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Effect of Nisin on the Quality and Antioxidant Activity of Fresh-Cut Pumpkins (*Cucurbita moschata* Duch.)

Ning Yuan [1], Yi Wang [1], Yuge Guan [2], Chen Chen [1,*] and Wenzhong Hu [1,*]

[1] College of Life Science, Dalian Minzu University, Dalian 116600, China; yn2822852617@163.com (N.Y.); w13936057663@163.com (Y.W.)
[2] School of Food and Health, Zhejiang Agricultural and Forestry University, Hangzhou 311300, China; gyg@zafu.edu.cn
* Correspondence: chenchen@dlnu.edu.cn (C.C.); hwz@dlnu.edu.cn (W.H.)

Abstract: Fresh-cut pumpkins refer to fresh pumpkin that has been graded, cleaned, peeled, sliced, preserved, and packaged. It has the qualities of freshness, nutrition, convenience, and being 100% edible. However, mechanical damages during the cutting processing can accelerate the quality deterioration, aging, and loss of nutritional values of fresh-cut pumpkins. Nisin, a natural preservative, has been widely used in fruits and vegetables with good preservation effects. To investigate the effect of different concentrations (0, 0.2, 0.4, and 0.6 g/L) of nisin on the quality of fresh-cut pumpkins, the critical indexes involved in weight loss, firmness, color, respiration intensity, reactive oxygen species (ROS) metabolism, ascorbate (AsA)—glutathione (GSH) cycle, and antioxidant capacity were monitored for fresh-cut pumpkins during storage at 4 °C for 10 days. The results showed that 0.4 g/L nisin was the best preservation concentration. Compared with 0 g/L nisin, 0.4 g/L nisin reduced the weight loss rate and whitening rate of fresh-cut pumpkins by 13.53% and 13.61%, inhibited respiration rate by 45.83%, and maintained hardness by 1.18 times. Meanwhile, 0.4 g/L nisin increased the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) and maintained higher contents of GSH and AsA. It prevented the rapid increase in ROS levels by improving antioxidant capacity, including DPPH, ABTS free radical scavenging rate, and T-AOC (total antioxidant capacity). The collected results showed that nisin has an obvious influence on the quality by regulating physiological and antioxidant activity metabolism. It is envisaged that the combination of nisin and physical and chemical preservation technology will further enhance the quality of fresh-cut pumpkins during storage in the future.

Keywords: fresh-cut pumpkins; nisin; reactive oxygen species; ascorbate-glutathione

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1. Introduction

Pumpkin is one of the most widely grown crops on the planet [1]. It is favored by consumers because of its sweet and mild flesh, as well as its high nutritional content [2]. Pumpkin polysaccharides [3], phenols (including flavonoids and phenolic acids), carotenoids [4], minerals (including potassium, phosphorus, magnesium, iron, and selenium), and vitamins (including B, C, E, and K) [5] are all found in pumpkin. These nutrients have antioxidant activity, which is useful for maintaining the body's immunological function and lowering the risk of some types of cancer, and they are especially vital for diabetics and those with vascular damage [6,7]. Thus, it is also regarded as a vegetable in many countries' healthy diets and traditional medicine. China is the world's largest producer of pumpkins. According to the Food and Agriculture Organization of the United Nations, China accounted for about one-third of the world's pumpkin production in 2021 [8]. Despite the fact that pumpkin is a store-tolerant crop, it is still wasted owing to quality degradation during the production, manufacture, transportation, retail, and home storage stages. Furthermore, the sheer size of pumpkins might make them difficult to transport and handle for the typical customer.

Fresh-cut pumpkins refer to fresh pumpkins that have been graded, cleaned, peeled, sliced, preserved, and packaged [9]. It has the qualities of freshness, nutrition, convenience, and being 100% edible. In line with consumer demands in the restaurants and retail markets for fresh-cut vegetables. However, mechanical cutting resulted in a number of quality changes that reduced the shelf life and marketability of fresh-cut pumpkins, including microbial infection, faster nutrient loss, yellowing and whitening of the cut surface, tissue softening, and flavor alterations [9]. For these reasons, food scientists have developed three different techniques. Firstly, the physical preservation method is the most widely employed for fresh-cut pumpkins because of its advantages of easy control of processing conditions and little influence on the structure and nutritional tastes, but it requires a relatively large investment in equipment [10]. Secondly, while the chemical preservation method has an excellent preservation effect, it uses chemicals that are easy to leave residue, which causes safety issues [11]. Finally, due to its safety, non-toxicity, and great efficiency, biological preservation technology has attracted special attention from scholars all over the world in recent decades, and it is utilized in a variety of foods [10].

Nisin, a bacteriocin produced by the Lactococcus lactis subspecies, is a safe and non-toxic natural biological preservative. Nisin has been widely used as a food preservative for more than 60 years. The U.S. Food and Drug Administration (FDA) classifies Nisin as Gras and other countries allow its use as a food preservative [12,13]. In addition, nisin can be rapidly digested and decomposed into various amino acids by protease, and it does not cause toxic side effects on the human body. Therefore, nisin has been widely used in the preservation of fruits and vegetables with good preservation effects [14,15]. Studies have shown that nisin combined with green tea (GTE), modified atmosphere packaging (MAP), and modified initial atmosphere (MIA) treatment on fresh-cut beet leaves greatly increased total polyphenol contents and antioxidant capacity [16]. Nisin and ε-polylysine with chitosan coating significantly ($p < 0.05$) inhibited respiration rate, decline of AsA, and white blush in fresh-cut carrots [17].

At present, there is no information available on the treatment of nisin on fresh-cut pumpkin to improve its quality and antioxidant activity during storage. Therefore, the purpose of this study is to investigate the effects of different nisin concentrations (0, 0.2, 0.4, and 0.6 g/L) on the weight loss, firmness, color, and respiratory intensity of fresh-cut pumpkins stored at 4 °C for 10 days. Additionally, a suitable concentration was chosen to measure the ROS level, antioxidant enzyme activity, antioxidant content, and antioxidant capacity of fresh-cut pumpkins in order to investigate how nisin affects their antioxidant activity. Simultaneously, the study provides a biological preservation strategy for maintaining the quality and extending the shelf life of fresh-cut pumpkins.

2. Materials and Methods

2.1. Processing and Treatments of Fresh-Cut Pumpkins

Pumpkins (*Cucurbita moschata*, Duch) of the cultivar "Luli" were obtained from a commercial farm near Wuchang City, Heilongjiang Province. The harvest area of "Luli" pumpkins was located in a high-cold and high-latitude area, with sufficient light in the growing season, long sunshine times, and a large temperature difference between day and night. The harvest area is fertile black soil, characterized by high organic content, fertile soil, and loose soil. The mature tropical pumpkins were harvested at the mature green 86 stage (65 days after flowering).

Pumpkins with uniform melon shapes and similar maturities, without diseases, pests, or mechanical damages, were selected and stored at 4 °C for later use. The pumpkins were soaked in 0.1% sodium hypochlorite water for 2 min, rinsed with distilled water, and then the skin and pulp were removed. The pumpkins were cut into 1/4 circular arc slices with a thickness of 0.5 cm and a width of 2 cm. Soak them in nisin (nisin A, purchased from Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China) solution at a concentration of 0, 0.2, 0.4, and 0.6 g/L for 10 min, and then make fresh-cut pumpkins after being drained. Then, fresh-cut pumpkins were packed inside a polypropylene container with polyethylene

films and stored at 4 °C for 10 days. Samples were taken every 2 days to determine the optimal concentration of nisin for weight loss, firmness, color, and respiration. Then the samples of the control group and the optimal concentration treatment group were ground into a powder after being frozen with liquid nitrogen, then stored at −80 °C for antioxidant index measurements.

2.2. Weight Loss

The weight of samples was weighed using an electronic balance, and three sets were set up in parallel; thus, the weight loss was expressed as the ratio of the mass difference value to the initial fresh mass (%) [18].

2.3. Firmness

The firmness of fresh-cut pumpkin slices was measured by carrying out texture profile analysis (TPA) using a TA-XT texture analyzer. The cylindrical P5 probe with a diameter of 5 mm was pressed down the slice at a rate of 1 mm/s, the trigger force was 0.5 N, and the depth of penetration was 4 mm [19]. Different parts of fresh-cut pumpkins in each treatment group were measured, and the maximum force (N) was measured as the firmness.

2.4. Color

The color (L*, a*, and b*) of fresh-cut pumpkin slices was measured by a CR-400 colorimeter, where the L* value defines brightness, the a* value defines red-green, and the b* value defines yellow-blue. The whitening index (WI) indicates the degree of whitening on the cut surface of fresh-cut pumpkins [20]. The formula is as follows:

$$WI = 100 - \sqrt{(100 - L*)^2 + (a*)^2 + (b*)^2)} \quad (1)$$

2.5. Respiration Intensity

The respiration intensity of fresh-cut pumpkins was measured by a gas analyzer (F-940 STORE II, Felix, Camas, WA, USA) [21]. Place 50 g of fresh-cut pumpkins in a 550-mL sealed crisper to store the sample at 4 °C for 1 h. Then the CO_2 content in the crisper was measured for 25 s, and repeated three times. The respiratory intensity was calculated according to the following formula:

$$\text{Respiration Intensity (mg/(kg·h))} = ((C_1 - C_2) \div 100 \times V) \div (m \times t) \times 1.96 \quad (2)$$

where C_1 represents the CO_2 content (%) in the sealed crimp box, C_2 represents the atmospheric CO_2 content (%), V represents the container volume (mL), m represents the sample mass (kg), and t represents the placement time (h).

2.6. Superoxide Anion (O_2^-·) Production Rate

Approximately 0.2 g of the frozen sample was used for the O_2^-· rate of production assay according to the protocol of the OFR measurement kit (SA-1-G, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the UV absorbances (A1) at 530 nm against a blank solution (A2) (ΔA = A1 − A2). A standard curve was generated for O_2^-· rate of production measurement (y = 0.0121x − 0.0027, R2 = 0.9980). Finally, the O_2^-· rate of production is measured by the following formula: (nmol/g·min) = (ΔA + 0.0027) ÷ 0.0121 × V1 ÷ (V2 ÷ V3 × W) × 2 ÷ T (V1: total response volume; V2: reaction sample volume; V3: extraction liquid volume; W: sample mass; 2: 2 molecules of O_2^- participating in the reaction to form 1 molecule of NO_2^-; T: reaction time.) Three independent replicates were used for each treatment.

2.7. H_2O_2 Content

Approximately 0.2 g of the frozen sample was used for H_2O_2 content assay according to the protocol of the H_2O_2 content measurement kit (H_2O_2-1-Y, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the UV absorbances (A1) at 415 nm against a blank solution (A2) (ΔA = A1 − A2). Regression curves were generated for the determination of H_2O_2 content under standard conditions (y = 0.3744x + 0.0006). Finally, the H_2O_2 content is measured by the following formula: (μmol/g) = [(ΔA − 0.0006) ÷ 0.3744 × V1] ÷ (W × V1 ÷ V2) (V1: sample volume in the reaction system; V2: extraction liquid volume; W: sample quality). Three independent replicates were used for each treatment.

2.8. Superoxide Dismutase Activity (SOD)

Approximately 0.2 g of the frozen sample was used for the SOD activity assay according to the protocol of the SOD measurement kit (SOD-1-Y, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the percentage inhibition was determined by measuring the UV absorbance at 560 nm for each sample (A1) and the light absorption of the control solution (A2) (inhibition rate = (A2 − A1) ÷ A2 × 100%). One unit of SOD activity was defined as SOD enzyme activity in the reaction system when the percentage inhibition was 50%. Three independent replicates were used for each treatment.

2.9. Catalase Activity (CAT)

Approximately 0.2 g of the frozen sample was used for the CAT activity assay according to the protocol of the CAT measurement kit (CAT-1-Y, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the initial UV absorbances (A1) at 240 nm against the absorbance value after 1 min (A2) (ΔA = A1 − A2). One unit of CAT activity was defined as catalyzing 1 nmol of H_2O_2 degradation per minute per g of tissue at 240 nm. Three independent replicates were used for each treatment.

2.10. Ascorbate Peroxidase Activity (APX)

The APX activity determination method refers to Yan et al. [23,24]. The frozen, fresh-cut pumpkin tissues (5 g) were added to 20 mL of 0.1 mol/L potassium phosphate buffer (pH 7.5), and the supernatant was centrifuged at 12,000× *g* for 30 min at 4 °C as the enzyme extraction solution, which was stored at a low temperature for later use. APX was determined by mixing a 2.6 mL reaction buffer (containing 0.1 mmol/L EDTA and 0.5 mmol/L ascorbic acid) with 0.1 mL of enzyme extract, then adding 0.3 mL of a 2 mmol/L H_2O_2 solution to start the reaction. The absorbance value at 290 nm was determined within 15 s. One unit of enzyme activity was defined as U/g when the absorbance value at 290 nm of the APX enzymatic reaction system decreased by 0.01 per gram of the fresh-cut pumpkin sample.

2.11. Glutathione Reductase Activity (GR)

Approximately 0.2 g of the frozen sample was used for the GR activity assay according to the protocol of the GR measurement kit (GR-1-W, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the initial UV absorbance (A1) at 240 nm against the absorbance value after 180 s (A2) (ΔA = A1 − A2). One unit of GR enzyme activity was defined as catalyzing the oxidation of 1 nmol of NADPH per minute per g of tissue at 340 nm at pH 8.0 at a specified temperature. Three independent replicates were used for each treatment.

2.12. Reduced Glutathione Content (GSH)

Approximately 0.2 g of the frozen sample was used for the GSH content assay according to the protocol of the GSH measurement kit (GSH-1-W, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [25]. After adding reagents, the light absorption for each sample

was determined by measuring the UV absorbances (A1) at 412 nm against the absorbance of the blank solution (A2) (ΔA = A1 − A2). A standard curve was generated for GSH content measurement (y = 0.75x). Finally, the GSH content is measured by the following formula: (μmol/g) = ΔA ÷ 0.75 × V1 ÷ (V1 ÷ V2 × W) (V1: accumulation of supernatant liquid in the reaction system; V2: total volume of supernatant; W: sample quality). Three replicates were used for each treatment.

2.13. Ascorbic Acid Content (AsA)

Approximately 0.2 g of the frozen sample was used for the AsA content assay according to the protocol of the AsA measurement kit (AsA-1-W, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the UV absorbances (A1) at 420 nm against the absorbance of the blank solution (A2) (ΔA = A1 − A2). A standard curve was generated for AsA content measurement (y = 0.0044x − 0.018, R2 = 0.9978). Finally, the AsA content is measured by the following formula: (μg/g) = (ΔA + 0.018) ÷ 0.0044 × V1 ÷ (W × V1 ÷ V2) (V1: reaction sample volume; V2: extraction liquid volume; W: sample quality). Three replicates were used for each treatment.

2.14. DPPH, ABTS Free Radical Scavenging Rate

Approximately 0.2 g of the frozen sample was used for the DPPH and ABTS clearance rate assays according to the protocol of the DPPH and ABTS measurement kits (DPPH-1-D and ABTS-1-D, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China), respectively [22]. After adding reagents, the light absorption of DPPH and ABTS for each sample was determined by measuring the UV absorbances (A1) at 515 nm against the absorbance of the blank solution (A2) (ΔA = A2 − A1), respectively. DPPH/ABTS free radical scavenging rate (%) = (A2 − A1) ÷ A2 × 100%.

2.15. Total Antioxidant Capacity (T-AOC)

Approximately 0.2 g of the frozen sample was used for the total antioxidant capacity assay according to the protocol of the FRAP measurement kit (FRAP-1-G, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the UV absorbances (A1) at 593 nm against the absorbance of the blank solution (A2) (ΔA = A1 − A2). A standard curve was generated for FRAP content measurement (y = 1.2416x + 0.0134, R2 = 0.9996). Finally, the FRAP content is measured by the following formula: (μmol/g) = (ΔA − 0.0134) ÷ 1.2416 × V1 ÷ (V1 ÷ V2 × W) (V1: reaction sample volume; V2: extraction liquid volume; W: sample quality). Three replicates were used for each treatment.

2.16. Statistical Analysis

The data were presented as the mean ± SD (standard deviation). Originpro 2021 software (OriginLab., Northampton, MA, USA) was used for the drawing of figures. Using SPSS Version 26 (IBM Corp., Armonk, NY, USA), one-way analysis of variance (ANOVA) and minimal significance difference (LSD) were used to differentiate mean values at the $p < 0.05$ level and the correlation between parameters, using Heml Version 2.0 (The CUCKOO Workgroup, Wuhan, China) software to draw a heat map.

3. Results

3.1. Weight Loss and Firmness

Due to direct contact with air, the transpiration of fresh-cut pumpkins is rapidly accelerated, resulting in increased weight loss [26]. As shown in Figure 1a, the weight loss of fresh-cut pumpkins in all treatment groups showed an increasing trend. The values in the nisin groups were significantly lower than those in the control group at 0–6 days, and the weight loss in the nisin group with 0.2 g/L, which was almost the same as that of the control group, was higher than that in the other two nisin groups from 8 to 10 days.

However, the 0.4 g/L nisin had the lowest weight loss and the most obvious inhibition of water loss during the whole storage period. Compared with the control group, the weight loss rate at the end of the storage period in the 0.4 g/L treatment group was reduced by 13.53%.

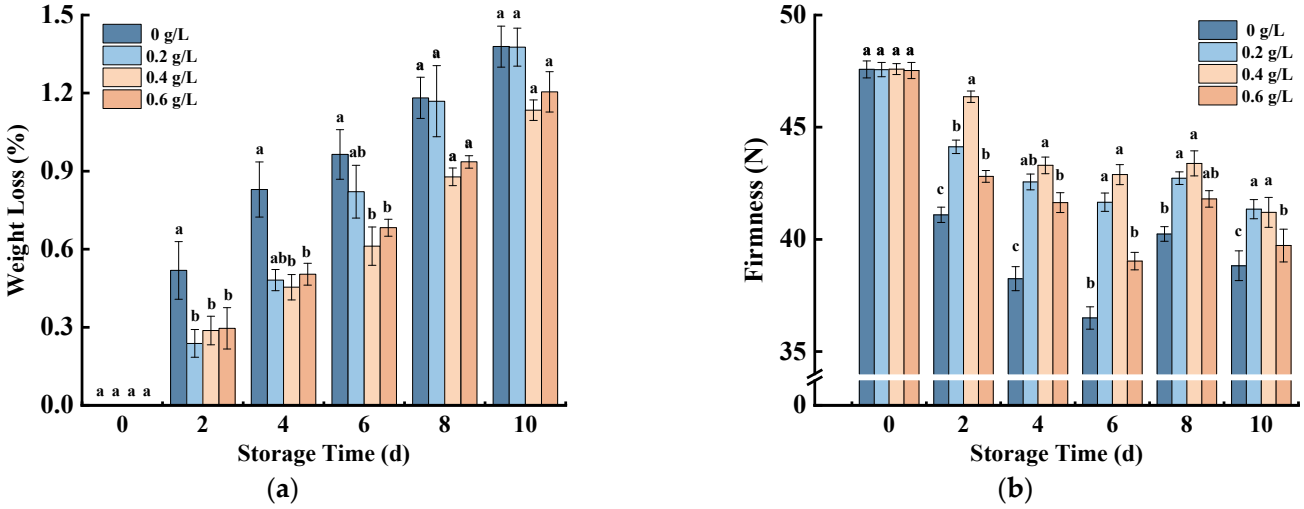

Figure 1. Effects of nisin treatment on weight loss (**a**) and firmness (**b**) in fresh-cut pumpkins. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

The firmness of fresh-cut pumpkins is easily affected by moisture content, lignin formation, and oxidation [27]. Figure 1b shows that the firmness of the four groups of fresh-cut pumpkins decreased rapidly and then increased slowly. During the whole storage period, the firmness of the 0.4 g/L nisin was higher than that of the other groups, and the value was the lowest in the control group. The 0.4 g/L Nisin most clearly preserved the hardness of fresh-cut pumpkin at 6 days, which was 1.18 times higher than the control group. The firmness increased slowly from 6 to 8 days, which may be due to the callus response of the pumpkin slices under the injury stress, which generated callus at the cut site, leading to the firmness increase of the fresh-cut pumpkins. Furthermore, applying a 1% nisin nano-coating to a white button similarly showed minimal weight loss and firmness [28].

3.2. Whitening Index (WI)

The surface of fresh-cut pumpkins easily produces a white substance when it loses water, and WI is used to indicate the degree of whitening [17]. Figure 2 shows an upward trend in the whitening index of fresh-cut pumpkins during storage, but nisin could effectively inhibit this change. It is possible that nisin reduced whitening on fresh-cut pumpkins by inhibiting surface weight loss. During the whole storage period, 0.4 g/L nisin-treated slowed the rise of the whitening index, and the values in all nisin-treated samples were lower than those in the control group. At the end of the storage period, the whitening index was reduced by 13.61% compared with the control group. Similar results were found in fresh-cut carrots [17] and white buttons [28], which indicated that nisin was extremely well maintained in the color of fruit and vegetables.

3.3. Respiration Intensity

Increased respiration rates can cause fruit and vegetable aging [29]. Figure 3 showed that the respiratory intensity of fresh-cut pumpkins in each treatment group decreased first and then increased during storage. The values reached their lowest intensity at 6 d and then rose. The respiratory intensity of all nisin-treated groups was lower than that of the control group, and the 0.4 g/L nisin group had a significant effect on the respiratory intensity

of fresh-cut pumpkins. Respiratory rates of fresh-cut pumpkins were greatly reduced by 0.4 g/L Nisin at 8 d and inhibited by 45.83% compared with the control group. Thus, it can effectively delay the aging process of fresh-cut pumpkins. In addition, similar results were found in fresh-cut carrots [17] and button mushrooms [30]. Those results indicated that nisin was extremely efficient in decreasing the respiration rates of fruits and vegetables.

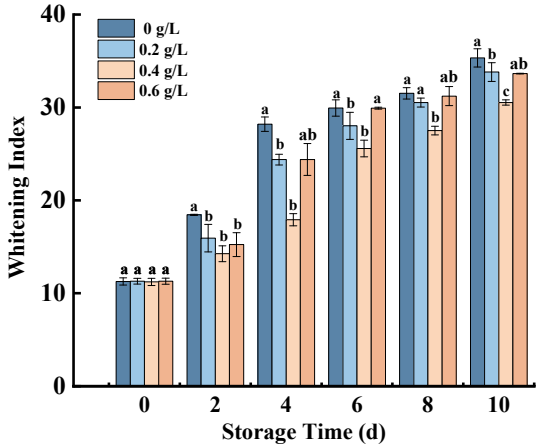

Figure 2. Effects of nisin treatment on WI in fresh-cut pumpkins. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

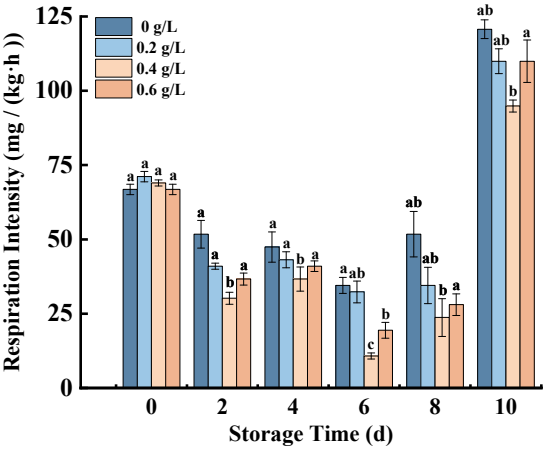

Figure 3. Effects of nisin treatment on respiration intensity in fresh-cut pumpkins. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

In conclusion, 0.4 g/L nisin treatment could effectively reduce the weight loss, hardness loss, whitening, and respiration intensity of fresh-cut pumpkins and better maintain the storage quality. Therefore, we selected the nisin concentration of 0.4 g/L for subsequent analysis of antioxidant activity indexes to explore the effect of nisin on the antioxidant capacity of fresh-cut pumpkins.

3.4. O_2^-· Production Rate and H_2O_2 Contents

Fresh-cut fruits and vegetables under cutting stress will trigger the production of a large number of ROS, such as O_2^-·and H_2O_2, in cells. However, excessive production and accumulation of ROS will seriously accelerate the aging of fruits and vegetables [31]. The O_2^-· production rate of fresh-cut pumpkins was at its highest rate at 0 d (Figure 4a). The control group showed a downward trend from 0 to 4 days and then slowly increased to 6 days and then decreased. After treatment with 0.4 g/L nisin, the O_2^-· production rate

of the nisin group was lower than that of the control group, except for the 4th day. Nisin reduced the $O_2^{-}\cdot$ production rate of fresh-cut pumpkins by 65.11% compared with the control group. Figure 4b showed that H_2O_2 content increased during the storage time, but the 0.4 g/L nisin effectively inhibited the accumulation of H_2O_2, compared with the control group. At day 8, 0.4 g/L nisin reduced the H_2O_2 content of fresh-cut pumpkins by 40% compared with the control. Similar results were found in mushrooms [30], indicating that nisin reduced $O_2^{-}\cdot$ production rate and H_2O_2 content.

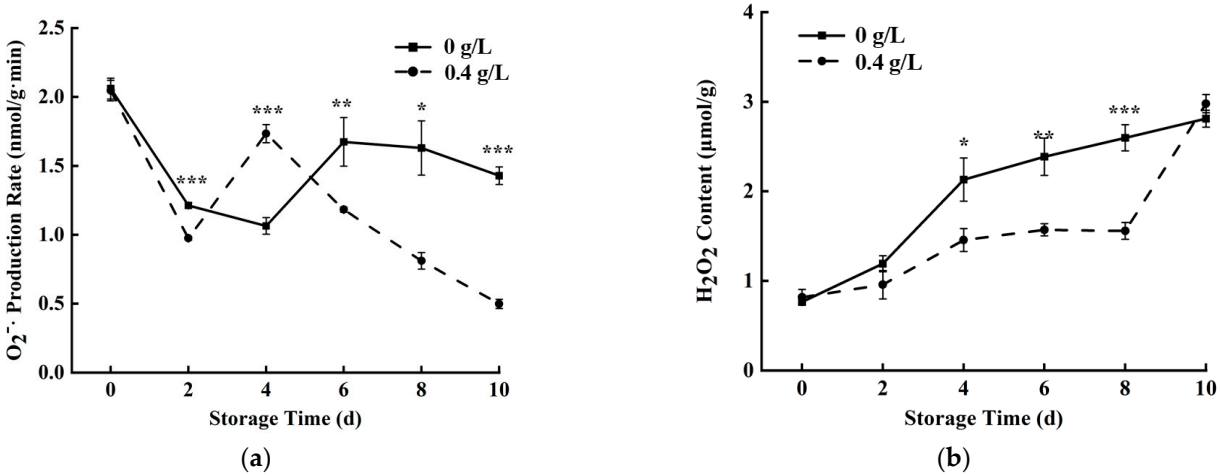

Figure 4. Effects of 0.4 g/L nisin treatment on $O_2^{-}\cdot$ (**a**) and H_2O_2 content production rate (**b**) in fresh-cut pumpkins (*, ** and *** indicate the significant difference between the 0.4 g/L nisin treatment and the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Vertical bars represent the standard deviation of the mean ($n = 3$)).

3.5. Antioxidant Metabolism-Related Enzyme Activities

SOD, CAT, APX, GR, and other enzymes work together to defend against the damage caused by reactive oxygen species or other peroxide free radicals to the cell membrane system, as well as maintain the balance of reactive oxygen metabolism and play an antioxidant role [32,33].

As can be seen from Figure 5a,c, the SOD and APX activities of fresh-cut pumpkins decreased first and then increased. Compared with the control group, 0.4 g/L nisin treatment could increase SOD activity at 2–4 days, while the trend in the later storage period was opposite to the early storage period. APX activity in the 0.4 g/L nisin group decreased from 0 to 4 days and then increased slowly from 4 to 10 days, whereas in the control group, it decreased to a nadir at 6 d and then increased, but it was always higher in the 0.4 g/L nisin group than that in the control group. Figure 5b showed that the CAT activity of fresh-cut pumpkins increased first and then decreased. CAT activity in the 0.4 g/L nisin group peaked at 6 d and in the control group at 4 d, but CAT activity was significantly higher in the 0.4 g/L nisin group at 0–6 days. Figure 5d showed that GR activity had been on the rise, and the activity of the 0.4 g/L nisin group was always higher than that of the control group during the storage. The activity of the 0.4 g/L nisin group was 759.51 U/g at 4 d, which was significantly higher than that of the control group.

3.6. Antioxidant Metabolism-Related Substances Contents

AsA and GSH are important antioxidants in the AsA-GSH cycle, and they complement each other in the process of scavenging reactive oxygen free radicals [34,35]. Figure 6a,b showed an increasing trend of both AsA and GSH in fresh-cut pumpkins. After 0.4 g/L nisin treatment, the AsA content of fresh-cut pumpkins accumulated rapidly from 4 to 10 days, which was significantly higher than that of the control group. In addition, the change trends of AsA content and APX enzyme activity were consistent, indicating that AsA can reduce the accumulation of H_2O_2 catalyzed by APX. Moreover, 0.4 g/L nisin treatment

could promote the accumulation of GSH content, which was higher than that of the control group during 4–10 days, which was conducive to the accumulation of antioxidants.

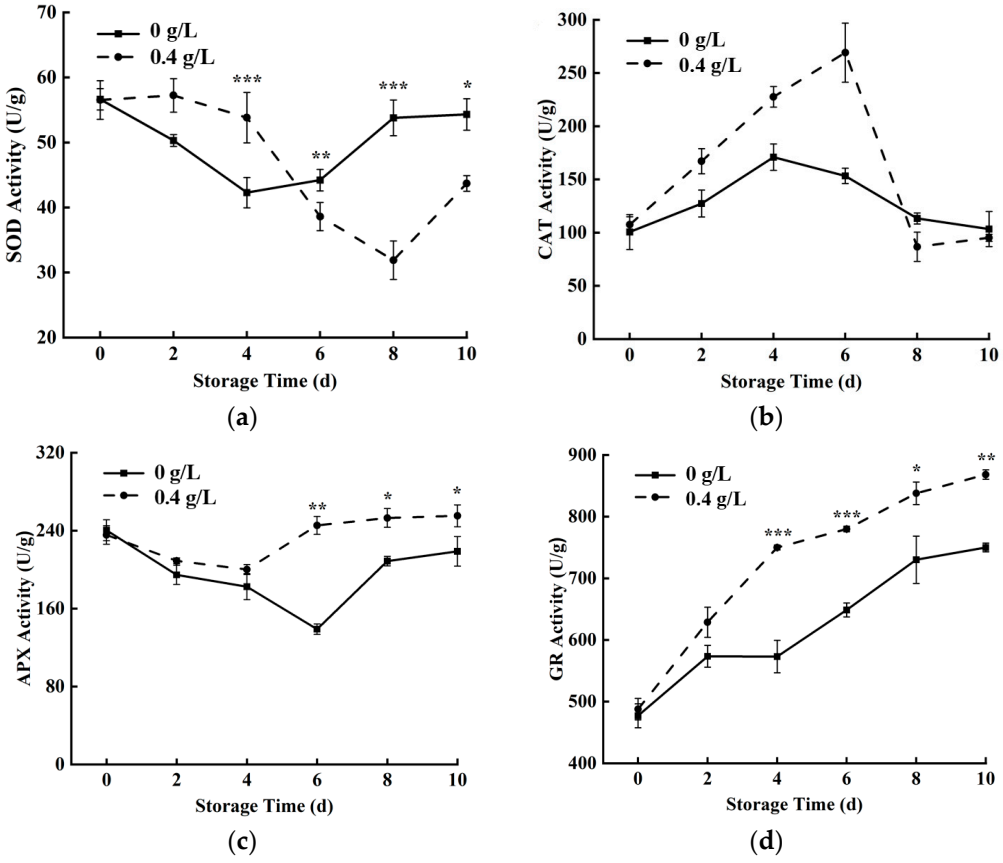

Figure 5. Effects of 0.4 g/L nisin treatment on SOD activity (**a**), CAT activity (**b**), APX activity (**c**), and GR activity (**d**) in fresh-cut pumpkins (*, **, and *** indicate the significant difference between the 0.4 g/L nisin treatment and the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Vertical bars represent the standard deviation of the mean ($n = 3$)).

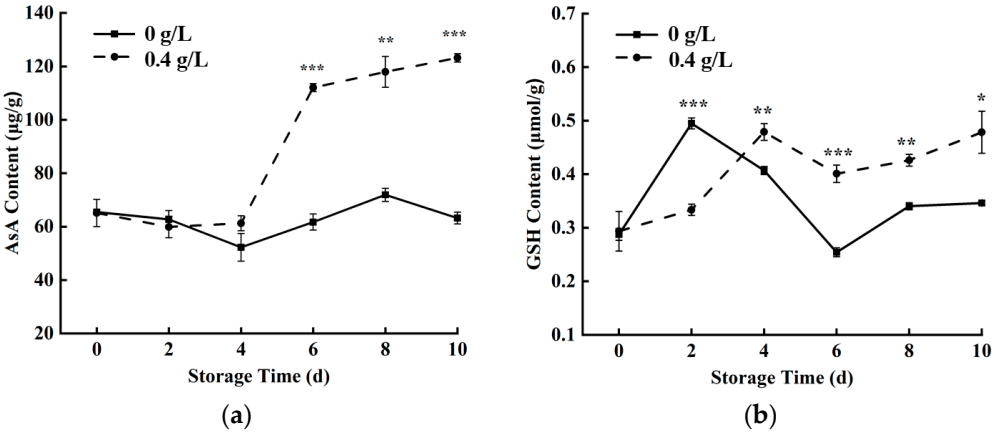

Figure 6. Effects of 0.4 g/L nisin treatment on AsA content (**a**) and GSH content (**b**) in fresh-cut pumpkins (*, **, and *** indicate the significant difference between the 0.4 g/L nisin treatment and the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Vertical bars represent the standard deviation of the mean ($n = 3$)).

3.7. Antioxidant Capacity

DPPH, ABTS, and T-AOC were used to evaluate the antioxidant capacity of fresh-cut pumpkins [32,36]. In the control group, DPPH, ABTS, and T-AOC of fresh-cut pumpkins showed an overall decreasing trend during storage (Figure 7a–c), and the scavenging ability of ROS decreased as a whole, but the antioxidant ability was improved after 0.4 g/L nisin treatment. The free radical scavenging rates of DPPH and ABTS reached their peaks on the second day, and the T-AOC was in an upward state, which was always higher than that of the control group, indicating that the 0.4 g/L nisin group had a higher antioxidant capacity.

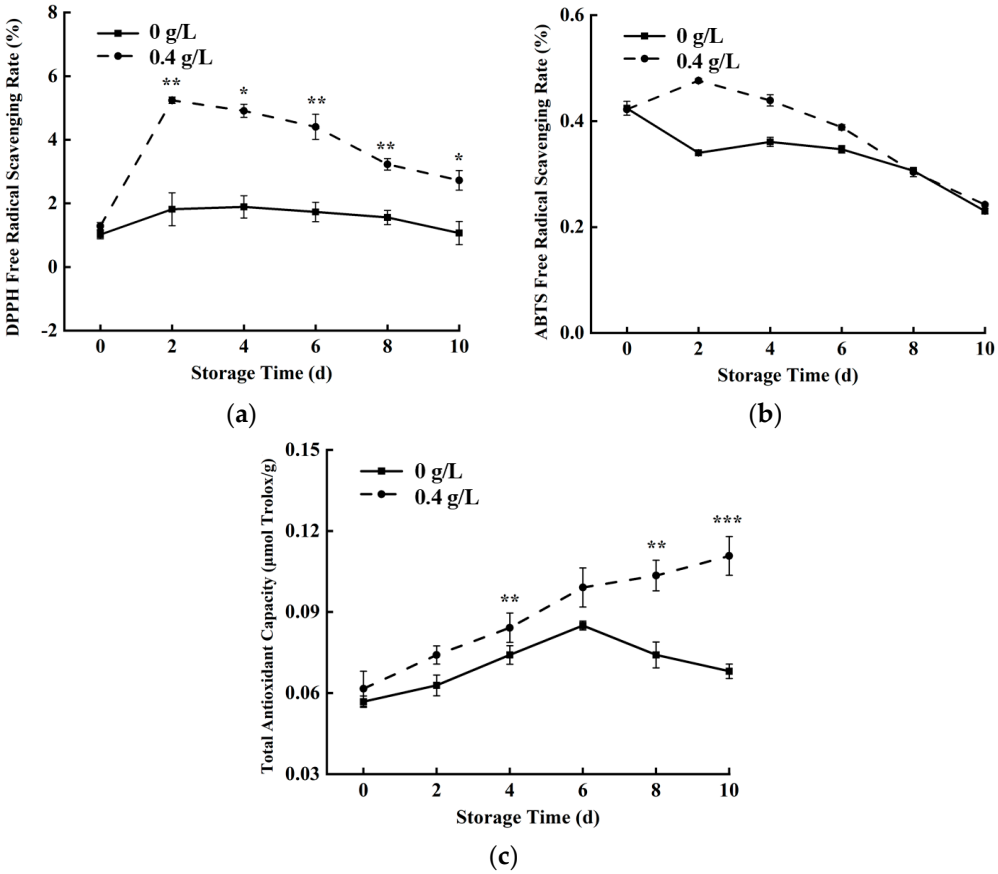

Figure 7. Effects of 0.4 g/L nisin treatment on DPPH (**a**), ABTS (**b**) free radical scavenging rate, and T−AOC (**c**) in fresh−cut pumpkins (*, **, and *** indicate the significant difference between the 0.4 g/L nisin treatment and the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Vertical bars represent the standard deviation of the mean ($n = 3$)).

3.8. Correlation Analysis

The Pearson correlation coefficient between different parameters of fresh-cut pumpkins after 0.4 g/L nisin treatment is shown in Figure 8. Firmness was significantly positively correlated with APX and ABTS ($p < 0.05$) and significantly negatively correlated with H_2O_2 ($p < 0.05$). H_2O_2 was extremely significantly positively correlated with weight loss rate and WI ($p < 0.01$), while ABTS was extremely significantly negatively correlated ($p < 0.01$). ABTS was extremely significantly negatively correlated with weight loss and WI ($p < 0.01$). These results indicated that the antioxidant system of fresh-cut pumpkins was closely related to their storage quality. In addition, $O_2^{-}\cdot$ was extremely significantly negatively correlated with GR, GSH, and FRAP ($p < 0.01$), as well as significantly negatively correlated with AsA ($p < 0.05$), indicating that the AsA−GSH cycle was conducive to $O_2^{-}\cdot$ clearance.

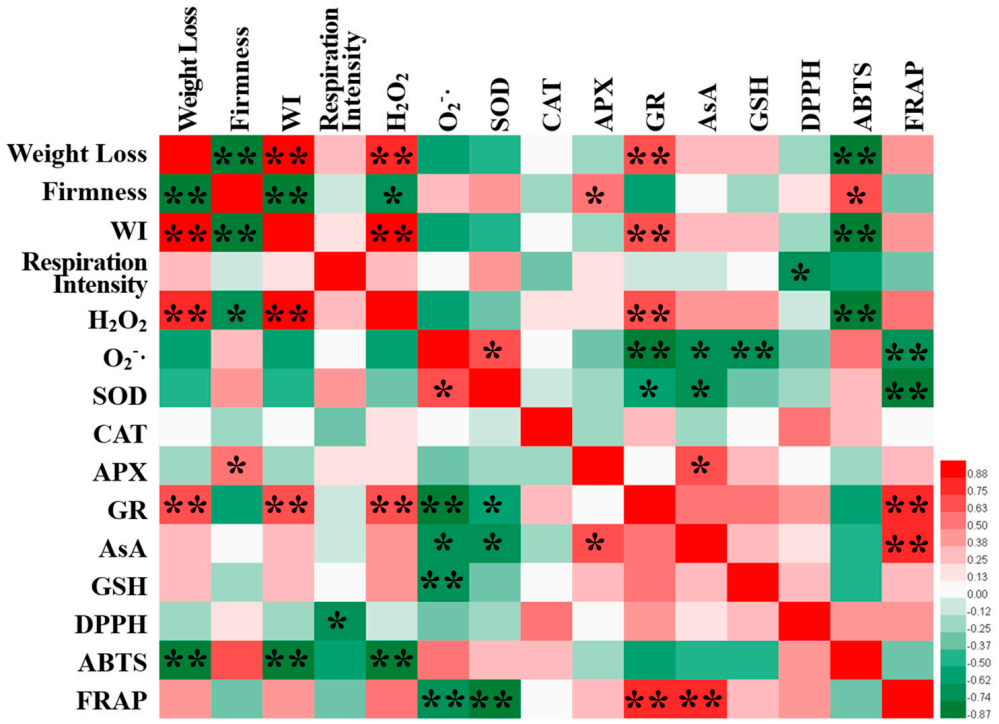

Figure 8. Pearson correlation matrix of each indicator. * and ** indicate that correlation between values reach the level of $p < 0.05$ and $p < 0.01$, respectively.

4. Discussion

Mechanically damaged pumpkin tissue is prone to a series of quality degradation problems such as cell rupture, water loss, and nutrient outflow, resulting in the weight loss rate decreasing, the tissue softening, the section turning white, and the respiration rate increasing [37,38]. Therefore, it is of great significance to explore safe and natural preservation technology to maintain the storage quality of fresh-cut pumpkins. Nisin, a safe food additive, is a safe and natural biological preservative agent, which has been widely concerned by researchers at home and abroad [39,40]. Many scholars have applied it to various foods and achieved good fresh-keeping effects, such as cucumber juice drinks [41], fresh-cut carrots [17], button mushrooms [30], fresh apple-kale blend juice [42], grape juice [43], fresh-cut beet leaves [16], fresh-cut onions [44], and fruit- and vegetable-based beverages [45]. In this study, it was found that nisin treatment could effectively inhibit the weight loss rate, the whiteness of the cut surface, the decrease in hardness, and the increase in respiration rate of fresh-cut pumpkins, as well as activate the antioxidant system and preserve the better quality of fresh-cut pumpkins.

Cutting stress leads to the accumulation of ROS and the aggravation of membrane lipid peroxidation, resulting in tissue oxidative damage and the acceleration of the aging process of fresh-cut pumpkins [46,47]. After broccoli was cut, high-intensity injury increased H_2O_2 content, low-intensity injury increased $O_2^-\cdot$ content, and SOD, POD, and CAT enzymes induced by the trauma accelerated the decomposition of ROS [48]. The antioxidant defense system is vital in the preservation of fruits and vegetables due to the fact that it can eliminate ROS and delay the aging process. SOD, CAT, APX, and GR are antioxidant enzymes that work together to maintain the metabolic equilibrium of ROS and function in an antioxidant role. SOD is a first-line defense system against ROS, capable of mutating $O_2^-\cdot$ into H_2O_2 and O_2. [49]. APX and GR serve as key enzymes in the AsA-GSH cycle [50]. APX and CAT enzymes catalyze the decomposition of H_2O_2 into O_2 and H_2O. GSH and AsA are important antioxidant substances in the ASA-GSH cycle, which are beneficial to maintain the activity of APX and provide ROS scavenging and reducing power [50,51].

In this study, ROS content was increased in the control group, while the production rate of $O_2^{-}\cdot$ and H_2O_2 content of fresh-cut pumpkins was decreased by nisin treatment almost during the storage period, which significantly inhibited ROS content. Additionally, nisin elevated SOD and CAT activities early in the storage period and APX activity later in the storage period. It is possible that SOD enzymes diminish $O_2^{-}\cdot$ production early in the storage period, whereas CAT and APX enzymes break down H_2O_2 later in the storage period, inhibiting ROS accumulation throughout the storage period. In the study of Agaricus bisporus using nisin in combination with chitosan and nano-silica, it was also found that the contents of $O_2^{-}\cdot$ and H_2O_2 could be maintained at the lowest levels, and the activities of SOD and CAT enzymes were significantly increased due to the addition of nisin [30]. Nisin treatment significantly raised the GR activity, GSH, and AsA contents of fresh-cut pumpkins, promoting the transport rate of the AsA-GSH cycle and increasing antioxidant capacity. Similar research has demonstrated that combining nisin and chitosan treatments could increase GR activity, GSH, and AsA contents in feijoa, improve fruit resistance, and so postpone plant senescence and fruit deterioration [52].

Furthermore, the correlation analysis results (Figure 8) show that $O_2^{-}\cdot$ was negatively correlated with GR, AsA, GSH, and FRAP, and H_2O_2 had a positive correlation with weight loss and the whitening index and was negatively correlated with hardness and ABTS. These findings revealed that ROS accumulation can lower the quality of fresh-cut pumpkins, but the antioxidant enzymes and substances are advantageous to ROS elimination.

In conclusion, fresh-cut pumpkins treated with nisin could increase the activity of the SOD enzyme in the early stage of storage to reduce the production rate of $O_2^{-}\cdot$ and activate the CAT and APX enzymes to remove H_2O_2, which results in reduced ROS levels. Meanwhile, GR activity and contents of GSH and AsA were also significantly increased, which promoted the transport rate of the AsA-GSH cycle and enhanced antioxidant capacity. Previous studies have also shown that nisin could enhance these antioxidant capacities in fresh-cut beet leaves [16], grape juice [43], fresh apple-kale blend juice [42], and Agaricus bisporus [30].

5. Conclusions

Nisin has a positive effect on the preservation of fresh-cut pumpkins. This study showed that 0.4 g/L nisin, compared with the control group, effectively reduced water loss, maintained firmness, inhibited whitening, and decreased the respiration rate for fresh-cut pumpkins to maintain a good appearance quality. Nisin could also reduce the accumulation of ROS ($O_2^{-}\cdot$ and H_2O_2) by enhancing the activities of SOD and CAT and regulating the conversion of antioxidant enzymes (GR and APX) and antioxidant substances (GSH and AsA) in the AsA-GSH cycle. In addition, combined with DPPH, the ABTS free radical scavenging rate, and T-AOC, nisin could effectively enhance the antioxidant activity of fresh-cut pumpkins. In conclusion, nisin treatment has the potential to maintain the preservation quality and delay the aging process during the storage period, which is a natural biological preservative that can effectively improve the quality of fresh-cut pumpkins.

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