Development of packaging systems to maximize quality retention and increase shelf life of *Pleurotus* mushrooms

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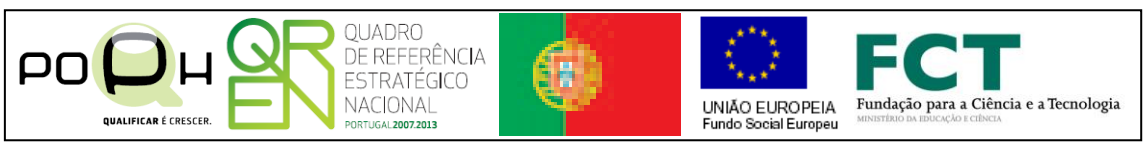

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Sílvia A.

ABSTRACT

Development of packaging systems to maximize quality retention and increase shelf life of *Pleurotus* mushrooms

Fresh horticultural commodities are excellent sources of vitamins, fiber and minerals for the human diet. They are also very perishable and highly dependent on the management of the postharvest environment surrounding the product. Therefore, quality maintenance over time relies on the success in the reduction of the physiological activity of the commodity.

Modified atmosphere packaging (MAP) used under strict temperature control is a postharvest technology that has been applied for many years in a wide range of commodities. MAP is known to directly affect commodity metabolism, with a positive effect on microbiological spoilage and, consequently, on the extension of the commodity shelf life.

MAP relies on the interplay between the respiration process of the product and the gas exchange through the package that contains the product. The movement of O_2 and CO_2 within the package creates a gradient that will, at equilibrium, lead to an atmosphere composition considered optimal for the preservation of the product.

In mushrooms, postharvest biological changes are particularly fast. Mushrooms have high respiration rate, tends to lose moisture rapidly and gets discoloured at a very fast rate. Predicting the kinetics of respiration and transpiration rate of oyster mushrooms (*Pleurotus ostreatus*), as influenced by temperature, relative humidity and storage time, is important in order to overcome the lack of information regarding the effect of storage conditions on oyster mushroom quality.

The main aim of this work is to estimate the respiration rate (RR) and the transpiration rate (TR) of oyster mushrooms in order to evaluate the response of postharvest technologies such as reduced temperatures, modified atmosphere and moisture absorbers in the retention of their quality and, ultimately, contribute for the development of novel packaging strategies.

The respiration rate of oyster mushrooms was evaluated under different constant storage temperatures (2, 6, 10, 14 and 18 ºC) and the impact of each storage temperature on quality attributes such as colour, solid soluble content, electrolyte leakage and mass loss, directly linked to the commodity metabolism, was also determined. Herein, it is shown that time had a significant effect on the oyster mushroom RR, decreasing over time. Storage temperature also had a significant effect in mushrooms quality. A gradual yellowness and high mass losses were also found throughout storage life, with an increase rate as storage temperature increases. Results show the importance of low temperature on oyster mushroom quality retention. Furthermore, results show that, under the conditions tested, storage of fresh oyster mushrooms at 2 °C has the potential to increase shelf life.

Given that respiration rate is an important indicator of postharvest senescence, reliable predictions of respiration rates are critical for the development of Modified atmosphere packaging (MAP). To study the influence of storage time and temperature on the respiration rate, oyster mushrooms were stored at constant temperatures of 2, 6, 10, 14 and 18 °C under ambient atmosphere. Respiration rate data was measured with eight hour intervals up to 240 h. Again, a decrease of respiration rate was found after the cutting of the carpophores. Therefore, time effect on respiration rate was modelled using a first order decay model. The results also show the positive influence of low temperatures storage on mushroom respiration rate and the primary model explaining the effect of time on oyster mushroom's RR also included the temperature dependence according to the Arrhenius equation. Moreover, the model included a parameter describing the decrease of the respiration rate, from the initial time until equilibrium. The overall model fitted well to the data and can be relevant for the choice of an appropriate packaging system for fresh oyster mushrooms.

Atmospheric composition (low O_2 and high CO_2) can affect respiration rate of horticultural commodities. To assess the potential benefits of MAP, the RR of fresh oyster mushrooms was evaluated under different concentrations of O_2 and CO_2 at a constant temperature (2 ºC) during storage. Six atmosphere were tested, according to a full factorial design, with 2 levels of O_2 (2 and 15 % v/v) and 3 levels of CO_2 (5, 10 or 20 % v/v) and with ambient air used as a control. Results showed that RR was affected by the conditions tested. Lower RR were found when mushroom were stored under 2 % O_2 and 20 % CO_2 (v/v), indicating that MAP under this levels may have potential in

increasing oyster mushroom shelf life. A response surface regression analysis was also used to determine the effect of O_2 and CO_2 on oyster mushrooms respiration rate.

Postharvest transpiration is also an important physiological process affecting storage life and overall quality. Although MAP of fresh produce can be used to restrain postharvest mass losses, its use in high metabolic products like mushrooms can lead to condensation inside the package, ultimately leading to quality losses and shelf-life shortening. Therefore, quantification of fresh produce transpiration rate (TR) and the use of predictive models could be useful to improve packaging design. To evaluate the impact of storage conditions (temperature and RH) on oyster mushroom transpiration rate over storage time, mushrooms were stored at 2, 6, 10, 14 and 18 °C and at 86, 96 and 100 % relative humidity (RH) under ambient atmosphere. Periodically, mushrooms mass losses were recorded over 248 h of storage. The results showed that both RH and temperature had a significant effect on oyster mushroom TR. Low temperatures and high RH decreases mass losses over storage time and therefore transpiration rate. An empirical mathematical model considering the effect of temperature and RH was developed. Temperature effect was explained using an Arrhenius model and the constants of the model were then fitted to linear equations to explain the effect of the relative humidity of storage on oyster mushrooms transpiration rate. The novel information obtained regarding oyster mushroom mass loss and the developed model may be useful for improving packaging design.

The usefulness of improving MAP of oyster mushrooms, by controlling the levels of moisture inside the package, was also tested using different mixtures of moisture absorbers in order to identify the mixture that matches the requirements of relative humidity inside oyster mushrooms package. Moisture holding capacity of the different mixtures was evaluated and a cubic model obtained. According to the model, a mixture containing 0.5, 0.26 and 0.24 (m/m) of calcium oxide, calcium chloride and sorbitol respectively yield a moisture holding capacity of 0.81 g water.g^{-1} desiccant and remains in powder form for at least 117 hours at 10 ºC presenting therefore, good perspectives for application of mixed desiccant for packaging of oyster mushrooms.

After gathering the necessary information regarding the *Pleurotus* metabolism and moisture holding capacity of the water absorber mixtures, a final validation experiment was developed. Locally grown oyster mushrooms were packaged in polystyrene trays and stored under different temperature regimes over a 240 h period.

Simulated commercial packages were compared with MAP and with MAP with moisture absorber obtained in the previous chapter. Levels of O_2 and CO_2 inside the packages and quality evaluation that included both physical (visual appearance, mass loss, colour of the cap and texture) and chemical (pH, SSC) parameters were determined. Quality of oyster mushrooms was affected by storage time, package and temperature regime. Temperature profile significantly affected the quality of oyster mushrooms, with mushrooms maintained under abuse temperature showing a fast rate of quality loss regarding colour and texture alterations and mass loss. The addition of the moisture absorber limited some condensation on mushroom surface.

This thesis provides novel information regarding the most accurate strategies to maintain the quality of oyster mushrooms namely by adjusting the storage time, type of package and temperature. The usefulness of the results here presented is also expected to motivate the development of other species-specific preservation systems that might be important for mushrooms with commercial significance.

KEY-WORDS: Postharvest technology, modified atmosphere packaging, moisture absorber, *Pleurotus ostreatus*, mass loss, respiration rate, mathematical modelling,

RESUMO

Desenvolvimento de sistemas de embalagem para maximizar a retenção da qualidade e aumentar o tempo de vida útil de cogumelos do género *Pleurotus*

Os produtos hortícolas frescos são excelentes fontes de vitaminas, fibras e minerais. Contudo, são muito perecíveis e muito dependentes do tipo de cuidados pós-colheita. Por conseguinte, a retenção da qualidade durante o armazenamento baseia-se principalmente no êxito na redução da atividade fisiológica do produto.

A embalagem em atmosfera modificada (EAM), utilizada sob rigoroso controlo de temperatura é uma tecnologia pós-colheita que tem sido aplicada desde há muitos anos numa vasta gama de produtos. A EAM é conhecida por afetar diretamente o metabolismo dos produtos hortícolas e pelo efeito positivo no controlo de microrganismos e, consequentemente, sobre a extensão da vida-útil do produto.

A EAM baseia-se na interação entre o processo de respiração do produto e a troca de gases através da embalagem que contém o produto. O movimento de O_2 e CO_2 no interior da embalagem cria um gradiente gasoso que, no estado de equilíbrio, origina uma composição da atmosfera considerada ideal para a conservação do produto.

A pós-colheita de cogumelos é caracterizada por mudanças biológicas particularmente rápidas. Os cogumelos têm alta taxa de respiração, perdem água rapidamente e sofrem alterações de cor a um ritmo muito rápido. A previsão das taxas de respiração e transpiração de repolga (*Pleurotus ostreatus*) e a influência da temperatura, humidade relativa e tempo de armazenamento é de extrema importância para colmatar, a falta de informação sobre as condições de armazenamento ótimas deste produto.

O principal objetivo do presente trabalho é estimar a taxa de respiração e a taxa de transpiração de *Pleurotus*, a fim de avaliar a resposta de tecnologias pós-colheita, tais como baixas temperaturas e atmosfera modificada na retenção da

qualidade e, assim contribuir para o desenvolvimento de novos sistemas de embalagem que aumentem o tempo de vida útil de cogumelos do género *Pleurotus*.

A taxa de respiração de *Pleurotus* foi avaliada sob diferentes temperaturas de armazenamento constantes (2, 6, 10, 14 e 18 ºC) e, o impacto da temperatura de armazenamento nos atributos de qualidade, tais como a cor, o teor em sólidos solúveis, a perda de eletrólitos e perda de massa, diretamente ligada ao metabolismo do cogumelo, também foi determinado. Quer o tempo de armazenamento quer a temperatura têm um efeito significativo na taxa de respiração do cogumelo que diminui com o tempo. A temperatura teve também influência na qualidade do cogumelo, especialmente no que se refere às perdas de massa obtidas e amarelecimento gradual do cogumelo. Os resultados mostram a importância da utilização de baixas temperaturas na retenção da qualidade do cogumelo. Além disso, os resultados demostram que, nas condições testadas, o armazenamento a 2 ºC pode aumentar a vida de útil do produto.

Tendo em conta que a taxa de respiração é um importante indicador de senescência pós colheita, as previsões das taxas de respiração são fatores críticos para o desenvolvimento de EAM. Para estudar a influência do tempo de armazenamento e da temperatura na taxa de respiração, os cogumelos foram armazenados a temperaturas constantes de 2, 6, 10, 14 e 18 ºC sob atmosfera ambiente. A taxa de respiração foi medida a cada oito horas até às 240 horas de armazenamento. Mais uma vez, verificou-se uma diminuição da taxa de respiração após o corte dos cogumelos. Como tal, o efeito do tempo na taxa de respiração de cogumelos foi modelado com uma cinética de primeira ordem. Os resultados mostram também o efeito positive das baixas temperaturas de armazenamento na taxa de respiração. Como tal, o modelo primário que explica o efeito do tempo na taxa de respiração incluiu também a dependência da temperatura de acordo com o modelo de Arrhenius. O modelo final inclui ainda um parâmetro que descreve a redução da taxa de respiração a partir do momento inicial, até ao equilíbrio. O modelo global ajustou-se bem aos dados e pode ser relevante para a escolha adequada de um sistema de embalagem para *Pleurotos* frescos.

A composição da atmosfera dentro da embalagem (níveis baixos de O_2 e altos de CO_2) pode também afetar a taxa de respiração de produtos hortícolas. Para avaliar esses potenciais benefícios para cogumelos, a taxa de respiração de cogumelos foi

avaliada sob diferentes concentrações de O_2 e CO_2 a uma temperatura constante de 2 ºC. Foram testadas seis atmosferas, de acordo com um delineamento fatorial completo, com 2 níveis de O_2 (2 e 15 % v/v) e três níveis de CO_2 (5, 10 ou 20 % v/v) e com o ar ambiente, utilizado como controlo. Os resultados mostraram que a taxa de respiração foi afetada pelas condições testadas. A taxa de respiração mais baixa foi observada para cogumelos armazenados a 2 % O_2 e 20 % de CO_2 (v/v), o que indica que a utilização destes níveis de O_2 e CO_2 pode ter efeitos positives no aumento da vida útil do produto.

A perda de água ou transpiração pós-colheita é também um processo fisiológico importante que afeta o tempo de armazenamento e a qualidade global do produto. Embora o uso de EAM possa restringir a perda de massa, a sua utilização em produtos de elevada taxa metabólico, como o cogumelo pode originar condensação no interior da embalagem, levando a perdas de qualidade e redução de vida útil. Portanto, a quantificação da taxa de transpiração e a utilização de modelos de previsão pode ser útil para melhorar o desenho da embalagem. Para avaliar o impacto das condições de armazenamento (temperatura e humidade relativa) na taxa de transpiração de cogumelos ao longo do tempo de armazenamento, estes foram armazenadas a 2, 6, 10, 14 e 18 ° C e a 86, 96 e 100% de humidade relativa em atmosfera ambiente. Periodicamente, as perdas de massa foram registadas durante um período máximo de 248 horas. Os resultados mostraram que quer a temperatura, quer a humidade relativa tiveram um efeito significativo sobre a taxa de transpiração. As baixas temperaturas e altas humidades relativas diminuíram as perdas de massa ao longo do tempo de armazenamento e, portanto, a taxa de transpiração. Um modelo matemático empírico considerando o efeito da temperatura e HR foi desenvolvido. O efeito da temperatura foi modelado através de uma equação de Arrhenius e as constantes do modelo foram modeladas com equações lineares para explicar o efeito da humidade relativa na taxa de transpiração de *Pleurotus*. As informações obtidas sobre a perda de massa de cogumelos e, consequentemente o modelo desenvolvido podem ser úteis para melhorar o desenho da embalagem para este produto.

A utilização de absorvedores de humidade para controlar os níveis de água dentro da embalagem foi também considerada. Para o efeito, avaliou-se a capacidade de retenção de água das diferentes misturas e obteve-se um modelo cúbico. De acordo com o modelo, uma mistura contendo 0.5, 0.26 e 0.24 (m/m) de óxido de cálcio, cloreto de cálcio e de sorbitol, respetivamente possui uma capacidade de

retenção de água de 0.81 $g_{água}.g^{-1}$, permanecendo na forma de pó durante pelo menos 117 horas a 10ºC. Por conseguinte, os resultados apresentam boas perspetivas para aplicação de um absorvedor de humidade para embalagem de *Pleurotus*.

Depois de reunir as informações necessárias sobre o metabolismo de P*leurotus* e da capacidade de absorção de água das misturas de dissecantes consideradas, a experiência final foi desenvolvida para validar uma embalagem para o armazenamento de cogumelo. Para o efeito, *Pleurotus* cultivados localmente foram embalados em bandejas de poliestireno e armazenadas sob diferentes regimes de temperatura por um período de 240 horas. As embalagens comerciais foram comparadas com as embalagens com modificação da atmosfera e com embalagens com modificação da atmosfera e absorvedor de humidade obtido no capítulo anterior. Os níveis de O_2 e CO_2 no interior da embalagem e a avaliação da qualidade, que incluiu parâmetros físicos (aparência visual, perda de massa, cor e textura) físicos e química (pH e teor em sólidos solúveis) foram determinados. A qualidade dos cogumelos foi afetada pelo tempo de armazenamento, pelo tipo de embalagem e pelo perfil de temperatura. A temperatura afetou significativamente a qualidade dos cogumelos, com estes mantidos sob temperaturas de abuso com perdas rápidas de qualidade no que se refere à cor, textura e perda de massa. A adição do absorvedor de humidade permitiu controlar de alguma forma a ocorrência de condensação na superfície do cogumelo.

Esta tese fornece novas informações sobre as estratégias mais precisas para manter a qualidade de cogumelos ostra nomeadamente ajustando o tempo de armazenamento, do tipo de embalagem e da temperatura. A utilidade dos resultados aqui apresentados também é esperado para motivar o desenvolvimento de outros sistemas de preservação específica de espécies que podem ser importantes para cogumelos com significado comercial.

PALAVRAS-CHAVE: Tecnologia pós-colheita, embalagem em atmosfera modificada, Absorvedor de humidade, *Pleurotus ostreatus*, perda de massa, modelação matemática.

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List of Symbols

Symbol	Designation	Unity
a*	CIE L*a*b* colour parameter (red/green)	Dimensionless
a_{Ea}	Model parameter (Eq. 6.4)	$kJ.mol^{-1}$
a_k	Model parameter (Eq. 6.4)	h^{-1}
b*	CIE L*a*b* colour parameter (yellow/blue)	Dimensionless
b_{Ea}	Model parameter (Eq. 6.4)	$kJ.mol^{-1}$
b_k	Model parameter (Eq. 6.4)	h^{-1}
C*	Chroma, colour parameter	Dimensionless
C_1	Conductivity after 1 minute	µs
C_1	Total Conductivity	µs
C_{60}	Conductivity after 60 minutes	µs
Ea	Activation energy	$kJ.mol^{-1}$
Ea_K	Activation energy for kinetic constant	$kJ.mol^{-1}$
Ea_{RR0}	Activation energy for initial respiration rate	$kJ.mol^{-1}$
Ea_{RReq}	Activation energy for equilibrium respiration	$kJ.mol^{-1}$
Ea_{RR}	Activation energy for respiration rate	$KJ.mol^{-1}$
Ea_{TR}	Activation energy for transpiration rate	$KJ.mol^{-1}$
H^o	Hue angle, colour parameter	Dimensionless
k	Kinetic constant	h^{-1}
k	Overall transpiration coefficient	$mg.s^{-1}.kg.kPa$ or $mg.s^{-1}.m^2.kPa$
L*	Lightness, CIE L*a*b* colour parameter (Black/white)	Dimensionless
M	Mass, product mass	kg or g
$\dot{m}$	Rate of moisture loss	$mg.s^{-1}$ or $mg.s^{-1}.m^2$
M_f	Mass at time t	g

M_i	Initial mass	g
ML	Mass loss	%
MHC	Moisture holding capacity	$g_{water}.g^{-1}$
P_∞	Water vapour pressure close to the product	kPa
P_s	Water vapour pressure at evaporating surface	kPa
Q_{10}	Respiration rate increase for a 10 ºC increase	Dimensionless
R	Ideal gas constant	$kJ.mol^{-1}.K^{-1}$
RQ	Respiratory quotient	Dimensionless
RR	Respiration rate	$mL.kg^{-1}.h^{-1}$
$RR^*_{O_2}$	Respiration rate estimated	$mL.kg^{-1}.h^{-1}$
RR_0	Respiration rate at time 0	$mL.kg^{-1}.h^{-1}$
$RR_{0.ref}$	Initial respiration rate at reference temperature	$mL.kg^{-1}.h^{-1}$
RR_{CO_2}	Rate of CO_2 production	$mL.kg^{-1}.h^{-1}$
RR_{eq}	Respiration rate at equilibrium	$mL.kg^{-1}.h^{-1}$
$RR_{eq.ref}$	Respiration rate at equilibrium at reference	$mL.kg^{-1}.h^{-1}$
RR_{O_2}	Rate of O_2 consumption	$mL.kg^{-1}.h^{-1}$
$RR_{T.t}$	Respiration rate at a given time and	$mL.kg^{-1}.h^{-1}$
T	Temperature	˚C or K
t	Time	h
TR	Transpiration rate	$g.kg^{-1}.h^{-1}$
T_{ref}	Reference temperature	K
TR_{ref}	Transpiration rate at reference temperature	$g.kg^{-1}.h^{-1}$
V	Volume	m^{-3}
V_f	Free volume	m^{-3}
ΔY_{CO_2}	Variation of CO_2	% (v/v)
ΔY_{O_2}	Variation of O_2	% (v/v)
Δt	Variation of time	h

α_1	Model parameter (Eq. 7.2)	Dimensionless
α_{12}	Model parameter (Eq. 7.2)	Dimensionless
α_{123}	Model parameter (Eq. 7.2)	Dimensionless
α_{13}	Model parameter (Eq. 7.2)	Dimensionless
α_2	Model parameter (Eq. 7.2)	Dimensionless
α_{23}	Model parameter (Eq. 7.2)	Dimensionless
α_3	Model parameter (Eq. 7.2)	Dimensionless
ρ	Commodity apparent density	$kg.m^{-3}$
τ	Model parameter (Eq. 4.7)	Dimensionless

List of Abbreviations

AA	Ascorbic acid
AC	After Christ
ACE	Angiotensin-I converting enzyme
acetyl-CoA	Acetyl coenzyme A
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BC	Before Christ
BI	Browning index
CFU	Colony forming units
CI	Confidence interval
CIE	Commission internationale de l'éclairage
COTR	Carbon dioxide transmission rate
DW	Dry weight
EMAP	Equilibrium modified atmosphere packaging
EMP	Glycolysis or Embeden-Meyerhof-Parnas pathway
FAO/WHO	Food and Agricultural Organization/World Health
FAOSTAT	Statistic division of Food and Agricultural Organization
FW	Fresh weight
IPRH	In-pack relative humidity
LDPE	Low-density polyethylene films
MA	Modified atmosphere
MAP	Modified atmosphere packaging
MHC	Moisture holding capacity
MHP	Modified-humidity packaging
n	Number
NAD	Nicotinamide adenine dinucleotide
SSC	Solid soluble content
OTR	Oxygen transmission rate

p	Significance level
PM-MAP	Perforation-mediated modified atmosphere packaging
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
R^2	Coefficient of determination
R^2 Adi	Adjusted coefficient of determination
REL	Relative electrolyte leakage
RH	Relative humidity
RQ	Respiration quotient
RR	Respiration rate
SD	Standard deviation
SE	Standard error
TCD	Total colour difference
TL	Total lightness
TR	Transpiration rate
WVP	Water vapour pressure
WVPD	Water vapour pressure deficit
WVTR	Water vapour transmission rate

List of Equations

$$C_6H_{12}O_6+6O_2\rightarrow 6CO_2+6H_2O+36\ ATP \quad \text{Eq. 2.1}$$

$$\dot{m}=k\times(P_s-P_\infty) \quad \text{Eq. 2.2}$$

$$RR_{O_2}=\frac{\Delta Y_{O_2}\times V_f}{\Delta t\times 100\times M} \quad \text{Eq. 3.1}$$

$$RR_{CO_2}=\frac{\Delta Y_{CO_2}\times V_f}{\Delta t\times 100\times M} \quad \text{Eq. 3.2}$$

$$V_f=V-\frac{M}{\rho} \quad \text{Eq. 3.3}$$

$$RQ=\frac{RR_{CO_2}}{RR_{O_2}} \quad \text{Eq. 3.4}$$

$$C^*=\left(a^{*2}+b^{*2}\right)^{1/2} \quad \text{Eq. 3.5}$$

$$H°=\text{arctg}\left(\frac{b^*}{a^*}\right) \quad \text{Eq. 3.6}$$

$$TL=\left[\left(\Delta L^*\right)^2\right]^{1/2} \quad \text{Eq. 3.7}$$

$$TCD=\left[\left(\Delta L^*\right)^2+\left(\Delta a^*\right)^2+\left(\Delta b^*\right)^2\right]^{1/2} \quad \text{Eq. 3.8}$$

$$BI=\frac{[100(x-0.31)]}{0.17} \quad \text{Eq. 3.9}$$

$$x=\frac{\left(a^*+1.75L^*\right)}{\left(5.64L^*+a^*-3.012b^*\right)} \quad \text{Eq. 3.10}$$

$$REL=\frac{(C_{60}-C_1)}{C_T}\times 100 \quad \text{Eq. 3.11}$$

$$ML = \frac{M_i - M_t}{M_i} \times 100 \quad \text{Eq. 3.12}$$

$$RR^*_{O_2} = \frac{RR_{CO_2}}{0.83} \quad \text{Eq. 4.1}$$

$$RR = \frac{RR_{O_2} + RR^*_{O_2}}{2} \quad \text{Eq. 4.2}$$

$$\frac{RR - RR_{eq}}{RR_0 - RR_{eq}} = \exp^{(-k \times t)} \quad \text{Eq. 4.3}$$

$$RR_0 = RR_{0,ref} \times \exp\left[\frac{E_{aRR_0}}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad \text{Eq. 4.4}$$

$$RR_{eq} = RR_{eq,ref} \times \exp\left[\frac{E_{aRR_{eq}}}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad \text{Eq. 4.5}$$

$$k = k_{ref} \times \exp\left[\frac{Ea_k}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad \text{Eq. 4.6}$$

$$RR_{eq} = \tau \times RR_0 \quad \text{Eq. 4.7}$$

$$RR = RR_{eq,ref} \times \exp\left[-\frac{Ea_{RR_{eq}}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] + \left\{\begin{array}{c} RR_{0,ref} \times \exp\left[-\frac{Ea_{RR_0}}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] - RR_{eq,ref} \times \\ \exp\left[-\frac{Ea_{RR_{eq}}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \end{array}\right\} \times \exp\left[-k_{ref} \times \exp\left[-\frac{Ea_k}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \times t\right] \quad \text{Eq. 4.8}$$

$$RR_{T,t} = RR_{0,ref} \times \exp\left[-\frac{Ea_{RR}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \times \left\{\tau + (1-\tau) \times \exp\left(-k_{ref} \times \exp\left[-\frac{Ea_k}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \times t\right)\right\} \quad \text{Eq. 4.9}$$

$$RR_{O_2} = 28.25 - 1.47 \times O_2 + 1.41 \times CO_2 - 0.09 \times (O_2)^2 + 0.09 \times O_2 \times CO_2 - 0.11 \times (CO_2)^2 \quad \text{Eq. 5.1}$$

$$RR_{CO_2} = 28.30 - 4.27 \times O_2 + 1.47 \times CO_2 + 0.20 \times (O_2)^2 + 0.077 \times O_2 \times CO_2 - 0.11 \times (CO_2)^2 \quad \text{Eq. 5.2}$$

$$TR = k \times \exp^{\left[-\frac{Ea_{TR}}{R_0} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right]} \quad \text{Eq. 6.1}$$

$$k = a_k - b_k \times RH \quad \text{Eq. 6.2}$$

$$Ea_{TR} = a_{Ea} + b_{Ea} \times RH \quad \text{Eq. 6.3}$$

$$\ln(TR) = \ln\left[a_k - b_k \times RH\right] - \left[\left(\frac{a_{Ea} + b_{E_a} \times RH}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)\right] \quad \text{Eq. 6.4}$$

$$MHC = \frac{M_t - M_i}{M_i} \quad \text{Eq. 7.1}$$

$$MHC = \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_{12} AB + \alpha_{13} AC + \alpha_{23} BC + \alpha_{123} ABC \quad \text{Eq. 7.2}$$

PART I -Thesis Framework

Chapter 1: General introduction

1.1. The importance of postharvest preservation

Fresh horticultural commodities are fundamental components in a balanced healthy diet, providing vitamins, minerals and phytochemicals. Additionally, horticultural commodities are recommended as a source of dietary fibber (Slavin and Lloyd, 2012).

Harvested horticultural products remain as living tissues subject to continuous metabolism. Before harvesting, when horticultural commodities are attached to the parent plant, losses of substrates and moisture due to respiration and transpiration are replaced. After harvest, however losses are not replaced and quality declines, limiting horticultural commodities shelf life.

Respiration involves a chain of oxidation–reduction reactions, each of which is catalysed by specific enzymes which breaks down organic reserves to simpler molecules releasing energy used for other metabolic processes of the cell (Kays, 1991; Fonseca *et al.*, 2002a). An inverse relation exists between respiration rate and storage life- higher respiration rates are associated with higher deterioration of commodities and consequently lower storage life (Brash *et al.*, 1995).

Moisture loss by transpiration in fresh products is associated with mass and quality losses and it is assumed to be a major cause of postharvest loss in some commodities such as leafy vegetables or citrus fruits (Ben-Yehoshua, 1969).

The goal of postharvest techniques is to maintain the level of quality found at harvest, so proper storage conditions are key factors to maintain maximum quality. One of the most important approaches for overall quality retention is proper control of temperature and relative humidity in the storage environment (Kader, 2002).

Although the management of temperature and relative humidity have a well-known positive effect, other hurdles may be used to obtain maximum quality. The low levels of O_2 and the high levels of CO_2 in modified atmospheres can potentially reduce respiration rate, ethylene sensitivity and production, decay and physiological changes, namely oxidation, with the resultant benefit of extending the storage life of the fresh produce (Kader, 1987).

The selection of the best storage conditions for a particular commodity helps to reduce postharvest losses, optimizing quality and maintaining fresh appearance and nutritional quality.

1.2. The relevance of mushrooms in human diet and health

In recent years, demand for fresh mushrooms is increasing due to their organoleptic, nutritional and pharmacological properties. The total number of species of mushrooms has been estimated as reaching about 140.000, though only a small fraction (10%) are currently known (Wasser, 2002).

The inclusion of mushrooms in human diet is a long lasting story as a key source of nutrients and proteins. Judging from archaeological finds, mushrooms and other fungi have been used since before recorded history (Moore and Chiu, 2001).

Several studies have been demonstrated that mushrooms have a high protein and carbohydrate content, low fat and many minerals and vitamins (Mattila *et al.*, 2001; Cohen *et al.*, 2002; Phillips *et al.*, 2012) indicating a balanced food that might have had an important role in human diet during evolution. In addition to their nutritional value, a significant role in human health is well supported by scientific research. Mushrooms protect human cells against oxidative damage (Yildirim *et al.*, 2012), enhance the immune response in the anti-inflammatory process (Jedinak *et al.*, 2011), protect against cardiovascular disease (Martin, 2010) and diabetes mellitus (Lo and Wasser, 2011), two of the most common human diseases. As well, mushrooms have antibacterial activity (Singdevsachan *et al.*, 2013) and perhaps the most promising therapeutic application of edible mushrooms is related with cancer prevention (Xu *et al.*, 2012). For example, the role of mushrooms in reducing cellular proliferation in human breast cancer cells was established recently (Martin and Brophy, 2010). Such a broad therapeutic spectrum deserves further investigation and, in parallel, the inclusion of this food in human diet should continue to be recommended.

One of the most common specie of mushrooms that are commercially available is *Pleurotus ostreatus*. The fruit bodies of *Pleurotus* species are considered a delicacy because of their flavour properties (Bano *et al*., 1988). This species presents a high

nutritional value and is especially effective against cancer cell proliferation (Martin and Brophy, 2010).

Aside from nutritive and medicinal values (Jedinak and Sliva, 2008), *Pleurotus* mushroom research have been focused in several bio potentialities such as the recycling of agricultural residues and bioconversion of lignocellulosic wastes, the production or improved of animal feed (Akinfemi *et al.*, 2010); the bioremediation and degradation of xenobiotics and industrial wastewaters (Morgan *et al.*, 1991; Faraco *et al.*, 2009).

1.3. Postharvest quality of fresh mushrooms

Mushroom quality and consumer acceptability of fresh mushrooms is strongly influenced by colour, texture and appearance (Vízhányó and Felföldi, 2000; Eastwood and Burton, 2002).

From a postharvest point of view, mushrooms are grouped with fruits and vegetables, being classified as highly perishable (Kader and Saltveit, 2003). Biological changes occurring after harvest are particularly fast in mushroom and prompt deterioration occurs (Ares *et al.*, 2007).

Mushrooms shelf life can end up due to: *i.* high rate of respiration; *ii*. high rate of dehydration; *iii*. browning and *iv*. texture changes (Burton and Noble, 1993; Braaksma *et al.*, 1994; Jolivet *et al.*, 1998; Mahajan *et al*., 2008a; Iqbal *et al.*, 2009a).

Mushrooms have high respiration rates, which is high when compared with other horticultural produce (Ares *et al.*, 2007).

Postharvest losses of water by transpiration are also significant in mushroom fruit body. Mahajan *et al.* (2008a) determined transpiration rates of button mushrooms and reported significant mass losses over time, with transpiration rate ranging from 0.29 $g.kg^{-1}.h^{-1}$ to 5.2 $g.kg^{-1}.h^{-1}$ for mushrooms stored at 4 ºC and 96 % RH and 16 ºC and 76 % RH.

Colour changes are also a factor known to affect postharvest quality of mushrooms and severe browning or discoloration may occur due to enzymatic and/or microbial activities.

In agreement with its delicate nature, reduction of all metabolic processes is a key factor for optimum storage of mushrooms. The general recommendations for the postharvest procedures in mushrooms, generally refers to the genus *Agaricus* and are then extrapolated to other species (Ares *et al.*, 2007; Singh *et al.*, 2010). However, differences in physiological activities as well as morphological characteristics of the mushroom cap may be important with regard to the maintenance of postharvest quality of particular specie.

Once proper conditions of temperature and relative humidity are established, modified atmosphere package can also be used, although no consensus regarding its effect in distinct genus of mushrooms exist. For instance, MAP is considered of little benefit to *Pleurotus* (Villaescusa and Gil, 2003), but Iqbal *et al.* (2009b) obtained an important metabolism reduction and an increase in the shelf life of *Agaricus*. Besides these discrepancies observed in the effects of MAP in mushrooms and their optimal levels of O_2 and CO_2, other constrain in the use of MAP has been described. The lack of packaging films with suitable permeability to O_2, CO_2 and moisture cause diverse problems related with the accumulation of phytotoxic CO_2 levels and/or the appearance of condensation within mushroom packages (Sapata *et al.*, 2004).

Due to these severe constraints, other strategies working synergetic with refrigeration temperatures and MAP must be developed to reduce postharvest deterioration in mushroom species. It has been suggested that placing mushrooms under controlled humidity atmosphere improve their quality and increases storability (Roy *et al.*, 1995a,b; Villaescusa and Gil, 2003). Nevertheless, the use of hygroscopic compounds to control the in-package relative humidity is only empirical and does not fulfil requirements regarding optimum relative humidity for mushrooms.

This work focused mainly in developing novel strategies that can be used in the storage of *Pleurotus ostreatus,* in order to prevent losses in quality and nutritional value and early degradation of the product. No other studies have addressed the problem. Hence, the results presented here are expected to be of major importance from the commercial and the nutritional points of view.

1.4. Research objectives

The central aim of the work presented in this thesis was the development of packaging systems that can maximize the quality and shelf life of *Pleurotus* mushrooms. To achieve this, several lines of research were developed and are here summarized as follows:

1. Evaluate postharvest temperature response in quality attributes of *Pleurotus* mushrooms over storage time;

2. Obtain experimental data on the respiratory behaviour of *Pleurotus* mushrooms in a wide range of temperatures relevant to the supply chain of fresh horticultural products;

3. Obtain experimental data on the transpiration behaviour of *Pleurotus* mushrooms in a range of temperatures and relative humidity relevant to fresh horticultural products;

4. Evaluate respiration and transpiration rates over time and apply mathematical models to describe the effects of the storage environment in those physiological characteristics;

5. Evaluate and model the kinetics of moisture absorbers to determine the best combination that match the transpiration characteristics of *Pleurotus* mushrooms;

6. Evaluate the effect of MAP and moisture absorber on different quality attributes of *Pleurotus* mushrooms at both constant and abuse temperature usually found in the supply chain of fresh horticultural products.

1.5. Thesis structure

The thesis is divided in three parts and nine chapters. The first part includes this chapter (**Chapter 1**) that presents the problem and the objectives of this work and **Chapter 2** that covers literature review with an overview about mushrooms, causes and effects of postharvest deterioration on commodities quality as well as overall strategies to control postharvest deterioration.

The second part includes the experimental work done according to the goals of the work. According to the general objective of the thesis, storage conditions on postharvest quality attributes were studied in **Chapter 3**.

In **Chapter 4**, a mathematical model describing the effect of temperature and storage time on oyster mushroom respiration rate was developed.

The effect of MA (lower O_2 and higher CO_2 than ambient air) on oyster mushroom rate was also evaluated (**Chapter 5**).

Chapter 6 includes the study performed to obtain and model transpiration rates of oyster mushrooms in a range of temperatures and relative humidities.

Kinetics of some mixture of moisture absorbers were also determined (**Chapter 7**) and finally the results obtained with the use of MAP and moisture absorbers in oyster mushrooms are presented (**Chapter 8**).

The third part of this thesis (**Chapter 9**) presents the final conclusions and suggestions for future work.

Schematic representation of the thesis outline is observed in Fig. 1.1.

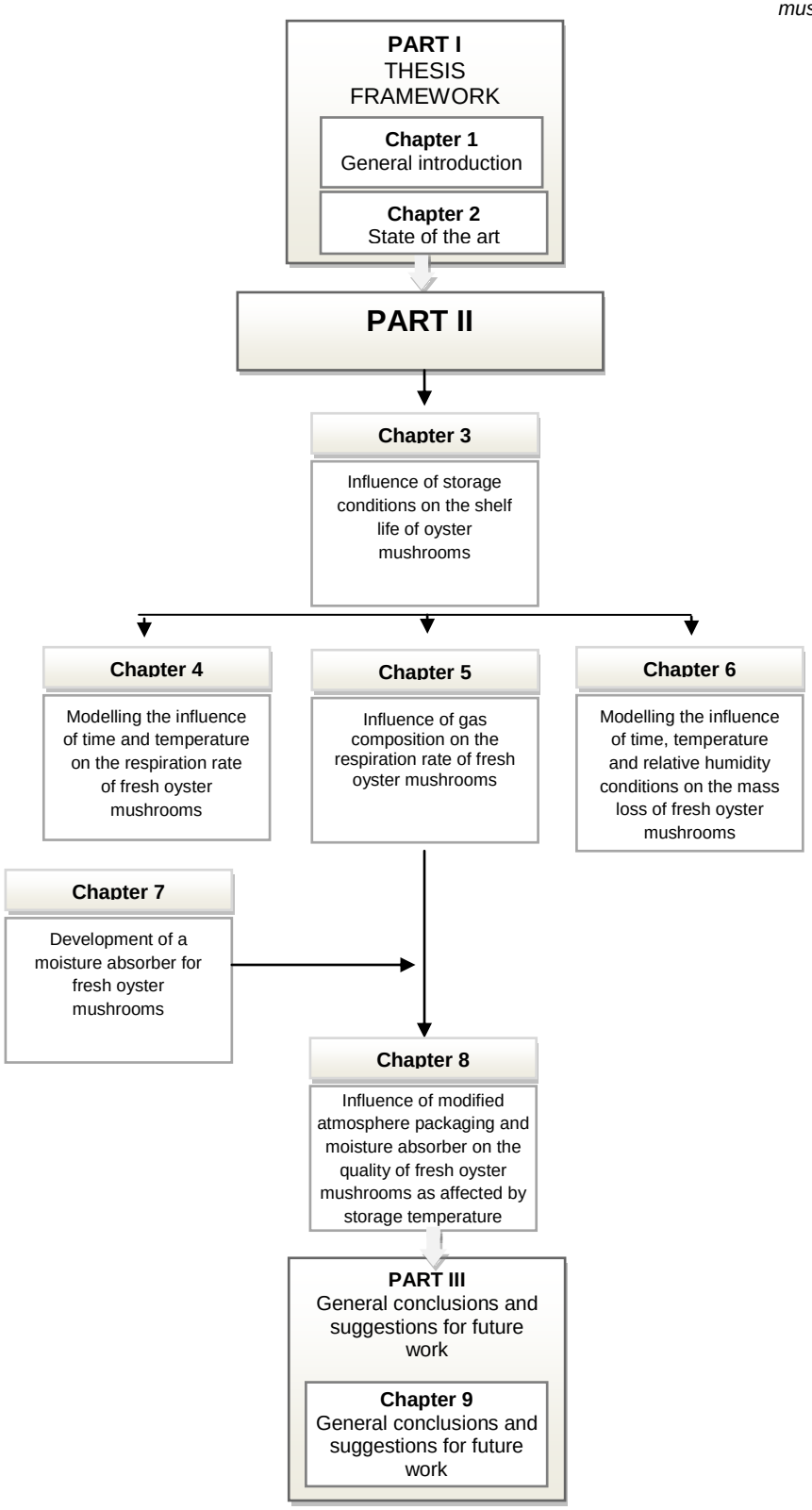

Fig. 1.1 - Schematic representation of the thesis outline.

Chapter 2: State of the art

2.1. Mushrooms taxonomy, morphology, cultivation, nutritional and medicinal properties and world production

Mushrooms have been appreciated since ancient times and have been used for several purposes. Their eating qualities are highly appreciated as well as their recognized health benefits.

Although *Agaricus bisporus* is the most popular mushroom, leading world production, other species such *as Lentinula edodes* and *Pleurotus ostreatus* are also becoming increasingly important. The genus *Pleurotus* comprises a number of different species. They are generally called oyster mushroom due to the sheel-like form of the cap. Due to the increasing importance of *Pleurotus* species as edible mushrooms throughout the world, this dissertation will focus on *Pleurotus ostreatus*.

In this sub-chapter, brief aspects of taxonomy and morphology of mushrooms are discussed. Nutritional value and health benefits of mushrooms, with special focus in *Pleurotus ostreatus* mushrooms will be covered. Mushrooms world production data is also presented.

2.1.1. *Mushrooms taxonomy and morphology*

Mushrooms were first classified as plants but due to their particularities like the lack of chlorophyll and presence of cellular wall, were placed in a different kingdom called Myceteae (Chang and Miles, 2004). According to Chang and Miles (2004), a mushroom is “a macrofungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand”.

Mushrooms are constituted by densely packed fine threads filaments - the hyphae – which together form a structure called mycelium. Under favourable conditions, the mycelium differentiates and produces the fruiting body (Boa, 2004; (Chang and Miles, 2004). The structure which is generally known as mushroom is the

fruiting body of the fungus. All mushrooms fructifications have a typical morphology as presented in Fig. 2.1.

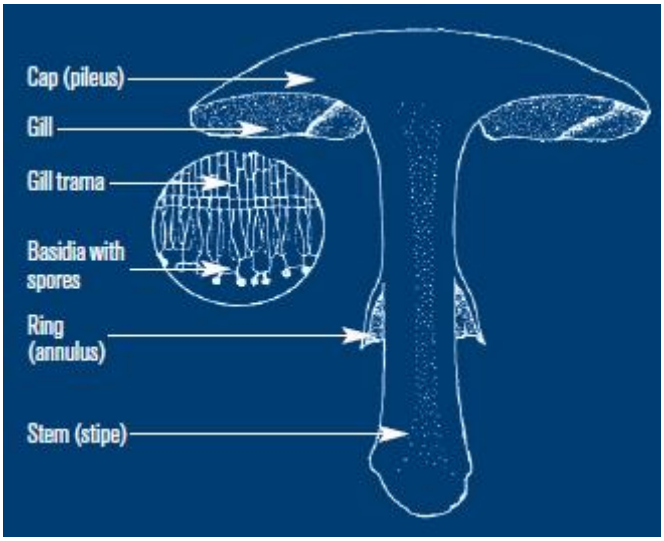

Fig. 2.1 - Mushroom structure.
Source: Eastwood and Burton (2002).

Generally, the majority of mushrooms consist of a stem (or stipe), and a fleshy umbrella-shaped cap (or pileus) that contains and bear the fungi fertile structure (spores).

Mushroom differentiation and classification are based in their macroscopic and microscopic features.

All mushrooms are included in two Phyla - Ascomycota and Basidiomycota. The phylum Ascomycota includes mushrooms like truffles and morels. Most of the known mushrooms such as *Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes* are included in phyla Basidiomycota and due to similarities in the morphological characteristics; most of the cultivated mushrooms are placed in the order Agararicale (Tab. 2.1).

Tab. 2.1 - Taxonomy of the main mushroom species cultivated.

	Agaricus bisporus (J.E. Lange)	***Pleurotus ostreatus (Jacq.)***	***Lentinula edodes (Berk.) Pegler***
Kingdom		Fungi	
Phylum		Basidiomycota	
Class		Basidiomycetes	
Order		Agaricale	
Family	Agaricaceae	Pleurotaceae	Marasmiaceae
Genus	*Agaricus*	*Pleurotus*	*Lentinula*
Specie	*A. bisporus*	*P. ostreatus*	*L. edodes*

A. *bisporus* – known as common mushroom, champignon or button mushroom – is, within the genus *Agaricus*, the most familiar and cultivated specie throughout the world.

The genus *Pleurotus* (the word pleurotus means side ear), encompass about 50-70 species, nearly all edible and very similar (Kong, 2004) and are characterized by a ear-like in shape cap attached to the wood from which they grow by means of a lateral, or side, stem. The genus is recognized by the white spores, the stem attached at the side of the cap, or at least off center, and the fleshy or tough texture of the cap (Christensen, 1943). Within the genus *Pleurotus*, only a few of those species are currently domesticated and exploited industrially. Among those, *P. ostreatus*, *P. sajor-caju* and *P. eryngii* (cardoncello) stands out as important industrial species (Chang and Miles, 2004). Visual aspect, scientific and common names of some oyster mushrooms species are presented in Fig. 2.2.

One specie of the genus *Pleurotus* (Fr.) Quel. is *P.ostreatus* (Jacq. et Fr.) Kummer, the most cultivated specie within the genus, and in which many commercial strains are developed and cultivated (Kong, 2004). It is common recognized as oyster mushrooms, shimeji or hiratake (Sánchez and Royse, 2002). In Portugal, oyster mushrooms are known as repolga (Martins, 2004).

An overview of *P. ostreatus* characteristics is provided in Tab. 2.2.

			
P. citrinopileatus (Golden Oyster Mushroom) *(a)*	*P. cystidiosus* (Abalone Mushroom) *(b)*	*P. djamor* (Pink Oyster Mushroom) *(c)*	*P. eryngii* (King Oyster Mushroom) *(d)*
			
P. florida (Florida Oyster Mushroom) *(e)*	*P. ostreatus* (Tree Oyster Mushroom) *(f)*	*P. pulmonarius* (Phoenix or Indian Oyster Mushroom) *(d)*	*P. pulmonarius* (Oyster Mushroom) *(g)*

Fig. 2.2 - Aspect of some important oyster mushroom species.
urce:(a) http://everythingmushrooms.com/grow-your-own-mushrooms/ ; (b) http://www.clovegarden.com/ingred/fungus.html; (c) http://mushroomersclub.blogspot.pt/2012/07/12-some-of-most-important-cultivated.html#.UwoJyU3itdg (d) Keizer (1998); (e) http://mushroomersclub.blogspot.pt/2012/07/12-some-of-most-important; (f) Authors photo(g) http://www.clovegarden.com/ingred/fungus.html

Tab. 2.2 – General characteristics of *P. ostreatus*.

Structure	Characterization
Cap	3 to 5 inches broad, shell-shaped, pale gray in colour, growing yellowish-white with age, margin very thin and turned in at first over the gills, later wavy. The flesh is thick, soft, white.
Gills	Running down on the stem, decurrent, somewhat distant, veined, broad, white, and yellowish when aged.
Stem	Short, growing from the side of the cap (lateral), firm elastic, smooth, thickening toward cap, whitish.
Ring	None
Volva	None
Spores	White
Odour	Quite mild
Taste	Rather strong, when cooked resembling the flavour of oysters

Source: Cole, 1910.

P. ostreatus has an oyster shape cap (Fig. 2.3), with a short stem tapering downward from one side. The upper side is smooth with variable colour. The gills are white, and the flesh is soft and spongy and varies between white to grey-white. It usually grows in clumps or cluster (Fig. 2.3) and it is quite adaptable to a range of climates and substrate materials. Some works also suggest that, in comparison with *P. sajor-caju* and *P. eryngii*, *P. ostreatus* is the most productive specie (Ramos *et al*., 2011).

Fig. 2.3 – Visual aspect of *Pleurotus ostreatus* mushrooms.

2.1.2. *History of mushroom cultivation*

For centuries, mushrooms have been used not only as food as well as a therapeutic agent. Both Chinese and Egyptian collected mushrooms in nature due to their knowledge of the benefits provided by mushroom consumption (Pérez-Armendáriz *et al.*, 2010).

Mushroom cultivation began in Asia around 600 BC with the production of *Auricularia auricula* (Chang and Miles, 2004). *Agaricus bisporus* was not cultivated until 1600 AC and it was with this specie that modern mushroom cultivation techniques began to emerge. In France, the cultivation of *Agaricus* initially done in limestone was transferred to Paris caves in the 18th century (Kües and Liu, 2000).

In comparison with *Agaricus bisporus*, the commercial cultivation of *Pleurotus* species is relatively recent. The first cultivation of oyster mushrooms was recorded in Europe in 1917 and cultivation increased rapidly and in 1959, a large scale production of oyster mushrooms was established in Hungary. In the mid-seventies, its commercial production was fully established in Europe (Sánchez and Royse, 2002). This rapid development was probably due to cultivation practices that were easier when compared to other species and the overall potentials of *Pleurotus* for the degradation of lignocellulosic substrates from agriculture and silviculture (Rajarathnam *et al.*, 1989).

2.1.3. *Mushroom nutritional composition*

Mushrooms are an attractive food item from the nutritional point of view due to their high protein and carbohydrate content, low fat and many minerals and vitamins (Manzi *et al.*, 1999; Mattila *et al.*, 2001; Cohen *et al.*, 2002; Pérez-Armendáriz *et al.*, 2010; Cheung, 2010; Phillips *et al.*, 2012).

Nutritional composition of mushrooms varies between species. Within the same species, strain, type of subtract used and cultivation techniques, maturity at harvest are important factors that contribute to the nutritional content of mushrooms (Beelman *et al.*, 1989).

The chemical composition of some mushroom species is shown as follow.

2.1.3.1. Moisture content

Moisture content of mushrooms ranges from 85 to 95 % of their fresh mass and is affected by the time of cropping, watering conditions during cultivation, postharvest period, and temperature and relative humidity during growth (Bano *et al.*, 1988). As a

reference, *A*. *bisporus* has moisture values within the range of 92.8 – 94.8 %, *L*. *edodes* between 81.8 and 90 % and *Flammulina velutipes* between 87.2 and 89.1 %. For *P. ostreatus*, moisture values between 85.2 and 94.7 % were found (Manzi *et al.*, 1999, Manzi *et al.*, 2001).

2.1.3.2. Carbohydrate content

Carbohydrates content of edible mushrooms varies with specie, and is the main component ranging from 35 to 70 % on dry weight (DW) basis (Manzi *et al.*, 2004). Most of the components of carbohydrate are in the form of polysaccharides like glycogen, dietary fiber, cellulose, chitin (mannans) and glucans (Manzi *et al.*, 2001). The dominant sugar is mannitol that constitutes about 80 % of the total free sugars (Tseng and Mau, 1999; Wannet *et al.*, 2000). Mannitol has an important role in volume growth and firmness of fruiting bodies (Barros *et al.*, 2007). In *P. ostreatus*, the carbohydrate content is 61.1 % DW (Longvah and Deosthale, 1998; Mau *et al.*, 2001). Trehalose is considered the major component of the soluble sugars in oyster mushrooms (Bano *et al.*, 1988; Mau *et al.*, 1997).

Dietary fiber in mushrooms results from the components of the cell wall and according to Mattila *et al.* (2002), *P*. *ostreatus* contains about 30 % DW of dietary fiber.

2.1.3.3. Protein content

Protein is an important component of the dry matter of mushrooms of edible mushrooms. Although deeply affected by factors such as specie and stage of development (Longvah and Deosthale, 1998), mushrooms have high protein content. *L*. *edodes* are reported to have protein content in the range of 15.2 to 23.0 % on DW basis (Longvah and Deosthale, 1998; Manzi *et al.*, 1999). Protein content of mushrooms also varies from flush to flush. In *P*. *ostreatus,* the protein content varies between 10 to 30 % on DW basis and in some cases reaches up to 40 % (Yang *et al.,* 2001).

According to Pérez-Armendáriz *et al.* (2010), the amino acids content presented on mushrooms represent 96 -110 % of the amino acids recommended by Food and Agricultural Organization/World Health Organization (FAO/WHO). The protein of edible mushrooms is rich in threonine and valine (Chang and Miles, 2004). However, it has been reported that lysine, leucine, isoleucine, and tryptophan are limiting amino acids in some edible mushrooms (Manzi *et al.*, 1999; Cheung, 2010). Although in terms of the amount of crude protein, mushrooms rank below animal meats, it ranks above other foods such as rice (7.3 %), wheat (12.7 %), soybean (38.1 %) and corn (9.4 %) (Bano *et al*., 1988).

2.1.3.4. Fat content

The lipid content of edible mushrooms is very low with a mean content of 4.0 % DW, although between species values diverge from 1.1 to 8.3 % DW (Chang and Miles, 2004). In *P. ostreatus*, lipid content is about 2.2 % g DW (Yang *et al.*, 2001). Linoleic acid is the predominant fatty acid and accounts for more than 70 % (68.8 – 84.0 %) of the total fatty acid content of the mushroom. Palmitic (19.2 % of the total fatty acid) and oleic acids are also important (8.3 % of the total fatty acid) (Longvah and Deosthale, 1998; Yang *et al*., 2001; Cheung, 2010). Besides the positive nutritional aspects, linoleic acid is also the precursor of 1-octen-3-ol, known as the alcohol of fungi, the principal aromatic compound that contribute to the characteristic mushroom flavour (Maga, 1981).

2.1.3.5. Mineral content

The ash content in edible mushrooms ranges from 6 to 10.9 % DW. In *P. ostreatus* the value of ash was 6.90 % DW, while for other species like shiitake, values varied between 5.27 and 5.85 % DW (Manzi *et al.*, 1999; Mau *et al.*, 2001). Mushrooms are considered an important source of minerals. The major minerals presented are potassium and phosphorus (2670 – 4730 mg/100 g DW and 493 – 1390 mg/100 g DW, respectively). Sodium, calcium, magnesium and selenium are also important (Zakhary *et al.*, 1983; Vetter, 1994; Bernaś *et al.*, 2006). The fruiting bodies of mushrooms are

characterized by a high level of well assimilated mineral elements. Elements like copper, zinc, iron, molybdenum, cadmium form minor constituents (Bano *et al.*, 1988).

2.1.3.6. Vitamin content

Vitamins also contribute to the nutritional importance of mushrooms. Edible mushrooms are a good source of vitamins such as riboflavin (vitamin B_2). Vitamin B_2 concentrations vary within the range 1.8 – 5.1 mg/100 g DW. Vitamin B content is higher than that generally found in vegetables, and some varieties of *A*. *bisporus* even have a higher level of vitamin B_2 than the content found in egg and cheese (Mattila *et al.*, 2001). The vitamin B_2 content of *P*. *ostreatus*, *A. bisporus* and *L*. *edodes* is 2.27 – 8.97, 3.70 – 5.10 and 0.90 – 1.80 mg/100 g DW, respectively (Crisan and Sand, 1978; Bano and Rajarathnam, 1986; Bano *et al.*, 1988; Mattila *et al.*, 2001). Concentrations of niacin are also high and vary from 33.8 – 109 mg/100 g DW for *P*. *ostreatus*, 11.9 – 98.5 mg/100 g DW for *L. edodes* and 36.2 – 57.0 mg/100 g DW for *A. bisporus* (Crisan and Sand, 1978; Bano and Rajarathnam, 1986; Bano *et al*., 1988) In addition, cultivated mushrooms also contain small amounts of vitamin C and vitamin B_1 and traces of vitamins B_{12} and D_2 (Crisan and Sands, 1978).

2.1.3.7. Energy content

The energy content of edible mushrooms is generally low, making them an interesting choice for low energy diets. Reference values for *A. bisporus* are 4.17 – 4.20 kcal/g DW, while in *P. ostreatus* values between 4.16 and 4.23 kcal/g DW were reported (Manzi *et al.*, 2001).

2.1.4. *Health promoting properties of mushrooms*

Besides its use as a food source, mushrooms are also known for medicinal or tonic purposes. In the last decades, scientific investigation studied the basic active principles of mushrooms which are health promoting (Wasser and Weis, 1999). Some

of the properties attributed to mushrooms are related with the content of bioactive products with antioxidant activity (Barros *et al.*, 2007; Ferreira *et al.*, 2009).

Mushrooms have been investigated for preventing diseases such as hypertension, hypercholesterolemia and cancer (Bobek *et al.*, 1995; Bobek and Galbavý, 1999; Martin and Brophy, 2010). Antibacterial, antimicrobial and antiviral activities have also been described (Hearst *et al.*, 2009; Singdevsachan *et al.*, 2013). Mushrooms can also be used as a treatment for diabetes mellitus (Lo and Wasser, 2011).

Although globally recognized as therapeutic agents, different species of mushrooms present different therapeutic characteristics. Some of the medicinal properties of *Pleurotus* are described as follow.

2.1.4.1. Antioxidant activity

Fruiting bodies of *Pleurotus* has good concentration of antioxidants (Mau *et al.,* 2001; Yang *et al*., 2002; Jayakumar *et al*., 2009; Jayakumar *et al*., 2011; Vamanu, 2012). Antioxidant properties in oyster mushrooms arise from the presence of polysaccharide pleuran (β - glucan) and it was reported to have a positive effect on rat colon with pre-cancerous lesions (Bobek and Galbavý, 2001) and on breast and colon cancer (Jedinak and Sliva, 2008). In humans, *P. ostreatus* reduced oxidative damage due to the increase of antioxidant enzymes (*viz.* superoxide dismutase, catalase and peroxidase) (Yang *et al.*, 2002).

2.1.4.2. Antimicrobial and antiviral activity

Pleurotus mushrooms contain substances that exert direct or indirect antiviral effects as a result of immune-stimulatory activity. Ubiquitin, an anti-viral protein was isolated and identified from fruiting body of oyster mushroom. Antimicrobial effect of an ethanolic extract of *Pleurotus ostreatus* also inhibited stains from the genus *Candida* observed by Vamanu (2012).

2.1.4.3. Hypoglycaemic activity

The inclusion of oyster mushrooms in the diet of diabetic patient may be a good choice due to the high fibre and proteins content and low fat content. Oral administration of aqueous extracts of *P. pulmonarius* decreased serum glucose level in alloxan-treated diabetic mice (Badole *et al*., 2006).

Similar, hypoglycaemic activity was found for *Pleurotus ostreatus* and *P. cystidiosus.* Moreover, polysaccharides extracted from *P. citrinopileatus* alleviated anti-hypoglycaemic effect by the elevation of the activity of glutathion peroxidase (Hu *et al*., 2006).

2.1.4.4. Hypotensive activity

Miyazawa *et al.* (2008) described a blood pressure lowering activity of *P. nebrodensis* in hypertensive rats. Ching *et al.* (2011) shown that protein fractions from *P. cystidiosus* possessed the highest angiotensin-I converting enzyme inhibitory activity that cause the contraction of blood vessels thereby raising the blood pressure.

2.1.4.5. Anti-Inflammatory

The polysaccharide pleuran (β - glucan) present in oyster mushrooms also possesses anti-inflammatory activity (Bobek and Galbavý, 2001; Jedinak *et al.*, 2011).

2.1.5. *Worldwide mushroom production*

Worldwide mushroom production has increased in the last decades from about 350 thousand tonnes in 1991 to 7700 thousand tonnes in 2011 (Fig. 2.4) (FAOSTAT, 2013).

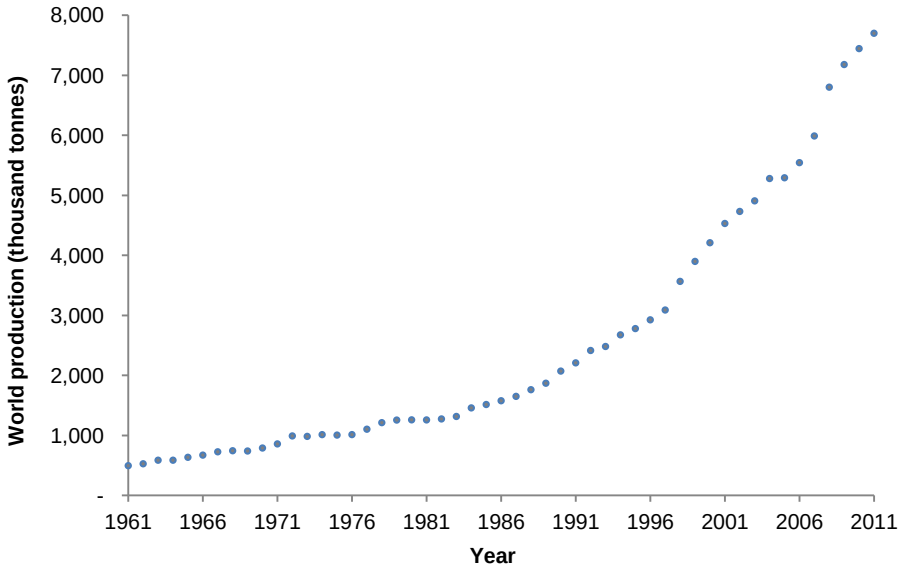

Fig. 2.4 - Evolution of world production of mushrooms and truffles.
Source: FAOSTAT (2013).

Mushroom cultivation has a long tradition in Asia and therefore it is not a surprise that the world production is leaded by China that contributes with 65 % of total world output (Fig. 2.5) (FAOSTAT, 2013).

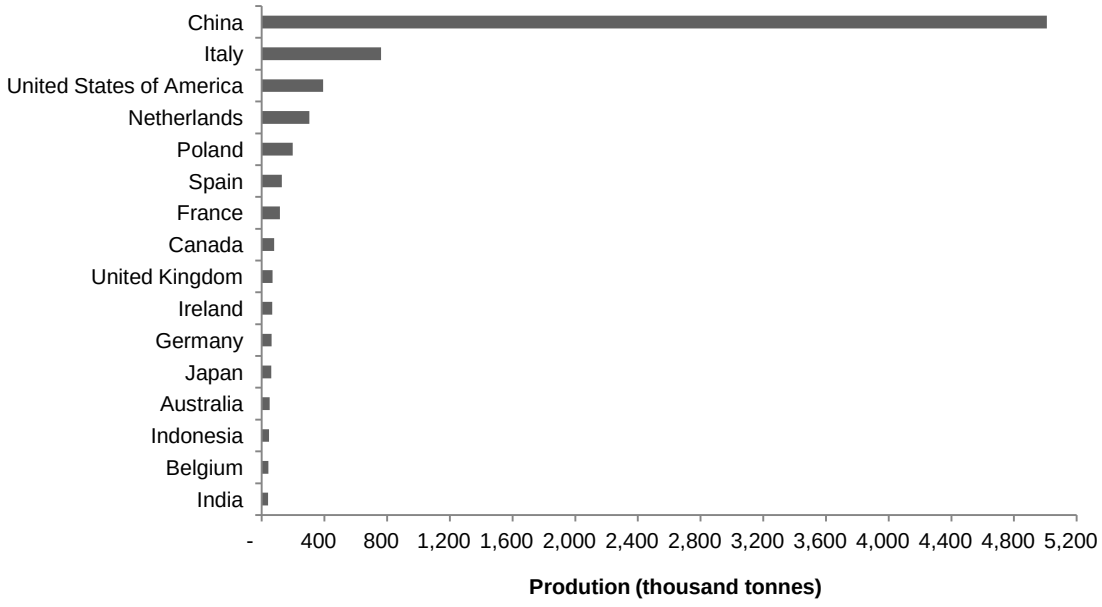

Fig. 2.5 - Production of leading producing countries of mushrooms and truffles in 2011.
Source: FAOSTAT (2013).

In 2011, the total of European countries produced 1850 thousand tonnes, contributing to 19.6 % of the total world production (FAOSTAT, 2013).

European production is led by Italy with a production of 1850 thousand ton, followed by the Netherlands (304 thousand tonnes), Poland (198 thousand tonnes), Spain (127 thousand tonnes) and France (116 thousand tonnes) (Fig. 2.6) (FAOSTAT, 2013). These five countries together are responsible for approximately 81 % of the European production.

Commercial markets are dominated by *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus spp*. accounting approximately 75 % of the cultivated mushrooms grown around the world (Chang, 1999).

Production of mushrooms in Portugal only began to be recorded since 1983 and has undergone some fluctuations over the years. The latest available data refer to 2011 with a total production of 1,240 tonnes (Fig. 2.7) (FAOSTAT, 2013).

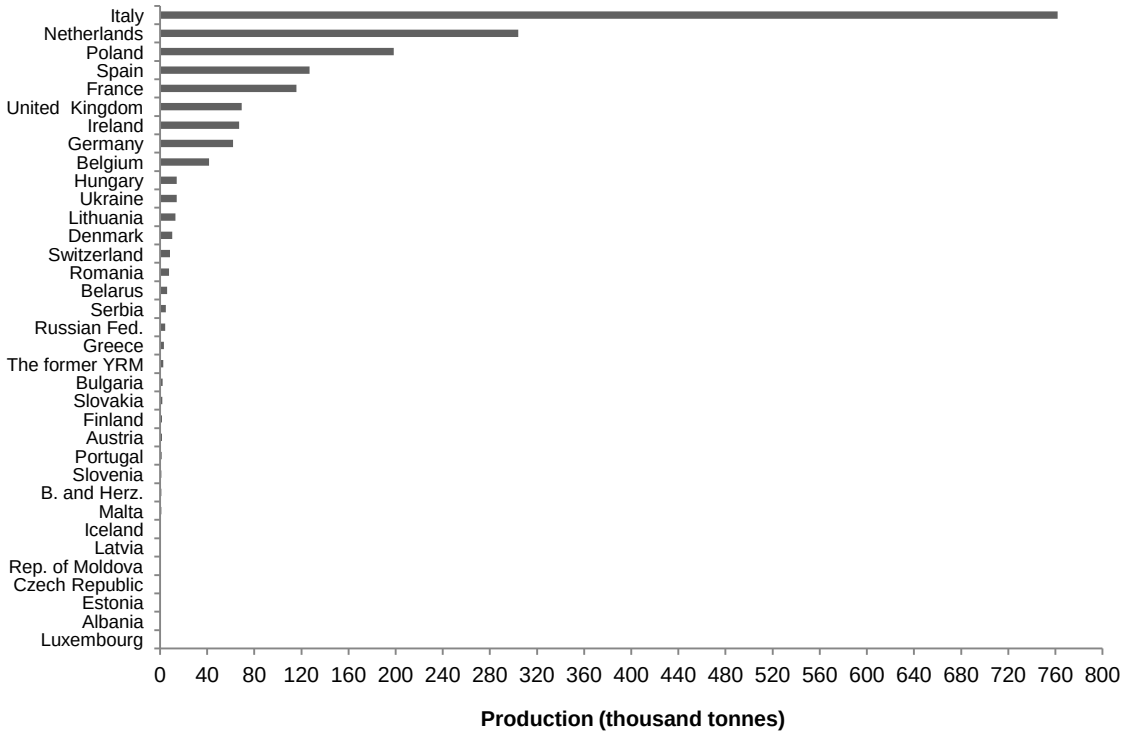

Fig. 2.6 - European mushroom and truffles producers and their outputs in 2011.
Source: FAOSTAT (2013).

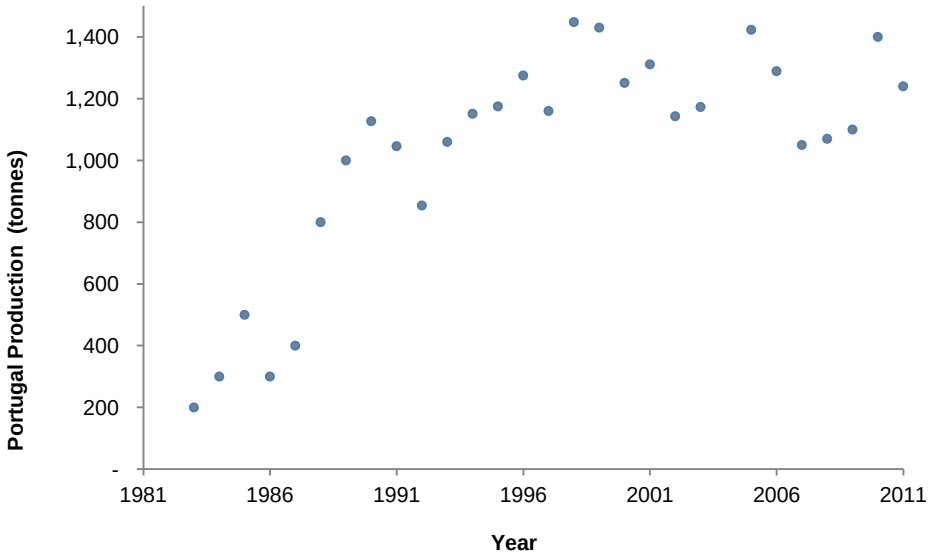

Fig. 2.7 - Production of cultivated mushrooms and truffles in Portugal, from 1981 to 2011.
Source: FAOSTAT (2013).

2.2. Main physiological processes responsible for quality deterioration in fresh commodities

Horticultural commodities deteriorate due to physical, chemical and microbiological processes. After harvest, horticultural products maintain active metabolism that results in product deterioration. The relative importance of each of these processes depends on the commodity and the storage techniques applied during postharvest period. Respiration and transpiration are the most important physiological processes that affect storage life and quality of horticultural products.

This sub-chapter presents an overview of the respiration and transpiration processes and the main factors that affect both processes.

2.2.1. *Respiratory metabolism*

Respiration is the oxidative catabolism of organic materials (carbohydrates, lipids and organic acids) that are broken down into water, carbon dioxide and energy through sequential enzymatic steps. Respiration constitutes, therefore a central process in living cells, providing energy and carbon skeletons that are vital components to support all reactions related with the postharvest developmental changes of the commodity (Wills *et al.*, 1998; Kader, 1987; Kays, 1991; Kader, 2002; DeEll *et al.*, 2003).

The process of respiration can be simplified by the following chemical reaction – Eq. 2.1 (Lee *et al.*, 1991):

$$CH_{12}O_6+6O_2 \rightarrow 6CO_2+6H_2O+36\ ATP \qquad \text{Eq. 2.1}$$

Despite the apparent simplicity, aerobic respiration is a complicated process that involves a series of enzymatic reactions taking place through three pathways (Kays, 1991; DeEll *et al,* 2003):

- Glycolysis or Embeden-Meyerhof-Parnas pathway(EMP);
- Tricarboxylic acid cycle (TCA) or Krebs cycle;

- Oxidative phosphorylation.

The first step – Glycolysis - occurs in cytoplasm of the cell and involves the oxidation of glucose to pyruvate, with the gain of two ATP molecules and reduced NAD. Then, the pyruvate is oxidized to acetyl coenzyme A (acetyl-CoA). In the presence of oxygen, acetyl-CoA enters the Krebs cycle to produce coenzymes with loss of carbon dioxide and water. After the acid citric cycle, occurs the oxidative phosphorylation that uses energy released to produce adenosine triphosphate (ATP) via a chain of electron carriers (Kader, 1987).

The ratio of the quantity of carbon dioxide (CO_2) produced and the amount of oxygen (O_2) consumed in respiration, referred to as respiratory quotient (RQ) is also important (Platenius, 1943). In aerobic respiration (described in Eq. 2.1), RQ ranges between 0.7 and 1.3. Within this range, variations may occur according with the type of substrates used for respiration. If carbohydrates are used in respiration, as seen in Eq. 2.1, RQ assumes a value of 1. On the other hand, if lipids (or proteins) or organic acids are the main substrates for respiratory metabolism, the RQ is expected to be below 1 and above 1, respectively (Kader *et al.*, 1989; Kader and Saltveit, 2003). To support the process of aerobic respiration in harvested produce, adequate levels of O_2 are required. In the absence or at very low O_2 levels, cells undergo anaerobic respiration. In such cases, oxidative phosphorylation (i.e. Krebs cycle and the hydrogen transport chain) does not take place, resulting in the formation of alcohol and consequently off-flavours development (Kader, 1987; Fonseca *et al.*, 2002a).

The association between respiration rate (RR), commodity metabolism and changes in quality or postharvest shelf life is broadly studied. Respiration uses substrates from the cells, ultimately leading to the substrate exhaustion, which gives an overall perspective of commodity metabolism. Commodities with high respiration rate have short shelf lives (Kader, 1987; Brash *et al.*, 1995; Kader and Saltveit, 2003).

Brash *et al.* (1995) observed that shelf life of asparagus at 20 °C is negatively related to respiration rate (as evaluated by CO_2 production) during storage. Due to this close link, several approaches relating gas exchange rates and quality changes in horticultural commodities are found. A close relationship between the rate of gas exchange and fruit softening was also reported for apples (Hertog *et al.*, 2001) and avocados (Hertog *et al.*, 2003).

Böttcher *et al.* (1999) also showed the importance of rate of respiration in quality maintenance of culinary herbs, while Fonseca *et al.* (2005) reported a direct relation between the rate of deterioration of shredded galega kale (measured as loss of chlorophyll a and b) and kale respiration rate.

Mushrooms have high respiration rate compared to other horticultural commodities (Kader, 2002; Fonseca *et al.* 2002a). At 10 °C and under air, respiration rate of fresh mushrooms ranges from 17.8 to 178 mL CO_2 kg $^{-1}$.s^{-1}, depending on mushroom species considered (López-Briones *et al.*, 1993; Varoquaux *et al.*, 1999; Villaescusa and Gil, 2003; Iqbal *et al.*, 2009a,b; Ares *et al.*, 2006; Parentelli *et al.*, 2007). After harvest, mushrooms undergo natural development, so its respiration respiratory behaviour is related with the maturation of the sporophore and two distinct phases can be observed. High respiration rates observed immediately after harvest are generally followed by a decrease in respiration rate few hours after harvest (Hammond and Nichols, 1975; Varoquaux *et al.*, 1999; Villaescusa and Gil, 2003).

2.2.2. *Factors affecting respiration rate*

2.2.2.1. Commodity related factors

Several effects related with commodity can have a great effect in respiration rate. Horticultural commodities include a wide range of botanical structures (plant organs and mushrooms) and each one is harvested at different developmental stage. Consequently, respiration rates vary widely between commodities (Wills *et al*., 1998).

High respiration rate are expected in young tissues, growing plants or immature fruits. On the other hand, organs like bulbs and tubers are known to have low respiration rate (Kader and Saltveit, 2003).

The same commodity can also show different respiration rates due to cultivar and varietal differences (Varoquaux *et al.*, 1996; Seljåsen *et al.*, 2001; Kim *et al*., 2004; Seefeldt *et al*., 2012). According to Pretel *et al*., (2000), in apricot, varietal differences accounted for up to 60 % of the total respiration rate differences. Kim *et al*. (2004)

reported that white salad savoy had higher respiration rates and lower quality scores than violet salad savoy.

Kiwifruit varieties also show differences among varieties (Manolopoulou and Papadopoulou, 1998). Cultivar differences were also found in broccoli and wild rocket salad as reported by Seefeldt *et al.* (2012). In sweet-cherry (Jaime *et al.*, 2001) and pomegranate, differences between cultivars were also found.

Seasonal effects in product respiration rate were also observed by Martínez-Sánchez *et al*. (2008) for a range of baby leafs and the influence of pre-harvest factors in postharvest respiration rate is also significant as observed for processed romaine lettuce (Luna *et al*., 2013).

2.2.2.2. Time after harvesting or processing

It is known that respiration rate it's not constant during shelf life. Differences in RR after harvest are due to natural product ageing and physical stress induced by harvest or processing and physical abuse during these operations and must be taking into account in MAP design.

Preparation of products to market or retail display include a series of operations that affect respiration rate. Operations such as peeling or cutting, causes a physiological response in the product, which causes an increase in RR over time. In general, wound-induced respiration is often transitory, reaching a maximum value that can last a few hours or days. Respiration then starts to decrease again until it reaches a stable level (Fonseca *et al*., 2002b; Torrieri *et al.*, 2010). Since this changes may have a major impact in the gas composition achieved in MAP (Fonseca *et al.*, 2002a,b), studies on this effect should be performed.

2.2.2.3. Environmental factors: temperature and atmospheric composition

Temperature is the most important environmental factor affecting respiration of fresh produce. This particular dependence has long been known, since temperature affects the rates of all biological reactions (Kader *et al.*, 1989; Wills *et al.*, 1998).

Within the range of temperature usually encountered in the distribution and marketing chain (4 – 30 °C), respiration increases 2 to 3 fold for every 10 °C rise in temperature (Zagory and Kader, 1988; Exama *et al.*, 1993; Varoquaux and Ozdemir, 2005). In some cases, these differences are significantly higher as shown for fresh-cut produce. These products present an increase in RR of 3.4 to 8.3 fold for every 10 °C rise in temperature (Watada and Qi, 1999). For temperatures higher than 50 °C, respiration decreases linearly with temperature, due to either a denaturing of respiratory enzymes or the lack of oxygen consequence of a limited rate of diffusion (Maguire *et al.*, 2004).

Respiration rate is also dependent on the amount of available oxygen and carbon dioxide present in the immediate surrounding environment of the commodity (Beaudry, 2000; Varoquaux and Ozdemir, 2005). Once optimum temperature are established, levels of O_2 and CO_2, different from air may have effect on the respiration rate of fresh produce and this effect have been studied for several commodities (Geysen *et al.*, 2005; Kim *et al.*, 2006; Escalona *et al.*, 2006; Conesa *et al.*, 2007a,b; Iqbal *et al.*, 2009b).

Oxygen has a major effect in respiratory metabolism. Respiration involves molecular oxygen (Wills *et al.*, 1998; Kays, 1991; Kader and Saltveit, 2003), so a decrease in contents of oxygen surrounding the produce is beneficial in order to maintain low aerobic respiratory metabolism. Lowering the O_2 level to 2-3 % (v/v) is considered the optimum to lower the RR of most of commodities, although some products like mushrooms withstand 1 % (v/v) of O_2 in order to found beneficial effects related to a decline in the respiration rate (Kader, 2002). The minimum oxygen level that has the ability to have a significant effect in the respiratory activity without causing anaerobic respiration is, as expected, commodity dependent.

The effect of CO_2 on horticultural commodities RR is less clear than the metabolic effect of O_2. In fact, depending on the commodity, concentration and time of exposure, CO_2 may allow a reduction on RR, have no effect or increase the respiration of fresh produce. Hertog *et al.* (1998) found that the effect of CO_2 in RR of apples, broccoli and Belgian endives was positive, lowering the metabolism. On the other hand, no effect of CO_2 was found in apples (Peppelenbos *et al.*, 1996; Peppelenbos and Van't Leven, 1996), strawberries (Li and Kader, 1989; Talasila *et al.*, 1992; Colelli and Martelli, 1995; Hertog *et al.*, 1999), onions, lettuce, and spinach (Mathooko, 1996). In raspberries, Joles *et al.* (1994) reported that partial pressures of CO_2 < 17 kPa did not affect RR and Beaudry (1993) reported a small reduction in O_2 uptake of blueberries stored under CO_2 > 20 kPa.

2.2.2.4. Mathematical modelling of respiration rate

Studies on the prediction of the respiration rate of horticultural commodities have been carried out with the intent of knowing postharvest characteristics and therefore to improve their storage characteristics.

As previous described (Chapter 2, § 2.2.2), the respiration rate of horticultural commodities is affected by many factors. Most of the respiratory models are attempts to depict the effect of environmental factors such as temperature and/or gas composition in the respiration process of a particular commodity. Extensive research has been done on the effect of temperature in RR and mathematical models describing that effect are currently available.

An approach to quantify the effects of temperature on chemical reactions, including respiratory rate is using the temperature quotient (Q_{10}). This coefficient explain how many times increases the rate of a reaction for each increase in temperature of 10 °C (Kader and Saltveit, 2003).

Although Q_{10} can be used to accurately describe the effect of temperature in RR in some commodity (Böttcher *et al.*, 1999), temperature quotient can vary considerably with temperature. For various commodities, Q_{10} values ranges from 1 to 4 (Kader, 1987; Talasila *et al.*, 1992; Exama *et al.*, 1993). At higher temperatures, the Q_{10} is usually smaller than at lower temperatures, e.g. at temperatures of 0-10 ºC, Q_{10}

assumes a value between 2.5 to 4.0, whereas a temperature quotient of 1.0 to 1.5 can is seen in commodities stored at higher temperatures (30 - 40 ºC) (Wills *et al.*, 1998; Saltveit, 1996).

As respiration involves many enzymatic reactions (Wills *et al.*, 1998; Kader, 2002), directly influenced by temperature, most of the models developed to explain the effect of temperature on the RR are based in the Arrhenius equation (Hertog *et al.*, 1999; Lakakul *et al.*, 1999; Mahajan and Goswami, 2001; Fonseca *et al.*, 2002b; Kaur *et al.*, 2011; Caleb *et al.*, 2012a,b). In this particular case, the effect of temperature increase is given by the activation energy (E_a) (Cameron *et al.*, 1995). As a simplification of the Arrhenius equation, the exponential model is also used to describe the effect of temperature on RR (Brash *et al.*, 1995).

Considering the fact that MAP design involves previous knowledge of the effect of O_2 and CO_2, many models describing this dependence are documented. Both Henig and Gilbert (1975) and Hayakawa *et al.* (1975) used a linear model to describe respiration as a function of the gas concentration.

The Michaëlis–Menten type equation to describe the relation between respiration and gas concentration was first introduced by Chevillotte (1973) *cit* Peppelenbos *et al*. (1996) in that considered the model on its simplest form, describing that CO_2 does not have an inhibitory effect on RR.

Later, Lee *et al.* (1991) included uncompetitive inhibition by CO_2 and validated the model using broccoli. Peppelenbos and Van't Leven (1996) evaluated the four types of inhibition for modelling the influence of CO_2 levels on RR as compared with the model that considers that CO_2 has no influence is fresh commodities RR. Most of the reported work describes the influence of CO_2 in RR using competitive inhibition.

Non-competitive inhibition has been described for mushrooms (Peppelenbos *et al.* (1993) and blueberry (Song *et al.*, 1992).

Ripening, aging or physical stress also affects respiration rate of a particular commodity. Therefore, time effect should be taken into account in order to predict the changes in gas composition achieved in a MAP system after harvest or processing (Fonseca *et al.*, 2002a,b; Kim *et al.*, 2004; Uchino *et al.*, 2004; Rocculi *et al.*, 2006; Caleb *et al.*, 2012a,b).

In order to predict respiration rate of tomato with storage time, Yang *et al*. (1988) used a quadratic function, with the previous inclusion of the effect of O_2 and CO_2 concentrations in fruit respiration rate.

Gong and Corey (1994) found that the best fit function for O_2 consumption was a polynomial of 2nd order equation. The Weibull model (Fonseca *et al.*, 2005; Waghmare *et al.*, 2014) or kinetic models (Waghmare *et al.*, 2013) have been suggested as good alternatives to describe the effect of time in the respiration rate of fresh commodities.

Mathematical models used to describe the effect of temperature, gas composition and time were reviewed by Fonseca *et al.* (2002a). Updates were performed by Caleb *et al.* (2012c).

2.2.3. *Transpiration or moisture loss*

Water has a vital role in the physiology of agricultural products during the growing season and postharvest period. After harvest, the moisture loss by transpiration cannot be replenished, which give rises to significant problems in quality retention of the commodity (Wills *et al.*, 1998).

Unique properties of water molecule and its physiochemical properties are discussed in detail in Ben-Yehoshua *et al.* (2003) and Shamaila (2005). From a simplified point of view, some of these properties are described next.

Water is a universal solvent and it is essential in hydrolytic reactions. It also has transportation and thermoregulatory functions in the cell. It cannot be overlooked the role in the maintenance of cell turgidity (cell expansion, physical and chemical integrity of cell walls) (Kramer and Boyer, 1995; Shamaila, 2005).

Water is a major constituent of horticultural products tissues. It accounts for at least 60 % of their fresh weight (FW), whereas others commodities hold about 84 to 96 % FW of water in their composition (Shamaila, 2005; Rodov *et al.*, 2010). In mushrooms, moisture content ranges from 85 to 95 % in FW (Manzi *et al.*, 1999, Manzi *et al.*, 2001).

The driving force for water loss by transpiration is the difference between the water vapour pressure (WVP) of the commodity and the WVP of the surrounding environment and it is called water vapour pressure deficit. While fresh, the WVP of the product is high due to the high water content of the commodities (near saturation). In the environment that surrounds the product, the WVP is generally lower which causes the movement of water from the product. The rate of moisture loss from a product is traditionally expressed in the single linear form expressed in Eq. 2.2 (Sastry, 1985):

$$\dot{m}=k\times(P_s-P_\infty) \quad \text{Eq. 2.2}$$

where $\dot{m}$ is the rate of moisture loss from product ($g.kg^{-1}.d^{-1}$); k is the transpiration coefficient of product ($g.kg^{-1}d^{1}.Pa^{-1}$); P_s is the WVP at evaporating surface and P_s is the ambient WVP close to the product surface.

Water loss from fresh commodities results in a significant reduction in saleable mass, with direct economic losses associated (Robinson *et al.*, 1975; Kays and Paull, 2004; Nunes and Emond, 2007). Furthermore, wilting, softening, colour alterations and enhancement of physiological disorders can also occur (Robinson *et al.*, 1975; Ben-Yehoshua *et al*., 2003; Kays and Paull, 2004; Nunes and Emond, 2007) with an equivalent decrease in quality and value of the commodity.

Harvested products remain with fresh appearance only as it retains water. The maximum permissible loss of water at which a commodity becomes unsalable has been reported (Robinson *et al.*, 1975). Reported maximum tolerable mass losses vary greatly, ranging from 5.0 % FW for apples and oranges to 37.0 % FW for green beans (Paull, 1999; Ben-Yehoshua *et al*., 2003). The greater the amount of water in the product, the lower is the amount of water that the product may lose without alterations in its appearance. In general, most commodities become unsalable as fresh produce after losing 3.0 to 10.0 % of their mass (Ben-Yehoshua *et al*., 2003).

Even so, even relatively small moisture losses are enough to cause marked loss of quality in many commodities. In fact, it should not be underestimated that the first symptoms of water stress appear long before the maximum permissible level of water loss (Ben-Yehoshua, 1987; Ben-Yehoshua *et al*., 2003), as shown in pepper where 2.0 to 4.0 % of loss of FW caused flaccidity and gloss compromising the overall appearance of the product (Lownds *et al.*, 1994).

The loss of water causes a set of symptoms that is typical for each commodity. As an example, in green vegetables, moisture loss causes changes that are similar to senescent breakdown, including the loss of membrane integrity, leakage of cell contents, more rapid degradation of surface tissues and colour degradation (Ben-Yehoshua, 1987). Mass losses can also cause significant loss of aroma and flavour, ultimately leading to the rejection by consumers as reported in lettuce (Agüero *et al.*, 2010). Additionally, water losses can also affect nutritional content of several commodities, as seen with the relationship found between the ascorbic acid (AA) content and moisture loss in strawberries (Nunes *et al.*, 1998). In climacteric fruits, it has been reported that moisture loss can also stimulate the synthesis of ethylene (Littmann, 1972 *cit. in* Paull, 1999) speeding up ripening of the commodity. In commodities susceptible to chilling injuries, moisture loss can further enhance that sensitivity (Paull, 1999; Kays and Paull, 2004; Maguire *et al.*, 2004). The resistance to pathogen invasion is also affected by moisture loss, possibly due to loss of membrane integrity and changes in cuticle structure of the cell (Van den Berg, 1987; Kays and Paull, 2004).

2.2.3.1. Factors affecting transpiration

2.2.3.1.1. Commodity related factors

Transpiration is a process dependent on the barriers existent in the commodity cell. Therefore, besides morphology, skin structures of horticultural products also have effect regarding the loss of water of the produce. Size, shape and surface area are among the commodity factors that affect the transpiration rate, due to the differences in the specific surface area of the commodity that is in contact with the surrounding air.

Moisture loss is affected by the ratio between surface area and volume of the commodity or by the surface area/mass ratio (Van den Berg, 1987; Ben-Yehoshua, 1987; Díaz-Pérez, 1988), although Burton (1982) observed that transpiration is a function of fruit surface area rather than fruit mass. An increase in surface area/volume increases the rate of moisture loss, as reported in capsicums (Lownds *et al.*, 1993). The type of tissue also tends to affect transpiration rates. It has been shown that about 67 % and 60 % of the total fruit transpiration is through the stem scar of tomato and the

calyx eggplant, respectively (Cameron and Yang, 1982; Díaz-Pérez, 1988) and not through the fruit itself.

Among the horticultural products, mushrooms present morphological particularities. They lack the specialized epidermal structure and are protected only by an epithelial layer which affects the rate of moisture loss and quality deterioration. San Antonio and Flegg (1964) analysed the loss of water from the fungal growth and found similarities between this process and an evaporative loss at a free water surface. Moreover, Nichols (1985) found that harvested mushrooms transpire at the same rate as non-harvested fruiting bodies.

2.2.3.1.2. Environmental factors

For a specific commodity, the rate of postharvest water loss is dependent on the storage conditions. Relative Humidity (RH - %) is defined as the ratio of water vapour present relative to the maximum amount of water vapour which can be present at the same temperature and atmospheric pressure) and temperature are the major storage factors influencing the rate of moisture loss (Kader, 2002).

It is generally assumed that internal atmosphere of horticultural commodities are in a saturated condition, due to the high water content of the cells. Unless the WVP in the storage atmosphere equals that on the produce surface, moisture will continue to evaporate from the produce surface (Shamaila, 2005).

Therefore, the WVPD or the difference in water vapour pressure between fresh products and the immediate surroundings gives an indication of the loss or gain of water by the product (Burton, 1982; Kays, 1991; Wills *et al.*, 1998; Thompson *et al*., 2002). Any increase in storage temperature increases the rate of transpiration even when humidity content of the air is constant since higher temperatures increase free energy of water molecules, which increase their movement and exchange potential (Ben-Yehoshua *et al.,* 2003).

2.3. Postharvest deteriorative changes in mushrooms

Among the horticultural commodities, mushrooms are one of the most perishable horticultural products and quality loss occurs rapidly after harvest. Quality losses in mushrooms are related with maturation process and in this sub-chapter the quality attributes related to the postharvest of mushroom, such as colour, texture and microbial changes will be outlined.

2.3.1. *Colour changes*

Colour is a major component related with quality in horticultural products and vital in the acceptability of the product by the consumer and highly related to senescence for most horticultural products (Kader, 2002). In mushrooms, colour is one of the most important parameters (González-Fandos *et al*., 2000). In mushrooms, especially for the white strains like button mushrooms, the whitest mushrooms reach the highest price (Singh *et al*., 2010). Colour evolution in mushrooms is related to ageing of the tissues and is generally characterized by a progressive darkening after harvest (Tano *et al*., 1999; Villaescusa and Gil, 2003; Sapata *et al*., 2004; Sapata *et al*., 2009a,b). Enzymatic browning is one of the main processes responsible for quality degradation in mushrooms (Braaksma *et al.*, 1994; Jolivet *et al.*, 1998). Browning is reported for button mushrooms (López-Briones *et al.*, 1993; Braaksma *et al.*, 1994), oyster mushrooms (Villaescusa and Gil, 2003; Sapata *et al.*, 2009a,b) or shiitake (Ares *et al.*, 2006; Parentelli *et al.*, 2007), therefore constitutes a general concern postharvest quality maintenance.

Browning occurs as a result of two distinct mechanisms of phenol oxidation: (*i*) activation of tyrosinase, an enzyme belonging to the polyphenol oxidase family, and (*ii*) spontaneous oxidation (Jolivet *et al.*, 1998).

Tyrosinase is an enzyme present at high levels in the mushroom surface tissue, and is normally found in a latent form (Soler-Rivas *et al.*, 2000). As tissue breakdown due to ageing, mechanical damage or bacterial activity (Beelman *et al*., 1989; Beaulieu *et al.*, 1999), the enzyme oxidizes phenolic compounds of mushroom into brown melanins, which results in brown discoloration (Boekelheide *et al.*, 1979; Soulier *et al.*, 1993; Jolivet *et al.*, 1998; Nerya *et al.*, 2006). Storage temperatures greatly affect

postharvest colour evolution in mushrooms, foremost due to the effect of temperature in enzymatic activity. Refrigeration temperatures can delay deleterious changes in mushrooms colour (Tano *et al.*, 1999; Villaescusa and Gil, 2003; Sapata *et al.*, 2009a,b).

In modified atmosphere packages the excessive accumulation of CO_2 can cause cell membrane damage and physiological injuries to the product, such as severe enzymatic browning (Burton *et al.*, 1987; López-Briones *et al.*, 1992; Varoquaux *et al.*, 1999).

Modified atmosphere packaging has the potential to slow down the rate of browning in mushroom (Ares *et al.*, 2007), although levels of O_2 and CO_2 outside the optimum range can have an opposite effect and induce severe browning (López-Briones *et al.*, 1992; Ares *et al.*, 2007).

2.3.2. *Textural changes*

The texture of horticultural commodities, a major quality attribute related to postharvest and therefore important in overall product acceptance is largely determined by the integrity of cell wall. When harvested, mushrooms are firm, crisp (resist deformation), and tender (easy to shear or chew), as has been described for button mushrooms.

Postharvest senescence is accompanied by changes in cell membrane, which leads to the loss of barrier function loss of turgor and senescence, ultimately resulting in the deterioration of mushroom (López-Briones *et al.* 1992; Villaescusa and Gil, 2003; Ares *et al.*, 2006; Parentelli *et al.*, 2007; Aguirre *et al.*, 2008; Mohapatra *et al.*, 2010).

Consequently, overall softening with elapsed harvest time is often seen in mushrooms of different species. That loss of firmness of mushrooms throughout postharvest storage is related to the degradation of protein and polysaccharide of the cell wall, hyphae shrinkage central vacuole disruption and expansion of intercellular space at the pilei surface (Zivanovic *et al.*, 2000). Softening can also occur due to the degradation of cell walls of postharvest mushrooms by bacterial enzymes (Jiang *et al.*, 2010).

Cohesiveness can also increase as storage progresses as seen in *Agaricus* and shiitake mushroom (Zivanovic *et al.*, 2000; Parentelli *et al.*, 2007; Jiang *et al.*, 2010). Zivanovic *et al.* (2000) reported that these specific changes are the result of the increase in chitin content and formation of covalent bonds between chitin and R-glucan, which increase the rigidity of the mushroom cell wall.

Changes in mushrooms texture are dependent on the storage conditions. Temperature has a major impact in mushroom firmness retention. Some studies suggest that in button mushrooms, texture is the quality parameter most affected by temperature (Mohapatra *et al.*, 2010). In accordance, Escriche *et al.* (2001) reported that temperature influenced firmness loss more than browning. The author analysed the sensitiveness of texture changes with increasing storage temperatures by calculating the activation energy and Q_{10}. Texture changes were more sensitive to the increase the temperature from 5 to 15 °C than from 15 to 25 °C, which results in an increase in reaction speed of 4.55 and 1.50, respectively.

2.3.3. *Mass loss*

Mass losses in fresh commodities are responsible for significantly quantitative losses and are a result of both respiration and transpiration processes. Transpiration is the process by which fresh commodities lose water to the surrounding environment, whereas mass losses from respiration arise from the exhaustion of substrates in the cell (Kader, 1987). Although both processes have the potential to promote mass losses in fresh commodities, transpiration is the most important factor involved in this process.

There are several factors that have effect in the rate of transpiration of fresh products (Chapter 2, § 2.2.3.1). The evaporative surface of the commodity has a major influence in the rate of transpiration. From a morphological point of view, epidermal structure of higher plant tissues is lacking in mushrooms. Instead, mushrooms are only protected by a thin and porous epidermal structure and therefore, water loss from mushroom can be compared to the water loss from a free water surface as reported by San Antonio and Flegg (1964) in *Agaricus* mushroom during growing. Given that it is hypothesised that freshly harvested mushroom transpires at the same rate as the fruiting sporophore (Nichols, 1985), mushrooms are very sensitive commodity regarding mass losses by transpiration. Water loss in mushrooms affects the main

quality characteristics such as saleable weight, appearance and texture (Mahajan *et al.*, 2008a).

Mass losses throughout storage time are linear (Burton and Noble, 1993; Mahajan *et al.*, 2008a). Stored at 5 ºC (73 % RH), Burton and Noble (1993) reported that mass losses from common mushrooms stored in open punnets were 4 % per day. When temperature increase from 5 ºC (73 % RH) to 18 ºC (90 % RH), the rate of mass loss increased to 6 % per day.

Increases in storage temperatures affect the rate of transpiration (from 0.29 g. $kg^{-1}.h^{-1}$ to 5.2 g. kg^{-1} $.h^{-1}$) when temperature increase from 4 to 16 ºC of *Agaricus bisporus* (Mahajan *et al.*, 2008a).

2.3.4. *Microbiological spoilage*

The rate of postharvest deterioration of fresh mushrooms has been directly related to the microbial load at harvest (Doores *et al.*, 1987; Singh *et al.*, 2010) and the presence of bacterial populations in mushrooms is a factor that significant diminishes quality of the produce throughout storage (Beelman *et al.*, 1989).Fresh mushrooms are considered an ideal medium for microbial growth. Their high moisture content, a water activity of 0.98 or higher and a neutral pH, provide good conditions for microbial growth. Total bacterial populations in fresh mushrooms are considered high, ranging from 6.3 to 7.2 log CFU g^{-1} (Doores *et al.*, 1987, Santana *et al.*, 2008; Venturini *et al.*, 2011). Significant levels of moulds and yeasts (3 log cfu and 6 log CFU g^{-1}, respectively (Chikthimmah *et al.*, 2006) have also been reported in fresh mushrooms. Most of the bacterial count present in mushrooms is within the genus *Pseudomonas* (Wells *et al.*, 1996; Doores *et al.*, 1987; Soler-Rivas *et al.*, 1999; Venturini *et al.*, 2011). Flavobacteria are also important in, comprising 10 % of the total bacteria count (Doores *et al.*, 1987). Within the genus *Pseudomonas*, *P. tolaasii* is the major specie reported and is responsible for bacterial blotch. Infections symptoms include the appearance of a brown, blotchy appearance during postharvest life of mushrooms (Beaulieu *et al.*, 1999). Other species such as *Pseudomonas fluorescens* have also been associated with mushroom spoilage (Masson *et al.*, 2002; Jiang *et al.*, 2010). Santana *et al.* (2008) identified *Pseudomonas putida* in minimally processed shiitake stored at 7, 10 and 15

ºC. Contamination of *Verticillium maltousei* also impairs mushrooms appearance, causing brown spots (Beaulieu *et al.*, 1999; Beaulieu *et al.*, 2002).

The evolution of microbial load profile during storage of all fresh horticultural commodities is related to prompt cooling, storage at low temperature, and prevention of physical injury. The effect of storage temperatures in microbial spoilage is well known. In mushrooms, Chikthimmah *et al.* (2006) and Doores *et al.* (1987) observed that bacterial populations tend to increase from 7.3 to 8.4 log $CFU.g^{-1}$ during 1 day at 4 ºC, while when stored at 13 ºC, for 10 days, mushrooms with an initial load of 7 log $CFU.g^{-1}$ presented an increase to almost 11 log $CFU.g^{-1}$.

In shiitake mushrooms stored at 7 ºC, 10 ºC and 15 ºC, the number of mesophilic bacteria had an increase of 3, 4 and 4.3 log cycles respectively, 15 days after storage (Santana *et al.*, 2008).

Postharvest treatment with hydrogen peroxide (Brennan *et al.*, 2000; Sapers *et al.*, 2001), citric acid (Brennan *et al.*, 2000) or sodium erythorbarte (Sapers *et al.*, 2001) have been used to extend the shelf life of fresh mushrooms by controlling or reducing the microbial populations. In this sense, Simón *et al.* (2010) determined a significant reduction of *Pseudomonas* in button mushrooms washed with citric acid before being sliced and stored for 17 days of storage at 5 ºC.

The use of low O_2 levels, generally recommended for mushrooms in modified atmosphere packaging (Kim *et al.*, 2006) may lead to the occurrence of anaerobic respiration. In those packages, pathogens such as *Clostridium botulinum* and *Staphylococcus aureus* have potential to grow (Farber *et al.*, 2003; Kim *et al.*, 2006; Parentelli *et al.*, 2007). As a result, several authors recommended that, inside mushroom packages, O_2 should not drop below 2 % (Varoquaux *et al.*, 1999; Ares *et al.*, 2006; Parentelli *et al.*, 2007).

The use of CO_2 enriched atmospheres has the ability to reduce decay, due to its direct antimicrobial activity (Phillips, 1996) but excessive accumulation of CO_2 can cause physiological injuries in mushrooms (Ares *et al.*, 2006; Parentelli *et al.*, 2007). On the other hand, the absence of CO_2 within mushrooms package caused by the use of CO_2 scavenger can also cause the growth of aerobic bacteria, yeast and moulds (Masson *et al.*, 2002; Oliveira *et al.*, 2012).

Recently, Venturini *et al.* (2011) evaluated the microbiological quality of fresh mushrooms (wild and cultivated) commercialized in Spain and did not found the occurrence of pathogens such as *Salmonella* spp, *E*. *coli* and *S*. *aureus*.

2.4. Postharvest technologies to extend the shelf life of fresh produce

Harvested fresh produce continues to perform its metabolic functions throughout storage, which greatly influences quality and shelf life.

To extend the shelf life of fresh produce and ensure the maximum quality for consumers, proper postharvest technologies for each commodity are required. Temperature and relative humidity management during storage is the most adopted technology to extend the shelf life of fresh commodities. Although several postharvest treatments are available, the use of modified atmosphere package can be used to reduce respiration rates and extend the shelf life. In this section, the effects of temperature and relative humidity control and modified atmosphere packaging will be presented.

2.4.1. *Temperature and relative humidity control*

Temperature and relative humidity control during postharvest life are the most important technologies to control deterioration and extend the shelf life of fresh horticultural commodities (Lee and Kader, 2000; Kader, 2002).

Storage temperature has a strong influence in postharvest quality maintenance due to its influence in the overall physiological and biochemical processes that affect commodity life, but also to the significant effect on the microbiological activity of the product (Kader, 2002).

Optimal temperature for storage and shelf life extension is product dependent. Despite varietal or cultural differences, it is generally accepted that the best temperature for any product is the lowest temperature possible that does not cause damage to produce. Low temperatures reduce respiration and ethylene production rates, water loss, pathogen growth and decay incidence (Kader, 2002). Nevertheless, the use of low refrigeration temperatures can cause some undesirable changes in some commodities. Problems caused by low temperature storages are known as chilling and freezing injuries and limit the use of low temperatures for some commodities (Saltveit and Morris, 1990).

Freezing injury occurs when the contents of the cell of the commodity freezes, resulting in water soaked damaged areas. Freezing is dependent on the commodity. While commodities with high content of sugars like sweet fruits are damaged by temperatures between - 2 and - 5 °C, for horticultural produces with lowest content of sugars, like leafy vegetables, freezing temperature is higher (Kader, 2002). Freezing injury may cause severe damage to the produce since it causes an immediate collapse of tissues and total loss of cellular integrity (Kader, 2002).

The most common form of injury related to low temperatures storage is chilling injury and occurs at temperatures above commodity freezing point causing severe physiological damage. The temperature at which such damage occurs is called the chilling threshold temperature and corresponds to lower safe limit for a stored produce throughout marketing chain (Kader, 2002). Manifestation of chilling temperatures includes loss of water, shrivelling, colour alterations and an increase in the susceptibility of postharvest rots (Saltveit and Morris, 1990).

For good quality maintenance, a precise control of temperature during entire postharvest chain is fundamental (Brecht *et al.*, 2003). Each product has an optimal range of storage temperatures that will maximize its storage life (Paull, 1999; Lurie, 2002; Thompson *et al.*, 2002). Recommended temperature conditions for storage of a particular product is available in numerous source of information (Kader, 2002). Fluctuations of temperature are common, which results in important quality losses (Paull, 1999; Jacxsens *et al.*, 2000; Brecht *et al.*, 2003). In some cases, water condensation on both film package and commodity surfaces may also occur. Consequently, while in the film package condensed water can cause alteration in the package permeability (Exama *et al.*, 1993), water in the commodity potentially increases the occurrence of fungal and bacterial decay (Nunes *et al.*, 2009).

Temperature management should start at the time of harvest. Prompt cooling is essential as the harvest causes a stress in the product increasing RR, water stress and a significant reduction in quality (Kader, 2002). Rapid cooling after harvest is a technique known as pre-cooling. It can be achieved by different ways. Forced air-cooling, hydro-cooling, hydro air-cooling and vacuum cooling are normally used (Kays, 1991; Kader, 2002).

Storage conditions are highly important for the quality of fresh mushrooms. The most favourable storage temperature is low temperature (0 – 2 ºC) combined with high

relative humidity of the air (Murr and Morris, 1975; López-Briones *et al.*, 1992, Escriche *et al.*, 2001). Storing mushrooms at low temperatures limits mass loss (Escriche *et al.*, 2001; Mahajan *et al.*, 2008a) and maintains freshness, as well as firmness and colour of the flesh (Burton and Noble, 1993).

Low temperatures contribute to a lower respiration rate in *Agaricus*. Iqbal *et al.* (2009a) obtained a reduction in respiratory rate of *Agaricus* in the order of 47 - 60 % at temperatures between 4 and 20 °C. Varoquaux *et al.* (1999) also reported that respiration rate of *Agaricus* mushrooms increased 2.9 fold for each 10 °C increase in temperature.

According to Escriche *et al.* (2001) and Mohapatra *et al.* (2010), storage temperature is the most important parameter affecting texture of *Agaricus* mushrooms. Besides the effect on the appearance, storage of fresh mushrooms under low temperatures also affects the level of sugars, free amino acids and 5'-nucleotides of mushrooms as seen for button mushrooms. Mushrooms stored at 12 °C for 12 days had a 36 % decrease in the level of total sugars; 42 % decrease in mannitol; 89 % decrease in fructose (Tseng and Mau, 1999), potentially decreasing its flavour.

Refrigeration temperatures significantly increase mushroom shelf life. At 0 - 1 °C button mushrooms can be stored for 7 - 9 days while at 15 °C for 2 - 3 days (Gormley, 1975). Oyster mushrooms held at 0 °C maintained quality for 8 - 11 days, but storage at 20 °C reduced the shelf life of about 1 - 2 days (Choi and Kim, 2002). RH management has a vast influence on moisture loss of mushrooms (Mahajan *et al.*, 2008a), and improper RH management causes losses in saleable mass, wrinkling and development of brown patches on the produce surfaces (Roy *et al.*, 1995a,b; Jayathunge and Illeperuma, 2005) As it has been shown for *Agaricus* (Cliffe-Byrnes *et al.*, 2007), moisture levels in the postharvest atmosphere will also have a direct effect in mushroom postharvest development, since higher levels slow down the maturation process, as indicated by the slower opening of mushroom cap.

On the other hand, saturated environment is generally seen in mushrooms packaging (Roy *et al.*, 1995a,b; Sapata *et al.*, 2009a,b), which favours the growth of microorganisms and consequent decay of the product (Roy *et al.*, 1995a,b).

2.4.2. *Packaging systems*

Packaging is a fundamental step to ensure quality of fresh produce. Package serves several purposes as described by Wills *et al.* (1998). Besides the main function of containment and protection of the produce, packages have been suffering a continuously evolution in agreement with development of new technologies to maintain quality and extend shelf life.

Modification of the atmosphere around the produce is a technique that, when used with temperature control, further reduce produce deterioration.

2.4.2.1. Modified atmosphere packaging

Modified atmosphere packaging (MAP) of fresh horticultural commodities refers to the technique of sealing the produce in polymeric film to modify the ambient conditions surrounding the produce (Kader, 1987; Church and Parsons, 1995). MAP is a dynamic process of altering gaseous composition inside a package and relies on the process of respiration and the gas flux throughout the package film (Kader, 2002). Due to the respiration process of fresh commodities and the characteristics of the package film, a gradient of O_2 and CO_2 between the package headspace and the storage environment is generated. If the film used has the proper barrier properties, a pre determined O_2 and CO_2 concentration will be achieved. At this equilibrium, the rate of gas exchange between the product and the packaging material equals, therefore, subsequently the gas composition surrounding the product is maintained for the rest of the storage life (Kader, 2002).

In a MAP system, gas compositions of the air (78.03 % of nitrogen (N_2), 20.99 % of oxygen (O_2); 0.03 % of carbon dioxide (CO_2), 0.94 % of Argon (Ar) and 0.01 % of Hydrogen (H_2) (Parry, 1993)) are changed to hold perishable products. The modification of the atmosphere generally implies a reduction in O_2 content and an increase in the CO_2 concentration. Other approaches are also available, like changing the level of carbon monoxide (CO), ethylene, ethanol or other compounds in the atmosphere can also contribute to shelf life extension.

The establishment of a desired gas mixture in a MAP system may be achieved in two different ways: passively or actively. In passive MAP, fresh produce is packaged with air as the initial gas concentration. Since over the initial gas composition no further control is made, some authors refer to this technology as passive atmosphere packaging (Farber *et al.*, 2003; Gorris and Peppelenbos, 2007). In an active MAP, a predetermined gas mixture is used to flush packages before sealing the package (Kader and Watkins, 2000; Farber *et al.*, 2003).

The first type of MAP is more economic and is used for products with high respiration rate (Gorris and Peppelenbos, 2007), that are able to reduce the in-pack O_2 level fast enough to lower levels that do not cause physiological or microbial deterioration. However, high perishable commodities have a relatively short shelf life and the time available to attain the steady-state is short. In such cases, active MAP may reduce or even suppress the length of the period required to achieve equilibrium (Gorris and Peppelenbos, 2007; Guillaume *et al.*, 2010).

Despite the initial gas composition, after closing the package, a decrease in the oxygen content and an increase in the carbon dioxide content occur due to the respiration of the product. This atmospheric composition inside the package causes a decrease in the respiration rate. After a transient period, in which interaction between the product respiration and gas exchange through the package occur, the system reaches equilibrium (Fonseca *et al.*, 2002a; Gorris and Peppelenbos, 2007). Therefore, the system is also known as equilibrium-modified atmosphere packaging. At this point, the atmospheric gases inside the package should be similar to the optimum gas concentration for that produce (Gorris and Peppelenbos, 2007).

Inside the package, the rate of atmosphere modification and the achievement of the steady-state are determined by three processes: i) respiration of the commodity; ii) gas diffusion through the commodity, and iii) gas permeation through the film. Each of these processes is strongly influenced by several factors as summarize in Tab. 2.3 (Gorris and Peppelenbos, 2007).

Tab. 2.3 - Variable involved in MAP design.

		Variables
Environmental conditions		Gas composition
		Temperature
Produce characteristics		Product mass
		Product density
		Product respiration rate
		Desired gas composition
Package	Film	Free volume
		Thickness of the film
		Area available for gas exchange
		Permeability to O_2 and CO_2
	Film with macroperforations	Free volume
		Thickness of the film
		Area of film available for gas
		Permeability to O_2 and CO_2
		Number of perforations
		Radius of holes
	Perforation-mediated packages	Free volume
		Number of tubes (perforations)
		Length of tubes (perforations)
		Diameter of tubes (perforations)
		Porosity of the tube packing

To achieve the optimum gas composition at the shortest period of time, a match between the RR and the film permeability should be achieved. As a consequence, permeability characteristics of the polymeric films used for packaging the produce are extremely important to achieve this target.

Most of the films commercially used for fresh produce packaging are low-density polyethylene (LDPE), polypropylene (PP) and polyvinyl chloride (PVC). Film used for these products have different permeabilities to O_2 and CO_2. OTR (Oxygen transmission rate) and COTR (carbon dioxide transmission rate) are used successfully for several commodities. The difficulty in the use of these films is to match the rate of permeability of films and the respiration rate of commodities with high respiration rates.

Most of these films have low permeability rates and their success in packaging high respiration commodities is limited. Under such conditions, anaerobic conditions within the package are developed (Exama *et al.*, 1993) with a subsequent deterioration in the quality of the commodity.

Since many of the films used in MAP, singly do not offer all the properties required for MAP, different approaches were developed to overcome the gas diffusion problems (Exama *et al.*, 1993; Mangaraj *et al.*, 2009).

A potential solution encountered for fresh produce package available to overcome the problem related to diffusion characteristics of the polymeric films is the introduction of perforations in films as proposed by several authors (Kader *et al.*, 1989; Exama *et al.*, 1993; Renault *et al.*, 1994a, b; Fishman *et al.*, 1996; Hirata *et al.*, 1996; Sanz *et al.*, 1999, Sanz *et al.*, 2000; Fonseca *et al.*, 2002c; Al-Ati and Hotchkiss, 2002; Mangaraj *et al.*, 2009). In fact, irrespective of the material used, any changes in the structure of the polymeric films imply changes on both O_2 and CO_2 transmission rate.

Perforations provide numerous advantages over non-perforated polymeric films (Exama *et al.*, 1993; Renault *et al.*, 1994a,b; Fishman *et al.*, 1996). Since gas flow through the perforations is much greater than gas movement through the film (Fishman *et al.*, 1996; Fonseca *et al.*, 2002c; Varoquaux and Ozdemir, 2005), perforations allows a significant increase in the total gas flow through the package.

Microperforated films provide a large range of O_2 permeabilities that can match the O_2 requirement of most produce (Varoquaux and Ozdemir, 2005). These films are applicable for products that can tolerate high CO_2 since CO_2 rate of diffusion is very similar to the rate of O_2. In this specific case, it is impossible to achieve low O_2 (1 – 5 %) concentration in the package headspace without accumulating high CO_2 levels (15 – 20 %) (Exama *et al.,* 1993). PM - MAP relies on the use of perforations in the form of tubes to control O_2 and CO_2 exchange. Gas flow through this type of perforations may be altered by the diameter and length of the tube (Fonseca *et al.*, 2002c).

The use of composite films, produced by blending polymeric films with inert inorganic material has also been explored mainly for high sensitive products, such as fresh –cut fruits and vegetables (Ahvenainen, 1996).

Mushrooms are among the produces with high RR that could benefit with a MAP system (Kader *et al.*, 1989; Iqbal *et al.*, 2009b). The use of modified atmosphere packaging to extend the shelf life of mushrooms has been extensively reported (López-Briones *et al.*, 1993; Roy *et al.*, 1995a; Tano *et al.*, 1999; Ares *et al.*, 2007; Singh *et al.*, 2010). Most of the literature, however, is related to the potential benefit on the use of MAP for button mushrooms (*Agaricus bisporus*) (Sveine *et al.*, 1967; López-Briones *et*

al., 1993). Other studies focusing on the positive effects of MAP in *Pleurotus* (Villaescusa and Gil, 2003; Sapata *et al.*, 2004; Sapata, 2005; Sapata *et al.*, 2009a,b; Sapata *et al*., 2010) and shiitake (Ares *et al.*, 2006; Antmann *et al.*, 2008) have also been reported.

The use of MAP on mushrooms is associated with a number of positive effects related to appearance of fresh produce. An important delay in maturation, reducing colour alterations and mass loss are among the factors described for fresh mushrooms resulting in higher quality (Nichols and Hammond, 1975; Kim *et al.*, 2006). In order to attain such benefits, recommended MAP conditions for mushrooms were 3 – 5 % (v/v) of O_2 and less than 12 % (v/v) of CO_2 (Ares *et al.*, 2006), but the range of O_2 and CO_2 recommended for each species are expected to be different.

First studies on MAP of *Agaricus* suggested the use of an atmosphere with simultaneous low O_2 and CO_2 to maintain an optimum quality of the fruit bodies. According to Murr and Morris (1975), lowering the O_2 levels to 0.1 % (v/v) in combination with 5.0 % (v/v) CO_2 (storage at 7 °C) increased the shelf life of *Agaricus* mushrooms by delaying cap maturation (as seen by both lower pileus growth and expansion of the stipe). In accordance, Gormley and MacCanna (1967), Nichols and Hammond (1975) and Sveine *et al.* (1967) also reported the use of low levels of O_2 (0 - 2 %, (v/v) maximum) to obtain optimum quality in button mushrooms. The optimum level of CO_2, although varying between studies, is in the range of 5 to 12 % (v/v). Atmospheres of 6 % (v/v) have demonstrated to delay maturity as seen by the significant reduction in cap development of *Agaricus* mushrooms (Roy *et al.*, 1995a).

Studies of the effect of MAP on *Pleurotus* mushrooms are also available. Popa *et al.* (1999) studied the effect of several combinations of O_2 and CO_2 and described that 1 kPa O_2 and 5 kPa CO_2 was ideal to maintain *Pleurotus* quality for 14 days at 4 °C, as seen for the positive effects in colour and texture. Villaescusa and Gil (2003) found that steady state MAP conditions with concentrations of 2 kPa O_2 and 12 kPa CO_2 maintained good visual quality of *Pleurotus ostreatus* for 7 days, but suggested the exploration of other types of atmosphere. Choi and Kim (2002) also evaluated the benefits of MAP utilization in the keeping quality of mushrooms. Packaging film was found to prevent or retard the deterioration of mushroom appearance, texture and discoloration.

Although the effects of MAP may positively affect several quality attributes in mushrooms, the effect of MAP in the RR of fresh mushrooms is founds no consensus. Peppelenbos *et al.* (1993) studied the effect of atmosphere modification in the RR of button mushrooms stored for 1–3 days. The authors found that, within the range of the combinations tested (0.81 – 2.6 % O_2 plus 0.18 – 9.7 % (v/v) CO_2) no effect was found in RR. In agreement, Varoquaux *et al.* (1999) also did not observe reduction in the respiration rate of *Agaricus* stored under O_2 and CO_2 partial pressure of 0.1 to 20 kPa and 0 to 20 kPa, respectively. On the other hand, during storage of *Pleurotus* mushrooms, Choi and Kim (2002) found a decrease in RR as a consequence of the levels of O_2 and CO_2 found in the package.

As previous stated, a setback in the successful use of MAP is the limits levels of O_2 and CO_2 that the commodity is able to support (Beaudry *et al.*, 1992; Beaudry, 1999). It has been reported that CO_2 concentrations higher than 12.0 % (v/v) causes a phytotoxic effect in mushrooms as seen in loss of firmness, browning, off-odours and a decrease in overall appearance of different mushroom species (Nichols and Hammond, 1973; Burton *et al.*, 1987; López-Briones *et al.* 1992; Varoquaux *et al.*, 1999; Ares *et al.*, 2006). Even levels as low as 5.0 or 6.0 % (v/v) caused severe browning in button mushrooms, as described by several authors (Nichols and Hammond; 1973; López-Briones *et al.*, 1992; Barron *et al.*, 2002). Ares *et al.* (2006) suggest that different species have different critical threshold values, since shiitake mushrooms are apparently more sensitive than other species.

Another setback in the use of MAP for mushrooms is that, in order to obtain the benefits previous described, such as a reduction in RR or an increase of the sensory quality, a very low concentration of O_2 must be used. Although mushrooms can benefit with low O_2 levels, at those concentrations, anaerobic respiration accompanied by off-odours was also reported (Burton *et al.*, 1987; Beit-Halachmy and Mannheim, 1992; López-Briones *et al.*, 1992; Tano *et al.*, 1999). Anaerobic conditions also have the potential for growth of food borne pathogens such as *Clostridium botulinum* as seen for *Agaricus* mushrooms (Sugiyama and Yang, 1975; Farber *et al.*, 2003). As consequence, for safety reasons, O_2 should not drop below 2.0 % (v/v) (Varoquaux *et al.*, 1999).

2.4.2.2. Controlling humidity inside fresh produce package

Controlling the in-pack relative humidity (IPRH) in MAP systems is becoming increasingly important and has been presented as an approach to improve effectiveness of MAP (Aharoni *et al*., 2008). In addition to the limitation on the transmission rates of O_2 and CO_2, package films generally used for fresh commodities have also limitations in the water vapour transmission rate (WVTR).

In general, most of the available polymers used in MAP have very low permeability to water vapour (Mahajan *et al.*, 2008a; Mangaraj *et al*., 2009). As a result, when used with produces with high transpiration rates, IPRH is generally maintained very high (~100 % RH) inside the package (Aharoni *et al*., 2008; Mangaraj *et al*., 2009). Although high humidity in packages may potential reduced weigh losses, some adverse effects may occur from these environmental conditions. At this humidity levels, even small fluctuations in temperature during storage result in water condensation on both film and produce. Besides the detrimental effects in package aspect, condensed water on the inner film surface may adversely affect the gas exchange, leading to an unfavourable internal atmosphere (Cameron *et al*., 1995; Aharoni *et al*., 2008). Even though the use of antifog additives combined with polymeric films is used to eliminate visible condensation, it does control the levels of humidity inside the package.

Shirazi and Cameron (1992) developed the concept of modified-humidity packaging (MHP) that is a type of equilibrium modified atmosphere packaging (EMAP), specially developed to control the levels of humidity inside a package.

Perforations are a relatively simple approach to reduce in-pack moisture and therefore condensation. However, matching the requirements of relative humidity, O_2 and CO_2 inside the packages is a specially challenging task. In fact, it has been shown that the extent of perforations necessary for a small change in the RH inside a package increased O_2 to the ambient level (Fishman *et al*., 1996).

Hydrophilic films may potentially reduce some of these issues, because they allow the desired MA as well as the humidity levels. Hydrophilic films combine different polymeric and non-polymeric compounds to achieve the optimum RH. The modified atmosphere is achieved with the microperforations of the combined film (Aharoni *et al*., 2008).

The use of moisture absorbers inside a package is also an important technology to control the IPRH. In this case, in order to achieve the optimum RH, three factors are important: the transpiration of the produce, the loss of water through the polymer film and the gain of water by the absorber (Shirazi and Cameron, 1992; Mahajan *et al*., 2008b).

Varoquaux *et al*. (1999) concluded that no extension of mushroom shelf life was attainable trough MAP and suggested a control of RH within the package as a more effective way to retain produce quality.

Roy *et al*. (1995a) have reported better colour retention and lower moisture loss for mushrooms packed under MA in combination with sorbitol pre-treatment. Use of small amounts of sorbitol was found to reduce condensation with $CaCl_2$ irrigated mushrooms (Anantheswaran *et al*., 1996). For *Pleurotus* mushrooms, Villaescusa and Gil (2003) also used sorbitol and silica gel (10–15 g/150 g of mushrooms) to control IPRH, concluding on one hand, that sorbitol deteriorated texture, whereas silica gel increased the mass loss of produce. On a different approach, Mahajan *et al*. (2008b) developed a moisture absorber for fresh mushrooms using different combinations of desiccants. The authors suggested a combination of bentonite, sorbitol and $CaCl_2$ (in proportions of 0.55, 0.25 and 0.2 $g.g^{-1}$) to fulfil mushrooms requirements (moisture holding capacity of 0.9 $g.g^{-1}$ mixed desiccant that remained in powder form during 120 h of storage at 10 ºC). Moreover, appearance of *Agaricus* mushrooms improved with the use of 5 g of mixed desiccant in 250 g of mushroom punnets when compared with produce packed without desiccant (Mahajan *et al.*, 2008b).

PART II- Experimental work

Chapter 3: Influence of storage conditions on the shelf life of fresh oyster mushrooms

3.1. Introduction

Mushrooms are a highly perishable food item, having a shorter postharvest life compared to other horticultural produces. At room temperature, quality losses can occur almost immediately (López-Briones *et al*., 1993; Tano *et al.*, 1999) significantly limiting their useful life, and causing problems in mushroom marketing as a fresh product (Antmann *et al.*, 2008). In fact, a shelf life of 1–3 days at ambient temperature has been described (Burton and Twining, 1989) which is known to be a natural consequence of their high metabolic activity and high water content (Villaescusa and Gil, 2003; Mahajan *et al.*, 2008a).

While other technologies may be used, temperature management is still the most important environmental factor related to postharvest quality retention (Kader, 2002). For highly perishable commodities like mushrooms, refrigeration is the most usual preservation method, reducing both physiological and microbial spoilage (Singh *et al.*, 2010).

Oyster mushrooms are characterized by high respiration and transpiration rates (Rajarathnam *et al.*, 1983; Villaescusa and Gil, 2003; Sapata, 2005; Jayathunge and Illeperuma, 2005). Moreover, the cap morphology is characterized by a thin and large surface which increases perishability (Rajarathnam *et al.*, 1983). To overcome the difficulty in preserving their quality, effective postharvest technologies, namely strictly storage temperature control is mandatory (Rajarathnam *et al.*, 1983; Ares *et al.*, 2007).

The objective of this study was to: (**i**) investigate the effect of storage temperature on different quality attributes of oyster mushrooms and (**ii**) to illustrate the importance of proper temperature management throughout the supply chain of oyster mushrooms.

3.2. Material and methods

3.2.1. *Sample preparation*

Fresh oyster mushrooms (*Pleurotus ostreatus*) were collected during the morning from a local producer in Gondomar region. Oyster mushrooms clusters were transported to the laboratory and stored at 2 °C until the beginning of the experiment at that same day.

3.2.2. *Experimental procedure*

Respiration rate and quality attributes of fresh oyster mushrooms were measured daily over a 96 hours period. Quality evaluation was based on colour, relative electrolyte leakage and mass loss.

3.2.3. *Respiration rate measurement*

Respiration rate was measured using the closed system methodology as reported in literature (Cameron *et al*., 1989; Gong and Corey, 1994; Fishman *et al*., 1996; Fonseca *et al*., 2002a,b; Song et al. 2002; Iqbal *et al*., 2008; Iqbal *et al*., 2009a,b; Torrieri *et al*., 2009; Caleb *et al*., 2012b,c).

Samples of separated carpophores were weighted (approximately 0.15 kg for each sample), placed in glass jars (volume of 1.9×10^{-3} m^3) used as respirometers and stored in temperature control chambers (Monte Branco Refrigerators, Ltd, Oporto, Portugal) at test temperatures (2, 6, 10, 14 and 18 °C ± 1 °C).

Respiration rate determination was performed daily. At each sampling time, jars were closed tightly, sealed with petroleum jelly and Parafilm, and hermeticity was verified. After finishing the measurements, jars were opened to allow air renewal, remaining opened overnight. To avoid excessive moisture loss, jar lids were kept slightly tilted over the jar tops.

For each sampling time, gas composition (volumetric fraction of O_2 and CO_2) of the headspace of each jar was monitored by withdrawing a 2 mL sample after inserting the analyser needle through a rubber septum on the jar lid. This was monitored over time using a gas analyzer (Checkmate, 9900, PBI Dansensor, Denmark O_2 /CO_2 gas analyser with an accuracy of 0.5 %). The interval of time used to calculate respiration rate was dependent on temperature, with up to four measurements by jar, varying from 30 minutes for 18 ºC to two hours for 2 ºC.

Respiration rate for each sample and for each measuring time in terms of CO_2 generation and O_2 consumption was determined from the slope of the fitted linear equation according to Equations 3.1 and 3.2.

$$RR_{O_2} = \frac{\Delta Y_{O_2} \times V_f}{\Delta t \times 100 \times M} \quad \text{Eq. 3.1}$$

$$RR_{CO_2} = \frac{\Delta Y_{CO_2} \times V_f}{\Delta t \times 100 \times M} \quad \text{Eq. 3.2}$$

where RR_{O_2} and RR_{CO_2} are O_2 consumption and CO_2 production rates (mL.kg.$^{-1}$.h^{-1}), ΔY_{O_2} and $\Delta Y_{CO_2}$are the variation % (v/v) of O_2 and CO_2 and Δt is the variation of time (h), M is the mass of the sample (kg), V_f is the free volume in the headspace jar (mL).

Free volume inside the glass jar was calculated from Equation 3.3:

$$V_f = V - \frac{M}{\rho} \quad \text{Eq. 3.3}$$

where V is the total volume of the jar (mL); ρ is the volumic mass (experimentally determined through a simple water displacement method and obtained the value of 656.67 kg.m^{-3}).

Respiratory quotient (RQ) was calculated according with Equation 3.4.

$$RQ = \frac{RR_{CO_2}}{RR_{O_2}} \quad \text{Eq. 3.4}$$

The determination of respiration rate was performed in triplicate for each combination storage time-temperature.

3.2.4. *Quality indicators analysis*

The colour of the upper surface of the mushrooms was evaluated using a reflectance colorimeter (CR 400, Minolta Corp., Osaka, Japan), with the illuminant D_{65}, previously calibrated with a standard white tile (X = 81.1, Y = 86.0 and Z = 91.8).

For each storage time, colour measurements were performed using an 8 mm diameter diaphragm, at ambient temperature and CIE $L^*a^*b^*$ parameters were recorded. (Appendix A). Chroma (C^*) and hue angle (H^o) values were obtained using the Eq. 3.5 and 3.6 (McGuire, 1992):

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad \text{Eq. 3.5}$$

$$H° = \text{arctg}\left(\frac{b^*}{a^*}\right) \quad \text{Eq. 3.6}$$

The original values of L^*, a^*, b^* were used to obtain the degree of overall colour change (total colour difference, TCD) and overall lightness change (total lightness,TL), throughout the produce storage period, using the Eq. 3.7 and 3.8.

$$TL = \left[\left(\Delta L^*\right)^2\right]^{1/2} \quad \text{Eq. 3.7}$$

$$TCD = \left[\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2\right]^{1/2} \quad \text{Eq. 3.8}$$

Browning index (BI) was calculated as described by Maskan (2001), according to Eq. 3.9 and 3.10:

$$BI = \frac{[100(x-0.31)]}{0.17} \quad \text{Eq. 3.9}$$

$$\text{in which, } x = \frac{\left(a^* + 1.75 \times L^*\right)}{\left(5.64L^* + a^* - 3.012b^*\right)} \quad \text{Eq. 3.10}$$

Colour measurements were made daily in the same mushrooms, directly on the cap surface, 3 times on 10 mushrooms from each condition.

Relative electrolyte leakage (REL, %) was assessed by cutting discs of fresh mushrooms (20 mm diameter, total 5 g) with a sharp cork borer.

Mushroom discs were placed in autoclaved jars (100 mL) with 50 mL of bi-deionised water. Conductivity (µS) was measured with a conductivimeter (510 CyberscanCON, Singapore) after 1 minute (C_1) and after 60 minutes (C_{60}), under agitation conditions (100 rpm. min^{-1}) in an electromagnetic stirrer (E Agimatic - C7001606, Spain).

The samples were then autoclaved for 15 minutes. After cooling, the total conductivity C_T of the solution in suspension was determined.

REL was calculated in accordance with that indicated by Fan and Sokorai (2005) (Eq. 3.11).

$$REL = \frac{(C_{60} - C_1)}{C_T} \times 100 \quad \text{Eq. 3.11}$$

Three replicates were performed for each time-temperature combination.

A small amount of the mushroom homogenized in a Ultra-Turrax (Basic T25, IKA, Germany) was squeezed through cheesecloth and a drop was used for SSC (expressed as ºBrix) determinations. SSC was determined in triplicate with a hand held refractometer (Milwaukee Instruments, Rocky Mount, U.S.A.).

Ten mushrooms of each time-temperature conditions were weighed individually and mass loss (ML, %) was determined by gravimetric difference in respect to the initial mushroom mass (Eq. 3.12).

$$ML\ (\%) = \frac{M_i - M_t}{M_i} \times 100 \quad \text{Eq. 3.12}$$

where M_i is the initial mushroom mass and M_t is the mushroom mass at time t (h). Results were expressed as percentage of mass loss.

3.2.5. *Data analysis*

Effect of time and temperature on quality indicators was inspected through the use of a two-way analysis of variance (ANOVA), using PASW Statistics for Windows,

Version 18.0 (SPSS Inc. Released 2009, Chicago: SPSS Inc. Multiple comparisons were performed using the Tukey´s test. All tests were applied at a 95 % confidence interval (CI), except if stated otherwise. Furthermore, results are presented as mean ± standard error of mean, unless otherwise stated.

3.3. Results and discussion

The respiration rate expressed as the rate of consumption of O_2 (RR_{O_2}, $mL.kg^{-1}.h^{-1}$) and rate of production of CO_2 (RR_{CO_2}, $mL.kg^{-1}.h^{-1}$) of oyster mushrooms throughout the storage period is shown in Fig. 3.1 a and b. Evolution of RQ is presented in Fig. 3.1 c.

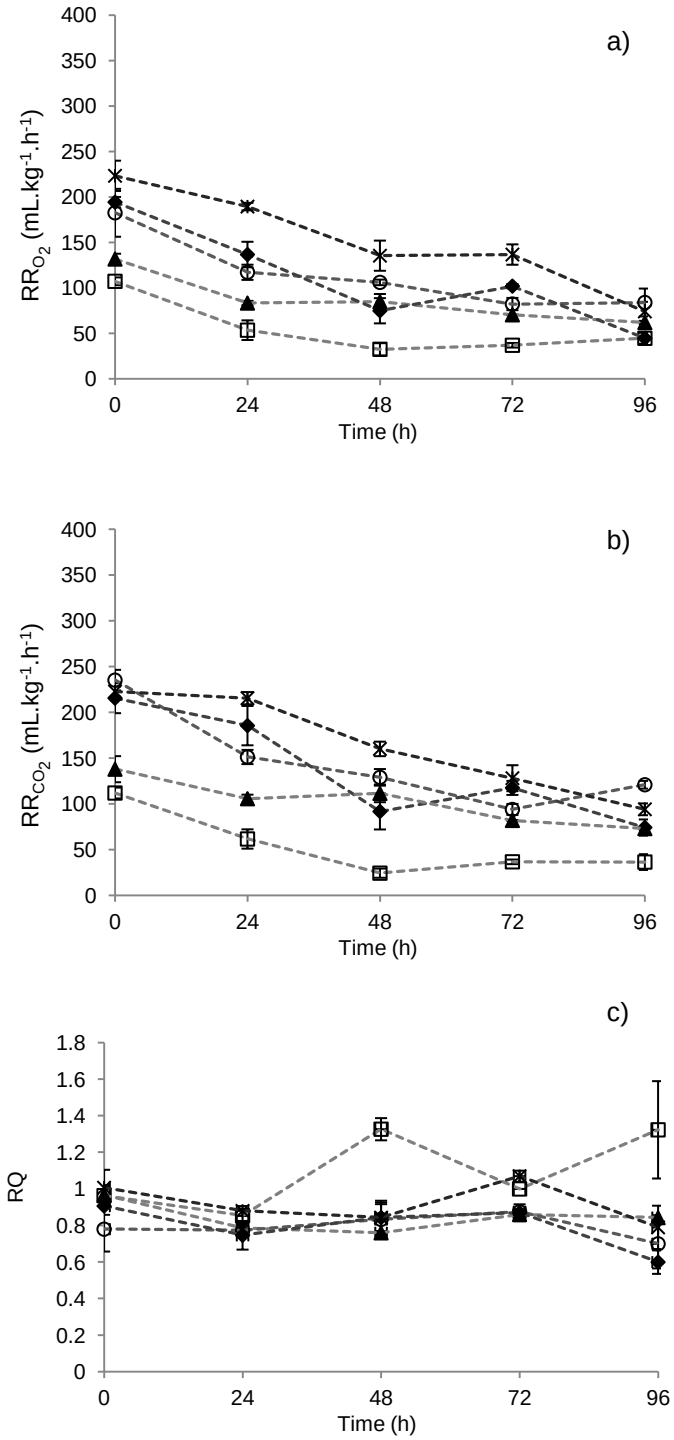

Fig. 3.1 - Changes in (a) RR_{O_2} (mL.kg^{-1}.h^{-1}) (b), RR_{CO_2} (mL.kg^{-1}.h^{-1}) and (c) RQ of *Pleurotus ostreatus* mushrooms over time stored at 2, 6, 10, 14 and 18 °C. Each point represents the mean of three replicates and vertical bars represent the standard error of the mean. □: 2ºC; ▲: 6ºC; ○:10ºC; ◆:14ºC; ✳: 18ºC. Dotted interpolation lines added for easiness in reading.

The initial O_2 consumption rate of oyster mushrooms (t = 0) is high, ranging from 111.93 $mL.kg^{-1}.h^{-1}$ (± 5.93 $mL.kg^{-1}.h^{-1}$) to 235.15 $mL.kg^{-1}.h^{-1}$ (± 11.24 $mL.kg^{-1}.h^{-1}$) in the range of temperatures tested. CO_2 also range between 107.13 and 223.17 $mL.kg^{-1}.h^{-1}$ (± 4.32 and 16.65 $mL.kg^{-1}.h^{-1}$, respectively) at the beginning of storage time.

Temperature had a significant effect on oyster mushroom RR, with values obtained for refrigeration temperatures of 2 and 6 ºC being significantly lower than the higher temperatures tested ($p < 0.05$; Appendix B, Table B.1 and B.2). Regardless of the storage temperature, respiration rate significantly declined over time ($p < 0.05$) and achieved the lowest value at the end of the experimental period (96 h) for all temperatures tested. In fact, for all temperatures, significant decreases ($p < 0.05$) were found for mushrooms stored after 24 and 48 h.

Regarding RQ, results show a mean value of 0.89 (± 0.02). Although no differences were found for RQ values throughout storage time ($p > 0.05$), slightly higher values were found for mushrooms stored at 2 ºC ($p < 0.05$; Appendix B, Table B.3).

Similar results for the respiration profile of mushrooms have been previously found for both oyster mushroom and other mushroom species. It has been suggested that postharvest elapsed time has an effect on respiratory activity of mushrooms and that respiration behaviour after harvest is similar to the respiration profile observed for non climacteric commodities, with a decline of respiration rate over time. In fact, on mushrooms, this metabolic behaviour is related with the maturity of the fruit bodies (Hammond and Nichols, 1975). Other authors suggest that the RR burst observed on mushrooms is a consequence of the harvest process or even related to the physiological response to wounding that occur in minimal processing operations, which implies a physiological response with a consequence increase on RR. Villaescusa and Gil (2003) found a decrease on respiration rate of oyster mushrooms after samples processing, whereas Iqbal *et al*. (2009a) reported that the respiration rate of button mushrooms showed a significant increase 24 h after harvest and then decreased with elapsed time. Moreover, although slicing increased the RR of button mushrooms, the respiration profile obtained for processed samples was similar to the obtained with whole mushrooms and suggested the influence of mushroom maturation on respiration rate.

Temperature is also an environmental factor that significantly increases the respiration of fresh produce. On mushrooms, it has been described that in the range of

temperatures generally found throughout the handling chain (4 to 16 ºC), button mushrooms respiration rate increased by 5 fold (Cliffe-Byrnes and O' Beirne, 2007). For temperatures between 10 and 20 °C, RR increased by 3 fold (Q_{10} of 2.9) (indicating that for each 10 ºC increasing in the storage temperature, RR increases 2.9 times) as reported by Varoquaux *et al.* (1999) for button mushrooms. Iqbal *et al.* (2009a) also reported comparable results for button mushrooms (the rate of O_2 consumption increased from 22 to 102 $mL.kg^{-1}.h^{-1}$ in the temperature range of 0 - 20 °C.

Respiratory quotient found is also in normal range of 0.7 to 1.3 reported in the literature for aerobic respiration (Kader *et al*., 1989), and in accordance with the type of substrate used for respiration rate. Varoquaux *et al*. (1999) reported values of 0.78 and 0.76 for *Agaricus bisporus* stored under ambient air at 10 and 20 ºC, respectively. Also for button mushrooms, Iqbal *et al*. (2009a) reported an RQ of 0.89 for mushrooms stored in air, while Cliffe-Byrnes and O'Beirne (2007) reported that RQ for mushrooms was 0.86.

Another important aspect that worth mentioning is that the respiration rate values obtained for oyster mushrooms under these experimental conditions are higher than values reported by other authors. As an example, RR of sliced button mushrooms were in the range of 105.5 ± 4.0 $mL.kg^{-1}.h^{-1}$ to 133.8 ± 2.0 $mL.kg^{-1}.h^{-1}$, during storage of 57 h at 20 **º**C and 59.2 ± 3.6 $mL.kg^{-1}.h^{-1}$ to 95.2 ± 3.6 $mL.kg^{-1}.h^{-1}$, during storage of 100 h at 12 **º**C (Iqbal *et al*., 2009a).

Considering this aspect, oyster mushrooms are more perishable than other mushrooms species. Moreover, increases in temperature that may occur within postharvest handling chain can sharply increase oyster mushroom respiration rate, affecting quality.

Colour is generally used for the assessment of horticultural commodities quality. For mushrooms, colour is considered one of the most important quality parameter (González-Fandos *et al.*, 2000). In Fig. 3.2, changes on colour parameters of oyster mushrooms at different storage temperatures over the storage period are presented.

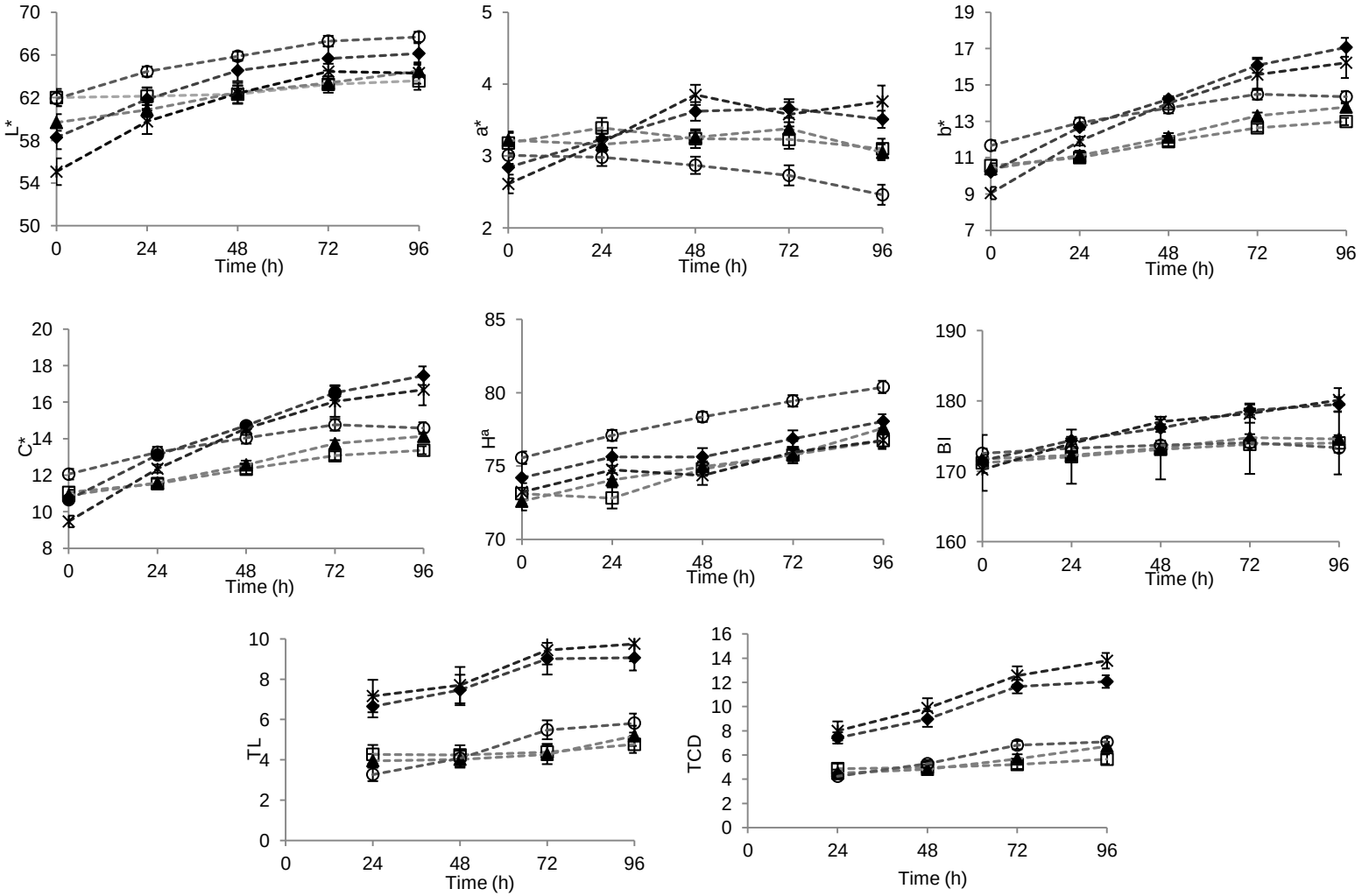

Fig. 3.2 - Evolution of CIE L*a*b* parameters, C*, Hº, browning index, total lightness and total colour differences of *Pleurotus ostreatus* mushrooms over time stored at 2, 6, 10, 14 and 18 °C. Each point represents the mean of three replicates and vertical bars represent SE. □: 2ºC; ▲: 6ºC; ○:10ºC; ◆:14ºC; ✳: 18ºC. Dotted interpolation lines added for easiness in reading.

Lightness of oyster mushroom, as indicated by L* value significantly increases ($p < 0.05$; Appendix B, Table B.4) throughout the storage period for all storage temperatures considered. Temperature also had a significant effect ($p < 0.05$) on oyster mushroom lightness at the end of storage life (Appendix B, Tab B.4). Oyster mushroom stored at 2 ºC did not show a significant increase of L* over storage time but at 6 ºC presented an increase in L* value from 59.67 (± 0.78) at the beginning of the storage period to 64.49 (± 0.59) after 96 h. At the highest temperature of 18 ºC, mushrooms had an increase in L* values from 55.06 (± 1.25) to 64.30 (± 1.00). With respect to the values of a* and b* (that represent redness and yellowness, respectively), ANOVA (Appendix B, Tab B.5 and B.6) shows that both temperature and time had important effect on the evolution of these parameters throughout storage life ($p< 0.05$).

At temperatures of 2 and 6 °C, the a* value remains relatively constant over the storage period, whereas at 14 and 18 ºC a significant increase in redness was observed for oyster mushroom (Appendix B, Tab B.5).

Yellowness (b* parameter) of oyster mushrooms significantly increases ($p < 0.05$) during 96 h for all temperatures tested. Even when stored at 2 ºC, the value of b* increases from 10.56 (± 0.30) to 12.99 (± 0.20) after 96 hours. Increase in b* values, however, were significantly higher for mushrooms stored at storage temperatures of 14 and 18 ºC (Fig. 3.2).

Values of hue angle (Hº) and chroma (C*) also present an increase as storage progresses ($p < 0.05$; Appendix B, Tab B.7 and B.8), with higher increase for mushroom stored at high temperatures. In accordance with the values obtained for a* and b*, an increase in Hº values with increase of storage time and temperature would also be expected.

Concerning the initial colour of the mushroom samples used in the present experiment, that displayed a greyish tone colour (presenting a value of a* near 3 and a b* value close to 10 respectively), all mushroom samples showed a progressive increase in yellowness (increase in b* value), regardless the storage temperature. Mushroom yellowing is a common postharvest phenomenon that occurs mainly due to the action of the enzyme tyrosinase (polyphenol oxidase). Bacterial infections, namely caused by *Pseudomonas tolassii* also cause oyster mushrooms yellowness during

postharvest storage. Colour alterations in mushrooms are considered a major biochemical event. With ageing, mechanical damage or bacterial activity (Beelman *et al*., 1989; Jiang *et al.*, 2010), the enzyme oxidizes mushroom phenolic compounds into brown melanins, which results in brown discoloration (Boekelheide *et al.*, 1979; Soulier *et al.*, 1993; Jolivet *et al.*, 1998). Infections by *Pseudomonas tolaasii* also affects mushroom natural colour causing brown blotch disease in *Agaricus bisporus* mushrooms and yellowing of *Pleurotus ostreatus* (Bessette *et al.*, 1985).

Similar results, pertaining yellowness of oyster mushrooms were previous reported. Villaescusa and Gil (2003) reported similar trend with an increase in yellowing after storage of oyster mushrooms for 7 days at 7 ºC. In previous works, Sapata *et al.*, (2004) and Sapata *et al.* (2009a,b) also reported an increase in yellowing of oyster mushrooms stored under MAP conditions at 4 ºC.

The colour parameters previous analysed can be transformed in browning index, total lightness and total colour changes. Browning index, related with enzymatic browning in foods, increases for all samples, but the rate of change was temperature dependent ($p < 0.05$; Appendix B, Tab B.9). Despite the storage temperature, mushrooms present a slightly increase in BI after 48 h of storage. From this time onwards, a rapid increase in BI was observed for samples stored at 14 and 18 ºC and by the end of storage time, no significant differences were found between these two temperatures. On the other hand, 2, 6 and 10 ºC also presented an increase although at a lower rate than the former temperatures.

Total lightness also increases significantly as storage progresses, with higher increase for higher temperatures ($p < 0.05$; Appendix B, Tab B.10).

Total colour differences significantly increased with time ($p < 0.05$; Appendix B, Tab B.11) for all temperatures, with the exception of oyster mushrooms maintained at 2 ºC. On the contrary to what one would expect, there was an increase in both TL and BI. This increase both colour parameters could be due to some water condensation on the mushroom surface.

Tab. 3.1 shows the changes in soluble solid contents over storage time as affected by storage temperatures of oyster mushrooms.

Tab. 3.1 - Soluble solid content (ºBrix) (mean ± SE) of *Pleurotus ostreatus* mushrooms stored at 2, 6, 10, 14 and 18 ºC for 96 hours.
The p-value for the main effects from two-way ANOVA is 0.001 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	4.33 (±0.58)^{a,A}	4.33 (±0.58)^{a,A,B}	4.33 (±0.00)^{a,A}	4.00 (±0.00)^{a,A}	4.00 (±0.07)^{a,A}	0.737
24	4.00 (±0.00)^{a,A}	4.00 (±0.00)^{a,A}	4.67 (±0.00)^{a,b,A}	5.00 (±0.00)^{b,A}	5.00 (±0.28)^{b,A}	0.001
48	4.33 (±0.58)^{a,b,A}	5.00 (±0.00)^{b,B}	4.00 (±0.00)^{a,A}	5.00 (±0.00)^{b,A}	5.00 (±0.05)^{b,A}	0.002
72	4.67 (±0.58)^{a,A}	4.33 (±0.58)^{a,A,B}	5.00 (±0.00)^{a,A}	5.00 (±0.00)^{a,A}	5.00 (±0.03)^{a,A}	0.171
96	4.33 (±0.58)^{a,A}	5.00 (±0.00)^{a,B}	4.33 (±0.00)^{a,A}	5.00 (±0.00)^{a,A}	5.00 (±0.03)^{a,A}	0.072
p-value (one-way ANOVA)	0.655	0.024	0.147	0.000	0.000	0.001*

a,b – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

The soluble solid content of oyster mushrooms was found to increase with storage time ($p < 0.05$) for storage temperatures of 6, 14 and 18 ºC. For 2 and 10 ºC, no significant differences were found regarding SSC with storage time ($p > 0.05$).

Similar results were found by Jafri *et al.* (2013) in which SSC of *Pleurotus Florida* presented an increase over storage at 4 ºC.

Sapata *et al.* (2004) and Sapata (2005) studied the effect of passive modified atmosphere and reported that SSC of oyster mushrooms did not change when stored at 4 °C for 14 days. Villaescusa and Gil (2003) did not found differences in the soluble solid content of oyster mushrooms stored at low temperatures (between 0 and 7 ºC).

Tao *et al.* (2006) reported an increase (reaching a peak after 5 days of storage) followed by a decline on the SSC of *Agaricus* mushrooms throughout storage. Li *et al.* (2007) reported an increase on the soluble solid content of *A. chaxingu* peaked at either the 8th and 12th storage day, and a concomitant increase on respiration rates on these storage days. After this time, the content decreased gradually.

Tab. 3.2 shows the relative electrolyte leakage of oyster mushrooms stored at different temperatures for 96 hours.

Tab. 3.2 – Relative electrolyte leakage (%) (Mean ± SE) of *Pleurotus ostreatus* mushrooms stored at 2, 6, 10, 14 and 18 ºC for 96 hours.
The p-value for the main effects from two-way ANOVA is 0.001 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	20.06 (± 3.61)^{a,B}	20.06 (± 3.61)^{a,B}	20.06 (± 3.61)^{a,B}	8.06 (± 6.57)^{a,A,B}	8.06 (± 6.57)^{a,A}	0.016
24	12.63 (± 10.09)^{a,A,B}	6.04 (± 5.06)^{a,A}	7.92 (± 1.61)^{a,A}	3.66 (± 1.47)^{a,A}	4.29 (± 1.88)^{a,A}	0.292
48	7.37 (± 4.78)^{a,A,B}	9.73 (± 2.31)^{a,A}	11.65 (± 4.18)^{a,A}	6.73 (± 1.29)^{a,A}	10.53 (± 0.87)^{a,A}	0.312
72	1.75 (± 1.47)^{a,A}	3.68 (± 0.41)^{a,A}	4.57 (± 1.46)^{a,A}	21.62 (± 1.48)^{b,B,C}	23.66 (± 5.33)^{b,B}	0.000
96	4.36 (±1.16)^{a,A}	5.43 (± 1.06)^{a,A}	9.27 (± 0.87)^{a,A}	23.63 (± 9.75)^{b,C}	31.31 (± 2.00)^{b,B}	0.000
p-value (one-way ANOVA)	0.012	0.000	0.000	0.003	0.000	0.000*

a,b – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

For storage temperatures of 14 ºC and 18 ºC, there was a tendency for an increase in REL over time. At the beginning of storage time, oyster mushrooms presented a value of 8.06 % (± 6.57 %) that significantly increased ($p < 0.05$) for 23.63 % (± 9.75 %) and 31.31 % (± 2.00 %), respectively.

This trend suggests an increase of oyster mushroom membrane vulnerability. Similar trends pertaining increases in the vulnerability of the membrane system with temperature throughout storage time have been previous reported by Li *et al*. (2007) for *Agrocybe chaxingu* mushrooms and Tao *et al.* (2006) for button mushrooms.

The mass loss under different storage temperatures over storage time is shown in Fig. 3.3.

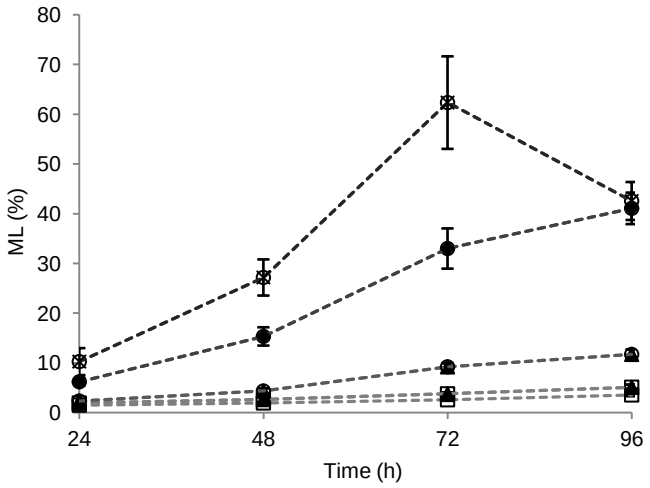

Fig. 3.3 - Evolution of mass loss of *Pleurotus ostreatus*, during storage at 2, 6, 10, 14 and 18 °C. Dots are the mean of ten replicates and vertical bar represent SE. □: 2ºC; ▲: 6ºC; ○:10ºC; ◆:14ºC; ✳: 18ºC . Dotted interpolation lines added for easiness in reading.

The mass loss increased with elapsed time and temperature; both factors have a significant effect on mass loss of ($p < 0.05$; Appendix B, Tab B.12).

Although a loss of 5 – 6 % of fresh mass in fresh horticultural commodities have the potential to decrease commodity commercial value (Paull, 1999; Guillaume *et al.*, 2010), Sveine *et al.* (1967) and Beit-Halachmy and Mannheim (1992) reported that for mushrooms, a fresh mass loss of 2 % is usually enough to cause marked deterioration.

Mass loss in mushrooms has proven to be important in the present experimental conditions. After 24 hours of storage, mushrooms presented important

amount of mass loss. By this storage time, two distinct groups regarding mass loss were found. Mushrooms stored at 2, 6 and 10 ºC lost 1.52 % (± 0.14 %), 1.94 % (± 0.17 %) and 2.34 % (± 0.19) of their fresh mass respectively. For higher temperatures, mushroom mass loss was significantly higher, attaining values of 6.21 % (± 0.79 %) and 10.27 % (± 2.72 %) for 14 and 18 ºC, respectively.

Moreover, considering the two percent limit for the maximum permissible mass loss for mushroom, the results show that even at 2 and 6 °C, this value was attained after 48 h after storage (1.96 % (± 0.16 %) and 2.66 % (± 0.20 %) for mushrooms stored at 2 and 6 ºC, respectively). By the end of the storage period considered (96 h), mushrooms had lost 3.57 % (± 0.26 %) and 5.12 % (± of 0.51 %) at 2 and 6 ºC, respectively, whereas significant higher mass losses were found for mushrooms stored at 14 and 18 ºC. At these storage temperatures, oyster mushrooms presented losses of 41.08 % (± 3.17 %) and 42.56 % (± 3.82 %) of their fresh mass when stored at 14 and 18 ºC, respectively.

The results presented suggest that, for mushrooms, mass loss is an important phenomena, affecting postharvest quality. Mass losses in horticultural commodities include moisture and dry matter by transpiration and respiration (Roy *et al.*, 1995a; Varoquaux *et al.*, 1999). The peculiarity of mushrooms is that, unlike fruits and vegetables that have a complex epidermal structure to restrain postharvest water loss, mushrooms are only protected by a thin epidermal structure. As a consequence, quick and high mass losses are generally found during mushroom postharvest period (Mahajan *et al.*, 2008a). In general, linear mass losses have been reported for mushrooms throughout storage (Burton and Noble, 1993; Varoquaux *et al.*, 1999; Mahajan *et al.*, 2008a; Guillaume *et al.*, 2010). The rate of mass loss is dependent of postharvest treatments, with temperature having an important effect. Burton and Noble (1993) reported a loss of 6 % (w/w) for button mushrooms stored at 18 °C in open punnets.

Roy *et al.* (1995a) stored mushrooms at 12 °C in polyethylene pouches and obtained mass losses in the range of 0.56 % (w/w) for each day of storage. In micro-perforated oriented polypropylene pouches, 0.17 % (w/w) by day for *Agaricus* mushrooms stored at 10 ºC was reported by Varoquaux *et al.* (1999).

When stored with a stretchable film, Guillaume *et al.* (2010) described mass losses of 0.18 % (w/w) day^{-1} at 20 ºC and under near saturation conditions. On day 3,

the author reported a loss of 0.7 % (w/w) of mushroom fresh mass stored in commercial stretchable film at 20 ºC. Jiang *et al.* (2011) reported that mass losses of button mushrooms (dipped in a nitric oxide) and in packages with biorientated polypropylene stored at 4 ºC for 16 days were below 3 %.

Villaescusa and Gil (2003) reported that at temperatures of 0 and 4 ºC and stored under highly humidified air, *Pleurotus* mushrooms lost 0.6 % of their fresh weight. Jayathunge and Illeperuma (2005) also found mass loss of 2.2 % for oyster mushrooms packed (with 3 and 5 g of magnesium oxide) after 12 d of storage at 8 °C and 70 % RH.

On the other hand, for oyster mushrooms (*Pleurotus florida*), Jafri *et al.* (2013) reported weigh losses values as high 21.27 % (± 0.60 %) for mushrooms stored in air at 4 ºC for 10 days.

As expected, mass loss was accentuated by storage temperature, in accordance with the previous results for other mushroom species (Burton and Noble, 1993; Kang *et al*., 2001; Mahajan *et al.*, 2008a). In fact, Escriche *et al.* (2001) reported that, amongst the quality attributes of button mushroom studied, mass loss was the most sensitive to temperature increase, highlighting that proper temperature management may be effective for the control of postharvest mass loss.

3.4. Conclusions

In the present study, quality attributes of oyster mushrooms were studied over a storage period of 96 h. Mushrooms were stored in air at five temperatures (2, 6, 10, 14 and 18 °C) and high relative humidity (85 – 95%). Surface colour, soluble solid content, mass loss, relative electrolyte leakage and respiration rate were assessed daily. Respiration rate of oyster mushrooms was high at the beginning of the experiments and decreased 24 hours after sample processing. Low temperatures significantly reduced respiration rate and consequent deterioration of produce.

Colour parameters of oyster mushrooms was affected by both temperature and storage time. Mushrooms underwent overall yellowness with storage time. Rate of colour changes was higher at higher storage temperatures.

These results clearly show the importance of temperature control on quality parameters of *Pleurotus ostreatus* mushrooms. Overall changes in metabolism and therefore physicochemical properties of oyster mushrooms can be delayed by employing proper temperatures throughout storage. Although overall quality was similar after 96 hours for refrigeration temperatures of 2 and 6 ºC, minimum mass losses were obtained for mushrooms stored at 2 ºC. Therefore, under the conditions tested, 2 °C is ideal temperature for maximum retention of quality attributes and has the potential to increase oyster mushroom shelf life.

Chapter 4: Modelling the influence of time and temperature on the respiration rate of fresh oyster mushrooms

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4.1. Introduction

Respiration is a central process in horticultural produce metabolism. It provides energy and carbon skeletons for cell maintenance during storage. Respiration rate (RR) is a measure of the metabolic activity of fresh horticultural products (Kader and Saltveit, 2003), therefore being an important component for shelf life studies.

As a complement to storage temperature control, other technologies can be used as a barrier to control postharvest quality depreciation. Modified Atmosphere Packaging (MAP) is one of them and it is considered an efficient technology to control postharvest degradation in several commodities (Fonseca *et al.*, 2002a; Kader, 2002).

The amount of product, size of packaging material and the permeability properties of the packaging film are adjusted for optimal packaging once RR is known (Fonseca *et al.*, 2002a; Mangaraj *et al.*, 2009).

When the respiration rates of fresh products does not match the permeability properties of the packaging film, concentrations of CO_2 will build up, leading to anaerobic respiration and ethanol accumulation inside the package, with a consequent development of off-flavours and decay of the produce (Caleb *et al.*, 2012c).

Because temperature is by far the most important factor in RR alterations, it has been investigated and included in most respiration models (Fonseca *et al.*, 2002a; Caleb *et al.*, 2012c). Other factors such as gas composition also determine changes in RR of several commodities (Fonseca *et al.*, 2002a,b; Caleb *et al.*, 2012c). Time elapsed from harvest can also affect RR of produce. In fact, for some commodities, respiration rate is not constant during shelf life. Since changes in RR may have a major impact in the gas composition achieved in MAP, postharvest respiration behaviour and the rate of change is also important to specify packaging conditions (Fonseca *et al.*, 2002a; Yang *et al*., 1988; Kim *et al.*, 2004; Uchino *et al.*, 2004; Rocculi *et al.*, 2006; Fonseca *et al.*, 2002a; Iqbal *et al.*, 2009b; Caleb *et al.*, 2012a).

Reported as one of the most perishable foods, mushrooms are expected to maintain their quality under MAP conditions (Burton *et al.*, 1987; Burton and Twining,

1989; López-Briones *et al.*, 1992, López-Briones *et al.*, 1993, Roy *et al.*, 1995a; Tano *et al.*, 1999).

While refrigeration retains quality conditions of fresh mushrooms (Murr and Morris, 1975; López-Briones *et al.*, 1992), MAP has been recognised as a simple, economical and effective technology in postharvest preservation of several types of mushrooms (Kader *et al.*, 1989; Kim *et al.*, 2006).

A vast amount of information related to the postharvest preservation of *Agaricus bisporus* is known, including RR modelling (Iqbal *et al.*, 2009a,b; Li *et al*., 2009). Nevertheless, information about storage conditions for other species is still scarce. Despite being one of the most commercially important edible mushrooms throughout the world (Smith *et al*., 2002; Sánchez, 2010), studies on the predictive modelling of the RR for MAP application on oyster mushrooms are lacking, and only a few studies on MAP were found for these species (Villaescusa and Gil, 2003; Sapata, 2005; Sapata *et al*., 2007; Sapata *et al.*, 2009a,b; Ramos *et al.*, 2011).

Therefore, the objectives pursued in this part of the study were (**i**) to investigate the effect of temperature and storage time on RR of fresh *Pleurotus*, and (**ii**) to develop a predictive model relating RR to temperature and storage time.

4.2. Material and methods

4.2.1. *Sample preparation*

Fresh mushrooms (*Pleurotus ostreatus*) cluster at commercial maturity stage were collected during the early morning from a producer in Trás-os-Montes, Portugal, transported to the laboratory in the early afternoon and stored at 2 ºC. Oyster mushrooms carpophores were carefully separated from the cluster using a sharp knife, and the ones with closer carpophores were selected and stored at 2 ºC approximately for one hour until the beginning of the experiment.

4.2.2. *Respiration rate measurement*

Respiration rate was measured using the closed system methodology as reported and described in Chapter 3, § 3.2.2.1.

Samples of separated carpophores were weighted (approximately 0.15 kg for each sample), placed in glass jars (volume of 1.9×10^{-3} m^3) used as respirometers and stored in temperature control chambers (Monte Branco Refrigerators, Ltd, Oporto, Portugal at test temperatures (2, 6, 14 and 18 ° C ± 0.5 ºC), and in a domestic type refrigerator (Bosch, Germany) at 10 ºC ± 2 ºC

Two sets of three jars were prepared for each of the five test temperatures. On an alternate procedure, each set was taken for respiration rate determination at every eight hours interval. At each sampling time, three selected jars were closed tightly, sealed with petroleum jelly and parafilm and hermeticity was verified. After finishing the measurements, jars were opened to allow air renewal, remaining opened overnight. To avoid excessive moisture loss, jar lids were kept slightly tilted over the jar tops.

For each sampling time, gas composition (volumetric fraction of O_2 and CO_2) of the headspace of each jar was monitored by withdrawing a 2 mL sample after inserting the analyser needle through a rubber septum on the jar lid. This was monitored over time using a gas analyzer (Checkmate, 9900, PBI Dansensor, Denmark O_2 /CO_2 gas analyser with an accuracy of 0.5 %). The interval of time used to calculate respiration rate was dependent on temperature, with up to four measurements by jar, varying from 30 minutes for 18 ºC to two hours for 2 ºC.

Respiration rate for each sample and for each measuring time in terms of CO_2 generation and O_2 consumption was determined from the slope of the fitted linear equation according to Eq. 3.1 and 3.2. RQ was calculated according with Eq. 3.4. (Chapter 3, § 3.2.2.1).

The determination of respiration rate was performed in triplicate for each combination storage time- temperature.

4.2.3. *Data analysis*

The constants of the model developed to describe the influence of time and temperature on respiration rate were estimated by fitting the model to the experimental data by nonlinear regression using the IBM SPSS Statistics Version 20.0 (IBM, 2011).

Model parameter estimates were obtained using the least square method that minimizes the sum of squares of the residuals between the experimental and the predicted data (Seber and Wild, 2003), using the Levenberg-Marquardt algorithm available in the software (IBM, 2011).

Respiration rate data, obtained under different temperature was analysed with a two-step method for non-linear regression. Through the two-step method, in a first step, parameters of the individual model, describing the time effect on oyster mushrooms respiration rate were determined individually for each temperature. Subsequently, the parameters estimated from the individual model were used as the initial values to obtain overall model parameters. This methodology has the advantage to allow the description of both the primary and global models and optimizes the quality of the fit (Cohen and Saguy, 1985; Haralampu *et al*., 1985; Cunha *et al*., 2006).

Unless otherwise stated, all results are presented as mean ± standard error of mean.

4.3. Results and discussion

4.3.1. *Respiration rate of fresh oyster mushrooms at ambient air*

Storage time is an important factor affecting respiration rate of fresh produce and thus it has to be take into account while developing packaging system (Fonseca *et al*., 2002a,b).

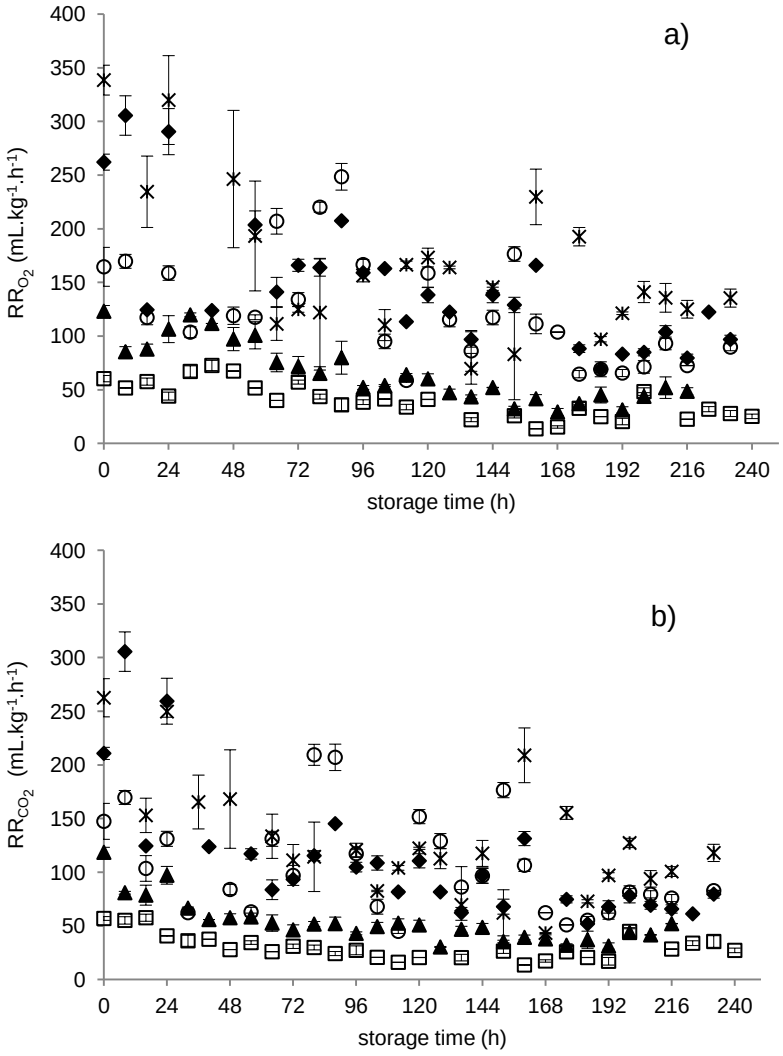

Fig. 4.1 - Evolution of a) RR_{O_2} and b) RR_{CO_2} (mL.kg^{-1}.h^{-1}) of oyster mushrooms stored at various constant temperatures under ambient air. Symbols represent the mean of 3 replicates and bars represent the standard error of the mean (□- 2 ºC; ▲- 6 ºC; ○-10 ºC; ◆-14 ºC; ✳- 18 ºC).

The O_2 consumption and CO_2 production rates of oyster mushrooms at the beginning of the storage experiment was very high and decreases with time for all temperatures (Fig. 4.1 a) and b)). These high initial respiration rates were probably caused by stress during the harvesting process just before storage or related with mushroom maturity (Villaescusa and Gil, 2003; Iqbal *et al*., 2009a).

The initial O_2 consumption rate of oyster mushrooms ranged from 60.30 $mL.kg^{-1}.h^{-1}$ (± 2.94 $mL.kg^{-1}.h^{-1}$) to values as high as 338.53 $mL.kg^{-1}.h^{-1}$ (± 13.88 $mL.kg^{-1}.h^{-1}$) for temperatures from 2 to 18 ºC, respectively.

The O_2 consumption rate of mushrooms decreased over the first three to four days of storage and then, remained relatively stable from that time onwards for all temperatures tested. Similar trends were observed by Villaescusa and Gil (2003) in which *Pleurotus* mushrooms also had high respiration rates at the beginning of the storage time at 0, 4 and 7 ºC, with a sharply decrease as storage progresses.

Respiration quotient (RQ) is measured as the ratio of CO_2 evolved and O_2 consumed and is a useful indicator of the respiration process occurring and of the substrate used for respiration.

RQ values obtained in the present study are within the normal range of 0.7 to 1.3 reported in the literature for aerobic respiration (Kader *et al*., 1989). The results are in accordance with previous results for RQ of mushrooms (Varoquaux *et al*., 1999; Cliffe-Byrnes and O'Beirne, 2007; Iqbal *et al*. 2009a).

No significant trend ($p > 0.05$) was found for the effect of both time and temperature on the respiratory quotient (RQ). Therefore, the mean RQ of 0.83 (± 0.09) was used to calculate an estimated value for RR_{O_2} ($RR^*_{O_2}$), according Eq. 4.1.

$$RR^*_{O_2} = \frac{RR_{CO_2}}{0.83} \qquad \text{Eq. 4.1}$$

where, $RR^*_{O_2}$ is the estimated O_2 consumption rate ($mL.kg^{-1}.h^{-1}$) and RR_{CO_2} is the experimental values obtained for CO_2 production ($mL.kg^{-1}.h^{-1}$).

O_2 consumption rate of oyster mushrooms used to access the effect of time and temperature on respiration rate were obtained considering the mean of the experimental values for RR_{O_2} and the estimated value ($RR^*_{O_2}$) obtained with Eq. 4.1, according Eq. 4.2 (Iqbal *et al.*, 2005).

$$RR = \frac{RR_{O_2} + RR^*_{O_2}}{2} \quad \text{Eq. 4.2}$$

where, RR is the estimated O_2 consumption rate ($mL.kg^{-1}.h^{-1}$).

4.3.2. *Effect of time on respiration rate*

According to experimental data obtained, the effect of time on oyster mushroom RR was described by a first order decay model (Eq. 4.3).

$$\frac{RR - RR_{eq}}{RR_0 - RR_{eq}} = \exp^{(-k \times t)} \quad \text{Eq. 4.3}$$

where, RR_{eq} and RR_0 are the respiration rates at the equilibrium and time 0, respectively ($mL.kg^{-1}.h^{-1}$), k is the kinetic parameter (h^{-1}) and t is time (h).

Model parameters (RR_{eq} RR_0 and k) of the Equation 4.3 were estimated from experimental data obtained for each storage temperature using nonlinear regression.

Since inconsistency between experimental and predicted data at 10 ºC was found, respiration data at this temperature was excluded from the subsequent analysis. This may be explained as the laboratory only had four temperature control chambers with the fifth experiment (10 ºC) being performed with a refrigerator with a weaker temperature control. Authors have chosen this approach to warrant that all experimental data were obtained from the same batch of mushrooms, avoiding higher produce variability. Using different batches would impart a larger error, as can be seen from Aguirre *et al.* (2008).

Estimated constants, statistical data and the quality of the fit of the first order decay model for each storage temperature are summarized in Tab. 4.1

Tab. 4.1 - Constant estimates (± SE) of the first order decay model describing the effect of time on respiration rate of fresh oyster mushrooms, according Eq. 4.3.

Temp.	RR_0 (mL.kg^{-1}.h^{-1})	RR_{eq} (mL.kg^{-1}.h^{-1})	k (h^{-1})	R^2
2	67.9 (± 5.5)	26.8 (± 3.8)	1.63×10^{-2} ($\pm 5.6\times10^{-3}$)	0.696
6	121.0 (± 6.3)	37.0 (± 6.7)	1.33×10^{-2} ($\pm 3.3\times10^{-3}$)	0.868
14	277.7 (± 27.2)	84.4 (± 23.2)	1.44×10^{-2} ($\pm 5.9\times10^{-3}$)	0.662
18	330.9 (± 38.1)	130.4 (± 14.2)	2.58×10^{-2} ($\pm 9.9\times10^{-3}$)	0.597

The fit of the individual model to the experimental respiration rate at each temperature ranged from reasonable to good, as indicated by the coefficient of determination (R^2) between 0.597 and 0.868, confirming an acceptable agreement between predicted and experimental values.

Fig 4.2 shows the fit of the individual model to the experimental respiration rate obtained at 2ºC and 18 ºC.

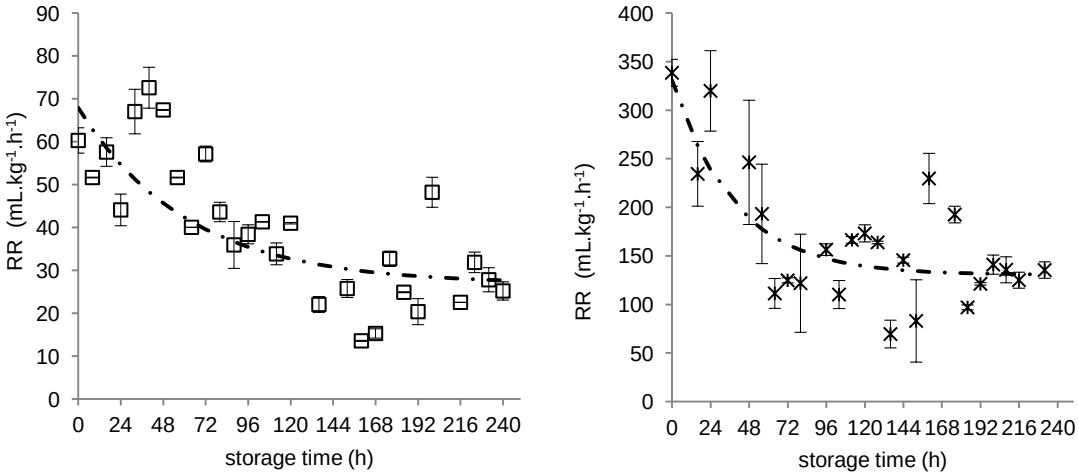

Fig. 4.2 – Respiration rate of oyster mushrooms stored under ambient air at 2ºC (□) and 18ºC (∗). The symbols represent the respiration rate data, vertical bars represent standard deviation and the dot and slash line represents the individual of the individual model according with Eq. 4.3.

As observed in the graph showing the fit of the first order decay model to the experimental data point for the lowest and highest temperature tested, a good agreement exists between the predicted and experimental values.

4.3.3. *Effect of temperature on respiration rate*

Temperature has been identified as the most important external factor influencing respiration rate of horticultural commodities. Biological reactions generally increase two to three-fold for every 10 ºC rise in temperature within the range of temperatures normally encountered in the distribution and marketing chain (Zagory and Kader, 1988).

The effect of temperature on kinetic parameters of the first order decay model (RR_0, RR_{eq} and k) was individually explained using the reparameterised Arrhenius equation (Eqs. 4.4, 4.5 and 4.6) as follows (Fonseca *et al*., 2002a; Uchino *et al*., 2004; Nei *et al*., 2006; Iqbal *et al*., 2008; Waghmare *et al*., 2013).

$$RR_0 = RR_{0,ref} \times \exp\left[-\frac{E_{aRR_0}}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad \text{Eq. 4.4}$$

$$RR_{eq} = RR_{eq,ref} \times \exp\left[-\frac{E_{aRR_{eq}}}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad \text{Eq. 4.5}$$

$$k = k_{ref} \times \exp\left[-\frac{Ea_T}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad \text{Eq. 4.6}$$

where, $RR_{0,ref}$, $RR_{eq,ref}$ and k_{ref} are the initial respiration rate, respiration rate at equilibrium and kinetic parameter at reference temperature (T_{ref}), respectively; Ea_{RR_0}, $Ea_{RR_{eq}}$ and Ea_k are the activation energies (kJ. mol^{-1}) for the initial and O_2 consumption rate at equilibrium and kinetic parameter, respectively; R is the universal gas constant (8.314×10^{-3} $kJ.mol^{-1}.K^{-1}$). Reference temperature was obtained by taking the average of the experimental temperature ranges (Tref = 10 ºC), as suggested in literature (Nelson, 1983; Van Boekel, 1996).

Dependence of temperature on the first order decay model parameters is presented in Fig. 4.3.

This reparameterisation of the Arrhenius equation was applied to improve regression convergence and to diminish model parameter colinearity (Nelson, 1983; Cunha *et al*., 2006).

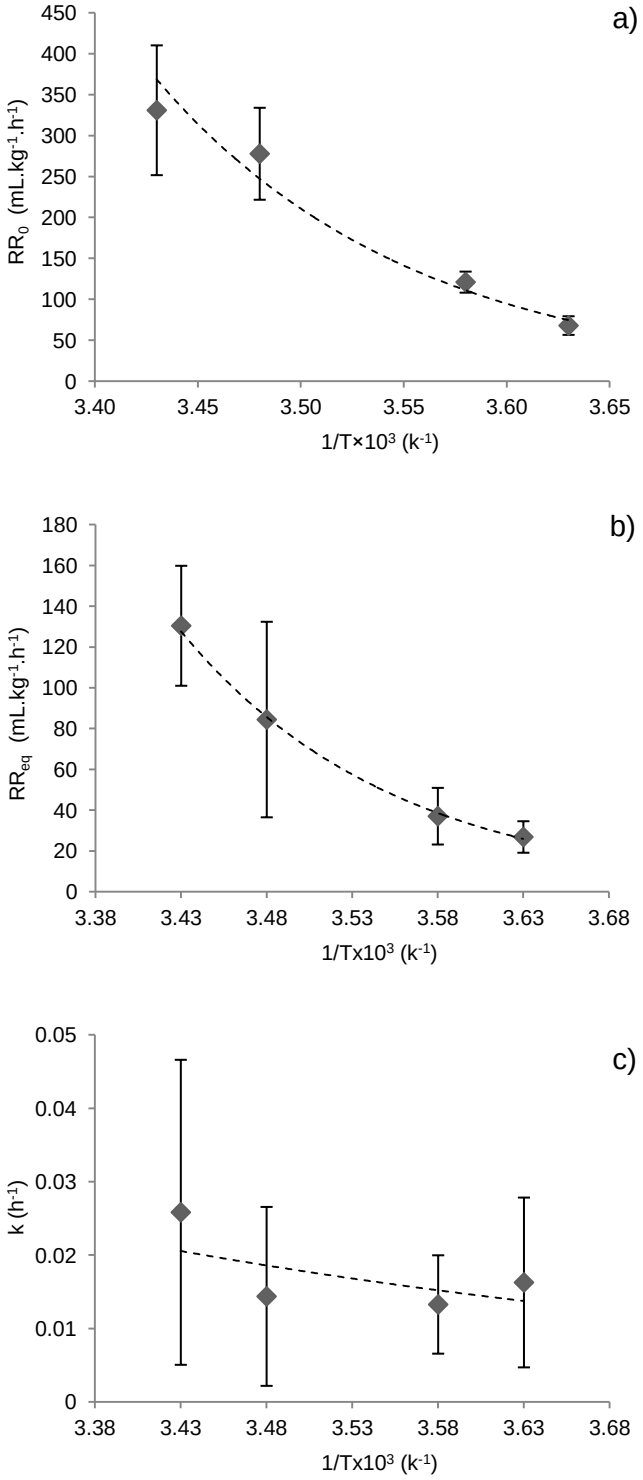

Fig. 4.3 - Dependence on temperature of the first order decay model parameters: a) $RR_{0,\,ref}$ ($mL.kg^{-1}.h^{-1}$); b) $RR_{eq,ref}$ ($mL.kg^{-1}.h^{-1}$) and c) k_{ref} (h^{-1}). The dots represent the individual estimates and the bars represent the asymptotic 95 % confidence intervals (Cunha *et al*., 2006) and the lines represent the fit of the model, according with Eqs. 4.4, 4.5 and 4.6, respectively.

As it may be observed in Fig. 4.3, the Arrhenius model adequately describes the effect of temperature on each of the parameters of the first order decay model.

Respiration rate of oyster mushrooms decreases with time, until equilibrium is attained. The relationship between RR_{O_2} and RR_{eq}, estimated at each of the temperatures used for the model, is shown Fig. 4.4.

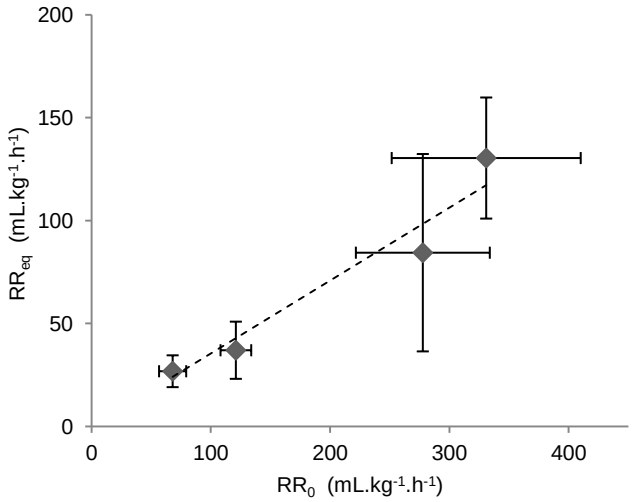

Fig. 4.4 - Relationship between individual parameter estimates of the initial respiration rate (RR_0) and respiration rate at equilibrium (RR_{eq}) at each temperatures used for model fitting (2, 6, 14 and 18 ºC). Bars represent the asymptotic 95 % individual confidence intervals. Dotted line represents the linear fit between RR_0 and RR_{eq} (R^2=0.940).

For each temperature, O_2 consumption rate after processing the samples and O_2 consumption rate at equilibrium shows a linear decrease described by

$$RR_{eq}=\tau \times RR_0 \qquad \text{Eq. 4.7}$$

where τ is the constant ratio between respiration rate at equilibrium and the initial RR (dimensionless).

4.3.4. *Overall model*

The Arrhenius relationship (Eqs. 4.4, 4.5 and 4.6) was then included in Eq. 4.5 to explain the variation of O_2 consumption rate of oyster mushroom with storage temperature, yielding:

$$RR=RR_{eq,ref}\times\exp\left[-\frac{Ea_{RR_{eq}}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]+\left\{RR_{0,ref}\times\exp\left[-\frac{Ea_{RR_0}}{R}\times\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]-RR_{eq,ref}\times\exp\left[-\frac{Ea_{RR_{eq}}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]\right\}$$

$$\times\exp\left[-k_{ref}\times\exp\left[-\frac{Ea_k}{R}\times\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]\times t\right] \quad \text{Eq. 4.8}$$

Model (Eq. 4.8) was fitted to all experimental data in its logarithm form to avoid heterocedasticity of residuals (Draper and Smith, 1981).

Resulting model parameter estimates showed that the activation energies related to O_2 consumption rate (Ea_{RR_0} (65.37 ± 8.47 kJ.mol^{-1}) and $Ea_{RR_{eq}}$ (64.83 ± 6.82 kJ.mol^{-1})) were not significantly different, therefore Ea_{RR_0} and $Ea_{RR_{eq}}$ were replaced by a single activation energy value (Ea_{RR}). A similar approach was used for other commodities like mushrooms (Iqbal *et al.*, 2005) and apples (Torrieri *et al*., 2009). Taking the linear relationship between $RR_{0,ref}$ and RR_{eq} (Eq. 4.7) into Eq. 4.1 and considering Eq. 4.4, Eq. 4.5 and Eq. 4.6, the overall model may be rewritten as (Eq. 4.9):

$$RR=RR_{0,ref}\times\exp\left[-\frac{Ea_{RR}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]\times\left\{\tau+(1-\tau)\times\exp\left(-k_{ref}\times\exp\left[-\frac{Ea_k}{R}\times\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]\times t\right)\right\} \quad \text{Eq. 4.9}$$

Model parameters estimates and relevant statistical data are summarized in Tab. 4.2. Correlation between parameters of the model is presented in Tab. 4.3.

Tab. 4.2 – Model parameter estimates of the global model, obtained to predict the effect of time and temperature on oyster mushrooms respiration rate, according with Eq. 4.9.

Parameter	Estimate	SE	95% Confidence Interval	
			Lower Bound	Upper Bound
$RR_{0,ref}$ (mL.kg^{-1}.h^{-1})	170.83	13.2	144.57	197.08
Ea_k (kJ. mol^{-1})	8.0	20.1	-31.94	47.87
$k_{ref} \times 10^{-3}$ (h^{-1})	18.4	3.5	11.5	25.4
Ea_{RR} (kJ. mol^{-1})	64.89	6.2	52.63	77.15
τ (dimensionless)	0.35	0.03	0.29	0.40

Tab. 4.3 – Correlations of model parameters estimates obtained to predict the effect of time and temperature on fresh oyster mushrooms O_2 consumption rate, according with Eq. 4.9.

	$RR_{0,ref}$	Ea_k	K_{ref}	Ea_{RR}	τ
$RR_{0,ref}$	1.00				
Ea_k	0.04	1.00			
K_{ref}	0.70	0.00	1.00		
Ea_{RR}	0.05	0.91	-0.04	1.00	
τ	-0.66	-0.10	-0.02	-0.15	1.00

Overall model fits the data well, showing a R^2 =0.879. Moreover, the correlation between parameters is small, with the exception for the correlation between Ea_k and Ea_{RR}. Nevertheless, this value is not high enough to hinder the predictability of the model (Cunha *et al*., 2006).

Fig 4.5 shows the fit of both the individual (Eq. 4.3) and the global model (Eq. 4.9) to the experimental O_2 consumption rate at each temperature tested.

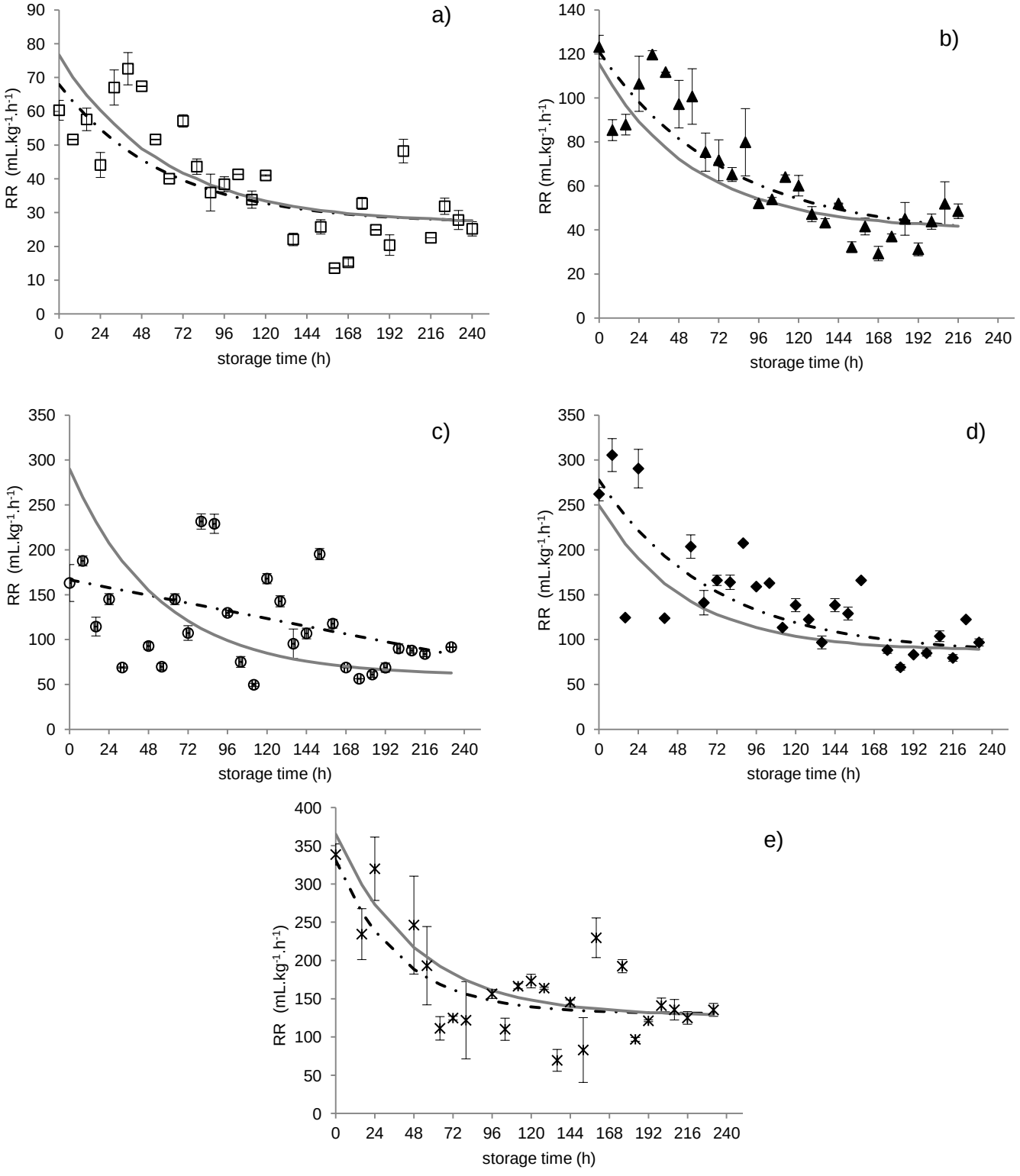

Fig. 4.5 –Respiration rate of oyster mushrooms at different constant temperatures under ambient air. Symbols depict the O_2 consumption rates data as calculated with Eq. 4.2 and vertical bars represent standard error for: a) 2 ºC; b) 6 ºC; c) 10 ºC; d) 14 ºC; e) 18 ºC). Lines (- - - and ---) represent the fit of the individual (Eq. 4.3) and global model (Eq. 4.9), respectively. Long lash dot line on Figure 5c represents the predicted O_2 consumption rate with time at 10 ºC according to the global model (Eq. 4.9).

Both results for model fitting are very close as depicted on Figure 4.5, indicating the quality of the overall model to predict O_2 consumption rate of fresh oyster mushrooms. Moreover, when using Eq. 4.9 to predict O_2 consumption rate at 10 ºC, one may see a reasonable prediction despite the experimental errors previous referred for this temperature.

Furthermore, Fig. 4.6 shows that the O_2 consumption rate model fits the experimental data well as seen by the good agreement between predicted and experimental values.

The residuals obtained presented a mean of 0.00 (SD of 0.24 for n = 107) and a normal distribution ($p > 0.05$), according the Kolmogorov-Smirnov test.

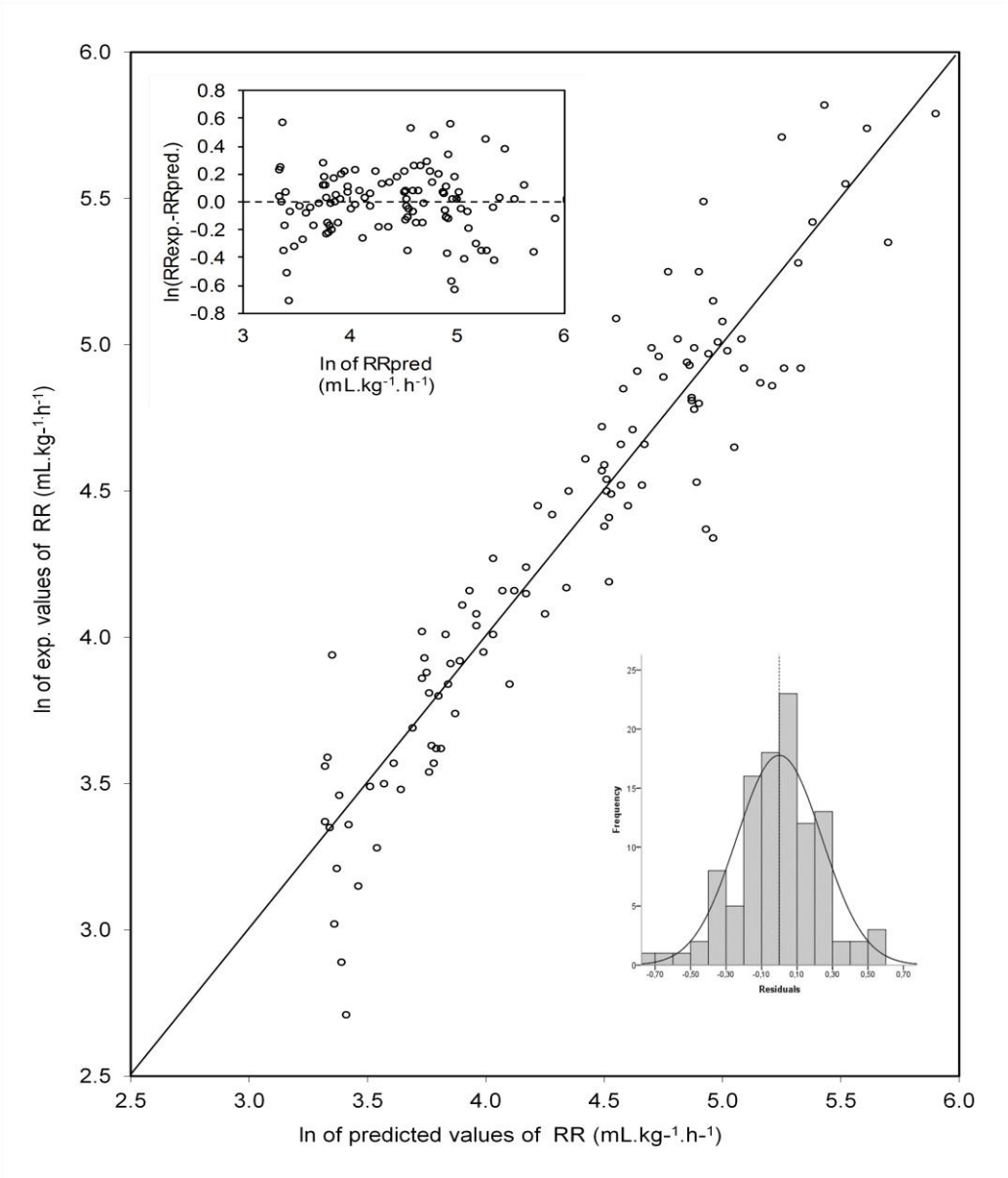

Fig. 4.6 - Relationship between experimental and predicted O_2 consumption rates using the first decay model and assuming an Arrhenius type dependence of the model parameters on temperature (global model – Eq. 4.9). Enclosed figures represent the residual versus predicted values (top corner) and the frequency distribution of residuals, with ¯ representing the expected normal distribution (bottom corner).

In order to estimate the respiration rate of fresh produce during postharvest handling chain, several mathematical models have been developed for a wide range of fresh and fresh-cut commodities. Those models have been reviewed by Fonseca *et al.* (2002a) and Caleb *et al.* (2012c). Regarding temperature dependence on RR, most of the developed models are based on Arrhenius or exponential effects and the values of

the activation energy can be compared. Exama *et al.* (1993) reported activation energies in the range of 29.0 to 92.9 kJ.mol^{-1} for the respiration rate of horticultural commodities stored in air. For mushrooms (*Agaricus bisporus*) stored in air, Exama *et al.* (1993) reported an Ea of 65.5 kJ.mol^{-1}. For Agaricus mushrooms, a significant effect of temperature on mushrooms respiration rate was found (Iqbal *et al.*, 2009). According to the authors, an activation energy of 54.38 (± 1.07) kJ.mol^{-1} and 56.04 (± 1.44) kJ.mol^{-1} was obtained for O_2 depletion and CO_2 evolution in the range of storage temperatures of 4 ºC to 20 ºC. Varoquaux *et al*. (1999) stated that the respiration rate of mushroom (*Agaricus bisporus* Lange, strain X25) under air at temperatures between 10 ºC and 20 ºC, also followed an Arrhenius' law with apparent activation energy of 43.40 kJ mol^{-1}. Although some information on RR parameters can be found for *Agaricus bisporus* mushroom, for other mushroom genus, no information is available. The values of the activation energy for the respiration rate obtained in the present study (67.27 kJ.mol^{-1} ± 6.93 kJ.mol^{-1}) are comparable to the values reported for horticultural commodities and higher when compared to the values obtained for Agaricus mushrooms.

In the particular case of the time effect on respiration rate, several models have been used. Time effect on respiration rate has been modelled using the Weibull model (and the Arrhenius for the temperature effect) in the case of galega kale (Fonseca *et al.*, 2005) and diced papaya (Waghmare *et al.*, 2014). Kinetic models (Waghmare *et al.*, 2013) have been suggested as good alternatives to describe the effect of time on the respiration rate of fresh commodities.

The values of the activation energy for the respiration rate obtained in the present study (67.27 ± 6.93 kJ.mol^{-1}) are comparable to the values reported for horticultural commodities. Exama *et al*. (1993) reported activation energies in the range of 29.0 to 92.9 kJ.mol^{-1} for the respiration rate of horticultural commodities stored in air. For mushrooms (*Agaricus bisporus*) stored in air, Exama *et al*. (1993) reported an Ea of 65.5 kJ.mol^{-1}.

4.4. Conclusions

The influence of storage time and temperature on respiration rate of oyster mushrooms was assessed by storing oyster mushrooms at 2, 6, 10, 14 and 18 $kJ.mol^{-1}$ under ambient atmosphere and measuring respiration rate with eight hour intervals for 240 h. Results are relevant to show the influence of temperature and storage time on mushroom respiration rate. Respiration rate of oyster mushroom decreased over time, following a first order kinetics model. Temperature dependence was explained by an Arrhenius type equation and the inclusion of a parameter describing the rate decrease of the respiration rate after sample processing and respiration at equilibrium fitted well to the experimental data. Furthermore, the predictive model developed is an important tool for the choice of the appropriate packaging system for fresh oyster mushrooms.

Chapter 5: Influence of gas composition on the respiration rate of fresh oyster mushrooms

5.1. Introduction

Modified atmosphere packaging (MAP), containing lower partial pressures of oxygen and higher levels of carbon dioxide than atmospheric air, is a technology successfully applied to many horticultural products. MAP have been shown to delay maturation, softening, colour degradation and to reduce microbiological spoilage in a broad range of fresh and fresh-cut horticultural products (Carlin *et al.*, 1990; Beaudry, 2000; Al-Ati and Hotchkiss, 2002; Roy *et al.*, 1995a; López-Briones *et al.*, 1992; Manolopoulou *et al.*, 2012; Caleb *et al.*, 2012a,b). The atmospheric modification occurring inside the package depends on the permeability of the film used and on the commodity respiration rate, among other factors (Fonseca *et al.*, 2002a).

Atmospheric composition is a factor that potentially affects respiration rate of horticultural commodities (Kader, 2002; Fonseca *et al.*, 2002a; Sandhya, 2010). The control of respiration rate brings advantages in maintaining products quality but implies a thorough knowledge of the effect of both O_2 and CO_2 on the respiration rate of the commodity.

Mushrooms, in general, have high respiration rates and deteriorate easily, causing problems in maintaining its freshness (Ares *et al.*, 2007; Antmann *et al.*, 2008). Therefore, a suitable packaging technology to retain freshness and increase the shelf life is important.

MAP is effective for mushrooms shelf life extension as reported for button mushrooms (Roy *et al.*, 1995a; Kim *et al.*, 2006) or oyster mushroom (Sapata *et al.*, 2004; Sapata, 2005). Nevertheless, selection of the optimal combination of O_2 and CO_2 becomes difficult, since available studies for mushrooms in MAP suggest very different conditions for optimal maintenance of quality of mushrooms (see section 2.4.2.1). Furthermore discrepancies are found when the effect of modified atmosphere on mushroom respiration rate is evaluated. The aim of this work was: (i) to evaluate the effect of different combinations of O_2 and CO_2 on oyster mushroom respiration rate and (ii) to select an optimum gas composition for MAP.

5.2. Material and methods

5.2.1. *Sample preparation and experimental procedure*

Fresh oyster mushrooms harvested in the early morning were collected from a local producer in Trás-os-Montes region. Mushrooms were transported to the laboratory in the early afternoon and stored at 2 ºC for cooling.

Mushrooms were then separated from the cluster and maintained refrigerated at 2 ºC in temperature control chambers (Monte Branco Refrigerators, Ltd, Oporto, Portugal). In the next day (24 hours after samples processing), mushrooms were separated for the jars used to measure respiration rate and flushed with the desired mixture. The concentrations of O_2 and CO_2 were selected according to the available literature. Six atmosphere were tested, according to a full factorial design, with 2 levels of O_2 (2 and 5 % v/v) and 3 levels of CO_2 (5, 10 or 20 % v/v). Ambient air was used as a control.

The determination of respiration rate for each storage condition and at 2 ºC was evaluated daily, in triplicate, for the next 3 days (t= 1, 2, 3 and 4 days after cluster cutting).

5.2.2. *Respiration rate measurement*

The O_2 consumption and CO_2 production rates were determined using a closed system (Chapter 5, §5.2.3). Mushroom samples were weighted (approximately 0.15 kg for each sample) and placed in glass jars (volume of 1.9×10^{-3} m^3).

Desired concentration of O_2 and CO_2 were mixed using a gas mixer (MAP Mix 9000, PBI-Dansensor), using N_2 as balance. Glass jars were tightly sealed and connected to a distribution jar with distilled water on the bottom. Gas mixture was bubbled through water to humidify the air introduced in jars containing mushrooms (Iqbal *et al.*, 2009b). Samples jars' flushing was made with constant flow, for 30 minutes to ensure that the ambient atmosphere was completely replaced inside the jars. After that period, inlet and outlet valves were closed and the atmosphere

composition was monitored, as previously described in Chapter 3, §3.2.3. O_2 consumption and CO_2 production rates were determined by Eqs. 3.1 and 3.2.

5.2.3. *Data analysis*

One-way ANOVA was performed for each mixture to study the effect of time on oyster mushrooms respiration rate. Since no significant difference ($p < 0.05$) was found for the effect of time on respiration rate for each of the gas mixtures tested, the four experimental times were analysed together. The effect of gas mixture on oyster mushroom RR was also performed throughout one-way ANOVA, using IBM SPSS Statistics Version 20.0 (IBM, 2011). Multiple comparisons were performed using the Tukey´s test. All tests were applied at a 95 % confidence interval, except if stated otherwise.

A Response Surface modelling (RSM) was used to test the interaction of O_2 and CO_2 on oyster mushroom respiration rate, using *Statistica* software (Version 12, Stat soft, Inc., Tulsa, Oklahoma, USA).

5.3. Results and discussion

As previously demonstrated (see chapter 4), the respiration rate of mushroom is not constant during storage. To minimize this effect, measurements of respiration rate were only started 24 h after processing, allowing for the major decrease to occur. Results for the respiration rate for each experimental time are shown Appendix C, Table C.1. Since no significant difference ($p > 0.05$) was found for the effect of time on respiration rate for each of the gas mixtures tested, the four experimental times were analysed together.

The mean RR of oyster mushrooms stored under air at 2°C at different O_2 and CO_2 concentration is shown in Fig. 5.1.

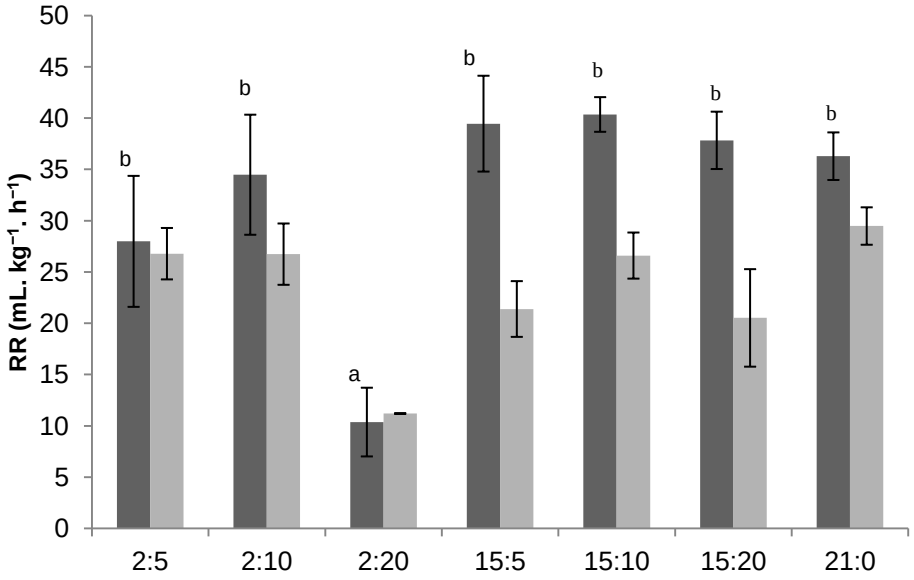

Fig. 0.1 – Oxygen consumption ( ) and CO_2 production rate ( ) ($mL.kg^{-1}.h^{-1}$) of oyster mushrooms stored in different O_2 and CO_2 concentrations at 2 °C. Vertical bars represent SE.
a,b – homogeneous groups for oxygen consumption according to the Tukey's test, at a 95 % CI.

Respiration rate of oyster mushrooms was significantly ($p \leq 0.05$) affected by the low O_2 and high CO_2 levels tested. According to the ANOVA (Appendix C, Table C.2), modified atmosphere, for the levels under evaluation, had a significant effect ($p<0.05$) on the respiration rate based on overall O_2 consumption and CO_2 consumption. Mushrooms stored in air at 2 ºC had a mean respiration of 36.28 $mL.kg^{-1}.h^{-1}$ (± 2.32 $mL.kg^{-1}.h^{-1}$) and 29.48 $mL.kg^{-1}.h^{-1}$ (± 1.89 $mL.kg^{-1}.h^{-1}$) for O_2 consumption and CO_2 production, respectively. This value was higher than the previous values found in the first two experiments (in chapter 3, §3.3.1, value obtained at 2 ºC after 24 h of storage was 61.76 $mL.kg^{-1}.h^{-1}$ (± 10.54 $mL.kg^{-1}.h^{-1}$) and after 48 h was 24.44 $mL.kg^{-1}.h^{-1}$ (± 5.01 $mL.kg^{-1}.h^{-1}$) and in chapter 4 §4.3.1, the estimated value for the RR_{eq} was 26.84 $mL.kg^{-1}.h^{-1}$ ± 3.75 $mL.kg^{-1}.h^{-1}$. These differences in respiration rate obtained are the result of the high variability of the raw material used for the experiments.

The lowest O_2 consumption rate was found for 2 % O_2 and 20 % CO_2 (Fig. 5.1). All other combinations were not significantly different, even form air.

In general, low levels of O_2 provided RQ values within the reported range for aerobic respiration (Kader *et al.*, 1989). The results are in accordance with previous results for RQ of mushrooms (Varoquaux *et al.*, 1999; Iqbal *et al.*; 2009a; Cliffe-Byrnes and O'Beirne, 2007) and were similar to RQ reported previously (Chapter 3). MAP has

been used with some success for mushrooms, although the mechanisms behind these beneficial effects are relatively ambiguous. Furthermore, concerning respiration parameters of mushrooms as influenced by O_2 and CO_2 concentrations, several publications can be found for button mushroom and less amount of information is found for other mushroom species. It has been reported that mushroom catabolism is aerobic under extreme lower levels of O_2. According to López- Briones *et al.* (1992) and Varoquaux *et al.* (1999), respiration rate of button mushrooms remained constant, even around 0.2 kPa of O_2, as seen from the respiratory quotient equal to unit whatever the level of O_2 used, with no effect on RR with low O_2 levels were found.

The results presented for oyster mushrooms are in accordance with previous reports for other mushrooms species. Tano *et al.* (1999) reported a decrease in RR of button mushrooms at 4 ºC as the gas composition was changed from air to an atmosphere of 5 % O_2 and 10 %. Iqbal *et al.*, (2009a,b) also observed a significant decreased on the respiration rate of button mushrooms stored under different concentrations of O_2 and CO_2.

Regarding CO_2 effect on mushroom respiration rate, it has been found that mushroom respiration follows a Michaëlis-Menten-type equation with no inhibition (Cliffe-Byrnes and O' Beirne, 2007; Varoquaux *et al.*, 1999) or uncompetitive (Cliffe-Byrnes and O' Beirne, 2007; Iqbal *et al.*, 2009b).

A response surface regression analysis was used to determine how O_2 and CO_2 affect the respiration rate of oyster mushrooms. Quadratic polynomial functions were selected as the best representing the data: (Eqs. 5.1 and 5.2).

$$RR_{O_2} = 28.25 - 1.47 \times O_2 + 1.41 \times CO_2 - 0.09 \times (O_2)^2 + 0.09 \times O_2 \times CO_2 - 0.11 \times (CO_2)^2 \quad \text{Eq. 0.1}$$

$$RR_{CO_2} = 28.30 - 4.27 \times O_2 + 1.47 \times CO_2 + 0.20 \times (O_2)^2 + 0.077 \times O_2 \times CO_2 - 0.11 \times (CO_2)^2 \quad \text{Eq. 0.2}$$

The plots obtained for the response surface modelling for RR_{O_2} and RR_{CO_2} are presented in Figs. 5.2 a) and b) respectively.

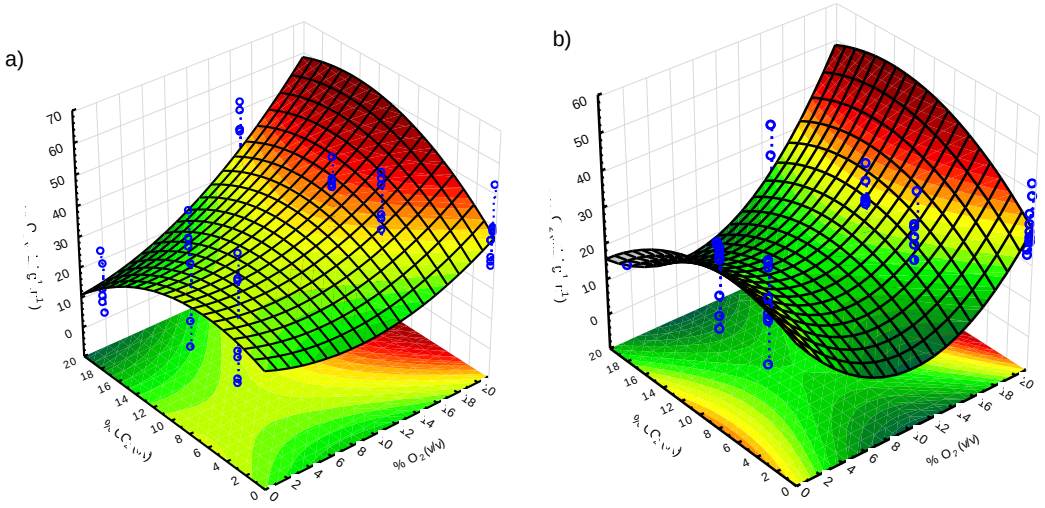

Fig. 0.2 – Response surface modelling for the effect of O_2 and CO_2 on oyster mushrooms respiration rate, according with Eqs. 5.1 and 5.2.

According to the results obtained it can be seen that in order to obtain the maximum decrease on the respiration rate, low levels of O_2 should be combined with high levels of CO_2.

5.4. Conclusions

Effect of O_2 and CO_2 levels (2 or 5 % and 5, 10 or 15 % respectively) on respiration ate of fresh oyster mushrooms was assessed. Mushrooms were stored at 2ºC for 4 days after sample processing. Every day, mushrooms were flushed with the gas mixture and the respiration rate was measured. Storage time did not affect the respiration rate of oyster mushrooms for each mixture tested. Levels of O_2 and CO_2 had significant effect on the consumption of O_2 and CO_2 production rate. If the minimum respiration rate is desired, a 2 % (v/v) and 20 (% v/v) should be maintained within the packaged mushrooms.

Chapter 6: Modelling the influence of time, temperature and relative humidity conditions on the mass loss of fresh oyster mushrooms

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6.1. Introduction

Horticultural commodities continue their active metabolism after harvest, which drives to severe consequences on produce quality and shelf life. Respiration and transpiration rates are the main physiological factors affecting postharvest quality retention (Kader, 2002). Harvested commodities release water vapour into the surrounding atmosphere by transpiration process and use reserves of organic materials, and also release water vapour by respiration process. Both processes are responsible for mass loss of fresh after harvest. Losses of water of fresh produce are known to affect several characteristics throughout storage. It includes loss of marketable mass and, loss of produce appearance, therefore decreasing overall value of fresh produce (Ben-Yehoshua *et al*., 2003).

Transpiration is related to the loss of water from fresh produce and involves the transport of water vapour from the produce surface to the surrounding air due to the difference between the water vapour pressure (WVP) of the produce and the WVP of the surrounding air (Ben-Yehoshua *et al*., 2003).

Postharvest water loss or transpiration is dependent on commodity characteristics such as surface area, respiration rate and air movement surrounding the commodity. Furthermore, external factors such as temperature and RH are also known to significantly affect the rate of transpiration of a commodity (Mahajan *et al*., 2008a).

Mass loss is an important parameter used to test the efficacy of various postharvest techniques. Quantification of transpiration rate and its mathematical modelling is useful to select appropriate packaging materials. The use of a packaging system such as modified atmosphere packaging is an effective technology for postharvest preservation of several mushroom species (Villaescusa and Gil, 2003; Illeperuma and Jayathunge, 2004; Sapata *et al*., 2004, Sapata *et al*., 2009a,b; Ramos *et al*., 2011; Kim *et al*., 2006). However, the choice of packaging film is usually based on O_2 and CO_2 transmission rates and not necessarily water vapour transmission rate in order to maintain the optimum relative humidity inside the package. Inappropriate selection of packaging film and also fluctuation of storage temperature encountered

during the cold chain will eventually lead to excessive humidity and condensation inside the package (Linke and Geyer, 2013). Therefore, knowledge of rate of mass loss and the development of reliable TR models could improve selection of packaging materials for fresh produce.

Song *et al*. (2001) and Song *et al*. (2002) developed a model for the transpiration rate of blueberry by the simultaneous application of mass and heat transfer processes. Mahajan *et al.* (2008a) developed a mathematical model based on Fick's law of diffusion to obtain an adequate description mass loss of mushroom under different combinations of temperature and relative humidity of storage environment. The model was later successfully adopted by Caleb *et al.* (2013) for pomegranate and pomegranate arils and strawberry (Sousa-Gallagher and Mahajan*,* 2013).

MAP has been recognized as a simple, economical and effective technology for postharvest preservation of several mushroom species (Villaescusa and Gil, 2003; Illeperuma and Jayathunge, 2004; Sapata *et al.*, 2004, Sapata *et al*., 2009a,b; Ramos *et al.*, 2011; Kim *et al.*, 2006), but the occurrence of rapid condensation inside the package have been also reported.

The objectives of this work were to (**i**) determine the transpiration rates under controlled environmental conditions (2, 6, 10, 14 and 18 ºC and 86, 96 and 100 % of RH) and (**ii**) to model the effect of these variables on the TR of fresh *Pleurotus ostreatus* mushrooms.

6.2. Material and methods

6.2.1. *Sample preparation*

Oyster mushrooms (*Pleurotus ostreatus*) grown in Wexford, Ireland were bought in a local retailer at Cork City and transported to the laboratory. Mushrooms were immediately sorted by appearance, prepared for mass loss measurements and maintained at each storage temperature for temperature equilibrium.

6.2.2. *Transpiration rate measurement*

To evaluate the transpiration rate of mushrooms, a mass loss approach was adopted (Leonardi *et al.*, 2000). Mushrooms were stored at 2, 6, 10, 14 and 18 ºC and 86, 96 and 100 % of RH in 6.5 L plastic containers.

In each test container, RH was controlled by using saturated salt solutions. Solutions of potassium chloride and potassium nitrate (providing 86 and 96 % RH, respectively) and distilled water (to create the saturated atmosphere) (Patel *et al.*, 1988), were placed on the bottom of the container. Lids were put in the container and sealed with petroleum jelly.

Plastic containers were stored in incubator (Sanyo MIR 253) maintained within 1ºC of the temperature tested. The temperature and RH inside the container was monitored using a data logger (HMP50, Campbell Scientific Inc., Utah). Furthermore, a gas analyzer (Checkmate, 9900, PBI Dansensor, Denmark) was used to control possible changes in the atmosphere inside each container as a result of respiration rate. The setup provided a constant temperature and relative humidity throughout the experimental run, without changes in gas composition due to mushroom respiration.

At regular intervals, mushrooms were weighed in an analytical scale (Precisa 1000C-3000 D), so mushroom mass could be known for each time, temperature and RH. Transpiration rate (TR, $g.kg^{-1}.h^{-1}$) was determined based on the slope of the graph of the changes in normalized mass loss (M/M_0) of oyster mushrooms over time (h).

For each storage condition, fours replicates were used and the entire set of experiments was replicated twice, with a total of 8 mushrooms for each condition.

6.2.3. *Data analysis*

The constants of the model developed to describe the influence of temperature and relative humidity on transpiration rate of oyster mushrooms were estimated by fitting the model to the experimental data by nonlinear regression using IBM SPSS Statistics Version 20.0 (IBM, 2011).

The Response Surface modelling (RSM) was used to apply the developed model to the transpiration rate ($g.kg^{-1}.h^{-1}$) of oyster mushrooms as a function of temperature and relative humidity, using *Statistica* software (Version 12, Stat soft, Inc., Tulsa, Oklahoma, USA).

6.3. Results and discussion

6.3.1. *Mass loss of oyster mushroom*

Mass loss of oyster mushrooms stored at 2 ºC and different relative humidities is shown in Fig. 6.1.

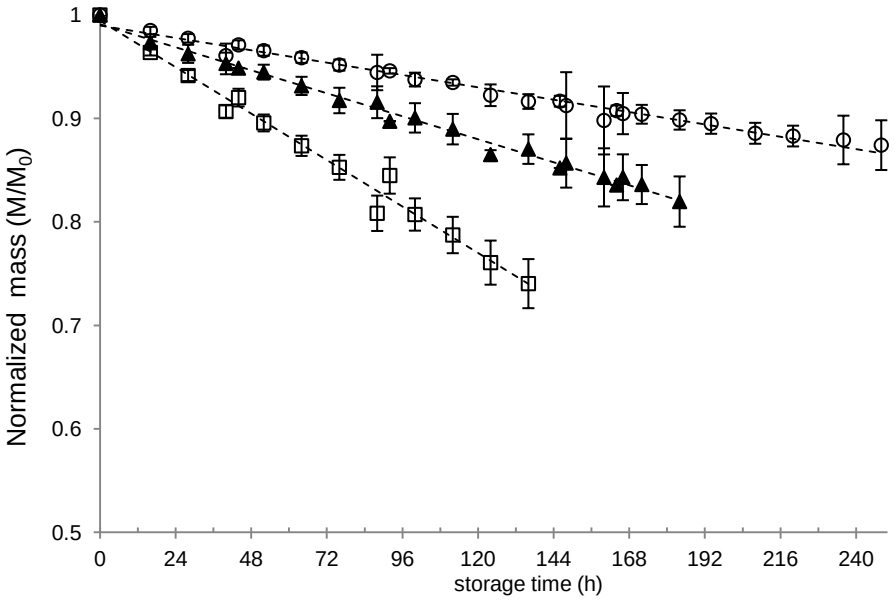

Fig. 6.1 – Changes in mass loss (M/M_0) of oyster mushrooms stored at 2 ºC over time. The values were normalized with respect to the initial mass of mushroom (M0). Each point represents the mean of eight replicates. Vertical bars represent the standard error of the mean. □- 86 % RH; ▲- 96 % RH; ○- 100 % RH.

There was linear decrease of mass of mushroom over time, therefore TR was calculated by the slope mass loss vs. time for all the storage conditions tested. Linear decrease of mass loss with time has been previously observed for button mushrooms (Burton and Noble, 1993; Varoquaux *et al*., 1999; Mahajan *et al*., 2008a; Guillaume *et*

al., 2010), although different rate of mass loss were found. Similar trend was also found for other commodities (Shirazi and Cameron, 1992).

Over a 248 h period, oyster mushrooms stored at 2 ºC, lost significant amounts of mass. Mushrooms stored under saturated condition, lost less mass (12.8 % FW (± 2.1 %)) compared with those stored under the lowest RH of 86 % that lost 26.0 % FW (± 2.4 %). These values were higher than the values obtained by Mahajan *et al*. (2008a) for *A. bisporus* mushrooms. From the results presented, it can be concluded that mass loss of oyster mushroom assume major importance for postharvest quality maintenance. Burton and Noble (1993) reported that mushrooms stored at 5 ºC and 73 % RH, had an mean mass loss of 4 % and 6 % per day for button mushrooms stored at 18 ºC and 90 % RH, respectively. Similar results were reported by Guillaume *et al*. (2010) that determined that button mushrooms in glass jar (maintained with RH near to 100 %) lost 0.18 % per day.

Tab. 6.1 presents the results for the transpiration rate of oyster mushrooms for each storage temperature and relative evaluated.

Tab. 6.1 – Transpiration rate ($g.kg^{-1}.h^{-1}$) (mean ± SE) of oyster mushrooms stored at 2, 6, 10, 14 and 18 ºC and 86, 96 and 100 % RH.

	RH (%)		
Temp. (ºC)	**86**	**96**	**100**
2	1.82 (± 0.20)	1.21 (± 0.41)	0.52 (± 0.04)
6	2.97 (± 0.16)	1.83 (± 0.21)	0.92 (± 0.10)
10	3.24 (± 0.38)	1.86 (± 0.21)	1.34 (± 0.36)
14	3.67 (± 0.54)	2.77 (± 0.56)	1.52 (± 0.11)
18	3.88 (± 0.41)	2.92 (± 0.37)	1.84 (± 0.14)

Transpiration rate for oyster mushrooms, ranged from 0.52 (± 0.04) to 3.88 (± 0.41) $g.kg^{-1}.h^{-1}$ across all the combinations of temperature and RH tested. In accordance with the trend observed for the influence of the storage conditions in mass loss, TR was higher for higher temperatures and lower relative humidities. Mahajan *et al.* (2008a) reported that TR of button mushrooms ranges between 0.27 and 3.83 $g.kg^{-1}.h^{-1}$ at 5, 10 and 15 ºC with RH of 76, 86 and 96 %. For apple, transpiration rate ~0.03 $g.kg^{-1}.h^{-1}$ (TR: 0.67 $g.kg^{-1}day^{-1}$) at 10 ºC and 86 % RH were reported by Patel *et al.*

(1988), whereas Caleb *et al.* (2013) reported values of 0.048 to values of 0.70 $g.kg^{-1}.h^{-1}$ for arils stored at 5, 10 and 15°C and relative humidity (RH) of 76, 86 and 96 %.

Fresh harvested commodities are living structures that keep their metabolic activities, namely respiration and transpiration. These processes are dependent on the food reserves and moisture content at harvest and both lead to a gradual mass loss after storage. Transpiration of fresh produce is a mass transfer process in which water vapour moves from the surface of the commodity to the surrounding air and occurs due to a gradient of water vapour pressure, generally explained by the Fick´s law of diffusion.

The partial pressure of water vapour of the commodity is very close to saturation (Burton, 1982) and also water vapour in fresh produce packaging is close to saturation level, mainly due to low water vapour permeability of packaging material and also water produced by the package commodity. When relative humidity of the air equals saturation levels, there is no difference in the partial pressures between the commodity and the environment and therefore there is no driving force for water loss to occur. In the present study, oyster mushrooms sustained continuous mass loss throughout storage, even when stored at 100 % RH. For that reason, other phenomena must be considered important for the mass loss of horticultural commodities. Mass loss due to respiration is due to the use of organic materials or water loss due to heat of respiration. Respiration is the metabolic process that generates energy to the commodity cell. The process consumes carbon reserves and produces CO_2, water and heat as by-products (Burton, 1982; Wills *et al*., 1989; Ben-Yehoshua *et al*., 2003). Carbon reserves consumption has a direct effect on the mass losses after harvest but respiration has also an indirect effect on transpiration. Some of the respiratory energy generated is fixed as ATP as a metabolic energy, whereas most of the respiratory energy released by the produce is dissipated as heat (Ben-Yehoshua *et al*., 2003). This heat produces can accumulate in the product, raising its temperature (Gaffney *et al*., 1985; Sastry, 1985), which leads to the evaporation of the saturated air film around produce (latent heat of vaporization) as well as temperature difference between the commodity and the surrounding air. In both cases, there is an increase in water vapour pressure deficit leading to water evaporation from the produce tissue (Ben-Yehoshua *et al*., 2003). For that reason, even at a saturated environment as often occurs in horticultural commodities packages, transpiration is assumed to occur. Although some authors consider that mass loss due to respiration process may be considered

negligible (Shirazi and Cameron, 1992), for produce with high metabolic rates like mushroom (Iqbal *et al*., 2009a), the contribution of the respiration process to the overall mass loss should be considered to the overall mass losses.

6.3.2. *Effect of temperature*

As previously stated, traditionally the rate of moisture loss from a product is expressed according to the Fick's law of diffusion (Sastry, 1985). The model considers that the driving force for transpiration is the difference in the partial pressures between commodity cells and surrounding environment.

Since the water content of the environment that surrounds the product is lower than water content of the commodities (that is considered near saturation), a water gradient is created leading to commodity mass losses. This model was successfully applied to button mushrooms, pomegranate and strawberry (Mahajan *et al*., 2008a; Caleb *et al*., 2013; Sousa-Gallagher and Mahajan, 2013). However, the model did not fit well with oyster mushrooms transpiration rate data due to RH saturation conditions analyzed in this work, the difference in WVP between the commodity surface and the surrounding environment would lead to a gain of mass in the product, which was not observed during the experimental work. Therefore, the Fick's model to explain the effect of relative humidity on oyster mushroom transpiration rate was dismissed. In alternative, according to the results obtained for the effect of temperature on oyster mushroom transpiration rate, a reparameterised Arrhenius type-model was fitted to experimental data, for each relative humidity, according with Eq. 6.1.

$$TR = k \times \exp\left[-\frac{Ea_{TR}}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad \text{Eq. 6.1}$$

where, TR is the transpiration rate ($g.kg^{-1}.h^{-1}$), k_0 is the reaction constant (h^{-1}), Ea_{TR} is the activation energy for the transpiration rate ($kJ.mol^{-1}$) for the transpiration rate; R is the universal gas constant (8.314×10^{-3}, $kJ.mol^{-1}.K^{-1}$), T is the storage temperature (ºC); T_{ref} is the reference temperature (considered the mean of the storage temperatures, 283.15, K).

The effect of temperature on oyster mushrooms TR for the particular case of 96 % RH is shown on Fig. 6.2. This reparameterisation of the Arrhenius equation was

applied to improve regression convergence and to diminish model parameter colinearity (Nelson, 1983; Cunha *et al*., 2006).

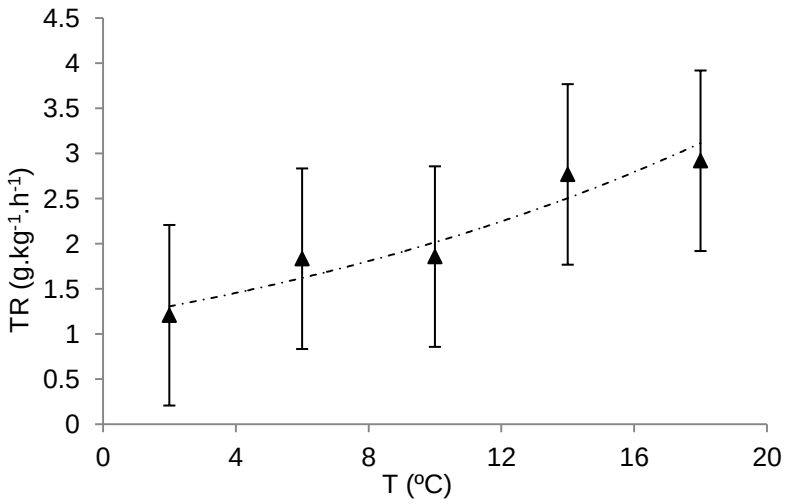

Fig. 6.2 – Effect of temperature on oyster mushrooms transpiration rate (g.kg-1 .h-1) stored at 96 % RH. Points are the mean of eight replicates, vertical bars represent the standard error of the mean and the line represents the fit of the primary model, according with Eq. 6.1.

Model parameters of the Eq. 6.1 were estimated from experimental data, using the linear form of the Arrhenius type equation, to stabilize variance (Draper and Smith, 1981). Estimated constants, statistical data and the quality of the adjust of the Arrhenius-type model at each relative humidity are summarized in Tab. 6.2.

Tab. 6.2 - Constant estimates (± SE) (k and apparent activation energy) of the Arrhenius type model (Eq. 6.1).

RH (%)	k (h^{-1})	Ea ($kJ.mol^{-1}$)	R^2
86	3.07 (± 0.17)	24.5 (± 6.5)	0.819
96	2.03 (± .0.19)	33.9 (± 10.6)	0.890
100	1.06 (± 0.1)	41.1 (± 10.7)	0.605

Despite the somehow low values of the determination coefficient (R^2), varying between 0.605 and 0.890, considering the functional relationship of TR with temperature, as depicted on Fig. 6.2, the model was accepted to explain the effect of temperature on oyster mushroom transpiration rate.

According to Fockens and Meffert (1972) mushroom surface is considered a wet surface covered with a thin layer of water or air saturated with water vapour, which contributes to high mass losses over time. Temperature has a significant effect on oyster mushroom transpiration rate due to the effect of temperature on water vapour partial pressures. Temperature of the commodity surface is a major determinant of the driving force for water loss. Exponential effect dependence of transpiration rate on temperature has been seen for *Agaricus bisporus* mushrooms (Mahajan *et al*., 2008a), pomegranate (Caleb *et al*., 2013) and strawberry (Sousa-Gallagher and Mahajan, 2013).

6.3.3. *Combined effect of temperature and relative humidity*

In a second step, the dependence of RH on the parameters k and Ea_{TR} of the Arrhenius model was studied. The effect of relative humidity on each parameter was described with the following model (Eq. 6.2 and 6.3).

$$k = a_k + b_k \times RH \quad \text{Eq. 6.2}$$

$$Ea_{TR} = a_{Ea} + b_{Ea} \times RH \quad \text{Eq. 6.3}$$

where, a_k (h^{-1}), b_k (h^{-1}) , a_{Ea} ($kJ.mol^{-1}$) and b_{Ea} ($kJ.mol^{-1}$) are the constants of the model.

The model fits the data well as observed in Fig. 6.3.

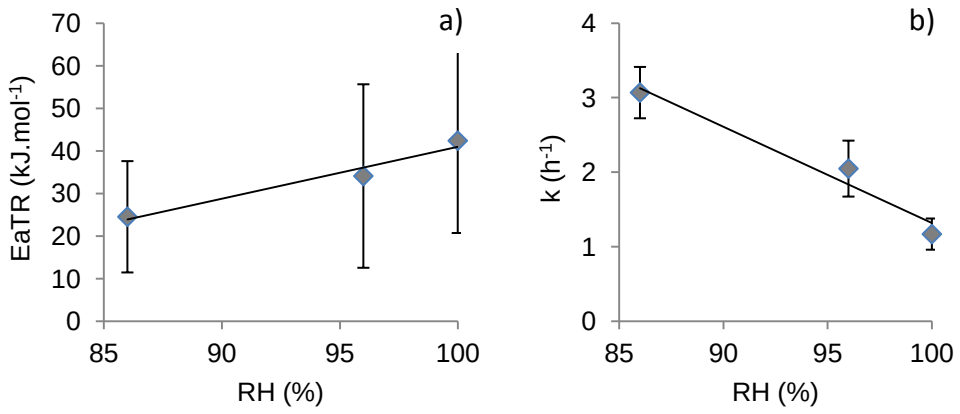

Fig. 6.3 – Effect of RH of the storage environment on the parameters of the Arrhenius model, (a) Ea_{TR} ($kJ.mol^{-1}$), and (b) k (h^{-1}). The symbols represent the estimates of the individual model parameters, the bars represent the 95 % asymptotic confidence intervals and the lines represent the fit of the model (Eq. 6.2 and 6.3, respectively).

The linear relationship (Eqs. 6.2 and 6.3) was then included in the Arrhenius-type equation (Eq. 6.1), yielding:

$$\ln(TR) = \ln\left[a_k - b_k \times RH\right] - \left[\left(\frac{a_{Ea} + b_{E_a} \times RH}{R} \times \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right)\right] \quad \text{Eq. 6.4}$$

where, ln(TR) is the natural logarithm of the transpiration rate of oyster mushrooms ($g.kg^{-1}.h^{-1}$).

Model (Eq. 6.4) was fitted to all experimental data in its logarithm form to avoid residuals heterocedasticity (Draper and Smith, 1981). Estimated constants, relevant statistical data and the quality of the fit of global model are summarized in Tab. 6.3.

Tab. 6.3 – Constant of the global model (Eq. 6.4) obtained to predict the effect of temperature and relative humidity in oyster mushroom transpiration rate.

Parameter	Estimate	Std. Error
a_{Ea} (kJ. mol^{-1})	-47.96	77.401
b_k (h^{-1})	0.152	0.016
b_{Ea} (kJ. mol^{-1})	0.885	0.825
a_k (h^{-1})	16.16	1.54

Fig. 6.4 shows that the transpiration rate model fits the experimental data well as seen by the good agreement between predicted and experimental values.

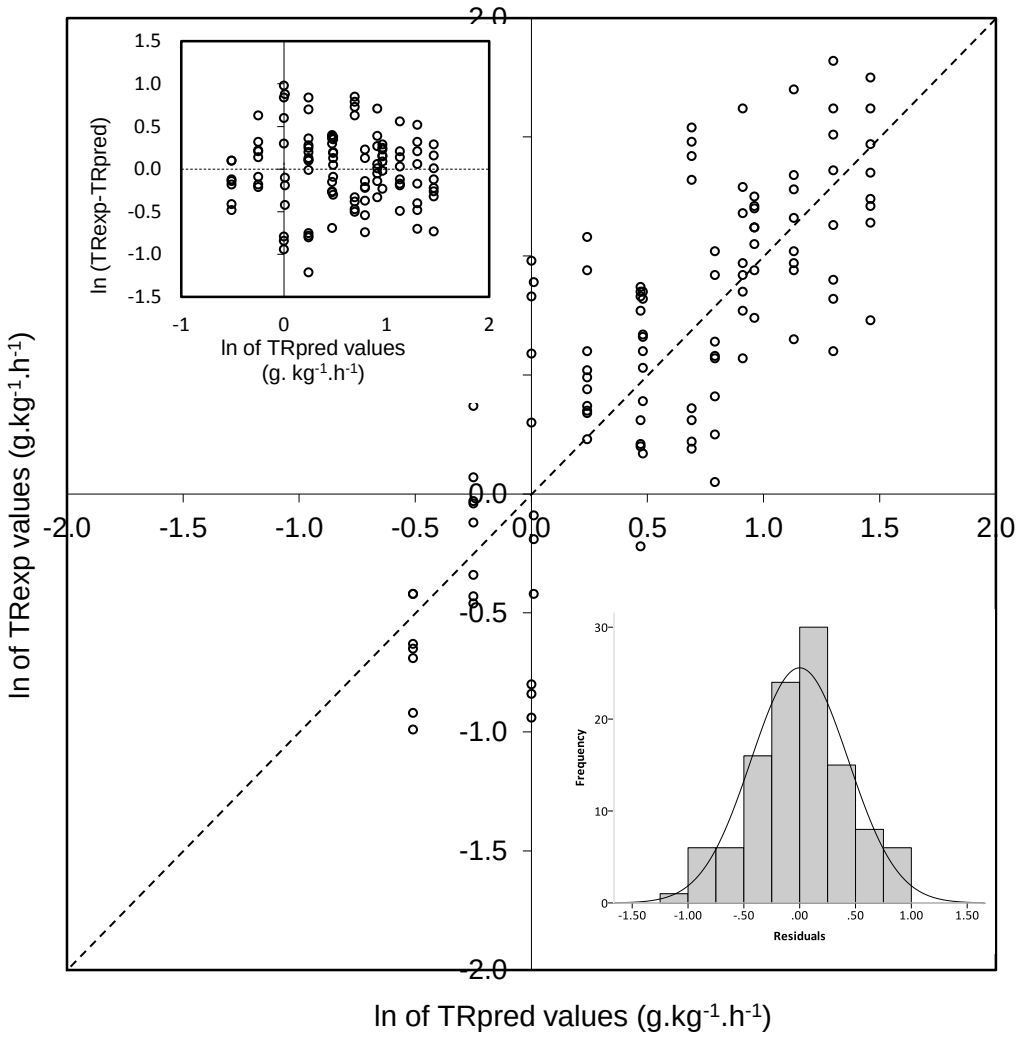

Fig. 6.4 - Relationship between transformed -natural logarithm- experimental (TRexp) and predicted (TRpred) transpiration rates, following Equation 6.4. Enclosed figures represent the residuals versus predicted values (top corner) and the frequency distribution of residuals with the solid line representing the expected normal distribution (bottom corner).

The residuals obtained presented a mean of 0.00 (SD of 0.44, for n=112) and a normal distribution ($p > 0.05$), according the Kolmogorov-Smirnov test, indicating that the distribution is not biased.

RSM was used as a mathematical approach to empirical model building (Box and Draper 2007). The surface curve representing Equation 4 is displayed on Figure 5.

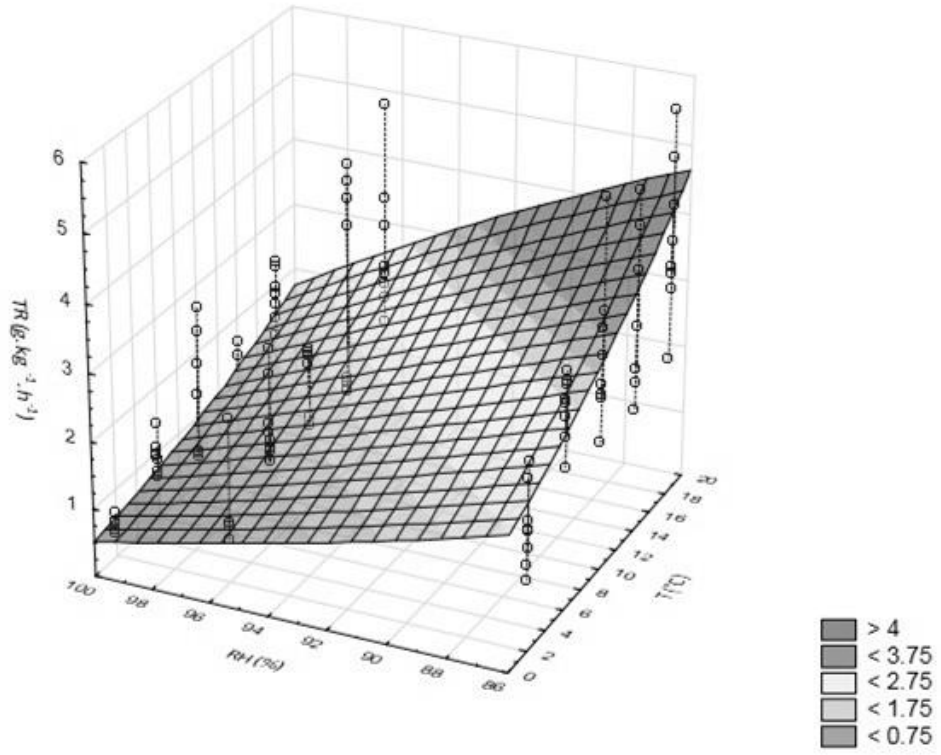

Fig. 6.5 – Response surface model for the effect of temperature (ºC) and relative humidity (%) on oyster mushrooms transpiration rate ($g.kg^{-1}.h^{-1}$), according with Equation 6.4.

Figure 6.5 shows the response surface the effects of the two studied environmental variables (temperature and relative humidity) on oyster mushroom transpiration rate. According to the Figure, no interaction exists between Temperature and Relative humidity. Similar findings were found for strawberry (Sousa-Gallagher and Mahajan, 2013). At a given temperature, losses of mass are minimized when the air is saturated with water vapour. Minimizing storage temperature also slows down mass losses due to carbon loss associated with respiration. Increasing the relative humidity of the package environment is significant in the reduction of water loss by transpiration. When the relative humidity of the surrounding environment becomes close to saturation, the percentage of mass loss due to respiration, considering the total mass loss, increases. Nevertheless, in saturated relative humidities packages, the control of temperature also becomes significant for the reduction of transpiration rate.

6.4. Conclusions

Quality of postharvest commodities is affected by transpiration rate that causes quantitative and qualitative losses. Estimation of transpiration rates of fresh produce may produce useful information for the development of new packaging systems, providing optimum in-package RH. Mass of fresh oyster mushrooms was measured throughout storage life at different storage conditions. Five temperatures (2, 6, 10, 14 and 18 ºC) and three relative humidities (86, 96 and 100 %) Experimental data revealed linear decreases with time in the mass of oyster mushrooms, despite the storage conditions. Both temperature and RH had a clear effect on the rate of mass loss.

A mathematical model, describing the effect of temperature on oyster mushroom transpiration rate, using an Arrhenius-type equation was developed. Parameters of the model were described by a linear relationship and the overall model presents a good fit and can be useful to predict transpiration rates of oyster mushrooms after harvest.

Chapter 7: Development of a moisture absorber for fresh oyster mushrooms

This chapter was part of the paper published as:

- Azevedo S, Cunha LM, Mahajan PV, Fonseca SC. Application of simplex lattice design for development of moisture absorber for oyster mushrooms. *Procedia Food Science.* 2011:184-189. DOI:10.1016/j.profoo.2011.09.029.

7.1. Introduction

MAP has been extensively studied for mushrooms, with positive effects on quality and shelf life (Exama *et al.*, 1993; Roy *et al.*, 1995a; Mangaraj *et al.*, 2009; Sapata *et al*., 2007; Sapata *et al.*, 2009a,b). Mushrooms however have high moisture content and lose large amounts of water causing condensation of moisture inside the packages (Mahajan *et al.*, 2008a). As a consequence of the low water vapour transmissions rates (WVTR) of the films generally used for horticultural commodities (Exama *et al.*, 1993; Roy *et al.*, 1995a,b; Sapata *et al.*, 2004; Mangaraj *et al.*, 2009). Condensation of moisture inside the packages turns the package unpleasant and when water reaches mushroom surface, creates the ideal conditions for microbial growth and subsequent decay of the product (Roy *et al.*, 1995a,b; Sapata, 2005).

To control the in-package relative humidity (IPRH) and therefore extend the shelf life of respiring product the use of moisture absorbers is an important reliable solution (Shirazi and Cameron, 1992; Anantheswaran *et al.*, 1996; Ben-Yehoshua *et al.*, 1983; DeEll *et al.*, 2006). The use of sorbitol, xylitol, sodium chloride and potassium chloride has already been applied for green tomatoes, increasing their shelf lives and suppressing mould growth (Shirazi and Cameron, 1992; Anantheswaran *et al.*, 1996; Ben-Yehoshua *et al.*, 1983; DeEll *et al.*, 2006). According to Ben-Yehoshua *et al.* (1983), the use of 5 g of calcium chloride to control IPRH of bell peppers packages controlled mass loss and maintained the RH inside the package between 80 and 88 %. In the work of DeEll *et al.* (2006), the addition of sorbitol in MAP allowed a better maintenance of general quality of broccoli heads when compared with control treatment.

Regarding mushrooms, very few studies were conducted on the use of desiccants for fresh produce packaging. Roy *et al.* (1995b) studied the use of sorbitol (15 g sorbitol/100 g mushrooms) to control IPRH of A*garicus* mushrooms at 10 °C, concluding that the desiccant application in the package increased the product shelf life and that higher sorbitol quantities increased product mass loss. Other commercially available food-grade moisture absorbers such as clay and silica were used in modified humidity packaging of fresh mushroom (Mahajan *et al.*, 2009b). The authors obtained

global better storage quality regarding maturity index and discoloration when 9 minipacks (3.5 g each pack) were used in a 225 g tray.

Once harvested, oyster mushrooms deteriorate rapidly and high mass losses are found during postharvest period. In particular with *Pleurotus* mushrooms, very few studies can be found regarding the use of moisture absorbers to achieve higher quality retention (Villaescusa and Gil, 2003; Sapata *et al.*, 2009a,b).

Existing moisture absorbers approved for use in food packaging have low absorption capacity or absorb moisture too quickly, making them unsuitable for food packaging. This study aims to develop a moisture absorber with the correct moisture holding capacity (MHC) for mushrooms. This will be achieved by combining three desiccants, calcium oxide, sorbitol and $CaCl_2$ in varying proportions and identifying the combination of the three desiccants which gives optimum performance. Simplex lattice design was used to design the experiments and optimize the proportion of ingredients for the mixed desiccant.

7.2. Material and Methods

7.2.1. *Measurement of the moisture holding capacity of the desiccant*

Three desiccants selected for the present study were calcium oxide (CaO fine powder, puriss Roedel-de Haen), calcium chloride ($CaCl_2$, Calcium chloride dehydrate, Merck) and sorbitol ($C_6H_{14}O_6$, D-Sorbitol, 99+%, Sigma-Aldrich).

Each desiccant was oven dried at 60 ºC for at least 1 h before being used in the mixture.

Mixture design experiment was used to determine the optimum mixture proportions based on moisture absorber kinetics. Simplex lattice design, available in Statistica software (Version 12, Stat soft, Inc., Tulsa, Oklahoma, USA) was used to determine the number of experimental runs and the proportion of three desiccants in each experimental run (Tab. 7.1). A simplex lattice design is a mixture design in which sum of the fractions of the desiccants is unity (Cornell, 1981).

Tab. 7.1 - Proportion of components used in each mixture of desiccant.
The proportion of components was obtained using Design of Experiments (DOE) tool in Statistica software. A 3 factor simplex-lattice design (Degree m=3) with interior points and overall centroid was used.

Mixture #	Component proportion		
	CaO	$CaCl_2$	Sorbitol
1	0.60	0.20	0.20
2	0.20	0.60	0.20
3	0.20	0.20	0.60
4	0.33	0.47	0.20
5	0.33	0.20	0.47
6	0.20	0.33	0.47
7	0.47	0.33	0.20
8	0.47	0.20	0.33
9	0.20	0.47	0.33
10	0.33	0.33	0.33
11	0.47	0.27	0.27
12	0.27	0.47	0.27
13	0.27	0.27	0.47
14	0.33	0.33	0.33

Moisture absorption of each mixture was measured at 10 ºC and 96% RH. Individual small plastic trays (1 mL) were used to hold 1 g of each of the 14 mixtures in an air tight 6.5 L plastic container (Fig. 7.1). In order to create 96 % RH, a saturated salt solution of potassium nitrate was placed at the bottom of the plastic container. Plastic containers holding the different mixtures at 96 % RH were sealed tightly with plastic cap and vaseline and then transferred to an incubator (Sanyo MIR 253), providing 10 ºC (± 0.2 ºC).

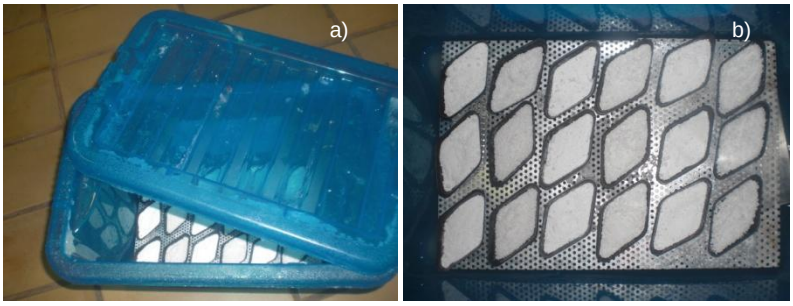

Fig. 7.1 – Experimental setup used to: (a) hold moisture absorber and of (b) the plastic trays used to hold each mixture.

The plastic trays were weighed at regular intervals and the moisture content (MHC) of desiccant was expressed in terms of $g_{water}.g^{-1}$ desiccant as Eq.7.1:

$$MHC = \frac{M_t - M_i}{M_i} \quad \text{Eq. 7.1}$$

where, MHC is the moisture holding capacity of desiccant at time t ($g_{water}.g^{-1}$ desiccant), t is time (h), M_i and M_t are the mass of desiccant (g) in the beginning and at time t. For each set of the desiccant, 3 replicates were performed and the entire set of experimental runs was replicated twice, with a total of 6 replicates for each mixture.

7.2.2. *Statistical analysis*

Results were analysed by non-linear regression with moisture content as the dependent variable, using *Statistica* software (Version 12, Stat soft, USA). In order to optimize the desiccant mixture, the proportion of $CaCl_2$ was fixed at 0.26 mass fractions and the maximum MHC at any combinations of CaO and sorbitol was obtained using the solver Tool in Microsoft Excel.

7.3. Results and discussion

The experimental data showed that the mass of the mixed desiccant increased over time (Appendix D, Figure D.1). Moisture was absorbed rapidly at first and then the slope began to level off indicating that moisture was gradually absorbed more slowly as the mixture reached equilibrium.

Considering that shelf life of oyster mushrooms is about 2 to 3 days at 10 ºC (Choi and Kim, 2002), moisture content of mixed desiccant up to maximum 117 h was considered as equilibrium moisture content (Appendix E, Figure E.2).

Tab. 7.2 – Moisture holding capacity (g_{water}. g^{-1} desiccant) of mixed desiccant at 117 hours.

Mixture #	Component proportion			MHC (g water g^{-1} desiccant) at 117 hours		
	CaO	**$CaCl_2$**	**Sorbitol**	**Mean**	**SD**	**n**
1	0.60	0.20	0.20	0.49	0.20	4
2	0.20	0.60	0.20	1.29	0.21	5
3	0.20	0.20	0.60	0.33	0.09	6
4	0.33	0.47	0.20	1.13	0.11	6
5	0.33	0.20	0.47	0.41	0.05	6
6	0.20	0.33	0.47	0.85	0.10	5
7	0.47	0.33	0.20	0.82	0.06	6
8	0.47	0.20	0.33	0.45	0.11	6
9	0.20	0.47	0.33	0.93	0.06	6
10	0.33	0.33	0.33	0.67	0.05	6
11	0.47	0.27	0.27	0.52	0.04	6
12	0.27	0.47	0.27	0.91	0.10	6
13	0.27	0.27	0.47	0.59	0.16	6
14	0.33	0.33	0.33	0.60	0.06	5

Moisture uptake for mixture #2 was faster than for mixture #13 (Appendix E, Figure D.2). Mixture #2 had a 0.6 of $CaCl_2$, whereas mixture #13 had a $CaCl_2$ of 0.27. The difference in proportion of this specific component could explain the rapid moisture uptake and higher moisture holding capacity (MHC) of mixture #2 could be due to the higher proportion of $CaCl_2$ in comparison with the other two desiccants.

Fig. 7.2 shows the Pareto chart accounting for the effect of mixing desiccants on MHC.

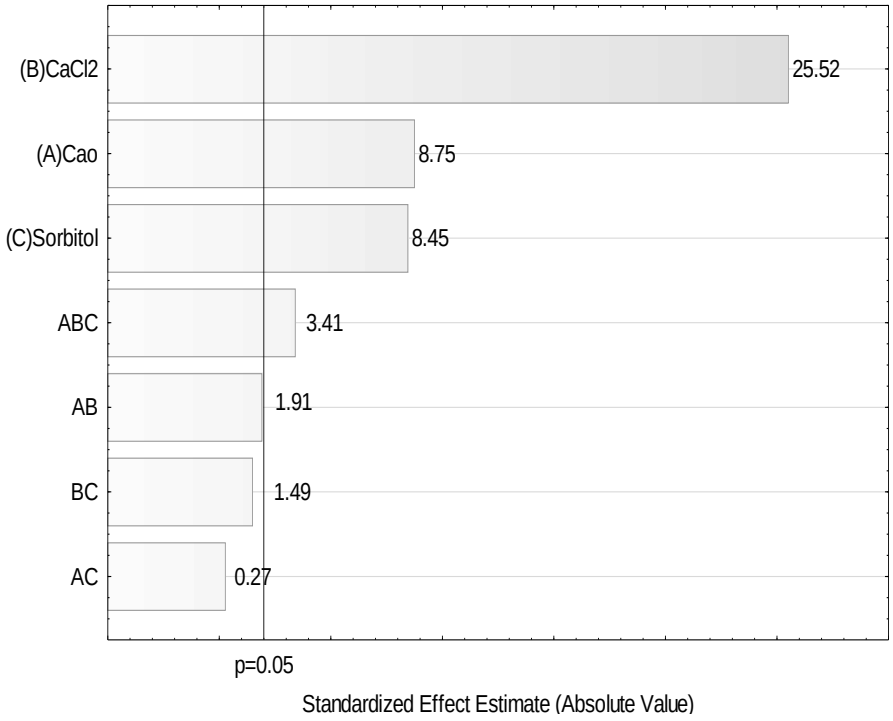

Fig. 7.2 - Standardized Pareto chart accounting for the effect of each of the individual components and the mixing component in the total moisture holding capacity. The dashed vertical line corresponds to the 95 % confidence limits.

According with the Pareto chart (see Fig. 7.2), the three components of the mixture (calcium oxide, calcium chloride and sorbitol) have a significant impact on moisture holding capacity of the mixed desiccant (p = 5 %).

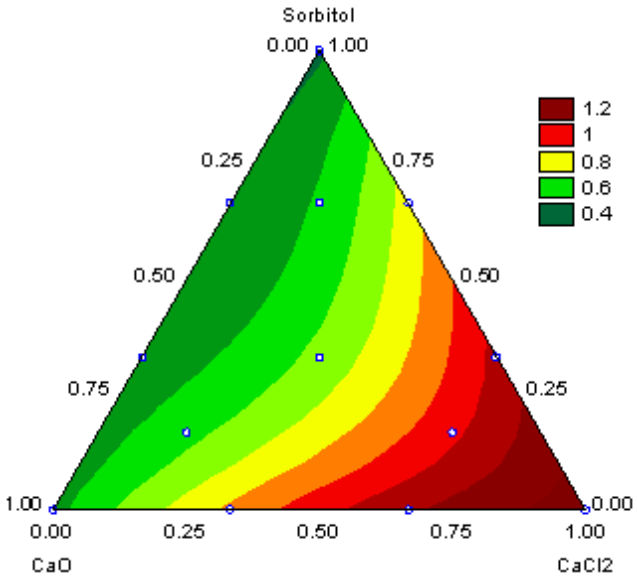

Fig. 7.3 - Contour plot for accounting the effect of each of the individual components and the mixing component in the total moisture holding capacity. The dashed vertical line corresponds to the 95 % confidence limits.

According to the results obtained, $CaCl_2$ had the most significant impact on moisture holding capacity of the mixed desiccant followed by CaO and sorbitol. These results are in agreement with previous studies (Mahajan *et al.*, 2008b; Song *et al.*, 2001) since $CaCl_2$ is known for its strong hydrophilic properties.

Moreover, it can be seen that the ternary mixture has also a significant effect on moisture holding capacity.

Since the 3 components have significant effect in the MHC, a cubic model (Eq. 7.2) was fitted to the experimental data.

$$MHC=\alpha_1 A+\alpha_2 B+\alpha_3 C+\alpha_{12} AB+\alpha_{13} AC+\alpha_{23} BC+\alpha_{123} ABC \quad \text{Eq. 7.2}$$

where A, B and C indicate CaO, $CaCl_2$ and sorbitol proportions in the mixture, respectively.

The coefficients (α's) of the cubic model and other statistical information are shown in Tab. 7.3 and 7.4.

Tab. 7.3 - Coefficients and respective standard errors of the cubic model (Eq. 7.2).

Coefficient	Value	SE
α_1	0.47	0.05
α_2	1.24	0.05
α_3	0.38	0.05
α_{12}	0.44	0.24
α_{13}	0.06	0.23
α_{23}	0.34	0.23
α_{123}	-4.35	1.28

The coefficient of determination (R^2) was 0.847 (R^2 Adj = 0.834) which indicated a good fit of the model.

Tab. 7.4 - Analysis of variance of the cubic model (Eq. 7.2).

	SS	df	MS	F	p - value
Model	5.600	6	0.933	66.29	< 0.001
Total Error	1.014	72	0.014		
Lack of Fit	0.232	6	0.039	3.27	0.007
Pure Error	0.781	66	0.012		
Total Adjusted	6.614	78	0.085		

$CaCl_2$ has the strongest influence on MHC, in contrast with sorbitol which showed the weakest effect on MHC. ABC is negative, meaning an antagonist blending effect, which will decrease the MHC of the mixture through the storage time.

From the contour plot accounting for the effect of each of the individual components and the mixing component in the total moisture holding capacity (Fig. 7.3), it can be observed that if high MHC is desired, higher levels of $CaCl_2$ should be used.

However, in order to develop a moisture absorber for respiring products, the selected moisture absorber must stay in the powder form throughout the storage life of the product. Therefore, addition of a specific amount of CaO and sorbitol is important to optimize moisture absorber performance. Calcium oxide is calcinated or recalcinated lime that removes water from a package very slowly and it has been used in primarily the packaging of dehydrated foods, remaining in the powder form for longer periods.

In order to optimize the desiccant mixture, considering the moisture holding characteristics of $CaCl_2$, the proportion of this component was fixed at 0.26 (m/m). The maximum MHC at any combinations of CaO and sorbitol was obtained using the solver Tool in Microsoft Excel.

The results show that in order to obtain a maximum MHC of 0.81 g water per g of desiccant, a mixture of CaO, $CaCl_2$ and sorbitol should be prepared with respective mass fractions of 0.50; 0.26 and 0.24, respectively.

7.4. Conclusions

This study was undertaken in order to develop a moisture absorber with optimum characteristics for fresh horticultural products. The experiment was designed according to a simplex lattice design, with moisture absorbers (calcium oxide, sorbitol and calcium chloride) being the factors. These three desiccants were mixed in varying proportions, according to the experimental design and stored 10 ºC and 96 % RH. Change in moisture content of each of the mixed desiccants was measured at regular intervals. Results showed that $CaCl_2$ had the most significant effect on final moisture content of mixed absorber, although to remain in powder, an optimum amount of CaO and sorbitol should be added.

According to the cubic model obtained for the MHC, a mixture containing 0.5, 0.26 and 0.24 of calcium oxide, calcium chloride and sorbitol respectively yield a moisture holding capacity of 0.81 g water g^{-1} desiccant and remains in powder form for at least 117 hours at 10 ºC. These results present good perspectives for application of mixed desiccant for packaging of oyster mushrooms.

Chapter 8: Influence of modified atmosphere packaging and moisture absorber on the quality of fresh oyster mushrooms as affected by storage temperature

8.1. Introduction

The maintenance of low temperatures and high levels of relative humidity are considered the most important techniques to retain quality and extend the shelf life of horticultural products (Kader and Saltveit, 2003). However, constant low temperatures are difficult to maintain through the generally long postharvest chain of fruits and vegetables (Paull, 1999; Jacxsens *et al.*, 2000; Brecht *et al.*, 2003), leading to problems regarding directly to the produce physiology as well as the package behaviour that contains the produce. In this context, the use of other hurdles rather than low temperature control is generally required.

The use of modified atmosphere packaging (MAP) for postharvest preservation of horticultural commodities has been recognised as an important technology to maintain quality throughout the distribution system (Kader *et al.*, 1989). The levels of O_2 and CO_2 reached inside the package can reduce respiration rate, ethylene sensitivity and production, decay and physiological changes, with the resultant benefit of extending the storage life of the fresh produce (Kader, 2002).

Mushrooms are delicate horticultural commodities that can benefit of the use of MAP throughout storage when stored at 5 % O_2 and under 12 % CO_2 (López-Briones *et al.*, 1993, Villaescusa and Gil, 2003; Ares *et al.*, 2007). Nevertheless, the use of films with lower water vapour transmission rate in combination with high respiration and transpiration rate of mushrooms (Mahajan *et al*., 2008a) may reduce the potential benefits of MAP. In fact, water retained inside the package moistens the product and therefore, shortens shelf life (Sapata *et al.*, 2004).

The use of moisture absorbers inside horticultural commodity package has been reported as a potential solution to address the problem of the excess of water inside commodity package. Although beneficial effects have been found in broccoli and tomato packages, its use in other products are still scarce. Though some beneficial effects in mushrooms were found by Sapata *et al*. (2009a,b), Villaescusa and Gil (2003) found no beneficial effects on the use of sorbitol in oyster mushroom packages.

The purpose of this study was to (**i**) evaluate quality of fresh *Pleurotus* mushrooms in modified atmosphere package with or without a selected moisture absorber and to compare with ones in actual commercial packages found in the market as affected by storage temperature.

8.2. Material and Methods

8.2.1. *Sample preparation*

Oyster mushrooms (*Pleurotus ostreatus*) were obtained from a commercial supplier one day after harvest and transported to the laboratory in a refrigerated transport. At the laboratory arrival, they were immediately placed in a refrigerated chamber and maintained at 4 °C. In the next day, mushrooms were carefully separated from the cluster and maintained refrigerated until the next day to overcome the respiration peak previous observed.

8.2.2. *Experimental procedure*

In order to test the effect of MAP and moisture absorber on oyster mushroom quality, 3 types of packages were evaluated: a commercial package, a modified atmosphere package and a modified atmosphere package with the addition of a moisture absorber.

For each experimental condition, 3 packages were prepared. In each package, about 200 g of mushrooms were placed in polystyrene trays (PS). In commercial packages (Control), mushrooms were placed in PS trays and wrapped with stretchable PVC film. MAP packages (MAP) consisted in PS trays heat sealed with a perforated film and an atmosphere of 5 % O_2 and 10 % CO_2 was injected. The choice of the packaging system was made based that at equilibrium the optimal preservation atmosphere were maintained. For packages with moisture absorbers (MAP+ ab), a combination of CaO, $CaCl_2$ and sorbitol (0.50: 0.26: 0.24) was used as moisture absorber as found in a previous chapter (Chapter 7). An amount of approximately 8 g were placed in 4 petri covered with filter paper (VWR, grade 413) (Fig. 8.1).

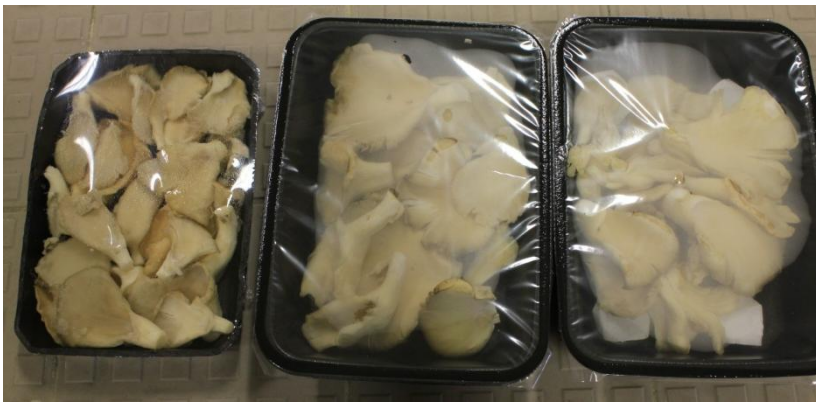

Fig. 8.1 – Examples of the packages used in the experiment. Commercial package; modified atmosphere package and modified atmosphere package with moisture absorber, respectively.

To evaluate the effect of temperature abuse on oyster mushroom quality, two different temperature profiles were used (Fig. 8.2).

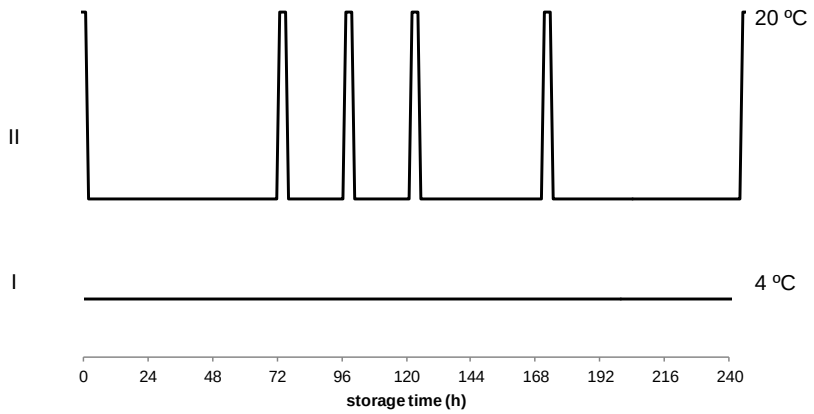

Fig. 8.2 - Temperature history of the storage ambient temperatures for the two temperature profile (I being constant and II with abuse of temperature).

For constant temperature (Profile I), mushrooms were stored in a cold room maintained at 4 °C. To simulate fluctuation and abuse of storage temperatures (Profile II), on each measuring day (0, 72, 120, 168 and 240 h), packages were taken out from refrigeration and left for 3 hours at ambient temperature (~20 ºC).

Quality parameters were evaluated at the beginning of experiment (0 h) and after 72, 120, 168 and 240 h.

8.2.3. *Headspace gas composition*

Changes in the package headspace gas composition were monitored by measuring the values of O_2 and CO_2 inside each package using an O_2 and CO_2 gas analyzer (PBI Dansensor, CheckMate 9900, Denmark) and withdrawing 5 mL of the package headspace atmosphere.

8.2.4. *Quality indicators*

The colour of the upper surface of the mushrooms was evaluated as described in Chapter 2; § 3.2.3). For each storage condition, 6 mushrooms were used and, in each mushroom 5 measurements were performed.

For texture analysis, a double penetration test was performed on the mushroom cap using a TA.XT2 texture analyser (Stable Micro Systems, Godalming, UK), with a load cell of 50 N. A 2 mm diameter cylindrical inox probe was used. The speed of the probe was 2.0 mm. s^{-1} during the pre-test, 2.0 $mm.s^{-1}$ during penetration, and 2.0 $mm.s^{-1}$ during relaxation. The relaxation time between penetrations was 2 s and samples were penetrated 5 mm, according to the methodology described in Parentelli *et al.* (2007) and Antmann *et al.* (2008).

For each experimental condition, 3 measurements were performed in the middle of the stipe of five mushrooms. From the force vs. time curves, firmness (N) was calculated as the maximum force of first peak. Resilience (how well a product fights to regain its original position) and cohesiveness (how well the product withstands a second deformation relative to how it behaved under the first deformation) were also determined to evaluate overall texture of oyster mushrooms. Resilience was determined as the as area during the withdrawal of the first penetration, divided by the area of the first penetration. Cohesiveness was measured as the area of the second penetration divided by the area of the first penetration on force vs. time plot.

Mushrooms were homogenized with a grinder and pH of a diluted solution (1:1 (w:w)) was measured using a digital pHmeter (Consort, Multi-parameter liquid analyser, Belgium). For each experimental condition, 3 replicates were performed.

Homogenized mushrooms were filtered and a drop of the obtained filtrate was used to measure the soluble solid content (SSC) using a digital refractometer (ABBE, mod. 315, Zuzi, Spain). Three replicates were performed and results were expressed as °Brix.

Mushroom mass loss was determined by weighing the content of the packages before and after each storage time. Mass loss (ML, %) was calculated by dividing the mass change during storage by the initial mass (t = 0), according with Eq. 3.12.

For each condition, 3 packages were used and results were expressed as percentage of mass loss.

The moisture absorption of the mixed desiccant was also calculated at the end of each experimental time and package and expressed in terms of $g_{water}.g^{-1}$ desiccant.

Mushrooms were taken out of each package, placed in 1.5 L glass jars and allowed to warm at room temperature. Sensory tests were performed in a test room based on NP4258. Samples were coded (three-digit random numbers) and provided simultaneous to panel. Each member of the panel (panel of 10 elements) was asked to rank the overall preference of the six mushroom samples (according to the odour and colour) (Appendix E, Figures E.1 and E.2).

Overall visual quality of packages and mushroom samples were also described according with odours and off-odours, colour, maturation, firmness and condensed water on the product.

8.2.5. *Statistical analysis*

Effect of storage time, temperature and type of package on quality indicators was inspected through the use of a three-way analysis of variance (ANOVA), using IBM SPSS Statistics Version 20.0 (IBM, 2011). Multiple comparisons were performed using the Tukey´s test. All tests were applied at a 95 % confidence interval, except if stated otherwise. Sensory analysis data was evaluated with non-parametric Friedman and Mann-Whitney-U tests, using the same software. Results are presented as mean and standard error of mean.

8.3. Results and discussion

8.3.1. *Atmosphere composition inside the package*

The evolution of O_2 and CO_2 concentration inside the package during the 240 h of storage are shown in Tab. 8.1.

Tab. 8.1 - Evolution of O_2 and CO_2 in *Pleurotus* mushroom stored under different storage conditions. Values are the mean (± SE) of 3 packages.

Temperature profile	**Temperature abuse**			**Constant Temperature**		
storage time (h)	**Control**	**MAP**	**MAP+ab**	**Control**	**MAP**	**MAP+ab**
			O_2 (%)			
0	20.90 (± 0.00)	5.84 (± 0.00)	5.84 (± 0.00)	20.90 (± 7.53)	5.84 (± 0.00)	5.84 (± 0.00)
120	16.90 (± 4.38)	0.13 (± 0.13)	5.26 (± 3.41)	19.03 (± 7.41)	4.84 (± 0.90)	6.19 (± 1.07)
240	14.33 (± 1.80)	10.69 (± 2.01)	5.80 (± 0.21)	19.20 (± 3.95)	5.87 (± 0.60)	11.40 (± 3.20)
			CO_2 (%)			
0	0.1 (± 0.00)	9.00 (± 0.00)	9.00 (± 0.00)	0.1 (± 0.10)	9.00 (± 0.00)	9.00 (± 0.00)
120	2.40 (± 2.30)	0.13 (± 0.76)	3.41 (± 0.10)	0.90 (± 1.50)	1.07 (± 14.43)	1.45 (± 0.10)
240	3.60 (± 1.19)	2.01 (± 1.99)	0.21 (± 0.99)	0.60 (± 0.87)	3.20 (± 11.23)	2.38 (± 0.03)

Commercial packages stored under constant temperatures remained with values of O_2 and CO_2 relatively constant throughout the 240 h of storage.

Temperature fluctuations during the storage of oyster mushrooms in commercial simulated packages created a gradual decrease in O_2 values that reach about 14 % at the end of storage life. Values of CO_2 increased inside the package and 3.6 % of CO_2 was found at the end of the storage period.

Differences in O_2 and CO_2 values are a consequence on respiration rate and film permeability used to package product. Moreover, both processes are affected by temperature, although the rate of change can vary. An increase in storage temperature promotes an increase in commodity metabolism which is generally higher than the increase observed on the permeability of packaging film (Exama *et al*., 1993; Cameron *et al*., 1995).

8.3.2. *Quality indicators*

Colour of mushroom fruiting body is an important quality parameter, related with consumer acceptance. Postharvest colour development of mushrooms has also a significant effect on quality evaluation throughout shelf life. The results obtained using CIE L*a*b* parameters clearly show the influence of storage time, temperature and gas composition of the headspace package in mushroom colour characteristics.

One of the CIE L*a*b* parameters related with mushroom colour is L*, that measures the lightness of the sample. The evolution of the L* parameter of oyster mushrooms stored under different conditions is shown in Fig. 8.3.

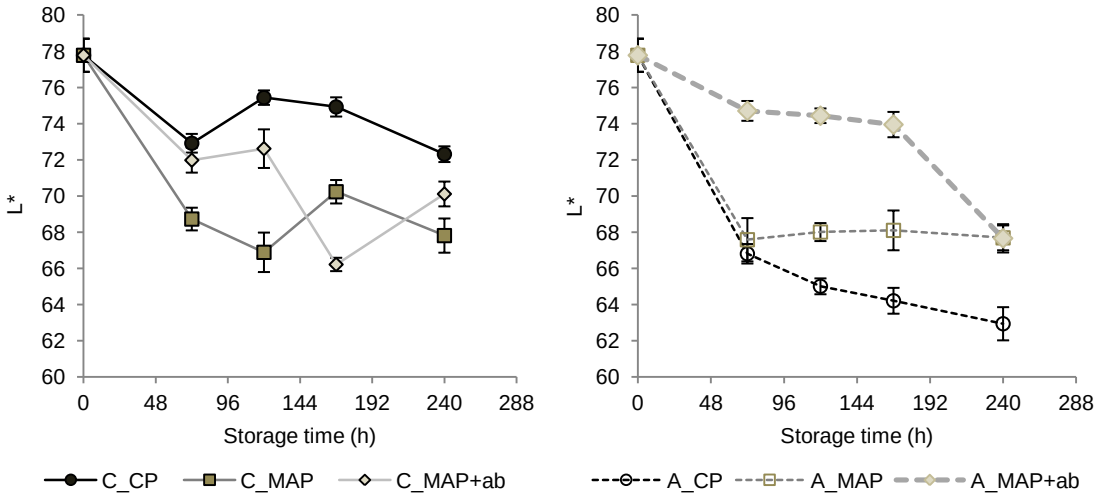

Fig. 8.3 - Evolution of L* parameter of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 30 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

L* value of oyster mushrooms gradually decreases during storage time from an initial value of 77.78 (± 0.91) to values between 62.94 (± 0.92) and 72.31 (± 0.43) obtained for commercial packages stored under abuse and constant temperatures, respectively. All conditions tested (package, temperature profile and storage time) had a significant effect on the L* value of the samples ($p < 0.05$) and interaction between factors were also significant for this colour parameter (Appendix E, Table E.1).

In the first 72 hours after sample packaging, a rapid decrease in L* value was found in all samples. By this storage time, lower values of L* were found for commercial packages stored at abuse temperatures, that displayed a value 66.81 (± 0.54). This value however, was not significantly different from the L* value obtained for oyster mushrooms stored under MAP ($p < 0.05$). Under abusive temperatures, oyster mushrooms stored in packages with moisture absorbers held significantly higher values ($p < 0.05$) for L* parameter when compared with other packages. When stored at fluctuating temperatures for 240 h, oyster mushrooms stored in commercial packages presented lower values of L* ($p < 0.05$; Appendix E, Table E.1), when compared with MAP and MAP with moisture absorber.

At constant temperatures of 4 ºC, oyster mushrooms stored for 72 h under MAP had the lowest value for L* value (Appendix E, Table E.1). Among the packaging conditions evaluated, by the end of storage time, significant lower values of L* were obtained for mushrooms stored in MAP.

Although mushroom colour has been commonly measured using the L* value (Jolivet *et al*., 1998; Brennan *et al.*, 2000; Cliffe-Byrnes and O'Beirne, 2007) because it is highly related with mushroom darkening, a* and b* values are also related to browning and may also be useful to describe the postharvest colour changes of mushrooms (Vízhányó and Felföldi, 2000; Villaescusa and Gil, 2003; Aguirre *et al.*; 2008; Mohapatra *et al.*, 2010). Values of a*, b* and BI are presented in Figs. 8.4, 8.5 and 8.6.

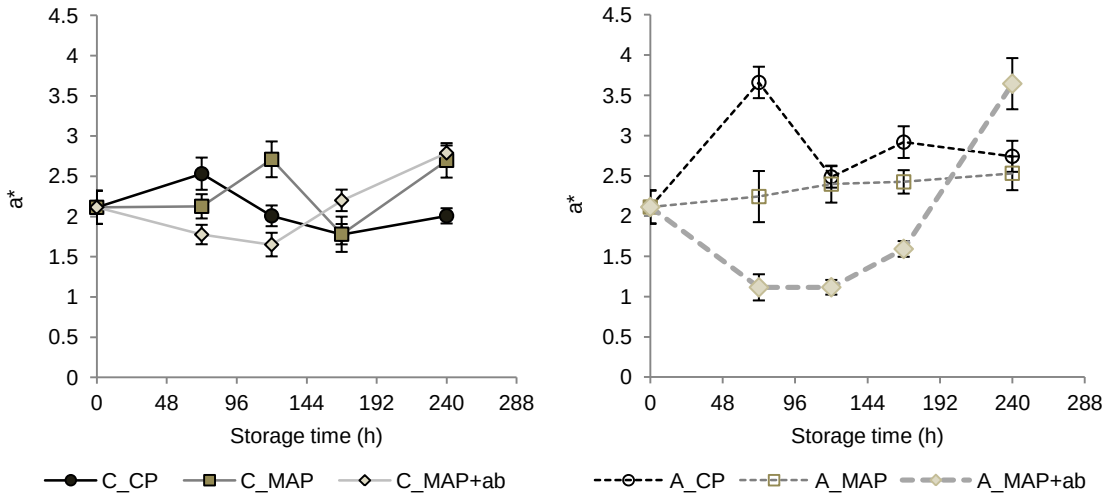

Fig. 8.4 - Evolution of a* parameter of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 30 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

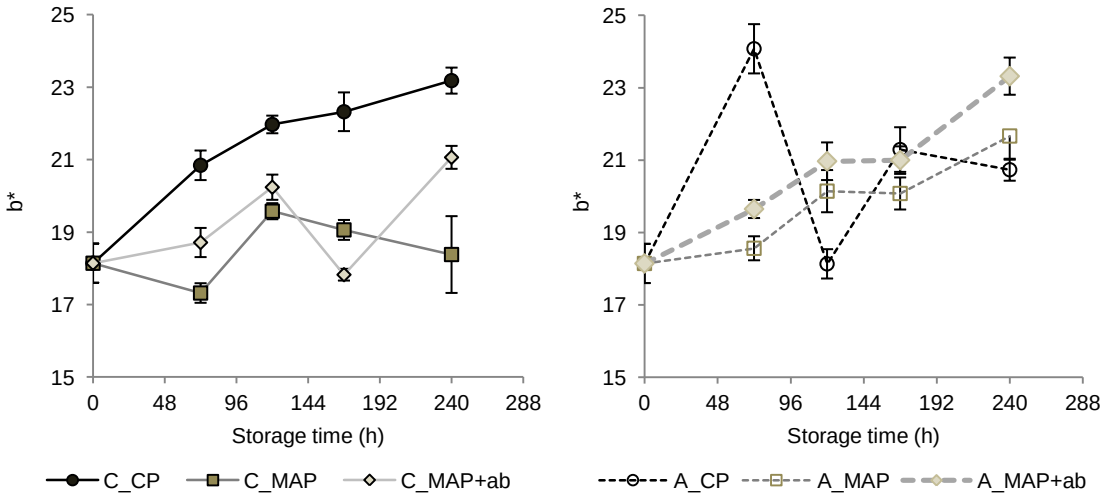

Fig. 8.5 - Evolution of b* parameter of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 30 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

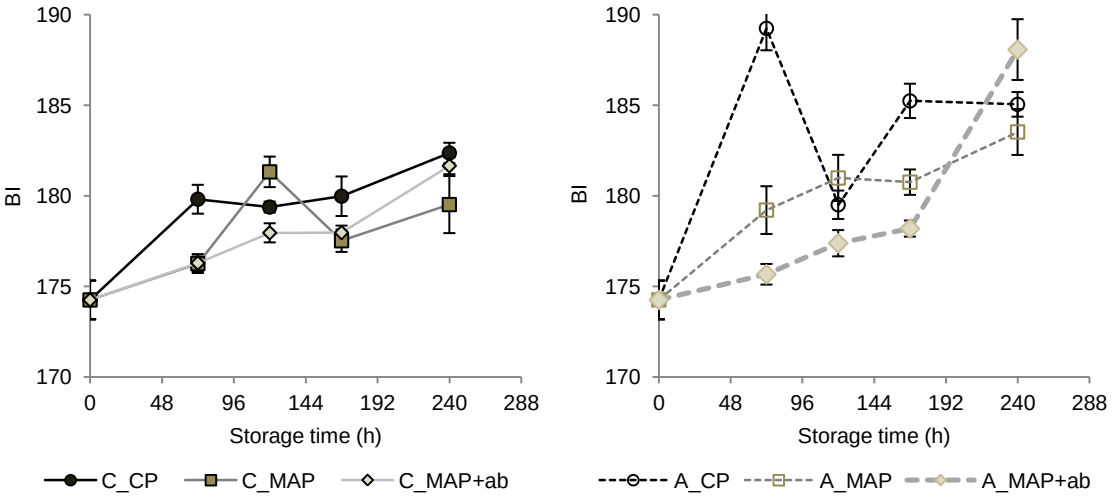

Fig. 8.6 - Evolution of BI parameter of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 30 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

At the beginning of the storage period, oyster mushrooms presented an a* value of 2.11 (± 0.21). This value significantly changed with time (Appendix E, Table E.2) for all conditions, with the exception for oyster mushrooms in MAP stored at fluctuating temperatures, in which changes on a* parameter were not significantly different ($p > 0.05$).

At the end of storage time, a* values ranged from 2.01 (± 0.09) for control packages stored at constant temperature to values of 3.64 (± 0.32) for packages stored under abusive temperatures in MAP with moisture absorbers.

Among the packages stored under constant temperatures, mushroom stored in commercial packages presented significantly lower values of a* ($p < 0.05$) than the other packaging conditions.

Regarding b* value (Fig. 8.5), a gradual increase in this coordinate was found for all samples throughout storage life, with significant effects found for all factors studied (Appendix E, Table E.3). Interactions between factors were also found (Appendix E, Table E.3). Higher increase in b* value was found in packages with atmosphere modification combined with moisture absorbers stored under abusive temperatures. In these packages, oyster mushrooms presented a significant increase

($p < 0.05$, Appendix E, Table E.3) in b* value from 18.14 (± 0.54) to 23.32 (± 0.51) after 240 h of storage.

When stored at constant temperatures, an increase in b* value was also observed, with the exception of the b* value of oyster mushrooms stored in MAP that remained relatively constant (values from 18.14 (± 0.54) to 18.38 (± 1.06).

Browning index can be defined as brown colour purity, is one of the most common indicators of browning in sugar containing food products (Lunadei *et al*., 2011. It can be used to capture the variations in CIE L*a*b* parameters that are related to a turn towards brown colour (Mohapatra *et al.*, 2010)

As observed in Fig. 8.6, BI measured as Maskan (2001) reported, increases over storage time from initial values of 174.26 (±1.07) to values of as high as 188.08 (± 1.68) obtained for mushrooms undergoing temperature fluctuations.

All parameters tested had influence in the evolution of browning of oyster mushrooms. Interactions among factors were also found (Appendix E, Table E.4).

Although BI increased for all package conditions, by the end of storage life, BI was lower for samples stored at constant temperatures. For mushrooms stored under these temperature conditions, *Pleurotus* stored under MAP had the lowest values regarding BI, although this value was not significant different from the other two package conditions ($p < 0.05$; Appendix E, Table E.4) For fluctuating temperatures, mushrooms stored in MAP with moisture absorber had significantly higher values for BI.

Numerical values of a* and b* can be converted and analysed as hue angle (H^o) and chroma (C*) (McGuire, 1992). Values for C* and H^o obtained for oyster mushrooms stored under different conditions are presented in Fig. 8.7 and 8.8, respectively.

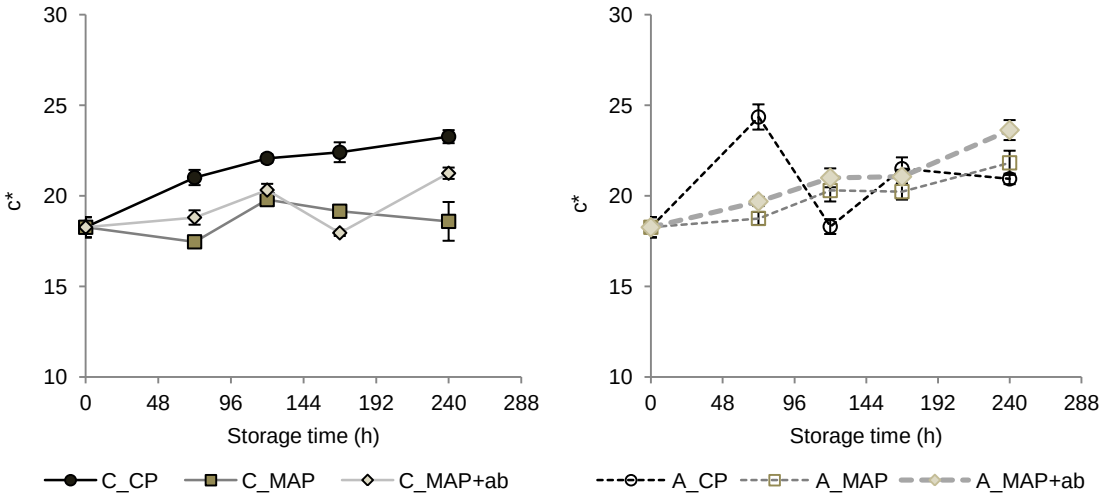

Fig. 8.7 - Evolution of C*of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 30 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

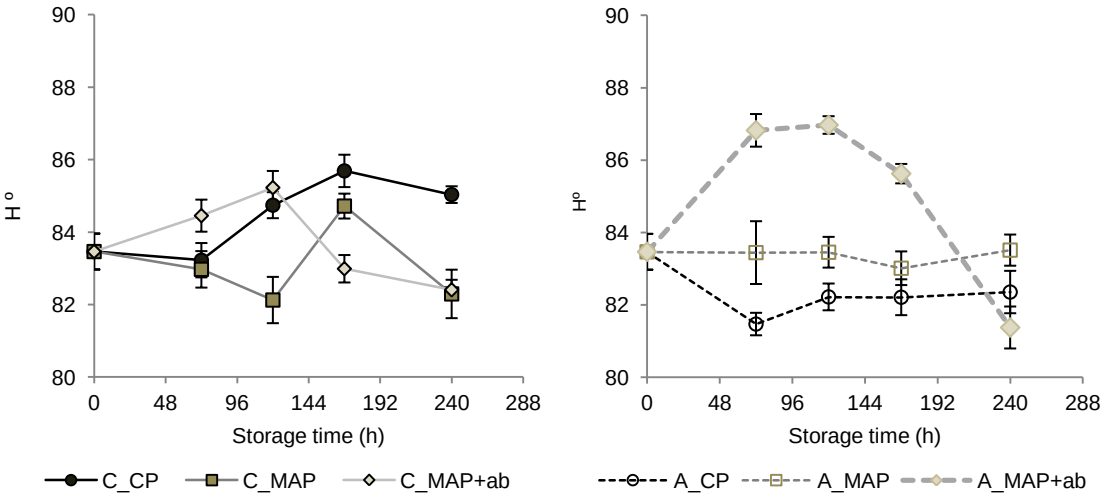

Fig. 8.8 - Evolution of hue values of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 30 replicates and bars are standard error of mean).
Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

In accordance with the values obtained for a* and b*, a significant increase in C* parameter was found at the end of storage time ($p < 0.05$) (Appendix E, Table E.5).

Slightly, non significant ($p > 0.05$, Appendix E, Table E.5) changes were found for mushrooms stored in MAP at constant temperatures (values changed from 18.27 (±

0.56) to values of 18.60 (± 1.07). on the other hand, mushrooms stored at fluctuating temperatures and MAP with moisture absorber had significantly higher values for C*.

Regarding Hº values (Fig. 8.8, Appendix E, Table E.6), higher increase were found for mushrooms stored at constant temperatures in commercial packages (under these conditions, Hº values increases from 83.46 ± 0.50 to 85.03 ± 0.23).

Mushrooms are very susceptible to enzymatic browning (Burton *et al.*, 1987; Jolivet *et al.*, 1998, Brennan *et al.*, 2000). Consequently, a progressive darkening is generally observed throughout storage life for different mushroom species (Tano *et al.*, 1999; Villaescusa and Gil, 2003; Sapata, 2005; Mohapatra *et al.*, 2010). In general, decreases in the value of L*, increase in a*, b* and BI are reported throughout storage life as seen for *Agaricus* (Mohapatra *et al.*, 2010) and *Pleurotus* (Choi and Kim, 2002; Villaescusa and Gil, 2003; Sapata *et al.*, 2004). Villaescusa and Gil (2003) reported an increase in yellowness after storage of oyster mushrooms for 7 days at 7 ºC. Similarly, the increase in H° and C*, as obtained under these experimental conditions also reflects this increasing trend in b* value during storage time.

Storage conditions used had a significant effect on colour changes of fresh oyster mushrooms. Temperature and modified atmosphere affect metabolism of fresh produce (Kader, 2002). Since colour changes in mushrooms, specially browning are governed by enzymatic activity, storage temperature affects the rate of colour changes. As observed in the present study, although colour changes could not be stopped, those changes were lower for mushrooms stored at constant temperatures, especially for L* and a* and BI.

Although changes were found for all samples, decreases in lightness and yellowing were always higher for mushrooms stored under abusive temperature. The fluctuating temperatures encountered in this experiment are not uncommon in the postharvest chain of some commodities. Consequently, even a small period of time at ambient temperature can impair product quality. These results highlight the importance of low temperature maintenance in colour retention of oyster mushrooms.

Texture is an important quality parameter for fresh horticultural commodities including different species of mushrooms (López-Briones *et al.*, 1992; Villaescusa and Gil., 2003; Ares *et al.*, 2007; Parentelli *et al.,* 2007; Oliveira *et al.*, 2012).

Evolution of the firmness during storage life of oyster mushroom is presented in Fig. 8.9.

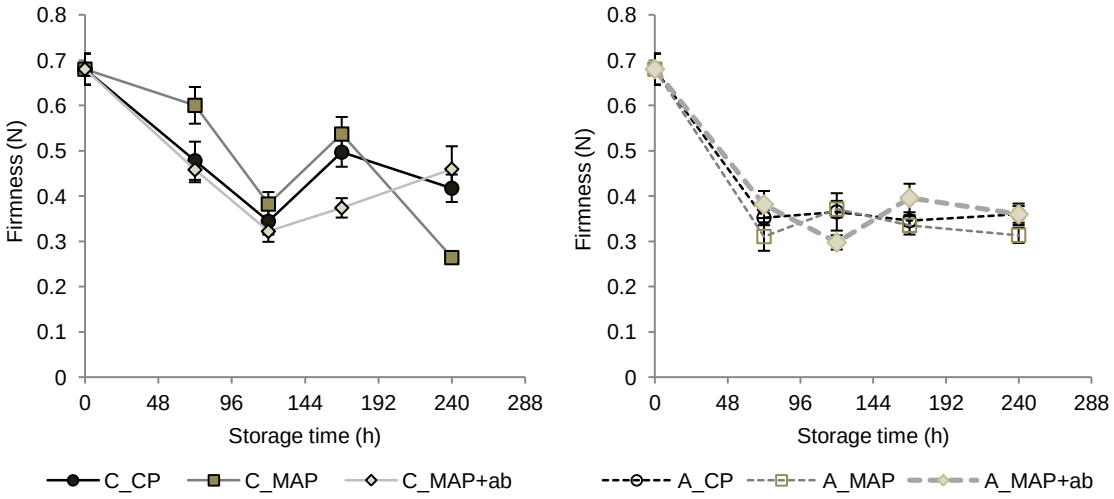

Fig. 8.9 - Evolution of firmness of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 30 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

Firmness of oyster mushrooms significantly decreases throughout storage time ($p < 0.05$; Appendix E, Table E.7) for all conditions tested, from initial value of 0.68 N (SE of 0.03) to values as low as 0.26 N (SE of 0.03) at the end of the storage period. On the other hand, no effect of package was found at the end of storage life for this textural parameter ($p > 0.05$; Appendix E, Table E.7).

Oyster mushroom underwent a rapid loss of firmness after 72 hours for all conditions studied ($p < 0.05$; Appendix E, Table E.7). When stored under temperature fluctuations, mushrooms firmnsess decreased from initial values of 0.68 N (± 0.13 N) to a minimum value of 0.31 N (± 0.03 N) when samples were stored in MAP, although no significant differences were found between packages ($p > 0.05$). At this storage time, constant temperatures also delayed softening ($p < 0.05$; Appendix E, Table E.7) of oyster mushrooms. As storage progresses, temperature profile had a significant effect ($p < 0.05$; Appendix B, Table E.7) on this textural parameter, whereas package did not affect firmness of oyster mushrooms ($p > 0.05$; Appendix E, Table E.7).

Evolution of resilience and cohesiveness of oyster mushrooms is presented in Fig. 8.10 and 8.11, respectively.

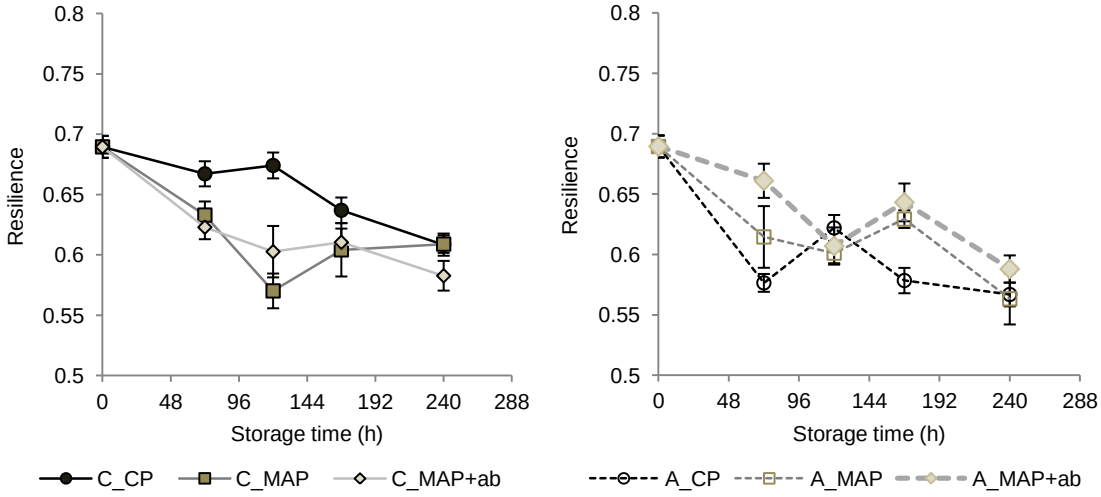

Fig. 8.10 - Evolution of resilience of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 30 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

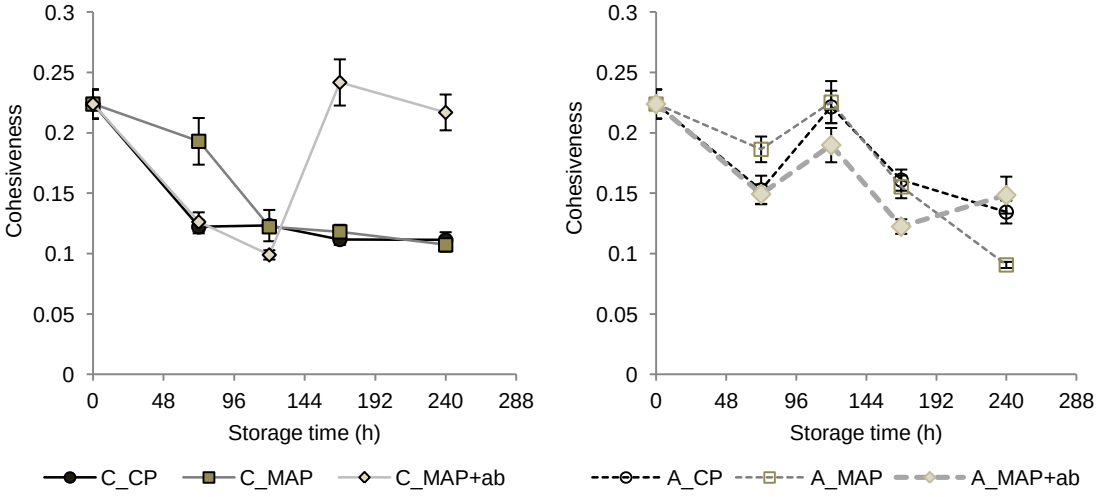

Fig. 8.11 - Evolution of cohesiveness of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 30 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

Resilience and cohesiveness (Figs. 8.10 and 8.11) of oyster mushrooms showed a trend similar to firmness, with a significant ($p < 0.05$; Appendix E, Tabs. 8 and 9) decrease throughout storage life. Both parameters show the effect of all factors studied and interactions between factors were also significant ($p < 0.05$; Appendix E, Tabs. 8 and 9).

This effect of elapsed postharvest time in mushroom textural parameters has been analyzed by several researchers. Reduction in firmness of mushroom has been reported (Antmann *et al*., 2008; Oliveira *et al*., 2012; Tano *et al.*, 1999; Villaescusa and Gil, 2003; Aguirre *et al.*, 2008). While fresh, mushrooms are firm, crisp (resist to deformation), and tender (easy to shear or crew) (Villaescusa and Gil, 2003; Oliveira *et al.*, 2012) and as maturation progresses, mushrooms softens (López-Briones *et al.*, 1992). This decrease in firmness is a result of the cellular wall degradation, hyphae shrinkage, central vacuole disruption and expansion of intercellular space at the pilei surface. Losses of firmness can also be enhanced by bacterial enzymes and increased activity of endogenous autolysins that appear throughout postharvest storage period (Zivanovic *et al.*, 2000).

Texture of mushrooms is affected by temperature (Mohapatra *et al.*, 2010). As observed in the present study, a single cycle of abusive temperature (3 h at ~20 ºC) was enough to decrease the values of firmness for about half of the initial values. Moreover, atmospheres saturated with water could have been responsible for the acceleration of mushroom softening as reported by Antmann *et al*. (2008).

A positive effect on firmness retention have been reported with the use of MAP (Villaescusa and Gil, 2003; Antmann *et al.*, 2008; Oliveira *et al.*, 2012). Sapata *et al.* (2009a), on the other hand, using texturometer Stable Microsystems TA-Hdi, with a load cell of 50 N, using a puncture, with a probe inox P6 (Ø 6 mm reported a significant decrease on postharvest firmness change, from 3.68 N to 1.90 N up to 11 days of storage under MAP. On the other hand, as mushroom maturation progresses, an increase in chitin content and formation of covalent bonds between chitin and R-glucan, increasing the rigidity of the hyphal wall may occur, increasing cohesiveness with storage time (Zivanovic *et al*., 2000; Parentelli *et al.,* 2007).

Values of SSC and pH of samples stored at different conditions are given in Figs. 8.12 and 8.13, respectively.

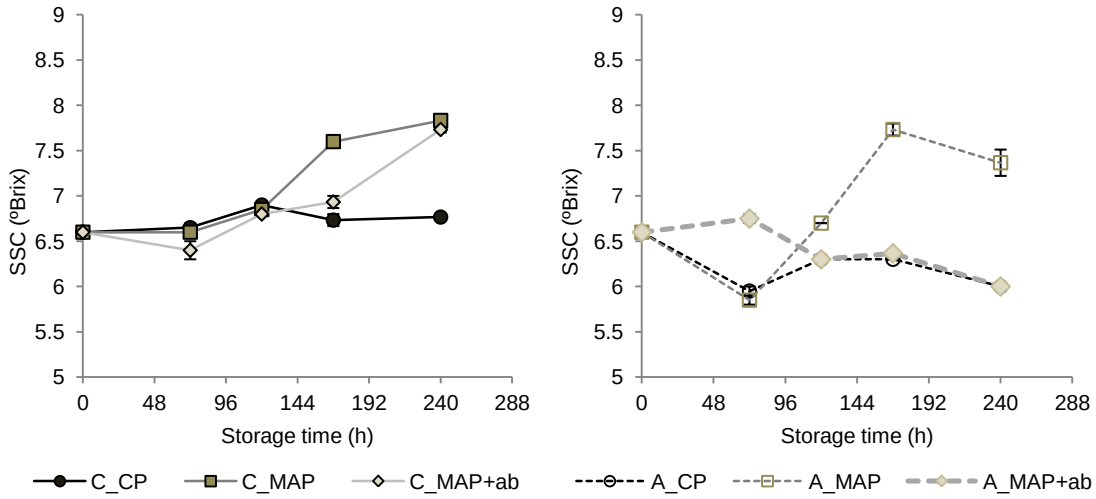

Fig. 8.12 - Evolution of SSC of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 3 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

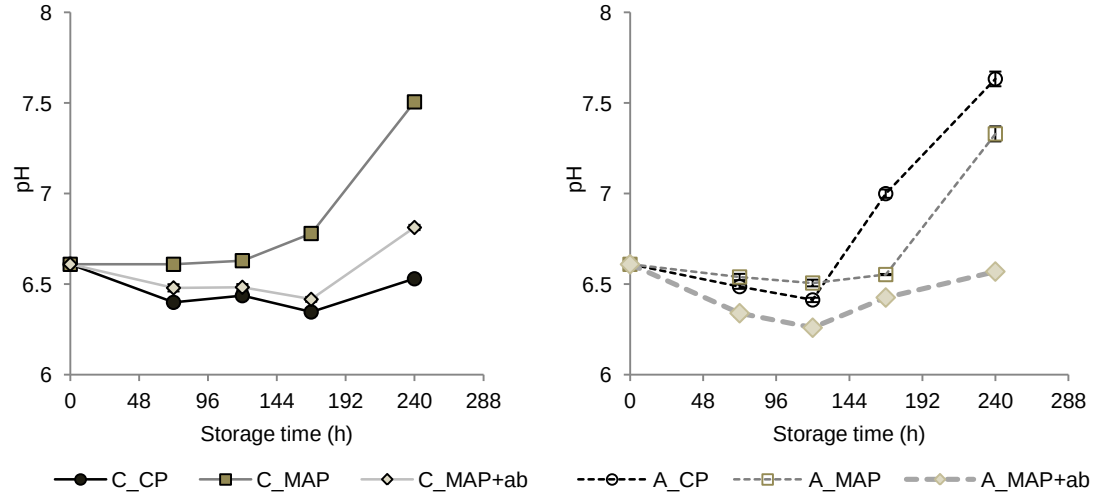

Fig. 8.13 - Evolution of pH of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 3 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

SSC of oyster mushrooms increase over time for most of the conditions studied (Appendix E, Table E.10). The exception was found for mushrooms stored at abusive temperature in commercial or MAP that show a significant decrease in SSC with time.

This decrease in SSC could be a consequence of mushrooms RR and therefore substrate depletion. The results are in accordance with those reported by Hammond and Nichols (1975). Villaescusa and Gil (2003) evaluated the content of soluble solids in oyster mushrooms stored at 4 °C and found a decrease in SSC of mushrooms stored under these conditions. On the other hand, an increase in SSC was observed for all other packages. This effect was more evident when mushrooms were stored at abuse temperatures, with the higher increase in SSC throughout storage life.

Initial oyster mushrooms pH (6.61 ± 0.00) stayed relatively stable during most of the storage time for all treatments. From 168 h onwards, a significant increase ($p < 0.05$; Appendix E, Table E.11) in pH was observed for all conditions tested. At the end of storage life, pH values of oyster mushrooms ranged from 7.63 (± 0.04) to 6.53 (± 0.01) for commercial packages stored at abuse and constant temperatures respectively. All the factors studied presented a significant effect ($p < 0.05$) on pH of oyster mushrooms (Appendix E, Table E.11).

Increases in pH as observed in the present study are generally related with increased in PPO activity, as previous seen in litchi fruits (Jiang and Fu, 1999). Sapata *et al.* (2009a) reported similar results for the pH evolution throughout the storage life of oyster mushrooms. According to the author, an increase in pH value was found (values of pH changed from 6.25 to 7.49) for mushrooms stored under modified atmosphere at 4 ºC for 11 days. On the other hand, Villaescusa and Gil (2003) did not found differences in pH levels for mushrooms stored at 0, 4 and 7 ºC. Evolution of pH throughout storage life of fresh horticultural commodities is related to a number a factors, although as a natural consequence of spoilage, a decrease in pH can be found. The growth of microorganisms in food products results in the production of organic acids, which decreases pH values during storage (Heard, 2002; Masson *et al.*, 2002).

Mass losses that occur in horticultural commodities are an important postharvest process and can significantly limit mushroom quality. Fig. 8.14 shows mass loss throughout storage time for oyster mushrooms.

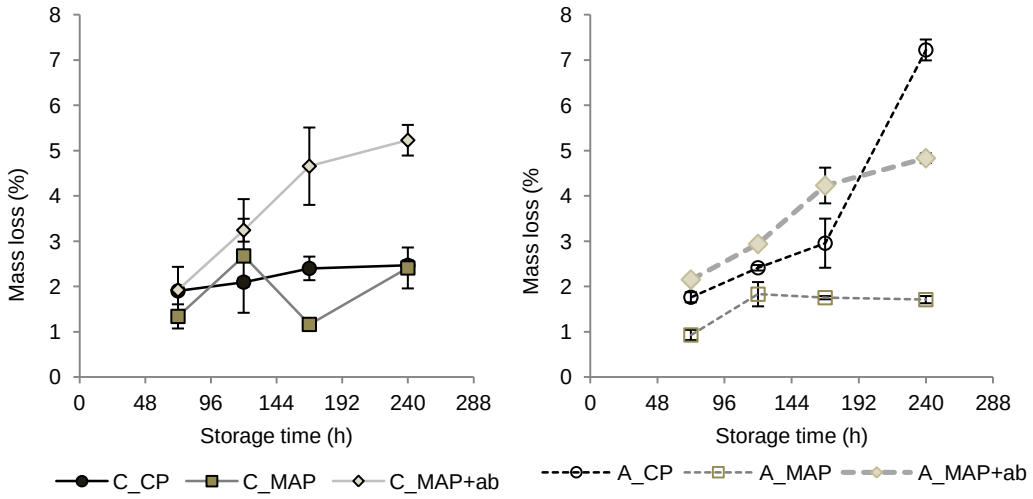

Fig. 8.14 - Evolution of mass loss of *Pleurotus* mushroom stored under different storage conditions (dots are the mean of 3 replicates and bars are standard error of mean).

Treatment identification: C_CP - Constant temperature, Commercial package; C_MAP-Constant temperature, Modified atmosphere packaging; C_MAP+ab-Constant temperature, Modified atmosphere packaging plus moisture absorber; A_CP - Abuse temperature, Commercial package; C_MAP-Abuse temperature, Modified atmosphere packaging; C_MAP+ab-Abuse temperature, Modified atmosphere packaging plus moisture absorber.

Mass loss of oyster mushrooms was influenced by storage time and temperature, although according to the statistical analysis, no effect of storage temperature was found or for the interaction of time and storage temperature.

By the end of storage time, at abusive temperatures, mushrooms had lost 7.22 % (± 0.23 %) of their initial mass when packaged in commercial conditions, which was significantly higher than the value obtained in the previous storage time (2.96 % ± 0.54 %). Mushrooms stored at constant temperature of 4 °C exhibited a maximum mass loss of 5.23 % (± 0.34 %) in the case of MAP with moisture absorbers. When MAP was used alone, mushrooms lost about 2.41 % (± 0.45 %), value very similar to the commercial package. Nevertheless, it should be noticed that, with the exception of the former storage time, MAP retained mass loss of mushrooms throughout the first 168 hours.

Mass loss is a consequence of moisture loss by transpiration and loss of carbon reserves by respiration, with only a minor contribution from respiration (Zagory and Kader, 1988; Maguire *et al.*, 2004). Mushrooms are subject to high water losses (Mahajan *et al*., 2008a), as a consequence of their delicate epidermic structure and high metabolic rate. Besides the obvious economic losses, water loss also influences mushroom deterioration rate due to effects on colour and texture.

Temperature has a major effect on the rate of water loss from fresh products. Moreover, with temperatures fluctuations encountered during postharvest handling chain, occurrence of condensate formation in film packaging or in commodity surface may occurs as a consequence of high air humidity in the head space of fresh commodities packaging (Ben-Yehoshua *et al*., 2003; Tano *et al.*, 2007). Although the lack of high relative humidities inside package can potentially cause severe losses, the excess of water also leads to problems in appearance (skin colour, surface structure, texture) (Feng *et al.*, 2003; Srinivasa *et al.*, 2004) and it may also encourage microbial growth (Brennan *et al.*, 2000).

Films used for oyster mushroom packaging restrained weigh losses throughout storage life, possibly due to the reduced transpiration and respiration rates. By the end of storage time, for mushrooms stored at constant temperature of 4 °C, mass loss value remained slightly below the acceptable mass loss for mushrooms, which is 2 % (Sveine *et al.*, 1967; Tano *et al.*, 1999). Nevertheless, even for control packages, that was not subjected to modify package atmosphere and thus, to potential reduced metabolism, reduced mushrooms dehydration.

By the end of storage period, moisture absorbers had gain 0.67 $g_{water}.g^{-1}$ desiccant (± 0.05 $g_{water}.g^{-1}$ desiccant) and 0.76 $g_{water}.g^{-1}$ desiccant (± 0.05 $g_{water}.g^{-1}$ desiccant) for abuse and constant temperatures respectively.

The addition of moisture absorber did not bring any positive effects regarding oyster mushrooms mass losses. No symptoms of severe mass loss were found although visually, packages had less condensation that the other ones packages conditions. Similar results were found by Roy *et al.* (1995b) for *Agaricus* mushrooms. Although silica gel provided a clear view through the package, no improvements on quality were found when comparing samples with or without silica gel.

Fresh mushrooms were judged by their appearance and odour. Scores obtained for the different samples are presented in Tab. 8.2.

Tab. 8.2 – Evolution of the scores of *Pleurotus* mushroom stored under different storage conditions.

	Storage time (h)			
Package condition	**72**	**120**	**168**	**240**
	Abusive temperature			
CP	1.40 (± 0.27)^{a}	1.20 (± 0.20)^{a}	1.40 (± 0.40)^{a}	1.11 (± 0.11)^{a}
MAP	2.80 (± 0.47)^{a,b}	3.00 (± 0.49)^{b}	2.90 (± 0.48)^{ab}	2.44 (± 0.24)^{b}
MAP + ab	3.60 (± 0.43)^{bc}	3.40 (± 0.43)b	4.60 (± 0.50)^{bc}	2.89 (± 0.35)^{b}
	Constant temperature			
CP	4.30 (± 0.65)^{bc}	4.30 (± 0.33)^{bc}	4.70 (± 0.40)^{c}	4.67 (± 0.33)^{c}
MAP	4.80 (± 0.36)^{c}	4.00 (± 0.39)^{bc}	3.60 (± 0.27)^{bc}	5.33 (± 0.17)^{c}
MAP + ab	4.20 (± 0.25)^{bc}	5.10 (± 0.46)^{c}	3.80 (± 0.49)^{bc}	4.56 (± 0.47)^{c}
p-value	0.001	0.000***	0.001***	0.000***
Chi-square	21.590	25.714	21.486	35.143

Values (mean of ten replicates) in the same column followed by the same letter (a - c) are not significantly different according to Mann-Whitney U test at $p < 0.05$; NS, not significant. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Treatment identification's - Commercial package; MAP- Modified atmosphere packaging; MAP+ab- Modified atmosphere packaging plus moisture absorber

Storage conditions lead to an overall statistically significant difference in the scores obtained for oyster mushrooms. Temperature had a significant effect on the sensory quality of oyster mushrooms. Mushrooms stored under abuse temperature had overall lower scores throughout shelf life, when compared to mushrooms stored under at constant refrigeration temperatures (4 ºC).

After 72 hours of storage, mushroom commercial packages stored under temperature fluctuations had the lowest score among all conditions. In comparison, samples packed with moisture absorber gathered a mean score of 4.40, value that was very similar to the score obtained for the other type of packages (4.30 and 3.60 for commercial packages and modified atmosphere package respectively). By the end of storage life, mushrooms stored under temperature fluctuations were scored with the worst quality and, among those, mushrooms packed in commercial packages had the lowest scores.

The visual quality of *Pleurotus* mushrooms was very good after 72 h of storage. From this time onwards, both temperature profile and package significantly affected mushroom visual quality (Appendix E, Figure E.1).

When packaging film was removed from commercial packages of *Pleurotus* maintained at constant temperature, a slightly strange odour was found that quickly

disappeared. Mushrooms presented characteristic odour, with a yellow colour in bottom surface. Broken lamellaes were also visible, indicating an increase in mushroom maturation. Moreover, mushrooms were soggy, but they were still within the range of marketability.

MAP packages at constant temperatures, presented a very good overall quality, although some moisture was found inside the package. By this storage time, MAP with moisture absorber also presented mushroom in good quality. Moreover, in comparison with other packages, *Pleurotus* stored under these conditions seemed less soggy.

At abusive temperatures, on the other hand, signs of deterioration were found in all packages. Intense off odours were found for control packages and mushroom were all very spongy as a consequence of high amount of water on the product surface. Mushroom stored under MAP or under MAP with desiccant had also off odours, severe yellowing and spongy texture. These characteristics impaired mushrooms acceptance.

Fluorescent pseudomonads (*e.g*. *Pseudomonas putida and Pseudomonas fluorescens*) also produce exopolysaccharides associated with the sliminess accompanying spoilage of *Agaricus* mushrooms (Fett *et al.,* 1995). Although some of the signs of mushrooms spoilage may be related to the presence of harmful microbial populations, Ares *et al*. (2006) reported that, in minimally processed shiitake, the rejection time was more related to the appearance (browning, slime aspect and the presence of fungi on their surface) of than the presence of harmful quantities of microorganisms on mushrooms.

Nevertheless, throughout all storage period considered, *Pleurotus* mushrooms quality was possible affected by the water content of the tissues and by the high condensation created inside the package

8.4. Conclusions

Fresh oyster mushrooms were stored under different packages at two different temperature regimes and their quality was studied over a 240 storage period. Levels of O_2 and CO_2 inside the packages and quality evaluation that included both physical (visual appearance, mass loss, colour of the cap and texture) and chemical (pH, SSC)

parameters were determined over a period of 240 h. Mushrooms were stored under commercial conditions, in a modified atmosphere packaging (active MAP) and a MAP with added moisture absorber. Temperature profile significantly affected the quality of oyster mushrooms, with mushrooms maintained under abuse temperature showing a fast rate of quality loss.

PART III - General conclusions and suggestions for future work

Chapter 9: General conclusions and suggestions for future work

9.1. General conclusions

Mushrooms have been gaining recognition as a source of unique properties that includes high nutritional qualities, functional and therapeutically properties. These amazing commodities are subjects of numerous scientific studies regarding biology, genetics, cultivation and also health promoting effects.

Edible mushrooms constitute a rich source of bioactive compounds, including non-starch polysaccharides, polysaccharide–protein and polysaccharide–peptide complexes, ribonucleases, proteases and lectins. Those components exhibit several recognized health promoting properties such as antitumor, hypocholesterolemic, immunosuppressive, antioxidant, antimicrobial and anti-inflammatory properties (Khan and Tania, 2012; Roupas *et al*., 2012; Cheung, 2013; Roy and Prasad, 2013).

In addition to the exceptional organoleptic characteristic and recognized culinary versatility, these unique features increase consumer's curiosity towards mushrooms and, nowadays mushrooms market is marked by an increase in mushroom species offered to the consumer. Under the common name of "oyster mushroom", several species of mushroom belonging to the genus *Pleurotus* are available and popularly consumed all over the world due to their taste and flavour. Within the genus, *Pleurotus ostreatus* (also recognized as oyster mushroom), is becoming increasingly important. Organoleptic quality (Cuppett *et al.*, 1998), undemanding cultivation conditions (Gregori *et al.*, 2007; Sánchez, 2010), high nutritional value (Manzi *et al.*, 1999; Mattila *et al.*, 2001; Lindequist *et al.*, 2005) and recognized therapeutic properties (Roy and Prasad, 2013), are possible explanations to the popularity of this specie.

Despite this potential interest in oyster mushroom production and consumption, they present a very short shelf life (Bano *et al.*, 1988; Villaescusa and Gil, 2003) and very little information about postharvest physiology and personalized postharvest technologies are known. In a work regarding postharvest characteristics of oyster mushrooms, Rajarathnam *et al*. (1983) reported a shelf-life of 12 hours for *P*.

flabellatus maintained at 25 ºC and a 30 % mass loss within 24 hours at the same temperature.

Physiological processes are important in determining deterioration rate and of oyster mushrooms (Rajarathnam *et al.*, 1983; Bano *et al.*, 1988; Villaescusa and Gil, 2003; Sapata *et al.*, 2009a,b). Besides the natural metabolism, absence of cuticle and large but thin mushroom surface also contribute to their extreme perishability (Bohling and Hansen, 1988). Accelerated postharvest metabolism of oyster mushrooms presents an important drawback for consumption of the produce in an ideal fresh state. In order to meet consumer demands and fulfil their expectations for fresh, tasteful and nutritious mushrooms, postharvest characteristics of each mushroom specie should be known. Although strictly storage temperature control is required to maintain optimum quality (Kader and Saltveit, 2003), knowledge of physiological postharvest specificities are useful to the development of other effective postharvest technologies.

Packaging is a fundamental tool in order to retain general quality and the use of MAP for the postharvest preservation of horticultural commodities has been recognised as an important technology to reduce losses, maintain quality and extend shelf life throughout the distribution chain (Kader *et al.*, 1989; Fonseca *et al.*, 2002a). MAP has been used for several mushrooms species, but leads to water accumulation resulting in produce sliminess and enhancement of microbial growth. The global goal of this thesis is to contribute for the knowledge of oyster mushrooms postharvest characteristics.

In a first approach of the experimental work of this thesis, the effect of storage temperature on fresh oyster mushrooms was analysed. Evaluation of temperature effect was performed on fresh oyster mushrooms stored at 2, 6, 10, 14 and 18 ºC with ~95 % RH. Physico-chemical attributes and respiration rates were assessed over a 96 h storage period (**Chapter 3**). Fresh mushrooms were separated from the cluster, allowed to equilibrate at each storage temperature and respiration rate and quality attributes were immediately analysed. On the day of harvest and after sample processing, high respiration rate for oyster mushroom were observed. A decrease on respiration rate with time was observed after the beginning of the experiment. Oyster mushroom quality also decreases with time. From the greyish natural colour, oyster mushrooms underwent yellowness (increase in CIE b* value) for all temperatures. As expected, increases were higher for higher temperatures. Mushroom membrane, evaluated by the relative electrolyte leakage, also suffered some damage over storage.

Severe mass losses were also found. In fact, oyster mushrooms presented severe dehydration for temperatures of 18 ºC, impairing the freshness of the produce. Considering the quality attributes studied, as storage temperature increases, the rate of deterioration speeds up, with temperatures higher than 6 ºC, affecting significantly the quality, even a few hours after harvest. For lower temperatures of 2 and 6 ºC, less deterioration was found.

Respiration rate studies and consequent quantification and modelling are a key factor in the design of the best strategies for storage and packaging. Several respiration rate models are available for a broad range of commodities.

Respiration of a produce depends on a wide range of environmental factors although temperature and atmospheric composition are the most important Respiration rate is also expected to change with elapsed time and therefore should be included on respiration rate studies. In **Chapter 4**, a mathematical model for the effect of time and storage temperature on *Pleurotus* mushroom respiration rate was developed. Respiration rates were measured in a closed system at 8 h intervals for 240 hours at 2, 6, 10, 14 and 18 ºC with ~95% RH. The respiration rate decrease with time was described with a first order kinetic model, with Arrhenius dependence for storage temperature. For each temperature, a clear relationship between initial respiration rate and respiration rate at equilibrium was also found and incorporated in the model. The developed model, should give reliable predictions of respiration rates of oyster mushrooms with storage time at any temperatures.

Most fresh fruits and vegetables maintain quality with high CO_2 concentrations and low O_2 concentrations longer than in air (Kader *et al*., 1989). Atmospheric composition also affects the metabolic rate of fresh produce. Oyster mushrooms were stored under different O_2 and CO_2 concentrations at 2 ºC (**Chapter 5**) for 4 days. Results show that respiration rate of oyster mushrooms was affect by the levels of O_2 and CO_2. Within the compositions tested, low levels of O_2 (2 % v/v) and high levels of CO_2 (20 % v/v) maintained respiration rate at low levels, without inducing fermentation.

Another physiological process strictly linked with respiration is the transpiration rate. Both respiration and transpiration rate result in mass losses of fresh produce.

In **Chapter 6**, oyster mushrooms, obtained one day after harvest were stored on relative humidity of 86, 96 and 100 % and on temperatures interval previous

defined. Results show linear mass losses with time and an empirical transpiration model describing the effect of storage temperature based on an Arrhenius type equation and linear relationships with relative humidity was developed.

High relative humidity diminishes dehydration and avoids wilting and shrivelling of the product. Nevertheless, the maintenance of a very high relative humidity can promote moisture condensation on the commodity, due to temperature fluctuation. Condensation creates conditions favourable for pathogen growth (Henig and Gilbert, 1975; Zagory and Kader, 1988). Thus, water condensation should be addressed when a MAP system is to be exploited in commercial scale.

Although the use of high relative humidity inside packages might bring benefits regarding a reduction in postharvest mass losses, other problems may occur. In fact, one of the main problems related with packaging of horticultural commodities with high metabolic rates is the condensation of moisture on the product and on the film used. To overcome this problem, the use of moisture absorbers has been suggested. Nevertheless, existing moisture absorbers don't have the requirement needed for packaging of fresh commodities. On **Chapter 7**, a simplex lattice design was used to studt different mixtures. CaO, $CaCl_2$ and sorbitol were mixed in different proportions and MHC for each mixture was determined. The optimized mixture containing 0.26, 0.24 and 0.50 of $CaCl_2$, CaO and Sorbitol, respectively has a moisture holding capacity of 0.814 g water. g^{-1} desiccant at 10 ºC and 96 % RH. This information suggests that the moisture absorber may the useful for the development of a novel packaging system for oyster mushrooms.

In the last chapter regarding experimental work of this thesis (**Chapter 8**), previous information was gathered and a package for oyster mushroom was validated. Commercial packages of oyster mushrooms were compared with active modified atmosphere (5 % O_2 and 10 % CO_2) and an active modified atmosphere with the selected moisture absorber. Since it is known that temperature abuses promote major damage in fresh produce, two temperature profiles were tested. Mushrooms packages were maintained at constant temperatures of 4 ºC, while other packages were maintained at 4 ºC, but with an abuse of temperature (3 h at ~20 ºC) every 48 h. After 240 h of storage, mushrooms presented some signs of deterioration. In commercial packages, mushrooms were very soggy as a result of the high water content in the

package. On the other hand, mushrooms in MAP with added moisture absorber had partially less amount of water inside the package.

9.2. Suggestions for future work

The development of a reliable MAP should be carefully design, since so many factors can affect the respiration rate. It has been recognized that, studies on respiration rate must consider the combined effect of temperature and gas composition (Fonseca *et al.* 2002a). Therefore, although respiration rate under different gas compositions was evaluated at the optimum temperature found for storage (2 ºC), with a positive effect on RR, it will be of utmost importance to verify the same trend under different storage temperatures.

Since tolerance limits for O_2 and CO_2 for a particular commodity may be affected by the concentration of the other gas (Kader *et al.*, 1989), studies on O_2 and CO_2 limits for aerobic respiration could provide some additional information on the dependence of O_2 and CO_2 on mushroom respiration rate and mushroom quality and for the safe levels of O_2 and CO_2 for MAP marketing of mushrooms.

Furthermore, since quality of fresh produce includes a combination of different quality attributes, studies on the effect of O_2 and CO_2 on mushrooms quality attributes is necessary.

Studies on the microbiological aspects of mushroom storage and the effect of postharvest quality should be developed to ensure quality and safety of the product.

Loss of water from products it's important and studies on the influence of relative humidity and temperature on other quality attributes should also be developed.

Regarding moisture absorbers, although they present potential for the development of a novel MAP system for mushrooms, the effect of time on each combination should be studied and other possible combination should be studied.

Although the contribution of some factors is not as significant as that of others, their combined or even synergetic effect (known as the hurdle technology) is important to preserve the overall product quality. From that point of view, the combination of

novel methods for mushroom packaging needs to be examined. Irradiation has been suggested as a reliable technology to control microbiological spoilage. Therefore, irradiation used with MAP should provide additional hurdles for oyster mushrooms.

List of publications and communications based on thesis work

Refereed papers

- Azevedo S, Cunha LM, Mahajan PV, Fonseca SC. Application of simplex lattice design for development of moisture absorber for oyster mushrooms. *Procedia Food Science.* 2011:1(0):184-189. DOI:10.1016/j.profoo.2011.09.029.

- Azevedo S, Cunha LM, Fonseca SC., Modelling the influence of time and temperature on the respiration rate of fresh oyster mushrooms, *Food Science and Technology International*. 2014. DOI: 10.1177/1082013214555925.

- Azevedo S, Cunha LM, Fonseca SC, Oliveira JC and Mahajan PV, Modelling the influence of time, temperature and relative humidity conditions on the mass loss of fresh oyster mushrooms (submitted to the *Journal of Food Science*).

- Azevedo S, Cunha LM, Fonseca SC., Influence of storage conditions on the shelf life of fresh oyster mushrooms (submitted to the *Journal of Food Science*).

Non- Refereed papers

- Azevedo S, Cunha LM, Fonseca SC (2011), Influência das condições de armazenamento na vida útil de cogumelos pleurotos (*Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm.)", Agrotec, (1), 44-46.

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- Azevedo, S. Cunha, LM, Mahajan PV, Fonseca, SC (2011), Application of simplex lattice design for development of moisture absorber for oyster mushrooms, work presented at *11th International Congress on Engineering and Food (11th ICEF) - "Food Process Engineering in a Changing World*" em Atenas, Grécia, 22-26 Maio, 2011.

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Appendices

Appendix A: CIE L*a*b* colour parameters

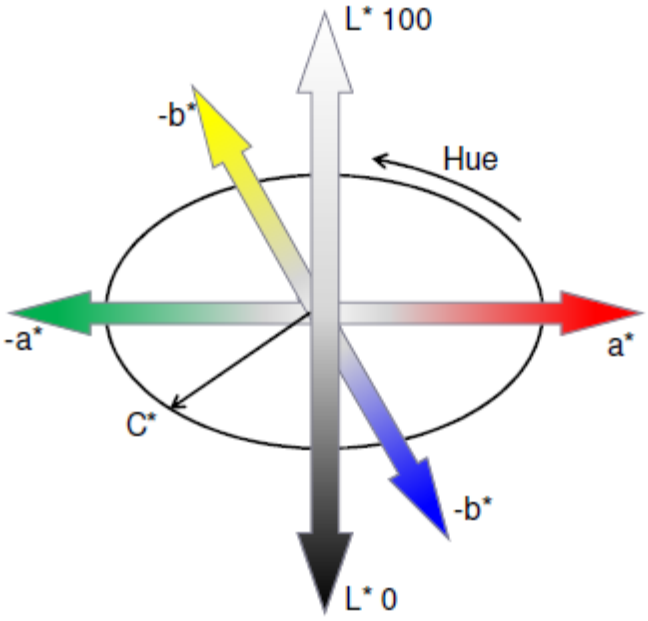


Figure A.1 - CIEL*a*b* chromaticity diagram. CIEL*a*b* space represent colour in a dimension rectangular space based on the opponent colours theory, where L* represent the Lightness that is related to the changes from white (+100) to black (0) in a food item. a* (vary on a green (-a*) to red (+a*) and b* represent yellowness. h (Hº) is an angle in a colour wheel of 360º, with 0º, 90º, 180º and 270º representing the hues red-purple, yellow, bluish-green and blue, respectively. Chroma is the intensity or purity of the hue, measures colour saturation and vary between 0 (pale colour) and +60 (vivid colour), source: Berns, 2000.

Appendix B: Supplementary material pertaining to Chapter 3.

Table B.1 - Evolution of respiration rate, RR_{O_2} ($mL.kg^{-1}.h^{-1}$) of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	111.93 (± 5.93)^{a,C}	137.97 (± 14.24)^{a,B}	235.15 (± 11.24)^{b,C}	215.45 (± 16.34)^{b,C}	222.67 (± 5.49)^{b,C}	0.000
24	61.76 (± 10.54)^{a,A,B}	105.73 (± 4.40)^{a,b,A,B}	151.28 (± 7.75)^{b,c,B}	185.51 (± 21.47)^{c,d,B,C}	215.49 (± 6.68)^{d,c,B,C}	0.000
48	24.44 (± 5.01)^{a,A}	111.54 (± 9.64)^{b,c,A,B}	129.04 (± 9.06)^{b,c,A,B}	91.80 (± 19.65)^{b,c,A}	160.09 (± 7.93)^{c,B}	0.000
72	36.82 (± 2.52)^{a,A,B}	82.01 (± 0.45)^{b,A}	94.00 (± 6.02)^{b,c,A}	117.54 (± 7.67)^{b,c,A,B}	128.14 (± 14.17)^{c,A,B}	0.000
96	36.56 (± 8.61)^{a,A,B}	73.12 (± 1.88)^{b,A}	120.95 (± 4.21)^{c,A,B}	74.30 (± 8.81)^{b,A}	94.15 (± 6.45)^{b,c,A}	0.000
p-value (one-way ANOVA)	0.000	0.004	0.000	0.000	0.000	0.000*

a,b,c – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B,C– homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.2 - Evolution of Respiration rate, RR_{CO_2} ($mL.kg^{-1}.h^{-1}$) of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	107.13 (± 4.32)^{a,B}	131.67 (± 5.84)^{a,b,C}	182.50 (± 26.35)^{b,c,B}	194.19 (± 5.76)^{b,c,D}	223.17 (± 16.65)^{c,C}	0.001
24	53.41 (± 10.81)^{a,A}	83.23 (± 2.40)^{a,b,A,B}	117.08 (± 8.36)^{b,c,B,A}	136.60 (± 14.03)^{c,C}	189.33 (± 4.13)^{d,C,B}	0.000
48	32.40 (± 6.72)^{a,A}	84.81 (± 8.39)^{a,B}	105.98 (± 3.06)^{b,c,A}	74.78 (± 13.89)^{a,b,A,B}	135.33 (± 16.59)^{c,A,B}	0.001
72	36.82 (± 2.52)^{a,A}	70.29 (± 0.39)^{b,A,B}	82.15 (± 6.74)^{b,c,A}	101.84 (± 2.39)^{c,B,C}	136.58 (± 11.21)^{d,A,B}	0.000
96	44.51 (± 6.76)^{a,A}	61.85 (± 1.80)^{a,A}	83.88 (± 15.29)^{a,A}	44.51 (± 6.05)^{a,A}	74.02 (± 10.59)^{a,A}	0.046
p-value (one-way ANOVA)	0.000	0.000	0.008	0.000	0.000	0.000*

a,b,c,d – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B,C, D – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.3 - Evolution of RQ of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.139 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	0.96 (± 0.05)^{a,A}	0.97 (± 0.05)^{a,B}	0.78 (± 0.12)^{a,A}	0.91 (± 0.05)^{a,A}	1.01 (± 0.10)^{a,A}	0.366
24	0.85 (± 0.04)^{a,A}	0.79 (± 0.01)^{a,A}	0.77 (± 0.04)^{a,A}	0.75 (± 0.08)^{a,A}	0.88 (± 0.03)^{a,A}	0.244
48	1.33 (± 0.06)^{b,A}	0.76 (± 0.01)^{a,A}	0.83 (± 0.08)^{a,A}	0.84 (± 0.09)^{a,A}	0.84 (± 0.08)^{a,A}	0.004
72	1.00 (± 0.00)^{a,b,A}	0.86 (± 0.00)^{a,B,A}	0.88 (± 0.02)^{a,A}	0.87 (± 0.05)^{a,A}	1.07 (± 0.03)^{b,A}	0.016
96	1.32 (± 0.27)^{b,A}	0.84 (± 0.01)^{a,b,B,A}	0.70 (± 0.13) ^{a,b,A}	0.60 (± 0.06)^{a,A}	0.79 (± 0.12)^{a,b,A}	0.044
p-value (one-way ANOVA)	0.100	0.010	0.798	0.059	0.246	0.024*

a,b – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.4 - Evolution of L* parameter of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	62.00 (± 0.81)^{c,A}	59.67 (± 0.78)^{c,b,A}	61.91 (± 0.69)^{c,b,A}	58.27 (± 1.10)^{a,b,A}	55.06 (± 1.25)^{a,A}	0.000
24	62.13 (± 0.83)^{a,b,A}	60.86 (± 0.69)^{a,A,B}	64.44 (± 0.47)^{b,B}	61.86 (± 1.05)^{a,b,A,B}	59.77 (± 1.18)^{a,B}	0.004
48	62.32 (± 0.83)^{a,A}	62.49 (± 0.62)^{a,B,C}	65.88 (± 0.43)^{b,B,C}	64.54 (± 1.00)^{a,b,B,C}	62.40 (± 0.98)^{a,B,C}	0.004
72	63.24 (± 0.78)^{a,A}	63.38 (± 0.60)^{a,B,C}	67.29 (± 0.50)^{b,C}	65.67 (± 1.14)^{a,b,B,C}	64.44 (± 0.73)^{a,b,C}	0.001
96	63.57 (± 0.84)^{a,A}	64.49 (± 0.59)^{a,C}	67.69 (± 0.48)^{b,C}	66.14 (± 0.96)^{a,b,C}	64.30 (± 1.00)^{a,C}	0.002
p-value (one-way ANOVA)	0.5640	0.0000	0.0000	0.0000	0.0000	0.000*

a,b,c – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.5 - Evolution of a* parameter of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	3.18 (± 0.14)^{b,A}	3.22 (± 0.12)^{b,A}	3.01 (± 0.12)^{a,b,B}	2.84 (± 0.15)^{a,b,A}	2.61 (± 0.13)^{a,A}	0.005
24	3.39 (± 0.14)^{a,A}	3.16 (± 0.10)^{a,A}	2.98 (± 0.12)^{a,B}	3.23 (± 0.12)^{a,A,B}	3.19 (± 0.12)^{a,A,B}	0.209
48	3.24 (± 0.13)^{a,b,A}	3.26 (± 0.09)^{a,b,A}	2.87 (± 0.12)^{a,A,B}	3.62 (± 0.13)^{b,c,B}	3.85 (± 0.14)^{c,C}	0.000
72	3.23 (± 0.13)^{a,b,A}	3.38 (± 0.09)^{b,A}	2.73 (± 0.14)^{a,A,B}	3.66 (± 0.13)^{b,B}	3.58 (± 0.17)^{b,B,C}	0.000
96	3.10 (± 0.14)^{b,A}	3.05 (± 0.11)^{b,A}	2.46 (± 0.14)^{a,A}	3.51 (± 0.12)$^{b\ c,B}$	3.76 (± 0.22)^{c,B,C}	0.000
p-value (one-way ANOVA)	0.654	0.219	0.016	0.000	0.000	0.000*

a,b,c – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.6 - Evolution of b* parameter of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	10.56 (± 0.30)^{b,A}	10.39 (± 0.30)^{b,A}	11.67 (± 0.28)^{c,A}	10.21 (± 0.16)^{b,A}	9.05 (± 0.34)^{a,A}	0.000
24	11.00 (± 0.27)^{a,A,B}	11.12 (± 0.26)^{a,A}	12.91 (± 0.29)^{c,B}	12.67 (± 0.14)^{b,c,B}	11.91 (± 0.24)^{a,b,B}	0.000
48	11.88 (± 0.25)^{a,B,C}	12.13 (± 0.22)^{a,B}	13.73 (± 0.29)^{b,B,C}	14.22 (± 0.16)^{b,C}	14.00 (± 0.36)^{b,B,C}	0.000
72	12.64 (± 0.22)^{a,C,D}	13.30 (± 0.18)^{a,b,C}	14.49 (± 0.30)^{b,c,C}	16.08 (± 0.42)^{c,D}	15.57 (± 0.85)^{c,C}	0.000
96	12.99 (± 0.20)^{a,D}	13.78 (± 0.20)^{a,C}	14.35 (± 0.30 ^{a,C}	17.07 (± 0.52)^{b,D}	16.22 (± 0.84)^{b,C}	0.000
p-value (one-way ANOVA)	0.000	0.000	0.000	0.000	0.000	0.000*

a,b,c – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.7 - Evolution of C* parameter of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	11.06 (± 0.30)^{b,c,A}	10.90 (± 0.30)^{b,A}	12.06 (± 0.29)^{c,A}	10.66 (± 0.13)^{b,A}	9.47 (± 0.32)^{a,A}	0.000
24	11.54 (± 0.27)^{a,A,B}	11.58 (± 0.27)^{a,A}	13.26 (± 0.30)^{b,B}	13.11 (± 0.13)^{b,B}	12.36 (± 0.22)^{a,b,B}	0.000
48	12.33 (± 0.26)^{a,B,C}	12.57 (± 0.23)^{a,C}	14.04 (± 0.31)^{a,B,C}	14.71 (± 0.15)^{b,C}	14.56 (± 0.35)^{b,B,C}	0.000
72	13.07 (± 0.24)^{a,c,D}	13.73 (± 0.19)^{a,D}	14.76 (± 0.32)^{a,b,C}	16.52 (± 0.41)^{c,D}	16.04 (± 0.84)^{b,c,C}	0.000
96	13.37 (± 0.22^{a,C}	14.13 (± 0.21)^{a,D,C}	14.58 (± 0.32)^{a,C}	17.45 (± 0.51)^{b,D}	16.68 (± 0.85)^{b,C}	0.000
p-value (one-way ANOVA)	0.0000	0.0000	0.0000	0.0000	0.0000	0.000*

a,b,c – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.8 - Evolution of Hº parameter of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	73.11 (± 0.71)^{a,A}	72.59 (± 0.63)^{a,A}	75.55 (± 0.43)^{a,A}	74.19 (± 0.98)^{a,A}	73.22 (± 1.00)^{a,A}	0.066
24	72.81 (± 0.72)^{a,A}	74.04 (± 0.46)^{a,b,A,B}	77.08 (± 0.37)^{c,B}	75.62 (± 0.61)^{b,c,A,B}	74.74 (± 0.66)^{a,b,A,B}	0.000
48	74.80 (± 0.48)^{a,A,B}	74.95 (± 0.36)^{a,B}	78.34 (± 0.34)^{b,B,C}	75.62 (± 0.59)^{a,A,B}	74.36 (± 0.66)^{a,A,B}	0.000
72	75.76 (± 0.46)^{a,B}	75.75 (± 0.33)^{a,B}	79.43 (± 0.39)^{b,C,D}	76.86 (± 0.56)^{a,B}	75.93 (± 0.76)^{a,A,B}	0.000
96	76.73 (± 0.49)^{a,B}	77.59 (± 0.35)^{a,C}	80.38 (± 0.42)^{b,D}	78.04 (± 0.48)^{a,B}	76.75 (± 0.60)^{a,B,B}	0.000
p-value (one-way ANOVA)	0.000	0.000	0.000	0.001	0.011	0.850*

a,b,c – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.9 - Evolution of BI of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.010 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
0	171.20 (± 0.63)^{a,b,A}	171.76 (± 0.62)^{a,b,A}	172.55 (± 0.67)^{b,A}	171.50 (± 0.50)^{a,b,A}	170.25 (± 0.49)^{a,A}	0.091
24	172.08 (± 0.61)^{a,A,B}	172.28 (± 0.55)^{a,b,A}	173.27 (± 0.64)^{a,b,A}	174.38 (± 0.52)^{b,A,B}	173.94 (± 0.47)^{a,b,A}	0.014
48	173.12 (± 0.67)^{a,A,B}	173.30 (± 0.53)^{a,A,B}	173.70 (± 0.66)^{a,A}	176.14 (± 0.55)^{b,B,C}	177.05 (± 0.69)^{b,A}	0.000
72	173.30 (± 0.53)^{a,B}	174.78 (± 0.50)^{a,B}	174.04 (± 0.67)^{a,A}	178.65 (± 0.98)^{a,C,D}	178.19 (± 1.29)^{a,A}	0.266
96	174.03 (± 0.71)^{a,B}	174.57 (± 0.56)^{a,B}	173.33 (± 0.68)^{a,A}	179.52 (± 1.03)^{b,D}	180.13 (± 1.69)^{b,A}	0.000
p-value (one-way ANOVA)	0.0120	0.0000	0.5880	0.0000	0.0550	0.000*

a,b,c – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.10 - Evolution of TL of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
24	4.27 (± 0.47)^{a,A}	3.95 (± 0.33)^{a,b,A}	3.27 (± 0.33)^{a,b,A}	6.64 (± 0.54)^{b,A}	7.16 (± 0.81)^{c,A}	0.002
48	4.24 (± 0.48)^{a,A}	4.02 (± 0.42)^{a,b,A,B}	4.04 (± 0.42)^{b,c,A,B}	7.46 (± 0.75)^{c,A,B}	7.70 (± 0.90)^{d,A}	0.000
72	4.38 (± 0.40)^{a,A}	4.27 (± 0.49)^{a,b,B}	5.48 (± 0.49)$^{a,b,B;C}$	9.01 (± 0.79)^{b,A,B}	9.43 (± 0.71)^{c,A}	0.000
96	4.76 (± 0.43)^{a,A}	5.18 (± 0.50)^{b,B}	5.82 (± 0.50)^{b,C}	9.06 (± 0.63)^{b,B}	9.75 (± 0.87)^{c,A}	0.000
p-value (one-way ANOVA	0.539	0.001	0.000	0.019	0.003	0.000*

a,b,c,d – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.11 - Evolution of TCD of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	p-value (one-way ANOVA)
24	4.87 (± 0.41)^{a,A}	4.51 (± 0.29)^{A}	4.24 (± 0.25)^{A}	7.40 (± 0.46)^{A}	7.98 (± 0.79)^{A}	0.000
48	4.96 (± 0.43)^{a,A}	4.82 (± 0.36)^{a,A}	5.28 (± 0.30)^{a,A}	8.96 (± 0.64)^{b,A}	9.88 (± 0.80)^{b,A}	0.000
72	5.21 (± 0.37)^{a,A}	5.68 (± 0.37)^{a,A,B}	6.82 (± 0.34)^{a,B}	11.65 (± 0.56)^{b,B}	12.56 (± 0.76)^{b,B}	0.000
96	6.65 (± 0.40)^{a,A}	6.72 (± 0.36)^{a,B}	7.08 (± 0.33)^{a,B}	12.06 (± 0.52)^{b,B}	13.78 (± 0.64)$^{b;B}$	0.000
p-value (one-way ANOVA)	0.528	0.000	0.000	0.000	0.000	0.000*

a,b – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Table B.12 - Evolution of ML (%) of *P. ostreatus* throughout storage time 2, 6, 10, 14 and 18 ºC for 96 hours. The p-value for the main effects from two-way ANOVA is 0.000 for temperature and 0.000 for time.

Storage time (h)	**2 ºC**	**6 ºC**	**10 ºC**	**14 ºC**	**18 ºC**	**P value**
24	1.52 (± 0.14)^{a,A}	1.94 (± 0.17)^{a,A}	2.34 (± 0.19)^{a,A}	6.21 (± 0.79)^{a,b,A}	10.27 (± 2.72)^{b,A}	0.000
48	1.96 (± 0.16)^{a,A,B}	2.66 (± 0.20)^{a,A,B}	4.36 (± 0.55)^{a,A}	15.34 (± 1.84)^{a,A}	27.18 (± 3.63)^{c,A,B}	0.000
72	2.60 (± 0.21)^{B}	3.79 (± 0.27)^{a,B}	9.21 (± 0.74)^{a,B}	33.01 (± 4.04)^{b,B}	62.35 (± 9.30)^{c,B}	0.000
96	3.57 (± 0.26)^{C}	5.12 (± 0.51)^{a,b,C}	11.72 (± 0.98)^{b,B}	41.08 (± 3.17)^{c,B}	42.56 (± 3.82)^{c,C}	0.000
p-value (one-way ANOVA)	0.000	0.000	0.000	0.000	0.000	

a,b – homogeneous groups of temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x temperature interactions from two-way ANOVA.

Appendix C: Supplementary material pertaining to Chapter 4.

Table C.1 – O_2 consumption and CO_2 production rates (Mean and SE) of fresh oyster mushrooms stored under different atmospheres, with t (h) being the time after samples processing.

O_2 (%, v/v)	CO_2 (%, v/v)	t (h)	RR_{O_2} (mL.kg^{-1}.h^{-1})		$RR_{C_{O_2}}$ (mL.kg^{-1}.h^{-1})	
			Mean	**SE**	**Mean**	**SE**
2	**5**	24	34.54	16.25	31.61	5.29
		48	36.92	5.52	30.05	7.74
		72	21.41	10.78	28.24	5.21
		96	16.91	3.21	16.96	6.18
2	**10**	48	35.35	14.02	30.20	7.99
		72	24.99	7.22	25.30	7.28
		96	38.43	9.62	35.64	9.99
2	**20**	24	14.57	7.76	15.15	1.39
		48	12.70	2.77	22.19	5.76
		72	27.69	6.60	35.66	15.7
15	**5**	24	39.07	7.32	21.60	4.27
		48	55.93	8.11	29.88	5.56
		72	48.50	5.67	26.0	1.17
		96	26.53	4.14	14.26	3.58
15	**10**	24	41.35	2.28	20.3	5.05
		48	44.15	4.09	31.99	3.38
		72	42.31	2.02	25.29	4.28
		96	33.59	2.06	27.66	5.44
15	**20**	24	31.9	5.56	21.99	5.50
		48	42.61	9.29	9.23	0.97
		72	41.10	4.88	19.0	7.84
		96	32.65	4.70	12.96	5.71
Ambient air		24	37.53	1.05	26.45	0.38
		48	57.91	2.98	45.14	5.37
		72	37.52	1.14	28.66	2.93
		96	28.35	0.73	26.40	2.43

Table C.2 – Results of one-way ANOVA for the effect of O_2 and CO_2 (% v/v) on oyster mushroom respiration rate.

		Sum of Squares	df	Mean Square	F	Sig.
RR_{O2} ($mL.kg^{-1}.h^{-1}$)	Between Groups	4556.909	6	759.485	6.192	0.000
	Within Groups	6623.307	54	122.654		
	Total	11180.216	60			
RR_{CO2} ($mL.kg^{-1}.h^{-1}$)	Between Groups	769.025	6	128.171	1.794	0.119
	Within Groups	3715.271	52	71.448		
	Total	4484.296	58			

Appendix D: Supplementary material pertaining to Chapter 7.

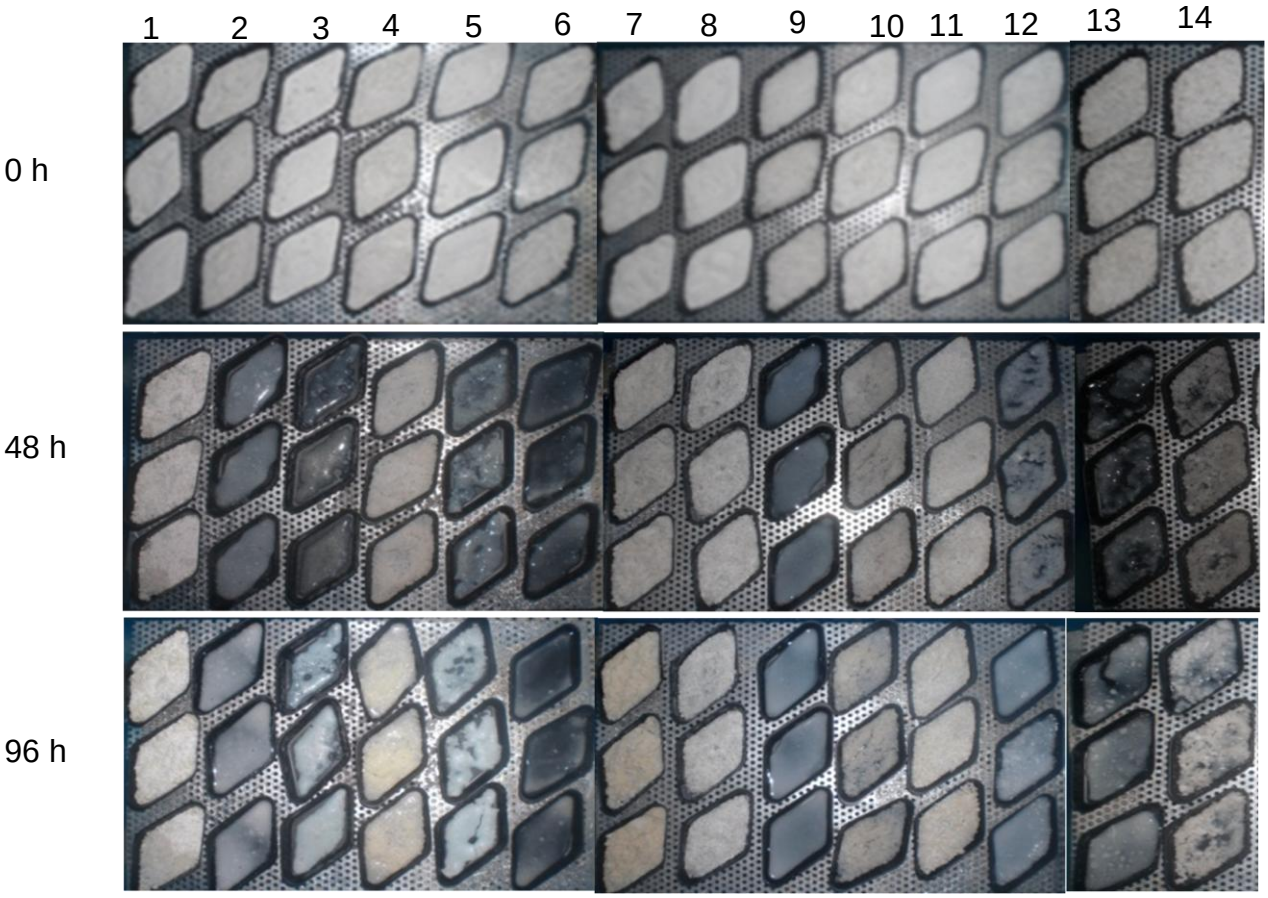


Figure D.1 - Visual appearance of the different mixtures at 10 ºC and 96 % RH over 5 days of storage. Mixtures (from 1 to 14) are presented in the following order: CaO: $CaCl_2$: sorbitol (w/w) -1- 0.60: 0.20: 0.20; 2 - 0.20: 0.60:0.20;3 - 0.20:0.20:0.60; 4 - 0.33:0.47:0.20; 5 - 0.33: 0.20:0.47; 6 – 0.20:0.33: 0.47; 7 - 0.47:0.33:0.20; 8 - 0.47:0.20:0.33; 9 - 0.20:0.47:0.33; 10 - 0.33:0.33:0.33; 11 - 0.47:0.27:0.27; 12 - 0.27:0.47:0.27; 13 - 0.27:0.27:0.47; 14 - 0.33:0.33:0.33.

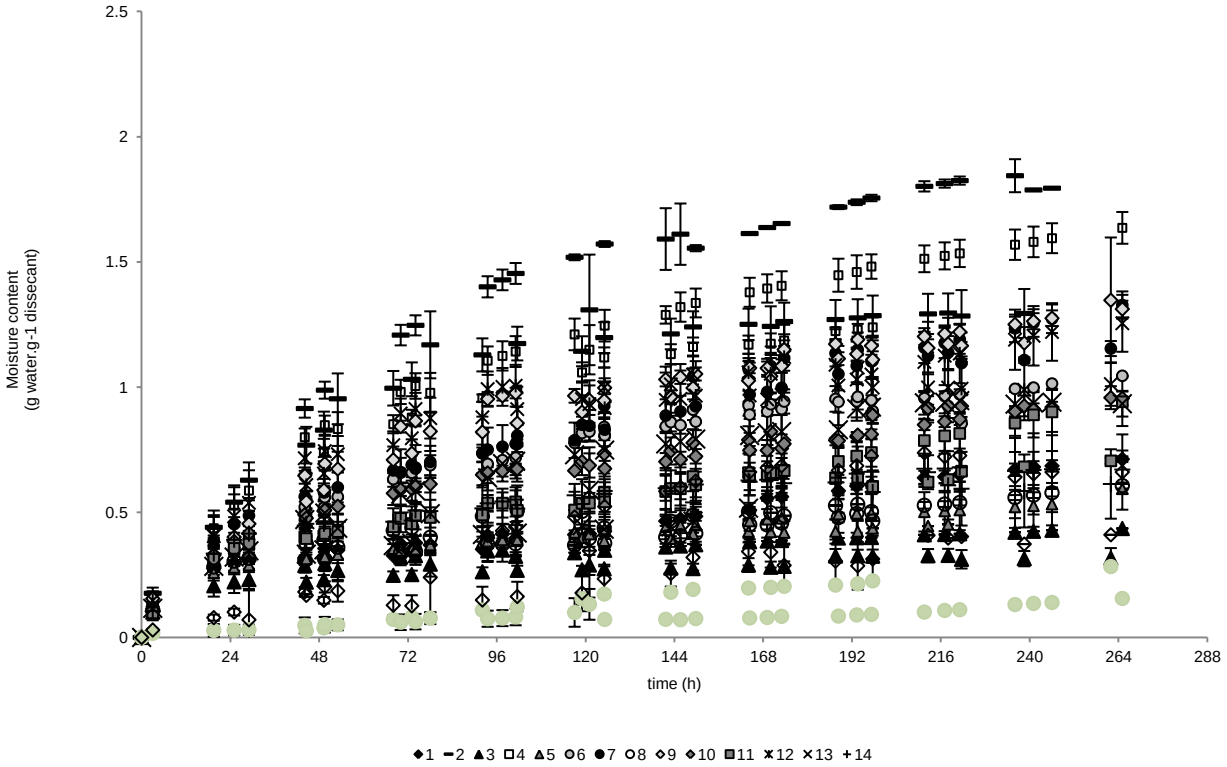


Figure D.2 – Evolution of moisture content of different mixtures at 10 ºC and 96 % RH over storage. Points are the mean of six replicates and vertical bars are the standard deviation. Mixtures are presented in the following order: CaO: $CaCl_2$: sorbitol (w/w) -1- 0.60: 0.20: 0.20; 2 - 0.20: 0.60:0.20;3 - 0.20:0.20:0.60; 4 - 0.33:0.47:0.20; 5 - 0.33: 0.20:0.47; 6 – 0.20:0.33: 0.47; 7 - 0.47:0.33:0.20; 8 - 0.47:0.20:0.33; 9 - 0.20:0.47:0.33; 10 - 0.33:0.33:0.33; 11 - 0.47:0.27:0.27; 12 - 0.27:0.47:0.27; 13 - 0.27:0.27:0.47; 14 - 0.33:0.33:0.33.

Appendix E: Supplementary material pertaining to Chapter 8.

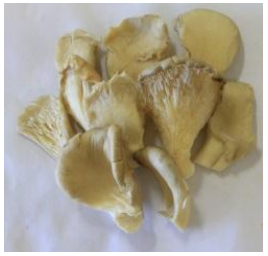
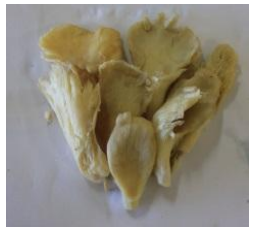
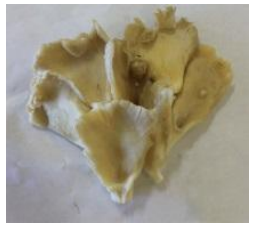
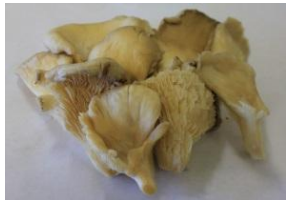
	Constant Temperature				abuse		
t (h)	**Commercial**	**MAP**	**MAP+ab**		**Commercial**	**MAP**	**MAP+ab**
120							
168							
240							

Figure E.1 **–** Appearance of *Pleurotus* mushrooms used for sensorial evaluation. Treatment identification: CP = Commercial package, control; MAP= Modified atmosphere package; MAP2= Modified atmosphere package and moisture absorber.

Sensory evaluation sheet -

Oyster mushroms

Name:_____Date:_____

Dear (a) taster (a), has its presence in 6 samples of mushrooms Pleurotus identified by a code, we ask you to sort them according to their global assessment (appearance and odour):

Code:

Worst Better

Figure E.2 – Sensory evaluation sheet provided for oyster mushrooms evaluation.

Table E.1 - Evolution of L*parameter of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.000; Package: 0.000; time x Temperature:0.000; time x Package:0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Constant**				**Abuse**			
Storage time (h)	**Commercial**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA)**	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA)**
0	77.78 (± 0.91)^{a,D}	77.78 (± 0.91)^{a,B}	77.78 (± 0.91)^{a,C}	1.000	77.78 (± 0.91)^{a,A,C}	77.78 (± 0.91)^{a,B}	77.78 (± 0.91)^{a,C}	1.000
72	72.92 (± 0.52)^{b,A,B}	68.73 (± 0.63)^{a,A}	71.98 (± 0.69)^{b,B}	0.000	66.81 (± 0.54)^{a,B}	67.58 (± 1.20)^{a,A}	74.70 (± 0.55)^{b,B}	0.000
120	75.44 (± 0.40)^{b,C}	66.89 (± 1.09)^{a,A}	72.62 (± 1.07)^{b,B}	0.000	65.01 (± 0.44)^{a,A,B}	68.01 (± 0.50)^{b,A}	74.44 (± 0.40)^{c,B}	0.000
168	74.92 (± 0.53)^{c,B,C}	70.24 (± 0.65)^{b,A}	66.22 (± 0.37)^{a,A}	0.000	64.21 (± 0.71)^{a,A,B}	68.10 (± 1.10)^{b,A}	73.95 (± 0.70)^{c,B}	0.000
240	72.31 (± 0.43)^{c,A}	67.81 (± 0.95)^{b,A}	70.11 (± 0.69)^{a,A,B}	0.000	62.94 (± 0.92)^{a,B}	67.69 (± 0.69)^{b,A}	67.66 (± 0.78)^{c,A}	0.000
p-value (one-way ANOVA)	0.000	0.000	0.000		0.000	0.000	0.000	0.000

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C,D – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.

Table E.2 - Evolution of a* of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.020; Package: 0.000; time x Temperature: 0.040; time x Package: 0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Constant**				**Abuse**			
Storage time (h)	**Commercial**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA)**	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA)**
0	2.11 (± 0.21)^{a,A,B}	2.11 (± 0.21)^{a,A,B}	2.11 (± 0.21)^{a,A}	1.000	2.11 (± 0.21)^{a,A}	2.11 (± 0.21)^{a,A}	2.11 (± 0.21)^{a,B}	1.000
72	2.53 (± 0.20)^{b,B}	2.13 (± 0.15)^{a,b,A,B}	1.77 (± 0.12)^{a,A}	0.010	3.66 (± 0.20)^{c,C}	2.24 (± 0.32)^{b,A}	1.11 (± 0.16)^{a,A}	0.000
120	2.01 (± 0.13)^{a,A,B}	2.71 (± 0.22)^{b,B}	1.65 (± 0.15)^{a,A}	0.000	2.49 (± 0.13)^{b,A,B}	2.40 (± 0.23)^{b,A}	1.12 (± 0.09)^{a,A}	0.000
168	1.78 (± 0.22)^{a,A}	1.78 (± 0.13)^{a,A}	2.20 (± 0.13)^{a,A,B}	0.420	2.92 (± 0.20)^{b,B,C}	2.42 (± 0.15)^{b,A}	1.59 (± 0.10)^{a,A,B}	0.000
240	2.01 (± 0.09)^{a,A,B}	2.70 (± 0.21)^{b,B}	2.79 (± 0.09)^{b,B}	0.000	2.74 (± 0.19)^{a,A,B}	2.53 (± 0.21)^{a,A}	3.64 (± 0.32)^{b,C}	0.000
p-value (one-way ANOVA	0.030	0.000	0.000		0.000	0.840	0.000	

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.

Table E.3 - Evolution of b* of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.020; Package: 0.000; time x Temperature: 0.040; time x Package: 0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Constant**				**Abuse**			
Storage time (h)	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**
0	18.14 (± 0.54)^{a,A}	18.14 (± 0.54)^{a,A}	18.14 (± 0.54)^{a,A}	1.000	18.14 (± 0.54)^{a,A}	18.14 (± 0.54)^{a,A}	18.14 (± 0.54)^{a,A}	1.0000
72	20.84 (± 0.41)^{c,B}	17.32 (± 0.27)^{a}	18.71 (± 0.40)^{b,A,B}	0.000	24.07 (± 0.68)^{b,C}	18.56 (± 0.33)^{a,A}	19.65 (± 0.25)^{a,A,B}	0.0000
120	21.97 (± 0.24)^{b,B,C}	19.58 (± 0.22)^{a}	20.24 (± 0.35)^{a,B,C}	0.000	18.13 (± 0.40)^{a,A}	20.14 (± 0.58)^{b,A,B}	20.97 (± 0.52)^{b,B}	0.0000
168	22.32 (± 0.53)^{b,B,C}	19.06 (± 0.27)^{a}	17.82 (± 0.16)^{a,A}	0.000	21.29 (± 0.62)^{a,B}	20.07 (± 0.44)^{a,A,B}	21.00 (± 0.38)^{a,B}	0.2000
240	23.18 (± 0.36)^{a,C}	18.38 (± 1.06)^{a}	21.06 (± 0.32)^{b,C}	0.000	20.73 (± 0.30)^{a,B}	21.66 (± 0.65)^{a,B,b}	23.32 (± 0.51)^{b,C}	0.0000
p-value (one-way ANOVA	0.000	0.060	0.000		0.000	0.000	0.000	

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C, D – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.

Table E.4 - Evolution of BI of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.020; Package: 0.000; time x Temperature: 0.040; time x Package: 0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Constant**				**Abuse**			
Storage time (h)	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**
0	174.26 (± 1.07)^{a,A}	174.26 (± 1.07)^{a,A}	174.26 (± 1.07)^{a,A}	1.000	174.26 (± 1.07)^{a,A}	174.26 (± 1.07)^{a,A}	174.26 (± 1.07)^{a,A}	1.000
72	179.82 (± 0.80)^{b,B}	176.26 (± 0.52)^{a,A,B}	176.30 (± 0.47)^{a,A,B}	0.000	189.25 (± 1.21)^{b,D}	179.22 (± 1.32)^{a,A,B}	175.68 (± 0.57)^{a,A}	0.000
120	179.39 (± 0.30)^{a,b,B}	181.33 (± 0.84)^{b,C}	177.96 (± 0.53)^{a,B}	0.000	179.51 (± 0.78)^{b,B}	180.99 (± 1.28)^{a,B}	177.39 (± 0.72)^{a,A}	0.030
168	179.98 (± 1.09)^{a,B}	177.53 (± 0.62)^{a,A,B,C}	177.98 (± 0.39)^{a,B}	0.110	185.25 (± 0.95)^{c,C,D}	180.76 (± 0.70)^{b,B}	178.20 (± 0.44)^{a,A}	0.000
240	182.37 (± 0.56)^{a,B}	179.52 (± 1.58)^{a,B,C}	181.66 (± 0.47)^{a,C}	0.120	185.05 (± 0.68)^{a,b,C}	183.53 (± 1.27)^{a,B}	188.08 (± 1.68)^{b,B}	0.040
p-value (one-way ANOVA	0.000	0.000	0.000		0.000	0.000	0.000	

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.
*p-value for overall time x Temperature interactions from two-way ANOVA.

Table E.5 - Evolution of C* of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.020; Package: 0.000; time x Temperature: 0.040; time x Package: 0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Constant**				**Abuse**			
Storage time (h)	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**
0	18.27 (± 0.56)^{a,A}	18.27 (± 0.56)^{a,A}	18.27 (± 0.56)^{a,A}	1.000	18.27 (± 0.56)^{a,A}	18.27 (± 0.56)^{a,A}	18.27 (± 0.56)^{a,A}	1.000
72	21.02 (± 0.42)^{c,B}	17.47 (± 0.27)^{a,A}	18.81 (± 0.40)^{b,A,B}	0.000	24.36 (± 0.70)^{b,C}	18.76 (± 0.36)^{a,A}	19.70 (± 0.25)^{a,A,B}	0.000
120	22.07 (± 0.24)^{b,B,C}	19.80 (± 0.22)^{a,A}	20.33 (± 0.34)^{a,B,C}	0.000	18.31 (± 0.41)^{a,A}	20.30 (± 0.61)^{b,A,B}	21.00 (± 0.52)^{b,B}	0.000
168	22.41 (± 0.55)^{b,B,C}	19.16 (± 0.28)^{a,A}	17.96 (± 0.17)^{a,A}	0.000	21.51 (± 0.62)^{a,B}	20.24 (± 0.44)^{a,A,B}	21.06 (± 0.38)^{a,B}	0.190
240	23.27 (± 0.36)^{b,B,C}	18.60 (± 1.07)^{a,A}	21.25 (± 0.31)^{b,C}	0.000	20.94 (± 0.29)^{a,B}	21.83 (± 0.67)^{a,B}	23.64 (± 0.55)^{b,C}	0.000
p-value (one-way ANOVA	0.000	0.060	0.000		0.000	0.000	0.000	

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.

Table E.6 - Evolution of Hº of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.240; Package: 0.000; time x Temperature: 0.080; time x Package: 0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Constant**				**Abuse**			
Storage time (h)	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**
0	83.46 (± 0.50)^{a,A,B}	83.46 (± 0.50)^{a}	83.46 (± 0.50)^{a}	1.000	83.46 (± 0.50)^{a,B}	83.46 (± 0.50)^{a,A}	83.46 (± 0.50)^{a,B}	1.000
72	83.22 (± 0.47)^{a,A}	82.97 (± 0.50)^{a}	84.45 (± 0.44)^{a}	0.070	81.47 (± 0.31)^{a,A}	83.44 (± 0.87)^{a,A}	86.82 (± 0.45)^{b,C}	0.000
120	84.74 (± 0.36)^{b,A,B,C}	82.13 (± 0.64)^{a}	85.23 (± 0.46)^{b}	1.000	82.22 (± 0.37)^{a,A,B}	83.45 (± 0.42)^{b,A}	86.97 (± 0.24)^{c,C}	0.000
168	85.69 (± 0.45)^{b,C}	84.72 (± 0.34)^{b}	82.99 (± 0.38)^{a}	0.000	82.21 (± 0.49)^{a,A,B}	83.01 (± 0.46)^{a,A}	85.62 (± 0.27)^{b,C}	0.000
240	85.03 (± 0.23)^{b,B,C}	82.29 (± 0.67)^{a}	82.41 (± 0.28)^{a}	0.000	82.35 (± 0.59)^{a,b,A,B}	83.51 (± 0.43)^{b,A}	81.37 (± 0.58)^{a,A}	0.020
p-value (one-way ANOVA	0.000	0.010	0.000		0.200	0.970	0.000	

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.

Table E.7 - Evolution of firmness (N) of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.020; Package: 0.300; time x Temperature: 0.040; time x Package: 0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Constant**				**Abuse**			
Storage time (h)	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**
0	0.68 (± 0.03)^{a,C}	0.68 (± 0.03)^{a,C}	0.68 (± 0.03)^{a,C}	1.000	0.68 (± 0.03)^{a,B}	0.68 (± 0.03)^{a,B}	0.68 (± 0.03)^{a,A}	1.000
72	0.48 (± 0.04)^{a,b,B}	0.60 (± 0.04)^{b,B,C}	0.46 (± 0.03)^{a,B}	0.020	0.35 (± 0.02)^{a,A}	0.31 (± 0.03)^{a,A}	0.38 (± 0.03)^{a,A}	0.180
120	0.34 (± 0.03)^{a,A}	0.38 (± 0.03)^{a,A}	0.32 (± 0.02)^{a,A}	0.330	0.37 (± 0.04)^{a,A}	0.37 (± 0.02)^{a,A}	0.30 (± 0.02)^{a,A}	0.130
168	0.50 (± 0.03)^{b,B}	0.54 (± 0.04)^{b,B}	0.37 (± 0.02)^{a,A,B}	0.000	0.35 (± 0.01)^{a,A}	0.34 (± 0.02)^{a,A}	0.40 (± 0.03)^{a,A}	0.160
240	0.42 (± 0.03)^{b,A,B}	0.26 (± 0.01)^{a,A}	0.46 (± 0.05)^{b,B}	0.000	0.36 (± 0.02)^{a,A}	0.31 (± 0.02)^{a,A}	0.36 (± 0.02)^{a,A}	0.180
p-value (one-way ANOVA	0.000	0.000	0.000		0.000	0.000	0.000	

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C– homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.

Table E.8 - Evolution of cohesiveness of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.020; Package: 0.000; time x Temperature: 0.040; time x Package: 0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Constant**				**Abuse**			
Storage time (h)	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**
0	0.69 (± 0.01)^{a,C}	0.69 (± 0.01)^{a,C}	0.69 (± 0.01)^{a,B}	1.000	0.69 (± 0.01)^{a,C}	0.69 (± 0.01)^{a,C}	0.69 (± 0.01)^{a,C}	1.000
72	0.67 (± 0.01)^{b,B,C}	0.63 (± 0.01)^{a,b,B,C}	0.62 (± 0.01)^{a,A}	0.010	0.58 (± 0.01)^{a,A}	0.61 (± 0.03)^{a,b,A,B}	0.66 (± 0.01)^{b,C}	0.000
120	0.67 (± 0.01)^{b,B,C}	0.57 (± 0.01)^{a,A}	0.60 (± 0.02)^{a,A}	0.000	0.62 (± 0.01)^{a,B}	0.60 (± 0.01)^{a,A,B}	0.61 (± 0.02)^{a,A,B}	0.450
168	0.64 (± 0.01)^{a,A,B}	0.60 (± 0.02)^{a,A,B}	0.61 (± 0.01)^{a,A}	0.310	0.58 (± 0.01)^{a,A}	0.63 (± 0.01)^{b,B,C}	0.64 (± 0.02)^{b,B,C}	0.000
240	0.61 (± 0.01)^{a,A}	0.61 (± 0.01)^{a,A,B}	0.58 (± 0.01)^{a,A}	0.110	0.57 (± 0.01)^{a,A}	0.56 (± 0.02)^{a,A}	0.59 (± 0.01)^{a,A}	0.460
p-value (one-way ANOVA	0.000	0.000	0.000		0.000	0.000	0.000	

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C– homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.

Table E.9 - Evolution of resilience of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.020; Package: 0.000; time x Temperature: 0.040; time x Package: 0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Control**				**Abuse**			
Storage time (h)	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**
0	0.22 (± 0.01)^{a,B}	0.22 (± 0.01)^{a,C}	0.22 (± 0.01)^{a,C}	1.000	0.22 (± 0.01)^{a,B}	0.22 (± 0.01)^{a,B}	0.22 (± 0.01)^{a,B}	1.000
72	0.15 (± 0.01)^{a,b,A}	0.19 (± 0.01)^{b,B,C}	0.15 (± 0.01)^{a,A,B}	0.030	0.12 (± 0.01)^{a,A}	0.19 (± 0.02)^{b,B}	0.13 (± 0.01)^{a,A}	0.000
120	0.22 (± 0.01)^{a,B}	0.23 (± 0.02)^{a,C}	0.19 (± 0.01)^{a,B,C}	0.190	0.12 (± 0.01)^{a,A}	0.12 (± 0.01)^{a,A}	0.10 (± 0.00)^{a,A}	0.080
168	0.16 (± 0.01)^{b,A}	0.16 (± 0.01)^{b,B}	0.12 (± 0.01)^{a,A}	0.000	0.11 (± 0.00)^{a,A}	0.12 (± 0.01)^{a,A}	0.24 (± 0.02)^{b,B}	0.000
240	0.13 (± 0.01)^{b,A}	0.09 (± 0.00)^{a,A}	0.15 (± 0.02)^{b,A,B}	0.000	0.11 (± 0.01)^{a,A}	0.11 (± 0.01)^{a,A}	0.22 (± 0.01)^{b,B}	0.000
p-value (one-way ANOVA	0.000	0.000	0.000		0.000	0.000	0.000	

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C, D – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.

Table E.10 - Evolution of SSC of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.020; Package: 0.000; time x Temperature: 0.040; time x Package: 0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Control**				**Abuse**			
Storage time (h)	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**	**Control**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**
0	6.61 (± 0.00)^{a,D}	6.61 (± 0.00)^{a,A}	6.61 (± 0.00)^{a,B}	1.000	6.61 (± 0.00)^{a,B}	6.61 (± 0.00)^{a,B}	6.61 (± 0.00)^{a,D}	1.000
72	6.48 (± 0.01)^{a,B}	6.40 (± 0.00)^{c,A}	6.49 (± 0.03)^{b,A}	0.000	6.48 (± 0.01)^{b,A}	6.54 (± 0.03)^{b,A,B}	6.36 (± 0.02)^{a,B}	0.000
120	6.44 (± 0.01)^{a,B}	6.63 (± 0.01)^{b,A}	6.48 (± 0.03)^{a,A}	0.000	6.41 (± 0.02)^{b,A}	6.53 (± 0.01)^{c,A}	6.26 (± 0.00)^{a,A}	0.000
168	6.35 (± 0.01)^{a,A}	6.78 (± 0.03)^{b,B}	6.42 (± 0.01)^{a,A}	0.000	7.00 (± 0.02)^{c,C}	6.55 (± 0.00)^{b,A,B}	6.43 (± 0.01)^{a,C}	0.000
240	6.53 (± 0.01)^{a,C}	7.51 (± 0.02)^{c,C}	6.81 (± 0.01)^{B,c}	0.000	7.63 (± 0.04)^{c,D}	7.33 (± 0.04)^{b,C}	6.57 (± 0.02)^{a,D}	0.000
p-value (one-way ANOVA	0.000	0.000	0.000		0.000	0.000	0.000	

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C, D – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.

Table E.11 - Evolution of pH of of *P. ostreatus* throughout storage time at different storage conditions. The p-value for the main effects from three -way ANOVA are as follows: time: 0.000; temperature: 0.020; Package: 0.000; time x Temperature: 0.040; time x Package: 0.000; Temp x Package: 0.000; time x Temp x Package: 0.000.

Temperature profile	**Control**				**Abuse**			
Storage time (h)	**Commercial**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**	**Commercial**	**MAP**	**MAP+ab**	**p-value (one-way ANOVA**
0	6.60 (± 0.00)^{a,A}	6.60 (± 0.00)^{a,A}	6.60 (± 0.00)^{a,A,B}	0.000	6.60 (± 0.00)^{a,D}	6.60 (± 0.00)^{a,B}	6.60 (± 0.00)^{a,C}	0.0000
72	6.60 (± 0.00)^{a,A}	6.65 (± 0.07)^{a,A}	6.40 (± 0.14)^{a,A}	0.135	5.95 (± 0.07)^{a,B}	6.75 (± 0.07)^{a,A}	5.85 (± 0.07)^{b,D}	0.0020
120	6.85 (± 0.07)^{a,B}	6.90 (± 0.00)^{a,B}	6.80 (± 0.00)^{a,B,C}	0.192	6.30 (± 0.00)^{B}	6.30 (± 0.00)^{B}	6.70 (± 0.00)^{B}	0.0000
168	7.60 (± 0.00)^{A,B}	6.73 (± 0.12)^{C}	6.93 (± 0.12)^{C}	0.000	6.30 (± 0.00)^{a,A}	6.37 (± 0.06)^{b,C}	7.73 (± 0.12)^{a,B}	0.0000
240	7.83 (± 0.06)^{a,A,B}	6.77 (± 0.06)^{b,D}	7.73 (± 0.06)^{a,D}	0.000	6.00 (± 0.00)^{C}	6.00 (± 0.00)^{C}	7.37 (± 0.25)^{A}	0.0000
p-value (one-way ANOVA)	0.0330	0.0000	0.0000		0.0000	0.0000	0.0000	

a,b,c – homogeneous groups of Temperature according to the Tukey's test, at a 95 % CI.
A,B,C, D – homogeneous groups of storage time according to the Tukey's test, at a 95 % CI.