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Effect of Aqueous n-Butanol Treatments on Shelf-Life Extension of Longkong Fruit during Ambient Storage

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Abstract: The pericarp of the Longkong fruit rapidly browns during ambient storage, typically reducing its shelf life to between 3 and 7 days. Recently, n-butanol has demonstrated a promising effect in preventing this deterioration, extending the shelf life of tropical fruits to more than a week. The present study exploited this opportunity to examine the exogenous application of aqueous n-butanol at various concentrations (0.2–0.6%) in controlling pericarp browning and suppressing different oxidoreductase enzymes in the pericarp under prolonged ambient storage conditions (8 days). Every two days, the fruit pericarps were tested for color (lightness (L*), redness (a*), and yellowness (b*)), browning index (BI), membrane permeability loss (MPL), malondialdehyde (MDA) content, total phenolic content (TPC), and reactive oxygen species (ROS). Enzymes including phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), phospholipase D (PLD), lipoxygenase (LOX), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) were also analyzed. All sample test results showed that increased storage significantly impacted color characteristics (decreased L*, b* and increased a*, and BI). MPL, MDA, and ROS also continuously increased. Furthermore, the browning-related enzymes (PAL and PPO), membrane-degrading enzymes (PLD and LOX), and antioxidant enzymes (SOD, CAT, and GPX) continuously increased in all pericarp samples throughout the storage. Among the samples, pericarp color, BI, MPL, MDA, PAL, PPO, PLD, and LOX were significantly high in the control samples, consequently adversely affecting the quality and shelf life of Longkong. On the other hand, the n-butanol-treated samples significantly controlled the loss and all problematic enzymes while improving the activities of SOD, CAT, and GPX in the pericarp. Furthermore, the positive effect of n-butanol application was dose-dependent; higher concentrations (0.4–0.6%) performed well in protecting the fruit from deterioration.

Keywords: longkong; pericarp; n-butanol; coating; shelf life; enzyme; quality

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1. Introduction

Longkong fruit is one of the commercial fruits cultivated widely in Thailand and in many other southeast Asian countries, including Malaysia, Indonesia, and the Philippines. It has a unique taste and flavor and is a tropical, non-climacteric and seasonal fruit. The cultivation and harvesting period of longkong fruits widely differs with the regions where it is produced [1]. Longkong fruit is completely consumed; the flesh is consumed as a fruit, and its seed and pericarp are used for medicinal purposes [2]. Longkong flesh is translucent with a unique combination of sweet and sour tastes, whereas the seeds are dark greenish and highly bitter [3]. In fully ripened longkong fruits, the fruits' pericarp color changes from dark green to bright yellow. This fruit contains abundant nutrition and phytochemicals [4]. Despite its uniqueness, this fruit suffers short shelf life due to

rapid biochemical and microbial degradations. Typically, the commercial value of the longkong fruit is decided by its external appearance, and the pericarp of the longkong turning from yellow to brown indicates that it has lost its commercial value [2]. The shelf life of this fruit under ambient conditions is limited to 3–7 days [5]. Several studies reported that the pericarp browning of longkong fruit is mainly attributed to the oxidoreductase enzymes, including polyphenol oxidase (PPO) and peroxidase (POD) [6]. Recently, several research reports suggested that the trigger of enzymatic browning occurs with the decompartmentalization of cell membranes, which is a major cause due to the activity of lipoxygenase (LOX) and phospholipase D (PLD) enzymes [7]. An essential function of PLD is to mediate the degradation of membrane phospholipids, which occurs rapidly and early in postharvest senescent tissues. PLD's catalyzed products (linolenic acid and phosphatidic acid) stimulate the oxylipin pathway and initiate cell signal transduction. This signal is believed to increase wound-induced activity, accumulate phenolics, and increase the browning pigments in the plant tissues [8]. Hence, regulating PLD activity may be essential to preserving fruit quality after harvest. Several chemicals have been shown to inhibit PLD, including butanol, acylethanolamines, lysophosphatidylethanolamine, diphosphoglycerate, and wortmannin [9]. The compound n-butanol inhibits PLD, which is responsible for producing free radicals within the cell membrane by signaling to initiate LOX activity from producing phosphatidic acid. According to recent studies, n-butanol inhibits phosphatidic acid production and membrane lipid degradation by acting as a substrate for transphosphatidylation reactions [10].

In the past, butanol was reported to extend the shelf life of many fruits; for example, the use of n-butanol to control lychee fruit quality was studied. Lychees were immersed in 0.1% (v/v) aqueous solution of n-butanol for 10 min at room temperature (25 °C) for 6 days and compared with untreated lychee fruits (control). The browning index and disease incidence index were lower than those of the untreated fruits [9]. Different concentrations of 2-butanol (0.05–0.15%) were applied after harvesting the longan fruit stored at ambient temperature. A positive effect was found in the 0.05% 2-butanol treatment. This treatment was found to affect the quality of the longan fruit most, slowing down browning, which is important to maintain the quality after harvest and prolong the shelf life of the fruit [11,12]. Mature 'Cripps Pink' apple fruit was treated with 10 or 25 μL L^{-1} n-butanol compared with the control and stored at 20 °C. The accumulation of liquid wax components was promoted in apple fruits treated with n-butanol on day 42 compared to the control group. The butyl ester content of the skin of 10 and 25 μL L^{-1} n-butanol-treated fruit was 1.7 and 2.9 times higher, respectively. The n-butanol had no effect on the ripening of the fruit or aging but promoted the development of apple skin greasiness [13]. In addition, n-butanol preserved the hardness of the fruit peel structure. Hami melon fruits were soaked with n-butanol with 0% (control group soaked in water), 0.5%, 1.0% and 1.5% for 30 min, dried naturally and stored at 3 °C for 42 days. The quality of Hami melon fruits was investigated. It was found that treatment with 1.0% n-butanol had a better inhibitory effect. It affects the function of membrane-associated membrane lipids in the epidermal cell membrane. Therefore, it enhances the cold resistance of the Hami melon fruits cell membrane [14,15]. However, numerous postharvest techniques were applied to control pericarp browning, extend longkong fruit shelf life, and most adversely affect flavor and taste. Till now, there has been no approach to using n-butanol as a postharvest chemical treatment to control longkong fruit pericarp browning. Therefore, our objective was to study the n-butanol effect on the physicochemical properties of longkong fruit pericarp during ambient storage. The insights from this research support the Sustainable Development Goals (SDGs) by enhancing agricultural productivity, commercial longkong producer income, food security, and sustainable agriculture.

2. Materials and Methods

2.1. Raw Materials and Treatments

Longkong (*Aglaia dookkoo* Griff) fruits, roughly at 85% maturity, were harvested from specific gardens and transported to the laboratory within a six-hours window for immediate preparation and experimentation. Upon arrival, all fruits underwent a comprehensive screening process to discard any specimens with visible signs of mechanical damage, insect-related injuries, or microbial attack. The selected fruits were then thoroughly cleaned with distilled water to remove any surface dirt, and subsequently separated from their bunches, maintaining the peduncle attachment, in preparation for subsequent treatments and storage. The treatment and storage protocol began with the careful separation of individual fruits from their respective bunches, each with the peduncle intact. These fruits were then randomly allocated to four different groups: a control group and three treatment groups (T1, T2, and T3). Each group consisted of 15 fruits per replication. The fruits in the control group were immersed in distilled water, while those in the T1 group were submerged in a 0.2% n-butanol aqueous solution. All submersion procedures occurred for 15 min at ambient temperature. The protocol for T2 and T3 was identical, with the exception of the n-butanol concentrations; T2 and T3 utilized 0.4% and 0.6% solutions, respectively. Post-treatment, all fruits were collected, and their surfaces were air-dried using an electric fan. The control and treatment groups were then separately placed into a punnet, the top of which was heat-sealed with perforated polyethylene films (0.03 mm thickness). Figure 1 provides a flowchart detailing the n-butanol treatment process for longkong fruit. Following the preparation of control and treated fruits, they were stored at ambient temperature for a period of 8 days. Every 2 days, a subset of fruits was selected from a range of quality assessments, as outlined in Section 2.2.

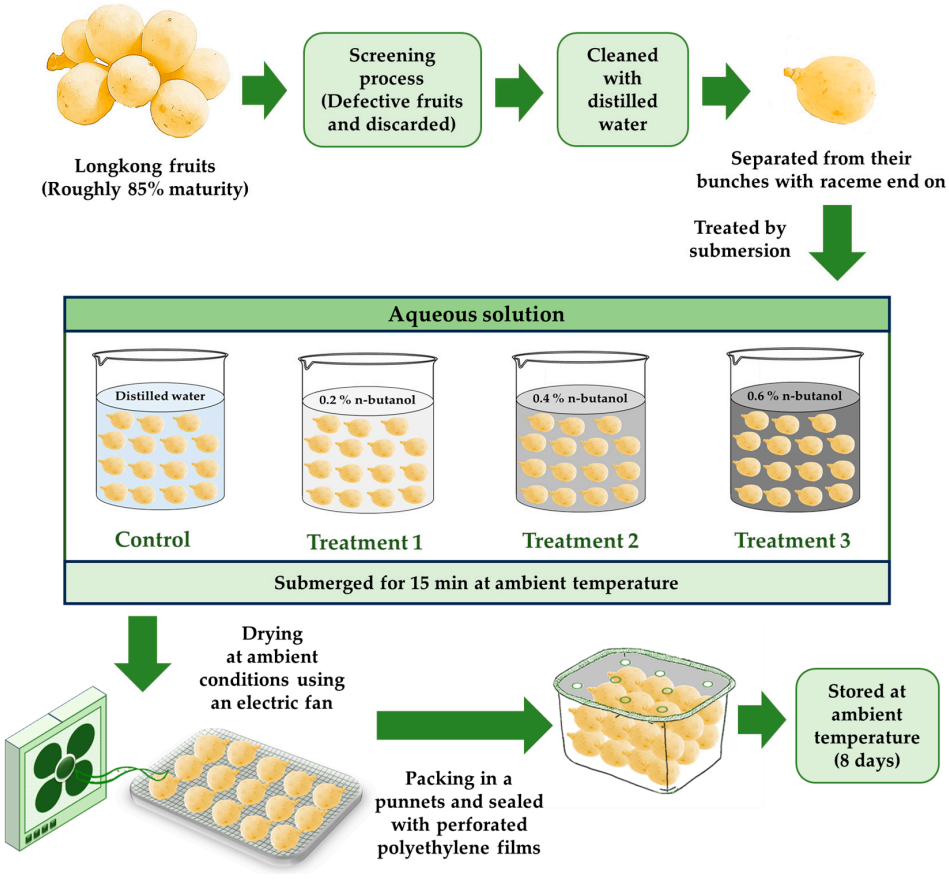

Figure 1. Illustration of Aqueous n-butanol treatment on longkong fruit.

2.2. Quality Assessment

2.2.1. Determination of Pericarp Color Characteristics

The color attributes of the fruit were quantified through the evaluation of 15 separate fruits extracted from each replication. The assessment included the determination of L*, a*, and b* color parameters of the fruit's pericarp using a colorimeter (Hunter Lab, Reston, VA, USA). This process was performed on four distinct randomly chosen surfaces of each individual fruit. The parameter L* signifies the level of brightness, while a* corresponds to a spectrum from "−green" to "+red" and b* signifies a range from "−blue" to "+yellow".

2.2.2. Determination of Pericarp Browning Index

A browning index was utilized to determine the extent of surface color change in the longkong fruit's peel. The visual analyses of the peel's browning index involved assessing the peel's surface to determine the degree of the color change, with the use of a specific ranking system: a grade of 0 implied no change in color, whereas grades of 1, 2, and 3 were associated with light brown, medium brown, and deep brown shades, respectively.

2.2.3. Determination of Membrane Permeability

The membrane permeability of the longkong pericarp was determined using electrolytic leakage (EL) as per the method of Wang et al. [16]. The pericarp was sliced into 10 mm diameter discs, which were then placed in a beaker containing 40 mL of distilled water. This setup was left to sit at ambient temperature for 3 h. After this incubation period, the initial EL (EL_0) was measured using a conductivity meter. Subsequently, the same aqueous solution containing the pericarp discs was boiled for 20 min, cooled to room temperature, and measured again to obtain the second EL (EL_1). These measurements were used in the following formula to calculate the relative membrane permeability of the longkong pericarps.

$$\text{Relative membrand permeability } (\%) = \frac{EL_0}{EL_1} \times 100$$

2.2.4. Determination of Malondialdehyde (MDA) Content

The content of MDA in the pericarp of the longkong fruit was quantified in alignment with the method suggested by Barman et al. [17], with minor adaptations. Approximately 5 g of pericarp tissue from the longkong fruit was homogenized in a solution of 5% trichloroacetic acid (TCA). Following homogenization, the mixture underwent centrifugation for 10 min at 15,000 rpm. After centrifugation, the supernatant was gathered and 2 mL of 6% tert-butyl alcohol (TBA) was added to it. This reaction mixture was then heated in a hot water bath at 90 °C for 10 min. Subsequently, the absorbance of the reaction mixture was recorded at 600 nm, 532 nm, and 450 nm wavelengths using a UV-Vis spectrophotometer. The results were calculated and expressed as nmol g^{-1} fresh weight (FW).

2.2.5. Determination of Pericarp Total Phenolic Contents (TPC)

The total phenolic content in the longkong pericarp was measured using the Singleton et al. [18] method. Initially, 5 g of longkong pericarp was homogenized in chilled (~4 °C) 80% ethanol. The homogenate was then centrifuged at 8000 rpm for 10 min. Subsequently, 0.1 mL of the supernatant was combined with a working reagent, which included 0.5 mL of Folin-Ciocalteu reagent and 2.9 mL of distilled water. Following this, 2 mL of 20% sodium bicarbonate solution was added. The mixture was incubated for 90 min, after which the absorbance was measured using a UV-Vis spectrometer at 760 nm against a blank. The results were expressed as gallic acid equivalents (μg GAE g^{-1} FW).

2.2.6. Determination of Reactive Oxygen Species (ROS)

The determination of ROS was performed using three methods using superoxide anion radical, hydrogen peroxide and hydroxyl radical contents.

For the determination of the superoxide anion radical [19], 10 g of longkong pericarp tissues were homogenized with 10 mL of 65 mmol/L sodium phosphate buffer (pH 7.8), which contained 0.09 mol/L polyvinyl pyrrolidone and 0.01 mol/L sodium diethyl dithiocarbamate trihydrate. Subsequently, the homogenate was centrifuged for 20 min at 12,000 rpm using a refrigerated centrifuge. After centrifugation, 1 mL of the supernatant was collected and combined with 1.8 mL of 50 mmol/L sodium phosphate buffer (pH 7.8), followed by the addition of 0.2 mL of 0.01 mol/L hydroxylamine hydrochloride. The reaction mixture was then incubated for 30 min at 25 °C. Following incubation, 1 mL was withdrawn from the mixture and combined with 1 mL of 17 mmol/L p-aminobenzenesulfonic acid and 1 mL of 0.05 mol/L α-naphthylamine. The mixture was then incubated again for 20 min at 25 °C. Subsequently, the absorbance of the reaction mixture was measured using a UV-Vis spectrophotometer at 530 nm. The absorbance value was calculated against the standard curve prepared with sodium nitrite, and the results were expressed as nmol g^{-1} FW.

For the determination of hydrogen peroxide radical [20], 1 g of longkong pericarp tissue was homogenized with 5 mL of 1% trichloroacetic acid (TCA) in a cold environment (4 °C) for 1 min. After that, the homogenate was centrifuged at 4 °C for 20 min at 20,000× *g*. Then, 0.5 mL of supernatant was collected and added to a test tube that contained 2.4 mL of 10 mM potassium buffer (pH 7) and 0.1 mL of 1 M Potassium Iodide. The reaction mixture was mixed well, and the absorbance was measured at 390 nm. Distilled water was used as a blank. The results were calculated using the standard curve made from hydrogen peroxide and expressed as μmol g^{-1} FW.

For the determination of hydroxyl radical content [21], 1 g of longkong pericarp tissue was homogenized with 15 mL of 20 mM phosphate buffer at pH 6.0 within a 4 °C environment for 1 min. The homogenate was then centrifuged at 3000× *g* for 20 min at 4 °C. Subsequently, 1 mL of the supernatant was incubated for 30 min at room temperature with 1.5 mL of 20 mM phosphate buffer (pH 6.0), which contained 20 mM of 2-deoxy-D-ribose. To this, 1 mL of 0.5% (*w*/*v*) 2-thiobarbituric acid (TBA) in 1.4% (*w*/*v*) TCA was added. The mixture was heated in a water bath for 10 min, then cooled to room temperature. The fluorescence intensity was then measured at excitation and emission wavelengths of 532 nm and 553 nm, respectively, using a reagent blank solution as a reference. Quantification was performed using the molar extinction coefficient of 155 mM^{-1} cm^{-1}, and the results were expressed as nmol g^{-1} FW.

2.2.7. Enzymatic Determinations

Determination of PAL Activity

To quantify the activity of PAL [22], 5 g of pericarp tissues were ground under refrigerated conditions (4 °C) using a mixture of 20 mL of 0.1 M sodium borate buffer (pH 8.0), inclusive of 0.5 g of polyvinylpyrrolidione, 5 mM β-mercaptoethanol, and 2 mM EDTA. After that, the homogenate was centrifuged at 19,000× *g* in a refrigerated centrifuge and the supernatant was collected for the enzyme assay. A volume of 0.1 mL of the crude extract was combined with 2.9 mL of the aforementioned buffer, including 3 mM of l-phenylalanine, and the reaction was ran for one hour at room temperature. The increase in the absorbance at 290 nm, due to the formation of trans-cinnamate, was measured using a UV-Vis spectrophotometer. One unit of enzyme activity was defined as the amount that caused an increase of 0.01 in absorbance per hour.

Determination of PPO Activity

To quantify the activity of PPO [23], 5 g of pericarp tissues were homogenized under cold conditions (4 °C) in a 20 mL solution of 0.05 M phosphate buffer (pH 7.0), that contained 0.5 g of insoluble polyvinylpyrrolidone. The homogenate was then strained

using a muslin cloth and then centrifuged for 20 min at 19,000× *g* and at 4 °C. Following this, the supernatant was collected to serve as the crude enzyme extract. A volume of 0.1 mL of crude extract was combined with 2.9 mL of 10 mM 4-methyl catechol, and the reaction mixture was measured every minute using a UV-Vis spectrophotometer at 420 nm. A single unit of enzymatic activity was described as the quantity leading to an alteration of 0.001 in the absorbance every minute.

Determination of PLD Activity

To quantify the activity of PLD [24], 5 g of pericarp tissues were finely ground using a mortar and pestle in a liquid nitrogen environment. The resulting powder, weighing 0.5 g, was then extracted with 2.5 mL of 0.1 M Tris-HCl buffer (pH 7.0) and centrifuged at 13,000× *g* for 30 min at 4 °C. The supernatant was collected post-centrifugation and used to assess the PLD activity. The reaction substrate was created by combining 0.05 g of phosphatidylcholine (lecithin) with a mixture of 3 mL of chloroform and 3 mL of purified water. This solution was evaporated to dryness using a rotary evaporator set at 40 °C. Subsequently, the substrate was rehydrated in 250 mL of 100 mM acetate buffer (pH 5.5, also containing 5 mM dithiothreitol and 25 mM $CaCl_2$). Then, 1 mL of the crude enzyme extract was mixed with 3 mL of the rehydrated reaction substrate. This mixture was vigorously stirred for an hour in ambient conditions before being treated with petroleum ether. The aqueous phase was collected, and 2 g of ammonium tetrarhodanatodiammonchromate in 100 mL of methanol was added to the reaction solution, causing a precipitate to form. Then, the precipitate was separated by centrifugation at 26,000× *g* and dissolved in 3 mL of acetone. The absorbance of the resulting clear supernatant was measured at 520 nm using a UV-visible spectrophotometer. A calibration curve for choline was generated by diluting 20 mg of choline chloride in 100 mL of 100 mM acetate buffer (pH 5.6). A single unit of PLD activity was defined as the micromoles of choline produced per minute on a fresh weight (FW) basis.

Determination of LOX Activity

To quantify pericarp LOX activity [25], 5 g of longkong fruit pericarp was homogenized in cold conditions (4 °C) in 10 mL of (50 mM, pH 7) potassium phosphate buffer. After that, the homogenate was centrifuged at 4 °C for 15 min at 15,000× *g*, and the supernatant was collected and used for LOX activity. Next, 0.2 mL of crude extract was mixed with 2.8 mL of potassium phosphate buffer containing 25 mM of sodium linoleate as a substrate, and then, the absorbance of the reaction mixture was measured at 234 nm using a UV-Visible spectrometer. The definition of LOX activity was as follows: one unit of LOX activity corresponded to the quantity of the enzyme that induced a change of 0.01 in absorbance within a minute. The resulting enzyme activity was expressed as U per g of FW.

Determination of SOD Activity

To quantify the SOD activity [26], 10 g of pericarp tissue was homogenized in a cold environment (around 4 °C) using 50 mL of (100 mmol/L, pH 6.4) sodium phosphate buffer that included 1% polyvinylpyrrolidone. After homogenization, the reaction mixture was centrifuged at 15,000× *g* for 20 min at 4 °C. The resulting supernatant was collected and used for the SOD assay. For the SOD assay, a reaction mixture of 2.9 mL was prepared, consisting of 0.1 mmol/L sodium EDTA, 13 mmol/L methionine, 75 µmol/L nitro blue tetrazolium chloride (NBT), and 0.5 µmol/L riboflavin. To initiate the reaction, 0.1 mL of the collected supernatant was added to the reaction mixture. The mixture was then incubated at room temperature under continuous fluorescent light illumination (60 µmol/(m^2·s)) for a duration of 10 min. After the incubation period, the absorbance of the reaction mixture was measured at 560 nm using a UV-Visible spectrophotometer. In this assay, one unit of SOD activity was defined as the quantity of SOD enzyme that inhibited the rate of NBT reduction by 50% under the specified assay conditions. The resulting enzyme activity was expressed as units per gram FW.

Determination of CAT Activity

The CAT activity within the pericarp of the longkong fruit was quantified through a method outlined by Dhindsa et al. [27]. A 10 g sample of the pericarp tissue was meticulously homogenized under cool conditions with 50 mL of a 50 mmol/L sodium phosphate buffer, which had a pH level of 7.8 and contained 1% polyvinylpyrrolidone. Following the homogenization process, the crude CAT extract was centrifuged at 15,000× *g* for 20 min at 4 °C. Subsequently, the supernatant was gathered for CAT analysis. A mixture was made using 0.1 mL of the raw enzyme extract and 3 mL of the identical extraction buffer with no added polyvinylpyrrolidone. This was then followed by the addition of 0.1 mL of 15 mmol/L hydrogen peroxide. Following this, the absorbance of the reaction mixture was evaluated at 240 nm every 40 s using a UV-Vis spectrophotometer. A single unit of CAT activity was determined by an increment of 0.1 absorbance unit per minute. The resulting enzymatic activity is expressed in terms of units per gram by fresh weight (FW).

Determination of GPX Activity

To quantify the GPX activity in the pericarp of the longkong fruit, extraction and determination were conducted following the method described by Modesti et al. [28]. A total of 10 g of pericarp samples were collected and subjected to homogenization in cold conditions using 50 mL of extraction buffer containing 50 mmol/L sodium phosphate buffer at pH 7, along with 1% polyvinylpyrrolidone (PVP). After the extraction, the homogenate was centrifuged at 15,000× *g* for 20 min at 4 °C. The resulting supernatant was collected for analysis of GPX activities. For the GPX assay, 0.1 mL of the crude GPX extraction was mixed with 2.7 mL of the extraction buffer without PVP. Following that, 0.1 mL of 20 mmol/L hydrogen peroxide and 0.1 mL of 20 mmol/L guaiacol were added to the mixture. The reaction mixture was then measured using a UV-Vis spectrometer at 470 nm. In this assay, one unit of GPX activity was defined as a 0.01 increase in absorbance units per minute. The enzyme activity is presented in units per gram by fresh weight (FW).

2.3. Statistical Analysis

All experiments were conducted in triplicates, and the results were presented as mean ± standard deviations (SD). To determine significant differences between the mean values of each treatment, an analysis of variance (ANOVA) test was performed. The significant differences between parameter means were assessed using the Least Significant Difference (LSD) test, with a significance level set at $p < 0.05$. The statistical analysis was carried out using SPSS software (v6) for Windows, provided by SPSS Inc., located in Chicago, IL, USA.

3. Results and Discussion

3.1. Pericarp Color Characteristics and Browning Index

Pericarp color characteristics, including lightness (L*), redness (a*), and yellowness (b*) of the longkong fruit that was treated with aqueous n-butanol at varying concentrations, are displayed in Figure 2A–C. The results demonstrated that an increased storage period under ambient conditions significantly affected the overall color characteristics of the longkong pericarp. A drastic decrease in the L* and b* values was observed in the control samples, whereas the application of n-butanol substantially mitigated color loss in comparison with the control (Figure 2A,B). Furthermore, the differences among samples treated with varying concentrations of n-butanol were significant; they suppressed color changes in a dose-dependent manner. On the other hand, a continuous increase in a* values was observed in all samples, with the 0.6% n-butanol-treated samples effectively limiting the rise in a* values (Figure 2C). Generally, it is well known that a decrease in L* and b* values, along with an increase in a* values, indicates increased pericarp browning in longkong fruit. Oxidoreductase enzymes, particularly PPO and LOX, are major contributors to the increasing browning in longkong fruit. Similarly, as depicted in Figure 2D, the browning level on the longkong fruit pericarp has continuously increased in both the control and n-butanol-treated samples. However, the control fruits experienced browning more quickly

than those treated with n-butanol. The n-butanol treatment effectively reduced surface browning, first noticeable on the seventh day of storage at ambient temperature. In contrast, the onset of browning was apparent in the control fruits as early as the third day. The concentration of n-butanol significantly influenced the anti-browning efficacy of the longkong fruit. Among the tested concentrations, higher levels of n-butanol were more effective in controlling browning than lower concentrations. Although the effects of n-butanol were dose-dependent, even at lower concentrations, it proved effective enough to control surface browning in longkong compared to the control. Surface browning is a primary indicator of longkong fruit's shelf life. Fruits treated with n-butanol were capable of mitigating the severity of shelf-life deterioration by controlling surface browning compared with the control fruits. These findings align with the study of litchi fruit [9], where n-butanol treatments significantly reduced browning during storage. Lichanporn et al. [29] reported that browning primarily contributes to the loss of longkong fruit quality, which is accelerated by external stimuli produced either by poor handling or postharvest disease infections. Noonim and Venkatachalam [5] reported that phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, and lipoxygenase significantly contribute to the increase in pericarp browning incidence in longkong fruit. Lin et al. [30] found that the reduced pericarp browning incidence during storage was primarily due to the increased imbalance in metabolic reactions, which generate reactive oxygen species and disrupt homeostasis and cell mitochondrial integrity. This leads to decreased energy charge exchange and decompartmentalization of the cell membrane, which results in browning incidence and subsequent pericarp browning. Li et al. [11] found that butanol treatment on litchi fruit significantly lowered senescence hormones and the respiration rate, suppressed membrane phospholipid degradation during storage, and consequently, lower pericarp browning was noticed compared with untreated fruits.

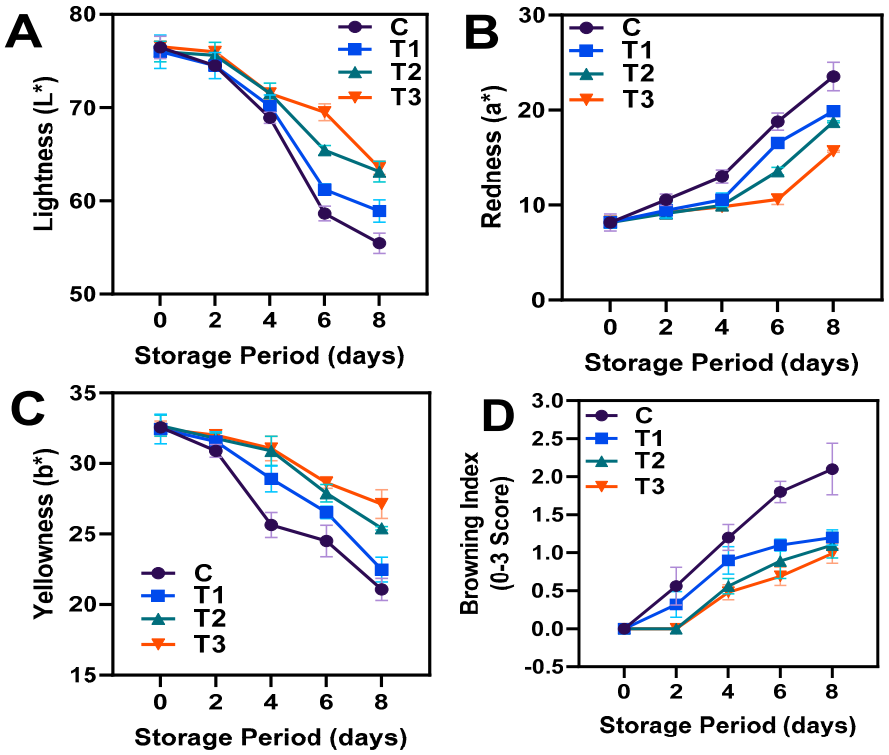

Figure 2. Color characteristics (**A**–**C**) and browning index of longkong fruit pericarp during storage under ambient conditions (**D**). Note: "C" represents the control, "T1" represents n-butanol treatment at 0.2%, "T2" represents n-butanol treatment at 0.4%, and "T3" represents n-butanol treatment at 0.6%.

3.2. Membrane Permeability and MDA Content

Cell membrane integrity and permeability in fruits are often evaluated through the relative conductivity rate. The data revealed that ambient storage had negative impacts on the cell permeability and integrity of longkong fruits. The control fruit pericarp showed a heightened level of relative conductivity compared to the n-butanol-treated samples (Figure 3A). Among the treated samples, higher concentrations of n-butanol effectively managed membrane changes in the longkong pericarp compared to lower concentrations. A decline in the plasma cell membrane integrity of the fruit cell during extended storage could contribute to the loss of cell turgor, followed by decompartmentalization and various permeability issues [31]. An increase in relative membrane permeability and the activity of membrane-degrading enzymes are typically linked with membrane lipid peroxidation [32]. However, it was observed that the membrane integrity of the longkong fruit was slightly improved by treatment with n-butanol. Furthermore, the membrane permeability results aligned with the MDA content as a continuous loss of membrane integrity which could be initiated by the lipid peroxidation and led to an increased accumulation of MDA, a by-product of membrane lipid peroxidation. MDA, a marker of membrane lipid peroxidation, is used to evaluate cell membrane lipid peroxidation and cell membrane disintegration [33]. The MDA content in both control and treated samples gradually increased during the initial days of storage (first 3 days), and afterward, it rapidly escalated, with control fruits accumulating high levels of MDA content (Figure 3B). Conversely, longkong fruit pericarp treated with n-butanol significantly controlled MDA accumulation throughout storage. A lower MDA content was found in the 0.4% and 0.6% n-butanol treated samples compared to those treated with lower concentrations. Motes et al. [34] reported that n-butanol application on plants could control various biochemical reactions involved in physiological changes, and one of the major roles of n-butanol is the inhibition of PLD. This aligns with our study, where the application of n-butanol significantly decreased PLD activity (see Figure 4).

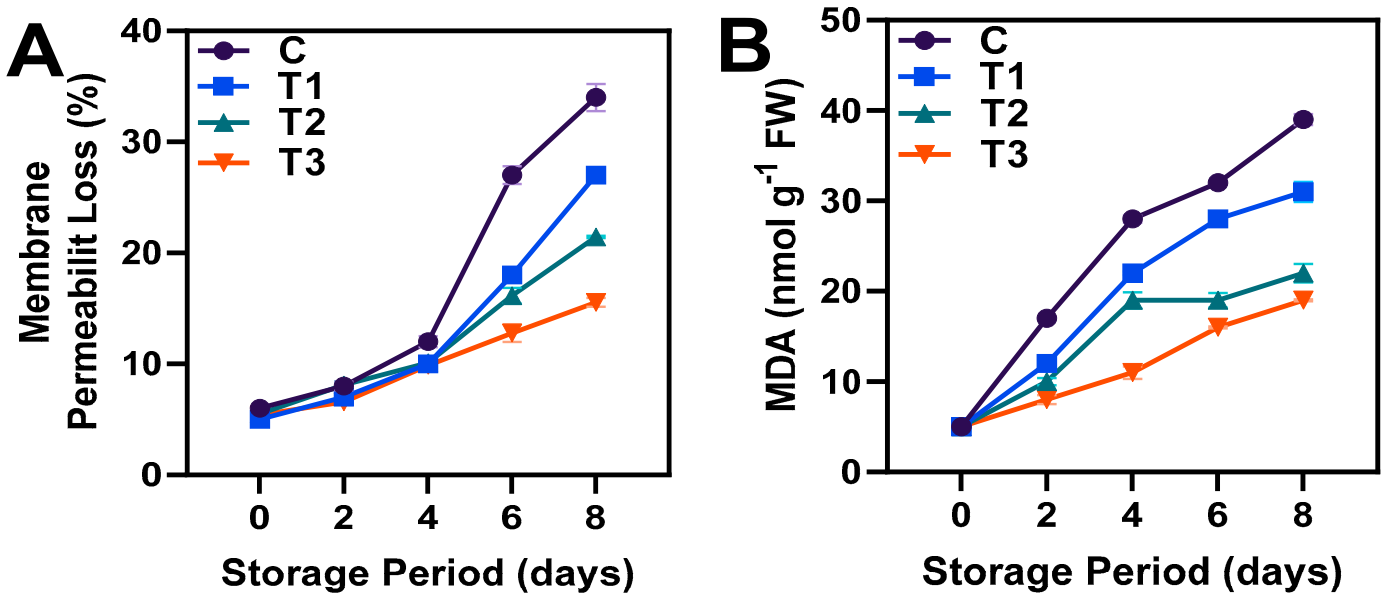

Figure 3. Membrane permeability loss (**A**) and MDA content (**B**) of longkong fruit pericarp during storage under ambient conditions. Note: "C" represents the control, "T1" represents n-butanol treatment at 0.2%, "T2" represents n-butanol treatment at 0.4%, and "T3" represents n-butanol treatment at 0.6%.

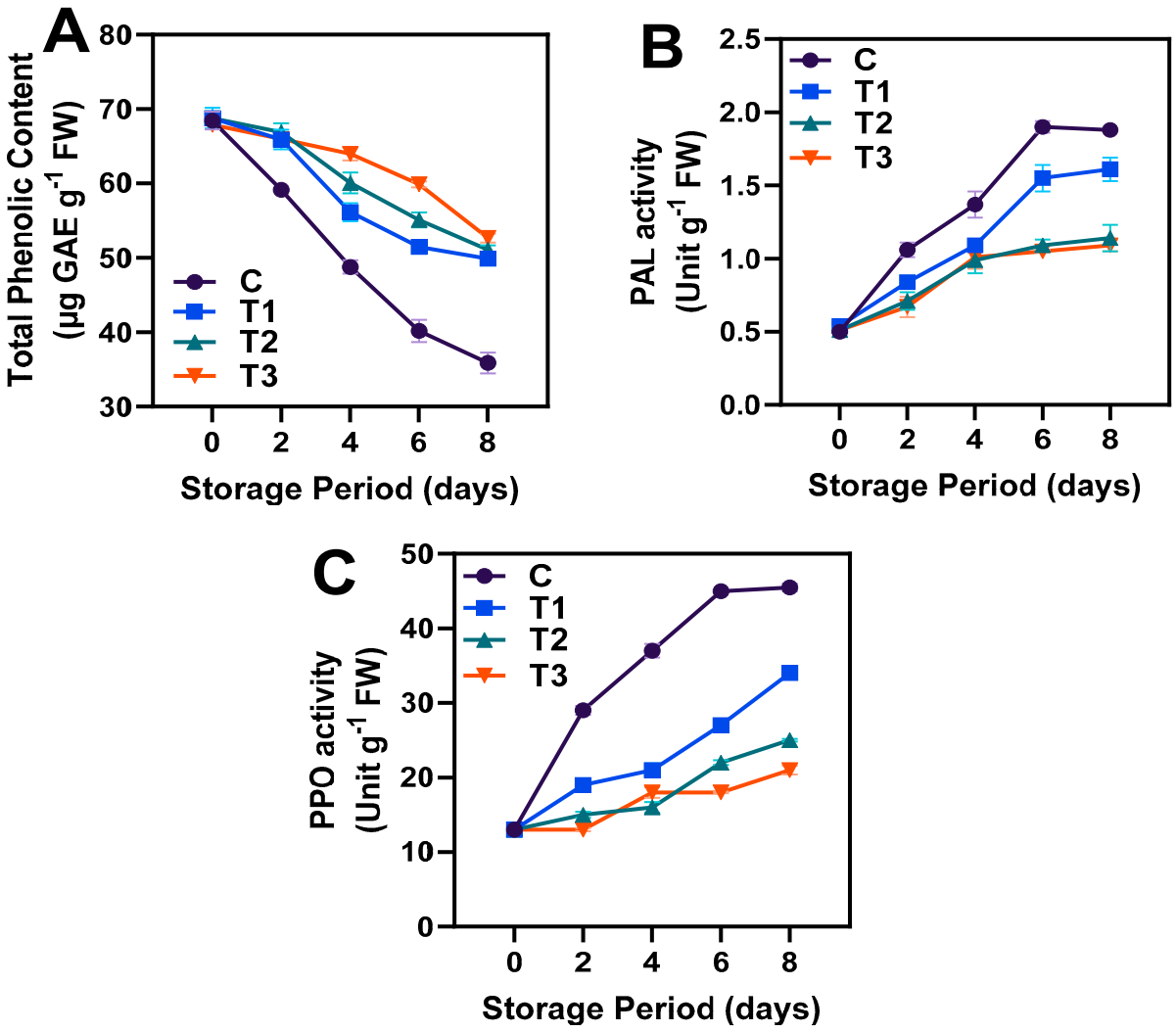

Figure 4. TPC content (**A**), PAL activity (**B**) and PPO activity (**C**) of longkong fruit pericarp during storage under ambient conditions. Note: "C" represents the control, "T1" represents n-butanol treatment at 0.2%, "T2" represents n-butanol treatment at 0.4%, and "T3" represents n-butanol treatment at 0.6%.

3.3. Total Phenolic Content and PAL and PPO Activity

The total phenolic content in the longkong fruit pericarp that was treated with n-butanol and stored under ambient temperature is shown in Figure 4A. The total phenolic content in the longkong pericarp gradually increased during the storage period. The control and treated fruits did not decrease much during the study period; however, the TPC levels were lost rapidly in the fruit samples after 3 days of storage, especially in the control fruits. On the other hand, the treated fruits were still able to maintain a high level of TPC until 7 days of storage as compared with the control fruits, and then the TPC decreased rapidly till the end of storage. Among the treated samples, the ones treated with n-butanol at 0.4 and 0.6% were able to retain more TPC than the others. Phytochemicals, especially phenolic acids and flavonoids, are abundant in the longkong fruit pericarp and its flesh. Phenolic acids in the longkong fruit are primarily produced by the enzyme called phenylalanine ammonia lyase (PAL) [35]. Phenylalanine ammonium-lyase (PAL), a key enzyme in the phenylpropanoid pathway, is involved in the defense response of plant cells, and it is generally used as a marker of environmental stress in different plant species. In this study, the longkong fruit pericarp had continuously increased levels of PAL activity during the beginning of the storage, and afterward, a slight decline in the activity

was noted at the end of storage. The control fruits had a larger amount of PAL activity as compared with the n-butanol-treated samples (Figure 4B). However, the differences were very minimal. Similarly, the controlling effect of n-butanol at higher concentrations did not significantly differ from each other. However, they were very significant when compared with other samples. Generally, PAL activity in plants could increase up to 5-fold when they are stored under stress conditions [36]. Lichanporn et al. [6] reported that longkong fruit stored under ambient temperature had significantly increased PAL activity followed by severe pericarp browning. A slight decrease in PAL activity at the end of storage could be the effect of the abundant accumulation of phenolic acid production, particularly chlorogenic acid, which is the direct inhibitor of the PAL enzyme [37]. On the other hand, the polyphenol oxidase activity increased in both samples over the storage period. In the initial storage period, the polyphenol oxidase activity in the samples was not high; however, at a later stage of storage, a consistent increment in activities was noticed in all the samples, and n-butanol-treated samples, especially at higher concentrations had significantly suppressed the polyphenol oxidase activity as compared with control (Figure 4C). A continuous decompartmentalization of the cell membrane could rapidly increase the activity of the PPO activity in phenolic-rich plants [38]. Numerous studies have reported that longkong pericarp browning is mainly attributed to PPO activity [39]. The n-butanol controlling effect on PPO activity was not direct; however, controlling the lipid peroxidation and its related activities could reduce the browning intensity in the longkong fruit during extended storage at ambient temperature. Sun et al. [40] observed that n-butanol-treated litchi fruit had lower PPO activity, and it was mainly caused by the inhibitory effect of PLD in the fruits, followed by reduced lipid peroxidation, which reduced the cell wall and cell membrane damage. Furthermore, Liu et al. [41] reported that PAL activity in plants could lead to the production of lignin precursor compounds. Lignin is a phenolic polymer that is mainly found in plant cells and protects the cell wall from various stress-induced destruction.

3.4. PLD and LOX Activities and ROS Production

PLD and LOX play crucial roles in lipid metabolism. PLD initiates the hydrolysis of phospholipids, followed by the oxygenation of polyunsaturated fats by the LOX. A loss of compartmentalization of the cell membrane system occurs because of peroxidation products attacking the cell membrane [42]. PLD and LOX activities in control and treated longkong fruit pericarps were tested during storage (Figure 5A,B). Overall, the membrane degrading enzymes tend to increase gradually in the longkong pericarp all over the storage. PLD activity in the control fruits had shown significantly higher activity than in the treated fruits. The treated fruits pericarp significantly controlled the PLD activity over the study period. Among the treated ones, the 0.6% n-butanol application established the least PLD activity in the longkong pericarp. Generally, phospholipids are the principal components in the plant cell membrane compositions, and they undergo significant changes by the hydrolytic enzymes under stress conditions. PLD activity is a direct indicator of phospholipid hydrolysis. PLD is a crucial enzyme that utilizes these phospholipids and converts them to various metabolic products, specifically phosphatidic acid, which promotes the destabilization of the cell membrane and is followed by damage and lipid peroxidation in the cell membranes [43]. This study showed that n-butanol significantly controlled PLD activity, mainly due to the inhibitory action of n-butanol, which disrupts the activity of PLD on converting the phospholipids to phosphatidic acid instead of converting them to phosphatidyl butanol. Similarly, LOX activity in the longkong pericarp was gradually increased throughout storage; at the beginning of the storage, LOX activities were not high in the pericarp; however, the onset of higher LOX activities was observed in the middle of storage and followed till the end of the storage. The longkong pericarp with n-butanol applied was slightly better in controlling LOX activity than in the control fruits. However, among the treated samples, the differences in LOX activities were not much ($p > 0.05$). It was found that throughout storage, a stable increment in LOX activity

was noticed; however, LOX activity was significantly deficient. It could be due to the lower activity of PLD in the pericarps, which suppresses the cellular damage and LOX activities. Several studies have demonstrated that PLD and LOX in plants are critical factors involved in phospholipid catabolism, which initiates a lipolytic cascade that results in membrane degeneration during senescence and stress [44]. Sun et al. [40] studied the n-butanol activity on the pear fruit's lipid metabolism, and their results found that the n-butanol treatment had significantly inhibited LOX activity and suppressed its gene expressions in the fruits, followed by the reduced accumulation of statured and unsaturated fatty acids, particularly, oleic acid, stearic and palmitic acids.

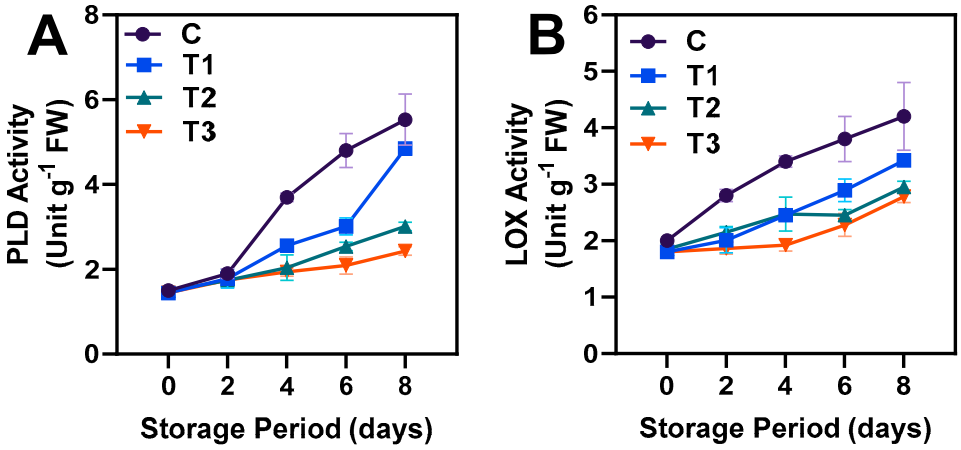

Figure 5. Changes in PLD activity (**A**) and LOX activity (**B**) of longkong fruit pericarp during storage under ambient conditions. Note: "C" represents the control, "T1" represents n-butanol treatment at 0.2%, "T2" represents n-butanol treatment at 0.4%, and "T3" represents n-butanol treatment at 0.6%.

ROS production in the longkong fruit pericarp during storage at ambient temperature is illustrated in Figure 6A–C. Typically, the production of reactive oxygen species (ROS) is a crucial aspect of aerobic respiration and metabolism [45]. Several types of ROS exist, including radicals such as superoxide, hydroxyl, peroxyl, and alkoxyl, and non-radicals that are either oxidizing agents or can be easily converted into radicals. These non-radicals include hypochlorous acid, ozone, singlet oxygen, and hydrogen peroxide [46]. In the present study, the longkong pericarp treated with n-butanol was tested for superoxide radical, hydrogen peroxide, and hydroxyl radical. The results showed that n-butanol-treated fruit significantly reduced the overall production of ROS, whereas the control fruits exhibited a higher production. Superoxide anion radical levels continuously increased in all samples. Initially, on the first day of storage, fruits did not show any increment in radicals; however, starting on the second day, a rapid increase in superoxide radicals was observed in the control samples, followed by the treated ones. The high concentration of n-butanol-treated samples exhibited better control of superoxide radical accumulations. Similarly, increases in hydrogen peroxide and hydroxyl radical were observed throughout storage in all samples. Nevertheless, n-butanol was able to control these radicals, with higher concentrations performing better than lower ones. Among the radicals tested, hydrogen peroxide was found in the highest concentration, followed by the hydroxyl radical and superoxide radicals. ROS reduction is vital in fruits, as it could induce rapid metabolic reactions related to enzymatic browning [47]. LOX activity could stimulate the production of NADPH oxidases (NOX), which have a significant role in ROS production in plants [48]. However, in this study, the n-butanol-treated fruits successfully reduced the activity of PLD, and as a result, the LOX and NOX activities and their metabolic by-products, including ROS, were substantially reduced. Rossatto et al. [49] reported that antioxidant enzymes, particularly superoxide dismutase, catalase, and ascorbate peroxidase, could also reduce ROS production in plants.

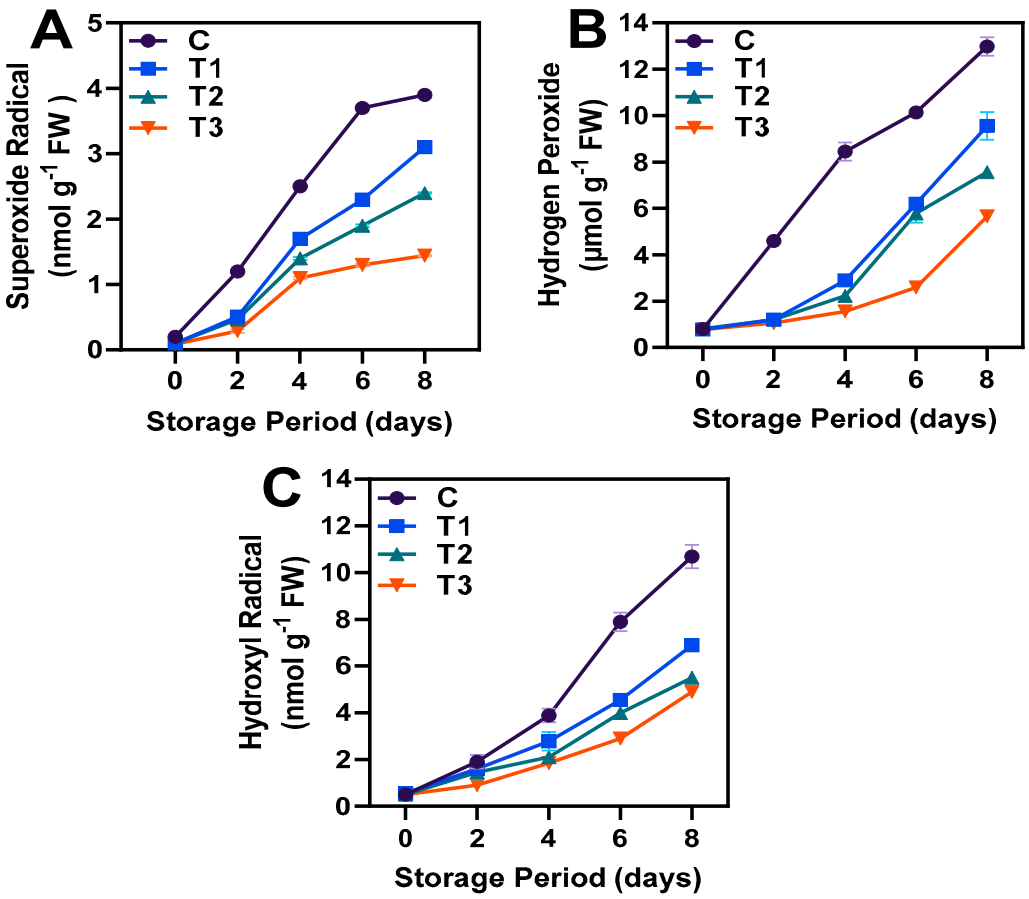

Figure 6. Production of ROS (superoxide radical (**A**), hydrogen peroxide (**B**) and hydroxyl radical (**C**)) in longkong fruit pericarp during storage under ambient conditions. Note: "C" represents the control, "T1" represents n-butanol treatment at 0.2%, "T2" represents n-butanol treatment at 0.4%, and "T3" represents n-butanol treatment at 0.6%.

3.5. Antioxidant Enzyme Activities

The antioxidant activities in the longkong pericarp treated with n-butanol are shown in Figure 7. Overall, the results showed that the antioxidant activities in both control and treated fruits pericarp were increased throughout the storage period. Notably, the control fruits had slightly lower antioxidant enzyme activities than the n-butanol-treated ones. The SOD activity in the treated samples was significantly higher than in control fruits (Figure 7A). During the testing period, the SOD activities did not differ between the control and treated fruits pericarp. However, when the storage period was prolonged, the activities were significantly higher in the treated fruits. Furthermore, the SOD activity was very high in the samples that were treated with high n-butanol concentrations. Overall, the results indicate that a prolonged storage period had adversely affected the membrane system and continuously produced the superoxide anion radicals, triggering the SOD enzymes and alleviating superoxide anion radicals. The CAT and GPX enzyme activities in the fruit pericarp are in accordance with the results of SOD activities, as CAT and GPX enzymes utilize hydrogen peroxide as substrate and generate water and oxygen as a by-product prohibition of ROS accumulation in the fruits. This study shows that CAT and GPX (Figure 7B,C) were continuously increased in the longkong pericarp during storage at the ambient temperature ($p < 0.05$). Generally, as a result of stress conditions in fruits, particularly by senescence and browning, ROS are extensively generated, causing the membrane lipids to degrade and resulting in loss of functions and integrity in the cell membranes [50]. Naturally, the plant develops a natural defense mechanism, including enzymatic and nonenzymatic defenses against ROS [51]. The different concentrations

of n-butanol treatment had somewhat increased the antioxidant enzyme activities in the longkong pericarp, and among them, a higher concentration of n-butanol induced more GPX activity as compared with the others. Tanko et al. [52] observed that the application of n-butanol had increased the antioxidant enzymes in the *Indiogfera pulchara* plants. In another study, Abu et al. [53] also found that the application of n-butanol had significantly increased the antioxidant potency of *Ficus glumosa* leaves. Chen et al. [54] reported that the application of n-butanol in Ligustrum robustum induced the production of various antioxidant genes to deal with MDA and hydrogen peroxide. Although several studies have shown an increment in antioxidant enzymes in various plants, the direct mechanism of n-butanol in inducing antioxidant enzymes in plants is still unclear.

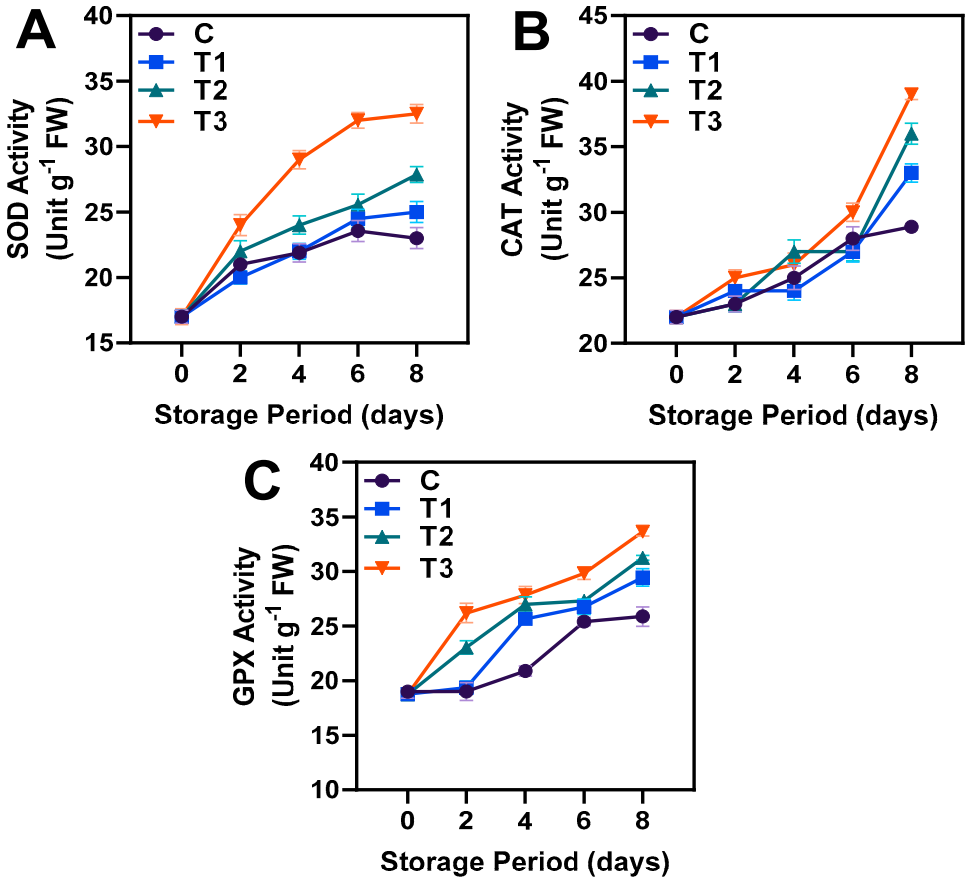

Figure 7. Changes in SOD activity (**A**), CAT activity (**B**) and GPX activity (**C**) of longkong fruit pericarp during storage under ambient conditions. Note: "C" represents the control, "T1" represents n-butanol treatment at 0.2%, "T2" represents n-butanol treatment at 0.4%, and "T3" represents n-butanol treatment at 0.6%.

4. Conclusions

The present study explored the efficacy of n-butanol in controlling pericarp browning and enzymatic and cellular degrading enzymes in the longkong pericarp during prolonged storage under ambient temperature. The results showed that the application of n-butanol at different concentrations had effectively controlled the degradation of cellular membranes, followed by a reduction in malondialdehyde and reactive oxygen species production. n-butanol at high concentrations, particularly at 0.4 and 0.6%, significantly inhibited phospholipids, and as a consequence, reduced activity of lipoxygenase and polyphenol oxidase was observed in the longkong pericarp. Browning incidences in the longkong pericarp were continuously noticed; however, the control fruit suffered at a high level compared to the treatment, supporting the n-butanol inhibitory effect against the cellular degradation enzymes. Prolonged storage had continuously accumulated the reactive oxygen species

in the samples. However, this was controlled in the treated pericarp, mainly attributed to the antioxidant enzyme activities. Overall, this study proved that n-butanol at 0.4 and 0.6% is a highly suitable and effective alternative to other harmful chemical applications for controlling longkong pericarp browning and browning-related enzymes and extending the shelf life at ambient temperature. Future research focused on enhancing the storage life and quality of longkong fruit, as well as advancing sustainable agriculture, will need to be investigated to provide a diversified strategy. This strategy will involve the use of n-butanol in synergy with other organic substances. Furthermore, the study will explore the implementation of both passive and active modified atmospheric packaging or hurdle technology, aligning with innovative preservation techniques. This comprehensive approach offers a promising pathway toward efficient long-term storage and a more sustainable agricultural future.

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