Article

Application of High Pressures in the Postharvest Conservation of Broccoli

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Abstract: Broccoli is a vegetable of high nutritional value, rich in bioactive compounds, but has a fast degradation after harvest. This work assesses the effect of hyperbaric pressure, at room temperature, on postharvest conservation of broccoli. The broccoli samples were subjected to the five hyperbaric pressures (100 control, 200, 400, 600, and 800 kPa) during three different times (1, 2, and 3 days), at 22 °C and 95% RH. The pressures of 400, 600, and 800 kPa provided the best conservation of broccoli quality. Respiratory rate, ethylene production, soluble solids content, and lipid peroxidation decreased at the highest-pressure treatments. Moreover, the highest pressures maintained fresh mass, green color, ascorbic acid content, and receptacle firmness. The hyperbaric treatments of 600 and 800 kPa increased catalase enzymatic activity and reduced peroxidase activity as a result of the reduction of oxidative stress, delaying the senescence of broccoli.

Keywords: *Brassica oleracea* var. Itálica; food quality; hyperbaric atmosphere; inflorescence

1. Introduction

Vegetable production increases by around 5 million tonnes per year [1]. The population's search for a healthy life may be the leading cause of this growth. Among these vegetables, broccoli has stood out because of its high nutritional value and nutraceutical properties, such as high levels of beta-carotene, vitamin C, selenium, fiber, lutein, zeaxanthin, vitamin K, folic acid, and minerals such as calcium, potassium, phosphorus [2–4].

Broccoli has high perishability, with a shelf life of only two days at room temperature [5]. This rapid senescence is due to the high production of ethylene and respiratory rates, requiring refrigeration to prolong the shelf life of this vegetable, making the production process more expensive.

Recent studies have shown the benefits of using hyperbaric pressures to retard the physiological mechanisms of maturation and senescence in vegetables. Hyperbaric pressure delayed maturation in tomatoes, as evidenced by the reduction in respiratory rate and maintenance of color and firmness [6–8]. Tomatoes submitted to pressures of 600 and 800 kPa at room temperature showed biochemical changes, such as the increased catalase activity and decrease in peroxidase [9].

The application of pressure treatment at room temperature can induce positive effects on the physiological activity of the vegetables in a similar way to refrigerated storage [7,8,10,11]. Since pressurizing uses only 3% of the energy required in the refrigeration, this technique allows for energy savings. The economy that occurs due to the pressurizing does not have to be instantaneous, and it takes little energy to maintain the pressure throughout the storage [8].

In this sense, this work aims to evaluate the effect of the application of hyperbaric pressures at room temperature on the postharvest quality of broccoli 'Legacy'.

2. Materials and Methods

2.1. Vegetable Material

The broccoli 'Legacy' were obtained in commercial crops of Taiúva county (21°7'24.87" S, 48°27'8.83" W, 634 m altitude), São Paulo, Brazil.

The inflorescences were harvested at commercial maturation stage (about 80 days after sowing, in the winter of 2018) and transported to the laboratory in 30 min. The sampling was homogenized by choosing inflorescences with similar shape and color, mean weight of 630 ± 60 g, and absence of diseases. All inflorescences were sanitized in a solution of Sodium Dichloro-s-triazinetriene Dihydrate (Sumaveg[®], Diversey, Socorro, SP, Brazil) at 200 mg L^{-1} for five minutes and allowed to dry in a cold room at 22 °C for 2–4 h at the place where the treatments were applied.

2.2. Hyperbaric System

The experiments were performed in a hyperbaric system as described by Inestroza-Lizardo [9].

The system comprised five steel chambers with 10.75 L each, interconnected to a closed circuit through which a constant flow of compressed air circulated, keeping constant the partial pressures of oxygen (21 kPa) and nitrogen (78 kPa). The previous passage of air adsorbed the CO₂ into a chamber containing calcium oxide.

The treatment chambers are equipped with a pressure regulator at the input and needle valve at the output of each vessel to regulate air pressure and airflow, respectively, in addition to a safety valve. The system was connected to a 250 L air compressor (Schulz, model MSV 20 MAX, Joinville, Santa Catarina, Brazil) that supplied compressed air. The air flow was measured using a flowmeter in a range of $5\text{--}2000 \text{ mL min}^{-1}$ (Bronkhorst[™], Ruurlo, The Netherlands). The CO₂ concentration was measured in an infrared gas analyzer (Guardian[®]Plus, Edinburgh Sensor's, Livingston, UK). The flowmeter and CO₂ analyzer were connected to the acquisition and control card (PersonnelDAQ 3000, Cleveland, OH, USA) and a computer. Pressure, airflow rate, and CO₂ level were recorded using DasyLab[®] software (Measurement Computing Corporation, Norton, MA, USA).

2.3. Experimental Procedure

Each replicate of the experiment comprised twelve inflorescences, two of which were used to characterize the initial quality (day 0) (Table 1) and ten were distributed in the five chambers of the system (two inflorescences per chamber). Then, the chambers were closed and pressurized at 100 (control), 200, 400, 600, and 800 kPa for periods of 1, 2, and 3 days. Data loggers (HOBO Prov2 U-23-001, Onset Corporation, Bourne, MA, USA) monitored temperature (22 ± 1 °C) and relative air humidity ($95 \pm 2.0\%$) within the chambers every 30 min. At the end of each treatment period, the chambers were automatically depressurized for two hours. The inflorescences were removed from the chambers and evaluated.

Table 1. Initial characterization of broccoli ‘Legacy’.

Variables	Values
Mass (g)	630 ± 60
Epidermis color index	1.04 ± 0.10
Receptacle firmness (N)	13.3 ± 1.02
Ethylene production (mL C ₂ H ₄ kg ^{−1} h ^{−1})	5.2 ± 1.2
Soluble solids (%)	6.17 ± 0.05
Titrateable acidity (g kg ^{−1})	0.14 ± 0.07
Ascorbic acid (mg kg ^{−1})	99.6 ± 2.40
Antioxidant activity (mmol kg ^{−1})	0.23 ± 0.20
Lipid peroxidation (mmol kg ^{−1})	19.22 ± 1.6
Catalase activity (U kg ^{−1} pt)	1.12 × 10 ^{−10} ± 0.1
Peroxidase activity (U kg ^{−1} pt)	0.03 ± 0.01

Values represent the mean ± standard deviation.

2.4. Physical, Metabolic, and Chemical Analyses

The weight loss was calculated by the difference between initial and final inflorescence weight divided by the initial weight. The samples were weighed on an electronic scale with a measuring range of 50–6000 g ± 2 g (BP6, Filizola, São Paulo, SP, Brazil). We expressed the result as a g kg^{−1}.

The color index (CI) included the luminosity parameters L*, a*, and b* (Minolta CR-400 colorimeter, Konica Minolta, New Jersey, NJ, USA) [12]. Readings were taken in five points on each inflorescence, and the CI was calculated using Equation (1):

$$CI = (100 \times a^*) / (L^* \times b^*) \quad (1)$$

The firmness was measured using a digital penetrometer with 8 mm tip (5–200 N ± 1 N, Impac, IP-90DI, São Paulo, SP, Brazil) and evaluated at the base of the receptacle (receptacle firmness). The results were expressed in Newton (N).

The respiratory rate (RR), CO₂ concentration, was measured using an infrared gas analyzer (Guardian®Plus, Edinburgh Sensors, Livingston, UK) connected to a data acquisition and control card (Personnel DAQ 3000, Cleveland, OH, USA) and a laptop computer. CO₂ levels were recorded using DasyLab® software (Measurement Computing Corporation, Norton, MA, USA) throughout the experiment and calculated automatically in real time during the whole trial, according to Equation (2) [13]:

$$RR = (\Delta CO_2 \times Q) / W \quad (2)$$

where ΔCO_2 is the difference between the concentration of CO₂ at the inlet and outlet of chambers, Q is the air flow rate, and W is the mass of broccoli. The results were expressed in mg CO₂ kg^{−1} h^{−1}.

The inflorescences were stored in 5 L sealed containers to estimate ethylene production. After one hour, 200 µL of the gas inside containers (headspace) was collected by a silicone septum with the aid of a gas chromatography syringe (SGE, Analytical Science, Ringwood, VIC, Australia). The samples were injected in a gas chromatograph (Trace GC Ultra, Thermo Scientific, Waltham, MA, USA) equipped with a flame ionization detector (FID), and a Porapack N capillary column (2 m in length) and set at 80 °C, with hydrogen as carrier gas (35 mL min^{−1}). The operating temperatures were 110 °C in the column, 250 °C in the detector, and 200 °C in the injector. Ethylene production was measured after removal of inflorescence from the chambers and was expressed in µg kg^{−1} s^{−1}.

Soluble solids (SS), titrateable acidity (TA), and ascorbic acid (AA) were determined according to AOC methodology [14]. The results were expressed as the percentage of SS, percentage of citric acid, and mg of ascorbic acid mg kg^{−1}.

Peroxidation of lipids was measured according to Heath and Packer [15]. Samples were frozen in liquid N₂, macerated, combined with 4 mL of TCA buffer (1% w/v), homog-

enized, and centrifuged for 10 min at $10,000 \times g$ at 4°C (Biofuge Stratos model, Heraeus, Hanau, Germany). A 1 mL aliquot was pipetted from the supernatant and transferred to a test tube, adding 3 mL of thiobarbituric acid at 0.5% (w/v), TCA at 20% (w/v), and incubating in a water bath at 95°C for 60 min. Subsequently, the tubes were held for 10 min in an ice bath to interrupt the reaction. The samples were then centrifuged again for 10 min at $10,000 \times g$. The absorbance of the supernatant was measured using a UV VIS spectrophotometer (Femto 700 plus, São Paulo-SP, Brazil) at 535 nm and 600 nm. We expressed the results as the malondialdehyde content (MDA) per mmol kg^{-1} .

The total antioxidant activity was estimated by the DPPH method according to Brand-Williams et al. [16]. The results were expressed in mmol kg^{-1} .

Catalase activity (CAT) (EC 1.11.1.6) was determined by the method of Kar and Mishra [17] with adaptations. We obtained the reaction combining 150 μL of sample in a potassium phosphate buffer + EDTA + DTT + PVPP at 100 mmol L^{-1} (pH 7.5). A total of 1950 μL of potassium phosphate buffer at 100 mmol L^{-1} (pH 7.5) was used as the determination buffer, 150 μL of potassium phosphate buffer + EDTA + DTT + PVPP at 100 mmol L^{-1} (pH 7.5) as the extraction buffer, and 750 μL of hydrogen peroxide solution at 50 mM as the enzymatic substrate. The readings were performed in a UV VIS spectrophotometer (Femto, São Paulo, SP, Brazil) at 240 nm absorbance. We expressed the specific activity of CAT as U kg^{-1} protein.

The activity of peroxidases (POD) (EC 1.11.1.7) was measured according to Lima et al. [18]. The reaction system comprised 1 mL of enzyme extract with 0.5 mL of 30% hydrogen peroxide in potassium phosphate buffer at 0.2 M (pH 6.7) and 0.5 mL of phenol and aminoantipyrine solution. The solution was immersed in a water bath at 30°C for 5 min. Absolute ethyl alcohol (2 mL) was added to stop the reaction, and we assessed the absorbance in a UV VIS spectrophotometer at 505 nm. We express the specific activity of POD as U kg^{-1} protein.

For the calculation of the specific activity of CAT and POD, the total soluble protein content was measured using the Bradford method [19].

2.5. Statistical Analysis

We applied a randomized block design in a factorial scheme (5 pressures $\times$ 3 exposure times). The data were submitted to analysis of variance (ANOVA) and Tukey tests, with 99% probability ($p < 0.01$), using AgroEstat software version 2014 [20]. When significant differences were found, we performed regression analyses.

3. Results

3.1. Physicochemical Analyzes

Weight Loss, Firmness, Color Index (CI), Soluble Solids (SS), Titratable Acidity (TA), Ascorbic Acid Content (AA)

The alteration in atmospheric pressure around the vegetable reduced the weight loss (WL) of broccoli 'Legacy' effectively. The higher the pressure, the lower the weight loss at all exposure times (1, 2, and 3 days) (Figure 1A). The inflorescences subjected to 100 kPa had the highest WL, and the longer the exposure time, the larger the WL. After three days of treatment, broccoli at 100 kPa lost 3% mass, while those under 800 kPa lost only 1.2%.

The increase in pressure promoted the maintenance of firmness (Figure 1B). The pressure of 800 kPa for one day maintained the firmness of the broccoli in 12.2 N, very close to the initial value of 13.3 N (Table 1) and higher than the control (9.5 N). Control broccoli had an average reduction of 11.4% in firmness compared to the initial values, but firmness values increased over the three days of storage. The high mass loss suffered by the broccoli that remained for three days in the chambers may have increased elasticity and resistance of tissues against the tip of the penetrometer.

In general, the color index (CI) increased with time of exposure to pressure, turning the broccoli yellowish (Figure 1C), which may be attributed to at least three factors: (1) the

degradation of chlorophylls by the enzyme chlorophyllase; (2) change in pH, following the change in titratable acidity (TA) and oxidation; and (3) ethylene production (Figure 1C).

Figure 1. Weight loss (A), firmness (B), color index (C), soluble solids (D), titratable acidity (E), and ascorbic acid (F) in broccoli ‘Legacy’ submitted to hyperbaric treatments during 1, 2, and 3 days at 22 °C and 95% RH. Color index: 0 = yellow; −2.0 = green. The error bar identifies the standard deviation of 5% of the mean.

The SS content decreased with increasing pressures, with the mean of the control being 20% higher than the inflorescences submitted to 800 kPa pressure (Figure 1D). The SS reduction was also observed in tomatoes subjected to 800 kPa pressure for six days [6]. On the other hand, the SS content in ‘Italy’ broccoli stored in different types of packages increases during the storage period [21]. Thus, the application of pressure probably improved the conservation of the vegetables, indicating a delay in senescence.

The TA contents ranged from 0.14 to 0.26 g kg⁻¹ (Figure 1E). The hyperbaric pressure improved organic acids preservation in broccoli compared to control inflorescences. The pressure of 800 kPa at three days of storage provided the highest levels of TA. The effect

of hyperbaric atmospheres on the increase in TA also occurs in fruits, as in mangoes, for example [22].

TA values of plants also depend on genetic, agronomic, climatic, and postharvest factors. For example, broccoli ‘Italia’ has higher TA values than those found in this study, reaching 0.53 to 0.77 g kg⁻¹ of pulp [21]. This divergence may be attributed to cropping conditions [23].

The AA content of control broccoli was reduced by 27% compared to the initial value (99.6 mg kg⁻¹, Figure 1F, Table 1). The 800 kPa treatment maintained the highest AA levels (94 mg kg⁻¹) regardless of the pressure time.

3.2. Physiological Phenomena

Production of Ethylene and Respiratory Rate

The increase in atmospheric pressure around broccoli effectively reduced the respiratory rate of broccoli ‘Legacy’ (Figure 2A). Broccoli submitted to hyperbaric pressures for three days had an average respiratory rate of 11 mg CO₂ kg⁻¹ h⁻¹, while broccoli at 100 kPa presented respiratory rates of 13.5 mg CO₂ kg⁻¹ h⁻¹ (Figure 3).

Figure 2. Relationship between respiration rate (A) and ethylene production (B) in broccoli ‘Legacy’ with pressures applied at times 1, 2, and 3 days at 22 °C and 95% RH. The error bar identifies the standard deviation of 5% of the mean.

Figure 3. Lipid peroxidation (A), antioxidant activity (B), catalase activity (C), peroxidase activity (D) in broccoli ‘Legacy’ submitted to hyperbaric treatments during 1, 2, and 3 days at 22 °C and 95% RH. The error bar identifies the standard deviation of 5% of the mean.

The production of ethylene in broccoli increased as a function of time of exposure to pressures, showing a quadratic relation. Increased pressure maintained ethylene production at low levels (Figure 2B). Broccoli submitted to pressures of 600 and 800 kPa for one day presented ethylene production about 16% lower than the control and very close to the initial values ($5.2 \text{ mg kg}^{-1} \text{ s}^{-1}$). After three days, control broccoli produced even more ethylene, and the difference for the best treatment (800 kPa) increased to 33.6% (Figure 1C).

3.3. Biochemical Analyses

Lipid Peroxidation, Total Antioxidant Activity (TAA), Activity of the CAT, and Activity of the POD

The maintenance of the AA contents under high pressures agrees with the increase in lipid peroxidation (Figure 3A), since the control presented the highest values of MDA regardless of the pressure period, with an average increment of 28% compared to the pressure of 800 kPa ($12.5 \text{ mmol kg}^{-1}$).

The content of TAA increased with the applied pressure, regardless of the period of exposure (Figure 3B). TAA values ranged from 0.30 to $0.38 \text{ } \mu\text{mol TEAC } 100 \text{ g}^{-1} \text{ FW}$ on one day of exposure to pressure, 0.37 to 0.43 on two days, and from 0.25 to 0.32 on three days (Figure 3B).

The hyperbaric pressure significantly influenced the activity of the CAT and POD enzymes of broccoli ($p < 0.01$; Figure 3C,D).

4. Discussion

4.1. Physicochemical Analyses

Weight Loss, Firmness, Color Index (CI), Soluble Solids (SS), Titratable Acidity (TA), Ascorbic Acid Content (AA)

The alteration of atmospheric pressure is a leading factor influencing the vapor pressure in the environment [6]. The difference between the vapor pressure of water in inter-cellular spaces of plant tissue and the surrounding air controls the rate of water loss in fresh vegetables. Thus, decreasing the difference in vapor pressure between vegetable and ambient air reduces water loss from plant tissue [24]. Such a process explains the higher mass losses in the broccoli under control treatment (100 kPa). This effect also occurs in other fruits and vegetables when submitted to high pressures [6,9,25,26].

The maintenance of firmness in fruits and vegetables depends on factors such as tissue turgor and cell wall degradation [27], which influence the weight loss in vegetables. Our data suggest that the difference in the firmness of broccoli relates to the weight loss because the broccoli with greater firmness presented a smaller loss of mass (Figure 1A). This correlation also occurs in several other vegetables, for example, the 'Debora' tomato [6] and lettuces [26].

Broccoli with firmness around 13.8 N are suitable for commercialization, as they maintain the visual characteristic of turgidity [21]. Thus, we can conclude that the broccoli stored under the pressures of 400, 600, and 800 kPa presented adequate firmness for commercialization until the third day of storage at 22 °C (Figure 1B).

The pressures of 400, 600, and 800 kPa preserved the green color of inflorescences, showing a lower color index than the inflorescences submitted to the pressures of 100 and 200 kPa (Figure 1C). The relationship between CI and TA was inversely proportional to third day of pressure, that is, as CI decreased, TA increased (Figure 1C,E). This correlation can be attributed to chlorophyll degradation due to enzymatic activity, causing the yellowing of inflorescences and the release of organic acids in the cellular environment, which increases the TA values.

The highest values of ethylene production and SS content occurred in the control inflorescences, indicating their advanced senescence when compared to the broccoli submitted to 800 kPa (Figure 1D). The increase of SS in control broccoli can be attributed to the high activity of enzymes involved in cell wall degradation [28] with consequent reduction of firmness (Figure 1B) and increased respiratory rate (Figure 2A).

Reduction of acidity during postharvest relates to ripening or senescence due to increased consumption of organic acids in the respiratory process or conversion of acids to sugars in glycogenesis. In the control broccoli, in addition to lower acidity (Figure 1E), they had a higher respiratory rate (Figure 2A), higher ethylene production (Figure 2B), and higher soluble solids (SS) content (Figure 1D), suggesting a greater metabolic activity than the other treatments.

Ascorbic acid content tends to decrease with maturation and storage time [29]. Possibly, the AA was consumed in metabolic reactions in the senescence process of control broccoli, whereas high-pressure application precluded such metabolic reactions (Figure 1E).

Tomatoes submitted to high pressures also present higher AA values when compared to fruits under control pressure [9].

Ascorbic acid is an antioxidant compound that plays a vital role in free radical suppression [30,31], with the ability to donate electrons to a wide range of enzymatic and non-enzymatic reactions [32]. The metabolism of AA acts on the defense to oxidative stress [33], that is, the higher the stress occurring in the vegetable, the lower the concentration of this compound. In this case, the stress was generated by the natural senescence of broccoli, which triggered reactions of degradation and oxidation, reducing postharvest life. The high pressures exerted on the vegetable acted in the metabolism as a whole, slowing the senescence and keeping the levels of AA elevated.

4.2. Physiological Phenomena

Production of Ethylene and Respiratory Rate

The increase in atmospheric pressure around broccoli effectively reduced the respiratory rate of broccoli 'Legacy'. Broccoli submitted to hyperbaric pressures for three days had an average respiratory rate of $11 \text{ mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$, while broccoli at 100 kPa presented respiratory rates of $13.5 \text{ mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (Figure 2A). The broccoli respiratory rate at 800 kPa pressure was 18.5% lower than the control. The reduction in respiratory rate due to the increase in atmospheric pressure also occurs in several fruits and vegetables, such as the fruits of *Prunus mume* [34], lettuce [26], and tomatoes [9].

The lower respiratory rates achieved by the treatments with high pressures may be due to the solubilization of CO_2 within the broccoli and its posterior dilution in the recipients as a result of the increase in the partial pressures of the air gases inside the hyperbaric chambers [25]. The CO_2 generated by the broccoli respiration was probably solubilized in the internal tissues until it reached the partial pressure of the CO_2 of the surrounding air. Subsequently, CO_2 was diluted inside the hyperbaric chamber, causing an increase in the CO_2 partial pressure of the air surrounding the vegetable with the rise in CO_2 concentration inside the chamber. This process of dilution and solubilization is continuous and stabilizes only when the amount of CO_2 exiting the chamber into the gas analyzer becomes equal to that generated by the respiratory rate of the plants [26]. However, the strength of the effect of hyperbaric pressures on the respiratory rates of plants depends on the type of material studied, exposure time, pressure intensity, and temperature.

Ethylene production should be controlled and kept low because it induces chlorophyll degradation with consequent yellowing of broccoli [35]. This effect was observed in the control inflorescences when compared to those submitted to 800 kPa, with the lowest ethylene production (Figure 2B) and the highest color index (Figure 1C), impairing appearance and decreasing shelf life [34,36].

Regardless of the pressure time, the ethylene values observed in broccoli subjected to pressures of 600 and 800 kPa were similar, indicating that the range between 600–800 kPa can be used to control ethylene biosynthesis in broccoli.

The inhibition of ACC oxidase (1-aminocyclopropane-1-carboxylate) activity, the enzyme responsible for converting ACC into ethylene, caused by the high pressure, may have kept low ethylene production. *Prunus mume* fruits submitted to 5 MPa pressure showed a 75% reduction in ACC oxidase activity compared to the control (100 kPa), while substrate levels (ACC) remained unchanged [37].

4.3. Biochemical Analyses

Lipid Peroxidation, Total Antioxidant Activity (TAA), Activity of the CAT and Activity of the POD

Oxidative stress comprises a central factor in biotic and abiotic stress phenomena, occurring during an imbalance between the reactive oxygen species (ROS) production and antioxidant defense in any compartment of a plant cell [38]. Oxidative stress can be measured by lipid peroxidation, which begins with the reaction of a free radical and unsaturated fatty acid, propagating by peroxy radicals, and resulting in the lipid hydroperoxides and aldehydes formation, such as malondialdehyde, 4-hydroxynonenal, and isoprostanes [39]. This process may occur in all living organisms and can trigger lesions in cells [30], altering membranes and leading to permeability disorders and cell death.

The lower levels of MDA in broccoli submitted to high pressures indicate the efficiency of this treatment in oxidative stress reduction. However, the influence of hyperbaric pressures on MDA is still unclear, with scarce reports in the literature. The three-day pressure treatment showed the lowest MDA values (Figure 3A). The MDA reduction was also observed in tomatoes preserved under high pressure [9,40].

The increase in TAA content as a function of pressure was also observed in 'Debora' tomatoes [9] and in mangoes [22]. This behavior may be attributed to a hormesis effect promoted by increased hyperbaric pressure.

Broccoli that remained at 800 kPa for 1 and 2 days had CAT activity 2.5 times higher than control broccoli (Figure 3C). CAT plays a crucial role in maintaining hydrogen peroxide homeostasis in plant cells. The enzyme disproportionates the H_2O_2 generated in peroxisomes by oxidases involved in β -oxidation of fatty acids, photorespiration, and purine catabolism [30,41]. Therefore, maintaining high CAT activity is a defense mechanism against stress factors [42]. In this sense, the hyperbaric treatments were efficient due to the storage under high pressure activating the CAT, which is directly related to the process of detoxification of H_2O_2 and indirectly to the protection of cells.

After two days of treatment, the control broccoli presented the POD activity 2.7 times higher than broccoli submitted to 800 kPa (Figure 3D). However, inflorescences treated with three days of pressure showed opposite behavior, since the highest values of POD activity were observed in inflorescences treated with 800 kPa (Figure 3D).

The higher pressures increased CAT activity and reduced POD activity (Figure 3C,D). Since the main function of CAT in plant metabolism is to rapidly convert hydrogen peroxide (toxic) into other compounds less harmful to the plant, and peroxide is the main substrate of POD, it was expected that the lower availability of peroxide in the intracellular medium would hinder POD activity.

The senescence may have induced the high POD levels of control inflorescences after two days of storage since high levels of POD are associated with the oxidative deterioration of fruits at an advanced stage of maturity [29]. In this aspect, the pressures of 600 and 800 kPa were efficient in keeping low enzymatic activity and propitiating the better conservation of the broccoli when compared to the control inflorescences (Figure 3D).

5. Conclusions

The hyperbaric treatments exerted efficient conservation on the postharvest of broccoli 'Legacy', mainly in the highest pressures. The pressures of 400, 600, and 800 kPa reduced the respiratory rate of the vegetables and the production of ethylene and maintained the fresh weight, firmness, and green color, which are essential characteristics for commercialization. The pressures of 600 and 800 kPa influenced antioxidant activity, leading to a reduction in lipid peroxidation and peroxidase enzyme, which preserved tissue integrity and delayed the senescence of broccoli.

Author Contributions: This study was conducted by E.M.P., who was responsible for conducting the study; carrying out the analyses; tabulating the data; and assembling the texts, figures, and tables. J.S.P.J., A.S.F., M.T.C., K.T.E.F.O. and I.N.F.C. assisted with experiment set-up, execution of the analyses, and the literature review necessary for writing this article. B.-H.M. was responsible for providing the resources for carrying out the experiment, including the necessary equipment and reagents. All authors have read and agreed to the published version of the manuscript.

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