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ORIGINAL PAPER

1 *H*-cyclopropa[*b*]naphthalene: a novel ethylene antagonist for extending storage life and maintaining quality of climacteric and suppressed climacteric plums

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Abstract
Ethylene is a key trigger that governs the rate of fruit ripening, storability, and postharvest quality of fresh fruit. Efficient inhibition of ethylene action is essential to slow down the postharvest ripening processes, extend storage life and maintain optimum fruit quality during storage. Ethylene antagonist compounds with the likelihood of application as liquid formulation would facilitate managing ethylene broadly as both preharvest and/or postharvest treatments. This study examined the effects of different aqueous formulations of 1 *H*-cyclopropa[*b*]naphthalene (NC) as an ethylene antagonist in the cold stored (1 °C) Japanese plums 'Angeleno' (suppressed-climacteric) and 'Tegan Blue', which exhibit climacteric peaks, respectively. NC was applied as a 2 µM spray solution prepared using only distilled water (NCA), 5% ethanol (NCE), 0.02% Tween® 20 (NCT), 5% β-cyclodextrin (NCD) or a 1 µM fumigant for 18 h at ambient conditions (20 ± 1 °C). Regardless of formulation, NC retarded ethylene production in both the suppressed-climacteric and climactic cultivars. The capacity of NC to suppress ethylene production was relatively higher in 'Angeleno' than in 'Tegan Blue'. Levels of bioactive compounds such as total phenols, total anthocyanins and total antioxidant capacity in fruit treated with NC were at par with the control. NC fumigation was the most effective treatment in suppressing ethylene production and maintaining fruit quality followed by NCE, NCT, NCD and NCA in both plum cultivars. Amongst the NC spray solutions, the ones with ethanol or Tween® 20 as adjuvants outperformed other solutions. Ethylene production positively correlated with weight loss, SSC, SSC: TA and concentrations of individual sugars, but was negatively correlated with firmness, titratable acidity (TA) and individual organic acids in both cultivars. The results also support the notion that ethylene has a role in the synthesis of phenolic compounds and anthocyanin depending on the sugar substrates present in the phenylpropanoid and flavonoid pathways.

Keywords Ethylene antagonist · Fumigation · Aqueous solutions · Fruit quality · Bioactive compounds · Fruit ripening

Introduction

Ethylene is a natural gaseous hormone and is primarily responsible for the postharvest quality deterioration and shortening of fresh fruit storage life (Burg and Burg 1962; Hu et al. 2019). During the fruit ripening process, in the case of the climacteric fruit, a steep rise of ethylene production called a 'climacteric peak' occurs, which usually appears along with an upsurge in respiration (Yang 1985). Climacteric ethylene production is linked with the acceleration of the ripening associated changes and senescence processes, which include fruit softening, conversion of starch to sugars, accumulation of sugars and organic acid synthesis and the development of flavour and aroma compounds (Burg

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and Burg 1962; Singh and Khan 2010; Hu et al. 2019). The undesirable consequences of ethylene in the fruit can be avoided by inhibiting ethylene biosynthesis in the fruit (e.g. using aminoethoxyvinylglycine (AVG), blocking ethylene action at the receptor level (e.g. using the 1-MCP), eliminating the external ethylene existing in the storage environment (e.g. using ozone or potassium permanganate ($KMnO_4$) or by gene editing for low ethylene production or sensitivity (e.g. down-regulation of ACC synthase (ACS) and ACC oxidase (ACO) (Hu et al. 2019; Tokala et al. 2021).

Plums are primarily categorized as climacteric fruit, though there are also diverse cultivars of non-climacteric and suppressed-climacteric plums (Singh and Khan 2010; Minas et al. 2015). Except for non-climacteric plum cultivars, other climacteric cultivars such as 'Jonna Red', as well as suppressed-climacteric cultivars such as 'Angeleno', exhibit accelerated ripening and associated changes with a significant climacteric rise in ethylene production (Minas et al. 2015). 'Angeleno', a suppressed-climacteric Japanese plum, is a transport- and storage-friendly cultivar due to its high flesh firmness. 'Tegan Blue', a climacteric Japanese plum, has a relatively large fruit size compared to other plum cultivars and possesses a dark purple-red skin which attracts consumers (Kyaw et al. 2021). Both climacteric and suppressed-climacteric plums are highly sensitive to ethylene resulting in a reduction of the storage life and marketability of the fruit, even at concentrations as low as 0.01 to 0.1 µL C_2H_4 (Khan et al. 2018).

The application of 1-methylcyclopropene (1-MCP), an ethylene antagonist, hinders ethylene action by irreversibly blocking the ethylene receptors in the endoplasmic reticulum (Sisler 2006). 1-MCP is one of the most effective postharvest management tools for delaying ripening processes and conserving the quality of plum fruit (Khan et al. 2018). Although 1-MCP is considered to be most efficient as a fumigant (Sisler 2006), a wide range of products with diverse delivery methods as such sachets, tablets, powder and even as a liquid is currently available in the market under different trade names like Harvista™ as a preharvest spray, Smartfresh™ as postharvest fumigation and Logfresh® as postharvest powder. However, the application of liquid 1-MCP is still limited to only certain fruit such as plums, apples, pears, cherries and berries (AgroFresh 2021). Lippert et al. (2004) made a formulation of 1-MCP for use as a pre-harvest spray on plums to facilitate mechanical harvesting and prolong storage and shelf-life. The development of new ethylene antagonist compounds with the likelihood of application as a liquid formulation would facilitate managing ethylene in both preharvest and/or postharvest situations (Seglie et al. 2010; Escribano et al. 2017). The ethylene antagonist, 1 *H*-cyclopropa[*b*]naphthalene (NC), has been identified by Singh et al. (2018). NC is a derivative of benzocyclopropene where a benzene ring is attached to a cyclopropene (Halton 1973). Having cyclopropene as a functional group, NC blocks the ethylene receptors through a mechanism similar to that of 1-MCP (Tokala et al. 2020). In contrast to 1-MCP, which is a volatile compound at room temperature (Sisler 2006), NC is a solid compound at room temperature and is partially water soluble with a high potential for liquid formulations (Halton 1973; Singh et al. 2018; Tokala et al. 2020). NC has been reported to be non-toxic for human consumption based on the initial toxicity studies, especially to VERO and KB cancer cell lines (> 100 µM) (Tokala 2019).

In formulating liquid agrochemicals, adjuvants play an important role as preconditioning compounds by enhancing the performance, solubility and permeability of active ingredients (Somervaille et al. 2012). Co-solvents such as ethanol (Otero-Diaz et al. 2017), non-ionic surfactants such as Tween® 20 (Singh et al. 2000) as well as cyclodextrins (Del Valle 2004) are documented as some of the effective adjuvants for the preparation of aqueous treatments. Ethanol also acts as a solubility promoter for hydrophobic organic compounds in polar solvents like water (Otero-Diaz et al. 2017) and as a penetration enhancer into the target sites of plants (Costa et al. 2012). Tween® 20 possesses both hydrophilic and lipophilic properties because of hydroxyl groups and hydrocarbon chains attached to its molecular structure (Kerwin 2008). This nature of Tween® 20 enhances the uptake of active ingredients by the fruit (Singh et al. 2000). Similarly, the cyclic molecular structure of cyclodextrins enables them to encapsulate guest molecules and regulate the slow release of the molecule while ensuring higher stability as well as water solubility (Del Valle 2004).

Previously, the effectiveness of NC as a fumigant to antagonize ethylene perception and hinder ethylene-dependent postharvest physiological changes has been reported in apple and pear fruit (Tokala et al. 2020, 2021, 2022). However, the antagonistic efficacy of liquid formulations of NC is yet to be studied. In addition, liquid formulations of 1 *H*-cyclopropabenzene (BC), which is the parent compound of NC (Halton 1973), suppressed ethylene production by plums by antagonizing ethylene perception (Kyaw et al. 2021). Being a derivative of BC, liquid formulations of NC should block ethylene perception to the same degree as, or more affirmatively than, its parent compound. Our preliminary research findings suggest that NC fumigation suppresses climacteric ethylene production and maintains the fruit quality of different plum cultivars (Kyaw et al. 2019). To the best of our knowledge, there is no detailed research work published on the effects of NC formulations on the ethylene production and fruit quality of 'Tegan Blue' and 'Angeleno' plums. A successful liquid NC formulation would facilitate pre-harvest application onto the tree as well

as a postharvest fruit dip or spray along the packing line. It was hypothesized that the NC aqueous solutions would be as effective as the fumigation treatment in blocking the receptors perceiving ethylene and in preserving the fruit quality attributes of plums. It is also hypothesized that the presence of adjuvants would improve the performance of NC aqueous formulations. The present study aims to investigate the effectiveness of different NC aqueous formulations as well as NC fumigation treatments in antagonizing the ethylene perception and preserving the fruit quality of plums following cold storage.

Materials and methods

Chemicals

NC was synthesized at the Chemistry Laboratory, School of Molecular and Life Sciences, Curtin University, Western Australia following the procedure described by Billups and Chow (1973). The concentrations to formulate aqueous solutions of NC and the treatment application time were based on the preliminary experiments conducted on plum fruit (Khan 2014).

For quality analysis, HPLC analytical grade standards of glucose, sucrose, fructose, sorbitol, malic acid, citric acid, fumaric acid, succinic acid, L-ascorbic acid, ethylenediaminetetraacetate acid (EDTA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium fluoride (NaF) and 6-hydroxy-2,5,7,8-tetramethylchoman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich, Sydney, Australia.

Treatment preparation and application

Just before the application of each treatment, four aqueous solutions of 2 µM NC were prepared using 100% distilled water (NCD), 5% ethanol (NCE), 0.02% Tween® 20 (NCT) or 5% β-cyclodextrin (NCB) following the procedure detailed in Kyaw et al. (2021). The 1 µM NC was also prepared and the fumigation treatment application was done according to the method described by Kyaw et al. (2021).

Japanese plum (*Prunus salicina* L. cvs. 'Angeleno' and 'Tegan Blue') fruit were harvested from the Eastwind Farm, Balingup (33°47'02.4" S 115°57'47.2" E), Western Australia. The plum fruit were harvested at the commercially mature stage (52.1 ± 3 N firmness, 10.5 ± 0.3% SSC and 2.2 ± 0.01% TA) from 20 and 25-year-old trees, respectively. The harvested fruit were immediately transported in an air-conditioned vehicle to Curtin Horticulture Research Laboratory, Perth, Western Australia. The fruit firmness, total soluble solids content and total titratable acidity of 'Angeleno' and 'Tegan Blue' plums on the day of the harvest were 38.4 ± 8 N and 37.1 ± 4 N, 15.6 ± 0.5% and 10.2 ± 0.5% and 2.8 ± 0.2% and 1.81 ± 0.2%, respectively. Two individual experiments were conducted following a completely randomized design with three replicates each.

Experimental details

Experiment 1: evaluating the influence of NC formulations on ethylene production rate and postharvest fruit quality attributes of 'Angeleno' plums

A total of 648 'Angeleno' plum fruit of relatively uniform size, free from defects were selected and divided into 6 groups of 108 fruit each. Following the treatment preparation, 4 groups of sample fruit were sprayed thoroughly with one of the aqueous NC formulations (NCD, NCE, NCT and NCB) at room temperature (20 ± 1 °C), making sure a thorough spread of the treatment chemical on the fruit surface. After spraying, the fruit were air-dried until no water droplets were observed on the fruit surface and then sealed in 60 L plastic containers for 18 h. One group of fruit was fumigated with NC in a 60 L plastic container for 18 h at ambient conditions (20 ± 1 °C) and the remaining untreated group was regarded as the control. A Petri dish filled with soda lime was kept in each plastic container to adsorb any carbon dioxide accumulated. A portable fan was also placed inside the containers to ensure uniform distribution of the NC fumes. Following the 18 h of treatment, the plastic containers were unsealed in an open space. Each group was then divided into two sub-lots and allocated to cardboard boxes, labelled with respect to treatments and storage period. The boxes were then stored for 25 and 40 d at 0 ± 1 °C and 90 ± 5% RH. At the end of the respective cold storage periods, the fruit were taken out of storage to assess fruit quality parameters and to determine the ethylene production rate at room temperature until the fruit exhibited a conspicuous climacteric ethylene peak. The weight loss, fruit firmness, soluble solid contents (SSC) and titratable acidity (TA), individual sugars, individual organic acids, total phenolic contents, ascorbic acid and total antioxidant capacity were estimated to investigate the effects of the NC formulations on fruit quality.

Experiment 2: evaluating the influence of NC formulations on ethylene production rate and postharvest fruit quality attributes of 'Tegan Blue' plums

A similar experimental procedure as in the first experiment was followed, except for the storage period. After treatment, the 'Tegan Blue' plum fruit was divided into two groups. The first group was stored for 10 d at 20 ± 1 °C and 85 ± 5% RH and the second group was stored for 40 d at 0 ± 1 °C and

90 ± 5% RH. The ethylene production rate was analysed for both storage periods; however, the fruit quality parameters were analysed only at the end of 40 d cold storage.

Analysis of ethylene production and fruit quality attributes

Rate of ethylene production

The ethylene production was estimated daily for 10 d at the end of the respective storage periods using an ethylene detector (ETD-300, Sensor sense B.V, Nijmegen, The Netherlands). To detect ethylene production, three fruit per replication were kept in air-tight containers of 1 L in volume which were set at a flow rate of 4 L h^{-1} following the continuous flow method for 20 min as described by Kyaw et al. (2021) and expressed as nmol kg^{-1} h^{-1}.

Weight loss

The initial weight before transferring to the storage and the final weight after completion of the storage period were recorded and the physiological weight loss was calculated using the formula below.

$$\text{Weight loss}\,(\%) = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100$$

Fruit firmness

At the end of each storage period, ten fruit per replication were used to determine the fruit firmness using a textural analyzer (TPA Plus, AMETEK Lloyd Instruments, UK) attached to an 8 mm probe. The fruit were peeled on two opposite sides of the cheeks and the firmness was determined on each side with 100 mm s^{-1} speed at 1 N trigger force (Kyaw et al. 2021).

Soluble solid concentration (SSC), titratable acidity (TA) and their ratio

Longitudinal slices were peeled and cut from ten fruit samples. The pulp of fruit samples were blended to obtain homogenous juice samples to determine SSC, TA and SSC: TA. The SSC was estimated from the pooled juice samples using a portable refractometer. To determine TA, 10 mL of the pooled juice was first mixed with 20 mL of distilled water. Then, 5 mL of diluted juice was titrated against 0.1 N NaOH until a pale pink endpoint in the presence of phenolphthalein indicator. The TA was calculated as a malic acid equivalent.

Individual sugars and organic acids

Aliquots (5 g) of pooled pulp sample prepared from 10 fruit of each replication were homogenized using an electronic homogenizer (Heidolph Homogeniser, DIAX 900, Sigma Aldrich, Australia). The samples were then diluted with degassed Milli-Q water and the volume was made to 50 mL. The diluted samples were centrifuged at 13,416 × g for 15 min and 0.22 µm nylon syringe filters (Thermo Fisher Scientific Pty Ltd., Australia) were used to filter the centrifuged aliquot. The filtered aliquots were then transferred into 1 mL clear glass HPLC vial shells with a polyethene plug (ThermoFisher Scientific Pty Ltd., Australia). To determine individual sugars and organic acids, a reversed-phase high-performance liquid chromatography (RP-HPLC) (Binary HPLC Pump, Waters 1525, Milford Corp., USA) was used (Kyaw et al. 2021). During the HPLC analysis, the flow rate was set at 0.6 mL min^{-1}. A refractive index detector (Waters 2414, Milford Corp., MA, USA) and a Fast Carbohydrate Analysis column (100 × 7.8 mm) were used to analyze individual sugars. A dual λ UV absorbance detector (Water 2487, Milford Corporation, USA) at 214 nm and an Organic Acid Analysis column (300 × 7.8 mm) was used for organic acids.

Total phenolic contents

Total phenolic compounds from the plum pulp samples were determined following the Folin-Ciocalteu reagent method explained by Robles-Sánchez et al. (2009) with some modifications as described by Kyaw et al. (2021).

Extraction of phenolic compounds Aliquots (20 g) of pooled pulp prepared from 10 fruit of each replication were homogenized with 15 mL of methanol (80%). The homogenized samples were sonicated for 15 min and centrifuged at 13,416 × g for 15 min. This protocol was repeated twice, and the volume of the final aliquot collected was made into 60 mL with distilled water. The extracted aliquot (50 µL), 250 µL of diluted Folin Reagent (1:2 ratio of FR and distilled water) and 3 mL of distilled water were mixed and the solution was kept in the dark for 5 min. Then, 250 µL of 7% sodium carbonate solution (Na_2CO_3) and 1450 µL of distilled water were added to the aliquot mixture and kept again in the dark for 90 min.

Estimation of phenolic contents After 90 min, the optical density (OD) of the aliquot mixture was recorded using a spectrophotometer (6405 UV/visible (190–1100 nm), Jenway, Dunmow, Essex, UK) at the wavelength of 750 nm. Total phenolic content was calculated using a standard

curve of gallic acid and expressed as g gallic acid equivalents (GAE) kg^{-1} fresh pulp weight.

Ascorbic acid content

Extraction of ascorbic acid Metaphosphoric acid (MPA, 6%) solution was prepared in distilled water along with ethylenediaminetetraacetate acid (EDTA) (1.8 g L^{-1}). The Folin reagent was diluted with distilled water to obtain a 1:5 concentration. Pooled pulp samples (5 g) prepared from 10 fruit of each replication were homogenised along with 20 mL of % MPA and centrifuged for 20 min at 3354 × g. To extract ascorbic acid, 400 µL of supernatant was mixed with 200 µL of % MPA solution, 200 µL of diluted Folin reagent and 1400 µL of distilled water and then, kept in the dark for 10 min.

Determination of ascorbic acid content Following 10 min incubation in the dark, the OD of the mixture was observed at 760 nm. To prepare a standard curve for calibration of the ascorbic acid content in the sample, analytical grade L-ascorbic acid was used, and the ascorbic acid content was expressed as g kg^{-1} (Tokala et al. 2021).

Total anthocyanin content

The total anthocyanin content in the fruit peel was estimated following the procedure of Whale and Singh (2007).

Extraction of total anthocyanin Thin slices of peel were collected from 10 fruit of each replication and mixed to get a homogenous sample. Aliquot (1 g) of peel samples were homogenized with 10 mL of extraction solution. The extraction solution was prepared by mixing 95% methanol with concentrated HCl in a ratio of 97: 3 (v/v). The homogenized samples were kept in the dark overnight at 2–4 °C. The decanted solution was centrifuged at 3354 × g for 20 min.

Determination of total anthocyanins The OD of the centrifuged supernatant sample was estimated at 530 nm using the spectrophotometer. To calculate the total anthocyanin content, cyanidin-3-glucoside was used as standard and expressed as g kg^{-1} (Siegelman and Hendricks 1958).

Total antioxidant capacity

Total antioxidant capacity was estimated using DPPH where the free radical scavenging capacity of the pulp sample was assessed following the procedure previously described by Tokala et al. (2021).

Extraction of antioxidant compounds DPPH (24 g) and pure methanol (100%) were mixed to prepare 100 mL of DPPH stock solution. A working solution of DPPH was then prepared by diluting the stock solution to 1: 4 with 100% methanol just before the assessment of antioxidant capacity. The OD of the working solution was adjusted to 1.1 at 515 nm in a spectrophotometer (by adding methanol when the OD was > 1.1 or adding DPPH stock when the OD was < 1.1). A sodium fluoride (NaF) solution (1 L) was prepared by mixing 84 mg of NaF and 80% methanol. Aliquot (1 g) of pulp sample was homogenized in 10 mL of NaF solution and centrifuged at 13,416 × g for 20 min.

Estimation of total antioxidant capacity The supernatant was mixed with 1900 µL of DPPH working solution and kept in the dark for 15 min. The amount of supernatant was adjusted until the OD of 0.6–0.7 at 515 nm was obtained. The calculated total antioxidant capacity was described as Trolox equivalent antioxidant activity (mmol kg^{-1}).

Statistical analysis

The statistical analysis was done using *GenStat* software version 14 (VSN International Ltd., UK) at ($P \leq 0.05$) LSD. The ethylene production data were subjected to a two-way analysis of variance (NC formulation treatments and ripening period (d) were the two variables) while the other fruit quality parameters were analyzed with one-way ANOVA (NC formulation treatments only). Treatment means were compared using Duncan's multiple-range tests. In addition, the principal component analysis (PCA) and the correlation matrices for each plum cultivar at the respective storage period were performed using R statistical software.

Results

Ethylene production

The rate of ethylene production varied depending on the cultivar. 'Tegan Blue' had significantly higher ethylene production (≈23 to 32-fold) and an earlier onset of the climacteric ethylene peak (≈5 d) as compared to 'Angeleno' (Fig. 1). It was observed that the effect of NC treatments in suppressing the ethylene production was much stronger in 'Angeleno' (suppressed climacteric) than 'Tegan Blue' (climacteric). In the 'Tegan Blue' plum, the NC fumigation treatment, followed by NCE and NCT, was the most

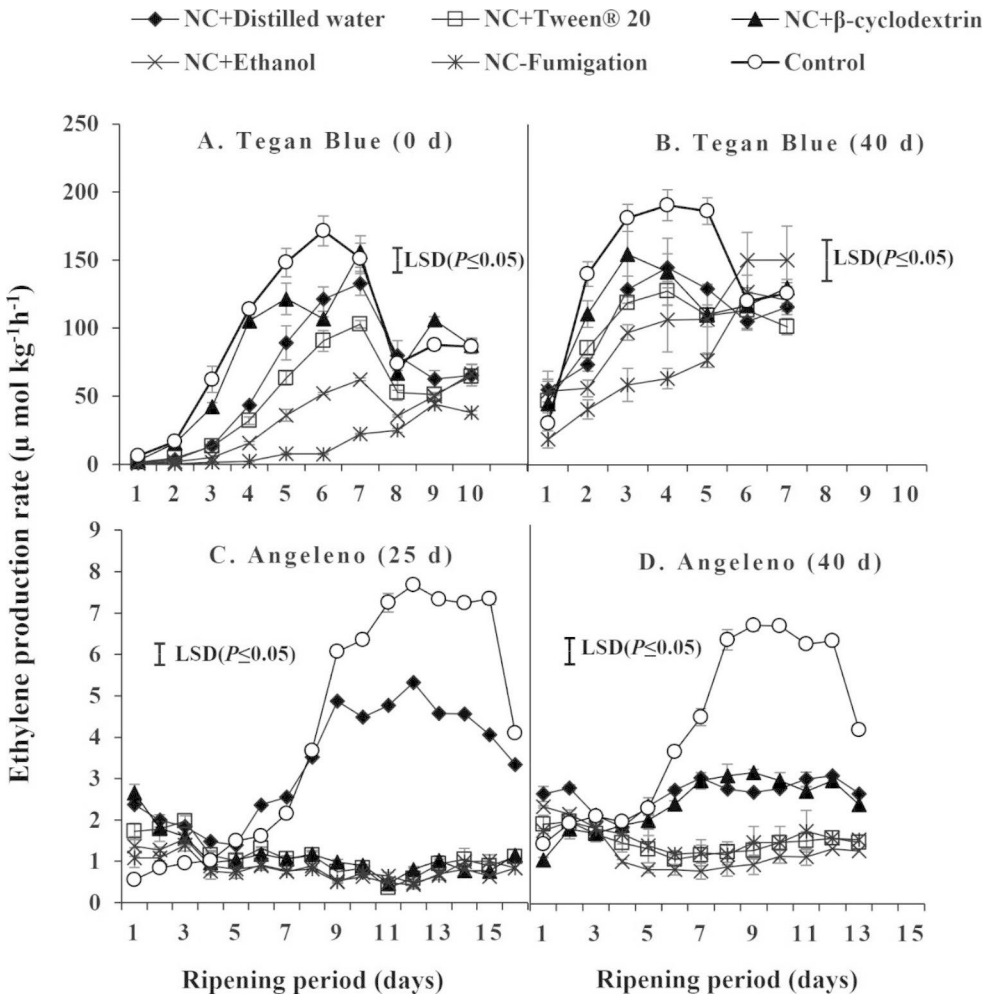

Fig. 1 Ethylene production of 'Tegan Blue' (stored for 0 and 40 d at 1 °C) and 'Angeleno' (stored for 25 and 40 d at 1 °C) plums treated with NC spray solutions (NC + distilled water (NCA), NC + Tween-20 (NCT), NC + β-cyclodextrin (NCD) and NC + ethanol (NCE)) and NC fumigation at room temperature. Vertical bars represent the SE of means of three replicates and are not visible when the values are smaller than the symbol

effective in suppressing ethylene production and in delaying the onset of the climacteric peak, irrespective of the storage period. NC fumigation delayed the onset of the climacteric ethylene peak of the 'Tegan Blue' plum by 3 d and 2 d compared to the control following 0 d and 40 d cold storage, respectively. Similarly, the ethylene production rate was also suppressed by 3.9-fold and 1.5-fold compared to control following 0 d and 40 d cold storage, respectively (Fig. 1A and B). The climacteric ethylene concentration of 'Tegan Blue' plum treated with NCE and NCT were significantly lower (2.8 and 1.3-fold, 1.7 and 1.5-fold, respectively) when compared to the controls following 0 d and 40 d cold storage, respectively (Fig. 1A, B). NCD-treated fruit also expressed relatively reduced (1.3-fold each) ethylene production compared to that of the controls in both the storage periods studied.

In the 'Angeleno' plum, when compared to the control fruit, NC treatments significantly suppressed the production of ethylene, regardless of formulation and the storage period. The climacteric ethylene peak onsets were suppressed by NC formulations, except in NCD and NCB-treated fruit after 25 d and 40 d storage (Fig. 1C and D). The ethylene production of 'Angeleno' plums treated with NC fumigation, NCE and NCT were not different from each other and were almost 5.1 and 2.7-fold reduced, respectively, as compared to control after 25 d and 40 d cold storage (Fig. 1C and D). After 40 d cold storage, the ethylene production of 'Angeleno' plum treated with NCB and NCD was considerably lower (1.2-fold and 1.6-fold, respectively) as compared to the control, although slightly higher than that of NC fumigation, NCE and NCT.

As visualized by the PCA biplots in Fig. 4A, B and C, ethylene production was on the negative side of PC2 and NC fumigation, NCE and NCT were on the positive sides of PC1, indicating that NC fumigation, NCE and NCT suppressed ethylene production effectively. In contrast, NCA and NCD were on the same side as ethylene production (negative sides of PC1) showing failure to suppress ethylene production in tested plum cultivars.

Weight loss and fruit firmness

Irrespective of formulation, NC treatments caused significantly lower weight loss as compared to control in 'Tegan Blue' fruit stored for 40 d (Fig. 2A), whilst there was no significant difference among the NC formulations. Following 25 d storage, the weight loss of the 'Angeleno' plum treated with NC, regardless of formulation, was considerably lower than the control (Fig. 2C). The weight loss of the fruit treated with NC fumigation, NCE and NCT were not significantly different from each other, and 2.3-fold lower compared to the control. Following 40 d storage, 'Angeleno' plums treated with NC fumigation and NCE, followed by NCT, had considerably lower weight loss (2.0, 1.6 and 1.3-fold, respectively) as compared to the rest of the treatments (Fig. 2D).

The firmness of 'Tegan Blue' plum stored for 40 d was higher after the NC fumigation (1.6-fold) treatment, followed by NCT (1.5-fold) and NCE (1.4-fold), as compared to the rest of the NC formulations and the control (Fig. 2B). A similar trend was observed in the 'Angeleno' plum stored for 40 d where the firmness of the fruit fumigated with NC and NCE, followed by NCT, were comparatively higher than for the other NC formulations and the control (Fig. 2F). After 25 d storage, 'Angeleno' plums treated with NC, regardless of formulation, retained notably higher firmness as compared to control (Fig. 2D). NC fumigated fruit exhibited the highest firmness retention followed by NCE and NCT which showed 1.3, 1.2 and 1.1-fold, respectively higher firmness than the control.

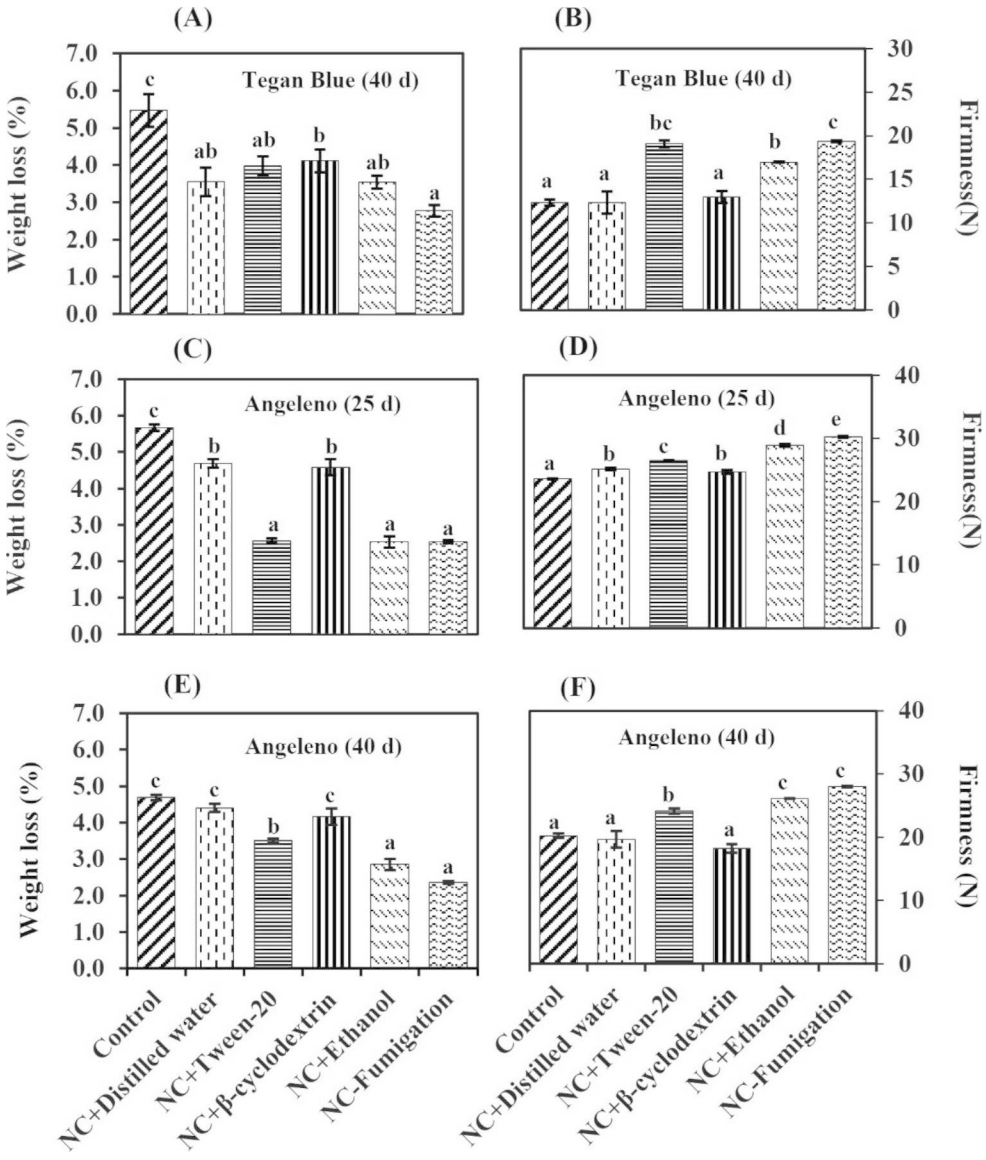

Fig. 2 Weight loss and firmness of 'Tegan Blue' plum stored for 40 d (**A** and **B**) and of 'Angeleno' plum stored for 25 d (**C** and **D**) and 40 d (**E** and **F**) at 1 °C as affected by NC spray solutions (NC + distilled water (NCA), NC + Tween-20 (NCT), NC + β-cyclodextrin (NCD) and NC + ethanol (NCE)) and NC fumigation. Vertical bars represent the SE of means of three replicates and are not visible when the values are small

SSC, TA and SSC: TA

NC fumigation and the NCE and NCT treatments maintained considerably lower SSC and SSC: TA and higher TA values in the fruit, as compared to control, regardless of cultivar and storage period (Fig. 3). The SSC and SSC: TA of 'Tegan Blue' fruit treated with all NC formulations, except NCA, were reduced up to 1.3 and 1.7-fold, respectively, as compared to control after 40 d cold storage (Fig. 3A and B). 'Tegan Blue' plum treated with NC fumigation, and NCT

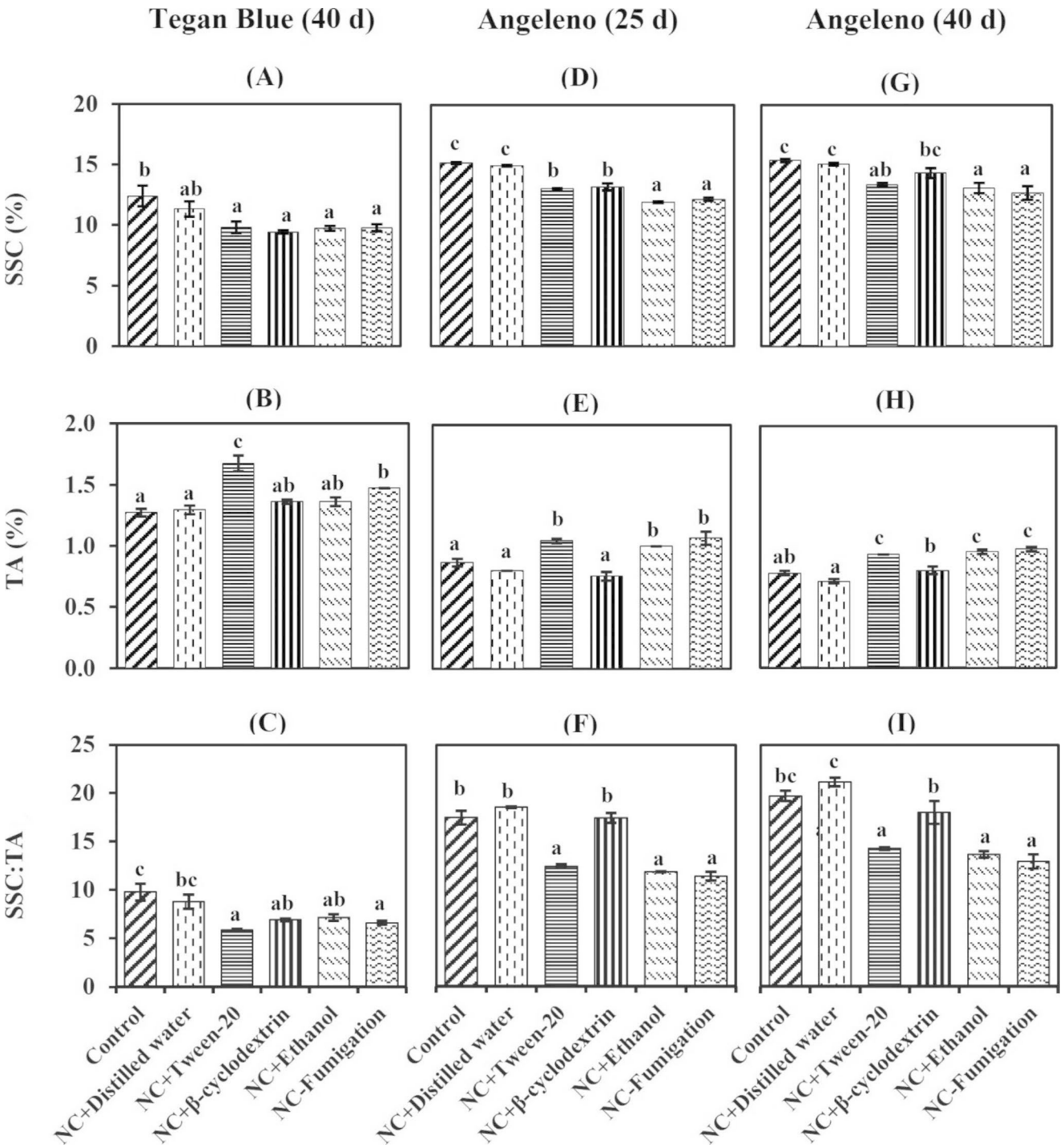

Fig. 3 SSC %, TA (%) and SSC: TA (%) of 'Tegan Blue' plum stored for 40 d (**A, B, C**) and of 'Angeleno' plum stored for 25 d (**D, E, F**) and 40 d (**G, H, I**) at 1 °C as affected by NC spray solutions (NC + distilled water (NCA), NC + Tween-20 (NCT), NC + β-cyclodextrin (NCD) and NC + ethanol (NCE)) and NC fumigation. Vertical bars represent the SE of means of three replicates and are not visible when the values are small

maintained higher TA (1.2 and 1.3-fold, respectively) than control after 40 d storage (Fig. 3C). The SSC of 'Angeleno' fruit treated with NC fumigation, NCE, and NCT were lower after both 25 d (1.3, 1.3, and 1.2-fold respectively) as well as after 40 d (1.2-fold each) cold storage periods when compared to the control (Fig. 3D and G). A similar trend of reduction was also observed in SSC: TA of 'Angeleno' after 25 d and 40 d cold storage (Fig. 3E and H). The TA of 'Angeleno' fruit treated with NC fumigation, NCE, and NCT were ≈ 1.2 and 1.3-fold higher as compared to the control after 25 d and 40 d cold storage, respectively (Fig. 3F and I). The SSC, TA and SSC: TA of the fruit sprayed with NCA and NCD, irrespective of cultivar and storage period, were not statistically different from the control.

Sugars and organic acids

Glucose, fructose, sucrose, and sorbitol were found in 'Angeleno' and 'Tegan Blue' fruit. Fructose was predominant among the individual sugars in both cultivars (Table 1). Individual sugars showed positive correlations with ethylene production while individual organic acids expressed negative correlations (Fig. 4D, E and F). NC formulations, except NCA, retained up to 21% lower levels of glucose, fructose and sorbitol as compared to the control for 'Angeleno' fruit stored for 25 d and 40 d. 'Angeleno' fruit fumigated with NC exhibited the lowest level of glucose, fructose and sorbitol, regardless of storage period. Similarly, the glucose (1.3-fold) and fructose (1.5-fold) levels in 'Tegan Blue' plum fruit sprayed with NCT and the sorbitol (1.5-fold) levels of 'Tegan Blue' fumigated with NC were significantly lower than control and the rest of NC formulations after 40 d storage. In contrast, 'Tegan Blue' plums sprayed with NCE had the highest level of sorbitol (32.5 g kg^{-1}) after 40 d storage. The sucrose content of 'Angeleno' plum fruit fumigated with NC after 25 d storage and sprayed with NCE after 40 d was higher (1.1-fold each) when compared to the untreated fruit and the fruit treated with other NC formulations. However, the level of sucrose in the 'Tegan Blue' fruit was not influenced by different NC formulation treatments.

Malic, citric, fumaric and succinic acids were quantified by RP-HPLC in fruits of both cultivars stored for 25 d and 40 d (Table 2). Malic acid was found to be the dominant in both 'Angeleno' and 'Tegan Blue', while fumaric acid was not detected in 'Tegan Blue' after 40 d storage. The levels of malic acid in 'Angeleno' fumigated with NC, sprayed with NCE and NCT were lower (up to 1.3-fold) than the untreated fruit and the fruit treated with the rest of the NC formulations after 25 d and 40 d storage. NC treatments, regardless of formulation, maintained higher succinic acid levels (up to 1.3-fold) in 'Angeleno' plum fruit stored for 25 d, whilst only NC fumigation and NCE maintained a higher

Table 1 Effect of different formulations of the ethylene antagonist (NC) on the sugars in 'Angeleno' and 'Tegan Blue' plum fruit stored for 25 d and 40 d at 1 °C

	Treatment	Glucose (g kg^{-1})		Fructose (g kg^{-1})		Sucrose (g kg^{-1})		Sorbitol (g kg^{-1})	
		25 d	40 d	25 d	40 d	25 d	40 d	25 d	40 d
Angeleno	Control	174.1 ± 1.6c	195.9 ± 2.3c	252.4 ± 1.8e	278.9 ± 3.2c	23.59 ± 1.1a	33.73 ± 0.4a	71.24 ± 3.7c	77.20 ± 1.9d
	NC + Distilled water	161.9 ± 3.9bc	190.1 ± 2.2bc	232.4 ± 5.8bd	273.5 ± 3.2bc	26.20 ± 2.1a	33.41 ± 0.3a	63.38 ± 2.3bc	67.00 ± 3.7bcd
	NC + Tween-20	149.2 ± 5.4ab	180.5 ± 1.6a	211.5 ± 6.9bc	257.1 ± 1.9a	25.01 ± 1.2a	32.12 ± 0.1a	59.98 ± 4.6bc	56.20 ± 1.9abc
	NC + β-cyclodextrin	155.5 ± 2.8ab	183.0 ± 1.9ab	219.1 ± 5.7bcd	260.3 ± 3.5ab	28.03 ± 2.5ab	33.63 ± 0.3a	52.44 ± 2.9ab	50.16 ± 4.3ab
	NC + Ethanol	147.0 ± 2.7a	186.7 ± 1.8ab	211.5 ± 4.7b	259.5 ± 3.3ab	24.00 ± 1.2a	36.06 ± 0.8b	42.76 ± 2.1a	73.88 ± 8.0 cd
	NC-Fumigation	143.5 ± 1.1a	180.7 ± 3.1a	189.1 ± 1.2a	256.6 ± 4.7a	34.95 ± 2.8b	33.65 ± 0.7a	52.91 ± 2.5ab	47.00 ± 3.8a
	LSD ($P \leq 0.05$)	13.42*	7.61*	19.88**	13.69*	ns	1.81*	12.95*	17.7*
Tegan Blue	Control		52.23 ± 1.1b		61.96 ± 1.1b		25.95 ± 1.9		27.80 ± 1.9bc
	NC + Distilled water		48.89 ± 1.1ab		53.54 ± 0.6b		28.35 ± 2.3		28.77 ± 2.0bc
	NC + Tween-20		40.67 ± 0.6a		40.63 ± 3.2a		28.52 ± 2.9		21.25 ± 1.1ab
	NC + β-cyclodextrin		51.89 ± 2.7b		53.99 ± 1.5b		36.73 ± 4.4		29.67 ± 2.0bc
	NC + Ethanol		52.64 ± 1.4b		62.17 ± 4.2b		27.32 ± 2.6		32.50 ± 0.6c
	NC-Fumigation		47.09 ± 3.2ab		57.23 ± 3.6b		23.75 ± 3.3		18.17 ± 3.5a
	LSD ($P \leq 0.05$)		7.89*		11.19*		ns		7.89*

Mean values followed by the similar letter are not different within the columns. The data for each plum cultivar and storage period were analysed separately. **, * = significant at 1% and 5% levels of LSD, ns = non-significance

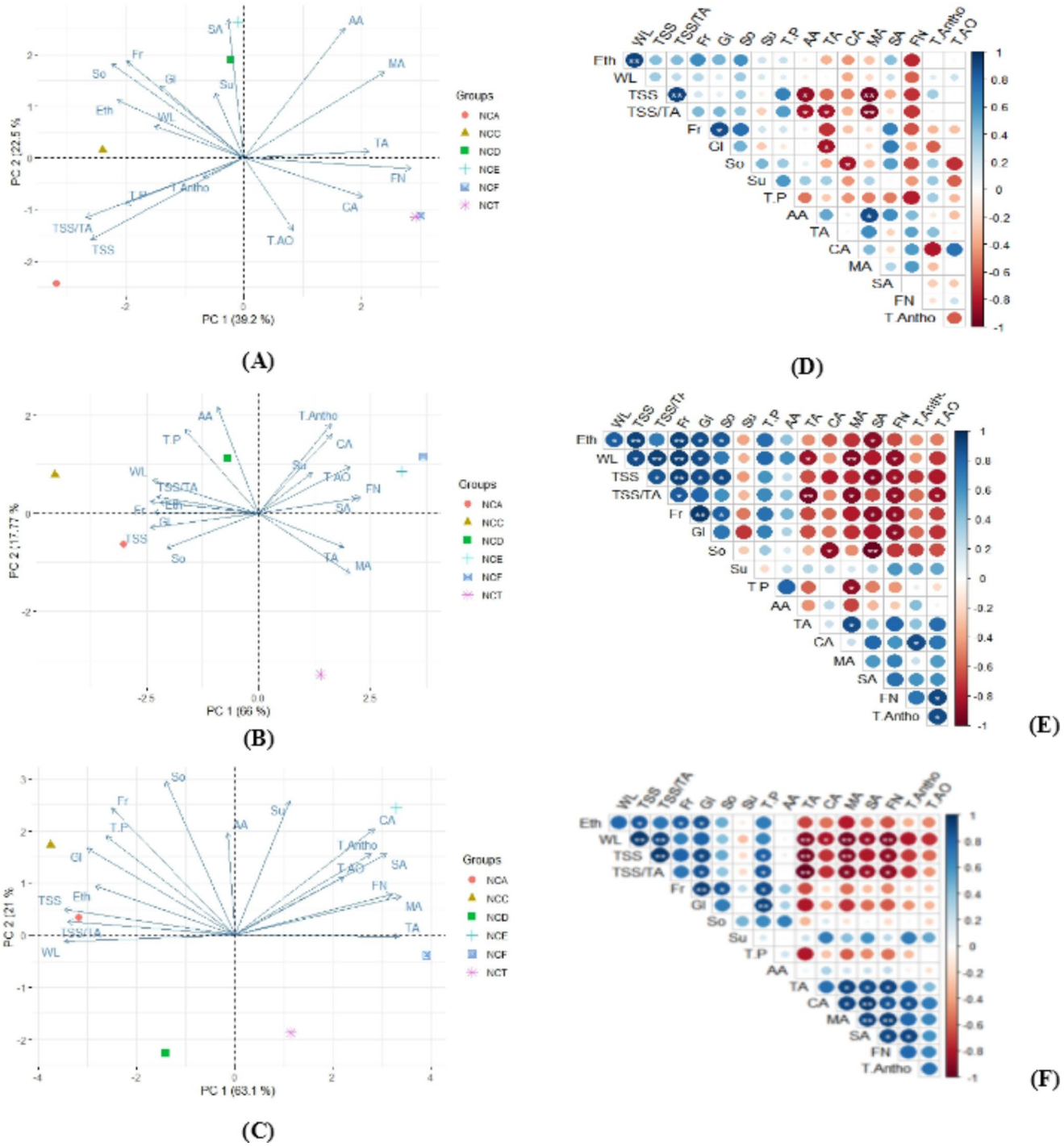

Fig. 4 PCA biplots and correlation matrices of 'Tegan Blue' plum cold-stored for 40 d (**A** and **D**) and of 'Angeleno' plum cold-stored for 25 d (**B** and **E**) and 40 d (**C** and **F**) at 1 °C. The PCA biplots were analyzed for the physicochemical attributes of respective plum cultivars and the different formulations of ethylene antagonist NC. Physicochemical attributes: Eth = ethylene production, WL = weight loss, TSS = total soluble solids, TSS/TA = ratio of TSS and TA, Fr = fructose, Gl = glucose, So = sorbitol, Su = sucrose, T.P = total phenolics, AA = ascorbic acid, TA = total titratable acidity, CA = citric acid, MA = malic acid, SA = succinic acid, FN = firmness, T.Antho = total anthocyanin, T.AO = total antioxidants. Groups (NC formulations): NCA = NC + Distilled water, NCC = control, NCT = NC + Tween-20, NCD = NC + β-cyclodextrin, NCE = NC + Ethanol, NCF = NC fumigation. The correlation matrices were analyzed for the physicochemical attributes of each plum cultivar at the respective storage period mentioned. **, * = significant at 1% and 5% levels of LSD.

Table 2 Effect of different formulations of the ethylene antagonist (NC) on the organic acids in 'Angeleno' and 'Tegan Blue' plum fruit stored for 25 d and 40 d at 1 °C

	Treatment	Malic acid (g kg^{-1})		Citric acid (g kg^{-1})		Fumaric acid (g kg^{-1})		Succinic acid (g kg^{-1})	
		25 d	40 d	25 d	40 d	25 d	40 d	25 d	40 d
Angeleno	Control	26.29 ± 0.2a	20.56 ± 0.5a	0.64 ± 0.0ab	0.93 ± 0.0ab	0.22 ± 0.0	0.30 ± 0.0	4.02 ± 0.2a	4.43 ± 0.0ab
	NC + Distilled water	26.21 ± 0.3a	21.45 ± 0.8ab	0.61 ± 0.1a	0.93 ± 0.0ab	0.21 ± 0.0	0.30 ± 0.01	4.49 ± 0.1ab	4.09 ± 0.2a
	NC + Tween-20	29.85 ± 1.1c	24.20 ± 1.5bc	0.62 ± 0.1a	0.96 ± 0.1ab	0.22 ± 0.0	0.29 ± 0.0	4.68 ± 0.1b	4.65 ± 0.1b
	NC + β-cyclodextrin	26.78 ± 0.1ab	21.06 ± 0.7a	0.76 ± 0.0ab	0.88 ± 0.0a	0.22 ± 0.0	0.29 ± 0.0	4.81 ± 0.1bc	4.21 ± 0.1ab
	NC + Ethanol	28.56 ± 0.4bc	26.72 ± 0.2c	0.84 ± 0.0b	1.15 ± 0.1c	0.21 ± 0.0	0.29 ± 0.0	5.22 ± 0.1c	5.45 ± 0.1c
	NC-Fumigation	28.64 ± 0.7bc	25.93 ± 0.3c	0.76 ± 0.1ab	1.06 ± 0.0bc	0.22 ± 0.0	0.31 ± 0.01	4.87 ± 0.1bc	5.12 ± 0.1c
	LSD ($P \leq 0.05$)	2.11*	2.85*	ns	0.15*	ns	ns	0.49*	**
Tegan Blue	Control		18.5 ± 0.17a		0.35 ± 0.02		ND		3.4 ± 0.03a
	NC + Distilled water		21.5 ± 0.65b		0.35 ± 0.01		ND		3.8 ± 0.07b
	NC + Tween-20		23.8 ± 0.22c		0.40 ± 0.01		ND		3.8 ± 0.07b
	NC + β-cyclodextrin		22.3 ± 0.63bc		0.36 ± 0.0		ND		3.8 ± 0.14b
	NC + Ethanol		23.7 ± 0.49c		0.37 ± 0.0		ND		3.9 ± 0.07b
	NC-Fumigation		23.3 ± 0.22c		0.38 ± 0.0		ND		3.9 ± 0.04b
	LSD ($P \leq 0.05$)		1.68**		ns				0.29*

Mean values followed by the similar letter are not different within the columns. The data for each plum cultivar and storage period were analysed individually. **, * = significant at 1% and 5% levels of LSD, ns = non-significance, ND = not detected

level of succinic acid (1.2-fold each) as compared to control in 'Angeleno' fruit stored for 40 d. Similarly, the malic acid and succinic acid levels in the 'Tegan Blue' fruit treated with NC, regardless of formulation, were also reduced up to 1.3 and 1.2-fold, respectively, as compared to the control after 40 d storage. The citric acid and fumaric acid levels in the fruit of both cultivars tested were not affected by NC treatments, except the citric acid level in 'Angeleno' fruit sprayed with NCE which were 1.2-fold higher as compared to control after 40 d storage.

Total phenols, ascorbic acid content, total anthocyanin and total antioxidant capacity

The concentrations of total phenols and ascorbic acid positively correlated, though not significantly, with ethylene production in 'Angeleno' and 'Tegan Blue' plums stored for 40 d at 1 °C, while total antioxidant capacity and total anthocyanin were negatively correlated with ethylene production (Fig. 4E and F). The levels of total phenols, ascorbic acid and total antioxidant capacity in 'Angeleno' and 'Tegan Blue' fruit were not affected by the application of NC formulations (Supplementary materials, Appendix 1, Table 1). However, total anthocyanin contents in 'Angeleno' plums treated with NC formulations, except NCA, were significantly lower by ≈1.2-fold as compared to control after 40 d storage.

Discussion

Japanese plums are a unique group of fruit cultivars with three different patterns of ripening behaviour: climacteric, suppressed-climacteric and non-climacteric (Abdi et al. 1998; Minas et al. 2015). Unlike normal climacteric plums, the suppressed-climacteric plums, e.g., Late Santa Rosa, produce negligible amounts of ethylene, and non-climacteric plums e.g. Sweet Miriam, are completely insensitive to external ethylene exposure (Minas et al. 2015). In the present study, the efficacy of NC in retarding ethylene production varied between cultivars. The fruit treated with different NC formulations suppressed ethylene production in the 'Angeleno' plum compared to 'Tegan Blue'. The rate of ethylene production in the 'Tegan Blue' fruit was ≈23-32-fold higher and the climacteric peak onset was ≈5 d earlier as compared to 'Angeleno'. Therefore, 'Angeleno' and 'Tegan Blue' express suppressed-climacteric and climacteric ethylene production natures, respectively. The application of greater concentrations of NC might have increased its ability to suppress ethylene production in plum cultivars with high ethylene production like 'Tegan Blue'. Measuring targeted gene expression of ethylene metabolism-related

genes with the NC treatments are warranted for future research to provide more technical evidence for its effectiveness.

1-MCP, the well-known commercial ethylene antagonist, inhibits the production and action of ethylene by binding the copper cofactor in the ethylene receptor ligand through the ring-opening reaction (Pirrung et al. 2008). Thus, it inactivates the receptors, blocks the action of ethylene and inhibits the cellular response (Sisler 2006; Pirrung et al. 2008). Similarly, NC suppresses ethylene production following a mechanism similar to that of 1-MCP (Singh et al. 2018). NC binds to the metal cofactor in the ethylene receptor of the targeted fruit tissues, triggers the ring-opening reaction, and reacts with a protein matrix of the receptor, leading to the inactivation of the ethylene receptor thereby blocking ethylene signal transduction.

Among the treatments, NC fumigation was most effective in retarding the ethylene production in the fruit of both plum cultivars. The longer exposure time (18 h) of NC to the fruit sample in the fumigation treatment and diffusion of the gaseous form of NC as compared to the spray application of different formulations may have contributed to the variation in the effectiveness of NC. There might also be some loss of active compounds during the process of spraying and drying in the NC spray solution treatments. In a study to delay banana fruit ripening with ethylene antagonists, Sisler et al. (2009) also concluded a higher amount of cyclopropene salt solutions and longer exposure time were required to inhibit the action of ethylene when compared to gaseous cyclopropenes. Escribano et al. (2017) also obtained a similar finding with liquid 1-MCP dip treatments, with the treatment concentration and the dipping time influencing the effectiveness of 1-MCP in delaying the ripening of 'Bartlett' pears.

The presence of adjuvants enhanced the performance of NC spray solutions in antagonising ethylene production. The comparatively higher ethylene suppression in the NCE and NCT treatments could be ascribed to the amphiphilic structures of ethanol and Tween® 20. The hydrophilic heads, the hydroxyl (-OH) groups, helped NC to be more soluble in aqueous solutions while the lipophilic tails assisted in disorganizing the structure of the cuticle giving NC more permeability across the fruit skin. Our previous research work (Kyaw et al. 2021) also confirmed that the adjuvants, ethanol and Tween® 20, play a significant role in improving the delivery of 1 *H*-cyclopropabenzene (BC), which is the parent compound of NC. Being a derivative of BC and having the same cyclopropene functional group as BC (Halton 1973), NC might have interacted with the adjuvants, ethanol and Tween® 20, in the same mechanism as that of BC. Ethanol enhances the surface binding and the infiltration of ethephon through the fruit cuticle, which increases the effectiveness of ethephon in promoting anthocyanin synthesis of blueberries (Costa et al. 2012). Similarly, increased calcium uptake was observed in the mangoes pretreated with 0.02% Tween® 20 before the dip treatment with a calcium solution (Singh et al. 2000). In the case of NCB, NC was likely to be encapsulated within the cyclic molecular structure of β-cyclodextrin, resulting in NC being more soluble in an aqueous solution and allowing the slow release of NC (Del Valle 2004). The lack of adjuvants in the NC solution with only distilled water resulted in a comparatively lower antagonistic effect on ethylene production in both plum cultivars studied.

The retention of fruit weight and firmness were higher in the plums treated with NC. Weight loss is primarily related to cumulative water loss which depends on the vapour pressure deficit during storage (Khan et al. 2018). However, a delay in the ripening mechanism by inhibiting the production of ethylene, a key triggering factor to fruit ripening, can slow down the physiological and biochemical changes in the fruit. In agreement with our findings, weight retention due to the application of an ethylene antagonist was also documented in different cultivars of plum such as 'Harrow Sun' plum (Manganaris et al. 2007), 'Santa Rosa' and 'Golden Japan' (Martinez-Romero et al. 2003) treated with 1-MCP, n 'Cripps Pink' apple (Tokala et al. 2020) and in 'Gold Rush' pear (Tokala et al. 2021) fumigated with NC. The reduction in fruit firmness is the result of increasing activities of softening enzymes (Khan and Singh 2007; Razzaq et al. 2016). The authors confirmed in 'Tegan Blue' plum that fumigation with 1-MCP suppresses the activities of enzymes related to fruit softening as such pectin esterase, endo-1,4-b-d-glucanase, exo-polygalacturonase and endo-polygalacturonase, and consequently resulted in higher fruit firmness.

The SSC, TA and the ratio of SSC: TA are the important sensory quality indices for consumer acceptance of plums (Khan et al. 2018). The plum fruit treated with NC formulations exhibited lower SSC and SSC: TA, and higher TA. The results were more pronounced with NC fumigation and the NCE and NCT treatments. In addition, malic acid and fructose were predominant among the individual organic acids and sugars identified in the plum fruit of both cultivars. Similar observations were previously reported in 'Formosa' (Bae et al. 2014), 'Angeleno' (Wang et al. 2014), 'Amber Jewel' and 'Black Amber' plums (Singh et al. 2009).

In our study, ethylene production relates positively correlated with SSC, glucose, fructose, and sorbitol, but negatively with TA, citric acid, malic acid and succinic acid, although the significance level of relations varied depending on the storage period and plum cultivar (Fig. 4D, E and F). Farcuh et al. (2018) found the relation between ethylene and the genes related to sugar metabolism of 'Santa Rosa' (climacteric) and 'Sweet Mariam' (non-climacteric) Japanese plums stored at 20 °C for 0, 1, 3, 5, 7, 10 and 14 d. The authors suggested that sucrose biosynthesis was induced by ethylene action and the breakdown of sucrose resulted in glucose and fructose formation. In our study, sucrose would have been broken down into glucose and fructose at the end of long cold storage at 1 °C,

which resulted in a negative correlation between ethylene and sucrose, not in agreement with Farcuh et al. (2018). The possibility of sugar synthesis is regulated by ethylene, which could be through the sorbitol catabolic pathway, as proposed by Kim et al. (2015) in the 'Sweet Miriam' plum. A similar influence of ethylene antagonism on SSC, SSC: TA and TA have also been reported previously in 'Songold' plum treated with 0.6 μL L^{-1} of 1-MCP for 24 h and stored at 0 and 8 °C (Velardo-Micharet et al. 2017). Fan et al. (2018) also reported that a post-cold-storage treatment with 1-MCP (0.5 μL L^{-1}) for 12 h to 'Shushanggan' apricot reduced the level of malic acid.

As visualised in the correlation matrices in Fig. 4D, E and F, total phenolic contents showed a positive correlation, though not significant, with ethylene production in both plum cultivars studied. The levels of total anthocyanins, total antioxidant capacity and ascorbic acid are affected by ethylene. According to Farcuh et al. (2022), in plums, ethylene regulates anthocyanin synthesis and phenolic contents depending on the sugar substrates that are present in the phenylpropanoid and flavonoid pathways. Sucrose may promote anthocyanin synthesis with the presence of ethylene; however, high concentrations of glucose or fructose or sorbitol with low levels of ethylene may suppress anthocyanin synthesis and lead to the phenylpropanoid pathway. In our study, as discussed earlier, sucrose might have already been broken down into glucose and fructose due to the exposure to long cold storage, resulting in higher concentrations of glucose and fructose in both plums. These higher concentrations of glucose and fructose may have suppressed anthocyanin synthesis and promoted the synthesis of more phenolic compounds.

Conclusions

The ethylene antagonist NC, irrespective of formulation, suppressed ethylene production in the fruit of both plum cultivars studied. The degree of effectiveness varied depending on the plum cultivar and NC formulation. Besides ethylene retardation, NC, either as a fumigant or as an aqueous formulation, also preserved the fruit quality of 'Angeleno' and 'Tegan Blue' plums during storage. The presence of ethanol or Tween® 20 as an adjuvant enhanced the performance of NC solutions in both ethylene retardation and fruit quality maintenance of plum fruit. Ethylene production expressed positive correlations with weight loss, SSC, SSC: TA and individual sugars of 'Angeleno' and 'Tegan Blue' plums while it negatively correlated with firmness, TA and individual organic acids. In addition, our results support the previous claim that ethylene regulates the synthesis of phenolic compounds and anthocyanin depending on the availability of sugar substrates in the phenylpropanoid and flavonoid pathways.

Abbreviations

1-MCP	1-Methylcyclopropene
NC	1*H*-cyclopropa[*b*]naphthalene
NCE	NC spray solution with 5% ethanol
NCT	NC spray solution with 0.02% ethanol
NCD	NC spray solution with 5% -cyclodextrin
NCA	NC spray solution with 100% distilled water

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit authorship contribution statement Zora Singh (ZS) and Poe Nandar Kyaw (PNK) jointly established the concept and constructed the experiments. PNK and Vijay Yadav Tokala (VYT) executed experiments with the supervision of ZS. PNK analyzed the data and prepared the first draft of the manuscript. ZS and VYT reviewed and improved the manuscript.

Chemical compounds 1 *H*-cyclopropa[*b*]naphthalene (PubChem CID: 136,126); ethanol (PubChem CID: 702); Tween® 20 (PubChem CID: 443,314); β-cyclodextrin (PubChem CID: 444,041).

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