

## COMPARISON OF VAPOR-PHASE ACETIC ACID AND VINEGAR EFFECTIVENESS IN MAINTAINING QUALITY OF SWEET BASIL (*Ocimum basilicum* Linn.)

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### ABSTRACT

Leafy shoots of sweet basil (*Ocimum basilicum* Linn.) were exposed to the vapor of either dilute acetic acid (AA) or of upland rice vinegar (URV) – both solutions were diluted to contain 4% of acetic acid – for 10 min and stored at 12°C. The sweet basil exposure to AA had a 16% increase in shelf life and those exposures to URV 35% increase compare to the control. There were no significant differences in fresh weight loss during storage between the AA and URV but both had significantly lower fresh weight losses than the control. The chlorophyll content of both AA and URV were significantly higher than the control. The radical scavenging was significantly higher in the URV than in the AA and the control. The electrolyte leakage was both significantly lower in the URV than in the AA and the control. The occurrence of lipid peroxidation was similar in both the AA and URV, and both were significantly lower than in the control. These results indicate URV has potential as an effective way of raising quality and extending postharvest storage of sweet basil shoots.

**Key words:** postharvest, electrolyte leakage, lipid peroxidation, chlorophyll content, antioxidant activity, sweet basil

### INTRODUCTION

Better understanding of the importance of healthy eating has increased consumer demand for fresh plant products. Accordingly, production volumes of these items have risen and supply chains have lengthened. This has put pressure on the industry infrastructure to adopt more sophisticated storage and transport systems that better maintain product quality through to the end user. Like many green, leafy vegetables, fresh herbs deteriorate rapidly after harvest, imposing major marketing limitations [Cantwell and Reid 2002]. Sweet basil, *Ocimum basilicum* Linn. is a widely-used

culinary herb in which postharvest losses occur due to a range of factors including fungal infection [Wells and Butterfield 1999] and chilling injury [Lang and Cameron 1994]. When stored at room temperature for 4–5 days from harvest, the leaves soon wilt and develop brown spotting in the interveinal areas rendering them unsaleable [Aharoni et al. 2010]. A number of techniques have been trialed with a view to extending the postharvest life of sweet basil. Costa et al. [2013] reported that daily treatment for 2 h with low-intensity light (30–37  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) during storage at 20°C

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is effective in delaying postharvest senescence. Hassan and Mahfouz [2010] found that treatment with 1-methylcyclopropane retarded chlorophyll and protein degradation, it also reduced weight loss and ethylene production.

Acetic acid is commonly used by food manufacturers for its antimicrobial properties and its safety. It has also been used as a hot vapor to slow postharvest decay in d'Anjou pear [Sholberg et al. 2004], strawberry [Hassenberg et al. 2010] and table grape [Venditti et al. 2017]. Vinegar is produced by the fermentation of sugar or starch in fruit, vegetable and seeds including rice. Vinegar consists mainly of acetic acid and water but it contains hundreds of secondary compounds, which contribute to its smell and taste. The secondary compounds present in vinegars vary significantly with the plant material from which they are derived, with the fermentation process and with the vinegar's age [Zhang et al. 2006]. Postharvest applications of vinegar as a fumigant have been shown to reduce decay in stone fruit, strawberry and apple [Sholberg et al. 2000], in strawberry [Krusong et al. 2015a] and in coriander leaf [Krusong et al. 2015b].

As above mentioned, many authors have reported on the effectiveness of acetic acid or vinegar vapor generated by heat on preservation of horticultural commodities. However, these can risk causing heat injury. Therefore, where acetic acid or vinegar is allowed to vaporize at room temperature would eliminate the risk of causing heat damage. In a previous report [Changsawake et al. 2017], we determined the optimum acetic acid concentration in upland rice vinegar (URV), and duration of exposure, in extending the shelf life of sweet basil. The results demonstrated that treatment with ambient temperature vapor of URV diluted to 4% acetic acid and exposure time of 10 min helped maintain greenness and freshness of sweet basil and thus markedly extended its storage life. We also showed that besides acetic acid, the ambient URV vapor also contained ethyl acetate, propane, pentanal, hexanol and butan-1-ol. Therefore this system was used to test ambient temperature vapor fumigation on sweet basil to compare the effects of acetic acid (only containing acetic acid vapor) and URV (containing acetic acid vapor and other volatile compounds) on its shelf life and biochemical changes taking place during subsequent storage at 12°C.

## MATERIALS AND METHODS

**Materials.** Leafy shoots of sweet basil were purchased from local commercial producers in Pathumthanee Province, Thailand. Only top-quality leafy shoots were used in this work, with leaves showing no signs of visible damage, yellowing, decay or bruising.

The acetic acid (AA) used was purchased from Merck KGaA (Darmstadt, Germany). Upland rice vinegar (URV) was produced at the Laboratory of Fermentation Technology, Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang and the percentage of acetic acid was determined by titration against NaOH to a pH 8.30 endpoint with a Brinkman titroprocessor ensemble (Metrohm, Herisau, Switzerland). It had the concentration of  $8 \pm 0.1\%$  (v/v) acetic acid so both the AA and the URV were diluted with distilled water to  $4 \pm 0.1\%$  acetic acid.

**AA or URV vapor treatment.** A plastic box ( $0.25 \times 0.30 \times 0.25$  m) with a sliding cover to prevent pressure build up during vapor treatment was used for exposure of the sweet basil to AA, URV or water vapor. To generate the vapor phase, ambient clean air at temperature of  $30 \pm 2.00^\circ\text{C}$  was bubbled at  $4 \text{ L min}^{-1}$  through a one Liter closed bottle containing 0.5 L of water, AA or URV. The vapor from the headspace of the bottle was delivered at the same rate ( $4 \text{ L min}^{-1}$ ) to the vapor exposure box via a spreader manifold as described by Krusong et al. [2015a]. Basil was exposed at temperature of  $30 \pm 2.0^\circ\text{C}$  for 10 min to the control or to one of two treatments: (1) distilled water vapor (control); (2) AA vapor or (3) URV vapor. Water vapor was supplied simultaneously at same rate as the treatment vapor to maintain the relative humidity at approximately  $80 \pm 2\%$  measured by a thermo-hygrometer (Model TH-302, Diichi, Japan). After exposure to the vapor for 10 min, the sweet basil was packed in polyethylene bags with eight holes (diameter 0.6 cm). All experiments consisted of four replicate plastic bags containing three leafy shoots for each treatment and each sampling time. All packed samples were stored in darkness at  $12^\circ\text{C}$  and relative humidity at  $80 \pm 2\%$  until the shelf-life assessment was made.

**Shelf life evaluation.** The shelf-life quality of the leaves was evaluated based on visible changes in the freshness and the presence of darkened, pitted lesions

on the leaves. A 5-step discontinuous quality scale was used visually, where 5 = no dark spots, 4 = several dark spots, 3 = black stains on 30% of the leaf surface, 2 = black stains on 30–50% of the leaf surface and 1 = black stains on more than 50% of the leaf surface [Meir et al. 1997]. Replicate scores were averaged to give a semi-continuous qualitative scale ranging from 1 to 5. Leaves were considered to have reached the limit of salability with a score of three.

**Fresh weight loss.** The leafy shoots were weighed using a Scout Pro balance (OHAUS Instruments, China) and the percentage weight loss was calculated as  $(A-B)/A \times 100$  where: A = weight before vapor treatment and B = weight after vapor treatment.

**Solute leakage.** Ten circular 60-mm-diameter leaf discs (4 replicates) were cut with a cork borer and weighed. Leaf discs were then floated on 10 mL of de-ionized water at room temperature (about 30°C) for 3 h [Benja-Tal and Borochoy 1994]. The electrical conductivity ( $S\ m^{-1}\ kg^{-1}$ ) of the solutions was determined using a conductivity meter (Consort, C830, Belgium).

**Lipid peroxidation.** The method of Heath and Packer [1968] was used to measure the malondialdehyde (MDA), a secondary product of lipid peroxidation. Results were expressed as  $\mu mol\ kg^{-1}$  on a fresh weight basis.

**Chlorophyll a content.** Fifteen circular 60-mm-diameter leaf discs (four replicates) were cut with a cork borer, carefully avoiding the main vein. These leaf discs were extracted in aqueous acetone (80%). The extracts were filtered through Whatman No. 1 filter paper. The absorbance of the acetone extracts was

measured at 647 and 663 nm using a Spectronic™ GENESYS 20 spectrophotometer (Thermo Electron Corporation, USA). The content of chlorophyll a, was calculated using Lichtenthaler's equation [Lichtenthaler 1987] and expressed as  $mg\ m^{-2}$ .

**DPPH radical-scavenging activity.** Radical-scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according to the procedure reported by Miliauskas et al. [2004]. A sample of 1 g of fresh leaves (4 replicates) was extracted with 95% ethanol for 72 h. Different dilutions of the ethanol extract (2 mL, triplicates) were added to 2 mL of DPPH (5.9 mg in 100 mL ethanol). Absorbance was measured at 517 nm after 30 min. Results were expressed as  $IC_{50}$  which is the concentration of ethanol extract which inhibited the DPPH radicals by 50%.

**Statistical analyses.** The experiment consisted of 3 treatments by 4 replicates in a completely randomized design. Analysis of variance was calculated for all the data and comparisons was carried out between treatments at the probability level of  $p \leq 0.05$  using Tukey's Studentized Range Test. The experiment was repeated two times with similar results and data presented in this paper were carried out for one time.

## RESULTS

**Shelf life.** The acetic acid contents of the vapor-phase of AA and URV were calculated based on the weight loss rates of the AA and URV solutions at the flow rate of  $4\ L\ min^{-1}$  during the 10 min exposure period (Tab. 1). Exposure to the acetic acid retained

**Table 1.** Postharvest shelf life of basil shoots pretreated with the vapor of dilute acetic acid (AA) or with that of upland rice vinegar (URV) for 10 min and then stored at 12°C

Treatment	Acetic acid content in vapor phase (g weight loss)	Acetic acid concentration in vapor phase** ( $g\ L^{-1}$ )	Shelf life*** (days)
Distilled water vapor	0	0	19.6 ± 1.67 c
AA* vapor	0.0124 ± 0.001	$6.6 \times 10^{-4} \pm 0.00005$	22.8 ± 0.84 b
URV* vapor	0.0128 ± 0.001	$6.8 \times 10^{-4} \pm 0.00005$	26.4 ± 1.14 a

\* The AA and URV were each diluted to contain  $4 \pm 0.1\%$  (v/v) acetic acid

\*\* The acetic acid concentrations of the vapor-phases AA and URV were calculated based on the weight loss of AA and URV solution, respectively, in the 18.75 L volume of the vapor exposure box. AA vapor =  $0.0124\ g \div 18.75\ L$ ; URV vapor =  $0.0128 \div 18.75\ L$

\*\*\* Mean ± one standard deviation. Means with different letters within a column were significantly different ( $P \leq 0.05$ ) based on Tukey's test

quality significantly ( $p \leq 0.05$ ) better throughout the storage period than the control (Fig. 1) and those that had been fumigated with URV retained their quality consistently better than AA, but this effect was not significant ( $p \leq 0.05$ ). The limit of salability (score 3) was reached after 19.6 days for the control and both the AA and URV extended shelf life significantly ( $p \leq 0.05$ ) with AA extending by 16% increase over the control and URV vapor extending by 35% increase over the control (Tab. 1).

**Fresh weight loss.** The weight loss of the sweet basil increased with the storage time in both control and treated samples, but was greater in control leaf. After 12 days storage, no differences in weight loss were observed between control and treated samples and from day 13 the losses were significantly ( $p \leq 0.05$ ) higher in the controls than in either AA or URV vapor. There were no differences between AA and URV vapor, both behaved similarly in that they delayed weight loss and slightly increased weight loss when storage time increased. By day 25 losses were 21.09% (controls), 9.49% (AA vapor) and 8.76% (URV vapor) – Figure 2.

**Electrolyte leakage and lipid peroxidation.** The electrical conductivity of leaf disc leachate is indicative of electrolyte leakage rate from the tissues. In AA and URV treatments, the leakage rate increased with the storage time from a low value on day 0 (conductivity  $78.42 \text{ S m}^{-1} \text{ kg}^{-1}$ ) – Figure 3 – after which the leakage rate accelerated markedly. By day 5, there were no significant differences electrical conductivity during storage between the AA and URV but both had significantly lower electrical conductivity than the control. After 10–25 days of storage, the electrolyte leakage was significantly lower in the URV than in the AA and the control. By day 25, conductivities of the leachates were  $215.91 \text{ S m}^{-1} \text{ kg}^{-1}$  for the control,  $209.01 \text{ S m}^{-1} \text{ kg}^{-1}$  for the AA and  $201.40 \text{ S m}^{-1} \text{ kg}^{-1}$  for URV.

The occurrence of lipid peroxidation by measurement of MDA content exhibited a similar pattern of increase MDA content in control and treated samples during storage. After storage for 5 days, no differences in MDA content were observed between control and treated samples. From day 10 to day 15 the content were significantly ( $p \leq 0.05$ ) higher in the controls than in URV vapor, but control and AA did not differ significantly ( $p \leq 0.05$ ). Thereafter, either AA or URV vapor treatments were significantly lower than in the

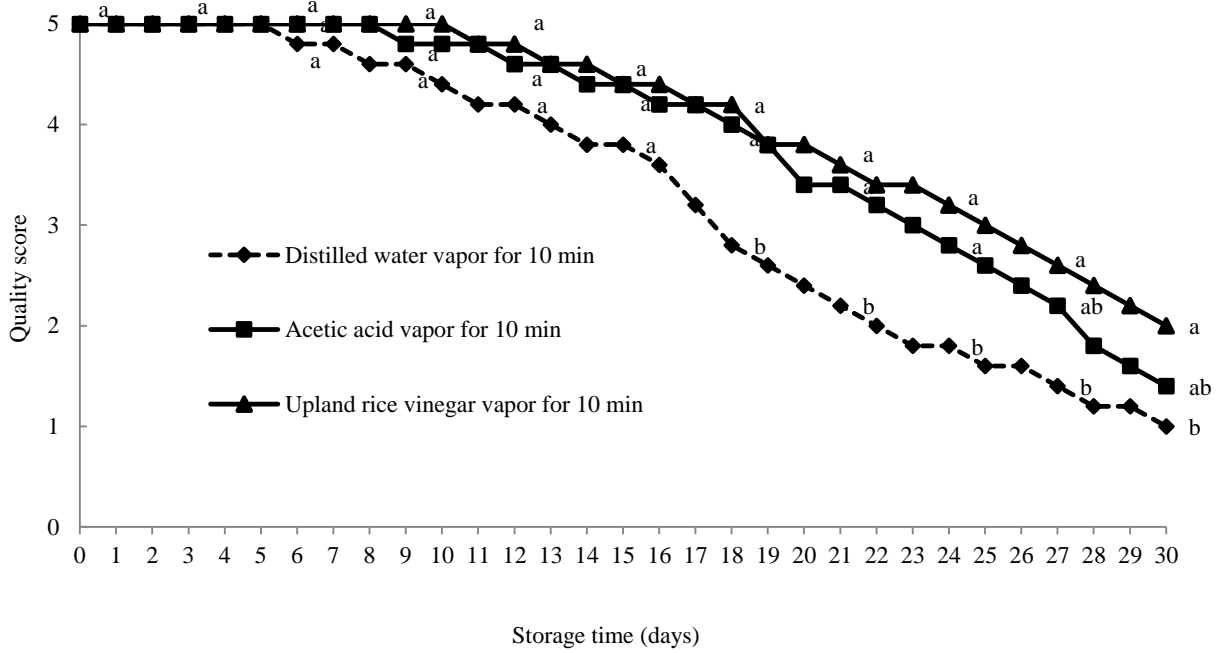
control after storage of 20 and 25 days. Lipid peroxidation increased with storage time from an initial value of  $3.39 \mu\text{mol kg}^{-1}$ . After 25 days storage the MDA content was  $6.10 \mu\text{mol kg}^{-1}$  (control),  $5.44 \mu\text{mol kg}^{-1}$  (AA) and  $5.32 \mu\text{mol kg}^{-1}$  (URV) – Figure 4.

**Chlorophyll a content.** The chlorophyll a content decreased during storage in all three treatments. After 15 days storage, no differences in chlorophyll a content were observed between control and treated samples and from day 20 to day 25, chlorophyll a was significantly ( $p \leq 0.05$ ) higher in the AA and URV vapor treatments than in the control. By day 25, chlorophyll a in the control had declined rapidly from  $159.32 \text{ mg m}^{-2}$  to  $125.22 \text{ mg m}^{-2}$  (–21.40%) while that of AA and URV decreased only slightly and simultaneously to  $135.25 \text{ mg m}^{-2}$  (–15.11%) and  $132.41 \text{ mg m}^{-2}$  (–16.89%), respectively (Fig. 5).

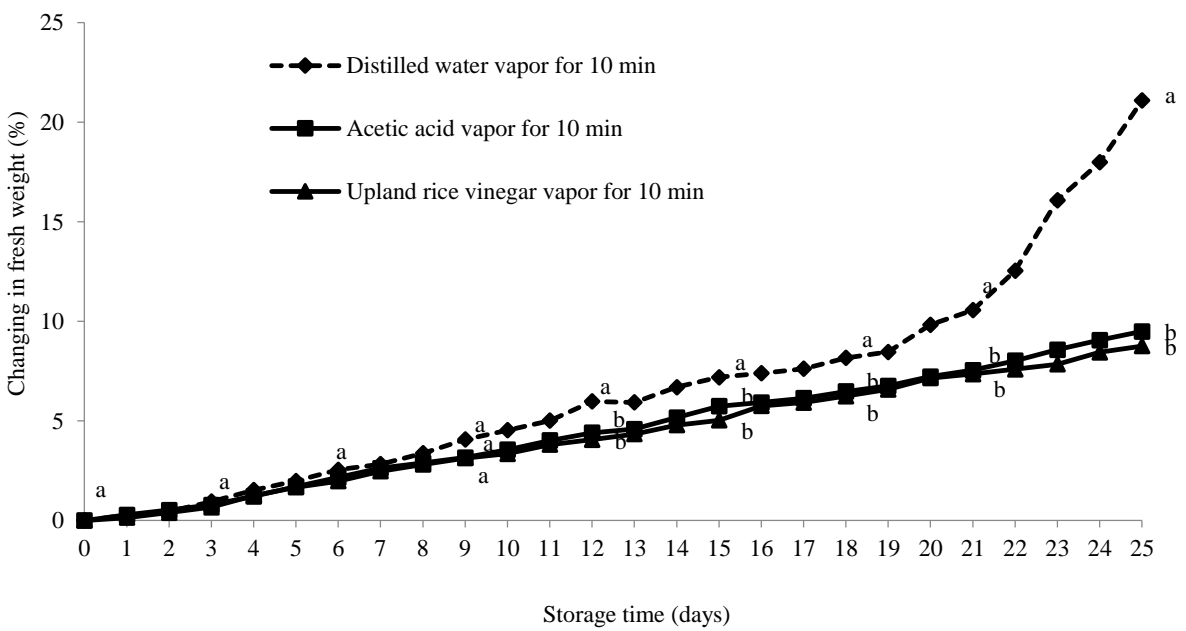
**Radical-scavenging activity.** Low values of radical scavenging activity  $\text{IC}_{50}$  (the extract concentration giving 50% inhibition) indicate high levels of antioxidant activity. Similar patterns of radical-scavenging were found in all three treatments (Fig. 6) showing a general decrease in radical-scavenging activity with time (rising  $\text{IC}_{50}$  values). At day 0 samples showed high antioxidant activity (low  $\text{IC}_{50}$ ,  $1094.45 \text{ mg L}^{-1}$ ) and by day 10, the values of the control were significantly ( $p \leq 0.05$ ) higher than those for AA or URV. Also, values for URV were consistently and significantly ( $p \leq 0.05$ ) higher than those for AA from day 10 to day 25, which indicates URV vapor is slightly more effective than AA vapor in maintaining radical scavenging activity (Fig. 6).

## DISCUSSION

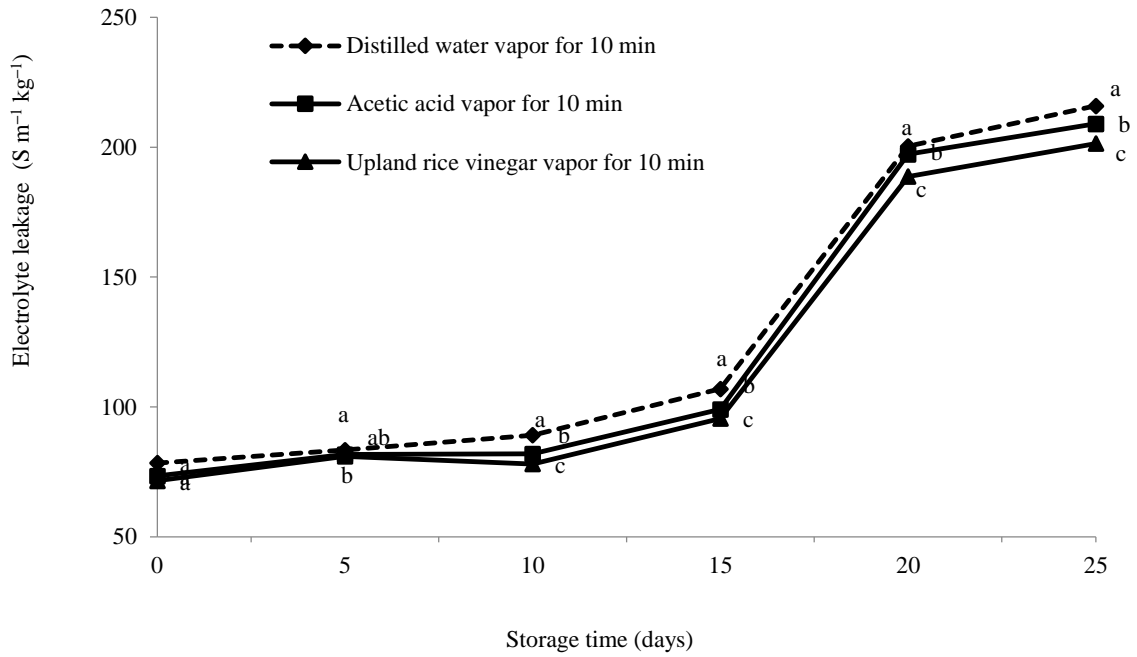
As seen in Figure 1 and Table 1, the shelf life of sweet basil leafy shoots packed in polyethylene bags and stored in darkness at  $12^\circ\text{C}$  was significantly increased by pretreatment with either AA or URV vapor for 10 min. Reduced fresh weight loss following AA or URV vapor pretreatment could be reduced water loss (Fig. 2) and hence represents a valuable gain in shelf life for sweet basil. Weight loss is a predominantly through loss of water through the stomata and the cuticle of leaves and is affected by the environment. The mechanism of AA or URV vapor, at appropriate concentration (no toxic effect on samples) may inhibit



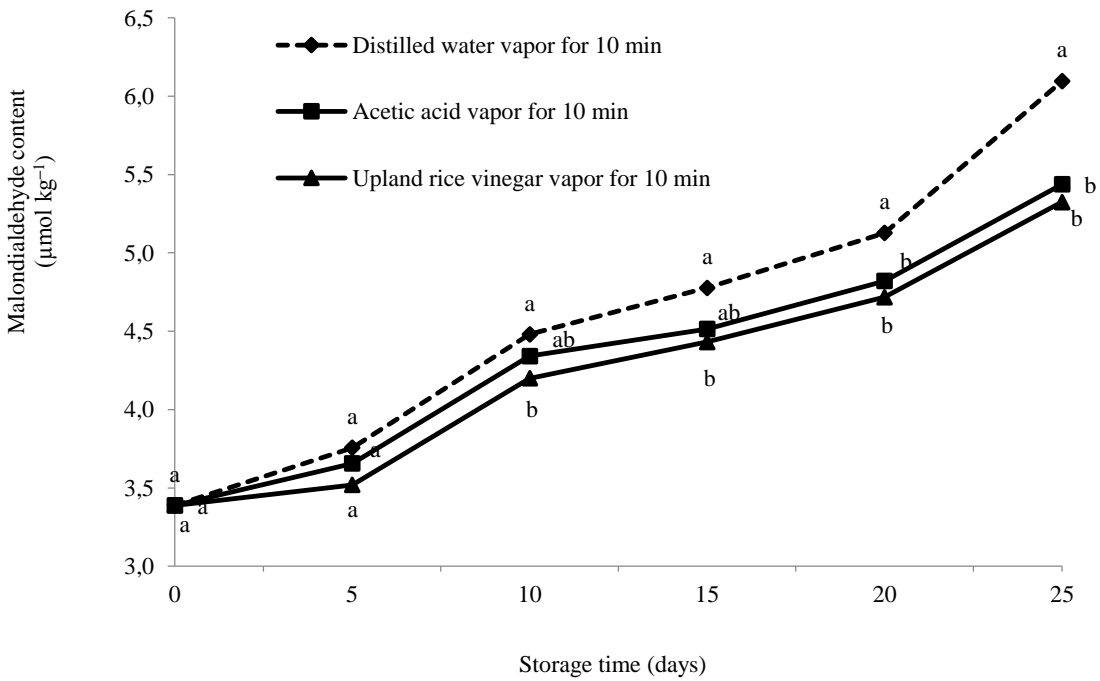
**Fig. 1.** Quality score of basil shoots treated with dilute acetic acid or upland rice vinegar vapor for 10 min and stored at 12°C for 30 days. Quality scale (the 5-step discontinuous quality scale was): score 5 – no damage, score 4 – several dark spots, score 3 – black stains on 30% of the leaf surface, score 2 – black stains on 30–50% of the leaf surface, and score 1 – black stains on more than 50% of the leaf surface. Product scoring <3 is deemed unsaleable



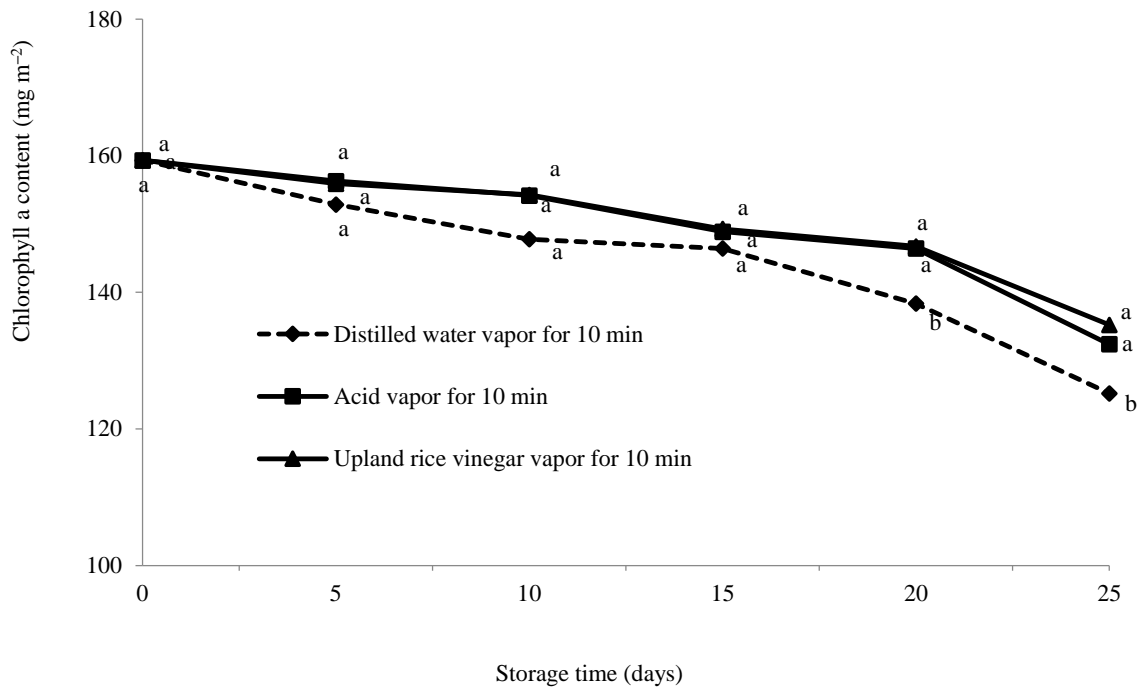
**Fig. 2.** Fresh weight loss of basil shoots treated with dilute acetic acid or upland rice vinegar vapor for 10 min and stored at 12°C for 25 days



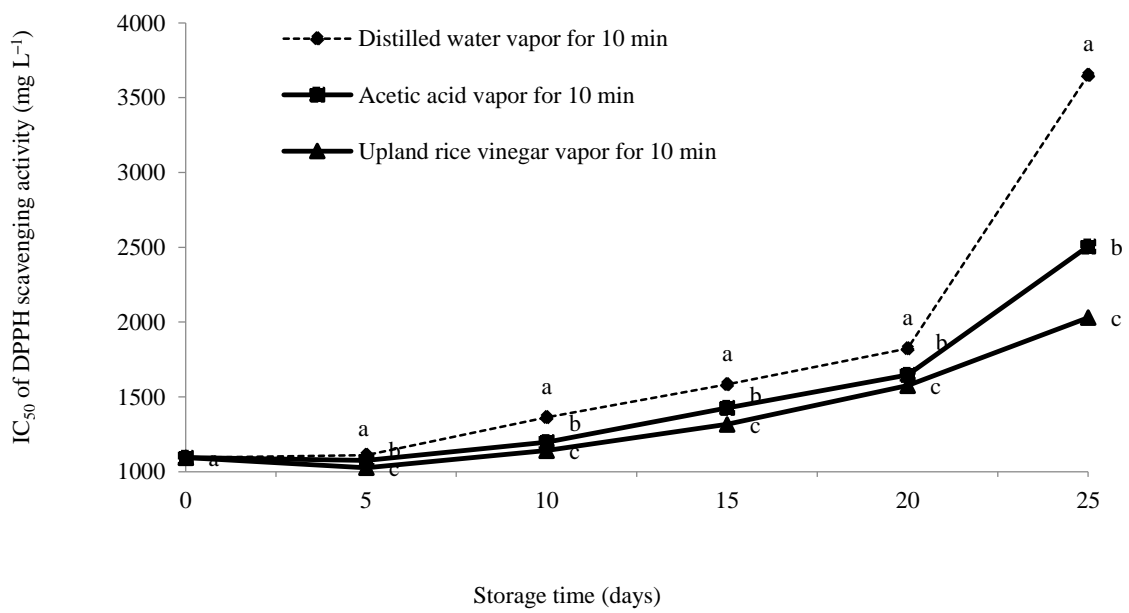
**Fig. 3.** Electrical conductivity of leaching solutions from sweet basil leaf discs treated with dilute acetic acid or upland rice vinegar vapor for 10 min and stored at 12°C for up to 25 days. Electrical conductivity is directly proportional to electrolyte leakage



**Fig. 4.** Malondialdehyde content of basil shoots treated with dilute acetic acid or upland rice vinegar vapor for 10 min and stored at 12°C for 25 days



**Fig. 5.** Chlorophyll a content of basil shoots treated with dilute acetic acid or upland rice vinegar vapor for 10 min and stored at 12°C for 25 days



**Fig. 6.** The IC<sub>50</sub> values of DPPH scavenging activity of sweet basil treated with dilute acetic acid or upland rice vinegar vapor for 10 min and stored at 12°C for 25 days

stomata opening. Opening was observed in relatively high concentration of acetic acid [Scarath 1932]. Acetic acid (AA and URV) declined leaf weight loss by inhibiting the stomata opening. Thus, good character and commercial value of postharvest sweet basil were effectively maintained.

Malondialdehyde, known as a toxic aldehyde, is one of the final products of membrane lipid peroxidation which reflects the injury degree of cell membrane system [Li et al. 2008]. The increasing propensity for solute loss in sweet basil during storage is presumably due to declining membrane integrity since postharvest increases in membrane leakage has previously been shown in Japanese radish [Dan et al. 1996], and spinach leaves [Glowacz et al. 2013]. Membrane deterioration can also affect the functioning of membrane-associated enzymes and lead to metabolic imbalances in the cells, manifested at the tissue level as visible symptoms of the disorder. The increase in MDA has been shown to be produced as a result of membrane damage in senescent broccoli [Zhuang et al. 1995] and cabbage [Cheour et al. 1992]. Here, basil shoots treated with AA or URV vapor showed delayed rises in MDA (Fig. 4) and also in membrane leakage (Fig. 3), indicating that the slowing of senescence was likely through the maintenance of membrane integrity and thus preserving the environment required by membrane-bound enzymes. Reduced lipid peroxidation and retained membrane stability have been demonstrated to be inversely proportional with the senescence process [Dan et al. 1996, Hassan et al. 2014].

Postharvest loss of chlorophyll in leaves has been shown to be mitigated by fumigation with 1-methylcyclopropene in sweet basil [Hassan and Mahfouz 2010], which is related to 1-MCP blocking the biosynthesis of ethylene that hastens chlorophyll degradation. Chlorophyll degradation is facilitated by enzymes and can be reduced with treatments other than blocking ethylene biosynthesis including chelators of copper and iron [Lüthy et al. 1984] and cyanide which has been shown to inhibit chlorophyll oxidase activity *in vivo* [Blackbourn et al. 1990]. From our results it appears that the mode of action of acetic acid could also be related to enzyme inhibition on the chlorophyll molecules in the chloroplasts of sweet basil. The improvement of chlorophyll retention after fumigation with acetic acid

(AA and URV) is an important positive benefit for the maintenance of quality of leafy vegetables.

Senescence is initiated by free radicals which cause protein degradation, lipid peroxidation and oxidative DNA damage [Arora et al. 2002]. Radical-scavenging activity is an important parameter for evaluating antioxidant activity via a non-enzymatic reaction [Duan et al. 2011]. Compared with the control, higher DPPH scavenging ability was observed with URV (Fig. 6). In confirmation of our findings, higher antioxidant capacities have also been reported in fresh broccoli florets treated after UV-C radiation and heat treatment [Lemoine et al. 2010] and also in 24-epibrassinolide treated lime fruit by vacuum infiltration [Tavallali 2018]. These all suggest the involvement of free radicals in senescence. Minimization of antioxidant loss is important not only to protect the tissues from free radicals, and thus delay senescence, but also to preserve the dietary antioxidant components that maintain the healthful properties of the product as a human food. A balance between free radicals and antioxidants is necessary for proper physiological functions and for blocking harmful events.

Interestingly, shelf life, membrane integrity and DPPH scavenging activity were all significantly and positively affected by URV vapor treatment more than by AA vapor treatment. These differences between URV and AA would seem to depend on the minor volatile components of URV i.e. other than just its acetic acid content. Changsawake et al. [2017] previously confirmed that URV vapor contained ethyl acetate, propane, pentanal as well as acetic acid. It seems probable therefore that acetic acid is the primary agent but other chemicals present in the URV contributed positively to its effects as a postharvest treatment. It is also possible to speculate that some of these chemicals may work synergistically to create the beneficial effects observed here. Another advantage of URV over similar vinegar products and over dilute acetic acid is its high vapor phase bioactivity, which makes it especially useful as a postharvest fumigant.

## CONCLUSIONS

Postharvest fumigation with AA or URV prior to storage had no negative effects on any of the quality



attributes of sweet basil. Their effects were either neutral or positive, with both treatments improving shelf life, membrane integrity and DPPH scavenging activity compared to the control. Moreover, URV improved some aspects of storage (radical scavenging ability and electrolyte leakage) compared to AA.

## ACKNOWLEDGEMENTS

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