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# Interaction of the hydrogen sulphide inhibitor, propargylglycine (PAG), with hydrogen sulphide on postharvest changes of the green leafy vegetable, pak choy

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ABSTRACT

Propargylglycine (PAG) is an inhibitor of hydrogen sulphide ($H_2S$) production and has been used to explore the mode of action of $H_2S$ in prolonging storage of horticultural produce but little attention has been given to how PAG and $H_2S$ interact when both are applied to produce. This study examined the effect of sequential application of PAG and $H_2S$ on a range of postharvest senescence factors of the leafy vegetable pak choy (*Brassica rapa* subsp. *Chinensis)* stored at 10 °C. The results showed differential responses between factors when compared to application of PAG or $H_2S$ alone. As expected, fumigation with $H_2S$ reduced the rate of loss of leaf green colour, respiration rate, ethylene production, ion leakage and enhanced antioxidant activity and leaves sprayed with PAG showing converse effects. If PAG acted solely by inhibiting endogenous $H_2S$ production then subsequent treatment with $H_2S$ should fully negate any effect induced by PAG. However, for the combined PAG + $H_2S$ treatment, respiration was similar leaves fumigated with $H_2S$, loss of green leaf colour was similar to the PAG single treatment and less than the untreated control, antioxidant activity was less than for PAG but greater than for control leaves, and ethylene production and ion leakage were similar to control leaves. Thus, the concept that PAG is exclusively an inhibitor of endogenous $H_2S$ production was not validated, with PAG having effects on metabolism that are not linked to the action of endogenous $H_2S$. The additional actions of PAG could be through its inhibition of pyridoxyal-5′-phosphate (PHP) which is a coenzyme for numerous enzyme systems.

## 1. Introduction

Hydrogen sulphide ($H_2S$) was considered a toxic gas, but about 10 years ago was found to be synthesised in mammalian tissues and to have a mediating role in a wide range of cellular physiology functions (Li and Moore, 2008). It is now also known to also act as gaseous plant growth regulator, impacting a diverse range of plant physiological functions such as germination, stomatal movement, root development and flower senescence (Jin and Pei, 2015; Hancock and Whiteman, 2016). A role for $H_2S$ in the regulation of postharvest senescence is quite recent. Zhang et al. (2011) reported delayed senescence in eight types of cut flowers and shoot explants treated with vase solutions of the $H_2S$ donor, sodium hydrogen sulphide (NaHS). Fumigation with $H_2S$ was subsequently extended to a range of postharvest fruit and vegetables including strawberry (Hu et al., 2012), broccoli, (Li et al., 2014, 2017a), peach (Wang et al., 2014), mulberry (Hu et al., 2014), and banana (Ge et al., 2017) with an extension in storage life achieved through inhibition of a wide range of senescence characteristics. Work examining the physiological role of $H_2S$ in postharvest produce is limited. Li et al. (2014) and Zheng et al. (2016) showed that exogenous $H_2S$ treatment of broccoli florets and apple slices, respectively, down regulated the expression of genes associated with ethylene biosynthesis. Ge et al. (2017) extended this understanding, showing that $H_2S$ fumigation also upregulated ethylene receptor expression. Further, Al Ubeed et al. (2017) identified that senescence characteristics in the green leafy vegetables, pak choy, basil and kale were delayed by fumigation with $H_2S$ and speculated that the mode of action of $H_2S$ was through inhibition of ethylene production and action.

The ability to inhibit endogenous $H_2S$ production has been a key tool for understanding the physiological actions of $H_2S$ in living systems. A range of inhibitor compounds have been identified including L-propargylglycine which has been widely employed in both animal and plant physiology to probe $H_2S$ activity. García-Mata and Lamattina (2010) utilised DL-propargylglycine (designated in this paper as PAG)

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to confirm $H_2S$ involvement in controlling stomatal closure in *Vicia faba* and *Aridopsis thaliana*. More recently, links between $H_2S$ homeostasis and the expression of senescence characteristics in postharvest produce has been investigated using PAG. Li et al. (2014) found the exogenous $H_2S$ treatment of broccoli florets enhanced endogenous $H_2S$ production leading to delayed onset of senescence characteristics and enhanced metabolic activity. They further showed PAG lowered the activity of L-cysteine desulfhydrase (LCD) and D-cysteine desulfhydrase (DCD), the enzymes that convert L- and D- cysteine, respectively, to $H_2S$ (Jin and Pei, 2015), thereby reducing endogenous $H_2S$ levels leading to accelerated senescence. Liu et al. (2017) identified similar effects on daylily with a range of senescence characteristics enhanced by spraying with PAG and inhibited by fumigation with $H_2S$.

If PAG acted solely on endogenous $H_2S$ production, it could be expected that the addition of exogenous $H_2S$ should negate the inhibitory effect of PAG. The only study where PAG and $H_2S$ have been applied sequentially produce was by Hu et al. (2015) with the leafy vegetable, water spinach (*Ipomea aquatica*). They reported that fumigation with $H_2S$ after spraying with PAG only partially negated the inhibition of $H_2S$ synthesis induced by PAG. In this study we further examined the interaction of PAG and $H_2S$ by measuring a range of postharvest changes of pak choy leaves sequentially treated with PAG and $H_2S$ and comparing these effects to responses of leaves treated with a single application of PAG or $H_2S$.

## 2. Materials and methods

### 2.1. Produce

Pak choy plants (*Brassica rapa* subsp. *Chinensis* cv. Shanghai) (also known as bok or pok choy, choi or tsoi)) were sourced from a local farm at Mangrove Mountain, New South Wales, Australia, and transported to the laboratory within two hours of harvest. 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, with each containing 24 leaves that weighed about 0.4 kg. Each treatment unit was placed into a sealable plastic container (4 L) fitted with an inlet and outlet tubes in the lid. All experiments were replicated by obtaining batches of plants on separate occasions with at least two weeks between batches with the various experiments conducted over two seasons.

### 2.2. Treatments

Treatments applied to a container of pak choy leaves at 20 °C were:

1 PAG: each side of each leaf in a treatment unit was sprayed with 0.1 mL of an aqueous solution containing 2 mM PAG (Sigma-Aldrich, Australia) without addition of a wetting agent, then left to air dry at 20 °C. This concentration was shown to give optimum inhibition of LCD and DCD activity in previous studies (Cui et al., 2014; Hu et al., 2015).
2 Control treatments: leaves in a treatment unit remained untreated or were similarly sprayed with water.
3 $H_2S$: treatment units were fumigated for four hours with 250 $\mu L\,L^{-1}$ $H_2S$, the optimum concentration reported by Al Ubeed et al. (2017) to inhibit senescence of pak choy. The gas was generated *in situ* by the addition of water to solid NaHS using the method described by Zhao et al. (2014).
4 PAG + $H_2S$: leaves were sprayed with PAG or water and left to dry for three hours then fumigated with $H_2S$ for four hours as per the above treatments.

After seven hours, all containers in an experiment were sealed at s10 °C and ventilated through the inlet tube with air containing 0.1 $\mu L\,L^{-1}$ ethylene or ethylene-free air at 0.75 $mL\,s^{-1}$. The ventilating gas streams were humidified by bubbling through water to ensure a high humidity of 97–99 % RH was maintained to minimise water loss.

### 2.3. Physio-chemical assessments

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 below. Respiration rate, as evolved carbon dioxide, and ethylene production were assessed at various times during storage.

#### 2.3.1. Visual leaf colour (market life)

The change in leaf colour from green to yellow of individual pak choy leaves was conducted daily by visual assessment using a scoring scale of 0–5 where 0 = green, 1 = 10%, 2 = 20%, 3 = 30%, 4 = 50% and 5 = > 70% loss of original green colour as proposed by Li et al. (2017b). A colour photograph was made of leaves at the various colour scores and used as a reference to maintain a uniform standard during the assessment process. Colour assessment was made by a single person but samples were coded so the observer was not aware of the treatments. The mean colour score of all leaves in a treatment unit was calculated daily. An average colour score of 3.0 was considered to be the limit of consumer acceptability and the time for leaves to reach a mean score of 3.0 was designated as the market life of that unit.

#### 2.3.2. Respiration rate and ethylene

After various times in storage, the respiration rate was measured as carbon dioxide evolution. A container containing a treatment unit of pak choy was sealed to allow the accumulation of a measureable concentration of carbon dioxide. A gas sample (5 mL) was collected in a syringe after four hours and the concentration of carbon dioxide in the sample was determined by injecting into a thermal conductivity gas chromatograph as described by Huque et al. (2013). The respiration rate was calculated as $\mu g\,kg^{-1}\,s^{-1}$ $CO_2$.

The concentration of ethylene in atmosphere was determined by a collecting a gas sample (1 mL) and analysing by flame ionization gas chromatography as described by Huque et al. (2013). Samples were obtained from ventilated treatment units just before sealing the container and again three hours after sealing. The difference between readings was used to calculate the rate of ethylene production as $ng\,kg^{-1}\,s^{-1}$.

#### 2.3.3. Ion leakage

Ion leakage was determined according to the method described by Lu (2007) after three days storage using five leaves selected from a treatment unit. This involved collecting two disks (50 mm diameter) from each leaf. The disks were immediately immersed in double distilled water (40 mL) in glass vials and incubated for two hours at 25 °C. The conductivity of the solution was then measured with a conductivity meter (Model 4071, Jenway, Staffordshire, UK). The solution was then boiled for 15 min. and after cooling to room temperature, the total conductivity was re-measured. Ion leakage was calculated as the percentage of the initial to final value.

#### 2.3.4. Antioxidant activity

Antioxidant activity was determined after three days of storage using two samples of leaf (each about 4 g) that were cut from the top part of five leaves from a treatment unit, ground using a mortar and pestle then mixed with 50% methanol (50 mL). The mixture was placed in an ultrasonic bath (Soniclean, Australia) set at 35 °C and 100 W for 30 min. before being filtered through filter paper (Whatman Grade 5). The filtrate was stored at –20 °C until analysed. Antioxidant activity was determined with 2,2-diphenyl-picrylhydrazine (DPPH), using the method described by Vuong et al. (2013). Briefly, a stock solution of DPPH (0.24 $g\,L^{-1}$ methanol) was prepared and stored at −20 °C until