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Research paper

Effects of 1-MCP in combination with Ca application on aroma volatiles production and softening of 'Fuji' apple fruit

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ABSTRACT

1-Methylcyclopropene (1-MCP) can maintain the physical quality of climacteric fruits, such as apple and pear, but inhibit aroma volatile production and fruit flavor. In this study, the effects of calcium (Ca) in combination with 0.6 and 1.0 μL L^{-1} 1-MCP on flesh firmness and aroma volatiles has been investigated on 'Fuji' apples stored at room temperature. Results from electronic nose detection and texture evaluation showed that 1-MCP of reduced concentration (0.6 μLL^{-1}), but not 1.0 μL L^{-1}, presented an interactive effect with Ca application on promoting volatile emission and reducing softening. The amount of branched and straight esters and total aroma volatiles, as well as related enzymes including aromatase-related acyltransferase (AAT), alcohol dehydrogenase (ADH), and pyruvate decarboxylase (PDC), were significantly higher in fruit treated with (1-MCP 0.6 + Ca) than 1-MCP 1.0 after 50 and 100 days storage. There was no significant difference between two treatments of (1-MCP 0.6 + Ca) and 1-MCP 1.0 in maintaining fruit firmness. Fruit treated with (1-MCP 0.6 + Ca) had higher aroma quality than 1-MCP 1.0 according to sensory evaluation, but showed no significant difference in terms of texture quality. In conclusion, 1-MCP of reduced concentration combined with Ca treatment had synergetic effect on the aroma formation and softening inhibition of apple fruit, resulting in advanced sensory quality.

1. Introduction

Volatile compounds that contribute to apple fruit aroma are an important component for consumer appeal (Daillant-Spinnler et al., 1996). Volatiles, particularly alcohols and esters, which originate primarily from lipids, proteins, and amino acids, increase during apple fruit ripening and their biosynthetic pathways are evidently regulated by ethylene (Heath and Reineccius, 1986; Fan and Mattheis, 1999). Using a transgenic line of apple with a high suppression of ethylene biosynthesis, Schaffer et al. (2007) demonstrated that some potential biochemical steps involved in the modulation of ester production are under ethylene regulation. 1-Methylcyclopropene (1-MCP), an ethylene action inhibitor, has been widely used in fruit fresh-keeping, such as apple, pear and kiwifruit, by inhibiting the formation of ethylene, and its application also confirms that volatile production, are linked to ethylene perception and signal transduction (Giovannoni, 2007; Fan and Mattheis, 1999; Marin et al., 2009; Ferenczi et al., 2006). Recent study on volatile production and gene expression of volatile biosynthesis in 'Golden Delicious' apples subjected to both ethylene and 1-MCP treatments, provides further evidence that regulation of these genes is ethylene dependent (Yang et al., 2016).

Apple volatile aroma plays an important role in improving eating quality and consumer acceptance (Yang et al., 2016). For example, in sensory evaluation on 'Pink lady' apple, sixty-five percent of consumers preferred fruit with higher emissions of aroma-active volatile compounds, despite the fact that these fruit displayed lower values for standard quality attributes (Villatoro et al., 2009). Studies on consumer acceptability of seven 'Fuji' apple strains suggested a high correlation between consumer acceptability and some of the volatile compounds emitted (Iglesias et al., 2012). 1-MCP treatment reduced flavor volatiles in 'Gala' apples and consumer difference tests showed people could distinguish between 1-MCP treated and untreated fruit (Marin et al., 2009).

Recent studies found that calcium treatment could promote fruit aroma generation. By pre-harvest calcium sprays or post-harvest calcium dipping, the activity of alcohol acyltransferase (AAT) and alcohol dehydrogenase (ADH) in 'Fuji' apples were significantly enhanced, which promoted the synthesis of ester precursor alcohols and acyl-COA, thereby increasing the emission of flavor-contributing volatile esters (Ortiz et al., 2009, 2010a). After postharvest calcium treatment, the contents of aroma substances increased in 'Golden Reinders' apples during medium-term storage (Ortiz et al., 2010b). Similar study also

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suggested that most volatile compounds contributing to overall flavor in ripe 'Nanguoli' fruit at harvest and post-harvest were enhanced in response to pre-harvest calcium applications (Wei et al., 2017).

A review of the literature shows 3.5 more studies on 1-MCP on physical rather than aroma-based criteria, which indicated that the current preservation technology generally paid more attention on keeping texture of apple fruit. However, above studies indicate that consumers also have a very positive demand for fruit aroma quality (Marin et al., 2009). Therefore, we proposed that whether calcium treatment could play a role in promoting the generation of volatile compounds in 1-MCP treated apple fruit. Thus, the objective of this study was firstly to investigate whether there was an interaction between calcium and 1-MCP applications on improving volatile aroma and reducing softening by means of electronic nose and texture evaluation, and secondly how the interactive effect occurred by chemical and enzymatic analysis.

2. Materials and methods

2.1. Samples and treatments

Experiments were carried out in 2014 and 2015, respectively. 'Fuji' apples (*Malus domestica Borkh*) were harvested at the commercial maturity from the orchard in Baishui, Shaanxi Province. Fruit were the transported to the laboratory on the same day, and visually sorted to obtain uniform size without defects. In 2014 (experiment 1), the apples were randomly divided into six groups of 30 fruit. Four groups of fruit were separately untreated (CK), dipped with 2% (w/v) $CaCl_2$ solution for 5 min (Ca), or treated with 0.6 (1-MCP 0.6) or 1.0 μL L^{-1} 1-MCP (1-MCP 1.0) for 24 h at room temperature (15 ± 2 °C). Another two groups were firstly dipped in 2% $CaCl_2$ for 5 min, and then after air-drying, exposed to 0.6 (1-MCP 0.6 + Ca) or 1.0 μL L^{-1} 1-MCP (1-MCP 1.0 + Ca) for 24 h, respectively. After treatment, all fruit were sealed with 0.04-mm-thick PE bags and stored at room temperature for 100 days. To prevent the water loss from fruit, a humidifier worked all day to keep the relative humidity to > 70%. Every 25 days, six fruit per treatment were randomly selected for firmness measurement. On day 50 and 100, the same six fruit were used for electronic nose detection.

For the year of 2015 (experiment 2), treatments of (1-MCP 0.6 + Ca), and 1-MCP 1.0 were selected for chemical and enzymatic analysis according to the results of experiment 1. Untreated fruit was set as control. Six fruit per replicate and three replicates per treatment were set for this experiment. Treated fruit were stored in the same condition as 2014 and on day 50 and 100, samples were selected for measurement of firmness, volatile compounds, and activities of ADH (EC 1.1.1.1), pyruvate decarboxylase (PDC) (EC 4.1.1.1), AAT (EC 2.3.1.84), polygalacturonase (PG) (EC3.2.1.15), pectin methylesterase (PEM) (EC3.1.1.11), and carboxymethyl-cellulase (Cx) (EC3.2.1.4), as well as for sensory evaluation.

2.2. Analytic methods

2.2.1. Flesh firmness

Flesh firmness was measured on two opposite sides of each fruit with a penetrometer (GY-4, Handpi, Wenzhou, China) equipped with an 11-mm diameter plunger tip; results were expressed in kg cm^{-2}.

2.2.2. Electronic nose measurement

Volatile compound emissions from samples in experiment 1 were monitored during storage by PEN 3 portable electronic nose (Win Muster Airsens Analytic Inc., Schwerim, Germany). The sensor array system is composed of 10 metal oxide semiconductors (MOS) of different chemical sensorsW1C (aromatic), W5S (broadrange), W3C (aromatic), W6S (hydrogen), W5C (aromatic aliphatics), W1S (broad methane), W1W (sulfur organic), W2S (broad alcohol), W2W (sulfur chlorinate), and W3S (methane aliphatics).The E-nose analyses were performed according to the methods by Rizzolo et al. (2012). The sensor response is given by the ratio of the conductivity response of the sensors to the sample gas (G) relative to the carrier gas (G0) over time (G/G0). For each treatment, six apples were randomly selected and each sample was evaluated three times.

2.2.3. Volatile compounds

In experiment 2, four intact fruits per replicate were used for sampling and each treatment had three replicates. For each replicate, four slices, with each cut from stem to blossom end from individual fruit were pulverized together in liquid N_2 into powder for enzyme extraction, and another four were mixed and homogenized for volatile compounds and aroma sensory evaluation. The rest part of each fruit was cut into pieces for texture sensory evaluation. The composition and concentration of volatile compounds was evaluated by solid-phase microextraction (SPME) analysis. 15 g slurry, 2.5 g NaCl and 10 μL of 1-octanol (2.49 mg L^{-1}, internal standard) were accurately measured and transferred to a 20 mL headspace vial. After stirring for 10 s, the mixed samples were equilibrated at 50 °C for 10 min and then exposed to a fiber coated with 50 μm of carboxen/Polydimethylsiloxane (CAR/PDMS, Supelco Co., Bellefonte) for 30 min. A GC–MS system (Trace1300-ISQ, Thermo Fisher, USA), equipped with a DB-WAX column (60 m × 0.25 mm × 0.25 μm film thickness), was used to separate and identify the volatile compounds. The volatile compounds were desorbed from the SPME fiber at 250 °C for 5 min in the GC injector under a splitless model. The chromatography conditions were as follows: injector, 250 °C for 5 min; initial oven temperature, 40 °C maintained for 2.5 min, increased at 5 °C min^{-1} to 150 °C and then increased by 10 °C min^{-1} to 230 °C and maintained for 5 min. Helium was used as a carrier gas at a rate of 1.0 mL min^{-1}. MS parameters were: source temperature of 230 °C, transfer-line temperature of 230 °C, and a scan range from 35 to 500 amu. There were three replicates of measurement for each treatment. NIST/WILEY MS Search 2.0 mass spectra libraries were used to identify the volatile compounds. Most compounds were confirmed by comparison of their linear retention indices and EI mass spectra with reference compounds. The identified compounds were quantified by the internal standard method and their concentration were normalized to that of 1-octanol.

2.2.4. Enzyme activity

For ADH and PDC extraction, 2 g of frozen powder were transferred to 10 mL tubes containing 4 mL extraction solution with 85 mol L^{-1} 2-(N-morpholino) ethane-sulfonic acid (MES) buffer, pH 6.0, 5 mmol L^{-1} dithiothreitol (DTT) and 1% (w/v) PVPP. The tubes were sealed and shaken continuously at 4 °C for 20 min. Then after centrifuged at 12,000 *g* for 20 min at 4 °C, the supernatant was recovered as crude enzyme extract. For AAT, the extraction solution contained 0.1 mol L^{-1} phosphate, pH 8.0, 1 mmol L^{-1} ethylene-diaminetetraacetic acid (EDTA), 0.1% (v/v) Triton X-100 and 1% (w/v) PVPP and other process was the same as mentioned above. ADH, PDC and AAT activities were assayed according to the methods described by Echeverrıa et al. (2004) and the results were expressed as specific activity (U mg^{-1} of protein).

For soften-related enzymes, 3 g of frozen flesh powder was stirred into 6 mL of cold 12% polyethyleneglycol containing 0.2% sodium bisulphite. After centrifuged at 12,000*g* for 10 min, the pellet was washed with 4 °C 0.2% sodium bisulfite. Then pellets were used for enzymes extraction with the solution of 0.1 M sodium acetate (pH 5.2), 100 mM NaCl, 2% (v/v) β-mercaptoethanol, and 5% (w/v) polyvinylpyrrolidone (PVP) at 4 °C for 1 h. After centrifugation as above, the supernatant was used to assay for enzyme activity according to the methods by Wei et al. (2010). All measurements were carried out in three replicates and each replicate contained four intact apples per treatment.

2.2.5. Descriptive sensory evaluation

The descriptive sensory analysis of fruit samples from each treatment were performed by a trained panel after 100 days storage,