Changes in quality parameters during growth, cold storage and shelf life of 'Conference' pears: from orchard to consumer

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PhD Thesis

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“Die Stimme der Vernunft ist leise”

Sigmund Freud

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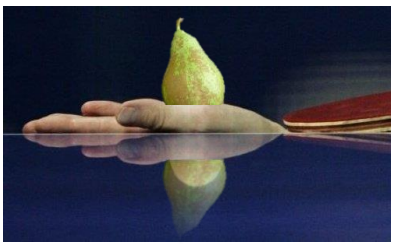

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SUMMARY

The area around Lleida is one of the main pear producing regions in southern Europe, being 'Conference' pear the main produced variety. Over the past 5 years, a decreasing trend in pear consumption in Europe has been reported. Among the reasons for the reported consumption decrease are quality factors, like the lack of flavor and excessive fruit firmness, and the growing consumer's exigence of chemical free products. These facts promoted the interest in understanding the factors affecting the evolution of fruit quality parameters during on-tree growth, the cold storage period, and the shelf-life until the end consumers.

During on-tree growth, both climatic and agronomic conditions influence fruit quality. During this stage, fruit undergoes important morphological and physiological changes until achieving the optimal maturity stage at harvest, either based on palatable quality or storage potential. Unfortunately, the most common techniques used to evaluate quality on the orchard are often based on destructive methods. In the first part of this thesis (Part A), such methods were described and correlations between destructive measurements and non-destructive modern technologies such as visible spectrometry and acoustic sensors were investigated both in apples and pears. Changes in quality parameters were related to changes in the fruit ethylene production capacity or fruit respiration. Using the emerging 3D scanning technology the shape and size of the fruit was digitized. The final fruit diameter was predicted one month in advance to the optimal harvest date.

Main factors responsible for the loss of commercial value during the cold storage period are loss of fruit firmness, development of off-flavors, appearance of physiological disorders, and weight-loss due to dehydration. With the growing consumer's demand for high quality and chemical-free fruit during all year round, the fruit industry has developed storage techniques capable of extending the commercial life of fruit without the use of chemicals. One of the recently implemented technique, known as dynamic controlled atmosphere (DCA), consists in cold storing the fruit under low oxygen atmospheres in order to slow down the fruit metabolism and then dynamically adjust the gases concentration depending on the fruit physiological state. The use of this type of atmospheres requires a precise control of the physiological response of the fruit, since the margin between the anaerobic compensation point and the beginning of fruit fermentation is very narrow. Different monitoring techniques of the physiological state of the fruit, such as analysis of the chlorophyll fluorescence evolution, continuous measurement of the respiratory quotient, and determination of ethanol content, both in fruit pulp and in the cold room's atmosphere, are currently available on the market. However, the use of these techniques has been surrounded by a certain controversy since results are not always reproducible.

In Part B of this thesis, three different studies were carried out during the fruit cold storage period. In the first study, a comparison of three commercially available DCA monitoring techniques was done. The second study assessed the effect of different low oxygen levels during cold-storage on the final pear quality and how these conditions affected the emission of volatile organic compounds (VOCs). It was found that 'Conference' pears stored for up to 8 months under such DCA conditions maintained the fruit firmness nearly to the values observed at harvest. Periodic analyses of the induced VOCs during the cold storage period pointed out that some of them could be used as indicators of the fruit's physiological state in order to control the gas concentrations appropriately. The third study was dedicated to the fruit weight loss during cold storage. A system to continuously monitor weight loss and fruit settlement inside the cold room was developed. A quite simple correlation between fruit weight loss and fruit mass settlement was found, opening the way to a simple continuous monitoring of weight loss.

Part C of this thesis includes two studies concerning the fruit quality evolution during shelf life after the cold storage period. In this period physiological changes occur rapidly, with a softening of the fruit and a loss of the organoleptic quality properties. The first study analyzed the relationship between measurable fruit quality parameters and the consumer's satisfaction provided by sensory analysis. It was found that esters and fruit firmness play a key role in defining the consumer's acceptance. The best consumer acceptance of pears stored under DCA for 8 months was found at day 3 of shelf life. The second study dealt with the spatial non-uniformity in the fruit flesh of the ripening processes. The spatial distribution of biochemical compounds, physiological activity and VOCs was measured at different flesh locations. It was observed that fructose content decreased inwards, from the skin to the core, while glucose and sucrose increased. Malic acid, together with antioxidant capacity and total phenolic compounds (TPC), had a minimum at mid-point in the flesh between skin and core. It was also found that naturally emitted VOCs could be applied as fungicides being able of inhibiting the growth of the pathogens *P. expansum* and *R. stolonifer*.

Results from this thesis provide detailed insights that could help to increase the quality of the fruit reaching the final consumer.

RESUM

L'àrea de Lleida és una de les principals regions productores de pera del sud d'Europa, essent la 'Conference' la principal varietat de pera produïda. En els darrers 5 anys s'ha detectat una tendència decreixent en el consum de pera a Europa. Entre les raons de la disminució del consum reportat es troben els factors de qualitat, com la falta de gust i l'excessiva fermesa de la fruita, i la creixent exigència dels consumidors per productes lliures de químics. Aquests fets han promogut l'interès per comprendre els factors que afecten la evolució dels paràmetres de qualitat de la fruita durant el creixement en l'arbre, el període d'emmagatzematge en fred i la vida útil fins arribar al consumidor final.

Durant el creixement en l'arbre, les condicions climàtiques i agronòmiques influeixen en la qualitat final de la fruita. Durant aquesta etapa, la fruita experimenta importants canvis morfològics i fisiològics fins a arribar a l'etapa de maduresa òptima en la collita, ja sigui en base una qualitat organolèptica o al potencial d'emmagatzematge. Desafortunadament, les tècniques més utilitzades per avaluar la qualitat en camp sovint es basen en mètodes destructius. A la primera part d'aquesta tesi (Part A), es descriuen aquests mètodes i s'investiguen les correlacions entre els mesuraments destructives i tecnologies modernes no destructives en pomes i peres, com l'espectrometria visible i els sensors acústics. Canvis en els paràmetres de qualitat es varen relacionar amb els canvis en la capacitat de producció d'etilè de les peres i amb la respiració. La forma i mida de la fruita es va digitalitzar mitjançant la tecnologia d'escaneig 3D. El diàmetre final del fruit es va predir un mes abans de la data òptima de collita.

Els principals factors que causen la desvaloració comercial durant el període d'emmagatzematge en fred són la pèrdua de fermesa, el desenvolupament de sabors desagradables, l'aparició de trastorns fisiològics i la pèrdua de pes a causa de la deshidratació. Amb el creixent interès de disposar de fruita d'alta qualitat i lliures de químics durant tot l'any, la indústria alimentaria ha desenvolupat tècniques d'emmagatzematge capaces d'estendre la vida comercial de la fruita sense l'ús d'additius químics. Una de les tècniques recentment implementades és l'atmosfera controlada dinàmica (DCA). La qual consisteix a emmagatzemar la fruita en atmosferes baixes d'oxigen per alentir el metabolisme de la fruita i ajustar dinàmicament les concentracions de gasos en funció de l'estat fisiològic de la fruita. L'ús d'aquest tipus d'atmosfera requereix un control precís de la resposta fisiològica de la fruita, ja que el marge entre el punt de compensació anaeròbic i l'inici de la fermentació de la fruita és molt petit. Actualment es comercialitzen diferents tècniques de monitoratge de l'estat fisiològic de la fruita basades en l'anàlisi de l'evolució de la fluorescència de la clorofil·la, el mesurament continu del quocient respiratori, el contingut d'etanol, tant en la polpa de la fruita com en l'atmosfera de la cambra freda. Malgrat això, l'ús d'aquestes tècniques ha estat envoltat d'una certa controvèrsia, doncs els resultats no són sempre reproduïbles.

En la Part B de la tesi es van dur a terme tres estudis durant el període d'emmagatzematge en fred de la fruita. En el primer estudi es va realitzar una comparació de tres tècniques comercials de monitoratge de DCA. En el segon estudi es va analitzar l'efecte sobre la qualitat final de la pera conservada sota diferents nivells baixos d'oxigen en fred i com tals condicions van afectar les emissions de compostos volàtils orgànics (VOC). Es va descobrir que la pera 'Conference' emmagatzemada durant 8 mesos sota tals condicions de DCA va mantenir la fermesa de la fruita pràcticament constant als valors observats en la collita. Les anàlisis periòdiques dels VOCs induïts durant l'emmagatzematge en fred van assenyalar que alguns d'ells podrien utilitzar-se com a indicadors de l'estat de la fruita i així poder regular les concentracions de gasos. El tercer estudi es va dedicar a la pèrdua de pes de la fruita durant l'emmagatzematge en fred. Es va desenvolupar un sistema per monitoritzar contínuament la pèrdua de pes i l'assentament de la fruita dins la cambra. Es va trobar una correlació entre la pèrdua de pes de la fruita i l'assentament de la massa de la fruita, obrint un nou camí al monitoratge continu de la pèrdua de pes.

La Part C de la tesi inclou dos estudis sobre l'evolució de la qualitat de la fruita durant la vida útil, després del període d'emmagatzematge en fred. Durant aquest període, els canvis fisiològics es produeixen ràpidament, amb un estovament de la fruita i una pèrdua de les propietats de qualitat organolèptica. El primer estudi va tractar d'aclarir la relació entre els paràmetres mesurables de qualitat de la fruita i la satisfacció del consumidor proporcionada per l'anàlisi sensorial. Es va descobrir que els èsters i la fermesa de la fruita juguen un paper clau en la definició de l'acceptació del consumidor. La millor acceptació per part dels consumidors es va trobar al tercer dia de vida útil en peres emmagatzemades durant 8 mesos sota condicions de DCA. El segon estudi va analitzar la no uniformitat espacial dels processos de maduració en la polpa del fruit. La distribució espacial dels components bioquímics, l'activitat fisiològica i els VOC es va mesurar en diferents ubicacions de carn. Es va descobrir que el contingut de fructosa va disminuir cap a l'interior del fruit, des de la pell fins al cor, mentre que la glucosa i la sucrosa van augmentar. L'àcid màlic, junt amb la capacitat antioxidant i els compostos fenòlics totals (TPC), van mostrar un mínim en el punt mig entre la pell i el cor. També es va trobar que els VOC emesos de forma natural per les peres podrien aplicar-se com fungicides capaços d'inhibir els agents patògens de *P. expansum* i *R. stolonifer*.

Els resultats d'aquesta tesi proporcionen informació detallada que pot ajudar a augmentar la qualitat de la fruita quan arriba al consumidor final.

RESUMEN

El área de Lleida es una de las principales regiones productoras de pera del sur de Europa, siendo 'Conference' la principal variedad de pera producida. En los últimos 5 años se ha detectado una tendencia decreciente en el consumo de pera. Entre las razones de la disminución del consumo reportado se encuentran los factores de calidad, como la falta de sabor y la excesiva firmeza de la fruta, y la creciente exigencia de los consumidores de productos libres de químicos. Estos hechos promovieron el interés en comprender los factores que afectan la evolución de la calidad de la fruta sin el uso de productos químicos durante el crecimiento en árbol, el período de almacenamiento y la vida útil hasta el consumidor final.

Durante el crecimiento en árbol, las condiciones climáticas y agronómicas influyen en la calidad final de la fruta. Durante esta etapa, la fruta experimenta importantes cambios morfológicos y fisiológicos hasta alcanzar la madurez óptima de cosecha, ya sea en base a una calidad organoléptica o potencial de almacenamiento. Desafortunadamente, las técnicas más comunes utilizadas para evaluar la calidad del fruto en campo a menudo se basan en métodos destructivos. En la primera parte de esta tesis (Parte A), se describen dichos métodos y se investigan las correlaciones entre las mediciones destructivas y las tecnologías modernas no destructivas en manzanas y peras, como la espectrometría visible y los sensores acústicos. Cambios en los parámetros de calidad se relacionaron con cambios en la capacidad de producción de etileno y con la respiración de la fruta. La forma y tamaño de la fruta fueron digitalizados mediante la tecnología emergente de escaneo 3D. El diámetro final se predijo con un mes de antelación a la fecha óptima de cosecha.

Los principales factores que causan la pérdida de valor comercial durante el período de almacenamiento son la pérdida de firmeza, el desarrollo de sabores desagradables, la aparición de trastornos fisiológicos y la pérdida de peso debido a la deshidratación. Con el creciente interés de disponer de fruta de alta calidad y libres de aditivos sintéticos durante todo el año, la industria alimentaria ha desarrollado técnicas de almacenamiento capaces de extender la vida comercial de la fruta sin el uso de aditivos químicos. Una de las técnicas recientemente implementadas es la atmósfera controlada dinámica (DCA). Esta consiste en almacenar la fruta en atmósferas con bajas concentraciones de oxígeno para ralentizar el metabolismo de la fruta y luego ajustar dinámicamente las concentraciones de gases dependiendo del estado fisiológico de la fruta. El uso de este tipo de atmósferas requiere un control preciso de la respuesta fisiológica de la fruta, ya que el margen entre el punto de compensación anaeróbico y el comienzo de la fermentación de la fruta es muy pequeño. Actualmente, se dispone en el mercado de diferentes técnicas de monitoreo del estado fisiológico de la fruta, como el análisis de la evolución de la fluorescencia de la clorofila, la medición continua del cociente respiratorio, o el contenido de etanol tanto en la pulpa de la fruta como

en la atmósfera de la cámara. Sin embargo, el uso de estas técnicas está rodeado de una cierta controversia, ya que los resultados no son siempre reproducibles.

En la Parte B de esta tesis se realizaron tres estudios diferentes durante el período de almacenamiento en frío de la fruta. En el primero se efectuó una comparación entre tres técnicas de monitoreo de DCA. En el segundo se analizó el efecto de niveles bajos de oxígeno durante la conservación en la calidad final de la pera y cómo tales condiciones afectaron las emisiones de compuestos orgánicos volátiles (VOCs). Se descubrió que la pera 'Conference' almacenada durante 8 meses bajo dichas condiciones de DCA mantuvo su firmeza casi a los valores observados en el momento de la cosecha. Análisis periódicos de los compuestos VOCs inducidos durante el almacenamiento en frío señalaron que algunos de ellos podrían usarse como indicadores del estado fisiológico de la fruta y ser utilizados para controlar la composición de los gases de la atmosfera. El tercer estudio se dedicó a analizar la pérdida de peso de la fruta durante el almacenamiento en frío. Se desarrolló un sistema para monitorizar continuamente la pérdida de peso y el asentamiento de la fruta dentro de la cámara. Se encontró una correlación entre la pérdida de peso y el asentamiento de la masa de la fruta, abriendo camino a una monitorización continua y simple de la pérdida de peso.

La Parte C de esta tesis incluye dos estudios sobre la evolución de la calidad de la fruta durante la vida útil. Durante este período, los cambios fisiológicos se producen rápidamente, con un ablandamiento de la fruta y una pérdida de las propiedades de calidad organoléptica. El primer estudio analizó relaciones entre los parámetros medibles de calidad de la fruta y la satisfacción del consumidor proporcionada por el análisis sensorial. Se observó que los ésteres y la firmeza de la fruta juegan un papel clave en la definición de la aceptación del consumidor. La mejor aceptación por parte de los consumidores se encontró al tercer día de vida útil en peras almacenadas durante 8 meses bajo condiciones de DCA. El segundo estudio se encargó de estudiar la heterogeneidad espacial en la pulpa del fruto en los procesos de maduración. La distribución espacial de los componentes bioquímicos, la actividad fisiológica y los VOCs se midió en diferentes ubicaciones de la pulpa. Se encontró que el contenido en fructosa disminuyó hacia el interior del fruto, des de la piel hasta el corazón, mientras que la glucosa y la sacarosa aumentaron. El ácido málico, junto con la capacidad antioxidante y los compuestos fenólicos totales (TPC), tuvieron un mínimo en el punto medio entre la piel y el corazón. También se encontró que, algunos de los VOCs naturales emitidos por las peras podrían aplicarse como fungicidas, siendo capaces de inhibir el crecimiento de *P. expansum* y *R. stolonifer*.

Los resultados de esta tesis proporcionan información detallada que pueden ayudar a aumentar la calidad de la fruta cuando llega al consumidor.

ABBREVIATIONS

AA	Acetaldehyde
ACC	1-Aminocyclepropane-1-carboxylic acid
ACP	Anaerobic Compensation Point
ACR	Advanced Control Respiration
ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
Afs	Acoustic firmness sensor
AFS	α-Farnesene Synthase
ANOVA	Analysis of Variance
AsA	Ascorbic Acid
C	Cluster
CA	Controlled Atmosphere
CF	Chlorophyll Fluorescence
c. i.	Confidence intervals
CO_2	Carbon dioxide
CoAs	Acyl coenzyme A
CS	Cold Storage
CSP	Cold Storage Period
CTIFL	Centre Technique Interprofessionnel des Fruits et Légumes
DA	Delta Absorvance
DAFB	Days After Full Bloom
DCA	Dynamic Controlled Atmosphere
DCR	Dynamic Controlled Respiration
DCS	Dynamic Controlled System
Dendr	Dendrometer
DLOS	Dynamic Low Oxygen Stress
DM	Dry Matter
DOP	Protected Designation of Origin
DPA	Diphenylamine
EC	European Commission

ET	Ethanol in fruit pulp
EtOH	Ethanol
F	Firmness
FIRM	Fluorescence Interactive Response Monitor
FO	Fruit Observer
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalent
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometer
H	Harvest
HPLC	High Performance Liquid Chromatography
HSD	Tukey's Honestly Significant Difference
HW	HarvestWatch
I_{AD}	Index of Absorbance Difference
ILOS	Initial low oxygen levels
ISO	International Organization of Standardization
IVOC	Induced Volatile Organic Compound
LOL	Lower Oxygen Level
LOX	Lipoxygenase
1-MCP	Methylcyclopropene
MRL	Maximum Residue Limit
MSD	Mass Spectrometer
M-T	Magness-Taylor
NA	Normal Air
NaOH	Sodium hydroxide
NaCl	Sodium Chloride
NaClO	Sodium Hypochlorite
NIPALS	Non-linear Iterative Partial Least Squares
NIR	Near-Infrared technology
O_2	Oxygen
OHD	Optimal Harvest Date

PAM	Pulse Amplitude Modulate
PCA	Principal Component Analyses
PC1	First Principal Component
PC2	Second Principal Component
PDA	Potato Dextrose Agar
PLS	Partial Least Square
PMI	Percentage of Mycelial Inhibition
RH	Relative Humidity
RLOS	Repeated Low Oxygen Stress
RQ	Respiration Quotient
S_i	Stress *i*
Sc	Scale
SL	Shelf Life
SPME	Solid-Phase Microextraction
T	Temperature
TPC	Total Phenolic Compounds
TSS	Total Soluble Solids
TTA	Total Titratable Acidity
ULO	Ultra Low Oxygen
US	Ultrasound
VIP	Variable Importance Plot
VOCs	Volatile Organic Compounds

NOMENCLATURE

Symbol	Units	Description
a	N	Ceiling firmness value.
D	mm	Fruit diameter measured by a dendrometer
d_c	cm	Growth average diameter of control.
d_t	cm	Growth average diameter of treatment.
h	mm	The high of fruit mass
I_{stress}	Pa d	Stress index
$k_{i_{CO_2}}$	-	CO_2 non-competitive inhibition constant.
$k_{m_{O_2}}$	-	Michaelis-Menten constant.
L	mm	Distance measured by the Ultrasound sensor
L_E	mm	Distance at the start of measurement
L_0	mm	Distance when the container is empty
m_c	%	Moisture content.
M_f	kg	Fruit weight.
P_{CO2}	kPa	Oxygen partial pressure.
P_{O2}	kPa	Carbon dioxide partial pressure.
r^2	-	Coefficient of determination.
r_{CO_2}	$mL_{CO2}\ h^{-1}\ kg^{-1}$	CO_2 production rate.
r_{eth}	$mol\ h^{-1}\ kg^{-1}$	Ethylene production rate.
r_m	$N\ d^{-1}$	Maximal firmness decay rate.
RQ	$mol_{CO2}\ mol_{O2}^{-1}$	Respiratory quotient.
t_{0_i}	d	Initial time of stress (i)
t_{f_i}	d	End time of stress (i)
V_m	$ml_{CO2}\ h^{-1}\ kg^{-1}$	CO_2 maximum production rate.
υ_{CO2}	v/v	Carbon dioxide air concentrations.
υ_{O2}	v/v	Oxygen air concentrations.
δ	%	Dimensionless mass loss
ξ	%	Dimensionless settlement
λ_d	d	Time at maximal firmness decay rate is achieve.

Chapter 1: INTRODUCTION

1.1 The economic importance of pears

Pears are considered worldwide one of the most delicious fruit due to their juicy texture and delicate flavor and aroma (Silva et al., 2014). There are two major species from the family Rosaceae commonly cultivated, European pear (*Pyrus communis L.*) and Asian pear (*Pyrus pyrifolia L.*).

In 2016 the world total gross production value of pear was estimated in 13 300 million USD, a production of $24 \cdot 10^6$ t (FAO, 2017). In 2017, the world's first pear producer was China with more than 16 million tones (Mt) produced, corresponding to 68 % of the world total production. The second country in order of production was Argentina (4 %) followed by Italy (3 %) while Spain was in position 7th of the list representing 2 % of the total pear production (Fig. 1.1).

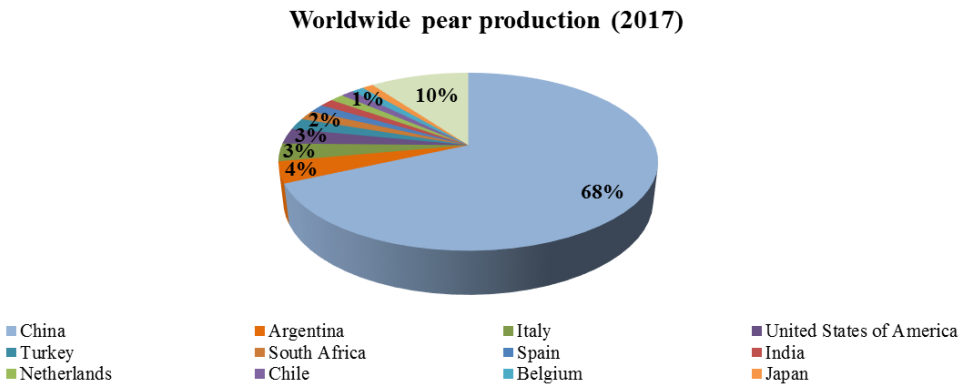

Figure 1.1 World pear production by country in 2017 (%) (FAO, 2017).

In Europe, pears are the most important produced fruit together with apples (Deckers and Schoofs, 2008). Total fresh pear production in EU was 2.24 Mt in 2017, and the forecast for 2018 predicted an increase of 4 %, due to the decrease in imports from overseas. In 2017, Italy lead the European pear production, 768 000 t (28 %), followed by Spain, 360 000 t (13 %) (Fig.1.2).

In Europe, the two most cultivated pear varieties in 2017 were 'Conference' and 'Abate Fetel', with a production of 873 and 328 Mt, respectively (Table 1.1) (Lieberz and Stange, 2018).

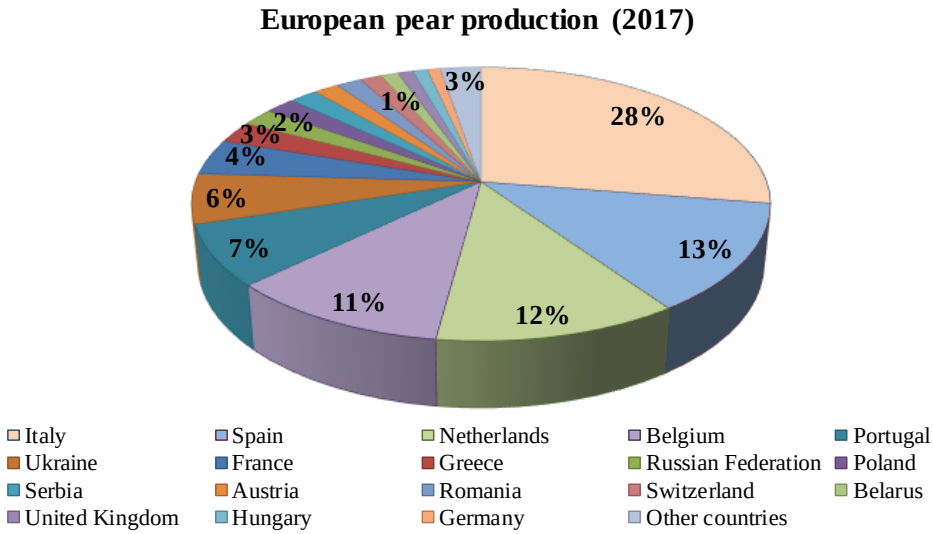

Figure 1.2 Relative European pear production by country in 2017 (%) (FAO, 2017).

Table 1.1 EU pear production by variety in Mt.

Variety	**2015**	**2016**	**2017**
Conference	967	910	873
Abate Fetel	333	296	328
Williams BC/Barlett	283	261	263
Rocha	134	113	186
Comice	87	81	59
Coscia-Ercollini	79	67	79
Guyot	74	59	65
Kaiser	45	38	43
Blanquilla	44	40	43
Passacrassana	12	11	9
Durondeau	5	3	2
Other	331	294	289
Total	2394	2173	2239

In Spain the pear production in the period of 2008-2018 decreased around 35 % (Fig. 1.3). This decreasing trend has also been observed in Portugal, Italy and France while in Turkey, Ukraine and The Netherlands the production of pear during the last decade has moderately increased. The main pear varieties cultivated in Spain are: 'Conference' (48 %), 'Blanquilla' (14 %), 'Ercolini' (12 %), 'Llimonera' (9 %) and 'Williams' (8 %) (Iglesias, 2018).

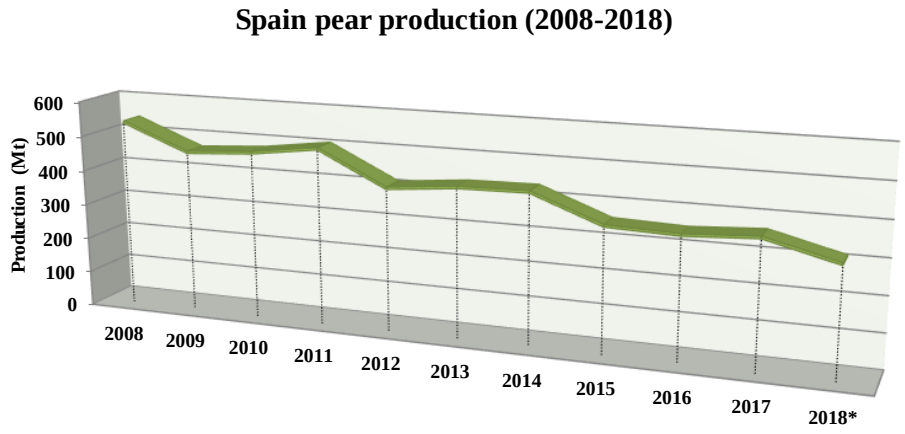

Figure 1.3 Spain pear production in the decade from 2008 to 2018 in Mt. (*) Forecast data (FAO, 2017).

Within Spain, in 2017, Catalonia (123 000 t), La Rioja (56 000 t) and Aragón (54 000 t) were the regions with the highest pear production (MAPA, 2017). The area around Lleida, (NE of Spain) represents more than 95 % of the Catalan fresh pear production, with a 'Conference' (50.4%) and Llimonera (15.2 %) being the most important varieties (Afrucat, 2019).

'Conference', 'Llimonera' and 'Blanquilla' pears from the Lleida area are officially recognized by the European Commission for their differentiated quality under the Protected Designation of Origin Pear (DOP) from Lleida.

The DOP project brings together 227 producers who cultivate around 580 ha of pear trees around the main irrigation areas of Lleida: Segrià, Noguera, Urgell, Pla d'Urgell and Garrigues.

As reported in DOP (2019), 'Conference' variety from Lleida is characterized by a medium-large caliber, having an elongated piriform shape with regular contours and more rounded than other European producing countries. Its skin is thick green to yellowish-green with moderate russeting, though always lower than those from other European countries, depending on the agro-climatic conditions. Its pulp is yellowish-white, very thin with, crunchy, juicy and sweet with an acidic flavor.

1.2 The optimal harvest date

The harvest date is an important factor that affects the final fruit quality. Fruit harvested too early is more susceptible to shrivel and to develop breakdown and superficial scald, while too late harvested fruit undergo a significant loss of firmness, acidity and sensitize the fruit to various postharvest

physiological disorders, some related to senescence processes and fungal diseases. The optimal harvest date (OHD) was traditionally determined counting days after full bloom (between 130 and 140 days in 'Conference' pear in the area of Lleida, but it is highly dependent on the season). However, other indicators are widely used, among them evaluating the fruit firmness or TSS (optimal values are 55-65 N and TSS>13 %) (Guneyli et al., 2015; Sugar and Einhorn, 2011).

Non-destructive techniques are currently available to monitor some fruit quality indicators during on-tree growth, which can be used to estimate physical properties of the fruit. Thus, the near-infrared technology (NIR), in the spectrum region of 780-2500 nm, may be considered as an indicator used to predict TSS (Wang et al., 2017). The Delta Absorbance (DA)-Meter is a non-destructive device used to determine the I_{AD}-index of the fruit, an index of the absorption difference between 670 and 720 nm, indicating chlorophyll content of the fruit skin. According to Rizzolo et al. (2015), I_{AD} index has a good correlation with ethylene production and acidity on 'Abate Fetel' pears, being a suitable marker to determine fruit maturity. Similarly, DA-meter is used to determine the apple ripening stage depending on fruit skin absorbance (Cocetta et al., 2017) whereas the acoustic impulse technique (acoustic firmness) is employed to evaluate the changes of firmness (Landahl et al., 2000). Such non-destructive techniques are widely used because of its simplicity and low cost, however, its correlation with fruit quality parameters is highly dependent on the fruit cultivar and its maturation stage.

Moreover, during on-tree growth, there are multiple factors that intervene on the quality of the product at the time of harvest, agroclimatic conditions, soil conditions and cultivation techniques such as pruning, fertilization, irrigation. Quality attributes evolution during on-tree growth and maturity indices derived from non-destructive techniques can be used as inputs into different mathematical models in order to predict the value of different fruit parameters at OHD (Li et al., 2018).

1.3 Fruit storage systems

To slow down the fruit metabolic activity upon harvest and thus to increase the commercial life of the fruit, the most common practice is to store pears at low temperatures.

By the end of the 19th century it was discovered that fruit ripen more slowly in an oxygen depleted atmosphere (Dalrymple, 1969). In 1925 the concept of controlled atmosphere (CA) was born to prolong and improve the conservation of the fruit (Yahia E., 1995). Kidd and West (1927) studied the

effects of low O_2 levels and high CO_2 levels in pome fruit and berries during ripening. However, until the 1950s, CA was not a worldwide commercialized technology (Prange, 2005). Since then, adjustments at CA have been introduced leading to different methods with a common goal: to prolong the storage of pome fruit with the highest quality.

In the 1970s, improvements of the CA promoted two different systems: Initial Low Oxygen Stress (ILOS) and Repeated Low Oxygen Stress (RLOS). ILOS consisted in producing an initial stress of the fruit by lowering the oxygen level (≈0.5 kPa O_2) at the beginning of the cold storage for two weeks, followed by storage in CA conditions (Eaves et al., 1969). Calvo et al. (2015) investigated ILOS (0.5 kPa O_2 and 0.1 kPa CO_2) conditions followed by ULO (1.0 kPa O_2 and 0.1 kPa CO_2) in 'Beurré d'Anjou' pears for 9 months and observed a reduction of superficial and internal disorders compared to pears treated with diphenylamine (DPA) and stored under normal air. Ramokonyane (2016) stored 'Packham's Triumph' pears in ILOS conditions at 0.7 kPa O_2 for 10 d followed by CA and effectively controlled superficial scald. The RLOS system consisted of performing stresses repeatedly. Ethanol content in fruit pulp was monitored during CA storage, in order to avoid fruit fermentation. (Prange et al., 2015). According to Fadanelli et al. (2015), stresses in apples should be applied 2-3 times throughout the storage period whereas Kawhena et al. (2018) stored 'Packham's Triumph' pears and lowered oxygen levels at 0.5 kPa for 7 days once per month during the whole storage period. Zanella and Stürz (2013) reported that RLOS conditions retained 'Red Delicious' apples quality when stored up to 6 months.

In 1996, Höhn et al. (1996) introduced a new concept, delayed CA, which consists of storing fruit between two to eight weeks under normal air at low temperatures before lowering atmosphere oxygen levels in order to avoid internal cavities and flesh breakdown. They found that a 21 days delayed CA reduced between 50-90 % the cavities formation in 'Conference' pears. Saquet et al. (2001) reported that delayed CA reduced browning disorders in 'Conference' pears. Saquet et al. (2017) investigated the delayed CA in 'Rocha' pear and observed that internal disorders depended on the oxygen partial pressure.

In 1982 appeared the first publication about the latest technology, dynamic controlled atmosphere (DCA), with the aim of long term fruit storage while maintaining postharvest quality and controlling physiological disorders (Alique and de la Plaza, 1982).

CA and ULO storage are static systems, which means that the atmosphere is set to an optimal level and does not vary according to the product response. The CA technique has evolved with the development of more accurate control systems, to dynamic controlled atmosphere (DCA storage). DCA storage aims for the lowest possible oxygen level, as per ULO, but adapts the gas concentrations dynamically on the basis of the changing physiological response of the produce. If the system detects low-oxygen stress, it increases the oxygen level until the commodity response is back to the optimal threshold.

1.3.1 Dynamic Controlled Atmosphere

DCA allows the customization of O_2 levels according to fruit physiological state during the storage period in order to maximize fruit quality retention. It consists of lowering the oxygen level of the atmosphere to the minimum tolerated by the fruit, also called lower oxygen level (LOL) and keeping it as close as possible to the anaerobic compensation point (ACP), but always above the fermentation threshold (Fig. 1.4). Ripening of pears kept at these oxygen levels, can be delayed more effectively than under regular CA storage. Under such conditions the oxidation and senescence processes are also slowed (Saquet, 2019).

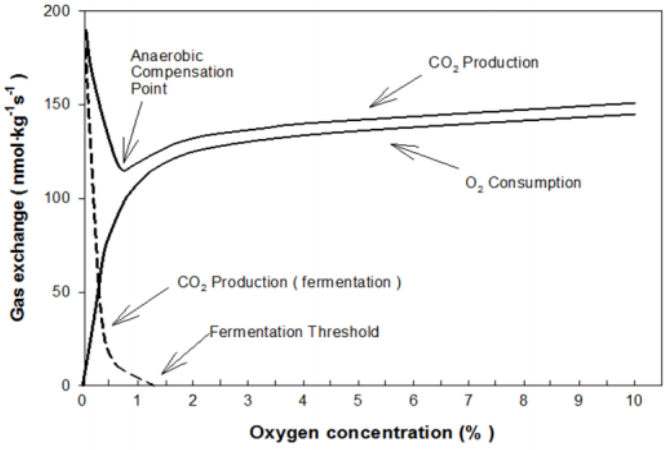

Figure 1.4 Oxygen consumption and carbon dioxide production (Prange et al., 2011).

DCA technology requires an accurate control of the gases concentrations and the monitoring of the fruit metabolic response, in order to ensure that the fruit fermentation process (anaerobic respiration) is not initiated. The fruit metabolic response is monitored by a sensor and the gas concentrations in DCA are regulated according to the signal provided by that sensor. Currently, on the market there are three types of sensors which are based on measurements of: chlorophyll fluorescence (DCA-CF), respiration quotient

(DCA-RQ) and ethanol content either in fruit pulp (DCA- EtOH) or at the cold room atmosphere (DCA-DCS).

1.3.1.1 Chlorophyll fluorescence sensor

The DCA-CF is used to continuously measure the fruit LOL during the cold storage period as fluorescence intensity changes rapidly when anaerobic respiration is triggered (Prange et al., 2013). The utilization of a fluorimeter with a pulse amplitude modulated (PAM) allows to determine the fruit pigment content in a non-intrusive way. Fruit is excited with a beam of light in the range of 420 nm (blue) to 660 nm (red) (Walker, 1990) then the response emission spectra by the fruit is captured. Chlorophyll excitation processes are useful for measuring tissue stress states that affect maturation, and allow detecting the stress before symptoms become visible (Prange et al., 2002).

Currently, there are two commercial sensors to monitor DCA-CF, the HarvestWatch (HW) (Isolcell, Canada) (Fig. 1.5 A) and the Fruit Observer (FO) (Besseling, The Netherlands) which was developed in 2013 (Fig. 1.5 B). Both sensors use a low power light sources to stimulate fruit photo systems based on a Fluorescence Interactive Response Monitor (FIRM) system. HarvestWatch was developed and patented in 2001 by a Canadian group (Prange et al., 2003). According to Mattheis and Rudell (2011), superficial scald in 'Anjou' pear was completely controlled with DCA-CF-HW but not black speck. Zerbini and Grassi (2010) reported that senescent scald was reduced in 'Conference' pears stored under DCA-CF-HW but was not reduced for 'Abbé Fétel' pears while 15% of 'Conference' pears and 10% in 'Abbé Fétel' pears showed superficial scald. However, Zerbini and Grassi (2010) reported that this system could be a problem when used in a large commercial chamber due to the small fruit sample on which the measurements are done.

Figure 1.5 A) Harvestwatch (HA) sensor (Isolcell, Canada). B) Fruit Observer (FO) sensor (Besseling, The Netherlands).

1.3.1.2 RQ sensor

It is known that the respiratory quotient (RQ) is a good marker of low O_2 stress of fruit inside a cold storage (Gasser et al., 2010). RQ is the ratio between the oxygen consumption rate (r_{O_2}) and the carbon dioxide production rate (r_{CO_2}) of a fruit (Eq. 1) (Gran and Beaudry, 1993).

$$RQ = \frac{-r_{CO_2}}{r_{O_2}} \quad (1)$$

The negative sign is due to the fact that in a respiration process is r_{O_2}<0.

In an aerobic respiration process glucose is combusted as a respiratory substrate according to the following chemical reaction (Eq. 2),

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad (2)$$

in which $RQ = 1$.

However, at very low oxygen levels fruit metabolism changes from respiration to fermentation, in this situation fruit does not consume oxygen and carbon dioxide is produced together with ethanol as a result of the anaerobic reaction (Eq. 3),

$$C_6H_{12}O_6 \Rightarrow 2CO_2 + 2C_2H_5OH \quad (3)$$

in which $RQ \rightarrow \infty$.

In practice there is a gradual transition from aerobic respiration to anaerobic fermentation and RQ increases its values above 1 at low oxygen levels (Fig. 1.4). The RQ sensor is based on direct measurements of the RQ: if the measured value lies between 1.3 and 1.5 the system does not modify any parameter of the CA. However, when a RQ value greater than 1.5 is detected then the system slightly increases P_{O2} in the room. The aim of respiratory quotient (RQ) monitoring is to keep the fruit close to the ACP but without shifting from aerobic respiration to fermentation. Storage of fruit at O_2 levels below the ACP could lead to physiological disorders and to fruit off-flavor (Prange, 2005).

DCA-RQ has been investigated in different apple cultivars, Weber et al. (2015) reported maintenance of quality for ‘Royal Gala’ apples after eight months of storage and Bessemans et al. (2016) reported the complete control of superficial scald in ‘Granny Smith’ apples during 9 months of storage under DCA-RQ. There is one commercial system the Advanced Control Respiration (ACR) (VanAmerongen, The Netherlands), patented as dynamic controlled

respiration (DCR) system (Veltman and Nijmege, 2015) which uses continuous measurements of the RQ.

The main drawback of this technology is that cold chambers must be completely airtight, then small leakiness of the cold chamber may allow small amounts of air to enter it, altering the gas partial pressures and causing a false RQ measurement. Commercial chambers have seals that can cause small losses (Persson and Yang, 2008). Bessemans et al. (2018) reported a method to correct real-time estimates of RQ in case of cold storage leakiness.

1.3.1.3 Ethanol sensor

Ke et al. (1990) discovered that ethanol content could be used as a marker of fruit stress. Even though, a methodology to analyze ethanol content in fruit pulp was previously developed by Beutler and Michal (1977). Ethanol content in fruit pulp can be analyzed by the Senzytec analyzer (Tectronik, Italy) (Fig. 1.6 A) or by gas chromatography (GC) (Deuchande et al., 2016).

The dynamic controlled system (DCS) technology, from now on (DCA-DCS) was developed and introduced by Wageningen University and Research (WUR) together with STOREX company in the Netherlands (Schouten, 1995). It consists of placing two boxes of fruit in a department connected with the environment of the chamber (Fig. 1.6 B). This department can be airtight closed when the ethanol measurement in the headspace takes place. Usually it is installed on the ceiling of the chamber.

Veltman et al. (2003) studied the viability of a DCS sensor in 'Elstar' apple and found better firmness maintenance and better retention of the green color than under normal CA (1.2 kPa O_2 and 2.5 kPa CO_2) and also to reduce skin spot development. However, to the best of our knowledge, no studies with pear cultivars have been done using this technology.

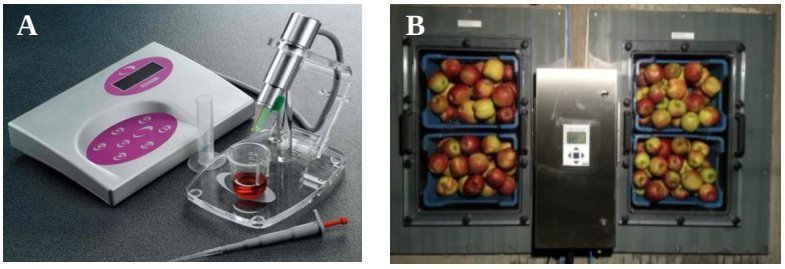

Figure 1.6 A) Senzytec analyzer (Tectronik, Italy). B) Dynamic control system (DCS) (STOREX, Netherlands).

1.3.2 DCA advantages and limitations

DCA-CF technology exerts positive effects in comparison with classical CA during the storage of apples, controlling different senescence related physiological disorders (Zanella et al., 2005, 2008). According to Prange et al. (2011), DCA-CF prevent superficial scald disorder in pear fruit. However, the number of sensors needed in one cold room must be chosen according to the heterogeneity of the room filling what can be very expensive. The number of fruit analyzed to determine the fermentation of the whole chamber is small.

DCA-RQ technology reported satisfactory results on stored apple fruit (Bessemans et al., 2016). However, chambers have to be completely airtight. The main advantage is that the sensor takes care of all fruit inside the chamber.

DCA-EtOH in fruit pulp is time consuming and no representative of the whole chamber. It is not an autonomous technology and it is necessary to collect fruit inside the chamber once the atmosphere is controlled.

DCA-DCS using the DCS technology analyzes a small sample, so measured values may not be representative of the whole chamber and the presence of a few rotted fruit can distortion the measurements. Moreover, the ethanol thresholds tolerated by the fruit are not yet well defined since part of the ethanol accumulated may be further metabolized by the fruit. Due to the high water solubility of ethanol, most of the produced through fermentation remains in the cells (Gupta et al., 2000), causing the measured ethanol concentration in the atmosphere to be lower, leading to an underestimation of the low oxygen stress experienced by the fruit (Pesis, 2005).

It is clear that the dilemma of choosing atmosphere optimal conditions can be overcome by continuously controlling the storage atmosphere composition based on product response. However, the available technologies on the market do not seem to be fully effective (Table 1.2).

Table 1.2 Comparison of commercial sensors used to control the oxygen levels in DCA.

Manufacturer	Sensor name	Measured parameter	Sample size	Measurement	Gases	Observations
Isolcell	HarvestWatch	Chlorophyll fluorescence	6 fruit/sensor 2-3 sensors/chamber	Continuous	Manual	• Fruit analyzed by sensor must be free from defects and representative of the chamber. • It is sensitive to light.
Besseling	Fruit Observer	Chlorophyll fluorescence	*ca*. 20-30 fruit	Continuous	Manual	• Fruit from first layer must to be free of defects and representative of the chamber. • Not definitive protocol.
Tectronik	Senzytec	Ethanol pulp content	6-10 fruit	Discontinuous	Manual	• Samples must be taken from the controlled chamber. • It is not a dynamic system. • It is not well known the ethanol content level tolerated by fruit.
Storex	Dynamic Control System	Ethanol in the atmosphere	20 kg/box 2 box/chamber	Analysis by cycles	Manual	• Fruit inside boxes must be free from defects and representative of the chamber. • Atmospheric conditions of the boxes may differ from those of the chambers. • The relationship between the ethanol levels in the atmosphere and inside the fruit may vary according to variety.
Van Amerongen	Advanced Control Respiration	Respiratory quotient	Whole chamber	Analysis by cycles	Automatic	• The chamber must be totally airtight. • It is difficult to obtain an average RQ with the variability within the chamber.

1.4 Shelf life period and consumer acceptance

After being removed from the cold room begins the shelf life (SL) period during which fruit retains an acceptable organoleptic quality (Sousa-Gallagher et al., 2011). The extension of the shelf life period depends on several factors and it can be as short as 5–15 d at room temperature (20 ºC), depending on the variety. However, it can be extended using chemical products, for example Methylcyclopropene (1-MCP) (Golding and Singh, 2017), modified atmosphere packaging (MAP) (Nath et al., 2012), storing fruit under low oxygen levels or using natural coatings (Misir et al., 2014).

The key aspect of SL is to evaluate the time at which the consumer acceptance is higher. The concept of quality in European pear fruit at the time of consumption is different from that of the quality of fruit at harvest, during and after storage. Consumer satisfaction will impact on a new purchase of the same fruit variety. Hájos (2012) determined external and internal preferences of pear using descriptive sensory analysis on four pear varieties just after long term storage and found that the total soluble solids and acids alone did not determine consumers liking level. Moya-León et al. (2006) evaluated the consumer preferences on 'Packham's Triumph' pear under different storage conditions and reported that 1-MCP treated pears stored during 6 months under normal air maintained textural characteristics and the capacity of volatile production as preferred by the consumers. Predieri and Gatti (2009), evaluated the effects of different cold storage periods and shelf-life on sensory quality and consumer acceptance of 'Abate Fetel' pears and they reported that consumer acceptance was higher in fruit stored under cold storage for 13 weeks, instead of 23 weeks.

Flavor plays a key role in determining the perception and acceptability of pears by consumers (Brückner, 2008). Understanding the relationships between the consumer preferences and pear quality attributes will give potential information to retailers at the time of sale. Delivering pears at the time of optimal quality in terms of consumer's criteria, namely ready-to-eat fruit, may provide useful information at the time to schedule fruit distribution.

1.5 Parameters defining the quality of fruit

The quality parameters described in this section can be applied both to apples and pears. 'Conference' pear (Fig. 1.7) is known as a juicy, sweet and crunchy fruit (Saquet, 2017).

Figure 1.7 Picture of a 'Conference' pear.

Quality of a fruit is an overall combination of its physical, chemical and sensory properties. Different authors have attempt in describing the concept of quality over the past years (Tijskens et al., 1994; Zerbini, 2002). The complexity is that quality means different things to the multiple stakeholders within the horticultural supply chain (Shewfelt, 1999). The International Organization of Standardization (ISO) defined quality as 'the sum of all characteristics, properties and attributes of a product or commodity which is aimed at fulfilling the established or presumed customer requirements'(ISO 8402, 1986). Quality is a dynamic term which evolves over the years adapting to society's changes such as gastronomic trends and health concerns (Jabs and Devine, 2006). Quality has been broadly defined by the firmness, sugars and acid content of the fruit. But recently, there has been a shift from a product orientation to a consumer orientation (Shewfelt, 2000).

Quality properties change during on-tree fruit growth as well as after harvest until the fruit is finally consumed (Shewfelt, 1999). At time of purchase the consumer decision will depend on the perceived quality rather than price (Harker et al., 2003).

1.5.1 Morphological aspects

Morphological aspects such as size and color are the basic quality attributes that consumers face when it comes to purchase decision (Opara and Pathare, 2014).

Fruit size and weight are the most important external aspects that influence the demand and price (Zhang et al., 2010). According to Kappel et al. (1995) the accepted pear size by North American consumers is in the range of

60-75 mm (equatorial diameter) and 150-250 g. However, these standards vary from country to country, for example, the most appreciate commercial size and weight for 'Conference' pears in Catalonia are in the range 64-84 mm and 178-358 g, as shown in Fig. 1.8.

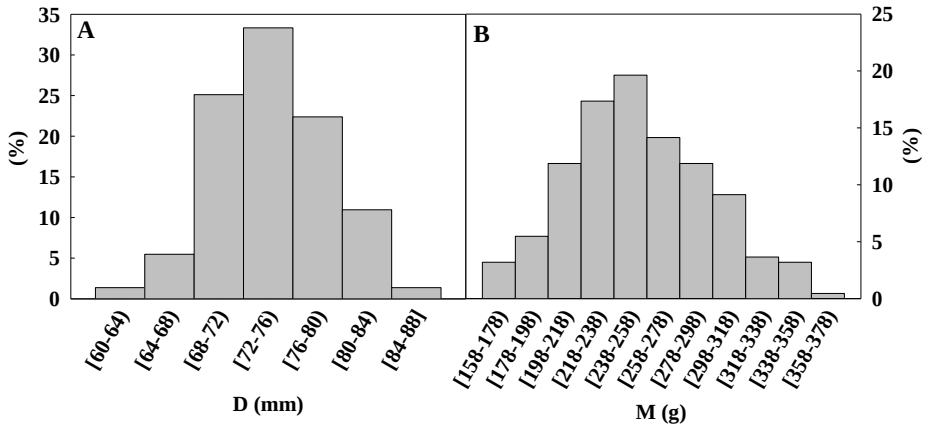

Figure 1.8 Ranges and distributions of 'Conference' pear from Lleida harvested in September 2017, A) diameter and B) weight.

Fruit is classified in categories mainly according to its equatorial diameter (Table 1.3). However, in recent years the color and presence of skin defects are being introduced as factors to be taken in consideration. Each category has a commercial price.

Table 1.3 Pear calibers classification.

Caliber	Diameter (mm)
Extra large	>80
Large	70-80
Medium	60-70
Small	<60

From the fruit diameter, mass can be estimated using a power function, $M = a \cdot D^b$, where M is the fruit mass (g) and D the fruit diameter in mm and a, b are constant parameters depending on the variety. Typical values for a and b in apples are a=338-394 and b=2.9 (Stajnko et al., 2013; Welte, 1990).

Fruit surface area, and volume are physical properties with a dominant role in the processes of gas exchange such as respiration and VOC emissions. Pears are irregular in shape so different attempts have been done in order to determine its surface and volume non-destructively. Schouten et al. (2004) used two methods, in the first one assumed that the shape of a pear can be considered as a cone placed on top of a semi sphere to calculate the surface of a

'Conference' pear. On the other hand, they developed a computer imaging (CI) technique and used it to calculate the surface area of a pear. They placed a pear at the bottom of a light box with three CCD color camera (JVC KY-F30 3CCD) attached to the top of the light box. Finally, they approximated the pear surface, assuming that a pear is rotationally symmetrical, by a finite series of slices with an equal, small thickness and a varying radius. The sphere-cone method underestimated the surface area by 2.2 ± 6.8 % whereas the CI method overestimated the surface by 3.5 ± 2.7 %. Babic et al. (2012) estimated surface area and volume of a quarter's pear using a mathematical model and algorithm based on pears length. New technologies such as 3D scanning together with 3D image analysis software determine the volume and surface are of irregularly shaped products with an accuracy higher than 98 % (Igathinathane et al., 2010).

Besides fruit shape, color is one of the main factors that determine the external appearance of the fruit and the visual quality by the consumer. Different varieties of pears have a wide range of colors yet the most accepted by consumers are the yellow-green skin pears (Jaeger, et al., 2003). Color can be measured using a hand-held spectrophotometer (Fig. 1.9A).

Changes related to chlorophyll breakdown are the most common color changes in fruit (Tijskens and Schouten, 2014). Color is used as an indicator to determine the optimal harvest date based on its maturity for some apple and pear varieties such as 'Abbe Fetel' and 'Kieffer' pears (Guneyli et al., 2015).

Figure 1.9 A) Portable spectrophotometer CM-2600d (Konica Minolta Sensing, Japan). B) DA-meter (Turoni, Italy).

In the maturity stage there are changes in pericarp chlorophyll content. Chlorophyll breakdown and the accumulation of large quantities of carotenoids in the plastids implies a change in the color of the fruit. During ripening, chloroplasts are transformed into chromoplasts (Bathgate et al., 1985). Peel chlorophyll content can be used as a maturity index in pears (Wang et al., 2015), and easily measured by using a DA-meter (Fig. 1.9B).

1.5.2 Firmness

Firmness is an important parameter for the evaluation of quality of pears and it is widely used for the determination of the optimal harvest date (OHD). As a maturity indicator fruit firmness changes considerably during ripening both during the last stages of fruit ripening on-tree as well as during postharvest handling (De Belie et al., 2000).

Firmness values at harvest depend on the variety and cultivar, even though European varieties are in the range of 50-65 N, except Barlett pears that are generally harvested at higher firmness values (87 N) (Table 1.4). However, European pears can be divided in summer and winter pears, winter pears required a chilling period in order to ripen with good quality (Knee, 1987). During cold storage firmness remains relatively constant but thereafter, in the SL period decreases rapidly (Fig. 1.10). This rapid decrease depends on several factors such as temperature (T), relative humidity (RH) and type of atmosphere at which has been exposed during the cold storage period. Pome fruit stored under low temperature and with a controlled atmosphere (CA) reduces firmness loss during SL (de Martin et al., 2017; Gwanpua et al., 2012).

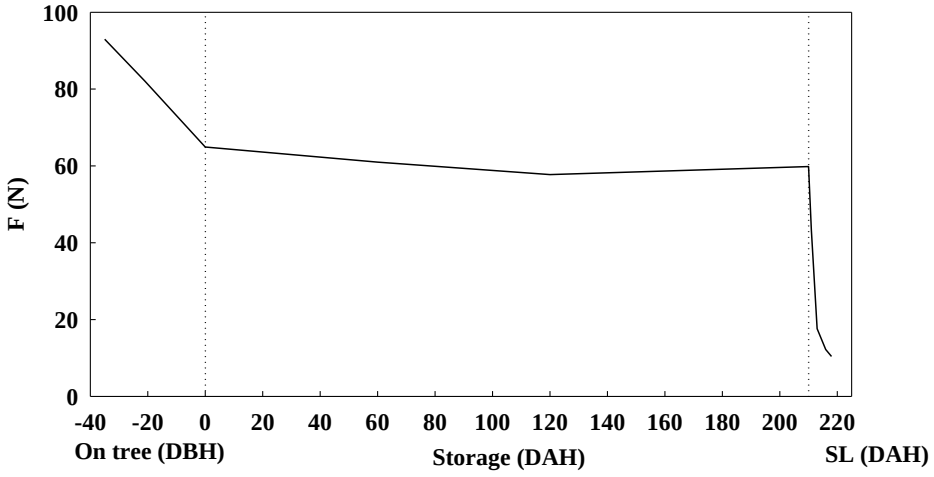

Figure 1.10 Firmness evolution during on tree growth in days before harvest (DBH), during storage and shelf life (SL) in days after harvest (DAH) in 'Conference' pears from Lleida region.

Some authors have developed models to predict firmness loss as function of the environmental conditions in different fruit. Tijskens et al. (1998) modeled firmness loss in peaches based on the enzyme polygalacturonase (PG) activity. Van Dijk et al. (2006) developed two models to describe the firmness loss in stored tomatoes, assuming that the softening is related to the breakdown of the pectin by the PG. De Ketelaere et al. (2006), used the Monte Carlo

method to predict the firmness evolution of mangoes during storage and Ochoa-Ascencio et al. (2009) used it to explain softening of avocado. Gwanpua et al. (2012) modeled the relation between the pectin degradation, the synthesis of pectin degrading enzymes and the ethylene production with the firmness loss in stored apples under different temperatures and under controlled atmosphere (CA). Gwanpua et al. (2013) used the Monte Carlo method to model fruit-to-fruit biological variation of firmness breakdown for three cultivars of apples as a function of time, temperature, controlled atmosphere conditions and endogenous ethylene concentration.

Fruit firmness is directly measured using the classic puncture test (Magness-Taylor (M-T)), by the means of a penetrometer fitted with an 8 mm or 11 mm diameter plunger for pears and apples, respectively (Fig. 1.11A). The semi-spherical plunger is introduced 8 mm, after removing the peel into each fruit, and the maximum force is measured.

Currently, there is a growing interest in developing non-destructive techniques to measure fruit firmness (De Belie et al., 2000; Gómez et al., 2005). Acoustic vibration technologies are cheap, reliable and fast (Aboonajmi et al., 2015), however, different indexes have been developed because resonance frequency depends on fruit shape and other internal physical characteristics of the fruit (Chen and DeBaerdemaeker, 1993). Jancsók et al. (2001) estimated the 'Conference' pear firmness introducing a shape descriptor and un-normalized resonant frequency in the calculations. Zhang et al. (2019) evaluated the acoustic firmness in different shaped pears through a resonance method (four frequency based-indices) and a propagation method (two velocity-based method). On the other hand, Kadowaki et al. (2012) used the contact of a piezoelectric transducer in order to detect core rot symptom of Japanese Pear. The resonance frequencies are related to acoustic elasticity, internal friction or damping, shape, size and density of the fruit. The firmer the pulp, the higher the resonance frequency for fruit with the same shape and size. This method consists in subjecting the fruit to a small blow and examining the vibrations it generates in response to internal excitation.

Nowadays, there is an Acoustic firmness sensor (Afs) commercial sensor which measures acoustic elasticity of the fruit (AWETA, Nootdorp, The Netherlands) (Fig. 1.11B). Each fruit is subjected to an impact in the equatorial zone through the action of a plastic piston with a semispherical termination. The acoustic signal transmitted inside is detected with a microphone close to the impact area and then the acoustic elasticity is calculated (Cooke and Rand, 1973).

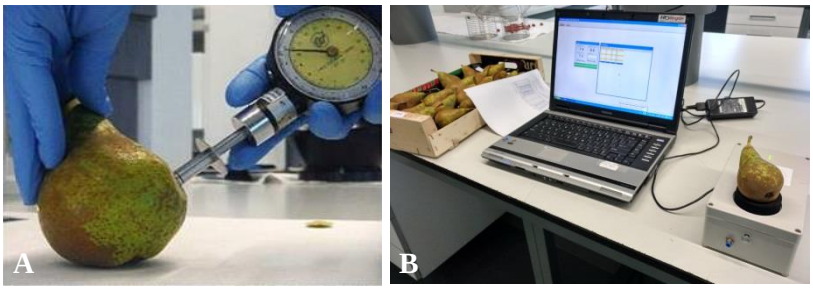

Figure 1.11 A) Hand-held penetrometer (Turoni, Italy). B) Acoustic elasticity measurement by an AWETA sensor (Afs AWETA, Nootdorp, The Netherlands) in a 'Conference' pear.

1.5.3 Total Soluble Solids

Total Soluble Solids (TSS) is one of the most important quality parameters used to quantify fruit sweetness. According to Hansen and Mellenthin (1979), a minimum of 10 % of soluble solids content are necessary in pears to guarantee a good quality and to prevent freezing during storage.

TSS (%) are measured by crushing together pieces of pear and filtered to obtain one juice, a drop is placed in the reader of a hand-held refractometer (Atago, Tokyo, Japan) (Fig. 1.12).

Figure 1.12 Hand-held refractometer (Atago, Tokyo, Japan).

1.5.4 Total Titratable Acidity

Total Titratable Acidity (TTA) is used, along with sugar content, as an indicator of maturity, the higher the maturity the lower the acid content. TTA increases during on-tree fruit growth and decline during storage and SL (Saquet, 2019). TTA is a fruit organoleptic characteristic of pears and together with TSS are the main responsible of the flavor.

TTA is measured by titration of 10 ml of juice with 0.1 N sodium hydroxide (NaOH) to pH 8.2 using phenolphthalein or measurements can be done using a potentiometric titrator, Titrex Act2 with AS23 micro auto-sampler (Steroglass, Perugia, Italy) (Fig. 1.13) or with a visual inspection. The result is expressed in milliliters of malic acid per liter of pear juice. TTA measures the total acidity but does not measure the strength of the acids.

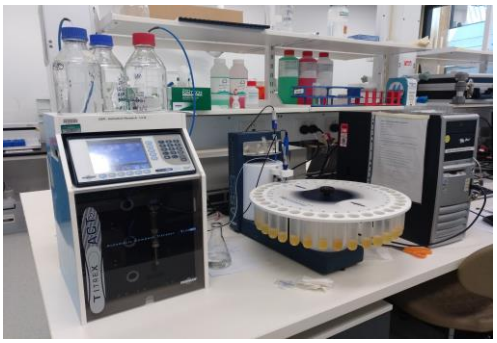

Figure 1.13 Potentiometric titrator, Titrex Act2 with AS23 micro auto-sampler (Steroglass, Perugia, Italy).

1.5.5 Reported data on firmness, TSS and TTA on pears

Firmness, TSS and TTA are used as indicative of the gustatory quality, maturity state and storage capacity of pears. Firmness values remain practically constant during the storage period, but decrease rapidly in the shelf life (Table 1.4). TSS in European pears at harvest are in the range of 12-17 %. 'Abate Fetel' cultivar have higher amounts of TSS content (17.2 %) at harvest than other reported cultivars (Table 1.4). 'Alexander Lucas' pears have the higher amount of TTA at harvest (5.3 mL malic acid L^{-1}) followed by 'Abate Fetel' (3.3 mL malic acid L^{-1}) while 'Conference' pears had the lowest (1.3 mL malic acid L^{-1}).

The TSS / TTA ratio is commonly used as indicative of the maturity in pears since it is related to the organoleptic characteristics of the fruit (Chen et al., 2007).

Table 1.4 Reported values of firmness (F), total soluble solids (TSS) and total titratable acidity (TTA) in different pear cultivars. The storage conditions refer to: Temperature (T), atmosphere relative humidity (HR) and contents of O_2 and CO_a. When treatment (Treat.) with 1-MCP was applied, the dose is indicated. Duration of storage period (SP) initiated at harvest (H) and the shelf-life period (SL) are also reflected.

Cultivar	Storage conditions				Treat.						Reference
	T(ºC)	HR (%)	%O_2	%CO_2	1-MCP (nL L^{-1})	SP (d)	SL (d)	F (N)	TSS (%)	TTA (mL malic ac.·L^{-1})	
Abate Fetel	0-1	95	21*	0*		H		54.2	17.2	3.33	(Predieri and Gatti, 2009)
						91	0	49.2	18.8	2.76	
							2	48.9	18.9	1.81	
							4	29.9	19.3	1.70	
							6	19.6	19.8	2.22	
							8	15.9	19.4	1.81	
							10	14.5	19.4	1.56	
						161	0	42.5	18.4	2.17	
							2	37.0	18.6	2.17	
							4	30.4	18.7	2.31	
							6	25.7	18.4	1.82	
							8	23.4	18.9	1.78	
							10	22.3	18.8	2.02	
Barlett	-1	--	21*	0*		H		87			(Zlatić et al., 2016)
			21*	0*		120	0	70			
							10	17			
	1		21*	0*		120	0	55			
							10	14			
	-1		0.8	<0.5		120	0	75			
							10	16			
	1		0.8	<0.5		120	0	74			
							10	17			

Table 1.4 Continuation.

Cultivar	Storage conditions				Treat.	SP	SL	F	TSS	TTA	Reference
	T(ºC)	HR (%)	%O_2	%CO_2	1-MCP (nL L^{-1})	(d)	(d)	(N)	(%)	(mL malic ac.·L^{-1})	
Conference	0±0.1	94±2	20.9	<0.04		H		56.0	13.7	1.3	(Hendges et al., 2018)
						210	7	19.1	15.9	0.94	
					300	210	7	24.3	16.6	1.28	
			2	<0.7		210	7	14.9	14.9	1.12	
					300	210	7	34.4	15.4	1.31	
			0.7	<0.7		210	7	15.5	15.3	1.27	
Alexander Lucas	0±0.1	94±2	20.9	<0.04		H		50.9	12.5	5.7	(Hendges et al., 2018)
						210	7	16.1	12.7	1.95	
					300	210	7	27.3	13.0	1.98	
			2	<0.7		210	7	16.2	12.8	2.21	
					300	210	7	29.7	12.8	2.58	
			0.7	<0.7		210	7	17.5	12.7	2.63	
Conference	0±0.3	--	21*	0*		H		57.6	13.3	2.3	(Saquet, 2018)
						180	0	42.1	14.2	0.7	
							7	11.0	14.2	0.9	
			0.5	0.5		180	0	50.1	14.4	1.3	
							7	12.5	15.0	1.6	
			1.5	1.5		180	0	50.0	14.5	1.1	
							7	11.5	15.0	1.6	
			2.0	1.0		180	0	52.9	13.8	1.3	
							7	15.5	14.1	1.6	
			3.0	6.0		180	0	49.0	14.0	0.9	
							7	12.4	15.1	1.7	
			0.5	6.0		180	0	49.1	14.5	0.9	
							7	10.9	15.2	1.7	

Table 1.4 Continuation.

Cultivar	Storage conditions				Treat.	SP	SL	F	TSS	TTA	Reference
	T(ºC)	HR (%)	$\%O_2$	$\%CO_2$	1-MCP (nL L^{-1})	(d)	(d)	(N)	(%)	(mL malic ac.·L^{-1})	
Conference						H		59.0	12.0	2.1	(Zerbini and Grassi, 2010)
Abate Fetel						H		48.0	13.0	3.8	
Rocha	0-0.5	90-95	21*	0*		180	0	65			(Galvis-Sánchez et al., 2004)
							6	31			
							9	22			
			4	0.5			0	71			
							6	27			
							9	19			
			4	1.5			0	72			
							6	28			
							9	15			
Rocha	0-0.5	90-95	2	0.5			0	68			(Galvis-Sánchez et al., 2004)
							6	30			
							9	20			
			2	1.5			0	68			
							6	25			
							9	12			
Rocha	2.5	90-95	21*	0*	312	H		54.9	12.9	2.1	(Gago et al., 2015)
						98	0	49.5	13.9	1.4	
						98	7	42.7	14.3	1.4	
						98	14	29.1	14.5	1.5	
						182	0	35.0	13.9	1.2	
						182	7	9.0	14.1	1.0	
						182	14	5.6	14.4	1.0	

Table 1.4 Continuation.

Cultivar	Storage conditions				Treat.	SP	SL	F	TSS	TTA	Reference
	T(ºC)	HR (%)	%O_2	%CO_2	1-MCP (nL L^{-1})	(d)	(d)	(N)	(%)	(mL malic ac.·L^{-1})	
Rocha						245	0	29.4	13.2	1.1	(Gago et al., 2015)
						245	7	13.9	13.6	0.7	
	2.5		3.04	0.91	312	98	0	54.3	13.5	1.4	
						98	7	57.8	13.8	1.4	
						98	14	48.7	13.8	1.4	
						182	0	34.7	14.2	1.3	
						182	7	44.5	13.9	1.2	
						182	14	20.1	15.0	1.3	
						245	0	41.0	13.8	1.4	
						245	7	15.4	14.3	1.1	
						245	14	6.0	14.4	1.0	
	0		21	0	312	98	0	56.6	14.1	1.6	
						98	7	47.5	14.1	1.4	
						98	14	32.8	14.1	1.3	
						182	0	53.1	13.9	1.1	
						182	7	40.4	14.2	1.2	
						182	14	21.0	14.7	1.1	
						245	0	55.1	14.0	1.3	
						245	7	32.6	14.5	1.3	
						245	14	10.5	14.4	1.3	
	0		3.04	0.91	312	98	0	57.3	14.3	1.7	
						98	7	60.6	14.1	1.8	
						98	14	50.8	14.3	1.8	
						182	0	55.0	13.9	1.4	
						182	7	53.2	14.0	1.6	
						182	14	37.3	14.3	1.4	

Table 1.4 Continuation.

Cultivar	Storage conditions				Treat.	SP	SL	F	TSS	TTA	Reference
	T(ºC)	HR (%)	%O_2	%CO_2	1-MCP (nL L^{-1})	(d)	(d)	(N)	(%)	(mL malic ac.·L^{-1})	
Rocha						245	0	57.6	13.4	1.5	(Gago et al., 2015)
						245	7	50.9	13.7	1.5	
						245	14	32.3	14.1	1.3	
Blanquilla	0.5	90	21*	0*		H		56.9	12.0	2.7	(Larrigaudière et al., 2004)
						90	0	50.0	12.7	1.9	
					100ppb	90	0	54.9	12.9	2.0	
						150	0	25.5	13.3	1.7	
					100ppb	150	0	50.0	13.0	2.0	
Blanquilla	-0.5	90	2.5	1.5		150	1	48.1	15.0	2.6	(Larrigaudière et al., 2019)
						150	3	24.5	14.6	2.5	
						150	7	14.7	14.9	1.6	
	-0.5	90	0.7	0.5		150	1	55.9	15.5	2.7	
						150	3	46.1	15.0	2.3	
						150	7	15.6	15.2	2.2	
	-0.5	90	2.5	1.5	300	150	1	54.9	15.1	2.7	
						150	3	55.9	14.8	2.3	
						150	7	54.9	14.7	2.4	

--Data not available. *Assumed values under normal atmosphere storage.

1.5.6 Starch

Starch can be used as a maturity indicator during the fruit growth. Pome fruit accumulate starch at early stages of maturation and it is degraded during ripening, in parallel to an increase of the fruit sweetness (Kvaale, 1986; Warrington et al., 1999).

Common starch measurements are made by applying an iodine solution in the pulp of the fruit, previously cut equatorially. Fruit pulp with starch produces a blue color, depending on the fruit maturity stage different patterns of starch degradation will appear (Fig. 1.14). In most European countries the reference CTIFL (Centre Technique Interprofessionnel des Fruits et Légumes) chart is used based on a 1 to 10 scale (Planton, 1994), 1 indicating no starch breakdown and 10 indicating complete breakdown, areas of the fruit that have lost starch will remain unstained.

Figure 1.14 CTIFL 1 to 10 starch content scale (Ctifl., 1993).

According to Johnson and Luton (1996), starch was a good maturity index marker for 'Conference' pears and it has been used for many years to indicate the harvest maturity. Saquet and Almeida (2017) dipped half surface of 45 'Rocha' pears in a solution of 1 % (w/v) iodine in 4 % (w/v) potassium iodine for 30 s and compared with the 10-point scale for 'Rocha' and they found a good reaction of the iodine solution test in relation to the starch content in fruit.

1.5.7 Biochemical parameters of quality

Quality is not only governed by color and firmness but rather by the consumer's perceived flavor. Flavor in pears is due to a complex interaction between sugars, acids, phenolic and a wide range of volatile organic compounds (VOCs) (Gonçalves et al., 2018), the concentration of which changes during fruit growth and is affected by postharvest handling.

The concentration of different compounds, such as sugars, acids, VOCs can be employed to determine the optimal harvest date and as indicators of the maturation stage (Baietto and Wilson, 2015; Kingston, 2010).

1.5.7.1 Sugars

Sugars increase during the fruit growth on tree. Once the fruit is harvested, sugars can increase due to the hydrolysis of polysaccharides or decrease if they are used as a respiratory substrate (Viñas et al., 2013). The main sugars in pears are fructose, sorbitol, glucose and sucrose (Moriguchi et al., 2019). In 'Conference' pears fructose is the main sugar followed by sorbitol while the amount of sucrose and glucose content depends on the agroclimatic conditions during fruit growth which may change from year to year (Table 1.5).

Table 1.5 Average content of sucrose, glucose, fructose and sorbitol in g kg^{-1} of fresh weight in different pear cultivars at Optimal Harvest Date (OHD).

Cultivar	Sucrose	Glucose	Fructose	Sorbitol	Reference
Abate Fetel	6.6	16.3	43.6	21.0	(Hudina and Stampar, 2000)
Burré Alexandre Lucas	13.2	6.9	34.9	17.6	
Conference	11.3	4.9	23.7	12.5	
Packham's Triumph	5.3	11.6	34.7	19.8	
Red Williams	2.8	5.8	32.8	16.7	
Bartlett	35.1	11.3	64	19.6	(Drake and Eisele, 1999)
Blanquilla	41	9	5	-	(Lindo-García et al., 2019)
Conference 2004	18.53	7.63	48.47	23.85	(Colaric et al., 2007)
Conference 2005	6.60	14.75	76.23	33.58	
Kuerle Fragant	20	40	60	-	(Ji et al., 2006)
Packham'sTriumph	5.3	18.2	56.9	24.7	(Fourie et al., 1991)
Radana	0.71	57.88	67.49	27.53	(Kolniak-Ostek, 2016)
Rocha	25	22.5	70	22.5	(Pais et al., 2008)
Williams	7.28	9.42	67.61	21.34	(Colaric et al., 2006)

1.5.7.2 Acids

Acidity is an organoleptic characteristic and together with the sugars are the main responsible for the fruit flavor. Acids that confer flavor to the fruit are those that are accumulated in the vacuoles, being the malic acid the most abundant in pears (Sha et al., 2011). Acids increase during fruit growth and

subsequently declines during ripening and the storage period. The individual content and composition of organic acids differ across cultivars, origin and year of production (Table 1.6).

Table 1.6 Average content of malic, citric, shikimic and fumaric acids in g kg^{-1} of fresh weight in different pear cultivars according different authors.

Cultivar	Malic	Citric	Shikimic	Fumaric	Reference
Abate Fetel	3.3	0.3	-	2.1	(Hudina and Stampar, 2000)
Burré Alexandre Lucas	4.1	0.0	-	0.6	
Conference	2.3	0.0	-	20.3	
Packham's Triumph	3.2	0.4	-	2.8	
Red Williams	1.5	2.1	-	0.8	
Blanquilla	1.65	3.06	0.38	0.0	(Drake and Eisele, 1999)
Conference 2004	4.25	0.22	0.09	3.09	(Colaric et al., 2007)
Conference 2005	1.97	0.21	0.05	2.42	
Kuerle Fragant	1.6	0.06	0.15	0.05	(Ji et al., 2006)
Williams	2.13	3.05	57.67	0.44	(Colaric et al., 2006)

1.5.7.3 Volatile Organic Compounds (VOCs)

More than 300 volatile compounds have been identified in pear (Table 1.7), including alcohols, aldehydes, ketones, esters, hydrocarbons, terpenes, acids and sulfur compounds (Rapparini and Predieri, 2003). Esters are known as the main responsible for fruit aroma. Methyl and hexyl esters of decadienoate are characteristic compounds of 'Conference' pears (Kahle et al., 2005; Rapparini and Predieri, 2003). In 'Conference' pears, hexanal, 2-methylpropyl acetate, ethyl acetate, hexyl acetate, 3-methylbutyl-2-methyl butanoate, ethyl butanoate and butanol have also been identified as impact volatiles (Rizzolo et al., 2005), the concentration of which is largely affected by the fruit maturity at the time of harvest as well as postharvest storage conditions. Pear flavor consists of both perception in mouth and on the odor, produced by several VOCs.

Volatiles profiles of pears are complex and depends on the cultivar (Qin et al., 2012), cold storage atmosphere composition (Goliáš et al., 2015; Saquet, 2017), ripening state (Torregrosa et al., 2019), fruit sample (either intact fruit, slices, or homogenized samples) (Chervin et al., 2000), and analytical methods used.

VOCs can be divided in two groups, depending on their formation. Primary volatiles are the ones produced in the entire fruit by controlled enzymatic reactions, mainly through the β-oxidation metabolic pathway

(Rapparini and Predieri, 2003). Secondary volatiles are formed through various uncontrolled enzymatic reactions when tissues are disrupted by slicing and cutting (El Hadi et al., 2013). Cellular disruption mixes the enzyme and substrate creating "new" volatiles which are mainly produced from fatty acids in disrupted tissues (Rapparini and Predieri, 2003). Secondary volatiles, such as hexanol and hexanal are generated during chewing. It should be noted that aroma evolves from the physiological ripening of the fruit until consumption (Viñas, et al., 2013).

Table 1.7 Main VOCs in pears at harvest (H), during the cold storage period (CSP) (days) under different atmospheres: NA (Normal Air), CA (Controlled Atmosphere), ULO (Ultra Low Oxygen) and after shelf life (SL) (days).

VOC	Cultivar	Stage	Value ranges	Units	Reference
Alcohols					
Ethanol	Conference	CSP (154) + SL (5)	24260	μg kg^{-1}	(Rizzolo et al., 2005)
	Anjou	CSP NA (240) + SL (7)	915	Nmol kg^{-1} h^{-1}	(Argenta et al., 2003)
1-Propanol	Anjou	CSP NA (240) + SL (7)	39	Nmol kg^{-1} h^{-1}	(Argenta et al., 2003)
1-Butanol	Doyenne du Comice	CSP CA (210) + SL (4)	11.71-28.15	μg kg^{-1}	(Lara et al., 2003)
2-Methyl-1-propanol	Doyenne du Comice	CSP NA (90) + SL(4) NA	1.7±0.4	μg kg^{-1}	(López et al., 2001)
1-Pentanol	Anjou	CSP NA (240) + SL (7)	2.6	Nmol kg^{-1} h^{-1}	(Argenta et al., 2003)
2-Methyl-1-butanol	Blanquilla	H + SL (6)	68.8 ± 10.57	μg kg^{-1}	(Lindo-García et al., 2019)
1-Hexanol	Doyenne du Comice	CSP CA (210) + SL (4)	2.09-3.94	μg kg^{-1}	(Lara et al., 2003)
(E)-2-Hexen-1-ol	Nanguoli	H	16.80±1.44	μg kg^{-1}	(Qin et al., 2012)
1-Heptanol	Nanguoli	H	0.89±0.41	μg kg^{-1}	(Qin et al., 2012)
1-Octanol	Nanguoli	H	4.10±2.56	μg kg^{-1}	(Qin et al., 2012)
Aldehydes					
Hexanal	Barlett	H + SL (10)	26.08	μg kg^{-1}	(Makkumrai et al., 2014)
	Packham's Triumph	CSP NA (60) + SL (12)	*		(Chervin et al., 2000)
(E)-2-Hexenal	Barlett	H + SL (10)	21.94	μg kg^{-1}	(Makkumrai et al., 2014)
	Ruanerli	H	27.37	μg kg^{-1}	(Li et al., 2016)
Nonanal	Anjou	CSP NA (240) + SL (7)	51	Nmol kg^{-1} h^{-1}	(Argenta et al., 2003)
Acetaldehyde	Conference	CSP NA (154) + SL (5)	4252	μg kg^{-1}	(Rizzolo et al., 2005)
Benzaldehyde	Anjou	CSP NA (240) + SL (7)	5.2	Nmol kg^{-1} h^{-1}	(Argenta et al., 2003)
Ketones					
Acetone	Conference	CSP (154) + SL (5)	75	μg kg^{-1}	(Rizzolo et al., 2005)
3-Hydroxy 2-butanone	Blanquilla	H + SL (7)	0.5-15.8	μg kg^{-1}	(López et al., 2015)

Table 1.7 Continuation.

VOC	Cultivar	Stage	Value ranges	Units	Reference
6-Methyl-5-hepten-2-one	Blanquilla	H	2.4	μg kg^{-1}	(López et al., 2015)
Esters					
Methyl acetate	Conference	CSP CA (180) + SL (7)	*		(Saquet, 2017)
	Barlett	CSP ULO (120) + SL (10)	2.46-3.31	μg L^{-1}	(Zlatić et al., 2016)
Ethyl acetate	Anjou	CSP NA (240) + SL (7)	68	Nmol kg^{-1} h^{-1}	(Argenta et al., 2003)
	Conference	CSP CA (180) + SL (7)	*		(Saquet, 2017)
Propyl acetate	Doyenne du Comice	CSP NA (90) + SL (4)	15.3±1.3	μg kg^{-1}	(López et al., 2001)
Butyl acetate	Abate Fetel	H	7.51± 6.29	μg g^{-1}	(Chen et al., 2018)
	Barlett	H	0.85± 0.49	μg g^{-1}	
	Doyenne du Comice	CSP NA (90) + SL (4)	1580.3±123.3	μg kg^{-1}	(López et al., 2001)
Pentyl acetate	Conference	CSP CA (180) + SL (7)	*		(Saquet, 2017)
Hexyl acetate	Conference	CSP CA (210) + SL (7)	470-1180	μg L^{-1}	(Hendges et al., 2018)
	Alexander Lucas	CSP CA (210) + SL (7)	10-630	μg L^{-1}	
Heptyl acetate	Beurre Bosc	H	4.22±2.97	μg g^{-1}	(Chen et al., 2018)
	Butirra Rosata Morettini	H	13.61±18.48	μg g^{-1}	
Octyl acetate	Blanquilla	H + SL (6)	175.28 ± 33.55	μg kg^{-1}	(Lindo-García et al., 2019)
Hexyl propanoate	Blanquilla	H	11.3	μg kg^{-1}	(López et al., 2015)
Methyl butanoate					
Ethyl butanoate	Conference	CSP NA (154) + SL (5)	2.3	μg kg^{-1}	(Rizzolo et al., 2005)
Ethyl-2 methylbutanoate	Nanguoli	H + SL (15)	7.48-37.2	μg kg^{-1}	(Li et al., 2013)
Butyl butanoate	Conference	CSP NA (154) + SL (5)	3.1	μg kg^{-1}	(Rizzolo et al., 2005)
Hexyl butanoate	Nanguo	CSP NA (90)	0.28	μg kg^{-1}	(Yao et al., 2018)
Ethyl hexanoate	Nanguo	CSP NA (90)	131	μg kg^{-1}	(Yao et al., 2018)
Butyl hexanoate					
Hexyl hexanoate	Blanquilla	H	16.9	μg kg^{-1}	(López et al., 2015)

Table 1.7 Continuation.

VOC	Cultivar	Stage	Value ranges	Units	Reference
Ethyl heptanoate	Nanguoli	H + SL (15)	0.93	μg kg^{-1}	(Li et al., 2013)
Methyl-3-hydroxyoctanoate	Barlett	H + SL (10)	0.06	μg kg^{-1}	(Makkumrai et al., 2014)
Ethyl octanoate	Barlett	H	0.52	μg kg^{-1}	(Li, 2012)
	Nanguoli	H	0.68	μg kg^{-1}	
Methyl decanoate	Barlett	H + SL (10)	0.01	μg kg^{-1}	(Makkumrai et al., 2014)
Ethyl decanoate	Barlett	H + SL (10)	0.69	μg kg^{-1}	(Makkumrai et al., 2014)
Methyl-(E,Z)-2,4-decadinoate	Barlett	H + SL (10)	5.30	μg kg^{-1}	(Makkumrai et al., 2014)
Methyl-(2E,4Z)-2,4-decadinoate	Barlett	CSP ULO (120) + SL (10)	0.68-1.35	μg L^{-1}	(Zlatić et al., 2016)
Ethyl-(E,Z)-2,4-decadinoate	Barlett	H + SL (10)	6.28	μg kg^{-1}	(Makkumrai et al., 2014)
Ethyl-(2E,4Z)-2,4-decadinoate	Barlett	CSP ULO (120) + SL (10)	0.20-0.31	μg L^{-1}	(Zlatić et al., 2016)
Ethyl benzoate	Conference	CSP (80) + SL (7)			(Goliáš et al., 2015)
Hydrocarbons					
Pentadecane	Nanguoli	H	0.27±0.24	μg kg^{-1}	(Qin et al., 2012)
Heptadecane	Nanguoli	H	0.10±0.05	μg kg^{-1}	(Qin et al., 2012)
Terpenes					
Limonene	Barlett	CSP ULO (120) + SL (10)	0.12-0.18	μg L^{-1}	(Zlatić et al., 2016)
α-Farnesene	Barlett	CSP ULO (120) + SL (10)	2.74-7.63	μg L^{-1}	(Zlatić et al., 2016)
(E,E)-α-Farnesene	Ruanerli	H	0.47	μg kg^{-1}	(Li et al., 2016)
(Z,E)-α-Farnesene	Nanguoli	H + SL (15)	2.85-9.94	μg kg^{-1}	(Li et al., 2013)

Table 1.7 Continuation.

VOC	Cultivar	Stage	Value ranges	Units	Reference
Acids					
Acetic acid	Ruanerli	H	5.81	µg kg^{-1}	(Li et al., 2016)
	Anjou	CSP NA (240) + SL (7)	17	Nmol kg^{-1} h^{-1}	(Argenta et al., 2003)
Sulphur compounds					
Ethyl-3-methylthio propanoate	Nanguoli	H + SL (15)	2.14	µg kg^{-1}	(Li et al., 2013)

*Authors presented values in percentage.

1.5.8 Nutritional composition

Pears are an excellent source of dietary fiber, a good source of vitamin C and micronutrients (Table 1.8) (Reiland and Slavin, 2015; Sinha, 2012).

Table 1.8 Nutritional values for 100 g of produce of European pears.

Water	g	83.96
Energy	kcal	57
Protein	g	0.36
Total fat	g	0.14
Carbohydrate	g	15.23
Dietary fiber	g	3.1
Total sugars	g	9.75
Sucrose	g	0.71
Glucose	g	2.6
Fructose	g	6.42
Calcium	mg	9
Iron	mg	0.18
Magnesium	mg	7
Phosphorus	mg	12
Potassium	mg	116
Sodium	mg	1
Vitamin A	µg	1
Vitamin C	mg	4.3

* **Source:** (USDA, 2019)

Phenolic compounds from 'Conference' pears can prevent human diseases due to their anti-inflammatory and antimicrobial activity (Liaudanskas et al., 2017). Pears are good for people on low-fat diets, as they contain negligible amounts of both saturated and unsaturated fats. Pears are rich in fiber, especially insoluble type, so they are considered a food with a mild laxative effect (Reiland and Slavin, 2015). They are also rich in minerals, especially potassium and tannins.

1.5.9 Ethylene production

Ethylene is a plant hormone regulating fruit ripening in climacteric fruit by coordinating the activation of many genes (Oetiker and Yang, 1995). The synthesis of volatiles as well as most biochemical changes occurring during fruit ripening and postharvest handling with the consequent softening and color changes are driven by ethylene. Ripening comprises several physical, chemical and biochemical changes, the easiest observable ones are the skin color displacement from green to yellow, the fruit softening or firmness loss

(Fig. 1.15), the increase of sugars, decrease of acid content and the production of aroma volatiles (Martínez-Romero et al., 2007). The ripening process is divided in a pre-climacteric stage and a climacteric stage. The pre-climacteric stage is characterized by low levels of ethylene production (Fig. 1.16A), while the climacteric stage is characterized by an increase in the ethylene production, reaching a maximum and a rapid decrease afterwards (Fig. 1.16B), and respiration increased (Oetiker and Yang, 1995). The burst displayed in the ethylene production is considered to control the aroma biosynthesis and other biochemical and physicochemical process (Moya-León et al., 2006; Rapparini and Predieri, 2003).

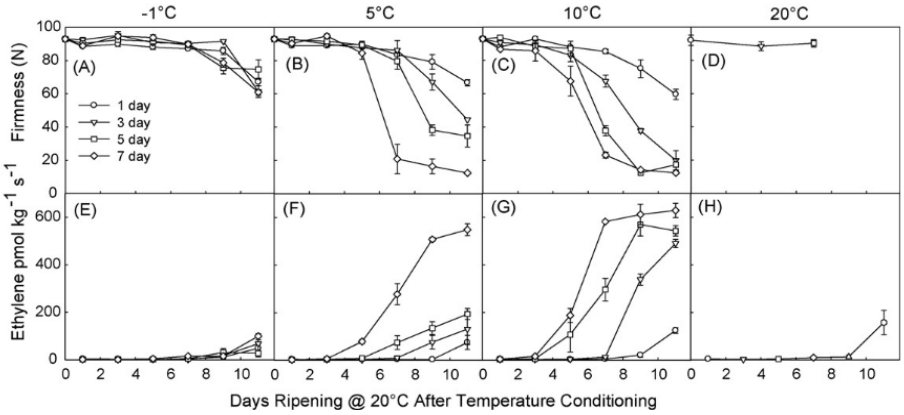

Figure 1.15 Firmness and ethylene production during Shelf Life (SL) in 'Bartlett' pears stored 1, 3, 5, and 7 d at −1 ºC (A and E), 5 ºC (B and F), 10 ºC (C and G) and 20 ºC (D and H) and subsequently ripened at 20 ºC (Villalobos-Acuña and Mitcham, 2008).

Ethylene stimulates the ripening process but an acceleration of it can lead to off-flavors and low texture. Most European pears must be subjected to cold temperatures or treated with ethylene to induce their capacity for ripening. This chilling requirement to initiate ripening largely depend on the cultivar but also on the fruit physiological maturity at the time of harvest. According to Chen and Mellenthin (1982), 'Anjou' pears harvested at 60-63 N required 60 d in cold storage while 'Bosc' pears harvested at 58-62 N required 10 d while when harvested at 53-58 N required 7 d.'Comice' pear required approximately 25–31d of cold storage when harvested at 53–58 N (Sugar et al., 2006). Generally, 'Conference' pears will require a chilling period after harvest, to initiate the autocatalytic ethylene production and thereby ripen (Villalobos-Acuña and Mitcham, 2008).

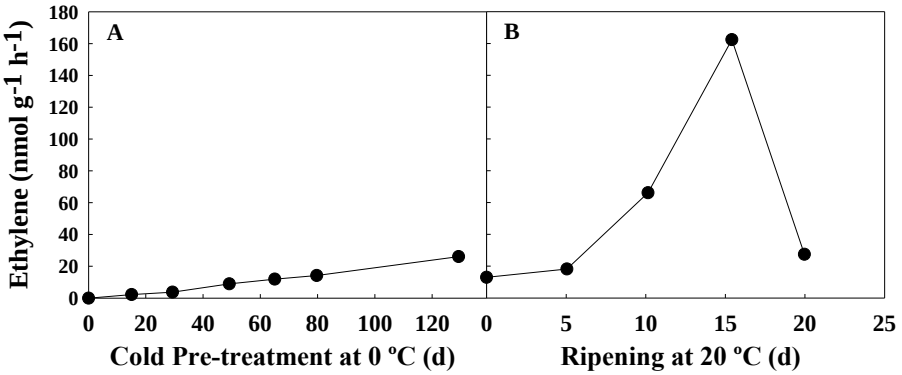

Figure 1.16 Ethylene production in 'Passe-Crassane' pear fruit during cold storage and ripening. Ethylene production of (A) pears during long-term cold storage; (B) pears during ripening at 20 °C after 80 d at 0 ºC (El-Sharkawy et al., 2003).

1.5.10 Respiration and transpiration

Pear respiration consists in the oxidative breakdown of labile compounds such as sugars, acids and starch, into simple end products, mainly water and CO_2 (see eq. 2). Respiration is affected by several factors such as temperature, atmosphere relative humidity and gases concentration, mainly O_2 and CO_2 (Xanthopoulos et al., 2017). Keeping the fruit at low temperature slows all biochemical processes and hence respiration, however, in order to avoid irreversible damages, the temperature should never reach the flesh freezing point. Low oxygen levels, together with low temperature, slow down respiration. However, there is a low oxygen level (LOL) tolerated by the fruit that should not be trespassed. Below it the anaerobic respiration pathway (eq. 3) is activated producing no tasty end products and ethanol tends to accumulate (Beaudry, 1993). The LOL varies along the storage period and is related to the physiological state of the fruit, hence the interest in determining it at every time.

Low oxygen and elevated CO_2 concentrations reduce the respiratory rate and inhibit the ethylene biosynthetic pathway (Villalobos-Acuña and Mitcham, 2008). The biochemical mechanism of that slowdown is complex, then low oxygen levels in pears (P_{O2}=0.4kPa) have been related to a reduction in cytosolic pH, adenosine triphosphate and adenosine diphosphate ratio (ATP/ADP) and Krebs cycle activity (Chervin et al., 1999; Nanos and Kader, 1993; Nanos et al., 1994). On the other hand, Kerbel et al. (1988) reported that CO_2 inhibits several respiratory enzymes of the Krebs cycle, particularly succinic dehydrogenase. According to Wild et al. (2003), CO_2 may inhibit the conversion from 1-aminocyclepropane-1-carboxylic acid (ACC) to ethylene by R oxidase.

Transpiration is a physical process that consists in the loss of water by evaporation through the skin of the fruit. Water diffuses through the pulp of the fruit towards the skin, were its cuticle and wax layer act as a barrier. On the skin surface water evaporates at a rate which is determined by the atmosphere relative humidity and air speed over the fruit surface (Tolesa and Workneh, 2018). It is well known that high levels of relative humidity do better preserve the water content inside the fruit but the adequate levels will depend on the variety and on the cultivar (Zagory and Kader, 1989). Fruit moisture loss causes an economic loss, because of the lower saleable weight of crop but also because of the fruit worse quality due to the skin shriveling (Nguyen et al., 2007).

To sum up, during the growing, maturation and ripening pears undergo changes in skin color, chlorophyll content, firmness, sugars, acids, VOCs, ethylene production and respiration (Fig. 1.17).

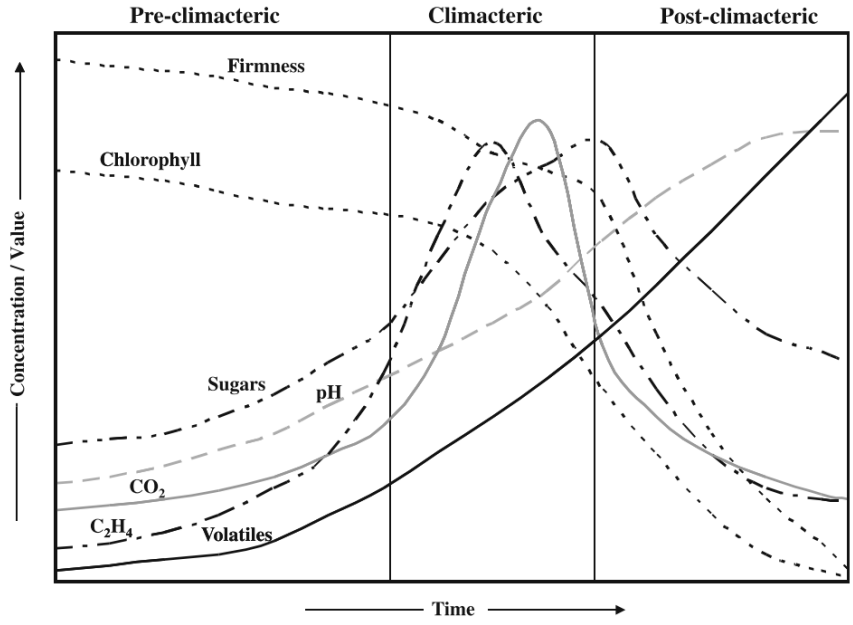

Figure 1.17 Chemical and biochemical changes during fruit ripening (Paul and Pandey, 2014).

1.6 New challenges in the cold storage of fruit

Pears are submitted to long-term cold storage periods to ensure their staggered marketing. During the cold storage period, under normal air or under controlled atmosphere (CA), a major problem is the appearance of physiological disorders. Diphenylamine (DPA) and ethoxyquin have been used as chemical treatments to control superficial scald in both, apples and pears, in combination with CA technologies (Chen et al., 1990).

In 2009 the European Commission (EC) agreed to ban the active substance DPA (Regulation (EC) No 1107/2009) and three years later, in 2012, entailed the prohibition of the use of ethoxyquin in fruit (Annex I of Directive 91/414/EEC which lists approved pesticides). In addition, on January 2015, the maximum residue limit (MRL) for DPA and ethoxyquin in fruit decreased to 0.05 mg/kg. The trend towards the reduction of synthetic chemical residues in fresh products has led, in recent years, to the search for alternatives to avoid physiological disorders. One strategy of the fight was the use of 1-MCP, a molecule that interferes with the metabolism of ethylene (Chiriboga et al., 2011). However, in some varieties of pear the use of this product may have some side effect in terms of delaying excessively the ripening process of fruit during the marketing period, referred as 'Evergreen' problem. According to Chiriboga et al. (2011), some European pears treated with 1-MCP often remain 'evergreen', meaning that their ripening process is blocked and remain firm and green even during the shelf life period after removal from cold storage, hence never reaching the optimal quality for consumption.

In recent decades, consumers are increasingly interested on free-chemical and local grown fruit, which is perceived as safer for consumption, and demand pears all year around with an optimal visual appearance, firmness and organoleptic characteristics. The potential of dynamic controlled atmospheres as a non-chemical treatment that maintains the postharvest quality of pears during long-term cold storage has attracted the attention of researchers during the last years. The research presented in this thesis aimed at improving the technology to ensure that pears reach the consumer with optimal shape, color, firmness, aroma and flavor and without physiological disorders.

The studies carried out covered the three phases of the commercial life of fruit: on-tree growth, cold storage and shelf life.

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Chapter 2: OBJECTIVES

The objectives of the present PhD thesis were stablished in order to respond to the technical needs of Industrial Leridana del Frío SL (ILERFRED), an enterprise located in Lleida who sponsored this research for three years. ILERFRED main activity is the implementation of fruit conservation technologies such as cold facilities and controlled atmosphere systems. Their after-sales technical assistance service has good knowledge on how to achieve the desired storage conditions regarding temperature, air relative humidity and gases concentrations, but they lack information on the physiological state of the fruit and on its evolution under different conservation conditions. Fruit storage facilities are equipped with computerized control and management systems of the different parameters and could easily incorporate real-time information on the state of the stored fruit. Consumers demand high quality fruit all year round and fruit industries want to meet the customer's needs.

The main objective of the present thesis is to investigate which actions can be undertaken during the growing season, cold storage and shelf life of fruit in order to extend the cold storage period and improve the quality of the fruit reaching the final consumer.

One of the main challenges during the on-tree growth is to determine the optimal harvest date (OHD) at which the state of the fruit is such that the best quality will be reached after the cold storage period. OHD is traditionally determined counting days after full bloom (DAFB) or based on firmness and color measurements.

During the cold storage period the main challenges are to extend its duration avoiding fruit physiological disorders and fruit dehydration.

The rapid metabolic processes set off during the shelf life shortens the commercial life of fruit. During that brief period the main challenge was to know the point at which the maximum consumer acceptance was reached and how undesired fungus pathologies could be avoided.

Obj A. On-tree quality parameters evolution and determination of the optimal harvest date in apples and pears.

Obj A1 Digitization of fruit surface and volume by means of a 3D scanner, as a first step to determine the evolution of biometric parameters.

Obj A2 Description of the on-tree growth phenomenon by means of mathematical equations with the aim to predict final fruit diameter and mass in advance of the optimal harvest date.

Obj A3 To determine correlations between destructive and non-destructive methods to measure fruit quality parameters.

Obj A4 To analyze the relationship between the main morphological and quality parameters during on-tree growth with the fruit ethylene production capacity and respiration rate.

Obj B. Viability of possible new sensors to determine the state of apples and pears during the cold storage period under dynamic controlled atmosphere.

Obj B1 To compare the viability of different commercial technologies to determine the lowest oxygen levels tolerated by pears, (I) chlorophyll fluorescence, (II) respiratory quotient and (III) ethanol accumulation in fruit pulp.

Obj B2 To evaluate the effect of the oxygen level in the cold room atmosphere on fruit quality parameters during the storage period.

Obj B3 To capture volatile organic compounds from the cold room atmosphere and look for markers of the fruit state during storage.

Obj B4 To check the viability of describing the influence of low oxygen and high CO_2 concentrations on the fruit respiration pattern upon removal from storage by means of a mathematical model.

Obj B5 Design and test of a new experimental device to continuously monitor the settlement of the whole mass of fruit inside a commercial container.

Obj B6 To find out possible relationships between fruit diameter shrinkage, fruit mass settlement and fruit mass loss in fruit stored in commercial cold rooms.

Obj C. To better understand the ripening behavior of ‘Conference’ pear during the shelf life period.

Obj C1 To monitor quality parameters and volatile organic compounds emission during the shelf life period.

Obj C2 To analyze the relationship between quality parameters and consumer's acceptance throughout the shelf life period of 'Conference' pear.

Obj C3 To analyze the spatial distribution of the main biochemical and flavor components in the 'Conference' pear flesh

Obj C4 To evaluate the protective effect of some volatile compounds naturally present in pears against two major pathogens (Penicillium expansum and Rhizopus stolonifer).

Chapter 3: WORKING PLAN

The thesis is structured in three parts (A, B and C) corresponding to the main phases of pear quality changes from fruit development on-tree to the final consumer: A) Fruit growth on tree, B) Cold storage period and C) Shelf life after the cold storage.

Part A is focused on the on-tree fruit growth. In this study (Chapter A1) the evolution of different fruit parameters were recorded during its growth on-tree, from 5 weeks after full bloom up to the optimal harvest date. The study was conducted in the 2016 season in orchards near Lleida and included the following cultivars: 'Golden' and 'Gala' apples and 'Conference' pears. The fruit quality parameters recorded at pre-established sampling dates can be classified into:

a) Morphological: fruit diameter, mass, surface and volume.
b) Physical: firmness, I_{AD} index, acoustic firmness and skin color.
c) Chemical: total soluble solids and total titratable acidity.
d) Physiological: ethylene production capacity and respiration rate.

Classical methods to evaluate the quality parameters were used. Interesting is the application of a relatively new technique, the 3D scanner, to non-destructively evaluate the surface area of the fruit and its volume evolution over fruit growth was investigated. A Gompertz-type function was used to fit time evolution of morphological parameters and a power function was used to establish a relationship between fruit mass and diameter. Gompertz fits were also applied to data from 'ERO' and 'Granny Smith' apples harvested during 2016 season and 'Conference', 'Blanquilla' and 'Williams' pears harvested during 2017 season.

The overall goal of the on-tree growth monitoring was to determine the optimal harvest date, the point at which the best fruit quality for storage is reached, as well as to predict the fruit quality at that time. The reliability of such a prediction may help producers to better introduce their production in the market. The different methodological steps followed during this study are depicted in Fig. 3.1.

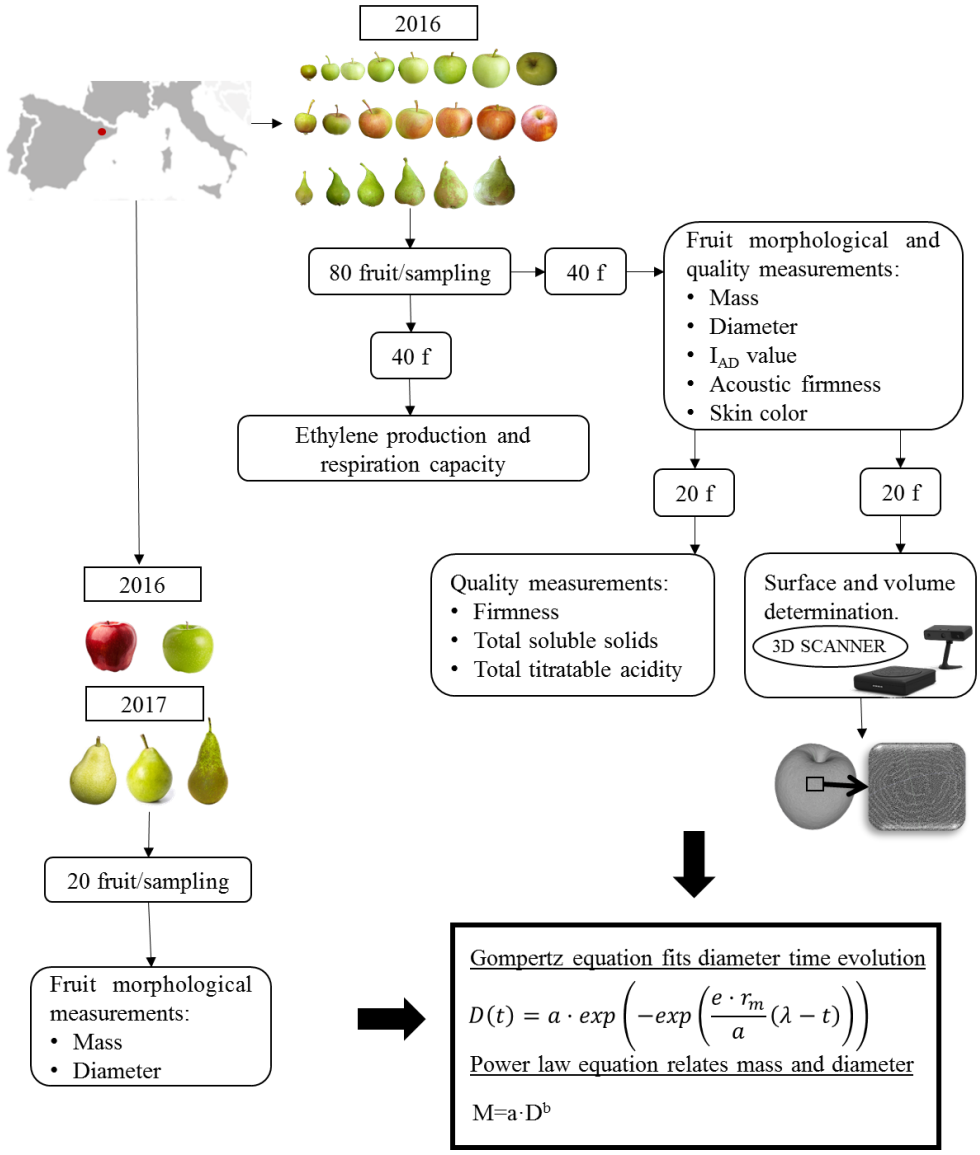

Figure 3.1 Flow diagram of the methodological steps followed after each sampling date during the on-tree fruit growth monitoring. Briefly, eighty fruit per cultivar were selected, forty of them underwent non-destructive measurements of fruit mass, fruit diameter, acoustic firmness, skin color and index of absorbance difference (I_{AD}). Thereafter, twenty of them were used for destructive quality measurements: firmness, TSS and TTA, the other 20 fruit were 3-D scanned to obtain its surface area and volume. The other group of 40 were used to measure ethylene production and respiration capacity.

Part B focuses on the cold storage period of the fruit. At ambient conditions fruit is a perishable product and it is necessary to store it in cold rooms in order to gradually deliver it to the market all the year around. In the 1950s it was found that keeping fruit in cold rooms under low oxygen levels (≈1 kPa O_2) enlarged its storage period without affecting its final quality, this technique was named as controlled atmosphere (CA) storage. Storage of fruit under CA conditions is not exempt of handicaps, then some physiological disorders such as core browning and superficial scald may appear. Sometimes these disorders were avoided applying chemical treatments with diphenylamine (DPA) and ethoxyquin. Recently it was discovered that such disorders can also be avoided keeping low oxygen levels in the cold room's atmosphere but adapting them to the physiological state of the fruit, what is known as Dynamic Controlled Atmosphere (DCA). In the DCA technique the oxygen level is kept as close as possible to the Anaerobic Compensation Point (ACP) but is slightly increased when a physiological response of the fruit is detected by a specific sensor. Nowadays, three different DCA techniques are available on the market, each based on detecting a different physiological response of the fruit: DCA-CF is based on detecting changes in the chlorophyll fluorescence of the fruit skin, DCA-RQ is based on detecting changes in the respiration quotient (RQ) and DCA-EtOH relays on changes both in fruit pulp and in the atmosphere. The DCA appears as an attractive technique now that the European directives, and consumer demands, establish greater restrictions on the use of chemical treatments.

Three different studies have been undertaken in part B of the thesis aiming at defining the best conditions for long-term storage of pears. In the first study (Chapter B1) the behavior of two different DCA sensors, namely the chlorophyll fluorescence sensor (DCA-CF) and the respiratory quotient sensor (DCA-RQ), installed in the same semi-commercial cold room was compared. Additionally, periodic fruit sampling every two months was planned in order to evaluate the evolution of ethanol content in fruit pulp (DCA-EtOH). Samples of the chamber atmosphere were extracted monthly with the aim of analyzing the presence of VOCs in order to determine markers describing fruit physiological state during the cold storage period. Fig. 3.2 shows an outline of the experimental plan. Briefly, 5.5 t of 'Conference' pears from different producers were kept during 8 months in a cold room at 0ºC. A chlorophyll fluorescence sensor (FO, Besseling) was installed in the cold room and monitored the chlorophyll profile of about 20 fruit. The cold room oxygen level was self-controlled by the ACR system. A check of the fruit quality after 8 months of storage plus 5 days in SL was done measuring the standard quality parameters.

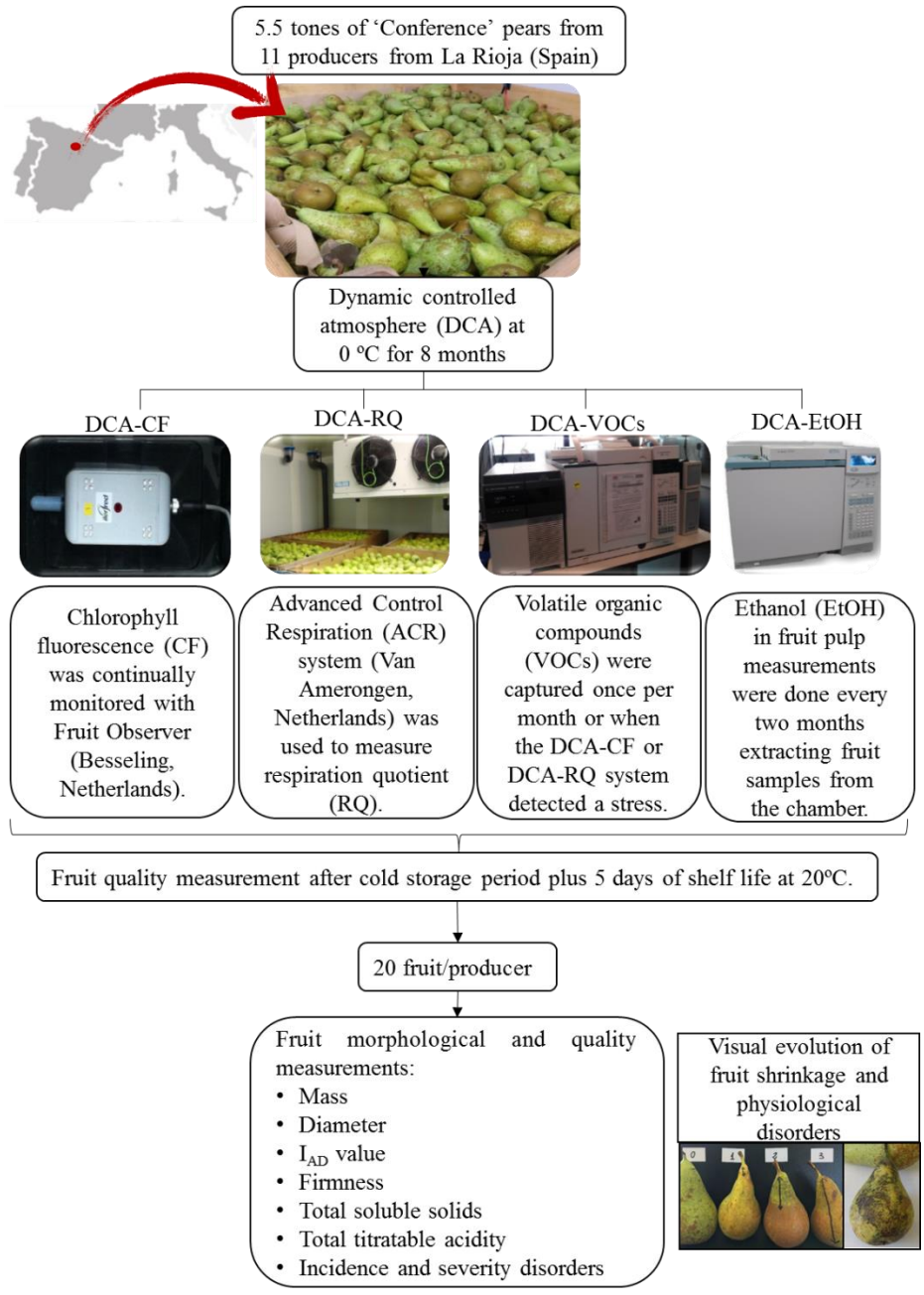

Figure 3.2 Flow diagram of the experimental procedure to compare different sensors to monitor the oxygen and CO_2 levels in a DCA cold room. The compared technologies were: Fruit Observer (Besseling, Netherlands) and the Advanced Control Respiration (ACR) system (Van Amerongen, Netherlands). Samples of the cold room atmosphere were captured regularly in order to analyze VOCs. Fruit samples were also taken and its pulp ethanol content was determined. Fruit morphology and quality was analyzed in 20 fruit per producer after 8 months cold storage plus 5 d of SL:

The second study (Chapter B2) aimed at clarifying the effects of three different storage atmosphere conditions, namely: Initial Low Oxygen Stress (ILOS), Dynamic Low Oxygen Stress ($DLOS_1$) and $DLOS_2$ on the final quality of 'Conference' pear. In the ILOS strategy only an initial oxygen lowering was applied and then the cold room atmosphere was kept at a constant concentration of oxygen and CO_2 during the whole storage period. The DLOS strategy applied several oxygen stresses by lowering the oxygen levels for a few days. In the $DLOS_2$ strategy the stresses were longer than in the $DLOS_1$ strategy.

The experiment was conducted placing three micro-chambers (350 L each) in a cold room. The atmosphere of each chamber could be independently regulated. Fruit sampling and gas extraction to analyze VOCs in the atmosphere of each chamber was done regularly. The standard quality parameters of the fruit were measured. See the overall experimental schedule in Fig. 3.3.

In the third study (Chapter B3) the known phenomenon of fruit weight loss during the cold storage period was analyzed. Fruit has a high content of water, and part of it can be lost by evaporation on the skin surface. The loss of water from the fruit represents an important economic loss, both, by the direct loss of weight of the stored fruit and by the low quality of the fruit, since water loss produces a shriveling and shrinkage of the fruit and its visual appearance worsens. The main cause of fruit weight loss is due to its moisture loss, as during the storage period the metabolism of the fruit is very low and the weight loss due to respiration, CO_2 emission, is much lower. Moisture loss depends on the water activity of the fruit, the skin permeability, the temperature and relative humidity of the environment air and on the air velocity around the fruit surface. In a cold room the air relative humidity is the main factor affecting water evaporation from the fruit. To keep the air RH at a constant level is quite difficult because of the natural oscillations caused by condensation when the air is forced through the evaporator of the cold room. When the air becomes too dry sprinkling it with water is a common commercial practice. Most industrial cold rooms lack of a direct monitoring system of the weight loss of the stored fruit.

In the experiments run along three seasons, direct continuous measurements of fruit weight and fruit shrinking, together with temperature and RH data, were recorded. Fruit weight was measured by placing a commercial fruit plastic container on a scale. Fruit shrinking was measured in two different ways, on individual fruit, using a dendrometer, and as a whole settlement of the fruit mass inside the container, by means of an ultrasound sensor. Measurements were carried out in commercial cold rooms under different

conditions and containing apples and pears. A schedule of the run experiments is shown in Fig. 3.4.

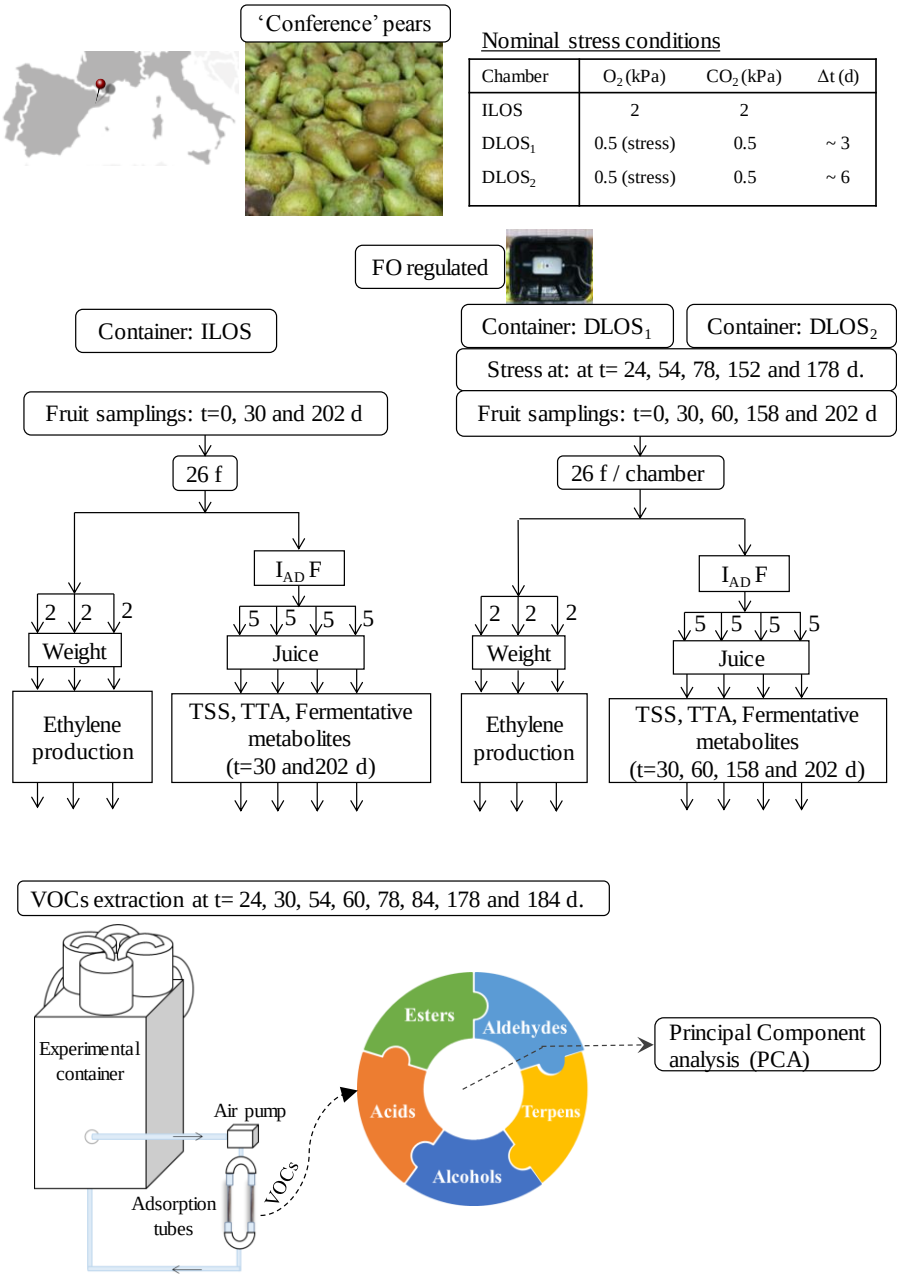

Figure 3.3 Flow diagram of the methodological steps followed to check the effects of different DCA strategies on the final fruit quality in 'Conference' pears as described in Chapter B2. Fruit was harvested at season 2016 and stored under three different storage conditions: initial low oxygen stress (ILOS) and two types of dynamic low oxygen stress (DLOS). At different time intervals quality parameters and volatile organic compounds (VOCs) in the cold room atmosphere were analyzed. VOCs data was then analyzed by the means of a PCA.

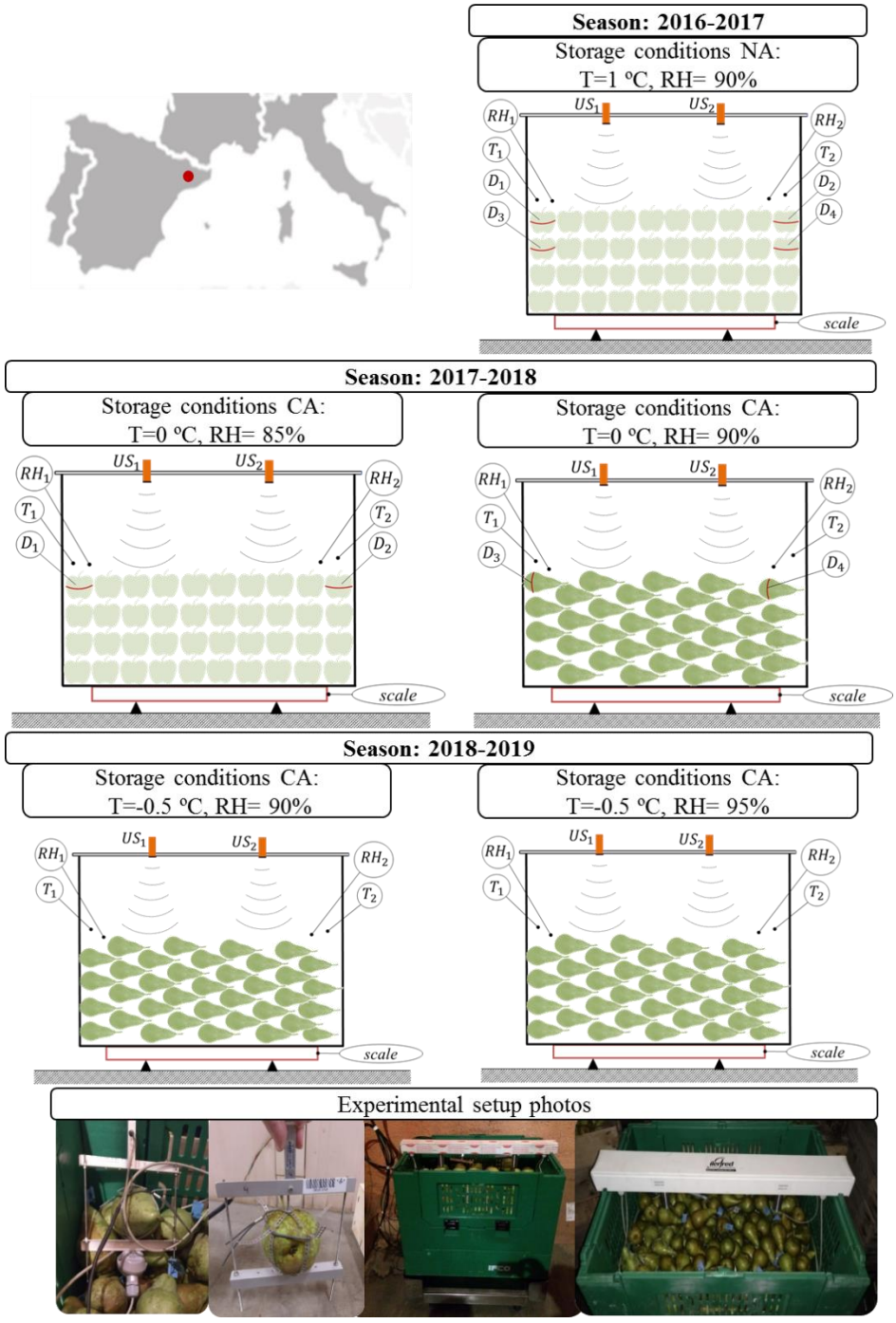

Figure 3.4 Experimental setups used during three consecutive seasons (from 2016 to 2019). Each setup installed inside a commercial cold room comprised: a commercial plastic container, a scale, two ultrasound sensors (US), two relative humidity (RH) and two temperature (T) probes. During the 2016-2017 and 2017-2018 seasons, four dendrometers (D) were used in order to evaluate individual fruit shrinking.

Part C is centered on the ripening of the fruit after cold storage, commonly referred as the shelf life period. Ripening of pears after the cold storage period is a chain of physiological processes that produce changes in the physical and biochemical parameters and determine the organoleptic properties of the fruit (such as texture, color, taste and aroma) which in turn, are responsible for the final consumer acceptance. These processes occur inside the flesh of the fruit and its rate is no uniformly distributed due to the transport phenomena of key metabolites like O_2 and CO_2 from and to the fruit surface. The spatial distribution of the maturation processes inside the fruit volume is not yet well known.

Two different studies were carried out in Part C. In the first study (Chapter C1) the SL behavior of 'Conference' pears from different orchards was studied and the relationship between fruit quality parameters, VOCs emission and consumer's acceptance were analyzed. See methodological steps in Fig. 3.5.

In the second study (Chapter C2) the spatial distribution of biochemical components (sugars, acids and antioxidants), physiological activity (respiration and ethylene production rates) and VOCs profile was measured at different locations of an equatorial cylinder of flesh extracted from the pear. The behavior of two important fungal pathogens of pear, *P. expansum* and *R. stolonifer*, on artificially inoculated flesh slices from different spatial locations, was evaluated. The fungistatic or fungicidal activity of four pour volatiles naturally present in the flesh of the pear was also evaluated by the means of an *in vitro* assay. An outline of the conducted experiments is shown in Fig. 3.6.

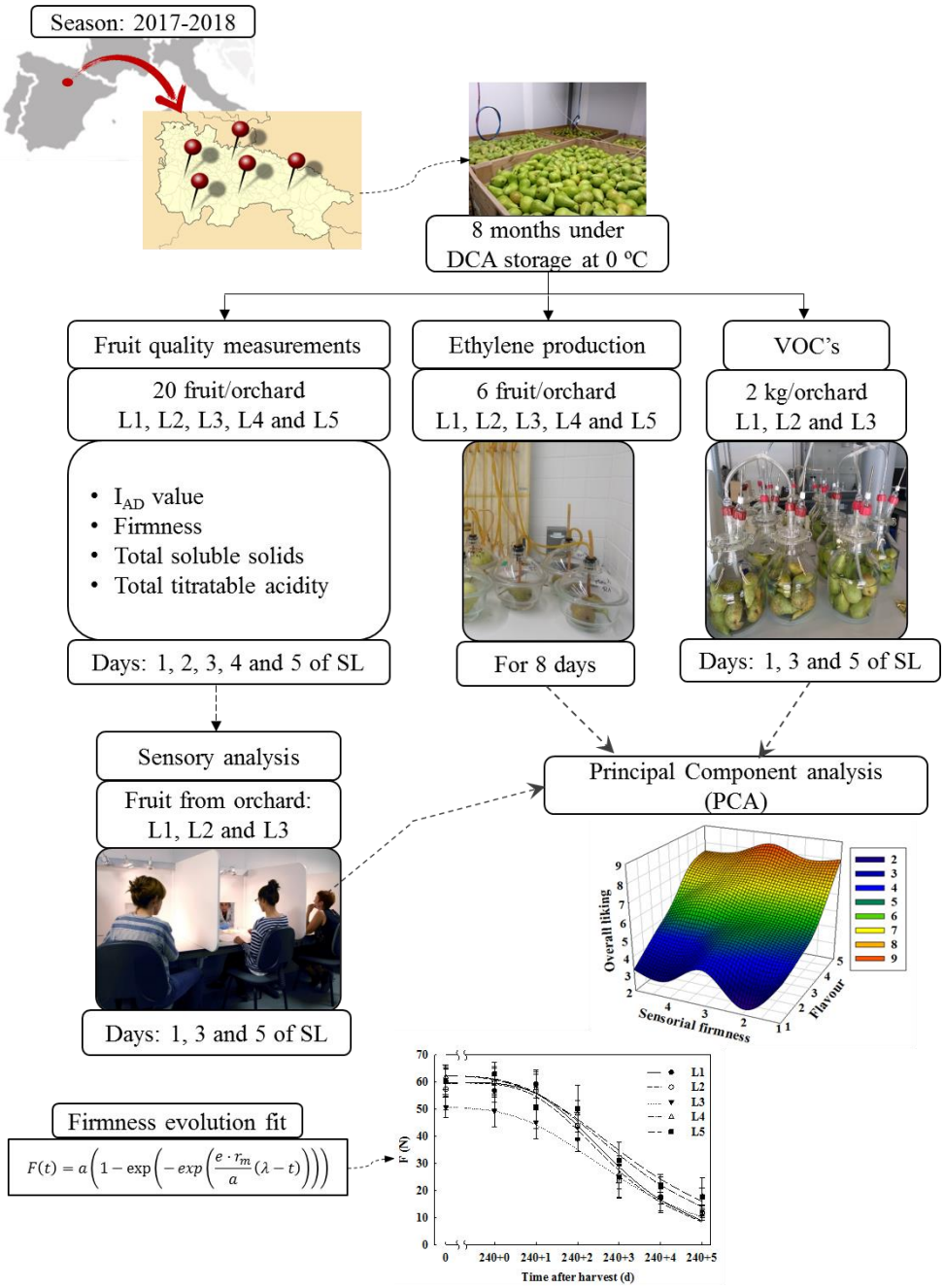

Figure 3.5: Flow diagram of the methodological steps followed used during the experimental assay in Chapter C1. Fruit from five different orchards from La Rioja (named L1, L2, L3, L4 and L5) was harvested and stored for 8 months under DCA conditions. Quality parameters, ethylene production capacity and volatile organic compounds emission were recorded during the shelf life period. Data was analyzed using a PCA. Firmness evolution during cold storage and shelf life was fitted as a function of time with a reverse Gompertz equation.

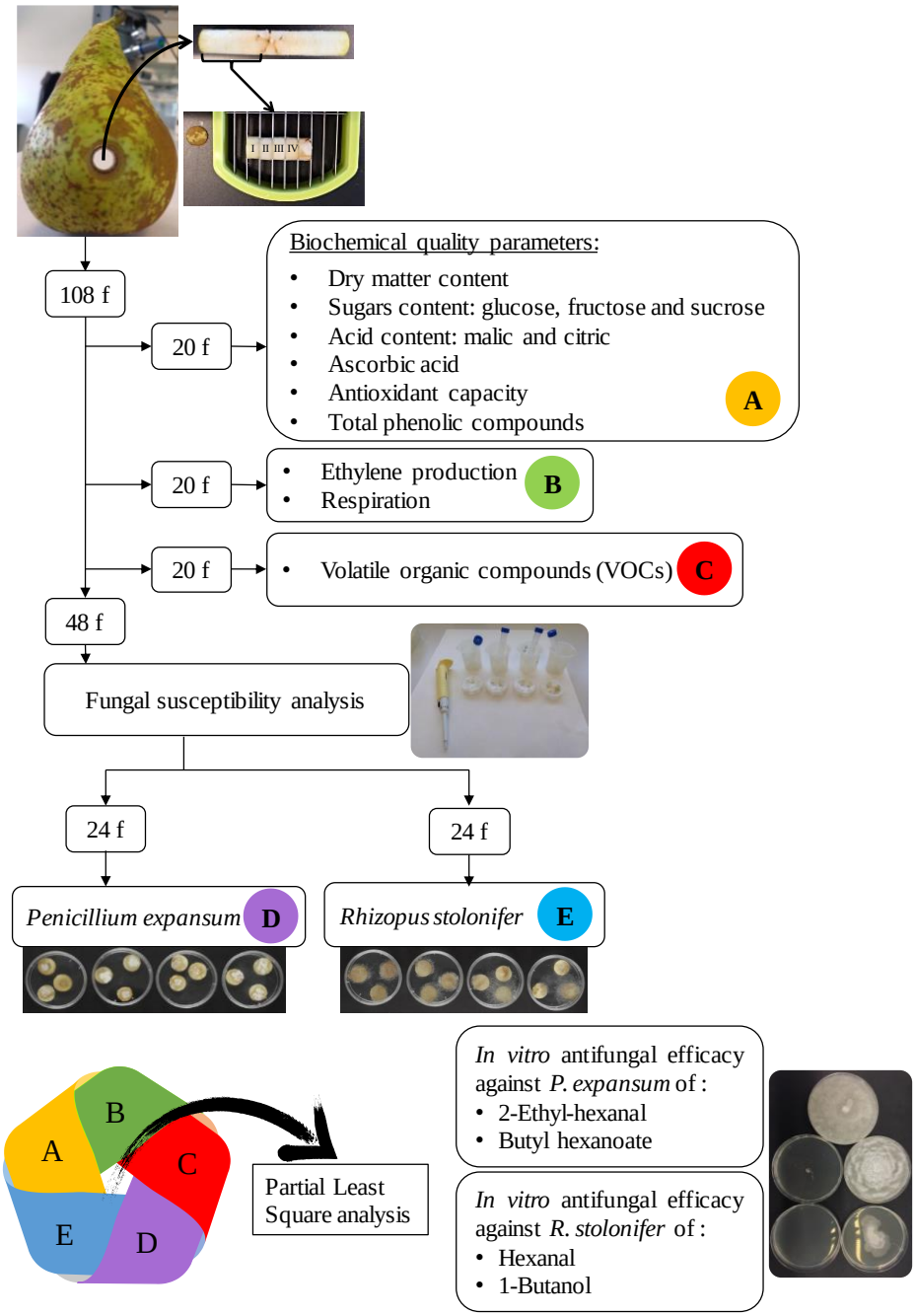

Figure 3.6 Flow diagram of the methodological steps used during the experimental assay in Chapter C2. Flesh cylinders extracted from different fruit were cut in four slices (I, II, III, IV) and biochemical quality parameters, ethylene, respiration and VOCs of each slice were analyzed. A fungal susceptibility analysis was performed at the different analyzed slices. With the data (A, B, C, D and E) of all slices a PLS analysis was done. The *in vitro* antifungal efficacy of characteristic volatiles, selected on the basis of the PLS analysis, was determined inside petri dishes.

Part A: QUALITY CHANGES DURING GROWTH DEVELOPMENT

CHAPTER A1: Fruit growth in apples and pears and its relationship with some major physiological and quality changes

Abstract

The present study investigated the relationship between quality and physiological changes during on-tree growth and ripening of 'Golden' and 'Gala' apples and 'Conference' pears.

Fruit surface and volume can be accurately determined using a 3D scanner. Pome fruit diameter and mass evolution curve from different species, varieties and seasons were successfully fitted to a 3-parameter Gompertz equation and the confidence intervals of each parameter were determined. The proposed equation was suitable to predict the final fruit size, with an error below 10%, 30 days prior to harvest. 'Conference' pears respiration had a quasi-linear relation with fruit surface/volume ratio. Ethylene production declined to the lowest levels after 60 days of full bloom (DAFB) to peak again after 110 DAFB. That later peak on the fruit ethylene production was well correlated with major quality changes such as the loss of firmness, the increase in total soluble solids (TSS) values and the decrease of total titratable acidity (TTA) and with some non-destructive measurements such as the index of absorbance difference (I_{AD}).

Results from this chapter showed that most morphological and physiological changes occurring during on-tree development of apples and pears can be correlated to specific measurements obtained by means of non-destructive techniques.

Keywords: Acoustic firmness, ethylene, Gompertz equation, respiration rate.

A1.1 Introduction

Lleida region (NE of Spain) is one of the main apple and pear producing areas in Southern Europe with around 220 000 and 129 000 tons produced, respectively, in 2015 (Iglesias et al., 2015). Among those, 'Golden' apples (60%) and 'Conference' pears (46%) are the main cultivated apple and pear varieties, respectively, due to their good adaptation to the agroclimatic conditions of the region and to their appreciated final quality. Quality and consumer acceptance of apples and pears are intimately related to the fruit external appearance (size, color and absence of defects) as well as to some intrinsic parameters such as firmness, crispness or sweetness (Bonany et al., 2014).

The effect of agronomical factors on the overall final fruit quality has been described in numerous studies (Àlvarez-Fernàndez et al., 2006; Dumas et al., 2003; Hossein Behboudian and Stephen Lawes, 1994). However few studies are available describing changes of quality parameters along fruit growth (Landahl et al., 2003; Zheng et al., 2012). Most fleshy fruit undergo appreciable morphological and physiological changes during growth and on-tree ripening leading to a final palatable produce. The most pronounced quality changes during fruit growth, which may strongly vary among species, include changes in skin color and softening as well as changes in the amounts of sugars and acids within the flesh tissue (Giovannoni, 2004). It is generally accepted that, in climacteric fruit, including apples and pears, such quality changes are driven by the autocatalytic ethylene production. Numerous studies have shown that apple and pear firmness after harvest decreases in parallel with the ethylene climacteric rise (Chiriboga et al., 2013; Gwanpua et al., 2012; Oetiker and Yang, 1995) and generally this phenomenon is accompanied by an increase in the total soluble solid content and a decrease of acidity. Other studies have investigated the relationship between ethylene production and major morphological or physiological changes occurring during fruit growth such as mass evolution in apples (Giné-Bordonaba et al., 2019; Walsh and Solomos, 1987), color changes in grapes (Chervin et al., 2004) or firmness loss in several stone fruit (Giné-Bordonaba et al., 2017; Pinto et al., 2016; Reig et al., 2017). Changes in respiration, ethylene production and other physiological traits during growth are undoubtedly related to final fruit quality at harvest (Giné-Bordonaba et al., 2019) and some studies are available describing fruit respiration pattern and ethylene production during apple or pear growth and development (Brandes and Zude-Sasse, 2019; Meigh et al., 1967). Monitoring morphological and physiological changes during fruit growth may assist on

predicting optimal harvest date while having some control of the final fruit quality.

With such a background, the aims of this study were 1) To monitor the evolution of morphological parameters during fruit growth and ripening of apples and pears 2) To identify possible mathematical functions describing such evolution. 3) To investigate potential relationships between major fruit quality attributes with fruit respiration and ethylene production pattern during growth.

A1.2 Material and methods

A1.2.1 Plant materials and experimental design

Apples and pears were harvested at different stages from different experimental orchards near Lleida during two consequently growing seasons 2016 and 2017. 'Golden' apples were harvested in Gimenells (41º39'N; 0º23'E), 'Gala' apples and 'Conference' pears were harvested in Mollerussa (41º38'N; 0º54'E), 'Early Red One' (ERO) and Granny Smith apples were harvested in Torregrosa (41º34'N; 0º50'E) in 2016 and 'Blanquilla', 'Conference' and 'Williams' pears were harvested in Alcoletge (41º38'N; 0º42'E) in 2017.

'ERO' and 'Granny' apples (2016) and 'Blanquilla', 'Conference' and 'Williams' (2017), were harvested at regular time intervals from 20 days after full bloom (DAFB) for apples and 10 DAFB for pears until the optimal harvest date (OHD), based on grower's recommendations. Weight and diameter measurements were done in 20 fruit at each sampling date.

From 'Golden', 'Gala' and 'Conference' in 2016, eighty fruit selected on the basis of free-defects, homogenous size and development stage, were collected at each sampling date, in the period extending from 40 DAFB until OHD, based on grower's. Eight trees were selected and then ten fruit per tree were harvested, five from the shadowed side and five from the sunny side.

Non-destructive measurements of fruit weight, fruit diameter, acoustic firmness, skin color, index of absorbance difference (I_{AD}) were determined in 40 fruit per cultivar at each evaluation point. In 20 of these fruit per cultivar, fruit surface and volume were determined. Destructive measurements of firmness, total soluble solids (TSS) and total titratable acidity (TTA) were carried out on the same fruit used for non-destructive evaluations per cultivar and sampling time (n=40). Forty additional fruit were used to measure ethylene production and respiration rate.

A1.2.2 Fruit morphological and quality measurements

Each fruit was individually weighted (AH-600, Blauscal, Spain) and the maximum fruit equatorial diameter recorded using an electronic digital caliper (Powerfix, 0-150 mm, Deutschland). For 'Golden', 'Gala' and 'Conference' (2016), acoustic firmness was evaluated at two opposite sides of each fruit using a portable sensor (Afs, AWETA, Nootdorp, The Netherlands) as described by Harker et al. (2008). External skin color was measured using a portable spectrophotometer CM-2600d (Konica Minolta Sensing, Japan) and the *L**, *a** and *b** values were recorded (Reay, 1998). The fruit apparent maturity of each individual fruit was measured based on the index of absorbance difference with a DA-Meter (TR Turoni, Forli, Italy), as described elsewhere (Giné-Bordonaba et al., 2016; Ziosi et al., 2008).

Finally, each fruit was scanned with an accuracy ≤ 0.1 mm by means of a 3D scanner (EinScan-S, Shining 3D, China) and its geometric shape was captured including color and texture information. The surface of a 77 mm diameter apple could be represented by a mesh of proximately 1 000 000 triangles (Fig. A1.1). From that information the surface and volume of the fruit was determined.

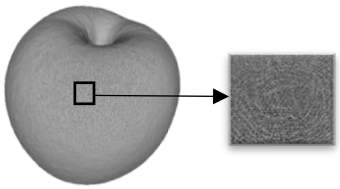

Figure A1.1 Scanning of a 'Golden' apple harvested in September 2016, with an area of 10940 mm^2, a volume of 94220 mm^3 and constituted by 975658 triangles.

Firmness was determined, on two opposite sides of each fruit after removing the peel, using a hand-held penetrometer (Turoni, Italy) fitted with an 11 mm diameter plunger for apples and with an 8 mm diameter plunger for pears. The semi-spherical plunger was introduced into each fruit and the maximum force was measured.

Five halves of fruit were crushed together and filtered to obtain one juice, from the forty experimental units, eight juices per cultivar at each collecting date were prepared. From the obtained juice, total soluble solids (TSS, º Brix) were measured using a digital hand-held refractometer (Atago, Tokyo, Japan), and acid content (TTA) was measured by titration of 10 ml of juice with 0.1 N sodium hydroxide (NaOH) to pH 8.2 using phenolphthalein. TTA results were expressed as g malic acid·L^{-1}.

A1.2.3 Ethylene production and fruit respiration rates

During the growth period ethylene production and respiration rate were measured, at each sampling date, by enclosing 5 fruit in airtight jars of known volume (8 replicates per cultivar at each sampling date) placed in an acclimatized chamber at 20 ºC during two hours. After that time, gas samples were taken to measure concentrations of ethylene, O_2 and CO_2, as described in Giné-Bordonaba et al. (2017). Ethylene concentration was measured by removing 1 mL of gas sample from the headspace of the jar and injecting it into a gas chromatograph fitted with a FID detector (Agilent Technologies 6890, Wilmington, Germany) and an alumina column 80/100 (2 m × 3 mm) (Teknokroma, Barcelona, Spain) as described elsewhere Giné Bordonaba et al. (2014). Oxygen and CO_2 concentrations within the jars were measured with an O_2/CO_2 gas analyzer (CheckPoint O_2/CO_2, PBI Dansensor, Ringsted, Denmark). Gas *i* (*i* = O_2, CO_2, ethylene) production rate, r_i ($mol_i \cdot kg^{-1} \cdot h^{-1}$), was then calculated using Eq. (1),

$$r_i = \frac{\Delta P_i \cdot V_g}{R \cdot T \cdot M_f \cdot \Delta t}, \quad (1)$$

where $\Delta P_i = P_i^t - P_i^0$ (Pa) is the difference between the initial partial pressure, P_i^0 and the partial pressure P_i^t after time Δt (h), $V_g = V_0 - V_f$ (m^3) is the gas volume inside the closed jar obtained as the difference of the jar capacity V_0 and the volume occupied by the fruit V_f, R=8.314 $J \cdot K^{-1} \cdot mol^{-1}$ is the universal gas constant, T (K) is the absolute ambient temperature and M_f (kg) is the mass of fruit inside the jar. Initial partial pressure of ethylene and CO_2 were assumed to be zero, while initial O_2 partial pressure was assumed to be $0.21 \cdot 10^5$ Pa. Fruit respiration rate was presented in terms of standard concentrations (i.e. $molCO_2 \cdot cm^{-2} \cdot h^{-1}$).

After commercial harvest date the dynamics of ethylene production were determined by measuring ethylene concentrations daily (during 23, 21 and 12 days for 'Golden', 'Gala' and 'Conference', respectively) in six 1.5L flasks, continuously aerated with humidified air at a flow rate of 1.5 $L \cdot h^{-1}$, kept at room temperature (20ºC), and containing each two weighted fruit. Ethylene production rate, r_{eth} ($\mu L \cdot kg^{-1} \cdot h^{-1}$) was calculated using Eq. (2),

$$r_{eth} = \frac{C_{eth} \cdot Q}{M_f} \quad (2)$$

where C_{eth} ($\mu L \cdot L^{-1}$) is the ethylene concentration measured by gas chromatography, Q ($L \cdot h^{-1}$) is the air flow and M_f (kg) is the mass of fruit inside the jar.

A1.2.4 Statistical and data analysis

Data were subjected to analysis of variance (ANOVA) tests using JMP 8.0.1 SAS Institute Inc. Least significant difference values (LSD; $P \leq 0.05$) were calculated for mean separation using critical values of t for two-tailed tests. Correlations between experimental variables were checked using Pearson's Correlations and, if required, presented as Pearson's Correlation Coefficient (r) and p value based on a two-tailed test. Unless otherwise stated, significant differences were $P \leq 0.05$.

The 3-parameter Gompertz function (Eq. 3) was used to fit the evolution of fruit diameter (D, mm) as a function of time (t, days) in apples and pears,

$$D(t) = a \cdot exp\left(-exp\left(\frac{e \cdot r_m}{a}(\lambda - t)\right)\right), \quad (3)$$

where e is the base of natural logarithms, parameter λ is the lag time, a is the position of the higher horizontal asymptote and r_m is the maximum slope value at $t = \lambda$. The least square sum of errors criterion was used in the fitting process and the Monte Carlo method was used to establish the 95% confidence interval on the estimated parameter values (Hauser, 2009). In summary, the method consists in generating additional synthetic data sets, each one being then processed with the same optimization routine as the experimental data set and, thus, obtaining a new set of parameters. From the generation and analysis of the synthetic data sets, the distribution of parameter values is then used to generate confidence intervals. The method is based on the assumption that the synthetic data sets deviate from the data predicted by the model in the same way as the measured data does. This was accomplished by generating synthetic data (n=1000) with the same standard deviation as the original data set. All routines for parameter estimation and Monte Carlo analysis were run in MatLab (The Mathworks, USA).

A1.3 Results and discussion

A1.3.1 Morphological changes during growth

Changes in the fruit shape along development were cultivar dependent. While 'Golden' and 'Gala' apples kept an almost spherical shape throughout the growing season, 'Conference' pears underwent remarkable changes in its shape (Fig. A1.2). Fruit surface and volume are important parameters to model

diffusive and convective mass transfer of water vapor and gases in fruit, however, accurate measurements of these parameters are not an easy task. Volume can be measured by the water displacement method (Xanthopoulos et al., 2017), but in many studies is estimated from the measured diameter assuming a spherical geometry (Ambaw et al., 2013).

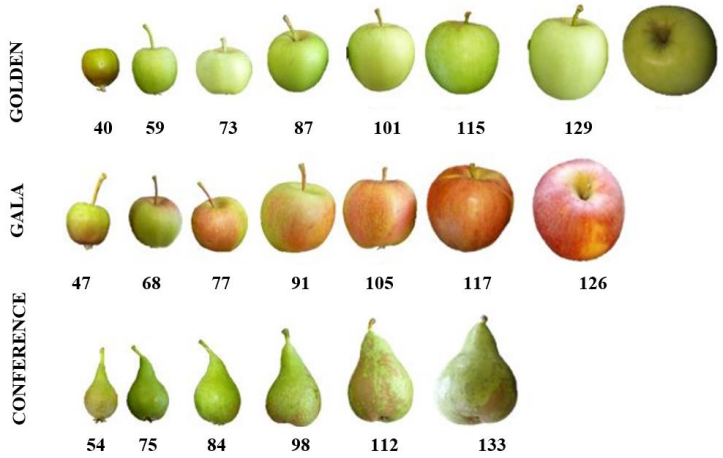

Figure A1.2 Image of the fruit at the different sampling dates during the season 2016. Date of sampling, in days after full bloom (DAFB), is given below each image.

In the present study volume and fruit surface were calculated from the triangle mesh generated by a 3D scanner. The time increase in the calculated fruit surface (Fig. A1.3A) exhibited a quasi linear pattern for all cultivars (1.55, 1.85 and 1.95 cm^2/day for 'Golden', 'Gala' and 'Conference', respectively), a hint that the fruit diameter evolution during growth could be better assimilated to a square root curve than to a straight line.

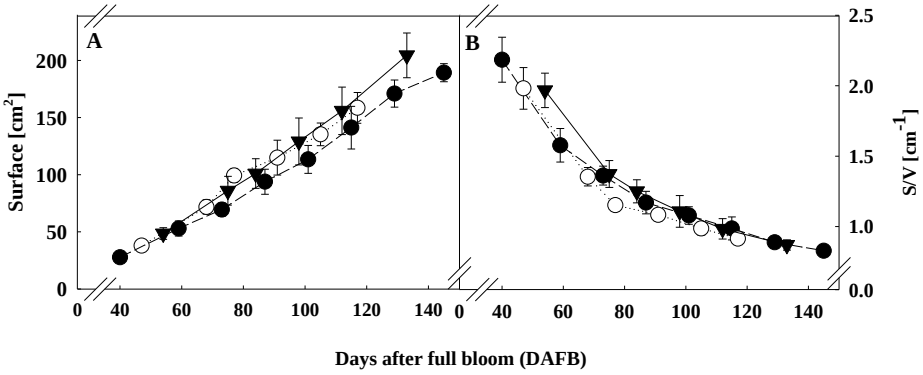

Figure A1.3 On-tree evolution (in days after full bloom (DAFB)) of fruit surface (A) and ratio fruit surface to volume (B) for 'Golden' (●) and 'Gala' (○) apples and 'Conference' (▼) pears from the 2016 season. Values represent the mean ± standard deviation (n=40).

The surface-to-volume (S/V) relationship has been investigated in bananas (Soltani et al., 2010), however, no much attention is focused on pome fruit. The obtained data (Fig.A1.3B) showed that the S/V ratio evolution presents a common decreasing trend in all three cultivars with a slight deviation of pears from 55 to 75 DAFB possibly due to its marked non-spherical shape (Fig. A1.2). The higher rate of decrease of that ratio at the early stages of development is consistent with the fact that in a spherical geometry the ratio S/V decreases inversely to diameter.

A power relationship between fruit mass (M) and diameter (D) in the form $M=a \cdot D^b$ was found to fit well the experimental data (Fig. A1.4A, A1.4B and A1.4C). That relationship was already reported by Welte (1990) in Jonagold apples (a=393.67, b=2.98) and by Stajnko et al. (2013) in 'Gala' apples (a=337.92, b=2.96) and the values of their parameters are quite close to our values (Fig. A1.4). Moreover, the same type of equation provided a good fit between fruit surface (S) and fruit mass (M) (Fig. A1.4D, A1.4E and A1.4F). It is worth to quote that the power relationship between mass and diameter obtained with data from the on-tree growth can also be used as a relationship between fruit of a sample after harvest.

The evolution of the fruit diameter throughout growth, both in apple and pears (Fig. A1.5), showed a sigmoid trend in accordance with previous studies in apples (Zheng et al., 2012), and with other fruit species, for example, in pineapples (Pauziah et al., 2013) or in logan fruit (Shi et al., 2016). Diameter and mass evolution could be successfully fitted as a function of time using the 3-parameter Gompertz function (Eq. 3) which has already been used to model 'Royal Gala' apple fruit growth (Stanley et al., 2000) and apple fruit mass (Gandar et al., 1996). In that equation parameter λ represents the time at which the maximum growth rate (r_m) is achieved and parameter *a* refers to the ceiling diameter (mass) value of the fruit. All diameter fits have own a determination coefficient higher than 0.99 (Table A1.1), thereby suggesting that this model may be employed to predict the final fruit diameter. Using diameter data up to 1 month before OHD to fit the Gompertz equation and then use it to predict the final diameter, deviations around 8% were found (Fig. A1.5). As expected, using diameter data up to 2 weeks before OHD the deviations were reduced to 2 %. The highest deviations occurred when the used data did not show the decreasing growth rate typical of final stage of maturation.

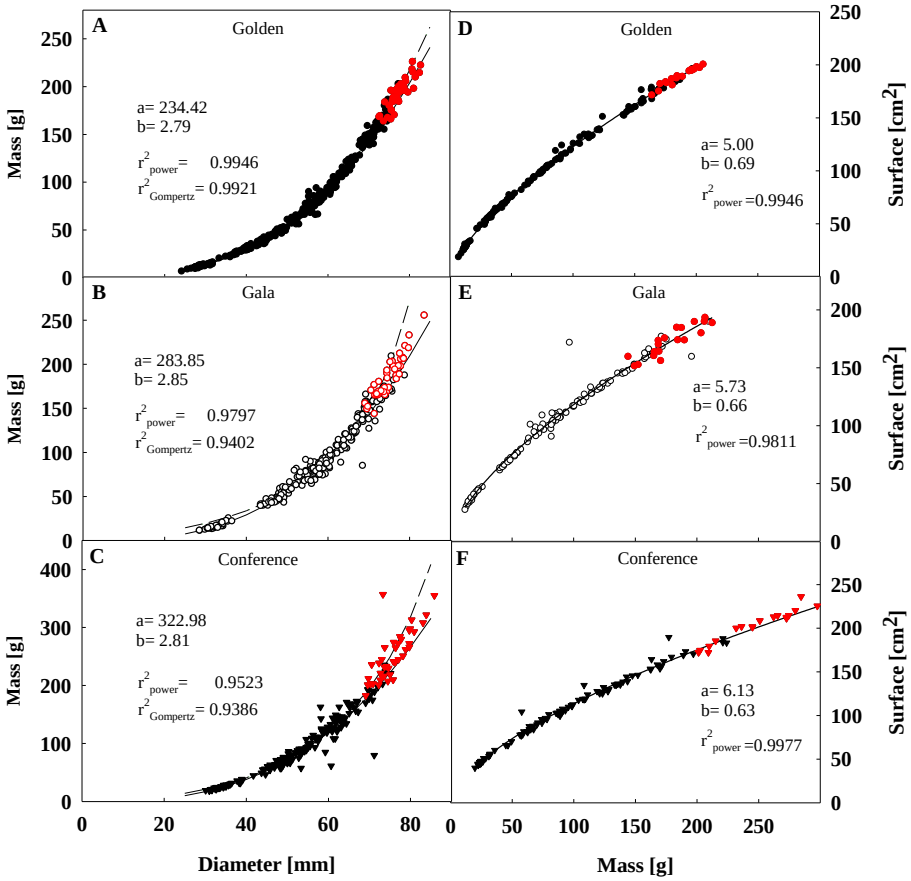

Figure A1.4 Relationship between fruit diameter and mass using: the power formula $M=a \cdot D^b$ (in continuous line), the fits with the Gompertz equation (in dashed line) and the corresponding coefficients of determination r^2_{power} and $r^2_{Gompertz}$. Values of parameters a,b of the power formula in SI units (A, B, C). Relationship between fruit mass and fruit surface with the power formula $S=a \cdot M^b$, values of parameters a, b in the units of the plot (D, E, F). Data points in red correspond to the last harvest at OHD.

Table A1.1 Estimated parameter values and corresponding confidence intervals (c.i.) at 95% confidence with the determination coefficient (r^2) when fitting fruit diameter as function of time with the Gompertz equation.

Cultivar	**λ (d)**	**λ (c.i.)**	***a*** **(mm)**	***a*** **(c.i.)**	$\mathbf{r_m}$ **(mm d^{-1})**	$\mathbf{r_m}$ **(c.i.)**	$\mathbf{r^2}$
Golden 2016	52.0	48.8-56.0	97.6	92.7-103.6	0.55	0.5-0.6	0.999
Gala 2016	46.1	42.4-54.0	88.2	80.6-103.5	0.70	0.6-0.8	0.993
ERO 2016	39.1	35.0-43.7	79.0	75.0-84.8	0.69	0.6-0.8	0.995
Granny Smith 2016	38.4	35.7-41.4	82.6	80.2-85.7	0.63	0.6-0.7	0.998
Conference 2016	56.9	50.5-76.3	99.7	87.0-129.1	0.61	0.5-0.7	0.995
Conference 2017	63.1	56.5-75.3	94.8	84.8-113.5	0.67	0.6-0.7	0.998
Blanquilla 2017	60.8	52.2-76.9	79.6	69.9-97.4	0.45	0.4-0.5	0.998
Williams 2016	58.2	53.2-65.7	82.1	76.0-90.3	0.53	0.5-0.6	0.999

Coefficients of determination greater than 0.98 for all varieties were found when fitting fruit weight as a function of time with the Gompertz equation. Although the fits are quite good, attention should be paid to the width of the confidence intervals, what gives an idea about the reproducibility of parameter values. Extreme cases are the c.i. when fitting the mass in 'Gala' 2016 and 'Conference' 2016 (Table A1.2). The width of these intervals are caused by the absence of experimental points in the stabilization zone (Fig. A1.5). That fact must be considered when comparing parameter values from different authors or data sets, although c.i. are scarcely reported in the literature.

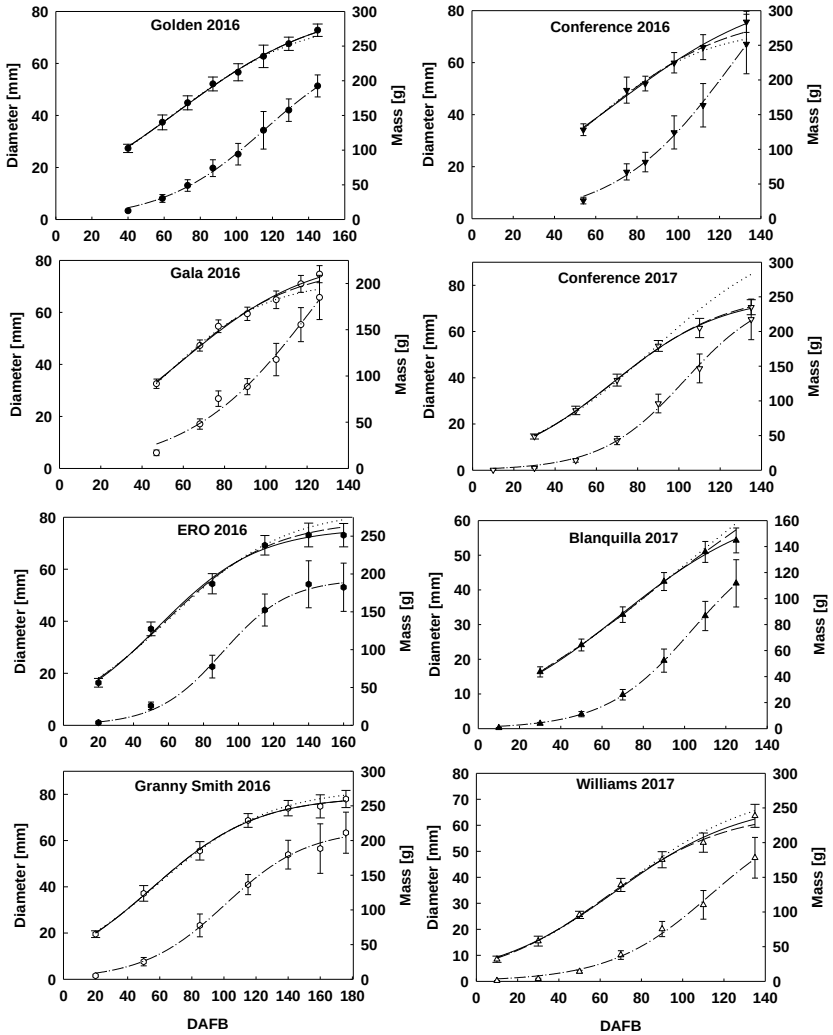

Figure A1.5 Fits of the fruit diameter (left axis) and fruit mass (right axis) as a function of time in DAFB with the Gompertz equation. Fruit diameter predictions using data up to the last but two harvest points (⋯⋯) and up to the last but one harvest point (- - -). Values represent the mean ± standard deviation (n=40).

The fits of diameter and mass evolution with the Gompertz equation for a cultivar can be seen as two functions: $D=f_D(t)$ and $M=f_M(t)$. From them a relationship between mass and diameter can be obtained: $M= f_M(f_D^{-1}(D))$. That relationship was evaluated and represented (Fig. A1.4A, A1.4B and A1.4C, dashed lines). A good agreement with the directly fitted power function was found in 'Golden' apples with a determination coefficient higher than 0.994, however, the curve fitting with the Gompertz equation had a similar determination coefficient (r^2=0.992). For 'Gala' and 'Conference' fruit the agreement was poorer (see coefficients of determination on Fig. A1.4A, A1.4B and A1.4C).

Table A1.2 Estimated parameter values and corresponding confidence intervals (c.i.) at 95% confidence with the determination coefficient (r^2) when fitting fruit weight as function of time with the Gompertz equation.

Cultivar	**λ (d)**	**λ (c.i.)**	***a* (g)**	***a* (c.i.)**	**r_m (g d^{-1})**	**r_m (c.i.)**	**r^2**
Golden 2016	128.9	116.5-147.4	428.3	358.1-545.0	2.20	2.1-2.4	0.999
Gala 2016	164.2	97.7-445.8	826.5	301.3-14050.0	3.30	2.3-23.8	0.988
ERO 2016	80.7	72.5-94.3	211.5	183.6-266.9	2.27	1.8-3.2	0.989
Granny Smith 2016	90.2	84.8-97.6	246.4	227.2-275.4	1.91	1.7-2.1	0.997
Conference 2016	174.2	132.5-275.2	1200.9	668.0-3876.7	4.81	3.7-10.0	0.998
Conference 2017	105.1	99.0-112.7	368.1	326.3-421.5	2.88	2.8-3.0	0.999
Blanquilla 2017	124.2	114.6-137.9	301.4	255.9-374.7	1.80	1.7-2.0	0.999
Williams 2017	134.4	112.7-173.0	477.0	335.6-818.6	2.55	2.3-3.3	0.998

A1.3.2 Quality changes during growth

Firmness is an important quality parameter in apples and pears which has a direct impact on the consumer's acceptance (Harker et al., 2008). During on-tree growth, a gradual loss of firmness was observed in the three studied cultivars after t=90 DAFB (Fig. A1.6A). Such loss was accelerated from 110 DAFB onwards. Acoustic firmness increased rapidly during the fruit development period and reached its maximum at 90 DAFB for apples and at 110 DAFB for pears with a slight decline thereafter until OHD (Fig. A1.6B). The obtained experimental data showed that there is a good correlation between acoustic firmness and destructive firmness once acoustic firmness has reached its maximum (Fig. A1.6A insert). The relatively high correlation coefficients, 0.96, 0.97 and 0.90 for 'Golden', 'Gala' and 'Conference', respectively, confirms that acoustic firmness, can be used as a non-destructive indicator of firmness in agreement with previous studies in pears (De Belie et al., 2000).

As a general trend, TSS increased during growth and ripening in apples (Fig. A1.6C), showing a marked increase at 110 DAFB while Conference pears did not show that pattern.

TTA presented a similar pattern in the three studied cultivars (Fig. A1.6D) with a progressive decline until OHD and a marked decrease at 110 DAFB in 'Gala' and 'Conference'. In 'Golden' apples that transition was not so well defined in part because of the greater dispersion of TTA data from 115 to 129 DAFB. A significant negative correlation was found between TSS and TTA in 'Gala' (Fig. A1.6C insert) with a correlation coefficient of -0.944 while that correlation in 'Golden' and 'Conference' was lower (correlation coefficients of -0.525 and -0.410, respectively) (data not shown).

The index of absorbance difference in the range of 670-720 nm at the fruit skin (I_{AD}) measures the light absorbance due to chlorophyll and is related to the fruit physiological maturity (Cocetta et al., 2017). The I_{AD} for 'Gala' apples (Fig. A1.6E) followed a soft linear decrease until 110 DAFB and a sharp decrease thereafter until the OHD, whereas 'Golden' and 'Conference' followed a soft decline throughout the studied period. A good correlation between I_{AD} and TTA in 'Gala' apples and 'Conference' pears was observed, but not in 'Golden' apples (Fig. A1.6D insert). However, I_{AD} showed a good correlation with softening in all cultivars (Fig. A1.6E insert), with lineal correlation coefficients (R^2) of: 0.920, 0.978 and 0.972 for 'Golden', 'Gala' and 'Conference', respectively. These results are in accordance with previous studies (DeLong et al., 2016) suggesting that the non-destructive measurement I_{AD} could be used as an indicator of firmness changes in the final period of on-tree ripening (≈ 40 days before OHD) and hence helping on deciding the OHD (Wang et al., 2015).

Values of color parameter *a** showed little change in 'Golden' apples and 'Conference' pears due to the small change in its greenish coloration during on-tree evolution (Fig. A1.6F). Some authors found that color parameter *a** for 'Gala' followed a sigmoid pattern (Sadar et al., 2016; Unuk et al., 2012) depicting changes from green to red coloration and determining the ripening stage of the fruit. Our data had a relatively high dispersion and could not confirm such pattern (Fig. A1.6F).

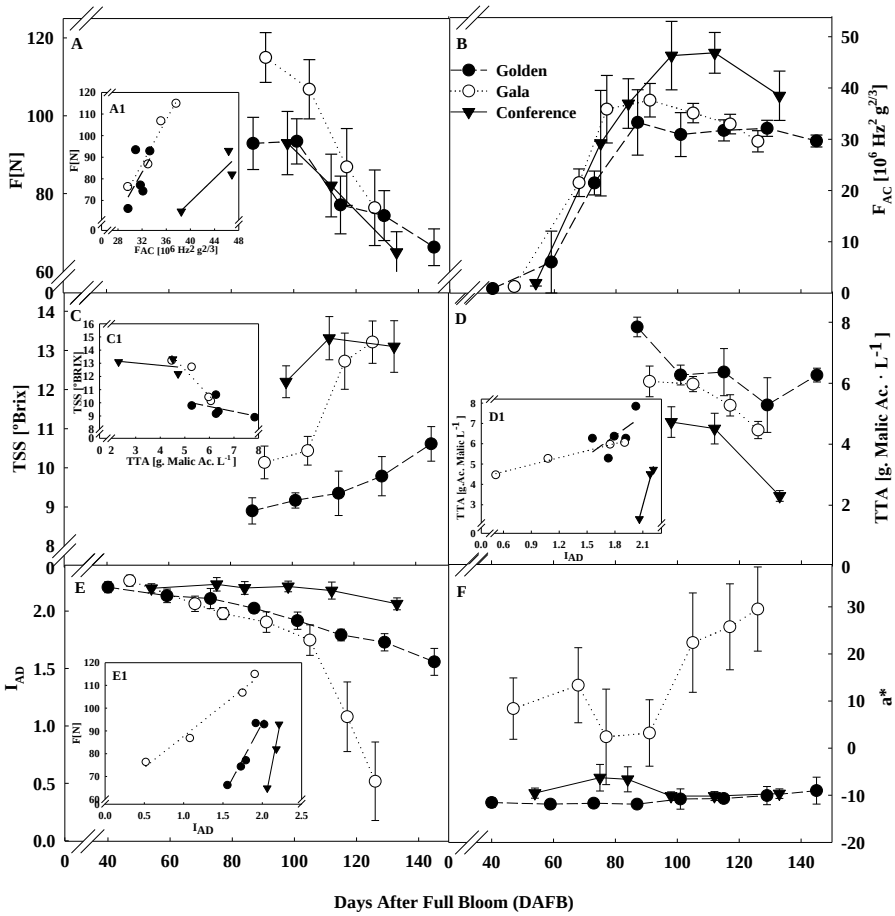

Figure A1.6 On-tree evolution (in days after full bloom (DAFB)) of fruit firmness (A), acoustic firmness (B), total soluble solids, TSS (C), titratable acidity, TTA (D), I_{AD} Index (E), color parameter a^* (F), ethylene production rate during growth (G), respiration rate on fruit basis (H) and ethylene production after harvest (G1) for 'Golden' (●) and 'Gala' (○) apples and 'Conference' (▼) pears. Inserts in A, C, D and E show the correlation between selected quality attributes. Values represent the mean ± standard deviation (n=40).

A1.3.3 Ethylene production and respiration

The three cultivars presented a common pattern with a moderate ethylene production rate declining after 60 DAFB to basal levels (0.02 µL kg^{-1} h^{-1}) until a prominent increase occurred at 110 DAFB. Thereafter each cultivar followed a completely different trend (Fig. A1.7A). Thus, ethylene production rate in 'Gala' apples reached the highest levels (2 µL kg^{-1} h^{-1}) among the three cultivars at the end of the ripening period, followed by 'Golden' and 'Conference', respectively. Other studies describing ethylene production of pome fruit during on tree growth have also found that a peak of ethylene occurs at later development stages (i.e. 130 DAFB for apple) (Cin et al., 2007; Whale and Singh, 2007). In apples, the increase in ethylene

production occurred simultaneously with the noticeable increase in TSS (Fig. A1.6C) and decrease of TTA values (Fig. A1.6D). In 'Gala' apples with reddish skin color, the increase in ethylene production was also accompanied by an increase in *a** values (Fig. A1.6F), which may be related to the increase in anthocyanins and degradation of chlorophyll, both phenomena known to be ethylene-dependent (Whale and Singh, 2007).

Differences in on-tree ethylene production among varieties or species were clearly projected into the capacity of the fruit to produce ethylene once harvested. As shown in Fig. A1.7A insert, the specific ethylene production rate in 'Gala' increased almost uniformly up to 100 µL kg^{-1} h^{-1} during the first 12 days after harvest, while ethylene production in 'Golden' and 'Conference' started to increase 7 and 12 days thereafter and reached much lower production rates. These results are in agreement with the fact that 'Conference' pears, as many other European pear varieties, depending on the maturity stage at harvest, require chilling to initiate their autocatalytic ethylene production (Chervin et al., 2004; Lelièvre et al., 1997).

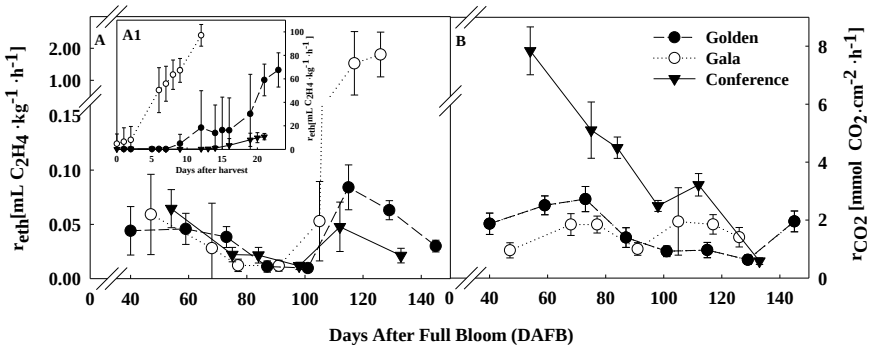

Figure A1.7 Ethylene production rate during growth (A), respiration rate on fruit basis (B) and ethylene production after harvest (A1) for 'Golden' (●) and 'Gala' (○) apples and 'Conference' (▼) pears. Values represent the mean ± standard deviation (n=6).

Fruit respiration rate (r_{CO2}) showed a common pattern in both apple cultivars with a uniform soft increase up to 75 DAFB followed by a slower peak at 110 DAFB, except in 'Golden' apples, and a final decrease until harvest (Fig. A1.7B). 'Conference' pears, on the other hand, showed initial values about 3 to 4-fold higher than in apples and steadily declined throughout its growth period. This decreasing pattern evolution in 'Conference' pears showed a quasi-linear correlation with fruit surface/volume ratio throughout growing period (r^2=0.954) while it was not seen in apples (data not shown). The small transient peak observed at 110 DAFB in 'Gala' coincides temporarily with the sharp increase in ethylene production referred earlier (Fig. A1.7A), as well as with the increase of TSS values in 'Gala' (Fig. A1.6C) and with the decline of

firmness, TTA and I_{AD} (Figs. A1.6A, A1.6D and A1.6E). Interestingly, 'Golden' apple did not show such a peak in fruit respiration. The observed higher respiration rate in pears than in apples may be related to the higher S/V ratio (Fig. A1.3B).

A1.4 Conclusions

The observed quality changes in 'Golden', 'Gala' and 'Conference' growth had a parallelism with changes in fruit respiration and ethylene production capacity. For both apples and pears, ethylene production rate, was high at initial developmental stages, declining thereafter and peaked again at 110 DAFB. That later peak was well correlated with major quality changes including fruit softening, the increase in TSS values and the decrease of TTA. From this time of fruit growth onwards our data suggest that: a) Acoustic firmness can be used as an indicator of firmness b) The index of absorbance difference I_{AD} clearly correlated with TTA in 'Gala' apples, but the correlation was much weaker in 'Golden' and 'Conference' and c) I_{AD} correlated with softening in all cultivars. Pears showed a higher respiration rate than apples during the growing season, which can be due to the higher S/V ratio.

Moreover, apple and pear growth determined by changes in the fruit equatorial diameter and mass could be successfully fitted using the 3-parameter Gompertz function for all tested cultivars and varieties. Thus, final fruit diameter could be predicted one month before commercial harvest with an error lower than 10 % in all the studied varieties. A power relationship between fruit diameter and mass was also established, with good agreement with the Gompertz fits.

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A1.5 References

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Part B: QUALITY CHANGES DURING THE COLD STORAGE PERIOD

[1] Torregrosa, L., Echeverria, G., Illa, J., Garcia, J., Giné-Bordonaba J., 2019. A comparative study between different sensors used to detect the lower oxygen level during dynamic controlled storage of Conference pears. Acta Hortic.

CHAPTER B1: A comparative study between different sensors used to detect the lower oxygen level during dynamic controlled storage of 'Conference' pears

Abstract

To guarantee the availability of high quality pears throughout the year, the new trend in storage is to reduce the oxygen levels within a chamber and continuously monitor the lower oxygen level (LOL) tolerated by the fruit prior to anaerobiosis (referred to dynamic controlled atmosphere (DCA)). LOL monitoring includes measurements of chlorophyll fluorescence (CF), respiratory quotient (RQ) and/or ethanol accumulation (EtOH), all of them well implemented in apples but not in pears. The objectives of this study were to explore the suitability of DCA to store 'Conference' pears, to reveal the best method to determine the LOL based on the fruit physiology and to check possible key volatiles emitted by the fruit into the storage atmosphere as a markers of LOL.

To do so, we used 'Conference' pears harvested at the optimal commercial maturity and stored them in a semi-commercial chamber at 0 ºC for up to 8 months under DCA conditions. The O_2 and CO_2 levels within the storage atmosphere were controlled by an Advanced Control Respiration (ACR) system which decreased O_2 levels until a consistent LOL signal (depicted by either RQ, CF or EtOH) was clearly observed. Changes in the volatiles concentration within the storage atmosphere were also recorded.

In general, DCA storage allowed to preserve 'Conference' pears at optimum quality. Our data showed a correlation between CF and RQ measurements during the first 6 weeks of the cold storage, having a CF peak when RQ values were higher than 1. However, this correlation was lost as the storage period increased, observing exclusively CF peaks that did not match either higher RQ values or ethanol levels. Overall both RQ and CF signals were only useful during the period of acclimatization of the fruit to the cold or when moving from relatively high (*ca.* 2%) to low O_2 levels. Further variables such as the emitted fruit volatiles can be used to monitor the LOL levels tolerated by the fruit.

Keywords: chlorophyll fluorescence, superficial scald, respiratory quotient, volatile organic compound

B1.1 Introduction

The region around Lleida (NE of Spain) is one of the main pear producing areas in Southern Europe with around 129 000 tones produced in 2015 (Iglesias et al., 2015). 'Conference' pear is by far the main cultivated variety (46%), due in part to its good adaptation to the agroclimatic conditions of the region and to their appreciated final quality. However, in the past years, consumers have been demanding pears with high quality all year around what has put pressure on the industry for developing novel storage techniques capable of extending the commercial life. Techniques such as controlled atmosphere allow to extend the storage period (up to 6 months), although such storage conditions may not control the appearance of certain physiological disorders. The new trend in long-term fruit storage is to reduce the oxygen in the chamber dynamically, keeping it at all time just above the lower oxygen level (LOL) tolerated by the fruit prior to anaerobiosis. Dynamic controlled atmosphere (DCA) is able to store at lower oxygen levels than controlled atmosphere and diminishes superficial scald in pip fruit (Prange et al., 2015). To determine the LOL there are different commercial sensors available either based on the chlorophyll fluorescence (Wright et al., 2012; Zerbini and Grassi, 2010), respiratory quotient (Bessemans et al., 2016) or ethanol accumulation (Deuchande et al., 2016a). For instance, technologies such as the Fruit Observer (Besseling, Netherlands) and HarvestWach (Isolcell, Italy) are used to evaluate the LOL through the chlorophyll fluorescence. Other commercial techniques such as the Advanced Control Respiration (ACR) system (Van Amerongen, Netherlands) is based on the respiratory quotient of the product while the DCS system (Storex, Netherlands) measures the ethanol emitted by the fruit on the storage atmosphere. While several studies are available describing the use of one of the technologies, less information exists comparing more than one system at the same time. Moreover, scarce information exists investigating the suitability of these techniques on pears.

The aims of this study were: 1) To evaluate the suitability of DCA storage in 'Conference' pears. 2) To investigate the potential relationship between the sensor's reaction under a poor-oxygen atmosphere. 3) To try to identify key volatiles emitted by the fruit into the storage atmosphere as a potential markers of the LOL.

B1.2 Materials and methods

'Conference' pears (5.5 t) were harvested at La Rioja region (North Spain) from eleven different producers at the commercial maturity stage according to growers' recommendations. Thereafter, fruit were transported to IRTA research institute and 20 fruit per producer were kept for initial quality

evaluations including (maturity (I_{AD}), firmness (F), total soluble solid (TSS) and total titratable acidity (TTA)) using the methodology described by Giné-Bordonaba et al., 2016. The rest of the fruit were stored in wooden bins (250 kg/bin) and two bins per producer, in a semi-commercial cold room (ca. 4x4x3m) at 0ºC during 8 months under a dynamic controlled atmosphere (DCA). The O_2 and CO_2 levels within the storage atmosphere and the RQ values (DCA-RQ), used as a measure of the LOL tolerated by the fruit, were controlled by an ACR system. The RQ values were compared with the values obtained by three other different systems: (I) Chlorophyll fluorescence (DCA-CF), (II) emitted volatiles (DCA-VOCs) within the storage chamber and (III) ethanol levels in the fruit flesh (DCA-EtOH).

Volatiles were captured once per month or when the DCA-CF or DCA-RQ system detected a stress. VOC's were captured on two parallel installed adsorption tubes filled with 350 mg Tenax TA (2, 6-dipheyl-1-*p*-henylene oxide), Carbograph 1TD and Carboxen 1003 through an in-house developed system equipped with a recirculating air pump. The air stream was set up at 300 ml/min during 30 min. The volatile compounds were desorbed using an automated UNITY Markes thermal desorption system (Markes International Ltd., Llantrisant, United Kingdom) at 275 ºC for 15 min. Identification and quantification were done with an Agilent 7890B gas chromatograph coupled to a 5977A mass spectrometer (MSD) (Agilent Technologies, Inc., Barcelona, Spain). Volatile compounds separation was performed with a capillary column with cross-linked free fatty acid as the stationary phase (FFAP; 50 m × 0.2 mm × 0.33 μm). Helium was used as the carrier gas, at a flow rate of 42 $cm \cdot s^{-1}$, with a split ratio of 60:1. Both the injector and detector were kept at 240 ºC. The analysis was conducted according to the following program: 40 ºC (1 min); 40-115 ºC (2.5 $ºC \cdot min^{-1}$); 115-225 ºC (8 $ºC \cdot min^{-1}$); 225 ºC (10 min). Mass spectra was obtained by electron impact ionization at 70 eV. Helium was used as the carrier gas (42 $cm \cdot s^{-1}$), following the same temperature gradient program described previously. Spectrometric data were recorded (Hewlett-Packard 3398 GC Chemstation) and compared with those from the original NIST HP59943C library mass spectra.

Samples for ethanol measurements were removed from the chamber every two months. Ethanol content was determined according to the protocol described by Iglesias et al., 2018.

Fruit apparent maturity of each selected fruit was measured based on the index of absorbance difference(I_{AD}) with a DA-Meter (TR Turoni, Forli, Italy), as described elsewhere (Giné-Bordonaba et al., 2016). Fruit firmness was determined, on two opposite sides of each fruit after removing the peel, using

a hand-held penetrometer (Turoni, Italy) fitted with an 8 mm diameter plunger. Five fruit were crushed together and filtered to obtain one juice. From the obtained juice, total soluble solids (TSS, º Brix) were measured using a digital hand-held refractometer (Atago, Tokyo, Japan), and acid content (TTA) was measured by titration of 10 ml of juice with 0.1 N sodium hydroxide (NaOH) to pH 8.2 using phenolphthalein. Quality parameters were evaluated at harvest, after DCA storage and after DCA storage plus 5 days of shelf life (SL). After 5 days of SL fruit dehydration, scald like disorders and internal breakdown were also visually analyzed (incidence and severity) in 40 fruit as reported by others (Deuchande et al., 2016a; Giné Bordonaba et al., 2013).

Data were subjected to analysis of variance (ANOVA) tests using JMP 13 SAS Institute Inc. Least significant difference values (LSD; $P \leq 0.05$) were calculated for mean separation using critical values of t for two-tailed tests.

B1.3 Results and discussion

B1.3.1 Quality at harvest and after removal from DCA storage

Quality indices measured and its statistical differences at harvest are shown in Table B1.1. On average fruit mass was 248.3±37.4 g, diameter 73.8±4.0 mm, firmness 57.4±6.2 N, I_{AD} 2.1±0.1, TSS 13.9±0.3 ºBrix and TTA 1.7±0.1 g Malic acid L^{-1}. Firmness values at harvest are suitable for long term storage (A. Rizzolo et al., 2015). Quality indices measured after storage and after 5 days of SL are shown in Table B1.2.

Dehydration was evaluated using 0-3 scale (Fig. B1.1A). All fruit showed symptoms of dehydration after 8 months of storage (Fig. B1.1B). However, most fruit were only affected partially at the top (Severity 1). Scald like disorders (Fig. B1.1C) were also present in most samples (45.0±9.7%). This disorder is characterized by similar appearance than typical superficial scald yet showing a completely different etiology (Larrigaudière, C. *personal communication*) and generally not leading to consumer rejection. Indeed, the physiology and biochemistry behind this disorder is not yet completely understood. The % of fruit with scald varied from 30% to 62.5%, depending on the producer. Internal breakdown (Fig. B1.1D) was found in almost all the producers (18.4±13.7%) but with incidence and severity not different to that generally found in fruit from the same region and harvest year stored under regular CA.

Table B1.1 Quality parameters of 20 fruit for each producer at harvest.

	Mass [g]	Ø [mm]	I_{AD}	F [N]	TSS [ºbrix]	TTA [g. Malic Ac. L^{-1}]
P1	251.6±45.1 ab	74.5±4.7 abcd	2.1±0.1 ab	60.1±6.6 ab	14.1±0.2 bc	1.8±0.1 abc
P2	247±35.4 b	74.2±3.3 abcd	2.1±0.1 a	57.4±8.4 abc	13±0.1 e	1.7±0.1 abcd
P3	228.7±37.4 b	73.1±3.3 bcd	1.8±0.1 d	50.7±4.2 c	14.6±0.4 a	1.3±0.2 f
P4	247.5±32.4 b	73.1±3.1 bcd	2±0.1 abc	61.8±5.8 a	13.8±0.3 cd	1.7±0 bcd
P5	292.8±44.1 a	78.4±4.5 a	2±0 abc	57.5±5.7 abc	13.1±0.4 e	1.5±0 e
P6	233.9±45 b	70.8±4 d	2±0 abc	51±3.5 c	13.3±0.2 e	1.2±0 f
P7	245.2±36.4 b	72.8±4.4 cd	1.9±0.1 c	53.3±11.1 bc	14±0.4 bcd	1.8±0 a
P8	248.2±51.6 b	73.1±5.9 bcd	2.1±0 ab	59.4±13.8 ab	14.2±0.1 b	1.6±0.1 d
P9	258.7±32.7 ab	73.8±3.8 bcd	2±0.2 bc	57.4±10.7 abc	14.5±0.3 a	1.7±0.1 cd
P10	267.6±41.5 ab	77.3±4.1 ab	2.1±0 ab	60.1±6.2 ab	13.7±0.3 d	1.5±0.1 e
P11	268.2±33.6 ab	76.8±3.4 abc	2±0 abc	54.2±5.2 bc	13±0.1 e	1.8±0.1 ab
AVG	248.2±37.4	73.8±4	2±0.1	57.4±6.2	13.8±0.3	1.7±0.1

Different letters indicate significant difference by LSD test $P<0.05$

Table B1.2 Quality parameters of 20 fruit for each producer after DCA treatment and after DCA treatment plus 5 days of (SL).

	I_{AD}		F[N]		TSS [ºbrix]		TTA [g. Malic Ac. L^{-1}]	
	Qf	**Qf+5dSL**	**Qf**	**Qf+5dSL**	**Qf**	**Qf+5dSL**	**Qf**	**Qf+5dSL**
P1	2±0.1 ab	1.7±0.2 a	56.8±4.8 abc	14.4±2.6 b	14.1±0.5 fg	16.6±1.3 a	1.8±0.4 a	1.3±0 b
P2	2±0.1 abc	1.5±0.2 abcd	60.3±5.7 ab	12.6±1.9 bc	14±0.5 g	14.9±0 e	1.3±0.2 def	1.3±0 ab
P3	1.6±0.4 e	1.1±0.3 e	49.3±6.2 d	13.9±2.1 bc	15.3±0.6 bc	16.2±0.1 ab	1±0.1 gh	0.9±0 fg
P4	1.8±0.1 bcde	1.5±0.2 abcd	60±6 ab	17.6±3.8 a	15.7±0 ab	15.7±0.1 cd	1.5±0.1 cd	1.3±0 b
P5	1.8±0.2 bcde	1.4±0.3 bcde	53.1±6.9 cd	12.6±3.2 bc	14.8±0.5 de	16±0.4 bc	1.2±0.1 efg	1.1±0 de
P6	1.7±0.3 de	1.3±0.2 de	48.3±4.5 d	11.7±3 c	15.6±0.1 abc	15.6±0.1 cd	1±0 h	0.9±0 fg
P7	1.8±0.2 cde	1.4±0.2 bcd	56±7.8 bc	14.2±2.3 b	15.4±0.2 bc	15.3±0.2 de	1.6±0 bc.1	1.2±0.1 c
P8	2±0.2 ab	1.6±0.2 ab	58.1±8.8 abc	13.9±2.7 bc	15.8±0.3 a	15.6±0.2 cd	1.8±0.3 ab	1.4±0.1 a
P9	1.9±0.2 abcd	1.4±0.2 abcd	57.7±7.8 abc	12.4±2.5 bc	14.8±0.9 de	16±0.3 bc	1.4±0.2 cde	1±0 ef
P10	2.1±0.1 a	1.6±0.3 abc	62.9±5 a	15±5.1 bc	15.2±0.3 cd	15.5±0.2 d	1.4±0.1 cde	1.1±0 d
P11	2±0.1 abc	1.3±0.2 cde	57.9±6.2 abc	12.9±2.1 bc	14.4±0.2 ef	14.2±0.5 f	1.1±0.1 fgh	0.9±0 g
AVG	1.9±0.2	1.4±0.2	57.7±6.2	13.9±2.6	15.2±0.3	15.6±0.2	1.4±0.1	1.1±0

Different letters indicate significant difference by LSD test $P<0.05$

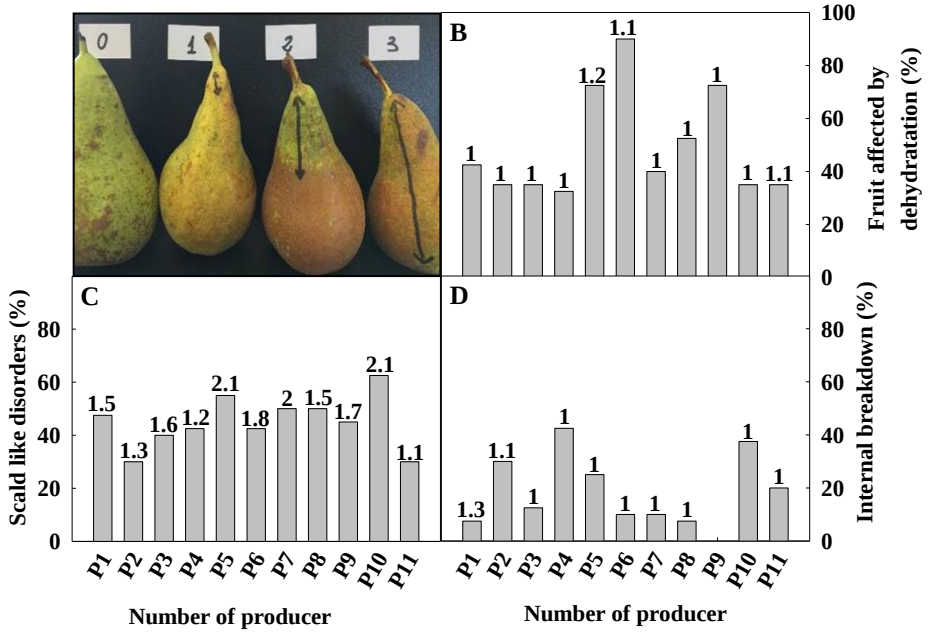

Figure B1.1 Incidence of external and internal disorders in % after 8 months under DCA storage plus 5 days in SL at 20 ºC. A) Dehydration visual scale used, B) (%) of affected fruit by dehydration, C) scald like disorders and D) internal breakdown. Numbers above the bars represent the average severity index of the affected fruit for each physiological disorder.

Overall, DCA storage maintains fruit quality similar to the values at harvest yet allowing the fruit to ripen normally when further stored at 20 ºC (Hendges et al., 2018).

B1.3.2 Correlation between different sensors

Considering the technical recommendations for long term storage, very low oxygen levels were used during the storage period (Fig. B1.2A). During the first 5 months (150 days) the room was self-controlled through the ACR system, afterwards the oxygen levels were increased manually and a final oxygen depletion (after t=205 d) was applied (Fig. B1.2A).

Results from the three different systems were compared (Fig. B1.2B). Data showed a good relationship between DCA-CF and DCA-RQ systems during the first 6 weeks of the cold storage trial, having a CF peak when RQ was higher than 1 (1.269 and 1.128). However, this correlation was lost as the storage period progressed, observing exclusively CF peaks that did not match with either higher RQ values or ethanol levels. These types of signals during the experiment were only useful up to 50 days of the storage period.

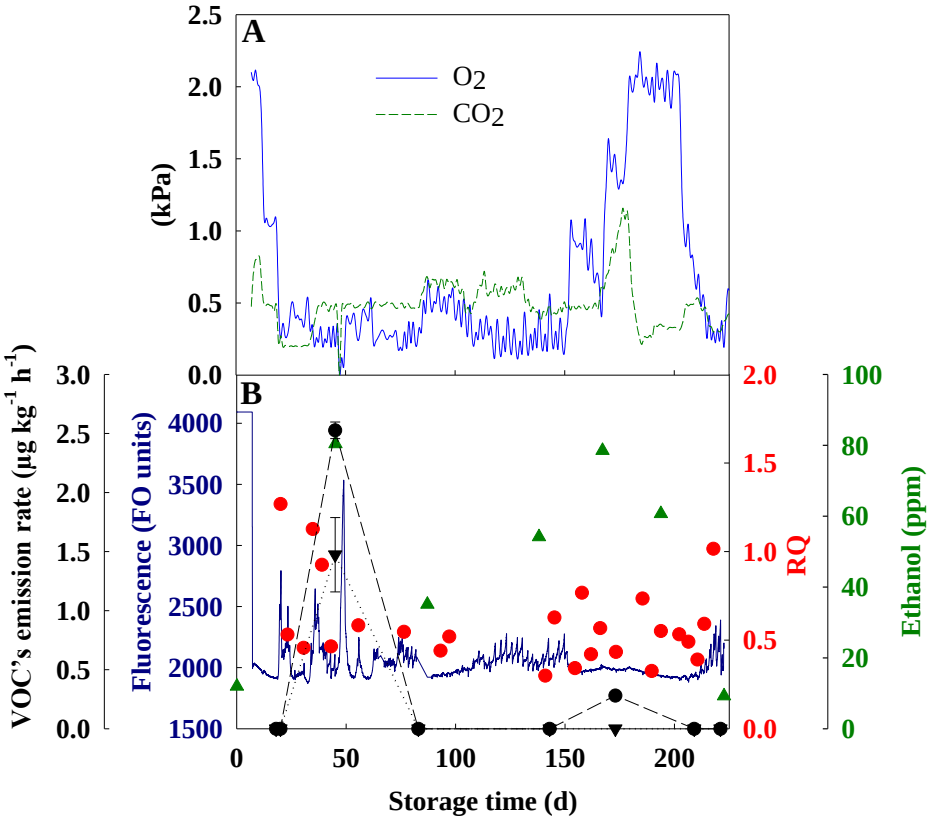

Figure B1.2 A) O_2 and CO_2 evolution among the storage period. B) Fluorescence response (____) of 'Conference' pears during storage in DCA for 8 months, RQ response (●) from ACR system and ethanol concentration (▲) in pulp from extracted samples and mean (n=2) values of VOC's emission rate (µg kg^{-1} h^{-1}) of α-pinene (▼,….) and β-pinene (●,- -).

B1.3.3 Volatiles as novel markers of the LOL during DCA storage of 'Conference' pears

Some volatiles such as, α-pinene and β-pinene, peaked after 50 days of storage showing a good relationship with the highest ethanol content and maximum fluorescence peak (Fig. B1.2B). These volatiles have been previously associated to biotic stress due to a fungal attack in 'Blanquilla' pears (López et al., 2015) or in tomato plants exposed to a single or combined stress (Catola et al., 2018). Although, further research is needed, these terpenes may be used as a stress marker of 'Conference' pear during cold storage under very low oxygen levels.

B1.4 Conclusions

DCA storage allowed to preserve ‘Conference’ pears at optimum quality (firmness, 57.7±6.2 N) and to limit the incidence or severity of some major physiological disorders (scald-like disorders and dehydration) typically observed in this pear variety. Our results demonstrate that ’Conference’ pears tolerated very low oxygen levels, actual commercial storage lower oxygen levels are about 1% O_2, without suffering typical fermentative issues, as revealed by the lower ethanol values within the flesh tissue, such as core browning. Under the conditions imposed in this study, it is feasible to speculate that the RQ or the CF signals measured herein are not suitable to correctly detect the LOL tolerated by ‘Conference’ pears. Based on the correlation with the ethanol flesh content or the CF signal, some terpenes may be employed as markers of the fruit stress under very low oxygen levels storage.

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CHAPTER B2: Effect of initial vs. dynamic low oxygen stresses on the storage potential of 'Conference' pear

Abstract

The effect of long-term storage of 'Conference' pears under very low oxygen levels (≤0.5 kPa) on fruit quality, ethylene emission, fermentative metabolites and volatile organic compounds was investigated. Pears were cold stored for seven months under three different gaseous conditions: initial low oxygen stress (ILOS), dynamic low oxygen stresses monitored with a chlorophyll fluorescence sensor ($DLOS_1$) and extended dynamic low oxygen stresses ($DLOS_2$).

'Conference' pears showed high resistance to anoxic conditions. The application of repeated low oxygen levels in the atmosphere composition did not affect the fruit firmness upon removal from cold storage. Fruit stored under more restrictive conditions ripened slower when placed at 20 ºC, as indicated by changes in $I_{AD,}$ ethylene production capacity and esters emission. After 5 days of shelf life (SL) fruit under ILOS showed significantly higher levels of ethanol and acetaldehyde confirming that these fruit were in a more advanced state of maturity than fruit from $DLOS_1$ and $DLOS_2$.

As observed in fruit from DLOS containers, esters and alcohols, could be used as maturity fruit state markers during the cold storage period of 'Conference' pear. Even though further research is needed, capturing fruit volatiles from the cold storage atmosphere provided key information about fruit preservation. Fruit respiration rates were modeled using Michaelis-Menten kinetics. However, after 202 d in storage pears respiration rates showed a lag which was not considered by the model.

Keywords: abiotic stress, chlorophyll fluorescence, ethanol, IVOCs, α-farnesene.

B2.1 Introduction

Cold storage (CS) of pear fruit is a common practice to satisfy the market demand of pears all year round. It is well known that the storage of pears at low temperatures reduces fruit metabolism and that high relative humidity avoids weight loss, helping to maintain optimal fruit quality (Mohapatra et al., 2013). However, cold storage can lead to the appearance of certain physiological disorders commonly known as chilling injuries (Lurie and Watkins, 2012). In this sense, the combination of CS with controlled atmosphere (CA) (i.e. 2 kPa O_2 and 3 kPa CO_2) can partially control the appearance of these disorders and further extent the storability of the fresh product. Nonetheless, pears are very sensitive to low P_{O2} and high P_{CO2} and under such conditions may develop some other physiological disorders such as core or internal breakdown (Lum et al., 2016). The development of these internal physiological disorders is mainly caused by anoxia and the induction of the fruit fermentative metabolism (Deuchande et al., 2016) together with an energy disruption (Ho et al., 2013).

To avoid the induction of fermentative metabolism, recent trends in CA storage aim to dynamically adjust the O_2 levels inside the cold room as close as possible to the lower oxygen level (LOL) tolerated by the fruit, but always above the fermentation threshold in order to avoid the shift from aerobic respiration to fermentation (Prange et al., 2011). Storing fruit under dynamic controlled atmosphere (DCA) conditions prevents physiological disorders (i.e. superficial scald) and fruit off-flavors and even extends further the produce storability (Deuchande et al., 2016). Currently, there are three commercial variants of this technology each based on monitoring a different biochemical parameter of the fruit: chlorophyll fluorescence, respiratory quotient and ethanol content, both in fruit pulp or in the cold room atmosphere. While DCA storage has been widely applied in apples (Mditshwa et al., 2018), less information is available on its use in pears (Prange et al., 2013; Saquet, 2019).

It is well recognized that low temperature and restricted or enhanced levels of O_2 and CO_2, respectively, during fruit storage act as important stress factors (Larrigaudiere et al., 2001). In response to biotic and abiotic stresses, fruit as well as plants, shift and alter their functional metabolic pathways leading to the synthesis of specific stress-induced volatile compounds (López et al., 2015; Spinelli et al., 2011). Induced volatile organic compounds (IVOCs) include alkenes, alkanes, carboxylic acids, nitrogen-containing compounds and alcohols, together with isoprene and terpenes (Holopainen and Gershenzon, 2010). A better understanding of the synthesis and emission of these

compounds in pear fruit exposed to very low oxygen levels during storage may assist on developing new DCA monitoring technologies capable of accurately determine the fruit physiological state prior and during the induced stress.

Very low oxygen levels inside the cold room slow down fruit respiration activity during storage (Soria and Recasens, 1998). However, respiration rates during the shelf life period at 20 ºC are affected by with the duration of storage (Chen et al., 1983) and by its temperature and atmosphere gaseous conditions. In recent years several studies have been undertaken in order to model fruit respiration under different storage temperatures such as Mahajan and Goswami (2001) in apple fruit, Wang et al. (2009) in guava fruit and Kandasamy et al. (2015) in tomato.

Accordingly, the objective of this study was to evaluate the quality parameters, ethylene production, fermentative metabolites and respiration of 'Conference' pears during the long-term storage under different imposed low oxygen stress storage conditions and to determine if specific volatile compounds are emitted or enhanced in response to such stresses.

B2.2 Materials and methods

B2.2.1 Plant material and storage conditions

'Conference' pears (*Pyrus communis* L.) were harvested in August 2016 at a commercial orchard near Lleida (NE of Spain). Fruit was picked up at optimum commercial maturity according to local growers' recommendations which are basically assessed in terms of firmness and sugars content (firmness ≈ 55-65 N and total soluble solids > 13 %). Thereafter, fruit was transported to IRTA research institute and stored in three experimental containers named as ILOS, $DLOS_1$ and $DLOS_2$, each with a volume of 350 L (Fig. B2.1) and located inside a semi-commercial cold room at 0ºC. Approximately 20 kg of fruit were stored in each experimental container and kept for up to seven months under the following atmosphere conditions:

- ILOS: 0.4 kPa O_2 and 1 kPa CO_2 for the first 14 d, thereafter storage at 2 kPa O_2 and 2 kPa CO_2 (Fig. B2.2A).

- Containers $DLOS_1$ and $DLOS_2$: set point was kept at 0.5 kPa O_2 and 0.5 kPa CO_2 although the system did not always reproduce it exactly. Oxygen partial pressure was lowered five times during the storage period simultaneously in containers $DLOS_1$ and $DLOS_2$ (Fig. B2.2B, B2.2C), however, in $DLOS_2$ container the low oxygen level (or stress) was kept for a longer time than in

$DLOS_1$ container. To compare the extension of the low oxygen stresses in $DLOS_1$ and $DLOS_2$ containers was used the stress index (I_{stress}) evaluated as,

$$I_{stress} = \int_{t_{0_i}}^{t_{f_i}} P_{O_2} dt \qquad \text{i=1, …, 5} \quad (1)$$

where P_{O_2} is the oxygen partial pressure during the *i* stress period (*i*= 1,…, 5) from the start time (t_{0_i}) to the end of the *i* stress (t_{f_i}) in days.

B2.2.2 Experimental setup

The three experimental containers were equipped with a volatile organic compounds (VOCs) extraction system which consisted in an air-recirculating pump that forced the air through two adsorption tubes in parallel (Fig. B2.1).

At the top of each container were installed three small chambers with a capacity of 21.4 L and filled with approximately 10kg of fruit each. The chambers were connected to each other and with the container's atmosphere in order to perform air circulation between the container and the chambers (Fig. B2 1). Connections were made through flexible pipes with a system of taps, in order to maintain the tightness of the container when removing the fruit for intermediate analysis at 30, 60 and 158 d (except ILOS, which was provided with one chamber and fruit was analyzed only after 30 d.

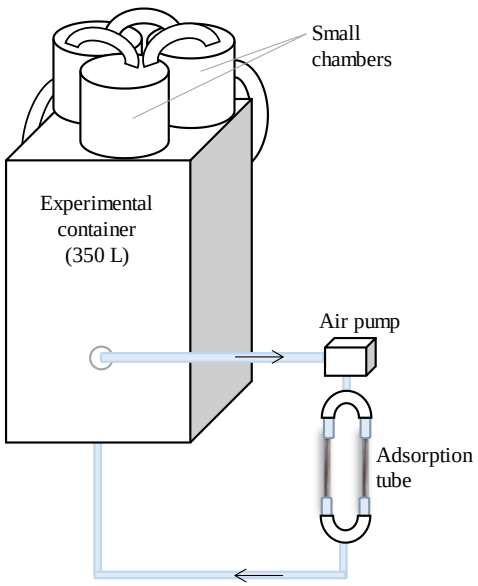

Figure B2.1 Scheme of the experimental setup used for the volatile organic compound's extraction from the experimental container atmosphere. At the top of the container are located the three small chambers.

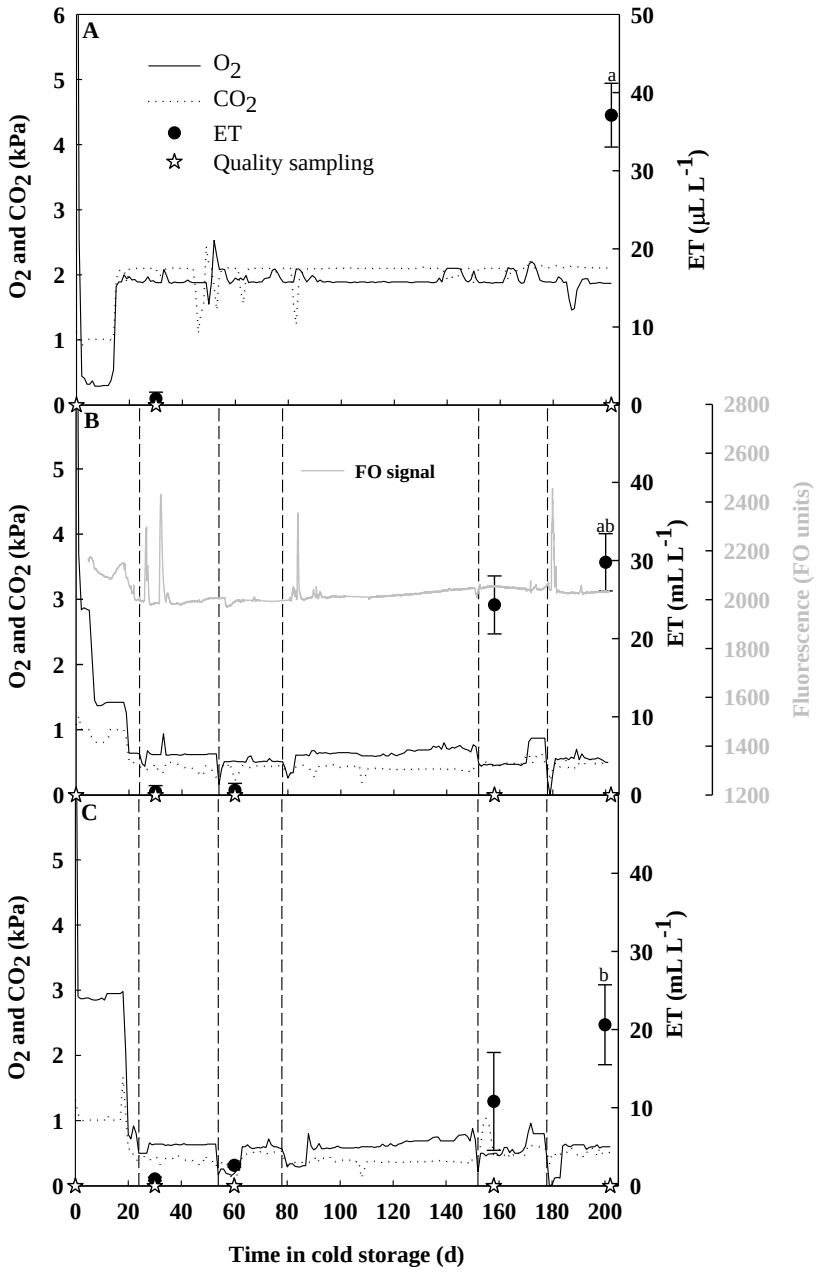

Figure B2.2 Oxygen and CO_2 partial pressure (left axis) and ethanol content (ET) in fruit pulp (right axis) in the three containers used for the cold storage. A) ILOS, B) $DLOS_1$ with chlorophyll fluorecence signal (right ofset axis) and C) $DLOS_2$. Discontinuos vertical lines indicate the time of application of the stresses (t=24, 54, 78, 152 and 178 d) in DLOS containers. (☆) Indicates the time at which samples of fruit were removed from the containers (t=0, 30, 60, 158 and 202), (●) indicates ethanol content in fruit pulp. Error bars indicate standard deviation for n=3, mean values with the same letter are not significantly different according to analysis of variance (ANOVA) and LSD test at $P \leq 0.05$. No letter indicates the absence of significant differences.

B2.2.3 Management of DLOS containers

The Fruit Observer (FO) chlorophyll sensor (Besseling, Netherlands) was installed inside the $DLOS_1$ container. The fluorescence monitoring system was used following Besseling's protocol with some modifications (Fig. B2.2). Briefly, the system was activated and the first pull down was applied, establishing P_{O2} and P_{CO2} at 3 and 1 kPa, respectively, over a 48h period, thereafter P_{O2} was reduced to 1.5 kPa and P_{CO2} to 0.8 kPa. After 17 days from harvest O_2 level was reduced to 0.5 kPa and after a week the first stress was applied lowering the P_{O2} and P_{CO2} levels to 0.2 and 0.4 kPa, respectively. Thereafter, the P_{O2} was increased up to 0.5 kPa and P_{CO2} to 0.5 kPa when the fluorescence signal presented a peak or after 48 h if the sensor did not register any stress signal. In the $DLOS_2$ container the oxygen level was initially reduced to 3 kPa and CO_2 to 1 kPa and after 17 days from harvest O_2 level was reduced to 0.5 kPa. A week later (t=24d) the first stress was applied lowering the P_{O2} and P_{CO2} levels to 0.4 and 0.2 kPa, respectively. Stresses lowering the oxygen and CO_2 levels were applied in both DLOS containers at days 24, 54, 78, 152 and 178 of storage. In the $DLOS_2$ container the stress levels were maintained longer.

B2.2.4 Fruit quality measurements

Fruit quality parameters (firmness (F), apparent maturity (I_{AD}), total soluble solids (TSS) and total tritatable acidity (TTA)) were measured as described elsewhere (Torregrosa et al., 2019). At harvest, 20 fruit were analyzed, afterwards 20 fruit from each chamber were analyzed 6 days after initiating the stresses (at days 30, 60 and 158), at the end of cold storage period (202 d) and after storage plus 5 d of shelf life (SL).

B2.2.5 Ethylene production and fruit respiration rates

At harvest and at each sampling date the fruit ethylene production capacity was measured daily during 15 days. Three 1.5L flasks were used per container, each containing two weighted fruit. The flasks were continuously aerated with humidified air at a flow rate of 1.5 $L \cdot h^{-1}$ and kept at room temperature (20 ºC). The amount of ethylene produced by the fruit was measured by taking a 1 mL sample of gas from the headspace of each flask and injecting it into a gas chromatograph fitted with a FID detector (Agilent Technologies 6890, Wilmington, Germany) and an alumina column 80/100 (2m ×3mm) (Teknokroma, Barcelona, Spain) as described by Torregrosa et al. (2019).

Fruit respiration was measured by enclosing 5 weighted fruit in airtight jars of known volume (3 replicates per each experimental condition) placed in an acclimatized chamber at 20 ºC. Oxygen and carbon dioxide concentrations within the jars were measured at each intermediate removal from cold storage. Measurement was done with an O_2/CO_2 gas analyzer (CheckPoint O_2/CO_2, PBI Dansensor, Ringsted, Denmark). Gas i (i = O_2, CO_2) production rate, r_i ($mol_i \cdot kg^{-1} \cdot h^{-1}$), was then calculated as previously described by Torregrosa et al. (2020).

B2.2.6 Determination of fermentative metabolites

Ethanol (ET) and acetaldehyde (AA) pulp content were determined at the same sampling dates as other quality measurements following the methodology described by Deuchande et al. (2017). Briefly, frozen juices were incubated in a water bath at 65 °C for 1 h, thereafter, 1 mL of headspace gas sample was taken with a 1 mL glass syringe for chromatographic determination. Nitrogen was used as the gas carrier, and the operating conditions were as follows: oven temperature: 90 °C; injector temperature: 250 °C; detector temperature: 220 °C. The in liquid concentrations were calculated using a standard curve generated by injecting standard solutions of known concentrations (acetaldehyde standards ranging between 0.5–15 μL L^{-1}; ethanol standards ranging between 2.5–250 μL L^{-1}).

B2.2.7 VOCs extraction and quantification

VOCs extraction was done just before each stress (t=24, 54, 78, 152 and 178 d) and one week after the stress initiation (t= 30, 60, 84, 158 and 184 d) simultaneously in the three containers.

The extraction was conducted by inserting the two adsorption tubes filled with 350 mg Tenax TA (2, 6-dipheyl-1-p-henylene oxide) and Carbograph 1TD outside of each container. Then an air stream of 250 ml/min was forced during 60 min. Adsorption tubes were kept at 4 ºC until were desorbed (Cano-Salazar et al., 2013). During the extraction the pump was turned on and the air of the container was forced to circulate through the adsorption tubes (Fig. B2.1).

Volatile compounds desorption was done using an automated UNITY Markes thermal desorption system (Markes International Ltd., Llantrisant, United Kingdom) at 275 ºC for 15 min. Identification and quantification were done with an Agilent 7890B gas chromatograph coupled to a 5977A mass spectrometer (MSD) (Agilent Technologies, Inc., Barcelona, Spain). Volatile

compounds separation was performed with a capillary column with cross-linked free fatty acid as the stationary phase (FFAP; 50 m×0.2 mm×0.33 μm). Helium was used as the carrier gas, at a flow speed of 42 cm s^{-1}. Both the injector and detector were kept at 240 ºC. The analysis was conducted according to the following program: 40 ºC (1 min); 40-115 ºC (2.5 ºC min^{-1}); 115-225 ºC (8 ºC min^{-1}); 225 ºC (10 min). Mass spectra was obtained by electron impact ionization at 70 eV, using the same flow of helium and following the same temperature gradient program as the ones used in the separation. Volatile compounds identification was carried out by comparing the spectrometric data recorded to those from the original NIST HP59943C library mass spectra. Quantification was performed using individual calibration curves, with correlation coefficient higher than 0.95, for each identified compound.

B2.2.8 Statistical and data analysis

Means were compared by analysis of variance (ANOVA), when the analysis was statistically significant, the Student t-test (LSD) at $P \leq 0.05$ was performed for separation of means using JMP® 13.1.0 SAS Institute Inc. (SAS, 2013).

A Principal Component Analyses (PCA) was conducted in order to establish a preliminary relationship between VOC's emitted by from the three experimental conditions, $DLOS_1$, $DLOS_2$ and ILOS, after the application of each stress.

B2.2.9 Respiration kinetics model

There are different models available in the literature to predict the respiration rate of different fruit varieties (Lee et al., 1991; Wang et al., 2009). In the present study, a model based on Michaelis-Menten kinetics, with non-competitive CO_2 inhibition and assuming a constant respiratory quotient was employed. The CO_2 production rate r_{CO2} (mL_{CO2} h^{-1} kg^{-1}) at a determined temperature as a function of the O_2 and CO_2 air concentrations (υ_{O2}, υ_{CO2} (V/V)) is given by eq. 2:

$$r_{CO_2} = \frac{V_m \, \upsilon_{O_2}}{k_{m_{O_2}} + \left(1 + \frac{\upsilon_{CO_2}}{k_{i_{CO_2}}}\right) \upsilon_{O_2}} \qquad (2)$$

where V_m (ml_{CO2} h^{-1} kg^{-1}) is the maximum production rate of CO_2 to the corresponding temperature, $k_{m_{O_2}}$ is the Michaelis-Menten constant, and $k_{i_{CO_2}}$ is the non-competitive CO_2 inhibition constant.

The used respiration model is then defined by the following ordinary differential equations set, eq. 3 and 4:

$$\frac{dv_{O_2}}{dt} = -M_f \frac{r_{CO_2}}{RQ} \tag{3}$$

$$\frac{dv_{CO_2}}{dt} = M_f r_{CO_2} \tag{4}$$

where M_f (kg) is the fruit mass and RQ (mol_{CO2} mol_{O2}^{-1}) the respiratory quotient. The four unknown parameters of the model are: V_m, $k_{m_{O_2}}$, $k_{i_{CO_2}}$ and RQ. Parameter fitting to the measured respiration data was done by non-linear least squares adjustment.

B2.3 Results and discussion

B2.3.1 Chlorophyll fluorescence and evolution of fruit ethanol content

Continuous evolution of oxygen, CO_2 and ethanol content in fruit at intermediate samplings in the three storage containers as well as the Fruit Observer (FO) signal evolution in $DLOS_1$ container during the cold storage period are shown in Fig. B2.2. Oxygen levels in both DLOS containers were lowered during the stresses initiated at days 24, 54, 78, 152 and 178. More extreme conditions were applied at $DLOS_2$ (Table B2.1).

Table B2.1 Duration in days of each stress and stress index in (Pa d), during the five applied stresses at $DLOS_1$ and $DLOS_2$ containers.

	Δt (d)		I_{stress} (Pa d)	
Stress	$DLOS_1$	$DLOS_2$	$DLOS_1$	$DLOS_2$
S_1	3.0	3.0	318.1	460.4
S_2	3.0	9.0	745.1	3777.3
S_3	5.0	9.5	1525.9	3668.2
S_4	10.5	10.5	5172.8	5732.8
S_5	3.5	6.5	1223.1	2790.8
	$\sum_{S_1}^{S_5} I_{stress}$		8985.0	16429.5

Fruit Observer signal exhibited a peak after the first oxygen pull down at t=24 d (stress S_1). At day 35 the FO sensor showed an unexpected peak which

could not be explained by O_2 or CO_2 variations nor by temperature variations. At day 54 (stress S_2) oxygen level was lowered again but no FO peak was observed. The third stress was applied at day 78 and the FO signal peaked 4 d afterwards. After 152 d (stress S_4) the oxygen level was lowered but FO did not show any peak, for this reason, levels of O_2 were maintained for 22 d. Since the fluorescence sensor was not peaking, at t =178 d the P_{O2} and P_{CO2} levels were increased to 1 kPa and 1 kPa, respectively, for 2 d and subsequently dropped again to 0.2 kPa of O_2 and 0.4 kPa of CO_2 (stress S_5). Then, the FO signal peaked 1 day afterwards.

Based on the data depicted in Fig. B2.2, FO not always peaked after an oxygen pull down, hence highlighting that pears harvested at the commercial maturity from the Lleida region are either very resistant to atmospheres with low oxygen level, as pointed out in recent studies (Torregrosa et al., 2019) or that the FO signal do not precisely monitor the low O_2-induced fruit stress.

Ethanol in fruit pulp is a common indicator of fermentative damage induced by low P_{O2} levels. At the first sampling, day 30, no ethanol accumulation in the fruit pulp was observed in any container even though the FO signal peaked at day 26 in parallel to the restriction in O_2 levels. After the second stress ethanol remained low in both DLOS containers, which is in accordance with the absence of the chlorophyll peak from the FO sensor. At the third sampling, after stress S_4, higher ethanol accumulation was found in fruit from $DLOS_2$ but with no significant differences between DLOS containers. Ethanol levels in fruit from $DLOS_2$ were above 20 ppm which was recently defined as the critical level for the induction of internal disorders in 'Rocha' pears by Deuchande et al. (2016). However, the ethanol levels registered in this study for $DLOS_2$-stored fruit, or any of the other conditions tested, were not accompanied by fruit internal damage (data not shown). After the last sampling, significant differences in ET content were found among the three containers. Fruit from the ILOS container had the higher ethanol content followed by $DLOS_1$ and $DLOS_2$ (37, 30 and 21 ppm, respectively). Hence, fruit stored under more restrictive oxygen levels tended to accumulate lower ethanol.

Pears accumulate ET not only in response to anoxia or high CO_2 but also during normal ripening (Nanos et al., 1992). Deuchande et al. (2016), reported the absence of internal browning disorders in 'Rocha' pears stored under dynamic controlled atmosphere with the aid of HarvestWatch monitoring sensor, which is also based on chlorophyll fluorescence. However, Saquet et al. (2000) reported that 'Conference' pears stored during 6 m at 0 ºC under different CA conditions developed internal disorders, and that the best results

in terms of avoidance of physiological disorders (less than 10 % of the fruit affected) was found when they stored 'Conference' pears at 1.5 kPa O_2, 1.5 kPa CO_2.

Fruit from different maturities and grown under different agroclimatic conditions are known to own a different susceptibility to both external and internal physiological disorders (Kadam et al., 1995), hence likely explaining the absence of physiological disorders observed in our experiments. Besides, no clear correlation was found between ET content and the chlorophyll signal, as given by the Besseling sensor, during the cold storage of 'Conference' pears. Similar results were obtained by Prange et al. (2003) who stored 'Summerland McIntosh' apples for 9 m under three different CA treatments and did not found a clear relationship between ethanol pulp content and changes in chlorophyll fluorescence. Our results strongly suggest that chlorophyll fluorescence, albeit representing alterations at the chloroplast level, do not always depict changes from aerobic to anaerobic respiration in 'Conference' pears stored under very low oxygen levels. Further research is needed to find more suitable markers of the LOL tolerated by the fruit under O_2-depleted atmospheres.

B2.3.2 Can emitted volatiles within the storage atmosphere be used as a marker of the LOL tolerated by the fruit?

It is well documented that low oxygen levels during the cold storage period affect the fruit metabolism, inhibiting the synthesis of some volatile esters and affecting their emission during the subsequent shelf life period (Chervin et al., 2000; Hendges et al., 2018; Rizzolo et al., 1991). This phenomenon may be associated to a reduced oxidation of lipids and a consequent lack of precursors for the biosynthesis of esters (Brackmann et al., 1993; Fellman et al., 1993; Lara et al., 2003). VOCs emission rates by 'Conference' pears inside the cold storage atmosphere (Fig. B2.3) were up to one thousand times lower than the ones emitted by the same pear variety during the shelf life period (Torregrosa et al., 2019). This result is not surprising but clearly showed that very sensitive equipment is needed when looking at the volatiles within cold-storage rooms (Harren and Cristescu, 2013).

In spite of their lower concentration, 22 active odor compounds were identified in our study inside the storage rooms (Fig. B2.3), including 12 esters, 3 aldehydes, 3 terpenes, 3 alcohols and 1 acid. Esters are the main contributors to the ripe pear aroma (El Hadi et al., 2013; Zlatić et al., 2016) and aldehydes generate a green and an herbaceous aroma which are typical for unripe fruit (El Hadi et al., 2013; Hendges et al., 2018).

A PCA model was developed to obtain a global view of the pear volatiles emission distribution after each of the five stresses provoked during the storage period at DLOS storage conditions. This PCA, which included the volatiles emissions, was used to characterize the different cold storage scenarios (three container's atmospheres sampled at t=30, 60, 84, 158 and 184 d, numbered from S_1 to S_5). The biplot of the two principal components captured 48.7 % of the total variability (Fig. B2.4). This relatively low explained variance was mainly due to an overlap in the information relating to volatile compounds included in the PCA yet it was sufficient for our qualitative purposes. The corresponding biplot showed that the main factor accounting for sample differentiation was the sampling dates; this finding is consistent with the higher concentration of hexanal, a-farnesene and hexyl propanoate of the pears stored for 184 d, in particular, those kept under the ILOS atmosphere. Hexanal was the main volatile compound counting for sampling date differentiation. The higher hexanal concentration found in pears from S_5 sampling date could be due to the fruit stress experienced for the low O_2 concentrations. It is known that the emission of the C_6 aldehydes, alcohols and esters derived from fatty acids through the action of lipoxygenases (Holopainen, 2004) may be increased during some biotic and abiotic stresses (Laothawornkitkul et al., 2008). However, the emission of the ester (Z)-2-hexen-1-yl acetate, other important C_6 ester, was higher after the two first stresses or sampling dates (Fig. B2.4), especially in pears from ILOS and $DLOS_1$ but was low for S_5 pears.

After the first stress S_1, $DLOS_2$ container appear located in the lower part of the cluster, what means that for this sampling date, the PC2 was important to differentiate pears stored under the three storage conditions. The pears from $DLOS_2$ showed higher concentrations of three esters (butyl propanoate, methyl butanoate and 2-methylpropyl acetate) (Fig. B2.3 and B2.4). These esters are characteristics of the pear aroma. The higher amounts of 2-methylpropyl acetate, methyl butanoate and butyl propanoate esters emitted by pears from $DLOS_2$ can be attributed to the higher oxygen level (3 kPa) observed in this container during the first 17 d of storage. It is known that the two main biosynthetic pathways of esters, that are the β-oxidation of fatty acids and LOX pathway, require oxygen. The β-oxidation of fatty acids, is the primary biosynthetic process providing alcohols and acyl coenzyme A (CoAs) for ester formation (Sanz et al., 1997). Pear esters are an example of ester formation through the β-oxidation pathway (Paillard, 1990). However, the LOX biosynthetic pathway has also the potential to provide substrates for ester production (De Pooter et al., 1983), acting as an alternative to β-oxidation of fatty acids.

After the second stress, only three acetates (2-methylpropyl, butyl and hexyl acetates) and the aldehyde hexanal showed significant differences between containers (Fig. B2.3). Pears from ILOS produced higher concentrations of 2-methylpropyl and hexyl acetates, while pears from $DLOS_1$ exhibited greater emissions of butyl acetate and hexanal. After the third stress differences along storage conditions PC2 were observed again, since the VOCs emitted by fruit stored under ILOS condition clearly differed from the ones stored under DLOS. DLOS fruit were mainly characterized by the emission of 2-ethylhexanal and 2-ethylhexanol (Fig. B2.4), even though significant differences between fruit from ILOS and DLOS were only detected in butyl acetate and hexyl 2-methylbutanoate, which showed higher concentration in DLOS pears, and in 2-methylpropyl acetate and α-farnesene, with higher emission in ILOS pears. The lower oxygen levels in $DLOS_1$ and $DLOS_2$, significantly inhibited α-farnesene emission which is consistent with the results from Chervin et al. (2000), suggesting again that the fruit maturity was more advanced in fruit stored under ILOS conditions. It is also clear that even though terpenes are considered as IVOCs, in this study α-farnesene emission seems not to be induced by the abiotic stress conditions applied. At t=152 d a prolonged stress was applied in both DLOS containers but no clear separation between storage containers were observed (Fig. B2.4), coinciding with the fact that no FO signal was detected upon the application of this stress. However, some volatile compounds showed statistical differences in their concentration (Fig. B2.3). Thus, only pears from DLOS containers emitted hexyl acetate and 2-ethylhexanol. After the fifth stress applied at t=178, figure B2.4 shows a clear separation between fruits from ILOS and the other two containers ($DLOS_1$ and $DLOS_2$) along the PC1. Fruit from ILOS container exhibited significantly higher amounts of some VOCs, such as ethyl and butyl acetate, which are typical ripening-related esters (Saquet, 2017; Torregrosa et al., 2019), hexyl propanoate, 2-methyl-1-butanol and α-farnesene. The high emitted amounts of ethyl and butyl acetate together with the lower I_{AD} values and the ethylene production pattern exhibited by fruit from ILOS container confirmed that the lower the oxygen levels during storage the higher the inhibition of the fruit ripening capacity upon removal.

Terpenes, such as α-farnesene are ethylene-dependent, since ethylene triggers the expression of AFS1, the gene encoding α-farnesene synthase (AFS), which is the enzyme responsible for the α-farnesene biosynthesis (Gapper et al., 2006). Even though, the presence of α-farnesene is reported to be related to scald disorder (Vanoli et al., 2015), no scald was observed in any fruit from the three storage conditions tested. As mentioned earlier, already from the second stress, pears from $DLOS_1$ and $DLOS_2$ exhibited lower

α-farnesene emission than those from ILOS, and thereby suggesting that the ripening inhibitory effect of low O_2 atmospheres was already noticeable during the second month of storage.

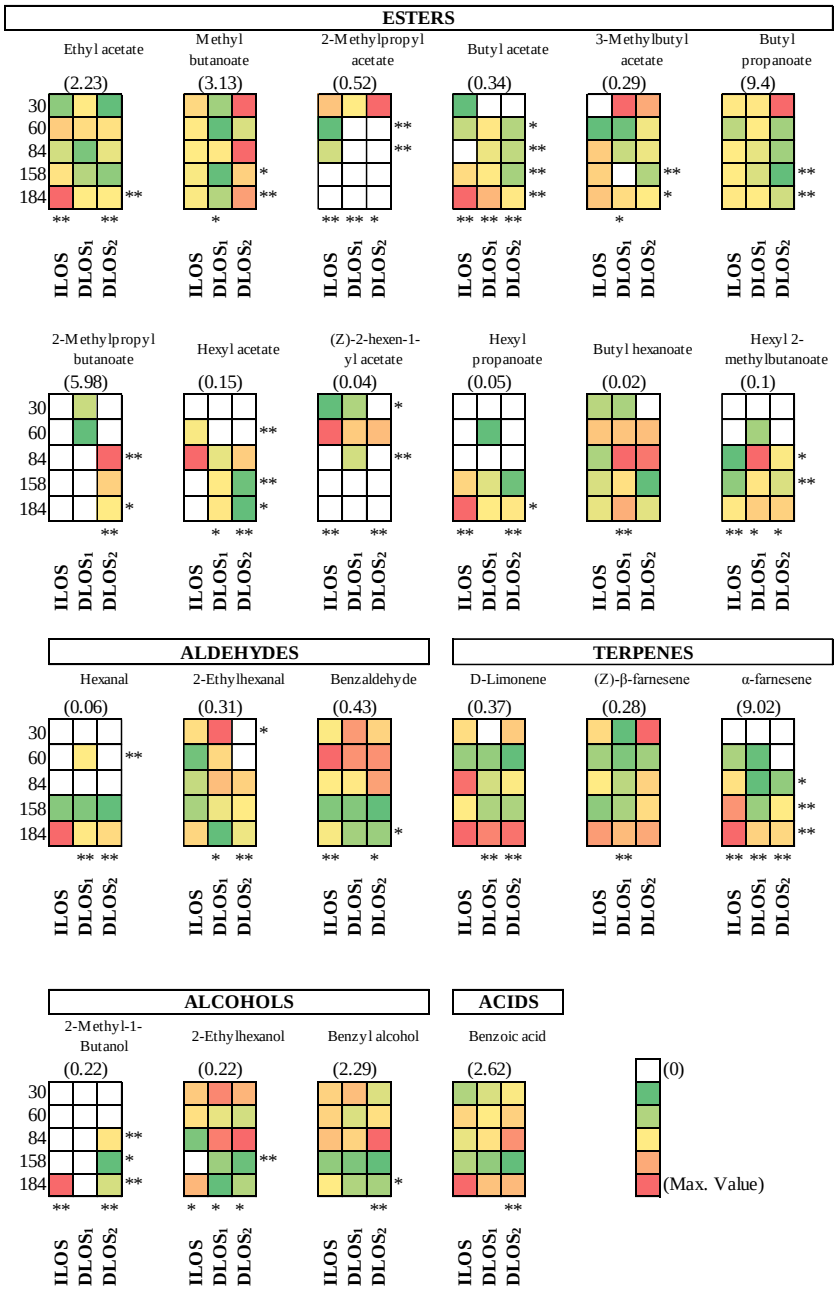

Figure B2.3 Heat map of VOCs grouped by esters, aldehydes, terpenes, alcohols and acids. Each row represents one sampling date during storage and each column represents one conservation condition (ILOS, $DLOS_1$, $DLOS_2$). Numbers in brackets under each VOC name represent the maximum emission rate in ng kg^{-1} h^{-1}. Variables of significance: * $P \leq 0.05$, ** $P \leq 0.01$ and the absence of asterisks means no significant differences, $P > 0.05$.

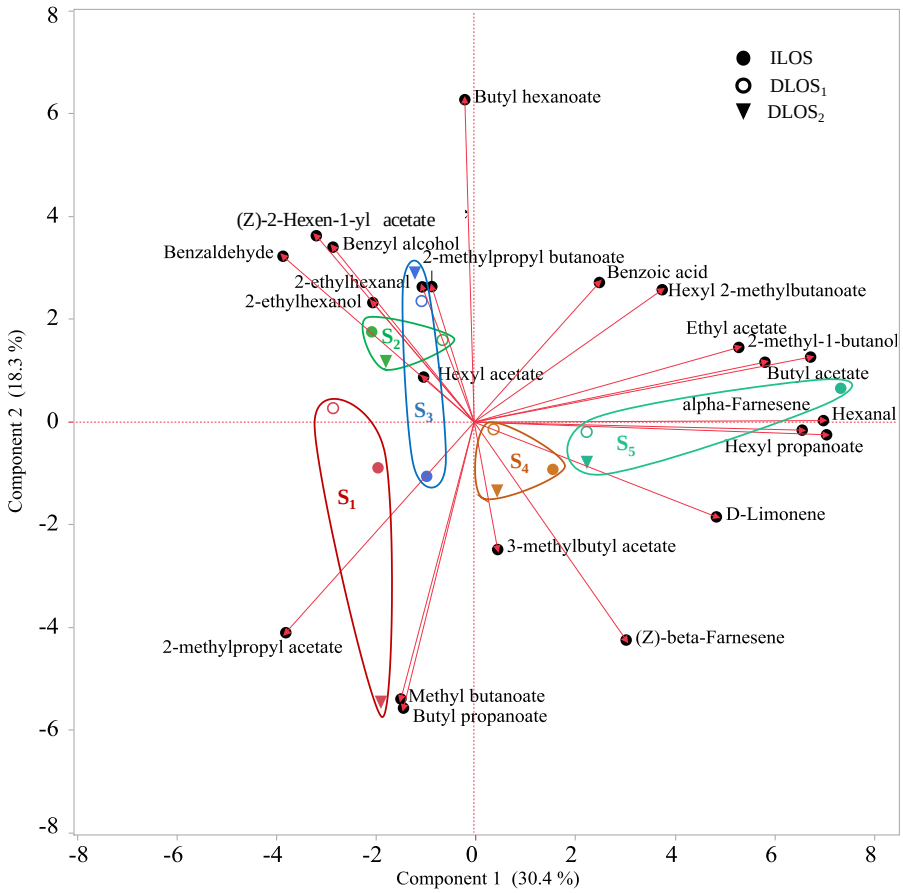

Figure B2.4 Biplot of PC1 and PC2 from a full data PCA model considering volatile organic compounds (n=22) after each stress. Data were identified in five different cluster groups, named S_1, S_2, S_3, S_4 and S_5, from three different atmosphere conditions: ILOS (●), $DLOS_1$ (o), $DLOS_2$ (▼).

Although oxygen level was forced to lower five times in $DLOS_1$ and $DLOS_2$ containers, none of the volatile substances detected showed a repeated maximum or minimum in parallel or after the fluorescence peaks. After the third stress (t=84), when ethanol accumulation in fruit pulp was higher, the emission of butyl hexanoate and 2-ethylhexanol were also higher in fruit from DLOS containers, while methyl butanoate and benzyl alcohol were higher emitted in fruit from the most restrictive container ($DLOS_2$) (Fig. 3). These results suggest that not only the amount of ethanol within the fruit pulp but also the concentration of emitted volatiles into the storage atmosphere may be employed as markers of abiotic stress (nearly to anoxia conditions) during storage of 'Conference' pears.

Although further research is needed, our results showed that IVOCs can be used not only to monitor the plant stress but also to fruit during storage.

Therefore, information on the physiological state of the fruit during storage period may be obtained with an adequate system capable of capturing and analyzing the volatiles emitted by the fruit.

B2.3.3 Impact of initial vs dynamic oxygen stresses on fruit quality and ripening capacity

Maturity at harvest determines the suitability of fruit for long-term storage (Kader, 1999), and in the case of pear fruit is measured in terms of firmness or I_{AD} index (Costa et al., 2016; Zerbini, 2002). In our study, the average firmness of pears at harvest was 61.3 N, in agreement with local recommendations as well as those published by others for long-term storage of 'Conference' pears (55-65 N; Rizzolo et al. 2015, Torregrosa et al. 2019). Firmness evolution throughout the storage period followed a similar trend in fruit stored under the three different conditions (Table B2.2). During the SL at 20 ºC after long term storage fruit firmness decreased from 65 N to approximately 17.5 N in 5 days regardless of the storage conditions (Table B2.2). Such firmness loss has also been reported in other studies (Torregrosa et al., 2019) and is linked with the highest liking degree (Kappel et al., 1995; Torregrosa et al., 2019). The I_{AD} index at harvest was 2.10±0.07, a range of values considered to be optimal in 'Conference' pears (Torregrosa et al., 2019) and 'Barlett' pears (Wang et al., 2015) for long term storage. In our experiments fruit stored under ILOS conditions had significantly lower I_{AD} values at day 5 of SL than fruit stored under DLOS conditions (Table B2.2).

The TSS/TTA ratio did not show significant differences during the storage period except after 5 d at SL, when fruit stored under more extreme conditions ($DLOS_2$) showed significantly lower values (8.5±0.4), suggesting that fruit were less ripe. This fact was confirmed with the volatile production capacity (Fig. B2.3) and ethylene data (Fig.B2.5).

Ethylene triggers the initiation of ripening in climacteric fruit with the associate physical and physiological changes in pears. 'Conference' pear, require a chilling period to start ripening (Villalobos-Acuña and Mitcham, 2008). In accordance with that, our results showed that pears harvested and maintained at 20 ºC without a cold period did not produce ethylene until day 15 (Fig. B2.5A).

Table B2.2 Physicochemical parameters evolution: Firmness (F), I_{AD} index and TTA/TSS ratio in 'Conference pears under different storage atmospheres: ILOS, $DLOS_1$ and $DLOS_2$, stresses were applied at t=24, 54, 78, 152 and 178 d in $DLOS_1$ and $DLOS_2$. Mean ± standard deviation (n=20 for F and I_{AD}) (n=3 for TSS/TTA). Different letters correspond to significant differences $P \leq 0.05$ (LSD test) between treatments. No letter indicates the absence of significant differences. *= not measured.

	F			I_{AD}			TTA/TSS		
Time (d)	**ILOS**	**$DLOS_1$**	**$DLOS_2$**	**ILOS**	**$DLOS_1$**	**$DLOS_2$**	**ILOS**	**$DLOS_1$**	**$DLOS_2$**
OHD t=0	61.3±6.2	61.3±6.2	61.3±6.2	2.1±0.07	2.1±0.07	2.1±0.07	6.1±0.8	6.1±0.8	6.1±0.8
30	67.7±7.1	71.2±7.6	70.3±4.8	^{b}2.02±0.08	^{a}2.22±0.10	^{a}2.16±0.10	5.2±0.8	5.2±1.0	6.0±0.6
60	*	73.2±11.1	71.5±6.7	*	2.07±0.05	2.11±0.07	*	5.8±0.8	5.4±0.5
158	*	64.0±7.0	67.8±5.4	*	1.95±0.13	1.93±0.15	*	6.9±0.5	6.8±1.0
202	^{a}70.1±6.0	^{b}63.5±5.3	^{b}63.7±6.7	^{b}1.69±0.20	^{a}2.00±0.13	^{a}1.93±0.19	7.5±0.7	7.0±0.9	7.1±1.0
202+5	17.9±3.4	17.7±1.9	16.6±2.6	^{b}1.30±0.27	^{a}1.57±0.20	^{a}1.60±0.21	^{a}9.6±0.8	^{a}9.5±0.6	^{b}8.5±0.4

After 30 d under cold storage, fruit from all containers started to produce ethylene at day 4 of SL, confirming the chilling requirement for ripening of 'Conference' pear. Fruit from ILOS container showed the climacteric peak one day earlier than DLOS containers (Fig. B2.5B). After the second sampling, at day 60 of cold storage, fruit started to produce ethylene just after 1 d in SL and fruit from $DLOS_1$ container reached the climacteric peak approximately two days before than fruit from $DLOS_2$ (Fig. B2.5C) thereby confirming that the lower the oxygen levels higher the inhibition of the fruit ripening capacity. After 202 d in cold storage fruit from all containers showed a postclimatreric behavior (Fig. B2.5D), characterized by a decrease in ethylene production just after the cold storage period which is typical for 'Conference' pear (Torregrosa et al., 2019).

Briefly, climacteric ethylene production rate peaks arrive earlier as the cold storage period increases, being the peak displaced to zero days (postclimateric behavior) when the cold storage period is about 200 d. Fruit stored the same period of time but under lower O_2 atmospheres showed a delay in the ethylene peak.

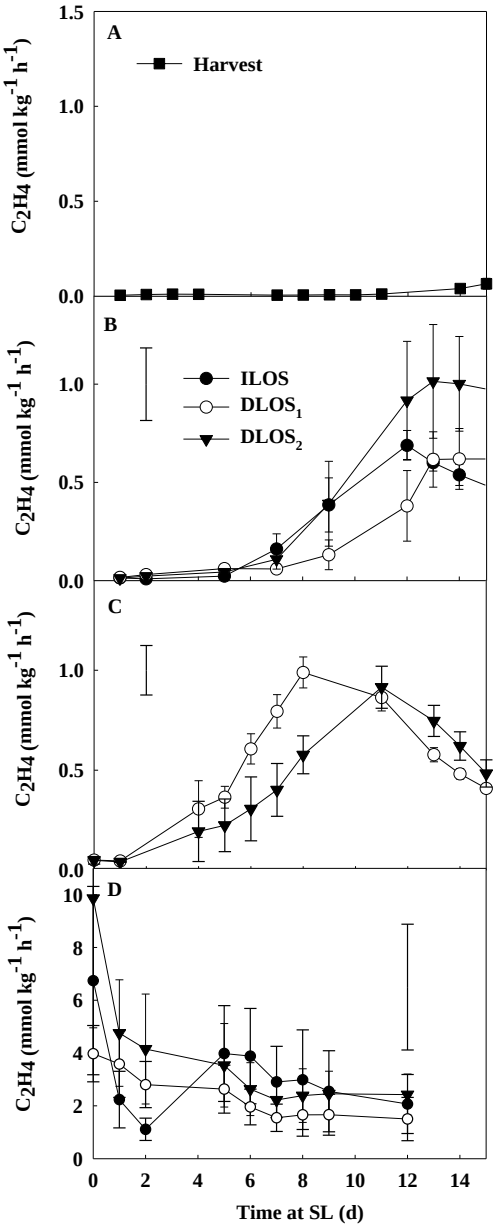

Figure B2.5 Ethylene production rate evolution of 'Conference' pears during SL at 20ºC without previous cold storage and after cold storage period of 30, 60 and 202 d under different atmospheres: ILOS (●), $DLOS_1$ (o), $DLOS_2$ (▼). A) Directly after harvest, B) after 30 d of cold storage, C) after 60 d of cold storage, D) after 202 d of cold storage. Error bars represent the mean ± standard error (n=3).

A side effect of storing pears under low oxygen level is the accumulation of ET and AA, which can result on fruit off flavors. Ethanol accumulates not only in response to anoxia (fermentation) but also during normal ripening (Pesis, 2005). ET accumulates through the fermentative

pathway, and it is an end-product while AA is an intermediate end-product (Veltman, 2002). Our results, showed an increase in ET content and AA concentrations after 5 d of SL after long-term (202 d) cold storage thereby highlighting that fruit was undergoing normal ripening. Significant lower ET levels were found in $DLOS_1$ and $DLOS_2$ containers, reflecting a slower SL ripening pattern of the fruit when stored under more restrictive storage conditions (Fig. B2.6). Our results are in accordance with the ones reported by Chervin et al. (1999), who found lower ethanol levels in Packham's Triumph pears stored for 2 m at 3 kPa O_2 and <0.2 kPa CO2 plus 18 d in SL (12.5 µmol g^{-1}) than under normal air (20 µmol g^{-1}). It has been reported that 'Conference' pears stored under different conditions produced AA levels in the range of 1-3 µL L^{-1} (Saquet and Streif, 2006), which was in accordance with our results during the whole storage period (0.5-3 µL L^{-1}, data not shown). However, after the day 8 of SL their pears produced half of AA (8-12 µL L^{-1}) than ours after 5 d of SL (20-26 µL L^{-1}, Fig. B2.6B).

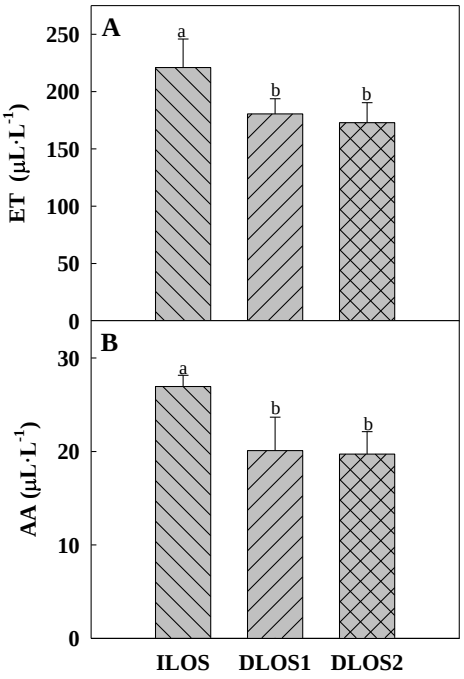

Figure B2.6 A) Ethanol content (ET) and B) acetaldehyde (AA) content in 'Conference' pears under different storage atmospheres: ILOS, $DLOS_1$ and $DLOS_2$ after 202 days of storage plus 5 days of SL. Error bars represent the mean ± standard deviation (n=3). Bars with small letters are significantly different on the bases of LSD test at $P \leq 0.05$.

Fruit respiration is an oxidation of substrate with O_2 consumption and CO_2 production. The velocity at which this process occurs is an indicator of the maturity state of the fruit. Respiration is a complex chain of reactions but can be successfully approximated with a simple enzyme kinetics model

(Kandasamy et al., 2015; Ravindra and Goswami, 2008; Wang et al., 2009). Respiration depends basically on fruit temperature and environmental gas conditions, O_2 and CO_2 concentrations (Hertog et al., 1998; Ho et al., 2018). Ravindra and Goswami (2008) analyzed the temperature effect on mature green mango respiration and they reported an increasing trend for V_m and k_{mO2} parameters and a decreasing tendency for k_{iCO2} as the surrounding temperature increased, indicating higher CO_2 production rate. Wang et al. (2009) reported a similar situation analyzing the guava fruit respiration at different temperatures.

The goodness of the used model (eq. 3 and 4) to fit the in jars measured O_2 and CO_2 concentrations after a period of 30 days and 202 days is shown in Fig. B2.7. Our results did not show differences in respiration rate depending on the storage conditions after 30 d or after 202 d. Differences in fruit ripening capacity were not visible in the respiration rate evolution. Similarly, Both et al. (2014) studied respiration rate in 'Royal Gala' apples after 8 m storage under different oxygen partial pressure (0.5, 0.7, 0.8 and 1 kPa) and only found significant differences one day after removal from cold room in apples stored at 0.5 kPa but no significant differences were found thereafter.

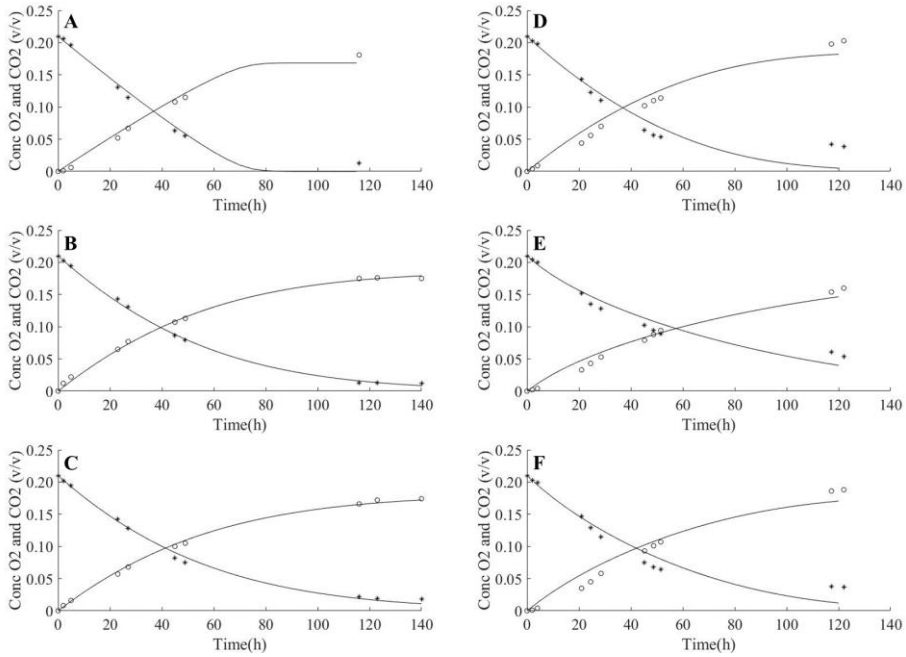

Figure B2.7 Respiration tests carried out in fruit after 30 d (left subplots) and after 202 d (right subplots) in cold storage under different conditions: A, D) ILOS; B, E) $DLOS_1$ and C, F) $DLOS_2$. Measured O_2 (*) and CO_2 (o) concentrations were fitted to the model (eq. 3 and 4) (continuous lines).

The estimated values of the kinetic parameters are shown in Table B2.3 with the corresponding coefficients of determination (r^2) for O_2 and CO_2, after four different cold storage periods. After 30 days in cold storage all coefficients of determination were higher than 0.98, however, after 202 d the goodness of the fit was reduced to 0.92. This is due to the fact that after 202 d of storage there was a respiration lag at the beginning of the fruit respiration as observed during the first 20 h when $\frac{\Delta co_2}{\Delta t}\Big)_{202\ d} < \frac{\Delta co_2}{\Delta t}\Big)_{30\ d}$ (Fig. B2.7). The model does not contemplate this lag and therefore the fits after 202 d of storage were weaker.

Table B2.3 Values of the best fit parameters of the used respiration model when the fruit was kept in air-tight jars at room temperature (20 ºC) after different cold storage periods in chambers under different atmospheres. Coefficients of determination for each variable of the model, υ_{O2} and υ_{CO2}, are also given ($r^2_{O_2}$ and $r^2_{CO_2}$, respectively).

Chamber	Storage period (d)	Vm (ml_{CO2} h^{-1} kg^{-1})	km_{O_2} (v/v)	ki_{CO_2} (v/v)	RQ (mol_{CO2}/mol_{O2})	$r^2_{O_2}$	$r^2_{CO_2}$
ILOS	30	9.32	0.01	1.40	0.80	0.992	0.988
$DLOS_1$		28.82	0.36	1.61	0.89	0.999	0.997
$DLOS_2$		33.16	0.51	2.05	0.87	0.996	0.998
$DLOS_1$	60	6.92	0.02	0.82	0.96	0.991	0.986
$DLOS_2$		8.06	0.03	1.15	0.92	0.994	0.987
$DLOS_1$	156	27.08	0.48	0.05	0.88	0.991	0.995
$DLOS_2$		7.84	0.02	0.99	0.98	0.996	0.995
ILOS	202	12.22	0.12	0.38	0.89	0.925	0.964
$DLOS_1$		35.45	0.52	0.02	0.86	0.952	0.977
$DLOS_2$		17.76	0.17	0.11	0.86	0.948	0.960

B2.4. Conclusions

The application of periodic low oxygen stresses, as generally done during dynamic controlled atmosphere storage, inhibits the ripening capacity of 'Conference' pears as indicated by the changes in the I_{AD} values and the ethylene production pattern or even the synthesis of some typical ripening related volatiles (ethyl and butyl acetate). Our data clearly show that the longer the oxygen stress period the higher the inhibitory effect. FO did not peak after all the oxygen pull downs, and was poorly correlated with the ethanol flesh content. FO peaks were to some extent in accordance with the induction of specific VOCs emission as highlighted by our multivariate data analysis. Based on our results, some esters such as methyl butanoate and butyl hexanoate and specific alcohols such as 2-ethylhexanol and benzyl alcohol can be considered as IVOCs as were highly emitted after the application of an imposed low oxygen stress and preceding the accumulation of ethanol within the fruit flesh. Accordingly, a reliable system to capture and to analyze VOCs periodically

during the dynamic controlled atmosphere storage could facilitate potential information about the LOL tolerated by the fruit. The oxygen consumption and CO_2 production of pears after 30 d in storage was accurately modeled using Michaelis-Menten kinetics whereas after 202 d the start of respiration was slower and the model did not contemplate this lag.

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CHAPTER B3: Using an ultrasound sensor for the estimation of fruit mass loss during cold storage

Abstract

An experimental device to continuously measure fruit diameter shrinkage, fruit settlement inside a container and fruit mass loss during the cold storage period was designed and tested. Temperature and air relative humidity (RH) evolution within the cold room were also recorded.

Individual fruit diameter shrinkage was measured using dendrometers and the shrinkage rate was affected by the cold room RH. Small fruit presented higher shrinkage rates (2.5 %) than bigger ones (1 %). The use of individual fruit shrinkage as an indirect measure of the moisture loss in a whole commercial cold room is not a reliable technique because of its lack of representativity of what is happening in the whole cold room.

The settlement of the whole mass of pome fruit inside a container during the long-term cold storage period was measured by means of an ultrasound sensor. It was found that this settlement was well correlated with fruit mass loss. Under all tested experimental conditions, including apples and pears in different seasons, the rate of mass loss and mass settlement presented a quasi linear relationship during the whole storage period. Changes in the air RH of the cold room induced changes in the mass loss rate.

According to our results the fruit mass settlement can be used as an indirect measurement of fruit moisture loss to monitor the RH in the cold room.

Keywords: dendrometer, fruit settlement, ultrasound sensor, weight loss, fruit shrinkage

B3.1 Introduction

Apples and pears are one of the most consumed fruit worldwide. In the region of Lleida (N 41º 36' 25"; E 0º 37' 51") 'Golden Delicious' apples and 'Conference' pears are the main cultivated varieties. Quality of pip fruit, such as apples and pears, depends on several factors, but in terms of consumer's choice, visual appearance is the main factor at the time of purchase (Barman et al., 2005).

In order to fulfil the consumer requirements all year round, pome fruit are traditionally harvested at the optimal harvest date (OHD) and stored up to 6 months in cold rooms at -1 to 0 ºC, depending on the cultivar, and at a relative humidity higher than 90 % under normal air atmosphere (NA) (Drake et al., 2004; Saquet, 2018; Wang and Sugar, 2013). Nowadays, there are different storage systems to prolong the storage period. One of the most used is the controlled atmosphere (CA) where low oxygen and CO_2 levels (2.5 kPa O_2, 0.7 kPa CO_2) are maintained in the cold room. The goal of the cold storage under CA is to delay metabolic processes and thus prevent the changes that occur in the quality parameters of the fruit during ripening (Wills et al., 2007). It has been reported (Torregrosa et al., 2019) that 'Conference' pears stored in cold rooms under DCA slowed down their metabolism in such a degree that after 8 months their firmness was reduced only in 5 %.

Moisture loss during long term storage represents a double economic loss, first because the saleable weight of the fruit is reduced and second, because the loss of fruit visual apparent quality due to shriveling (Nguyen et al., 2007). Moisture loss can be reduced with a proper control of the storage conditions, mainly temperature and air relative humidity (RH) (Tolesa and Workneh, 2018). It is well known that high levels of relative humidity do better preserve the water content of the fruit but too high levels can promote the development of undesired fungus, thus the proper levels will depend on the cultivar (Zagory and Kader, 1989). To maintain the RH at an established level is not an easy task, since air humidity condenses and air dries when passing through the evaporator of the cold room. Lower evaporation temperatures reduce the heat transfer area required by the evaporator but increases condensation and reduces air relative humidity. Evaporation at high temperatures, close to 0 ºC, reduces the air drying but bigger and more expensive equipment is required. In practice, RH of the air in a cold room oscillates and direct water spraying is necessary when too low levels are reached.

There are several methods to monitor dehydration in practice, such as monitoring the amount of defrosting water (Van Schaik et al., 2005), or fruit

hand weighting at regular periods during storage (Tu et al., 1996). Once an excessive dehydration of fruit is detected, the relative humidity in the room is raised by direct spraying of water. However, to our knowledge scarce information exists describing a system to continuously monitor fruit weight loss during storage.

Traditionally, fruit are stacked inside a commercial container (1.0x1.2x0.76 m) up to a high around 0.5-0.8 m. In practice it is well known that during the storage period the mass of fruit inside a container settles about 4-8 % of its initial height. The main factors affecting that settlement are the viscoelastic behavior of fruit when submitted to the compression stress caused by its own weight and the shrinkage of fruit due to moisture loss (Kim et al., 2008). For a given container and fruit variety the first factor is relatively constant and the second is directly linked to the air RH inside the cold room. To our knowledge there are no studies in the literature reporting the relation between mass settlement and weight loss during the cold storage period of the fruit.

With such a background, the aims of this study were: 1) To register, during the fruit storage in different commercial cold rooms, the evolution of: weight loss, fruit diameter, global settlement of the fruit mass inside a container, temperature and air relative humidity. 2) To explore the relationship between global settlement and fruit weight loss.

B3.2 Materials and methods

B3.2.1 Fruit varieties

All experiments were conducted on ‘Golden Delicious’ apples and ‘Conference’ pears which were harvested from different commercial orchards near Lleida and transported to the commercial packinghouse. ‘Golden’ apples were harvested in October 2016 and 2017 with an average firmness of 66.7 N and ‘Conference’ pears in August 2017 and 2018 with an average firmness of 70.0 N.

B3.2.2 Storage conditions

Fruit was stored in commercial cold rooms according to the common practice in the region. The cold rooms were approximately 8.5 m wide, 14.5 m deep and 7.5 m high and filled with about 150 metric tons of fruit, leaving a separation between the door in the front wall and the stowage of about 3 m. Each room was refrigerated with one evaporator with two fans located above the door and had a water spraying system to control the air relative humidity located next to the air outflow of the evaporator.

Table B3.1 Storage conditions of the fruit in each of the three seasons included in the present study. Place refers to the commercial packinghouse with corresponding cold room. Installed sensors: Dendrometer (Dendr) on a fruit; Scale (Sc); Ultrasound sensor (US); Temperature sensor (T); Air relative humidity sensor (RH). Atmosphere conditions type refer to: Normal air (NA); Controlled atmosphere (CA). Set point values of: Temperature (T); air relative humidity (RH); oxygen concentration (O_2); CO_2 concentration (CO_2).

			Storage period				Atmosphere conditions				
Season	**Fruit**	**Cold room**	**Start date**	**End date**	**Duration (d)**	**Installed sensors**	**Type**	**T (ºC)**	**RH (%)**	**O_2 (%)**	**CO_2 (%)**
2016-2017	Golden D	BF	24/11/2016	19/05/2017	177	4 Dendr+Sc+2US+2T+2RH	NA	1.0	90-95	21	0.0
2017-2018	Golden D	BFC12	11/10/2017	09/03/2018	126	2 Dendr+Sc+2US+2T+2RH	CA	0.0	85	2.5	2.0
	Conference	BFC13	30/08/2017	26/01/2018	148	2 Dendr+Sc+2US+2T+2RH	CA	0.0	90	2.5	1.0
2018-2019	Conference	SCFC16	29/08/2018	09/01/2019	133	Sc+2US+2T+2RH	CA	-0.5	90	1.5	0.6
	Conference	SCFC17	29/08/2018	14/01/2019	138	Sc+2US+2T+2RH	CA	-0.8	95	1.0	0.5

In the experiments conducted during the 2016/17, season apples were stored under normal air (NA) atmosphere at 1.0 ºC and 90 % RH during the first 4 months; afterwards the air humidity was increased up to 95 %. In the experiments executed during the two following seasons fruit was stored under controlled atmosphere (CA) at 0 ºC, apples at 85 % RH and pears at 90 % RH (Table B3.1). Defrosting of the evaporators, one of the main cause of water loss from cold rooms, was carried out every 3 hours of cold operation, which means 1-2 times a day once rooms were stabilized, and it was performed with water. During the 2018/19 season, moisture contribution was made by spraying 0.59 L/time of water at ambient temperature for 5 minutes every hour.

B3.2.3 Experimental setup

Once fruit arrived to the packinghouse, a plastic container (800x600x620 mm) was filled out up to 10 cm below the upper edge and placed inside a commercial cold room on a stainless-steel scale (600x600 mm) IP-65 (Bàscules Mor, Lleida) which had a maximal weight capacity of 150 kg. The scale was wired to a stainless-steel weighing terminal provided with a RS-232 output which was placed outside the cold room. Two distance ultrasound sensors (US) were mounted on a rigid transversal bar leaning on the edge of the container (Fig. B3.1). The ultrasound sensor UC2000-30GM-IUR2-V15 (Pepperl-Fuchs, Germany) had a distance detection range between 80 and 2000 mm with an accuracy ≤0.1 % of full-scale value, IP-65, with a working temperature range from -40 to 85 ºC, and was provided with a 4-20 mA output. Inside the container and on the fruit were placed two temperature sensors T03 (JUMO, Germany) with a 2 % accuracy, and two air relative humidity sensors (JUMO, Germany) with 2.3 % accuracy.

Four DF type dendrometers (Ecomatik, Germany) embracing one fruit each around the equatorial zone, were installed during the 2016/17 season. Dendrometers were used to continuously measure fruit diameter evolution with an accuracy of 0.2-0.5 µm, and had a working temperature range from -30 to 40 ºC and a RH range from 0 to 100 %. All sensors were connected to a computer outside the cold room and data were recorded every 5 minutes.

The sensors were placed in a symmetrical arrangement with respect to the vertical central plane of the container forming two independent groups (G1 and G2), each group consisting of a US distance sensor, one temperature sensor (T), one RH sensor and two dendrometers (D). Oxygen and CO_2 concentration measurements in CA rooms were registered by the room's management system.

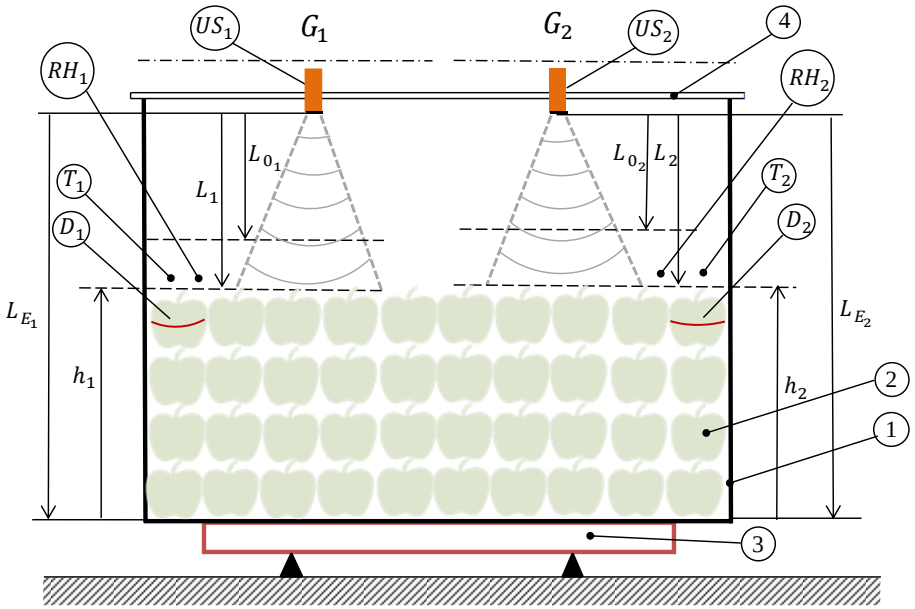

Figure B3.1 Scheme of the experimental setup used to measure fruit weight loss and fruit settlement inside a commercial cold room: 1) Plastic container, 2) Mass of fruit stacked inside the container, 3) Scale, 4) Supporting rigid bar, US) Ultrasound sensor, T) Temperature sensor, RH) Air relative humidity sensor, D) Dendrometer embracing one fruit. *L* denotes the distance measured by the US sensor, being L_0 the distance at the start of the measurement (at t=0) and L_E the measured distance when the container was empty. The height of the fruit mass (*h*) is then the difference $h = L_E - L$. Two independent groups of sensors (G_1 and G_2) were used.

B3.2.4 Data analyses

Data was downloaded to a spread sheet (Excel) and then read from Matlab (The Mathworks Inc., Natick, USA). Data from the scale and the US were filtered. Each data time-series was first fitted to a second order polynomial, then the points which separated more than 5 % of the fit were disregarded.

Dimensionless mass loss was calculated as: $\delta\,(\%) = \frac{M_0 - M_t}{M_0} \cdot 100$, were M_0 is the initial mass of fruit at t=0 on the container and M_t is the mass of fruit at time *t*. Dimensionless settlement of the fruit inside the container was calculated as: $\xi\,(\%) = \frac{L_t - L_0}{L_E - L_0} \cdot 100$, were L_E was the US measurement when the container was empty, L_0 was the US measurement with the container full of fruit at t=0, and L_t was the US measurement at an arbitrary time *t*.

B3.3 Results and discussion

B3.3.1 Diameter shrinkage in 'Golden' apples

Several studies in fruit crop management have used digital dendrometers, for example, to measure tree trunk diameter fluctuations caused by water irrigation (Corell et al., 2014; Ginestar and Castel, 1998), or to measure fruit diameter evolution during on-tree growth (Domínguez et al., 2012; Link et al., 1998). In the present study dendrometers were used to register the fruit diameter evolution during the cold storage period (Fig. B3.2).

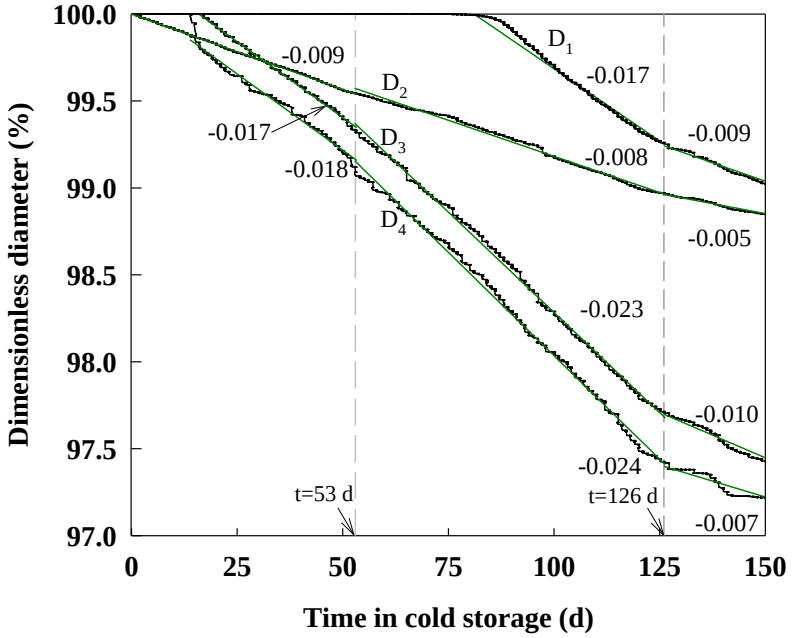

Figure B3.2 Dimensionless diameter ($D/D_0 \cdot 100$) evolution (D_1, D_2, D_3 and D_4) during the cold storage period of each of the four apples provided with a dendrometer during the 2016/17 season. D_1 and D_2 correspond to fruit with initial diameter between 80-85 mm, D_3 and D_4 correspond to fruit with initial diameter between 60-65 mm. Vertical dashed lines separate periods with different air RH. Straight lines are the linear fits in each RH period and its slope is given by black numbers.

Due to unknown reasons two of the used dendrometers (D_1 and D_3 Fig. B3.2) got stuck at the beginning of the experiment, D_1 for nearly half of the storage period, D_4 for only 3 weeks. In all of them a progressive diameter reduction was recorded and the highest reduction rate correspond to the periods with lower RH in the cold room (Fig. B3.3A). It is interesting to observe the parallelism of the lines corresponding to the smallest fruit (D_3 and D_4, Fig. B3.2). Small apples reached a maximal reduction of 2.5 % and bigger ones only 1 %. The fact that the smaller fruit has higher relative diameter shrinkage is in accordance with the observation that smaller fruit has also higher weight

loss (Van Schaik et al., 2005) which is due to the higher surface/volume ratio (Banaras et al., 1994).

Fig. B3.3A shows the evolution of temperature and RH of the air in the cold room. The temperature measurements recorded by the two sensors showed a maximal difference of 0.2 ºC and the RH measurements did not differ in more than 1 percentage point. It can be observed that the RH set point was raised from 90 to 95 % at t=126 d. That sudden increase in the air RH was reflected in the decreasing rate of the fruit diameter shrinkage (D_2, D_3 and D_4, Fig. B3.2) stating the inverse relationship between RH inside the cold room and diameter shrinkage rate. Measuring the moisture loss of the fruit in a cold room based only on the shrinkage of a few fruit does not seem a reliable technique, since in the experiment one of the four dendrometers presented a malfunction (D_1, Fig. B3.2). Such lack of representation of the on-one fruit direct measurements was also observed during season 2017/18 both in 'Golden' apples and in 'Conference' pears and consequently were not used in the following season (Table B3.1).

B3.3.2 Weight loss and settlement in 'Golden' apples

As shown in Fig. B3.3A, the temperature of the cold room during the 2016/17 season remained between 1 and 1.5 ºC except for some peaks caused by the opening of the door and by a power outage. The relative humidity evolution can be divided into 3 different periods, from t=0 to t=53 d, from t=53 to t=126 d and after t=126 d (Fig. B3.3A) with average RH values of 90, 88 and 94 % respectively. The RH increase in the third period was caused by the activation of the cold room humidification at t=126 d when the RH set point was raised from 90 to 95 %.

Fruit mass and settlement were measured every 5 min during the whole storage period (Fig. B3.3B). One of the US sensors got stuck and provided the same measurement during the first 21 d of storage.

Fruit mass had an almost linear decreasing trend during the first 126 d, thereafter, when the RH of the room was raised, the rate of mass loss slowed down (Fig. B3.3B). The observed global tendency was confirmed by measurements in 'Golden' apples stored under CA conditions during the 2017/18 season (Fig. B3.3C and B3.3D). In that experiment only two RH periods were identified, with a small average RH difference, prior to t=54 d RH average was 89 %, thereafter was 92 %. Some malfunctions appeared during that experiment: at t=54 d a power outage stopped the scale readings until t=68 d; at t=108 d the US registration was lost during nearly 2 weeks and, due to unknown reasons, the US measurements manifested weekly oscillations.

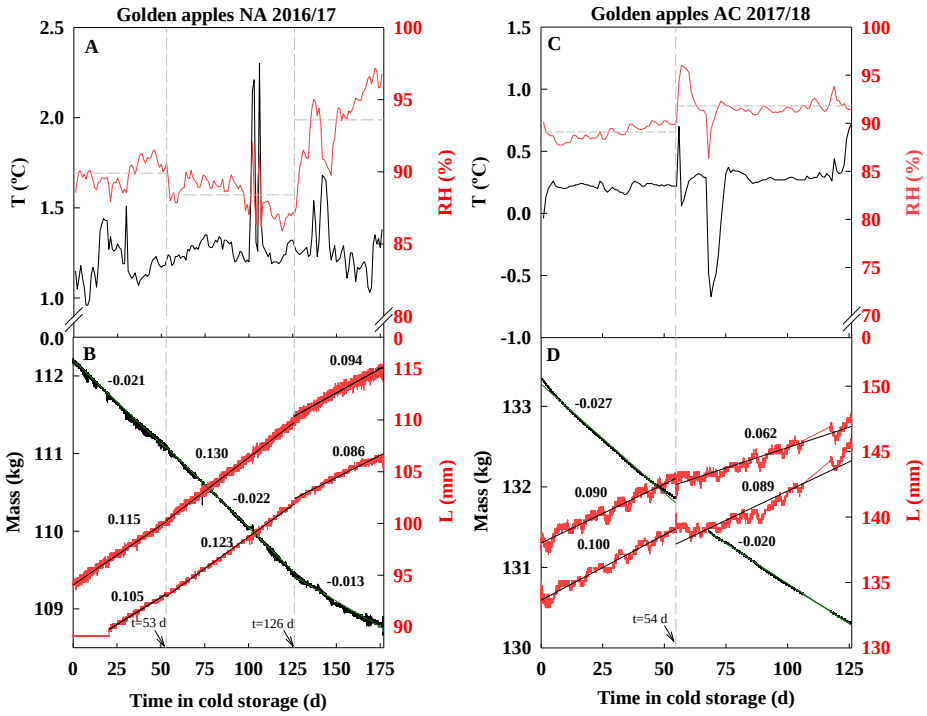

Figure B3.3 Time evolution of storage conditions, mass loss and settlement of 'Golden' apples during the storage seasons 2016/17 (under Normal Air atmosphere NA) and 2017/18 (under Controlled Atmosphere CA). A, C) Cold room's temperature (T) and relative humidity (RH). B, D) Fruit total mass and US measurement (L). Discontinuous vertical lines separate periods with different RH average and discontinuous horizontal lines represent the average RH value in each period. Black numbers correspond to the slope of the linear fitting in each period.

A linear negative correlation was found between mass and settlement in all the analyzed periods. During the 2016-2017 season correlation coefficients for the two first periods were higher than 0.99 and decreased to 0.97 after 126 d when the humidification was activated. During the 2017-2018 season lower correlation coefficients were found. Until day 54 of the storage period the coefficient was 0.96 and decreased thereafter to 0.86 (data not shown).

The relationships between dimensionless mass loss and dimensionless settlement in apples corresponding to the consecutive seasons 2016/17 and 2017/18 are shown in Fig. B3.4. The cloud of points correspond to one measurement every 5 min (Fig. B3.3). It is worth to observe that the linear relationship was maintained even when the mass and settlement curves deviate from the initial straight line after t=126 d when the air RH was increased by the sprayers (Fig. B3.3B). The weekly perturbations in the US measurements recorded during the 2017/18 season are responsible for the greater dispersion of the cloud.

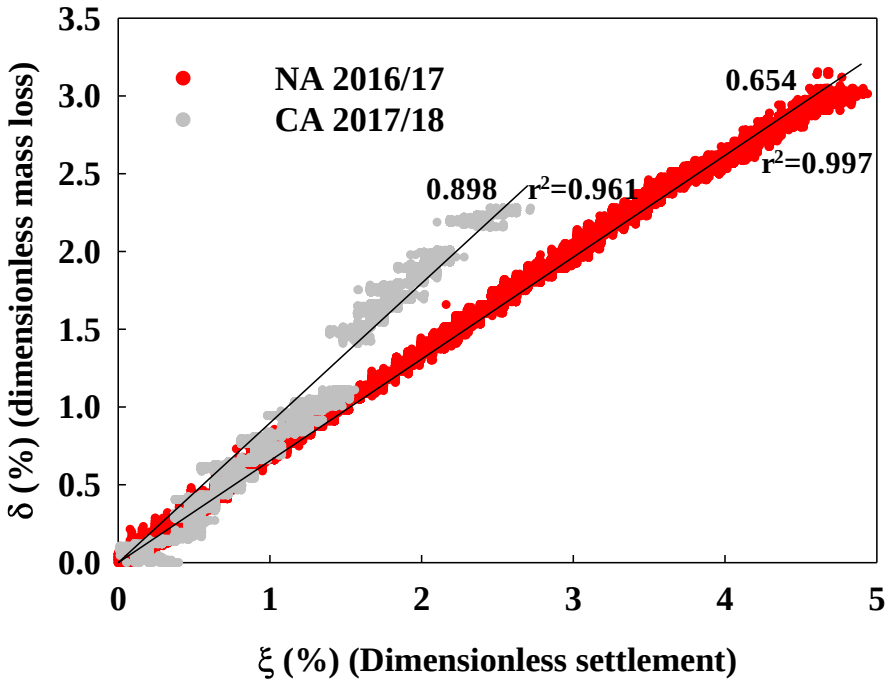

Figure B3.4 Relationship between dimensionless mass loss and settlement during the storage of 'Golden' apples. Linear fits were forced to pass through the origin. Black numbers above the linear fit correspond to the slope of the fitting and numbers underneath represent the correlation coefficient. The fits contain about 50000 points, 175 d season 2016/17 (36000 points, 125 d season 2017/18) corresponding to one point every 5 min.

B3.3.3 Weight loss and settlement in 'Conference' pears

In the measurements carried out during the 2017/18 season 'Conference' pears were stored under CA conditions at an average temperature of 0 ºC while the air RH increased from 83 % at the beginning of the storage up to 93 % by the end (Fig. B3.5A). Two different periods can be distinguished, the first, until day 54, with an average RH of 86 % and the second, up to the end of the storage, in which the average RH was 90%. The registered scale signal shows a quite smooth decreasing trend with only a small perturbation, at day 134, simultaneous with the sudden peak of temperature (Fig. B3.5B) caused by an opening of the door. The signal provided by the US sensors was not so stabile and was filtered. These two periods could also be identified in fruit mass and settlement evolution (Fig. B3.5B), with a steepest trend during the first period caused by lower air RH in the cold room. In all trials the two US sensors showed a parallel trend.

In the 2018/19 season the study was conducted in two commercial chambers under CA conditions. Average cold room temperatures were -0.5 and

-0.8 ºC and the air RH increased during the storage period from 85 % to 95 % approximately (Fig. B3.5C and B3.5E). Two different RH periods could also be distinguished, each with a corresponding different slope in the mass and settlement evolution (Fig. B3.5D and B3.5F).

Fruit mass loss showed a gradual decrease in both cold rooms, with a lower slope after 54 d in storage caused by the highest RH in that second period. This tendency was also observed in the settlement evolution (Fig. B3.5D and B3.5F).

The relationship between dimensionless mass loss and settlement during the storage of 'Conference' pears is shown in Fig. B3.6. The first 10 days of storage were disregarded due to the fluctuation in the US measurements at the beginning of 2018/19 season (Fig. B3.5D and B3.5F). It should be noted that in our experiments the average dimensionless settlement was higher in pears (5-7 %) than in apples (3-5 %) and that in average, for a given settlement, the dimensionless mass loss in apples was higher than in pears (Fig. B3.4 and B3.6). However, with our data, we could find no clear explanation for the different slope of the fits. In further experiments, it would be interesting to measure the firmness of the fruits and parameters describing its maturity.

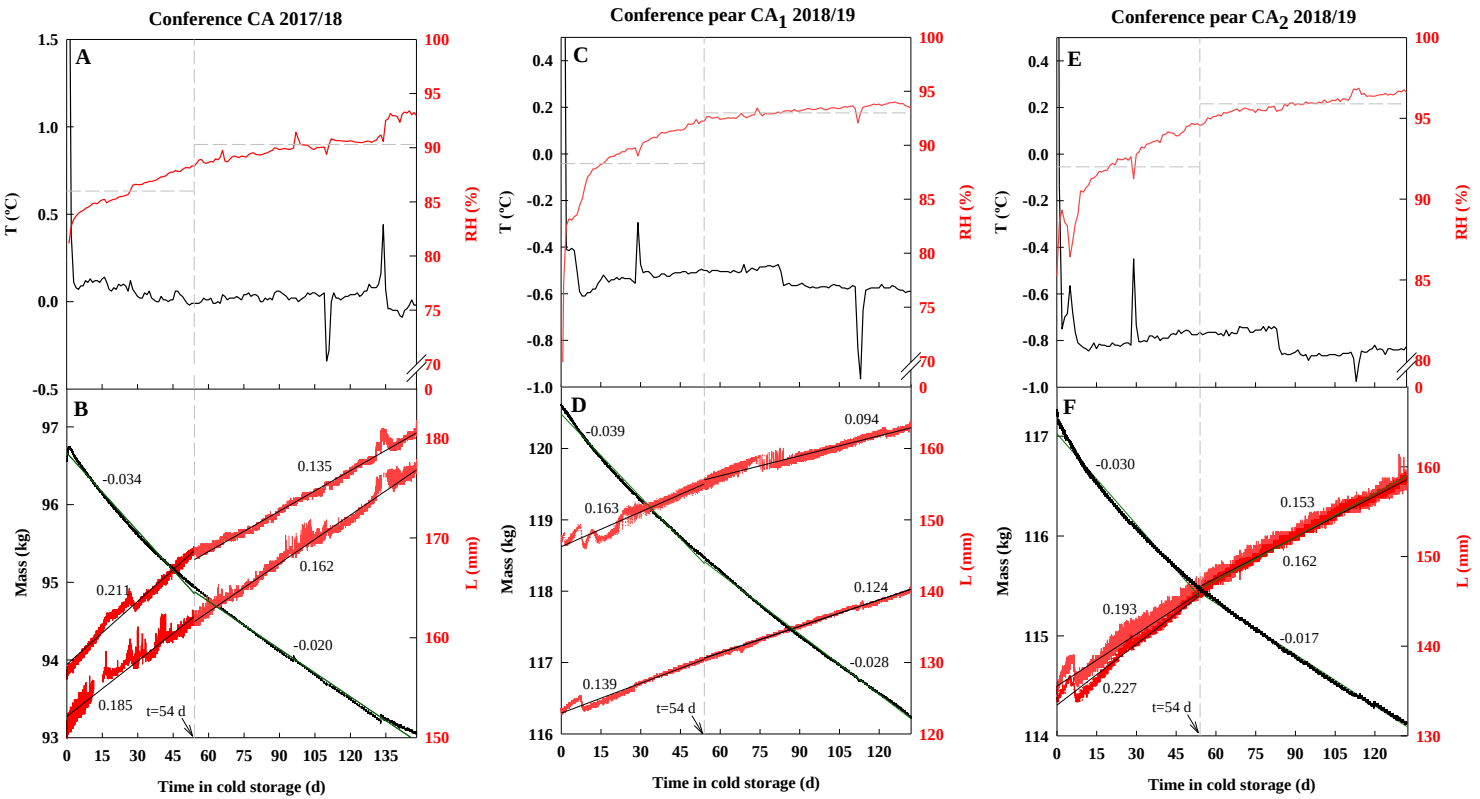

Figure B3.5 Time evolution of storage conditions, mass loss and settlement of 'Conference' pear during the storage seasons 2017/18 and 2018/19 under Controlled Atmosphere. A, C, E) Cold rooms' temperature (T) and relative humidity (RH). B, D, F) Fruit total mass and US measurement (L). Discontinuous vertical lines separate periods with different RH average and discontinuous horizontal lines represent the RH average value in each period. Black numbers correspond to the slope of the linear fitting in each period.

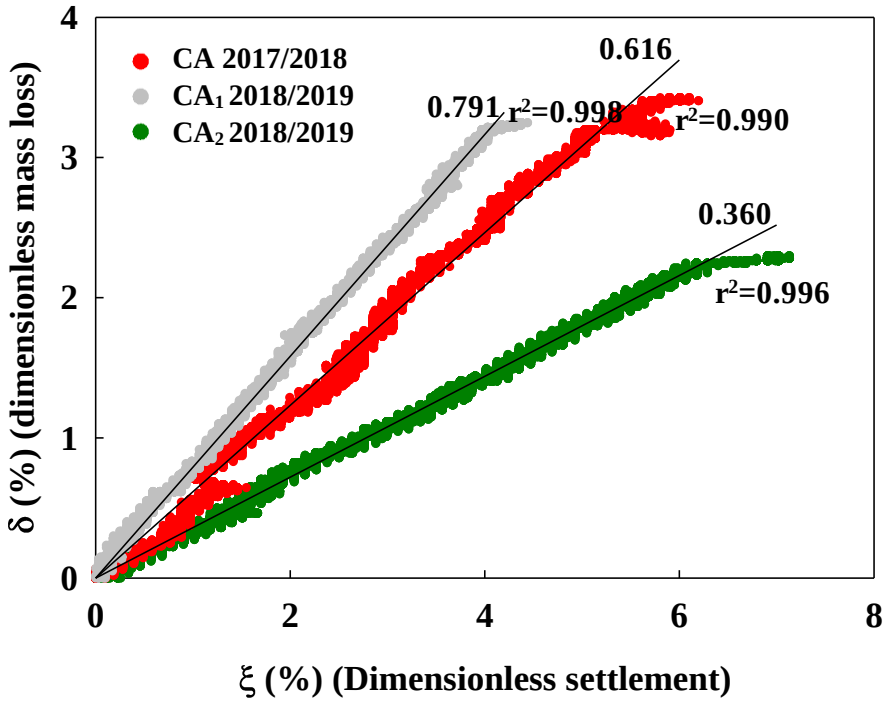

Figure B3.6 Relationship between dimensionless mass loss and settlement during the storage of 'Conference' pears. Linear fits were forced to pass through the origin. Each cloud contains about 36000 points, one measured every 5 min, 10 first days were disregarded. Black numbers above the linear fit correspond to the slope of the fitting and numbers underneath represent the correlation coefficient.

B3.4. Conclusions

Fruit diameter shrinkage reflects relative humidity changes inside the cold room. However, the diameter measurement based on individual fruit is not a reliable technique to account for the moisture loss of the fruit in the whole cold room due to the lack of representation.

Measurements of fruit mass loss and mass settlement during the three analyzed seasons showed a linear correlation, both in apples and pears. The measurement of the settlement using an ultrasound sensor is a quite simple technique that can be used to monitor the dehydration of fruit in a commercial cold room and hence to control the humidification system. Modifications in the relative air humidity directly affected both, fruit mass loss and settlement rates.

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Part C: QUALITY CHANGES DURING THE SHELF LIFE PERIOD

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CHAPTER C1: Ripening behavior and consumer acceptance of 'Conference' pears during shelf life after long term DCA-storage

Abstract

With the increasing demand for ready to eat fruit, understanding how pear quality evolves during shelf life (SL) is of paramount importance for retailers. Accordingly, the relationships between physicochemical quality parameters, the emission of volatile compounds and consumer satisfaction were investigated in 'Conference' pears from different orchards and stored at 20 ºC following 8 months of cold storage (-0.5 ºC) under dynamic controlled atmosphere (DCA). Our results showed that DCA storage strongly inhibits firmness loss (<5 %) without negatively affecting other quality traits. Upon removal from cold storage and ripening at 20 ºC, 'Conference' pears loss nearly 80 % of its initial firmness in only 5 d. Firmness evolution from harvest to 5 d of SL was successfully fitted with a reverse Gompertz equation ($R^2 > 0.96$). Prolonged DCA storage of 'Conference' did not completely impede ripening as indicated by the reducing trend of I_{AD} and the ethylene postclimacteric behavior of the fruit during SL. In parallel to the decrease of firmness during SL, there was a consistent increase in most ester-type volatiles and especially in hexyl acetate and butyl acetate. Generally, the highest consumer satisfaction after DCA cold storage of 'Conference' pears was reached after 3 d at 20 ºC. In this sense, the most appreciated pears by consumer were those showing high flavor in combination with firmness values in the range of 10-30 N. The Partial Least Square (PLS) model showed that total soluble solids (TSS), the ratio TSS/TTA (total titratable acidity), consumer flavor perception and some particular volatile compounds (i.e. methyl, ethyl and hexyl acetates as well as ethyl trans,cis-2,4-decadienoate) were positively correlated to consumer's overall liking while firmness, TTA and index of absorbance difference (I_{AD}) had a negative correlation and higher prediction capability.

Keywords: esters, overall liking, physicochemical parameter, PCA, PLS, reverse Gompertz, VOC.

C1.1 Introduction

'Conference' is the most grown pear variety in Europe, representing more than 30 % of the pear yield (Chiriboga et al., 2013) . Pear production in Spain in 2017 was higher than 360 000 t (MAGRAMA, 2018). Pears are climacteric fruit and most European pear varieties require a chilling period after harvest, that may vary from a few days to months depending on the variety, to initiate the autocatalytic ethylene production and thereby ripen (Lindo-García et al., 2019). Under this scenario and to guarantee the supply of pears all year round, long-term cold storage under controlled atmosphere conditions are common practices employed by the pear industry. Storage under dynamic controlled atmosphere is undoubtedly the new storage trend in most pear producing countries (Saquet, 2019). Long term cold storage can reduce pear volatile compounds emission (Zlatić et al., 2016) and has been reported to damage to some extent the aroma of some pear varieties such as 'Passe-Crassane' (Rizzolo et al., 1991), 'Packham'sTriumph' (Chervin et al., 2000) and 'Doyenne du Comice' (Lara et al., 2003).

Pear consumption has been steadily decreasing over the past 5 years (MAGRAMA, 2018). The lack of flavor is among the main reasons for the reported decrease in consumption. Consumers demand a closer relationship between the visual appearance, firmness and organoleptic characteristics (Zerbini, 2002). In this sense, the flavor of pears consist of a complex interaction between taste and odor (Yao et al., 2018) where esters play an important role (Lara et al., 2003; López et al., 2001). In relation to the odor or aroma, methyl and hexyl esters of decadienoate are characteristic compounds of European pears such as 'Conference' (Kahle et al., 2005; Rapparini and Predieri, 2003). In addition, hexanal, 2-methylpropyl acetate, ethyl acetate, hexyl acetate, 3-methylbutyl-2-methyl butanoate, ethyl butanoate, and butanol were also identified as impact volatiles in "Conference" pears (Rizzolo et al., 2005), the concentration of which was largely affected by the fruit maturity at the time of harvest as well as postharvest storage conditions.

Several studies are available describing consumer acceptance of pears just after harvest (Brückner, 2008b; Kappel et al., 1995), or how consumer acceptance is affected by long term storage (Hájos, 2012; Moya-León et al., 2006). However, scarce information is found about the temporal variations in the fruit quality and the levels of consumers satisfaction during post-cold storage ripening of pears (Zlatić et al., 2016), a period that under regular shelf life conditions (20 ºC) may be as short as 5 to 15 d depending on the

variety. A better understanding of the post-cold storage ripening of 'Conference' pears may provide crucial information for retailers to schedule the distribution of ready-to-eat fruit in order to deliver it at the time of optimal quality in terms of consumer acceptance.

Accordingly, this study aimed at: 1) To assess the evolution of quality attributes such as physicochemical parameters, aroma volatile compounds emission and consumer's overall liking during shelf life at 20 ºC under long term DCA storage, and 2) To find out which of these experimentally measured quality attributes have the greatest influence on consumer's satisfaction.

C1.2 Materials and methods

C1.2.1 Plant material and storage conditions

'Conference' pears (*Pyrus communis* L.) were harvested in august 2018 from five different commercial orchards (L1, L2, L3, L4 and L5) in la Rioja (Spain). Fruit was picked up at optimum commercial maturity according to local growers recommendations which are basically assessed in terms of firmness and sugars content (firmness≈ 55-65 N and total soluble solids>13 %). Thereafter, fruit was transported to IRTA research institute with a refrigerated lorry at 0 ºC and stored at -0.5 ºC for 8 months (34 weeks) under a dynamic controlled atmosphere (DCA) at 90-95 % of relative humidity (RH).The initial set values were 1 kPa O_2 and 0.5 kPa CO_2. An ACR system (Van Amerongen, Netherlands) was used to measure the respiration quotient (RQ) every 4 d. When RQ was higher than 2, the O_2 levels were increased by 0.1 kPa, when the RQ was between 1.5 and 2 the O_2 levels were maintained and when it was lower than 1.5 the O_2 level was lowered 0.1 kPa. After storage, fruit was kept at 20 ºC, 70 % RH, and physicochemical parameters, ethylene production, aroma volatile compounds emission, consumer overall liking and some sensory attributes were determined.

C1.2.2 Physicochemical parameters

Physicochemical parameters (firmness (F), apparent maturity (I_{AD}), total soluble solids (TSS) and total tritatable acidity (TTA)) were measured each sampling day on 20 fruit from each orchard. Samples were taken upon arrival of fruit to IRTA at harvest, after 8 months of cold storage (0 d) and at 1, 2, 3, 4 and 5 d of shelf life (SL) at 20 ºC. Consumer's satisfaction tests were carried out at 1, 3 and 5 d of SL. On these days each fruit was divided in two

halves, one was used to measure physicochemical parameters and the other half for the consumer evaluation test.

Firmness was determined on two opposite sides of each fruit after removing the peel, using a hand-held penetrometer (Turoni, Italy) fitted with an 8 mm diameter plunger. The semi-spherical plunger was introduced into each fruit and the maximum force was measured. The apparent maturity of each fruit was measured with a DA-Meter (TR Turoni, Forli, Italy), based on the index of absorbance difference ($I_{AD} = A_{670} - A_{720}$), as described by Turpin et al. (2016).

At each sampling date four juices per orchard were prepared crushing together five halves of fruit. From each obtained juice, total soluble solids (TSS, %) were measured using a digital hand-held refractometer (Atago, Tokyo, Japan), and acidity content (TTA) was measured by titration of 10 ml of juice with 0.1 N sodium hydroxide (NaOH) to pH 8.2 using phenolphthalein. TTA results were expressed as g malic acid L^{-1}.

C1.2.3 Ethylene production

Fruit ethylene production capacity upon removal from cold storage (0 d) was measured daily in 15 flasks (3 flasks per orchard) for 8 d. In each 1.5 L flask, 2 weighted fruit were introduced. Flasks were continuously aerated with humidified air at a constant flow rate of 250 mL min^{-1}, and kept in an acclimatized chamber at 20 °C.

The amount of ethylene produced by the fruit was measured by taking a 1 mL sample of gas from the headspace of each flask and injecting it into a gas chromatograph fitted with a FID detector (Agilent Technologies 6890, Wilmington, Germany) and an alumina column 80/100 (2 m × 3 mm) (Teknokroma, Barcelona, Spain) as described by Giné Bordonaba et al. (2014).

C1.2.4 VOCs analysis

Volatile organic compounds (VOCs) emission was determined at 1, 3 and 5 d during the SL period on fruit from orchards L1, L2 and L3. About 2 kg (6-7 fruit per container) of selected fruit free from defects were introduced in an 8-L Pyrex container. A total of 9 containers (3 per orchard) were kept at 20 ºC up to 5 d. A nitrogen stream (150 mL min^{-1}) was forced for 1 h for the VOC's acquisition. The resulting effluent circulated through an adsorption tube filled with 350 mg Tenax TA (2, 6-dipheyl-1-p-henylene oxide) and

Carbograph 1TD (Markes International Ltd., Llantrisant, United Kingdom). Adsorption tubes were kept at 4 ºC until were desorbed (Cano-Salazar et al., 2013).

Volatile compounds desorption was done using an automated UNITY Markes thermal desorption system (Markes International Ltd., Llantrisant, United Kingdom) at 275 ºC for 15 min. Identification and quantification were done with an Agilent 7890B gas chromatograph coupled to a 5977A mass spectrometer (MSD) (Agilent Technologies, Inc., Barcelona, Spain). Volatile compounds separation was performed with a capillary column with cross-linked free fatty acid as the stationary phase (FFAP; 50 m×0.2 mm× 0.33 μm). Helium was used as the carrier gas, at a flow speed of 42 cm s^{-1}, with a split flow of 20 mL min^{-1}. Both the injector and detector were kept at 240 ºC. The analysis was conducted according to the following program: 40 ºC (1 min); 40-115 ºC (2.5 ºC min^{-1}); 115-225 ºC (8 ºC min^{-1}); 225 ºC (10 min). Mass spectra was obtained by electron impact ionization at 70 eV, using the same flow of helium and following the same temperature gradient program as the ones used in the separation. Volatile compounds identification was carried out by comparing the spectrometric data recorded to those from the original NIST HP59943C library mass spectra. Quantification was performed using individual calibration curves, with correlation coefficient higher than 0.95, for each identified compound.

C1.2.5 Sensory analysis

As explained in the physicochemical parameters section, consumer evaluation tests were carried on the remaining halves from fruit used for the physicochemical analysis. Briefly, sensory evaluations were conducted as described by Echeverría et al. (2008). Each half of the fruit was peeled and cut into pieces which were used for the sensory evaluation and evaluated separately by one consumer. All of them were regular consumers of pear. Each plate was therefore presented with five pieces of fruit at one time (one from each orchard). Pieces were identified using three digits and were presented to each consumer in a randomized order. The panel of consumers consisted of 56 experienced volunteers from the staff working at the IRTA research institute. Nearly 70 % of the members own more than 15 years of experience in this type of tests. In this sense, this may actually be considered as a semi-trained panel. Consumers (30 % men, 70 % women) were asked to rate the overall liking according to a nine-point hedonic scale (1, dislike extremely; 5, neither like nor dislike; 9, like extremely) (Lopez et al., 2011) and to evaluate firmness and flavor separately

through a five-point hedonic scale (1 very low intensity; 2 low; 3 regular; 4 moderate; 5 very high intensity) (Echeverría et al., 2015).

C1.2.6 Statistical and data analysis

Means were compared by analysis of variance (ANOVA), when the analysis was statistically significant, the Tukey's Honestly Significant Difference (HSD) test at $P \leq 0.05$ was performed for separation of means using JMP® 13.1.0 SAS Institute Inc. (SAS, 2013). Correlations between experimental variables were checked using Spearman's rank correlation and, if required, presented as Spearman's correlation coefficient (r) and p-value based on a two-tailed test. Unless otherwise stated, significant differences were $P \leq 0.05$.

A Principal Component Analyses (PCA) was conducted in order to establish a preliminary relationship between physicochemical parameters and VOC's. The analyzed data included all measured variables along days of SL (1, 3 and 5) and orchards (L1, L2, L3). A Partial Least Square (PLS) model was used to correlate physicochemical parameters and volatile compounds with sensory evaluation. The physicochemical parameters, volatile compounds, sensorial firmness and flavor were selected as X variables in the PLS model. This model contained consumer's overall liking as response variables (Y). The non-linear iterative partial least squares (NIPALS) algorithm was used for computing the first few factors. KFold validation was used to select the number of factors that minimize the Root Mean PRESS statistic. As a pre-treatment, data were centered and weighed by the inverse of the standard deviation of each variable in order to avoid dependence on measured units. All analyses were carried out with the PLS platform of JMP® 13.1.0 SAS Institute Inc. (SAS, 2013).

The reverse Gompertz function (Eq. 1) was used to fit the evolution of fruit firmness F (N) as a function of time t (d),

$$F(t) = a\left(1 - \exp\left(-exp\left(\frac{e \cdot r_m}{a}(\lambda - t)\right)\right)\right), \quad (1)$$

where e is the base of natural logarithms, λ (d) represents the time at which the maximal firmness decay rate r_m (N d^{-1}) is achieved and parameter a (N) refers to the ceiling firmness value of the fruit. The confidence intervals for the estimated parameters were obtained by the Monte-Carlo method as described by Illa et al. (2012).

C1.3 Results and discussion

C1.3.1 Physicochemical parameters evolution during shelf life

According to Kappel et al. (1995) physicochemical parameters of pears such as firmness, I_{AD}, total soluble solids content, total titratable acidity and ethylene are important parameters affecting consumer preferences.

In our study, the initial firmness at harvest was in the range 50-62 N (Fig. C1.1). Fruit from L3 had the lowest firmness values at harvest, yet after 240+3 d of SL differences only existed between L4 and L5 that showed slightly but significantly higher firmness than the rest of orchards (P<0.05). After 8 months of cold storage under DCA only fruit from orchard L1 lost 5 % of the initial firmness while no significant losses were observed in the other orchards. In contrast, Saquet (2018) reported a firmness loss higher than 10 % in 'Conference' pears after only 6 months of storage under different CA conditions (0.5 to 3 kPa O_2 and 0.5 to 6 kPa CO_2). It is therefore likely that our more restrictive storage conditions (DCA with average 0.5 kPa O_2 and 0.5 kPa CO_2) better preserve the firmness of Conference pears. Results reported by Goliáš et al. (2015) on 'Conference' pears stored under regular air for 80 d at 1 ºC and 90 % RH showed a firmness decrease around 50 % during the storage period. Their reported values at 7 d of SL were in line to the ones found in our experiment at 5 d of SL. We found that DCA storage, at the conditions described above, better preserves the fruit firmness of 'Conference' pears during long term storage without negatively affecting other quality attributes or leading to fermentative-related physiological disorders. Indeed, fruit did not show a significant incidence of internal breakdown disorders (data not shown). Further investigation regarding which are the O_2 threshold levels supported by the fruit under DCA storage is warranted since our storage conditions were far more restrictive than those recommended by Saquet (2019) (2 kPa O_2 and lower than 0.7 kPa CO_2).

The overall evolution of fruit firmness reported herein followed an inverted sigmoidal pattern with a clear inflexion point during the SL (Fig. C1.1). Predieri and Gatti, (2009) analyzed firmness decrease on 'Abate Fetel' pears during SL after 13 and 23 weeks storage in regular air at (0-1 ºC) and 95 % RH. They reported that pears stored for 13 weeks also followed an inverted sigmoid curve; however, most curves after 23 weeks did not show the inflexion point. Galvis-Sánchez et al. (2004) reported a similar yet slower firmness loss and no inflexion points during the SL of 'Rocha' pears stored during 9 months under different controlled atmospheres (2 and 4 kPa O_2 with

0.5 and 1.5 kPa CO_2) at a temperature between 0-0.5 ºC and RH in the range of 90-95 %. Differences between both studies are likely related not only to cultivar differences but also to the different storage conditions and different data points being measured.

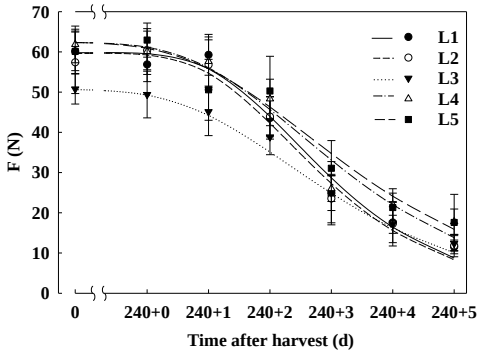

Figure C1.1 Fits of the mean measured fruit firmness at harvest (0), after 8 months of cold storage (240+0) and during SL period (1 to 5 d) as a function of time with the reverse Gompertz equation (lines) of fruit from orchards: L1, L2, L3, L4 and L5. Error bars represent the mean ± standard deviation (n=20).

Table C1.1 shows the best fit parameter values for Eq. 1 and its confidence intervals. All firmness fits had a determination coefficient higher than 0.96. To fit the function, firmness values from harvest, after cold storage and during SL were used. When the fitting was performed without the harvest point, a maximum deviation in the functions of 1.3 % at the time t=240+0 d was found in orchard 5. It should be highlighted that in that case the fitted parameter values were not significantly different from the ones shown in Table 1 but the confidence intervals were wider than when including data point from harvest.

Table C1.1 Estimated parameter values of the reverse Gompertz equation and corresponding confidence intervals (c.i.) at 95 % confidence when fitting firmness evolution of 'Conference' pears during 8 months of cold storage and 5 d of SL at 20ºC as function of time. Coefficient of determination r^2 reflects the goodness of the fits.

Orchard	λ (d)	λ (c.i.)	*a* (N)	*a* (c.i.)	r_m (N d^{-1})	r_m (c.i.)	r^2
L1	2.4	2.1-2.7	59.8	56.1-63.9	15.6	12.9-19.7	0.984
L2	2.3	2.0-2.7	59.6	56.0-64.0	15.4	12.7-19.4	0.984
L3	2.3	2.5-2.7	50.6	48.6-52.7	10.4	9.3-11.7	0.994
L4	2.5	1.9-2.9	62.3	57.0-68.4	12.7	9.9-17.4	0.966
L5	2.6	2.0-3.1	62.3	55.9-67.4	11.7	9.1-15.8	0.966

The index of absorbance difference in the range of 670-720 nm at the fruit skin (I_{AD}) measures the light absorbance due to chlorophyll. The I_{AD} for 'Conference' pears presented a clear decrease trend during the 8-month cold storage and followed a soft decline throughout the shelf life period (Fig. C1.2A). Costa et al. (2016) reported that I_{AD} values were a useful tool for

assessing postharvest ripening of 'Abbé Fétel' pear fruit. Similar observations were made by Saquet (2019) when reviewing the use of this non-destructive parameter as a quality indicator during postharvest storage of pears.

In a study carried out by Jaeger et al. (2003), consumers described the ideal pear as juicy and sweet, the key characteristics of ripeness. Sweetness is mostly related to TSS concentration and the balance between TSS/TTA. In our study, TTA values clearly decreased during the storage period in fruit from all orchards. During the SL period the non-uniform evolution in each orchard followed a global slightly decreasing trend (Fig. C1.2B). TSS/TTA ratio increased during the DCA cold storage in all orchards, but no clear trend was observed during the SL period (Fig. C1.2C). The unsteady trend in TSS/TTA ratio during SL has already been reported by Bolte-Lombardiz et al. (2000) in 'Shinsseiki' pears and was attributed to TTA variations.

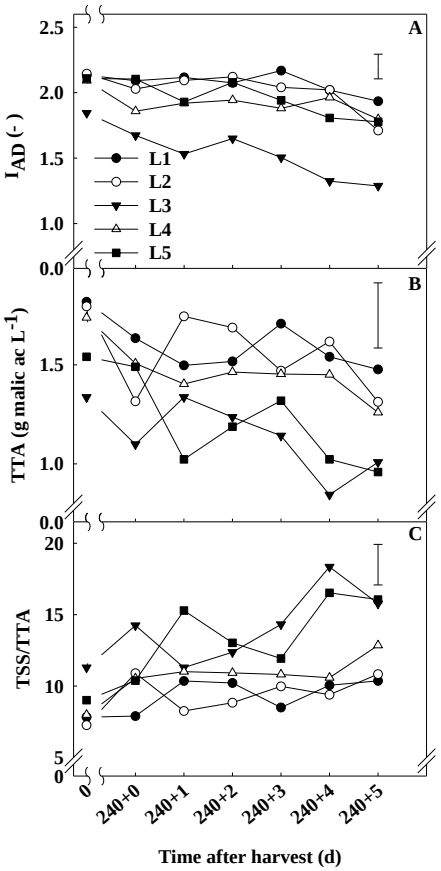

Figure C1.2 Postharvest evolution of physicochemical parameters in 'Conference pears: A) I_{AD} Index; B) titratable acidity, TTA; C) total soluble solids, TSS/TTA ratio at harvest (0), just after 8 months of cold storage (240+0) d and during SL period (1, 2, 3, 4 and 5 d). The vertical bar at the upper right corner represents the significant difference length according to the Tukey HSD test value.

Ethylene is known to be a major factor regulating fruit ripening, and its sharp increase is considered to control the aroma biosynthesis and other biochemical and physicochemical process (Moya-León et al., 2006; Rapparini and Predieri, 2003). Similar ethylene production rates to those described herein have been previously reported in Spanish 'Conference' pears (Chiriboga et al. 2013). In our study, fruit had a postclimateric behavior with the highest ethylene production rate immediately upon removal from cold storage and a decline thereafter, except for the fruit from L3 orchard (Fig. C1.3). In 'Conference' pears stored under regular air, a typical climacteric behavior during post-cold storage ripening has been observed up to 90-120 d following cold-storage but not later (Chiriboga et al. 2013).

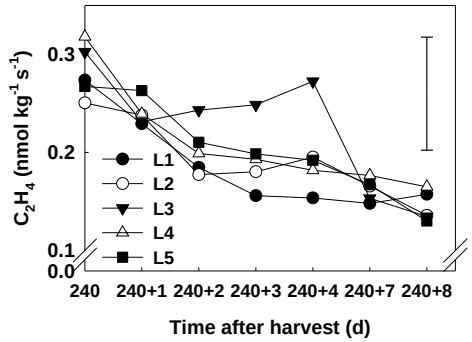

Figure C1.3 Ethylene production rate evolution during SL at 20 ºC of 'Conference' pears from different orchards: L1, L2, L3, L4 and L5. The vertical bar at the upper right corner represents the significant difference length according to the Tukey HSD test value.

Table C1.2 Mean (n=3) values of major VOC's emission rate (μg kg^{-1} h^{-1}) by 'Conference' pears from orchards L1, L2 and L3 at 1, 3 and 5 d of SL. Means within the orchard and days of SL preceded by the same small letters are not significantly different at $P \leq 0.05$ (HSD test). No letter indicates the absence of significant differences. (-, values under the detection threshold).

	L1			L2			L3		
	1	**3**	**5**	**1**	**3**	**5**	**1**	**3**	**5**
Methyl acetate	-	^{ab}1.228	^{a}1.868	-	^{cd}0.183	^{d}0.107	^{d}0.141	^{bc}0.963	^{ab}1.524
Ethyl Acetate	2.329	3.353	5.508	0.802	1.418	1.775	0.956	3.233	5.527
Butyl acetate	^{bc}2.992	^{ab}11.392	^{a}14.371	^{c}1.578	^{c}2.195	^{bc}3.407	^{bc}3.267	^{abc}7.137	^{abc}9.951
Pentyl acetate	^{b}0.344	-	^{a}1.022	^{b}0.033	^{b}0.084	b0.150	-	^{b}0.097	^{b}0.179
Butyl butanoate	-	0.489	0.136	-	0.056	0.017	-	0.034	-
Ethyl hexanoate	-	0.017	0.023	-	0.002	0.006	-	0.008	0.012
Hexyl acetate	-	^{ab}2.898	^{a}3.412	^{a}b0.312	^{ab}0.569	^{ab}0.641	^{ab}0.307	^{ab}2.698	^{a}3.313
Butyl hexanoate	0.055	-	0.114	-	0.035	0.046	-	0.027	0.077
Hexyl butanoate	-	0.014	0.035	-	0.042	0.017	-	0.037	0.063
Methyl trans,cis-2,4-decadienoate	-	^{a}3.896	^{c}0.859	-	^{c}0.109	^{c}0.465	-	^{bc}0.943	^{ab}2.649
Ethyl trans,cis-2,4-decadienoate	-	^{bc}2.088	^{ab}3.233	-	^{d}0.573	^{d}1.166	^{d}0.267	^{c}3.074	^{a}5.132
Ethanol	3.780	2.402	2.962	1.973	1.384	2.214	1.584	1.548	1.753
3-Hydroxydodecanoic acid	-	^{a}0.345	^{abc}0.178	-	^{bc}0.060	^{bc}0.037	^{ab}0.257	^{abc}0.134	^{bc}0.120

C1.3.2 Volatile organic compounds emission

Thirty-four volatile compounds were identified and quantified during the SL period (1, 3 and 5 d) of 'Conference' pears previously stored under DCA conditions for 8 months (data not shown). These volatile compounds included 20 esters, 4 alcohols, 1 aldehyde, 4 terpenoids, 2 hydrocarbons and 3 acids. However, only those quantitatively more important and following some remarkable trend over the shelf-life period are shown in table C1.2.

According to previous works, the aroma of pears is mainly caused by esters (El Hadi et al., 2013; Kahle et al., 2005; Maarse, 1991; Zlatić et al., 2016). The main esters detected in our study (Table C1.2) were butyl, ethyl and hexyl acetates as straight esters, and methyl and ethyl trans, cis-2,4-decadienoate as branched esters. Similar results were obtained by Rapparini and Predieri (2003) and Kahle et al. (2005), who reported that the methyl esters of decadienoate were the characteristic compounds of European pears. Further, hexyl and butyl acetates were also found important volatile compounds in the pear aroma (Rapparini and Predieri, 2003). High concentrations of these acetates were reported by Saquet (2017) on 'Conference' pear after 2 months of storage plus 7 d of SL. An increase in the ethyl acetate concentration was observed as SL period lengthened (Table C1.2). Other authors also identified ethyl acetate and hexyl acetate as impact volatiles in 'Conference' pears stored for up to 22 weeks in air and controlled atmosphere (Rizzolo et al., 2005).

In our study, the highest emission rates of ethyl trans, cis-2,4-decadienoate were detected after 5 d of SL in fruit from all the orchards. Similarly, Hendges et al. (2018) observed a high content of this volatile compound in 'Conference' pears treated with 1-MCP after 7 months of storage under normal air and controlled atmosphere plus 7 d at 20 ºC and 60 % RH.

Ethanol was the main alcohol present in the headspace from 'Conference' pears, however, it did not contribute to the fruit odor pattern, owing to its very high odor threshold concentration. Zerbini, et al. (1993) also reported that in 'Conference' pear ethanol was the main alcohol. Ethanol is a marker of fermentative paths if produced in high amounts (Perata and Alpi, 1993). However, the concentrations detected in this work were well below its odor threshold which is 10 000 □g L^{-1} The ethanol emission rates detected in this work during SL period for the three orchards were similar. In our experiments, 3-hydroxydodecanoic acid was also found as a characteristic acid

of 'Conference' pears and thereby agree with the results from Heinz and Jennings (1966) on other pear varieties ('Barlett').

C1.3.3 The relationship between physicochemical parameters and VOC production

A PCA model was used to obtain a global overview of the relationship between physicochemical parameters and the profile of volatile compounds in a reduced dimension plot. In this data set, 19 variables were used for the PCA: 5 physicochemical parameters (F, I_{AD}, TSS, TTA TSS/TTA ratio), ethylene production and the 13 volatile compounds showed in Table 2. The biplot of the two principal components (PC1 and PC2) captured 71.9% of the total variability (Fig. C1.4). This biplot showed three groups along the first component, differentiating samples from different SL periods. On the left of the first component are located the samples at 1 d of SL, which were mainly characterized by higher values of firmness, titratable acidity, I_{AD} and ethylene production, meaning that these fruit were less mature. In the middle of the graph are situated the samples at 3 d of SL and on the right the ones at 5 d of SL. These last samples, especially the pears from L1 and L2, were related to high concentrations of some of the most important volatile compounds (hexyl butanoate, ethyl trans, cis-2,4-decadienoate, hexyl and butyl acetate), together with high TTS and TSS/TTA ratio values. The variability among samples increased with time, samples at 1 d of SL were quite homogenous compared with samples at 3 and 5 d of SL. All the volatiles emissions were positively correlated among themselves, while were negatively correlated with the fruit firmness. Similar aromatic volatiles were described for Barlett pears by Li (2012). The observed increase of the variability seems to be mainly due to the biosynthesis of some particular volatile compounds, to the erratic pattern changes in the TSS values and thereby also by the TTS/TTA ratio. The volatile compounds emitted by fruit of the L1 orchard, located in the upper part of the two groups (at 3 and 5 d of SL period), showed higher concentrations of butyl butanoate, methyl trans, cis-2,4-decadienoate and butyl acetate, and less TTS and TTS/TTA content. All these compounds own a strong "pear-like" aroma (Suwanagul, 1996). The second component discriminated the three different sources. At the top, lied the samples from orchard L1, which were more immature at harvest based on the I_{AD} index. In the middle of the plot, were located fruit from L2. Finally, at the bottom there were samples from orchard L3. The variability among orchards was lower than among days of SL, since the later cluster was the one that represented most of the variability along the PC1.

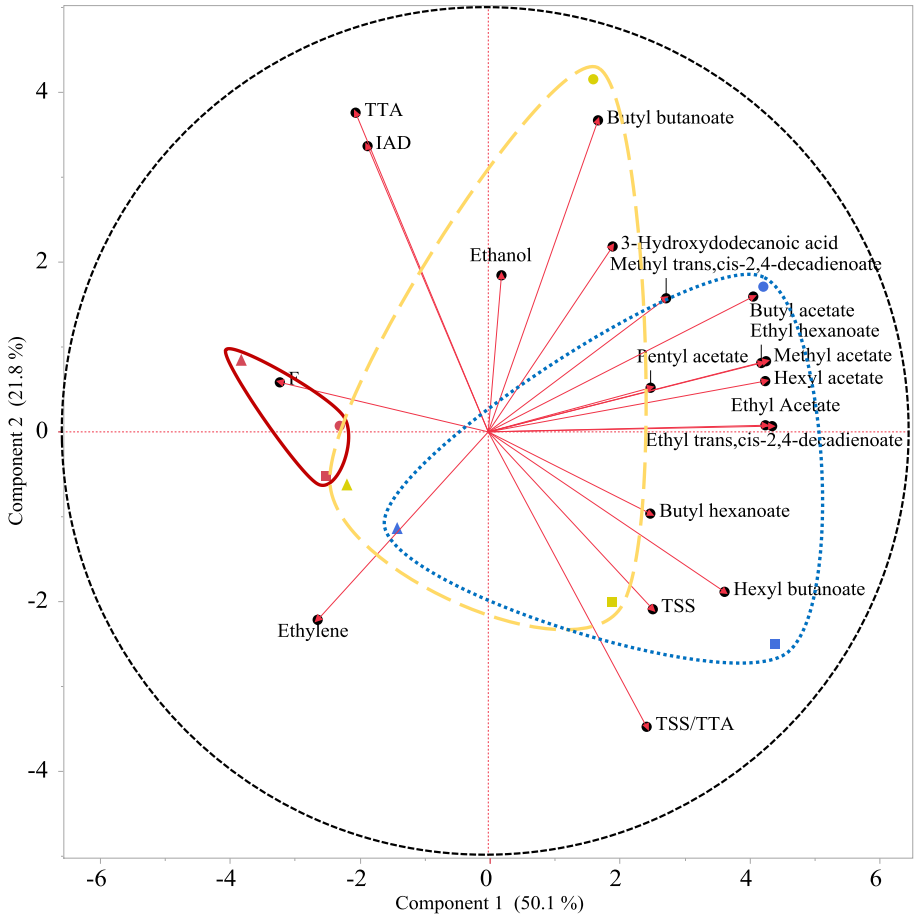

Figure C1.4 Score plot of PC1 and PC2 from a full data PCA model considering instrumental quality and VOC's (n=19). Data were identified in three different cluster groups: 1 d of SL (red continuous line), 3 d of SL (yellow dashed line) and 5 d of SL (blue dotted line). Data representing three different orchards: L1 (●), L2 (▲) and L3 (■) is contained in the circumference of the correlation circle (black dashed circle).

C1.3.4 Consumer acceptance

Figure C1.5A shows that consumer's overall liking depended on the interaction between the sensorial firmness and flavor. In our study, the most pleasing pears, or the ones that obtained higher overall liking scores, were those with a moderate-low sensorial firmness (consumers rated from 1 to 3 in a 5-points hedonic scale) and with a high flavor (consumers rated as 5 in a 5-points hedonic scale). This higher overall liking was obtained between 3 and 5 d of SL (Fig. C1.5B), regardless of the orchard. Thus, for long term stored 'Conference' pears higher marketability will be reached after being 3 d in retail.

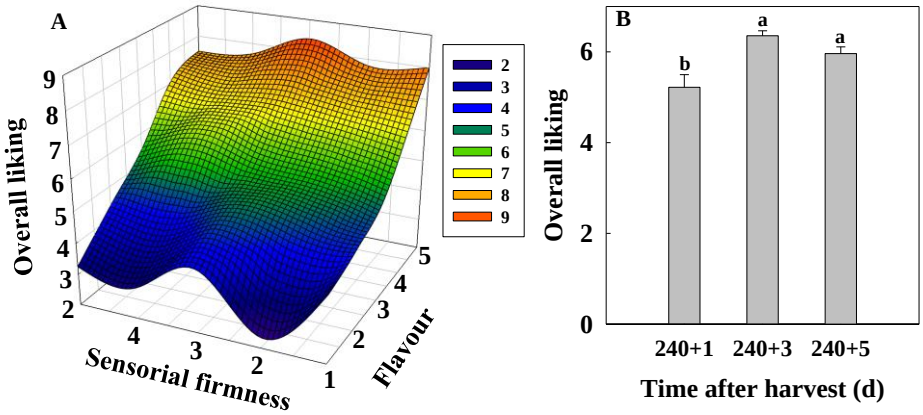

Figure C1.5 A) 3D plot of the interaction between sensorial firmness (Y) and flavour (X) through a five-point hedonic scale (1, very low intensity; 5, very high intensity) with consumers overall liking (Z) based on a nine-point hedonic test (1, dislike extremely; 5, neither like nor dislike; 9, like extremely). B) Overall liking during the SL period of 'Conference' pears at 240+1 d, 240+3 d, 240+5 d. Error bars represent the standard error. Different letters indicate significant differences ($P < 0.05$) for each day of SL.

A PLS model is a useful tool to identify which are the indicators that a consumer value more in terms of overall liking (Abdi, 2003). Similar approaches have been done with other fruit including apples (Altisent et al., 2011) and peaches (Cano-Salazar et al., 2013), but to the best of our knowledge this information is lacking for pears. We used a PLS to correlate consumer overall liking (Y variable) with a set of potentially explanatory variables: physicochemical parameters, ethylene production, volatiles organic compounds and sensory attributes (X variables).

Based on PLS method, the X data set was reduced to two principal factors. The first factor explained the 74.58 % of the variation while the second explained the 9.01 %. Thus, the cumulative variation explained by two principal factors was 83.6 % (Fig. C1.6). The correlation between measured and predicted overall liking was R^2=0.836, demonstrating the goodness of the model (Fig. C1.6 insert). This figure showed that consumers preferred fruit at 5 d of SL from orchards L3. Interestingly, those fruit were harvested at I_{AD} values of 1.8 which is the reported optimal harvest values to maximize consumer acceptance in other pear varieties ('Abbé Fétel'; Costa et al. (2016)). The variable importance plot (VIP) (Fig. C1.7) showed that TSS, TSS/TTA ratio, methyl, ethyl and hexyl acetates, hexyl butanoate, ethyl hexanoate, ethyl trans,cis-2,4-decadienoate and flavor (sensory attribute) were variables positively correlated, with a high weight, to consumers overall liking. In contrast, fruit firmness, TTA and sensorial firmness were negatively correlated to consumer global satisfaction. All of them, were among the most powerful X

variables in the determination of the PLS model. All these variables had values above 1 and therefore were the greater contributors that explained the variation (Chong and Jun, 2005).

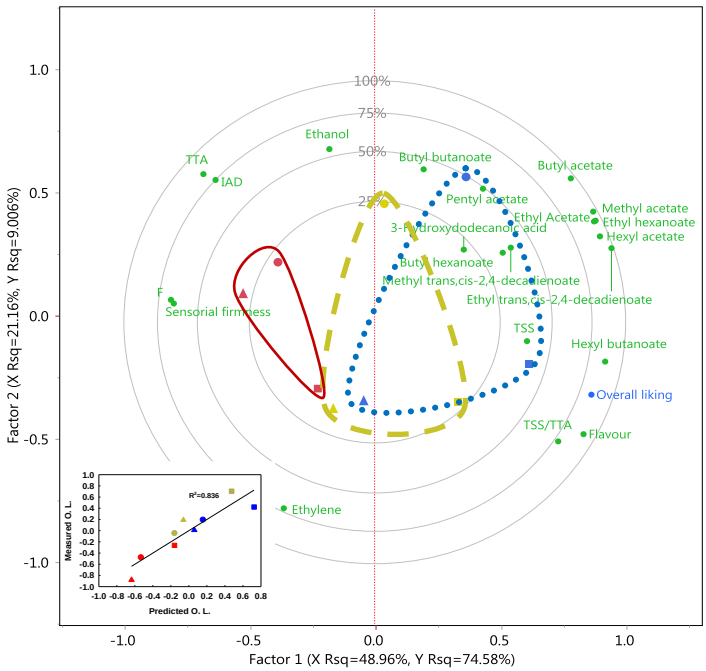

Figure C1.6 Partial Least Squares (PLS) correlation loading plots of the 2 factors. Data was identified in three different groups: 1 d of SL (red continuous line), 3 d of SL (yellow dashed line) and 5 d of SL (blue dotted line), representing fruit from three different orchards: L1 (●), L2 (▲) and L3 (■). The measured vs the predicted overall liking through the model and its correlation coefficient is given in the insert.

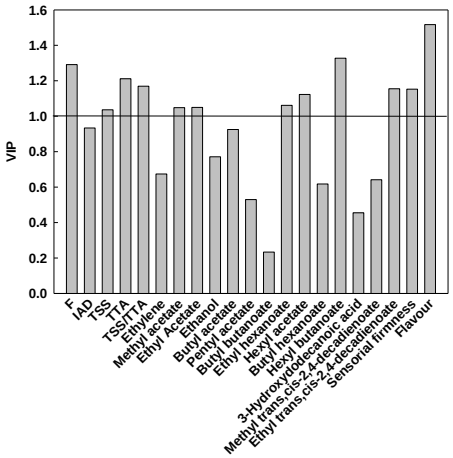

Figure C1.7 Variable importance plot (VIP), the number of VIP>1 (continuous black line) indicates that the indicators are influential in determining the two factors used in the model.

C1.4 Conclusions

Long term storage of 'Conference' pears at -0.5 ºC under DCA reduces the decay of firmness at levels below 5 % without significantly altering other quality traits yet without completely impeding fruit ripening. Accordingly, upon removal from cold storage and ripening at 20 ºC, eating quality, in terms of flesh firmness, is reached in no longer than 5 d. The decrease in the fruit firmness during shelf-life is parallel by an increase in most ester-type volatiles and especially in butyl acetate and ethyl-trans,cis-2,4-decadienoate (5-fold higher at 5 d of SL than at 1 d). The highest consumer appreciation of 'Conference' pears during SL occurred at 3 d of SL when pears had a moderate-low sensorial firmness (equivalent to 25 N of instrumental firmness) and high flavor. The PLS model showed that TSS, TSS/TTA ratio, consumer flavor perception and some particular volatile compounds (i.e. methyl, ethyl and hexyl acetates, ethyl trans,cis-2,4-decadienoate) were positively correlated to consumer's overall liking while firmness, TTA and I_{AD} had a negative correlation yet with higher prediction capability.

Overall, the results from this study may be of paramount importance for retailers aiming to distribute ready-to-eat 'Conference' pears at the time of optimal quality in terms of consumer acceptance.

Acknowledgments

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CHAPTER C2: Spatial distribution of flavor components and antioxidants in the flesh of 'Conference' pears and its relationship with postharvest pathogens susceptibility

The spatial distribution of dry matter, ethylene production, respiration rate, organic acids, sugars, antioxidants, volatiles and fungal (*Penicillium expansum* and *Rhizopus stolonifer*) growth was evaluated analyzing four different slices of 'Conference' pear flesh taken along an equatorial radius. A common spatial distribution trend was found for ethylene emission, CO_2 production, antioxidant capacity and total phenolic compounds with a minimum in the slice under the skin and a maximum in the slice near the core. Fructose, which was the dominant sugar followed by sucrose and glucose, showed a quasi-linear decreasing profile from the outer slice towards the core. Malic and ascorbic acid had the highest content in the outer slice while citric remained practically constant over the different slices. Twenty-nine volatile organic compounds (VOCs) were identified using solid-phase microextraction (SPME), yet only six of them showed significant differences between flesh slices. The content in VOCs was further related to the tissue susceptibility to the above-mentioned postharvest pathogens using a multivariate approach. Fruit flesh from inner sections was more prone to *P. expansum* whereas flesh from the slice under the skin presented the highest incidence of *R. stolonifer*. A Partial Least Square (PLS) model showed that *P. expansum* growth was negatively correlated with malic acid, dry matter content, 2-ethyl-hexanal and butyl hexanoate concentrations and *R. stolonifer* was negatively correlated to sucrose and some volatiles such as hexanal and 1-butanol. Based on the results from the PLS, selected volatiles naturally present in the pear flesh were tested *in vitro*, at different concentrations, in order to investigate their effectiveness to control blue mold caused by *P. expansum* and soft rot caused by *R. stolonifer*. A completely control of *P. expansum* was found with 2-ethyl-hexanal application and hexanal while 1-butanol showed a total fungicide effect against *R. stolonifer*. This study is a step towards a better understanding of how biochemical compounds are spatially distributed among different slices of 'Conference' pears as well as in the development of natural compounds to fight major postharvest pathogens in pear fruit.

Keywords: 2-ethyl-hexanal, Fungicide**,** *Penicillium expansum*, Phenolic compounds, *Rhizopus stolonifer*, VOCs.

C2.1 Introduction

Pear is one of the most important fruit produced in Europe, with 'Conference' cultivar as the most commonly grown in north east of Spain. 'Conference' is highly appreciated by consumers due to its flavor, juiciness and aroma (Saquet, 2018).

'Conference' pear as a climacteric fruit is a highly perishable product. The climacteric phase is characterized by a peak in ethylene production accompanied by a peak in fruit respiration. The burst displayed in the ethylene production is considered to set off biochemical and physicochemical processes (Moya-León et al., 2006; Rapparini and Predieri, 2003) leading to the biosynthesis of aroma compounds and stablishing the nutritional properties of the fruit.

The variability in aroma compounds of pear fruit is known to largely depend on the cultivar (Qin et al., 2012), maturity stage (Zerbini et al., 1993), agro-climatic conditions (Li et al., 2013) and storage conditions or postharvest handling (Zlatić et al., 2016). Volatile compounds, together with sugars and organic acids content (Defilippi et al., 2009), play an important role in fruit flavor. The major sugars in pears are fructose, glucose and sucrose (Colaric et al., 2006; Kolniak-Ostek, 2016; Lindo-García et al., 2019; Moriguchi et al., 2019) while malic and citric are the predominant organic acids in most pear cultivars. The ratio of sugar to organic acids is generally referred as a good indicator of flavor (Sha et al., 2011). However, scarce information is available on how volatile compounds, sugars and organic acids, are spatially distributed within the pear flesh. In other species such as peach, the volatiles concentration has been reported to notably differ from skin to flesh (Aubert and Milhet, 2007).

Despite present at relatively low concentration, pears are also a source of ascorbic acid (AsA) (Galvis Sánchez et al., 2003) and other bioactive compounds, including polyphenols, which positively contribute to human health. AsA content in 'Conference' pears changes during the fruit development and postharvest handling (Veltman et al., 2000) and higher concentration of this compound within the pear flesh has been linked to lower incidence of core browning in 'Conference' (Veltman et al., 1999) as well as superficial scald in 'Blanquilla' pears (Larrigaudière et al., 2016). Phenolic compounds also contribute to the fruit aroma and flavor (Imeh and Khokhar, 2002) and thanks to their anti-inflammatory and antimicrobial activity, can help to prevent human diseases (Liaudanskas et al., 2017).

Pear major losses take place during the postharvest phase being mainly caused by physical, physiological and pathological induced-changes. The main postharvest diseases of pears are caused by *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* (Sardella et al., 2016). Traditionally, pears have been treated with chemical fungicide in order to control postharvest decay. In the last years, new alternatives to curtail fungal growth such as the application of natural compounds, including those emitted by pears, have also been studied. Neri et al. (2006b), applied 2-hexanal vapors to satisfactorily control blue mold growth caused by *P. expansum* and, Alla et al. (2008) applied cinnamaldehyde vapors to control soft rot caused by *R. stolonifer*. Indeed, the antifungal or fungistatic activity of a range of volatiles is well documented (Mari et al., 2016, 2002; Neri et al., 2006a; Sivakumar and Bautista-Baños, 2014). However, whether the concentration of these 'antifungic' compounds along the pear flesh can account to improve resistance to certain fungal postharvest pathogens is still elusive.

Accordingly, the aims of the present study were: 1) To investigate the spatial distribution of the main flavor components and antioxidants in the flesh of 'Conference' pears. 2) To determine the behavior of flesh samples from different spatial positions artificially inoculated with *P. expansum* and *R. stolonifer* 3) To evaluate the protective effect of some naturally occurring volatile compounds against both pathogens.

C2.2 Materials and methods

C2.2.1 Plant material and experimental design

'Conference' pears (*Pyrus communis L.*) were harvested in August 2018 from a commercial orchard near Lleida (NE of Spain). Fruit was picked up at optimum commercial maturity according to local growers recommendations which are basically assessed in terms of firmness and sugars content (firmness≈ 55-65 N and total soluble solids >13 %). No pre-harvest fungicide treatments were applied later than 30 d prior the commercial harvest. Thereafter, fruit were transported to IRTA facilities where 108 fruit free from defects and uniform size were selected and divided in 3 groups of 20 fruit each plus 2 groups of 24 fruit each. One group of 20 fruit was used to evaluate the dry matter content, sugars, organic acids, antioxidant capacity and phenols. Another group was used to evaluate ethylene production and respiration, and the last group of fruit was used to evaluate the VOCs content. The 2 groups of 24 fruit were used to evaluate the growth ability of *P. expansum* and *R. stolonifer* along different spatial locations.

From each fruit a pulp cylinder in the radial direction, equatorial zone, from the outside of the fruit to the heart was extracted (Fig. C2.1). Each cylinder was 11 mm in diameter and 24 mm in length. Then, the peel was removed, and the cylinder was cut into 4 equal slices, 6 mm high each, named I, II, III and IV and corresponding to the 4 spatial positions considered in this study (Fig.C2.1; Outer slice (slice 'I') until the core (slice 'IV')).

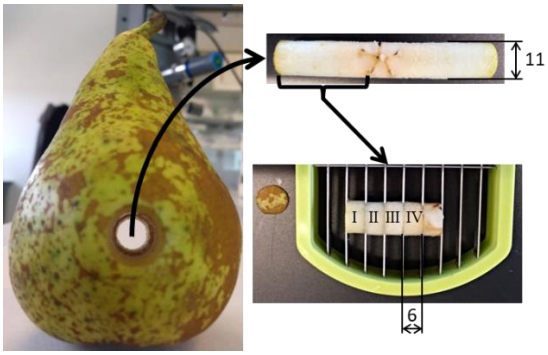

Figure C2.1 Methodology used for the equatorial cylinder extraction and slices division in 'Conference' pear. Fruit skin was adhered to the left side of slice I.

C2.2.2 Dry matter content

The dry matter content profile was determined in 20 fruit, 4 replicates of 5 fruit each. Five slices per each location were placed in a petri dish, weighted (m_{0i}) and immediately frozen with liquid nitrogen. Slices were lyophilized for 72 h. After this time, each petri dish was weighted (m_{1i}) and the dry matter content was evaluated according to the formula: $(m_{1i}/m_{0i})\cdot 100$.

C2.2.3 Ethylene production and respiration

Ethylene production and respiration were measured by enclosing 5 slices per each location in airtight tubes of a known volume (4 replicates) and placed in an acclimatized chamber at 20 ºC for two hours. After that time, ethylene concentration was measured by removing 1 mL of gas sample from the headspace of the tube and injecting it into a gas chromatograph fitted with a FID detector (Agilent Technologies 6890, Wilmington, Germany) and an alumina column 80/100 (2 m × 3 mm) (Teknokroma, Barcelona, Spain) as described by (Giné-Bordonaba et al., 2014). Oxygen and carbon dioxide concentrations within the tubes were measured with an O_2/CO_2 gas analyzer (CheckPoint O_2/CO_2, PBI Dansensor, Ringsted, Denmark). Gas i (i = O_2, CO_2, ethylene) production rate, r_i ($mol_i\ kg^{-1}\ h^{-1}$), was then calculated using Eq. (1),

$$r_i = \frac{\Delta P_i \cdot V_g}{R \cdot T \cdot M_f \cdot \Delta t}, \quad (1)$$

where $\Delta P_i = P_i^t - P_i^0$ (Pa) is the difference between the initial partial pressure, P_i^0 and the partial pressure P_i^t after time Δt (h), $V_g = V_0 - V_f$ (m^3) is the gas volume inside the closed tube obtained as the difference of the tube capacity V_0 and the volume occupied by the slices V_f, R=8.314 J K^{-1} mol^{-1} is the universal gas constant, T (K) is the absolute ambient temperature and M_f (kg) is the mass of slices inside the tube. Initial partial pressure of ethylene and CO_2 were assumed to be zero, while initial O_2 partial pressure was assumed to be $0.21 \cdot 10^5$ Pa. The respiratory quotient, RQ, was calculated as the molar ratio of CO_2 produced to O_2 consumed by the fruit, $RQ = -r_{CO2} / r_{O2}$.

C2.2.4 Determination of fruit sugar content

Lyophilized slices used in dry matter content determination were ground with a stainless-steel blender and 100 mg of the powder were used for sugar content determination. Glucose, fructose and sucrose were extracted from lyophilized material as described by Giné-Bordonaba and Terry (2010). Briefly, 100 mg of lyophilized sample were dissolved in 2 mL of 62.5 % (v/v) aqueous methanol solvent and placed in a thermostatic bath at 55 °C for 15 min, mixing the solution with a vortex every 5 min to prevent layering. Then, samples were centrifuged at 20 000 *g* for 7 min at 20 °C. The supernatant from each extraction was recovered and used for enzyme-coupled spectrophotometric determination of glucose and fructose (hexokinase/phosphoglucose isomerase) and sucrose (β-fructosidase) as described by Famiani et al. (2012) using commercial kits (BioSystems S.A., Barcelona, Spain) and following the manufacturer instructions. All results are expressed on a fresh weight basis.

C2.2.5 Determination of fruit organic acid content

Extracts for malic and citric acids determination, were prepared as described in Giné-Bordonaba and Terry (2010) with some modifications. One hundred mg of lyophilized frozen fruit tissue from each location were added to 2 mL of HPLC-grade water. Samples were kept at room temperature (20 °C) for 10 min and then centrifuged at 20 000 *g* for 7 min at 20 °C. The supernatant from each extraction was recovered and used for enzyme-coupled spectrophotometric determination of malic (L-malate dehydrogenase) and citric (citrate lyase / malate dehydrogenase) acids as described by Famiani et al., (2012) using commercial kits (BioSystems S.A., Barcelona, Spain) and following the manufacturer instructions.

Ascorbic acid (AsA) was determined using the freeze-dried material described above. One hundred mg of freeze-dried fruit slices were diluted in 2 mL of 3% (v/v) meta-phosphoric acid (MPA) and 8% (v/v) acetic acid aqueous solvent, mixing the solution for 1 min with a vortex. Then, the samples were centrifuged at 24 000 *g* for 22 min at 4 °C. The supernatants of each sample were filtered through a 0.45 µm filter for High Performance Liquid Chromatography (HPLC) (Millipore, Bedford, MA, USA) and used for HPLC-UV determination as described by Collazo et al. (2018). All results are expressed on a fresh weight basis.

C2.2.6 Determination of fruit antioxidant capacity and total phenolic content

Fruit antioxidant capacity and total phenolic compounds (TPC) were quantified from the freeze-dried material used in the dry matter content determination, as described earlier (Giné-Bordonaba and Terry, 2008). One hundred mg of freeze-dried fruit sample were mixed with 2 mL of 79.5% (v/v) methanol and 0.5% (v/v) HCl aqueous solvent. Sample extraction was held at 20 °C, mixing the solution every 15 min with a vortex (Giné-Bordonaba and Terry, 2016). From the same extract, TPC was measured by means of the Folin-Ciocalteu method calculated from a Gallic Acid Equivalent (GAE) curve and total antioxidant capacity was measured by the Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Szeto (1999). All results are expressed on a fresh weight basis.

C2.2.7 Spatial distribution of volatiles in pears

Headspace solid-phase microextraction (HS-SPME) was used to extract and to determine the concentrations of volatile compounds along the cylinder of pear flesh. SPME fibers coated with a 65-µm layer of polydimethylsiloxane–divinylbenzene (65 µm PDMS/DVB; Supelco Co., Bellefonte, PA, USA) were used. Fibers were activated before sampling according to the manufacturer's instructions.

Five slices per each spatial location and per replicate (4 replicates) were frozen in liquid nitrogen, crushed together and immediately transferred to −80 °C storage until the volatile compounds were analyzed. For each extraction, 5 g of homogenized sample per location were placed in a 20 mL screw-cap vial containing 2 g of NaCl to facilitate the release of volatile compounds. Prior to sealing the vial, 2 µL of 0.03 mL L^{-1} 3-nonanone was added as an internal standard, and the solution was mixed with a glass rod. The

mixture was incubated and agitated at 40 ºC during 20 min. Afterwards, the SPME fiber was injected into the headspace and exposed for 30 min at 40 ºC to absorb the volatiles as described by Qin et al. (2012). Volatile compounds were subsequently desorbed as described by Iglesias et al. (2018) and results expressed on a fresh weight basis.

C2.2.8 Fungal growth evaluation in pear tissue

Both strains used in this study, *P. expansum* (CMP-1) and *R. stolonifer* (RSF) belong to the collection from the Postharvest Pathology group of IRTA (Lleida). They were the most aggressive isolates capable of infecting pome fruit, respectively. Conidial suspensions were prepared by rubbing the surface of 7 to 10-day-old cultures grown on potato dextrose agar (PDA) with sterile water containing 0.01 % (w/v) Tween-80 using a sterile glass rod. Concentration of each fungus was determined using a hemocytometer and prepared to obtain $3 \cdot 10^4$ conidia mL^{-1} of *P. expansum* and $1 \cdot 10^3$ conidia mL^{-1} of *R. stolonifer*.

Two groups of 24 fruit (8 replicates, 3 fruit each) were used to evaluate the growth of fungi. The first group was used to evaluate the severity and incidence of *P. expansum* and the second the incidence of *R. stolonifer*. Fruit were disinfected with 0.525% (v/v) sodium hypochlorite (NaClO) for 5 min and cleaned five times with tap water. Once dried, a pulp cylinder of the fruit was extracted and cut into 4 slices as explained in the plant material and experimental design section. Each slice of the first group was inoculated with 5 µL of *P. expansum* and the ones of the second group were inoculated with 5 µL of *R. stolonifer*.

P. expansum incidence was evaluated by measuring the diameter of fungus growth and severity infection was evaluated as the % of mycelial presence on slices regarding the total of infected samples. *P. expansum* incidence was evaluated after 72 h post the inoculation while *R. stolonifer* incidence was measured after 44 h post the inoculation.

C2.2.9 Evaluation of fungistatic or fungicide activity of synthetic pear volatiles *in vitro*

Fungistatic and fungicide activity of the four most representative VOCs found in the Principal Component Analysis (PCA) of detected pear volatiles was evaluated as reported by Gotor-Vila et al. (2017) with some modifications. Briefly, pure standards of these four volatiles were purchased from Sigma-

Aldrich (Madrid, Spain) and individually tested for suppressing mycelial growth of target pathogens. For this purpose, 10 µL of conidial suspension containing each pathogen were placed in the center of petri dishes containing PDA. Then, a paper filter (85 mm diameter) containing an aliquot of pure compound was positioned inside the cover of the dishes and the petri dishes were immediately sealed with parafilm and incubated at 25 °C. The aliquots of pure compounds introduced in the petri dishes were: 5, 10, 20, 40, 80, 160 and 320 µL corresponding to 0.027, 0.055, 0.11, 0.22, 0.44, 0.88, 1.76 mL L^{-1} headspace, respectively. Measures for *P. expansum* were made after three, four, five and seven days post the inoculation and *R. stolonifer* after one, two and three days. The sample unit was represented by four replicates for each dose and pathogen and dishes with paper filter with water at 1.76 mL L^{-1} were used as control. The percentage of mycelial inhibition (PMI) of fungal growth was calculated after 5 and 3 d from inoculation for *P. expansum* and *R. stolonifer*, respectively. Percentage mycelial inhibition (PMI) was determined according to the formula $(\%)=[(d_c-d_t)/d_c)]\cdot 100$, where d_c is the diameter growth average of control and d_t is the treatment diameter average (Li et al., 2016). The effect of VOC's on fungus were tested by determining the effective concentration values that reduced the mycelial growth by 50% (EC_{50}) as reported by Alexander et al. (1999).

C2.2.10 Statistical analyses

Means were compared by analysis of variance (ANOVA). When the analysis was statistically significant, the Tukey's Honestly Significant Difference (HSD) test at $P \leq 0.05$ was performed for separation of means.

A hierarchical cluster analysis dendrogram was done applying Ward method of minimum variance. The objective function was the error of the sum of the squares or variance (Ward, 1963). The dendrogram and the constellation graph were constructed in order to establish a preliminary relationship between sugars, organic acids and antioxidants among different pear 'Conference' slices spatially distributed. The analyzed data included the 4 slices along the spatial distribution (I, II, III and IV) and 40 variables representing the components being analyzed.

Two partial least square (PLS) regression models were used to correlate organic acids, sugars, antioxidants and volatile compounds (as X variables or explanatory variables) with fungal infections as response variables, *P. expansum* as (Y_1) and *R. stolonifer* as (Y_2). The non-linear iterative partial least squares (NIPALS) algorithm was used for computing the first few factors.

KFold validation was used to select the number of factors that minimize the Root Mean PRESS statistic. As a pre-treatment, data were centered and weighed by the inverse of the standard deviation of each variable in order to avoid dependence on measured units. All analyses were carried out with the PLS platform of JMP® 13.1.0 SAS Institute Inc. (SAS, 2013).

C2.3 Results and discussion

C2.3.1 Dry matter content, ethylene emission and respiration

Dry matter (DM) of pip fruit is basically formed by carbohydrates (90 %) (Travers et al., 2014), in soluble and insoluble forms, and the remaining part are mainly organic acids (Suni et al., 2000). Our results showed that the DM content was minimum in slice II and III but with no significant differences between them ($p=0.1891$) (Fig. C2.2A). The average of DM content reported herein (17.8 %) was in accordance with the ones reported by Costa et al. (2015) in pear fruit from four different varieties (average 17.9 %). The moisture content profile, which is its complementary (m_c=100-DM), had thus a maximum in slice II, which can be explained by the fact that moisture diffuses outwards to the fruit surface at a higher flux rate than it does inwards, to the core of the fruit, hence resulting in a lower gradient towards the center.

Several studies have already analyzed the ethylene emission of whole pears at different maturities, temperatures and storage periods (Knee, 1987; Lindo-García et al., 2019; Villalobos-Acuña and Mitcham, 2008) as well as its respiration rate (Ho et al., 2018; Lammertyn et al., 2001; Saquet and Streif, 2017). To our knowledge no studies are available investigating the spatial distribution of ethylene production and respiration rates in pears. The ethylene production profile (Fig. C2.2B) presented a minimum at intermediate slices, II and III, with a significant increase towards the core. A similar profile, but with a better defined minimum at slice III, was found in the respiration rate (Fig. C2.2C).

Our results showed a relatively poor correlation between respiration rate and ethylene production ($r^2=0.546$) likely due to the different diffusivity of both compounds (ethylene and CO_2) within the pear flesh. Rudell et al. (2000) found that ethylene production had a maximum in the carpellary tissue in 'Fuji' apple at all harvest dates, which is in accordance to our results found for the inner slice (referred as IV). Moreover, Rudell et al. (2000) reported a minimum in CO_2 production in the hypanthial tissue, hence also in accordance with our data (Fig. C2.2C).

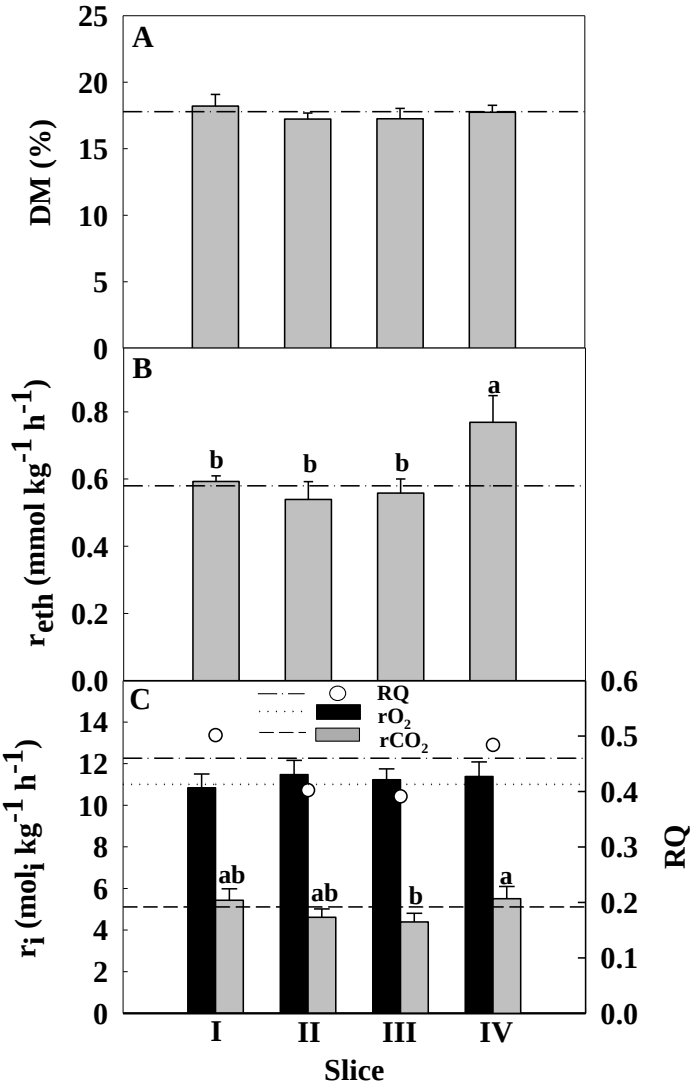

Figure C2.2 A) Spatial distribution among slices of dry matter content, B) ethylene production rate, C) O_2 consumption rate (black bars, left axis), CO_2 production rate (grey bars, left axis) and RQ (○, right axis). Error bars indicate standard deviation for n=4. For each graph, mean values with the same letter are not significantly different according to analysis of variance (ANOVA) and Tukey's HSD test ($P < 0.05$). Horizontal lines represent weighted averages, and were calculated weighting the value at each location by the difference of spherical volumes corresponding to the radius of both extremes of the sample.

C2.3.2 Sugar and organic acid composition

Fructose, glucose and sucrose are known to be the main sugars in 'Conference' pear fruit, and according to Colaric et al. (2007), in general, fructose represents more than 50 % of the pear sugar content. Our results are in accordance with this statement, since fructose accounted for 60 % of the total

sugar content, but clearly showed that these sugars were not uniformly distributed within the flesh of the fruit. Fructose content showed a quasi-linear decreasing profile with content values in the inner slice (slice IV Fig. C2.3A) about 40 % lower than in the outer slice, while sucrose showed an opposite trend with its lowest values under the fruit skin. Glucose content was minimum at slice II (Fig. C2.3A) and significantly higher ($p > 0.022$) in the slice near the core (slice IV). Measured fructose values, 46.3 g kg^{-1} as weighted average, were similar to the ones reported by Colaric et al. (2007) for 'Conference' pears harvested in 2004, however, these values were 1.5-fold lower than the ones obtained in the same study for fruit harvested in 2005. The measured glucose content (11.6 g kg^{-1}, weighted average) was nearly 2-fold higher than the values reported by Colaric et al. (2007) in fruit harvested in 2004 and Hudina and Štampar (2004) in Williams pears. Hudina and Štampar (2004) reported that the fruit sugar content was affected by climatic and soil conditions leading to differences as high as 50 %.

Malic acid is the predominant organic acid in 'Conference' pears followed by citric acid (Hudina and Stampar, 2000). The ratio between malic acid content and citric correlates with sensory perception of fruit taste (Colaric et al., 2007). In our measurements (Fig. C2.3B) malic was the predominant acid (3.6 g kg^{-1} as weighted averages) and its distribution profile presented a minimum in slice II. Hudina and Štampar (2004) reported similar results (3.7 g kg^{-1}) for 'Conference' pears harvested at south-east of Slovenia. Kou et al., (2014) reported that malic acid content in the peel (3.6 g kg^{-1}) of 'Huang guan' pear was higher than in the pulp (2.2 g kg^{-1}) which is in line with our findings. The spatial distribution of citric acid followed a similar trend than the one observed in malic acid content although no significant differences were found between slices (Fig. C2.3B). Citric acid (1.2 g kg^{-1} as weighted average) was 2.5-fold lower than malic acid in all slices.

In our study, only slice 'IV' had the lowest AsA content and showed significant differences if compared to the other slices ($p=0.0393$) (Fig. C2.3B). Johnson et al. (2013) found that AsA content in pulp (0.093 g kg^{-1}) of '*Citrullus Lanatus*' watermelon was higher than in rind and seed (0.076 and 0.053 g kg^{-1}, respectively). AsA content and fructose showed a quite good correlation with $r^2=0.764$. This result was in agreement with that found by Franck et al. (2003) who reported that AsA and fructose content had a similar pattern in 'Conference' pear, suggesting a close relationship between both components.

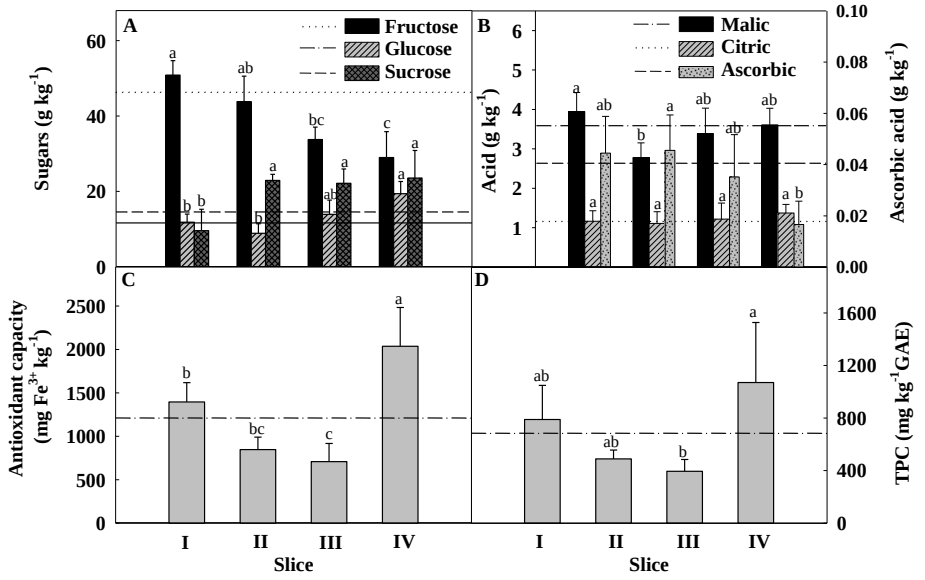

Figure C2.3 Contents, referred to unit of pulp fresh mass, of: A) sugars: fructose, glucose and sucrose, and B) acids: malic, citric (black and grey with diagonal lines bars, left axis) and ascorbic (grey dotted bars, right axis), C) antioxidant capacity measured by the FRAP assay and D) total phenolic compounds in different slices of 'Conference' pears spatially distributed. Error bars indicate standard deviation for n=4. For each graph, mean values with the same letter are not significantly different according to analysis of variance (ANOVA) and Tukey's HSD test ($P < 0.05$). Horizontal lines represent weighted averages.

C2.3.3 Antioxidant capacity and total phenolic compounds

According to different studies, pear fruit has beneficial health effects, protecting against different diseases, thanks to its antioxidant properties (Imeh and Khokhar, 2002; Kolniak-Ostek, 2016; Liaudanskas et al., 2017). Even though antioxidant capacity and total phenolic compounds in pears are low when compared to other fruit such as berries (Määttä-Riihinen et al., 2004), orange, kiwifruit and apples (Wang et al., 1996), the contribution of pear to the daily consumption of antioxidants and phenolic is relatively high (Chun et al., 2005). If compared to apples, total phenolic content in pear flesh is 3-fold lower (Leontowicz et al., 2002) and great variability exist among different pear cultivars (Brahem et al., 2017).

To our knowledge, little information is available about how antioxidant capacity and TPC are distributed along the flesh of fruit, and especially in pear. The fruit antioxidant capacity (1210.5 mg Fe^{3+} kg^{-1} as weighted average) had a minimum in slice III with a sharp increase in the slice near the core (Fig.C2.3C).

A similar profile was also found for TPC content (Fig. C2.3D). Imeh and Khokhar, (2002) analyzed TPC in different apple, pear and stone fruit cultivars and reported that 'Conference' pear had the lowest values (3023 mg kg^{-1} GAE) among the studied cultivars. However, their values were two-fold higher than that obtained in this study. This could be because in their analysis they included the peel, which is reported to have higher amounts of TPC.

C2.3.4 Volatiles spatial distribution

While several studies have been focusing on 'Conference' pear volatiles emission under different circumstances (Goliáš et al., 2015; Hendges et al., 2018; Saquet, 2017) no information is available describing the VOC's concentration in different locations inside the pear flesh. Aubert and Milhet (2007) investigated the distribution of VOCs in different parts of a white-fleshed peach (cv. Maura) and found that volatiles content in skin were significantly higher than in flesh.

In our study twenty-nine volatile compounds were identified and quantified in the different locations of the slices in 'Conference' pear (Table C2.1). These volatile compounds included 16 esters, 6 alcohols, 3 aldehydes, 2 terpenoids, 1 acid and 1 ketone. Indeed, esters play an important role providing a characteristic fruity aroma (Zlatić et al., 2016) when these volatiles are released from intact fruit. However, when fruit is cut or crushed different enzymatic processes can be activated, some of which are extremely rapid once cellular disruption begins (Rapparini and Predieri, 2003). In this context, aldehydes are major components in pulp extracts, but not in the headspace of intact pears.

Our research showed that hexanal was the main volatile detected with its highest concentration in the 'II' slice (140 μg kg^{-1}) but with no significant differences between slices ($p=0.1278$). Aldehydes are known to be the main responsible of grassy aroma (Zlatić et al., 2016) and green flavor (Rapparini and Predieri, 2003). Besides being a typical fruit volatile, hexanal is also formed when cellular structures are disrupted (Clark et al., 2014) and hence this compound is detected at its highest concentrations in fresh-cut fruit or when using similar methodologies to the one described herein (SPME);. For instance, Rizzolo et al. (2005), found that hexanal was one of the main volatile in 'Conference' pears under controlled atmosphere and it was the most prominent in odor units. Lindo-García et al. (2019) also found that hexanal was the principal aldehyde in 'Blanquilla' pears during on and off-tree ripening.

Similarly, Makkumrai et al. (2014) reported that hexanal was the main aldehyde in 'Barlett' pears stored at 20 ºC for 11 d and Horvat et al. (1992) found that hexanal was one of the main volatiles in five Asian pear cultivars. All these studies used similar methodologies as the one described in this study.

The main ester detected was butyl butanoate which has been already reported as an impact volatile in 'Conference' pears (Rizzolo et al., 2005). Even though, no significant differences between locations of the slices were found, its maximum concentration was found in slice II. Butyl butanoate is largely known to contribute to sweet or fruity odors.

From the 29 identified volatiles only six presented significant differences between locations of the slices; butyl acetate, 2-ethy-hexanal, 3-methylbutyl 3-methyl-butanoate, (E)-2-hexenyl acetate, hexyl butanoate and hexyl 2-methylbutanoate. Some of these compounds have been previously identified as important character-impact volatiles in whole 'Conference' pears (El Hadi et al., 2013; Saquet, 2017; Torregrosa et al., 2019) contributing, among others, to sweet and fruity odors. The spatial distribution of flavor components and antioxidants along the flesh of pear fruit may be of use to the fresh-cut industry to supply fruit with improved flavor and nutritional value by selecting not only the appropriate fruit but also specific parts of it.

Table C2.1 Mean ± standard deviations (n=4) values of VOC's concentration (µg kg^{-1}) of equatorial slices of 'Conference' pear from different radial locations. Means within the slices preceded by the same small letters are not significantly different at $P \leq 0.05$ (HSD test). No letter indicates the absence of significant differences.

	Slice			
Volatile compounds	**I**	**II**	**III**	**IV**
Esters				
Ethyl Acetate	7.1±0.8	6.6±0.1	6.4±0.1	5.0±3.3
Tert-Butyl propionate	3.4±3.0	2.4±3.5	3.7±2.5	2.4±2.9
Methyl butanoate	1.6±2.9	2.4±3.5	2.4±2.9	1.2±2.5
Butyl acetate	[a]3.6±3.2	[a]5.5±0.2	[a]5.5±0.3	[b]0.0±0.0
Pentyl acetate	1.2±2.2	3.7±0.0	1.0±2.1	1.3±2.7
Butyl butanoate	27.2±8.8	33.4±3.8	29.1±20.9	24.6±17.0
Hexyl acetate	1.3±2.3	3.8±0.0	2.7±1.9	0.0±0.0
3-Methylbutyl 3-methyl-butanoate	[b]0.0±0.0	[b]0.0±0.0	[a]2.7±1.8	[b]0.0±0.0
(E)-2-Hexenyl acetate	[c]0.0±0.0	[c]0.0±0.0	[a]4.1±0.2	[ab]2.7±1.8
Butyl hexanoate	4.2±0.1	2.0±2.9	2.0±2.4	3.4±2.4
Hexyl butanoate	[b]0.0±0.0	[b]0.0±0.0	[b]0.0±0.0	[a]2.3±1.6
Hexyl 2-methylbutanoate	[b]0.0±0.0	[a]3.5±0.0	[b]0.0±0.0	[b]0.0±0.0
Ethyl octanoate	0.0±0.0	0.0±0.0	2.9±2.0	2.7±1.9
Octyl acetate	2.0±0.0	1.0±1.4	2.0±0.1	1.5±1.0
Pentyl hexanoate	0.0±0.0	0.0±0.0	1.2±2.4	1.1±2.3
Hexyl hexanoate	3.0±2.7	4.1±0.4	4.4±0.5	2.2±2.6
Alcohols				
1-Butanol	2.7±2.3	4.1±0.0	4.0±0.1	3.0±2.0
2-Methyl-1-butanol	3.0±2.7	4.6±0.5	3.6±2.4	3.2±2.2
1-Hexanol	3.3±0.1	3.6±0.2	2.7±1.8	3.4±0.1
2-Ethyl-1-hexanol	5.0±1.0	5.1±0.3	5.0±0.8	4.2±0.5
1-Octanol	1.1±1.9	0.0±0.0	1.6±1.9	0.8±1.6
Benzyl alcohol	1.4±2.5	0.0±0.0	3.2±2.2	1.0±2.0
Aldehydes				
Hexanal	104.4±91.9	140.2±15.5	128.8±29.7	128.7±86.3
2-Ethyl-hexanal	[a]5.1±1.5	[b]0.0±0.0	[b]0.0±0.0	[ab]2.2±2.7
Benzaldehyde	2.8±2.5	4.2±0.2	4.2±0.1	2.1±2.4
Terpenoids				
(Z)-β-farnesene	6.5±6.8	5.4±0.0	5.9±4.4	6.0±4.3
α-farnesene	4.7±0.3	4.4±0.1	3.6±2.4	3.3±2.2
Acid				
Acetic acid	8.9±6.0	2.8±4.0	16.1±27.2	5.4±6.4
Ketone				
6-Methyl-5-hepten-2-one	2.5±2.3	3.8±0.2	3.8±0.2	2.7±1.8

C2.3.5 Susceptibility to *P. expansum* and *R. stolonifer* along the pear flesh

P. expansum and *R. stolonifer* fungus are important destructive fungal pathogens of pome fruit. Many studies have analyzed blue mold and soft rot in entire pears (López et al., 2015; Neri et al., 2010). However, no information is available about the fungal growth on flesh from different locations within the flesh of 'Conference' pear.

P. expansum showed an incidence of 100 % in all locations of the evaluated slices, in contrast severity was significantly different between slices ($P < 0.001$), slice (I) close to the peel had the lower fungal severity (Fig. C2.4A). Rot incidence was evaluated in inoculated slices with *R. stolonif*er since measuring severity for this type of pathogen is not an easy task mainly due to the black and loose mycelium with white aerial fruiting structures (Sardella et al., 2016). Slice 'I' had the highest incidence of *R. stolonifer* (Fig. C2.4B).

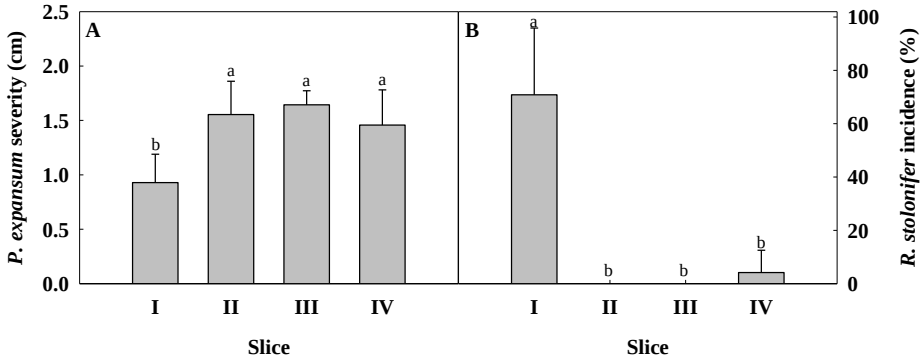

Figure C2.4 Fungal susceptivility, A) blue mold (*Penicillium expansum*) severity and B) soft rot (*Rhizopus stolonifer*) incidence in the different locations of 'Conference' pear flesh. For each graph, mean values with the same letter are not significantly different according to analysis of variance (ANOVA) and Tukey's HSD test ($P < 0.05$).

C2.3.6 Relationship between tissue composition and susceptibility to major postharvest pathogens

In order to know which variables were characteristics of each slice and determine those that were key to differentiate slices, a first multivariate analysis considering all the analyzed variables, except those of fungal susceptibility to *P. expansum* and *R. stolonifer*, was done. A dendrogram graph was used to further obtain a global overview of the relationship between ethylene emission, respiration, sugars, organic acids, antioxidants, phenols and the profile of volatile compounds in a reduced dimension plot. In this data set, 42 variables

were used (Fig. C2.5A). The hierarchical heatmap showed that slices 'I' and 'IV' had similar amounts of the components in cluster 1 (C1), except for sucrose and hexanal (Fig. C2.5B). This cluster encompasses some major pear character-impact compounds such as butyl butanoate. Components encompassed in cluster 3 (C3) had a similar behavior in slices 'II' and 'III', except for 1-hexanol.

On the other hand, and given the different susceptibility of the different slices locations to blue mold and soft rot, two partial least square regression (PLS) models were performed in order to identify which variables had higher correlation with the susceptibility of *P. expansum* and *R. stolonifer* growth. The PLS models were done to correlate respectively *P. expansum* growth (Y_1 variable) and *R. stolonifer* growth (Y_2 variable) with a set of potentially explanatory variables: sugars and organic acids content, ethylene production, respiration, dry matter, volatiles compounds, antioxidant capacity and total phenolic content (X variables). Based on PLS method, the X data set was reduced to two principal factors. The first factor explained more than 99% for both fungi, *P. expansum* (Fig. C2.6A) and *R. stolonifer* (Fig. C2.7A). The correlation between measured and predicted blue mold severity and soft rot incidence were higher than 0.99, demonstrating the goodness of the model (Fig. C2.6B, C2.7B). *P. expansum* growth showed a positively correlation with the sucrose content and some VOC's such as (E)-2-hexenyl acetate, ethyl octanoate, pentyl hexanoate, hexanal, 1-butanol, 2-methyl-1-butanol and 6-methyl-5-hepten-2-one (Fig. C2.6C). With such a background, 'II' and 'III' slices followed by 'IV' and 'I' were more prone to the growth of this fungus. However, *R. stolonifer* was positively correlated with fructose, malic acid and dry matter content and with ethyl acetate, butyl hexanoate, 2-ethyl-hexanal, butyl hexanoate, (Z)-b-farnesene and α-farnesene (Fig. C2.7C). 'I' is the most suitable slice for its fungus to growth.

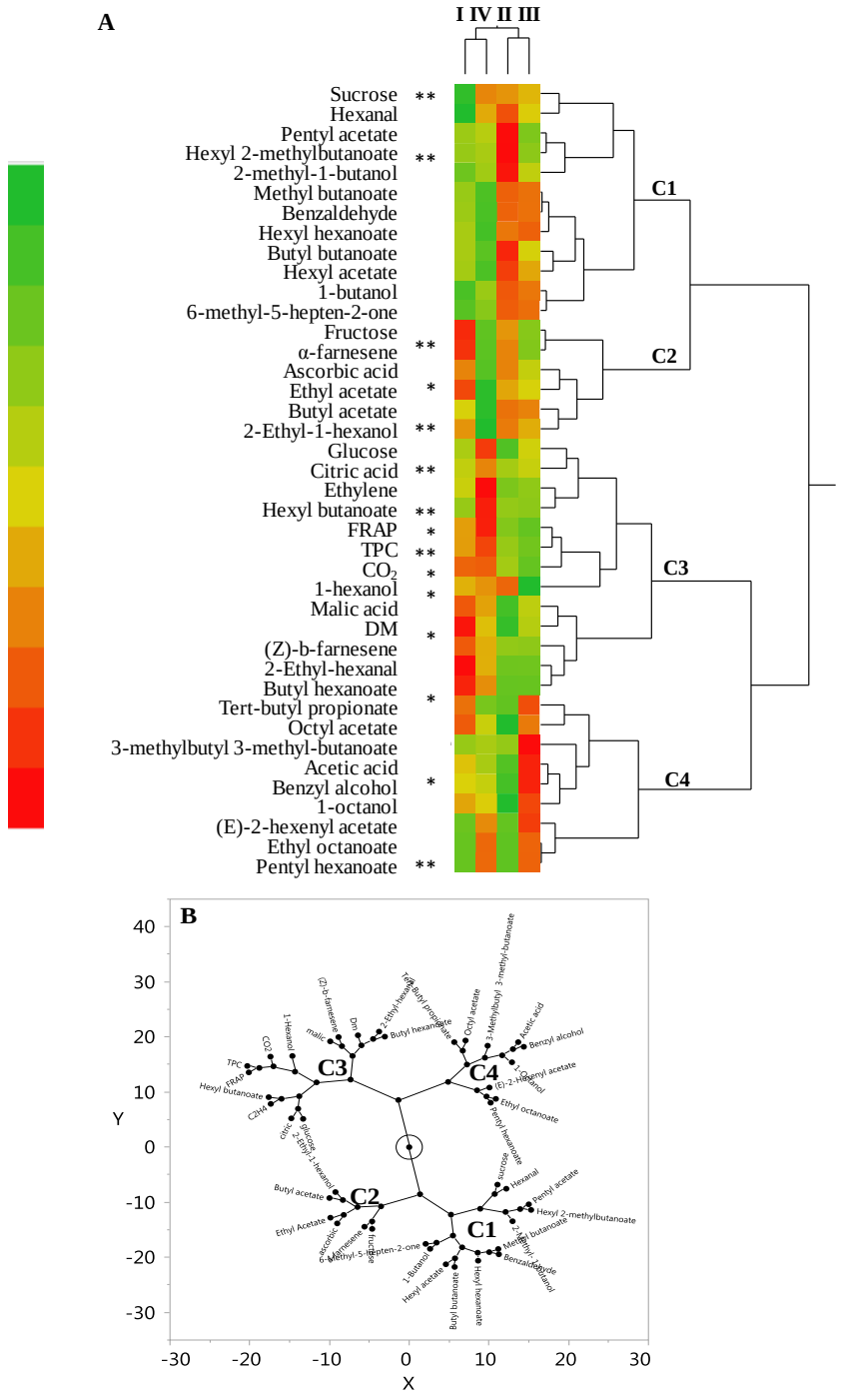

Figure C2.5 A) Hierarchical heatmap based on the normalized quantities of the analyzed elements and identified volatiles in each 'Conference' section. The lowest content is in the lightest green and the highest in the darkest red. * indicate significant differences ($P < 0.05$) and ** indicate significant differences ($P < 0.01$) between sections. B) Constellation plot of the different clusters.

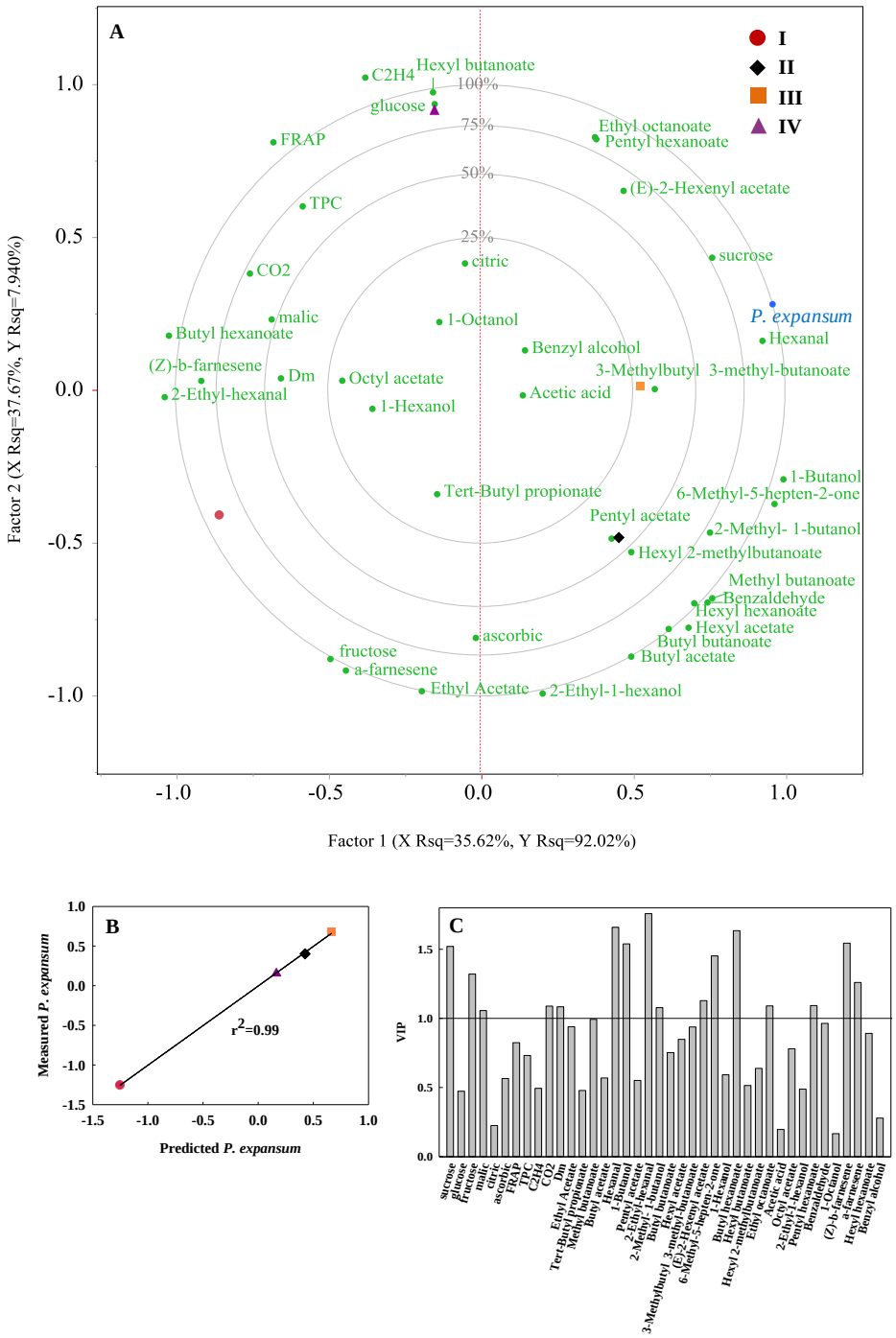

Figure C2.6 A) Partial Least Squares (PLS) correlation loading plots of the 2 factors of *P. expansum* severity. B) The measured vs the predicted *P. expansum* severity through the model and its correlation coefficient. C) Variable importance plot (VIP), the number of VIP>1.

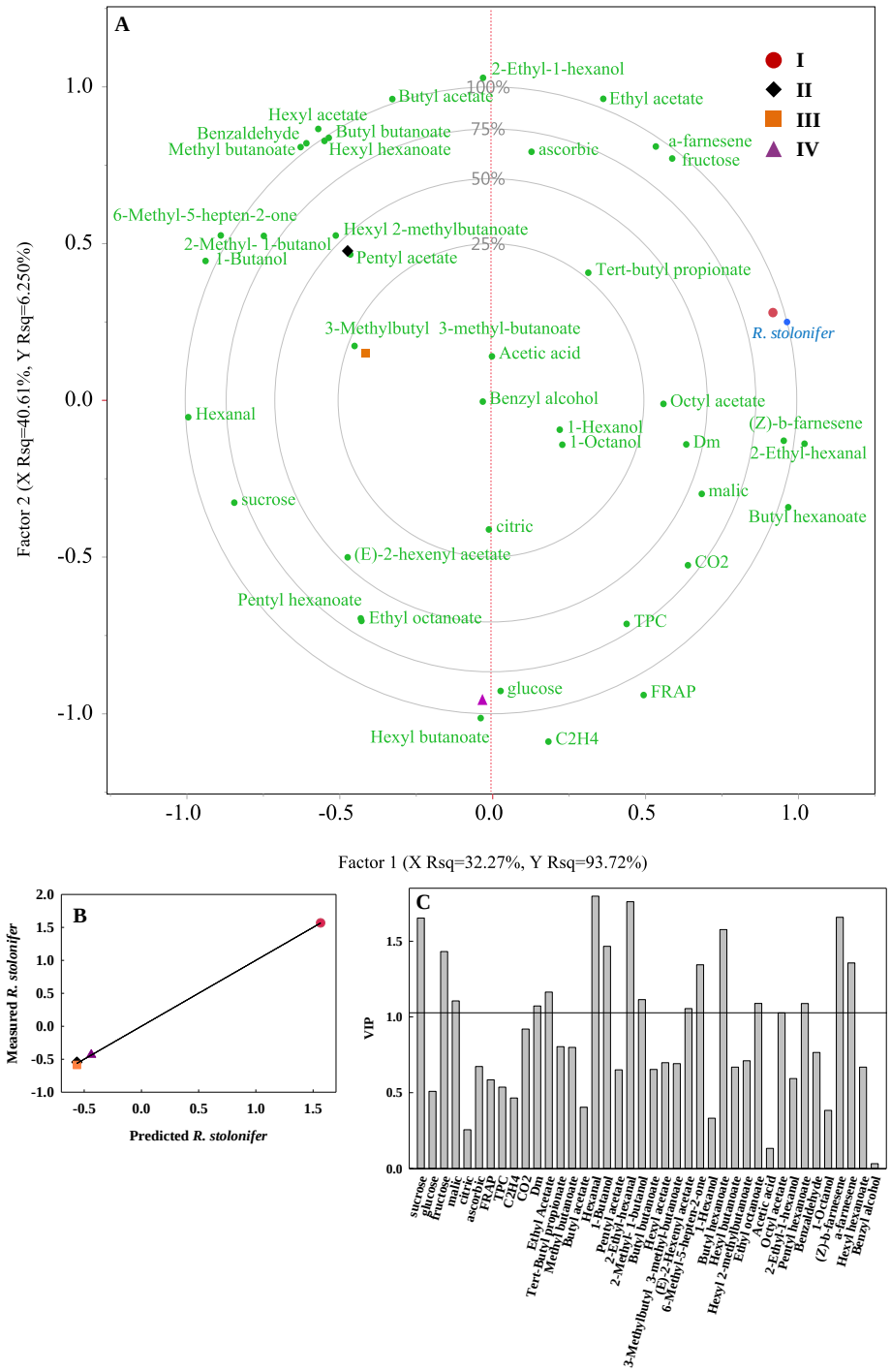

Figure C2.7 A) Partial Least Squares (PLS) correlation loading plots of the 2 factors of *R. stolonifer* incidence. B) The measured vs the predicted *R. stolonifer* incidence through the model and its correlation coefficient. C) Variable importance plot (VIP), the number of VIP > 1.

C2.3.7 Antifungal efficacy *in vitro* of VOCs against *P. expansum* and *R. stolonifer*

Based on our PLS results (Fig. C2.6 and C2.7), 2-ethyl-hexanal and butyl hexanoate were the most effective compounds against *P. expansum* whereas hexanal and 1-butanol were the most effective against *R. stolonifer*. The effect of these volatiles was further studied *in vitro* with different concentrations (Fig. C2.8). The *in vitro* results of exogenous applied compounds, commonly emitted by 'Conference' pears, and their capacity to suppress the mycelial growth of both pathogens is shown in Table C2.2. All tested concentrations of 2-ethyl-hexanal, completely controlled *P. expansum* growth while control fruit had a diameter growth of 3 cm after 3 d (Fig. C2.8A). Moreover, any used concentration of butyl hexanoate was capable to completely control mycelial growth (Fig. C2.8B). A concentration of 0.22 µL mL^{-1} of hexanal completely controlled the infection (Fig. C2.8C) and hexanal had an EC_{50} of 0.055 µL mL^{-1} on *R. stolonifer* growth (Table C2.2). Soft rot was completely controlled by 1-butanol application at 1.76 µL mL^{-1} (Fig. C2.8D). These results agreed with those found by Neri et al. (2006), who investigated the effect of nine plant volatiles *in vitro* and *in vivo* trials against blue mold on pears and found that *trans*-2-hexanal and carvacol had prominent effects, while hexanal had a less marked effect. Sáenz-Garza et al. (2013) also reported that the hexanal released from microcapsules on the surface of PDA inhibit blue mold growth and it was viable to preserve apple slices. As reviewed by Mari et al. (2016), other aldehydes and alcohols such as benzaldehyde and ethanol have shown promising results controlling different fungal growth in a wide range of fruit and vegetables and hence future studies are warrant.

Table C2.2 Antifungal activity of pure volatile organic compounds at different concentrations on the in vitro mycelial growth inhibition (%) tests against *P. expansum* after 5 d and *R. stolonifer* after 3 d. When possible, EC_{50} values were calculated according to Alexander et al. (1999) (µL mL^{-1} headspace).

Pathogen	**Compound**	**Concentration (mL L^{-1} headspace)**	**Mycelial growth inhibition (%)**	**EC_{50} (µL mL^{-1})**
P. expansum	2-Ethyl hexanal	0.027	100.0	-
		0.055	100.0	
		0.11	100.0	
		0.22	100.0	
		0.44	100.0	
		0.88	100.0	
		1.76	100.0	
	Butyl hexanoate	0.027	9.5	0.61
		0.055	29.7	
		0.11	10.9	
		0.22	26.6	
		0.44	37.6	
		0.88	56.1	
		1.76	54.9	
R. stolonifer	Hexanal	0.027	4.4	0.055
		0.055	50.0	
		0.11	95.9	
		0.22	100.0	
		0.44	100.0	
		0.88	100.0	
		1.76	100.0	
	1-Butanol	0.027	ni	0.48
		0.055	ni	
		0.11	ni	
		0.22	9.1	
		0.44	43.4	
		0.88	97.6	
		1.76	100.0	

ni: no mycelial growth inhibition observed

-: insufficient data to calculate EC_{50} values.

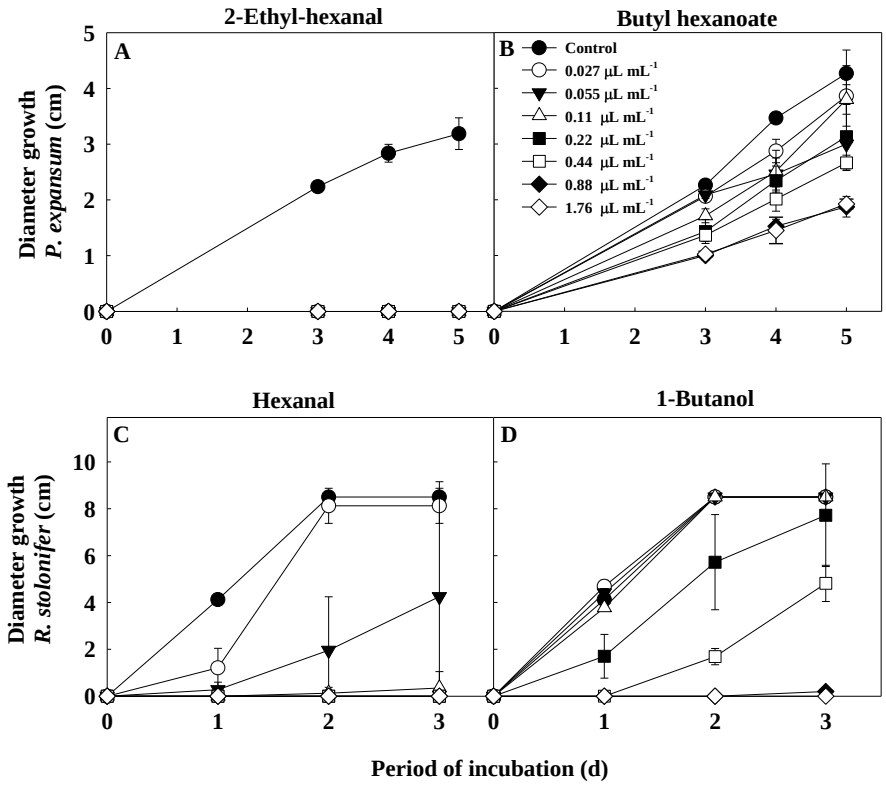

Figure C2.8 Effects of different concentrations of VOCs, A) 2-ethyl-hexanal and B) butyl hexanoate on mycelia diameter (cm) of *P. expansum* growth during 5 d and C) hexanal and D) 1-Butanol on mycelia diameter (cm) of *R. stolonifer* growth during 3 d. Error bars indicate standard deviation for n=4. For each graph, mean values with the same letter are not significantly different according to analysis of variance (ANOVA) and Tukey's HSD test ($P < 0.05$).

C2.4 Conclusions

The results from this study demonstrate that flavor components including sugars and organic acids are non-uniformly distributed along the flesh of 'Conference' pears. Not only components but also the capacity of the tissue to produce ethylene and CO_2 was different along the equatorial location. Some VOCs also presented significant differences among slices and such differences may partly explain the different susceptibility to major postharvest pathogens. *In vitro* experiments showed that components naturally present along the pear flesh had antifungal activity. Thus, 2-ethyl-hexanal revealed an antifungal effect against *P. expansum* while hexanal and 1-butanol acted against *R. stolonifer*. Overall, the results presented herein give added value to the fresh-cut industry (aiming to attain fruit with improved nutritional quality and flavor) and could improve food security using natural compounds capable of inhibiting major postharvest pathogens.

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Chapter 4: GLOBAL DISCUSSION AND CONCLUSIONS

In Catalonia, in 2017 the fresh fruit sector (excluding citrus) accounted for 23 % of the final agricultural production (Idescat, 2019). The area around Lleida, (NE of Spain) is one of the main pear production areas in south Europe and represents more than 95 % of the Catalan fresh pear production, with 'Conference' (50.4%) and 'Llimonera' (15.2 %) being the most important varieties (Afrucat, 2019). This thesis focus on the time-evolution of 'Conference' pear quality parameters and how the fruit quality may be influenced by environmental conditions along each of the three consecutive commercial periods: on-tree growth, cold storage and shelf life. Fruit quality is affected by different parameters which can be measured objectively. Table 4.1 reflects the parameters studied at every stage.

Table 4.1 Quality parameters experimentally measured in each of the three stages of the fruit life.

	On-tree	**Cold storage**	**Shelf Life**
Diameter	x	x	x
Mass	x	x	x
Firmness	x	x	x
Acoustic firmness	x	x	
I_{AD}	x	x	x
Color	x	x	x
TSS	x	x	x
TTA	x	x	x
Ethylene rate	x	x	x
Respiration rate	x	x	x
VOCs		x	x
Ethanol		x	x
Acetaldehyde		x	x
Sugars			x
Acids			x
FRAP			x
TPC			x
Fungus susceptibility			x

As quoted in Chapter 2, one of the main challenges during the on-tree growth is to determine the optimal harvest date (OHD) at which the state of the fruit is such that the best quality will be reached after the cold storage period. OHD is traditionally determined counting days after full bloom (DAFB) or based on firmness and color measurements. In the developed studies (Chapter A1) the evolution of quality parameters was checked, presenting correlations between different measurements, a 3D scanner technique to measure morphological parameters was introduced and a function to predict the fruit final diameter up to one month prior to harvest was proposed.

During the cold storage period the main challenges are to extend its duration avoiding fruit physiological disorders and fruit dehydration. In a first study (Chapter B1) the comparison of three commercial technologies to monitor DCA chambers was presented. The second study (Chapter B2) focused on the conditions under which the fruit was best preserved and how the emission of VOCs could be used to detect the states of stress of the fruit. The third study (Chapter B3) tackled the problem of fruit dehydration, a new measuring device was designed and the obtained measurements were correlated with fruit mass loss.

The rapid metabolic processes set off during the shelf life shortens the commercial life of fruit. During that brief period the main challenge was to know the point at which the maximum consumer acceptance was reached and how undesired fungus pathologies could be avoided. In one study (Chapter C1) the relationship between quality parameters and consumer acceptance was unveiled. In a second study (Chapter C2) the spatial distribution of biochemical compounds inside the fruit and how different natural compounds could be used to avoid fungus growth was investigated.

4.1 Part A. Evolution of apples and pears quality parameters during on-tree growth and OHD determination

- 'Conference' pears undergo remarkable shape and size changes during on-tree growth. Fruit surface and volume are important parameters to model diffusive and convective mass transfer of water vapor and gases in fruit, however, accurate measurements of these parameters are not an easy task (Khojastehnazhand et al., 2009). Traditionally, fruit surface is estimated by measuring the area of adhesive tape required to cover the fruit (Clayton et al., 1995) and volume is measured using the water displacement method (Xanthopoulos et al., 2017). Both methods are associated with different errors (Moreda et al., 2009). In apples volume can be approximated assuming that fruit has a spherical shape (Ambaw et al., 2013), what is a good approximation due to their rounded shape but that is not reliable in pears. Babic et al. (2012), developed a mathematical model for the estimation of a pear quarter's surface area and volume on the basis of just the pear's length, an accurate technique for well-formed fruit but not for the misshapen ones. In this thesis the geometric shape of the fruit was captured by means of a 3D scanner (Chapter A1). The coordinates of each point from a triangular mesh covering the fruit surface were determined with an accuracy $\leq$0.1 mm (Fig. 4.1). From that mesh the fruit surface and volume were calculated. The digitized information can be used for further shape analysis and to model the fruit maturation process as a solid-state reactor (Herremans et al., 2015).

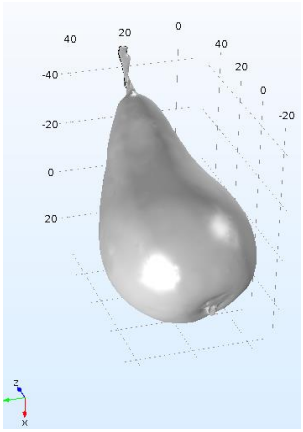

Figure 4.1 Screenshot of a 'Conference' pear 3D geometric shape on 25th July 2016, using EinScan-S, Shining 3D, China. Surface area was 109.4 cm^2 and volume 245.0 cm^3.

The use of 3D scanning techniques in fruit sorting lines can provide added value when discretizing fruit by size and shape, distinguishing different size categories and well-formed and misshapen fruit (Moreda et al., 2012). Fruit sorting is considered one of the most important steps of handling (Amer Eissa and Abdel Khalik, 2012). Nowadays, sorting is mainly made based on fruit caliper. A step forward is the application of image, texture and shape acquisition techniques at the sorting lines (Mahendran et al., 2015).

- Pear size at the time of harvest, measured as fruit diameter, is one of the main factors to establish the market price (Garriz et al., 2005). The evolution of the fruit diameter throughout the growth period showed a sigmoid trend in accordance with previous studies in apples (Zheng et al., 2012), and with other fruit species, such as pineapples (Pauziah et al., 2013) and logan fruit (Shi et al., 2016). The capacity to predict the final fruit diameter some weeks prior to harvest can facilitate key information to producers in order to decide the optimal harvest date.

During on-tree growth 'Conference' pear equatorial diameter and mass followed a sigmoid trend that could be successfully fitted with a 3-parameter Gompertz equation (eq. 3, Chapter A1) which has already been used to model 'Royal Gala' apple fruit growth (Stanley et al., 2000) and apple fruit mass (Gandar et al., 1996). The fits using growth data, from 'Golden' and 'Gala' apples and 'Conference' pears harvested in 2016, up to one month prior to OHD allowed to predict the harvest diameter with an error lower than 10 %. If the fits used data up to two weeks before OHD then the error could be reduced to 4 %. The same methodology was also successfully applied to the diameter and mass evolution in other pear cultivars ('Conference', 'Blanquilla' and 'Williams' harvested in 2017) and in different apple varieties ('ERO' and

'Granny Smith' harvested in 2016). In all cases the error in the diameter prediction two weeks in advance to harvest was lower than 4 % when data used in the fit contained points in the growth decelerating phase (Fig. A1.4).

Although different studies reported values for the 3 Gompertz parameters, confidence intervals are scarcely reported in the literature. Monte Carlo method was used to provide meaningful estimations (Illa et al., 2012). All the reported fits in Chapter A1 were quite good ($r^2 \geq 0.98$), but attention should be paid to the large width of some confidence intervals, which gives an idea about the reproducibility of parameter values. That fact must be considered when comparing parameter values from different authors or data sets.

- Non-destructive techniques are currently available to monitor some fruit quality indicators. Indicators are referred as non-destructive measurements that can be used to estimate physical properties of the fruit whereas parameters are those measurements, destructive or not, that denote fruit attributes. Such non-destructive techniques are widely used because of its simplicity and low cost, however, its correlation with fruit quality parameters is highly dependent on the fruit cultivar and its maturation stage.

During on-tree growth destructive measurements of fruit firmness were recorded simultaneously with non-destructive measurements of acoustic firmness and I_{AD} index, based on visible spectrometry (Chapter A1). A good correlation was found between traditional destructive firmness and acoustic firmness in 'Conference' pears (Fig. 4.2), confirming that the acoustic impulse technique is a valuable method to monitor firmness evolution during fruit growth (De Belie et al., 2000). On the other hand, DA meter based on visible spectrometry has already been used to determine the OHD on different fruit species such as pears (Rizzolo et al., 2015; Wang et al., 2015), apples (DeLong et al., 2016) and in stone fruit (Bonora et al., 2014; Ziosi et al., 2008). A good correlation between I_{AD} values and destructive firmness was found during the 40 days prior to the OHD (Fig. 4.2).

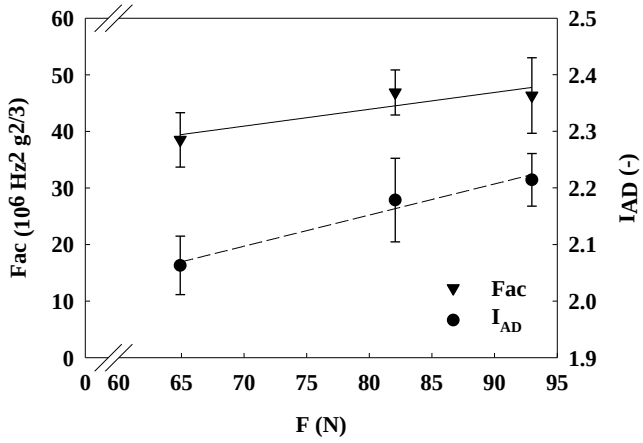

Figure 4.2 Relationship between destructive firmness (F) and acoustic firmness (F_{ac}) and relationship between F and index of Delta Absorbance (I_{AD}) in 'Conference' pears (n=40).

- It is well known that morphological and quality changes during on-tree growth show a parallelism with changes in ethylene production and respiration rates (Chiriboga et al., 2013; Gwanpua et al., 2012; Oetiker and Yang, 1995). In Chapter A1 was explored the relationship between major quality changes and fruit physiology as determined by fruit ethylene production capacity and respiration rate. The ethylene production rate in 'Conference' pears showed a transient peak at 110 DAFB which was well correlated with major changes in: fruit softening, increase in TSS values and decrease of TTA. These results confirm that ethylene plays a key role in controlling the initiation of quality changes on-tree in climacteric fruit (Lelièvre et al., 1997) like pears. A quasi-linear correlation was found between the fruit respiration rate and the surface/volume ratio throughout the growing period (Fig. 4.3). That is consistent with the fact that respiration triggers a diffusion process of oxygen through the fruit skin and fruit pulp in which the skin permeability is the key parameter (Nguyen et al., 2007).

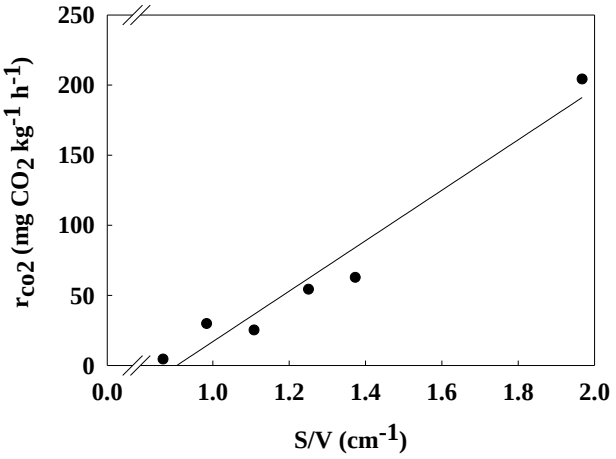

Figure 4.3 Relationship between respiration rate (r_{CO2}) and surface/volume ratio during on-tree growth in 'Conference' pears.

Changes in respiration, ethylene production and other physiological traits during growth are undoubtedly related to final fruit quality at harvest (Giné-Bordonaba et al., 2019). Monitoring morphological and physiological changes during fruit growth may assist producers on predicting optimal harvest date while having some control of the final fruit quality.

4.2 Part B. DCA as a chemical-free alternative to extend the pear commercial life. Different criteria to determine the state of the fruit

- Cold storage under low oxygen levels can enlarge the storage period, however excessive oxygen depletion can trigger fruit internal browning (Saquet et al., 2001). There is not a definite oxygen threshold under which internal browning appear, as it depends on several factors such as environmental conditions during on-tree growth, the fruit maturity stage at harvest, storage duration, cultivar and season (Boeckx et al., 2019). Nevertheless, the determination of the lowest oxygen level tolerated by the fruit at any time during the cold storage period is based on different physiological responses of the fruit. Nowadays there are three commercial techniques available, namely: I) chlorophyll fluorescence, II) respiratory quotient and III) ethanol accumulation in fruit pulp. During the last decade industries have been improving their installations and implementing the DCA technology. In Fig. 4.4 it is shown the rapid growth of the number of cold storage rooms under DCA in Girona region (northeast Catalunya). In 2016-2017 season, Lleida region had 210 commercial cold rooms working with DCA, mainly based on chlorophyll fluorescence technology. However, it is not clear the most effective way to evaluate fruit stress during cold storage.

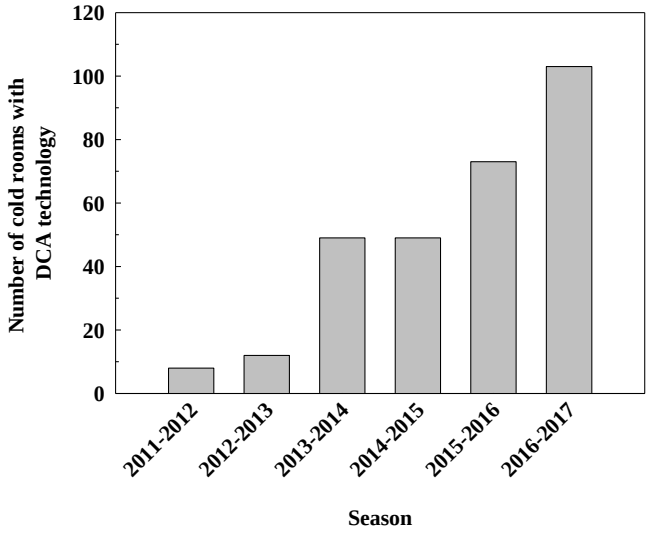

Figure 4.4 Evolution of the number of commercial chambers under DCA conditions in Girona. (Adapted from Dr. Elena Costa, STP, IRTA).

Previous studies have demonstrated that, unlike other low oxygen technologies such as CA which must be complemented with chemical treatments, DCA can be used as a non-chemical postharvest treatment (Prange et al., 2011). Each DCA technique has reported different results depending on the fruit variety, for example, DCA-CF was an effective technology to avoid internal breakdown in 'Rocha' pear (Deuchande et al., 2016), DCA-RQ was an efficient method to detect the LOL of 'Fuji Suprema' apple (Weber et al., 2017) whereas, DCA-EtOH was associated with production of high levels of fermentative metabolites and did not avoid internal breakdown incidence in 'Rocha pear (Deuchande et al., 2016).

In order to compare the response of these techniques 'Conference' pears were stored for 8 months during the 2017-18 season and were monitored by the RQ system and compared with chlorophyll fluorescence evolution and with ethanol in fruit pulp content (Chapter B1). Volatile organic compounds from the cold room atmosphere were captured once per month.

At the beginning of storage, when O_2 levels dropped below 0.1 % there was a peak in the CF signal, paralleled by an increase in the levels of EtOH as well as the content of some VOCs in the storage atmosphere (i.e. α-pinene, and β-pinene). In contrast, neither a clear trend was found in the RQ values nor a clear relationship between the signals provided by different sensors after 6 weeks of storage (Fig. B1.2). In summary, regardless of the DCA-monitoring system employed, fruit was successfully stored under DCA conditions for 8 months without symptoms of internal disorders. In contrast, other authors

reported high incidence of brown heart and quality losses when storing 'Conference' pears under low oxygen and high CO_2 concentrations (Saquet et al., 2000; Saquet and Streif, 2002).

- To determine the effects of different low oxygen strategies during the cold storage on the final fruit quality the following experiment was planned and executed. In a cold room were kept for 7 months three different chambers of 350 L each, filled with 'Conference' pear (Chapter B2). The atmosphere in each chamber was independently controlled. The fruit in one of the chambers (ILOS) was exposed to an initial low oxygen stress and a constant oxygen and CO_2 levels afterwards. The other two chambers ($DLOS_1$ and $DLOS_2$) were exposed to five low oxygen stresses during the conservation period, in the $DLOS_2$ the stresses were longer. After each stress a gas sample from each chamber was extracted in order to analyze VOCs.

The application of repeated stresses has been previously applied when storing apples. Fadanelli et al. (2015) stored 'Red Delicious' apples during 8 months and applied 3 stresses during the whole period (Fig. 4.5), analyzing the ethanol content in fruit pulp by Senzytec sensor and found high amounts of ethanol accumulation after the first stress whereas it was low in the following stresses.

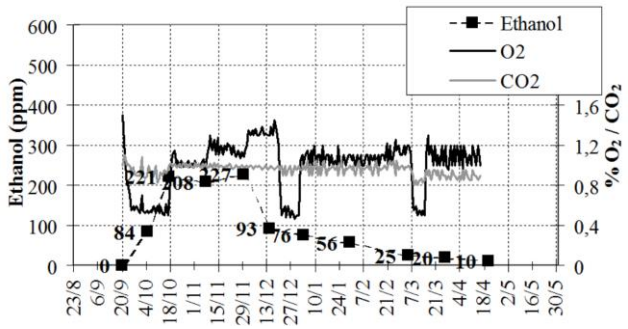

Figure 4.5 Atmosphere oxygen levels evolution and ethanol content in 'Red Delicious' apples during 8 months in a cold room under DCA conditions (Fadanelli et al., 2015).

Deuchande et al. (2016b) recently defined that 20 ppm of ethanol in 'Rocha' pears pulp as a critical level for the induction of internal disorders. However, our results showed higher amounts of ethanol and 'Conference' pears did not developed any disorder.

After the storage period plus 5 days in SL it was observed that fruit stored under the ILOS conditions had lower I_{AD} values and higher ethanol and acetaldehyde content, suggesting that fruit was more mature than fruit stored under DLOS chambers. No significant differences in the quality parameters

were observed between $DLOS_1$ and $DLOS_2$ chambers, both after storage and after 5 days in SL.

- It is known that volatile organic compounds emitted by fruit into the atmosphere carry key information about the fruit physiological state (López et al., 2015; Vandendriessche et al., 2012).When comparing the characteristic VOCs detected in the three chambers it could be observed that some esters such as methyl butanoate and butyl hexanoate and alcohols such as 2-ethylhexanol and benzyl alcohol increased their concentration in the chamber atmosphere after the third low oxygen stress. That is a hint that they can be used as biomarkers of fruit to the stresses, since it is known that esters are synthesized in plant, and likely in fruit, as a result of abiotic stress exposure (Spinelli et al., 2011).

- Fruit respiration after two storage periods, 30 and 202 days, was approximated by an enzyme kinetics model (Wang et al., 2009). After 30 d in storage the coefficient of determination of the fit was higher than 0.98 and declined after 202 d when a lag in respiration was observed (Fig. B2.7). The used kinetic model did not include the mechanisms to simulate that lag. Interesting is, that differences in fruit ripening capacity were not observed in the respiration rate evolution. Similarly, Both et al. (2014) evaluated 'Royal Gala' respiration capacity after 8 months stored under different oxygen levels and found significant differences one day after storage but not thereafter.

- The loss of water from fruit negatively affects its visual appearance because the skin shrinks and forms a rougher surface. To prevent excessive water loss fruit is cooled down as soon as possible after harvest (Amarante et al., 2001) and water is sprayed in cold rooms in order to keep a high RH in the atmosphere. However, excessive RH in cold rooms should be avoided because can promote the growth of undesired fungal pathogens. Nowadays it is not a commercial practice to continuously monitor the fruit mass loss during the storage period given that scales are expensive and very sensible to mechanical perturbations. A new device to indirectly measure the mass loss in commercial chambers based on measuring the mass settlement was developed and tested (Chapter B3). Settlement was continuously measured with an ultrasound distance sensor simultaneously with fruit diameter and the whole fruit container mass during three seasons on 'Golden' apples and on 'Conference' pears. Air temperature and relative humidity were also recorded (Fig. B3.1).

- Diameter shrinkage of individual fruit was correlated with mass loss, however such measurements are based only on few fruit and are not reliable in order to represent a whole commercial room (Fig. B3.2). A linear correlation

was found between settlement and mass loss both in apples and pears, so that fruit mass loss due to moisture loss can be estimated from data obtained by an ultrasound sensor (Fig. B3.4 and B3.6). A clear relationship between mass loss rate and air relative humidity was observed (Fig. B3.3 and B3.5).

The developed technique of monitoring fruit mass and settlement allows the estimation of mass loss due to dehydration, providing information to packinghouses to properly control the chamber's RH. The industrial implementation of this technique will allow to reduce fruit losses.

4.3 Part C. Evolution of quality parameters during the shelf life period

- With consumers more aware about the beneficial contribution of pears in human health (Li et al., 2014; Nieman et al., 2015), stakeholders face increased pressure to bring sophisticated ripening protocols that can provide consumers with fruit of optimum sensorial and nutritional quality.

After the cold storage period fruit metabolism reacts quickly when placed under ambient atmosphere at 20 ºC. The firmness loss of 'Conference' pears during 8 months under DCA was less than 5 % of its initial value at harvest, but was reduced in 80 % in just 5 days of shelf life. That firmness evolution from harvest to day 5 of shelf life could be successfully fitted with a 3-parameter reverse Gompertz function (eq. 1, Chapter C1). The softening evolution during the SL period did not show any dependence with the firmness level at harvest. The rapid fruit softening during shelf life was accompanied by an increase in VOCs emission. In detail, 34 VOCs were identified and quantified at days 1, 3 and 5 of SL (Table C1.2) and the main detected compounds were esters, among them, hexyl and butyl acetates had the highest concentrations.

- There are many factors affecting the consumer's acceptance of pear. At the time of shopping pear external factors are key to determine the consumers choice (Gamble et al., 2006). 'Conference' pears are highly appreciated by consumers due to its flavor, juiciness and aroma (Saquet, 2018). It was found that the overall consumer's liking depends on the interaction between sensorial firmness and flavor, with esters playing a key role in flavor. Sensory evolutions were conducted by a panel of 56 volunteers who were asked to rate the overall liking according to a 9-hedonic point scale and to evaluate firmness and flavors separately through a 5-point hedonic scale. It was found that the overall liking increased with flavors but presented a maximum around the mid-point of the sensorial firmness scale (Fig. C1.5).

- Traditionally quality parameters of the fruit are measured in a way that reflect the average values in fruit pulp. However, a fruit is a volume occupied by a porous medium in which biochemical and diffusion processes take place. Few studies are focused on how the concentration of biochemical components are spatially distributed within the fruit flesh.

In Chapter C2, the spatial distribution of qualitative parameters as well as the emission of VOCs were evaluated in different slices along the equatorial diameter of the pear flesh, from the slice just below the skin until the slice near the core. It was found that the innermost slice of the fruit had ethylene production and respiration rates significantly higher than the rest, indicating that this slice had a higher metabolic activity. This trend was also observed when analyzing the antioxidant capacity and the total phenolic compounds, however, minimum production rate was found at intermediate slices. In contrast, sugars and acids showed more variable trends. These results give added value to the fresh-cut industry, aiming to attain fruit with improved nutritional quality and flavor.

- The spatial distribution of VOCs can lead to a spatial distribution of the flesh tissue susceptibility to some postharvest pathogens. Slices from different locations were inoculated with *P. expansum* and *R. stolonifer*, two major postharvest pathogens of pear fruit (Sardella et al., 2016), and differences in the mycelial diameter growth were found between flesh locations (Fig. 4.6). By performing a multivariate data analysis relating organic acids, sugars, antioxidants and volatile compounds with fungal infections of *P. expansum* and *R. stolonifer*, it was found that some biochemical compounds within the pear flesh may favor or limit pathogens growth. For example, higher amounts of fructose and malic acid were likely to favor *R. stolonifer* growth.

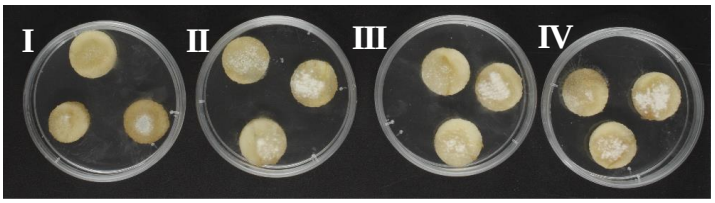

Figure 4.6 'Conference' pear flesh slices, from (I) slice just below the peel until (IV) slice next to the core, after 70 h of inoculation with *P. expansum*.

It is well known that natural compounds present in the fruit flesh can have fungicide or fungistatic effect against different pathogens (Mari et al., 2016; Sivakumar and Bautista-Baños, 2014). Our results showed that 2 ethyl-hexanal and butyl hexanoate were the most effective compounds

against *P. expansum* whereas hexanal and 1-butanol were the most effective against *R. stolonifer*.

Bio fumigation consists in the application of natural produced VOCs as a gaseous treatment (Mari et al., 2016). In the last years there has been a growing interest on their application since they are naturally produced (Li et al., 2015).

To explore this possibility, *in vitro* assays were carried out using different concentrations of 2-ethyl hexanal, butyl hexanoate, hexanal and 1-butanol (Fig. 4.7). It was found that all tested concentrations of 2-ethylhexanal completely controlled the expansion of *P. expansum* and that 0.22 µL mL^{-1} of hexanal completely stopped the growth of *R. stolonifer*.

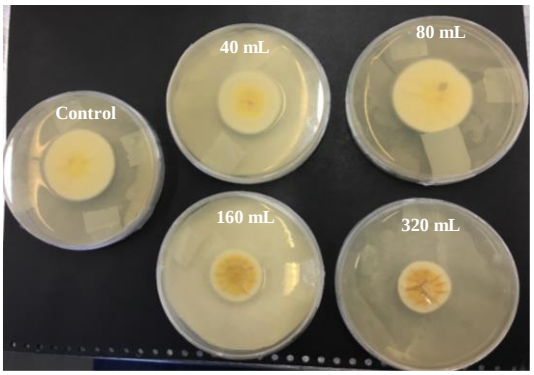

Figure 4.7 Photo of the *in vitro* assay, petri dishes inoculated with *P. expansum*. Different concentrations of butyl hexanoate vapors were applied to the paper filter.

Even though, studies carried out under *in vitro* conditions showed promising results, it is necessary to conduct *in vivo* studies to be able to extrapolate the results observed herein and perform conclusive hypotheses. Confirmation of these results can lead to an improvement of food security because using natural compounds capable of inhibiting major postharvest pathogens.

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