How to cite: Ekinci, N., M. A. Gündoğdu & M. Şeker, 2021. Effects of different calcium oxide dipping concentrations in aroma profiles of pink lady apple cultivar during cold-storage, Ege Univ. Ziraat Fak. Derg., 58 (3):305-313, https://doi.org/10.20289/zfdergi.771770

Research Article
(Araştırma Makalesi)

Neslihan EKİNCİ[1*]

Mehmet Ali GÜNDOĞDU[1]

Murat ŞEKER[1]

[1] Çanakkale Onsekiz Mart University, Agricultural Faculty, Horticulture Department, Çanakkale/Turkey

* Corresponding author:
nekinci@comu.edu.tr

Keywords: Aroma, Volatile Compounds, Chromatography, Post-Harvest, Esters

Anahtar sözcükler: Aroma, Uçucu bileşenler, Kromatografi, Hasat sonrası, Esterler

Ege Üniv. Ziraat Fak. Derg., 2021, 58 (3):305-313
https://doi.org/10.20289/zfdergi.771770

Effects of different calcium oxide dipping concentrations in aroma profiles of pink lady apple cultivar during cold-storage

Farklı kalsiyum oksit konsantrasyonlarında daldırma uygulamasının depolama süresince pink lady elma çeşidinde aroma profiline etkisi

Received (Alınış): **20.07.2020** **Accepted (**Kabul Tarihi): **19.11.2020**

ABSTRACT

Objective: The aim of this research was to assess the profile of volatile compounds changes during the cold storage in 'Pink Lady' apples.

Material and Methods: Six-year-old Cripps Pink (Pink Lady) fruits of apple trees grafted onto M9 rootstock were used as a plant material for this study. The trees were in a commercial orchard in Çanakkale-Turkey. Fruits were carefully picked up by hand at the commercial harvesting time. The fruits were dipped into calcium oxide solutions (concentrations of 2 and 4%) during three minutes. Control fruits were exposed to distilled water. Fruits were stored at 0°C and 90% relative humidity for 6 months. The aroma profiles of apples determined by GC/MS (Gas Chromatography/Mass Spectrometry) analysis followed by liquid-liquid extraction. Stored fruits were analyzed for their volatile contents at the 2nd, 4th and 6th months.

Results: Fresh fruits at harvest period contained 15 volatile compounds in total. However, higher levels of aroma volatile compounds were detected in 6 months of cold storage. According to the chromatography results, control fruits had 24 volatile compounds, 2% CaO treated fruits had 25 volatiles and 4% CaO treated fruits had 27 volatile compounds at the end of the 6 months of cold storage. Apples at the initial stage produced an abundance of hexyl acetate (4.56%), butyl acetate (3.83%), and hexyl hexanoate (1.68%), which confer typical apple aroma characteristics. Mostly, ester production exhibited a fairly broad peak, declining as fruit aged. Isobutyric acid, allyl ester (initial 0.55%) was lower at the beginning of the storage compare to the end of storage (control fruits 0.06%, 2%CaO 0.13%, 4% CaO 0.20%).

Conclusion: CaO applications especially 4% concentration had deformation effect on epidermal layer cells on fruit surface. As a result, CaO applications had a significant effect on aroma enhancement in Pink Lady apples during cold storage.

ÖZ

Amaç: Çanakkale'de yürütülen çalışmada, 6 yaşında, M9 anacı üzerine aşılanmış Cripps Pink (Pink Lady) çeşidi meyveler kullanılmıştır.

Materyal ve Yöntem: Üretici meyve bahçesinden hassas bir şekilde hasat edilen meyveler, %2 ve %4 dozlarında kalsiyum oksit solüsyonuna, 3 dakika süresince daldırılmıştır. Kontrol meyveleri, aynı süre, saf suya daldırılmıştır. Meyveler, 0°C ve %90 oransal nemde 6 ay depolanmıştır. Çalışmanın amacı, kalsiyum oksit uygulamalarının Pink Lady elma çeşidi meyvelerinin depolama süresince, aroma bileşenleri üzerine etkisini belirlemektir. Aroma bileşenleri, GC/MS (Gas Chromatography/Mass Spectrometry) ile sıvı-sıvı ekstraksiyon metoduna göre yapılmıştır. Analizler, depolamanın başlangıç, 2. ay, 4. ay ve 6. ayında yapılmıştır.

Araştırma Bulguları: Taze meyvelerde başlangıçta toplam 15 aroma bileşeni görülürken, 6. ayın sonunda çok daha yüksek bileşen elde edilmiştir. Kontrol meyveleri 24 bileşen, %2 CaO uygulanan meyveler 25, ve %4 CaO uygulaması 27 aroma bileşeni göstermiştir. Depolamanın başlangıcında hekzil asetat (%4.56), bütil asetat (%3.83) ve heksanoik asit heksil ester (%1.68) gibi tipik meyve aromalarını vermiştir. Meyvelerin yaşlanmasıyla, ester üretimi de azalış göstermektedir. Propanoik asit 2-metil-2- propenil asetat (başlangıçta %0.548) yüksek değerdeyken, 6. ayın sonunda dah düşük değerler göstermiştir (kontrol %0.064, %2 CaO %0.127,% 4 CaO %0.203).

Sonuç: CaO uygulamaları, özellikle %4 CaO, epidermal yüzeyde deformasyon meydana getirmektedir. Sonuç olarak CaO uygulamalarının Pink Lady elma çeşidinde aroma gelişimi üzerine etkisi önemli olmuştur.

INTRODUCTION

Production of aroma volatile compounds is an important factor determining quality of fruit produce and is directly influenced by fruit maturity (Mattheis et al., 1991). Flavor is one of the most important criteria of fruit quality. The formation of flavor and aroma compounds in fruits is a dynamic process, volatile substances are continuously synthesized and developed during fruit growth and ripening. Volatile composition changes qualitatively and quantitatively (Amira, 2011). Aroma compound analysis plays an important role in the process of quality apple breeding. The aroma of a fruit is the result of a complex mixture of esters, alcohols, aldehydes, terpenoid compounds and etc. (Nie et al., 2006).

Increment in tissue calcium (Ca) content by postharvest Ca treatment reduces disorders and maintains storage quality of whole fruit including apple (Saftner et al., 1998; Mordoğan and Ergun, 2001), ethylene production (Conway and Sams, 1987), to inhibit respiration and ethylene production rates and to persistent inhibitory effect on volatile production (Brackmann, 1993), the factor considered most likely responsible for diminished apple flavor (Knee and Hatfield, 1981). Postharvest injection of $CaNO_3$ solution into the core of apples has been shown to non-significant inhibit quality-associated volatile production (Wills, 1972). 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). Aromatic compound analysis plays an important role in the process of quality apple breeding. The aroma of a fruit is the result of a complex mixture of esters, alcohols, aldehydes, terpenoid compounds, etc. The concentration of volatile compounds and their types show great changeability in apple under different ecological conditions of Turkey (Duran, 2013).

'Pink Lady', is very popular, late maturing apple (*Malus* × *domestica* Borkh.) cultivar increasingly grown in many apple-producing areas of the world owing to its excellent flavor and sensory attributes. Commercial interest is thus focused on developing suitable criteria for harvest maturity as well as appropriate storage procedures in order to assure quality of final produce (Villatoro et al., 2008). Fruits of 'Pink Lady' apple cultivar has flesh and excellent flavor and it is firm, crisp and juicy (Corrigan, 1997). Production of aroma volatile compounds is an important factor determining final sensory quality of fruit produce and hence consumer satisfaction and influenced by maturity (Mattheis, 1991). Whereas defficient aroma production in immature fruit, suggested to arise from low rates of precursor synthesis, is gradually overcome as fruit approach the optimal harvest date (Song and Bangerth, 1994),

In this study, 2% and 4% CaO treated fruits are stored at the cold storage during the 6 month. 2nd, 4th and 6th month aroma volatile compounds were detected by GC-MS. It is investigated differences aroma volatile content among treatments and storage period.

MATERIAL and METHOD

Plant material

Six year old Cripps Pink® (Pink Lady) apple trees which grown in a commercial orchard (40˚24'04'' N and 26˚24'35'' E, just 35 m above sea level) in Çanakkale (Turkey), were used as plant material. The trees were randomly selected from unblemished trees showing general characteristics of the apple cultivar. The fruits were carefully harvested by hand. Starch content of the harvested apples was determined by standard procedures using a starch index (1 to 8, Generic Starch Iodine Index Chart for Apples). When the average starch index reached 5, the fruits were picked up for the experiment.

Control fruits of 'Pink Lady' apple cultivar were exposed to distilled water. Experiment fruits were dipped into solutions of calcium oxide (both at the concentrations of 2 and 4%) containing 0.5% of Tween 20 during three minutes. The fruits stored later at 0ºC and 90% relative humidity for 6 months. Aroma volatiles contents were analyzed on the fruits taken out of at the 2nd, 4th and 6th months.

Determination of Volatile Compounds

The volatile compounds of the stored apples were determined by GC/MS analysis followed by liquid-liquid extraction. Liquid-liquid extraction was performed by using of diethyl ether solvent. Each extraction

included two replications and each replication contained 100 g apple puree obtained by using a liquidizer (homogenizer). 100 mL diethyl ether solvent was added into the Erlenmeyer flask with 100 g apple puree. After solvent treatment, the extract was concentrated to 1 mL with a centrifuge and concentrator. Then the solvent was directly injected into a gas chromatograph for volatile compounds (Solis-Solis et al., 2007).

The amount of the aroma volatile determined with a gas chromatograph-mass spectrometer (Shimadzu QP2010 GC/MS) fitted with a DB-WAX column (30 m x 0.25 mm ID, 0.25 µm film thickness; J & W, USA). Identification of volatile content was carried out by mass spectrometry using a mass spectrometer set at 280ºC of capillary direct interface temperature, the ionization energy of the mass spectrometer was programmed for 70 eV. Besides the ion source temperature was set at 250˚C and 40-350 amu of mass interval and 666 amu s^{-1} scan rate. WILEY and NIST libraries were used for identification of compounds. One microliter samples were injected in 1:50 split ratio (with 280ºC injection temperature) by an auto injector. Initially, the column temperature was set at 40 ºC for 2 min. After the column reached at 280˚C by 10˚C min^{-1} and held for 10 min.

RESULTS and DISCUSSION

15 volatile constituents including 5 alcohols (61.06%), 6 esters (11.68%), 1 aldehyde compound (0.54%), 1 alkane compound (0.85%) and 2 other compounds (25.87%), were detected at harvest stage of Pink Lady apple fruits. According to the obtained results, 55 volatile compounds including 19 alcohol, 24 ester, 6 aldehydes, 3 alkanes and 3 other compounds were identified in fruits of Pink Lady cultivar at this research (Table 1, Table 2 and Table 3).

Table 1. Effects of dipping of different CaO concentrations on volatile compounds of 'Pink Lady' apple cultivar (alcohol compounds) during the storage

Çizelge 1. *Hasat sonrası farklı dozlarda CaO uygulamalarının 'Pink Lady' elma çeşidinin depolama süresince aroma bileşenlerine (alkol bileşenleri) etkileri*

Aroma Volatiles	**Harvest Time**	**2nd Month**			**4th Month**			**6th Month**		
Alcohols		**Control**	**2% CaO**	**4% CaO**	**Control**	**2% CaO**	**4% CaO**	**Control**	**2% CaO**	**4% CaO**
Ethyl alcohol	0.04	1.22	0.64	1.03	20.87	13.76	19.36	64.19	63.11	56.78
1-Propanol	0.69	0.95	0.59	0.82	0.74	0.86	0.76	0.06	0.11	0.11
1-Hexanol	5.41	14.44	11.53	12.33	6.28	3.77	4.36	2.55	3.04	4.07
2-Butanol	0.00	0.00	0.53	0.59	0.33	0.38	0.51	0.00	0.00	0.00
1-Butanol	0.00	8.79	8.00	7.46	4.12	3.34	5.06	0.00	0.00	0.00
3-Phenyl-2-butanol	0.00	0.00	0.26	0.00	0.26	0.00	0.00	0.00	0.00	0.00
2-Methyl-1-butanol	0.00	2.57	2.22	2.24	0.93	0.79	1.02	0.00	0.00	0.00
2-Pentanol	0.00	0.00	0.36	0.00	0.00	0.04	0.00	0.00	0.00	0.00
1-Pentanol	0.00	0.00	0.26	0.18	0.00	0.07	0.00	0.00	0.00	0.00
2-Hexenol	0.00	1.30	1.04	1.20	0.37	0.12	0.00	0.05	0.03	0.03
3-Hexanol	0.00	0.00	0.00	0.00	0.23	0.12	0.36	0.07	0.03	0.07
1-Undecanol	0.00	0.00	0.00	2.01	0.00	0.00	0.00	0.09	0.08	0.08
1,3-Octanediol	2.32	17.63	13.46	14.88	18.11	12.61	17.76	0.00	0.00	0.00
Phenol,2,6-bis(1,1-dimethylethyl) -4-methyl	0.00	0.00	0.67	0.00	0.36	0.00	0.00	8.25	2.56	5.13
1-Hexen-3-ol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.04
Phenol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.67	2.67	2.62
Farnesol	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tridecanol	0.00	1.24	1.82	1.75	1.74	2.16	0.71	0.00	0.00	0.00
10-Nonadecanol	52.60	0.00	0.00	0.00	1.04	0.00	2.87	1.62	0.48	0.81
Total Alcohol Areas (%)	**61.06**	**48.14**	**41.98**	**44.49**	**55.38**	**38.02**	**52.77**	**78.55**	**72.11**	**70.74**

*Percentage obtained by MS peak area normalization.

The alcohols were ethyl alcohol (0.04%), 1-propanol (0.07%), 1-hexanol (5.41%), 1,3-octanediol (2.32%), and 10-nonadecanol (52.60%). The esters were butyl hydroxyacetate (0.68%), butyl aceate (3.86%), hexyl acetate (4.60%), 2-methyl-2-propenyl ester (0.55%), propanoic acid 1-methyl ester (0.31%), hexanoic acid hexyl ester (1.68%). The aldehyde was trans-2-hexenal (0.54%) and the other compounds were toluene (1.03%) and alpha farnasene (24.84%).

According to the obtained results of storage periods, volatile compounds were changed in both of qualitative and quantitatively. While initial alcohol content was around 61.06%, its content decreased at the 2nd month of storage. Control fruits had more alcohols levels than CaO treatments (Table 1). Total alcohol content increased at the end of 4th and 6th month storage. However, among the treatments resulted similarly. Ethyl alcohol was increased significantly, during the storage period. Especially, 4th and 6th month resulted higher level of ethyl alcohol. Control fruits were resulted 64.19% while 2% CaO 63.11%, 4% CaO 56.78% showed the lowest content of ethyl alcohol. Initially, 10-nonadecanol was observed at very high amounts. However, according to analysis 2nd month were not detected for 10-nonadecanol. 4th and 6th month increased too slight. Alcohol precursors paralleled that of the corresponding esters for 'Gala' (Fellman, 2000) and 'Fuji' (Lara, 2006; Gur, 2019).

Some alcohol compounds such as tridecanol, 1-butanol and 2-hexanol were not detected in initial stage of study, when they were appeared in 2nd and 4th month. These alcohol compounds synthesized were detected at 2nd and 4th months, as they are components that are synthesized upon maturation and form the desired aroma compounds in fruits. However, these compounds were not identified in 6th month of cold-storage, because of over-ripe (Dixon and Hewett, 2000).

The levels of methanol and ethanol sources in fruit increased steadily throughout ripening, with esters formed from ethyl alcohol predominating from the half-ripe through the senescence phases. The alcohol dehydrogenase (ADH) activity in the mesocarp increased dramatically during the early ripening stages, whereas ATT was active throughout ripening (Fuggate et al., 2010; Yang et al., 2011).

Most alcohols and carbonyls tended to continuously decrease during ripening. 2-methyl 4-pentyl 1,3-dioxane is the condensation product between acetaldehyde generated from fermentation and 1,3 octanediol, an unusual alcohol which occurs naturally (and also in glycosidic form) in apples and pears (Beurle et al 1996; Beurle and Schwab, 1997). Therefore, according to Kavvadis et al. (1999); the 1,3-dioxanes were chemically formed from the natural apple ingredients (R)-octane-1,3-diol, (R)-5(Z)-octene-1,3-diol, (3R,7R)- and (3R,7S)-octane-1,3,7-triol and the respective aldehydes and ketones, which were produced either by the apples (Paillard 1990) or by the yeast fermentation of the apple juice. The dioxanes exhibit a weak "green note" flavor so they might contribute to the overall flavor of cider by decreasing the concentration of undesirable aldehydes and ketones by converting them into compounds with pleasant arom (Kavvadias et al., 1999).

Ester compounds were largely predominant in the aroma profile. According to many researchers ester compounds are the most important compounds that are contribute to the aroma of ripe apples (Dimick et al., 1983; Rowan et al., 1996; Rowan et al., 1999; Dixon and Hewett, 2000; Vallat et al., 2005; Mattheis et al., 2005; Espino-Diaz et al., 2016). In this research, ethyl acetate were not determined at harvest time. 2nd and 4th month increased. However end of the 6 month haven't observed (Table 2). The maximum level of ester compounds obtained at 4% CaO (3.79%) end of the 4th month. Control fruits (3.32%) and 2% CaO (3.11%) were lower. 1-chloroethyl acetate is determined only, end of the storage. 4% CaO, 2% CaO and control (0.12%, 0.10% and 0.09%) fruits were given respectively.

The beta-oxidation pathway provides alcohols and acyl co-enzyme, acyl (CoA), which are the main precursors for volatile ester production. Acyl CoAs are reduced by acyl CoA reductase to produce aldehydes, which in turn are reduced by alcohol dehydrogenase (ADH) to form alcohols that are converted to esters via the action of aspartate amino transferase (AAT) (Song and Bangerth, 1994; Dixon and Hewett, 2000; Espino-Diaz, 2016). In the previous researches, it was explained that 2-methylbutyl acetate, butyl acetate, ethyl-2-methylbutanoate, hexyl-2-methylbutanoate were the main ester compounds in 'Royal Gala', 'Golden Delicious', 'Fuji', 'Mondial Gala' and 'Pink Lady' apple cultivars (Song et al., 1996; Young et al., 1996; Lara et al., 2007; Lopez et al., 2007; Echeverria et al., 2008; Salas et al., 2011; Gur, 2019).

Researchers (Espino-Diaz et al., 2016) were stated that hexyl acetate as red apple and pear flavor, butyl acetate as banana and red apple flavor and 2-methylbutyl acetate as apple flavor. According to De Pooter et al. (1983), 'Golden Delicious' apples treated with hexanal and hexanoic acid vapors had increased hexyl, butyl, and ethyl esters (Dixon and Hewett, 2000). However, yellow-skinned apple varieties have been reported to produce mainly acetate esters and red-skinned varieties mostly butanoate esters; butyric acid is rapidly transformed by β-oxidation into acetic acid, forming acetate esters (Paillard, 1979).

Table 2. Effects of dipping of different CaO concentrations on volatile compounds of 'Pink Lady' apple cultivar (ester compounds) during the storage.

Çizelge 2. *Hasat sonrası farklı dozlarda CaO uygulamalarının 'Pink Lady' elma çeşidinin depolama süresince aroma bileşenlerine (ester bileşenleri) etkileri*

Aroma Volatiles	Harvest Time	2nd Month			4th Month			6th Month		
Esters		Control	2% CaO	4% CaO	Control	2% CaO	4% CaO	Control	2% CaO	4% CaO
Ethyl acetate	0.00	3.35	2.88	3.61	3.32	3.11	3.79	0.00	0.00	0.00
Butyl hydroxyacetate	0.68	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.10	0.16
Propyl acetate	0.00	0.33	0.39	0.30	0.00	0.00	0.00	0.00	0.00	0.00
1-Chloroethyl acetate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.10	0.12
Propanoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.06
Butyl acetate	3.86	1.37	1.72	2.09	1.06	1.36	1.51	0.11	0.36	0.28
Isoamyl acetate	0.00	0.87	1.02	1.16	0.43	0.44	0.34	0.00	0.00	0.00
2-Methylbutyl acetate	0.00	0.00	0.00	0.25	0.23	0.24	0.34	0.00	0.00	0.00
2-Propenyl hexanoate; 2-propenyl ester	0.00	0.00	0.20	0.10	0.00	0.05	0.00	0.00	0.00	0.00
Hexyl acetate	4.60	5.21	4.67	5.82	2.85	2.40	2.48	0.00	0.00	0.00
Isobutyric acid, allyl ester	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.13	0.20
2-Hexenyl Acetate	0.00	0.33	0.32	0.54	0.14	0.04	0.00	0.63	0.36	0.18
Hexyl propionate	0.00	0.37	0.53	0.51	0.14	0.10	0.00	2.37	4.59	4.66
Isopropyl propionate	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Butyl hexanoate	0.00	0.68	0.79	0.68	0.74	0.87	0.91	0.00	0.00	0.00
Hexyl butanoate	0.00	1.15	1.03	1.01	0.94	0.77	0.94	0.00	0.00	0.00
Hexyl 2-methylbutanoate	0.00	2.13	1.78	1.44	0.59	0.62	0.66	0.00	0.00	0.00
Hexyl hexanoate	1.68	2.93	3.05	2.90	3.29	3.79	4.01	0.27	0.57	0.53
Isobutyl caprylate	0.00	0.00	0.00	0.35	0.44	0.41	0.00	0.00	0.00	0.00
Hexyl Octanoate	0.00	0.00	0.00	0.43	0.49	0.41	0.48	0.15	0.00	0.00
Pentyl propanoate	0.00	1.61	1.19	1.18	1.22	0.85	1.25	0.00	0.00	0.00
11,13-Tetradecadien-1-ol acetate	0.00	0.00	0.00	0.00	0.52	0.00	0.00	0.00	0.30	0.81
Ethyl linoleate	0.00	0.00	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00
2-Methylcyclohexyl propionate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.92	0.56	0.18
Total Esters Area (%)	**11.68**	**20.33**	**19.57**	**22.37**	**17.00**	**15.46**	**16.71**	**6.74**	**7.25**	**7.18**

*Percentage obtained by MS peak area normalization.

At harvest time, individual straight chain 2-methylbutyl acetate were high (3.86%). During the cold storage, butyl acetate content slightly decreased. 2nd and 4th month 4% CaO higher than 2% CaO and control fruits. End of the storage, 2% CaO more than, 4% CaO and control fruits (Table 2). CaO treatments had not effective on reducing of butyl acetate. On the contrary, it caused increasement of ester. Isoamyl acetate was not determined at harvest time. 2nd and 4th month was seen in the experiment. 2nd month 4% CaO, 4th month 2% CaO applications were higher compound.

2-methylbutyl acetate was not determined at harvest time. 2nd month only 4% CaO application gave a low level of compound (0.25%). 4th month 4% CaO (0.34%), 2% CaO (0.24%) and control fruits (0.23%) were given 2-methylbutyl acetate, respectively.

2-Propenyl hexanoate; 2-propenyl ester, was not determined at harvest time. 2nd month, 2% CaO and 4% CaO 4th month, only 2% CaO treatments gave to 2-propenyl hexanoate; 2-propenyl ester.

Hexyl acetate, at harvest time determined 4.60%. 2nd month increased of compound. 4% CaO treatment gave to high percentage (5.82%), control fruits (5.21%), 2% CaO (4.67) treatments respectively. 4th month, ester content decreased, 6th month, was not determined to hexyl acetate.

2-hexenyl acetate was not determined at harvest stage. 2nd month 4% CaO higher compound content. 4th and 6th month, control fruits gave to higher 2-hexenyl acetate.

Hexyl propionate was not determined at harvest time. 6th month, 4% CaO the highest compound hexyl propionate ester determined. 2% CaO and control fruits gave to lower content. Isopropyl propionate is determined only at initial.

Butyl hexanoate was not determined at harvest time and 6th month. Second month 2% CaO, 4th month, 4% CaO treatments determined higher ester.

Hexyl butanoate was not determined at harvest time and 6th month. 2nd and 4th month control fruits were given higher ester.

Hexyl 2-methylbutanoate was not determined at harvest time and 6th month. 2nd month control fruits, 4th month, 4% CaO treatments gave to higher butyric acid 2-methyl-hexyl ester.

At the harvest time, 1.68% hexyl hexanoate determined. 2nd month 2% CaO (3.05%), 4th month, 4% CaO (4.01%), 6th month, 2% CaO (0.57%) treatments were higher than 4% CaO (0.53%) and control fruits (0.27).

Iso butyl caprylate was determined at the end of the 2nd month, only 4% CaO treatments and 4th month, control and 2% CaO treatments.

Hexyl octanoate, 2nd month, 4% CaO, 4th month, control fruits were higher than 4% CaO and 2% CaO treatments. 6th month only control fruits showed to hexyl octanoate.

2-Methylcyclohexyl propionate was determined only end of the cold storage.

Total ester content examined in this research. 2nd month gave the 4% CaO, 4th month, control fruits, 6th month 2% CaO showed to higher content of esters during the cold storage.

Trans-2-hexenal (E-2-hexenal) compound was the only identified aldehyde component at the harvest time (%0.54) (Table 3). Ratio of total aldehyde compounds in 2nd month 4% CaO, 2% CaO and control treatments were determined 2.19%, 0.83% 0.52%, respectively. Aldehydes in 4th month of storage 4% CaO, 2% CaO and control treatments were quantified 0.88%, 0.76%, 1.37%, respectively. Last storage period (at the 6th month) total aldehyde ratio increased dramatically in all treatments, 2% CaO (9.90%), control (6.53%), 4% CaO (4.37%), respectively. The higher concentration of aldehydes was found to correspond with increased green/grassy odor of apple juice (Komthong et al., 2007). E-2-hexenal and the increased content of acetate esters which were well corresponded with the green and sweet aroma intensity, respectively (Komthong et al., 2006).

Initially hexatriacontane compound was the only alkane and too low content (0.85%) (Table 3). Ratio of total alkane compounds in 2nd month 4% CaO, 2% CaO and control treatments were quantified 8.63%, 19.36% and 11.05%, respectively. 4th month of storage 4% CaO, 2% CaO and control treatments were specified 9.12%, 24.27% and 9.36%, respectively. Ratio of alkane compounds changed dramatically in 2% CaO (15.05%), control (6.26%), 4% CaO (4.90%) treatments at the last storage period (at the 6th month).

Table 3. Effects of dipping of different CaO concentrations on volatile compounds of 'Pink Lady' apple cultivar (aldehydes, alkanes and other compounds) during the storage.

Çizelge 3. *Hasat sonrası farklı dozlarda CaO uygulamalarının 'Pink Lady' elma çeşidinin depolama süresince aroma bileşenlerine (aldehitler, alkanlar ve diğer bileşenlere) etkileri*

Aroma Volatiles	**Harvest Time**	**2nd Month**			**4th Month**			**6th Month**		
Aldehydes		**Control**	**2% CaO**	**4% CaO**	**Control**	**2% CaO**	**4% CaO**	**Control**	**2% CaO**	**4% CaO**
E-2-hexenal	0.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexanal	0.00	0.52	0.83	1.85	0.88	0.76	1.37	0.00	0.00	0.00
2-Hexyl-2-decenal	0.00	0.00	0.00	0.34	0.00	0.00	0.00	0.15	0.07	0.08
13-Tetradecenal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.10	0.00	2.67
Pentadecanal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.48	1.26
14-Heptadecenal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.36	0.36
Total Aldehydes Area (%)	**0.54**	**0.52**	**0.83**	**2.19**	**0.88**	**0.76**	**1.37**	**6.54**	**9.91**	**4.37**
Alkanes	**Harvest Time**	**2nd Month**			**4th Month**			**6th Month**		
		Control	**2% CaO**	**4% CaO**	**Control**	**2% CaO**	**4% CaO**	**Control**	**2% CaO**	**4% CaO**
2-Butanone	0.00	0.00	0.00	0.00	0.00	16.53	0.00	0.00	0.00	0.00
Hexatriacontane	0.85	0.00	19.36	8.63	0.00	0.00	9.12	0.00	0.00	0.28
Dotriacontane	0.00	11.05	0.00	0.00	9.36	7.74	0.00	4.90	6.26	14.77
Total Alkanes Area (%)	**0.85**	**11.05**	**19.36**	**8.63**	**9.36**	**24.27**	**9.12**	**4.90**	**6.26**	**15.05**
Other Compounds	**Harvest Time**	**2nd Month**			**4th Month**			**6th Month**		
		Control	**2% CaO**	**4% CaO**	**Control**	**2% CaO**	**4% CaO**	**Control**	**2% CaO**	**4% CaO**
Toluene	1.03	1.59	2.17	2.32	0.81	0.78	0.94	0.00	0.00	0.00
Xylene	0.00	0.87	1.17	1.49	0.00	0.21	0.27	0.00	0.00	0.00
Alpha-Farnesene	24.84	17.50	14.94	18.53	16.57	20.53	18.84	3.26	4.48	2.67
Total Others Area (%)	**25.87**	**19.96**	**18.28**	**22.34**	**17.38**	**21.52**	**20.05**	**3.26**	**4.48**	**2.67**

*Percentage obtained by MS peak area normalization.

Toluene, xylene and alpha farnesene were identified as other compounds (Table 3). Especially, alpha farnesene was determined too high ratio (24.84%) in harvest maturity. Its concentration was reduced during the storage. Ratio of α-farnesene compound in 2nd month of storage 4% CaO, 2% CaO and control treatments were determined 18.53%, 14.94% and 17.50%, respectively. Content of α-farnesene on 4% CaO, 2% CaO and control treatments in 4th month of cold-storage were quantified 18.84%, 20.53% and 16.57%, respectively. Ratio of α-farnesene compound decreased dramatically in 2% CaO (4.48%), control (3.26%), 4% CaO (2.67%) treatments at the last storage period (at the 6th month). Toluene and xylene compounds were changed at low ratios. Toluene and xylene reached the highest rate of 2.32% and 1.49% in treatment of %4 CaO at 2nd month, respectively.

α-Farnesene as an aromatic compound is primarily synthesized in apple skin (Kondo et al., 2005). The accumulation of α-farnesene increases during 8 to 12 weeks of cold storage, especially in scald-susceptible apple cultivars such as Granny Smith, Law Rome, and Delicious apples. These varieties typically exhibit a relatively highrate of α-farnesene synthesis shortly after they are placedin cold storage, which results in marked accumulation ofsesquiterpene, including α-farnesene in the skin (Anet, 1972; Whitaker et al., 1997, 1998; Lurie and Watkins, 2012). The concentration of α-farnesene subsequently decreases and is converted to conjugated triene oxidation products during 16–24 weeks of cold storage, as pointed out by several authors (Huelin and Coggiola, 1970; Anet, 1972; Whitaker et al., 1997, 1998; Pechous et al., 2005).

CONCLUSION

CaO solutions were not resulted in reduced total volatile levels. 2% CaO and 4% CaO treatments beyond 6 months storage, cause higher total volatile levels as those in the corresponding control fruit.

The two concentrations of CaO used in this study had a similar effect on total volatile levels during cold storage. It can be concluded that CaO applications especially 4% concentration had deformation effect on epidermal layer cells on fruit surface. According to the results, 2% CaO application may be suggested for aroma enhancement due to conservative effects of fruit texture.

REFERENCES

Amira, E.A., F. Guido., S.E. Behija., I. Manel., Z. Nesrine., F. Ali., H. Mohamed., H.A. Noureddine., and A. Lotfi. 2011. Chemical and aroma volatile compositions of date palm (*Phoenix dactylifera* L.) fruits at three maturation stages. Food Chemistry, 127:1744-1754.

Anet, E. F. 1972. Superficial Scald, A Functional Disorder of Stored Apples. IX. Effect of Maturity and Ventilation. J Sci Food Agric 23: 763–769.

Beuerle, T., P. Schreier, P. Brunerie; C. Bicchi, W. Schwab. 1996. Absolute Configuration of Octanol Derivatives in Apple Fruits. Phytochemistry, 43, 145-149.

Beuerle, T., W. Z. Schwab. 1997. Octane-1,3-diol and its derivatives from pear fruits. Lebensm. Unters. Forsch. A, 205, 215-217.

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

Conway, W.S. and C.E. Sams. 1987. The effects of postharvest infiltration of calcium, magnesium, or strontium on decay, firmness, respiration, and ethylene production in apples. Journal of the American Society for Horticultural Science, 112:300-303.

Corrigan, V.K., P.L. Hurst., G. Boulton. 1997. Sensory characteristics and consumer acceptability of 'Pink Lady' and other late- season apple cultivars. 25:1175-8783.

De Pooter, H.L., J.P. Montens., G.A. Willaert., P.J. Dirinck., N.M. Schamp. 1983. Treatment of Golden Delicious apples with aldehydes and carboxylic acids: effect on the headspace composition. Journal of Agricultural and Food Chemistry, 31:813–818.

Dixon, J., E.W. Hewett. 2000. Factors affecting apple aroma/flavour volatile concentration: A Review. New Zealand Journal of Crop and Horticultural Science, 28:3, 155-173.

Duran, O. 2013. Çanakkale Yöresinde Yetiştirilen Elma Çeşitlerinde Aromatik Maddelerin Belirlenmesi, Çanakkale Onsekiz Mart Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, 52 sayfa.

Echeverría, G., J. Graell., I. Lara., M.L. López. 2008. Physicochemical measurements in 'Mondial Gala®' apples stored at different atmospheres: influence on consumer acceptability. Postharvest Biology and Technology, 50:135–144.

Espino-Diaz, M., D.R. Sepulveda., G. Gonzalez-Aguilar., G.I. Olivas. 2016. Biochemistry of Apple Aroma: A Review. Food Technology and Biotechnology, 54 (4):375–394.

Fellman, J.K., T. Miller., D. Mattinson., J. Mattheis. 2000. Factors that influence biosynthesis of volatile flavor compounds in apple fruits. HortScience, 35: 1026-1033.

Fuggate, P., C. Wongs-Aree, S. Noichinda, S. Kanlayanarat. 2010. Quality and Volatile Attributes of Attached and Detached "Pluk Mai Lie" Papaya During Fruit Ripening. Sci. Hortic. 126, 120–129.

Gur, E. 2019. The effects of different rootstocks on aroma volatile constituents in the fruits of 'Fuji' apples (Malus domestica Borkh.). Applied Ecology and Environmental Research 17(5):11745-11756.

Huelin, F. E., I. M. Coggiola. 1970. Superficial Scald, a Functional Disorder of Stored Apples. V. Oxidation of α-farnesene and Its Inhibition by Diphenylamine. J Sci Food Agric 21: 44–48.

Kavvadias, D., T. Beuerle, M. Wein, B. Boss, T. König. 1999. Novel 1,3-Dioxanes from Apple Juice and Cider. J. Agric. Food Chem. 47, 5178-5183.

Knee, M., S.G.S. Hatfield. 1981. The metabolism of alcohols by apple fruit tissue. Journal of the Science of Food and Agriculture, 32:593-600.

Komthong, P., N. Igura and M. Shimoda. 2007. Effect of ascorbic acid on the odours of cloudy apple juice. Food Chemistry, 100: 1342–1349.

Komthong, P., T. Katoh., N. Igura., M. Shimoda 2006. Changes in the odours of apple juice during enzymatic browning. Food Quality and Preferance, 17: 497-504.

Kondo, S, S. Setha, D. R. Rudell, D. A. Buchanan, J. P. Mattheis. 2005. Aroma Volatile Biosynthesis in Apples Affected by 1-MCP and Methyl Jasmonate. Postharvest Biol Technol 36: 61–68.

Lara, I., G. Echeverría., J. Graell., 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.

Lara, I., J. Grael., M.L. López., G. Echeverría. (2006). Multivariate analysis of modifications in biosynthesis of volatile compounds after CA storage of 'Fuji' apples. Postharvest Biology and Technology, 39: 19–28.

Lopez, M.L., M.T. Lavilla., M. Riba., M. Vendrell. 1997. Comparison of Volatile Compounds In Two Seasons In Apples: Golden Delicious And Granny Smith. Journal of Food Quality, 21: 155-166.

Lurie, S., C. Watkins. 2012. Superficial Scald, Its Etiology and Control. Postharvest Biol Technol 65: 44–60.

Mattheis, J.P., J.K. Fellman., P.M. Chen., M.E. Patterson.1991. Changes in headspace volatiles during physiological development of 'Bisbee Delicious' apple fruit. J. Agric. Food Chem. 39:1902–1906.

Mordoğan, N., S. Ergun. 2001. Elma Meyvesinin Organik Asit İçerikleri ile Bitki Besin Elementleri Arasındaki İlişkiler. Journal of Agriculture Faculty of Ege University, 38(2-3):111-118

Nie, L.C., J.S. Sun., H.J. Chen., X.W. Zou. 2006. Study on fruit aroma of different apple cultivars. Scientia Agricultura Sinica, 39: 641-646.

Paillard, N. M. M. 1990. The flavour of apples, pears and quinces. In Food Flavours Part C. The Flavour of Fruits; Morton, I. D., MacLeod, A. J., Eds.; Elsevier: Amsterdam, The Netherlands, 1-42.

Paillard, N.M. 1979. Biosynthesis of apple volatiles: formation of alcohols and esters from fatty acids. Phytochemistry. 18: 1165–1171.

Pechous, S. W., C. B. Watkins, B. D. Whitaker. 2005. Expression of α-farnesene Synthase Gene AFS1 in Relation to Levels of α-farnesene and Conjugated Trienols in Peel Tissue of Scald Susceptible 'Law Rome' and Scald Resistant 'Idared' Apple Fruit. Postharvest Biol Technol 35: 125–132.

Saftner, R.A., W.S. Conway., C.E. Sams. 1998. Effects of postharvest calcium and fruit coating treatments on postharvest life, quality maintenance, and fruit-surface injury in 'Golden Delicious' apples. American Society for Horticulture Science, 123: 294–298.

Salas, N.A., F.J. Molina-Corral., G.A. Gonzalez-Aguilar., A. Otero., D.R. Sepulveda., G.I. Olivas. 2011. Volatile production by 'Golden Delicious' apples is affected by preharvest application of aminoethoxyvinylglycine. Scientia Horticulturae (Amsterdam), 130 (2):436–444.

Solis-Solis, H.M., M.C. Santoyo., S.S. Golindo., G.L. Solano., and J.A.R. 2007. Charaterization of aroma potential of apricot varieties using different extraction techniques. Food Chemsitry, 105:829-837.

Song, J., F. Bangerth. 1994. Production and development of volatile aroma compounds of apple fruits at different times of maturity. Acta Horticulturae. 368: 150-159.

Song, J., 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.

Villatoro, C., R. Altisent., G. Echeverria., J. Graell, J., M.L. Lopez, 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.

Whitaker, B.D., T. Solomos, D. J. Harrison. 1997. Quantification of α-farnesene and Its Conjugated Trienol Oxidation Products from Apple Peel by C18-HPLC with UV Detection. J Agric Food Chem 45: 760–765.

Whitaker, B.D., T. Solomos, D. J. Harrison. 1998. Synthesis and Oxidation of α-farnesene During High and Low O2 Storage of Apple Cultivars Differing in Scald Susceptibility. Acta Hort 464: 165–171.

Wills, R. B. H. 1972. Effect of calcium on the production of volatiles by apples. Journal of the Science of Food and Agriculture, 23:1131–1134.

Yang, C.X.; Y. J. Wang, B. H. Wu, J. B. Fang, S. H. Li. 2011. Volatile Compounds Evolution of Three Table Grapes with Different Flavor During and After Maturation. Food Chem. 128, 823–830.

Young, H., J.M. Gilbert., S.H. Murray., R.D. Ball, 1996. Causal Effects of Aroma Compounds on Royal Gala Apple Flavours. Journal of the Science of Food and Agriculture, 71: 329-336

Young, J.C., C.L.G. Chu, X.W. Lu., H.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.