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Changes in Flavour-related Volatile Production during Post-harvest Handling of Apple and Pear Fruit

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ABSTRACT

Flavour is a major attribute determining consumer acceptance of apples and pears. Although flavour results from a complex combination of taste and odour, it is largely determined by the production of aroma volatiles, which gives fruit much of their perceived quality. Biosynthesis of aroma volatile compounds and its contribution to eating quality of fruit are very intricate. The final aroma profile of a fruit results from the properties of each individual compound as well as from a fine balance among all compounds emitted. In addition, the involved biosynthetic pathways are influenced by many pre- and post-harvest factors, including harvest maturity and post-harvest handling. Thus any change in this balance will cause alterations in the volatile profile and hence will give rise to modifications in fruit flavour and acceptability. Novel post-harvest technologies have focused mostly on appearance and decay resistance of fruit, and have often neglected flavour, despite the relevance of this attribute for sensory quality. For instance, controlled-atmosphere storage is known to decrease volatile production and thus to be detrimental to aroma. The low eating quality of these fruit results accordingly in consumer dissatisfaction, frequently preventing repeated purchases. This problem is exacerbated by the fact that the understanding of the fundamental mechanisms controlling changes in flavour is limited, and that many biochemical pathways determining this quality trait are still unknown. In order to minimise loss of flavour, a deeper knowledge of the involved pathways, mechanisms and factors is required. This review aims at summarising current information available on the production of aroma volatiles in apple and pear, and on how different factors affect the biochemical pathways involved in this process.

Keywords: aroma, biosynthesis of volatile compounds, post-harvest treatments

Abbreviations: AAT, alcohol o-acyltransferase; **ADH**, alcohol dehydrogenase; **AVG**, aminoethoxivinylglycine; **$CaCl_2$**, calcium chloride; **CA**, controlled atmosphere; **DACP**, diazocyclopentadiene; **2,4-DP**, 2,4-dichlorophenoxypropionic acid; **DPA**, diphenylamine; **EST**, expressed sequence tag; **HPL**, hydroperoxide lyase; **LOX**, lipoxygenase; **1-MCP**, 1-methylcyclopropene; **MJ**, methyl jasmonate; **PBZ**, paclobutrazol; **PDC**, pyruvate decarboxylase

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INTRODUCTION

Apples (*Malus* × *domestica* Borkh.) and pears (*Pyrus communis* L.) are among the major classes of fruits in terms of world production. Sensory quality of fruit includes all those attributes (colour, flavour, texture) directly affecting enjoyment by the consumer. Attributes showing most influence on consumer acceptance in apple are texture and flavour (Acree and McLellan 1988; Stow 1995), the latter being the result of a complex mixture of stimuli in which both taste and smell are involved. Due to the seasonality and large extent of apple and pear production and to high perishability of fruit, their commercialisation as a fresh produce is limited in time by over-ripening and senescence, by the incidence of physiological disorders and by post-harvest decay, all of which are the cause of low eating quality and important economic losses. In order to maintain availability for as long as feasible and/or economically desirable, the bulk of the produce is harvested commercially before becoming fully ripe, and stored subsequently for several months. Early harvest date improves storage potential by preserving firmness, thus increasing withstanding of handling and marketing procedures while reducing susceptibility to bruising and rots. Unfortunately, fruit often fail to reach full flavour if harvested before optimal maturity. In addition, post-harvest treatments and storage technologies have foc-

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used mainly on firmness and appearance, and have often neglected flavour, despite the relevance of this attribute for sensory quality. Disregard of this attribute along the post-harvest handling chain often leads to unsatisfactory eating quality when produce reaches the final consumer. This review aims at summarising available information on how different post-harvest procedures affect the biochemical pathways involved in the production of flavour-related compounds.

THE COMPOUNDS

Fruit aroma is the consequence of those sensations driven by perception of volatile compounds after chewing. Volatile compounds thereafter dissolve in mouth and pharyngeal mucosa, volatilise due to warm temperature in mouth, and arrive to olfactory receptors. Abundant literature is available reporting on the volatile compounds emitted by different apple and pear cultivars. In all instances, volatile profiles have been shown to consist of a complex blend of a number of different compounds, the total number and the identity of which being species- and cultivar-specific (Dixon and Hewett 2000a). The contribution of each particular compound to the characteristic flavour of a fruit is dependent upon its odour threshold and on its concentration, which in turn is dependent on the activity of key enzymes in its biosynthetic pathway and on substrate availability (Sanz *et al.* 1997). The quotient between concentration and odour threshold determines its aromatic intensity in terms of odour units (Buttery 1993). Further complexity arises from the fact that sensory interactions have been observed among different compounds (Bult *et al.* 2002). Therefore the presence of other flavour-related substances can also modulate the contribution of each individual volatile, and thus the final perceived aroma is not a simple linear combination of the separate odour intensities of each individual odorant. Moreover, other maturity attributes such as firmness, soluble solids content or acidity also contribute to the perception of fruit flavour in spite of the specific blend of volatiles being emitted (Eccher-Zerbini *et al.* 1999; Harker *et al.* 2002, 2006).

The actual aroma profile thus results from a fine-tuning metabolic balance, any change in this balance leading to changes in fruit flavour and probably in sensory acceptance. Although more than 200 aroma volatile substances have been detected in apple, including alcohols, aldehydes, ketones, acids, esters and others (Dixon and Hewett 2000a), the major compounds in both quantitative and qualitative terms are of the ester type, which can account for up to 98% of total volatiles emitted by the fruit (Young *et al.* 1996; López *et al.* 1998a, 1998b; Lavilla *et al.* 1999; Lo Scalzo *et al.* 2001; Fellman *et al.* 2003; Lara *et al.* 2006; Mehinagic *et al.* 2006; Villatoro *et al.* 2008a). As for pear, esters are also especially prominent both quantitatively and qualitatively. A total of 112 volatile compounds were identified in a study conducted on eight cultivars, including 'Bartlett', 'Packham's Triumph', 'Anjou', 'Comice', 'Bosc', 'Seckel', 'Vicar of Winkfield' and 'Forelle' (Suwanagul and Richardson 1998). Esters comprised 60 to 98% of total volatiles emitted, which also included alcohols, hydrocarbons, aldehydes and ketones, with hexyl acetate as the major compound. In 'Bartlett' pear, ethyl *trans*-2, *cis*-4 decadienoate has been reported as a character impact compound (Jennings *et al.* 1964) and hexyl acetate as an important contributing flavour compound (Jennings and Sevenants 1964). Genotype is a major factor accounting for the phenotypic differences in the composition of the volatile fraction emitted by fruit, as significant qualitative and quantitative variation is found among apple and pear cultivars (Young *et al.* 2004; Holland *et al.* 2005, and references therein). Also, some volatiles reported to be constituents of apple and fruit aroma may be in fact derived from fruit-infecting microorganisms (Schumacher *et al.* 1998; Vikram *et al.* 2004).

THE PATHWAYS

Aroma of a ripe apple or pear is a very complex attribute owing to the diversity of compounds emitted and to the wide range of their chemical nature. This complexity is reflected in the large number of biosynthetic pathways involved, in final metabolite accumulation and in its regulation. The major esters produced by ripening fruit can be broadly separated into straight-chain and branched-chain types (Dixon and Hewett 2000a). In general, fatty acids are considered to be major precursors of straight-chain volatile esters in apples (Brackmann *et al.* 1993; Yahia 1994; Sanz *et al.* 1997; Rowan *et al.* 1999; Defilippi *et al.* 2005a). Branched-chain esters are thought to arise from the metabolism of particular amino acids (Sanz *et al.* 1997; Wyllie and Fellman 2000). The concentration and composition of the pool of both lipid- and amino acid-derived substrates is strictly regulated during fruit development. Previous investigations have shown that the supply of fatty acids may be a major limiting factor for the production of aroma volatiles (Song and Bangerth 2003), and transgenic modification of fatty acid biosynthesis in plant tissues leads to significant changes in emitted volatiles (Wang *et al.* 2001). As for amino acids, the most important precursors are alanine, valine, leucine, isoleucine, phenylalanine and aspartic acid, and their addition to apple slices has been shown to increase total aroma volatile production (Rowan *et al.* 1996).

Because of the significant contribution of ester-like compounds to the aroma profiles of apple, pear and other fruits, most studies on volatile biosynthesis have been concerned with alcohol o-acyltransferase (AAT; EC 2.3.1.84), which catalyses the esterification reaction between a range of alcohols and acyl-CoAs, and is thus the direct responsible for the regulation of the final step in volatile ester biosynthesis (Dixon and Hewett 2000a). Some genes encoding for apple AATs have been isolated and characterised (Defilippi *et al.* 2005b; Souleyre *et al.* 2005; Li *et al.* 2006, 2007). Although AAT expression and activity has been shown to follow an ethylene-dependent pattern in 'Greensleeves' apple fruit (Defilippi *et al.* 2005b), in agreement with observations for 'Golden Delicious' (Li *et al.* 2006) and 'Royal Gala' (Souleyre *et al.* 2005), results of that work also indicated that there are ethylene-independent regulatory processes involved in aroma production. Actually, ester biosynthesis is also limited by alcohol availability (Berger and Drawert 1984), suggesting a key role for enzymes located upstream in the pathway. Maturity stage at harvest is critical in this regard, as gene expression and activity of the involved enzymes, and thus production of the corresponding intermediates, depends in part on the developmental stage of tissues (Lara *et al.* 2008; Villatoro *et al.* 2008b). The availability of precursors and the supply of alcohol and acid substrates may thus play a major role in determining the specific esters emitted by fruit, provided AAT activity is present at sufficient levels (Lara *et al.* 2003, 2007; Villatoro *et al.* 2008a). This is also supported by numerous feeding experiments in which the addition of intermediates enhanced the production of specific volatile compounds. For instance, treatment of apple fruit or tissue sections with the vapours of alcohols, aldehydes, or carboxylic acids significantly increased concentrations of the corresponding volatile esters (Berger and Drawert 1984; Bartley *et al.* 1985; Kollmannsberger *et al.* 1992; Harb *et al.* 1994; Li *et al.* 2006). Similarly, incorporation of deuterium into both straight- and branched-chain ester volatiles has been demonstrated after the incubation of apple fruit tissue with deuterated flavour precursors (Rowan *et al.* 1996, 1999; Matich and Rowan 2007).

Composition of esters finally emitted can be thus controlled by selectivity and activity of involved enzymes and/ or by substrate availability. Fatty acid precursors are metabolised through two main routes: β-oxidation and the lipoxygenase pathway (LOX; EC 1.13.11.12). LOX catalyses hydroperoxidation of polyunsaturated fatty acids, and reaction products are metabolised through a number of different pathways, one of them leading to aldehydes and oxo-acids and involving the action of hydroperoxide lyase (HPL; EC 4.2.1.-). Although the LOX pathway is thought to remain inactive in unripe fruit due to different sub-cellular localisation of enzymes and substrates, cell walls and membranes become increasingly permeable in the course of ripening, intensifying the contribution of this pathway to volatile production (Sanz *et al.* 1997). As for the amino acid precursors, their conversion into branched-chain alcohols requires previous transformation into oxo-acids by an aminotransferase. Oxo-acids derived from both fatty acids and amino acids are reduced to aldehydes by an oxo-acid decarboxylase, the major of which being pyruvate decarboxylase (PDC; EC 4.1.1.1), and aldehydes are in turn reduced to alcohols by alcohol deshydrogenase (ADH; EC 1.1.1.1). AAT can subsequently act upon alcohols provided by these or other pathways to synthesise volatile esters. Since substrate specificity of all AAT isoforms reported to date is reportedly wide, alterations in the activity of enzymes located upstream in the biosynthetic pathway can give rise to changes in compounds emitted, as a given alcohol or aldehyde may out-compete the preferential substrate if the respective concentrations are sufficiently different as a result of the alterations induced (Rudell *et al.* 2002). These differences in substrate specificity among AAT isoforms are believed to contribute to the varietal differences in volatile ester profiles (Holland *et al.* 2005).

Recent genomic approaches have helped shedding light on the regulation of this important quality attribute. Expressed sequence tag (EST) available in public databanks have been intensively analysed to identify apple genes likely to be involved in the biochemical pathways leading to the synthesis of precursors for volatile esters during fruit maturation and ripening (Park *et al.* 2006). A subset of genes potentially participating in the generation of flavour and aroma-related compounds in mature fruit has been identified, including one *LOX*, two *ADH*s, and some other genes involved in ß-oxidation and fatty acid metabolism, most of which are specific of fruit tissues. A microarray approach has also been used to identify the ethylene-regulated transcriptional control points of aroma production in ripening apple fruit (Schaffer *et al.* 2007), and resulted in the identification of 17 candidate genes likely to be ethylene control points for aroma production in apple, mostly at the last step of each biosynthetic pathway. High-density cDNA microarrays have been constructed and used in order to elucidate molecular events associated to pear fruit development and ripening (Fonseca *et al.* 2004). A set of genes was found to activate at the onset of the climacteric period, which included transcripts encoding for cell wall modifications, and pigment and aroma biosynthesis. The latter comprised two short-chain type *ADH*s and a *PDC*.

THE EFFECT OF POSTHARVEST HANDLING

Consumers often complain about altered sensory quality in stored fruit in comparison with those just harvested, and indeed the concentration and composition of volatile esters in fresh and stored apples and pears is affected by several factors. This problem is exacerbated by the fact that the understanding of the fundamental mechanisms controlling changes in flavour is limited, and that many biochemical pathways determining this quality trait are still unknown. Apple fruit maturity at harvest is a major factor for aroma development during the post-harvest period, and thus for produce manipulation (Song and Bargerth 1996; Fellman *et al.* 2000; Lara *et al.* 2008; Villatoro *et al.* 2008b). Too early a harvest, aimed at improving storage potential and handling possibilities, will result in deficient development of the cultivar-specific aroma profile and thus in important quality loss in terms of flavour (Fellman *et al.* 1993; Herregods and Goffings 1994; Vanoli *et al.* 1995). The effect of crop load and of cultural practices such as deficit irrigation, paclobutrazol (PBZ) applications, or organic and integrated production, has also been reported (Rizzolo *et al.* 1997; Mpelasoka *et al.* 2000; Mpelasoka and Behboudian 2002; Róth *et al.* 2007). In addition, all post-harvest treatments or procedures that have been considered to date reportedly affect, to a larger or lesser extent, the emission of volatile aroma-contributing compounds. The specific effect is dependent upon the pathway preferentially altered by the procedure and the general effect of the treatment on fruit metabolism.

Controlled atmosphere storage

Because most apple and pear production is stored with the purpose of lengthening the commercialisation period, controlled atmosphere (CA) storage has been widely adopted in order to maintain acceptable fruit quality for much longer. Long-term storage of these fruits usually involves the use of CA in the presence of low or very low oxygen concentrations and increased carbon dioxide, which is one of the best options to control ripening- and senescence-related events. Yet in spite of these benefits on overall quality preservation, detrimental effects on volatile production and thus on aroma quality have been reported for a number of apple and pear cultivars, including ‘Cox's Orange Pippin', ‘McIntosh', ‘Jonagold', 'Golden Delicious', ‘Fuji Nagafu-6', ‘Granny Smith', ‘Pacific Rose', ‘Red Delicious', ‘Royal Gala', ‘Splendour', ‘Southern Snap', ‘Delicious', ‘Bartlett', ‘Passe-Crassane', ‘Packham's Triumph', ‘Conference' and ‘Doyenne du Comice' (Knee and Sharples 1981; Yahia *et al.* 1985; Streif and Bangerth 1988; Mattheis *et al.* 1991; Rizzolo *et al.* 1991; Hansen *et al.* 1992; Nanos *et al.* 1992; Brackmann *et al.* 1993; Chervin *et al.* 2000; Dixon and Hewett 2000b; López *et al.* 2000; Lara *et al.* 2003; Rizzolo *et al.* 2005; Chen *et al.* 2006a, 2006b; Lara *et al.* 2006, 2007; Moya-León *et al.* 2007; Villatoro *et al.* 2008a). These previous reports informed that CA storage causes a decrease in the emission of volatile compounds with a resulting loss of sensory acceptance. Undesirable responses in aroma development also include the induction of fermentation and the development of disagreeable flavours (Beaudry 1999).

The severity of these detrimental effects is largely dependent on O_2 and CO_2 concentrations in the storage atmosphere and on storage period. Significant variability in the response to modifications in atmosphere composition among cultivars has been also reported (Dixon and Hewett 2000b), as well as the dependence on the temperature of the hypoxic treatment (Dixon and Hewett 2001). In general, lower O_2 and higher CO_2 levels and/or longer storage periods will result in stronger suppression of volatile emission. However, the influence of CA storage on the biosynthesis of these compounds is also dependent on their metabolic origin: a decrease in straight-chain esters (fatty acid-derived) has been observed in response to CA (Fellman *et al.* 1993), whereas emission of branched-chain esters (amino acid-derived) remains apparently unaffected (Mattheis *et al.* 1998). Therefore, the aroma profile of CA-stored fruit may display both decreased intensity and alterations owing to imbalances among compounds derived from different precursors. In addition, low O_2 and high CO_2 concentrations can lead to disruption of respiratory metabolism and to the onset of fermentative processes, resulting in pyruvate accumulation, which is subsequently converted into acetaldehyde and ethanol by PDC and ADH (Forney *et al.* 2000). High CO_2 levels tend to decrease cytoplasm pH thus activating both enzyme activities, whose optimum pH is 6, and leading to ethanol accumulation and to an increase in the emission of ethyl esters, and particularly of ethyl acetate. Excess of ethyl acetate, acetaldehyde and ethanol may result in the development of off-flavours (Ke *et al.* 1994). ADH activity in ‘Packham's Triumph' pears stored under 3 kPa O_2 at -1°C for two months was approximately twice that in air-stored samples (Chervin *et al.* 1999), and activity remained higher throughout the post-storage period at 18°C, although only slightly higher levels of ADH transcripts were observed. Since no significant differences were found for acetaldehyde, ethanol, methanol or PDC activity levels were found between treatments, it was concluded that, in

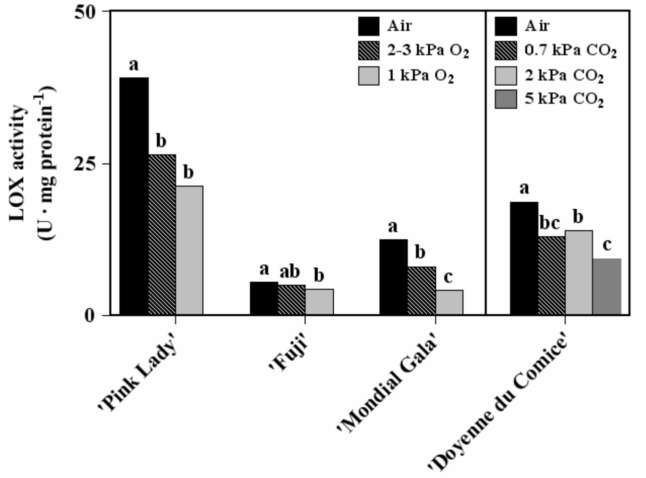

Fig. 1 LOX specific activity in flesh of apple and pear fruit after CA storage + 7 days at 20°C (data from Lara *et al.* 2003, 2006, 2007; Villatoro *et al.* 2008a). Fruit were stored for 3 ('Pink Lady', 'Mondial Gala'), 4 ('Fuji') or 5 ('Doyenne du Comice') months. For 'Doyenne du Comice', O_2 concentration in all three CA conditions was 2 kPa. Values represent means of three replicates. Means with the same letter for a given cultivar are not significantly different at $p < 0.05$ (LSD test).

ripening pears, ADH does not limit ethanol production, and that the expression of this enzyme comprises post-transcriptional regulations.

Other volatile-related enzyme activities are also altered in response to CA conditions. Oxygen is important for the bioconversion of unsaturated fatty acids into volatile aldehydes (Almosnino *et al.* 1996). Accordingly, LOX activity, and thus the supply of fatty-acid derived precursors, has been reported to be partially inhibited in CA-stored apples and pears, causing abnormal development of fruit aroma after removal from hypoxia to air (Lara *et al.* 2003, 2006, 2007; Villatoro *et al.* 2008a) (**Fig. 1**). Decreased production of volatile esters by these fruit appeared to be more dependent on lessened substrate availability than on inhibited ester-forming capacity. Moreover, an extra period in cold air after CA storage has been reported to help recovery of ester-synthesising capacity during the marketing period of apple fruit (Altisent *et al.* 2008), and this partial regeneration has been shown to be related to enhanced LOX activity (Altisent *et al.* 2009). Likewise, improved ester biosynthesis after dynamic or 'delayed' CA storage reported for apple (Mattheis *et al.* 1998) and pear (Saquet *et al.* 2000) has been suggested to arise from better preservation of ATP and fatty acid levels (Song and Bangerth 2003). The formation of acetyl CoA, derived from pyruvate through oxidative processes, is also inhibited under anaerobic conditions, leading to decreased acetate ester production (Wendakoon *et al.* 2004).

Ethylene and ethylene inhibitors

Ethylene regulates and coordinates many ripening-related events in climacteric fruit species, although the inductive concentration appears to be different for different ripening-related events. For instance, although ethylene treatments have a positive impact on aroma volatile production in 'd'Anjou' pears during ripening at 20°C, the threshold required for the stimulation of aroma production biosynthesis was higher than that required for the induction of fruit softening (Wang *et al.* 1972), which suggested that continuous ethylene treatment might provide better fruit flavour in comparison with short-term exposure. Continuous exposure to ethylene for 7 days at 20°C has been reported to stimulate volatile production in 'Gala' apples after storage under CA for up to 28 weeks (Mattheis *et al.* 2005), but in contrast did not consistently counteract the inhibiting effect of previous ethylene suppression treatments in 'd'Anjou' pear fruit (Argenta *et al.* 2003).

Thus suppression of ethylene biosynthesis or action is expected to have inhibitory effects on these modifications, including the production of flavour-related volatile compounds. A dramatic inhibition of the biosynthesis of volatile esters has been observed in apple fruit silenced for ethylene production, although no significant suppression was found for the aldehyde and alcohol precursors of these esters (Dandekar *et al.* 2004), which indicated differential regulation of the different flavour-contributing compounds and suggested a possible ethylene-mediated regulation of LOX or HPL expression. Particularly, the ethylene-silenced apples displayed a sharp reduction in hexyl and butyl acetates, whereas the suppression was less dramatic for other volatile esters. Similar results were found by Defilippi *et al.* (2004), reporting a reduction in ester and alcohol reduction in ethylene-suppressed apple lines, but no major differences in the concentrations of aldehyde volatiles. When volatile production, enzyme activity levels, and precursor availability were studied in detail, differential ethylene regulation was found (Defilippi *et al.* 2005a). Whereas AAT activity showed a clear ethylene-dependent pattern, ADH and LOX seemed independent of ethylene modulation. Isoleucine, an important precursor of branched-chain volatile esters, responded significantly to ethylene regulation, while the production of linoleic and linolenic acids, important precursors of straight-chain esters, was associated to increases in aldehydes.

These observations on ethylene regulation of AAT expression and activity are in agreement with other work on apple fruit in which ethylene sensitivity was reduced by means of an ethylene action inhibitor (Fan and Mattheis 1999; Lurie *et al.* 2002; Defilippi *et al.* 2004, 2005b; Moya-León *et al.* 2007) as well as in other fruit species (Yahyaoui *et al.* 2002; El-Sharkawy *et al.* 2005). Specifically, 1-methylcyclopropene (1-MCP) has been reported to affect the expression and activity of AAT, with concomitant alterations in the capacity for ester biosynthesis. However, 1-MCP treatments may be useful to prolong eating quality after middle-term cold storage: 1-MCP-treated 'Conference' pears displayed better sensory quality than untreated fruit after cold storage in air for 14 or 22 weeks, partially resulting from better preservation of fresh flavour (Rizzolo *et al.* 2005). Similarly, 1-MCP treatment has been reported to preserve aroma quality of 'Packham's Triumph' pears after long-term storage (Moya-León *et al.* 2006).

Not all isogenes in the AAT family are apparently affected equally by 1-MCP applications: treatment led to selected inhibition of gene expression of specific AAT family members in both 'Granny Smith' and 'Golden Delicious' apple fruit (Zhu *et al.* 2008). Little impact was observed on the expression of *MdAAT1*, whereas substantial suppression was found for *MdAAT2*. Cultivar-related differences were also found: *MdAAT2* expression was suppressed in 'Golden Delicious' but increased slightly in 'Granny Smith' following treatment. These results indicate differential ethylene regulation for the different members of the AAT family, and are in accordance with work on 'Royal Gala' apple, in which ethylene-independent or even ethylene-inhibited patterns were observed for some *AAT* isogenes (Souleyre *et al.* unpublished). Increased levels of *AAT* transcripts, with correspondingly enhanced AAT activity, have been also found for 1-MCP-treated 'Delbard Estivale' apples in comparison with untreated samples in spite of dramatically reduced biosynthesis of most straight-chain esters, and similar results have been observed for fruit treated with aminoethoxyvinylglycine (AVG), an inhibitor of ethylene production (Harb *et al.* 2010). Apples treated with AVG have reduced production of ethylene and aroma volatile compounds (Halder-Doll and Bangerth 1987). Decreased volatile ester production in 1-MCP- and AVG-treated samples must have arisen from limited supply of alcohol and acyl-CoA precursors for AAT action, as the activity of some related enzyme activities was inhibited in treated fruit (**Fig. 2**), which highlights the relevance of substrate availability for volatile biosynthesis and illustrates the complexity of the process. The

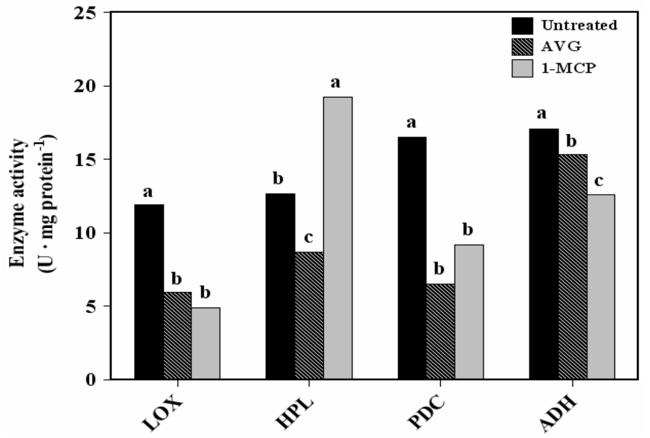

Fig. 2 Aroma volatile-related enzyme activities (U · mg protein^{-1}) in untreated and 1-MCP-treated 'Delbard Estivale' apple fruit one week after harvest. AVG (ReTain®) (125 µL L^{-1} a. i.) was applied about 3 weeks before harvest. 1-MCP (0.625 µL L^{-1}) treatment was done immediately after harvest, at 10°C during 24 h. Values represent means of three replicates. Means with the same letter for a given enzyme activity are not significantly different at $p < 0.05$ (LSD test).

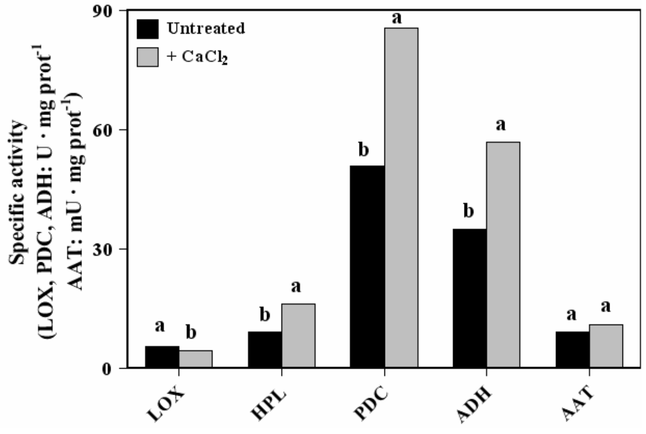

Fig. 3 Aroma volatile-related enzyme activities (U · mg protein^{-1}) in untreated and calcium-treated 'Fuji' apple fruit after storage at 1°C for 4 months + 7 days at 20°C. AAT activity is given as mU · mg protein^{-1}. Fruit were dipped in $CaCl_2$ (2%, w/v) at ambient temperature for 5 min. Values represent means of three replicates. Means with the same letter for a given enzyme activity are not significantly different at $p < 0.05$ (LSD test).

inhibition of ADH activity by ethylene suppression treatments disagrees with previous reports that 1-MCP-treated apples had severely reduced emission of total volatiles but retained more alcohols and aldehydes (Lurie *et al.* 2002) and that activity and expression levels of ADH were not affected by changes in the levels of endogenous ethylene (Defilippi *et al.* 2005b), and supports the existence of significant cultivar-related differences.

Other compounds that modify ethylene production or action also reportedly affect flavour-related volatile emission. Diazocyclopentadiene (DACP), an ethylene action inhibitor, has been reported to inhibit production of ethylene and of some volatile compounds in apple (Fan *et al.* 1998). Compounds that modulate ethylene production, such as methyl jasmonate (MJ), enhance production of volatile alcohols and esters in preclimacteric apple fruit (Fan *et al.* 1997), although reportedly inhibit volatile ester production by fruit stored in CA prior to MJ treatment (Olías *et al.* 1992). The combination of MJ and 1-MCP treatments has been shown to inhibit the production of many volatile alcohols and esters in 'Fuji' apples, although the emission of particular compounds appeared to be differentially inhibited by MJ or 1-MCP (Fan and Mattheis 1999). The combined treatment affected not only the formation of esters from alcohols, but also alcohol production, indicating that the inhibition affected different points of the biochemical pathway. The combination of ethephon with MJ treatments reduced volatile production by 'Delicious' apple fruit compared with ethephon only, but the combined treatment enhanced the production of volatile compounds other than esters by 'Golden Delicious' apples (Kondo *et al.* 2005). The application of ethephon restored volatile production of AVG-treated 'Jonagold' apples to control levels, and improved volatile emission in CA-stored fruit (Mir *et al.* 1999). The application of the synthetic auxin 2,4-dichlorophenoxypropionic acid (2,4-DP), which induces ethylene production and fruit ripening, has been shown to increase the production of alcohols, esters, ketones and aldehydes in 'Bartlett' pears (Kondo *et al.* 2006).

Calcium treatments

Prestorage calcium treatment of apples has been shown to reduce the incidence of physiological disorders, softening rates and losses due to decay-causing fungi (Yuen 1994), and to increase overall acceptability scores (Abbott *et al.* 2000), but only a few works have been published on the effects of calcium treatments on aroma volatile production (Song and Bangerth 1993; Saftner *et al.* 1999; Ortiz *et al.* 2009). Song and Bangerth (1993) reported that the infiltration with 0.15 M $CaCl_2$ decreased significantly total aroma production in 'Golden Delicious'. Because this reduction was similar to all the main volatiles determined individually (butyl acetate, hexyl acetate, and 1-butanol), it was suggested that treatment affected the biosynthesis pathway at a rather early, common step. Similarly, the use of increasing concentrations of $CaCl_2$ for the infiltration of 'Golden Delicious' apples resulted in progressively reduced total volatile levels, respiration, ethylene production, and internal O_2 levels and increased CO_2 levels following storage in air at 0°C for 2 to 4 months (Saftner *et al.* 1999), although the composition of the volatile profile was not altered in comparison to untreated controls. It was suggested that reduced production of volatile compounds arose from treatment-induced formation of a transient barrier to CO_2 and O_2 exchange between the fruit tissue and the surrounding atmosphere. Additionally, calcium plays an important role in maintaining structural integrity of the cell membranes (Picchioni *et al.* 1998), thus delaying lipid catabolism and probably also the production of lipid-derived precursors of aroma volatiles. This is in accordance with recent results (Ortiz *et al.* 2009) showing decreased LOX activity in calcium-treated 'Fuji' fruit after 4 months of cold storage in air as compared to untreated samples (**Fig. 3**). However, in contrast to earlier reports on 'Golden Delicious', calcium treatment of 'Fuji' apples led to increased production of aroma volatile compounds, probably arising from enhanced supply of alcohol precursors for ester production, including ethanol, 1-butanol, 2-methyl-1-butanol and 1-hexanol, as a consequence of increased HPL, PDC and ADH activities (**Fig. 3**). Enhanced activity levels of PDC and ADH, in turn, could have resulted from treatment-induced changes in O_2 and CO_2 levels inside the fruit (Hewett and Thompson 1992; Rajapakse *et al.* 1992; Saftner *et al.* 1999).

Heat treatments

Prestorage heat treatments have been found to maintain apple fruit firmness, colour, soluble solids content and acidity while promoting resistance to many physiological disorders and fungal diseases (Klein and Lurie 1991; Conway *et al.* 1994; Fallik *et al.* 1995), but there have not been many reports on the effects of such treatments on volatile production. Fallik *et al.* (1997) reported that heat treatment of 'Golden Delicious' apples strongly inhibited the emission of total volatile esters immediately after treatment. However, after extended refrigerated storage heat-treated fruit recovered and produced more total volatiles than untreated samples after six weeks of storage at 1°C, suggesting that treatment caused a transient inhibition of the volatile biosynthesising system but actually enhanced the enzyme systems catalysing the production of flavour-related volatile compounds. Although data on related enzyme activities were not reported, these results suggest that pre-storage heat treatments have the potential to improve or overcome the decrease in the emission of aroma volatiles after storage, and are worth of further research.

Ionising radiation

Exposure to ionising radiation inhibits ripening and senescence, extends shelf life, and reduces spoilage of many fruits and vegetables, but its commercial use has been limited because of adverse effects on some fruit quality attributes (Thomas 1986). Because of treatment effectiveness for insect disinfestation and enhanced food safety (Thayer and Rajkowski 1999), some studies have been conducted to assess the effects of irradiation on important fruit quality attributes such as texture or colour (Massey et al. 1964; Bhushan and Thomas 1998). In spite of beneficial effects on some quality aspects, development of off-flavours and reduced biosynthesis of aroma volatiles have been found for irradiated 'Boskoop' apples (Tobback *et al*. 1973). In 'Gala' fruit (Fan *et al*. 2001), irradiation inhibited the production of most, but not all, esters and of some alcohols. This inhibiting effect on the ability to produce volatile compounds increased with applied dose, and was not recoverable for doses higher than 0.44 kGy.

Miscellaneous

Some physiological disorders are also associated to a reduction in the ability for flavour-volatile emission. Superficial scald affects chloroplast-containing fruit cells, and fatty acid biosynthesis takes place in chloroplasts (Ohlrogge and Jaworski 1997), and indeed ester production in scald-developing 'Cortland' apple fruit was reduced reportedly by 50% (Mir and Beaudry 1999). This decrease was particularly noticeable for hexyl esters, consistent with partial inhibition of the biosynthesis of fatty-acid derived volatile esters through the LOX pathway. Interestingly, the application of the antioxidant diphenylamine (DPA), a scald-preventing agrochemical, helped flavour preservation in addition to scald prevention.

THE POTENTIAL FOR GENETIC MANIPULATION

Current knowledge of the biosynthetic pathways leading to the production of aroma-related volatile compounds, and of the modifications induced by intrinsic and extrinsic variables, if yet incomplete, shows a very complex scenario. Aroma is much more challenging to manipulate than other quality attributes. The possibilities in this regard are restricted by the great specificity of profiles, the complexity of involved pathways and of their regulation, and by limited understanding. Ethylene is considered to control volatile ester production, but not necessarily the upstream steps in the biosynthetic pathway involved in ester biosynthesis (Fellman *et al*. 2000; Lurie *et al*. 2002; Dandekar *et al*. 2004). In addition, both ethylene-independent and ethylene-dependent AAT isoforms have been found to operate in fruit (Flores *et al*. 2002; Zhu *et al*. 2008; Souleyre *et al*., unpublished).

The expression of some aroma-related genes has been modified in tomato, and the results have been useful as a source of information on the biosynthetic pathways involved in the generation of aroma volatiles by fruit. For instance, overexpression of a yeast Δ-9 desaturase gene in tomato increased the concentrations of fatty acids, some of which are precursors of important flavour volatiles (Wang *et al*. 1996). Enhanced concentrations of LOX substrates in transformed fruit led to higher levels of hexanal and hexanol, thus underlying the relevance of this pathway for the final aroma profile of fruit. In a later study, the genetic manipulation of an ADH isogen (*ADH2*) expression was reported to affect the balance of some flavour-related aldehydes and alcohols (Speirs *et al*. 1998). Fruit modified to display increased levels of ADH expression and activity exhibited correspondingly higher production of hexanol and *cis*-3-hexenol and, interestingly, were rated higher in "ripe fruit" flavour by a sensory panel.

Silencing of two tomato LOX isogenes (*TomloxA* and *TomloxB*) failed to reduce significantly flavour volatiles in ripening fruit (Griffiths *et al*. 1999), suggesting that LOX may have other physiological functions in addition to volatile production, and that a third LOX isoform could be the one actually involved in the process. Indeed, a more recent paper (Chen *et al*. 2004) has reported that the specific depletion of *TomloxC* in transgenic tomatoes led to a marked reduction in the levels of some flavour volatiles, including hexanal, hexenal, and hexenol, to as little as 1.5% of those of wild-type controls. This series of works adds further complexity to a hypothetical genetic manipulation of flavour, arising from the fact that many of the genes involved in volatile generation belong to multigene families, and that they may be related to completely different physiological processes than the one targeted. In addition, due to the close interconnection among different pathways, the attempts to modify the expression of a particular gene may produce undesirable changes in the emission of other volatiles. The effects of cultural and environmental factors should be also taken into account.

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REFERENCES

Abbott JA, Klein JD, Campbell TA, Conway WS, Sams CE (2000) Sensory and firmness measurements of calcium- and heat-treated apples. *Journal of Texture Studies* **31**, 109-121

Acree TE, McLellan MR (1988) Flavour components and quality attributes. In: Downing DL (Ed) *Processed Apple Products*, Van Nostrand Reinhold, New York, USA, pp 323-341

Almosnino AM, Bensoussan M, Belin JM (1996) Unsaturated fatty acid bioconversion by apple pomace enzyme system. Factors influencing the production of aroma compounds. *Food Chemistry* **55**, 327-332

Altisent R, Graell J, Lara I, López ML, Echeverría G (2008) Regeneration of volatile compounds in 'Fuji' apples following ultra low oxygen atmosphere storage and its effect on sensory acceptability. *Journal of Agricultural and Food Chemistry* **56**, 8490-8497

Altisent R, Echeverría G, Graell J, López ML, Lara I (2009) Lipoxygenase activity is involved in the regeneration of volatile ester-synthesizing capacity after ultra-low oxygen storage of 'Fuji' apple. *Journal of Agricultural and Food Chemistry* **57**, 4305-4312

Argenta LC, Fan XT, Mattheis JP (2003) Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. pear fruit. *Journal of Agricultural and Food Chemistry* **51**, 3585-3864

Bartley IM, Stoker PG, Martin ADE, Hatfield SGS, Knee M (1985) Synthesis of aroma compounds by apples supplied with alcohols and methyl esters of fatty acids. *Journal of the Science of Food and Agriculture* **36**, 567-574

Beaudry RM (1999) Effect of O_2 and CO_2 partial pressure on selected phenolmena affecting fruit and vegetable quality. *Postharvest Biology and Technology* **15**, 293-303

Berger RG, Drawert F (1984) Changes in the composition of volatiles by postharvest application of alcohols to Red Delicious apples. *Journal of the Science of Food and Agriculture* **35**, 1318-1325

Bhushan B, Thomas P (1998) Quality of apples following gamma irradiation and cold storage. *International Journal of Food Sciences and Nutrition* **49**, 485-492

Brackmann A, Streif J, Bangerth F (1993) Relationship between a reduced aroma production and lipid metabolism of apple after long-term controlled atmosphere storage. *Journal of the American Society for Horticultural Science* **118**, 243-247

Bult JHF, Schifferstein HNJ, Roozen JP, Dalmau Boronat E, Voragen AGJ, Kroeze JHA (2002) Sensory evaluation of character impact components in an apple model mixture. *Chemical Senses* **27**, 485-494

Buttery RG (1993) Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In: Acree TE, Teranishi R (Eds) *Flavor Science: Sensible Principles and Techniques*, ACS Professional, Washington DC, USA, pp 259-286

Chen G, Hackett R, Walker D, Taylor A, Lin Z, Grierson D (2004) Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the generation of fatty acid-derived flavor compounds. *Plant Physiology* **136**, 2641-2651

Chen JL, Yan S, Feng Z, Xiao L, Hu XS (2006a) Changes in the volatile compounds and chemical and physical properties of Yali pear (*Pyrus bertschneideri* Reld) during storage. *Food Chemistry* **97**, 248-255

Chen JL, Wu JH, Wang Q, Deng H, Hu XS (2006b) Changes in the volatile compounds and chemical and physical properties of Kuerle Fragrant pear (*Pyrus serotina* Reld) during storage. *Journal of Agricultural and Food Chemistry* **54**, 8842-8847

Chervin C, Truett JK, Speirs J (1999) Alcohol dehydrogenase expression and alcohol production during pear ripening. *Journal of the American Society for Horticultural Science* **124**, 71-75

Chervin C, Speirs J, Loveys B, Patterson BD (2000) Influence of low oxygen storage on aroma compounds of whole pears and crushed pear flesh. *Postharvest Biology and Technology* **19**, 279-285

Conway WS, Sams CF, Wang CY, Abbott JA (1994) Additive effects of postharvest calcium and heat treatment on reducing decay and maintaining quality in apples. *Journal of the American Society for Horticultural Science* **119**, 49-53

Dandekar AM, Teo G, Defilippi BG, Uratsu SL, Passey AJ, Kader AA, Stow JR, Colgan RJ, James DJ (2004) Effect of down-regulation of ethylene biosynthesis on fruit flavour complex in apple fruit. *Transgenic Research* **13**, 373-384

Defilippi BG, Dandekar AM, Kader AA (2004) Impact of suppression of ethylene action or biosynthesis on flavour metabolites in apple (*Malus domestica* Borkh) fruits. *Journal of Agricultural and Food Chemistry* **52**, 5694-5701

Defilippi BG, Dandekar AM, Kader AA (2005a) Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissues. *Journal of Agricultural and Food Chemistry* **53**, 3133-3141

Defilippi BG, Kader AA, Dandekar AM (2005b) Apple aroma: alcohol acyltransferase, a rate limiting step for ester biosynthesis, is regulated by ethylene. *Plant Science* **168**, 1199-1210

Dixon J, Hewett EW (2000a) Factors affecting apple aroma/flavour volatile concentration: a review. *New Zealand Journal of Crop and Horticultural Science* **28**, 155-173

Dixon J, Hewett EW (2000b) Exposure to hypoxia conditions alters volatile concentrations of apple cultivars. *Journal of the Science of Food and Agriculture* **81**, 22-29

Dixon J, Hewett EW (2001) Temperature of hypoxic treatment alters volatile composition of juice from 'Fuji' and 'Royal Gala' apples. *Postharvest Biology and Technology* **22**, 71-83

Eccher Zerbini P, Pianezzola A, Grassi M (1999) Poststorage sensory profiles of fruit of five apple cultivar harvested at different maturity stages. *Journal of Food Quality* **22**, 1-17

El-Sharkawy I, Manríquez D, Flores FB, Regad F, Bouzayen M, Latché A, Pech JC (2005) Functional characterization of a melon alcohol acyl-transferase gene family involved in the biosynthesis of ester volatiles. Identification of the crucial role of a threonine residue for enzyme activity. *Plant Molecular Biology* **59**, 345-362

Fallik E, Grinberg S, Gambourg M, Klein DJ, Lurie S (1995) Prestorage heat treatment reduces pathogenicity of *Penicillium expansum* in apple fruit. *Plant Pathology* **45**, 92-97

Fallik E, Archbold DD, Hamilton-Kemp TR, Loughrin JH, Collins RW (1997) Heat treatment temporarily inhibits aroma volatile compound emission from Golden Delicious apples. *Journal of Agricultural and Food Chemistry* **45**, 4038-4041

Fan X, Mattheis JP, Fellman JK, Patterson ME (1997) Effect of methyl jasmonate on ethylene and volatile production by Summerred apples depends on fruit developmental stage. *Journal of Agricultural and Food Chemistry* **45**, 208-211

Fan X, Mattheis JP, Buchanan D (1998) Continuous requirement of ethylene for apple fruit volatile synthesis. *Journal of Agricultural and Food Chemistry* **46**, 1959-1963

Fan X, Mattheis JP (1999) Impact of 1-methylcyclopropene and methyl jasmonate on apple volatile production. *Journal of Agricultural and Food Chemistry* **47**, 2847-2853

Fan X, Argenta L, Mattheis J (2001) Impacts of ionizing radiation on volatile production by ripening Gala apple fruit. *Journal of Agricultural and Food Chemistry* **49**, 254-262

Fellman JK, Mattheis JP, Patterson MF, Mattinson DS, Bostick BC (1993) Study of ester biosynthesis in relation to harvest maturity and controlled-atmosphere storage of apples (*Malus domestica* Borkh.). In: Blanpied GD (Ed) *Proceedings of the 6th International CA Research Conference*, Cornell University, Ithaca, NY, pp 500-507

Fellman JK, Miller TW, Mattison DS, Mattheis JP (2000) Factors that influence biosynthesis of flavor compounds in apple fruits. *HortScience* **35**, 1026-1033

Fellman JK, Rudell DR, Mattinson DS, Mattheis JP (2003) Relationship of harvest maturity to flavour regeneration after CA storage of 'Delicious' apples. *Postharvest Biology and Technology* **27**, 39-51

Flores F, El Yahyaoui I, de Billerbeck G, Romojaro F, Latché A, Bouzayen M, Pech JC, Ambid C (2002) Role of ethylene in the biosynthetic pathway of aliphatic ester aroma volatiles in Charentais Cantaloupe melons. *Journal of Experimental Botany* **53**, 201-206

Fonseca S, Hackler L, Zvara A, Ferreira S, Baldé A, Dudits D, Pais MS, Puskás LG (2004) Monitoring gene expression along pear fruit development, ripening and senescence using cDNA microarrays. *Plant Science* **167**, 457-469

Forney CF, Kalt W, Jordan MA (2000) The composition of strawberry aroma is influenced by cultivar, maturity, and storage. *HortScience* **35**, 1022-1026

Griffiths A, Prestage S, Linforth R, Zhang JL, Taylor A, Grierson D (1999) Fruit-specific lipoxygenase suppression in antisense-transgenic tomatoes. *Postharvest Biology and Technology* **17**, 163-173

Halder-Doll H, Bangerth F (1987) Inhibition of autocatalytic C_2H_4 biosynthesis by AVG applications and consequences on the physiological behaviour and quality of apples fruits in cool storage. *Scientia Horticulturae* **33**, 87-96

Hansen K, Poll L, Olsen CE, Lewis MJ (1992) The influence of oxygen concentration in storage atmospheres on the post-storage volatile production of 'Jonagold' apples. *Lebensmittel Wissenschaft und Technologie* **25**, 457-461

Harb J, Streif J, Bangerth F (1994) Synthesis of aroma compounds by controlled atmosphere (CA) stored apples supplied with aroma precursors: Alcohols, acids, and esters. *Acta Horticulturae* **368**, 142-149

Harb J, Lara I, Saleh O, Khraiwesh B, Streif J (2010) Ethylene suppression modifies gene expression and activity of aroma volatile-related enzymes in 'Delbard Estivale' apples. *Acta Horticulturae* in press

Harker FR, Marsh KB, Young H, Murray SH, Gunson FA, Walker SB (2002) Sensory interpretation of instrumental measurements 2: sweet and acid taste of apple fruit. *Postharvest Biology and Technology* **24**, 241-250

Harker FR, Amos RL, Echeverría G, Gunson FA (2006) Influence of texture on taste: Insights gained during studies of hardness, juiciness, and sweetness of apple fruit. *Journal of Food Science* **71**, 77-82

Herregods M, Goffings Y (1994) The storage of 'Jonagold' apples in U.L.O.-circumstances. *Acta Horticulturae* **343**, 148-154

Hewett EW, Thompson CJ (1992) Modification of internal carbon dioxide and oxygen levels in apple fruit by postharvest calcium application and modified atmospheres. *Postharvest Biology and Technology* **1**, 213-219

Holland D, Larkov O, Bar-Ya'akok I, Bar E, Zax A, Brandeis E, Ravid U, Lewinsohn E (2005) Developmental and varietal differences in volatile ester formation and acetyl-CoA: alcohol acetyl transferase activities in apple (*Malus domestica* Borkh.) fruit. *Journal of Agricultural and Food Chemistry* **53**, 7198-7203

Jennings WG, Sevenants MR (1964) Volatile esters of Bartlett pear III. *Journal of Food Science* **29**, 158-163

Jennings WG, Creveling RK, Heinz DE (1964) Volatile esters of Bartlett pear IV. Esters of *trans*:2-*cis*:4-decadienoic acid. *Journal of Food Science* **29**, 730-734

Ke D, Yahia EM, Mateos M, Kader AA (1994) Ethanolic fermentation of 'Bartlett' pears as influenced by ripening stage and atmospheric composition. *Journal of the American Society for Horticultural Science* **119**, 976-/982

Klein JD, Lurie S (1991) Postharvest heat treatment and fruit quality. *Postharvest News and Information* **2**, 15-19

Knee M, Sharples RO (1981) The influence of controlled atmosphere storage on the ripening of apples in relation to quality. In: Goodenough PW, Atkins RK (Eds) *Quality in Stored and Processed Vegetables and Fruit*, Academic Press, London, UK, pp 341-352

Kollmannsberger H, Berger RG (1992) Precursor atmosphere storage induced flavor changes in apple cv. 'Red Delicious'. *Chemie, Mikrobiologie, Technologie der Lebensmittel* **14**, 81-86

Kondo S, Setha S, Rudell DR, Buchanan DA, Mattheis JP (2005) Aroma volatile biosynthesis in apples affected by 1-MCP and methyl jasmonate. *Postharvest Biology and Technology* **36**, 61-68

Kondo S, Isuzugawa I, Kobayashi S, Mattheis J (2006) Aroma volatile emission and expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase genes in pears treated with 2,4-DP. *Postharvest Biology and Technology* **41**, 22-31

Lara I, Miró RM, Fuentes T, Sayez G, Graell J, López ML (2003) Biosynthesis of volatile aroma compounds in pear fruit stored under long-term controlled-atmosphere conditions. *Postharvest Biology and Technology* **29**, 29-39

Lara I, Graell J, López ML, Echeverría G (2006) Multivariate analysis of modifications in biosynthesis of volatile compounds after CA storage of 'Fuji' apples. *Postharvest Biology and Technology* **39**, 19-28

Lara I, Echeverría G, Graell J, López ML (2007) Volatile emission after controlled atmosphere storage of 'Mondial Gala' apples (*Malus × domestica*): Relationship to some involved enzyme activities. *Journal of Agricultural and Food Chemistry* **55**, 6087-6095

Lara I, Ortiz A, Echeverría G, López ML, Graell J (2008) Development of aroma-synthesising capacity throughout fruit maturation of 'Mondial Gala' apples. *Journal of Agricultural Science and Biotechnology* **83**, 253-259

Lavilla T, Puy J, López ML, Recasens I, Vendrell M (1999) Relationships between volatile production, fruit quality, and sensory evaluation in Granny Smith apple stored in different controlled atmosphere treatments by multivariate analysis. *Journal of Agricultural and Food Chemistry* **47**, 3791-3803

Li D, Xu Y, Xu G, Gu L, Li D, Shu H (2006) Molecular cloning and expression of a gene encoding alcohol acyltransferase (*MdAAT2*) from apple (cv. Golden Delicious). *Phytochemistry* **67**, 658-667

Li D, Xu G, Xu Y, Wu T, Shen J, Li D, Shu H (2007) Prokariotic expression, purification, and sub-cellular localization of a novel alcohol acyltransferase from apple. *Biotechnology Letters* **29**, 1363-1368

López ML, Lavilla MT, Riba M, Vendrell M (1998a) Comparison of volatile compounds in two seasons in apples: 'Golden Delicious' and 'Granny Smith'. *Journal of Food Quality* **21**, 155-166

López ML, Lavilla MT, Recasens I, Riba M, Vendrell M (1998b) Influence of different oxygen and carbon dioxide concentrations during storage on production of volatile compounds by 'Starking Delicious' apples. *Journal of Agricultural and Food Chemistry* **46**, 634-643

López ML, Lavilla T, Recasens I, Graell J, Vendrell M (2000) Changes in aroma quality of 'Golden Delicious' apples after storage at different oxygen and carbon dioxide concentrations. *Journal of the Science of Food and Agriculture* **80**, 311-324

Lo Scalzo R, Testoni A, Genna A (2001) 'Annurca' apple fruit, a southern Italy apple cultivar: textural properties and aroma composition. *Food Chemistry* **73**, 333-343

Lurie S, Pre-Aymard C, Ravid U, Larkov O, Fallik E (2002) Effect of 1-methylcyclopropene on volatile emission and aroma in cv. Anna apples. *Journal of Agricultural and Food Chemistry* **50**, 4251-4256

Massey LM, Parsons GF, Smock RM (1964) Radiation processing of foods: Some effects of gamma radiation on the keeping quality of apples. *Journal of Agricultural and Food Chemistry* **12**, 268-274

Matich A, Rowan D (2007) Pathway analysis of branched-chain ester biosynthesis in apples using deuterium labeling and enantioselective gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry* **55**, 2727-2735

Mattheis JP, Buchanan DA, Fellman JK (1991) Change in apple fruit volatiles alter storage in atmospheres inducing anaerobic metabolism. *Journal of Agricultural and Food Chemistry* **39**, 1602-1605

Mattheis JP, Buchanan DA, Fellman JK (1998) Volatile compounds emitted by 'Gala' apples following dynamic atmosphere storage. *Journal of the American Society for Horticultural Science* **123**, 426-432

Mattheis JP, Fan X, Argenta LC (2005) Interactive responses of Gala apple fruit volatile production to controlled atmosphere storage and chemical inhibition of ethylene action. *Journal of Agricultural and Food Chemistry* **53**, 4510-4516

Mehinagic E, Royer G, Symoneaux R, Jourjon F, Prost C (2006) Characterization of odor-active volatiles in apples: Influence of cultivars and maturity stage. *Journal of Agricultural and Food Chemistry* **54**, 2678-2687

Mir NA, Beaudry R (1999) Effect of superficial scald suppression by diphenylamine application on volatile evolution by stored Cortland apple fruit. *Journal of Agricultural and Food Chemistry* **47**, 7-11

Mir NA, Pérez R, Schwallier P, Beaudry R (1999) Relationship between ethylene response manipulation and volatile production in Jonagold variety apples. *Journal of Agricultural and Food Chemistry* **47**, 2653-2659

Moya-León MA, Vergara M, Bravo C, Montes ME, Moggia C (2006) 1-MCP treatment preserves aroma quality of 'Packham's Triumph' pears during long-term storage. *Postharvest Biology and Technology* **42**, 185-197

Moya-León MA, Vergara M, Bravo C, Pereira M, Moggia C (2007) Development of aroma compounds and sensory quality of 'Royal Gala' apples during storage. *Journal of Horticultural Science and Biotechnology* **82**, 403-413

Mpelasoka BS, Behboudian MH, Dixon J, Neal SM, Caspari HW (2000) Improvement of fruit quality and storage potential of 'Braeburn' apple through deficit irrigation. *Journal of Horticultural Science and Biotechnology* **75**, 615-621

Mpelasoka BS, Behboudian MH (2002) Production of aroma volatiles in response to deficit irrigation and to crop load in relation to fruit maturity for 'Braeburn' apple. *Postharvest Biology and Technology* **24**, 1-11

Nanos GD, Romani RJ, Kader AA (1992) Metabolic and other responses of 'Bartlett' pear fruit and suspension-cultured 'Passe-Crassane' pear fruit cells held in 0.25% O_2. *Journal of the American Society for Horticultural Science* **177**, 934-940

Ohlrogge JB, Jaworski JG (1997) Regulation of fatty acid synthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**, 109-136

Olías JM, Sanz LC, Ríos JJ, Pérez AG (1992) Inhibitory effect of methyl jasmonate on the volatile ester-forming enzyme system in Golden Delicious apples. *Journal of Agricultural and Food Chemistry* **40**, 266-270

Ortiz A, Echeverría G, Graell J, Lara I (2009) Calcium dips enhance aroma volatile emission of cold-stored 'Fuji Kiku-8' apples. *Journal of Agricultural and Food Chemistry* **57**, 4931-4938

Park S, Sugimoto N, Larson MD, Beaudry R, van Nocker S (2006) Identification of genes with potential roles in apple fruit development and biochemistry through large-scale statistical analysis of expressed sequence tags. *Plant Physiology* **141**, 811-824

Picchioni GA, Watada AE, Conway WS, Whitaker BD, Sams CE (1998) Postharvest calcium infiltration delays membrane lipid catabolism in apple fruit. *Journal of Agricultural and Food Chemistry* **46**, 2452-2457

Rajapakse NC, Hewett EW, Banks NH, Cleland DJ (1992) Vacuum infiltration with calcium chloride influences oxygen distribution in apple fruit flesh. *Postharvest Biology and Technology* **1**, 221-229

Rizzolo A, Sodi C, Polesello A (1991) Influence of ethylene removal on the volatile development of 'Passe Crassane' pears stored in controlled atmosphere. *Food Chemistry* **42**, 275-285

Rizzolo A, Visai C, Vanoli M (1997) Changes in some odour-active compounds in pacrobutrazol-treated 'Starspur Golden' apples at harvest and after cold storage. *Postharvest Biology and Technology* **11**, 39-46

Rizzolo A, Cambiaghi P, Grassi M, Eccher Zerbini P (2005) Influence of 1-methylcyclopropene and storage atmosphere on changes in volatile compounds and fruit quality of Conference pears. *Journal of Agricultural and Food Chemistry* **53**, 9781-9789

Róth E, Berna A, Beullens K, Yarramraju S, Lammertyn J, Schenk A, Nicolaï B (2007) Postharvest quality of integrated and organically produced apple fruit. *Postharvest Biology and Technology* **45**, 11-19

Rowan DD, Lane HP, Allen JM, Fielder S, Hunt MB (1996) Biosynthesis of 2-methylbutyl, 2-methyl-2-butenyl, and 2-methylbutanoate esters in Red Delicious and Granny Smith apples using deuterium-labeled substrates. *Journal of Agricultural and Food Chemistry* **44**, 3276-3285

Rowan DD, Allen JM, Fielder S, Hunt MB (1999) Biosynthesis of straight-chain ester volatiles in Red Delicious and Granny Smith apples using deuterium-labeled precursors. *Journal of Agricultural and Food Chemistry* **47**, 2553-2562

Rudell DR, Mattinson DS, Mattheis JP, Wyllie SG, Fellman JK (2002) Investigations of aroma volatile biosynthesis under anoxic conditions and in different tissues of 'Redchief Delicious' apple fruit (*Malus domestica* Borkh.). *Journal of Agricultural and Food Chemistry* **50**, 2627-2632

Saftner RA, Conway WS, Sams CE (1999) Posharvest calcium infiltration alone and combined with surface coating treatments influence volatile levels, respiration, ethylene production, and internal atmospheres of 'Golden Delicious' apples. *Journal of the American Society for Horticultural Science* **124**, 553-558

Sanz C, Olías JM, Pérez AG (1997) Aroma biochemistry of fruits and vegetables. In: Tomás Barberán FA, Robins RJ (Eds) *Phytochemistry of Fruits and Vegetables*, Clarendon Press, Oxford, UK, pp 125-155

Saquet AA, Streif J, Bangerth F (2000) Changes in ATP, ADP and pyridine nucleotide level related to the incidence of physiological disorders in 'Conference' pears and 'Jonagold' apples during controlled atmosphere storage. *Journal of Horticultural Science and Biotechnology* **75**, 243-249

Schaffer RJ, Friel EN, Souleyre EJF, Bolitho K, Thodey K, Ledger S, Bowen JH, Ma JH, Nain B, Cohen D, Gleave AP, Crowhurst RN, Janssen BJ, Yao JL, Newcomb RD (2007) A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. *Plant Physiology* **144**, 1899-1912

Schumacher K, Asche S, Heil M, Mittelstädt F, Dietrich H, Mosandl A (1998) Methyl-branched flavour compounds in fresh and processed apples. *Journal of Agricultural and Food Chemistry* **46**, 4496-4500

Song J, Bangerth F (1993) The effect of calcium-infiltration on respiration, ethylene and aroma production of 'Golden Delicious' apple fruits. *Acta Horticulturae* **326**, 131-137

Song J, Bangerth F (1996) The effect of harvest date on aroma compound production from 'Golden Delicious' apple fruit and relationship to respiration and ethylene production. *Postharvest Biology and Technology* **8**, 259-269

Song J, Bangerth F (2003) Fatty acids as precursors for aroma volatile biosynthesis in preclimacteric and climacteric apple fruit. *Postharvest Biology and Technology* **30**, 113-121

Souleyre EJF, Greenwood DR, Friel EN, Karunairetnam S, Newcomb RD (2005) An alcohol acyl transferase from apple (cv. Royal Gala), MpAAT1, produces esters involved in apple fruit flavour. *The FEBS Journal* **272**, 3132-3144

Speirs J, Lee E, Holt K, Yong-Duk K, Steele N, Loveys B, Schuch W (1998) Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavour aldehydes and alcohols. *Plant Physiology* **117**, 1047-1058

Stow J (1995) Quality measurements of apples. *Postharvest News and Information* **6**, 32-33

Streif J, Bangerth F (1988) Production of volatile aroma substances by 'Golden Delicious' apple fruits after storage for various times in different CO_2 and O_2 concentrations. *Journal of Horticultural Science* **63**, 193-199

Suwanagul A, Richardson DC (1998) Identification of headspace volatile compounds from different pear (*Pyrus communis* L.) varieties. *Acta Horticulturae* **475**, 605-622

Thayer DW, Rajkowski KT (1999) New development in irradiation of fresh fruits and vegetables. *Food Technology* **53**, 62-65

Thomas P (1986) Radiation preservation of foods of plant origin. Part V. Temperate fruits: Pome fruits, stone fruits, and berries. *CRC Critical Reviews in Food Science and Nutrition* **24**, 357-400

Tobback PP, Glandian RE, Blockeel A, Maes E, Herregods M (1973) Influence of gamma irradiation on the production of aroma compositions in apple (variety Boskoop) during ripening and storage. In: *Radiation Preservation of Food*, International Atomic Energy Commission, Vienna, Austria, pp 137-152

Vanoli M, Visai C, Rizzolo A (1995) The influence of harvest date on the volatile composition of 'Starkspur Golden' apples. *Postharvest Biology and Technology* **6**, 225-234

Vikram A, Prithiviraj B, Hamzehzarghani H, Kushalappa AC (2004) Volatile metabolite profiling to discriminate diseases of McIntosh apple inoculated with fungal pathogens. *Journal of the Science of Food and Agriculture* **84**, 1333-1340

Villatoro C, Echeverría G, Graell J, López ML, Lara I (2008a) Long-term storage of 'Pink Lady®' apples modifies volatile-involved enzyme activities: Consequences on production of volatile esters. *Journal of Agricultural and Food Chemistry* **56**, 9166-9174

Villatoro C, Altisent R, Echeverría G, Graell J, López ML, Lara I (2008b) Changes in biosynthesis of aroma volatile compounds during on-tree maturation of 'Pink Lady®' apples. *Postharvest Biology and Technology* **47**, 286-295

Wang CY, Mellenthin WM, Hansen E (1972) Maturation of 'Anjou' pears in relation to chemical composition and reaction to ethylene. *Journal of the American Society for Horticultural Science* **97**, 9-42

Wang C, Chin CK, Ho CT, Hwang CF, Polashock JJ, Martin CE (1996) Changes of fatty acids and fatty acid-derived flavor compounds by expressing the yeast d-9 desaturase gene in tomato. *Journal of Agricultural and Food Chemistry* **44**, 3399-3402

Wang C, Xing J, Chin CK, Ho CT, Martin CE (2001) Modification of fatty acids changes the flavour volatiles in tomato leaves. *Phytochemistry* **58**, 227-232

Wendakoon SK, Ueda Y, Imahori Y, Ishimaru M (2004) Inhibition of acetate ester biosynthesis in banana (*Musa sapientum* L.) fruit pulp under anaerobic conditions. *Journal of Agricultural and Food Chemistry* **52**, 1615-1620

Wyllie S, Fellman J (2000) Formation of volatile branched chain esters in bananas (*Musa sapientum* L.). *Journal of Agricultural and Food Chemistry* **48**, 3493-3496

Yahia EM (1994) Apple flavour. *Horticultural Reviews* **16**, 197-234

Yahia EM, Liu FW, Acree TE, Butts R (1985) Odor-active volatiles in McIntosh apples stored in simulated low ethylene controlled atmosphere. In: Blankenship SM (Ed) *Controlled Atmosphere for Storage and Transport of Perishable Agricultural Commodities. Proceedings of the 4th National CA Research Conference*, North Carolina State University, Raleigh, NC, pp 70-81

Yahyaoui FEL, Wongs-Aree C, Latché A, Hackett R, Grierson D, Pech JC (2002) Molecular and biochemical characteristics of a gene encoding an alcohol acyl-transferase involved in the generation of aroma volatile esters during melon ripening. *European Journal of Biochemistry* **269**, 2359-2366

Young H, Gilbert JM, Murray SH, Ball RD (1996) Causal effects of aroma compounds on Royal Gala apple flavours. *Journal of the Science of Food and Agriculture* **71**, 329-336

Yuen CMC (1994) Calcium and fruit storage potential. In: *Postharvest Handling of Tropical Fruits*, ACIAR Proceeding Series (Vol 50), International Conference on Postharvest Handling of Tropical Fruits, 19-23 July, 1993, Chiang Mai, Thailand, pp 218-227

Zhu Y, Rudell DR, Mattheis JP (2008) Characterization of cultivar differences in alcohol acyltransferase and 1-aminocyclopropane-1-carboxylate synthase gene expression and volatile ester emission during apple fruit maturation and ripening. *Postharvest Biology and Technology* **49**, 330-339