Mouldy, musty earthy off-odour of apple fruits – Short communication

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Abstract

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Chemical compounds identification that cause the mouldy, musty and earthy off-odours of apple fruit stored in controlled atmosphere at low temperature for several months has been studied. Compounds with off-odour were extracted from cv. Golden Delicious apple peel using ether and purified on a silica-aluminium column with washing using a sodium carbonate solution. Presence of compounds with mouldy, musty and earthy off-odour in extracts and washings was verified using a sensory panel with four members intensively trained in identification and description of the odour throughout the whole analytical process. Separation of compounds in the final extract by gas chromatography showed that the odour was associated with five peaks associated with oxidation products of α-farnesene.

Keywords: α-farnesene; oxidation; 1-octen-3-one; scald; peel; disease

The aroma of cv. Golden Delicious apples from long term controlled atmosphere (CA) storage has often been described as mouldy, musty and earthy (Kaack 1977), without freshness and a very low apple fruit flavour characteristics (Kaack 1977, 1992). An uncontrolled level of oxidation and formation of off-odours was promoted by Anet and Coggiola (1974) with long term storage at low oxygen levels. Abnormal oxidation of the hydrocarbon α-farnesene has been reported to result in a disordered accumulation of oxidised compounds in the apple skin during storage (Huelin, Kennett 1958; Filmer, Meigh 1971) with α-farnesene being oxidised easily, at 1°C, to a conjugated triene hydroperoxide, its isomer and two peroxides (Anet 1969). Subsequently, Filmer and Meigh (1971) identified a long oxidation chain resulting in a major end product 6-methyl-5-heptene-2-one with an off-favour, confirmed by Spicer et al. (1993). Hashizume et al. (2007) also reported that 1-octen-3-one imparts a metallic, mouldy and mushroom odour while 2-methyl-1-pentene-3-one creates a rubber, sulphur and sweaty aroma in apple juice in glass bottles under fluorescent light.

α-Farnesene autooxidises easily at 1°C to 2,6,10-trimethyldodeca-2,7(*E*),9(*E*),11-tetraen-6-ol, 6-hydroperoxy-2,6,10-trimethyldodeca-2,7(*E*),9(*E*)-11-tetraene, its 7*E*,9*Z* isomers and 6-methyl-5-hepten-2-one (Anet 1969; Rowan et al. 1995, 2001). On this basis it was concluded that the mouldy, musty and earthy off-odour of cv. Golden Delicious apple fruits that develops during CA storage cannot be completely explained at the present time because of a variety of aroma compounds that arise from the complicated fruit physiology that occurs during storage.

The aim of this research was therefore to identify compounds that contribute significantly to the mouldy, musty, earthy off-odour of cv. Golden Delicious apples.

MATERIALS AND METHODS

Cv. Golden Delicious apples 60–80 mm in diameter were harvested from trees grown on a sandy loam soil (JB6 of the Danish classification system) at the optimum maturity for controlled atmosphere storage. The fruit were mixed carefully before packing in net sacks with 25 apples (8 kg) each. The nets were stored at one meter below the surface of the bulk of apples in a commercial CA-storage at 1°C at 3% O_2 and 3% CO_2 for six months.

After storage five apples from each net sack were maturated at 20°C for five days prior to cutting each apple into 12 longitudinal pieces. These pieces were then evaluated for mouldy, musty and earthy odours by four intensively trained panellists using a ten point scale with 1 representing no off-odour and 10 for a very strong off-odour.

Cuticle components were extracted from the remaining fruit by dipping them in one litre of diethyl ether (Merck, Darmstadt, Germany) for 3 min at 22°C. The ether was removed using an evaporator and one gram of the apple sample was dissolved in ether and transferred to a 2 × 20 cm column with a mixture of silica acid (Fluka, Buchs, Schwitzerland) and aluminium oxide (Merck, Darmstadt, Germany). The off-odour compounds were eluted using pentane (Bie & Berntsen, Roedovre, Denmark). Free fatty acids were removed from the eluate by extraction using 8% Na_2CO_3 (Merck, Darmstadt, Germany) dissolved in water and the hydrocarbons were precipitated using acetone. The extraction was repeated five times using fruit from five other samples of apple from the CA storage. Presence of off-odour compounds in samples at each step in the extraction process were followed using test samples prepared by placing 50 µl of the liquid onto a filter paper and waiting five minutes until the extraction liquid was evaporated. The panel members evaluated the odour of compounds eluted from the GC-column five times (n = 5).

The extracted compounds were separated using a FID gas chromatograph (Model 64; Pye Unicam, Cambridge, UK) equipped with an integrator (HP 3370; Hewlett Packard, Palo Alto, USA), and a 2.5 m stainless steel column 2 mm in diameter packed with 3% OV-1 on diatomite CQ. Nitrogen was used as the carrier gas (15 ml/min). The injector and detector temperatures were 130°C and 300°C and the oven temperature increased from 150°C to 300°C at 1°C for 10 min and then 2°C/min for separation of the extracted compounds. To sensory panel members sniffed the odour of each compound emitted from the GC-column, with the detector flame extinguished. To determine the identity of the odorous compounds a Varian Walnut Creek Saturn 2000 ion trap mass spectrometer operated at an ionisation potential of 70 eV and interfaced to a Varian Star 3400 CX gas chromatograph GC (both Varian, Walnut Creek, USA) using the column and conditions described above was as used for production of mass spectre.

RESULTS AND DISCUSSION

Combined gas chromatograph and mass spectrometry (GC-MS) resulted in six peaks eluted from the GC-column after 23.1, 30.5, 33.9, 40.0, 53.7 and 61.2 min for compounds with a decrease in molecular oxygen from four in endoperoxide to none in α-farnesene. For the first five of these peaks the sensory panel identified that their aroma had a very strong off-odour (score 10) that was characterized as mouldy, musty and earthy off-odour similar to the stored and sliced apples.

The compound eluted after 61.2 min, encompassing 57.0% of the total peak area, contained molecule fragments corresponding to spectrums for α-farnesene published previously (Rowan et al. 1995; Whitaker et al. 1997; Rudell et al. 2009). The odour of α-farnesene has been characterised as freshly green and herbaceous (Arvisenet et al. 2008).

The compound eluted after 53.7 min, encompassing 1.0% of the total peak area, (minor variant) and the compound eluted after 40.0 min, with a peak area of 7% (major variant), had a characteristic spectra pattern for 2,6,10-trimethyldodeca-2,7*E*,9*E*,11-tetraen-6-ol and its *Z*-isomer 2,7*E*,9*Z*,11 (Rowan et al. 1995; Whitaker et al. 1997; Fielder et al. 1998; Krings et al. 2006; Whitaker 2007). In this research the peak area of the major variant was seven times higher in comparison to the peak area of the minor variant, while Whitaker et al. (2007) found that the level of the major variant was 10 times higher in comparison to the minor variant.

The compound eluted after 33.9 min and encompassing 32.0 % of the total peak area was identified as 6,7-hydroperoxy-2,6,10-trimethyldodeca-2,7*E*,9*E*,11-tetraen. This compound has previously been found in the skin of the five apple cvs Red Delicious, Fuji, Gala, Golden Delicious and Granny

Smith (Anet 1969; Rowan et al. 1995; Fielder et al. 1998).

The peak eluted from the GC column after 31.2 min, with a peak area 1,6 % of the total peak area, was identified as 6,7-epoxy-3,7,11trimethyldodeca-1,3*E*,10*E*-triene (Anet 1969; Brimble et al. 1994; Fielder et al. 1998).

The last compounds found in cv. Golden Delicious associated with a mouldy, musty and earthy off-odour in this research were two endoperoxides with molecular weights 236 and 252, respectively, as described previously (Rowan et al. 1995, 2001). These compounds were eluted after 27.5 and 30.5 min and encompassed 0.5% and 0.7% of the peak area, respectively.

In the literature it is reported that 1-octen-3-one found in apple juice processed from cvs Golden Delicious and Fuji apples has a strong metallic mushroom-like odour (Glindemann et al. 2006) and other aroma compounds reported including pentanal, 2-methyl-1-penten-3-one, hexanal, (*E*)-2-heptenal, 6-methyl-5-hepten-2-one and (*E*)-2octenal contribute to this off-odour (Hashizume et al. 2007). 6-methyl-5-heptene-2-one has been described as having a distinctive mouldy, musty and earthy odour (Anet 1972; Spicer et al. 1993) and this compound was identified as the final product from oxidation of trienols (Anet 1972; Mir et al. 1999; Whitaker, Saftner 2000; Rudell et al. 2009).

The aim of this study was to identify the aromatic compounds in stored cv. Golden Delicious apples that impart a mouldy, musty or earthy off-odour to the fruit. Using a trained sensory panel and gas chromatograph procedures this research identified five compounds, derived from the oxidation of α-farnesene with potential to contribute to this aroma. In increasing order of oxidation the compounds were identified as 2,6,10-trimethyldodeca-2,7*E*,9*E*,11-tetraen-6-ol and its *Z*-isomer 2,7*E*,9*Z*,11; 6,7-hydroperoxy-2,6,10-trimethyldodeca-2,7*E*,9*E*,11-tetraen; 6,7-epoxy-3,7,11trimethyldodeca-1,3*E*,10*E*-triene and two endoperoxides.

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