

1-methylcyclopropene effects on volatile profile and quality of 'Royal Gala' apples produced in Southern Brazil and stored in controlled atmosphere

Efeito do 1-metilciclopropeno no perfil volátil e qualidade de maçãs 'Royal Gala' produzidas no Sul do Brasil e armazenadas em atmosfera controlada

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ABSTRACT

Apple volatile aroma depends of complex interaction among organic compounds. The aim of the present study was to evaluate the effect of 1-methylcyclopropene (1-MCP) application on volatile profile and quality on 'Royal Gala' apples produced in Southern Brazil and stored under controlled atmosphere condition (CA; 1.0kPa O₂+1.2kPa CO₂; 0.5±0.1°C; 94±1% relative humidity). Volatile profile was determined after eight month of CA storage plus 7 days of shelf life via solid-phase microextraction (HS-SPME) and analyzed by gas chromatography coupled to mass spectrometer. In the volatile profile were identified 8 esters, 11 alcohols, 6 aldehydes, 6 acids, 2 ketones, 1 ether and 1 terpene. 1-MCP application reduced significantly the main volatile compounds production by 'Royal Gala' apples produced in Southern Brazil. Its application decreases significantly the esters production, especially 2-methyl-butyl acetate and hexyl acetate, compared to 'Royal Gala' apples storage under CA. 1-methylcyclopropene application decrease ethylene production and respiration rate. Apples treated with 1-MCP exhibit higher hexanal and 2(E)-hexenal production.

Key words: *Malus domestica*, solid phase microextraction, fruits, GC/MS.

RESUMO

O aroma de maçãs depende de uma complexa interação entre compostos orgânicos. O objetivo do presente estudo foi avaliar o efeito da aplicação de 1-metilciclopropeno (1-MCP) sobre o perfil volátil e a qualidade de maçãs 'Royal Gala' produzidas no Sul do Brasil e armazenadas em atmosfera controlada (AC; 1,0kPa O₂+1,2kPa CO₂; 0,5±0,1°C; 94±1% umidade relativa). O perfil volátil foi determinado após oito meses de armazenamento mais seis dias de vida de prateleira através de micro extração em fase sólida (HS-SPME) e analisado por cromatografia gasosa acoplado a espectrofotômetro de massa. No perfil volátil, foram identificados 8 ésteres, 11 alcoóis, 6 aldeídos, 6 ácidos, 2 cetonas, 1 éter e 1 terpeno. Aplicação de 1-MCP reduziu

significativamente os principais compostos voláteis produzidos pela maçã 'Royal Gala'. Sua aplicação reduziu significativamente a produção de ésteres, especialmente 2-metil-butil acetato e hexil acetato, quando comparado à maçã 'Royal Gala' produzidas no Sul do Brasil e armazenada em AC. Aplicação de 1-metilciclopropeno reduziu a produção de etileno e respiração dos frutos. Maçãs tratadas com 1-MCP apresentam maior produção de hexanal e 2(E)-hexenal.

Palavras-chave: *Malus domestica*, micro extração em fase sólida, frutos, GC/MS.

INTRODUCTION

In Brazil over 50% of apple production stands out to 'Gala' and its mutants, such as 'Royal Gala'. Harvest of this cultivar is carried out in a short period, so its production must be stored to offer high quality apples throughout the off season. Nowadays, the most commonly apples storage technique is the controlled atmosphere (CA) (BRACKMANN et al., 2013; WEBER et al., 2013). However, even during apples storage under CA, may occur quality losses and physiological disorders. Thus, other postharvest techniques need to be employed during storage to maintain apple quality.

As well as CA storage, the 1-methylcyclopropene (1-MCP) application is a worldwide supplementary technique employed on apple storage. The 1-MCP is a competitive ethylene action inhibitor (WATKINS, 2006; LEE et al., 2012).

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With the ethylene action inhibiting, the fruit softening reactions are hindering, keeping flesh firmness (MOGGIA et al., 2010), titratable acidity and soluble solids, as well reducing superficial scald (MOGGIA et al., 2010) and mealiness (WATKINS, 2006). On the other hand, the 1-MCP application may negatively affect on the volatile profile of 'Delicious', 'Golden Delicious' (KONDO et al., 2005), 'Anna' (LURIE et al., 2002) and 'Empire' apples (LEE et al., 2012), but, there is a lack of information of 1-MCP effect on 'Royal Gala' volatile profile.

The volatile compounds exert a very important influence on the consumer intension of purchase a product, especially apples. Apple aroma depends of complex interaction between amounts of organic compounds. These organic compounds are significantly affected by the cultivar, duration and storage method employed (BOTH et al., 2014), preharvest treatment (SALLAS et al., 2011) and 1-MCP application (KONDO et al., 2005). For apples of 'Gala' group, such as 'Royal Gala', the 2-methyl-butyl acetate is the most important contributor to sensorial quality (YOUNG et al., 1996). It is possible that 1-MCP application affect significantly on the production of ethylene dependent volatile compounds, like 2-methyl-butyl acetate, once actually is proven that 1-MCP application decrease the ethylene production (WATKINS, 2006; LEE et al., 2012). However, the effect of 1-MCP application on the volatile profile after a long time storage of Southern Brazil produced 'Royal Gala' apples is not well studied yet. Thus, the aim of the present study was to determine the effect of 1-MCP application on volatile profile and quality of 'Royal Gala' apples produced in Southern Brazil and stored under a CA condition.

MATERIALS AND METHODS

'Royal Gala' apples were harvested in February, 19, 2011 from a commercial orchard of Vacaria county, Rio Grande do Sul State, Brazil. At harvest, the fruit were submitted to a selection aiming to eliminate fruit with any injury and standardize fruit size. Immediately after this selection, fruit were transported to the Postharvest Research Center of the Universidade Federal de Santa Maria. A new selection was carried out aiming to eliminate fruit with any mechanical injury due to the transportation. Thereafter, the samples, with 25 fruits each, were done and put into experimental minichambers for 1-MCP application. Each treatment was composed by three replicates (25 fruits each replicate) totalizing 75 fruits per treatment.

In order to 1-MCP application, fruit were put into a 230 liters chamber and 1-MCP was applied at a dose of $0.625\mu\text{L L}^{-1}$. Immediately after the 1-MCP application, the chamber was hermetically closed during 24 hours, after this period the fruit were taken out and stored in a CA chamber together with fruits without 1-MCP application. The 1-MCP was obtained from the commercial SmartFresh® product, with a concentration of 0.14%.

Samples with and without 1-MCP were put in a same storage chamber with 230 liters, which allows hermetic closing. After the chamber closing, the oxygen level was reduced down to 1.0kPa by chamber flushing with N_2 down to the desired concentration and the carbon dioxide level was increased up to 1.2kPa by its injection from a high pressure cylinder that contained this gas up to the pre-established concentration. The CA condition was monitored daily throughout the storage period according to WEBER et al. (2013). The storage temperature was seated at $0.5\pm 0.1^\circ\text{C}$ and the relative humidity at $94\pm 1\%$.

After eight months of storage the ethylene production ($\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$) and respiration rate ($\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) were accessed at chamber opening (12 hours at 20°C), 2, 4 and 6 days of shelf life ($20\pm 1^\circ\text{C}$ and relative humidity $82\pm 5\%$), according to WEBER et al. (2013). ACC oxidase enzyme activity: evaluated according to the methodology proposed by BUFLER (1986). Results were expressed as $\eta\text{L C}_2\text{H}_4 \text{ g}^{-1} \text{ h}^{-1}$. Physical quality parameters (decay incidence, mealiness, healthy fruit and flesh firmness) were evaluated after eight months of storage plus 6 days of shelf life, according to BRACKMANN et al. (2013).

In order to make the samples and determine the volatile profile a methodology proposed by BOTH et al. (2014), using solid phase microextraction (HS-SPME) in the headspace of samples. The volatile compounds were only evaluated in the samples after 8 months of storage and 7 days of shelf life at 20°C . After this period, fruit were cooled down to 0°C (pulp temperature) and endocarp and seeds were eliminated. Then, samples were ground and centrifuged. All time the samples do not reach temperature upper 5°C , to prevent any chemical or enzymatic oxidation of samples. Juices were placed inside amber glass flasks and immediately frozen down to -30°C .

Samples for volatile compounds evaluation were obtained from the headspace of a 20mL vial, where was placed an aliquot of 10mL of apple juice, 3g NaCl and $10\mu\text{L}$ 3-octanol ($82.2\mu\text{g mL}^{-1}$) as internal standard solution. Frozen juice samples were thawed for 24h, at 5°C temperature, before being inserted in the vial that

was than sealed with PTFE-coated silicone lid seals. A Divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (Supelco, 50/30 μm \times 20mm) was then inserted in the lid of the vial, for volatile absorption. Thereafter, the vial was inserted in a water bath, at 35°C, for 5 minutes and then the fiber was exposed to the vial headspace for 60 minutes under constant stirring of the sample.

After extraction, the fiber was immediately inserted into the injection port of a Varian Star 3400CX (CA, Palo Alto, CA, USA) gas chromatograph, equipped with a flame ionization detector (GC-FID), for thermal desorption of the compounds. The fiber remained exposed during 10min, at 250°C, in a splitless mode for 2 initial min. Volatile compounds were separated and quantified in a polar fused silica capillary column CP-WAX 52 CB (Chrompack, USA; 60m \times 0.25mm \times 0.25 μm) according to BOTH et al. (2014). In the same conditions of the sample analyses, a series of homologous n-alkanes was analyzed, to calculate the Kovats Index (KI). Concentration of each volatile compound was determined according to the concentration of the internal standard 3-octanol. The qualitative analyses of volatile compounds were carried out according to BOTH et al. (2014).

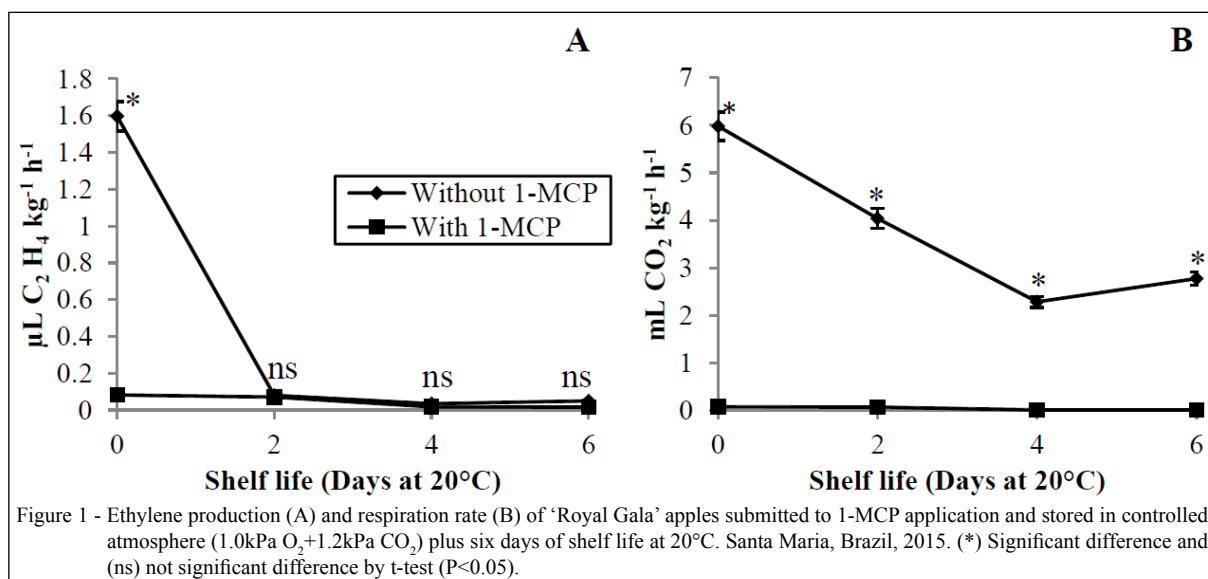
Data were analyzed with two independent sample t-test and treatment means were compared with the protected least significant difference (LSD) test ($P < 0.05$). Statistical software package Sisvar, version 5.3 was used to run the analyses. Data expressed in percentage were transformed by the formula $\arcsin \sqrt{x/100}$ before variance analysis. The experiment was conducted in a completely randomized design, with three replicates per treatment.

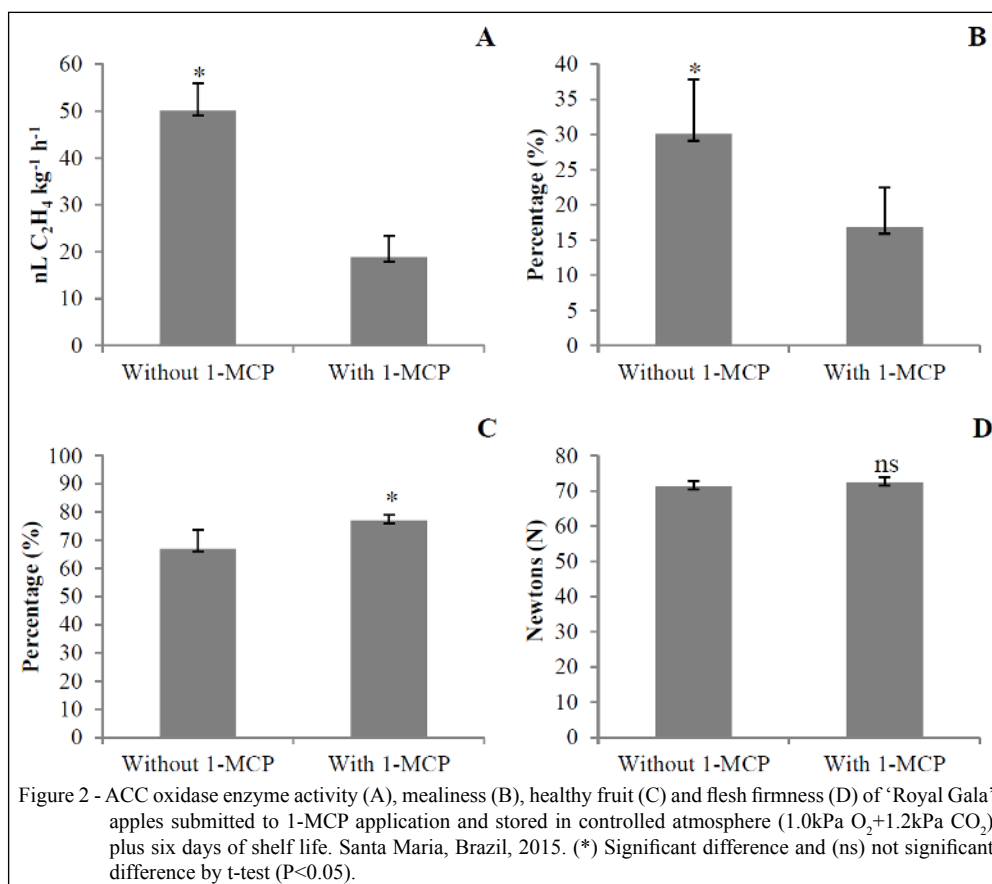
RESULTS AND DISCUSSION

Ethylene is a plant hormone responsible for start the fruit ripening. Thus, its reduction delays a series of physiological events that culminate in fruit softening. Right after the chamber opening (0 days at 20°C), the ethylene production was lower on fruits submitted to 1-MCP treatment, but after 2, 4 and 6 days of shelf life the ethylene production was similar on the two treatments (Figure 1). A noteworthy fact is that fruits without 1-MCP application exhibited a dramatic ethylene reduction from the opening of chamber up to 2 days of shelf life. This response to the shelf life was not observed on fruit submitted to 1-MCP application. Some studies realized previously have also suggest that the 1-MCP decrease significantly the ethylene production and the biochemical step ethylene-dependent, such as softening (WATKINS, 2006; BRACKMANN et al., 2013) and volatile profile, especially the esters pathway (LURIE et al., 2002; DEFILIPPI et al., 2005).

The lower ethylene production in fruits with 1-MCP after chamber opening (Figure 1A) is a result of the lower ACC oxidase enzyme activity (Figure 2A). This enzyme catalyzes the last step of ethylene production, when ACC (1-aminocyclopropane-1-carboxylate) is converted in ethylene. Probably, the lower ACC oxidase enzyme activity is a result of the lower genes expression related to this enzyme by fruit that received 1-MCP application, once some previously researches suggested that the 1-MCP application reduced the *MdACO1* gene expression (WAKASA et al., 2006).

Fruit respiration rate shows a similar response to ethylene production (Figure 1A),





confirming that respiration is an ethylene-triggered event. The figure 1B shows that fruit submitted 1-MCP application have a lower and constant respiration rate throughout shelf life, according with BRACKMANN et al. (2013). However, on fruit without 1-MCP application the respiration rate decreased up to the fourth day of shelf life. The lower respiration rate, by fruit submitted to 1-MCP application, may have reduced the NADPH supply and result again in lower de novo biosynthesis of fatty acid and also decreased the aroma biosynthesis (BANGERTH et al., 2012).

Together, the lower ethylene production and respiration rate of fruit submitted to 1-MCP, culminated in higher fruit quality. The higher fruit quality is represented by the lower mealiness incidence (Figure 2B) and the higher healthy fruit amount (Figure 2C). The higher ethylene production and respiration rate, by fruit without 1-MCP application, resulted in higher cell wall enzyme activity thus reducing adhesion among cells and turning the flesh mealy and decrease fruit quality. However, the higher mealiness by fruit without 1-MCP not results in lower flesh firmness (Figure 2D). This result disagree with some previously studies that found higher flesh

firmness on fruit submitted to 1-MCP application (MOGGIA et al., 2010; BRACKMANN et al., 2013).

Among the total esters determined in the experiment, no significant difference was observed for isobutyl acetate, butyl acetate and 3(Z)-hexenyl acetate for fruits with and without 1-MCP (Table 1). All the other esters were significantly (P<0.05) affected by 1-MCP application, being reduced with the ethylene inhibitor application. This result is in agreement with some previously studies with 1-MCP (DEFILIPPI et al., 2005; KONDO et al., 2005). The reduction in ester production by apples treated with 1-MCP is a result of the lower ethylene production of fruit of the same treatment (Figure 1A), as soon as researches suggest that the ester production is an ethylene-dependent pathway (LURIE et al., 2002; DEFILIPPI et al., 2005). Authors found a reduction, lesser than 10%, in esters production for 'Greensleeves' apples by 1-MCP application (DEFILIPPI et al., 2005), but at the present research the ester suppression was greater than 50% (Table 1). Probably, this difference is a result of the oxygen level employed during storage, once on the present study the fruit were stored under 1.0kPa of O₂ and the researches cited previously

Table 1 - Volatile compounds emitted by 'Royal Gala' apples after 8 months of storage under CA with 1.0kPa O₂+1.2kPa CO₂ condition followed by 7 days of shelf life at 20°C. Santa Maria, Brazil, 2015.

Compound	KI	----- 1-methylcyclopropene -----		Significance
		Without	With	
-----Esters-----				
-----Concentration (µg L ⁻¹) ± SD-----				
Ethyl acetate	903	19.90 ± 6.80	8.15 ± 1.63	*
Isobutyl acetate	1028	2.34 ± 0.59	1.97 ± 0.45	ns
Butyl acetate	1078	0.33 ± 0.05	2.73 ± 1.59	ns
2-Methyl-butyl acetate	1122	48.36 ± 5.31	8.52 ± 2.22	*
Hexyl acetate	1246	43.68 ± 9.17	19.43 ± 1.14	*
3(Z)-Hexenyl acetate	1317	8.92 ± 0.96	11.70 ± 3.07	ns
2-Hexenyl acetate	1328	14.91 ± 3.24	7.70 ± 0.71	*
Benzyl acetate	1714	4.09 ± 0.33	2.16 ± 0.10	*
-----Alcohols-----				
2-Propanol	936	5.68 ± 1.37	4.48 ± 0.26	ns
Ethanol	942	22.75 ± 0.58	13.04 ± 1.96	*
2-Butanol	1039	1.94 ± 0.07	2.68 ± 1.46	ns
2-Pentanol	1137	27.95 ± 2.86	19.90 ± 0.38	*
1-Butanol, 2-methyl	1197	12.22 ± 2.92	20.47 ± 4.63	ns
1-Hexanol	1353	136.04 ± 13.90	91.35 ± 9.80	*
3(Z)-Hexen-1-ol	1378	7.26 ± 1.78	6.12 ± 0.43	ns
2(Z)-Hexen-1-ol	1401	15.40 ± 3.29	15.56 ± 3.37	ns
6-Methyl-hept-5-en-2-ol	1458	0.86 ± 0.41	1.11 ± 0.10	ns
2-Ethyl-1-hexanol	1486	3.75 ± 0.45	3.65 ± 0.19	ns
1-Octanol	1557	2.21 ± 0.30	1.27 ± 0.05	*
-----Aldehydes-----				
Acetaldehyde	650	16.51 ± 1.70	3.58 ± 0.71	*
Butanal	889	1.52 ± 0.18	0.62 ± 0.10	*
3-Methyl-butanal,	924	0.83 ± 0.37	0.30 ± 0.05	ns
Hexanal	1087	405.82 ± 61.05	640.72 ± 134.51	*
2(E)-Hexenal	1212	416.13 ± 124.93	651.96 ± 52.09	*
2,4-Hexadienal	1411	1.48 ± 0.48	0.77 ± 0.15	ns
-----Acid-----				
Phosphorodithioic acid	1847	4.99 ± 0.37	4.63 ± 0.68	ns
Hexanoic acid	1856	1.88 ± 2.18	2.04 ± 0.80	ns
2-Ethylhexanoic acid	1953	0.73 ± 0.06	0.36 ± 0.03	*
Heptanoic acid	1957	2.61 ± 0.77	2.53 ± 1.58	ns
Octanoic acid	2065	3.54 ± 1.58	2.27 ± 0.49	ns
Nonanoic acid	2172	4.47 ± 3.46	2.71 ± 0.70	ns
-----Ketones-----				
2-Propanone	833	16.94	3.55	ns
Geranyl acetone	1863	2.04	2.07	ns
-----Ether-----				
Allyl ethyl ether	920	1.43	0.89	ns
-----Terpene-----				
Menthol	1653	2.03	1.04	ns

(*) Significant difference and (ns) not significant difference by t-test (P<0.05). KI = experimental Kovats Index. Concentrations were calculated relative to an internal standard (3-octanol).

under cold storage (20.9kPa of O₂). BRACKMANN et al. (1993) evaluating the effect of precursor adding on the volatile emission of 'Golden Delicious' apples stored under ultralow oxygen, have not found a

significant increasing of the main esters produced by apples, like 2-methyl-butyl acetate and hexyl acetate.

Esters are the main volatile compounds produced by apple. Among the esters, the 2-methyl-

butyl acetate and hexyl acetate were related as the highest key odor impact in apple, once they have an importance on aroma of 'Gala' group apples volatile profile (YOUNG et al., 1996). Reduction on hexyl esters by 1-MCP, such as hexyl acetate and 2-hexenyl acetate (Table 1), could be attributed to the low precursor concentration, such as acids (SONG & BANGERTH, 2003) and/or to the low alcohol acyltransferase (AAT) enzyme activity (DEFILIPPI et al., 2005). AAT catalyzes the last step ester formation by linking an acetyl moiety from acetyl CoA to the appropriate alcohols (DEFILIPPI et al., 2005). On the present study, the reduced hexyl ester production can be a result of the lower AAT enzyme activity and lower alcohols concentration, once the 1-MCP application besides reduces the alcohol concentration (LEE et al., 2012) reduces the expression of genes related to the AAT enzyme transcription (DEFILIPPI et al., 2005).

Together with the esters, the alcohols are the more abundant volatile compounds produced by apples (Table 1). Among the 11 alcohols detected on the present study, significant difference was observed only for ethanol, 2-pentanol, 1-hexanol and 1-octanol (Table 1). All the four alcohols were significantly reduced by the 1-MCP application, this behavior were previous reported in apples by KONDO et al. (2005) and LEE et al. (2012). This result suggests that 1-MCP application reduces the anaerobic metabolism culminating in lower alcohol production, especially 1-hexanol, a hexyl ester precursor. In turn, it explains the lower hexyl ester production, by fruit exposed to 1-MCP application, as soon as the 1-hexanol is a precursor of these esters production (KONDO et al., 2005). The lower ethanol production by fruit submitted to 1-MCP application also can be attributed to the lower acetaldehyde production (Table 1) by fruits of the same treatment, as soon as ethanol is produced from acetaldehyde in a reaction catalyzed by the alcohol dehydrogenase enzyme (ADH) (PESIS, 2005; LEE et al., 2012). This lower ethanol production also results in lower ethyl acetate production (LEE et al., 2012), once this ester is synthesized from the linkage of acetyl and ethanol by AAT enzyme.

Aldehydes are very important ester precursors, due to the reason that they are the first step in these volatile compounds formation (SALLAS et al., 2011). These compounds can have three different origins: from fatty acids, proteins and carbohydrates (DIMICK et al., 1983). An important enzyme on this step is acyl CoA reductase, which reduces acyl CoA to aldehyde, in the ester formation pathway (DEFILIPPI et al., 2005). In the present study 3-methyl-butanol,

and 2,4-hexadienal were not significantly affected by 1-MCP application (Table 1). A noteworthy fact is that hexanal and 2(E)-hexenal production increase with the 1-MCP application, contrasting to the remaining aldehyde identified in this research. This result disagrees with a result obtained in 'Jonagold' apples, on which the 1-MCP application decreased the hexanal and 2(E)-hexenal production (CONTRERAS & BEAUDRY, 2013). Probably, the higher hexanal and 2(E)-hexenal is a result of higher lipoxygenase (LOX) activity, once this enzyme transform fatty acids in hexanal and 2(E)-hexenal (DIMICK et al., 1983; SONG & BANGERTH, 2003; CONTRERAS & BEAUDRY, 2013). On the other hand, the higher hexanal and 2(E)-hexenal could be a result of the lower ADH enzyme activity, that reduced the conversion to alcohols, especially 1-hexanol, in fruits treated with 1-MCP (Table 1). However, another research (ORTIZ et al., 2010) found significant effect of the 1-MCP application on ADH enzyme activity in peaches and its application also reduced the alcohol acetyl transferase (AAT) genes transcription (DEFILIPPI et al., 2005), that could be explain the lower ester production by fruit submitted to 1-MCP.

The aldehydes produced via LOX generally have 6 and 9 carbon molecules, so the acetaldehyde and butanal probably are not derived from fatty acids. These two aldehydes are closely related to the anaerobic metabolism of fruit throughout the storage (PESIS, 2005). 1-MCP application significantly reduced the production of these aldehydes (Table 1). Thus, it can supposed that fruit submitted to 1-MCP application, besides exhibited a lower aerobic metabolism (Figure 1B), also show a lower anaerobic metabolism products, due to the lower acetaldehyde and butanal concentration. The lower production of these aldehydes is a result of the lower pyruvate decarboxylase (PDC) enzyme activity, as soon as this enzyme is responsible for convert pyruvate in acetaldehyde (PESIS, 2005) and its activity is inhibiting by 1-MCP application (ORTIZ et al., 2010).

Fatty acids are one of the main esters precursors (SONG & BANGERTH, 2003). On our research, the major part of acids are not affected by the 1-MCP application, with the exception of the reduction of 2-ethylhexanoic acid (Table 1). According a previously research, the short chain fatty acids can be originated by two ways: the first from the membrane catabolism or other lipids and the second by the de novo fatty acids biosynthesis via LOX (SONG & BANGERTH, 2003). Nowadays, the second way is the most accepted for acids biosynthesis by apple (SONG & BANGERTH, 2003).

In relation to the ketones concentration in fruits, there was not observed significant difference (Table 1). Another study found lower ketones production by 'Delicious' and 'Golden Delicious' treated with 1-MCP (KONDO et al., 2005). The same author attributes the lower ketones production due to the lower ethylene, once on fruit that received ethephon application showed high ketones production. On the other hand, on our works, the 1-MCP application did not affect on the ether and terpene compounds (Table 1).

CONCLUSION

The 1-methylcyclopropene application decreases significantly the ester production, especially 2-methyl-butyl acetate and hexyl acetate, of 'Royal Gala' apples produced in Southern Brazil after eight months of storage under controlled atmosphere. These fruits also exhibit higher hexanal and 2(E)-hexenal production. So, 1-MCP may have produced some effect on the step of hexanal to hexanol conversion and, thus, decreased the concentration of hexyl esters.

1-methylcyclopropene application decrease ethylene production, respiration rate, physiological disorders and maintain higher healthy fruit amount after eight months of storage plus shelf life at 20°C.

REFERENCES

- BANGERTH, F. et al. Physiological impacts of fruit ripening and storage conditions on aroma volatile formation in apples and strawberry fruit: a review. **Hortscience**, v.47, p.4-10, 2012. Available from: <<http://hortsci.ashspublications.org/content/47/1/4.full.pdf+html>>. Accessed: Oct. 10, 2014.
- BRACKMANN, A. et al. Effect of initial low oxygen stress combined with 1-methylcyclopropene in 'Royal Gala' apple quality stored under ultralow O₂. **Semina: Ciências Agrárias**, v.34, p.1185-1194, 2013. Available from: <<http://www.uel.br/revistas/uel/index.php/semagrarias/article/viewFile/13233/pdf>>. Accessed: Oct. 09, 2014. doi: 10.5433/1679-0359.2013v34n3p1185.
- BOTH, V. et al. Effect of storage under extremely low oxygen on the volatile composition of 'Royal Gala' apples. **Food Chemistry**, v.156, p.50-57, 2014. Available from: <http://ac.els-cdn.com/S0308814614001265/1-s2.0-S0308814614001265-main.pdf?_tid=09a6d664-debe-11e4-9743-00000a0f6c&acdnat=1428587031_310f6a041e5ce69ad7bffcb3b598194>. Accessed: Oct. 05, 2014. doi: 10.1016/j.foodchem.2014.01.094.
- BUFLER, G. Ethylene-promoted conversion of 1-aminocyclopropene-1-carboxylic acid to ethylene in peel of apple at various stages of fruit development. **Plant Physiology**, v.80, 539-543, 1986. Available from: <http://www.jstor.org/stable/4269783?seq=1#page_scan_tab_contents>. Accessed: Sep. 28, 2014.
- CONTRERAS, C.; BEAUDRY, R. Lipxygenase-associated apple volatile and their relationship with aroma perception during ripening. **Postharvest Biology and Technology**, v.82, p.28-38, 2013. Available from: <<http://www.sciencedirect.com/science/article/pii/S0925521413000501>>. Accessed: Sep. 15, 2014. doi: 10.1016/j.postharvbio.2013.02.006.
- DEFILIPPI, B.G. et al. Apple aroma: Alcohol Acyltransferase, a rate limiting step for ester biosynthesis, is regulated by ethylene. **Plant Science**, v.168, p.1199-1210, 2005. Available from: <<http://www.sciencedirect.com/science/article/pii/S0168945204005254>>. Accessed: Nov. 01, 2014. doi: 10.1016/j.plantsci.2004.12.018.
- DIMICK, P.S. et al. Review of apple flavor—State of the art. **Critical Review in Food Science and Nutrition**, v.18, p.387-409, 1983. Available from: <http://www.tandfonline.com/doi/abs/10.1080/10408398309527367?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed#.V5aFLvnF8io>. Accessed: Oct. 11, 2014. doi: 10.1080/10408398309527367.
- KONDO, S. et al. Aroma volatile biosynthesis in apples affected by 1-MCP and methyl jasmonate. **Postharvest Biology and Technology**, v.36, p.61-68, 2005. Available from: <<http://www.sciencedirect.com/science/article/pii/S0925521404002649>>. Accessed: Nov. 02, 2014. doi: 10.1016/j.postharvbio.2004.11.005.
- LEE, J. et al. Metabolic change in 1-methylcyclopropene (1-MCP)-treated 'Empire' apple fruit during storage. **Metabolomics**, v.8, p.742-753, 2012. Available from: <<http://link.springer.com/article/10.1007%2Fs11306-011-0373-5#page-1>>. Accessed: Sep. 15, 2014. doi: 10.1007/s11306-011-0373-5.
- LURIE, S. et al. Effect of 1-methylcyclopropene on volatile emission and aroma in cv. Anna apples. **Journal of Agricultural and Food Chemistry**, v.50, p.4251-4256, 2002. Available from: <<http://pubs.acs.org/doi/abs/10.1021/jf0200873>>. Accessed: Sep. 11, 2014. doi: 10.1021/jf0200873.
- MOGGIA, C. et al. Effect of DPA and 1-MCP on chemical compounds related to superficial scald of 'Granny Smith' apples. **Spanish Journal of Agricultural Research**, v.10, p.178-187, 2010. Available from: <<http://revistas.inia.es/index.php/sjar/article/view/1157>>. Accessed: Nov. 03, 2014. doi: 10.5424/sjar/2010081-1157.
- ORTIZ, A. et al. Volatile ester-synthesizing capacity in 'Tardibelle' peach fruit in response to controlled atmosphere and 1-MCP application. **Food Chemistry**, v.123, p.698-704, 2010. Available from: <<http://www.sciencedirect.com/science/article/pii/S0308814610005972>>. Accessed: Oct. 01, 2014. doi: 10.1016/j.foodchem.2010.05.037.
- PESIS, E. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. **Postharvest Biology and Technology**, v.37, p.1-19, 2005. Available from: <<http://www.sciencedirect.com/science/article/pii/S0925521405000608>>. Accessed: Sep. 12, 2014. doi: 10.1016/j.postharvbio.2005.03.001.
- SALLAS, N.A. et al. Volatile production by 'Golden Delicious' apples is affected by preharvest application of aminoethoxyvinylglycine. **Scientia Horticulturae**, v.130, p.436-444, 2011. Available from: <<http://www.sciencedirect.com/science/article/pii/S030442381100375X>>. Accessed: Sep. 16, 2014. doi: 10.1016/j.scienta.2011.07.017.
- SONG, J.; BANGERTH, F. Fatty acids as precursor for aroma volatile biosynthesis in pre-climacteric and climacteric apple fruit. **Postharvest Biology and Technology**, v.30, p.113-121, 2003. Available from: <<http://www.sciencedirect.com/science/article/pii/S092552140300098X>>. Accessed: Oct. 02, 2014.

WATKINS, C.B. The use of 1-methylcyclopropene (1-MCP) on fruit and vegetables. **Biotechnology Advances**, v.24, p.389-409, 2006. Available from: <<http://www.sciencedirect.com/science/article/pii/S073497500600022X>>. Accessed: Nov. 02, 2014. doi: 10.1016/j.biotechadv.2006.01.005.

WAKASA, Y. et al. Low expression of an endopolygalacturonase gene in apple fruit with long-term storage potential. **Postharvest Biology and Technology**, v.39, p.193-198, 2006. Available from: <<http://www.sciencedirect.com/science/article/pii/S0925521405002152>>. Accessed: Sep. 20, 2014. doi: 10.1016/j.postharvbio.2005.10.005.

WEBER, A. et al. Controlled atmosphere to the storage of 'Maxi Gala' apples. **Ciência Agronômica**, v.44, p.294-301, 2013. Available from: <http://www.scielo.br/scielo.php?pid=S1806-66902013000200011&script=sci_arttext>. Accessed: Sept. 10, 2014. doi: 10.1590/S1806-66902013000200011.

YOUNG, H. et al. Causal effect of aroma compounds on 'Royal Gala' apple flavours. **Journal of the Science of Food and Agriculture**, v.71, p.329-336, 1996. Available from: <<http://2010.igem.org/wiki/images/1/15/%E3%83%AA%E3%83%B3%E3%82%B4%E5%85%83.pdf>>. Accessed: Nov. 02, 2014.