# Effect of Alginate Oligosaccharides on Oxidative Reactions in Sweet Basil During Low-Temperature Storage

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## Abstract

　　This study aimed to investigate the antioxidant effect of alginate oligosaccharides (AOS) on sweet basil leaves preserved at low temperatures. This investigation was divided into 3 treatments and 3 replications using a completely randomized design (CRD) experiment. Specifically, the effects of dipping basil leaves in AOS solutions at different concentrations (100 and 200 mg $L^{-1}$) and in distilled water (control) for 1 min were compared. Cell deterioration due to cold storage was investigated based on the variation in oxidative reactions in the cells, causing an accumulation of free radicals. The AOS-treated sweet basil leaves had a 12-day shelf life, compared to just 9 days for those in the control group. Those treated with AOS at 100 mg $L^{-1}$ exhibited the lowest levels of phenylalanine ammonia-lyase, lipoxygenase, hydrogen peroxide, peroxidase and malondialdehyde content. On the contrary, the contents of phenolic compounds, polyphenol oxidase, catalase and superoxide dismutase increased in the treated leaves during storage compared to those observed in the control treatment.

**Keywords:** Sweet basil, Alginate oligosaccharides, Chilling injury, Antioxidation, Postharvest quality

## Introduction

　　*Ocimum basilicum* Linn., or sweet basil, is a widely used herb and important ingredient in Thai cuisine. Toxic residues, pesticide residues, microbiological contamination and short shelf life are the main challenges affecting basil handling. This are caused by substandard postharvest management and result in economic losses. In addition to the issue of microbiological contamination, it is also a significant concern that basil leaves that are kept at low temperatures for extended periods of time, eventually turn brown. Due to its tropical origins, sweet basil is susceptible to chilling injury (CI) at temperatures below 10 °C [1], which also results in lipid degradation. According to Maalekuu *et al.* [2], lipoxygenase (LOX), which is usually activated during CI, may be partially involved in the breakdown of lipids. Malondialdehyde (MDA) and peroxide ions are produced during the degradation of polyunsaturated fatty acids. MDA accumulation is commonly used as a CI indicator. The low activity of antioxidant enzymes such superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) is also frequently associated with CI [3].

　　Alginate oligosaccharides (AOS) are linear low-molecular-weight polymers composed of 2 - 25 monosaccharides linked to α-L-glucuronic acid (G) and/or β-D-mannuronic acid (M) in accordance with alginate through 1,4-glycosidic connections. M and G are each other's C5 epimers [4]. AOS are naturally occurring immunogenic and biodegradable compounds with low toxicity, and their attributes have drawn a lot of interest from researchers. As a result, AOS are now used for a wide range of applications in processing food, medicine, animal nutrition and agriculture. According to reports, AOS have important qualities,

including antioxidant, antibacterial and anti-inflammatory properties [5,6]. Initial studies have investigated the use of alginate-derived oligosaccharides (ADOs) to potentially treat dehydration stress in cucumbers (*Cucumis sativus* L.). ADOs were shown to reduce the harm caused by dehydration in this plant and also to boost photosynthetic efficiency as well as POD and SOD activities [7].

A 2019 study on the use of AOS to extend the shelf life of strawberries revealed that AOS had an effect on the accumulation of abscisic acid in this fruit and were able to delay its softening and deterioration [8]. Later in 2020, a study on the efficacy of AOS in extending the shelf life of kiwifruit stored at 25 °C reported that these compounds can maintain fruit quality and extend shelf life owing to their ability to stimulate the activity of antioxidant substances, including CAT and SOD [9].

Prebiotics, a group of oligosaccharides that includes chitosan oligosaccharides, alginate oligosaccharides and pectin oligosaccharides, have also been used to improve fruit storage after harvest. Studies have shown that these oligosaccharides enhance fruit quality and extend the shelf life of fruits, slow down the breakdown of cell walls, increase antioxidants, control hormone synthesis and promote the activity of helpful microbes [10]. Consequently, the aim of the present study was to further clarify how AOS affect oxidative responses during low-temperature storage, particularly in terms of the postharvest biochemical alterations associated with POD, LOX and CAT.

## Materials and methods

### Plant material and treatments

Sweet basil (*Ocimum basilicum*) was harvested early in the morning from local farms in the Phetchaburi district of Thailand and carried by truck at room temperature (35 ± 2 °C) to the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University. A commercial maturity of basil plant harvest should be completed just before the plants start to flower. Cut the entire plant 4 inches above the ground to promote regrowth. Leaves without defects or disease symptoms were selected and cleaned using tap water and then allowed to air dry in room at 25 °C. 3 different treatments were conducted by submerging the sweet basil leaves in the following 3 solutions for 1 min: 1) distilled water (control), 2) 100 mg. $L^{-1}$ AOS, and 3) 200 mg. $L^{-1}$ AOS. Each treatment group was packed (approximately 200 g) into an 80 μ linear low density polyethylene bag (bag size, 7 × 24 cm, 144 bags in total) and stored at 10 °C for 12 days. Samples were randomly collected from each treatment group at 0, 3, 6, 9 and 12 days after treatment for microbial and chemical assays. All parameters were measured in triplicate.

### Phenolic compounds

Determination of phenolic compounds used the method of Ketsa and Atantee. [11]. Tissue powder (0.5 g) was extracted using 1 mL of 80 % ethanol, and the mixture was centrifuged at 15,000 rpm for 20 min at 4 °C. The assay reaction mixture consisted of 150 μl of 7.5 % sodium carbonate, 100 μl of 10 % Folin-Ciocalteu reagent, and 20 μl of supernatant. Absorbance was recorded at 765 nm using a UV-vis microplate reader. The overall quantity of phenolic compounds was determined using the standard curve of gallic acid.

### Phenylalanine ammonia-lyase (PAL) activity

PAL activity was measured using a method modified from Ke and Saltveitis [12]. 1 mL of 50 mM borate buffer (pH 8.6) containing 2 % polyvinylpyrrolidone and 5 mM 2-mercaptoethanol was used to homogenise a 0.5 g sample of tissue powder. The homogenised mixture was centrifuged at 15,000 rpm and 4 °C for 30 min. The reaction was detected at 290 nm using a microplate reader, and the mixture was then

incubated for 1 h at 30 °C after adding 10 µl of supernatant, 150 µl of 0.05M borate buffer, and 40 µl of 10 mM L-phenylalanine. An enzyme activity unit was defined as the quantity of PAL mmol of cinnamic acid produced in 1 h.

### Polyphenol oxidase and peroxidase (PPO and POD) activities

The PPO and POD enzyme extracts were prepared by homogenising 0.5 g of sample with 1 mL of 50 mM potassium phosphate buffer (pH 6.5) containing 0.5 % polyvinylpyrrolidone for 30 min at 15,000 rpm and 4 °C. The enzymes were then extracted to determine their activity.

PPO activity was determined by measuring an increase in absorbance at 480 nm for 3 min, as described in Galeazzi and Sgarbieri [13]. A total of 20 µl of enzyme extract, 50 µl of 50 mM potassium phosphate buffer (pH 6.5), and 200 µl of 25 mM catechol solution were added to start the reaction. 1 unit of enzyme activity was defined as the amount of enzyme causing a change of 0.001 in absorbance per min.

POD activity was measured based on the method described in Castillo *et al.* [14]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 6.5), 16 mM guaiacol, 2 mM hydrogen peroxide and the enzyme extract. The reaction rate increased the absorbance at 470 nm for 3 min. The extinction coefficient of 26.6 $mM^{-1}$ $cm^{-1}$ was used to determine POD activity. The activities of PPO and POD were expressed as units $mg^{-1}$ protein. The protein content was calculated using bovine serum albumin as a standard based on the Bradford technique (1976) [15].

### Malondialdehyde (MDA) content

The thiobarbituric acid (TBA) reaction demonstrated that MDA was a byproduct of the oxidation of polyunsaturated fatty acids [16]. A total of 0.5 g of sample was homogenised with 1 mL of 80 % ethanol and centrifuged at 15,000 rpm for 30 min at 4 °C. After centrifugation, 1 mL of the supernatant was mixed with 1 mL of 0.65 % TBA solution containing 20.0 % (w/v) trichloroacetic acid (TCA) and 0.01 % butylated hydroxytoluene (BHT) or added to a solution containing 20.0 % (w/v) TCA and 0.01% BHT after that being incubated for 30 min at 95 °C and immediately chilled in an ice bath. Absorbance was measured at 532, 440 and 600 nm. An extinction coefficient of 157 $mM^{-1}cm^{-1}$ was used to calculate the MDA content.

### Hydrogen peroxide content

A total of 0.5 g of sample was extracted using 1 mL of 1 % trichloroacetic acid (TCA), and centrifuged at 15,000 rpm for 30 min. The supernatant was mixed with 50 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide, and the absorbance was measured at 390 nm. The results were expressed based on the standard curve of hydrogen peroxide [17].

### Lipoxygenase (LOX) activity

LOX activity was determined based on Maalekuu *et al.* [2]. To prepare the enzyme extract, 0.5 g of sample was homogenised with 1 mL of 50 mM potassium phosphate buffer (pH 7.0), and the mixture was centrifuged at 15,000 rpm for 30 min at 4 °C. The reaction was initiated by adding linoleic acid and enzyme extract. LOX activity was calculated using an extinction coefficient of 25.00 $mM^{-1}$ $cm^{-1}$, and absorbance was then measured at 234 mM.

### Catalase (CAT) activity

A total of 0.5 g of sample was extracted using 1 mL of 0.1 M phosphate buffer (pH 7.0) and centrifuged at 15,000 rpm for 30 min. The reaction mixture was mixed with 0.1 M phosphate buffer (pH

7.0) and 30 mM hydrogen peroxide, and the absorbance was then measured at 240 nm. CAT activity was determined via hydrogen peroxide decomposition (extinction coefficient, 39.4 mM$^{-1}$ cm$^{-1}$) [16].

### SOD activity

SOD activity was determined based on the method described in Ukeda *et al.* [18]. The reaction mixture included 0.75 mM XTT (2,3-bis(2methoxy-4-nitro-5-sulophenyl)-2H-tetrazolium-5-carboxanilide), 3 mM xanthine, 3 mM EDTA and 0.14 units of xanthine oxidase. Sodium phosphate buffer (pH 8.0) was also present. The enzyme activity was measured using solutions containing and excluding the enzyme extract by tracking the rate of XTT reduction at 470 nm. The amount of enzyme that produced a half-maximal inhibition was defined as the unit of SOD.

### Experimental design and statistical analysis

A completely randomised design was used for the entire experiment. One-way analysis of variance (ANOVA) was used to evaluate all the data. Tukey HSD (Tukey's honestly significant difference) was used to perform mean separations. Differences were considered significant at $p \leq 0.01$.

## Results and discussion

### Variations in phenolic compounds, PAL activity, and polyphenol oxidase (PPO) activity

The shelf life of the AOS-treated sweet basil leaves was 12 days while that of untreated leaves was just 9 days. The browning and rotting of the untreated basil leaves indicated CI, which had a negative impact on shelf life. Consequently, for the control sweet basil leaves, only the data collected over the 9-day period of storage were considered. Variations in phenolic compounds, PAL activity, and PPO activity were determined to identify the effect of browning in the cells during storage.

Phenolic compounds are classified as antioxidants and are found in abundance in nature, for example in vegetables, fruits, spices, herbs, dried beans and whole grains. Phenolic compounds play a crucial role in the health benefits and sensory properties of basil. These chemicals have antibacterial, anti-inflammatory, and antioxidant properties that improve the flavour and aroma of the plant while also providing a number of health benefits. They protect organisms against damage caused by various stresses. Stress-induced damage generates free radicals, including hydrogen peroxide, superoxide anion radicals, hydroxyl radicals and peroxyl radicals, which harm cells and cause the loss of their functions. These factors alter cells either chemically or physically, ultimately resulting in cell degeneration [19,20]. **Figure 1** shows the phenolic compounds found in the sweet basil leaves immerged in distilled water (control) and in the 100 mg L$^{-1}$ and 200 mg. L$^{-1}$ AOS solutions. In the treated leaves, the synthesis of phenolic chemicals was promoted during the first days of storage (at 3 days after treatment). However, after 6 days, this activity rapidly decreased and was rarely steady until the end of the storage period. Compared to the other treatments, the 100 mg. L$^{-1}$ AOS treatment exhibited the highest accumulation of phenolic compounds after 9 days of storage.
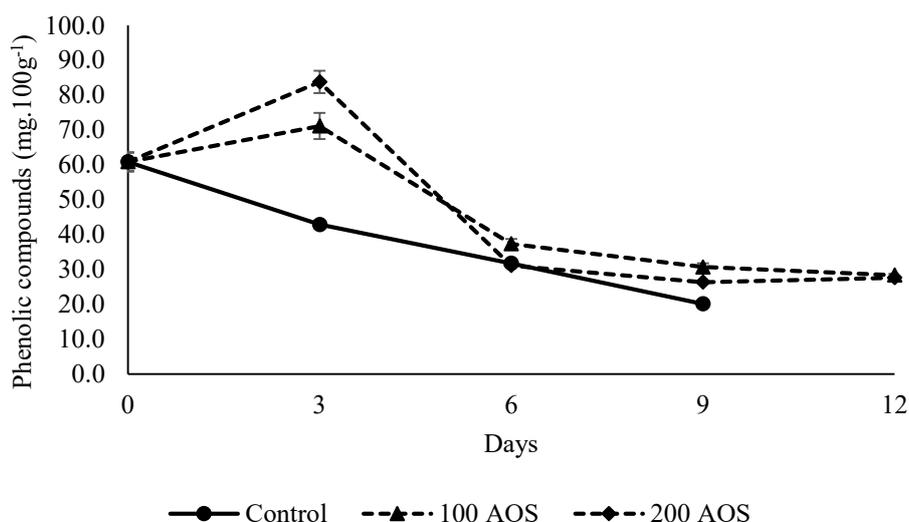
**Figure 1** Effect of phenolic compounds in the sweet basil leaves immersed in distilled water (control) and in alginate oligosaccharide (AOS) solutions (100 AOS = 100 mg. $L^{-1}$ and 200 AOS = 200 mg. $L^{-1}$) during storage at 10 °C. Values are expressed as means ± standard errors (replication = 3). Different letters indicate significant differences among treatments for each sampling time at $p \leq 0.01$.

In the AOS-treated sweet basil leaves, PAL activity was shown to rapidly decrease during the first 3 days of storage and then to stabilise throughout the rest of the storage period [*please rephrase after this point*] which was statistical significance (**Figure 2**). PAL activity was the highest in the control treatment and the lowest in the 100 mg. $L^{-1}$ AOS treatment. Similarly, PAL activity was higher in the 100 mg. $L^{-1}$ AOS treatment than in the 200 mg. $L^{-1}$ AOS treatment. PAL is activated in stressed or wounded plants and stimulates the synthesis of phenolic compounds [21,22]. According to previous reports, AOS promote an increase in the activity of enzymes involved in stimulating plant immunity, such as PAL, PPO, CAT and SOD [23,24].

**Figure 3** shows the variations in PPO activity. During the first 3 days of storage, PPO activity in the control treatment rapidly dropped. In contrast, under the 100 and 200 mg. $L^{-1}$ AOS treatments, it decreased on days 9 and 6, respectively. Compared with the other treatments, the 100 mg. $L^{-1}$ treatment resulted in the highest PPO activity, suggesting that AOS had an impact on the activity of this enzyme. According to Han *et al.* [25], the application of AOS in *Meyerozyma guilliermondii* increased the activity of several enzymes, including SOD, CAT, PPO, POD and PAL.
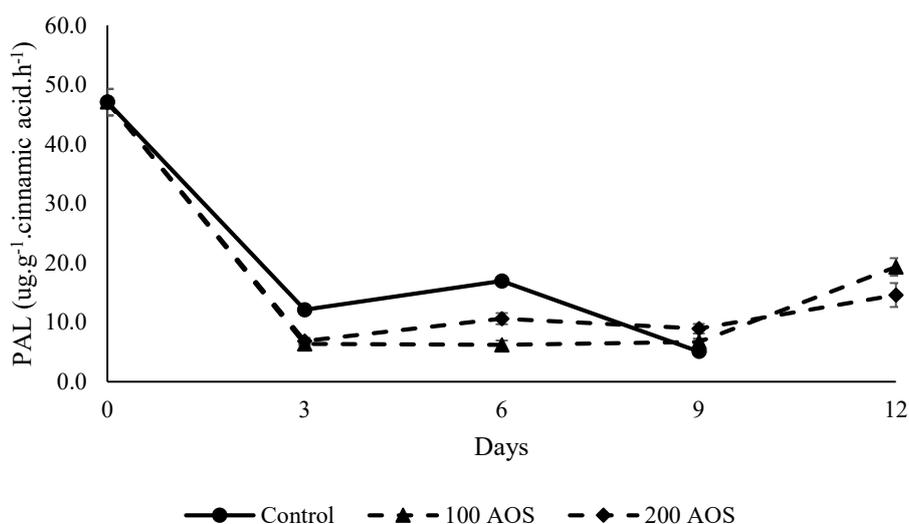
**Figure 2** Synthesis of phenylalanine ammonia-lyase in sweet basil leaves immersed in distilled water (control) and in alginate oligosaccharide (AOS) solutions (100 AOS = 100 mg. $L^{-1}$ and 200 AOS = 200 mg. $L^{-1}$) during storage at 10 °C. Values are expressed as means ± standard errors (replication = 3). Different letters indicate significant differences among treatments for each sampling time at $p \leq 0.01$.
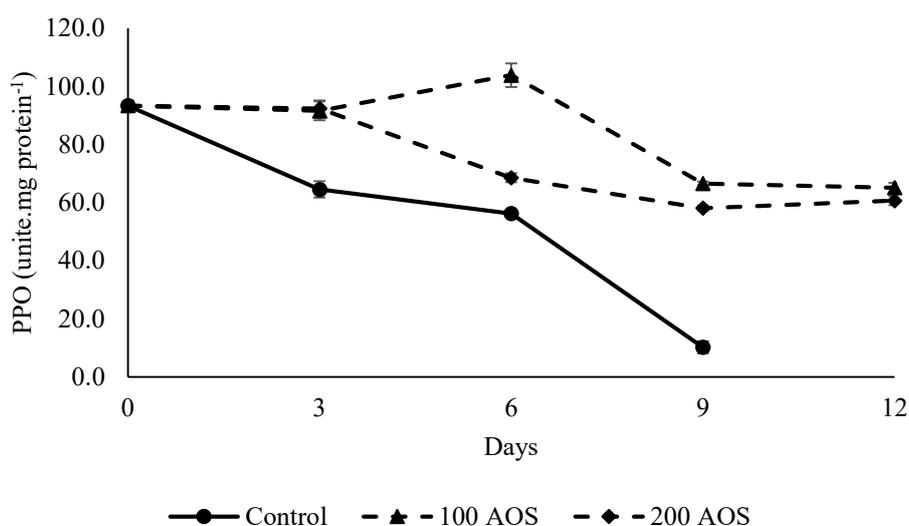


**Figure 3** Synthesis of polyphenol oxidase in sweet basil leaves immersed in distilled water (control) and in alginate oligosaccharide (AOS) solutions (100 AOS = 100 mg. $L^{-1}$ and 200 AOS = 200 mg. $L^{-1}$) during storage at 10 °C. Values are expressed as means ± standard errors (replication = 3). Different letters indicate significant differences among treatments for each sampling time at $p \leq 0.01$.

### Variation in lipid peroxidation

The variation in lipid peroxidation is an indicator of peroxidase (POD) activity, malondialdehyde (MDA) content, hydrogen peroxide content and lipoxygenase (LOX) activity. **Figure 4** specifically illustrates the statistically significant changes in POD activity. This parameter increased in sweet basil leaves (*please indicate which*) on day 3 and remained relatively constant throughout the rest of the storage period. In contrast to the other treatments, the sweet basil leaves treated with 100 mg. $L^{-1}$ AOS exhibited

the lowest levels of POD activity. It is possible that plant stress can be managed using 100 mg. L$^{-1}$ AOS, which would reduce POD activity. According to previous reports, POD activity contributes to the reduction of stress in plants and, in some of them, it is associated with browning. Variations in POD activity depends on the type of plant and the duration of stress exposure. However, free radicals are produced when plants are under stress. Particularly, the antioxidant hydrogen peroxide, which is often produced spontaneously, reduces intracellular stress. The purpose of POD is to prevent the production of hydrogen peroxide [26,27].
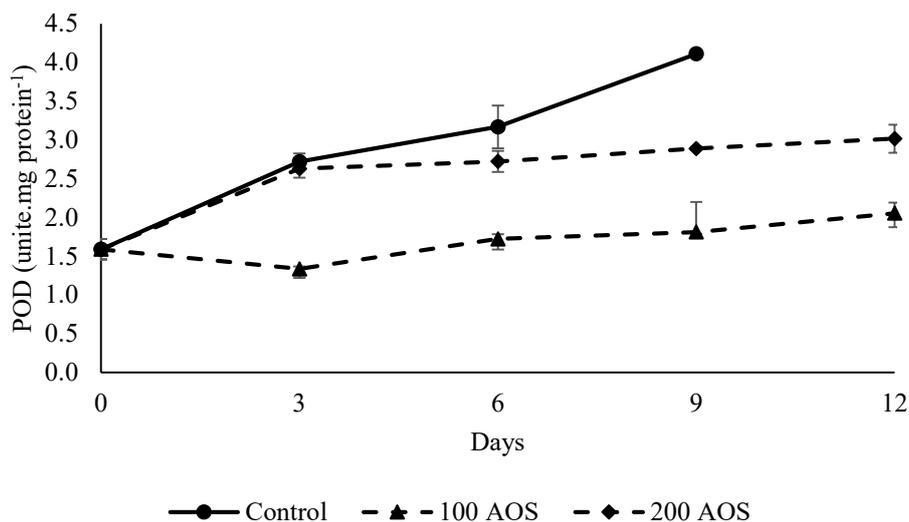


**Figure 4** Synthesis of peroxidase in sweet basil leaves immersed in distilled water (control) and in alginate oligosaccharide (AOS) solutions (100 AOS = 100 mg. L$^{-1}$ and 200 AOS = 200 mg. L$^{-1}$) during storage at 10 °C. Values are expressed as means ± standard errors (replication = 3). Different letters indicate significant differences among treatments for each sampling time at $p \leq 0.01$.

Sweet basil leaves immersed in distilled water (control) showed a noticeable increase in MDA content over the course of 6 days of storage, whereas those immersed in AOS showed no change in MDA content. The sweet basil in 100 mg. L$^{-1}$ AOS treatment was the highest MDA content (**Figure 5**). Plant stress or senescence produces MDA, a byproduct of lipid oxidation [28]. Consequently, because the lowered MDA measurements may be an indication of cell degeneration, the usage of 100 mg. L$^{-1}$ AOS can prevent stress during storage.
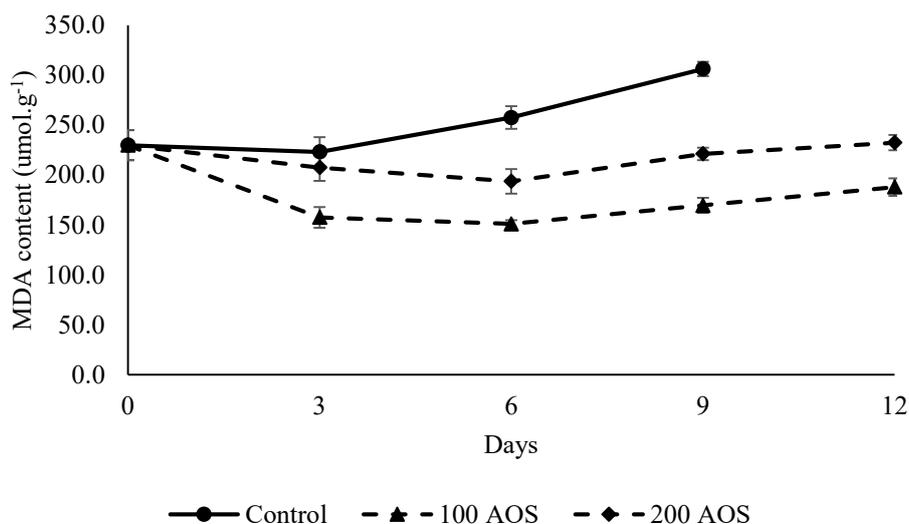
**Figure 5** Effect of malondialdehyde content in sweet basil that immersed in distilled water (control), in a solution of alginate oligosaccharide (100 AOS = 100 mg. L$^{-1}$ and 200 AOS = 200 mg. L$^{-1}$) during storage at 10 °C. Values are the means ± standard errors (replication = 3). Different letters indicate significant differences among treatment for each sampling time at $p \leq 0.01$.

The concentration of hydrogen peroxide was 3.55 μmol g$^{-1}$ at the start of the storage period. The results indicated that, compared to the control group, the use of AOS decreased the accumulation of hydrogen peroxide. On day 6, the hydrogen peroxide concentration in the control group increased and stabilised, while that in the AOS group declined (**Figure 6**). A previous study showed that AOS treatment could enhance the activities of ROS-scavenging enzymes such as SOD and CAT, thereby reducing hydrogen peroxide levels in the cells [29].

LOX is involved in the digestion of fatty acids in cell membranes. Specifically, it catalyses the oxidation of fatty acids with double bonds on the membrane, which results in the modification of the physical properties of the membrane cell. The membrane loses its fluid mosaic model properties [30,31]. The oxidation reaction also causes the synthesis of various free radicals, such as hydroxyl radical, superoxide anion radical, hydrogen peroxide and oxygen singlet.

The sweet basil leaves immerged in distilled water and those immerged in 100 mg. L$^{-1}$ AOS showed the highest and lowest enzyme activities during the experiment, respectively (**Figure 6**). MDA is an oxidation product, and it was suggested that AOS can limit LOX activity. This parameter was consistent with the increase in MDA concentration during storage (**Figures 5** and **6**) [32]. Additionally, increases in LOX activity are an indicator of cell membrane deterioration associated with CI or cell senescence during storage [9,33,34]. The results of this study revealed a correlation between the increase in CI and that in LOX activity.
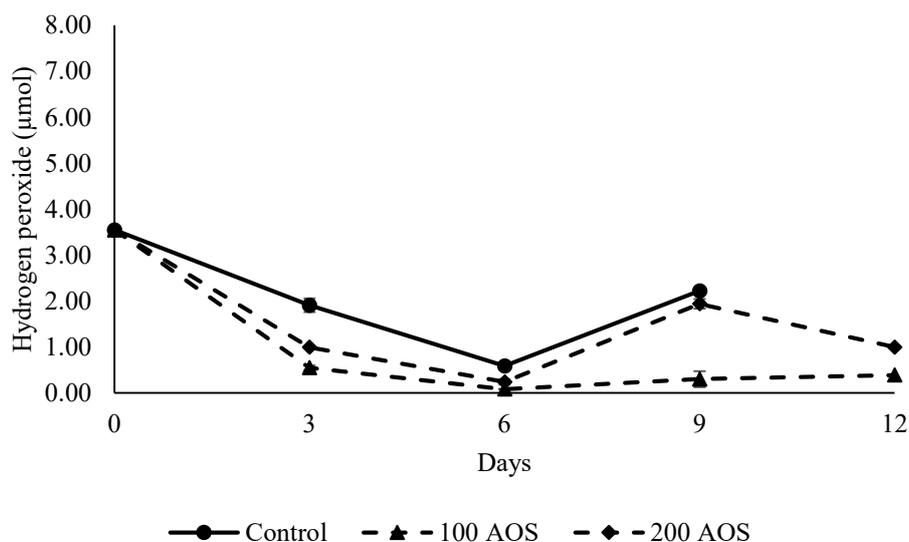
**Figure 6** Hydrogen peroxide content in sweet basil leaves immersed in distilled water (control) and in alginate oligosaccharide (AOS) solutions (100 AOS = 100 mg. $L^{-1}$ and 200 AOS = 200 mg. $L^{-1}$) during storage at 10 °C. Values are expressed as means ± standard errors (replication = 3). Different letters indicate significant differences among treatments for each sampling time at $p \leq 0.01$.

### Variation in antioxidant enzymes such as catalase (CAT) and SOD

CAT is an antioxidant that catalyses the breakdown of hydrogen peroxide ($H_2O_2$) into water and oxygen. In this study, the sweet basil leaves immersed in AOS exhibited an increase in CAT activity on day 3 and a decrease thereafter. The highest levels of CAT activity were observed in the leaves immersed in 100 mg. $L^{-1}$ AOS, followed by those immersed in 200 mg. $L^{-1}$ AOS and distilled water, in this order. Previous studies have also demonstrated that AOS increase the activity of the CAT enzyme, and that their application to plants can promote the production of enzymatic and non-enzymatic antioxidants [9,24,29].
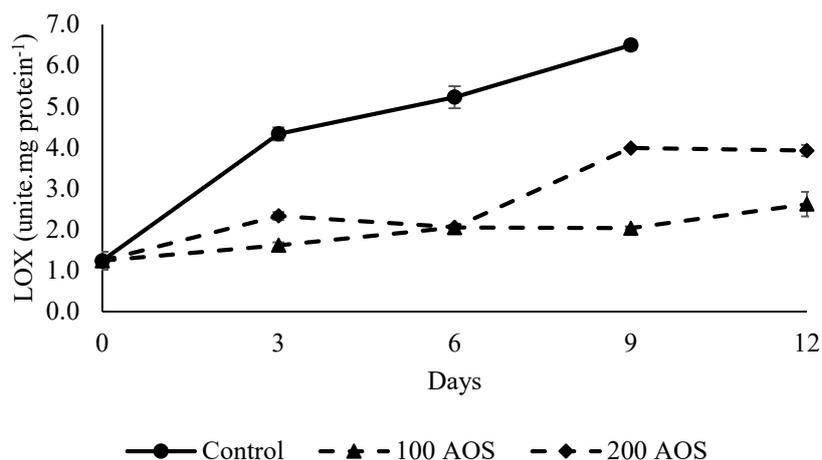


**Figure 7** Synthesis of lipoxygenase in sweet basil leaves immersed in distilled water (control) and in alginate oligosaccharide (AOS) solutions (100 AOS = 100 mg. $L^{-1}$ and 200 AOS = 200 mg. $L^{-1}$) during storage at 10 °C. Values are expressed as means ± standard errors (replication = 3). Different letters indicate significant differences among treatments for each sampling time at $p \leq 0.01$.
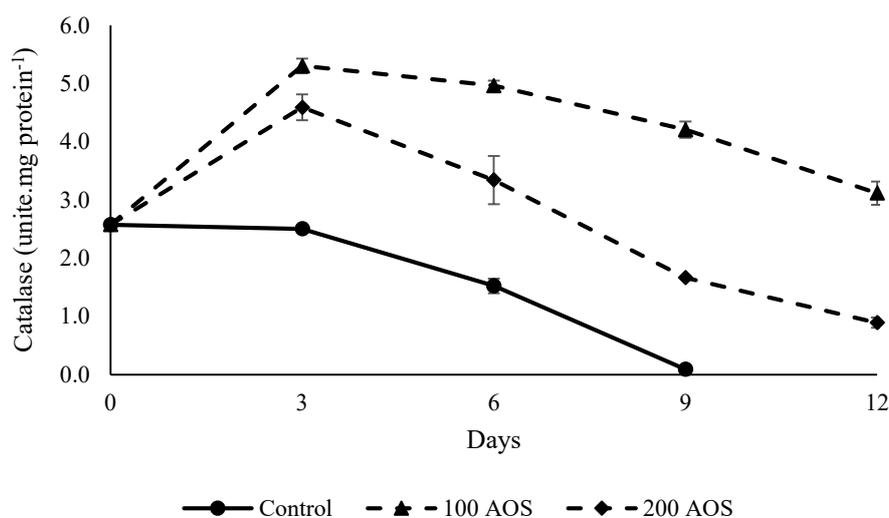
**Figure 8** Synthesis of catalase in sweet basil leaves immersed in distilled water (control) and in alginate oligosaccharide (AOS) solutions (100 AOS = 100 mg. L$^{-1}$ and 200 AOS = 200 mg. L$^{-1}$) during storage at 10 °C. Values are expressed as means ± standard errors (replication = 3). Different letters indicate significant differences among treatments for each sampling time at $p \leq 0.01$.

The variation in SOD activity is shown in **Figure 9**. The sweet basil leaves treated with both 100 and 200 mg. L$^{-1}$ AOS exhibited higher SOD levels than the control group. SOD is an important enzyme in plants that plays a crucial role in protecting cells against oxidative stress, which occurs when there is an imbalance between the production of ROS and the ability of cells to remove them. ROS, such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals, are generated as byproducts of various metabolic processes in plants [29,35]. In the present study, the high SOD activity observed was shown to be related to the accumulation of hydrogen peroxide in sweet basil leaves (**Figure 6**).
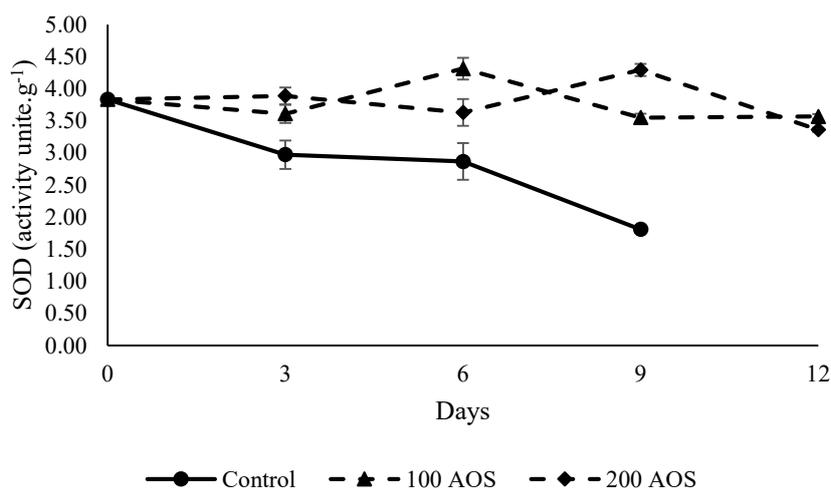


**Figure 9** Synthesis of superoxide dismutase in sweet basil leaves immersed in distilled water (control) and in alginate oligosaccharide (AOS) solutions (100 AOS = 100 mg. L$^{-1}$ and 200 AOS = 200 mg. L$^{-1}$) during storage at 10 °C. Values are expressed as means ± standard errors (replication = 3). Different letters indicate significant differences among treatments for each sampling time at $p \leq 0.01$.

## Conclusions

The shelf life of sweet basil leaves was extended the most by using 100 mg. $L^{-1}$ AOS, as evidenced by the reduced CI. The leaves with CI had higher MDA concentrations and LOX activity, which indicated a connection between this type of damage and the 2 compounds. The increase in hydrogen peroxide and MDA concentrations indicated the level of CI. Specifically, high concentrations meant that LOX-induced lipid peroxidation had severely damaged cell membranes. Furthermore, AOS were shown to stimulate the synthesis of phenolic compounds. These are associated with increases in the activity of specific enzymes, including PPO and PAL, which are in turn linked to CI-induced browning. AOS can stimulate the activity of CAT, POD and SOD, which are involved in the resistance to free radicals.

## Acknowledgements

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