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Impact of exogenous arginine, cysteine and methionine on the postharvest senescence of six green leafy vegetables

Muhammad Sohail[1], Ron B. H. Wills[1*], Michael C. Bowyer[1] and Penta Pristijono[1]

1, *School of Environmental and Life Sciences, University of Newcastle, Ourimbah, Australia*

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***Corresponding author:**

School of Environmental and Life Sciences, University of Newcastle, Ourimbah, Australia.

Email: ron.wills@newcastle.edu.au

ABSTRACT

Purpose: This study examined the efficacy of aqueous dips containing the amino acids, L-arginine, L-cysteine and L-methionine, to inhibit the senescence of six leafy green vegetables pak choy, coriander, choy sum, spinach, parsley and rocket. **Research method:** Pak choy was dipped in amino acid solutions from 2-100 mM to determine the optimum concentration that inhibited senescence. The other vegetables were dipped in solutions with the optimal concentration. Senescence of the vegetables was assessed during storage at 10 °C in air containing 0.1 µL L^{1} ethylene by determining loss of green colour (designated as green life), ethylene production and respiration rates. **Findings:** For each amino acid, a dipping concentration of 5 mM was found optimal to inhibit senescence as shown by an extended green life and reduced ethylene production and respiration rates of all vegetables to a similar extent, except methionine which did not show a significant effect with rocket, and for spinach only reduced ethylene production. **Limitations:** No limitations were encountered. **Originality/Value:** Arginine and cysteine showed considerable potential for commercial use to extend the market life of many green vegetables and with their Generally Recognized As Safe (GRAS) status the amino acids should be a safe, consumer-acceptable treatment.

INTRODUCTION

Green leafy vegetables are a common inclusion in the diet of most people and provide a range of essential nutrients. A major limitation to their marketing is the loss of green colour during senescence as appearance is the major quality criteria available to consumers in purchase decisions (Zhang & Jiang, 2019). Research on a wide range of physical and chemical techniques to extend market life has included the use of nitric oxide (NO) and hydrogen sulphide (H_2S), which are naturally occurring signalling agents that have been shown to extend the postharvest life of many horticultural produce including various green leafy vegetables (Al Ubeed et al., 2017; 2019b; Li et al., 2014; Wills et al., 2007). Methods of applying NO and H_2S to horticultural produce include direct fumigation or through a donor compound that degrades to release the gas. Such techniques are acceptable for laboratory research but face obstacles in adaptation for commercial use. Firstly, NO and H_2S are highly toxic, biologically active gases and pose logistical, health and safety issues during fumigation (Jones, 2014; Paquay et al., 2000). In addition, NO is an unstable, free radical species with a relatively short half-life (Wills et al., 2007). Given that metabolism of NO and H_2S in plants is not identical to animals (Corpas et al., 2019), further safety studies would be required to gain regulatory approval. There is also the question of whether consumers would accept adding a known toxic gas to foods.

An alternate strategy to gain the benefit of NO and/or H_2S is to utilise compounds with Generally Recognised as Safe (GRAS) status, or are accepted as posing no safety issue (either real or perceived) by consumers. In this respect, the amino acids, arginine and cysteine present a potential solution as they are known metabolic precursors for endogenous NO (Morris, 2007) and H_2S (Liu et al., 2012) production. The limited evaluation of arginine and cysteine as a postharvest treatment has mostly been with fresh-cut or exposed tissues to inhibit enzymatic browning, for example, arginine with potato (Ali et al., 2016) and cysteine with banana (Dussán et al., 2017). The few published studies on intact produce have been with fruit where arginine inhibited *Botrytis cinerea* growth of tomato (Zhang et al., 2017), and the onset of chilling injury in pomegranate (Babalar et al., 2018). The effect of arginine and cysteine on intact green vegetables has been assessed by Al Ubeed et al. (2019a). They found that treatment with cysteine delayed general senescence, including loss of green colour in pak choy, parsley and peppermint, while Wang et al. (2017) observed delayed senescence, including loss of chlorophyll of asparagus spears with arginine solutions. More recently, Sohail et al. (2021) found that dipping in a solution containing arginine and cysteine reduced postharvest senescence of broccoli florets stored at 10 °C, with inhibition of green colour loss one of the many beneficial changes determined. Methionine was also included in this study, as it is a biosynthetic precursor of the polyamines spermidine and spermine, both of which have been shown to inhibit senescence (Serrano et al., 2016; Sharma et al., 2017). All three amino acids were found to be equally effective at inhibiting senescence.

The aim of the present study was to determine whether the beneficial effect of the three amino acids, L-arginine, L-cysteine or L-methionine, also applied to six other green leafy vegetables. Thus, pak choy, coriander, choy sum, spinach, parsley and rocket leaves were dipped in a solution of amino acid and the loss of green colour along with changes in endogenous ethylene production and respiration rate were observed during storage at 10 °C.

MATERIALS AND METHODS

Produce handling and treatment

Freshly harvested coriander (*Coriandrum sativum*), choy sum (*Brassica chinensis* var. *parachinensis*) plants, pak choy (*Brassica rapa* var *chinensis*), English spinach (*Spinacia oleracea*), flat parsley (*Petroselinum crispum*), and wild rocket (*Diplotaxis tenuifolia*) leaves were collected from a vegetable outlet located near the laboratory at the University of Newcastle Ourimbah campus, Australia. Within one hour of arrival, leaves from each vegetable of uniform size and free of defects were selected and randomly distributed into the required number of treatment units, each comprising approximately 150 g of leaves.

A treatment unit was dipped for 5 min in a solution of deionized water containing a specified concentration of L-arginine, L-cysteine or L-methionine (Sigma Aldrich, Australia) and a wetting agent Tween-20 at 0.5 g L^{-1}. A control unit was dipped in deionised water with Tween 20. For each batch of produce, each treatment was applied to three replicate units.

The dipped leaves were air dried for two hours using a pedestal fan at 20 °C and treatment units were packed into separate 4 L plastic containers, the lids of which were fitted with an inlet and outlet port to allow ventilation of the contents with humidified air (about 99% RH) containing 0.1 µL L^{-1} ethylene at 90 mL hr^{-1}. The concentration of ethylene was achieved by mixing ethylene from a cylinder (1000 µL L^{-1}) (Coregas, Sydney) with compressed air. The gas mixture was humidified by bubbling through water in a tall (height 225 cm) 2 L glass jar. The ethylene concentration was added to simulate an environment around the produce that is commonly found in commercial storage and transport operations (Wills et al., 2000) and humidified to minimise water loss from the produce. All containers were stored at 10 °C. The ethylene concentration in the gas streams was monitored regularly as described below.

Postharvest quality assessment

Green life

The green colour of each leaf in a treatment unit was visually assessed daily by a single observer using a scoring scale from 1 to 5 where: 1= full green colour, 2 = 20% loss of green colour, 3 = 40% loss of green colour, 4 = 60% loss of green colour, 5 = >70% loss of green colour (Al Ubeed et al., 2019a). To avoid any systematic bias by the observer, each unit was pre-coded by an independent person and decoded after all units had been assessed for colour. The time for the leaves in a unit to reach an average of score 3.0 was taken as the green life for that unit.

Ethylene production and respiration rate

Endogenous ethylene production and respiration rate were determined according to the method described by Al Ubeed et al. (2019a). Ethylene production and respiration (as evolved carbon dioxide) rates were assessed 6 hours after treatment and then after 3, 6 and 9 days using gas chromatography (GC) (Nexis GC-2030 Shimadzu Kyoto). The GC was fitted with a flame ionization detector (FID) and thermal conductivity detector (TCD) for the determination of ethylene production and respiration rate respectively.

Temperature of the FID was 275 °C while for TCD, the temperature was 230 °C. Each container of vegetables was sealed and a gas sample (1 mL) withdrawn from the internal atmosphere in a syringe for analysis. After three hours, another gas sample was withdrawn and analyzed. The difference between the two levels of gas was used to calculate ethylene production as µL $C_2H_4.kg^{-1}.hr^{-1}$ and respiration rate as mL CO_2 kg^{-1} hr^{-1}.

Statistical analyses

The data was statistically analysed by analysis of variance (ANOVA) using Statistical Analysis System - version 9.4 (SAS Institute, Cary, NC, USA). To determine significant difference between treatments, the least significant difference (LSD) at P = 0.05 was calculated from the variance and applied to each set of data.

RESULTS

Effect of amino acid dip concentration on senescence of pak choy

The initial study was to identify the optimum dip concentration of the amino acids to inhibit loss of green colour, ethylene production and respiration rate of pak choy. The first experiment examined the effect of arginine and cysteine dip concentrations of 5, 10, 25 and 100 mM. The data in Figure 1 showed that the green life of pak choy treated with 5, 10 and 25 mM arginine and cysteine was significantly greater than the control while 100 mM arginine was not significantly different to control and 100 mM cysteine had a shorter green life than control. The green life of pak choy dipped in 5 mM arginine and cysteine was significantly greater than leaves dipped in 10 and 25 mM solutions. Figure 1 also showed that endogenous ethylene production was significantly reduced in pak choy dipped in 5, 10 and 25 mM arginine and cysteine. Leaves dipped in 100 mM arginine and cysteine showed no significant reduction in ethylene production compared to control. Respiration rate was only significantly decreased in pak choy dipped in 5 mM arginine and cysteine.

Since the greatest inhibition of senescence was achieved by the lowest concentration of 5 mM arginine and cysteine, in second experiment pak choy leaves were dipped in lower concentrations of 2 and 3 mM as well as 5 mM arginine and cysteine. The experiment also included methionine at the same concentrations. Data for the effect of the three amino acids on the green life, ethylene production and respiration rate are shown in Figure 2. Relative to control, green life was significantly enhanced in pak choy treated with 3 and 5 mM arginine, 5 mM cysteine and 2, 3 and 5 mM methionine. Ethylene production and respiration rate were significantly reduced by dips of 3 and 5 mM arginine, cysteine and methionine as well as by 2 mM methionine. Comparison within the concentrations of each amino acid showed that a 5 mM dip was significantly more beneficial than dipping in 2 and 3 mM.

It is noted that Sohail et al. (2021) also found that, for broccoli, 5 mM was the optimal amino acid dip concentration for extending green life and decreasing ethylene production and respiration rate. While broccoli is a floret rather than a leafy vegetable, the result suggests that a 5 mM dip could give beneficial results for a range of green produce. This finding differs from Al Ubeed et al. (2019a), who found 10 mM cysteine sprayed onto pak choy, parsley and peppermint leaves was slightly more effective than a 5 mM spray. However, it is considered that dipping would be a more efficient method for uniform delivery of amino acid than spraying, and thus an optimal effect could be achieved with a lower concentration dip.

Effect of a 5 mM dip of amino acids on senescence of leafy vegetables

Five leafy vegetables, coriander, choy sum, spinach, parsley and rocket, were dipped in solutions containing 5 mM of arginine, cysteine or methionine and assessed for green life, ethylene production and respiration rate during storage at 10 °C. Data for pak choy dipped in 5 mM amino acids were extracted from in the previous study and included in Table 1, hence values are presented for six green leafy vegetables.

Considering the six vegetables as a group, the mean values in Table 1 show that green life was significantly increased by the three amino acids. Statistically, cysteine was more effective than methionine, with arginine not significantly different from cysteine and methionine, but the magnitude of the difference in green life between the amino acids was relatively small. From a practical perspective, cysteine, arginine and methionine can be considered equally effective in extending green life. Examining the effect of amino acids on individual vegetables, green life for pak choy, coriander and choy sum was significantly increased over control by dipping in all three amino acids.

Using coriander as an example, Figure 3 illustrates the inhibition of loss of green colour generated by the three amino acids compared to control leaves after nine days of storage. For parsley, cysteine and methionine significantly increased green life, while for spinach and rocket only arginine and cysteine significantly increased green life. Thus, only cysteine increased the green life of all vegetables.

Table 1. Effect of dipping in solutions of arginine, cysteine and methionine on the green life, ethylene production and respiration rate of green leafy vegetables during storage at 10 °C

Treatment	Pak choy	Coriander	Choy sum	Spinach	Parsley	Rocket	Mean
n*	2	4	3	3	3	3	
	Green life (days)						
Control	10.3^{a}	11.1^{a}	18.8^{a}	23.3^{a}	11.4^{a}	9.6^{a}	14.1^{a}
Arginine	13.9^{b}	13.3^{bc}	20.6^{b}	27.5^{c}	11.7^{a}	10.5^{b}	16.3^{bc}
Cysteine	14.3^{b}	13.7^{c}	22.8^{c}	26.2^{b}	13.1^{b}	11.3^{c}	16.9^{c}
Methionine	15.8^{b}	12.5^{b}	20.6^{b}	23.8^{a}	13.7^{b}	10.1^{ab}	16.1^{b}
	Ethylene production (μL kg^{-1} hr^{-1})						
Control	0.32^{a}	0.57^{a}	0.56^{a}	0.34^{a}	0.44^{a}	0.89^{a}	0.52^{a}
Arginine	0.20^{b}	0.37^{b}	0.47^{b}	0.20^{c}	0.35^{b}	0.80^{a}	0.40^{b}
Cysteine	0.20^{b}	0.34^{b}	0.41^{c}	0.22^{bc}	0.38^{b}	0.69^{b}	0.38^{b}
Methionine	0.20^{b}	0.41^{b}	0.46^{b}	0.27^{b}	0.32^{b}	0.86^{a}	0.42^{b}
	Respiration (mL CO_2 kg^{-1} hr^{-1})						
Control	72^{a}	166^{a}	153^{a}	121^{a}	132^{a}	83^{a}	121^{a}
Arginine	38^{b}	133^{b}	133^{bc}	89^{b}	120^{b}	74^{b}	98^{c}
Cysteine	38^{b}	128^{b}	125^{c}	97^{b}	118b	73^{b}	97^{c}
Methionine	38^{b}	145^{b}	138^{b}	116^{a}	113b	85^{a}	106^{b}

* n = number of batches of produce.

Values for green life are the mean of the number of batches × 3 replicates per batch. Values for ethylene production and respiration are the mean of analyses conducted on four different days of storage, hence is 24 for pak choy, 48 for coriander and 36 for the other vegetables. Different superscript letters for each vegetable show a significant difference at P= 0.05.

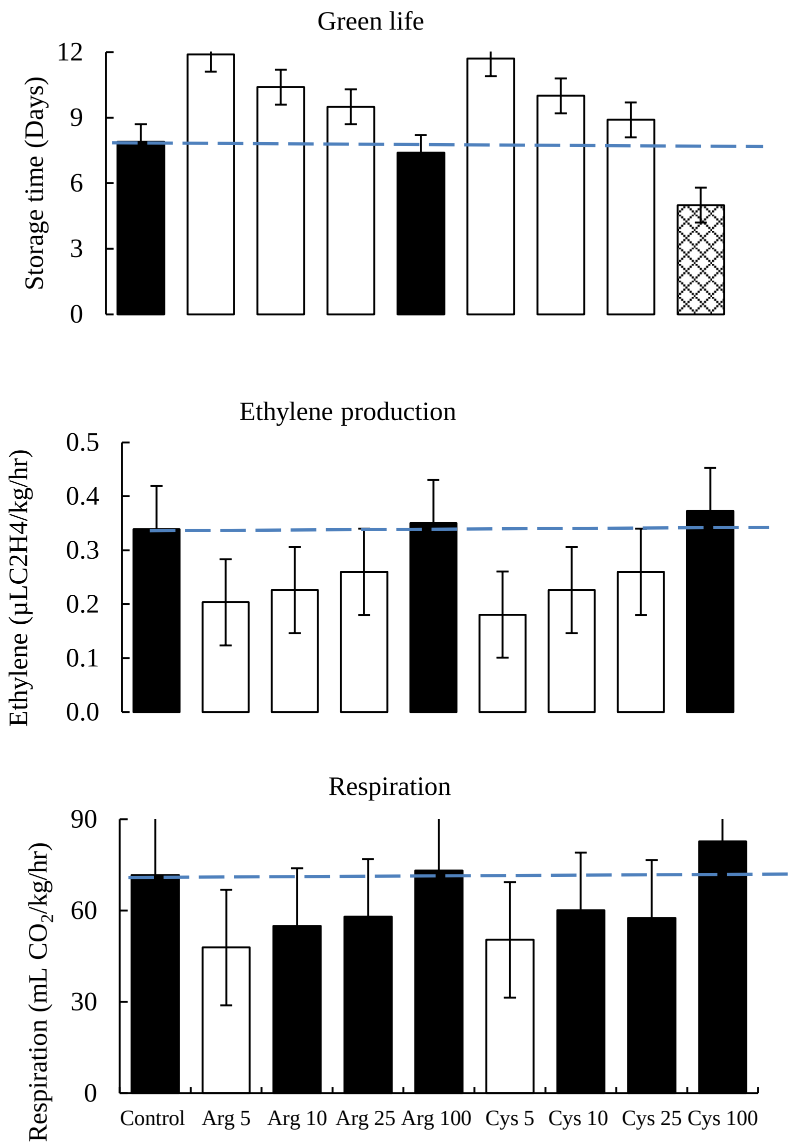

Fig. 1. Green life, ethylene production and respiration rate of pak choy leaves dipped in solutions of 5-100 mM arginine (Arg) and cysteine (Cys) during storage at 10 °C.

Each green life value is the mean of 3 replicate units. Ethylene and respiration values are the mean of 12 analyses (3 replicates × 4 storage times). Green life bars with no fill are significantly greater than control while ethylene and respiration bars with no fill are significantly lower than control. Solid bars are not significantly different to the respective control. For green life, the hatched bar is significantly less than control. Error bars indicate the LSD at P=0.05 which for green life is 0.8, for ethylene is 0.08 and for respiration is 19.4.

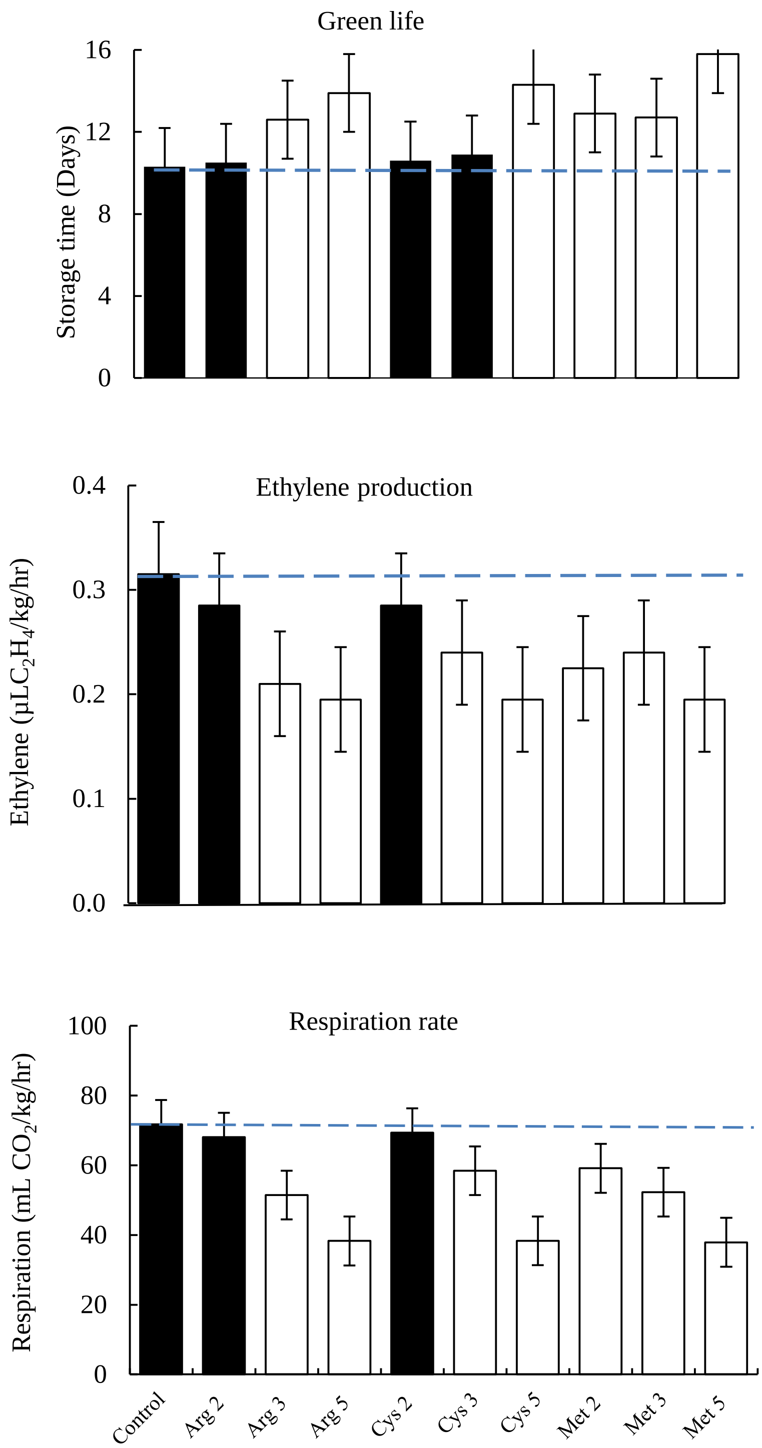

Fig. 2. Green life, ethylene production and respiration rate of pak choy leaves dipped in solutions of 2, 3 and 5 mM arginine (Arg), cysteine (Cys) and methionine (Met) during storage at 10 °C.

Green life values are the mean of 6 units (2 batches x 3 replicates). Ethylene and respiration values are the mean of 24 analyses (2 batches × 3 replicates x 4 storage times). Green life bars with no fill are significantly greater than control while ethylene and respiration bars with no fill are significantly lower than control. Solid bars are not significantly different to control. Error bars indicate the LSD at P=0.05 which for green life is 1.9, for ethylene is 0.05 and for respiration is 7.1.

Fig. 3. Appearance after storage for nine days at 10 °C of coriander that had been dipped in 5 mM of arginine, cysteine or methionine.

Data in Table 1 for ethylene production and respiration rate for the six vegetables are the mean values of analyses taken at four storage times (0.6, 3, 6 and 9 days), as statistical analysis showed that while there was a significant effect of treatment for each vegetable there was no significant interaction between treatment and time in storage. For ethylene production, the combined data for all six vegetables showed that the three amino acids inhibited ethylene production compared to that of control leaves but there was no significant difference between amino acids. For individual vegetables, there was a similar significant uniformity of inhibition by all amino acids for pak choy, coriander and parsley. However, for choy sum and spinach, while all amino acids generated a significantly lower ethylene production, for choy sum cysteine induced a significantly lower ethylene production than arginine and methionine, and for spinach arginine was more inhibitory than methionine. For rocket, the only significant reduction in ethylene production was achieved with cysteine.

The respiration rate showed a mostly similar effect of amino acids as ethylene production (Table 1). Pak choy, coriander, parsley and choy sum showed all three amino acids significantly inhibited respiration to the same extent except for choy sum where cysteine was significantly more inhibitory than methionine. For spinach and rocket, arginine and cysteine showed a similar inhibition of respiration but methionine did not significantly reduce respiration. The mean respiration of the six vegetables was significantly inhibited by all three amino acids, but the lack of effect of methionine on spinach and rocket resulted in methionine being significantly less inhibitory than arginine and cysteine.

DISCUSSION

The findings in this paper that 5 mM arginine, cysteine and methionine dips were effective in inhibiting senescence of pak choy, coriander, choy sum, spinach, parsley and rocket as seen through inhibition of loss of green colour, ethylene production and respiration are consistent with the results obtained by Sohail et al. (2021) for the same amino acids on broccoli florets. They are also consistent with Wang et al. (2017) who observed a longer postharvest life for asparagus treated with arginine. Broccoli and asparagus are not leafy vegetables, but the overall findings support a hypothesis that the amino acids are able to inhibit the loss of chlorophyll in a wide range of green produce. This warrants further study to see if there is commercial potential for dipping in an amino acid to extend market life of leafy vegetables. While, on average, arginine, cysteine and methionine were effective at a similar level of green life extension, cysteine and arginine seemed to be more consistent as they extended the green life of all six vegetables in this study and broccoli (Sohail et al., 2021), whereas methionine did not give a significant extension to the green life of spinach and rocket. The high ratio of surface area to weight in leafy vegetable should allow ready uptake of the amino acids but this may not be so rapid in bulkier produce such as fruits but trials on fruits are warranted even if some method of increasing solution uptake such as vacuum infiltration may be required.

The most likely mode of action of arginine and cysteine is conversion to NO and H_2S, respectively (Morris, 2007; Aroca et al., 2018), and both compounds have been found to inhibit endogenous ethylene production (Al Ubeed et al., 2019b). Sohail et al. (2021) studied the time course changes of a wide range of senescence factors of broccoli exposed to the amino acids and reported that endogenous ethylene production was the earliest factor to be affected. Given the known role of ethylene in promoting general senescence (Wills, 2015), they postulated that reduced internal ethylene could be an important trigger for the longer retention of green colour. This is supported by this study where a reduction in ethylene production occurred soon after application of the amino acids. The associated reduction in respiration rate is an indicator of reduced general metabolism. It could be a direct consequence of the reduction in ethylene production, but it may arise from inhibition of some other as yet unknown step in the respiratory cycle.

CONCLUSION

The ability of arginine and cysteine, and to a lesser extent, methioine, to inhibt loss of green colour in many green vegetables has potential commercial use given the importance of green colour as a purchase signal to consumers. Their primary mode of action is possibly through inhibition of ethylene production.

Conflict of interest

The authors have no conflict of interest to report.

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