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Interaction of exogenous hydrogen sulphide and ethylene on senescence of green leafy vegetables

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ABSTRACT

Hydrogen sulphide (H_2S) gas has been found to delay the appearance of many characteristics associated with senescence of horticultural produce but little attention has been given to its physiological role or its interaction with ethylene. This study used the green leafy vegetable, pak choy (*Brassica rapa* subsp. *Chinensis*) as the principal test commodity and examined the interaction of fumigation with hydrogen sulphide and storage at 10 °C in the presence of controlled levels of ethylene on endogenous ethylene production and a range of factors associated with postharvest deterioration. It was found that hydrogen sulphide inhibited ethylene production, chlorophyll loss, respiration, weight loss, various antioxidant factors and ion leakage. Hydrogen sulphide also inhibited chlorophyll loss and respiration of other green leafy vegetables/herbs, sweet Italian basil (*Ocimum basilicum*) and green curly kale (*Brassica oleracea* var. *sabellica).* The results suggest that the mode of action of hydrogen sulphide in delaying senescence could be by inhibiting both the production of ethylene and the action of ethylene. The substantial reduction in the rate of loss of chlorophyll following short-term treatment with hydrogen sulphide may have potential commercial benefit for extension in market life of green leafy vegetables.

1. Introduction

Ethylene is well known as a gaseous plant growth regulator that regulates numerous aspects of plant growth and development including promoting senescence and fruit ripening (Abeles et al., 1992; Wills, 2015). A concentration of 0.1 $\mu L\,L^{-1}$ was generally cited as the threshold level for the activity of ethylene on postharvest behaviour (Burg and Burg, 1962; Knee et al., 1985) but this related more to analytical capability than empirical evidence. It has now been shown that a wide range of climacteric and non-climacteric produce are incrementally responsive to ethylene at concentrations greater than 0.001 $\mu L\,L^{-1}$ (Wills et al., 1999, 2001).

In addition to ethylene other gaseous plant growth regulators such as nitric oxide (NO) have now been identified. Nitric oxide has been shown to extend the postharvest life of horticultural commodities (Leshem and Haramaty, 1996; Leshem et al., 1998) with the proposed mode of action being through interaction with ethylene. Nitric oxide has been reported to regulate endogenous ethylene production through suppression of pathways linked to ethylene synthesis and by direct stoichiometric inhibition (Manjunatha et al., 2012).

Another more recently identified gaseous plant growth regulator is hydrogen sulphide (H_2S) (Jin and Pei, 2015). Hydrogen sulphide has been linked to diverse plant physiological functions such as germination, stomatal movement, root development and flower senescence (Hancock and Whiteman, 2016). Recognition of the role of hydrogen sulphide as a regulator of postharvest senescence is quite recent. Zhang et al. (2011) reported delayed senescence in cut flowers and shoot explants treated with solutions of the hydrogen sulphide donor sodium hydrogen sulphide (NaHS). Fumigation with hydrogen sulphide was subsequently extended to postharvest fruit and vegetables with an extension in storage life achieved through inhibition of a wide range of senescence characteristics. Horticultural produce that have been shown to benefit from treatment with exogenous hydrogen sulphide include strawberry (Hu et al., 2012), broccoli, (Li et al., 2014), peach (Wang et al., 2014), kiwifruit (Zhu et al., 2014), pear (Hu et al., 2014b), mulberry (Hu et al., 2014a) sweet potato (Tang et al., 2014), lotus root (Sun et al., 2015), water spinach (Hu et al., 2015), grape (Ni et al., 2016) and apple (Zheng et al., 2016). Despite this plethora of recent studies, little work has examined the physiological role of hydrogen sulphide in postharvest behaviour and given the established link between ethylene and postharvest senescence, little attention has been given to understanding the interaction of hydrogen sulphide and

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ethylene. Li et al. (2014) and Zheng et al. (2016) did show that exogenous hydrogen sulphide treatment of broccoli florets and apple slices, respectively, down regulated the expression of genes associated with ethylene biosynthesis. However, these and other published studies did not monitor the concentration of ethylene around produce following exposure to hydrogen sulphide, nor maintain different levels of ethylene around produce during storage. In this study, we have used the green leafy vegetable, pak choy as the principal test commodity and examined the interaction of fumigation with hydrogen sulphide and storage at 10 °C in the presence of controlled levels of ethylene on postharvest deterioration.

Pak choy (*Brassica rapa* subsp. *Chinensis*) (also known as bok or pok choy, choi or tsoi) is widely grown in south and east Asia but is now also available in most Western countries. As with many green leafy vegetables, quality is dependent on leaves retaining their green colour and there being no other visible sign of deterioration such as wilting, rotting or browning (Able et al., 2005). It is commonly claimed (Anon, 1989; Able et al., 2005; Luo, 2016) that pak choy is not particularly sensitive to ethylene but Li et al. (2017) showed that any reduction in the atmospheric ethylene concentration in the range of 1.0 to 0.001 μL L^{-1} at temperatures from 0 to 20 °C resulted in a substantial increase in market life. The experiments in this study report on the interaction of hydrogen sulphide in the presence of continuous ethylene in the storage of pak choy. The effect of hydrogen sulphide on ethylene production and quality factors associated with senescence of non-climacteric produce such as chlorophyll retention, respiration, weight loss, various antioxidant factors and ion leakage were assessed. In addition, the effect of hydrogen sulphide on chlorophyll retention and respiration of two other green leafy herb vegetables, sweet Italian basil (*Ocimum basilicum*) and green curly kale (*Brassica oleracea* var. *sabellica),* was also examined.

2. Materials and methods

2.1. Produce and experimental designs

2.1.1. Produce

Pak choy plants (cv. 'Shanghai') were harvested from a local commercial farm at Mangrove Mountain, NSW and transported to the laboratory within two hours. All plants selected for each experiment were of uniform size (10 cm length) and colour and without damage to leaves or stem. Pak choy heads were cut and a specific number of outside leaves (the number varying between different experiments) were selected and gently cleaned with tap water. The leaves from each head were randomly distributed into the required number of treatment units each containing the same weight of produce. All experiments were replicated by obtaining batches of plants on three separate occasions with at least two weeks between batches.

Fresh cut basil and kale plants were obtained from a local market. All the leaves selected for an experiment were uniform in size and colour and without damage and similarly distributed into treatment units as for pak choy.

2.1.2. Effect of ethylene concentration

Each treatment unit consisted of eight leaves (about 150 g) from different pak choy heads placed into a 4 L plastic container that was fitted with inlet and outlet ports in the lid. Containers were placed into a temperature controlled cabinet at 10 °C and polyethylene tubing (5 mm diam.) was connected to the inlet port through which flowed humidified air containing four concentrations of ethylene. The experiment comprised 12 containers and groups of three containers were respectively ventilated with 1, 0.1, 0.01 and < 0.001 μL L^{-1} ethylene at 45 mL min^{-1}. The < 0.001 μL L^{-1} concentration can be considered as ethylene-free as the analytical limit of detection was 0.001 μL L. The desired concentrations of ethylene were achieved by mixing compressed air that was made ethylene-free by passing through a tube filled with potassium permanganate adsorbed onto alumina pellets with a regulated flow of ethylene from a cylinder (1 mL L^{-1} ethylene in air, BOC Gases, Sydney). The gas mixtures were humidified by bubbling through water in a 2 L glass jar (height 225 cm) to ensure a high humidity of 97–99% RH was maintained in the gas stream to minimise water loss. The ethylene concentration in gas streams was monitored at regular intervals at the inlet port with a gas sample (1 mL) that was analysed by flame ionization gas chromatography as described by Huque et al. (2013). Leaves in each unit were visually assessed daily for green colour and the time for each unit to develop an unacceptable colour (denoted as the market life) was determined using the scoring scale given in Section 2.2.1.

2.1.3. Effect of hydrogen sulphide concentration

Similar as described above, a treatment unit consisted of eight leaves from different pak choy heads with the experiment comprising a total of 24 containers. The 4 L containers were placed at 10 °C and after two hours, groups of three units were fumigated with hydrogen sulphide vapour at 0, 50, 100 and 250 μL L^{-1}. Hydrogen sulphide gas at the desired concentration inside a sealed container was generated *in situ* by placing the required weight of solid $NaHS{\cdot}H_2O$ into a dry beaker that was sealed in a container along with the produce to be treated. Water (2 mL) was then injected into the beaker through a septum in the container lid to generate the hydrogen sulphide. Using this method, the liberation of hydrogen sulphide gas has been shown to be quantitative and immediate (Zhao et al., 2014). The containers remained sealed for 4 h after which time the containers were ventilated with ethylene-free air (< 0.001 μL L^{-1}) or 0.1 μL L^{-1} ethylene at 45 mL min^{-1}. Leaves in all units were visually assessed daily for green colour and the respiration rate of units was analysed by gas chromatography at periodic intervals during storage.

Basil and kale were similarly evaluated for the effect of hydrogen sulphide but only with three concentrations (0, 50 and 100 μL L^{-1}). A preliminary experiment showed that a concentration of 250 μL L^{-1} damaged the leaves, particularly of basil. Damage symptoms were brown-black patches typical of chemical injury. A treatment unit consisted of six plant leaves with a total unit weight of about 125 g. Leaves were assessed for green colour and respiration rate as previously described.

2.1.4. Effect on ethylene production

The effect of hydrogen sulphide on ethylene production of pak choy was examined by fumigating three 4 L containers of pak choy (150 g) with 250 μL L^{-1} hydrogen sulphide with three control containers. Containers were then stored at 10 °C in and ventilated with ethylene-free air or air containing 0.1 μL L^{-1} ethylene. At periodic intervals, the containers were sealed and a gas sample (1 mL) was immediately collected in a syringe and analysed for ethylene concentration by gas chromatography. After two hours, another gas sample was collected and analysed for ethylene concentration. The difference in the two readings was used to calculate the rate of ethylene production for each unit of pak choy. After three and six days, produce were visually assessed for green colour and weight loss.

2.1.5. Evaluation of changes in physio-chemical attributes

Analysis during storage of a number of physio-chemical factors required a larger sample size of pak choy. In these experiments, a total of 36 treatment units comprising 30 leaves (about 400 g) from different pak choy heads were placed in 4 L containers which were stored at 10 °C. Half the containers were sealed and fumigated with hydrogen sulphide (250 μL L^{-1}) for four hours as detailed above. The remaining 18 containers were sealed for 4 h and became the control treatment. After four hours, all containers were ventilated with humidified air containing 0.1 μL L^{-1} ethylene at 45 mL min^{-1}. Eight leaves in each treatment unit were randomly assigned to be visually assessed daily for leaf colour throughout the storage period while 12 leaves were analysed