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Ethanol and Methyl Jasmonate Fumigation Impact on Quality, Antioxidant Capacity, and Phytochemical Content of Broccoli Florets during Storage †

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† This paper is a part of the Ph.D. thesis of Arturo Duarte-Sierra presented at University Laval entitled: Abiotic stress hormesis: hormetic stresses to maintain quality and enhance glucosinolates and phenolic compounds in broccoli (*Brassica oleracea* var. italica) during storage.

Abstract: Broccoli is a vegetable that offers valuable components, such as glucosinolates (GLS), flavonoids, and hydroxycinnamic acids (HCA), for our daily food intake. These substances have been associated with reducing the risk of cancer and cardiovascular diseases (CVD). Broccoli florets are also highly perishable, given their elevated respiration rates and their sensitivity to ethylene. Experiments have been carried out on broccoli to investigate the consequences of abiotic stress post-harvest treatments in retarding the senescence process. Nevertheless, the influence of these treatments on the phytochemicals of broccoli has not been extensively examined. Florets of broccoli (*Brassica oleracea*) were exposed to an atmosphere consisting of 10,000 $\mu L.L^{-1}$ ethanol at room temperature for 30 min and 120 min. The exposure to methyl jasmonate (MeJA) treatments was carried out at room temperature using 1 $\mu L.L^{-1}$ for 45 min and 180 min. The yellowing of florets was delayed using 10,000 $\mu L.L^{-1}$ of ethanol at both exposure times as compared to untreated florets, and the chlorophyll titers were also superior with both doses over the control. The total phenols of the florets increased by 15% and 18% with the application of the hormetic and high doses, respectively, throughout the storage period compared to unexposed broccoli. The GLS and HCA yields were also increased by both ethanol doses. The exposure of florets to 1 $\mu L.L^{1}$ MeJA for 45 min resulted in delayed yellowing of florets; however, longer exposures resulted in yellowing after 21 d and significantly ($p < 0.05$) increased respiration rates relative to untreated florets. Overall antioxidant capacity of the florets was significantly reduced with both doses of methyl jasmonate; however, HCA titers were increased at both doses. The amount of total glucobrassicins within broccoli was increased following exposure of florets to both doses, but no significant differences in glucoraphanin content were observed. As a conclusion, the ethanol treatment could indeed delay senescence and lead to the induction of phytochemicals. In contrast, MeJA's effect on quality is not quite substantial; it can, however, be used to improve the phytochemical content of florets, particularly indole-type GLS.

Keywords: broccoli; ethanol; methyl jasmonate; glucosinolates; hydroxycinnamic acids

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1. Introduction

Broccoli (*Brassica oleracea* var. Italica) florets are an excellent source of phytochemicals, including glucosinolates (GLS) and flavonoids. Glucobrassicin and glucoraphanin are the most important glucosinolates in broccoli, while quercetin and kaempferol are the most abundant flavonoids [1]. Both GLS and flavonoids have been recognized for the prevention of degenerative diseases such as cancer and cardiovascular diseases (CVD) [2]. At low temperatures, these substances tend to remain quite stable during storage. However, if stored at 20 °C, the amount of glucoraphanin, which is a precursor to a cancer-preventative

isothiocyanate termed sulforaphane (4-methylsulfinylbutyl isothiocyanate), tends to decrease significantly by up to 55% during the initial three days [3]. Moreover, this vegetable is susceptible to chlorophyll loss, fungal growth, and odor generation during storage [4,5]. The difficulty in preserving broccoli florets during postharvest storage is further complicated by the need to maintain the optimal conditions of temperature, humidity, and packaging, which can vary depending on the cultivar and storage duration.

To prolong the storage time of broccoli florets, storage conditions involve a combination of low temperatures, specifically 0 °C (32 °F), and high relative humidity ranging from 98% to 100% [6]. These conditions are suitable to preserve the quality of broccoli florets for up to three weeks. Furthermore, in recent times, consumers have been placing greater emphasis not only on the appearance of produce but also on its nutritional and health-promoting properties. Apart from their impact on human health, GLS is the primary defense mechanism of the *Brassicaceae* family, which includes broccoli [7]. Typically, the production of these substances is prompted by an attack from herbivores during the plant's growth phase in the field [8]. Abiotic stresses such as UV-B irradiation are also known to induce the production of GLS and phenylpropanoids in broccoli sprouts [9]. Induction of defense mechanisms in postharvest commodities by physical agents has been used as an adjunct to cold storage to reduce phytochemical losses [10–12]. Abiotic stresses and plant signal modulators could have a potential commercial application, especially those that have already been Generally Recognized As Safe (GRAS) by the U.S. Food and Drug Administration (FDA). One example is the combination of methyl jasmonate (MeJA) and ethanol (EtOH) that reduced decay, maintained quality, and enhanced antioxidant activity in Chinese bayberries [13].

This approach could also be used for fresh broccoli, and besides, both are GRAS compounds. Moreover, EtOH is frequently employed as a disinfectant in the food industry, and it has also been utilized to enhance the storability of produce. The most prevalent methods of application are dips and vapor treatments, which are used to prevent discoloration, decrease decay, and inhibit ethylene biosynthesis [14–16]. The suppression of ethylene synthesis is one of the implied effects of ethanol in stored produce [16]. Its suppression is mainly due to the loss of the activities of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase [17]. The delay in chlorophyll loss and the prevention of off-odor synthesis in broccoli may also be attributable to the reduction of ethylene production [18]. Ethanol is also associated with the protection of reduced compounds, such as those associated with the ascorbate-glutathione cycle in broccoli florets [19]. Conversely, it is known that the consumption of ethanol by animals can lead to the production of reactive oxygen species (ROS) [20], as well as oxidative damage to membrane lipids, proteins, and DNA [21]. Likewise, the use of MeJA in crops has been focused on plant protection against microbial development and enhancement of phytochemical compounds [22]. Jasmonates trigger defense-related responses in plants, such as the production of toxic secondary metabolites, the development of physical barriers, and alterations in the rate of vegetative growth [23]. Jasmonates have been shown to boost the production of secondary metabolites in crops, such as resveratrol in grapevines [24] and the accumulation of glucosinolates in pak choi [25]. Similar to the EtOH treatment, the application of MeJA to plants was observed to generate ROS in chloroplasts [26].

This study explores how fumigation with EtOH and MeJA prior to storage, which is a relatively easy method to conduct, affects senescence, quality, antioxidant capacity, and secondary metabolites (specifically GLS and hydroxycinnamic acids (HCA)) in broccoli florets while stored at 4 °C. The above assumes the hypothesis that low doses of oxidative stress, or plant growth regulators modify the redox state of plant cells, leading to the production of secondary defense metabolites that have beneficial functional properties related to human health.

2. Materials and Methods

2.1. Broccoli

The broccoli used in this study, specifically the Diplomat cultivar of *Brassica oleracea* L., was freshly harvested from a commercial farm (Ile d'Orléans, QC, Canada). The florets, which were of uniform size (about 7 cm), were separated from the heads and kept under dark conditions overnight at 4 °C/90% RH prior to performing any experiments.

2.2. Selection of the Optimal Dose of Ethanol and MeJA

To reduce any effects of stress, the broccoli was kept in a storage facility at 4 °C with 90–100% relative humidity for one night before treatments were applied. The application of ethanol and MeJA was performed through fumigation in a hermetic chamber made of Plexiglas, which was 59 × 59 × 60 cm in size with a total volume of 0.2 m^3. To saturate the atmosphere with the desired concentration of ethanol, a solution of 2 L of 25% ethanol/water (v/v) was placed in the hermetic chamber for 2 h before exposing the broccoli florets to it. A concentration of 10,000 $\mu L.L^{-1}$ (v/v) of ethanol was chosen after conducting preliminary experiments. The concentration of ethanol in the chamber's atmosphere was measured using gas chromatography. A standard curve was prepared using a concentration range of 1 $\mu L.L^{-1}$ to 100 $\mu L.L^{-1}$, and the measurements were repeated four times for each concentration using GC-MS (Hewlett-Packard (HP) 6890 Network GC system (Wilmington, DE, USA)) coupled to a HP 5973 mass spectrometer with an injection volume of 300 µL and a split ratio of 200. Broccoli florets were exposed to a concentration of 10,000 $\mu L.L^{-1}$ of ethanol for different time intervals ranging from 0 to 540 min. For the MeJA treatment, a solution of 10 mL of MeJA (Sigma Aldrich, Oakville, ON, Canada) was introduced into the chamber 24 h prior to the treatment to saturate the atmosphere with MeJA vapor. The concentration of MeJA used was 1 $\mu L.L^{-1}$, which was determined based on the vapor pressure of MeJA at 23 °C (1.28 × 10^{-4} mm Hg) [27]. The broccoli florets were exposed to MeJA for varying lengths of time, ranging from 0 to 720 min. To do this, a standard curve was prepared with MeJA concentrations ranging from 1 to 10 $\mu L.L^{-1}$, and this process was repeated four times using an injection volume of 200 µL. The concentrations were measured by a GC-MS (Hewlett-Packard (HP) 6890 Network GC system (Wilmington, DE, USA) coupled to a HP 5973 mass spectrometer. The minimum ΔE value, which determined the hormetic dose, was evaluated by measuring this parameter on a daily basis for either 5 or 10 d, depending on the treatment.

2.3. Treatments and Storage Conditions

In Section 2.2, a description of how several EtOH and MeJA treatments were applied to determine the optimal dosage of each of the compounds during low-temperature storage has been provided. This hormetic dose corresponded to an application of 10,000 $\mu L.L^{-1}$/30 min of EtOH and 1 $\mu L.L^{-1}$/45 min of MeJA. These treatments were contrasted with higher doses by a factor of 4, i.e., 120 min for ethanol and 180 min for MeJA. Once all the treatments had been applied, the broccoli florets were placed randomly in small plastic punnets weighing 500 mL each. The punnets were then placed inside larger plastic containers that held 5 L of air and had perforations for ventilation. The containers also had a layer of water at the bottom to maintain a high humidity level (98–100%). The entire setup was then stored at 4 °C.

2.4. Color and Respiration Rate of Broccoli Florets

Each individual floret of broccoli was labeled, and its color was measured using a CR-400 colorimeter from Minolta (Osaka, Japan), which had an 8 mm measuring head and a D 65 illuminant. The L^*, a^*, and b^* values were obtained to calculate the total color change (ΔE) value $\sqrt{(L_0^* - L_t^*)^2 + (a_0^* - a_t^*)^2 + (b_0^* - b_t^*)^2}$. All the measurements were recorded in triplicate, with each batch containing 3–4 florets. The color was assessed at uniform intervals of 7 days over a period of 21 d, specifically on days 0, 7, 14, and 21 [28].

The rate at which the broccoli florets were respiring was determined using a headspace analyzer (CheckMate 9900, Cambridge, ON, Canada). The measurements were recorded

four times, with each batch consisting of 3–4 florets. The florets were kept in an airtight plastic container at 4 °C, and measurements were taken after one hour. The respiration rate of the florets was measured every 24 h for 21 d, and the results were reported at regular intervals (0, 7, 14, and 21 d).

2.5. Chemical Assays

All chemical tests were performed on freeze-dried tissue powders obtained from six flower buds frozen in liquid nitrogen per replicate. The amount of chlorophyll in the broccoli sample was measured using a method described by Warren [29], which is a quick and reliable way to measure chlorophyll levels using a microplate reader. To do this, 0.01 g of freeze-dried broccoli was mixed with 2 mL of methanol, and the amount of chlorophyll was determined by measuring the absorbance at 665 nm and 652 nm. This measurement was recorded three times for each sample. The methodology for chlorophyll measurements and other chemical assays, such as total phenols and total flavonoids, ascorbic acid, and ORAC, have been previously described by Duarte-Sierra, Forney, Michaud, Angers, and Arul [28].

Briefly, for the determination of total phenols, samples were extracted using deionized water, and the resulting supernatants were pooled and mixed with a Folin-Ciocalteau reagent and sodium carbonate. The absorbance was measured with a spectrophotometer (Benchmark Plus, Bio-Rad, Philadelphia, PA, USA) at 765 nm, and the phenolic content was expressed as mg gallic acid equivalents on a dry mass basis. The same extracts were used for the determination of flavonoids using the aluminum chloride colorimetric method at 415 nm. Total and reduced ascorbic acid samples were extracted using trichloroacetic acid, and the absorbance was measured at 525 nm. Finally, for the ORAC (Oxygen Radical Absorbance Capacity) assay, the extracts were obtained using acetone and diluted 80 times. The ORAC values were measured using a microplate reader (FluoStar Galaxy DMG, Vienna, VA, USA) with an excitation wavelength of 485 nm and an emission wavelength of 530 nm at 37 °C, and Trolox was used as a standard. The total antioxidant capacity was determined in triplicate and expressed as mg Trolox equivalents on a dry mass basis. All measurements were performed every 7 days in triplicate on three broccoli samples for 21 days.

2.6. Glucosinolates and Hydroxycinnamic Acid Analysis

To extract GLS, HCA, and kaempferol from dried samples (0.5 g), 5.5 mL of 70% methanol and 80 µL (800 µg) of sinigrin (10 mg mL^{-1}) diluted in 70% methanol/water were used. The mixture was placed in an ultrasound bath at 70 °C, vortexed for 10 s after 5 min in the bath, and maintained at the extraction temperature for a total of 10 min. After the extraction, the mixture was vortexed again, cooled on ice, and centrifuged at 4528 g for 5 min at 4 °C. The resulting supernatant was filtered through a 0.2 µm PVDF syringe filter and evaporated in a rotavapor at 40 °C in a 50 mL flask. The extract was then resuspended in 1800 µL of 10 mM ammonium acetate/formic acid at pH 4.4 (mobile phase) and filtered with a 0.22 µm PVDF syringe filter. Finally, 15 µL of the extract was injected into a vial containing 285 µL of mobile phase to achieve a concentration of 5 $\mu g.\mu L^{-1}$. The procedure for separating and quantifying GLS and HCA has been previously explained by Duarte-Sierra, Forney, Michaud, Angers, and Arul [28]. The measurements were carried out in triplicate on three broccoli samples on days 0, 3, 7, and 14.

2.7. Statistical Analysis

A complete randomized design was used for the experiment, and the data were analyzed using one-way analysis of variance (ANOVA) with a significance level of 0.05. The statistical analysis was conducted using the statistical analysis system version 9.3 from SAS Institute Inc. (2011, Base SAS® 9.3 Procedures Guide, Cary, NC, USA). For chemical analysis, an average was calculated for equidistant periods of 0, 7, 14, and 21 days for the ORAC, ascorbic acid, total phenols, total flavonoids, chlorogenic acid, and kaempferol assays.

3. Results and Discussion

3.1. Hormetic Dose

To minimize the amount of ethanol or MeJA needed for treatment, the broccoli florets were fumigated in an atmosphere containing them since their use in the vapor phase requires less consumption compared to their application in the liquid phase [30].

The hormetic dose of ethanol was determined by exposing broccoli florets to an atmosphere containing 10,000 μL.L^{-1} at various durations (ranging from 0 min to 540 min) and observing their color retention by the total color difference (ΔE) for 10 days at 10 °C (Figure 1). Results indicated that an exposure time of 30 min was beneficial for color retention compared to both the control and longer exposure times. Increasing the exposure time of florets to ethanol caused the ΔE value to rise due to the yellowing of the florets, reaching a peak at 240 min. However, the ΔE value decreased and became comparable to the control for exposure times between 240 and 420 min, before increasing again for longer exposure times (Figure 1a). Such a bimodal pattern in color retention in broccoli was also observed in florets exposed to UV-B, ozone, and hydrogen peroxide [31–33]. When exposed to an atmosphere containing 1 μL.L^{-1} of MeJA, broccoli florets showed a decrease in the ΔE value, which reached a minimum at an exposure time of 45 min (Figure 1B). Unlike ethanol, the ΔE value increased continuously with longer exposure times. Therefore, exposure of the florets to an atmosphere containing 1 μL.L^{-1} of MeJA for 45 min was considered hormetic.

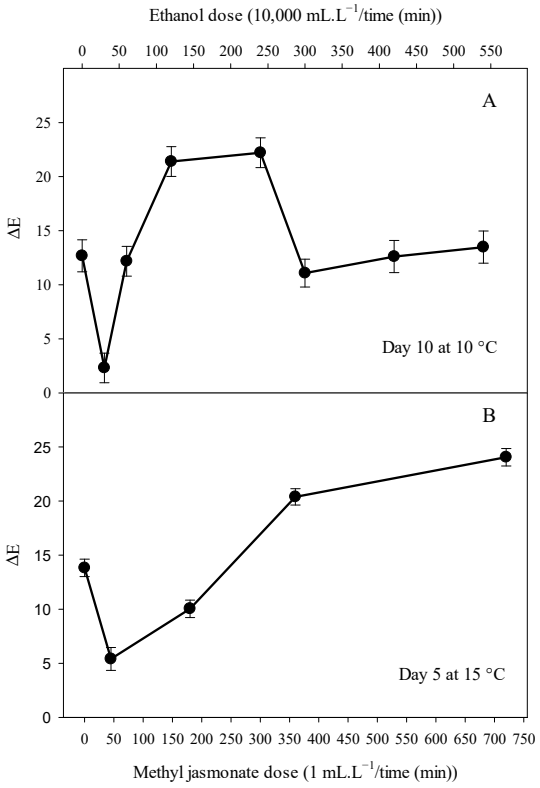

Figure 1. Hormetic dose determination for ethanol and methyl jasmonate. The determination of the hormetic dose of the stress chemicals ethanol (**A**) and methyl jasmonate (**B**) was based on the total color difference (ΔE) at the end of the storage period. Data points are means + standard deviation of the mean for n = 9.

3.2. Color Evolution and Respiration Rate during Storage

Exposing florets to 10,000 μL.L^{-1} of ethanol for 30 min resulted in improved color retention compared to untreated florets when stored at 4 °C (Figure 2A). Previous studies have demonstrated that ethanol-treated broccoli exhibited little to no change in the activities of chlorophyll-degrading enzymes such as magnesium dechelatase and chlorophyll-degrading peroxidases [2]. Yet, ethanol vapor treatment appears to inhibit ethylene biosyn-

thesis and its action in broccoli [17]. In line with the above, the increase in the shelf life of carnation cut flowers with ethanol has been shown to be reversed when ethanol oxidation is blocked, suggesting that the enhanced shelf life of the cut flower is probably due to the oxidation of ethanol to acetaldehyde, which is naturally present in plants at a ratio of 1 part acetaldehyde to 100 parts ethanol [34]. Such a mechanism can be expected to operate in a broccoli floret as well as a flower body. The accumulative intake of ethanol in broccoli florets and its possible conversion to acetaldehyde have also been discussed previously [18].

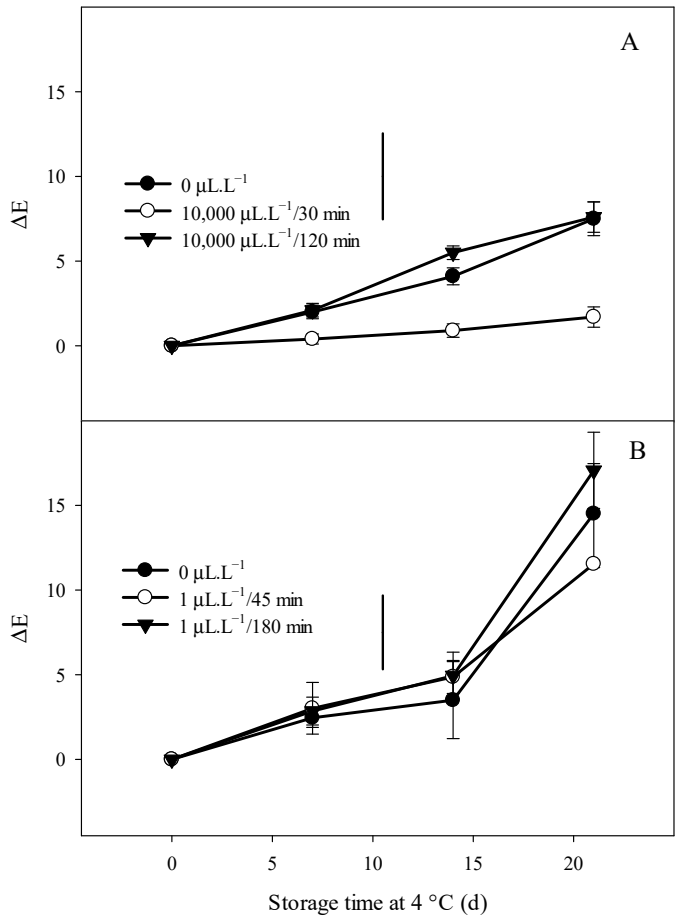

Figure 2. Evolution of total color change in broccoli florets exposed to ethanol and methyl jasmonate. Broccoli florets were treated with ethanol (**A**) at three different doses: (●), control; (○), 10,000 µL.L^{-1}/30 min (hormetic dose); and (▼), 10,000 µL.L^{-1}/120 min (high dose). Methyl jasmonate (**B**) (●), control; (○), 1 µL.L^{-1}/45 min (hormetic dose); and (▼), 1 µL.L^{-1}/180 min (high dose). Color change (ΔE) was observed during 21 d of storage in darkness at 4 °C. Vertical bars represent the standard deviation of the mean (n = 9). LSD (0.05), EtOH = 4.36; LSD (0.05), MeJA = 5.09.

The conversion of ethanol to acetaldehyde causes an imbalance in the NAD+/NADH ratio, shifting the equilibrium towards NADH production. In yeast, this shift results in various outcomes, including the stimulation of pyruvate to lactate conversion, the inhibition of fatty acid oxidation and respiration rate, and the reduction of the ATP/ADP ratio [35]. Moreover, ethanol is known to have a bactericidal effect, disrupting cell membranes, denaturing proteins, and reducing water activity (A_w) in the medium. This effect can be enhanced by the oxidation of ethanol to acetaldehyde. Indeed, the aldehydes exhibit bactericidal effects, which increase with decreasing chain length, with formaldehyde and acetaldehyde being the most toxic [35].

The effect of acetaldehyde on ethylene action has been proven to be more pronounced compared to ethanol. For instance, the inhibition of ACC-treated grapes and mango disks was

more effective with acetaldehyde compared with ethanol at equal concentrations [36,37]. However, this is not the only response of fruits and vegetables to ethanol and acetaldehyde exposure.

High doses of ethanol and the correspondingly high levels of acetaldehyde delay ripening and ethylene production, while low concentrations of ethanol can induce ethylene synthesis. In addition, at high concentrations, these compounds can be phytotoxic and induce off-flavors and increased respiration by the decomposition of organic acids [38]. The rates of respiration of broccoli florets treated with the two doses of ethanol used in this experiment, however, were very similar to those of untreated florets and did not exhibit statistically significant differences immediately after ethanol exposure (Figure 3A). Further, no unpleasant odors or higher chlorophyll levels were detected at the end of storage (Figure 4A), suggesting any irreversible damage to the tissue was not caused by the concentrations of ethanol used.

The treatment with MeJA was less successful in retaining the color of broccoli florets compared to ethanol, even though the hormetic dose induced better color retention compared to untreated florets (2-fold). However, the longer exposure times of florets to MeJA showed significantly less color retention when compared to the control (Figure 1B). Increasing exposure time of florets to MeJA was commensurate with color change (Figure 1B). Jasmonates are a group of compounds that include methyl jasmonate and jasmonic acid. These compounds, which are cyclopentanones, play a role in signal transmission and trigger defensive responses against pathogens in plants [39]. The link between the signaling pathways of jasmonate and ethylene is highly important in defense responses, such as plant-microbe and plant-insect interactions, as well as wound healing. Additionally, jasmonates can stimulate the production of ethylene in plants [40].

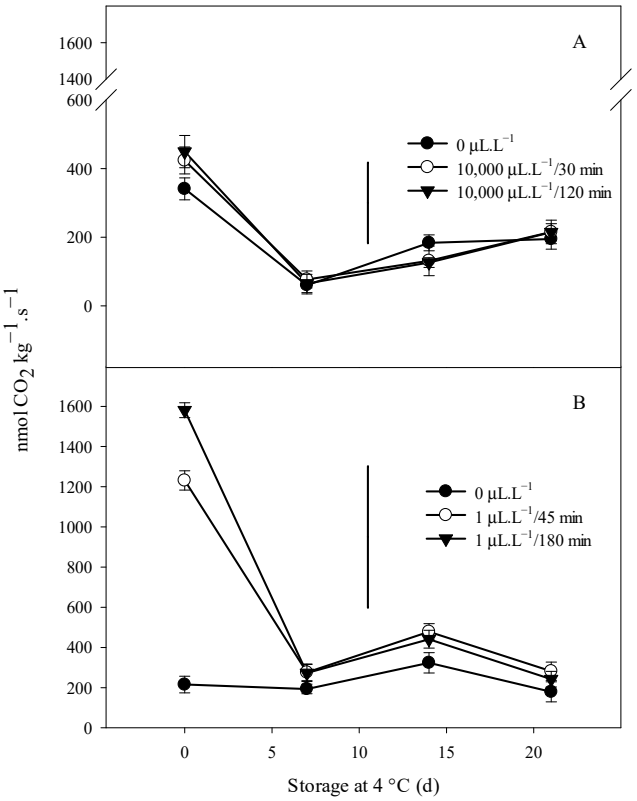

Figure 3. Evolution of the respiration rate of broccoli florets exposed to ethanol and methyl jasmonate. Broccoli florets were treated with ethanol (**A**) at three different doses: (●), control; (○), 10,000 µL.L^{-1}/30 min (hormetic dose); and (▼), 10,000 µL.L^{-1}/120 min (high dose). Methyl jasmonate (**B**) (●), control; (○), 1 µL.L^{-1}/45 min (hormetic dose); and (▼), 1 µL.L^{-1}/180 min (high dose). Color change (ΔE) was observed during 21 d of storage in darkness at 4 °C. Vertical bars represent the standard deviation of the mean (n = 4). LSD (0.05), EtOH = 235.22; LSD (0.05), MeJA = 724.56.

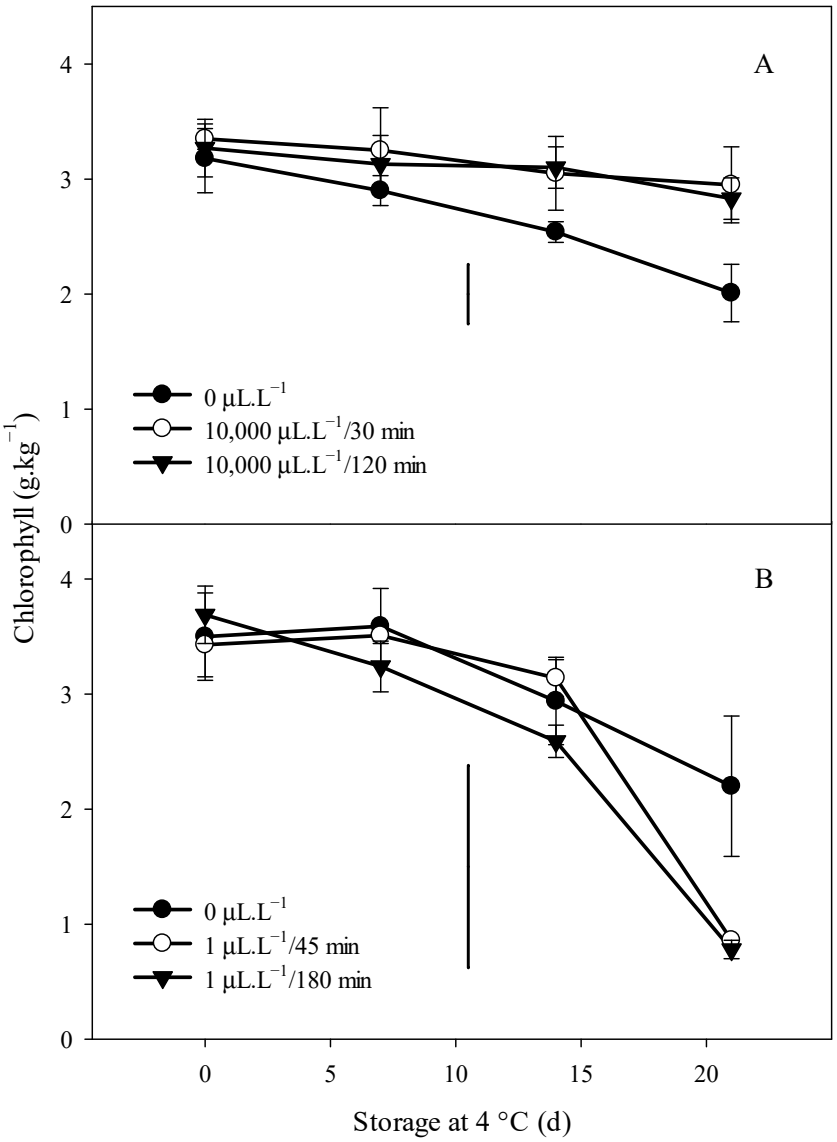

Figure 4. Evolution of chlorophyll content of broccoli florets exposed to ethanol and methyl jasmonate. Broccoli florets were treated with ethanol (**A**) at three different doses: (●), control; (○), 10,000 µL.L^{-1}/30 min (hormetic dose); and (▼), 10,000 µL.L^{-1}/120 min (high dose). Methyl jasmonate (**B**) (●), control; (○), 1 µL.L^{-1}/45 min (hormetic dose); and (▼), 1 µL.L^{-1}/180 min (high dose). Color change (ΔE) was observed during 21 d of storage in darkness at 4 °C. Color change (ΔE) was observed for 21 days of storage in darkness at 4 °C. Vertical bars represent the standard deviation (n = 4). LSD (0.05), EtOH = 0.52; LSD (0.05), MeJA = 1.76.

Exposure of broccoli florets to ethylene causes a dose-dependent decline in green color [41]. The gradual intensification of color transformation in broccoli florets as they are exposed to MeJA over an extended period implies that there is a factor, possibly ethylene, that is stimulated by MeJA and contributes to this change. Such a possible increase in ethylene may be related to the accelerated metabolism evident in the high respiration rates of broccoli florets (Figure 3B). In contrast to ethanol, the concentration of MeJA was relatively low, but its impact on the yellowing of florets was observed to increase with exposure time. Nevertheless, the color retention of the florets was slightly better in those treated with a low dose of MeJA, which exhibited hormetic effects, compared to the control or high dose (Figure 2B). Furthermore, the chlorophyll contents of the florets treated at both doses were significantly lower ($p < 0.05$) compared to unexposed florets (Figure 4B). Similar effects of MeJA in apples have been previously observed. Fan and Mattheis [42] found

that treatment with MeJA stimulated respiration. They have proposed two mechanisms by which MeJA can modulate color change: (1) by promoting ethylene biosynthesis or (2) by MeJA itself, regardless of ethylene action, as the apples were previously treated with 1-MCP and underwent degreening.

3.3. Secondary Metabolism Modification by Ethanol and Methyl Jasmonate

MeJA, which is a signaling molecule, is recognized for its impact on the secondary plant metabolism and has been found to stimulate the production of phenolic compounds [13], anthocyanins [43], resveratrol [24], and indole GLS [44]. In contrast, ethanol can disturb the membrane by making it more fluid [45,46] and can alter enzyme activity by causing changes in protein conformation [47]. Furthermore, ethanol can be broken down into CO_2, organic acids, and amino acids in various tissues, such as storage organs, fruits, stems, and leaves, over a relatively brief period [48].

The florets were analyzed for both individual and total GLS, as well as HCA content, following exposure to methyl jasmonate and ethanol. The total amount of glucobrassicin present was calculated as the sum of glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin, and 4-hydroxyglucobrassicin. Similarly, the total HCA content was determined by adding up the contents of 1,2-disinapoylgentiobiose (DSG), 1-sinapoyl-2-feruloylgentiobiose (SFG), 1,2-diferuoylgentiobiose (DFG), 1,2,2-trisinapoylgentiobiose (TSG), and 1,2-disynalpoyl-2-feruloylgentiobiose (DSFG).

The total amount of glucobrassicins in broccoli florets was significantly increased ($p < 0.05$) by exposure to ethanol during storage, as shown in Figure 5A. Both the hormetic and high doses of ethanol resulted in a 10–11% increase in total glucobrassicins compared to unexposed florets after 30 and 120 min, respectively. However, there were no significant differences observed in the content of individual glucobrassicins between ethanol-exposed and unexposed florets, as seen in Table 1. Only the content of glucoraphanin was increased by ethanol treatments, with a 20–24% increase in titers in both the hormetic and high doses compared to untreated florets, as shown in Figure 6A. The total content of HCA in broccoli was generally enhanced by 17–15% with the hormetic and high doses of ethanol, respectively, compared to the control, as shown in Figure 7A. Additionally, the content of SFG in florets was increased by 20% with both doses of ethanol compared to unexposed florets, as seen in Table 2.

Exposure of broccoli florets to MeJA resulted in a significant increase in total glucobrassicin content. Specifically, an increase of 14% and 15% was observed after exposure to 1 $\mu L.L^{-1}$ of MeJA for 45 min and 180 min, respectively, compared to unexposed florets. (Figure 5B). Moreover, the titers of neoglucobrassicin and 4-hydroxyglucobrassicin were substantially enhanced by MeJA exposure. The titer of neoglucobrassicin was 26% and 20% higher in exposed florets to the hormetic and high doses of MeJA, respectively, compared with unexposed florets. Similarly, the titer of 4-hydroxyglucobrassicin was 38% and 44% higher in exposed florets to the hormetic and high doses of MeJA, respectively, compared with unexposed florets (Table 1). However, there were no significant differences observed in glucoraphanin content (Figure 6). The concentration of HCA in florets significantly increased by 5% with 1 $\mu L.L^{-1}$ of MeJA applied for 45 min and by 11% with 1 $\mu L.L^{-1}$ of MeJA applied for 180 min (Figure 7B). As with ethanol exposure, SFG titers in broccoli florets treated with the high dose of MeJA increased by 10% (Table 2).

The increase in secondary metabolites (SM) observed in broccoli florets exposed to ethanol may be due to the increased production of NADH resulting from the oxidation of ethanol into acetaldehyde. Additionally, ethanol is metabolized in the tissue to form acetate, organic acids, and amino acids, which serve as substrates for the production of glucosinolates and HCA [49]. In contrast, exposing plants to methyl jasmonate appears to elicit a response similar to that observed in insects or mechanical wounding, leading to significant systemic increases in the concentration of indole-type glucosinolates in *Brassicas* [44,50–52].

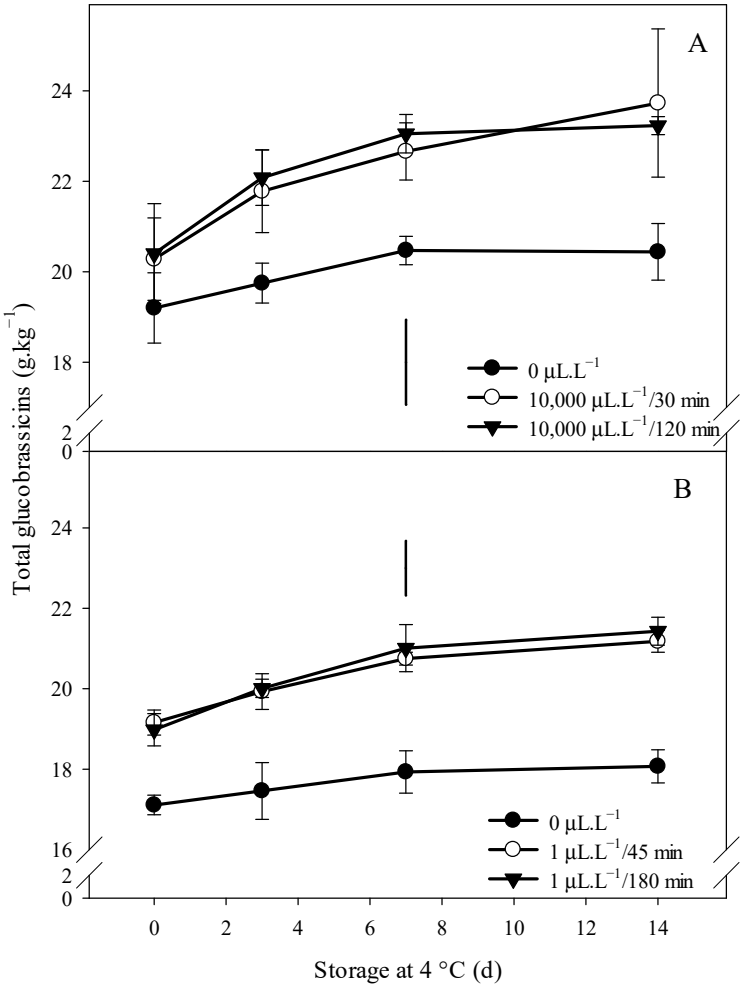

Figure 5. Total glucobrassicin content of florets exposed to ethanol and methyl jasmonate. Broccoli florets were treated with ethanol (**A**) at three different doses: (●), control; (○), 10,000 µL.L^{-1}/30 min (hormetic dose); and (▼), 10,000 µL.L^{-1}/120 min (high dose). Methyl jasmonate (**B**) (●), control; (○), 1 µL.L^{-1}/45 min (hormetic dose); and (▼), 1 µL.L^{-1}/180 min (high dose). Color change (ΔE) was observed during 21 d of storage in darkness at 4 °C. The content of total glucobrassicins (glucobrassicin + neoglucobrassicin + 4-hydroxyglucobrassicin + 4-methoxyglucobrassicin) was monitored for 14 days of storage in darkness at 4 °C. Vertical bars represent the standard deviation (n = 4). LSD (0.05), EtOH = 1.88; LSD (0.05), MeJA = 1.36.

Table 1. Glucosinolate content of ethanol and methyl jasmonate-treated broccoli. Florets were exposed to three different doses of ethanol (control: 0 µL.L^{-1}; hormetic: 10,000 µL.L^{-1}/30 min; high: 10,000 µL.L^{-1}/120 min) and methyl jasmonate (control: 0 µL.L^{-1}; hormetic: 1 µL.L^{-1}/45 min; high: 1 µL.L^{-1}/180 min), stored for 14 days in darkness at 4 °C, and the obtained values were time averaged (0, 7, and 14 d).

	Ethanol	**MeJA**
	Glucobrassicin (g.kg^{-1})	
Control	10.9 ± 0.8	8.4 ± 0.8
Hormetic dose	11.9 ± 1.5	8.9 ± 0.2
High dose	11.8 ± 0.9	8.9 ± 0.5
	Neoglucobrassicin (g.kg^{-1})	
Control	4.3 ± 0.3	4.9 ± 0.3
Hormetic dose	4.8 ± 0.6	6.2 ± 0.4 *
High dose	4.9 ± 0.4	5.9 ± 0.4 *

Table 1. *Cont.*

	Ethanol	MeJA
	4-Hydroxyglucobrassicin (g.kg^{-1})	
Control	1.7 ± 0.1	1.8 ± 0.0
Hormetic dose	1.9 ± 0.3	2.5 ± 0.3 *
High dose	2.0 ± 0.2	2.6 ± 0.3 *
	4-Methoxyglucobrassicin (g.kg^{-1})	
Control	3.1 ± 0.3	2.6 ± 0.1
Hormetic dose	3.5 ± 0.3	2.8 ± 0.2
High dose	3.4 ± 0.2	2.9 ± 0.2

The asterisk indicates that the value is significantly different from the corresponding control at $p < 0.05$.

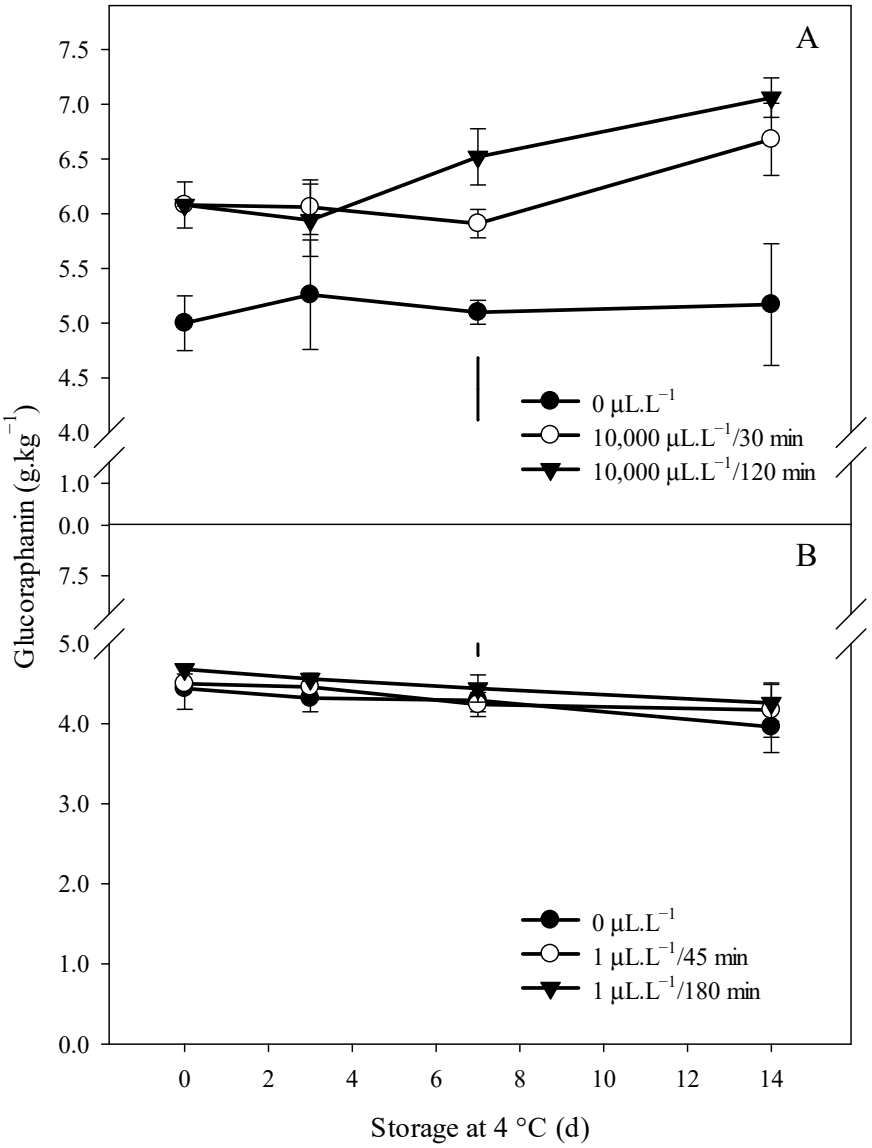

Figure 6. Total glucoraphanin contents of florets exposed to ethanol and methyl jasmonate. Broccoli florets were treated with ethanol (**A**) at three different doses: (•), control; (○), 10,000 µL.L^{-1}/30 min (hormetic dose); and (▼), 10,000 µL.L^{-1}/120 min (high dose). Methyl jasmonate (**B**) (•), control; (○), 1 µL.L^{-1}/45 min (hormetic dose); and (▼), 1 µL.L^{-1}/180 min (high dose). Color change (ΔE) was observed during 21 d of storage in darkness at 4 °C. The content of glucoraphanin was monitored for 14 days of storage in darkness at 4 °C. Vertical bars represent the standard deviation (n = 4). LSD (0.05), EtOH = 0.57; LSD (0.05), MeJA = 0.29.

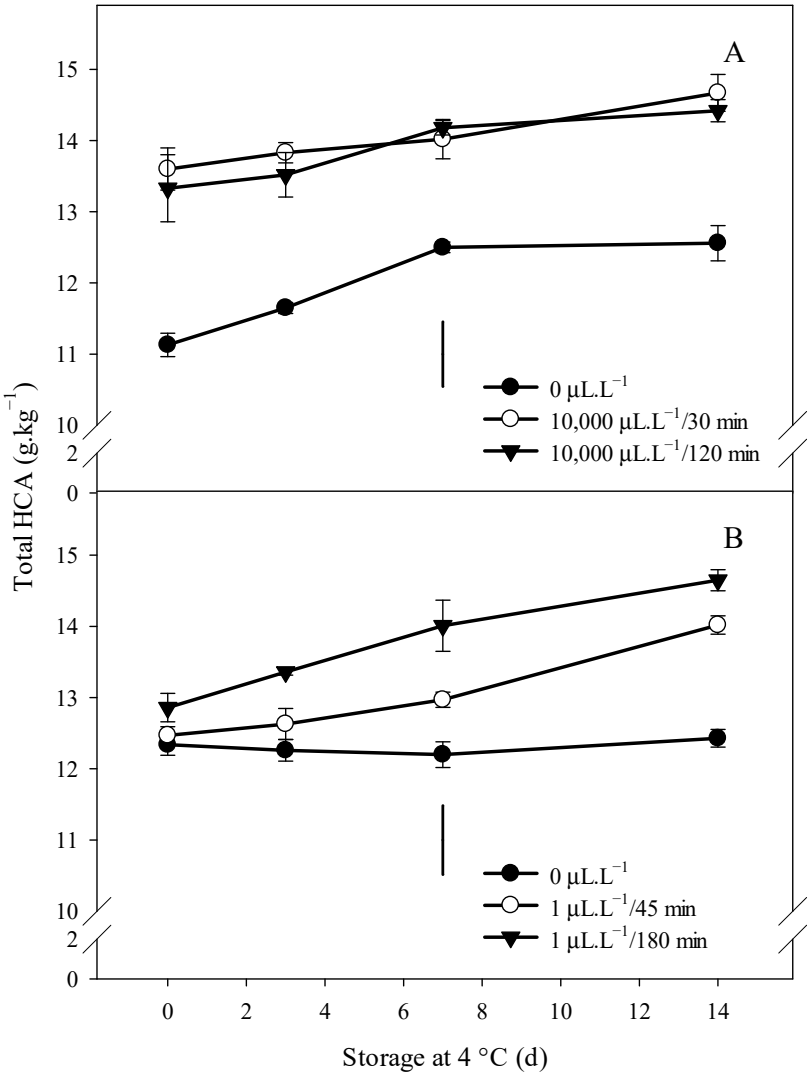

Figure 7. Total hydroxy-cinnamic acid (HCA) content of florets exposed to ethanol and methyl jasmonate. Broccoli florets were treated with ethanol (**A**) at three different doses: (●), control; (○), 10,000 µL.L^{-1}/30 min (hormetic dose); and (▼), 10,000 µL.L^{-1}/120 min (high dose). Methyl jasmonate (**B**) (●), control; (○), 1 µL.L^{-1}/45 min (hormetic dose); and (▼), 1 µL.L^{-1}/180 min (high dose). Color change (ΔE) was observed during 21 d of storage in darkness at 4 °C. The content of total HCA (1,2-disinapoyl gentibiose + 1-sinapoyl-2-feruloyl gentibiose + 1,2-diferuloyl gentibiose + 1,2,2-trisinalpoyl-gentibiose + 1,2-disynalpoyl-2-feruloyl gentibiose) was monitored for 14 days of storage in darkness at 4 °C. Vertical bars represent the standard deviation (n = 4). LSD (0.05), EtOH = 0.91; LSD (0.05), MeJA = 0.07.

Table 2. Hydroxycinnamic-acid content (HCA) of ethanol and methyl jasmonate-treated broccoli. Florets were exposed to three different doses of ethanol (control: 0 µL.L^{-1}; hormetic: 10,000 µL.L^{-1}/30 min; high: 10,000 µL.L^{-1}/120 min) and methyl jasmonate (control: 0 µL.L^{-1}; hormetic: 1 µL.L^{-1}/45 min; high: 1 µL.L^{-1}/180 min), stored for 14 days in darkness at 4 °C, and the obtained values were time averaged (0, 7, and 14 d).

	Ethanol	**MeJA**
	1,2-Disinapoylgentibiose (g.kg^{-1})	
Control	2.2 ± 0.1	2.3 ± 0.2
Hormetic dose	2.5 ± 0.1	2.4 ± 0.1
High dose	2.5 ± 0.3	2.5 ± 0.1

Table 2. *Cont.*

	Ethanol	MeJA
1-sinapoyl-2-feruloylgentibiose (g.kg^{-1})		
Control	4.9 ± 0.2	4.6 ± 0.1
Hormetic dose	5.9 ± 0.4 *	4.8 ± 0.2
High dose	5.9 ± 0.4 *	5.1 ± 0.3 *
1,2-Diferuoylgentiobiose (g.kg^{-1})		
Control	1.3 ± 0.1	1.1 ± 0.1
Hormetic dose	1.5 ± 0.1	1.1 ± 0.1
High dose	1.5 ± 0.1	1.2 ± 0.2
1,2,2-Trisinapoylgentibiose (g.kg^{-1})		
Control	1.5 ± 0.1	1.8 ± 0.1
Hormetic dose	1.7 ± 0.1	1.9 ± 0.0
High dose	1.7 ± 0.1	2.0 ± 0.1
1,2-Disynalpoyl-2-feruloylgentiobiose (g.kg^{-1})		
Control	2.0 ± 0.2	2.6 ± 0.1
Hormetic dose	2.4 ± 0.2	2.7 ± 0.1
High dose	2.3 ± 0.1	2.9 ± 0.2

The asterisk indicates that the value is significantly different from the corresponding control at $p < 0.05$.

The primary defense mechanism in *Brassicas* is the glucosinolate-myrosinase system, which converts glucosinolates into isothiocyanates to protect the tissue against herbivores [8]. While sulforaphane and 4-methylsulfinylbutyl isothiocyanates are potent inducers of phase 2 enzymes that protect against carcinogenesis in humans [53], tryptophan-derived indole glucosinolates are more responsive to wounding, insect feeding, phytohormone treatments, and fungal infection than aliphatic glucosinolates [50,54]. Previous research has shown that MeJA can increase the content of glucoraphanin, glucobrassicin, and neoglucobrassicin in cauliflower, which were enhanced by 1.5, 2.4, and 4.6 times, respectively, compared to control [54]. In contrast, the indole-type glucosinolates were enhanced by 1.25 times in the florets treated with MeJA in this study, which was significant but to a lesser extent (Figure 5B).

MeJA and ethylene are two phytohormones that normally act in conjunction with the elicitation of defense responses. However, the enhancement of the aliphatic glucoraphanin in the florets exposed to ethanol and the lack of such enhancement by MeJA treatment raises the question regarding the role of ethylene in glucosinolate biosynthesis. As mentioned before, the inhibition of yellowing of florets by ethanol could be attributable to reduced ethylene production and/or action by possible denaturation of the proteins involved in ethylene production (ACC oxidase) and its action (ethylene receptor). Thus, it would seem that reduced ethylene in the tissue caused by ethanol leads to the enhancement of glucoraphanin but not in response to the exposure of florets to MeJA. In this case, higher concentrations of ethylene can be expected [55,56], and it is likely the consequence of increased yellowing of florets exposed to MeJA. It would seem likely that the presence of both MeJA and ethylene will interact, leading to the synthesis of indole glucosinolates, which are effective defense compounds. Or it may also suggest that MeJA alone transduces the synthesis of indole glucosinolates. In this respect, Brader, Tas, and Palva [50] observed that the elicitors of *E. carotovora* triggered specific induction of indole glucosinolates in *Arabidopsis*, which was mainly jasmonic acid-dependent, and that the role of either ethylene or salicylic acid was of minor importance.

Improvement of HCAs was also observed in ethanol- and MeJA-treated florets compared with the controls (Figure 7). Ethanol can inhibit the activity of peroxidase (POD), and the accumulation of phenolic compounds is one of the consequences [57]. In this work, enhancement of HCA and total phenols in florets was observed by the application of both doses of ethanol (Table 3). Even if total phenolic compounds were accumulated after exposure to ethanol, no differences were observed in the ascorbic acid or total flavonoid

content of broccoli. Only the amount of kaempferol was slightly enhanced by both doses of ethanol (Table 3). The accumulation of total phenols and HCA can also be attributed to the possible utilization of ethanol as a carbon source for the synthesis of sugars and amino acids [48]. Ethanol may be converted into amino acids such as phenylalanine, which can be transformed into phenolic compounds by enzymes including phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and others. Another possibility is that the increase in concentration of phenols in tissues after ethanol exposure could be associated with weight loss. When comparing various hormetic stresses, ethanol caused significant weight loss (unpublished data). Weight loss due to ethanol can be explained by the disruption of the osmotic balance of the cell as well as the disturbance of water structure, which can affect the structure of membranes. Thus, dehydration of tissue could have concentrated HCAs, which could have also been possible for the observed augmentation of GLS in the florets exposed to ethanol.

Table 3. Oxygen radical absorbance capacity (ORAC, Trolox equivalents), ascorbic acid (oxidized, reduced, or total), total phenols (gallic acid equivalents), total flavonoids (quercetin equivalents), chlorogenic acid, and kaempferol (sinigrin equivalents) contents in broccoli florets exposed to ethanol and methyl jasmonate. Florets were exposed to three different doses of ethanol (control: 0 $\mu L.L^{-1}$; hormetic: 10,000 $\mu L.L^{-1}$/30 min; high: 10,000 $\mu L.L^{-1}$/120 min) and methyl jasmonate (control: 0 $\mu L.L^{-1}$; hormetic: 1 $\mu L.L^{-1}$/45 min; high: 1 $\mu L.L^{-1}$/180 min), stored for 14 days in darkness at 4 °C, and the obtained values were time averaged (0, 7, and 14 d).

	Ethanol	**MeJA**
	ORAC ($g.kg^{-1}$)	
Control	149.5 ± 5.7	180.6 ± 11.8
Hormetic dose	150.1 ± 3.9	137.3 ± 12.8 *
High dose	147.7 ± 4.1	134.6 ± 7.7 *
	Total ascorbic acid ($g.kg^{-1}$)	
Control	14.1 ± 0.4	8.9 ± 0.5
Hormetic dose	13.7 ± 0.5	8.7 ± 0.5
High dose	14.1 ± 0.7	8.7 ± 0.6
	Reduced ascorbic acid ($g.kg^{-1}$)	
Control	10.1 ± 0.3	5.7 ± 0.3
Hormetic dose	9.9 ± 0.3	5.5 ± 0.4
High dose	9.9 ± 0.3	5.4 ± 0.3
	Oxidized ascorbic acid ($g.kg^{-1}$)	
Control	4.0 ± 0.1	3.2 ± 0.2
Hormetic dose	3.8 ± 0.2	3.3 ± 0.1
High dose	4.2 ± 0.4	3.3 ± 0.3
	Total phenols ($g.kg^{-1}$)	
Control	12.8 ± 0.3	14.5 ± 0.6
Hormetic dose	14.8 ± 0.4 *	14.5 ± 0.5
High dose	15.1 ± 0.2 *	14.7 ± 0.8
	Total flavonoids ($g.kg^{-1}$)	
Control	3.7 ± 0.4	4.0 ± 0.4
Hormetic dose	3.7 ± 0.1	3.9 ± 0.2
High dose	4.0 ± 0.2	4.5 ± 0.4
	Chlorogenic acid ($g.kg^{-1}$)	
Control	2.2 ± 0.1	1.9 ± 0.1
Hormetic dose	2.5 ± 0.2	2.0 ± 0.1
High dose	2.5 ± 0.2	2.0 ± 0.1
	Kaempferol ($g.kg^{-1}$)	
Control	1.6 ± 0.1	1.4 ± 0.1
Hormetic dose	2.0 ± 0.2 *	1.5 ± 0.1
High dose	2.0 ± 0.1 *	1.5±0.1

The asterisk indicates that the value is significantly different from the corresponding control at $p < 0.05$.

On the other hand, the induction of PAL, which is the key enzyme for the synthesis of phenylpropanoids, can be mediated by jasmonates [58]. Results of this investigation

revealed a significant enhancement of HCAs with the hormetic and high dose of MeJA. The augmentation of HCAs was directly related to the exposure time of florets to MeJA. However, a substantial decrease in ORAC was not related to the exposure time of broccoli to the phytohormone (Table 3). This observation differs from the results of studies where MeJA enhanced antioxidant activity in blackberries and raspberries [59,60].

Since the total phenols, flavonoids, and ascorbic acid of exposed MeJA florets were not very different from those of the control (Table 3), it should be expected that the antioxidant capacity should also remain the same as that of the control. Furthermore, the oxidized ascorbic acid content of the MeJA-treated florets was also nearly the same as that of the control, suggesting a low ROS generation. It is also different from the observations in response to oxidative stresses in broccoli florets. However, MeJA also caused yellowing of the florets in a dose-dependent manner. It raises the question of whether redox reactions to protect chlorophyll occur mainly in the chloroplast, involving the antioxidants protecting the photosystem such as glutathione or α-tocopherol.

4. Conclusions

The study findings demonstrate the impact of postharvest storage treatments using EtOH and MeJA on broccoli florets. The EtOH doses administered helped sustain the visual appearance of the florets by decreasing yellowing during storage at 4 °C while simultaneously elevating the level of GLS in the plant. Similarly, the application of the hormetic dose of MeJa resulted in the production of secondary metabolites, including phenolic compounds and GLS. However, the plant tissue quality parameters were compromised as a result.

It is important to note that the results were achieved under controlled conditions involving the application of gases, which may not be easily implemented in practical settings. Additionally, it is worth mentioning that the detection of secondary metabolites was performed on florets rather than on broccoli heads, which are typically sold commercially. Finally, it is important to consider that our study simulated storage conditions in a household rather than a commercial packaging environment, where the storage temperature is typically 0 °C instead of 4 °C.

Although consumers are increasingly interested in the nutritional value of their food, taste remains a key factor when choosing foods to consume. Consequently, the induction of secondary metabolites in vegetables usually results in a bitter taste; future studies in this area should include sensory analysis. Additionally, it is important to identify the specific components involved in the oxidative stress response caused by both biotic and abiotic stresses, rather than solely attributing it to reactive oxygen species. This is crucial in a more fundamental context.

Author Contributions: M.T. participated in conducting experiments related to chemical analysis. J.A., M.T. and A.D.-S. were involved in designing the experiments and reviewing the manuscript. J.A. conceptualized the research and supervised its activities and the publication review process. A.D.-S. assisted in writing the manuscript and supervised M.T. All authors have read and agreed to the published version of the manuscript.

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