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Aqueous formulations of 1*H*-cyclopropabenzene modulate ethylene production and fruit quality in Japanese plums

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Abstract

The efficacy of aqueous formulations of 1*H*-cyclopropabenzene (BC) containing different adjuvants to retard ethylene production and maintain fruit quality of Japanese plums (*Prunus salicina* Lindl. cvs. 'Angeleno', 'Fortune' and 'Tegan Blue') following 25 and 40 d cold storage (1 °C) was evaluated. Plum fruit were sprayed with different solutions of 2 μM BC (i.e., aqueous solutions containing distilled water only or 5 % ethanol or 0.02 % Tween® 20 or 5 % β-cyclodextrin) or fumigated with 1 μM BC at ambient temperature. Plum fruit without any treatment were regarded as control. Regardless of the cultivars tested, all formulations of BC remarkably suppressed the ethylene production, while the fumigation was most effective treatment, when compared to control. Effects of BC on fruit firmness, weight loss and all other fruit quality parameters varied among formulations and cultivars. The fruit treated with BC had lower total anthocyanins levels than control whilst, total phenolic content and total antioxidant capacity did not differ significantly. BC solutions prepared containing 5 % ethanol or 0.02 % Tween® 20 outperformed other BC aqueous formulations in impeding production of ethylene and maintaining quality of cold stored Japanese plums.

Keywords: Ethylene antagonist; ethanol; Tween® 20; spray formulations; ripening; bioactive compounds

Chemical compounds: ethanol (PubChem CID: 702); β-cyclodextrin (PubChem CID: 444041); Tween® 20 (PubChem CID: 443314); 1*H*-cyclopropabenzene (PubChem CID: 138310)

1. Introduction

Plums are one of the commercially important temperate fruit crops in Australia and are mostly exported to Asian countries, especially to Hong Kong and Singapore, while generating more than A$ 80 million revenue annually (Hort Innovation, 2017). In Australia, over two-thirds of the total plum fruit produced are consumed fresh and remaining fruit is used for processing (Hort Innovation, 2017). The fresh plum fruit has a relatively limited postharvest life and is highly susceptible to quality losses. Maintaining the nutritional quality, fruit firmness, sensory as well as aesthetic properties of fruit, along the supply chain, is a crucial challenge for the plum fruit industry. Ethylene initiates and promotes fruit ripening, chlorophyll degradation (Rozo-Romero et al., 2015), sugar metabolism (Farcuh et al., 2018), fruit softening (Khan and Singh, 2007), flavor and aroma compounds formation (Cheng et al., 2016). Ethylene also accelerates the senescence processes, shortens storage life and deteriorates quality of the fruit (Blanke, 2014). Therefore, ethylene management during postharvest handling is one of the key factors to prolong the storage life and maintain optimum fruit quality in plums. The negative effects of ethylene can be managed by suppressing its biosynthesis, removing from the storage environment or by blocking its perception at the receptor site (Zhang et al., 2017). Ethylene antagonist compounds irreversibly fuse to the receptors of ethylene in plant cells and consequently block ethylene action. The ethylene antagonist application is considered as the most effective approach to mitigate the adverse impact of ethylene in plum fruit (Sisler, 2006, Khan and Singh, 2007 and 2009). Several compounds such as substituted cyclopropenes, 1-methylcyclopropene (1-MCP), diazocyclopentadiene (DCPA), *trans*-cyclooctene and 2,5-norbornadiene (2,5-NBD) have been reported to antagonize the ethylene action in plant parts (Sisler, 2006). 1-MCP is relatively more effective than other substituted cyclopropenes, in antagonizing ethylene action and is being used commercially to manage ethylene and quality of fruit and vegetables after harvest (Watkins, 2015; Zhang et al., 2017). However, pure 1-MCP at the room temperatures is in gaseous form and this property makes it difficult to prepare effective spray formulations (Sisler et al., 2006). The dip formulation of 1-MCP was as effective as fumigation only when seven hundred times the concentration of fumigation was used, which is higher than permissible limits (Argenta et al., 2007). Various delivery methods of 1-MCP as such fumigants, powders, dusts and liquids have been developed by different companies such as AgroFresh Solutions Inc., USA (SmartFresh™ products), Hazel Technologies Inc., USA (Hazel® products) and Shandong Aoweite Biotechnology Co., Ltd, China (Logfresh® products). The liquid form of 1-MCP, Harvista™, developed by AgroFresh Solutions Inc., USA, is presently available in the market but its application is still limited as pre-harvest spray in crops like apples and cherries (AgroFresh, 2020). The ethylene antagonistic property of 1*H*-cyclopropabenzene (BC), as well as it's capacity to suppress ethylene production in fruits, delay ripening and inhibit abscission of waxflowers was discovered by Singh et al. (2018). BC belongs to the cycloproparenes group and is the end-product of the binding of a benzene ring with cyclopropene (Halton, 1973). BC, unlike 1-MCP, is basically liquid and slightly water soluble at room temperature which allow to formulate diverse application methods as ethylene management tools.

Adjuvants are co-solvent or surfactant compounds, which enhance the function of the active ingredients or expand the contact area of a solution on the targeted plant parts. Co-solvent or surfactant compounds have been reported as one of the prerequisite components in preparing the aqueous agrochemical solutions (Somervaille et al., 2012). Ethanol is a co-solvent which enhance the solubility of the ethylene antagonists (Grichko, 2006). It has also been evidenced to intensify the penetration capacity of the active compound (Farag et al., 1992). Tween® 20, a non-ionic surfactant, has been reported to improve the absorption of active ingredients by the fruit when applied as a surface spray (Singh et al., 2000). The cyclodextrins are used as inclusion compounds in different marketed products of 1-MCP as such EthylBloc®, SmartFresh™ and SmartTabs™ to deliver 1-MCP slowly over the time of application (Watkins, 2015). The ability to apply aqueous solutions of ethylene antagonists would enable their application as pre-or postharvest spray, dip or coating and bypass the need for sealed rooms for fumigation treatments (Argenta et al., 2007). Previously, the effects of BC fumigation in suppressing ethylene action in plum, nectarine and apple fruits as well as in wax flowers have been reported by Khan (2014), Singh et al. (2018) and Tokala et al. (2020 and 2021 a, b). In ‘Cripps Pink’ apple stored for 90 and 120 d at 0 ± 2 °C (90 ± 5 % RH), applying BC either as fumigant or dip solution showed positive response on ethylene production and fruit quality (Tokala et al., 2020). Whilst no research has been documented on different liquid formulations of BC in antagonizing the ethylene action, retarding ripening associated quality changes and prolonging the storage life of stone fruits and warrants to be investigated. It was hypothesised that the BC fumigation as well as spray treatments of different aqueous formulations containing adjuvants, will inhibit the production of ethylene and consequently maintain the fruit quality of Japanese plums, during cold storage. This study aims at evaluating the comparative efficacy of aqueous formulations of BC prepared with three different adjuvants i.e., 5 % ethanol, 0.02 % Tween® 20 and 5 % β-cyclodextrin, as well as BC fumigation suppressing production of ethylene and preserving fruit quality of cold-stored Japanese plums.

2. Materials and methods

2.1 Preparing aqueous formulations of BC

BC was synthesized at the Chemistry laboratory, Curtin University following the procedure of Davalian et al. (1980) and previously detailed by Singh et al. (2018). Different 2 µM aqueous spray solutions of BC (i.e., solutions containing distilled water only; 5 % ethanol; 0.02 % Tween® 20 or 5 % β-cyclodextrin) were prepared according to the procedure described by Tokala et al. (2020). The concentrations of ethanol, Tween® 20 and β-cyclodextrin were determined according to Farag et al. (1992), Singh et al. (1999) and Del Valle (2004), respectively, with some modifications based on the chemical properties of BC. All the aqueous spray solutions of BC were prepared fresh on the day of the treatment application.

2.2 Plant materials, fruit and experiments

The ethylene antagonistic potency of various aqueous formulations and fumigation of BC was evaluated in Japanese plum (*P. salicina* Lindl.) cvs. 'Fortune', 'Tegan Blue' and 'Angeleno' by conducting three independent experiments. The fruit were collected from Eastwind Farm, Balingup, Western Australia (33°47' S 115°57' E). The fruit at commercial harvest maturity were harvested and immediately transported to Horticultural Research Laboratory, Curtin University using an air-conditioned van. The relatively uniform-sized fruit with no visible symptoms of disease or pest damage, physiological disorders and mechanical injuries were used in the experiments.

2.2.1 Experiment 1: Effectiveness of different aqueous formulations and fumigation of BC on ethylene production and fruit quality of cold stored 'Fortune' plum

The first experiment was conducted during January 2017 using 'Fortune' plum fruit, an early-maturing cultivar. The fruit with commercial maturity (53.1 ± 4 N firmness, 10.4 ± 0.3 % SSC and 2.3 ± 0.01 % TA) were harvested from the 20 years old trees, planted at 1.5 m × 4 m spacing in North to South row direction. In the spray treatment, the fruit were sprayed with the respective aqueous formulation of BC (2 µM) using a hand sprayer (Nylex 500 ml Trigger Garden Sprayer, Doncaster, Victoria, Australia) ensuring uniform spread of droplets on the surface of fruit at room temperature (20 ± 1 °C, 80 ± 5 % RH). The spray volume was 100 mL per replicate. The fruit were then air-dried till no water droplets was observed on the surface of fruit. For the fumigation treatment, the fruit were placed in 60 L hermetically sealable storage containers and the calculated amount of 1 µM BC (v/v) was then applied using a Petri-plate with filter paper according to the procedure detailed by Tokala et al. (2020). The containers were then immediately sealed and left for 18 h at room temperature (20 ± 1 °C, 80 ± 5 % RH). Along with the fruit, a portable fan for uniform distribution of BC fumes and a Petri-dish of 30 g soda-lime for adsorption of excess carbon dioxide accumulated, were placed inside the container. The fruit with no treatment were regarded as control. Along with the fumigation treatment, rest of fruit untreated or treated with BC aqueous formulations were kept at the same environmental condition at room temperature (20 ± 1 °C, 80 ± 5 % RH). After the treatment, the containers were unsealed in an open-space and fruit were then arranged in the corrugated cardboard boxes. The boxes were labelled appropriately with respect to the treatments and kept at cold storage (0 ± 1 °C and 90 ± 5 % RH) for 25 and 40 d. Following each cold storage period, the fruit were allowed to ripen for 10 days at ambient temperature (20 ± 1 °C) to determine ethylene production and climacteric ethylene peak. Quality parameters such as weight loss, firmness, SSC, TA, SSC: TA, individual sugars and organic acids, total phenolic content, ascorbic acid, total anthocyanins and total antioxidant capacity were evaluated right after the cold storage period. The completely randomized design (CRD) with four replications was followed and each replication included 15 fruit.

2.2.2 Experiment 2: Effectiveness of different aqueous formulations and fumigation of BC on ethylene production and fruit quality of 'Tegan Blue' Japanese plum

The second experiment was conducted during February 2017 using 'Tegan Blue' plum, a mid-season maturing cultivar. The fruit at commercial maturity (37.1 ± 4 N firmness, 10.2 ± 0.5 % SSC and 1.81 ± 0.2 % TA) were harvested from the 25 years old trees, planted at 1.5 m × 4 m spacing and in North to South row direction. The fruit were treated following similar procedures as described in experiment 1. After the treatment, the duly labelled fruit boxes were kept for 40 d in cold storage (0 ± 1 °C and 90 ± 5 % RH). Following each cold storage period, the fruit were allowed to ripen for 10 days at ambient temperature (20 ± 1 °C) for determination of ethylene production and climacteric ethylene peak. The experiment was carried out with CRD and three replications (15 fruit in each replication). The parameters evaluated were the same as detailed in experiment 1.

2.2.3 Experiment 3: Effectiveness of different aqueous formulations and fumigation of BC on ethylene production and fruit quality of 'Angeleno' Japanese plum

The third experiment was conducted during March 2017 using 'Angeleno' plum, a late-maturing cultivar, following all the treatments and the procedure as described in experiment 1. The fruit were harvested at commercial maturity (38.4 ± 8.0 N firmness, 15.6 ± 0.5 % SSC and 2.8 ± 0.2 % TA) from the 20 years old trees planted at 1.5 m × 4 m spacing and in North to South row direction. The experiment was carried out with CRD and three replications (15 fruit in each replication). The boxes were labelled according to the treatment and cold stored for 25 and 40 d at 0 ± 1 °C and 90 ± 5 % RH). Following each cold storage period, the fruit were allowed to ripen for 15 days at ambient temperature (20 ± 1 °C) for determination of ethylene production and climacteric ethylene peak. On completion of respective storage periods, fruit quality parameters were determined as explained in experiment 1.

2.3 Ethylene production

Ethylene production of 'Fortune' plum was determined using a gas chromatogram (GC) (6890N Network GC, Agilent Technologies, USA). The GC was fitted with a stainless column and a flame ionization detector (Porapak-Q, Supelco, CA, USA). The ethylene production was determined following the procedure described by Khan and Singh (2008). Three fruit per replication were randomly selected and were then sealed as a group in a glass jar (1 L) equipped with a rubber septum for 1 h. Four replications per treatment was measured. The headspace gas sample (1 mL), following 1 h, was drawn using a syringe and injected into the GC. The peaks were compared to the standard gas sample of ethylene (1.15 ± 0.06 μL L^{-1} ethylene in N_2) (BOC Gases, Australia Ltd., WA). The rate of ethylene production was calculated using the following formula and mentioned as μmol kg^{-1} h^{-1}.

$$\text{Ethylene production} = \frac{\text{ethylene concentration by GC } (\mu L\ L^{-1}) \times \text{headspace volume (L)}}{\text{Fruit weight (kg)} \times \text{incubation time (h)}}$$

'Tegan Blue' and 'Angeleno' plum fruit produce very low amounts of ethylene, undetectable by GC. Therefore, a laser-based ethylene detector (ETD-300, Sensor Sense B.V, Nijmegen, The Netherlands) was used to determine their ethylene production rates following the procedure previously reported by Cristescu et al. (2013). Three fruit per replication were weighed and then, sealed in the air-tight glass jars connected to a valve controller, which allows computerized multi-sample detection (six samples simultaneously). The headspace gas sample passes through the catalyzer to remove hydrocarbons other than ethylene, before entering into the ethylene detector. The detection time was set for 20 min with the continuous flow method at a flow rate of 4 L h^{-1}. The ethylene production rate was expressed as nmol kg^{-1} h^{-1}.

The number of days for the climacteric ethylene peak onset and the concentration of ethylene at climacteric peak were determined from the dates at which the maximum amount of ethylene was produced during the ripening period at room temperature.

2.4. Determination of fruit quality attributes

2.4.1 Physiological weight loss and firmness

The initial fruit weight (IW) and final fruit weight (FW) of each replicate (fifteen fruit) were recorded before and after the respective storage duration. The physiological weight loss (PWL) was calculated using the following formula as explained by Tokala et al. (2020) and expressed as % weight loss.

$$\text{PWL (\%)} = \frac{\text{(IW - FW)}}{\text{IW}} \text{ x } 100$$

Fruit firmness was determined using a texture analyzer fitted with 8 mm probe (TPA Plus, AMETEK Lloyd Instruments, UK) according to the procedure described by Tokala et al. (2020). Twelve fruit per replication were peeled on two opposite cheeks of fruit. Firmness was detected using a trigger force of 1 N, 100 mm s^{-1} probe speed, 8 mm sample depth and expressed as Newtons (N).

2.4.2 Soluble solid content (SSC), titratable acidity (TA) and SSC: TA

The percent SSC, TA and SSC: TA were estimated following the procedure mentioned by Tokala et al. (2020) using the pooled juice sample from twelve fruit per replication. A portable digital refractometer (Atago-Palette PR 101, Japan) was used for SSC determination and expressed as %. For determination of TA, 5 mL of diluted fruit juice (10 parts juice: 20 parts distilled water) was titrated against 0.1 N NaOH and phenolphthalein was used as an endpoint indicator. The per cent TA was calculated as malic acid equivalent. The SSC value was divided by the respective TA value to calculate SSC: TA.

2.4.2 Individual sugars and organic acids

Individual sugars and organic acids were estimated using a reverse-phase HPLC system following the procedure previously detailed by Tokala et al. (2020). 5 g of pulp was collected from the homogenized sample of the longitudinal sections cut from twelve fruit per replication. Pulp sample was diluted and volume made to 50 mL using degassed Milli-Q water. The samples were then centrifuged at 10000 × g for 15 min. 1 mL of supernatant was filtered using a 0.22 µm nylon filter and the filtered samples were used for HPLC analysis. The individual sugars were quantified using a reverse-phase HPLC system fitted with a Fast Carbohydrate Analysis column (100 × 7.8 mm) and a Refractive Index Detector (Waters 2414, Milford Corp., MA, USA). At the flow rate of 0.6 mL min^{-1}, individual organic acids were estimated with a Dual λ UV absorbance detector at 214 nm fitted with an Organic Acid Analysis column (300 × 7.8 mm) (Water 2487, Milford Corporation, USA). The estimated levels of individual sugars and organic acids were expressed as g kg^{-1}.

2.4.3 Ascorbic acid

The 5 g of homogenized pulp sample collected from the longitudinal sections cut from twelve fruit per replication was used. The pulp sample was diluted in 20 mL extraction solution, prepared with metaphosphoric acid (6 % MPA) and ethylenediaminetetraacetate acid (0.18 % EDTA). The homogenized sample was centrifuged for 20 min at 5000 × g. The supernatant (400 μL) was then mixed with 200 μL of 3 % MPA solution, 200 μL of diluted Folin Reagent (1:3 in water) and 1400 μL of distilled water. The mixture was kept in the dark for 10 min and the absorbance values were recorded at 760 nm absorbance using a spectrophotometer (6405 UV/visible (190-1100 nm, Jenway, Dunmow, Essex, UK). The levels of ascorbic acid were quantified following the method previously described by Tokala et al. (2021) and expressed as g kg^{-1}.

2.4.4 Total anthocyanins content

The content of total anthocyanins was quantified according to the procedure described by Whale and Singh (2007). The 1 g of homogenized pulp sample collected from the longitudinal sections cut from twelve fruit per replication was used. Anthocyanins were extracted from the pulp sample using 10 mL of 97:3 (v/v) 95 % methanol and concentrated HCl as extraction solution. The aliquot was kept overnight in the dark at 2 to 4 °C. The spectrophotometric assay of anthocyanin was undertaken at 530 nm wavelength and expressed as g kg^{-1}.

2.4.5 Total phenolic content

The content of total phenolic was determined following the method previously described by Cantin et al. (2009). The 20 g of homogenized pulp sample collected from the longitudinal sections cut from twelve fruit per replication was used. For the extraction of phenols, the pulp sample was homogenized with 15 mL of methanol (80 %), then sonicated for 15 min and centrifuged for 15 min at 10000 × g. The aliquot was used for the phenol estimation in the presence of Folin reagent and 7 % sodium carbonate solution. The absorbance was recorded at the wavelength of 750 nm after 90 min in dark. The total phenolic content was measured in gallic acid equivalent (GAE) and expressed as g kg^{-1}.

2.4.6 Total antioxidant capacity

The total antioxidant capacity was quantified by estimating the free radical scavenging capacity of the fruit pulp following the DPPH (2,2-diphenyl-1-picrylhydrazyl) method described earlier by Tokala et al. (2021). The 1 g of homogenized pulp sample collected from the longitudinal sections cut from twelve fruit per replication was used. The pulp sample was mixed with 10 mL of sodium fluoride (NaF) and then centrifuged for 20 min at 10,000 × g. The required amount of supernatant was mixed with 1900 µL of diluted DPPH solution and kept in the dark for 15 min. The absorbance value was recorded at 515 nm using a spectrophotometer (6405 UV/visible (190 to 1100 nm, Jenway, Dunmow, Essex, UK). This spectrophotometric assay was repeated until the absorbance value was in the range of 0.6 to 0.7 at 515 nm. Total antioxidant capacity was measured in Trolox equivalent capacity and expressed as mol kg^{-1}.

2.5 Statistical analysis

GenStat software version 14.0 was used to analyze the data. The data were presented as means ± standard errors (SE) of means at LSD ($P \leq 0.05$) level. Duncan's multiple range test was done for the mean comparison of the treatments.

3. Results

3.1. Ethylene production

The climacteric ethylene production varied depending upon the plum cultivars. Early season plum 'Fortune' produced relatively higher ethylene level when compared to the midseason 'Tegan Blue' and the late season 'Angeleno' fruit. In all the plum cultivars tested, the ethylene production was comparatively lower in the fruit kept in cold storage for 40 d. BC, regardless of formulations, significantly suppressed the climacteric ethylene production in the plum cultivars tested, as compared to control, except in Fortune and Tegan Blue plum fruit stored for 40 d (Fig. 1, A-E). The climacteric ethylene production of the plum fumigated with BC was the lowest, whilst that of control fruit was the highest (in Fig. 2 C, D and E, the web radars incline more towards control) regardless of cultivars and storage periods. Next to BC fumigation, BC aqueous solution prepared with 5 % ethanol was effective in reducing climacteric ethylene production of all the plum cultivars tested as compared to control and rest of BC aqueous formulations (Fig 2). When averaged the ethylene production along the ripening period, BC aqueous solutions with 0.02 % Tween® 20 reduced ethylene production in 'Angeleno' plum on completion of 25 and 40 d of cold storage periods (4.9 and 14.3 fold lesser than control, respectively) (Fig. 1, C and D). The aqueous BC solutions containing 5 % β-cyclodextrin, and distilled water alone suppressed ethylene production throughout the storage period only in the 'Fortune' plum following 25 d storage and 'Angeleno' plum stored for 40 d. The effect of BC formulations on the onset of climacteric ethylene peaks varied depending on the cultivars. BC fumigation delayed the onset of climacteric ethylene peaks in 'Fortune' plum for 2 d as compared to control following 25 d storage (Fig 2). BC fumigation and BC aqueous solution containing ethanol delayed the onset of climacteric ethylene peaks by 2 d each in 'Tegan Blue' plum stored for 40 d, when compared to control fruit (Fig 2). However, BC formulations did not show any significant effect on the onset of climacteric ethylene peak in 'Angeleno' plum regardless of storage period (Fig 2).

3. 2 Physiological weight loss (PLW) and firmness

BC fumigation and aqueous formulations treatments reduced PLW in 'Fortune' plum cold-stored for 25 d and there was no significant difference the among treatments and control in 40 d cold-stored fruit (Fig 3 A and B). BC fumigation and spray treatments of aqueous BC formulations with 5 % ethanol have reduced (3.4-fold each) the weight loss in 'Angeleno' fruit stored for 25 d as compared to the control (Fig 3 C). In 40 d cold-stored 'Angeleno' fruit, the weight loss was lower (2.3-fold) in the fruit fumigated with BC as well as aqueous formulations containing 5 % ethanol (2.2-fold lower) or 0.02 % Tween® 20 (1.8-fold lower), when compared to control (Fig 3 D). In 40 d cold-stored 'Tegan Blue' fruit, the PLW of the fruit fumigated with BC was the lowest and were 2.5-fold lower in comparison with the control and all other treatments but the spray of aqueous BC containing 5 % ethanol was at par with the fumigation treatment (Fig. 3 E).

Following 25 d cold storage, 'Fortune' plum fruit sprayed with BC containing 5 % ethanol maintained highest firmness (39.4 N) when compared to control and other treatments. Whereas the BC fumigation and spray treatment with 0.02 % Tween® 20 as an adjuvant were at par with BC containing 5 % ethanol (Fig. 3 F). The fruit fumigated with BC showed the highest firmness (32.1 N) in 40 d cold-stored 'Fortune' plum when compared to the control and other treatments. Whilst, BC containing 5 % ethanol, 0.02 % Tween® 20 and distilled water only were at par with BC fumigation treatment

(Fig 3 G). The fruit fumigated with BC showed significantly highest firmness in 'Angeleno' plum after 25 and 40 d cold storages (30.4 and 28.3 N, respectively) (Fig 3 H, I). Similarly, the 'Tegan Blue' plum fumigated with BC and cold-stored for 40 d resulted highest firmness (20.2 N) in comparison to all other treatments and control (Fig 3 J). Irrespective of cultivar and storage period, the firmness of the plum sprayed with BC aqueous solution containing 5 % β-cyclodextrin or only distilled water was lower than other formulations as well as respective control.

3.4 SSC, TA and SSC: TA

The levels of SSC in 'Fortune' plum fruit after 25 d cold storage were considerably lower with the fumigation and all the aqueous formulations of BC treatments, except in distilled water only, compared to the control (Table 1). Th levels of SSC did not differ among treatments and control in 40 d cold-stored 'Fortune' plum. 'Angeleno' plum fruit treated with an aqueous formulation of BC containing 0.02 % Tween® 20 showed substantially lowest levels of SSC following 25 d and 40 d cold storage (11.5 % and 13.9 %, respectively), as compared to the other treatments and control (Table 1). 'Tegan Blue' plum treated with an aqueous formulation of BC containing 5 % ethanol and fumigation exhibited lower levels of SSC (7.4 % and 6.5 %, respectively) as compared to the control and all other treatments following 40 d cold storage.

The 'Fortune' plum fruit treated with an aqueous formulation of BC containing 5 % ethanol showed higher levels of TA after 25 d and 40 d of cold storage (1.7 % and 1.4 %, respectively) as compared to control and the fruit sprayed with an aqueous formulation of BC containing distilled water only (Table 1). 'Angeleno' plum fruit sprayed with an aqueous formulation of BC containing distilled water only and control showed lower levels of TA as compared to all other treatments after 25 d and 40 d cold storage (Table 1). The levels of TA were highest (1.5 %) in the BC fumigated 'Tegan Blue' as compared to all other treatments and control after 40 d cold storage.

SSC: TA was lower in 'Fortune' plum fruit treated with an aqueous formulation of BC containing 5 % ethanol and fumigation after 25 d and 40 d cold storage, as compared to control and all other treatments (Table 1). ‘Angeleno’ plum fruit treated with an aqueous formulation of BC containing 0.02 % Tween® 20 showed lowest SSC: TA following 25 d and 40 d cold storage (11.4 and 15.5, respectively) as compared to the other treatments and control. Whilst the treatments of an aqueous formulation of BC containing 5 % ethanol and fumigation were at par with the values of BC with 0.02 % Tween® 20 (Table 1). The ‘Tegan Blue’ plum fruit fumigated with BC showed substantially lowest SSC: TA (4.4) as compared to aqueous formulation of BC containing distilled water and control, after 40 d cold storage.

3.5 Individual sugars and organic acids

‘Fortune’ plum fumigated with BC showed the least levels of glucose (2.2 g kg^{-1}) when compared to the control and all other treatments after 25 d storage. The aqueous formulation of BC containing 5 % ethanol and 0.02 % Tween® 20 were at par with the fumigation treatments (Table 2). The levels of fructose were lowest (3.0 g kg^{-1}) in ‘Fortune’ plum fruit fumigated with BC following 25 d cold storage, as compared to all other treatments and control (Table 2). The levels of glucose and fructose in 40 d cold stored ‘Fortune’ and ‘Tegan Blue’, 25 d and 40 d cold stored ‘Angeleno’ plum did not differ noticeably among treatments and control. Irrespective of 25 d or 40 d cold storage period, the treatments of fumigation and a spray of aqueous formulations of BC did not markedly affect the levels of sucrose and sorbitol in Fortune’ plum fruit. Fumigation and a spray of all aqueous formulations of BC, except aqueous formulations of BC containing distilled water only, exhibited reduced levels of sucrose in 25 d and 40 d cold stored ‘Angeleno’ plum fruit (Table 2). The ‘Angeleno’ plum fumigated with BC exhibited lowest sorbitol levels following 25 d and 40 d of cold storage (5.7 g kg^{-1} and 5.6 g kg^{-1}, respectively). Whilst, in 40 d cold-stored ‘Tegan Blue’ plum fruit, the control fruit had the lowest level of sucrose (1.6 g kg^{-1}) when compared to fumigation and spray of aqueous formulations of BC (Table 2). The levels of sorbitol in the fruit treated with BC fumigation did not differ clearly with that of the fruit sprayed with different aqueous formulations of BC and control in 40 d cold-stored ‘Tegan Blue’ plum fruit.

Among the individual organic acids quantified, malic acid was the predominant one in all three plum cultivars tested (Table 3). Fumaric acid was not detected in ‘Tegan Blue’ plum cold-stored for 40 d. The fumigation and all aqueous formulations of BC treatments did not affect the levels of malic acid and succinic acid in the 25 d and 40 d cold stored ‘Fortune’ and ‘Angeleno’ plum fruit. All BC treatments did not affect the levels of citric acid and fumaric acid in the 40 d cold-stored ‘Fortune’ and ‘Angeleno’ plum fruit. All the BC treatments showed no remarkable effect on the levels of malic acid, citric acid and succinic acid in Tegan Blue plum following 40 d cold storage.

3.6 Total phenols, ascorbic acid, total anthocyanins and total antioxidant capacity

The levels of total phenols and antioxidant capacity were not influenced by BC fumigation and different aqueous formulations, regardless of cultivars and storage periods tested. The levels of ascorbic acid in ‘Fortune’ plum treated with BC fumigation and aqueous formulations were considerably higher as compared to control after 25 d cold storage (Supplementary Table 1). ‘Fortune’ plum fruit fumigated with BC showed highest levels of ascorbic acid (19.3 g kg^{-1}) following 40 d cold storage (Supplementary Table 1). ‘Fortune’ plum fruit fumigated with BC exhibited lowest levels of total anthocyanins as compared to the control and all other treatments following 25 d and 40 d cold storage (26.4 g kg^{-1} and 27.8 g kg^{-1}, respectively) (Supplementary Table 1). The application of all BC formulations reduced the levels of total anthocyanins in ‘Angeleno’ plum as compared to control after 40 d cold storage (Supplementary Table 1). The ‘Tegan Blue’ plum fruit sprayed with an aqueous solution containing BC and 0.02 % Tween® showed the lowest levels of total anthocyanins (22.5 g kg^{-1}) when compared to all other treatments and the control. The anthocyanin values in the ‘Tegan Blue’ plum treated with the aqueous formulation of BC containing 5 % ethanol (25.2 g kg^{-1}) as well as BC fumigation (24.2 g kg^{-1}) were at par with BC and 0.02 % Tween® formulation (Supplementary Table 1).

4. Discussion

The effects of different aqueous formulations, fumigation of BC and cold storage period on climacteric ethylene production as well as on fruit quality parameters of early, mid-season and late-maturing cultivars of Japanese plum have been investigated for the first time. Ethylene production in early maturing cultivar ‘Fortune’ was relatively higher than that of mid-and late-maturing cultivars ‘Tegan Blue’ and ‘Angeleno’ indicating two distinct patterns of ethylene production i.e., suppressed-climacteric and climacteric fruit ripening (Minas et al. (2015). Ethylene production of all the Japanese plum cultivars tested declined as the cold storage period extended from 25 d to 40 d. The prolonged cold storage may cause damage to the enzymatic system involved in biosynthesis of ethylene such as 1-amino-cyclopropane carboxylic acid oxidase (ACO) and 1-aminocyclopropane-1-carboxylate synthase (ACS). It was also previously reported that the capacity of ‘Laetitia’ plum fruit to produce ethylene decreased with prolonged exposure to low temperature (Argenta et al., 2003).

Aqueous formulations of BC, regardless of the adjuvant applied, suppressed ethylene production in all the Japanese plum cultivars tested, following both cold storage periods. BC formulations suppressed climacteric ethylene production in all the plum cultivars tested following both cold storage periods, except in ‘Fortune’ plum sprayed with BC solution containing only distilled water after 40 d storage (Fig 2). BC fumigation and BC aqueous solution containing ethanol was effective in delaying the climacteric ethylene peak onsets, depending on cultivar and storage period (Fig 2). Ethylene antagonists irreversibly bind with a copper co-factor of ethylene receptors and subsequently suppress the ethylene production as well as inhibit the actions of ethylene in plant (Sisler et al., 2006). According to Pirrung et al. (2008), 1-MCP, a 1-substituted cyclopropene, binds with the copper co-factor of ethylene receptors through the ring-opening mechanism and inhibits the action of ethylene. BC has a cyclopropene fused to a benzene ring (Halton, 1973) and the mechanism of BC in blocking the ethylene receptors is anticipated to be similar to the ethylene receptor blocking mechanism of 1-MCP (Singh et al., 2018). The reduction in production of ethylene through the application of 1-MCP has been previously reported in ‘Tegan Blue’ (Khan and Singh, 2007) and in ‘Black Amber’, ‘Black Splendor’ and ‘Yummy Beaut’ plums (Minas et al., 2013). The ethylene antagonistic potency of 1-

MCP is highest when applied as a fumigant (Sisler, 2006). Similarly, the BC fumigation outperformed the spray of aqueous formulations of BC containing different adjuvants in suppressing the ethylene production in all the tested Japanese plum cultivars. Aqueous formulations of BC containing 5 % ethanol or 0.02 % Tween® 20 were comparatively more efficient in reducing climacteric ethylene production, than the rest of BC aqueous formulations. The presence of ethanol enhances the delivery of active ingredient by increasing its solubility (Grichko, 2006) and by reducing the barrier properties of fruit cuticle which is composed of lipid compounds. Farag et al. (1992) reported that ethanol enhanced the diffusion of ethephon through the fruit cuticle resulting in increased anthocyanin accumulation in cranberries. Having the amphiphilic molecular structure, Tween® 20 also increases the water solubility of BC as well as promotes infiltration of the active compound into the fruit by increasing the permeability of cuticle (Castro et al., 2014). Considering the facts mentioned, possibly the adjuvants, especially ethanol and Tween® 20 could have improved the penetration of BC compounds to reach the targeted fruit cells where the ethylene antagonistic actions occur as depicted in Figure 4.

As the consequential effects of suppressed ethylene production, the reduction in weight loss and higher fruit firmness were also surpassed in the fruit fumigated with BC and treated with aqueous formulations of BC containing 5 % ethanol and 0.02 % Tween® 20. The activity of enzymes responsible for the breakdown of cell wall structure during fruit ripening process is initiated by ethylene (Khan and Singh, 2007). The higher fruit firmness retention in BC treated plum fruit may be attributed to the reduction in ethylene production and/or its action, leading to the lowered activities of enzymes responsible for fruit softening. Similarly, commercial ethylene antagonist 1-MCP slow down the reduction of fruit firmness by downregulating the activity of enzymes such as *endo*- and *exo*- polygalacturonase pectin esterase, pectinesterase and *endo*-1,4-β-*D*-glucanase, which responsible for fruit softening, in ‘Tegan Blue’ plum (Khan and Singh, 2007). Likewise, the effects of ethylene antagonist on lowering weight loss and maintaining fruit firmness have also been documented in 1-MCP treated ‘Santa Rosa’ and ‘Golden Japan’ plums (Martinez-Romero et al., 2003).

The lower levels of SSC, SSC: TA and higher TA resulted in the fruit treated with BC could be the after-effects of the retarded fruit ripening process associated with the suppressed ethylene production. Earlier, Martinez-Romero et al. (2003) reported that regulation of ethylene using 1-MCP delays the accumulation of SSC levels and reduction of TA levels in *Prunus* species, during cold storage. The application of the ethylene antagonist 1-MCP (0.6 μL L^{-1}) for 24 h at low temperature (0 or 8°C), noticeably reduced the levels of SSC, SSC: TA in ‘Sungold’ plum (Velardo-Micharet et al., 2017). While higher TA was maintained in cold stored ‘Red Lane’ and ‘Black Amber’ plums fumigated with 0.5 μL L^{-1} of 1-MCP (Minas et al., 2013).

Fructose was the major sugar, while malic acid was predominant organic acid in ‘Fortune’, ‘Tegan Blue’ and ‘Angeleno’ Japanese plum cultivars studied. The concentrations of individual sugars and organic acids varied among the cultivars. The present results are in agreement with the previously reported results of Singh et al. (2009) that concentrations of individual sugars varied among ‘Black Amber’, ‘Angeleno’ and ‘Amber Jewel’ plums. The individual sugars and organic acids responded differently to the BC treatment without any specific trend and the significance of the treatments varied depending upon the formulations, cultivars, and cold storage periods. The role of ethylene in sugar biosynthesis varies depending on the type of sugar in plums, without any regard to their ripening behaviour. Ethylene induces anabolism of sucrose while it hastens the catabolism of sorbitol in both climacteric and non-climacteric types of plums (Farauch et al., 2020). In the present study, BC might have interfered the biosynthesis processes of individual sugars resulting lower contents of glucose, fructose and sucrose. Sun et al. (2021) also reported that the preharvest regulation with liquid 1-MCP retarded the biosynthesis process, but in storage-period-dependent manner, of glucose, fructose, sucrose and reducing sugars in ‘Starkrimson’ apple. Earlier, Watkins (2015) explained that the effect of ethylene antagonist on the quality parameters of fruit differed with different genotypes, concentration applied and exposure time.

In the present study, BC treatments did not have a noticeable effect on the levels of total phenol and antioxidant regardless of cultivars and cold storage periods. Defilippi et al. (2004) also revealed a similar trend in the levels of total phenol in 'Greensleeves' apples treated with 1-MCP. The ascorbic acid levels in 'Fortune' plum fruit treated with BC fumigation and aqueous formulation of BC containing 0.02 % Tween® 20 remained high following both the cold storage periods. The ripening process involves several oxidative reactions and depletes antioxidant compounds such as phenols and ascorbic acid. The retainment of higher ascorbic acid content is a result of the delayed ripening process affected by ethylene antagonist (Masia, 1998). Irrespective of the adjuvants, the concentrations of anthocyanins lowered with the application of aqueous formulations of BC, but the responses were diverse depending on the cultivars. The up regulation of the genes associated with anthocyanin biosynthesis is influenced by ethylene (Cheng et al., 2016). Therefore, the reduced anthocyanin levels in the plum treated with BC could be ascribed to the consequent action of ethylene antagonist in retarding the production and action of ethylene in the fruit.

5. Conclusion

BC fumigation, as well as aqueous solutions of BC containing adjuvants, have the potential to maintain the postharvest quality by retarding ethylene production in Japanese plum fruit following cold storage. BC fumigation outperformed the aqueous formulations of BC in suppressing ethylene production. Among the aqueous formulations of BC, the ones with 5 % ethanol or 0.02 % Tween® 20 as adjuvant were relatively more effective in antagonizing ethylene action in the plum cultivars studied. The effect of BC on the quality parameters such as fruit firmness, weight loss, SSC, TA, SSC: TA, ascorbic acid, total anthocyanins, individual sugars and organic acids varied with the type of adjuvant applied, plum cultivars and storage period. Aqueous formulations of BC could therefore be an alternative option for ethylene management along the different stages of supply chain or as preharvest application in plum fruit industry.

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Figure (s):

Figure 1. Kyaw *et al.*

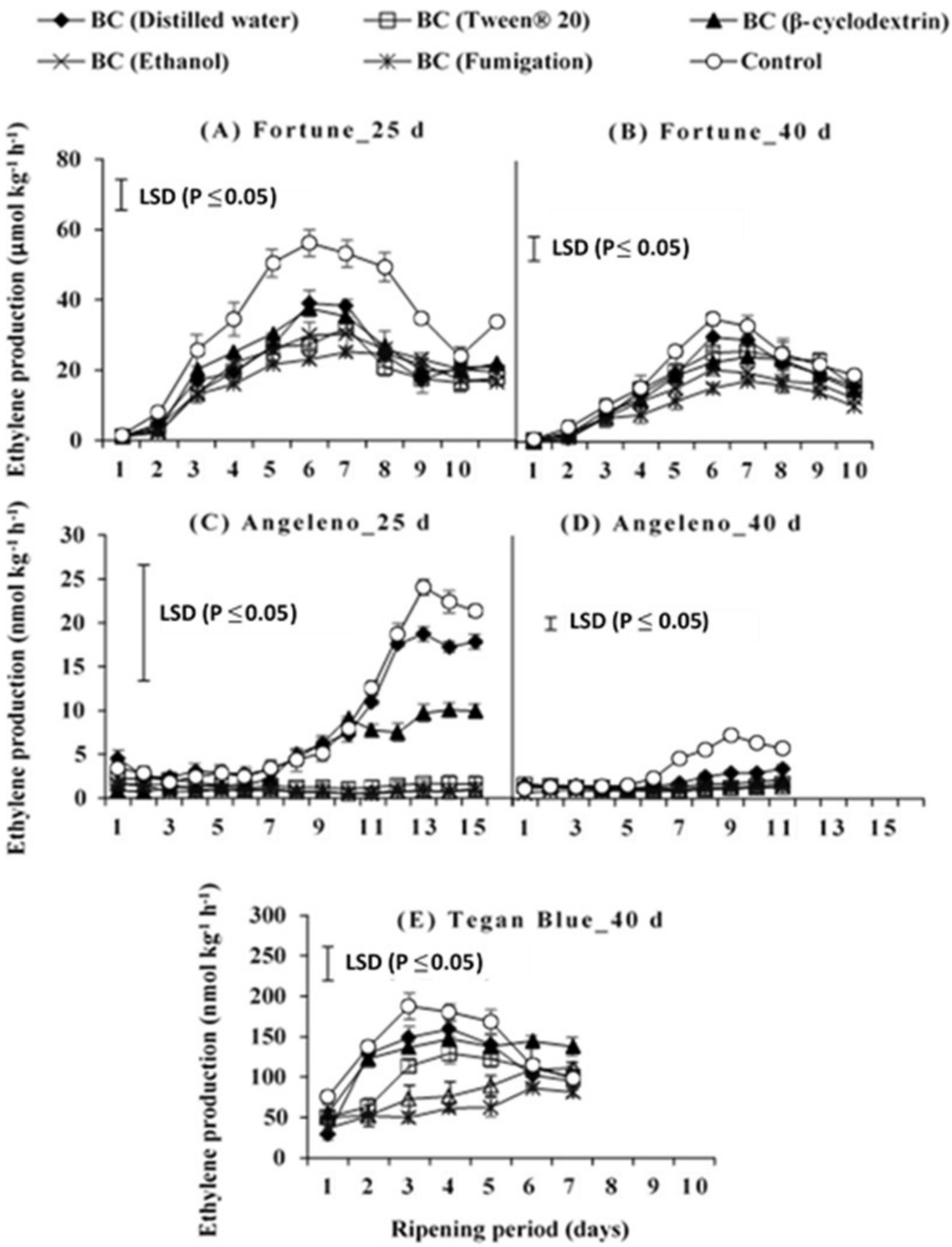

Figure 1. Ethylene production of 'Fortune' (A) cold-stored for 25 d and (B) for 40 d, 'Angeleno' (C) cold-stored for 25 d and (D) for 40 d, and 'Tegan Blue' (E) cold-stored for 40 d, and treated with different BC formulations during the ripening period at 20 °C. Vertical bars are SE of means of three replicates.

Figure 2. Kyaw *et al.*

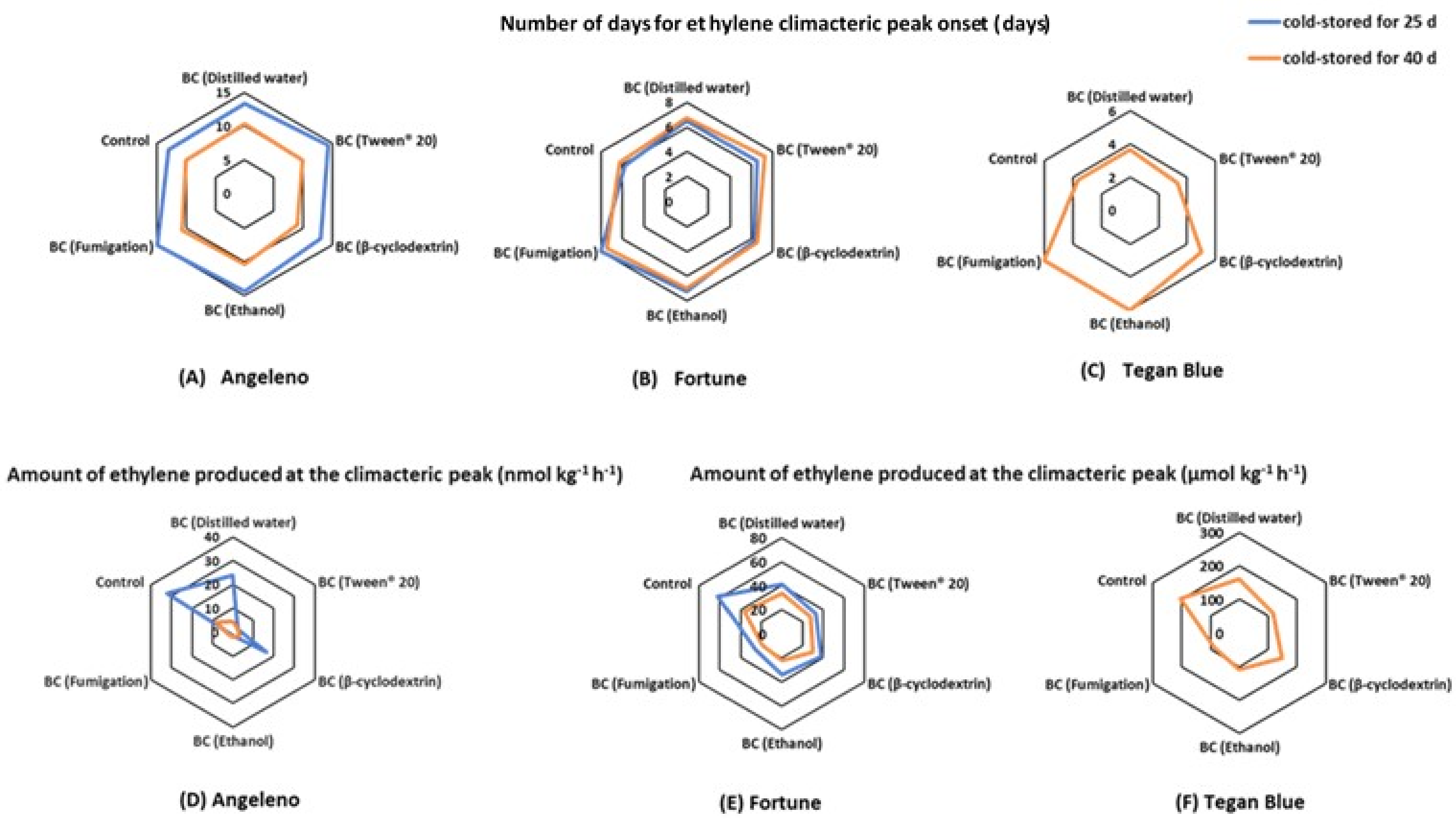

Figure 2. Number of days for ethylene climacteric peak onset (A, B, C) and amount of ethylene produced at the climacteric peak (D, E, F) of cold-stored ‘Angeleno’, ‘Fortune’ and ‘Tegan Blue’ plums treated with different BC formulations during the ripening period at 20 °C. In case of ‘Fortune’ plum cold-stored for 25 d which was represented with blue line in Fig. 2 (A), it needs average 15 d to occur the onset of ethylene climacteric peak in BC fumigation treatment, whilst it needs only 13 d in control. It means that control fruit ripened 2 d earlier than the plums treated with BC fumigation.

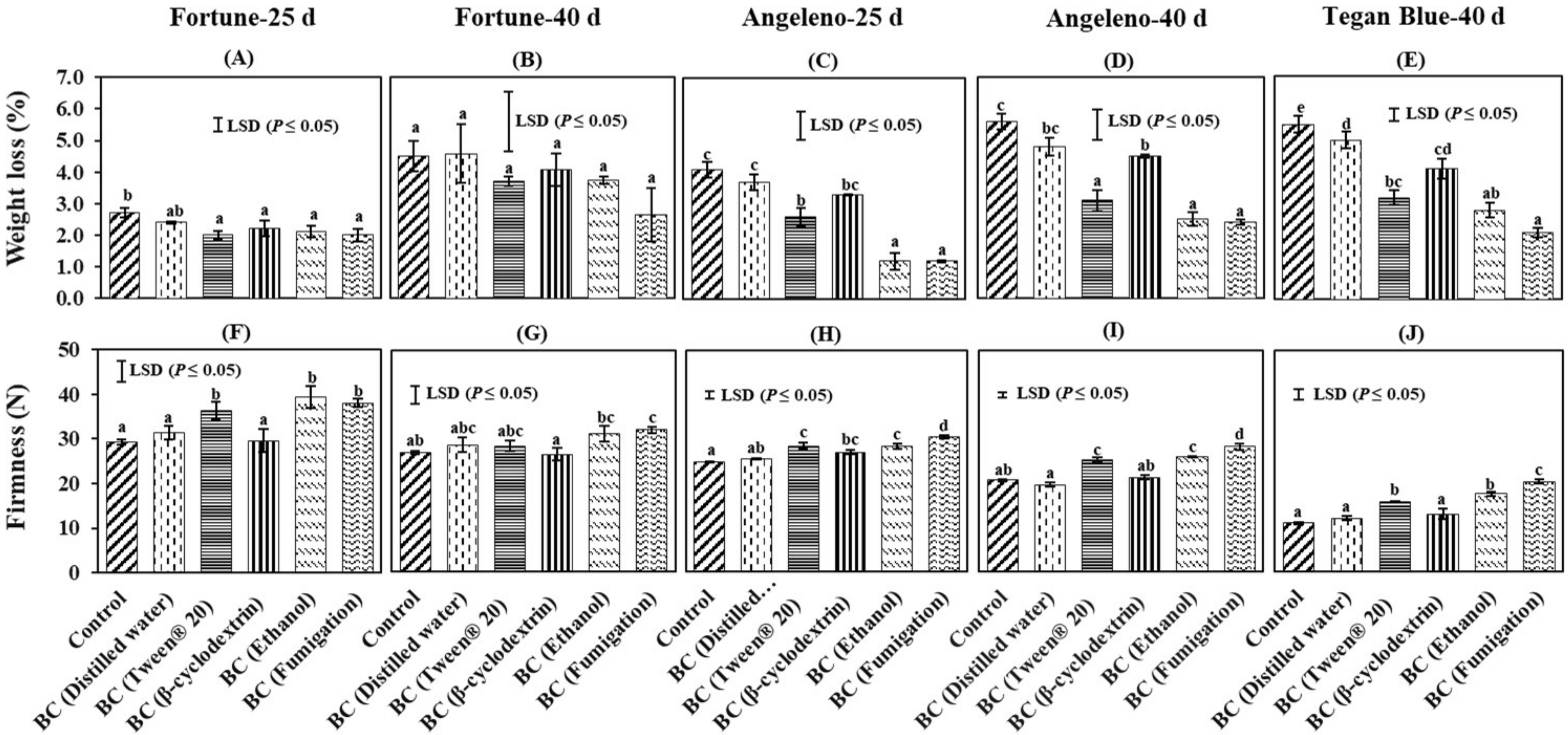

Figure 3. Physiological weight loss (A to E) and firmness (F to J) of 'Fortune', 'Angeleno' and 'Tegan Blue' plums treated with different formulations of BC following 25 and 40 days cold storage. Vertical bars are SE of means of three replicates. The treatments with the same letter are not significantly different from each other.

Figure 4. Kyaw *et al.*

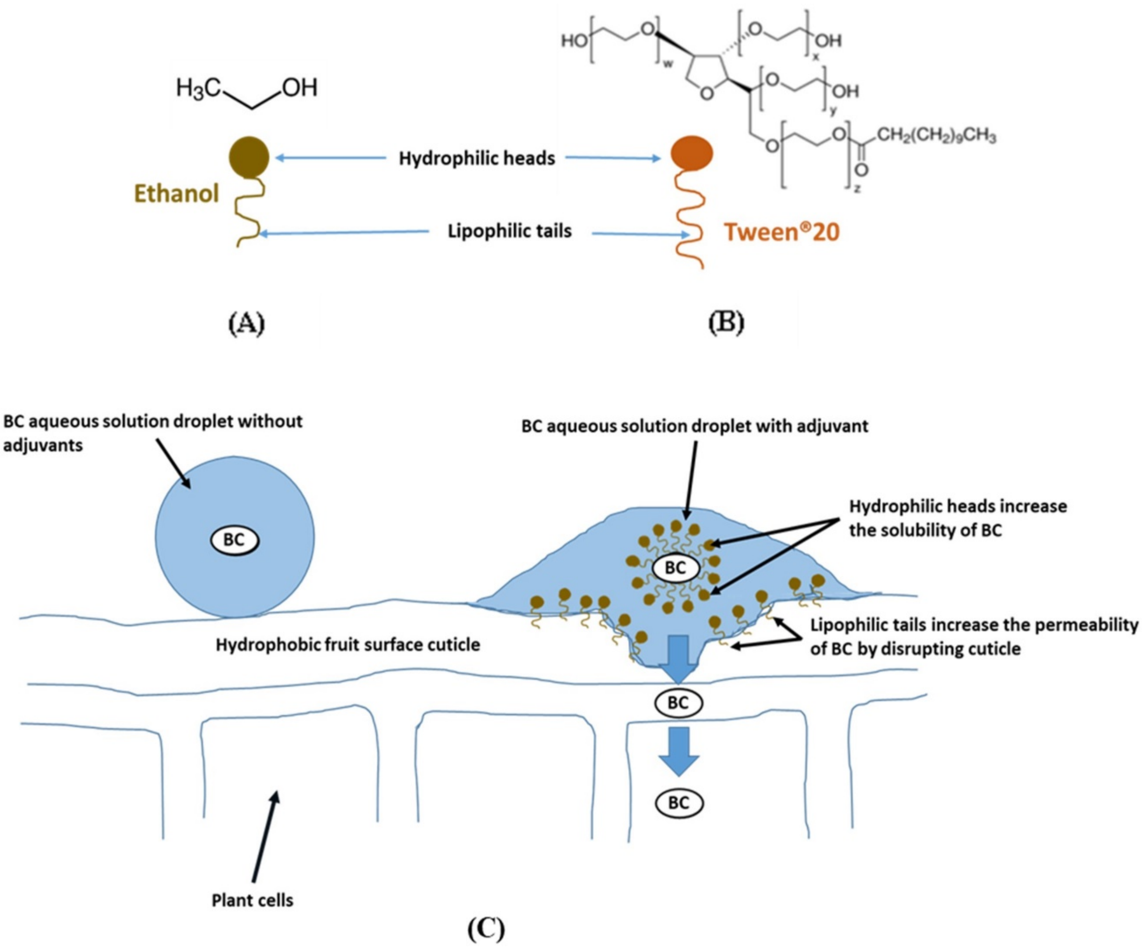

Figure 4. Structures of ethanol (A) and Tween® 20 (B) and the proposed BC-performance enhancing mechanism (C). The hydrophilic heads and the lipophilic tails of ethanol and Tween® 20 may have facilitated to increase the solubility of BC in aqueous solutions and the permeability of BC through the fruit surface cuticle. Which, in turn, might have allowed the higher infiltration and better action of BC.

Table (s):

Table 1. SSC, TA and SSC: TA of Fortune, Angeleno and Tegan Blue plums affected by different formulations of BC following 25 and 40 d cold storage at 1 °C .

Cultivar	Treatment	SSC (%)		TA (%)		SSC: TA	
		25 d	40 d	25 d	40 d	25 d	40 d
Fortune	Control	11.3±0.2b	10.9±0.2	1.5±0.0ab	1.3±0.02ab	7.5±0.1b	8.3±0.2ab
	BC (Distilled water)	10.8±0.1ab	11.1±0.1	1.5±0.1 a	1.3±0.02a	7.3±0.2b	8.9±0.2b
	BC (Tween® 20)	10.3±0.4a	10.7±0.2	1.6±0.1bc	1.3±0.04ab	6.5±0.2a	8.0±0.2ab
	BC (β-cyclodextrin)	10.3±0.1a	10.8±0.1	1.5±0.0abc	1.2±0.02a	6.7±0.2a	8.8±0.2b
	BC (Ethanol)	10.2±0.3a	10.7±0.2	1.7±0.0c	1.4±0.07b	6.2±0.1a	7.5±0.4a
	BC (Fumigation)	10.3±0.2a	10.3±0.3	1.6±0.0bc	1.3±0.02ab	6.4±0.1a	7.7±0.3a
	LSD ($P \leq 0.05$)	0.60*	ns	0.11*	0.11*	0.51**	0.82*
Angeleno	Control	14.1±0.1d	16.1±0.1e	0.7±0.02a	0.6±0.03a	19.7±0.4c	24.2±1.0c
	BC (Distilled water)	13.4±0.1cd	15.9±0.1de	0.7±0.03a	0.7±0.05ab	18.3±0.9c	21.2±1.3bc
	BC (Tween® 20)	11.5±0.5a	13.9±0.2a	1.0±0.00c	0.9±0.07c	11.4±0.5a	15.5±1.2a
	BC (β-cyclodextrin)	13.3±0.5cd	15.5±0.2cd	0.8±0.03b	0.8±0.02bc	15.3±0.3b	18.8±0.6ab
	BC (Ethanol)	12.1±0.5ab	14.9±0.1bc	0.9±0.02c	0.8±0.03bc	12.6±0.6a	17.3±0.7a
	BC (Fumigation)	12.7±0.2bc	14.8±0.2b	1.0±0.02c	0.8±0.02bc	12.3±0.2a	18.0±0.6ab
	LSD ($P \leq 0.05$)	1.01*	0.54**	0.08**	0.14*	2.05**	3.12*
Tegan Blue	Control		11.8±0.1c		1.1±0.03a		11.0±0.2e
	BC (Distilled water)		9.9±0.4b		1.1±0.03ab		8.7±0.6d
	BC (Tween® 20)		9.6±0.5b		1.3±0.03b		7.6±0.3c
	BC (β-cyclodextrin)		10.2±0.3b		1.2±0.03ab		8.4±0.1d
	BC (Ethanol)		7.4±0.2a		1.3±0.02b		5.7±0.1b
	BC (Fumigation)		6.5±0.1a		1.5±0.08c		4.4±0.3a
	LSD ($P \leq 0.05$)		1.10**		0.16*		0.86**

The mean values of Fortune, Angeleno and Tegan Blue are independent of each other. Mean values followed by the same letter within the columns are not significantly different. ** and * = significant at 1% and 5% level of LSD, ns=non-significant.

Table 2. Levels of individual sugars in Fortune, Angeleno and Tegan Blue plums influenced by different formulations of BC following 25 and 40 d cold stored storage at 1 °C.

Cultivar	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
Fortune	Control	4.9±0.1c	6.4±0.3	3.5±0.2abc	5.4±0.2	4.9±0.5	2.8±0.3	2.2±0.2	2.1±0.1
	BC (Distilled water)	3.9±0.3bc	7.4±0.2	4.5±0.1d	5.9±0.2	3.6±0.4	3.0±0.4	2.2±0.1	2.1±0.1
	BC (Tween® 20)	3.2±0.3ab	5.7±0.4	3.2±0.2ab	5.0±0.2	2.6±0.4	2.7±0.6	1.5±0.2	1.9±0.1
	BC (β-cyclodextrin)	4.5±0.5c	6.2±0.3	4.3±0.2cd	5.7±0.2	3.8±0.4	3.9±0.1	2.4±0.1	2.3±0.1
	BC (Ethanol)	2.8±0.3ab	5.8±0.6	4.0±0.2bcd	5.4±0.2	3.9±0.5	3.7±0.8	2.2±0.1	2.3±0.3
	BC (Fumigation)	2.2±0.4a	5.5±0.5	3.0±0.4a	5.4±0.3	4.1±0.7	3.9±0.5	2.0±0.3	2.4±0.2
	LSD ($P \leq 0.05$)	1.1**	ns	0.8*	ns	ns	ns	ns	ns
Angeleno	Control	17.0±0.3	18.4±0.3	25.5±1.1	26.4±0.5	2.9±0.1cd	3.5±0.4b	7.3±0.2c	6.8±0.2b
	BC (Distilled water)	17.0±0.4	18.7±0.2	25.4±0.6	26.9±0.2	3.1±0.0d	3.3±0.3ab	7.1±0.2bc	6.9±0.2b
	BC (Tween® 20)	15.9±0.1	17.9±0.1	23.7±0.2	25.7±0.2	2.7±0.0bc	3.3±0.4a	6.3±0.1ab	5.6±0.2a
	BC (β-cyclodextrin)	17.3±0.2	18.6±0.1	26.2±0.3	26.6±0.3	2.5±0.1b	3.2±0.5a	6.4±0.2ab	7.1±0.2b
	BC (Ethanol)	16.3±0.4	18.7±0.3	24.3±0.7	26.8±0.5	2.5±0.1b	3.1±0.3a	6.1±0.2a	7.5±0.3b
	BC (Fumigation)	16.8±0.1	18.5±0.3	23.3±0.3	25.3±0.3	2.0±0.1a	3.2±0.7a	5.7±0.2a	5.6±0.3a
	LSD ($P \leq 0.05$)	ns	ns	ns	ns	0.3**	0.2*	0.8*	0.9*
Tegan Blue	Control		5.7±0.2		4.7±0.0		1.6±0.1a		2.5±0.1
	BC (Distilled water)		5.7±0.8		4.6±0.0		2.1±0.2ab		2.4±0.4
	BC (Tween® 20)		5.1±0.2		4.4±0.0		2.2±0.2ab		2.0±0.3
	BC (β-cyclodextrin)		5.4±0.5		4.7±0.0		2.9±0.1bc		2.6±0.2
	BC (Ethanol)		4.9±0.2		4.0±0.0		3.2±0.5c		2.7±0.2
	BC (Fumigation)		3.7±0.1		4.3±0.0		2.3±0.2abc		2.1±0.2
	LSD ($P \leq 0.05$)		ns		ns		0.9*		ns

The mean values of Fortune, Angeleno and Tegan Blue are independent of each other. Mean values followed by the same letter within the columns are not significantly different. ** and * = significant at 1% and 5% level of LSD, ns=non-significant.

Table 3. Levels of individual organic acids in Fortune, Angeleno and Tegan Blue plums influenced by different formulations of BC following 25 and 40 d cold stored storage at 1 °C.

Cultivar	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
Fortune	Control	3.5±0.4a	3.4±0.3	0.04±0.0a	0.04±0.0	0.03±0.0a	0.03±0.0	0.4±0.0a	0.3±0.3
	BC (Distilled water)	3.8±0.1ab	3.4±0.2	0.04±0.0ab	0.05±0.0	0.03±0.0a	0.02±0.0	0.4±0.0a	0.3±0.1
	BC (Tween® 20)	4.3±0.1b	3.9±0.2	0.05±0.0abc	0.05±0.0	0.03±0.0a	0.02±0.0	0.4±0.0a	0.4±0.1
	BC (β-cyclodextrin)	3.6±0.2a	3.5±0.1	0.05±0.0bc	0.05±0.0	0.03±0.0a	0.02±0.0	0.4±0.0a	0.3±0.1
	BC (Ethanol)	3.6±0.2a	3.7±0.2	0.05±0.0c	0.04±0.0	0.03±0.0b	0.02±0.0	0.4±0.0a	0.3±0.3
	BC (Fumigation)	4.1±0.1ab	3.8±0.2	0.05±0.0c	0.04±0.0	0.03±0.0a	0.02±0.0	0.4±0.0a	0.3±0.1
	LSD ($P \leq 0.05$)	ns	ns	0.01*	ns	0.003 **	ns	ns	ns
Angeleno	Control	2.9±0.3	2.7±0.1	0.06±0.0	0.07±0.0	0.02±0.0ab	0.02±0.0	0.5±0.0	0.5±0.2
	BC (Distilled water)	2.9±0.1	2.9±0.1	0.06±0.0	0.06±0.0	0.02±0.0a	0.02±0.0	0.5±0.0	0.5±0.1
	BC (Tween® 20)	3.0±0.1	3.0±0.0	0.07±0.0	0.06±0.0	0.02±0.0b	0.02±0.0	0.5±0.0	0.5±0.2
	BC (β-cyclodextrin)	2.8±0.1	2.9±0.0	0.06±0.0	0.07±0.0	0.02±0.0a	0.02±0.0	0.5±0.0	0.5±0.1
	BC (Ethanol)	3.0±0.1	3.0±0.2	0.07±0.0	0.06±0.0	0.02±0.0a	0.02±0.0	0.5±0.0	0.5±0.2
	BC (Fumigation)	3.1±0.0	3.1±0.0	0.07±0.0	0.06±0.0	0.02±0.0ab	0.02±0.0	0.6±0.0	0.5±0.2
	LSD ($P \leq 0.05$)	ns	ns	ns	ns	0.001*	ns	ns	ns
Tegan Blue	Control		2.1±0.0		0.02±0.0		nd		0.09±0.0
	BC (Distilled water)		2.1±0.0		0.02±0.0		nd		0.08±0.0
	BC (Tween® 20)		2.1±0.0		0.02±0.0		nd		0.09±0.0
	BC (β-cyclodextrin)		2.1±0.0		0.02±0.0		nd		0.09±0.0
	BC (Ethanol)		2.1±0.0		0.02±0.0		nd		0.09±0.0
	BC (Fumigation)		2.1±0.0		0.02±0.0		nd		0.08±0.0
	LSD ($P \leq 0.05$)		ns		ns				ns

The mean values of Fortune, Angeleno and Tegan Blue are independent of each other. Mean values followed by the same letter within the columns are not significantly different. ** and * = significant at 1% and 5% level of LSD, ns=non-significant, nd=not detected.

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Supplementary Table 1. Levels of total phenols, ascorbic acid, antioxidant capacity and anthocyanin in Fortune, Angeleno and Tegan Blue plums treated with different formulations of BC and stored for 25 and 40 d at 1°C.

Cultivar	Treatment	Total phenols ($g\ kg^{-1}$)		Ascorbic acid ($g\ kg^{-1}$)		Antioxidant capacity ($mol\ kg^{-1}$)		Anthocyanin ($g\ kg^{-1}$)	
		25 d	40d	25 d	40d	25 d	40 d	25 d	40 d
Fortune	Control	72.3±2.9	84.3±5.4	11.9±0.3a	16.3±0.3ab	0.02±0.0	0.02±0.0	36.4±1.9c	37.1±1.7b
	BC (Distilled water)	74.9±2.2	85.9±5.9	13.8±0.8b	15.6±0.7a	0.02±0.0	0.02±0.0	35.4±2.0c	35.6±1.3b
	BC (Tween® 20)	83.0±7.6	78.0±2.5	16.7±0.6d	17.8±0.6c	0.02±0.0	0.02±0.0	30.6±1.0ab	29.3±1.2a
	BC (β-cyclodextrin)	68.6±2.3	78.7±8.8	15.5±1.1cd	16.1±0.7a	0.02±0.0	0.02±0.0	34.4±0.6bc	32.6±0.7ab
	BC (Ethanol)	69.2±3.9	71.7±8.1	15.6±0.5cd	17.7±0.5bc	0.02±0.0	0.02±0.0	33.6±0.6bc	30.6±0.8a
	BC (Fumigation)	67.5±5.8	70.3±2.8	14.9±0.8bc	19.3±0.6d	0.02±0.0	0.02±0.0	26.4±0.2a	27.8±0.9a
	LSD ($P \leq 0.05$)	ns	ns	1.54**	1.45**	ns	ns	4.13*	4.51*
Angeleno	Control	86.3±4.6	87.8±3.5	11.1±0.8	12.3±1.1	0.02±0.0	0.02±0.0		13.4±0.5b
	BC (Distilled water)	77.1±4.2	84.3±5.3	11.3±0.5	12.6±0.1	0.02±0.0	0.02±0.0		9.72±0.5a
	BC (Tween® 20)	78.9±2.9	83.6±7.7	11.2±0.1	12.2±0.1	0.02±0.0	0.02±0.0		9.51±0.6a
	BC (β-cyclodextrin)	82.3±3.3	92.6±3.9	11.1±0.3	11.7±0.5	0.02±0.0	0.02±0.0		8.94±1.0a
	BC (Ethanol)	72.5±5.7	78.7±2.9	11.2±0.6	12.3±0.2	0.02±0.0	0.02±0.0		9.95±1.0a
	BC (Fumigation)	79.5±6.8	94.8±2.7	11.3±0.5	12.6±0.5	0.02±0.0	0.02±0.0		8.02±1.1a
	LSD ($P \leq 0.05$)	ns	ns	ns	ns	ns	ns		2.55*
Tegan Blue	Control		70.0±9.8		10.4±0.5		0.02±0.0		30.4±1.2c
	BC (Distilled water)		76.2±2.9		10.0±0.2		0.02±0.0		36.4±3.3d
	BC (Tween® 20)		71.5±11.2		11.2±0.2		0.01±0.0		22.5±1.4a
	BC (β-cyclodextrin)		75.6±10.9		10.8±0.6		0.02±0.0		29.0±1.4bc
	BC (Ethanol)		82.2±15.8		10.6±0.5		0.02±0.0		25.2±2.4ab
	BC (Fumigation)		80.6±6.4		10.9±1.1		0.02±0.0		24.2±0.8a
	LSD ($P \leq 0.05$)		ns		ns		ns		4.50**

The mean values of Fortune, Angeleno and Tegan Blue are independent of each other. Mean values followed by the same letter within the columns are not significantly different. ** and * = significant at 1% and 5% level of LSD, ns=non-significant.