Non-destructive aroma production of a climacteric near-isogenic line of melon obtained by headspace stir-bar sorptive extraction

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A climacteric aromatic near-isogenic line (NIL) of melon (*Cucumis melo* L.) SC3-5-1 contained an introgression of the non-climacteric Korean cultivar "Shongwan Charmi" accession PI 161375 (SC) in the genetic background of the non-climacteric cultivar "Piel de Sapo" (PS). The aroma production was monitored during ripening at $22^{o}C$ in intact fruit using headspace sorptive bar extraction (HSSE). Bars were composed of polydimethylsiloxane (PDMS) and aromas were desorbed and analysed by gas-chromatography mass-spectrometry. The aromatic profile was comprised 66 aromatic compounds with a predominance of esters, particularly acetate (2-methylbutyl acetate, 2-methylpropyl acetate, hexyl acetate, phenylmethyl acetate). Some compounds were severely affected by postharvest time. The acetate esters (3-methylbutyl acetate, butan-2-yl acetate or phenylmethyl acetate) decreased with ripening and sulfur-derived compounds (S-methyl butanethioate and S-methyl 3-methylbutanethioate) increased gradually with ripening. A few compounds increased at the senescence phase (propyl ethanoate). Other compounds such as hexadecanoic acid showed a marked decrease after harvest, some declining from a relative maximum at harvest at different extent (2-methylpropyl hexanoate; n-hexadecanoic acid; nonanoic acid).

INTRODUCTION

Climacteric or non-climacteric behaviour is an interesting topic in fruit ripening with potential implications for insect attraction, seed dispersal and readiness for predation, or human consumption (Rodríguez et al., 2012). The production of volatiles associated with climacteric behaviour is common to many fruit but only a few of them such as melons or plums have cultivars showing a differential behaviour permits the in-depth

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study of these traits (Ezura and Owino, 2008; Obando-Ulloa et al., 2008; Paul et al., 2012; Pech et al., 2008). Based on differences in the intensity of climacteric behaviour, Obando et al. (2009) proposed at least two QTLs controlling this character, one at least (*eth3.5*) previously mapped in LG III (Fernández-Trujillo et al., 2008; Moreno et al., 2008).

The climacteric behaviour of this NIL is strongly associated with a typical aromatic profile (Obando-Ulloa et al., 2008) and softening associated with cell wall degradation and accelerated ripening compared with the non-climacteric inbred "Piel de Sapo" parental (Dos-Santos et al., 2011; Gomes et al., 2010; Vegas et al., in press). Recently, Vegas et al. (in press) showed that SC3-5-1, a climacteric NIL of melon, have two introgressions in melon linkage groups III and VI, respectively, of the Korean accession PI 161375 in a "Piel de Sapo" genetic background. In intact fruit of SC3-5-1 harvested close to the climacteric peak, Fernández-Trujillo et al. (2012) showed an increase of total acetate esters, and a sudden decrease in alcohols, accompanied by an upsurge in non-acetate esters and maximum ethylene production lasting around 3 days. However, the main individual aroma volatiles of NIL SC3-5-1 fruit during ripening have not been reported.

The goal of this paper is to characterize the main individual changes in volatiles associated with SC3-5-1 climacteric fruit ripening and senescence, particularly those revealing potential ethylene-dependent behaviour in intact fruit after harvest.

MATERIAL AND METHODS

Plant material and experimental design

Fruits obtained from melon (*Cucumis melo* L.) plants of the NIL SC3-5-1 were harvested in full-ripe stage of maturity in mid July 2009 in Cartagena, Murcia, SE Spain. Harvest indices, experimental design and flesh sampling followed the methodology reported by Obando-Ulloa et al. (2009a). The inbred parents showed non-climacteric behavior, while SC3-5-1 is a climacteric NIL (Fernández-Trujillo et al., 2012; Moreno et al., 2008).

For ripening experiments, fruit were stored at $21\pm1^{o}C$ and $93\pm3\%$ RH for 10 d. For aroma volatile analysis, four selected fruits (one single fruit per replicate) harvested at the end of the season for SC3-5-1 were used. Fruit weight and density (mean±SE, n=4) were 1636±88 g and 992 kg·m^{-3}, respectively. All the fruit samples were sampled at harvest and during ripening for1 hour within hermetic containers of 5283 cm^{3}.

The method reported by Obando-Ulloa et al. (2008) was used for sampling carbon dioxide (after 1 h) and ethylene (after 45 min) with two 4-mL syringes in order to monitor respiration rates and ethylene production after injecting 0.5 mL into different gas chromatographs.

For sampling aroma volatiles non-destructively, the Gerstel twister used was of 0.5 mm thickness, 10 mm length, 24 µL volume of polydimethylsiloxane (PDMS; Gertsel GmbH, Mülheim an der Ruhr, Germany). The bars were stacked onto the metallic wall of the container for absorbing aroma headspace. Also, they followed the conditioning process before or after analysis previously reported by Fernández-Trujillo et al. (2012). Volatile analysis was performed as in Fernández-Trujillo et al. (2012). The chromatograms and mass spectra were evaluated using ChemStation software (G1701DA D.02.00.275, Agilent Technol.). The peaks were registered using a mass spectrometer (5973 Network Mass Selective Detector, Agilent Technol.) coupled to the GC. Volatile compounds were obtained by comparing the experimental spectra with those of the National Institute for Standards and Technology (NIST05a.L) data bank (Obando-Ulloa et al., 2008). The compounds with a match quality (MQ) higher than 50% in the NIST database were considered and the rest of the areas discarded. In order to suppress compounds not associated with melon aroma, a thorough literature and internet search was also performed. Levels of volatile compounds were expressed as a percentage of the total area counts recorded in each chromatogram and the data were then averaged.

Raw data or data transformed into their respective logarithm were analyzed by analysis of variance with ripening time as fixed factor and, when significant, mean differences were separated by LSD test with type-I error $\alpha \leq 0.05$. Only compounds showing a significant effect of the ripening time are reported as time-dependent.

RESULTS AND DISCUSSION

The NIL SC3-5-1 showed a climacteric behaviour that started after the 3rd day post harvest, with a peak in ethylene production after 9 days of ripening of 57.1±2.5 pmol·kg^{-1}·s^{-1} (Fig. 1), accompanied by a respiration rate of 100-150 nmol·kg^{-1}·s^{-1} (data not shown).

Twister technology is appropriate for monitoring changes in melon volatiles non-destructively because most of the well-known aromas in the climacteric melon NILs flesh were also recovered here (Obando-Ulloa et al., 2008 and 2009).

The aroma profile commonly found during ripening in NIL SC3-5-1 was constituted by 66 volatile compounds, mostly esters (eighteen acetate, sixteen non-acetate, six thioesters), six organic acids, five aldehydes, five ketones, one alcohol, five terpenes, and another four compounds of other chemical groups (data not shown).

The profile was composed mainly of esters with well-know odour descriptors: for example, acetate esters (2-methylbutyl acetate, apple; hexyl acetate, pine; phenylmethyl acetate, pine; 2-methylpropyl acetate, fruity) (Figs. 2A, 2C, 2G and 2H; data not shown), followed by non-acetate esters such as the 3-methylbutyl propanoate, of fruity odour (data not shown). This last compound did not show significant changes over time. The most abundant compounds in SC3-5-1 that also increased during ripening were 2-methylbutyl acetate, representing 30% of the total area counts at the climacteric peak after 9 d of ripening (Fig. 2A), followed by phenylmethyl acetate (Fig. 2H) and others (Fig. 2). The 2-methylbutyl acetate is a medium odorant. It has an odour description to apple and have also been identified in Jiashi melon (Pang et al., 2012), and its amino acid precursor is L-isoleucine (Gonda et al., 2010). This compound is very abundant in Cantaloupe and Charentais melons (Kourkoutas et al., 2001) and is predominant together with butyl acetate and hexyl acetate in Galia melons (Fallik et al., 2001). Other non-acetate esters, such as pental-2-yl propanoate (Fig. 2L), also peaked at the climacteric peak.

The large amount of esters is also consistent with the strong dependence on ethylene biosynthesis of most of the ester and thioesters catalyzed by several alcohol acetyl transferases (Luchetta et al., 2007) and with methionine or other amino acids being precursors (Bauchot et al., 1998; Gonda et al., 2010). In fact, the pattern of many volatile compounds (e.g. Figs. 2A, 2B, 2C 2E, 2I and 2L) was concomitant with the upsurge in ethylene production (Fig. 1), sometimes having important aromatic values at harvest (Figs. 2A). In contrast, other volatiles followed the opposite trend to ethylene production (Figs. 2D, 2F and 2H).

The volatile compounds were classified according to their pattern during postharvest ripening time. For example, four acetate esters (3-methylbutyl acetate 1-methylpropyl acetate; 2-methylpropyl acetate; phenylmethyl acetate) decreased during ripening (Figs. 2D, 2F, 2G and 2H). However, some thioesters (S-methyl butanethioate or S-methyl 3-

methylbutanethioate; Figs. 2E and 2I, respectively), 2-methyl-2-methylsulfanylbutane (from 0.8 to 1.2% after the 6th day), or non-acetate esters (i.e. ethyl butanoate from 0 to 1.4% after 10 d of ripening), among other compounds, followed the opposite pattern The data confirm that all these compounds can be either detected in climacteric NILs either nondestructivfely or destructively (in the flesh) (Fernández-Trujillo et al., 2012; Obando-Ulloa et al., 2008 and 2009). Because they were detectable in intact fruit most of them are not apparently artefacts. The data also show (Fig. 1) that during the postharvest climacteric events aroma biosynthesis and degradation still coexists.

A few compounds rapidly declined after a relative maximum attained at harvest and were classified as typical harvest aroma compounds, such 2-methylpropyl hexanoate (from 1% to 0.2-0.5% after 1-2 d of ripening), or n-hexadecanoic acid (from 4.3% to levels below 0.3% after 1 d of ripening). Other compounds decreased slowly during ripening, such a nonanoic acid (from 0.6% to less than 0.2% after 3 d of ripening) or n-hexanoic acid (Fig. 2J). Probably the fast decline in some volatiles was particularly associated with melon plant detachment while, in general, the decline of some compound may be considered as indication of its role as intermediate-acting compounds for the biosynthesis of others.

Finally, other volatiles were typical of melon senescence, though in some cases with similar levels at harvest and very close to the maximum ethylene peak, such as propyl ethanoate (Fig. 2K). These compounds can be considered good candidates for validating optimum ripeness or for selecting fruit for immediate consumption or processing.

CONCLUSION

The acetate and thioesters, particularly 2-methylbutyl acetate, predominated in the SC3-5-1 profile. Aroma volatiles identified during ripening of the climacteric NIL SC3-5-1 followed different patterns but apparently following an ethylene-dependent pattern due to their biosynthesis or degradation.

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TABLES AND FIGURES
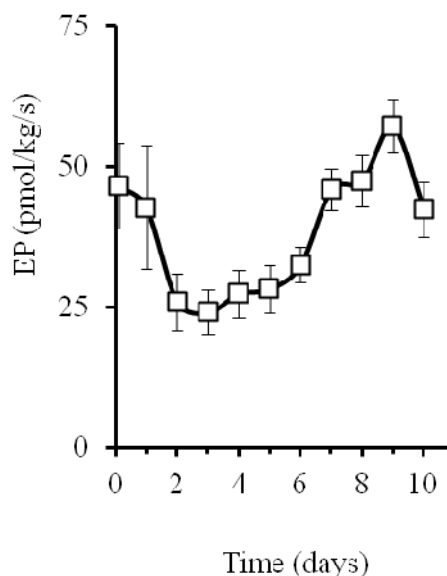
Fig. 1. Ethylene production (EP) during ripening at 21±1°C and 93±3% RH of intact fruit of near-isogenic line SC3-5-1 (mean±SE, n=4).
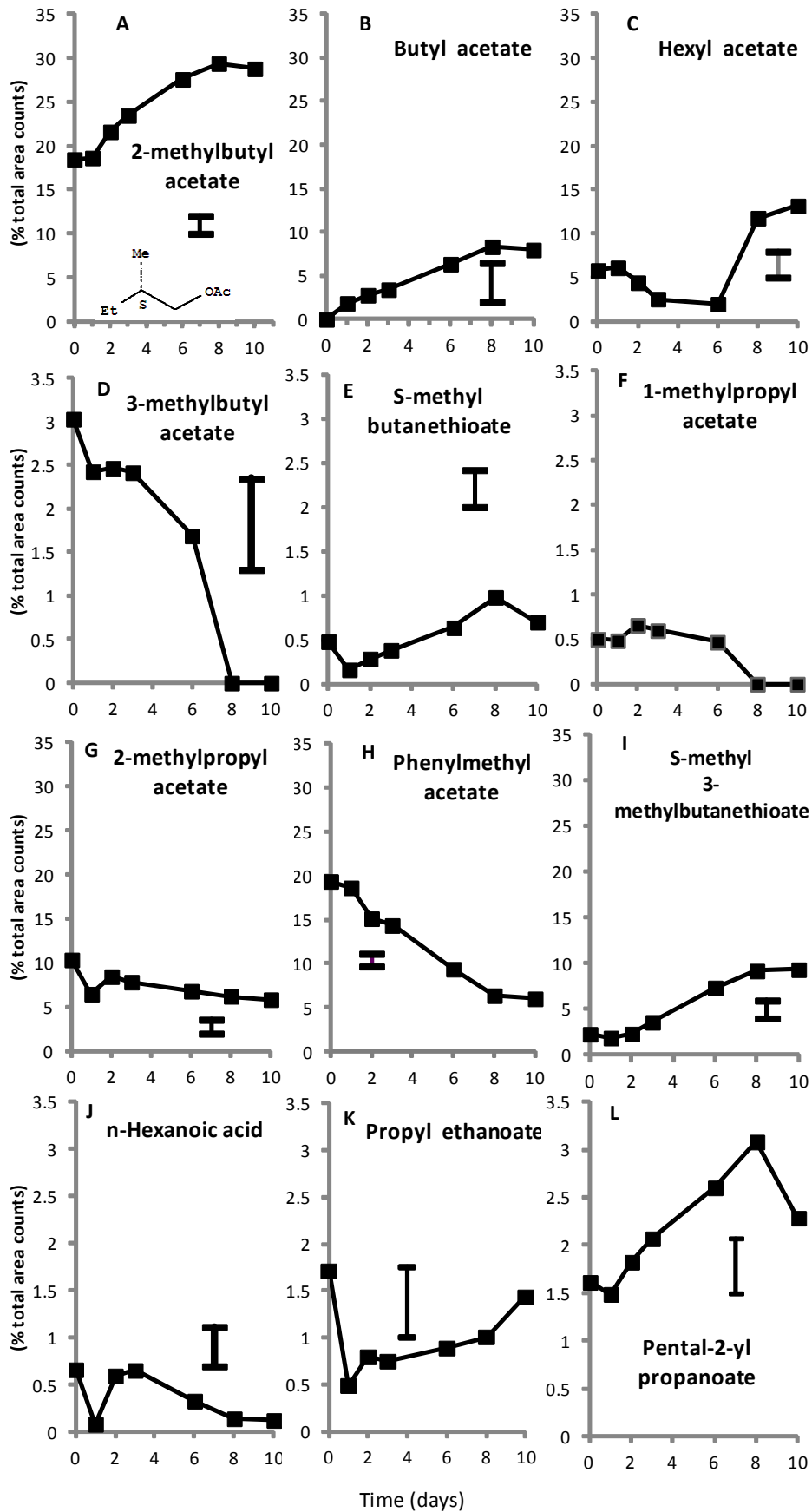
Fig. 2. Individual aroma volatiles expressed as mean percentage of total area counts of the compounds identified per chromatogram during ripening at $21 \pm 1^{\circ}C$ and $93 \pm 3\%$ RH of intact fruit of near-isogenic line SC3-5-1 (mean±SE, n=4).