Rev. Fitotec. Mex. Vol. 39 (2): 159 - 173, 2016

VOLATILE COMPOUNDS IN GOLDEN DELICIOUS APPLE FRUIT (*Malus domestica*) DURING COLD STORAGE

COMPUESTOS VOLÁTILES DE MANZANA (*Malus domestica*) GOLDEN DELICIOUS DURANTE ALMACENAMIENTO

N. A. Salas[1], G. A. González-Aguilar[3], J. L. Jacobo-Cuéllar[4], M. Espino[2], D. Sepúlveda[2], V. Guerrero[1] and G. I. Olivas[2]*

[1]Facultad de Ciencias Agrotecnológicas, Universidad Autónoma de Chihuahua. Cuauhtémoc, Chih., México. [2]Laboratorio de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en Alimentación y Desarrollo. Cuauhtémoc, Chih., México. [3]Centro de Investigación en Alimentación y Desarrollo, A.C., Km 0.6 Carretera la Victoria. Hermosillo, Son., México. [4]Campo Experimental Sierra de Chihuahua, INIFAP. Av. Hidalgo No. 1213,Cuauhtémoc, Chih. México.

**Corresponding autor*: golivas@ciad.mx

SUMMARY

Biosynthesis of volatile compounds (VC), as well as activity of related enzymes (lipoxygenase LOX, alcohol acyltransferase AAT, and alcohol dehydrogenase ADH), and fatty acids (palmitic, stearic, oleic, linoleic and linolenic acids) were assessed in Golden Delicious fruit apples (*Malus domestica* Borkh.) during 1 ºC storage at different atmosphere conditions. Three atmosphere conditions were used: 21 % O_2 and > 1 % CO_2 (Regular Atmosphere, RA), 3 % CO_2 and 2 % O_2 (Controlled Atmosphere, CA), and CA, with 7 d under RA conditions (CA + RA), to evaluate the effect of shorts periods under air storage. CA conditions inhibited the production of butyl acetate and hexyl acetate esters, and increased hexanol concentration. Production of the branched ester 2-methyl butyl acetate did not decrease under CA conditions. As a result of 7 d under RA, butyl acetate and hexyl acetate in CA + RA increased, mainly after one month of storage. Storage under CA conditions inhibited LOX and AAT activity at some stages whereas ADH activity increased during CA storage. LOX activity showed high correlation with production of aldehydes (r^2 = 0.85) and cis-2-hexenal (r^2 = 0.94), during storage of apples under CA conditions. Good correlation was found between AAT activity and total esters and butyl acetate content under CA storage of apples (r^2 = 0.92 and r^2 = 0.93, respectively). While most fatty acids increased in concentration during RA and CA storage, linolenic acid content decreased. No correlation between volatile compounds content and fatty acid production was found.

Index words: Controlled atmosphere, enzymes, fatty acids, *Malus domestica*.

RESUMEN

La biosíntesis de compuestos volátiles, así como la actividad de las enzimas involucradas (lipooxigenasa LOX, alcohol aciltransferasa AAT y alcohol deshidrogenasa ADH), y los ácidos grasos (palmítico, esteárico, oléico, linoléico y linolénico) fueron evaluados en manzana (*Malus domestica* Borkh.) var. Golden Delicious durante almacenamiento en refrigeración (1 ºC) con diferentes condiciones de atmósfera: 21 % O_2 y >1 % CO_2 (Atmósfera Regular, RA), 3 % CO_2 y 2 % O_2 (Atmósfera Controlada, CA), y atmósfera controlada más 7 d en refrigeración bajo atmosfera regular, para evaluar el efecto de un corto periodo de almacenamiento en aire. La condición de CA inhibió la producción de ésteres como acetato de butilo y acetato de hexilo e incrementó la concentración de hexanol. El ester ramificado acetato de 2-metil butilo no fue afectado negativamente en condiciones de CA. Como resultado de 7 d en RA, en CA + RA el acetato de butilo y el acetato de hexilo se incrementaron, principalmente después del primer mes de almacenamiento. La actividad enzimática de LOX y AAT fueron inhibidas en algunas etapas durante el almacenamiento con CA. La actividad enzimática de la ADH incrementó durante el almacenamiento con CA. La actividad enzimática mostró correlación con el total de aldehídos (r^2 = 0.85) y con la producción de cis 2-hexenal (r^2 = 0.94), durante el almacenamiento en condiciones de CA. También se encontró correlación entre la actividad enzimática de AAT con el total de ésteres y acetato de butilo en condiciones de CA (r^2 = 0.92 y r^2 = 0.93, respectivamente). En tanto, la mayoría de los ácidos grasos incrementaron su concentración durante el almacenamiento en RA y CA, el ácido linolénico disminuyó. No se encontró correlación entre los compuestos volátiles y la producción de ácidos grasos.

Palabras clave: Atmósfera controlada, enzimas, ácidos grasos, *Malus domestica*.

INTRODUCTION

Golden Delicious is the most cultivated apple (*Malus domestica* Borkh.) variety in Chihuahua, México (SAGARPA 2010). Flavor is the main quality attribute of apples from Chihuahua (Bismark, 2002; Olivas *et al*., 2007). Volatile compounds are essential and confer a complex combination of taste and odor (Defilippi *et al*., 2009).

More than 300 different volatile compounds have been identified in apples to date (Dixon and Hewett, 2000). The main precursors of volatile compounds in apple are fatty acids which are catabolized through β-oxidation and lipoxygenase (LOX) pathway (Pérez and Sanz, 2008), that produce straight chain aldehydes, alcohols, and esters. Aldehydes are predominant in immature apples (De Pooter *et al*., 1987), whereas alcohols and esters prevail in ripe fruits (Flath *et al*., 1967). Alcohol biosynthesis involves enzymes such as alcohol dehydrogenase (ADH; EC 1.1.1.1) and lipoxygenase (LOX; EC 1.13.11.12) (Defilippi *et al*., 2005; Echeverría *et al*., 2004a). Availability of alcohols is a limiting factor for ester biosynthesis (Berger and Drawert, 1984; Defilippi *et al*., 2005), since they are derived from a reaction catalyzed by alcohol acyltransferase (AAT; EC 2.3.1.84) involving esterification of alcohols and acyl-CoA (Sanz *et al*., 1997). Esters are qualitatively and quantitatively predominant in most

Recibido: 18 de Junio del 2014
Aceptado: 29 de Noviembre del 2015

apples, accounting for 80 % of the total volatile content in Golden Delicious apples (López *et al*., 1998).

A small percentage of the fruit is commercialized immediately after harvest, while most of it is stored. Apples stored for a long periods of time are usually kept under controlled atmosphere (CA) conditions (Brackmann *et al*., 1994). Composition of the atmosphere under CA (1.5 - 1.7 % O_2, 2 - 2.2 % CO_2) differs from cold storage under regular atmosphere (RA) conditions (78.08 % N_2, 20.95 % O_2, 0.03 % CO_2) (Kader, 2002). Recent research has shown that apple storage under CA may suppress production volatile compounds that create the typical aroma (Fellman *et al*., 2003; Lara *et al*., 2007; Singh *et al*., 2010; Starr *et al*., 2010; Lumpkin *et al*., 2015).

Fellman *et al*. (2000) found that Gala apples stored for long periods under a 1 % O_2 and 1 % CO_2 atmosphere suppressed flavor production. Echeverría *et al*. (2004b) found that CA (3 % O_2 and 2 % CO_2) significantly suppressed volatile production after 5 months of storage, compared to apples cold-stored under RA. However, López *et al*. (2000) found that volatile compound emission in Golden Delicious apples increased after storage for 5 months under a low oxygen atmosphere; sampled apples kept acceptable levels of firmness, acidity, total soluble solids content, color, and high concentrations of branched-chain esters that intensified fruit flavor.

Since flavor depends on volatile biosynthesis, and Golden Delicious apples from Chihuahua, México are primarily recognized by their flavor, this study focused on volatile biosynthesis of fruit stored under CA, RA, and CA after 7 d under RA. Other variables like fatty acids quantification and measurement of activity of the enzymes lipoxygenase (LOX), alcohol acyltransferase (AAT), and alcohol dehydrogenase (ADH) were also determined.

MATERIALS AND METHODS

Plant material and storage conditions

Thirty-five-year old Golden Delicious apple trees from a commercial orchard located in Cuauhtémoc, Chihuahua, México (28º 23' 51.43'' N, 106º 49' 05.79'' W, at 2062 masl) were selected for this study. Apples were harvested 176 days after full bloom, when internal ethylene content (IEC) was 0.9 ppm. Ethylene production was used as a harvest index, according to Dhall (2013). Apples were selected according to color and weight to ensure uniformity in maturity and size, as well as consistent skin-pulp ratio in the analyzed samples. Apples were stored under CA (2 % O_2 and 3 % CO_2), RA (78.08 % N_2, 20.95 % O_2, 0.03 % CO_2) (Kader, 2002), and CA followed by seven days at RA (CA + RA) at 1 ºC. Volatile compound content, specific activity for the enzymes lipoxygenase (LOX), alcohol dehydrogenase (ADH), and alcohol acyltransferase (AAT), and fatty acid composition were evaluated at harvest and after 1, 3, 5, and 7 months of storage.

Aroma volatiles

Volatiles concentration in apples was determined by gas chromatography-mass spectrometry (GC-MS) using the solid phase microextraction (SPME) technique, as described by Maya-Meraz *et al*. (2014). Apple juice from eight apples per treatment was obtained with a food processor (Turmix, México). The juice (20 mL) was placed in a 20 mL PTFE (polytetrafluoroethylene) vial, frozen in liquid nitrogen, and kept at -70 ºC until analysis. An aliquot of 2 mL of thawed apple juice was placed in a 4 mL vial containing 0.7 g of sodium chloride, and stirred while a SPME fiber (65 µm, PDMS-DVB, Supelco, USA) was exposed to the headspace of the sample for 1 h at room temperature (25 ºC). The fiber was desorbed by splitless injection for 5 min at 200 ºC into a GC-MS system (Varian Saturn 2100D GC/MS; California, USA) equipped with an Equity-1 column (60 m × 0.25 mm ID × 0.25µm film thickness; Supelco, USA).

Chromatographic conditions were, initial oven temperature of 33 ºC held for 5 min, increased to 50 ºC at 2 ºC min^{-1}, then increased to 250 ºC at 5 ºC min^{-1}, and held for 6.5 min. Helium was used as carrier gas with a flow rate of 1 mL min^{-1}. Mass spectra were obtained by electron impact ionization at 70 eV. Transfer line and ion source temperatures were 250 and 180 ºC, respectively. Spectra were recorded with a Saturn GC/MS workstation (Varian).

Volatile organic compounds (VOCs) of interest were identified by spectral match to the National Institute of Standards and Technology (1998), Mass Spectral Library (NIST 98 MS) and by comparison of retention times against high purity standards (ethanol, 2-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, 1-pentanol, 3-hexen-1-ol (Z), 2-hexen-1-ol (E), 1-hexanol, 1-heptanol, 1-octanol, 2-ethyl 1-hexanol, acetaldehyde, butanal, 2-methyl butanal, pentanal, hexanal, 2-hexenal, benzaldehyde, octanal, nonanal, decanal, ethyl acetate, 1-methyl ethyl acetate, propyl acetate, 2-methyl propyl acetate, butyl acetate, 2-methyl butyl acetate, pentyl acetate, 2-buten-1-ol, 3-methyl acetate, 3-hexen-1-ol acetate, hexyl acetate, ethyl propanoate, propyl propanoate, butyl propanoate, hexyl propanoate, methyl butanoate, methyl-2-methyl butanoate, ethyl butanoate, ethyl-2-methyl butanoate, butyl butanoate, butyl 2-methyl butanoate, hexyl butanoate, hexyl 2-methyl butanoate, ethyl pentanoate, ethyl hexanoate, propyl hexanoate, hexyl hexanoate, and ethyl octanoate) (Sigma-Aldrich and ChemService).

Quantification was accomplished by external standard calibration curves using peak areas. All values represent the average of triplicated samples consisting of eight apples each. Acidity and soluble solids contents were measured in the same juice.

Lipoxygenase specific activity

Peel and cortical tissue from eight apple fruits per treatment were freeze-dried with a Labconco, Freezone 12 (Labconco, Corporation, USA). A 200 mg aliquot of the freeze-dried tissues mentioned above was homogenized three times for 20 s with 5 mL of an extraction solution (0.5 M sodium phosphate buffer pH 6.5, 4 mM dithiothreitol, 1 mM EDTA, 0.2 % Triton X-100, and 1 % polyvinylpolypyrrolidone) using an UltraTurrax T25 homogenizer (IKA Labortechnik, Staufen, Germany). The slurry obtained was filtered through two cheesecloth layers and centrifuged at 25,000 ×g for 15 min. The pellet was discarded, and the supernatant was used as crude extract.

LOX activity was assayed spectrophotometrically at 234 nm and 30 ºC by monitoring the formation of conjugated dienes from linoleic acid, according to Wang *et al*. (2004). The assayed mixture (3 mL) consisted of 2.75 mL sodium phosphate buffer (100 mM, pH 6.5), 50 µL sodium linoleic acid solution (10 mM), and 0.2 mL crude extract. Each determination was done in triplicate, and one activity unit (U) was defined as the increment in one unit of absorbance per minute. Results were expressed as specific activity (U mg^{-1} of protein) (Wang *et al*., 2004).

Alcohol acyltransferase specific activity (AAT)

AAT activity was assayed according to the modified Pérez *et al*. (1996) method. A 10 mg sample of freeze-dried apple (containing peel and cortical tissue) was homogenized in 1 mL of extraction solution (0.1 M sodium phosphate buffer pH 8.0, 1 mM EDTA, 0.1 % Triton X-100, and 1 % PVPP) utilizing an UltraTurrax T25 homogenizer. The homogenate was centrifuged at 20,800 ×g for 20 min at 4 ºC. The supernatant was recovered and set on ice as crude enzyme extract. AAT activity was assayed by mixing 2.5 mL $MgCl_2$ solution (5 mM $MgCl_2$ in 0.1 M sodium phosphate buffer pH 8.0), 150 µL of acetyl-CoA solution (2.5 mM acetyl-CoA in 0.1 M sodium phosphate buffer pH 8.0), 50 µL butanol solution (200 mM butanol in 0.1 M sodium phosphate buffer pH 8.0), and 200 µL crude extract. The mixture was incubated at 35 ºC for 15 min. After that 100 µL of 10 mM 5,5-dithiobis (nitrobenzoic acid) (DTNB) were added, and the mixture allowed to stand at room temperature for 10 min.

AAT activity was measured spectrophotometrically by the increment in absorbance at 412 nm as a yellow thiophenol complex of DTNB and free Coenzyme A(CoA) liberated during the catalytic reaction formed. Each determination was carried out in triplicate: one activity unit (U) was defined as the increment in one absorbance unit at 412 nm per minute. Results were expressed as AAT specific activity (mUmg^{-1} of protein) (Echeverría *et al*., 2004a).

Alcohol dehydrogenase specific activity (ADH)

The method used for extraction of ADH was described by Chang *et al*. (1982). A 100 mg freeze-dried apple (containing peel and cortical tissue) sample was homogenized three times for 20 s with 5 mL extraction solution (10 mM sodium phosphate buffer pH 8.0, 5 mM dithiothreitol and 0.5 % polyvinylpolypyrrolidone). The homogenate was centrifuged at 15,000 ×g for 15 min at 4 ºC. The pellet was discarded, and the supernatant was used as crude extract. The reduction of acetaldehyde was followed spectrophotometrically at 25 ºC by measuring the change in absorbance at 340 nm for 2 min of a reaction mixture containing 800 mL of a 25 mM MES (2-(N-morpholino) ethanesulfonic acid) buffer at pH 7.2, 50 mL of nicotinamide adenine dinucleotide (NADH) (5 mM), 100 mL of enzyme extract, and 50 mL of acetaldehyde (80 mM). Each determination was done in triplicate; one activity unit (U) was defined as the decrease in one unit of absorbance at 340 nm per minute, and results were expressed as specific activity (U mg^{-1} protein).

Fatty acid analysis

Fatty acids content was determined by fatty acid methyl ester (FAME) analysis according to slight modifications to Defilippi *et al*. (2005). A 0.15 g sample of freeze-dried apple (containing peel and cortical tissue) was mixed with 1 mL of toluene and shaken overnight (100 rpm) at room temperature on an orbital shaker. Subsequently, 500 µL of methanolic 0.5 N sodium methoxide was added and shaken for 1 h (100 rpm) at room temperature. After the hour, the transesterification reaction was terminated by addition of 50 µL of a 10 % NaCl solution. Finally, 400 µL of heptane was added, mixed and centrifuged for 5 min at 1400 ×g. After phase separation, an aliquot of the upper phase was transferred to a vial and refrigerated for further analysis.

Fatty acid composition was determined by gas chromatography using a 7820 Agilent instrument equipped with a flame-ionization detector. The instrument was fitted with a DB-Wax capillary column (30 m, 0.25 mm I. D. 0.25 µm). Injector port and detector temperatures were 250 and 300 ºC, respectively. Oven temperature was initially set at 50 ºC, increased to 200 ºC at a rate of 25 ºC min^{-1}, increased to 230 ºC at a rate of 3 ºC min^{-1} and held in that condition for

4 min. The carrier gas was helium with a flow rate of 1 mL min^{-1}. Identification of FAMEs was done by comparing the retention times to those of high purity standards analyzed under identical chromatographic conditions. Each determination was done in triplicate.

Statistical analysis

For statistical analysis, a mixed model design was used with storage conditions, storage period, and replication as fixed factors, and replications nested in treatments as random factors. Analyses were carried out using SAS Version 9 (SAS Institute, Cary, NC). Means were separated by (Least Squares) LS means test at $P \leq 0.05$. Volatile compounds data collected were analyzed by PROC MIXED for the analysis of a repeated measures factorial ANOVA and the statistical comparison of means was Tukey's range test method.

RESULTS AND DISCUSSION

Volatile compounds

In this study, 35 volatile compounds (VC) were identified and quantified during storage of Golden Delicious apples. The compounds identified included 18 esters: ethyl acetate, n-propyl acetate, 2-methyl propyl acetate, butyl acetate, 2-methyl butyl acetate, ethyl pentanoate, butyl propanoate, pentyl acetate, butyl butanoate, 3-hexen-1-ol acetate, hexyl acetate, butyl 2-methyl butanoate, propyl hexanoate, hexyl propanoate, hexyl butyrate, ethyl octanoate, hexyl 2-methyl butyrate, hexyl hexanoate; seven aldehydes: butanal, butanal 2-methyl, pentanal, cis 3-hexenal, hexanal, 2-hexenal, nonanal; and ten alcohols: 1-butanol, 2 methyl 1-propanol, 2 methyl 1-butanol, 1-pentanol, 3-hexen-1-ol (Z), 2-hexen-1-ol (E), 1-hexanol, 1-heptanol, 2-ethyl 1-hexanol, 1-octanol. Storage under CA caused a decrease on VC development (Figure 1). Mattheis *et al*. (1995), Fellman *et al*. (2003) and Saquet *et al*. (2003) found similar results for Bisbee Delicious, Redchief Delicious and Jonagold apples, respectively.

The main VC found in Golden Delicious apples at harvest time were (in decreasing order) 2-hexenal, 2-methyl 1-butanol, hexanal, butyl acetate, 2-methyl 1-propanol, 2-methyl butyl acetate, cis 3-hexenal, and hexyl acetate (Table 1). Significant interaction between different atmospheric conditions and storage time was detected. CA and CA + RA apples showed their highest total VC values after one month of storage, with no significant differences among CA, CA + RA, and RA ($P < 0.05$) (Figure 1). However, although total VC values were similar among treatments after one month of storage, their specific composition was different for each treatment (Figure 1, Table 1).

Treatment RA presented considerably higher concentration of esters (mainly butyl acetate) when compared to CA + RA and CA apples, after one month of storage ($P < 0.05$). CA + RA treatment induced 78 % higher ester values than CA-treated apples, essentially butyl acetate, after one month of storage. On the other hand, CA-treated apples had 27.3 % higher aldehyde concentration and 58 % more alcohol levels than RA and CA + RA apples (mainly hexanol and 2-metyl-1-butanol) after one month of storage (Figure 1, Table 1).

Butyl acetate (66.7 %, 46.9 ppm), hexanal (8.6 %, 6.0 ppm), and 2-hexenal (6.5 %, 4.5 ppm) make up about 82 % of the total VC produced by apples, after one month of RA storage. Compounds 2-hexenal (34.7 %, 17.7 ppm), 1-hexanol (30 %, 15.3 ppm) and 2-methyl 1-butanol (14.5 %, 7.4 ppm) account for 80 % of the total VC by CA stored apples after one-month storage. Butyl acetate (34.5 %, 13 ppm), 2-hexenal (27.9 %, 10.5 ppm), and 1-hexanol (13.5 %, 5 ppm) amount to about 76 % of total VC by CA + RA stored apples, after month one of storage.

CA conditions inhibited the production of butyl acetate and hexyl acetate and increased hexanol concentration, after one month of storage. At this moment, branched ester, 2-methyl butyl acetate, increased on CA apples. According to López *et al*. (1998) branch chain esters are not affected by CA storage, since these come from the amino acid pathway. Fellman *et al*. (1993) found greater concentration of 2-methyl butyl acetate on apples stored under CA conditions, when compared to RA apples.

Seven days of RA after CA caused a regeneration of volatiles to get a composition resembling RA apples at month one of storage: ester biosynthesis was present, mainly butyl acetate, and concentration of alcohols and aldehydes (mainly 1-hexanol and 2-hexenal) decreased (Figure 1, Table 1). These results have important sensory implications: studies on commercial apple odor have correlated 'unwanted essences' with high levels of alcohols like hexanol which give an earthy unpleasant flavor, and 'desirable essences' with high levels of hexanal, 2-hexenal, and butyl acetate (Dürr and Schobinger, 1981; Petró-Turza *et al*., 1986). Altisent *et al*. (2011) found that the emission of 26 volatile compounds increased on Golden Reinders apples, after a regeneration period (air storage) of 2 and 4 weeks after ultralow-oxygen storage. According to Dixon and Hewett (2000), after hypoxia apples increase ester concentration. Young *et al*. (2004) indicated that low molecular weight esters increase more rapidly than their counterparts.

RA apples showed a four-fold value on total VC when compared to CA and CA + RA apples after three months of

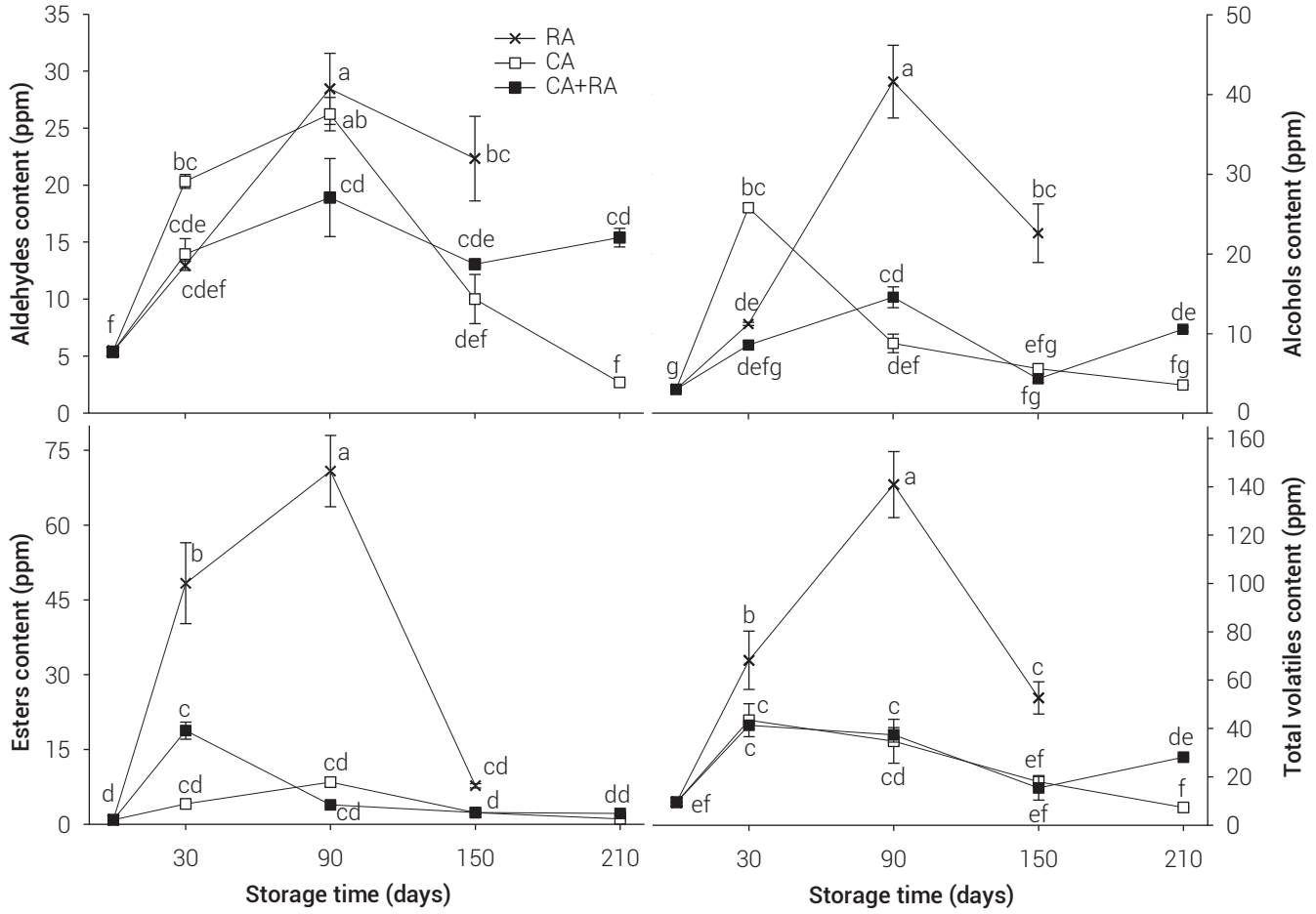

Figure 1. Aldehyde, alcohol, ester and total volatile content in Golden Delicious apple fruits under different storage conditions. Values represent mean of three repetitions. Vertical bars represent ± SE. Means showing different letters are significantly different (Tukey, 0.05).

Table 1. Production of volatiles compounds (ppm) by Golden Delicious apples under different storage conditions.

Aroma compound	At harvest	Months	Storage condition		
			RA	CA	CA+RA
			Aldehydes		
Butanal	Traces	1	0.067 aA	0.045 aA	0.279 aB
		3	0.217 bA	0.410 bB	0.670 bC
		5	0.08 aA	0.0256 aA	Traces
		7	-	Traces	Traces
Butanal 2-methyl	ND	1	0.002 a	ND	ND
		3	0.005 b	ND	ND
		5	0.002 a	ND	ND
		7	-	ND	ND
Pentanal	Traces	1	0.002 a	Traces	Traces
		3	0.002 a	Traces	Traces
		5	0.001 a	Traces	Traces
		7	-	Traces	Traces

Table 1. Continuity.

Aroma compound	At harvest	Months	Storage condition		
			RA	CA	CA+RA
cis 3-hexenal	0.154	1	0.096 aA	0.709 aB	0.371 aC
		3	0.098 aA	0.386 bB	0.398 aB
		5	0.149 aA	0.424 bB	0.206 bAC
		7	-	0.148 cA	0.104 bA
Hexanal	1.576	1	6.033 aA	2.546 aA	2.848 aA
		3	15.740 bA	4.990 aB	6.384 aB
		5	18.164 bA	5.492 aB	4.495 aB
		7	-	2.536 aA	3.098 aA
2-hexenal	3.640	1	4.571 aA	17.659 aB	10.452 abC
		3	12.427 bA	19.441 aB	11.452 abA
		5	3.922 aA	4.051 bA	7.320 aA
		7	-	Traces	12.185 b
Nonanal	0.0007	1	0.001 aA	0.002 aA	0.002 aA
		3	0.002 aA	0.002 aA	0.001 aA
		5	0.002 aA	0.002 aA	0.002 aA
		7	-	0.002 aA	0.002 aA
			Alcohols		
1-butanol	0.033	1	3.822 aA	0.969 abB	1.250 aB
		3	7.541bA	2.071bB	1.910 aB
		5	1.560 cA	0.145 aB	0.003 aB
		7	-	Traces	Traces
2-methyl 1-propanol	0.448	1	0.455 aA	1.832 aB	0.721 aA
		3	0.674 aA	1.435 aB	0.685 aA
		5	0.542 aA	2.469 bB	1.274 bC
		7	-	1.599 aA	2.673 cB
2-methyl 1-butanol	2.525	1	2.170 aA	7.371 aB	1.446 aA
		3	31.314 bA	0.849 bB	1.514 aB
		5	20.165 cA	0.676 bB	1.160 aB
		7	-	0.690 bA	2.541 aA
1-pentanol	0.012	1	0.011 aA	0.388 aB	0.141 aC
		3	0.014 aA	0.110 bB	0.107 aB
		5	0.003 aA	0.067 bcA	0.018 bA
		7	-	0.006 cA	0.010 bA
3-hexen-1-ol (Z)	0.003	1	Traces	0.004 aA	0.001 aB
		3	Traces	0.001 bA	0.001 aA
		5	Traces	0.002 bA	0.002 bA
		7	Traces	0.001 bA	0.002 bA
2-hexen-1-ol (E)-	0.005	1	0.005 aA	0.002 B	ND
		3	Traces	Traces	1.909
		5	0.0006 b	Traces	Traces
		7	-	Traces	Traces

Table 1. Continuity.

Aroma compound	At harvest	Months	Storage condition		
			RA	CA	CA+RA
1-hexanol	0.061	1	4.781 aA	15.246 aB	5.038 aA
		3	2.303 bA	4.330 bB	10.361 bC
		5	0.348 cA	2.280 cB	1.910 cAB
		7	-	1.280 cA	5.331 aB
1-heptanol	0.003	1	0.002 aA	0.002 aA	0.003 aA
		3	0.004 bA	0.002 aB	0.003 aA
		5	0.003 aA	0.002 aB	0.003 aA
		7	-	0.001 bA	0.002 bA
2-ethyl 1-hexanol	0.0005	1	0.0006 a	Traces	Traces
		3	0.0009 b	Traces	Traces
		5	0.001 bA	Traces	0.002 aB
		7	-	Traces	0.002 a
1-octanol	0.001	1	0.0006 aA	Traces	0.0009 aB
		3	0.0008 aA	0.0005 A	0.004 bB
		5	0.0008 aA	Traces	0.0007 aA
		7	-	Traces	Traces
			Esters		
Ethyl acetate	0.002	1	0.006 aA	0.004 aA	0.024 aB
		3	0.014 bA	0.005 aB	0.007 bB
		5	0.012 bA	0.002 aB	0.008 bC
		7	-	0.002 aA	0.007 bB
n-Propyl acetate	0.002	1	0.002 aA	0.005 aA	0.002 aA
		3	0.125 bA	0.011 aB	0.002 aB
		5	0.151 b	Traces	Traces
		7	-	Traces	Traces
2-methyl propyl	0.006	1	0.011 abA	0.025 aB	0.005 aC
Acetate		3	0.009 aA	0.012 bA	0.002aB
		5	0.015 bA	0.016 cbA	0.011bA
		7	-	0.018 cA	0.029 cB
Butyl acetate	0.774	1	46.938 aA	1.945 aB	12.945 aC
		3	68.420 bA	6.715 aB	2.797 bB
		5	5.908 cA	1.388 aA	1.482 bA
		7	-	0.895 aA	1.407 bA
2-methyl butyl acetate	0.236	1	0.487 aA	1.617 aB	0.681 aA
		3	1.298 bA	1.078 bA	0.134 bcB
		5	0.570 aAB	0.713 cA	0.354 bdB
		7	-	0.020 dA	0.528 adB
Ethyl pentanoate	Traces	1	0.002 a	Traces	Traces
		3	0.004 b	Traces	Traces
		5	0.002 a	Traces	Traces

Table 1. Continuity.

Aroma compound	At harvest	Months	Storage condition		
			RA	CA	CA+RA
		7	-	Traces	Traces
Butyl propanoate	0.002	1	0.006 aA	0.004 aA	0.0019 aB
		3	0.018 bA	0.002 bB	0.0006 aB
		5	0.013 c	Traces	Traces
		7	-	Traces	Traces
Pentyl acetate	0.007	1	0.023 aA	0.051 aB	0.059 aA
		3	0.027 aA	0.040 aB	0.035 bAB
		5	0.029 aA	0.024 bA	0.026 bcB
		7	-	0.016 bA	0.020 cB
Butyl butanoate	0.002	1	0.029 aA	0.009 aB	0.025 abA
		3	0.061 bA	0.015 aB	0.016 bcB
		5	0.035 aA	0.003 aB	0.007 cB
		7	-	0.003 aA	0.014 cB
3-hexen-1-ol acetate	0.0008	1	Traces	Traces	Traces
		3	Traces	Traces	Traces
		5	Traces	Traces	0.001 a
		7	-	0.015 A	0.002 aB
Hexyl acetate	0.034	1	0.826 aA	0.409 abB	1.164 aA
		3	0.838 aA	0.554 bA	0.894 aA
		5	0.958 aA	0.183 aB	0.461 bB
		7	-	0.099 aA	0.153 bB
Butyl 2-methyl	0.0006	1	0.0005 a	Traces	Traces
butanoate		3	0.002 b	Traces	Traces
		5	0.003 b	Traces	Traces
		7	-	Traces	Traces
Propyl hexanoate	0.001	1	0.001 a	Traces	Traces
		3	0.001 a	Traces	Traces
		5	0.003 b	Traces	Traces
		7	-	0.003	Traces
Hexyl propanoate	0.0007	1	0.001 aA	0.002 aA	0.0007 aA
		3	0.001 aA	0.002 aA	0.0008 aA
		5	0.003 bA	0.002 aB	0.003 bA
		7	-	0.002 aA	0.001 aA
Hexyl butyrate	Traces	1	0.011 aA	Traces	0.003 aB
		3	0.017 bA	Traces	0.002 aB
		5	0.010 a	Traces	Traces
		7	-	Traces	Traces
Ethyl octanoate	0.004	1	0.004 a	Traces	Traces
		3	0.006 b	Traces	Traces
		5	0.011 c	Traces	Traces

Table 1. Continuity.

Aroma compound	At harvest	Months	Storage condition		
			RA	CA	CA+RA
		7	-	Traces	Traces
Hexyl 2-methyl	Traces	1	0.0008 a	Traces	Traces
butanoate		3	0.002 b	Traces	Traces
		5	0.001 c	Traces	Traces
		7	-	Traces	Traces
Hexyl hexanoate	Traces	1	0.002 a	Traces	Traces
		3	0.004 b	Traces	Traces
		5	0.002 a	Traces	Traces
		7	-	Traces	Traces

Values are mean of three repetitions. Means within the same storage period followed by different capital letters are significantly different at P ≤ 0.05 (LS means test). Means within the same storage conditions followed by different small letters are significantly different at P ≤ 0.05 (LS means test). Traces are values below 0.0005 ppm. ND, not detected.

storage (Figure 1) (P < 0.05); however, by the third month of RA storage, apples had the highest ester content (70.8 ppm, mainly butyl acetate), but CA and CA + RA apples showed 88 % lower ester values, with no significant differences among them (Figure 1) (P < 0.05).

An increase on aldehydes content was observed in all treatments, without statistical differences between CA and RA, for the three-month storage. However, aldehyde composition among CA and RA apples varied: hexanal dominated RA-treated apples, while 2-hexenal prevailed in CA apples (Table 1). A 79 % increase in alcohol content was found in RA apples compared to CA apples, mainly 2-metyl-1-butanol, 1-butanol and 1-hexanol, after the same storage period. For the same period, a higher concentration of alcohols was observed on CA + RA apples when compared to CA apples, mainly due to hexanol production (Figure 1, Table 1).

At the third month of storage, RA apples showed the highest content of total VC's, mostly composed by by butyl acetate (68 ppm, 48.5 %), 2-methyl-1-butanol (33.1 ppm, 22.2 %), hexanal (15.7 ppm, 11.2 %) and 2-hexenal (12.4 ppm, 8 %). In contrast, CA-stored apples had a VC profile made up of 2-hexenal (19.4 ppm, 45.8 %), butyl acetate (15.8 %, 6.7 ppm), hexanal (11.8 %, 5.0 ppm) and 1-hexanol (4.3 ppm, 10 %). CA + RA apples showed a VC profile with 2-hexenal (11.4 ppm, 29.2 %), 1-hexanol (10.4 ppm, 26.4 %) and hexanal (6.4 ppm, 16.2 %). Butyl acetate ester accounted for almost 70 % of total VC in RA apples, and 2-hexenal, an aldehyde, accounted for almost 35 % of total VC content in CA apples.

This behavior demonstrates the delaying ripening effect in CA-stored apples, since aldehydes are precursors of alcohols, and in turn, alcohols are ester precursors. Butyl acetate ester was the main compound produced under RA (66.7 %) and under CA + RA apples (34.5 %), which agrees with Drawert (1973), who found this compound to be the prevailing ester in Golden Delicious apples under RA-stored conditions. The higher ester concentration on RA apples (mainly butyl acetate) modifies sharply aroma, compared to CA apples, since butyl acetate is an impact compound in Golden Delicious apple (Kakiuchi *et al*., 1986), while hexanal and 2-hexenal are aldehydes related to 'unripe' flavors in Golden Delicious apples (Flath *et al*., 1967; Rizzolo *et al*., 1989).

After five months of storage, total VC content decreased sharply on RA apples, being 74 % lower than after three months of storage, although still considerably higher than in CA and CA + RA apples. A significant decrease in the concentration of aldehydes on CA apples was observed (55 % lower values than on RA conditions), mainly due to a major decrease in 2-hexenal. CA + RA apples showed higher aldehyde concentration when compared to CA after five months of storage (Figure 1) (P < 0.05). A considerable decrease in ester concentration was found in RA apples on the fifth month of storage, an 89 % lower ester content than at three months of storage. No significant difference on esters profiles was found among RA, CA, and CA + RA apples (Figure 1).

At the fifth month of storage, the main VC's on Golden Delicious apples under RA were 2-methyl-1-butanol (38 %, 20.2 ppm), hexanal (34 %, 18.2 ppm) and butyl acetate (11 %, 6.0 ppm), while the main VC occurring on CA apples were hexanal (31 %, 5.5 ppm), 2-hexenal (23 %, 4.0 ppm), and 2-methyl 1-propanol (14 %, 2.5 ppm). On CA + RA apples the compounds 2-hexenal (39 %, 7.3 ppm), hexanal (24 %, 4.5 ppm), and 1-hexanol (10 %, 1.9 ppm) were the major VC produced after five months of storage.

Finally, after seven months of storage, CA + RA apples showed a volatile´s recovery, presenting 82.5 % more aldehydes and 66 % more alcohol concentration than CA apples (Figure 1). The main VC produced on CA+RA apples were 2-hexenal (43 %, 12 ppm), 1-hexanol (19 %, 5.3 ppm) and 2-methyl-1-propanol (10 %, 2.7 ppm), while in CA apples the major VC were hexanal (35 %, 2.5 ppm), 2-methyl-1-propanol (22 %, 1.6 ppm) and hexanol (17 %, 1.3 ppm). RA apples were not evaluated at seven months of storage, since fruit did not maintain the required quality.

LOX, AAT, and ADH activity

Lipoxygenase (LOX) may play a key role in determining the composition of volatile compounds in apple (Fellman *et al*., 2000). In this study an increase in LOX specific activity was observed from harvest to the first month of storage (Figure 2). No significant difference in LOX activity was found among RA and CA apples at one month of storage. After the first month, storing fruit under CA caused a decrease in the specific activity of the enzyme (Figure 2). Lara *et al.* (2007) found similar results attributing this effect to LOX-O_2 requirements. Figure 2 shows the relationship between LOX specific activity and total aldehydes production during apple storage under RA and CA conditions. Determination coefficient (r^2) between aldehydes and LOX activity was 0.25 for RA apples and 0.85 for CA apples. These details, along with decreased LOX specific activity observed on CA apples, could indicate that LOX activity plays an important role in controlling aldehyde production when oxygen concentration is limited.

Ester-like volatile compounds are generated by the esterification of alcohols and acyl-CoA catalyzed by the enzyme alcohol acyltransferase (AAT) (Sanz *et al*., 1997). The effect of storage conditions on the AAT specific activity is shown in Figure 3. The highest AAT activity was observed at harvest (208.26 mU mg^{-1} protein). After the first month

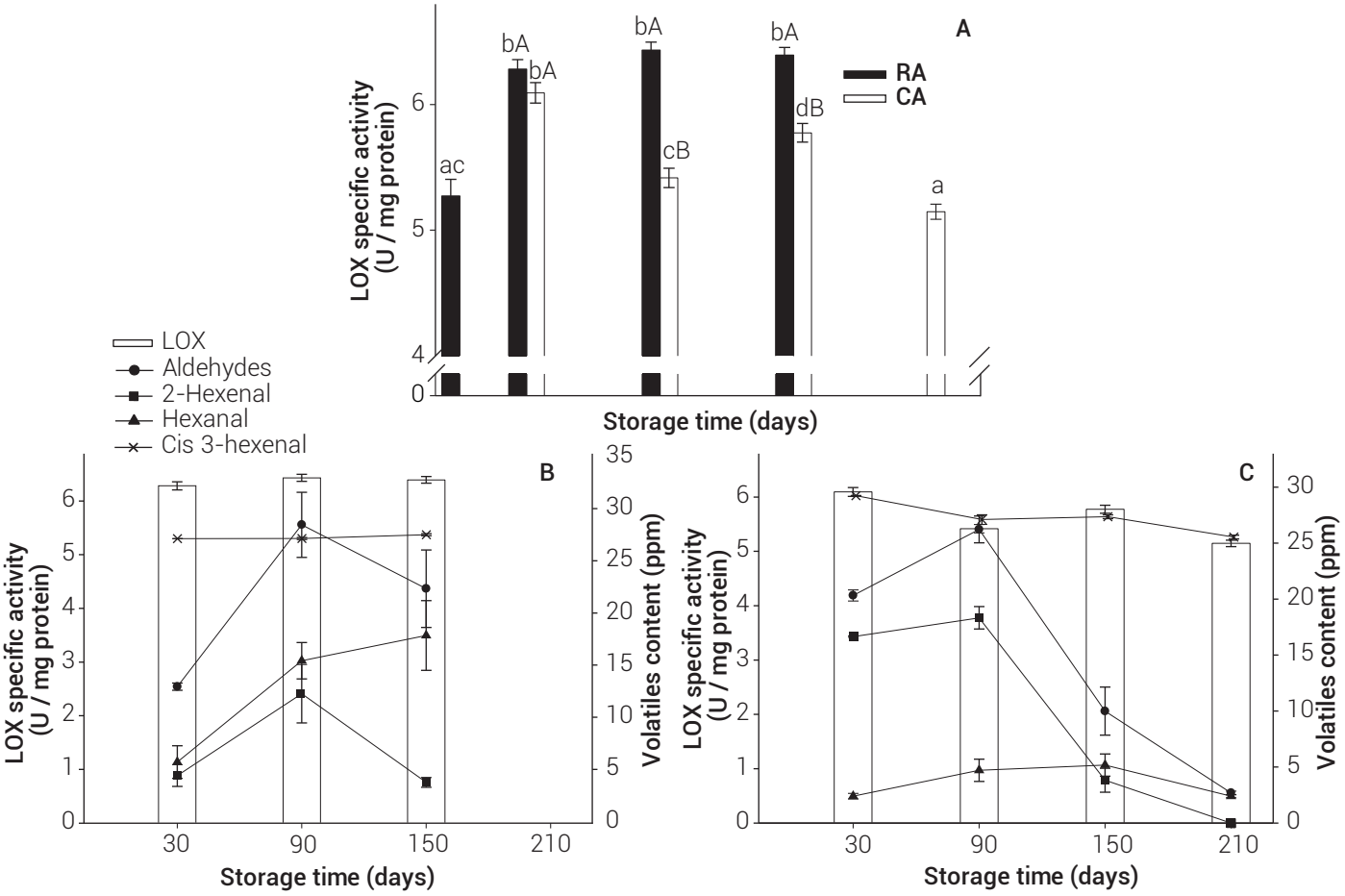

Figure 2. LOX specific activity in Golden Delicious apples at different storage conditions (A), LOX specific activity at RA (B) and at CA (C) compared to important aldehydes. Values represent mean of three repetitions. Vertical bars represent ± SE. Means within the same storage period followed by different capital letters are significantly different at P ≤ 0.05 (LS means test). Means within the same storage conditions followed by different small letters are significantly different at P ≤ 0.05 (LS means test).

of storage, higher enzyme specific activity was found in RA stored fruit when compared to CA conditions (P < 0.05). Fellman *et al*. (2000) noted this same effect in Law Rome and 262 Rome apples. On RA apples, as the storage period advanced AAT specific activity decreased, suggesting enzymatic activity was affected by storage conditions.

After three months of storage there was an increase in AAT specific activity of CA apples that was not significantly different from RA apples (P < 0.05). Lara *et al*. (2007) found higher AAT specific activity in fruit stored under CA than in RA stored fruit. The highest AAT specific activity for CA apples occurred during the third month of storage, which coincides with their highest ester production. A decrease in AAT specific activity was observed in both treatments (RA and CA) after five months of storage; RA showed significantly higher AAT activity than CA (P < 0.05).

Figure 3 shows the relationship between AAT activity and esters (total esters, butyl acetate, 2 methyl butyl acetate, and hexyl acetate) on RA and CA stored apples. RA-stored apples did not show a clear connection between AAT specific activity and the ester synthesis. However, CA apples showed high correlation between AAT specific activity and total esters (r^2 = 0.92). These findings could indicate that under CA conditions, ester production is correlated with AAT enzyme activity. In the case of RA apples, ester production did not show to be dependent on AAT enzyme activity. Echeverría *et al*. (2004a) and Villatoro *et al*. (2008) noted that modifications in AAT specific activity could not explain the observed behavior in the production of esters.

Figure 4 shows ADH specific activity in apples stored under CA and RA (P < 0.05). CA apples showed higher ADH activity during storage, when compared to RA apples (P < 0.05). The highest ADH activity in CA stored apples was

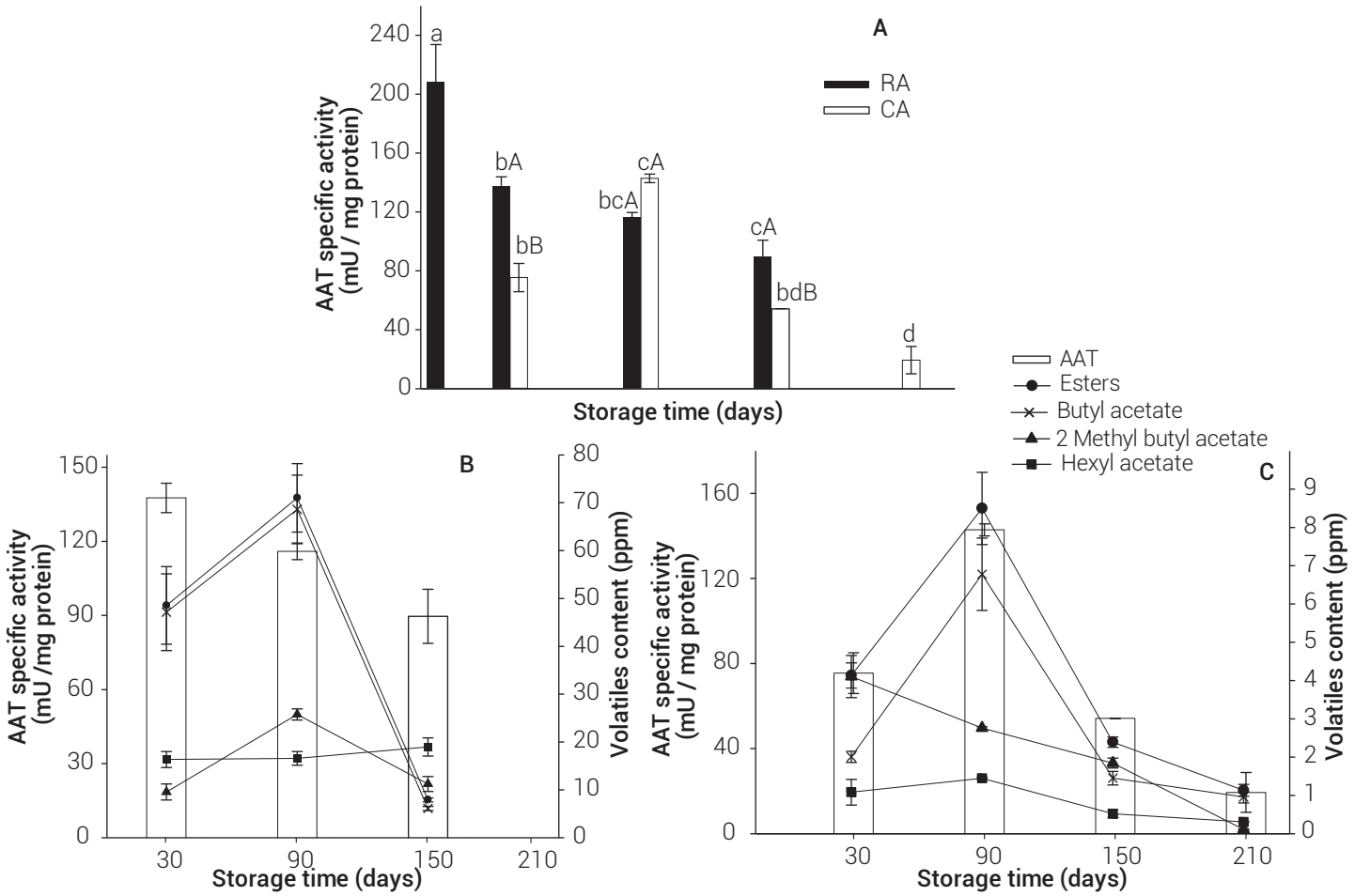

Figure 3. AAT specific activity in Golden Delicious apples at different storage conditions (A), AAT specific activity at RA (B), and at CA (C) compared to important alcohols and esters. Values represent mean of three repetitions. Vertical bars represent ± SE. Means within the same storage period followed by different capital letters are significantly different at P ≤ 0.05 (LS means test). Means within the same storage conditions followed by different small letters are significantly different at P ≤ 0.05 (LS means test).

observed after three months of storage, having values four-fold higher than RA apples. Higher ADH activity in the CA-stored apples coincided with a lower VC production; it is suggested that this enzyme activity may be related to the onset of fermentative processes after extended storage under hypoxia. Furthermore, AAT activity after extended storage under CA conditions decreased (Figure 3), thus preventing esterification of alcohol precursors (Lara *et al*., 2007).

Fatty acids

Fatty acids are considered the main precursors of volatile compounds; they are important structural compounds and metabolic constituents of fruit cells (Marangoni *et al*., 1996). They are detached and metabolized by lipase, β-oxidation enzymes and/or lipoxygenase, and they produce volatile aroma substances (Fellman *et al*., 2000). Figure 5 shows fatty acids obtained from RA and CA apples. Linoleic acid was found in higher concentration (85 - 125 ppm), followed by palmitic acid (27 - 45 ppm), linolenic acid (5 - 17 ppm), oleic acid (3 - 12 ppm) and stearic acid (5 - 8 ppm).

Linoleic acid is considered one of the main precursors of volatile compounds in apples (Yahia, 1994). Linoleic acid concentration increased during storage in both treatments, but no significant differences were found between RA and CA ($P < 0.05$). Linoleic acid concentration, as well as the VC of the fruit stored under RA-conditions, increased continuously until the fifth month of storage, which is associated with fruit climacteric ripening (Song and Bangerth, 1996; Song and Bangerth, 2003; Defilippi *et al*., 2005).

Palmitic acid concentration increased on CA apples after five months of storage. Palmitic acid concentration remained steady on RA apples through storage ($P < 0.05$). Similar results were shown by Song *et al*. (2003) who found higher concentration of palmitic acid on CA apples after 6 months of storage. Linolenic acid concentration decreased thorough storage, more markedly in RA apples; this was the only fatty acid that decreased in concentration steadily during storage. Previous research found that linolenic acid in apples decreases with ripening, as a result of breakdown of fatty acids in chloroplasts to produce straight C-chain esters (Meigh and Hulme, 1965; Galliard, 1968). Oleic acid concentration increased after one month of storage for RA and after three months of storage in CA apples. Stearic acid content steadily increased during storage, and it reached its highest value after five months of storage. CA apples reached higher values of stearic acid after three months of storage, when compared to RA apples. Stearic acid in Jonagold apples showed similar behavior under CA conditions (Saquet *et al*., 2003).

No significant decrease of the fatty acid content in CA-stored apples with respect to that observed in RA-stored apples was observed in this study, except for oleic acid during the first month of storage. These results differed with those found by Saquet *et al*. (2003), who reported that fatty acid concentrations in Jonagold apple pericarp tissue are lowered under CA storage. This suppression effect on fatty acid concentration in CA apples may be dependent upon atmospheric composition and apple variety under study,

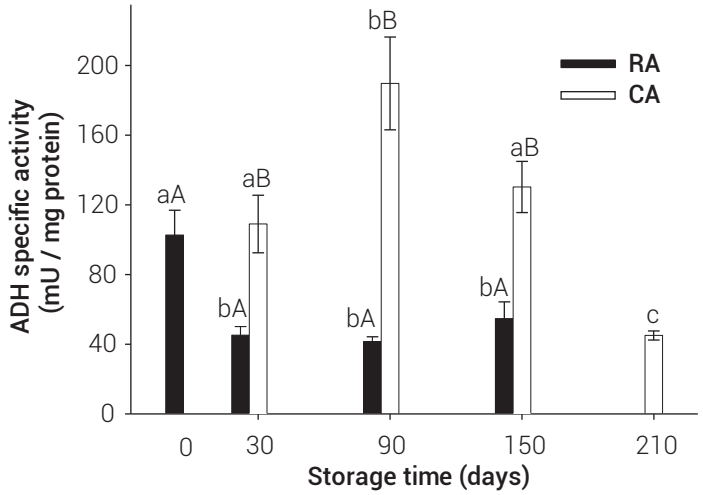

Figure 4. ADH specific activity in Golden Delicious apples at different storage conditions. Values represent mean of three repetitions. Vertical bars represent ± SE. Means within the same storage period followed by different capital letters are significantly different at P ≤ 0.05 (LS means test). Means within the same storage conditions followed by different small letters are significantly different at P ≤ 0.05 (LS means test).

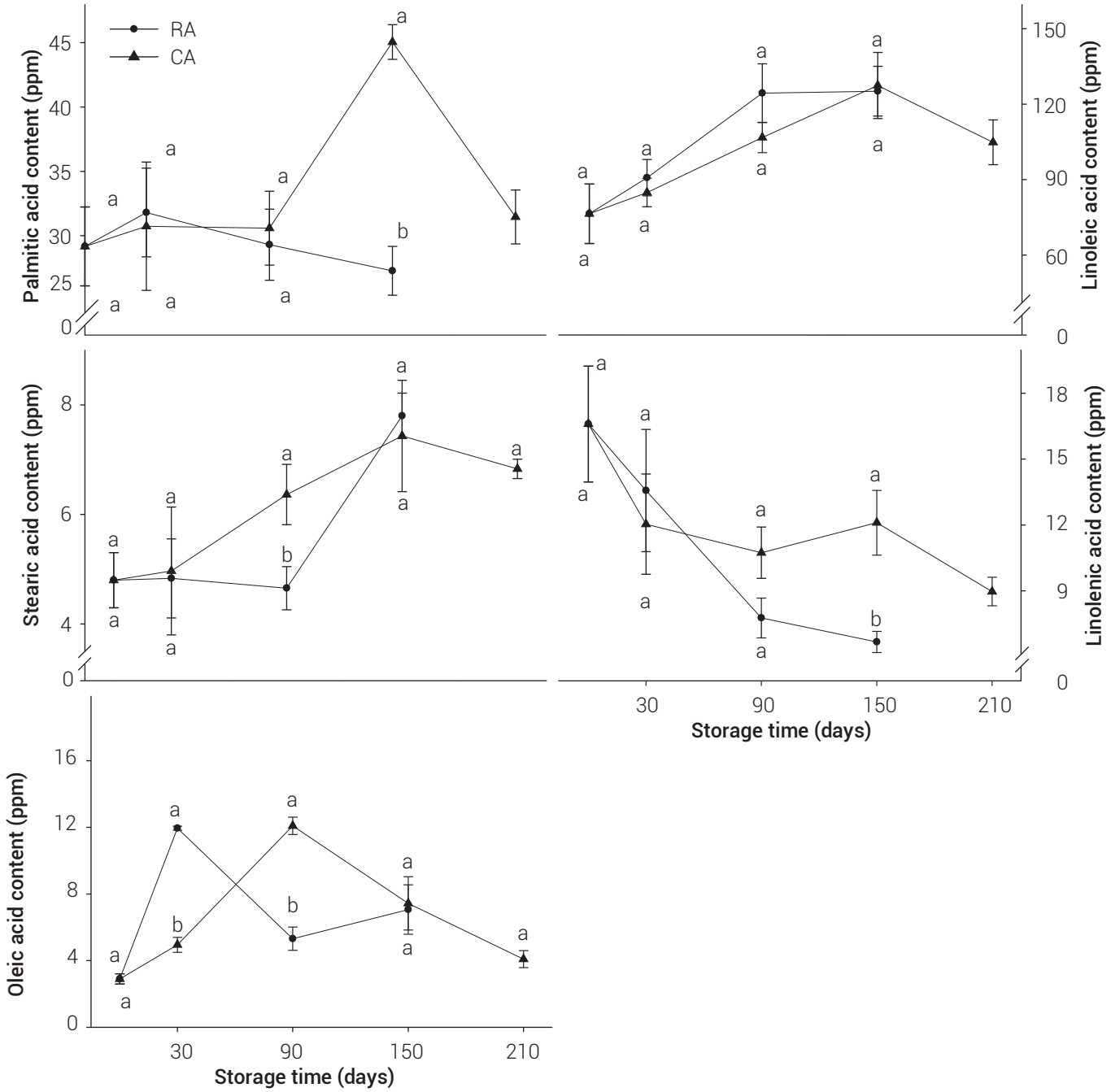

Figure 5. Fatty acids in Golden Delicious apples at different storage conditions. Values represent mean of three repetitions. Verticals bars represent ± SE. Means showing different letters are significantly different at P ≤ 0.05 (LS means test).

among other conditions.

CONCLUSIONS

Storage conditions affected composition and concentration of volatile compounds in apples. Volatile compounds synthesis decreased under CA storage. This storage conditions inhibited the production of esters butyl acetate and hexyl acetate, two key apple aroma compounds. We propose that the main cause of this reduction is the decrease in the activity of LOX and AAT enzymes at some stage during CA storage.

ACKNOWLEDGMENTS

This research was supported by the Mexican National Council of Science and Technology (CONACyT) and Chihuahua State Government, through the CHIH-2010-C01-146966 FOMIX grant. Authors thank Grupo La Norteñita S.A., for providing fruit samples for this study and Unión Agricola Regional de Fruticultores del Estado de Chihuahua UNIFRUT for support.

BIBLIOGRAPHY

Altisent R., J. Graell, I. Lara, L. López and G. Echeverría (2011) Comparison of the volatile profile and sensory analysis of "Golden Reinders" apples after the application of a cold air period after ultralow oxygen (ULO) storage. *Journal of Agricultural and Food Chemistry* 59:6193-6201.

Berger R. G. and F. Drawert (1984) Changes in the composition of volatiles by post-harvest application of alcohols to 'Red Delicious' apples. *Journal of the Science of Food and Agriculture* 35:1318-1325.

Bismark, Banco de Investigación y Marketing (2002) Manzano: Las Variedades de más Interés. 1ª ed. Irta, Barcelona, España. pp:1-63.

Brackmann A., J. Streif and F. Bangerth (1994) Influence of CA and ULO storage conditions on quality parameters and ripening of preclimateric and climateric harvested apples. Effect on colour, firmness, acidity and SS. *Gartenbauwissenschaft* 59:243-257.

Chang L. A., L. K. Hammett and D. M. Pharr (1982) Ethanol, alcohol dehydrogenase and pyruvate decarboxylase in storage roots of four sweet potato cultivars during simulated flood-damage and storage. *Journal of the American Society for Horticultural Science* 107:674-677.

Defilippi G. B., A. M. Dandekar and A. A. Kader (2005) 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 G. B., D. Manríquez, K. Luengwilai and M. González-Agüero (2009) Aroma Volatiles: Biosynthesis and Mechanisms of Modulation During Fruit Ripening. *Advances in Botanical Research* 50:1-37.

De Pooter H. L., M. Van Acker and N. M. Schamp (1987) Aldehyde metabolism and the aroma quality of stored "Golden Delicious" apples. *Phytochemistry* 26:89-92.

Dhall R. K. (2013) Advances in edible coatings for fresh fruits and vegetables: a review. *Critical Reviews in Food Science and Nutrition* 53:435-450.

Dixon J. and E. W. Hewett (2000) Factors affecting apple aroma/flavor volatile concentration: A review. *New Zealand journal of Crop and Horticultural Science* 28:155-173

Drawert F., R. Tressl, W. Heimann, R. Emberger and M. Speck (1973) Über die Biogenese von Aromastoffen bei Pflanzen und Früchten XV; Enzymatisch-oxidative Bildung von c6-Aldehyden und Alkoholen und deren Vorstufen bei Äpfeln und Trauben. *Chemie Mikrobiologie Technologie der Lebensmittel* 2:10-22.

Dürr P. and U. Schobinger (1981) The contribution of some volatiles to the sensory quality of apple and orange juice odor. *In*: Flavour 81. P Schreier (ed). Walter de Gruyter, Berlin. pp:179-193.

Echeverría G., J. Graell, I. Lara, M. L. López and I. Lara (2004a) Volatile production, quality and aroma-related enzyme activities during maturation of 'Fuji' apples. *Postharvest Biology and Technology* 31:217-227.

Echeverría G., T. Fuentes, J. Graell, I. Lara and M. L. López (2004b) Relationship between volatile production, fruit quality, and sensory evaluation of 'Fuji' apples stored in different atmospheres by means of multivariate analysis. *Journal of the Science of Food and Agriculture* 84:5-20.

Flath R., D. R. Black, D. G. Guadagni, W. H. McFadden and T. H. Schultz (1967) Identification and organoleptic evolution of compounds in 'Delicious' apple essence. *Journal of Agricultural and Food Chemistry* 15:29-35.

Fellman J. K., D. S. Mattinson, B. Bostick, J. P. Mattheis and M. Patterson (1993) Ester biosynthesis in Rome apples subjected to low-oxygen atmospheres. *Postharvest Biology and Technology* 3:201-214.

Fellman J. K., T. W. Miller, D. S. Mattinson and J. P. Mattheis (2000) Factors that influence biosynthesis of volatile flavor compound in apple fruits. *HortScience* 35:1026-1033.

Fellman J. K., D. R. Rudell, D. S. Mattison and J. P. Mattheis (2003) Relationship of harvest maturity to flavor regeneration after CA storage of "Delicious" apples. *Postharvest Biology and Technology* 27:39-51.

Galliard T. (1968) Aspects of lipid metabolism in higher plants-II. The identification and quantitative analysis of lipids from the pulp of pre- and post-climacteric apples. *Phytochemistry* 7:1915-1922.

Kakiuchi N., T. Moriguchi, H. Fukuda, N. Ichimura, Y. Kato and Y. Banba (1986) Composition of volatile compounds of apple fruits in relation to cultivars. *Journal of the Japanese Society for Horticultural Science* 55:280-289.

Kader A. A. (2002) Modified atmospheres during transport and storage. *In*: Postharvest Technology of Horticultural Crops. A. A. Kader (ed). Univesity California., Oakland, Calif. pp:135-144.

Lara I., G. Echeverría, J. Graell and M. L. López (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.

López M. L., T. Lavilla, M. Riba and M. Vendrell (1998) Comparison of volatile compounds in two seasons in apples: 'Golden Delicious' and 'Granny Smith'. *Journal of Food Quality* 21:155-166.

López M. L., T. Lavilla, I. Recasens, J. Graell and M. Vendrell (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.

Lumpkin C., J. K. Fellman, D. R. Rudell and J. P. Mattheis (2015) 'Fuji' apple (*Malus domestica Borkh*) volatile production during high pCO_2 controlled atmosphere storage. *Postharvest Biology and Technology* 100:234-243.

Marangoni A. G., T. Palma and D. W. Stanley (1996) Membrane effects in postharvest physiology. *Postharvest Biology and Technology* 7:193-199.

Mattheis J. P., D. A. Buchanan and J. K. Fellman (1995) Volatile compound production by "Bisbee Delicious" apples after sequential atmospheres storage. *Journal of Agricultural and Food Chemistry* 43:1902-1906.

Maya-Meraz I. O., Espino-Díaz M., Molina-Corral F. J., González-Aguilar G. A., Jacobo-Cuellar J. L., Sepulveda D. R. and Olivas G. I. (2014) Production of volatiles in fresh-cut Apple: Effect of applying alginate coatings containing linoleic acid or isoleucine. *Journal of Food Science* 79:C2185-C2191.

Meigh D. F. and A. C. Hulme (1965) Fatty Acid Metabolism in the apple fruit during the respiration climacteric. *Phytochemistry* 4:863-871.

NIST (1998) National Institute of Standards and Technology, Mass Spectral Library. Database and Software. Version 1.7, USA.

Pérez A. G. and C. Sanz (2008) Formation of fruit flavor. *In*: Fruit and Vegetable Flavour. B. Bruckner and W. S. Grant (eds.). Cambridge. England. pp:41-70.

Pérez A. G., C. Sanz, R. Olías, J. J. Ríos and J. M. Olías (1996) Evolution of strawberry alcohol acyltransferase activity during fruit development and storage. *Journal of Agricultural and Food Chemistry* 44:3286-3290.

Petró-Turza M., I. Szarföldi-Szalma, E. Madarassy-Mersich, G. Teleky-Vamossy and K. Füzesi-Kardos (1986) Correlation between chemical composition and sensory quality of natural apple aroma condensates. *Die Nahrung Food* 30:765-774.

Rizzolo A., A. Polesello and G. Y. Teleky-Vamossy (1989) CGC/Sensory analysis of volatile compounds developed from ripening apple fruit. *Journal of High Resolution Chromatography* 12:824-827.

Sanz C. J., M. Olias and A. G. Pérez (1997) Aroma biochemistry of fruits and vegetables. *In*: Phytochemistry of Fruit and Vegetables. F. A. Tomas-Barberan and R. J. Robins (eds.). New York, Oxford University. pp:125-155.

Saquet A. A., J. Streif and F. Bangerth (2003) Impaired aroma production of CA-stored "Jonagold" apples as affected by adenine and pyridine

nucletotide levels and fatty acid concentrations. *Journal of Horticultural Science and Biotechnology* 78:695-705.

SIAP, Servicio de Información Agroalimentaria y Pesquera (2010) Secretaría de Agricultura, Ganadería y Recursos Pesqueros, México. www.siap.sagarpa.gob.mx.

SAS (2009) Statistical Software, version 9 (SAS Institute, Cary, NC, USA).

Singh H. P., D. P. Murr, G. Paliyath and J. R. DeEll (2010) Aroma volatile biosynthesis in 'Gala' apples stored in controlled atmosphere. *ISHS Acta Horticulturae* 857:115-122.

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

Starr C., S. Mattinson and J. Fellman (2010) Analysis of the control of the most important flavor production process occurring in apples. WSU Academic Showcase Posters (64). http://hdl.handle.net/2376/2574.

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

Wang Y. S., S. P. Tian, Y. Xu, G. Z. Qin and H. J. Yao (2004) Changes in the activities of pro-and anti-oxidant enzymes in peach fruit inoculated with *Cryptococcus laurentii or Phenicillium expansum* at 0 or 20 ºC. *Postharvest Biology and Technology* 34:21-28.

Yahia E. M. (1994) Apple flavor. *Horticultural Reviews* 16:197-234.

Young J. C., G. Chu, X. Liu and H. Zhu (2004) Ester variability in apple varieties as determined by solid-phase microextraction and gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry* 52:8086-8093.