food ingredients for industrial purposes came from their impressive phenolic content and antioxidant capabilities.

ACKNOWLEDGE

This research is funded by Vietnam National University Ho Chi Minh City (VNU-HCM) under grant number C2023-16-02.

DATA AVAILABILITY

Datasets from the current study are available from the corresponding author upon request.

DECLARATIONS

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

Open Access

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

REFERENCES

Abeysuriya, H. I., Bulugahapitiya, V. P., Loku Pulukkuttige, J. (2020). Total vitamin C, ascorbic acid, dehydroascorbic acid, antioxidant properties, and iron content of underutilized and commonly consumed fruits in Sri Lanka. Int. J. Food Sci., ID 4783029. https://doi.org/10.1155/2020/4783029

Adelakun, O. E., Oyelade, O. J., Ade-Omowaye, B. I. O., Adeyemi, I. A., Van de Venter, M. (2009). Chemical composition and the antioxidative properties of Nigerian Okra Seed (*Abelmoschus esculentus* Moench) Flour. Food Chem. Toxicol., 47(6), 1123–1126. https://doi.org/10.1016/j.fct.2009.01.036

Adetuyi, F. O., Osagie, A. U., Adekunle, A. T. (2008). Antioxidant degradation in six indigenous okra *Abelmoschus esculentus* (L) Moench varieties during storage in Nigeria. J. Food Technol., 6(5), 227–230.

Ahmed, B. T., Kumar, S. A. (2016). Antioxidant and Antidiabetic properties of *Abelmoschus esculentus* extract–an in vitro assay. Res. J. Biotechnol., 11(3), 34–41.

Akoja, S. S., Coker, O. J. (2018). Physicochemical, functional, pasting and sensory properties of wheat flour biscuit incorporated with Okra powder. Int. J. Food Sci. Nutr., 3(5), 64–70.

Alves, S. M., Freitas, R. S., do Val, D. R., Vieira, L. V., de Assis, E. L., …, Chaves, H. V. (2018). The efficacy of a lectin from *Abelmoschus esculentus* depends on central opioid receptor activation to reduce temporomandibular joint

hypernociception in rats. Biomed. Pharmacother., 101, 478–484. https://doi.org/10.1016/j.biopha.2018.02.117

AOAC (2005). Official Methods of Analysis, 18th ed. Washington, DC: Association of Analytical Communities.

Barnwal, A. K., Pal, A. K., Tiwari, A., Pal, S., Singh, A. K. (2017). Effect of picking stages on fruit and seed development in okra [*Abelmoschus esculentus* (L.) Moench] cultivars kashi pragati and kashi kranti. Int. J. Agric. Environ. Biotech., 10(6), 695–702. https://doi.org/ 10.5958/2230-732X.2017.00086.9

Bini, N. (2003). Post harvest quality evaluation of okra (*Abelmoschus esculentus* (L.) Moench). Doctoral dissertation, Department of Processing Technology. Vellayani: College of Agriculture.

Biswas, A. K., Sahoo, J., Chatli, M. K. (2011). A simple UV-Vis spectrophotometric method for determination of β-carotene content in raw carrot, sweet potato and supplemented chicken meat nuggets. LWT-Food Sci. Technol., 44(8), 1809–1813. https://doi.org/10.1016/j.lwt.2011.03.017

Bramley, P. M. (2013). Carotenoid biosynthesis and chlorophyll degradation. Mol. Biol. Biochem. Fruit Ripening, 75–116. https://doi.org/10.1002/9781118593714.ch4

Cachero, T., Belonias, B. S. (2017). Morpho-anatomical and physiological changes of okra ((L.) Moench var. *Abelmoschus esculentus* 'Smooth Green') fruit and seed during development. Sci. Humanit. J., 11, 40–63. https://doi.org/10.47773/shj.1998.110.3

Castro, W., Oblitas, J., De-La-Torre, M., Cotrina, C., Bazán, K., Avila-George, H. (2019). Classification of cape gooseberry fruit according to its level of ripeness using machine learning techniques and different color spaces. IEEE Access, 7, 27389–27400. https://doi.org/10.1109/ACCESS.2019.2898223

Chandra, R. D., Prihastyanti, M. N. U., Lukitasari, D. M. (2021). Effects of pH, high pressure processing, and ultraviolet light on carotenoids, chlorophylls, and anthocyanins of fresh fruit and vegetable juices. EFood, 2(3), 113–124. https://doi.org/10.2991/efood.k.210630.001

Chao, P. Y., Lin, S. Y., Lin, K. H., Liu, Y. F., Hsu, J. I., Yang, C. M., Lai, J. Y. (2014). Antioxidant activity in extracts of 27 indigenous Taiwanese vegetables. Nutrients, 6(5), 2115–2130. https://doi.org/10.3390/nu6052115

Chen, Q., Shen, P., Bock, R., Li, S., Zhang, J. (2022). Comprehensive analysis of plastid gene expression during fruit development and ripening of kiwifruit. Plant Cell Rep., 41(4), 1103–1114. https://doi.org/10.1007/s00299-022-02840-7

Chen, Y., Zhang, B. C., Sun, Y. H., Zhang, J. G., Sun, H. J., Wei, Z. J. (2015). Physicochemical properties and adsorption of cholesterol by okra (*Abelmoschus esculentus*) powder. Food Funct., 6(12), 3728–3736. https://doi.org/10.1039/C5FO00600G

Dantas, T. L., Alonso Buriti, F. C., Florentino, E. R. (2021). Okra (*Abelmoschus esculentus* L.) as a potential functional food source of mucilage and bioactive compounds with technological applications and health benefits. Plants, 10(8), 1683. https://doi.org/10.3390/plants10081683

de Alvarenga Pinto Cotrim, M., Mottin, A. C., Ayres, E. (2016). Preparation and characterization of okra mucilage (*Abelmoschus esculentus*) edible films. Macromol. Symp., 367, 1, 90–100. https://doi.org/10.1002/masy.201600019

Durazzo, A., Lucarini, M., Novellino, E., Souto, E. B., Daliu, P., Santini, A. (2018). *Abelmoschus esculentus* (L.): Bioactive components' beneficial properties – Focused on antidiabetic role – For sustainable health applications. Molecules, 24(1), 38. https://doi.org/10.3390/molecules24010038

Gajadhar, S. (2023). The prebiotic effects of amadumbe (Colocasia Esculenta) and okra (*Abelmoschus esculentus*) mucilage. Doctoral dissertation. Durban, South Africa: Durban University of Technology. https://doi.org/10.51415/10321/4803

Gemede, H. F., Ratta. N., Haki. G. D., Woldegiorgis. A. Z., Beyene. F. (2015). Nutritional quality and health benefits of "okra" (*Abelmoschus esculentus*): A review. Glob. J. Med. Res., 14(5), 28–37. DOI: 10.11648/j.ijnfs.20150402.22

Girma, E., Worku, T. (2016). Extraction and characterization of pectin from selected fruit peel waste. Int. J. Sci. Res., 6(2), 447–454.

Habtemariam, S. (2019). The chemical and pharmacological basis of okra (*Abelmoschus esculentus* (L.) Moench) as potential therapy for type 2 diabetes. Medicinal Foods as Potential Therapies for Type-2 Diabetes and Associated Diseases. Amsterdam: The Netherlands Elsevier, 307, 332. https://doi.org/10.1016/B978-0-08-102922-0.00010-9

Heller, R., Esnault, R., Lance, C. (2004). Physiologie végétale II, développement. Paris: Dunod, 64–240.

Jain, V. M., Karibasappa, G. N., Dodamani, A. S., Mali, G. V. (2017). Estimating the carbohydrate content of various forms of tobacco by phenol-sulfuric acid method. J. Educ. Health Promot., 6. https://doi.org/10.4103/jehp.jehp_41_17

Jarret, R. L., Wang, M. L., Levy, I. J. (2011). Seed oil and fatty acid content in okra (*Abelmoschus esculentus*) and related species. J. Agric. Food Chem., 59(8), 4019–4024. https://doi.org/10.1021/jf104590u

Kpodo, F. M., Agbenorhevi, J. K., Alba, K., Bingham, R. J., Oduro, I. N., Morris, G. A., Kontogiorgos, V. (2017). Pectin isolation and characterization from six okra genotypes. Food Hydrocoll., 72, 323–330. https://doi.org/10.1016/j.foodhyd.2017.06.014

Kulkarni, A. P., Aradhya, S. M. (2005). Chemical changes and antioxidant activity in pomegranate arils during fruit development. Food Chem., 93(2), 319–324. https://doi.org/10.1016/j.foodchem.2004.09.029

Liu, Y., Zhou, X., Zhang, M., Zhou, Y., Fan, Y., Zhang, X., Yu X., Xiong G., Li, J. (2021). A novel meat quality improver composed of carrageenan and superfine smashed okra powder and its application in chicken meatballs. Int. J. Food Eng., 17(5), 377–384. https://doi.org/10.1515/ijfe-2020-0299

Meena, V. K., Dubey, A. K., Jain, V. K., Tiwari, A., Negi, P. (2017). Effect of plant growth promoters on flowering and fruiting attributes of okra [*Abelmoschus esculentus* (L.) Moench]. Crop Res., 52(1,2&3), 37–40.

Minh, N. P., Bach, L. G., Loan, L. Y., Tram, V. T. B., Van Truyen, T. (2019). Production of dried tea from okra (Abelmoschus Esculentus). J. Pharm. Sci. Res., 11(2), 279–283.

Moharram, H. A., Youssef, M. M. (2014). Methods for determining the antioxidant activity: A review. Alex. J. Food Sci. Tech., 11(1), 31–42.

Mui, N. V. (2007). Biochemistry practice. Hanoi: Hanoi National University Publishing.

Nampuak, C., Tongkhao, K. (2020). Okra mucilage powder: A novel functional ingredient with antioxidant activity and antibacterial mode of action revealed by scanning and transmission electron microscopy. Int. J. Food Sci. Technol., 55(2), 569–577. https://doi.org/10.1111/ijfs.14308

Ngangbam, P., Jahangir, K. (2011). Effect of age of harvest on fruit quality of okra (*Abelmoschus esculentus* L.). J. Environ. Res. And Dev., 5(3). 615–622.

Olivera, D. F., Mugridge, A., Chaves, A. R., Mascheroni, R. H., Viña, S. Z. (2012). Quality attributes of okra (*Abelmoschus esculentus* L. Moench) pods as affected by cultivar and fruit size. J. Food Res., 1(4), 224–235. http://dx.doi.org/10. 5539/jfr.v1n4p224

Omoniyi, S. A., Idowu, M. A., Francis, P. N., Adeola, A. A. (2021). Nutrient Composition and functional properties of okra seed flour and some quality attributes of its soups. J. Culin. Sci. Technol., 19(4), 285–293. https://doi.org/10.1080/15428052.2020.1759169

Petropoulos, S., Fernandes, Â., Barros, L., Ferreira, I. C. (2018). Chemical composition nutritional value and antioxidant properties of Mediterranean okra genotypes in relation to harvest stage. Food Chem., 242, 466–474. https://doi.org/10.1016/j.foodchem.2017.09.082

Rai, D. R., Balasubramanian, S. (2009). Qualitative and textural changes in fresh okra pods (*Hibiscus esculentus* L.) under modified atmosphere packaging in perforated film packages. Food Sci. Technol. Int., 15(2). 131–138. https://doi.org/10.1177/108201320810620

Reddy, M. T., Haribabu, K., Ganesh, M., Reddy, K. C., Begum, H. (2012). Genetic divergence analysis of indigenous and exotic collections of okra (*Abelmoschus esculentus* (L.) Moench). J. Agric. Technol., 8(2), 611–623.

Romdhane, M. H., Chahdoura, H., Barros, L., Dias, M. I., Corrêa, R. C. G., …, Ferreira, I. C. (2020). Chemical composition, nutritional value, and biological evaluation of Tunisian okra pods (*Abelmoschus esculentus* L. Moench). Molecules, 25(20), 4739. https://doi.org/10.3390/molecules25204739

Samakradhamrongthai, R. S., Nortuy, N., Jannu, T., Supawan, T., Chanakun, P., Yimkaew, Y., Renaldi, G. (2022). Influence of three drying methods on physicochemical properties of okra (*Abelmoschus esculentus* L.) powder. J. Food Process. Preserv., 46(3), e16381. https://doi.org/10.1111/jfpp.16381

Samnotra, R. K. (2002). Seed Development in Okra (*Abelmoschus esculentus* (L.) Moench) cv Parbhani Kranti. Env. Iron. Ecol., 20(1), 230–232.

Sengkhamparn, N., Verhoef, R., Schols, H. A., Sajjaanantakul, T., Voragen, A. G. (2009). Characterisation of cell wall polysaccharides from okra (*Abelmoschus esculentus* (L.)). Carbohydr. Res., 344(14), 1824–1832. https://doi.org/10.1016/j.carres.2008.10.012

Shen, D. D., Li, X., Qin, Y. L., Li, M. T., Han, Q. H, …, Wu, D. T. (2019). Physicochemical properties. phenolic profiles. antioxidant capacities. and inhibitory effects on digestive enzymes of okra (*Abelmoschus esculentus*) fruit at different maturation stages. J. Food Sci. Tech., 56, 1275–1286. https://doi.org/10.1007/s13197-019-03592-1

Teixeira, B., Marques, A., Ramos, C., Serrano, C., Matos, O., …, Nunes, M. L. (2013). Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil. J. Sci. Food Agric., 93(11), 2707–2714. https://doi.org/10.1002/jsfa.6089

Trang, P. Q., Sy, D. T., Quang, T. H., Bich, H. T., Minh, P. T. H., Quang, V. M., Huong, N. T. (2021). Study

on extraction process of chlorophyll from silkworm fertilizer. J. Sci. Technol., 57(1), 117–120.

Trong, L.V., Phuong, H. T., Quyen, L. T. (2023). Research on physiological and biochemical transformations according to developmental age of "Nhan Hau" persimmon (*Diospyros kaki* L.f.) grown in Thanh Hoa. J. Agric. Dev., 22(1), 54–61. https://doi.org/10.52997/jad.6.01.2023

Tufaro, D., Bassoli, A., Cappa, C. (2022). Okra (*Abelmoschus esculentus*) powder production and application in gluten-free bread: Effect of particle size. Food Bioprocess Technol., 15(4), 904–914. https://doi.org/10.1007/s11947-022-02784-6

Wu, D. T., Nie, X. R., Shen, D. D., Li, H. Y., Zhao, L., Zhang, Q., Lin, D. R., Qin, W. (2020). Phenolic compounds, antioxidant activities, and inhibitory effects on digestive enzymes of different cultivars of okra (*Abelmoschus esculentus*). Molecules, 25(6), 1276. https://doi.org/10.3390/molecules25061276