Postharvest Biology and Technology 131 (2017) 55–67

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Relationships between cuticular waxes and skin greasiness of apples during storage

Yanqing Yang, Bin Zhou, Jing Zhang, Cong Wang, Cuihua Liu, Yanli Liu, Xianbo Zhu, Xiaolin Ren*

College of Horticulture, Northwest Agricultural & Forestry University, Yangling, Shaanxi 712100, China

ARTICLE INFO

Keywords:
Apples
Cuticular wax
Skin greasiness
During storage
Greasy esters
Wax morphology

ABSTRACT

Certain apple (*Malus domestica* Borkh.) cultivars develop a greasy surface when overripe and the wax changes are considered to be responsible for this disorder, but the contributing wax composition for skin greasiness remains unclear. Cuticular wax composition and wax morphologies of three cultivars were analyzed during storage at 20 °C. 'Jonagold' rapidly became greasy over a 20 d storage period but 'Red Delicious' did not. 'Cripps Pink' showed a slower greasiness development than 'Jonagold' over 70 d of storage. Alkanes, principally nonacosane, and fatty alcohols, principally nonacosan-10-ol contributed to the formation of the three-dimensional structure of waxes. Accumulation of more fluid wax constituents, which consist mainly of linoleate and oleate esters of (E,E)-farnesol and short-chain alcohols (C_3-C_5), led to the solid-liquid phase change of waxes, ultimately causing the greasy feeling on 'Jonagold' and 'Cripps Pink' apples. Compared with 'Jonagold', the increase of liquid fractions in 'Red Delicious' was much smaller and the wax crystals remained intact after the same storage period. The intact farnesyl esters were profiled for the first time using GC–MS. The results for 'Cripps Pink' suggest that the increase in solid fractions (alkanes and fatty alcohols) may lessen the effects of the fluid fractions on greasiness by maintaining wax structure. Finally, a hypothesis is proposed for ester production associated with greasiness development.

1. Introduction

The outer surfaces of all land plants are covered by a continuous hydrophobic layer-the cuticle. This layer consists of both cuticular waxes and cutin. The wax layer forms the first barrier against the environment and is effective in minimizing water loss and protecting against physical, chemical and biological attack, as well as mechanical support to maintain organ integrity (Lara et al., 2014). The cuticular waxes are in two layers: the intracuticular waxes which are embedded within the cutin polymer matrix and the epicuticular waxes which lie on the outer surface of the cutin polymer (Koch and Ensikat, 2008). The self-assembly of the epicuticular waxes result in a wide range of crystal types, including plates, platelets, ribbons, rodlets, threads and crusts (Barthlott et al., 1998; Jeffree, 2008). Most waxes are composed of aliphatic compounds (C_{20}-C_{34}) which originate from the elongation of C_{16} and C_{18} free fatty acids, including alkanes, primary and secondary fatty alcohols, aldehydes, ketones, and esters (Kunst and Samuels, 2003). Large amounts of terpenoids with ring structures are also found in the intracuticular waxes (van Maarseveen and Jetter, 2009).

In apples (*Malus domestica* Borkh.), the C_{29} homologues are the most abundant components of the wax layer (Belding et al., 1998; Verardo et al., 2003). The composition of cuticular waxes varies in different apple cultivars (Belding et al., 1998; Bringe et al., 2006) and also changes with external preharvest conditions including wind (Gardingen et al., 1991), temperature (Lurie et al., 1996), and light (Letchamo and Gosselin, 1996). Waxes reduce transpiration (Blanke and Holthe, 1997), limit surface permeability (Baur et al., 1996) and prevent the invasion of microorganism (Marcell and Beattie, 2002; Markstädter et al., 2000). The physical and chemical properties of wax composition also determine the apple's appearance during the postharvest storage (Glenn et al., 1990), one of the most important quality components determining consumer demand. Therefore, changes in wax properties can have economic consequences.

Despite its importance, limited information is available on variations in the chemical composition of apple waxes during storage, especially in relation to specific fruit surface characteristics. The surface waxes of some apple cultivars, such as 'Jonagold', 'Royal Gala' and 'Granny Smith' may become unpleasantly greasy during storage (Curry, 2008; Fan et al., 1999; Veraverbeke et al., 2001). This disorder is among the most unappealing quality attributes of fresh fruit at point of

* Corresponding author.
E-mail addresses: renxl@nwsuaf.edu.cn, caihou2014@163.com (X. Ren).

http://dx.doi.org/10.1016/j.postharvbio.2017.05.006
Received 26 January 2017; Received in revised form 12 May 2017; Accepted 12 May 2017

sale (Richardson-Harman et al., 1998). The most accepted view about greasiness development is related to changes in the production of wax lipids in stored apples (Curry, 2008; Dadzie et al., 1995; Fan et al., 1999; Veraverbeke et al., 2001). A few researchers have examined wax changes in apple fruit during storage, but fruit were held under cold storage conditions which inhibits greasiness development and many compounds were still not unidentified, so compounds potentially relating to greasiness development remained unknown (Curry, 2008; Dong et al., 2012; Veraverbeke et al., 2001). Recent work by Christeller and Roughan (2016) correlates the induction of apple skin greasiness with accumulations of novel long-chain unsaturated fatty acid esters of farnesol. However, the compounds leading to greasiness were not identified just by comparing wax composition differences between non-greasy and greasy apples. In addition to chemical composition, wax structure also determines fruit appearance (Koch and Ensikat, 2008; Veraverbeke et al., 2001). These two aspects can not be considered independently. Hence, a more comprehensive study is required to increase our understanding of greasiness development.

The objective of this study was to reveal the relationship between cuticular waxes and skin greasiness by evaluating the changes in wax compounds and wax morphology of three apple cultivars stored at 20 °C.

2. Materials and treatment

2.1. Plant materials

Mature apples (*Malus domestica* Borkh.) of 'Jonagold' and 'Red Delicious' were picked from the Baishui apple experimental farm of Northwest A & F University, China. 'Jonagold' apples were picked on 20 September 2014 and 18 September 2015. 'Red Delicious' apples were picked on 14 September 2014. 'Cripps Pink' apples were picked on 4 November 2013 and 24 October 2014 from an orchard in Fufeng County, Shaanxi Province, China.

Apples of uniform size without any physical damage were randomly selected and packaged in PVC (polyvinyl chloride) bags. The fruit diameters of 'Jonagold' and 'Red Delicious' were about 80 mm and for 'Cripps Pink' about 70 mm. Fruit of 'Jonagold' and 'Red Delicious' were stored at 20 °C for 20 d. 'Cripps Pink' apple fruit were stored at 20 °C for 70 d.

2.2. Assessment of skin greasiness and fruit firmness

During storage, eight fruit of each cultivar were used for assessment of skin greasiness and fifteen fruit for assessment of fruit firmness. 'Jonagold' (2014) and 'Red Delicious' apples were sampled on days 0, 5, 10, 13 and 20. 'Cripps Pink' apples (2013) were sampled on days 0, 8, 15, 25, 36, 45 50, 60 and 70.

Skin greasiness was assessed quantitatively using the greasiness score of Dadzie et al. (1995), with some modifications. Grease score was obtained by rubbing the fruit against the hand. The level of greasiness was assessed subjectively at four levels and quantified numerically as: *none* ($0.5 >$ grease score ≥ 0), *slight* ($1 >$ grease score ≥ 0.5), *moderate* ($2 >$ grease score ≥ 1) or *severe* (grease score ≥ 2.0). The discontinuous greasiness score were combined statistically to give a semi-continuous greasiness variable.

Fruit firmness was measured with GS-15 fruit texture analyzer (Guss Manufacture, Republic of South Africa). A 7.9-mm diameter probe was chosen for the measurement. The penetration depth was 5 mm and the penetration rate was 1 mm s^{-1}. Measurements were made at the fruit equator after removal of a 1 mm thick slice of skin.

2.3. Cuticular wax extraction

Based on the results for the assessment of greasiness, 'Jonagold' apples (2014 and 2015) with different greasiness levels were sampled on day 0 (no greasiness), day 10 (moderate greasiness) and day 20 (severe greasiness) for extraction of cuticular waxes. 'Red Delicious' apples were sampled similarly. For 'Cripps Pink' apples in 2013, the cuticular waxes were extracted on day 0 (no greasiness), day 25 (light greasiness), day 45 (moderate greasiness) and day 70 (severe greasiness). In 2014, the cuticular waxes were extracted at the same sampling periods as in 2013.

A group of five apples were eluted by three successive 45 s-washes of clean chloroform (400 mL) (Belding et al., 1998; Wang et al., 2014) and then the solvent was combined. Three replicates were taken. After extraction, the solvent was concentrated by a rotary evaporator under vacuum at 40 °C. The remaining waxes were transferred to a vial, then all samples were dried under a nitrogen flow. Dried samples were stored at −40 °C until analyzed.

2.4. Wax preparation and chemical analysis of testing condition with GC–MS

The waxes were re-dissolved in 20–40 mL of chloroform: methanol (10:1, v/v) with internal standard of *n*-heptadecane (50 mg L^{-1}, Alfa Aesar, Heysham, UK). 1 mL of the sample was dried under a stream of nitrogen and then derived with 400 μL of bis-N,N-(trimethylsilyl) trifluoroacetamide (BSTFA, Alfa Aesar, Heysham, UK) for 30 min at 70 °C. After removing BSTFA under a nitrogen flow, those derivatives were dissolved in 1 mL chloroform for GC–MS analysis (Belge et al., 2014; Vogg et al., 2004; Wang et al., 2014).

Both quantitative analysis and qualitative analysis were carried out by GC–MS. A volume of 1 μL of sample was analyzed using a TRACE GC Ultra GC coupled with an ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, USA). The GC was fitted with a DB-5 MS column (30 m × 0.25 mm × 0.25 μm film thickness, Agilent, Palo Alto, USA). Helium was used as the carrier gas, with a split ratio of 10:1, at a flow rate of 1 mL min^{-1}. The temperatures of the injection port, ion source, and MS transfer line were 250 °C, 280 °C, and 250 °C, respectively. The oven temperature program was as follows: Initial temperature was 70 °C for 1 min, followed by a ramp of 10 °C min^{-1} to reach 200 °C, then increased to 300 °C at a rate of 4 °C min^{-1}, and finally kept at 300 °C for 20 min. The MS was operated in positive electron ionization mode at 70 eV, obtaining spectra with an m/z range of 45−650. Wax compounds were identified by matching their mass spectra with those from standards and the NIST 14 MS library. Semi-quantitative determination of wax compounds was carried out using the internal standard method, where the concentrations of various wax compounds were normalized to that of *n*-heptadecane (Li et al., 2016; Yeats et al., 2012)

The surface area of apple was calculated according to Yuan et al. (1995). The amount of wax composition was expressed as per unit of fruit surface area (mg m^{-2}).

2.5. Chemical analysis of epicuticular waxes rubbed from apple surfaces

Epicuticular waxes of 'Jonagold' (2014, day 0 and day 20) and 'Cripps Pink' (2013, day 0 and day 70) were obtained by rubbing fruit thoroughly with absorbent gauze (the absorbent gauze had been extracted by chloroform with Soxhlet extractor for 48 h). The absorbent gauze was immersed in 500 mL of chloroform and extracted by ultrasonic extraction for 20 min. Then the solvent was removed by rotary evaporation at 40 °C. Wax preparation and chemical analysis were the same as those of cuticular waxes. Results were expressed as relative%. Two replicates of 5 fruit each were used for the analysis of epicuticular waxes.

2.6. Separation of cuticular wax constituents

Cuticular waxes extracted from severely greasy 'Jonagold' fruit (2014, day 20) were loaded on a glass column (30 × 1.5 cm) packaged with silica gel and alumina (V/V = 3:1) (Yin et al., 2011). The wax