Aroma volatiles of 'Conference' pear and their changes during regular air and controlled atmosphere storage

Voláteis do aroma da pera 'Conference' e suas alterações durante o armazenamento em frio convencional e atmosfera controlada

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ABSTRACT

The aim of this research was to investigate the aroma profile and changes of individual volatiles during regular air (RA) and controlled atmosphere (CA) storage of 'Conference' pear during six months at 0 °C. Gas combinations used were: RA; 0.5 kPa O2 + 0.5 kPa CO2; 1.5 kPa O2 + 1.5 kPa CO2; 0.5 kPa O2 + 6.0 kPa CO2 and 3.0 kPa O2 + 6.0 kPa CO2. The main compounds found during ripening of 'Conference' pear were butyl acetate (34 %), ethyl acetate (16 %), hexyl acetate (12 %), ethanol (11 %) and methyl acetate (5 %). Fruits under RA produced the highest amounts of volatiles and intermediary emissions were measured under 1.5 kPa O2 + 1.5 kPa CO2 and 3.0 kPa O2 + 6.0 kPa CO2. The storage under 0.5 kPa O2 + 0.5 kPa CO2 and 0.5 kPa O2 + 6.0 kPa CO2 induced the strongest inhibition in total aroma production. Under CA, the emission of ethyl acetate was not significantly suppressed even under 0.5 kPa O2 + 6.0 kPa CO2. Ethanol release increased during shelf-life in fruits of all storage conditions. Butyl acetate was only slightly suppressed even under 0.5 kPa O2 + 6.0 kPa CO2 and hexyl acetate, was significantly reduced only under 0.5 kPa O2 combined with 6.0 kPa CO2.

Keywords: Carbon dioxide, fruit quality, low oxygen, odor volatiles, Pyrus communis L.

RESUMO

O objetivo desta pesquisa foi de investigar o perfil de aroma da pera 'Conference' e alterações dos voláteis individuais durante o armazenamento em frio convencional e atmosfera controlada (AC) durante seis meses a 0 °C. As combinações de gases usadas foram: frio convencional (AR); 0,5 kPa O2 + 0,5 kPa CO2; 1,5 kPa O2 + 1,5 kPa CO2; 0,5 kPa O2 + 6,0 kPa CO2 e 3,0 kPa O2 + 6,0 kPa CO2. Os principais componentes do aroma encontrados durante o amadurecimento da pera 'Conference' foram o butil acetato (34%), etil acetato (16%), hexil acetato (12%), etanol (11%) e metil acetato (5%). Frutos armazenados em AR produziram as maiores quantidades de aroma, sendo as emissões intermediárias medidas sob 1,5 kPa O2 + 1,5 kPa CO2 e 3,0 kPa O2 + 6,0 kPa CO2. O armazenamento em AC com 0,5 kPa O2 + 0,5 kPa CO2 e 0,5 kPa O2 + 6,0 kPa CO2 induziu a maior supressão na produção total de aroma da pera. Em AC, a emissão de etil acetato não foi suprimida significativamente mesmo durante o armazenamento sob 0,5 kPa O2 + 6,0 kPa CO2. A produção de etanol aumentou durante o período de shelf-life em frutos de todos os tratamentos avaliados. A produção de butil acetato foi suprimida levemente mesmo sob 0,5 kPa O2 + 6,0 kPa CO2, já a produção de hexil acetato foi reduzida significativamente somente quando os frutos foram armazenados AC com 0,5 kPa O2 + 6.0 kPa CO2.

Palavras-chave: Baixo oxigênio, dióxido de carbono, Pyrus communis L., qualidade da fruta, voláteis.

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1 Introduction

'Conference' pear is one of the most important pear cultivar grown in Europe. It is known as a juicy, sweet and crunchy pear fruit that can be consumed in any maturity stage. It is frequently during fruit quality evaluation to consider priority to the fruit firmness, color changes and taste as main quality attributes, however, one very important fruit quality trait in pears dissembled and normally not intensive studied is the aroma composition and changes during normal ripening immediately after harvest and/or during long-term CA-storage (SAQUET, 2001).

The aroma evolution occurs in various stages during the fruit ripening of pome fruits. Immediately after harvest, at the pre-climacteric stage, pome fruits produce only very low amounts of aroma volatiles (BRACKMANN et al., 1993; HARB et al., 2008). Only during the climacteric phase with the ethylene and respiration burst is that the aroma production increases substantially and turns fruits aromatic and more suitable for consumption (SONG and BANGERTH, 1996; SAQUET et al., 2003a).

The aroma volatiles play a very important role for consumption of fruits because they act as stimulant of appetite and are constitutive of the flavor of fruits and vegetables (WEHNER, 1992). It is known more than 300 volatile compounds in pears and apples (DIMICK and HOSKIN, 1983; RAPPARINI and PREDIERI, 2003) however, methyl and hexyl esters of decadienoate are considered the impact compounds in European pears (KAHLE et al., 2005). Other volatile esters such as hexyl acetate, 2-methylpropyl acetate, butyl acetate, butyl butanoate, pentyl acetate, and ethyl hexanoate show strong pear-like aromas (RAPPARINI et al., 2003; EL HADI et al., 2015). Ethyl octanoate and ethyl-(E)-2-octenoate contribute to sweet or fruity odors in pears (RIZZOLO et al., 1991). In addition, hexanal, 2-methylpropyl acetate, ethyl acetate, hexyl acetate, 3-methylbutyl-2-methyl butanoate, ethyl butanoate, and butanol were characterized as impact aroma volatiles in 'Conference' pear (RIZZOLO et al., 2005).

Pears are, in general, more difficult to keep fruit quality during long-term CA-storage due to their higher susceptibility to internal browning disorders (IBD) than apples (GARCIA and STREIF, 1993; SAQUET et al., 2001). This is not necessarily the case of 'Conference' pear that can be stored for up to 6 months at -0.5 °C when used moderate CA-conditions with CO_2 below 0.5 kPa combined with O_2 higher than 2.0 kPa (SAQUET et al., 2003b). As is usually observed in apples, one detrimental effect of the long-term CA-storage is the impairment of the aroma production during storage and further shelf-life (PATTERSON et al., 1974; BRACKMANN et al., 1993; SAQUET et al., 2003a; HARB et al., 2008; BANGERTH et al., 2012). However, not much is known on the composition of the aroma volatiles in 'Conference' pear and much less on the effects of long-term CA-storage on the emission and changes in aroma compounds in 'Conference' pear.

Therefore, the aim of this research was to investigate the composition and changes in aroma volatiles emission during six months of CA-storage of 'Conference' pear followed by a 7 d shelf-life at 20 °C.

2 Material and Methods

2.1 Plant material and storage procedures

At-harvest, pre-climacteric ‹Conference› pears were selected for maturity, size, colour and freedom from damage and/or defects. Each experimental treatment used 30 fruits in three replicates.

Over two consecutive years, fruits were stored for 6 months under various CA-conditions and RA at 0 °C followed a 7 d shelf-life at 20 °C. The CA-conditions were: 0.5 kPa O_2 + 0.5 kPa CO_2; 1.5 kPa O_2 + 1.5 kPa CO_2; 0.5 kPa O_2 + 6 kPa CO_2 and 3 kPa O_2 + 6 kPa CO_2. SAQUET et al. (2000) describe storage procedures.

2.2 Aroma sampling

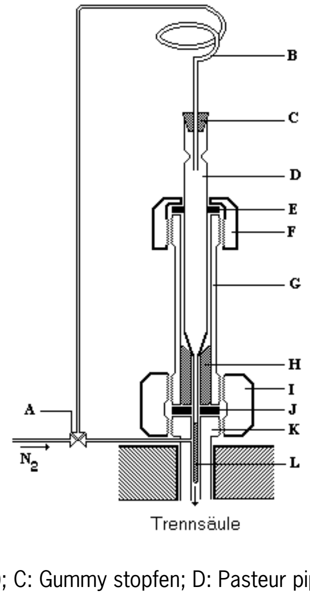
Aroma volatiles were measured after storage during shelf-life at 20 °C at the third and seventh day according to STREIF (1981). Immediately after storage, 4 fruits each treatment in 3 replicates were transferred in to 4.2 L sealed glass jars at 20 °C, which were continuously ventilated with fresh air in a flow rate of 18 L h^{-1}. After 24 h pear fruit samples reached 20 °C and the air of headspace was collected as follows: a especial air pump was used to suck 3 L of headspace through a Pasteur pipette containing inside the tip powdered mineral activated charcoal and the aroma volatiles adsorbed. It was necessary to calculate the exact flow rate of each pipette in order to have exact 3 L headspace air from each glass jar for all pipettes. After the adsorption of volatiles inside the Pasteur pipette tips, they were analyses immediately at the same day or maximal 24 h later. During this waiting time, Pasteur pipettes were stored in a sealed glass jar at 20 °C containing silica gel in order to keep the humidity very low.

2.3 Aroma volatiles measurements

For desorption and injection of the aroma samples adsorbed inside the tip of Pasteur pipettes in to gas chromatograph (GC), each Pasteur pipette was inserted inside of a modified injector as shown in Figure 1. The GC used was a Carlo Erba, Series 2150, Italy, fitted with a packed column (4.2 m x 1/8") filled with 2% carbowax 20 M on chromosorb W (80-100 mesh). A flame ionization detector and injector at 250 °C were used. The carrier gas as nitrogen at a flow rate of 50 L h^{-1}. Column temperature program used was: 2 min at 40 °C; 40 to 100 °C at a rate of 4 °C min^{-1}; 100 to 125 °C at a rate of 6 °C min^{-1}; and 15 min at 125 °C.

The identification of the aroma volatiles was carried out injecting many times various pure substances in to GC and observed the retention time of each chemical. From the peak area of the integrator and fruit weight of samples was calculated the aroma production.

Figure 1 - Device for injection of aroma samples in the gas chromatograph (STREIF, 1981)



A: Three way valve; B: Teflon tube (Æ 2 mm); C: Gummy stopfen; D: Pasteur pipette; E: Silicone seal with Teflon sleeve; F: Open screw cap; G: Glass tube; H: Seal septum; I: Two metal nut soldered together for glass tube fixation in to GC; J: Seal septum; K: Injector heating; L: Powdered active mineral charcoal inside the Pasteur pipette tip.

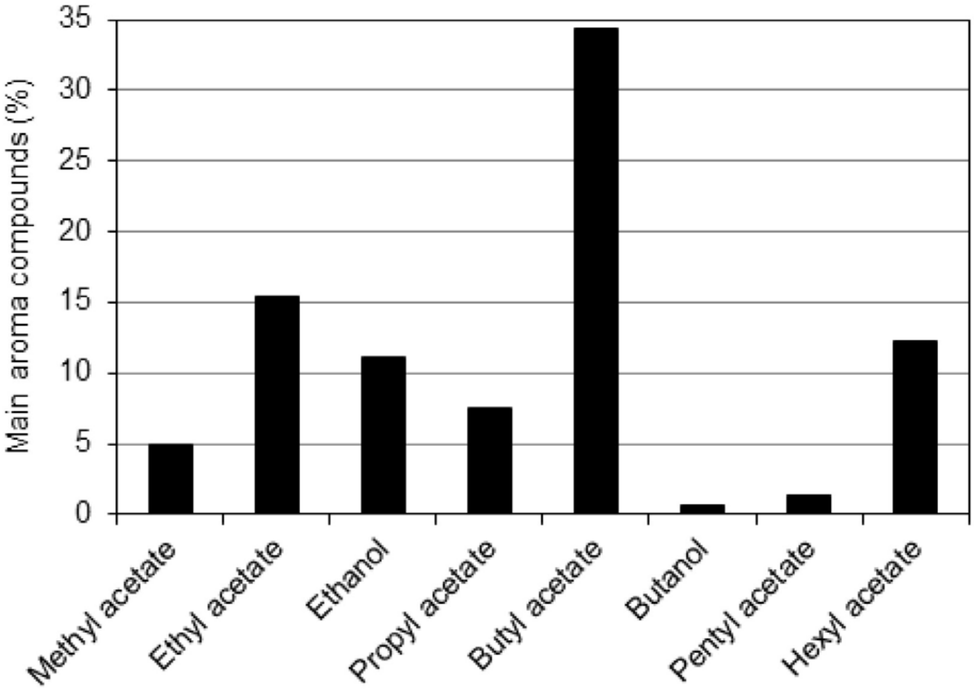
2.4 Statistical analysis

For all analyses investigated it were used, at least, three true replicates as described in each parameter analyzed. For statistical comparison, it was calculated de standard deviation (SD) of replicates (n=3).

3 Results and Discussion

The aroma profile of 'Conference' pear measured immediately at each 2-months storage intervals during shelf-life at 20 °C showed predominantly five main compounds: butyl acetate making up 34%, ethyl acetate 16%, hexyl acetate 12%, ethanol 11% and methyl acetate 5% of the total volatiles found in headspace (Figure 2).

Figure 2 - Main aroma volatiles during ripening of 'Conference' pear in air at 20 °C

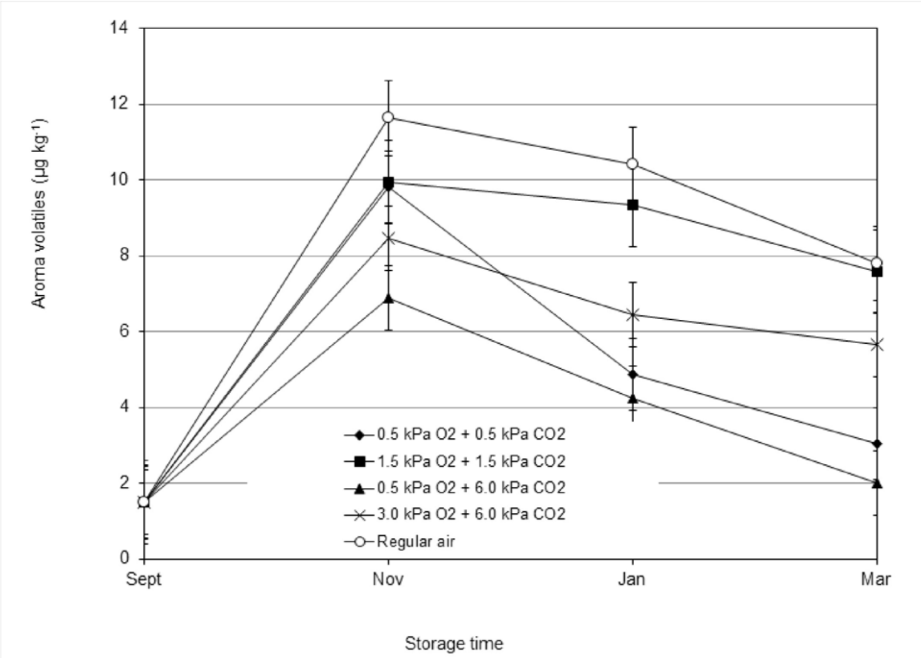


NEUBELLER and BUCHLOH (1982) measured a similar profile during ripening of 'Conference' pear. The esters butyl and hexyl acetate are normally found in higher amounts in other pear cultivars such as 'Rocha' (AVELAR et al., 1994), 'Bartlett' (JENNINGS and TRESSL, 1974) and 'La France' (SHIOTA, 1990). According to RIZZOLO et al. (2005) the headspace volatile compounds from 'Conference' pear further includes alcohols, aldehydes, ketones, and esters, the major volatile compounds being 2-methylpropyl acetate, methyl acetate, butyl acetate, and 3-methylbutyl 2-methylbutanoate among esters, and ethanol, butanol, acetaldehyde and hexanal among other volatiles.

During storage period, the highest amounts of total aroma compounds (Figure 3) in fruits of all storage conditions were measured at the second month storage agreeing with the maximum ethylene production and respiration rates at this time (data not shown). RA fruits produced the highest emissions, however, not much different of the moderated CA-condition of 1.5 kPa O_2 plus 1.5 kPa CO_2. Intermediary emissions were measured in fruits stored under 3.0 kPa O_2 plus 6.0 kPa CO_2 and only under the extreme CA-condition with 0.5 kPa O_2 combined with 6.0 kPa CO_2 was the aroma emission considerably suppressed. According to these trends, it can be stated that the low O_2 kPa was decisively in reducing the aroma production during CA-storage of 'Conference' pear. HÖHN et al. (1996) report about a general low aroma production of 'Conference' pear under either RA or CA--storage and not observed significant differences among moderate CA-condition and RA stored pear fruits. In the present research only with the use of high CO_2 as high as 6.0 kPa combined with 0.5 kPa O_2 reduced significantly the total aroma production in 'Conference' pear.

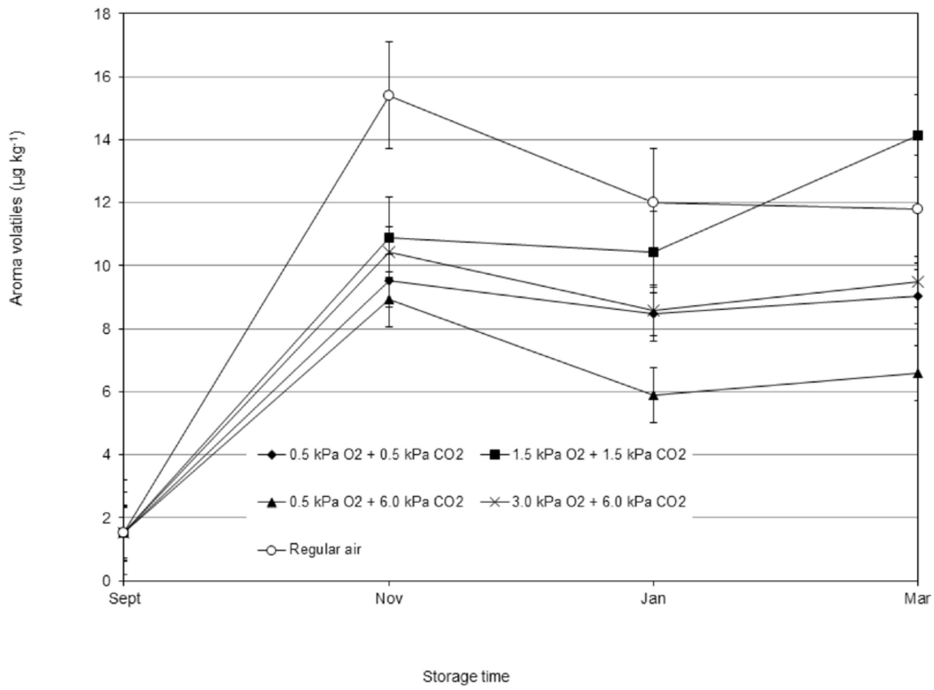
Changes in individual compounds during CA-storage were relatively low and similar to those measured by AVELAR et al. (1994) in 'Rocha' pear and CHERVIN et al. (2000) in 'Packham´s' Triumph' pear.

Figure 3 - Total release of aroma volatiles of 'Conference' pear during storage period



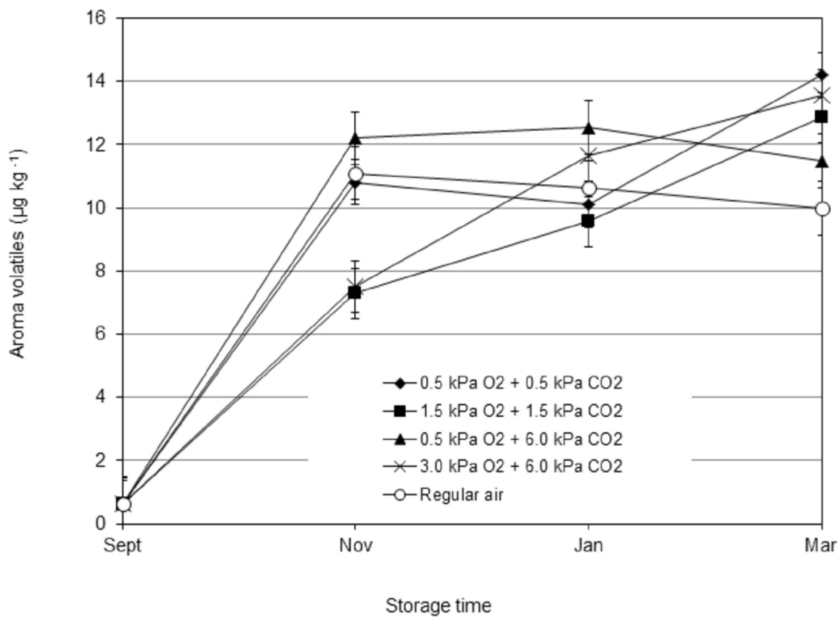
Ethyl acetate emission (Figure 4) followed a similar behavior of the total aroma production discussed early, however, in this case, even under the extreme CA-condition with 0.5 kPa O_2 plus 6.0 kPa CO_2, ethyl acetate was not strongly suppressed. It is well known that pear fruits produce ethyl acetate as one of the end products of the anaerobic respiration (NANOS et al., 1992; KE et al., 1994) and this can explain the relatively high release of ethyl acetate even under 0.5 kPa O_2 plus 6.0 kPa CO_2. Results of GOLIÁS et al. (2015) investigating 'Conference' pear found similar results in which it were measured significant amounts of ethyl acetate during ripening of pear fruits in air after long-term CA-storage.

Figure 4 - Ethyl acetate release by 'Conference' pear during storage period



Ethanol emission (Figure 5) increased continuously during the full time of shelf-life in all storage conditions. Both compounds ethyl acetate and ethanol are normally found as products of fermentation in pear fruits, but surprisingly 'Conference' pear was able to produce both compounds during the exposition in air at 20 °C even after long-term CA-storage. In this same trial, using another protocol of measurement, it was also found increasing amounts of acetaldehyde during ripening of 'Conference' pear in air after CA-storage (data not shown). The significant presence of ethanol and acetaldehyde volatiles found in the present study are markers of fermentative pathways when produced in high amounts (SAQUET and STREIF, 2006). These volatiles were also detected in high amounts in 'Conference' pear either at harvest or after storage under CA with 2 kPa O_2 plus 0.7 kPa CO_2 in previous research (ECCHER-ZERBINI et al., 2000). Various researches studying the biochemistry of pears found ethanol production during normal ripening in air at room temperature. These are the cases of 'Bartlett' (NANOS et al., 1992), 'Conference' (RECASENS et al., 1997) and 'Packham´s Triumph' (CHERVIN et al., 1999) pears. In contrast, both acetaldehyde and ethanol concentrations in 'Doyenne du Comice' pear were markedly low during fruit during ripening in air after CA-storage with 2.0 kPa O_2 (LARA et al., 2003).

Figure 5 - Ethanol release by 'Conference' pear during storage period



Butyl acetate (Figure 6) was only slightly suppressed during the 6-months storage of 'Conference' pear even under 0.5 kPa O_2 plus 6.0 kPa CO_2. The release of hexyl acetate (Figure 7) on the other side was significant, but only under 0.5 kPa O_2 combined either with 0.5 or 6.0 kPa CO_2. Investigation of CHERVIN et al. (2000) studying the pear cultivar 'Packham's Triumph' during a 2-months storage period under 3.0 kPa O_2 could not find significant inhibition in the emission of butyl and hexyl acetate. LARA et al. (2003) investigated the production of butyl and hexyl acetates and the contents of ethanol and acetaldehyde in 'Doyenne du Comice' pear during ripening at 20 °C, following long-term of regular air and under different CA-conditions and found a decrease in volatile production in fruits stored under low O_2 upon return to ambient conditions. The authors concluded that the inhibition of volatile biosynthesis by CA storage in pear was caused mainly by the limited precursor/substrate supply to the related enzymes rather than by enzyme degradation or inactivation. These results are confirmed by QIN et al. (2014) who observed, especially with esters, which were significantly increased, both qualitatively and quantitatively, in pear fruits fed on fatty acid metabolic precursors.

Figure 6 - Butyl acetate release by 'Conference' pear during storage period

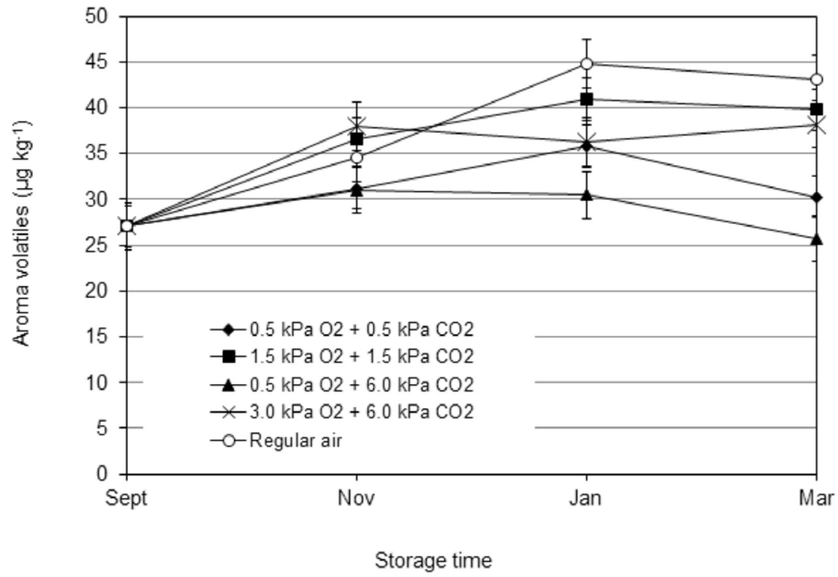
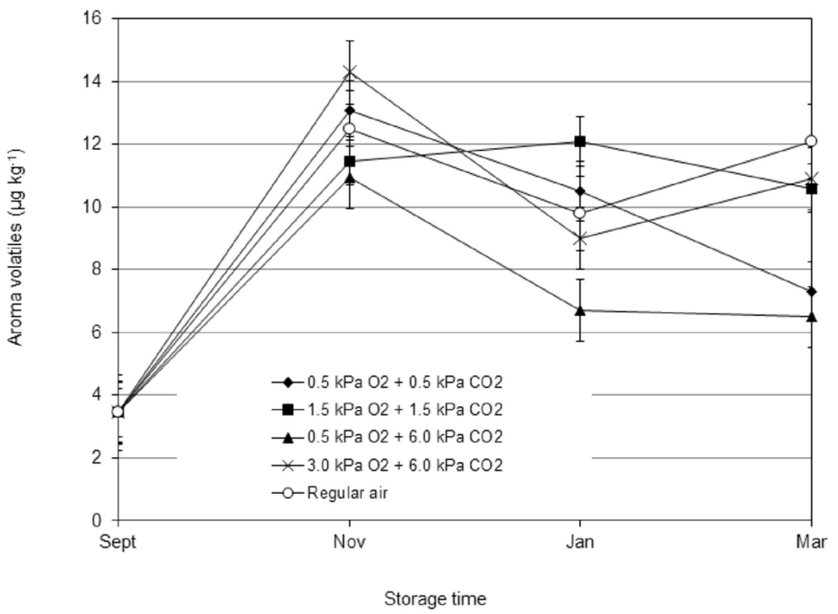


Figure 7- Hexyl acetate release by 'Conference' pear during storage period



According to this behavior of 'Conference' pear behaves similar like to the apples, which belong to the so-called group of the "alcohol type" apples. The aroma production of such apples are not so strong suppressed by low O_2 kPa such as the cultivars of the group of the "ester type" as is the case of 'Golden Delicious' (BRACKMANN et al., 1993) and 'Jonagold' apples (SAQUET et al., 2003a). The observed high amounts of ethanol, ethyl acetate and acetaldehyde produced by 'Conference' in air and even the only slightly suppressed emission of some esters contributed to the maintenance of aroma production of 'Conference' pear after storage under extreme CA-conditions. As well known, esters may be produced by the esterification of alcohols and acids, catalyzed by the enzyme alcohol acetyl transferase as was early observed in banana (HARADA et al., 1985), tomato (BALDWIN et al., 2000) and strawberry fruits (FORNEY et al., 2000) and could contribute to maintain this metabolism active.

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