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Multivariate analysis of modifications in biosynthesis of volatile compounds after CA storage of 'Fuji' apples

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Abstract

Emission of aroma volatile compounds and some related enzyme activities (LOX, PDC, ADH, and AAT) were assessed in 'Fuji' apples (*Malus* × *domestica* Borkh.) during shelf life at 20 °C following cold storage under air or under three different CA conditions (3 kPa O_2:2 kPa CO_2; 1 kPa O_2:1 kPa CO_2; or 1 kPa O_2:2 kPa CO_2). Data were used for principal component analysis (PCA) and partial least-square regression (PLSR) analysis of results. LOX activity was partly inhibited by hypoxic conditions, and thus could have contributed to differentiation between air- and CA-stored fruit. Accordingly, emission of straight-chain esters was also higher in air- than in CA-stored fruit. In contrast, PDC activity was responsible for part of the differences between low (3 kPa) and ultra-low (1 kPa) O_2 storage conditions, probably by providing substrates for AAT action. AAT activity afforded no satisfactory differentiation between samples, and therefore it is suggested that substrate availability is a more decisive factor than enzyme activity for volatile production after storage. The PCA and PLSR models developed in this work were not useful for discrimination between the two studied ultra-low O_2 conditions.

Keywords: Aroma; Alcohol dehydrogenase; Alcohol *o*-acyltransferase; Controlled atmospheres; *Malus* × *domestica*; Multivariate analysis; Pyruvate decarboxylase; Lipoxygenase; Volatile compounds

1. Introduction

Apple (*Malus* × *domestica* Borkh.) cultivar 'Fuji' stands out for the excellent organoleptic quality after long-term storage (Yoshida et al., 1995). Controlled atmosphere (CA) storage is widely applied in fruit-producing areas, in order to preserve fruit quality and decrease the incidence of physiological disorders. In spite of the beneficial effects of CA storage conditions on a number of quality aspects such as firmness, acidity, and sugars (Brackmann et al., 1994), detrimental effects on volatile production, and thus on fruit aroma, have also been reported for some apple cultivars (Knee and Sharples, 1981; Yahia et al., 1985; Streif and Bangerth, 1988; Hansen et al., 1992; Brackmann et al., 1993; López et al., 2000), including 'Fuji' (Echeverría et al., 2003, 2004b).

Aroma profile of apples is complex, the most significant contributors being ester-like compounds, which have been reported to account for up to 80, 88, 90, and 98% of total volatiles in 'Golden Delicious' (López et al., 1998a), 'Granny Smith' (Lavilla et al., 1999), 'Fuji' (Echeverría et al., 2003), and 'Starking Delicious' (López et al., 1998b) cultivars, respectively. The major esters produced by ripening apple fruit are thought to arise primarily from lipid and amino acid catabolism (Sanz et al., 1997; Dixon and Hewett, 2000). The contribution of each individual compound depends on its odour threshold (minimum concentration for a compound to be detected by smell) as well as on its concentration (Buttery, 1993), which in turn is dependent on the activity of related enzymes and on substrate availability (Fellman et al., 1993; Sanz et al., 1997). Therefore, the final aroma profile of a fruit is the result of a fine balance among all compounds emitted, and consequently any change in this balance will result in changes in fruit flavour.

In addition to season-to-season variability (López et al., 1998a; Echeverría et al., 2004b), a number of factors influence volatile emission after apple storage, including maturity stage at harvest (Dirinck and Schamp, 1989; Fellman et

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al., 2003; Echeverría et al., 2004b) and, very prominently, storage treatment including period as well as O_2 and CO_2 concentrations in the storage atmosphere (Fellman et al., 2000; Echeverría et al., 2003). Alterations in volatile profile caused by any of these factors may give rise to modifications in fruit acceptability. For instance, CA storage is known to reduce the capacity for ester synthesis in apples (Streif and Bangerth, 1988), and indeed higher consumer acceptance has been reported to correlate with production of some esters in 'Fuji' apples (Echeverría et al., 2003).

In order to optimise storage conditions for flavour development, a better knowledge of modifications caused in the biosynthesis of volatile compounds is required, which makes it necessary to integrate a large amount of information. Bilinear multivariate procedures avoid collinearity effects found in conventional multiple linear regression approaches (Martens and Naes, 1989; Brockhoff et al., 1993), and thus can help in extracting interpretable and statistically reliable information, especially when a large set of data is involved. Multivariate analysis has been applied in previous work to the analysis of relationships between different variables affecting sensory quality (Echeverría et al., 2003) and volatile production during fruit maturation (Echeverría et al., 2004a) of 'Fuji' apples. In this work, the same approach was taken for assessing modifications in biosynthesis of volatile compounds after CA storage. Different storage conditions were studied in relation to their influence on volatile emission and related enzyme activities.

2. Materials and methods

2.1. Plant material and storage conditions

Apple fruit (*Malus* × *domestica* Borkh., cv. Fuji) were harvested in 2000 at commercial maturity (185 days after full bloom) in a commercial orchard near Lleida, NE Spain. Quality parameters at harvest date averaged 73.5 N firmness, soluble solids content of 16.9 g × 100 gFW^{-1}, and malic acid content of 30.6 mmol l^{-1}. Immediately after harvest, fruit were selected for uniformity of size and absence of defects, and stored in chambers at 1 °C and about 92% relative humidity, under either air or different CA conditions: 3 kPa O_2:2 kPa CO_2, 1 kPa O_2:1 kPa CO_2, or 1 kPa O_2:2 kPa CO_2. O_2 and CO_2 concentrations were monitored and automatically corrected using N_2 from a tank and by scrubbing off excess CO_2 with a charcoal system. Fruit samples were taken from each storage atmosphere after 3, 5 or 7 months of storage, and analysed after 1, 4 or 10 days at 20 °C.

2.2. Analysis of aroma volatile compounds

The extraction of volatile aroma compounds from a sample (2 kg × four replicates) of intact fruit was performed according to the method of dynamic headspace as described by Rizzolo et al. (1989), with slight modifications. Briefly, each fruit sample was placed in a 10-l Pyrex glass container, and an air stream (900 ml min^{-1}) was passed through for 4 h; the effluent was then passed through an ORBO-32 adsorption tube filled with 100 mg of activated charcoal (20/40 mesh), from which volatile compounds were de-adsorbed by agitation for 40 min with 0.5 ml of diethyl ether. Identification and quantitation of volatile compounds were achieved on a Hewlett Packard 5890 gas chromatograph equipped with a flame ionisation detector and a cross-linked free fatty acid phase (FFAP; 50 m × 0.2 mm i.d. × 0.33 μm) as the capillary column, where a volume of 1 μl from the extract was injected in all the analyses. Helium was used as the carrier gas (42 cm s^{-1}), with a split ratio of 1:40. The injector and detector were held at 220 and 240 °C, respectively. The analysis was conducted according to the following programme: 70 °C (1 min); 70–142 °C (3 °C min^{-1}); 142–225 °C (5 °C min^{-1}); 225 °C (10 min), as described elsewhere (Echeverría et al., 2002). Volatile compounds were identified by comparing retention indexes with those of standards and by enriching apple extract with authentic samples. The quantification was made using butylbenzene (assay >99.5%, Fluka) as internal standard. A GC–MS system (Hewlett Packard 5890) was used for compound confirmation, in which the same capillary column was used as in the GC analyses. Mass spectra were obtained by electron impact ionisation at 70 eV. Helium was used as the carrier gas (42 cm s^{-1}), with a split ratio of 1:40, according to the same temperature gradient program as described above. Spectrometric data were recorded (Hewlett Packard 3398GC Chemstation) and compared with those from the NIST HP59943C original library mass-spectra. Results were expressed as μg kg^{-1}.

2.3. Analysis of acetaldehyde and ethanol concentrations

Juice from 20 fruit per treatment (atmosphere × storage period × shelf life period) was individually obtained and frozen at −20 °C until analysis of ethanol and acetaldehyde contents as described by Ke et al. (1994b). Frozen juice from each fruit was thawed, and a 5-ml sample was introduced in a 10-ml test tube, which was closed with an elastic cap and incubated at 65 °C for 1 h. A 1-ml headspace gas sample was taken with a syringe and injected into a Hewlett Packard 5890 gas chromatograph, equipped with a column containing Carbowax (5%) on Carbopack (60/80, 2 m × 2 mm i.d.) as the stationary phase, and a flame ionisation detector. Nitrogen was used as the carrier gas (24 cm s^{-1}), and operating conditions were as follows: oven temperature 110 °C, injector temperature 180 °C, detector temperature 220 °C. Acetaldehyde and ethanol were identified and quantified by comparison with external standards, and results were expressed as μl l^{-1}.

2.4. Enzyme extraction and assays

For extraction and analysis of enzyme activities, samples of both peel and pulp tissue were taken separately, frozen in