

ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND CHLOROPHYLL CONTENT OF KENINGAU GROWN *CUCUMIS SATIVUS* L. AT TWO GROWTH STAGES

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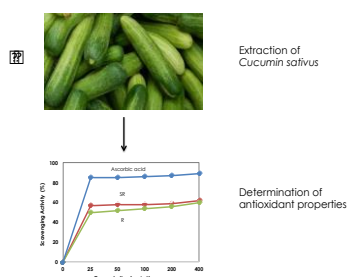
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Graphical abstract



Abstract

The maturation stage of *Cucumis sativus* is among the important factors affecting its composition and quality. Hence, this study monitored the differences in total phenolic content (TPC), antioxidant activity, pigment and colour of Keningau-grown cucumbers (*Cucumis sativus* L.) at two stages of maturities, namely the semi-ripe (SR) and ripe (R). The colourimetric and spectroscopic findings revealed significant differences in the assessed variables ($P < 0.05$) in the two growth stages except for the pigment. The colour of semi-ripe cucumbers was of lower L^* (33.39 ± 4.26) and a^* (-10.00 ± 1.74) mean values, while the ripe cucumbers registered the corresponding mean values of 36.71 ± 2.85 and -8.90 ± 1.85 . R cucumbers gave a higher mean b^* coordinate (16.38 ± 3.16) over the SR ones (14.52 ± 2.52). Compositions of pigments, namely, chlorophyll-a (SR: 4.86 ± 0.01 $\mu\text{g/mL}$, R: 3.55 ± 0.00 $\mu\text{g/mL}$), chlorophyll-b (SR: 2.12 ± 0.02 $\mu\text{g/mL}$, R: $1.79, 0.02$ $\mu\text{g/mL}$) and total chlorophyll were higher in SR (6.98 ± 0.02 $\mu\text{g/mL}$) than R (5.34 ± 0.02 $\mu\text{g/mL}$) cucumbers, except for the composition of carotenoids (SR: 0.82 ± 0.01 $\mu\text{g/mL}$, R: 1.78 ± 0.01 $\mu\text{g/mL}$). The TPC in SR was higher (424.21 ± 5.32 mg/g) than the R ones (185.51 ± 4.62 mg/g), with the corresponding antioxidant activity (IC_{50}) for SR and R at 157.98 ± 1.57 and 191.66 ± 2.58 $\mu\text{g/mL}$, respectively. TPC and antioxidant activity between the SR and R cucumbers were negatively correlated (-0.992), which meant that not all phenolic compounds were involved in free radical scavenging.

Keywords: *Cucumis sativus* L, total phenolic content, antioxidant activity, pigment

Abstrak

Tahap pematangan *Cucumis sativus* adalah antara faktor penting yang mempengaruhi komposisi dan kualitasnya. Kajian ini memantau perbezaan kandungan fenolik (TPC), aktiviti antioksidan, pigmen dan warna timun Keningau (*Cucumis sativus* L.) pada dua tahap kematangan, iaitu semi matang (SR) dan matang (R). Penemuan kolorimetrik dan spektroskopi menunjukkan perbezaan yang signifikan dalam pemboleh ubah yang dinilai iaitu ($P < 0.05$) pada dua peringkat pertumbuhan kecuali pigmen. Warna timun separuh masak mempunyai nilai L^* (33.39 ± 4.26) dan nilai a^* (-10.00 ± 1.74), sementara timun masak mencatatkan nilai b^* yang bersamaan dengan 36.71 ± 2.85 dan -8.90 ± 1.85 . Timun R memberikan koordinat b^* min yang lebih tinggi (16.38 ± 3.16) berbanding yang SR (14.52 ± 2.52). Komposisi pigmen, iaitu, klorofil a (SR: $4.86 \pm 0.01 \mu\text{g} / \text{mL}$, R: $3.55 \pm 0.00 \mu\text{g} / \text{mL}$), klorofil b (SR: $2.12 \pm 0.02 \mu\text{g} / \text{mL}$, R: $1.79, 0.02 \mu\text{g} / \text{mL}$), jumlah klorofil (SR: 6.98 ± 0.02 , R: $5.34 \pm 0.02 \mu\text{g} / \text{mL}$) lebih tinggi dalam SR daripada timun R, kecuali untuk komposisi karotenoid (SR: $0.82 \pm 0.01 \mu\text{g} / \text{mL}$, R: $1.78 \pm 0.01 \mu\text{g} / \text{mL}$). TPC dalam SR lebih tinggi ($424.21 \pm 5.32 \text{ mg} / \text{g}$) daripada yang R ($185.51 \pm 4.62 \text{ mg} / \text{g}$), dengan aktiviti antioksidan yang sesuai (IC50) untuk SR dan R pada 157.98 ± 1.57 dan $191.66 \pm 2.58 \mu\text{g} / \text{mL}$. TPC dan aktiviti antioksidan antara timun SR dan R berkorelasi negatif (-0.992), yang bermaksud bahawa tidak semua sebatian fenolik terlibat dalam pembersihan radikal bebas.

Kata kunci: *Cucumis sativus* L, kandungan fenolik, aktiviti antioksidan, pigmen

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1.0 INTRODUCTION

The maturation of fruits involves a complex process that causes changes in colour, texture, taste, and aroma [1], alongside changes in nutrient content. Hence, the appropriate harvest time must be established so that consumers can maximally benefit from the highest nutrient composition in the fruit [2]. Fruits can be divided into two groups, the climatic and non-climatic, characteristically defined by the increase in respiratory-related to ethylene production, and contrariwise, in the latter [1]. The fruit of interest in this study is the cucumber, scientifically known as *Cucumis sativus* L., a widely grown vegetable from the family of *Cucurbitaceae*, which also includes other fruit-producing vegetables [3]. Although cucumber is eaten as a vegetable, it is scientifically a fruit because of the closed seeds that developed from the flower [4].

According to Brasil & Siddiqui (2018), the cucumber should be harvested in its almost full-sized, semi-mature phase, but well before the fruit fully matures and turn hard. The cucumbers also have a short lifespan of less than 14 days. The fruit quality deteriorates after harvest due to a relatively high moisture loss, alongside other damages due to bumps and the general loss of shape during transportation storage [6]. The maturation stage is among the important factors affecting the composition and quality of fruits and vegetables [7]. This is because different maturation stages affect the composition of fruits, including antioxidant elements, and impart variable biochemistry, physiology, and structure that directly influence the phytochemical content [8]. For instance, Rodriguez et al. (2016) discovered the total

phenolic content and polyphenolic composition of interspecific hybrid palm oil extracted from *Elaeis oleifera* × *Elaeis guineensis* (O × G, Coari × La Mé cultivar) during the fruit ripening process 18, 20, 22, and 24 weeks after anthesis showed marked differences in radical scavenging activity in relation to total phenolic content, with the highest at 18 weeks.

A study by Osorio et al. (2013) revealed that during maturation, the tomato changes the degradation of chlorophyll-and lycopene formation, where the green color turns red and increased in ethylene production, as well as the synthesis of sugar, acids, and aromatic compounds. This is because changes in the quality and phytochemical compositions in plants can occur appropriately during maturity [10]. Moreover, the aforesaid features tend to vary within the same species and between species [11]. Likewise, this study's findings may prove useful to consumers, where the suitable time to harvest the cucumber showing the highest phytochemical contents and antioxidants can be made known. By analysing cucumbers in the SR and R maturation stages, more information on the biological nature of this crop may be brought to light, and plausibly the data can be made available to farmers in deciding the best time to harvest the cucumbers. *Ipsa facto*, a rationally harvested batch of cucumbers will impart maximal nutritional benefits to the consumer.

That said, this study aimed to quantify the antioxidant activity, total phenolic content (TPC), pigment, and colours in semi-ripe (SR) (five to six weeks old) and ripe (R) (ten weeks old) Keningau-grown cucumbers. The cucumber seeds were planted and harvested according to the above-said durations from the first day of emergence. It is important to note

that the aforesaid factors have yet to be explored in such cucumbers, and therefore should be investigated. Lastly, the study compares the TPC and antioxidant activity in cucumbers of the SR and R stages, with the use of Pearson's product-moment correlation. The findings in this study will augment our comprehension on the nutritional differences in cucumbers at the two maturation stages and also open doors to scientific endeavours that can further improve their nutritional quality.

2.0 METHODOLOGY

2.1 Materials

The main materials used in this study are semi-ripe (SR) and ripe (R) *Cucumis sativus* L., grown in Keningau, Sabah, Malaysia. Chemicals, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, gallic acid (C₇H₆O₅), ascorbic acid (C₆H₈O₆), petroleum ether, ethanol and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (St. Louis, USA). Copper sulphate (CuSO₄), potassium sulphate (K₂SO₄), sodium hydroxide (NaOH), sulphuric acid (H₂SO₄), boric acid (H₃BO₃), hydrochloric acid (HCl), hydrogen peroxide (H₂O₂), and nitric acid (HNO₃) were purchased from Merck (Darmstadt, Germany). The chemicals used in this study were all analytical grade.

2.2 Sampling Method

A sampling of Keningau cucumbers was done using a completely randomised design method to ensure representative results. All 30 cucumber samples were collected on the same day in October 2019. The cucumbers were rinsed under running tap water to remove dirt and then wiped dry by laboratory tissue. The 30 randomly-collected cucumbers were then categorised into 2 groups, namely 15 cucumbers each for the semi-ripe (SR) and ripe (R) stage. Each group was further divided into three individual composites. The R cucumbers were from 10 weeks old harvest from anthesis [12], while SR cucumbers were collected at five weeks old. Average of the size and weight of the cucumbers were 10 cm and 500 g, respectively. Figure 1 shows the location of the vegetable farm for cucumbers sampling.

2.3 Analysis of Colour

The colours of the fresh cucumbers were using the colourimetric method using the CR-400 Chroma Meter (Minolta Co., Ltd., Osaka, Japan). The difference in the colour of the cucumber skin was ascertained by dividing each sample into three equal parts, where the colour in this section is recorded. It is pertinent to indicate here that the colour observed in this study is represented in L*, a*, b* (CIELAB) colour area coordinates. The L* coordinates define the darkness or lightness of the colour and range from black (0) to

white (100), whereas the a* and b* coordinates indicate the direction of the colour. Coordinates +a*, -a*, +b* and -b* lean toward the red, green yellow and blue color, respectively [13].

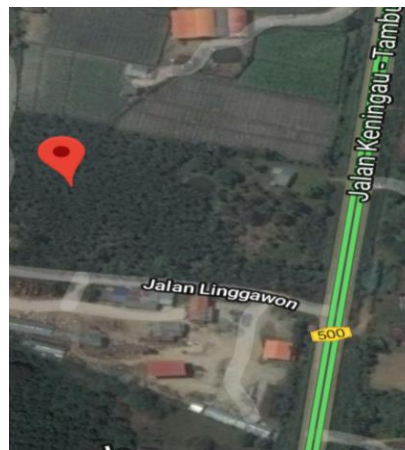


Figure 1 A map of cucumber sampling is carried out in Keningau, Sabah

2.4 Analysis of Pigment

The analysis was done according to the method of Costache et al. (2012) with minor modifications. The cucumber skin was first peeled and left to dry in an oven for three days at 40°C. Next, the skin (10 g) was ground into a fine powder, and then the powder (2 g) was transferred on an Erlenmeyer flask containing methanol (100 mL) and agitated for 30 mins at 200 rpm, and the mixture was centrifuged (10, 000 xg, 15 min). The supernatant was collected and then read under a UV-Vis spectrophotometer at 666 nm, 653 nm and 470 nm, to quantify the contents of chlorophyll-a, chlorophyll-b, and carotenoids, respectively [14]. Estimations of Chlorophyll a and b, and chlorophyll contents were illustrated in Equations 1–3.

$$\text{Chl a} = 15.65 A_{666} - 7.340 A_{653} \dots\dots\dots (1)$$

$$\text{Chl b} = 27.05 A_{653} - 11.21 A_{666} \dots\dots\dots (2)$$

Where, A signifies the absorption at 666 nm and 653 nm, Chl a is the concentration of chlorophyll-a (µg/mL), Chl b represents the concentration of chlorophyll-b (µg/mL), and the chlorophyll content is calculated as the amount of chlorophyll-a and chlorophyll-b (µg/mL).

$$C_{x+c} = (1000A_{470} - 2.270 \text{ Chl a} - 129.2 \text{ Chl b})/245 \dots\dots\dots (3)$$

Where, A is the absorption at 470 nm and, C_{x+c} refers to the concentration of carotenoids (µg/mL)

2.5 Analysis of Total Phenolic Content

Powdered cucumber sample was transferred into a 2 L Erlenmeyer flask containing methanol and was left to soak for two days in darkness at room temperature (1 000 mL methanol: 50 g powdered cucumber). Next, the mixture was filtered through a Whatman No. 1 paper. The solution was vacuum-evaporated at 40°C using a rotary evaporator until the sample volume was reduced to ~50 mL. The concentrated liquid sample was lyophilized and stored at -20°C until further analysis [15]. The powdered cucumber samples' total phenolic content (TPC) was ascertained using the Folin-Ciocalteu method that used gallic acid as the standard. The lyophilized sample was dissolved in distilled water to a concentration of 0.4 mg/mL. Then, an aliquot of each sample (0.5 mL) was mixed with the Folin-Ciocalteu reagent (0.5 mL) and distilled water (1.0 mL) and left to stand for 5 minutes. Then, sodium carbonate solution (0.5 mL, 10% w/v) was added, the mixture vortexed and was further incubated for 1 h at room temperature. The UV absorption was measured at 760 nm using distilled water as the control. Varying concentrations of gallic acid, 0.06, 0.08, 0.1, and 0.12 mg/mL, were prepared in distilled water to obtain the standard curve. The TPC value was expressed as a milligram equivalent of the gallic acid (mg GAE/g extract) [15] according to the following Equation 4:

$$C = \frac{cV}{m} \dots\dots\dots (4)$$

Where C signifies the TPC (mg/g) c is the concentration of gallic acid from the standard calibration curve (mg/mL), V is the volume of extract (mL) and m is the mass of the cucumber extract (m).

2.6 Analysis of Antioxidant activity

The scavenging free radical activity of the SR and R extracts was estimated using the DPPH method. DPPH stock solution was prepared by dissolving 1 mg of DPPH powder into an Erlenmeyer flask containing 98% methanol (100 mL) in a dark room. Ascorbic acid was used as the standard where 4 mg of ascorbic acid was added to 98% methanol (5 mL) and then made up to a concentration of 800 g/mL. The solution was

incubated for 30 min in room temperature, and the absorption was measured at 517 nm [15]. Percentage of scavenging activity was calculated using Equation %:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100 \dots (3.5)$$

Where, Abs control = DPPH radical absorption + methanol and Abs of sample = DPPH radical absorption + sample extract.

2.7 Statistical Analysis

The data collected in this study were assessed for significance and correlation using the Statistical Package for the Social Sciences (SPSS) version 26.0. Results of colour analysis, pigments, TPC, and antioxidant activity, were analysed using the Independent T-test to compare the mean difference between the cucumbers at the two different maturation stages [16]. The association between the SR and R cucumbers' TPC and antioxidant activity was established using Pearson's product-moment correlation.

3.0 RESULTS AND DISCUSSION

3.1 Colour

Colour analysis among the unripe and ripe cucumber was carried out using the colorimetry method and CR-400 chroma meter to measure the sample colour. In general, the L* coordinates defined the color's darkness or lightness and have a range of black (0) to white (100). The coordinate a* also states the colour direction where +a* is towards the red colour, while the -a* is towards the green colour. The b* coordinates, +b*, indicate that the color is towards the yellow color and -b* towards the blue color. Table.1 shows the colour parameters L*, a*, and b* in unripe and ripe cucumber (*C. sativus* L.).

Table 1 The colour coordinates for the SR and R stage cucumbers (*C. sativus* L.).

Colour	Mean ± standard deviation	
	SR	R
L	33.39 ± 4.26 ^a	36.71 ± 2.85 ^b
a*	-10.00 ± 1.74 ^a	-8.90 ± 1.85 ^b
b*	14.52 ± 2.52 ^a	16.38 ± 3.16 ^b

The values with the same superscript on each row indicate that the values were not significantly different from each other (p < 0.05).

As can be seen, the L* coordinates for the SR and R cucumber were 33.39 ± 4.26 and 36.71 ± 2.85 , respectively, which signified that cucumber samples of the former were significant of a darker colour than the latter ($p < 0.05$). The same was observed for coordinates a* and b* in the SR (-10.00 ± 1.74 , 14.52 ± 2.52) compared to the R cucumbers (-8.90 ± 1.85 , 16.38 ± 3.16). The SR cucumbers were significantly greener (a*) on average over cucumbers of the R stage, whereby the colour intensity (b*) of the skin reduced with maturation ($p < 0.05$). The higher intensity in the R cucumbers was characteristically a more yellow colour than those of the SR.

The outcome is explicable by the fact that cucumbers harvested at a later stage are greener from the degradation of chlorophyll and the increased exposure of carotenoid (yellow hue), a phenomenon commonplace in matured fruits [17]. The decline in chlorophyll content with maturation is because of senescence, reducing the green colour's intensity [18]. The higher mean intensity and darker green colour in SR cucumbers have to do with the chlorophyll in the cells being predominantly at the bottom of the epidermis but is less so with increasing maturation as the cells move closer to the surface of the cucumber skin [19]. Results seen here were supported findings of previous work by Nambi *et al.* (2015) that investigated unripe and ripe mangoes, where the L*, a*, and b* coordinates increased with increasing maturation. The trend seen here was apparent from the corresponding mean values of coordinates, L*, a*, and b*, for which unripe mangoes registered averages of 51.86 ± 2.13 , -11.32 ± 2.55 , and 27.75 ± 3.95 , respectively. In contrast, ripe mangoes were observed at 65.05 ± 2.02 , 16.43 ± 2.41 , and 48.81 ± 4.1 , respectively.

3.2 Pigment

Chlorophyll is a pigment found in almost every green plant, and it can be divided into two types, namely, chlorophyll-a and chlorophyll-b. Generally, chlorophyll-a is found in nearly all photosynthetic organisms and aids in converting light energy to chemical energy through photosynthesis. Conversely, chlorophyll-b has a different function, mainly as an assisting role alongside chlorophyll-a in the photosynthesis process [14,20]. The mean \pm standard deviation of the SR and R cucumbers' spectrophotometric measurements are tabulated in Table 2.

The SR cucumbers recorded chlorophyll-a, chlorophyll-b and carotenoid contents that were significantly different between the groups, corresponding to $4.86 \pm 0.01 \mu\text{g/mL}$, $2.12 \pm 0.02 \mu\text{g/mL}$ and $0.82 \pm 0.01 \mu\text{g/mL}$ ($p < 0.05$), respectively. On the other hand, the R stage of cucumbers showed lower contents of chlorophylls a and b at 3.55 ± 0.00 and $1.79 \pm 0.02 \mu\text{g/mL}$, respectively, except for the higher carotenoids content ($1.78 \pm 0.01 \mu\text{g/mL}$). The mean total chlorophyll for the SR cucumbers was significantly higher at 6.98 ± 0.02 than the R ones at 5.34 ± 0.02 (p

< 0.05). Mean contents of chlorophyll-a and b were higher in the SR cucumber and declined steadily with maturity as the carotenoid increased. This has to do with chloroplast change into a chromosome that ensues the subsequent degradation of the chlorophyll pigment and synthesis of carotenoids [22].

Table 2 Contents of chlorophyll-a, chlorophyll-b, chlorophyll, and carotenoid ($\mu\text{g/mL}$) in the SR and R stage cucumbers (*C. sativus* L.)

Pigment	Mean \pm standard deviation ($\mu\text{g/mL}$)	
	SR	R
Chlorophyll-a	4.86 ± 0.01^a	3.55 ± 0.00^b
Chlorophyll-b	2.12 ± 0.02^a	1.79 ± 0.02^b
Total chlorophyll	6.98 ± 0.02^a	5.34 ± 0.02^b
Carotenoids	0.82 ± 0.01^a	1.78 ± 0.01^b

The values with the same superscript on each row indicate that the values are not significantly different ($p < 0.05$).

Moreover, post-harvest storage tends to affect pigmentation on the cucumber skin. It is said that changes in surrounding conditions can affect the contents of chlorophyll. The degree of available light also plays an important role in deciding the chlorophyll content on the cucumber's skin and the contents of chlorophyll-a and b. The presence of both types of chlorophylls in the skin of cucumbers of different growth stages is also affected by changes in temperature and amount of ethylene, where temperatures between 20–28 °C, and high quantities of ethylene expedite the de-greening of fruits [22]. The higher content of carotenoids in the R cucumber with the concomitant decrease in the total chlorophyll with maturity is expected. The same observation was reported by Sun *et al.* (2019) [23], and in our case, the cucumber skin switched colour from green to yellow at a later stage of maturity.

3.3 Total Phenolic Content

The TPC for cucumber extract using the Folin-Ciocalteu reagent is expressed in the gallic acid equivalent (mg/g of GAE), in which the obtained standard curve equation was $y = 1.298x - 0.0021$, $R^2 = 0.9948$. The standard calibration of gallic acid and the TPC of the SR and R cucumbers are shown in Figure 2 and Table 3, respectively.

According to Table 3, the mean TPC of the SR and R stage cucumbers was 424.21 ± 5.32 mg/g of GAE and 185.51 ± 4.62 mg/g of GAE, respectively. Independent T-test results for the TPC in the SR and R stage cucumber extracts indicated a significant difference between the two maturity stages ($P < 0.05$). The mean TPC in the SR stage cucumber is significantly higher than in the R stage cucumbers, largely because the latter has a richer content of biologically active ingredients, i.e., such as phenolic compounds [24]. A study by Puteri Nor Zulaikha (2019) similarly

reported the decline in the mean TPC in R stage cucumbers. However, the SR cucumbers showed twice the amount of phenolics than R cucumbers. This is supported by Alexandros *et al.* (2018), which also described a trend in declining TPC in the SR fruit with increasing maturity due to the gradual reduction in phenolic acids with increasing fruit maturity [27].

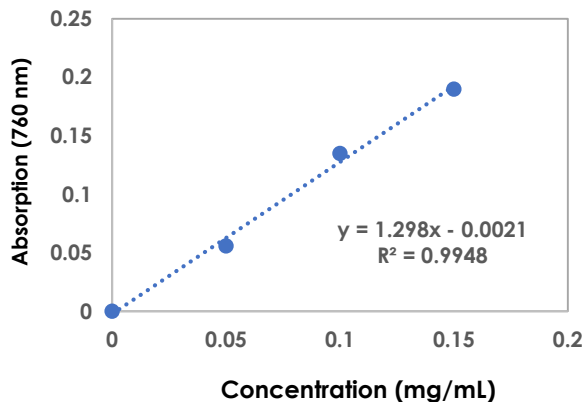


Figure 2 Gallic acid standard calibration curve

Table 3 The TPC of SR and R stage cucumbers expressed in the equivalent gallic acid (mg/g GAE)

TPC (mg/g GAE)	Mean \pm standard deviation	
	SR	R
	424.21 \pm 5.32 ^a	185.51 \pm 4.62 ^b

The values with the same superscript on each row indicate that the values are not significantly different from each other ($p < 0.05$)

Comparatively, a study by Yunusa *et al.* (2018) recorded a mean TPC for R stage cucumbers at 19.16 ± 1.07 mg GAE/g, significantly lower than the TPC in the Keningau-grown R cucumbers in this study (185.51 ± 4.62 mg of GAE/g) ($p < 0.05$). The notable difference may be due to the chemical treatment used to extract the phytochemicals from the cucumbers. This is because the diversity in solvent-selective power can variably extract different kinds of hydrophobic phenols or hydrophilic compounds to varying degrees of efficacy [28], aside from other factors, for instance, pH, temperature, extraction time, and sample composition [29]. Similarly, the TPC for the R stage cucumbers reported by Olajire & Azeez (2011), at 101.33 ± 13.05 mg GAE/g, was also lower than that reported in this study. Possible reasonings for this are likely to be genetic and the influence of environmental factors such as climate, soil mineral composition, transport condition, and the cucumbers' storage condition. Plus, high enzyme-catalyzed conversions of phenolic compounds to other secondary metabolites occur as the fruit matures [31], as previously reported by Fernando *et al.* (2013), which were linked to elevated pigment degradation for reabsorption into the plant.

The decrease in the total TPC as the fruit matures is also associated with an increased reduction of organic acids in the cells, thus directly affects the antioxidant activity. This is because several factors greatly influence polyphenols compositions; a notable one is maturation level [33]. The same was observed for the snake-skin fruit and the rambai fruit, in which their mean TPCs decreased proportionally as the fruits mature [34]. Wang *et al.* (2018) indicated that the chemical structure's instability might cause a further reduction in phenolic contents, as the compound is inadvertently converted to other substances during biosynthesis. Despite this, there are also exceptions to certain fruits that bear higher TPC at the R stage than the SR stage. Good examples are strawberries and tomatoes, but not apples which TPC is higher at the SR stage. This feature is particularly important for consumers to benefit from optimal nutrition when consuming natural food [36].

The Pearson's product-moment correlation analysis between the total chlorophyll and TPC a significant positive correlation ($r = 1.000$ $P < 0.05$). The amount of chlorophyll content correlated well with the TPC of two stages of cucumber extracts. Carotenoid content and the TPC were negatively correlated ($r = -1.000$ $p < 0.05$) and significant for the SR and R stage cucumbers. This shows that the two variables in cucumbers harvested between the two maturity stages were inversely correlated, and for the consumer to enjoy both benefits in the plant may not be possible. Thus, a halfway option may be available where the Keningau grown cucumbers is best harvested at 7-8 weeks old, i.e., between the SR and R growth stage.

3.4 Antioxidant Activity

Figure 3 depicts the DPPH radical scavenging activity, calculated at different concentrations (25, 50, 100, 200, and 400 $\mu\text{g/mL}$). At the same time, Table 4 shows the value of IC_{50} , the half-maximal inhibitory concentration, for the extract of *Cucumis sativus L.*, collected at the SR and R stage. As can be seen, the mean radical scavenging activity of the SR cucumber extract was higher compared to the R cucumber extract and the ascorbic acid standard. A significant difference was noted between the IC_{50} value of the SR and R cucumbers, in which the SR cucumber has a lower IC_{50} value (157.98 ± 1.57 $\mu\text{g/mL}$) compared to the R cucumber extract (191.66 ± 2.58 $\mu\text{g/mL}$). Notably, a lower IC_{50} value indicates a higher antioxidant activity [34].

The T-test results indicated a significant difference in the SR and R stage cucumber extracts' antioxidant activity ($P < 0.05$). As shown in Table 4, the lower IC_{50} value in the SR cucumber extract over that of the R stage ones represents the higher antioxidant capacity, associated with increased scavenging activity of DPPH [37]. For comparison, Yunusa *et al.* (2018) reported the cucumber's antioxidant activity extracted using ethanol as the solvent material, at 20.18 ± 6.07 %, lower than that seen in this study. In

another study, the antioxidant activity of mango decreased from $66.23 \pm 0.43\%$ to $51.93 \pm 0.62\%$ when the fruit reached maturity. The same was also observed for fruits, snake-skin and rambai, which exhibited higher antioxidant activity than the SR stage fruits (84.45 ± 0.19 , over the R ones [34]. The difference between antioxidant activity seen in Keningau grown cucumbers harvested at the SR and R stage can also be influenced by different climatic conditions [38]. However, the lack of clear trends on the aforesaid factors' phenolic compounds could be linked to their varying decomposition rate, influenced by changes in the surrounding condition [39].

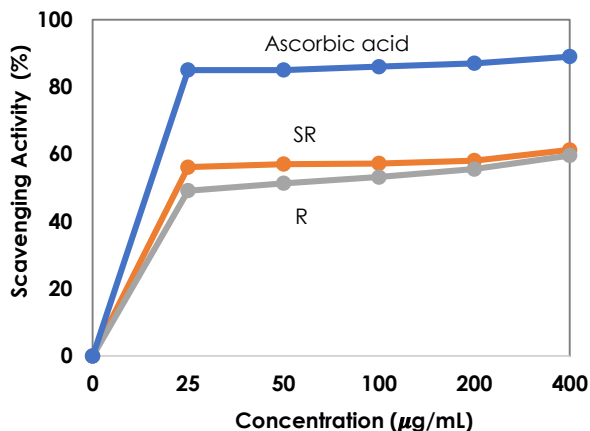


Figure 3 The DPPH radical scavenging activity of SR and R cucumber (*C. sativus* L.)

Table 4 The antioxidant activity in the SR and R cucumber (*C. sativus* L.)

IC ₅₀ (µg/mL)	Mean ± standard deviation	
	SR	R
	157.98 ± 1.57 ^a	191.66 ± 2.58 ^b

The values with the same superscript on each row indicate that the values are not significantly different on $p < 0.05$.

Pearson's product-moment correlation analysis between antioxidant activity and TPC revealed that the two variables were significantly negatively correlated ($r = 0.992$, $p < 0.05$). The outcome seen here meant that not all of the phenolic compounds in the SR and R stage cucumber extracts participated in the observed radical scavenging activity. The same may apply to explain the negative and significantly correlated chlorophyll pigment and antioxidant activity ($R = -0.994$, $p < 0.05$). Lastly, the content of carotenoids in the SR and R stage cucumbers exhibited positive correlations ($r = 0.993$, $p < 0.05$) with antioxidant activity. This outcome undoubtedly supported carotenoids' reactivity relationship in the SR and R cucumber extracts to scavenge for free radicals.

4.0 CONCLUSION

Thus, it was demonstrated that the SR cucumber has a darker green colour, but the intensity increases towards yellow in the R cucumbers. While a higher mean amount of chlorophyll was observed in SR cucumbers, while the carotenoid content was higher in the R cucumbers. Meanwhile, the colour change seen in SR to R cucumbers could be associated with the pigment content. The outcome conveyed that the mean total chlorophyll in the cucumbers decreases as the fruit matures, consequently forming more yellow-pigmented carotenoids. The mean TPC in the SR cucumber may be higher value, but the study found that phenolic acid contents decreased as the cucumber reached maturity. Interestingly, the significant negatively correlated ($r = 0.992$, $p < 0.05$) Pearson's product-moment between antioxidant activity and TPC meant that not all phenolic compounds in the SR and R stages cucumber extracts participated in radical scavenging activity. The same trend was observed for the chlorophyll pigment and antioxidant activity ($R = -0.994$, $p < 0.05$). Lastly, the positive correlations ($r = 0.993$, $p < 0.05$) between antioxidant activity and carotenoid content in the SR and R stage cucumber extracts strongly supported the compounds' reactivity relationship to scavenge free radicals.

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