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Different Cutting Methods Affect the Quality of Fresh-Cut Cucumbers by Regulating ROS Metabolism

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Abstract: Fresh-cut cucumbers (*Cucumis sativus* L.) are appreciated by consumers for their convenience and freshness. In the process of home cooking and in the food industry, different cutting methods for cucumbers are needed. In order to explore the effect of cutting methods on the quality of fresh-cut cucumbers, cucumbers were cut into slices, pieces, and strips and whole cucumbers were used as the control. The results indicate that the vitamin C content of the sliced, pieced, and stripped cucumbers was gradually reduced, while the glutathione content increased significantly ($p < 0.05$) compared with the whole cucumbers. Furthermore, this study reveals that the fresh-cutting operation induced the production of ROS ($O_2^{-}\cdot$ and H_2O_2). Simultaneously, cutting activates phenylalanine ammonia-lyase and peroxidase activity, which enhanced the total phenol content by 1.35 times, 1.51 times, and 1.78 times in the pieced, stripped, and sliced cucumbers, respectively. This combines with the enhancement in the ascorbate peroxidase, glutathione reductase, superoxide dismutase, and catalase activity, contributing to the antioxidant capacity increasing by 1.14–1.95 times compared with the control. In conclusion, the degree of quality indexes was sliced > pieced > stripped. Therefore, this study provides useful information to illuminate the mechanism of the quality change in fresh-cut cucumbers subjected to different cutting methods and makes suggestions on the appropriate cutting style for the commercial or home use of cucumbers.

Keywords: fresh-cut cucumber; cutting methods; reactive oxygen species; quality

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1. Introduction

Fresh-cut fruits and vegetables are ready-to-eat fresh fruits and vegetables that have undergone various procedures such as classification, trimming, cleaning, cutting, coring, dressing, preservation, and packaging; these procedures make them convenient, safe, nutritious, and fresh to consume [1]. Additionally, the waste materials generated during the fresh-cutting process, such as skin residue and cores, can be recycled and reused, contributing to the reduction in urban domestic waste and environmental protection [2,3]. In recent years, fresh-cut fruits and vegetables have been increasingly favored by consumers due to their convenient characteristics of being ready-to-eat, ready-to-use, and ready-to-cook [4]. During fresh-cut produce processing, cutting operations may induce the explosion of reactive oxygen species (ROS) in the injured and surrounding tissues; this can lead to a faster deterioration process including oxidative browning, tissue softening, and development of off-flavors, thus limiting the shelf-life of fresh-cut produce [3,5].

Simultaneously, wounded products would activate various responses to regulate ROS levels, which allows for physiological modifications that aid in survival and protect the wounded tissue further. Studies have shown that the ascorbic acid–glutathione (AsA–GSH) cycle plays an important role in enhancing stress resistance and maintaining product quality [4,6,7]. Moreover, wounding stress would activate the phenylpropanoid metabolic

pathway to produce secondary metabolites, including phenolic compounds and lignin. This, in turn, enhances the adaptability of the organism to adapt to the stress caused by wounding. This phenomenon has been observed in various fresh-cut products such as apple [8], pitaya [9], potato [10], broccoli [11], carrot [12], lettuce [13], mushroom [14], onions [15], and celery [16].

Cucumber (*Cucumis sativus* L.) is an important vegetable crop with a vast cultivation area. It has become a very popular vegetable in people's daily diet, not only because of its sensorial properties and economic importance but also for its antioxidants, vitamins, pantothenic acid, and minerals that are important to maintaining human health [17]. In recent years, fresh-cut cucumbers have gained extensive worldwide attention due to their convenience and health benefits, and their application in the market has gradually increased [18,19]. Generally, during the processing of fresh-cut cucumbers, different cutting methods including cutting into pieces, strips, and slices were necessary for western vegetable salads and the prefabricated food industry or home use.

Previous studies have demonstrated that cutting styles have an obvious influence on the quality of fresh-cut carrots [20], onions [15], potatoes [10], and pitaya [9]. Interestingly, a previous study on fresh-cut broccoli revealed that the cutting type had no significant difference on the chlorophyll, total soluble solid, and reduced glutathione content [20]. However, previous research on fresh-cut cucumbers has primarily focused on preservation technology [18,19], and there is still a lack of scientific certification and systematic research on whether different cutting methods affect the quality of fresh-cut cucumbers.

Therefore, this study aimed to explore the mechanism of quality change based on the comparative analysis of the lightness, whiteness index, total soluble solid content, weight loss, respiration rate, firmness, and antioxidant substance content in fresh-cut cucumbers subjected to slicing, piecing, and stripping. Moreover, this study takes the ROS mechanism as the core, is focused on investigating the ROS content, ROS metabolism-related enzyme activities, and antioxidant substances content, in order to illuminate the mechanism of ROS in regulating the quality of fresh-cut cucumbers.

2. Materials and Methods

2.1. Sample Preparation and Treatment

Cucumber (*Cucumis sativus* L.) variety named "Zhongnong" was harvested in June 2022 and transported to the laboratory within 2 h. Fresh cucumbers, uniform in size, color, ripeness, and free of physical damage, were thoroughly washed with tap water and then sterilized in sodium hypochlorite (0.2 mL L^{-1}). After being rinsed twice with water, the selected cucumbers were cut into strips, pieces, and slices with a sharp knife. The wounded surface diameter of the strips, pieces, and slices was manually measured, and the wounding intensity (A/W) was defined as the ratio of the new surface area created by wounding in cm^2 divided by the tissue weight in g according to the method of Surjadinata and Cisneros-Zevallos [21]. The wounding intensities (A/W) of the whole, stripped, pieced, and sliced cucumbers were calculated as 0, 1.65, 1.76, and 3.20 m^2 kg^{-1}, respectively.

Fresh-cut cucumbers (100 g) were loaded into a polypropylene container and packaged with polyethylene films (Miuge Chemical Commodities Science and Technology Co., Ltd., Hangzhou, China). The whole cucumber was also packaged with polyethylene films at 4 °C, which was used as the control in this experiment. The polyethylene film sheets were 200 mm × 300 mm, and 0.01 μm thick, and the CO_2 transmission rate, O_2 transmission rate, water vapor transmission rate, and resistant temperature of the film were 363,000 cm^3 m^{-2} d^{-1} atm^{-1}, 10,030 cm^3 m^{-2} d^{-1} atm^{-1}, 25 g m^{-2} d^{-1}, and −60~110 °C. The samples including the whole cucumber and fresh-cut cucumbers were stored in the dark at 4 °C and 80–90% relative humidity for 5 d. The experiment was repeated using separate batches of "Zhongnong" cucumbers on two different occasions, with three replicates each time.

2.2. Colour Parameters, Weight Loss, Respiration Rate, and Firmness Assay

The color including lightness (L^*), redness (a^*), and yellowness (b^*) was evaluated according to the CIELAB colorimetric system using a CR400 colorimeter (Konica Minolta Inc., Tokyo, Japan). Three samples from each group were chosen to measure the surface color, taking an average of three surface measurements per sample. The whiteness index (WI) was used to evaluate the color of the sample; it was calculated as the following: $WI = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$ [22]. Weight loss was measured according to the differential method [10]. The respiration rate was determined according to Zhou et al. [23], and the result was expressed as mg kg^{-1} h^{-1}. The firmness was determined according to the method reported by Hu et al. [10]. Briefly, the test speed was 1.0 mm s^{-1}, the penetration distance was 8 mm, and the measurement was averaged over six replicates corresponding to the maximum force (N).

2.3. Total Soluble Solid (TSS), Ascorbic Acid (AsA), and Glutathione (GSH) Content Assay

A total of 4 g of cucumber was ground with 20 mL distilled water and then filtered by filter paper (30–50 μm). The cucumber juice was used for the determination of the TSS content by using a hand-held refractometer (PAL-1, Aiago Co., Ltd., Tokyo, Japan) and the result was expressed as %.

The AsA content was analyzed according to the procedure used by Guan et al. [11]. Briefly, 5 g cucumber powder was mixed with 20 mL 2% oxalic acid solution. Then, the mixture was filtered and the supernatant was used to determine the AsA content (mg kg^{-1}) through 2,6-dichlorindophenol titration.

The method for the GSH content (μmol kg^{-1}) assay was performed according to the instructions of commercially available kits (Nanjing Jiancheng Technology Co., Ltd., Nanjing, China). Frozen cucumber powder (0.1 g) was mixed with 1 mL buffer and then centrifuged at 8000× *g* for 10 min at 4 °C, and the supernatant was used to measure the GSH content, and the GSH content was expressed as μmol kg^{-1}.

2.4. Total Phenols, Lignin Content, and PAL Activity Assay

The frozen cucumber sample (5 g) was mixed with 20 mL 80% ethanol, shaken well, and extracted in the dark at 40 °C for 40 min. After centrifuging at 15,000× *g* for 30 min, the collected supernatant was used to determine the total phenol content. The reaction system was used according to the method reported by Hu et al. [24], which consisted of 1 mL Folin-Ciocalteu reagent, 1 mL supernatant, 10 mL Na_2CO_3 solution, and 14 mL water, then, the absorbance value was determined at 750 nm. The result of the total phenol content was expressed as mg kg^{-1} from the gallic acid standard curve ($y = 0.0051x + 0.0178$, $R^2 = 0.999$).

The frozen powder of the fresh-cut cucumber was put into the air oven at 80 °C for the drying operation to constant weight, then passed through a 40-mesh sieve prepared for the lignin content assay. The lignin content was determined using assay kits (Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) by measuring the absorbance at 280 nm, and the result was expressed as g kg^{-1}.

PAL activity was assayed as described by Hu et al. [24]. The crude enzyme was extracted by sodium borate buffer (50 mM, pH 8.5, containing 5 mM b-mercaptoethanol and 2 mM EDTA), and then L-phenylalanine (20 mM) was added before incubating for 60 min at 37 °C. The reaction was stopped by adding 0.1 mL of 6 M HCl, and the absorbance value was determined at 290 nm; 1 unit of PAL activity was equal to a change of 0.01 at 290 nm per min, and expressed as U kg^{-1} protein.

2.5. $O_2^{-}\cdot$ and H_2O_2 Content Assay

Frozen tissues (0.10 g) were homogenized with 1 mL of buffer coming from the $O_2^{-}\cdot$ and H_2O_2 kit (Suzhou Keming Biotechnology Co., Ltd., Suzhou, China). After centrifuging at 20,000× *g* at 4 °C for 15 min, the supernatants were collected and used for the $O_2^{-}\cdot$ and H_2O_2 content assay. The $O_2^{-}\cdot$ and H_2O_2 contents of the cucumbers were measured by the absorbance at 415 and 530 nm, respectively. The $O_2^{-}\cdot$ content was expressed

as mmol kg^{-1} based on the sodium nitrite standard curve, and the H_2O_2 content was expressed as mmol kg^{-1} based on the fresh weight.

2.6. Antioxidant Enzymes Activity Assay

The ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) enzyme extractions were performed according to the instructions on the plant kits (Suzhou Keming Biotechnology Co., Ltd., Suzhou, China). The reaction system of the APX, GR, POD, SOD, and CAT activity determined the absorbance at 290, 340, 470, 560, and 240 nm, respectively. The antioxidant enzyme activities were expressed as U kg^{-1}, where $U_{APX} = 0.01 \times \Delta A_{290\,nm}$ per min, $U_{GR} = 0.01 \times \Delta A_{340\,nm}$ per min, $U_{POD} = 0.01 \times \Delta A_{470\,nm}$ per min, $U_{SOD} = 0.01 \times \Delta A_{560\,nm}$ per min, and $U_{CAT} = 0.01 \times \Delta A_{398\,nm}$ per min.

2.7. Antioxidant Activity Assay

The DPPH and ABTS free radical scavenging assays were performed according to the procedure of Chen et al. [8] with some modifications. The extraction method was the same as the extracts prepared for the total phenol assay, four times dilution of ethanol extraction solution for the detection of the DPPH and ABTS free radical scavenging capacity, and the results were expressed as nmol Trolox equivalents per kg (nmol Trolox kg^{-1}) of fresh tissue. The OH· scavenging activity (in nmol Trolox kg^{-1}) and ferric-reducing antioxidant power (FRAP) (in Trolox mmol kg^{-1}) were determined according to the instructions of the relevant kits (Suzhou Keming Biotechnology Co., Ltd., Suzhou, China).

2.8. Statistical Assay

IBM SPSS 20 (IBM Corp., Armonk, NY, USA) was applied to perform an analysis of variance (ANOVA). A significant difference was calculated by using Duncan's test with 95% level of confidence ($p < 0.05$) among the treatments.

3. Results

3.1. Colorimetric Values

Generally, the fresh-cutting process with wounding stress induces a variety of physiological responses and affects the quality of fresh-cut products [25]. Among these, the color and appearance quality are the important parameters that affect consumers' willingness to accept and buy fresh-cut products. Among these, the lightness (L^*) indicates the color depth, and the larger the lightness, the brighter the fresh-cut products. Furthermore, the WI could reflect the extent of the fade due to physiological disorders in the fresh-cut cucumber; the larger the WI, the more severe the fading phenomenon. In this study, the L^* and WI change in fresh-cut cucumbers subjected to three cutting methods, including stripping, piecing, and slicing, were explored. The results revealed that the L^* in the cutting treatment groups decreased, and the degree of the L^* level was stripped > pieced > sliced > whole (Figure 1a).

On the contrary, the WI of the fresh-cut cucumbers increased, especially after 2 days, when the WI increased sharply, and the degree of the WI value was sliced > pieced > stripped > whole (Figure 1b). At 5 day, the WI values of the sliced, pieced, and stripped cucumbers increased by 8.13%, 4.85%, and 3.95% compared with the whole cucumber, respectively, which demonstrated that the WI value increased as the wounding intensity increased. The above results proved that there was an obvious influence on the lightness and whiteness index in fresh-cut cucumbers with different cutting methods, which was similar to a previous study of fresh-cut potatoes [10].

3.2. Weight Loss and Total Soluble Solid

Cucumber is a vegetable with a high water content, thus, fresh-cutting and subsequent storage were easy to cause water loss and further resulted in a loss of quality in the fresh-cut cucumbers [18]. This study found that the weight loss in all the groups showed an upward trend during the storage period, among which, the maximum weight loss appeared in the

stripped group (Table 1). At 5 day, the weight loss in the stripped, pieced, sliced, and whole cucumbers was 1.26%, 1.13%, 0.72%, and 0.38%, respectively.

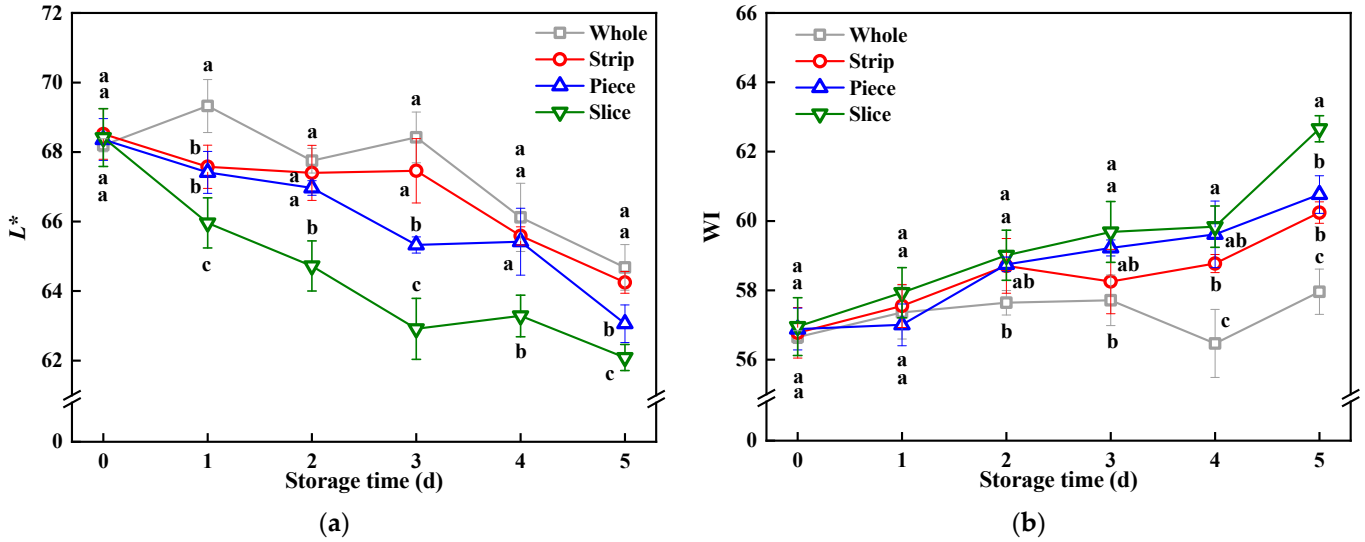

Figure 1. Effect of cutting methods on lightness (*L**) (**a**) and whiteness index (WI) (**b**) of fresh-cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

Table 1. The effect of different cutting styles on the quality of fresh-cut cucumbers stored at 4 °C.

Storage Time (Day)	Treatment	Total Soluble Solid (%)	Weight Loss (%)	Respiration Rate (mg kg^{-1} h^{-1})	Firmness (N)	Ascorbic Acid (mg kg^{-1})	Glutathione (mg kg^{-1})
0	Whole	0.81 ± 0.03 a	0	51.64 ± 1.68 a	21.76 ± 0.90 a	19.97 ± 1.04 a	0.11 ± 0.004 a
	Stripped	0.82 ± 0.05 a	0	53.36 ± 0.98 a	21.83 ± 0.84 a	20.81 ± 0.94 a	0.12 ± 0.007 a
	Pieced	0.77 ± 0.09 a	0	52.09 ± 2.31 a	20.10 ± 1.21 a	21.93 ± 0.80 a	0.12 ± 0.008 a
	Sliced	0.82 ± 0.05 a	0	52.66 ± 0.75 a	20.64 ± 0.95 a	20.44 ± 1.31 a	0.11 ± 0.004 a
1	Whole	0.75 ± 0.05 b	0.09 ± 0.01 c	47.69 ± 1.39 b	19.84 ± 0.47 a	20.17 ± 0.75 a	0.10 ± 0.002 c
	Stripped	0.98 ± 0.04 a	0.29 ± 0.05 a	36.11 ± 1.20 c	17.05 ± 0.61 b	21.05 ± 1.41 a	0.13 ± 0.002 b
	Pieced	0.98 ± 0.04 a	0.24 ± 0.05 ab	44.80 ± 2.44 b	16.83 ± 1.02 b	20.64 ± 1.51 a	0.13 ± 0.005 b
	Sliced	0.98 ± 0.05 a	0.12 ± 0.08 b	56.18 ± 0.92 a	14.88 ± 1.49 c	15.62 ± 0.97 b	0.15 ± 0.007 a
2	Whole	0.78 ± 0.08 c	0.16 ± 0.02 d	55.30 ± 0.66 b	18.71 ± 0.76 a	18.69 ± 0.91 a	0.11 ± 0.003 c
	Stripped	1.03 ± 0.03 ab	0.60 ± 0.07 a	40.07 ± 0.70 c	15.82 ± 0.80 b	10.26 ± 0.81 c	0.13 ± 0.004 b
	Pieced	1.09 ± 0.04 a	0.46 ± 0.05 b	51.81 ± 2.25 b	15.09 ± 0.90 b	15.04 ± 1.32 b	0.14 ± 0.005 a
	Sliced	0.98 ± 0.06 b	0.31 ± 0.01 c	59.47 ± 1.86 a	11.93 ± 0.89 c	16.02 ± 1.41 b	0.15 ± 0.004 a
3	Whole	0.73 ±0.04 d	0.25 ± 0.04 d	36.32 ± 0.94 c	19.49 ± 0.94 d	19.34 ± 1.07 a	0.11 ± 0.004 b
	Stripped	0.88 ± 0.04 c	0.83 ± 0.09 a	59.49 ± 1.02 b	33.84 ± 0.83 a	14.91 ± 0.90 b	0.13 ± 0.005 a
	Pieced	1.08 ± 0.07 a	0.65 ± 0.03 b	62.31 ± 1.40 b	32.04 ± 0.65 b	15.00 ± 1.06 b	0.13 ± 0.006 a
	Sliced	0.92 ± 0.07 b	0.43 ± 0.09 c	72.30 ± 0.98 a	25.68 ± 0.84 c	16.20 ± 1.29 b	0.13 ± 0.007 a
4	Whole	0.82 ± 0.07 b	0.33 ± 0.04 c	30.15 ± 0.90 c	20.35 ± 1.28 b	18.61 ± 1.21 a	0.11 ± 0.004 c
	Stripped	1.01 ± 0.03 a	1.01 ± 0.10 a	59.04 ± 1.28 a	24.17 ± 0.67 a	15.43 ± 0.73 b	0.13 ± 0.003 b
	Pieced	1.01 ± 0.04 a	0.90 ± 0.07 a	54.20 ± 1.09 b	16.04 ± 0.84 c	16.58 ± 1.41 ab	0.12 ± 0.004 b
	Sliced	0.93 ± 0.06 a	0.57 ± 0.09 b	59.62 ± 2.06 a	15.36 ± 1.19 c	18.17 ± 0.98 a	0.14 ± 0.006 a
5	Whole	0.78 ± 0.04 c	0.38 ± 0.03 d	32.17 ± 0.93 c	19.34 ± 1.23 a	17.85 ± 1.47 a	0.10 ± 0.002 d
	Stripped	1.10 ± 0.03 a	1.26 ± 0.03 a	35.87 ± 1.72 b	15.76 ± 1.12 b	16.99 ± 1.03 a	0.11 ± 0.003 c
	Pieced	1.01 ± 0.04 b	1.13 ± 0.03 b	37.65 ± 0.92 b	12.69 ± 0.68 c	15.86 ± 0.85 a	0.12 ± 0.005 b
	Sliced	0.93 ± 0.08 b	0.72 ± 0.01 c	66.46 ± 2.16 a	11.54 ± 1.00 c	17.76 ± 1.22 a	0.13 ± 0.004 a

Note: Data are expressed as the mean ± SD (n = 3). Values with different letters are significantly different at $p < 0.05$ among the treatments.

Soluble solid is one of the important indexes to judge the nutritional quality of fruits and vegetables [26]. As shown in Table 1, compared to the whole sample, the fresh-cutting treatment enhanced the total soluble solid (TSS) content in the cucumbers. After storage for 5 day, the TSS values of the stripped, pieced, and sliced cucumbers were 1.10%, 1.01%, and 0.93%, which was 41.02%, 29.49%, and 19.23% higher than that in the control group in the same period, respectively. Interestingly, the trend in the TSS content was consistent with the trend in the weight loss, and the significant positive correlation between the weight

loss and TSS content confirmed that the fresh-cutting operation increased the weight loss and then resulted in a higher concentration of soluble solids in the samples. Furthermore, previous studies in fresh-cut broccoli [7], onion [27], and ginger [28] reported that the cutting treatment accelerates the decomposition of macromolecular substances into small carbohydrate substances to provide adequate energy required in the metabolic process. Therefore, we extrapolated the degradation of substances such as protein and starch which may be another reason for the TSS content enhancement in fresh-cut cucumbers.

3.3. Respiration Rate and Firmness

Respiratory intensity is an important condition to maintain the freshness of agricultural products, and also a necessary index which affects the shelf life of fresh-cut fruits and vegetables [29]. The present study of fresh-cut cucumbers revealed that the respiration rate was decreased during 1–2 day of storage in the stripped and pieced samples, then increased and decreased again in the later storage time (Table 1). Whereas the respiration rate of the sliced cucumbers was active through cutting in the entire storage period, and the highest respiration rate appeared in the sliced samples subjected to the highest wounding intensity. With the extension in the storage time, the respiration rate of the sliced, pieced, and stripped cucumbers reached the peak value on the third day, and they increased by 99.04%, 71.56%, and 63.79% compared with the whole sample. The above results revealed that the cutting resulted in physiological stress on the plant tissues, triggering the respiratory metabolism in the fresh-cut cucumbers [1,23]. The respiration rate levels in the fresh-cut cucumbers were sliced > pieced > stripped > whole during the later storage time, which indicated that different cutting methods have a significant influence on the respiratory metabolism in fresh-cut cucumbers, and this may be an important reason that directly affected the cucumber tissue senescence [30].

The firmness showed the same downward trend from the beginning to the first 2 day of storage; this may be because the cell tissue in the fresh products was destroyed by cutting and then led to the increase in pectinase activity, which accelerated the decomposition of the cell wall and tissue softening in the fresh-cut cucumbers [31,32]. In addition, the process of hydrolysis of starch and degradation of pectin may be another reason for the declining firmness of the sample [33]. However, at a storage time of 3 day, the firmness of the sliced, pieced, and stripped samples increased rapidly to 1.32, 1.64, and 1.74 times that before storage. The reason for this result may be that the wound-induced callus and closing layer at the wound site then contributed to the substantial increase in firmness at a storage time of 3 day [34].

3.4. Reactive Oxygen Species (ROS) Content

In this study, to evaluate the different cutting methods for the production of ROS, we measured the $O_2^{-}\cdot$ and H_2O_2 content in fresh-cut cucumbers. The results showed that fresh-cutting induced the production of $O_2^{-}\cdot$, and the highest values were found in the sliced sample at 2 day; it was increased by 3.07-folds compared with the whole group (Figure 2a). However, the highest levels in the pieced and stripped samples appeared at 1 day and 3 day, respectively, which indicated that the different cutting styles have a significant influence on the ROS production in fresh-cut cucumbers.

Similarly, the H_2O_2 content was enhanced obviously in the cucumbers after the cutting operation, and the maximum values of the sliced, pieced, and stripped samples were increased by 11.20 times, 7.35-times, and 4.89 times, respectively, compared with the control group. During the whole storage time, the H_2O_2 values were sliced > pieced > stripped > whole (Figure 2b), which revealed that the H_2O_2 content increased as the wounding intensity increased. This may be caused by the cutting operations attacking the unsaturated fatty acids in the cell plasma membrane and organelle membrane, leading to the decrease in the activity of cell membrane-binding enzymes, which destroys the normal redox balance of cells, thus causing the explosion of ROS [3,35]. Furthermore, the correlation analysis showed that there was a significant positive correlation between the H_2O_2 content and

WI, whereas there was a negative positive correlation between the H_2O_2 content and L^* (Figure 3, demonstrating that the H_2O_2 content plays an important role in the whiteness phenomenon of fresh-cut cucumbers. The results in fresh-cut pitaya [2], apple [36], carrot [5], potato [23], and onion [15] have also proved the accumulation of ROS could further influence the quality such as browning, softening, and secondary metabolites synthesis.

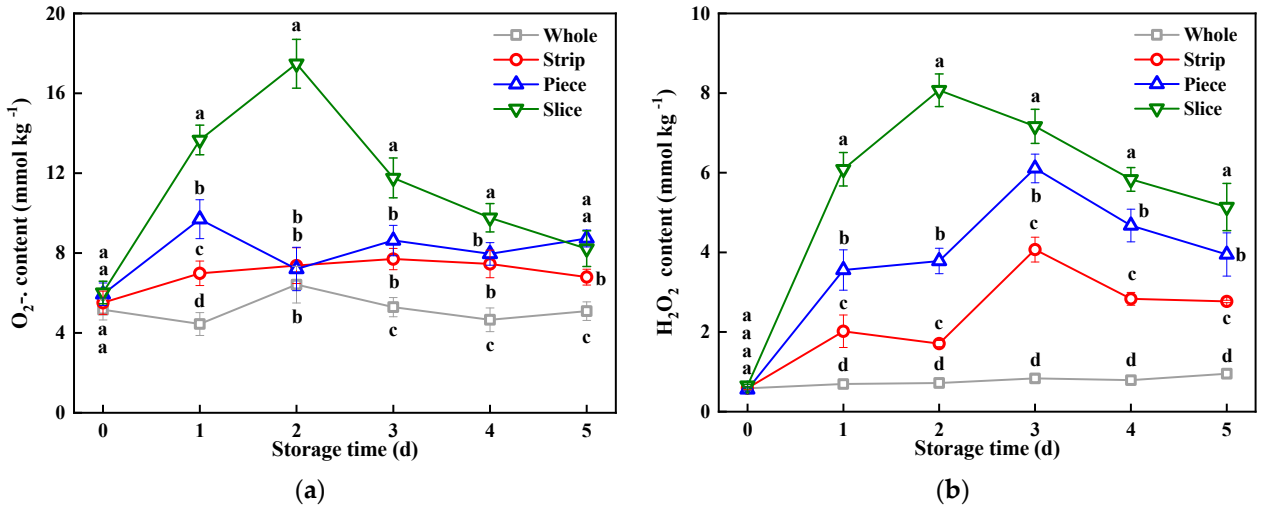

Figure 2. Effect of cutting methods on $O_2^{-}\cdot$ content (**a**) and H_2O_2 content (**b**) in fresh-cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

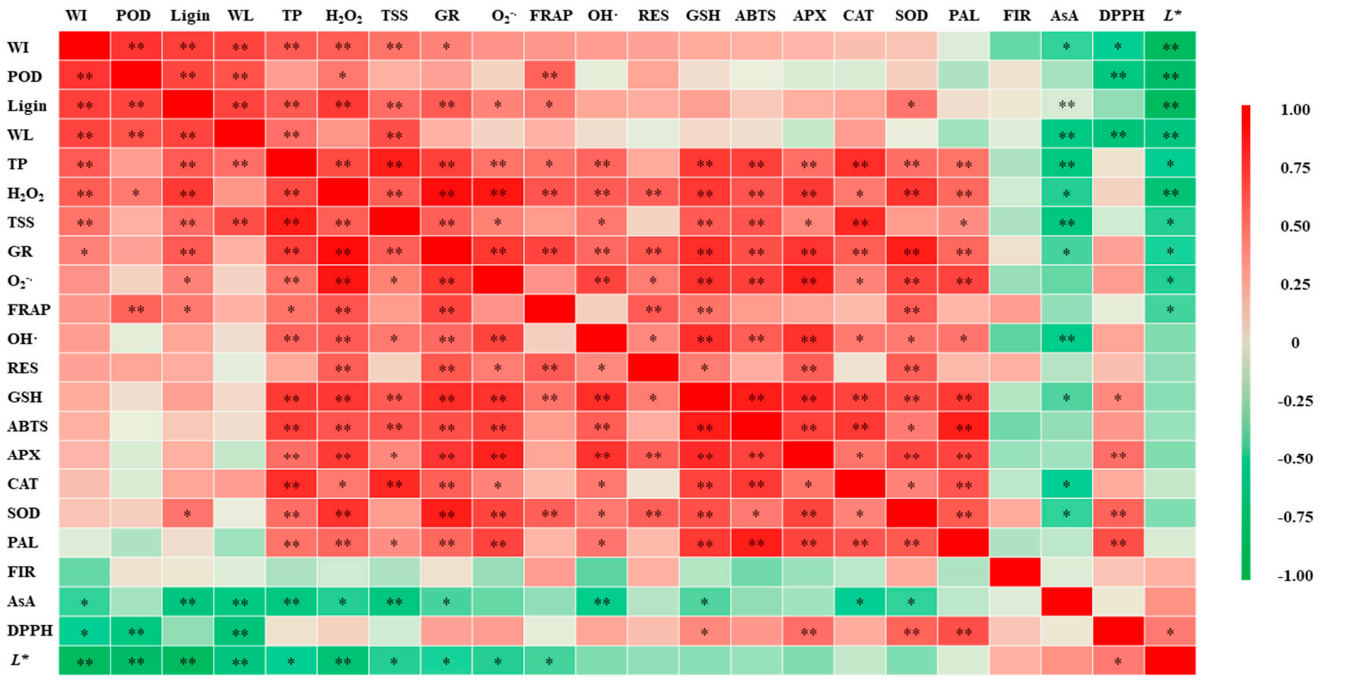

Figure 3. Pearson correlation matrix for each index. * $p < 0.05$, ** $p < 0.01$. WI, whiteness index; POD, peroxidase; WL, weight loss; TP, total phenols content; TSS, total soluble solid; GR, glutathione reductase; FRAP, ferric−reducing antioxidant power; RES, respiration rate; GSH, glutathione; APX, ascorbate peroxidase; CAT, catalase; SOD, superoxide dismutase; PAL, phenylalanine ammonia−lyase; FIR, firmness; AsA, ascorbic acid; L^*, lightness.

3.5. Ascorbic Acid–Glutathione Cycle-Related Indexes

According to Table 1, the ascorbic acid (AsA) content in the cutting-treatment cucumbers exhibited a downward trend until 2 day of storage, and the AsA content declined by

45.07%, compared with the original values. The reason for the decrease in the AsA content may be that the injured tissue of the fresh-cut cucumbers is exposed to the air, which will increase the contact area between the wounded tissue and air, and accelerate the loss rate of the AsA content [4]. On the other hand, the fresh-cutting instantly triggers the burst of ROS and then activates the decomposition of AsA to enhance the resistance to wounding stress and further protect the wounded tissue, which resulted in the decrease in the AsA content [12]. It is worth mentioning that the AsA content of the fresh-cut cucumbers was increased slightly after 2 day of storage, and the sliced samples had the highest AsA content (17.76 mg kg^{-1}) at 5 day, and there was no significant difference with the control group.

As another important component of AsA–GSH in plants, glutathione (GSH) is a tripeptide-containing sulfhydryl group with a strong antioxidant capacity [7]. Contrary to the changing trend in the AsA content, the GSH content increased during 1–2 day of storage, then decreased (Table 1). The enhancement in the GSH content in the early stage may be used to clear the ROS in the fresh-cut cucumbers and then reduce the oxidative stress [37]. Hu et al. reported that the cutting operation enhanced the GSH content generation triggered by activating the antioxidant defense mechanism in fresh-cut potatoes, thereby increasing the ability to resist wounding stress [37]. Meanwhile, Xia et al. [38] revealed that exogenous GSH treatment alleviates the aging damage of oat seeds by regulating the GSH content to maintain the integrity of the mitochondrial structural and functional systems. In our study, we found that the GSH content is significantly negatively correlated with the AsA content, which indicates the antioxidant enzymes catalyze the rapid decomposition of AsA in the process of scavenging ROS and then provide the necessary prerequisite for GSH synthesis, and further increase the GSH content [39].

In the AsA–GSH cycle, ascorbate peroxidase (APX) and glutathione reductase (GR) are the major enzymes [40]. In this study, we found that the APX activity was enhanced in the first 2 day of storage, and it increased by 118.97%, 68.97%, and 12.07% in the sliced, pieced, and stripped cucumbers, respectively, compared with the whole cucumber in the same period (Figure 4a). The APX activity decreased after 3–5 day of storage, and this changing trend was opposite to that of the AsA content, which indicated that APX catalyzes the conversion of AsA to dehydroascorbic acid with the reduction reaction of H_2O_2, thereby further scavenging free radicals in fresh-cut cucumber cells [41].

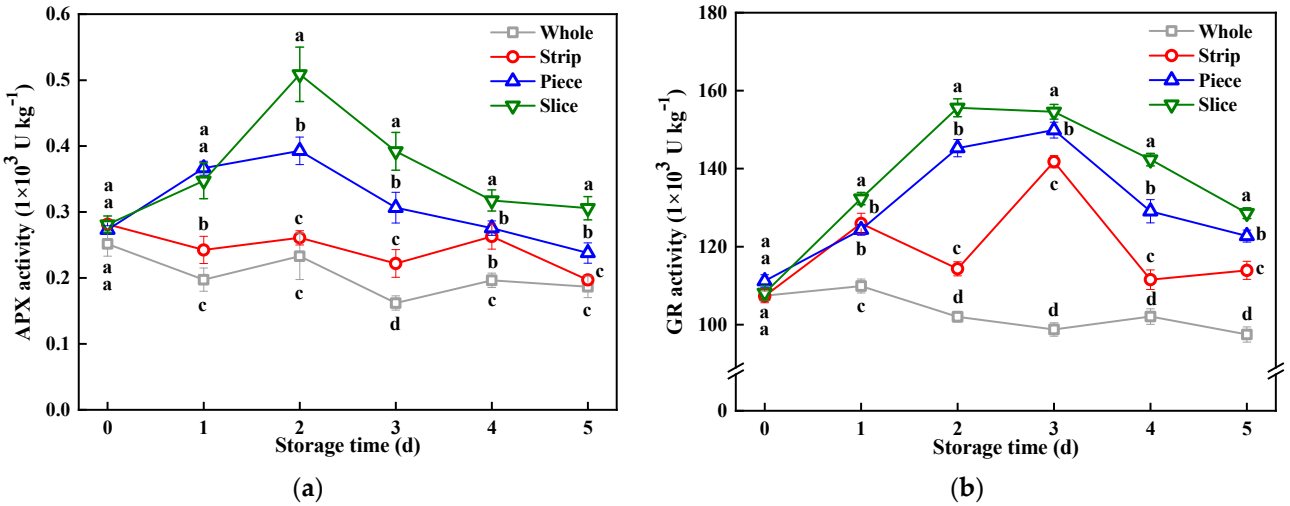

Figure 4. Effect of cutting methods on ascorbate peroxidase (APX) (**a**) and glutathione reductase (GR) (**b**) activity of fresh−cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

Generally, GR catalyzes the conversion of oxidized GSH into reduced GSH with the participation of NADPH, thus limiting the operation efficiency of the AsA–GSH cycle. As shown in Figure 4b, the GR activity in the fresh-cut cucumbers increased and then

decreased with the extension in the storage time, and the levels of the GR activity were the same as the APX activity, that is, sliced > pieced > stripped > whole during the storage time. At 3 day, it reached 154.59, 149.88, and 141.79 U kg^{-1} in the sliced, pieced, and stripped samples, respectively. In the early storage period, the increase in the GR activity may promote the conversion of oxidized GSH into reduced GSH, thereby increasing the accumulation of reduced GSH [40,42].

3.6. Phenylpropanoid Metabolism-Related Indexes

As the most important antioxidants in fruits and vegetables, phenolic compounds are synthesized through the phenylpropanoid metabolic pathway [43,44]. A previous study has reported that wounding stress induces the accumulation of phenols intended to achieve healing and defense [5]. In this study, we found that the total phenol (TP) content increased following all treatments and the highest value was found in the pieced samples at the earlier storage time (Figure 5a). However, the TP contents were arranged as whole < pieced < stripped < sliced later in the storage duration. At 5 d of storage, the TP content increased by 1.35 times, 1.51 times, and 1.78 times in the pieced, stripped, and sliced samples, respectively.

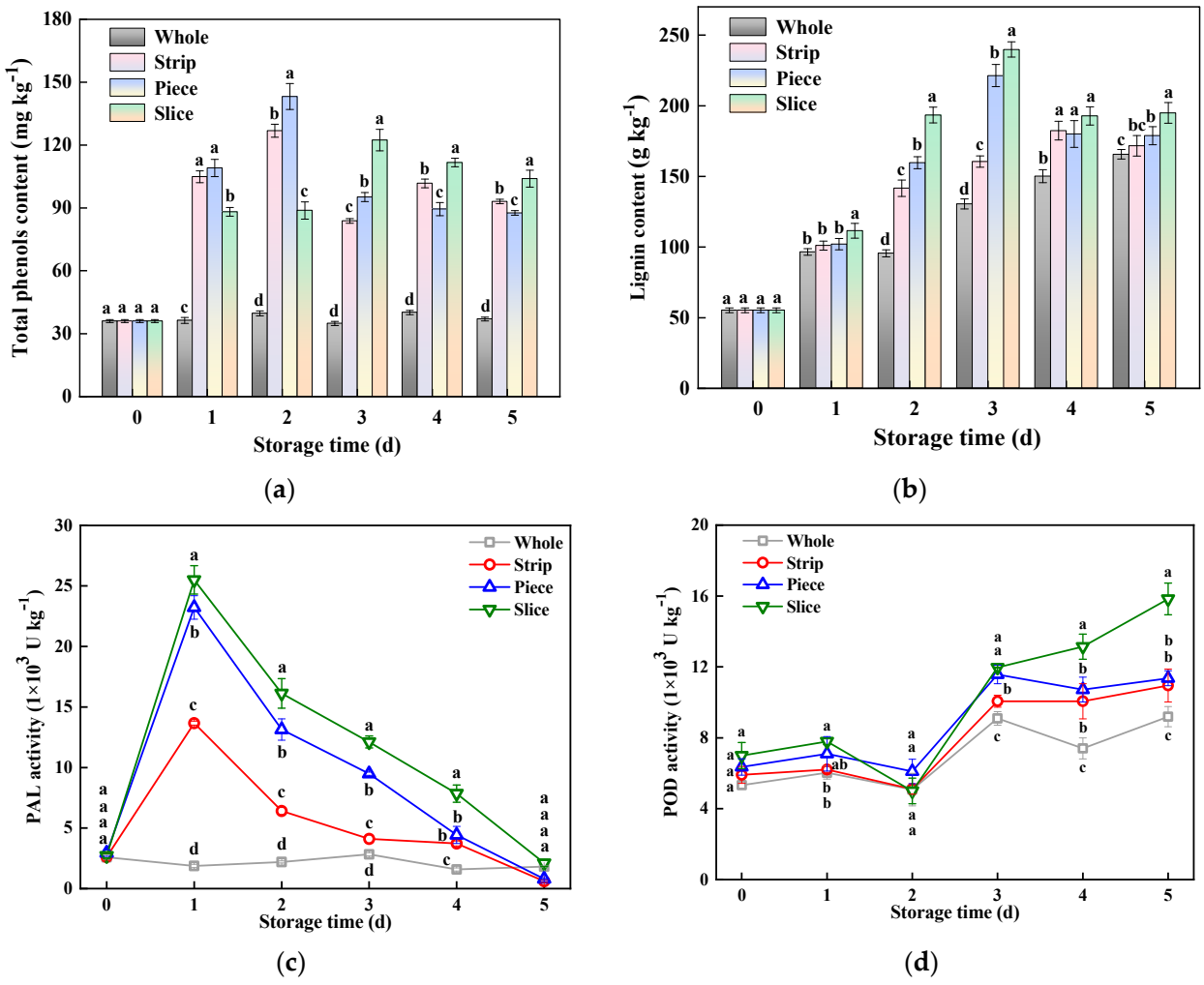

Figure 5. Effect of cutting methods on ascorbate peroxidase (APX) (**a**) glutathione reductase (GR) (**b**) phenylalanine ammonia−lyase (PAL) (**c**) and peroxidase (POD) (**d**) activity in fresh−cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

According to Figure 5b, the lignin content increased in all the cutting treatment groups, and it was higher than that in the control samples. The highest lignin content in the control samples was 165.69 g kg^{-1}, while the contents in the stripped, pieced, and sliced samples were 182.38 g kg^{-1}, 221.43 g kg^{-1}, and 239.90 g kg^{-1} ($p < 0.05$), respectively. Meanwhile, we found that there was a significant positive correlation between the lignin content and TP content (Figure 3). In addition, the lignin content was positively correlated with the WI, whereas there was a significant negative correlation with the L^* (Figure 3), which demonstrated that the synthesis of lignin accelerated the occurrence of the whiteness phenomenon in the fresh-cut cucumbers. A previous study of fresh-cut potatoes also proved that lignin could enhance the resistance of injured parts to mechanical damage [23,45].

Phenylpropanoid metabolism plays an active role in improving the resistance of fresh-cut fruits and vegetables [46]. In this pathway, phenylalanine ammonia-lyase (PAL) was the first critical enzyme which could catalyze the deamination of phenylalanine to produce phenolics [47]. As the precursor of lignin synthesis, the phenolic substances were synthesized at the site of injury and then converted into lignin with the catalysis of the peroxidase (POD) enzyme [48]. According to Figure 5c, the PAL activity increased sharply during the first 1 day of storage and then decreased. At 1 day, the PAL activity in the stripped, pieced, and sliced samples increased by 5.26, 7.95, and 9.44 times, respectively, compared with the initial value.

The POD activity showed an upward trend during the whole storage period (Figure 5d). At 5 day of storage, the POD activity in the stripped, pieced, and sliced samples increased by 19.15%, 23.50%, and 72.25%, respectively, compared with the whole cucumber. These results indicated that the cutting methods had a significant influence on the PAL and POD activity in the fresh-cut cucumbers ($p < 0.05$), and the PAL and POD activity was enhanced as the wounding intensity increased.

Furthermore, the result of the correlation reveals that the H_2O_2 content was positively correlated with the phenylpropanoid metabolism-related indexes including the TP content, lignin content, PAL, and POD activity, which illustrates that the H_2O_2 signal molecule plays an important role in regulating the phenylpropanoid metabolism to synthesize phenolic compounds and lignin [47]. A similar result was found in fresh-cut potatoes which showed that following mechanical wounds, ROS are overproduced, which contributes to the process of lignification [23]. Meanwhile, Han et al. [49] reported that ROS can be used as a second messenger to participate in the defense response against wounding stress and plays a role in signal transmission in the synthesis and accumulation of phenols in fresh-cut carrots.

3.7. Antioxidant Enzymes Activity

In this study, we evaluated the antioxidant-related enzyme activity including superoxide dismutase (SOD) and catalase (CAT). As shown in Figure 6a, fresh-cut processing causes an increase in the SOD activity in the first 3 day and a decrease later on. The CAT activity showed a similar variation compared with that of the SOD; the three kinds of fresh-cut tissues showed a higher enzyme activity compared with the control ($p < 0.05$, Figure 6b). Meanwhile, the cutting methods have an obvious effect on the SOD and CAT activity during the whole storage period.

The results of the correlation analysis between the antioxidant enzyme activity (SOD, CAT) and AsA–GSH cycle-related indexes (AsA, GSH, APX, and GR) showed a significant correlation between them. Moreover, there was also a significant positive correlation between the antioxidant enzyme activity and ROS level; these results demonstrate that the SOD, CAT complexed with the AsA–GSH cycle could finely modulate the ROS level [4]. It is worth mentioning that the resistance mechanisms of antioxidant enzymes in fruits and vegetables, with different biological characteristic responses to oxidative damage induced by wounding stress, are different. For instance, the SOD and CAT activity was enhanced sharply in fresh-cut tomatoes at the beginning of storage [50], while in a study of fresh-cut pitaya fruit and fresh-cut broccoli, it was found that the fresh-cutting treatment inhibited the

APX activity [9,51]. In our experiment, we found that the fresh-cutting treatment induces the activity in SOD, CAT, APX, and GR and further enhances the ability to scavenge free radicals in cucumbers.

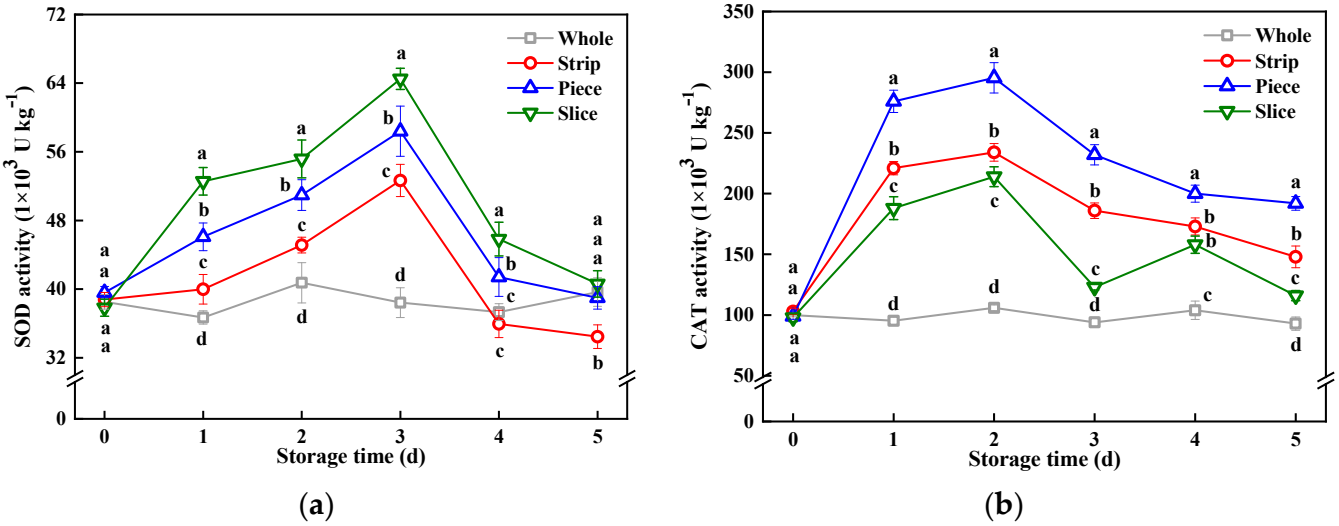

Figure 6. Effect of cutting methods on superoxide dismutase (SOD) (**a**) and catalase (CAT) activity (**b**) in fresh−cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

3.8. Antioxidant Capacity

As shown in Figure 7a,b, the DPPH and ABTS$^+$ radical scavenging capacity of the sliced, pieced, and stripped samples firstly increased and then decreased slowly, reaching a maximum at 2 day and 1 day, respectively; the DPPH radical scavenging capacity of these three groups showed increases of 42.63%, 32.93%, and 14.55%, respectively; the ABTS$^+$ radical scavenging capacity of these three groups showed increases of 40.75%, 29.39%, and 23.27%, respectively, compared with the whole cucumber. Similar to the changing trend in the DPPH and ABTS$^+$ radical scavenging capacity, the OH· radical scavenging capacity of the sliced and pieced samples increased and then decreased, whereas the OH· radical scavenging capacity of the stripped samples decreased at 1 day; it was 22.05% and 9.39% lower than the levels in the sliced and pieced groups (Figure 7c). The ferric-reducing antioxidant power (FRAP) of all the treatment groups reached a maximum at 3 day; the FRAP values of the sliced, pieced, and stripped samples increased by 95.31%, 89.06%, and 61.72%, respectively, compared with the control (Figure 7d).

In this study, the correlation between the ABTS$^+$, OH· radical scavenging capacity and the SOD, CAT activity was higher, indicating that SOD and CAT play an important role in scavenging free radicals [52]. Furthermore, we found that a higher antioxidant capacity (ABTS$^+$, OH· radical scavenging capacity, and FRAP) was observed in the sliced than in the pieced and stripped samples at the later storage time, probably because the cutting method with a high wounding intensity stimulates the antioxidant system enzymes and improves the ability to resist ROS [53]. A similar result was also found in fresh-cut pitayas [9]. However, there was no significant correlation between the antioxidant capacity and POD activity; this result may be because peroxidase family proteins (POD) are composed of proteins with different biological functions corresponding to dual biological functions of scavenging ROS and catalyzing lignin synthesis in fruits and vegetables [54,55]. A previous study showed that wounding stress upregulated POD42 and downregulated POD37 in fresh-cut broccoli, which also proved that POD proteins have various functions in oxidative metabolic processes and secondary metabolic pathways [48].

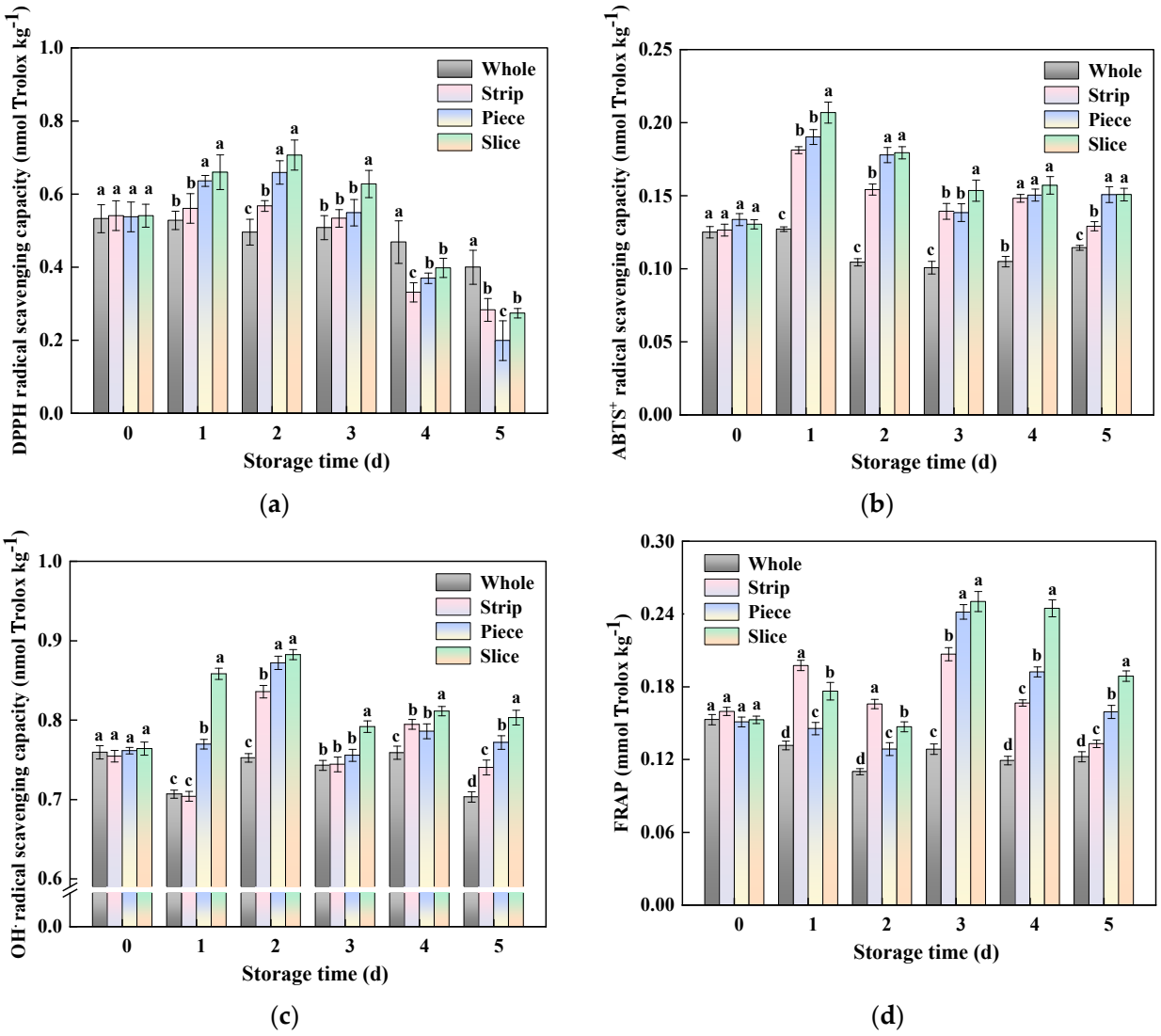

Figure 7. Effect of cutting methods on DPPH radical scavenging capacity (**a**), ABTS^{+} radical scavenging capacity (**b**), OH· radical scavenging capacity (**c**), and ferric−reducing antioxidant power (FRAP) (**d**) in fresh−cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

4. Conclusions

Cutting induced the enhancement in the weight loss, respiration rate, whiteness index, and total soluble solid content in fresh-cut cucumbers during storage, and there was a significant difference in cutting methods on these parameters. Meanwhile, activity in PAL and POD, which are associated with the phenylpropanoid metabolism, was also induced by the fresh-cutting treatment, and this resulted in the synthesis of phenols and lignin in the wounded cucumbers. The total phenol content increased by 1.35 times, 1.51 times, and 1.78 times in the pieced, stripped, and sliced samples, respectively. Furthermore, the cutting operation induced the production of ROS (O_2^{-}· and H_2O_2), then motivated the activity in antioxidant-related enzymes, including SOD, CAT, APX, and GR, leading to a 1.14–1.95 times increase in the FRAP, DPPH, ABTS^{+}, and OH· radical scavenging capacity. These phenomena are more obvious in the cutting method with the enhancement in the wounding intensity degree. The collected results reveal that different cutting methods have an obvious influence on the ROS metabolism, AsA–GSH cycle, and phenylpropanoid metabolism, which further affect the quality of fresh-cut cucumbers.

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Conflicts of Interest: The authors declare no conflict of interest.

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