Bulletin of the *Transilvania* University of Brasov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 16(65) No. 1 – 2023
https://doi.org/10.31926/but.fwiafe.2023.16.65.1.11

# CHANGES IN THE PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF CUCUMBER (*CUCUMIS SATIVUS* L.) DURING FRUIT DEVELOPMENT

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***Abstract:*** *The cucumber (Cucumis sativus L.) is an economically important vegetable crop cultivated worldwide, including Vietnam. This study aims to determine the physiological ripening time of cucumber fruit to establish a scientific basis for optimizing fruit quality, harvest, and preservation. Changes in the physiological and biochemical parameters during the growth and development of cucumbers from 2 to 12 days after anthesis (DAA) were observed and analyzed. The fruit reached its maximum size in length, diameter, volume, and weight at 11 DAA. The chlorophyll content of the fruit reached its maximum value at 8 DAA and then decreased. The carotenoid content was low during fruit formation and increased until fruit ripening. The vitamin C content and reducing sugar content increased continuously and reached a maximum at 11 DAA before decreasing slightly. The starch content, total organic acid content, and tannin content reached a maximum at 9 DAA and then gradually decreased. The pectin content increased from fruit formation until fruit ripening. Our results indicate that cucumbers should be harvested at 11DAA to maximize the nutritional value of the fruit for consumption and processing.*

***Key words:*** *cucumber, fruit development, maturation, post-harvest, fruit ripening.*

## 1. Introduction

The cucumber (*Cucumis sativus* L.) belongs to the *Cucumis* genus, a part of the Cucurbitaceae family [35]. Of the 30 species of *Cucumis*, *C. sativus* has the greatest economic significance [35]. Originating in India, today cucumbers are produced globally, with the largest growing area and production volume in China [28]. There are close to 100 cucumber varieties, but the most common include the English, garden, Persian, mini, and lemon varieties [28]. Cucumbers are naturally monoecious, with the cucumber fruit considered a “false berry”. Cucumber

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fruits are round or triangular and covered with a hard, thick outer rind. The oblong fruits can be up to 60 cm long and 10 cm in diameter [1].

The cucumber is an important vegetable crop worldwide [13]. It is a common ingredient in salads, valued mainly for its crisp texture and juiciness [34]. Cucumbers have a mild flavor and aroma depending on the variety, partly due to the unsaturated aldehydes present in the fruit such as (E,Z)-nona-2,6-dienal, and *cis*- and *trans*- isomers of 2-nonenal [21]. Cucumbers contain high levels of vitamin K, cucurbitacins and their derivatives (triterpenoids), flavonoids (apigenin, luteolin, quercetin, and kaempferol), antioxidants such as β-carotene, vitamin C, vitamin B, and minerals [16]. Furthermore, cucumber seeds are high in protein, fat, carbohydrates, and crude fiber [1]. The cucumber also has significant medicinal effects, including reducing cholesterol in the body, improving skin and hair, benefitting people with diabetes, and reducing the risk of cancer [22], [28].

In Vietnam, many cucumber varieties are grown and harvested without a scientific basis, reducing the quality of the fruit sold in the market. Physiological and biochemical changes during fruit development are important criteria that can be used to determine fruit maturity and ideal harvest stage [20], [24, 25], 27]. This study aims to examine the physiological and biochemical changes of the cucumber fruit throughout its development to determine physiological ripening time and establish a scientific basis for harvest.

## 2. Materials and Methods

### 2.1. Experimental Materials

Cucumber fruits (*Cucumis sativus* L., cv. Fadia) were harvested from a greenhouse in Thanh Hoa province, Vietnam (20°08′28″N and 105°18′34″E) in January 2021. The fruit on the experimental plants was marked using tape once it formed. The fruit samples were harvested from twenty plants in eight development stages: 2, 4, 6, 8, 9, 10, 11, and 12 days after anthesis (DAA). The fruits were collected in the morning according to the mixed sampling method [26] and immediately transported to the laboratory of Hong Duc University, Vietnam.

### 2.2. Measurement of Physiological and Biochemical Parameters

After each sampling, the length and diameter of the fruit were measured using a digital Vernier caliper. The digital weighing balance (PCB 1000-2, KERN, China) with 0.01 g precision was used for measuring the weight of the fresh fruit. The volume of the fruit was measured following the water displacement method described by Akbolat et al. [2].

The pigment content was determined by the spectrophotometric method as described by Trong et al. [26]. Approximately 5 mg of peel sample was used for pigment extraction using acetone (80% v/v). Wavelengths of 662.0 and 644.0 nm for chlorophyll and 440.5 nm for carotenoids were used to determine the pigment content. All pigments were expressed as mg/g fresh weight.

Reducing the sugar and starch contents was determined by the Bertrand method [25]. Five grams of flesh sample were homogenized using a mortar and a pestle

with 25 mL distilled water before increasing the volume to 100 mL with distilled water. The solution was then filtered through filter paper and the filtrate was obtained for analysis. 10 mL of sample solution was put in a 1000 mL conical flask and 10 mL of Fehling solution was added. The mixture was boiled for 3 minutes, at which point precipitate appeared and was filtered into a Buchner vacuum filter. The flask and the filter funnel were cleaned with hot distilled water 3-4 times. The resulting sediment of $Cu_2O$ in the Buchner filter was completely dissolved using 5 mL of $Fe_2(SO_4)_3$ in $H_2SO_4$ and stirred with a glass rod. The resulting solution was titrated with $KMnO_4$ 1/30N until a light pink color appeared within 20-30 seconds. A control experiment was conducted at the same time in which the sugar solution was replaced with distilled water. The amount of $KMnO_4$ used for titration was calculated and a reference table was used to determine the equivalent amounts of reducing sugar and starch.

The protein content was determined using the Lowry method [31]. 0.5 mL of 1% $CuSO_4$ was added to 1 mL of the sample solution in a test tube, mixed thoroughly, and incubated at room temperature for 10 minutes. The solution was mixed with 0.5 mL of Folin 1N, incubated for 30 minutes, and measured by colorimeter at a wavelength of 750 nm. The protein content was calculated by the standard graph.

The lipid content was determined using the Soxhlet method [25]. A flask was put on a bain-marie and ether equal to half the volume of the flask was added. The material bag was put in the extraction thimble connected to the flask. Solvent was added to submerge the material bag to above the upper part of the siphon arm of the thimble. A cooling tube was installed and the material was soaked in the solvent for 4 hours. A Soxhlet extractor was put inside the bain-marie to set the condensation rate for the solvent to approximately 10-15 drops per hour. After the extraction, the flask was removed and a welding tube was installed to distill the ether. The flask containing lipids was dried to constant mass to determine the lipid content.

The total organic acid content was calculated as previously described by Trong et al. [26]. 5g of flesh sample was ground to a fine powder and placed in a 50 mL flask before adding distilled water and mixing well. 10 mL of the extracted filtrate was transferred into a 100 mL conical flask and a few drops of phenolphthalein reagent were added. The solution was titrated with NaOH 0.1N until a persistent pink color appeared.

The vitamin C content was determined by the titration method according to Trong et al. [26]. 5 g of flesh sample was crushed with 5 mL of 5% HCl and put into a 100 mL flask, before adding distilled water and mixing well. The solution was titrated with liquid $I_2$ until a blue color appeared.

The tannin content was determined by the titrimetric method [14]. 5 mL of sample solution was mixed with 12.5 mL of indigo-carmine solution and 375 mL of distilled water, then titrated with $KMnO_4$ solution. The tannin concentration was determined using the following relationships: 1 mL of standard $KMnO_4$ = 0.595 mL of oxalic acid 0.1N; 1 mL of oxalic acid 0.1 N = 0.0042 g of tannin.

The pectin content was calculated by the calcium pectate precipitation method [29]. 0.16 g of sample was placed in the

flask and 100mL NaOH 1N was added, before leaving the mixture for 7 hours to completely saponify the pectin to pectic acid. 50 mL of acetic acid 0.1N was added, and 5 minutes later 50 mL of $CaCl_2$ 2N was added, before sitting for 1 hour. The solution was boiled for 5 minutes and filtered through insoluble filter paper, which had been dried to constant mass. The calcium pectate precipitate was washed with hot distilled water and after washing, the filter paper with precipitate was dried at 150°C to constant weight. The weight of the precipitate was used to determine the calcium pectate content.

### 2.3. Statistical Analysis

All analyses were performed in triplicate. The data were processed and subjected to analysis of variance (ANOVA) by IRRISTAT 5.0 software (International Rice Research Inst., Manila, Philippines). Mean comparisons were done using the LSD test at a 5% level of probability.

## 3. Results and Discussion

### 3.1. Changes in Length, Diameter, Volume and Fresh Weight during Fruit Development

After fertilization and fruit formation, a cucumber fruit grows and develops, resulting in changes in the fruit's length and diameter. Monitoring these changes allows for the determination of the time it takes the fruit to reach its maximum size, and therefore its ripening period [5, 12]. The length and diameter of the fruit increased rapidly between 2 DAA and 11 DAA (Figure 1). From 11 DAA, cucumber fruit size increased slowly and remained almost unchanged (Figure 2). At 12 DAA, the fruit reached the maximum size with a length of 12.53 cm and a diameter of 3.03 cm. The fruit grows initially from rapid cell division, increasing the number and size of cells in the fruit, and later, from cell elongation [5]. At 11 DAA, the fruit has prepared enough nutrients to enter the ripening stage, so the rate of size increase slows down.

The volume and fresh weight of the cucumber fruit also increased proportionally withage. The average cucumber volume reached 0.82 mL at 2 DAA, and then increased gradually (Figure 1). After 11 DAA, the fruit volume continued to increase but at a slower rate, reaching its maximum volume of 501.33 mL at 12 DAA. The period from 2 to 11 DAA is a period of strong cell growth where cells increase water absorption due to the vacuole volume rapidly increasing and the dilation of the primary cell wall. Cell wall elongation is caused by the action of H+-ATPase, which breaks the hydrogen bonds between the cellulose microfibrils and is regulated by the phytohormone auxin [9]. The fruit weight was 1.54 g at 2 DAA and then increased rapidly, reaching 124.19 g at 11 DAA (Figure 1). After this period, the fruit weight increased at a slower rate and reached 126.32 g by 12 DAA. The change in fruit weight was consistent with the changes in fruit volume, length, and diameter during fruit growth.

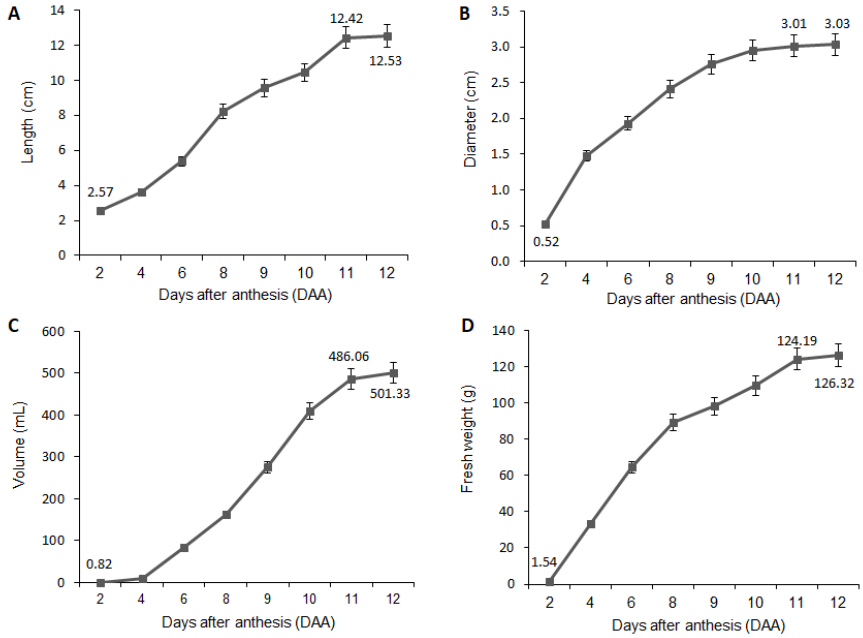


Fig. 1. *Changes in length (A), diameter (B), volume (C) and fresh weight (D) of cucumber fruit during development*


Fig. 2. *Development stages of cucumber fruit. DAA: Days after anthesis*

### 3.2. Changes in Chlorophyll and Carotenoid Content during Fruit Development

At 2 DAA, the chlorophyll content in the cucumber peel was low, with a chlorophyll *a* content of 0.02 mg/g and a chlorophyll *b* content of 0.04 mg/g. From 2 to 8 DAA, the chlorophyll *a* and chlorophyll *b* contents increased rapidly and reached maximums at 8 DAA (chlorophyll *a* was 0.14 mg/g and chlorophyll *b* was 0.25 mg/g). After 8 DAA, the chlorophyll content gradually decreased (Figure 3). During cucumber fruit development, a visible degreening process associated with chlorophyll degradation was noticed, which is consistent with some studies that chlorophyll decomposition is related to fruit maturity [7, 11, 30]. The carotenoid content was 0.01 mg/g at 2 DAA, it increased slowly from 2 to 6 DAA, and then increased rapidly as the fruit ripened (Figure 3). At 12 DAA, the carotenoid content reached 0.12 mg/g. At an early stage, the fruit is primarily green because of the large amount of chlorophyll that obscures the carotenoids, but the yellow color of the carotenoids becomes more apparent during the ripening process due to chlorophyll breakdown [4].

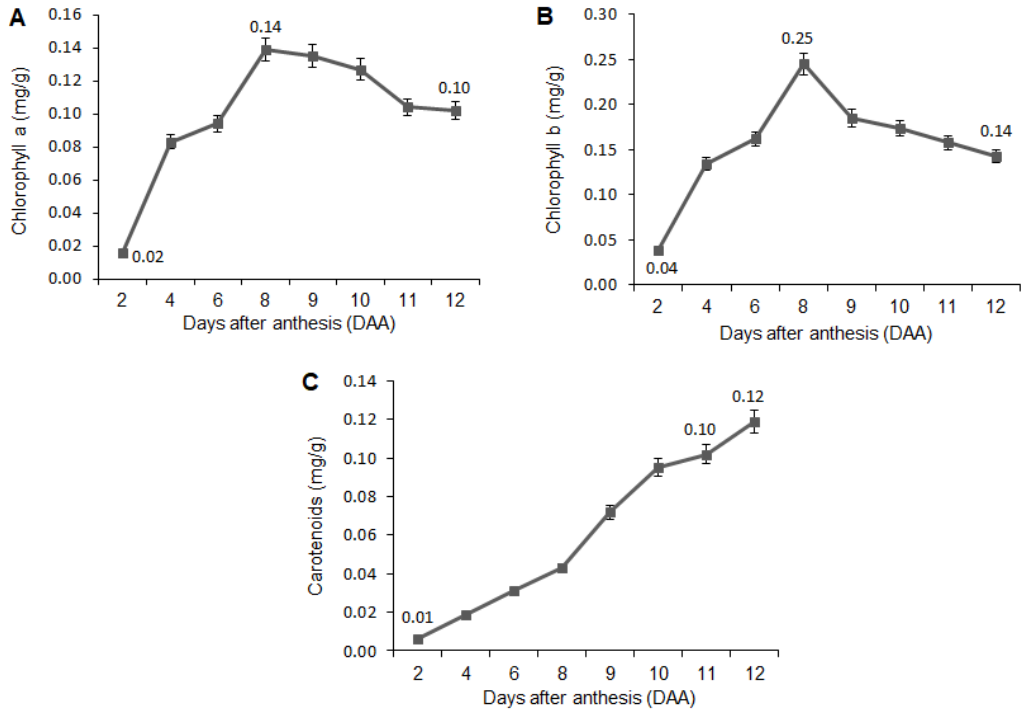


Fig. 3. *Changes in the content of chlorophyll a (A), chlorophyll b (B) and carotenoids (C) of cucumber fruit during development*

### 3.3. Changes in Reducing Sugars and Starch Content during Fruit Development

The reducing sugar content in the early period of cucumber fruiting at 2 DAA was relatively low at 0.88%. From 2 to 11 DAA, the content of reducing sugar in the fruit increased rapidly and reached 1.77% at 11 DAA (Figure 4; $P< 0.05$). This result is consistent with other observations of rapidly increasing total sugar in the late fruit development stage [17]. However, the reducing sugar content decreased after 11 DAA, which is attributed to a rapid increase in respiration during cucumber ripening. Reducing sugars are used directly during respiration for energy and can be used by cells for synthetic reactions [32].

After initial cucumber fruit formation, the minimum value of starch content was 1.53% at 2 DAA, while the maximum value was 2.32% at 9 DAA (Figure 4). During this time, the fruit accumulates nutrients in preparation for ripening. After 9 DAA, the content of starch in the fruit decreased due to the strong metabolism in the fruit. At 12 DAA, the content of starch decreased to 1.32% ($P< 0.05$) and the activity of the α-amylase enzyme increased. Under the action of the α-amylase enzyme, starch is converted to sugar as a material for energy-generating respiration [33].

### 3.4. Changes in Protein and Lipid Content during Fruit Development

The protein content in cucumber fruit was relatively high at 2 DAA and decreased from 0.97% to 0.36% in the period from 2 to 12 DAA (Figure 5). Changes in the protein content indicated a change in metabolic activities during the development of the fruit. The protein content of the fruit decreased during growth and development because the protein in fruit mainly acts as enzymes rather than reserves [8].

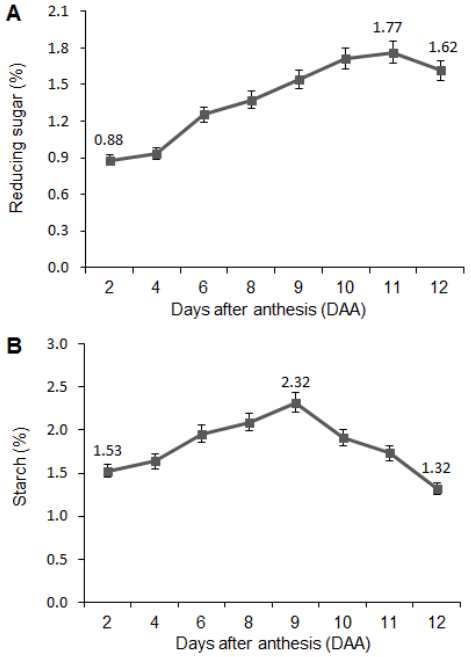


Fig. 4. *Changes in the content of reducing sugars (A) and starch (B) of cucumber fruit during development*

At 2 DAA, the lipid content in cucumber fruit was relatively high at 0.26%, then increased as the fruit grew and reached a maximum value of 0.83% at 9 DAA (Figure 5). After 9 DAA, the lipid content decreased and reached 0.33% at 12 DAA ($P< 0.05$). The decrease in lipids is due to the strong metabolism in the fruit. Under the action of the lipase enzyme, lipids are hydrolyzed to provide materials and energy for respiration when the fruit begins to ripen [25].

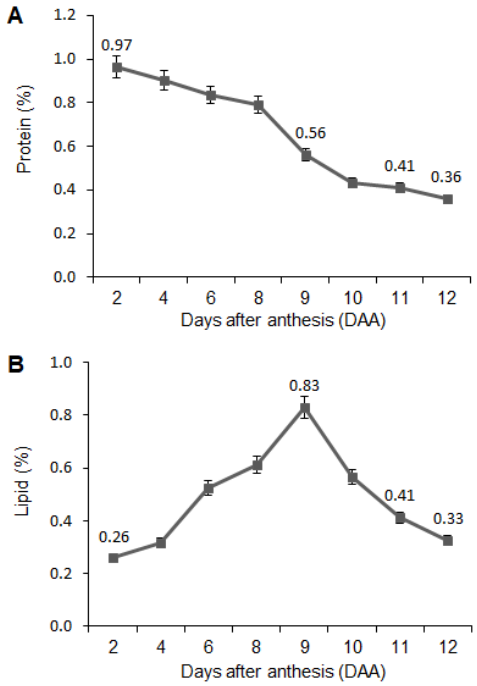


Fig. 5. *Changes in the content of protein (A) and lipid (B) of cucumber fruit during development*

### 3.5. Changes in Total Organic Acids and Vitamin C Content during Fruit Development

After initial cucumber fruit formation, the total organic acid content was 36.58 mg/100g at 2 DAA. The total organic acid content increased gradually from 2 to 9 DAA, reaching a maximum of 53.13 mg/100g at 9 DAA (Figure 6). From 9 to 12 DAA, the total organic acid content decreased since organic acids are used in respiration to provide energy for starch synthesis. The plant also requires energy for the biosynthesis of fruit-specific ripening substances, such as enzymes for hydrolysis, esters to create an aroma for the ripening fruit, and synthesis of sugars to create sweetness in the fruit, resulting in a decrease in total acid content [19].

Vitamin C is synthesized in the plant and is a key nutritional component of humans, making vitamin C content an important indicator to assess the nutritional value of many fruits [18]. The vitamin C content increased from 2 to 11 DAA, reaching a maximum value of 4.52 mg/100g at 11 DAA (Figure 6). After 11 DAA, the vitamin C content decreased due to its use in the biosynthesis of ethylene, oxalate, and tartrate in the fruit [23].

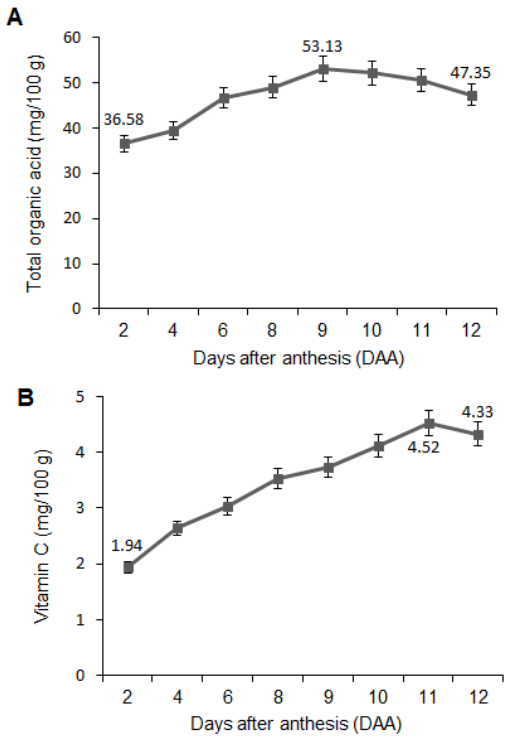


Fig. 6. *Changes in the content of total organic acids (A) and vitamin C (B) of cucumber fruit during development*

### 3.6. Changes in Tannin and Pectin Content during Fruit Development

Tannin compounds are abundant in many plant species and act as plant growth regulators. Eating unripe fruit with

acrid tannins produces unpleasant feelings of dryness and puckering in the mouth [15]. The tannin content increased rapidly from 0.92% at 2 DAA to 1.94% at 9 DAA and decreased from 1.94% at 9 DAA to 1.50% at 12 DAA (Figure 7). The decline from 9 to 12 DAA is due to the decomposition of tannins into pyrogallol and $CO_2$ when the fruit enters the physiological ripening stage [6].

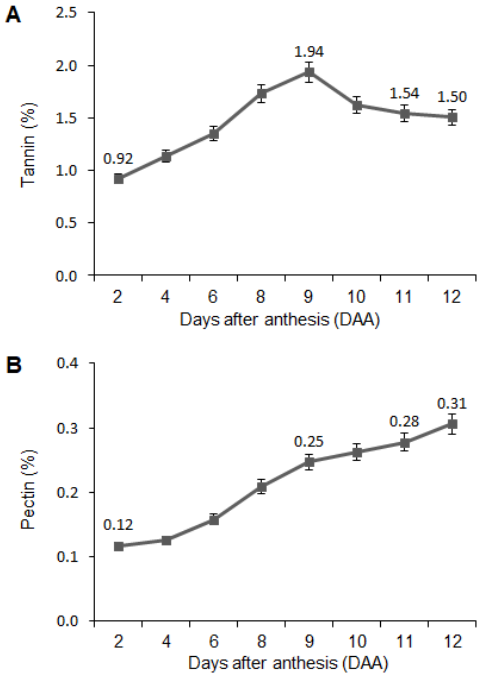


Fig. 7. *Changes in the content of tannin (A) and pectin (B) of cucumber fruit during development*

Pectin is found in many fruits, especially within the peel. During fruit development and ripening, the pectin content varies depending on the type of fruit and its characteristics as it ripens [3]. The pectin content in cucumber was relatively low at 2 DAA (0.12%), then increased slowly with the growth of the fruit until it reached 0.31% at 12 DAA (Figure 7). The slow change in fruit pectin content supports the common observation of cucumbers slowly softening in texture from formation to maturity. The fluctuations of pectin content in cucumber fruit were also consistent with the fluctuations of pectin content observed throughout the growth and development of oranges [10].

## 4. Conclusions

At 11 DAA, cucumber fruits reached nearly their maximum size (in length, diameter, volume, and weight) and were green due to their high chlorophyll and low carotenoid content. Components such as starch, total organic acids, proteins, lipids, pectin, and tannins varied with fruit growth and development. At 11 DAA, cucumber fruit had maximum reducing sugar and vitamin C contents. After 11 DAA, these components of fruit decreased, indicating that cucumber fruit should be harvested at 11 DAA to maximize the nutritional value of the fruit. However, more studies of the physiological and biochemical relationships among different cucumber varieties need to be undertaken.

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