

Impact of polylactide (PLA) on the quality of bio-products in contact

Romulo Vinicio Salazar Gonzalez

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Impact du polylactide (PLA) sur la qualité des bioproduits au contact

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Symbols and abbreviations

ASE	Accelerated solvent extraction
BP	Boiling point
BZA	Benzaldehyde
C	Concentration of a substance in the polymer matrix
CAR/PDMS	Carboxen-polydimethylsiloxane
d	Film density
D	Diffusion coefficient
DSC	Differential scanning calorimetry
DVB/CAR/PDMS	Divinylbenzene-Carboxen-polydimethylsiloxane
E2MB	Ethyl-2-methylbutanoate
EA	Ethyl acetate
EB	Ethyl butanoate
EH	Ethyl hexanoate
EVOH	Poly(ethylene-co-vinyl alcohol)
FID	Flame ionization detector
GC	Gas chromatography
HS	Headspace
K	Partition coefficients
$K_{hs/liq}$	Headspace-liquid partition coefficient
$K_{polym/hs}$	Polymer-headspace partition coefficient
$K_{polym/liq}$	Polymer-liquid partition coefficient
l	Film thickness
LDPE	Low- density polyethylene
LOD	Limit of detection
Log P	Measure of hydrophobicity
LOQ	Limit of quantification
M_∞	Mass sorption at equilibrium
M_0	Initial mass
MHE	Multiple headspace extraction
MHE-SPME	Multiple headspace – solid phase micro extraction

Mn	Number average molecular mass
MS	Mass spectrometry
Mw	Weight average molecular mass
OPP	Oriented polypropylene
P	Permeability coefficient
PA	Polyamide
PE	Polyethylene
PET	Polyethylene terephthalate
PLA	Polylactide
P_p	Partial pressure
PP	Polypropylene
PS	Polystyrene
PVDC	Polyvinylidene chloride
P_{vs}	Saturated vapour pressure
RH	Relative humidity
S	Solubility coefficient
SFE	Supercritical fluid extraction
SPME	Solid Phase MicroExtraction
Tc	Crystallization temperature
Tg	Glass transition temperature
Tm	Melting temperature
VOC	Volatile organic compound
WVTR	Water vapor transfer rate
Xc	Degree of crystallinity
δ_D	Contribution of non polar interactions
δ_H	Contribution of hydrogen bonding forces
δ_P	Contribution of polar interactions
δ_T	Total solubility parameter
θ	Time-lag
2NNE	2-nonanone

Introduction

New biodegradable materials based on renewable resources are being developed for food packaging, which market uses large tonnages of plastics materials, offering thus the possibility of reducing its environmental impact. Polylactide (PLA) forms part of this new generation of polymers.

According to European regulations, packaging material must be suitable for food contact. However, polymer packaging materials are not inert and mass transfer occurs between packaging and foodstuffs, which can lead to quality issues. Permeation of gas, such as oxygen and vapours of water or volatile organic compounds through the packaging may lead to degradation of the packaged food or unbalance the aroma profile. Additives, polymer stabilizers, volatile compounds from degradation products of additives or polymers may migrate into food and cause off-flavors. Conversely constituents of food, such as aroma compounds, can be sorbed in significant quantities by the plastic packaging materials, involving modifications of the foodstuff flavor. Furthermore, some aroma compounds present in food can have a plasticizing effect on food packaging, changing its thermal, mechanical and barrier properties during storage.

PLA is coming into packaging applications now. Only a few literature studies exist about food-PLA interactions and the major part has been focused on the evaluation of food quality during storage. Because of the large number of different food products used in these studies, from fruits to cheeses, generalization of interaction effects is difficult. Furthermore, the evolution of the packaging polymer PLA itself was not studied. One of the reasons might be that PLA is a glassy polymer at room temperature and in general literature about interactions between flavor molecules and glassy polymers is very scarce. Indeed, one of the principal difficulties in dealing with sorption studies in glassy polymers such as PLA is the low mass

transfer rate which challenges analytical methods. So, due to methodological constraints, the most of studies about this subject have been made using single aroma compound at high concentration or high partial pressure, which is far from real service conditions of the considered polymers. Furthermore, the polymer can undergo thermal degradation during its processing into food containers, which can give rise to newly formed volatile compounds. Those compounds present in the headspace of the product or in the food itself potentially cause sensorial defects. Although literature of the degradation of PLA during processing exists, it did not focus on the occurrence of such volatile compounds. Therefore, no methodology was developed up to date to trace the appearance of molecules with potentially negative impact on the sensorial qualities of food.

The principal aim of this thesis was therefore better understanding the impact of PLA on the quality of packaged products, either by the sorption in PLA of aroma compounds or by the presence in PLA of volatile organic compounds (VOCs) potentially migrants. In that aim, the approach undertaken in the present work was on the one hand the measurement of aroma compounds under similar conditions to those of a real food, which means at low vapour activity, and on the other hand, the identification and quantification of VOCs present after the thermal processing of PLA. In the case of the measurement of aroma compounds, the difficulty was working with a glassy material at room temperature using low concentrations of aroma compounds. To deal with this problem two ways were followed. A way used a model food that is close to the real service conditions, where the equilibrium state between the polymer and the model food was not expected. The other way used model conditions where a model solution of aroma compounds at concentrations usually present in food products allowed expect the steady state. For studying sorption of aroma compound mixtures at low activities in a glassy polymer and also to probe the volatile compounds generated during thermal processing of PLA a particular methodology was developed and applied to PLA.

Additionally, morphological changes in the packaging polymer exposed to volatile aroma compounds and during storage of food were analyzed to be able to provide structural explanations of observed behavior.

This thesis is developed inside the Joint Research Unit 1145 Food Process Engineering (*UMR 1145 Ingénierie Procédés Aliments*) joining researchers from AgroParisTech, INRA and CNAM. The aim of the research unit is the understanding of the transformations of foodstuff undergone during its industrial processing from the agricultural raw materials to commercial food products. More precisely, the work was carried out inside the I2MC team (*Interactions Matériaux – Milieu au Contact*) aiming on understanding the transport mechanisms of small molecules inside solid polymer matrices. One of the applications of this understanding is the control and prediction of barrier, sorption and migration properties of food packaging during its service life. The thesis project was furthermore associated to the European project SENSOPLAST (*l'aspect émissif et SENSOriel des matériaux PLASTique*). SENSOPLAST was focused on the emission of VOCs from plastic materials and its sensorial impact. This project was supported by the European Regional Development Fund (ERDF) and involved the *Centre des resources technologiques en chimie* (Certech) and the *Département Technologie des Polymères et Composites &Ingénierie Mécanique du Centre Commun ARMINES/Ecole des Mines de Douai* (ARMINES-EMD). In the field of packaging, the study of the Food/ Aroma/ Packaging interactions is carried out by the research team I2MC.

The organization of this thesis is as follows:

- Chapter 1 provides a literature overview about the food packaging interactions covering the sorption and permeation phenomena and the factors affecting aroma transport in packaging materials. The methods used to study the mass transfer and to analyze the

volatile organic compounds are presented. The properties and synthesis of PLA are shown and, with the purpose of review, the organic compounds present in PLA that probably could migrate to foodstuffs, thermal degradation of PLA is discussed.

- The Chapter 2 presents the materials and methods used throughout this work and the methodological development of the measure of sorption of organic compounds at low levels of activity in PLA samples. It presented furthermore the methodology developed to identify and to quantify the VOCs, additives and monomers.

- In Chapter 3, the sorption of aroma compounds in PLA and the plasticization effect of polymer are investigated at low levels of activity using two headspace methods and the effect of the sorption on morphological changes of the polymer is studied.

- The sorption of organic compounds in PLA from model foodstuff is studied in Chapter 4. Two food models were used: a sponge cake and a food emulsion. For the assessment of mixture effects, results were compared to a model solution containing a mixture of aroma compounds.

- In Chapter 5, the additives and monomers present in different PLA grades are investigated. Furthermore, identification and quantification of VOCs formed during processing are shown and discussed in the light of known degradation reactions.

- Finally, Conclusions and Perspectives summarize the results of this work and provide recommendations in this field.

Chapter 1

State of the art

1 State of the art

Food packaging is one of the main actors in preservation of food safety and quality during shelf life. However, polymeric packaging materials are not inert and mass transfer occurs between packaging polymer and foodstuff (Lau and Wong 2000) leading to safety and quality issues. Plastic packaging materials can absorb a significant quantity of aroma compounds from food which can involve modifications of the flavor composition, decrease of intensity, unbalance flavor and modifications of packaging material properties (Ducruet et al. 2001, Ducruet et al. 2007, Tawfik et al. 1997, Togawa et al. 2001). Indeed, the packaging materials contain additives to improve the polymer properties, such as plasticizers, antioxidants, ultraviolet light absorbers, slip agents. Other molecules may also be present in the package as residual monomers or low molecular weight oligomers and even non-intentionally added substances (Vandenburg et al. 1997). Moreover, volatile organic compounds (VOCs) formed during the process of forming (extrusion, thermoforming, etc.), can migrate to the food in contact (Bach et al. 2012), changing its sensorial properties by giving off-taste and/or undesirables flavors (Ezquerro et al. 2003).

In the last decades, the increasing environmental problems such as the decreasing fossil resources have generated a major interest on the biopolymers. New materials from alternative resources, with lower energy consumption, biodegradable and non-toxic to the environment, have been developed (Bordes et al. 2009). One of the most promising bio-based polyesters aimed for food packaging is polylactide (PLA) (Auras et al. 2004b, Avérous 2008, Sinclair 1996) due to its ease of processing using standard equipment and its good mechanical and barrier properties.

In this context, it is necessary to better understand the food – PLA based packaging interactions and to know the levels of organic compounds in the polymer to control the quality

of the products and to ensure consequently that bio-based packaging materials can achieve the purpose for which they were created.

1.1. Food packaging interactions

1.1.1. Mass Transfer of small organic molecules through polymers

Food packaging is one of the main actors in preservation of food safety and quality during shelf life. However, all polymers interact with the packed foodstuff during storage time. This interaction concerns mainly mass transport through the packaging and between both partners (Figure 1.1). Used terms are sorption, meaning the mass transfer of molecules from the food to the packaging material, and migration, being the transfer of compounds from the packaging to the foodstuffs. The permeation is the transfer of molecules or gases through the package in both directions.

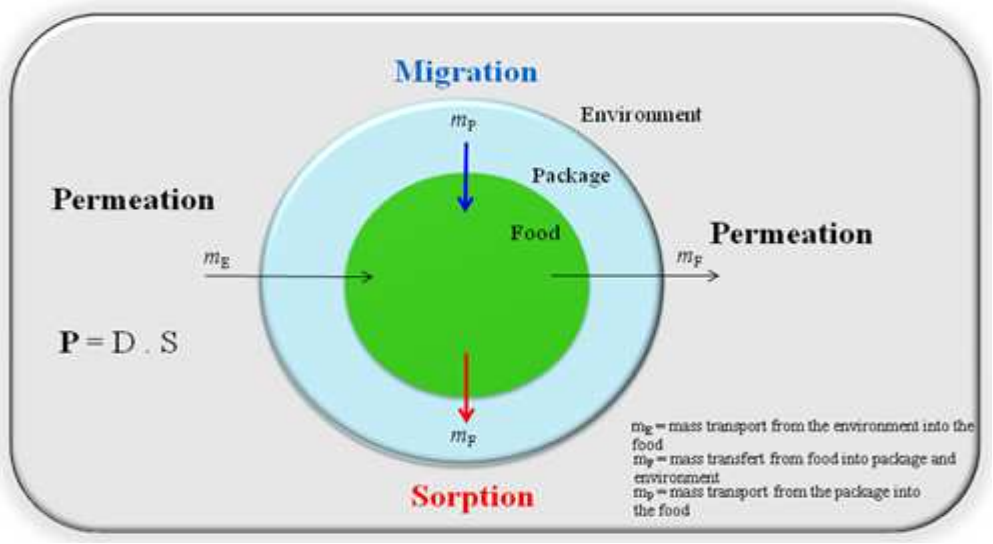


Figure 1.1. Interactions between packaging and food, adapted from Piringer and Baner (2008).

The interactions above mentioned can cause various changes in the quality of the product in contact and/or modifying the structure of the packaging material. The migrant molecules can be residues from the polymerization (monomers, oligomers, solvents), stabilizers (additives,

plasticizers, pigments) and printing inks. If the package contains foodstuff, the molecules that can be sorbed from headspace or from the environment in the package are volatile organic compounds, water vapor and gases from food. In direct contact with the product, the package can sorbe food compounds as fat, pigments and vitamins. In the case of permeation, volatile compounds (flavors, water vapor and gases) from food, its headspace or from the environment can pass through packaging (Dury-Brun et al. 2007).

The transport of molecules in continuous polymeric materials or dense membranes (micropores < 2 nm) follows the solution-diffusion mechanism (Wijmans and Baker 1995).This mode permeation proceeds in three steps: sorption of the compounds at the film surface, diffusion of molecule through the polymer film and desorption of compounds on the other side of the film (Almenar and Auras 2010, Dury-Brun et al. 2007, Mark 2003).

The phenomenon of permeation can be represented by the permeation coefficient or permeability, *P*, in terms of more fundamental properties:

$$P = S \times D$$

(Equation 1.1)

Where *S* is the solubility coefficient and *D* is the diffusion coefficient. The variations in the permeation coefficient *P* depend on temperature, the structural parameters of the polymer and the form of permeants.

1.1.2. Sorption

Sorption is the general term that expresses the penetration and dispersion of compounds in a polymer matrix and that includes the adsorption, absorption, clustering of aggregates or trapping in micro-voids (Dury-Brun et al. 2007). The adsorption is physical when van der Waals or electrostatic interactions are implicated or is chemical when chemical bonds between the adsorbent (film or polymer structure) and the adsorbed compound are involved.

The absorption expresses the dissolution of the permeants in the polymer matrix in rubber state.

The sorption is a phenomenon controlled by the thermodynamic of polymer-permeant system and is characterized by the solubility coefficient, which quantifies the permeant molecules sorbed in the polymer matrix at the equilibrium. There are four classical modes of sorption to describe the sorption in the polymer matrices (Figure 1.2), they were reviewed by Klopffer and Flaconneche (2001).

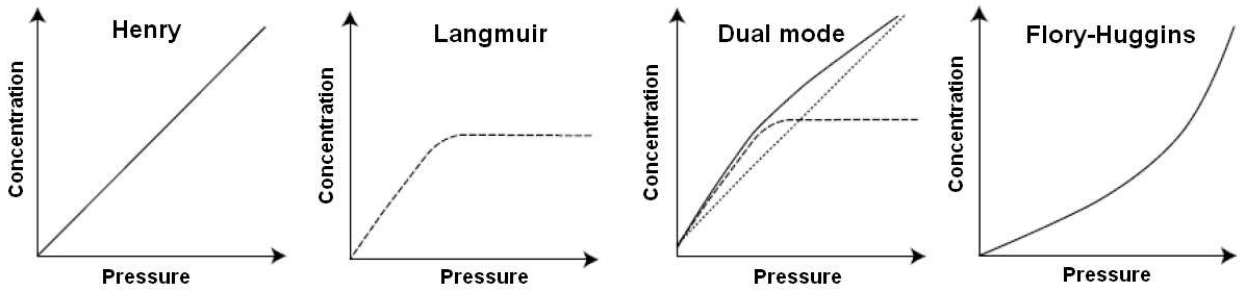


Figure 1.2. Types of isotherms showing the concentration of sorbed molecules versus vapor pressure (Klopffer and Flaconneche 2001).

1.1.2.1. Henry's law sorption

In the case of the isotherm following Henry's law, the volatile compound solubility is independent of the concentration. There is a linear relation between the concentration of a substance (C) in the polymer matrix and its partial pressure (p), under equilibrium conditions:

$$\boldsymbol{C = k_D . p}$$

(Equation 1.2)

where k_D is the solubility coefficient, S, at a given temperature. In this sorption mode, the gas is dispersed randomly in the matrix, which means that neither polymer-permeant interactions nor the permeant-permeant interactions are preferred. This sorption mode is mainly observed at low pressures and when permeant-permeant and permeant-polymer interactions are low

compared with polymer-polymer interactions. This case is observed for sorption of permanent gases in apolar polymers as polyolefins or even for sorption of condensable organic compounds at low activity. For example, literature showed that limonene at concentration below 3.5 ppm was sorbed in Henry mode in OPP (Apostolopoulos and Winters 1991).

1.1.2.2. Langmuir mode sorption

In the case of Langmuir–type sorption mode, the permeant-polymer interactions are predominant. The diffusing molecules occupy specific sites in the polymer until they become saturated. The concentration of the permeant in the matrix (C_H) can be expressed by:

$$C_H = \frac{C'_H . b . p}{1 + b . p}$$

(Equation 1.3)

Where C'_H is the hole saturation constant, p is the partial pressure and b is the hole affinity constant. This mode is common for polymers with specific sites of sorption in particular for cellulosic material with water molecules.

1.1.2.3. Dual sorption model

In the case of sorption in glassy polymers, the dual sorption model combines Henry's law and Langmuir modes and assumes that there are two populations of diffusing compounds in the polymer. It is only applicable when no strong interactions as swelling or plasticization between polymer and permeant take place. According to this model, the concentration of gas compounds in the polymer matrix is expressed by:

$$C = k_D . p + \frac{C'_H . b . p}{1 + b . p}$$

(Equation 1.4)

Where k_D is the solubility coefficient, S, at a given temperature, p is the partial pressure, C'_H is the hole saturation constant and b is the hole affinity constant.

1.1.2.4. Flory-Huggins model

If the interactions between permeants are stronger than permeant-polymer interactions, the solubility coefficient of the permeant increases constantly with pressure, and a non-linearly growing sorption isotherm is observed. This curve form is characteristic of the dissolution of the penetrant vapors in the polymer above its glass transition where clustering of penetrant molecules occurs leading to swelling of the polymer at high penetrant activity. It can described by the Flory-Huggins equation:

$$\ln(a) = ln\frac{p}{p_0} = ln\phi_1 + (1 - \phi_1) + \chi(1 - \phi_1)^2$$

(Equation 1.5)

Where the thermodynamic activity of the permeant a is the ratio of the pressure p and the saturation vapor pressure p_0 at the experimental temperature; ϕ is the volume fraction of the permeant in the polymer and χ the enthalpic interaction parameter between the permeant and the polymer.

An example for polymer swelling and plasticization was shown by Apostolopoulos and Winters (1991) for sorption of limonene in polypropylene when the concentration of limonene exceeds 35 ppm.

1.1.3. Diffusion

Diffusion is the phenomenon that describes the mass transport within a polymer membrane by random molecular motions. The molecules diffuse through the polymer from the higher chemical potential side to the other side until equilibrate the chemical potential on both sides. The diffusion coefficient is the term used to describe kinetics in the polymeric matrix and giving indication on the time required to reach the steady state, i.e. the flux is constant with time and the compound concentration does not vary with time.

The general transport expression was derived by Adolf Fick in 1855 and is commonly known as Fick's second law, reading:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$$

(Equation 1.6)

where x is the thickness of the membrane, C the permeant concentration, and t the time.

The diffusion coefficient of organic species in dense polymer materials is a function of molecular geometry of diffusant component and polymer type. Berens (1981) showed in his pioneering work that the diffusion coefficient D decreases with the increase of the size of the organic compound (Figure 1.3).

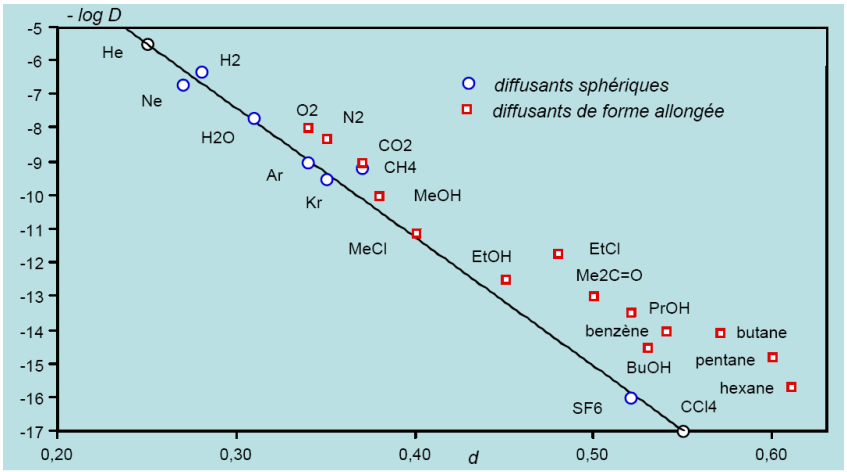


Figure 1.3. Influence of Van der Waals diameter (d) and the form of organic compounds on their diffusion coefficient (D) in PVC (Berens 1981).

Another way to approximate the geometric effects is the use of the number of carbon-atoms of a homologous series of flavor compounds on diffusion, permeation and sorption, which was reported by Shimoda et al. (1987) for the example of polyethylene films. Figure 1.4 shows that D decreases with the increase of the number of carbon atoms of the compounds.

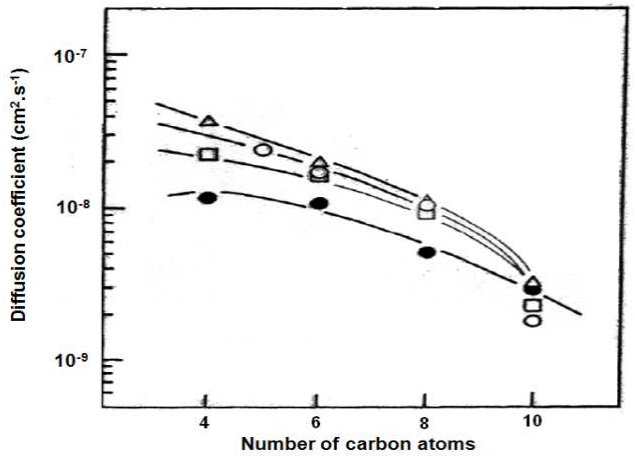


Figure 1.4. Diffusion coefficient of organic compounds of three homologous series in LDPE: ○ Hydrocarbons; **Δ** ethyl esters; □ n-aldehydes; ● n-alcohols (Shimoda et al. 1987).

The geometry effect of the molecules can however in most cases be successfully described by the molecular mass. For example, Reynier et al. (2004) showed the effects of molecular weight of some aroma compounds on the diffusion coefficient in different polymers materials. For a given polymer, they showed that the effect of molecular weight of aroma compounds leads to a *D* variation of less than 1 order of magnitude, while for a given compound, polymer matrix changes lead to variations of *D* up to 4 orders of magnitude (Figure 1.5).

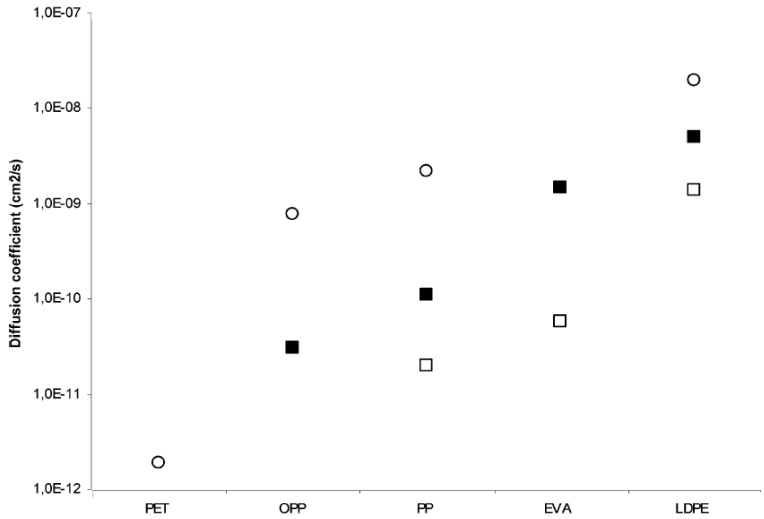


Figure 1.5. Diffusion coefficients at 23 °C of ethanol ○, ethyl butyrate ■, and nonanal □ (Reynier et al. 2004).

1.1.4. Factors affecting aroma transport in packaging materials

Large literature has reported on the factors affecting the mass transfer of small molecules in packaging materials. Comprehensive reviews can, for example, be found in literature (Dury-Brun et al. 2007, Giacin 1995, Mark 2003, Strandburg et al. 1990). The main factors that affect the aroma compounds transport in polymer packaging materials, with an emphasis on aroma sorption, can be grouped in factors related to packaging, factors related to aroma compounds, factors related to food matrix and factors related to environment.

1.1.4.1. Factors related to packaging

The composition and structure of a polymer affect directly the transport of small, little interacting molecules in polymer matrix. The parameters related to packaging that have a major role on mass transfers through polymeric matrices are free volume, glass transition temperature and crystallinity of the polymer. The free volume is an intrinsic property of the polymer defined as the molecular void volume that is trapped in the matter. For transport, the permeating molecule needs to jump from one void in the amorphous phase to the other. In general, sorption of molecules increases with the increase of free volume.

In a semi-crystalline polymer the permeant molecules cannot be sorbed or diffuse in crystalline phase, transport takes place uniquely in the amorphous phase. In the presence of crystallites the penetrant molecules need to compass the impermeable structures, which lengthen their pathway by making it more tortuous. The macroscopic consequence is a decrease in the diffusion coefficient. This hypothesis was put by Michaels and Bixler (1961) in their pioneering work on the gas diffusion through polyethylene.

Recently, Kanehashi et al. (2010) showed an extensive literature overview on the relationship between crystallinity degree of semicrystalline polymers and barrier properties. Figure 1.6b

shows that the oxygen permeability of PE decreases in 4 orders of magnitude with the increase of crystallinity degree. However, this relation does not hold for all polymers (Figure 1.6a). Interestingly, PLA is one of the polymers where the influence of crystallinity on the barrier properties gives contradictory results. Courgneau et al. (2012) reported different types of behaviour of PLA according to the studied molecule, as helium and oxygen. They showed that the helium permeability coefficient decreases with crystallization. In the oxygen case, the permeability coefficient slightly decreases whereas the diffusion coefficient increases with the crystallinity. A similar result was observed by Guinault et al. (2012) for PLA recrystallized at different crystallinity degrees and in different crystalline forms. The authors showed that the diffusion coefficient of PLA increased with crystallinity degree while the sorption coefficient remained constant. They concluded that under specific crystallization conditions a continuous pathway of increased diffusivity was created around PLA crystals (Guinault et al. 2012).

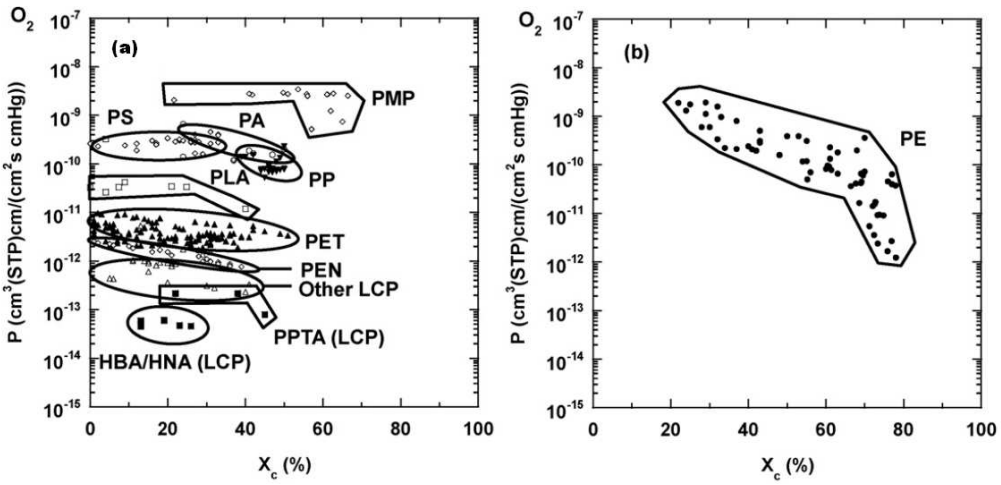


Figure 1.6. Oxygen permeability of semi-crystalline polymers in function of their degree of crystallinity (Kanehashi et al. 2010).

1.1.4.2. Factors related to aroma compounds

Aroma compounds are generally much more interacting molecules than permanent gases. In that case transfer in polymers can be highly affected by factors linked to the physico-chemical

properties of aroma molecules themselves, such as molecular size, structure, hydrophobicity and polarity. Table 1.1. summarizes the effects of these factors on the aroma compound solubility in packaging materials.

One of the main factors is the low condensation temperature of organic vapors, compared to permanent gases. For example, Shimoda et al. (1987) reported the effects of the number of carbon-atoms of three homologous series of aroma compounds on the sorption in polyethylene films. They concluded that the solubility coefficient *S* increases with the increase of the number of carbon atoms of the compounds, as shown in Figure 1.7.

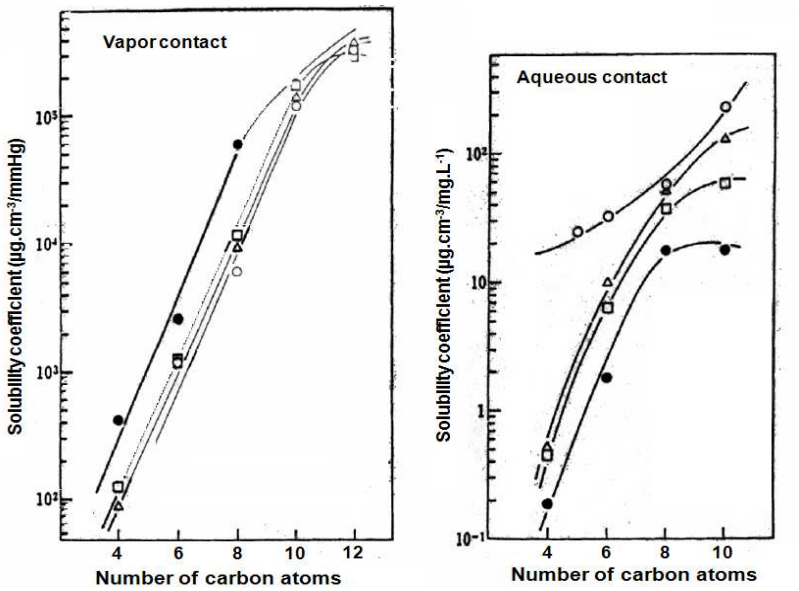


Figure 1.7. Solubility coefficient of organic compounds of three homologous series in LDPE: ○ Hydrocarbons; **Δ** ethyl esters; □ n-aldehydes; ● n-alcohols (Shimoda et al. 1987).

Regarding the hydrophobicity factor and polylactide, Auras et al. (2006) studied the sorption of different volatile compounds and showed that PLA had more affinity to more hydrophilic molecules, such as ethyl acetate, compared to hydrophobic one such as limonene. Colomines et al. (2010) reported also a high affinity of PLA for ethyl acetate, the most hydrophilic compound in the homologous series of ethyl esters. So, since the majority of the aroma

compounds are more hydrophobic than ethyl acetate, good performance can be expected for the aroma barrier properties of PLA.

Table 1.1. Summary of factors related to aroma molecules and their effects on solubility in packaging materials*.

Factors	Variable	Effect on Solubility
Molecular weight	Increase	Increase – effect of condensation temperature
Carbon number	Increase	Increase – effect of condensation temperature
Structure	Linear molecules	Sorbs more easily
	Cyclic molecules	Sorbs less easily except PET
Polarity and hydrophobicity	Apolar molecules	More easily absorbed in lipophilic polymers as polyolefins
	Polar molecules	More easily sorbed in moderately polar polymers as polyesters
Concentration	Low (≤ 0.1 to 10 ppm)	Amount sorbed proportional to the concentration of the sorbents
		The concentration of the aroma compounds in a range from did not affect the partition
	High	Sorption may alter the polymer matrix (plasticization).
Mixing effect	Synergy effect	Higher sorption rate in mixtures compared to the individual aroma compound
	Antagonist effect	Lower sorption rate in mixtures compared to the individual aroma compounds

*from (Caner 2011, Dury-Brun et al. 2007, Mohney et al. 1988, Nielsen et al. 1992a, Shimoda et al. 1987).

Furthermore, aroma compounds are generally present in mixtures and molecules can have antagonistic or synergistic effects. Antagonistic effects can be expected in the low concentration range where polymer morphology remains unchanged while synergy of sorption between aroma compounds is expected in the higher concentration range (approx. > 1 ppm) where sorption causes swelling of the polymer. In the first case the permeation is

predicted to follow the laws of permanent gases, which are based on competitive sorption and diffusion (Story & Koros, 1989).

1.1.4.3. Factors related to food matrix

The food matrix composition has an important role in the sorption of aroma compounds by polymer materials. Oil, polysaccharides, proteins and ethanol can interact with aroma compounds, retaining the aroma compounds in the food matrix and therefore changing the sorption behavior in the packaging materials.

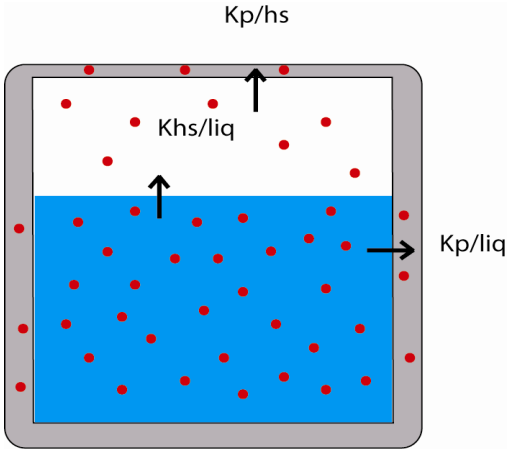


Figure 1.8. Partition coefficients (*K*) of aroma compounds present in food matrix and in contact with packaging material.

The partition coefficient is defined as the mass concentration relation of a molecule between two phases at equilibrium. Figure 1.8 shows the different partition coefficients involved in the transfer of aroma compound between food matrix and the packaging material in contact.

The retention of aroma compounds can be studied by comparing the headspace – liquid partition coefficients (*Khs/liq*) in different food matrices and in water. A large literature has been reported about *Khs/liq* (Ettre et al. 1993, Jouquand et al. 2004, Landy et al. 1996, Savary et al. 2006, Seuvre et al. 2006) and some data regarding an ethyl ester series are presented in Table 1.2. Table 1.2 shows that *Khs/liq* of ethyl acetate, the most hydrophilic compound, is almost three times higher in aqueous medium than in lipophilic medium.

Table 1.2. Headspace/matrix partition coefficients (*Khs/liq*) from different liquid matrix.

Liquid matrix	T (°C)	Ethyl acetate	Ethyl butanoate	Ethyl hexanoate	Refereces
Water A	25		1.35E-02	3.40E-02	(Landy et al. 1996)
Water B	30	1.62E-02	1.83E-02	3.02E-02	(Savary et al. 2006)
Water C	30		4.50E-03	2.75E-02	(Juteau et al. 2004)
Water D	35		3.00E-02	6.90E-02	(Jouquand et al. 2004)
Water E	37	10	25	41	(Philippe et al. 2003)
Water F	37	10		42	(Seuvre et al. 2006)
Water G	25		1.25E-02	2.20E-02	(Landy et al. 1996)
Water H	25		1.22E-02	1.85E-02	(Landy et al. 1996)
Iota-carrageenan I	30		4.50E-03	2.10E-02	(Juteau et al. 2004)
Xanthan gum J	35		2.82E-02	4.76E-02	(Jouquand et al. 2004)
Carbohydrate matrix K	37	11		34	(Seuvre et al. 2006)
Carbohydrate matrix L	37	10	22.5	33.6	(Philippe et al. 2003)
Complex matrix M	37	9.0		1.50	(Seuvre et al. 2006)
Complex matrix N	37	9.1	8.5	1.64	(Philippe et al. 2003)
Triolein O	25		2.20E-04	3.00E-05	(Landy et al. 1996)
Triolein P	37	9.1	9.25	1.64	(Philippe et al. 2003)
Triolein Q	37	3.2	0.5	0.41	(Philippe et al. 2003)

A aroma solution in water
B aroma solution in water
C aroma solution (20 uL/L) in water with 0.3% NaCl (w/w) after 4 hours
D aroma solution in water + NaCl 0.01M
E aroma solution (50 ppm) in water after 48 hours
F aroma solution (50 ppm) in water after 48 hours
G aroma solution in water with 5g/L of sodium caseinate
H aroma solution in water with 5g/L of sucrose stearate
I aroma solution (20 uL/L) in water with 0.3% NaCl (w/w)+ 0.2% ioata-carrageenan after 4 hours
J aroma solution in xanthan gum (0.1% w/w) + NaCl 0.01M
K aroma solution (50 ppm) in carbohydrate matrix (2.4% modified waxy corn starch, 1.3% pectin, 1.8% D-glucose, 0.15% citric acid, 0.05% trisodium citrate and 0.03% tricalcium citrate) (w/w) after 48 hours
L aroma solution (50 ppm) in carbohydrate matrix (2.4% waxy corn starch, 1.3% pectin, 1.8% D-glucose, 0.15% citric acid, 0.05% trisodium citrate and 0.03% tricalcium citrate) (w/w) after 48 hours
M aroma solution (50 ppm) in complex matrix (2.4% modyfied waxy corn starch, 1.3% pectin, 1.8% D-glucose, 0.15% citric acid, 0.05% trisodium citrate and 0.03% tricalcium citrate) + 3.5% triolein (w/w) after 48 hours
N aroma solution (50 ppm) in complex matrix (2.4% waxy corn starch, 1.3% pectin, 1.8% D-glucose, 0.15% citric acid and 0.05% trisodium citrate) + 3.5% triolein (w/w) + 0.4% soybean lecithin after 48 hours
O aroma solution water with triolein (volumetric fraction 15% v/v)
P aroma solution (50 ppm) in water emulsion 3.5% triolein (w/w) + 0.4% soybean lecithin after 48 hours
Q aroma solution (50 ppm) in purified triolein after 48 hours

However, *Khs/liq* of ethyl hexanoate, a hydrophobic compound, is more of 100 times higher in aqueous medium than in oily medium and only 1.5 times higher in water medium than that in water medium with 0.1% of xanthan gum. Furthermore, ethyl butanoate and ethyl

hexanoate show a dramatic drop of *Khs/liq*in water/triolin phase compared with that in water phase. So, it is clear that partition coefficients of aroma compounds can be affected by composition of liquid phase and so that interaction of aroma compound with macromolecules, assessed with the *Khs/liq,* could play a positive role against sorption by packaging.

The polymer-liquid partition coefficient, *Kpolym/liq* of aroma compound can be calculated as the ratio between the concentration of the compound in the polymer and the concentration of the compound in the matrix, generally a liquid at equilibrium. So, a large partition coefficient indicates that the compound has a higher affinity for the packaging material than for the matrix, and consequently, large amount of compound is sorbed by the polymer material.

Several studies have reported the partition coefficients (*Kpolym/liq)* in food-packaging systems (Ducruet et al. 2007, Gavara et al. 1996, Hernandez-Muñoz et al. 2001, Johansson and Leufven 1997, Nielsen et al. 1992b, Piringer and Baner 2008, Reynier et al. 2004). For instance, Nielsen et al. (1992) showed that the *Kpolym/liq* value for ethyl butanoate in LDPE was affected significantly by the matrix composition, being 3.0 when matrix was water, and 10.5 when was oil.

Table 1.3 shows data of *Kpolym/liq* for some aroma compounds contained in different matrices that were in direct contact with polymers materials.

Table 1.3. Partition coefficients data (*Kpolym/liq)* for some aroma compounds between various packaging materials and different liquid matrices.

Aroma compounds	PP	PS	PET	LDPE
Ethyl acetate	-	1.9 [d]	-	-
Benzaldehyde	-	-	-	1.5[h]
Ethyl butanoate	0.9 [a]; 2.5 [i]	-	0.6 [j]	3.0[b]; 10.5[c]
Ethyl 2-methylbutanoate	6.5 [n]	-	0.65×10^{-4}[g]; 0.3 [j] 0.034 [l] 0.15 [m]	9.4[b]; 17.1[c] 75 [l] 100 [m]
Ethyl hexanoate	-	-	0.8 [e]; 0.15 [f]; 2.2×10^{-4}[g]	0.45[f] ; 0.55[f]
2-nonanone	40 [i] 18.6 [n]	-	0.21 [l] 1.1 [m]	20 [i] 212 [l] 290 [m]
D-limonene	60 [i]	5100 [d]	0.9 [e] 0.05[f] 2.1 [k]	1.5[e] 0.9[f] 40 [i]

[a] from a mixture of 6 aroma compounds (50 µg/cm^3 each) in ethanol/water (40%) at 23°C for 14 days, in Johansson and Leufven (1997)
[b] from a mixture of 10 aroma compounds (1mg/Kg each) in water at 25°C for 7 days, in Nielsen, Jagerstad, Oste and Wesslen (1992)
[c] from a mixture of 10 aroma compounds (1mg/Kg each) in oil at 25°C for 7 days, in Nielsen et al. (1992)
[d] from an aroma compound (Ethyl acetate = 400 mg/L; D-limonene = 0.6 mg/L) in water at 25°C for 60 days, in Gavara, Hernandez and Giacin (1996)
[e] from an aroma compound (500 ppm) in ethanol at 25°C for 30 days for LLDPE or 90 days for m-PET, in Hernandez-Muñoz, Catalá and Gavara (2001)
[f] from an aroma compound (500 ppm) in oil at 25°C for 30 days for LLDPE or 90 days for m-PET, in Hernandez-Muñoz et al. (2001)
[g] estimated from a pasteurized strawberry syrup (surcrose syrup + strawberry, elder and lemon juices) at 20 ºC after 90 days, in Ducruet et al. (2007)
[h] in water + 10% (w/w) ethanol, at 23 ºC, from (Piringer and Baner 2008).
[i] in water (≤300 ppm), at 23 ºC for 15 days, from (Reynier et al. 2004)
[j] in water + 10% (w/w) ethanol, at 23 ºC, from (Piringer and Baner 2008).
[k] in 50% (w/w) Aqueous ethanol, at 23 ºC, from (Piringer and Baner 2008).
[l] From a mixture of 10 aroma compounds (each 100 µL/L) in 6 g/L aqueous Tween 80® (pH = 4.2 ± 0.2) at 20ºC for 21 days, calculated from (Van Willige R. et al. 2002).
[m] From a mixture of 10 aroma compounds (each 100 µL/L) in 6 g/L aqueous Tween 80® (pH = 4.2 ± 0.2) at 40ºC for 21 days, calculated from (Van Willige R. et al. 2002).
[n] From a mixture of 10 aroma compounds (each 100 µL/L) in 6 g/L aqueous Tween 80® (pH = 4.2 ± 0.2) at 20ºC for 14 days, calculated from (Van Willige R. et al. 2002).

Moreover, the group of van Willige has done an extended work on the influence of proteins, carbohydrates, oil and real food products on the sorption of flavors compounds by linear low-density polyethylene (LLDPE) (van Willige Remco WG et al. 2000b, a). These authors showed that the aroma sorption in LLDPE of limonene, decanal, linalool and 2EMB in water solutions decreased significantly with the increase of oil concentration in the emulsion after

14 days of contact at 4°C. Specific interaction of aldehydes was evidenced with β-lactoglobulin reducing their sorption into LLDPE.

1.1.4.4. Factors related to environment

The most important factors affecting the mass transfer and related to environment are temperature and relative humidity (RH). In the case of permanent gases, literature has reported for PET and PLA higher values of oxygen permeability and diffusion coefficients with the increase of temperature from 5 to 40 ºC at 0 %RH, whereas the oxygen solubility coefficients decreased (Auras et al. 2004a).

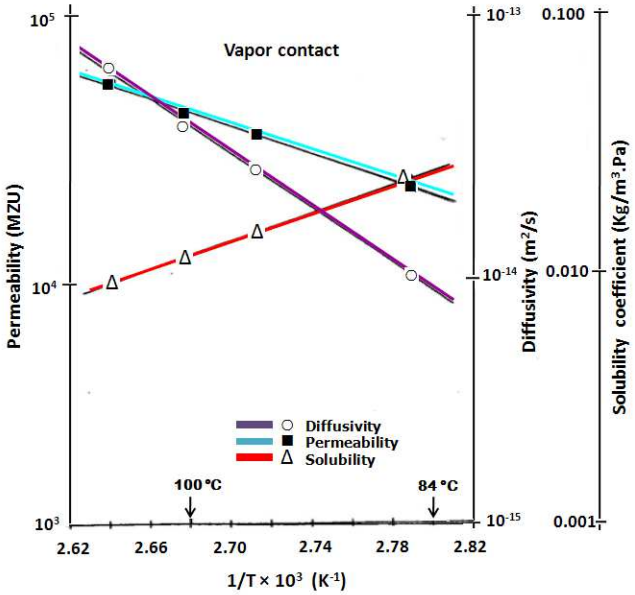


Figure 1.9. Effect of temperature on permeability, diffusion and solubility of ethyl hexanoate in PVDC (Delassus et al. 1988).

In the case of gases and organic compounds, the permeability and diffusion in polymers increases with increasing temperature, whereas solubility of organic compounds decreases, according to the model described by the Van't Hoff – Arrhenius equations (Van Krevelen and Te Nijenhuis 2009). This was shown by Delassus et al. (1988) on PVDC with ethyl hexanoate in vapor contact (Figure 1.9).

Auras et al. (2006) reported that while the permeability and diffusion coefficients of ethyl acetate in PLA increased with the increase of the temperature from 30 to 45ºC, the solubility coefficient decreased (Auras et al. 2006).

In the case of vapors such as aroma compounds, Hirata and Ducruet (2006) showed that the solubility coefficients of ethyl acetate and ethyl hexanoate in PE increased with the increase of temperature from 20 to 35 °C (Hirata and Ducruet 2006). Several reasons can explain the increasing aroma compound sorption at higher temperatures such as the increased mobility of the aroma molecules, change in polymer configuration, such as swelling or decrease of crystallinity (Tehrany and Desobry 2004).

The effects of relative humidity on the barrier properties of polymers have been largely studied for the case of oxygen permeation. Generally, the presence of water has a significant effect on the barrier properties of the hydrophilic polymers, such as poly(ethylene-co-vinyl alcohol) (EVOH) and most polyamides, accelerating the gas diffusion and consequently increasing the O_2 permeability (Caner 2011, Dury-Brun et al. 2007). In contrast, under high %RH, the oxygen permeability of polyolefins and PET remains almost constant (Almenar and Auras 2010). For PLA case, literature has reported that the oxygen permeability decreased with the increase of water activity from 0 to 0.9 (Auras et al. 2004a). The authors explained this behavior due to the relatively hydrophobic nature of PLA.

1.2. Methodology

In the aim of studying the interaction of a packaging polymer with foodstuff, and more precisely with organic vapors, the solubility and diffusion coefficients need to be measured. The food packaging interactions have been studied during the last decades using different methods. The choice of a methodology depends on several factors, such as the type of analyte (aroma compounds, VOCs, additives, etc.). Therefore, a brief overview of some

methodologies used in literature to identify and quantify organic compounds involved in food and packaging interactions is presented in the following paragraph.

1.2.1. Quantification of the VOCs and aroma compounds into polymer

1.2.1.1. Methods of extraction using solvents

With the purpose of quantifying the sorption or migration of compounds in food/package systems, different methods have been used, the majority of them being extractive methods (Gavara et al. 1996) and quantification is generally carried out by gas chromatography (GC).

The conventional extraction methods can be classified in two categories: i) dissolution of the polymer (mostly under reflux) and precipitation and ii) liquid–solid extraction methods. Both of them have been largely used in the extraction of additives from polymeric matrices (Vandenburg et al. 1997). The first one consists in the dissolution of polymer in a solvent, generally under reflux, following by the precipitation of the polymer by addition of a non solvent. In the second one, the compound is extracted from the solid medium by a liquid, which is then separated usually by filtration. Many methods for carrying out liquid-solid extractions have been used including Soxhlet, ultrasounds and shake-flask extractions. To use these methods is important to select the most appropriate solvent for the extraction, without interaction with the target compounds and ease of eliminate after extraction.

Some techniques, such as Accelerated Solvent Extraction (ASE) and Supercritical Fluid Extraction (SFE) have been commonly used to replace conventional extraction methods, presenting the possibility of automating the process and requiring less solvent and less time of extraction. Accelerated Solvent Extraction (ASE) is a method where the sample is placed into an extraction cell and solvent liquid is pumped inside the cell, which is heated in an oven at

conditions of pressure and temperature programmed. After a given extraction time, more solvent is pumped through the cell into the collecting vessel and the remaining solvent is purged into a collecting vessel with nitrogen. The equipment is automated, allowing sequential extractions to be programmed (Vandenburg et al. 1997).

Supercritical Fluid Extraction (SFE) is an extraction method that uses the properties that fluids have at a pressure and temperature above their critical point. Due to its critical temperature (31.3 °C) and pressure (7.4 MPa), safe to use, non-explosive character and low cost, CO_2 is the solvent most used. SFE has been applied in many solid matrices, including food, polymers, natural products. SFE can also provide a method for extracting environmental contaminants and additives from polymers, being much faster than Soxhlet extraction (Vandenburg et al. 1997) due to the higher diffusivity of in its critical state than for a liquid solvent . The use of supercritical carbon dioxide extraction and gas chromatography to study the sorption of aroma compounds in polymer packaging materials (i.e. polyethylene terephthalate, polycarbonate and recycled polypropylene) have been largely reported in literature (Sajilata et al. 2007). The main advantage is the lack of concentration step after extraction as CO_2 evaporates when pressure returns to atmospheric pressure.

When the target components are present in low amounts, the extracts obtained should be concentrated by distillation or vacuum evaporation of the solvent, allowing a higher detection and sensitivity by further analytical procedures. Although these methods have been shown to be efficient, they are often tedious and time-consuming, using large volumes of often toxic solvents and requiring also a further concentration phase, which may result in the loss of volatile compounds. Moreover when the extraction of sorbed aroma compounds is carried out using solvent, oligomers and additives coming from polymer are co-extracted leading to a complex gas chromatography profile. Thus to gain selectivity by the extraction procedure of

volatile and aroma compounds into polymer, it is more judicious to choose methodology profiting from volatility of these molecules.

1.2.1.2. Methods using volatility of molecules

Many methods to extract and quantify the organic compounds in solid matrices have been established thanks to their volatility. Between them, dynamic headspace (HS) purge-and-trap and direct HS methods are mainly used. The first one allows the analysis of low concentrations by enriching volatile components on a polymer bed and therefore improve the sensitivity, whereas the second one is largely used in samples with high concentration of aroma compounds (Kataoka et al. 2000).

The headspace methods allow measuring the transfer or release of volatile compounds in solid matrix, by static or dynamic ways. The static headspace method uses a closed system sampling the headspace at thermodynamic equilibrium, whereas the dynamic headspace method uses an open system and the measurements are taken when the equilibrium is not reached.

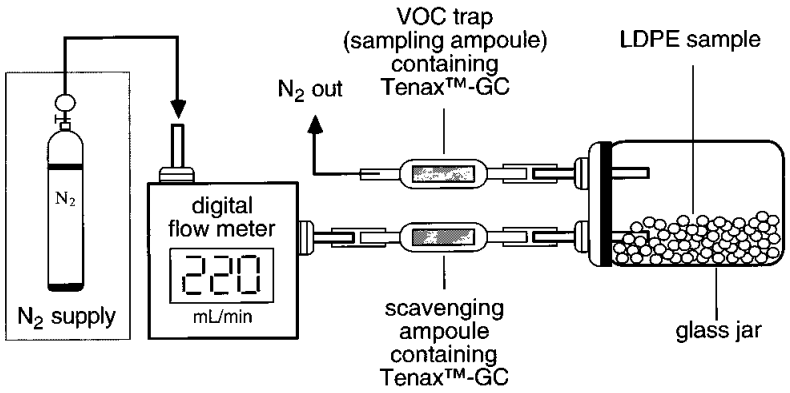


Figure 1.10. Schematic diagram of trapping of VOCs on Tenax^{TM}, from (Hodgson et al. 1998).

The dynamic headspace extraction system is a purge and trap technique proceeding with a pre-concentration step on adsorbent for sampling VOCs. Figure 1.10. shows a schematic

diagram of this technique. Its principle is the adsorption of the volatile molecules on a chemically inert sorbent material, generally Tenax^{TM} (2,6-diphenyl-*p*-phenylene oxide polymer), followed by thermal desorption of the adsorbent and its subsequent analysis by GC-MS (Hodgson et al. 1998).

In 1981 - 1982, Kolb, Auer and Pospisil presented the theoretical principles of multiple headspace extraction, the purpose of which was to eliminate the matrix effect produced by the calibration phase when different matrix standards are used. This method is a dynamic gas extraction carried out stepwise on a same sample, allowing quantify the volatiles compounds in solid or complex liquid samples. At each extraction step, the overall equilibrium of the analyte in the system must be first established then analyte present in the headspace volume is removed and analyzed as GC area. Various extractions of the same sample, generally five, are performed in order to reach an exponential decay of the analyte present in the sample. MHE calculates, thanks to a mathematical extrapolation, the total peak area which is proportional to the total amount of the analyte present in the original sample, which can be determined by external calibration (Kolb and Ettre 1991).

Multiple headspace extraction has been used for determination of volatile compounds in solid matrixes such as polymers (Kolb et al. 1981) and cellulose based packaging materials (Wenzl and Lankmayr 2000). This method has also been used for determination of monomer solubilities (i.e. styrene and acylonitrile) in water (Chai et al. 2005).

Solid Phase MicroExtraction (SPME), being intermediate method between dynamic and static headspace technique, was introduced in 1990 by Arthur and Pawliszyn as a solvent-free sample preparation technique (Arthur and Pawliszyn 1990). This technique uses a small volume of sorbent dispersed on the surface of small fibres to isolate and concentrate analytes from sample matrix either by immersion in the liquid matrix or in the headspace above solid

matrix (HS-SPME). Depending on the nature of the fibre coating, the compounds are absorbed or adsorbed by the fibre until the equilibrium is reached in the system (Pawliszyn 2000). The Figure 1.11 shows the design of a commercial SPME device. Because the amount of analyte extracted by the fibre is linked to the partition coefficient HS/fiber and is not a mirror picture of the molecules present in sample, SPME is mainly used as non-quantitative extraction technique. Internal or external calibration techniques are used to quantifying, however they can be affected by the matrix effects, causing significant differences in the partition coefficients and release rates for different compounds and consequently satisfactory results are rarely produced. Despite this difficulty of calibration, SPME has proven a performing technique for the trace analysis of volatiles offering more sensitivity and selectivity than a simple static headspace technique.

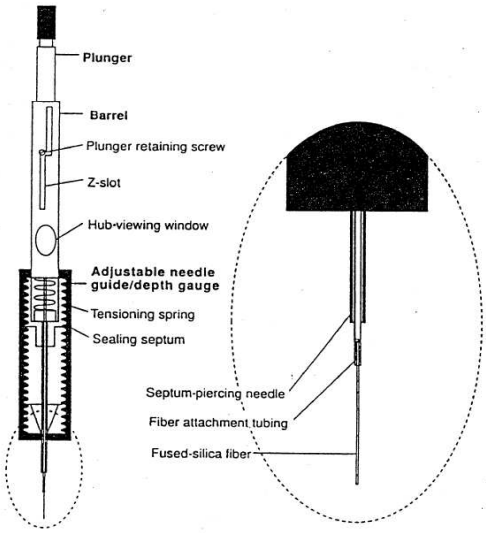


Figure 1.11. Design of the commercial SPME device (Harmon 1997).

In the field of food science, SPME technique has been used to investigate the volatile organic compounds in packaging materials (Hakkarainen 2008) and coupled to mass spectrometry (MS) has been reported as a powerful tool for screening purposes concerning volatile compounds (Felix et al. 2012). Additionally, SPME has also been used for the analysis of

components and contaminants including flavors, off-flavors, pesticides and other contaminants. Many different food types have been studied by SMPE, such as fruits, vegetables, plants, beverages and dairy products (Kataoka et al. 2000). For example, Van Aardt et al. (2001) reported the use of CAR/PDMS solid phase microextraction in static headspace at 45 °C for 15 min as an effective method for the recovery of acetaldehyde in milk and water media with detection levels as low as 200 and 20 ppb, respectively (van Aardt et al. 2001).

In the last decade, with the purpose of quantifying analytes in different matrices and increasing the sensitivity, multiple headspace extraction was coupled with solid phase microextraction. The theory of this method was presented by Ezquerro et al. (2003). Since the amount of compound extracted by the fibre is proportional to the initial amount and the peak area decays exponentially with the number of extractions of the same sample, it is possible to estimate the total peak area carrying out three or four successive extractions by HS-SPME. The total peak area allows the quantification of analyte using an external calibration. This method eliminates the matrix effect in the quantitative analysis of solid samples and it is a solvent-free method. Its use to quantifying the volatiles compounds in polymers packaging materials (Ezquerro et al. 2003), in cork stoppers (Ezquerro and Tena 2005), in wine (Carrillo and Tena 2006), in tomatoes (Serrano et al. 2009) has already been reported.

1.2.1.3. Determination of transport coefficients

The permeability and diffusion coefficients of small molecules through a polymer membrane can be determined by two measurement methods: permeation or sorption-desorption. Both methods give theoretically equivalent results, if interactions of organic compound with the polymer matrix are negligible which means at low concentrations. However, the limits of

detection of some methodologies don't allow working at low concentration. Therefore the values reported in the literature present large variability, particularly in the case of diffusion coefficient (Cayot et al. 2008) when the change in the structure of the polymer is not controlled in parallel.

However Dury-Brun et al. (2008) compared two methods of measure (sorption-desorption or permeation) for the case of ethyl hexanoate and packaging materials. When compared with literature, their results showed that the variability of the solubility coefficient (S) for a given aroma compound and controlled packaging material is lower than the variability of the diffusion coefficient (D). They concluded that the determination of S is more reliable because it is performed at equilibrium state.

The study of transport of small molecules through a polymer membrane can be realized by 3 main methods: integral permeation and differential permeation sorption-desorption, (Dury-Brun et al. 2007).

The permeation approaches allow obtaining the permeability coefficient (P) and determining the diffusion coefficient (D) while the solubility coefficient (S) is inferred using the relation $P=S\times D$ (Equation 1.1).

In the integral permeation method, the material is placed between two chambers with the same total pressure but with different permeant partial pressure, producing the mass transfer through the polymer membrane (Cayot et al. 2008). In this method, the permeant is accumulated in a closed receiving chamber. When the permeation curve reaches the equilibrium, the transport coefficients P and D can be determined thanks to the time-lag method.

The differential permeation method is based on the same principle than the integral permeation method but the upstream chamber is opened and the pressure is constant. In this case, a gas stream sweeps the downstream chamber to move the permeant to a detector.

The sorption-desorption method measure the weight variation of a polymer sample with time. This measurement can be direct (gravimetric methods) or indirect (pressure or volume measurement). At the beginning all molecules are removed from the chamber, then the permeant gas is gradually introduced in the chamber until the given pressure and finally the measurement of the weight variation begins. Due that this method measure directly the sorption, the solubility coefficient measure is more accurate than deduced from permeation measurements. Then, the apparent diffusion coefficient can be calculated from the kinetic of sorption.

Two examples of methodologies used for the determination of *P*, *D* and *S* were presented below.

Permeation associated to Purge and Trap Injector (PTI)

To work with low concentrations of organic compounds, permeation experiment was assessed with the coupling of purge and trap injector (PTI) by (Lebossé et al. 1997). This set-up consists of a permeation cell containing the packaging material and the mixture of organic compounds to be analyzed. A flow of carrier gas passes through the downstream part of the cell and sweep the volatiles into a cryogenic trap held at -120 °C with liquid nitrogen. After the purge time, the trapped volatiles are thermally flash desorbed and directly injected in the GC column. A water condenser at -10 °C is used between the purge cell and the cryogenic trap to prevent blocking of the latter by ice crystals when aqueous samples were assessed.

The measurement of permeability is carried out until to reach the steady state of each volatile compound studied. The permeability coefficients (P, $Kg.m^{-1}.s^{-1}.Pa^{-1}$) are calculated from the pressure gradient between the compartments of the purge cell (Δp), the quantity of compound having passed through the film (ΔQ), the exchange surface (A), the thickness of the film (l) and the time (Δt), according to the equation 1.7:

$$P = \frac{\Delta Q}{\Delta t \times A} \times \frac{l}{\Delta p}$$

(Equation 1.7)

The diffusion coefficient (D) of each organic compound is calculated by the method of time-lag. This is measured from the plot of the sum of the chromatographic peak areas of each compound having permeated versus time. The Figure 1.12 shows an example of the described plot.

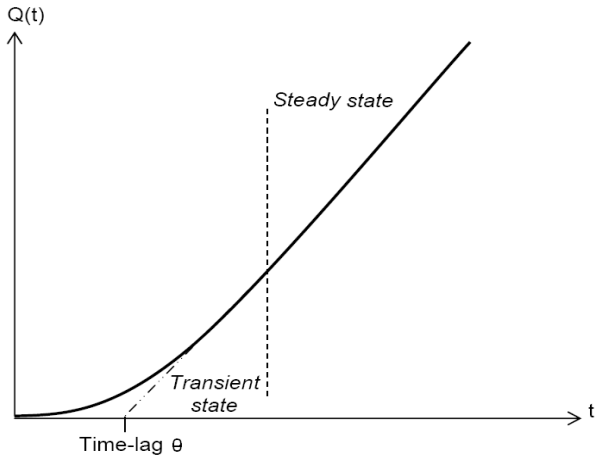


Figure 1.12. Theoretical curve of unidirectional diffusion through a flat membrane (time-lag method), from (Dury-Brun et al. 2007).

The point of intersection of the abscissa axis and the curve in linear growth phase is defined as the time-lag (θ), which is the time required to reach the steady state. Then, D ($m^2.s^{-1}$) is calculated according to Equation 1.8 and S is deduced from Equation 1.1.

$$D = \frac{l^2}{6 \times \theta}$$

(Equation 1.8)

Sorption-Desorption methodology

The gravimetric method is commonly used to determine the solubility of volatile compounds and permanent gases. In this method, the measurement of mass gain or mass loss of

permeates are recorded during time, until equilibrium occurs under the given experimental conditions (temperature, molecule activity, system geometry and homogeneity, aerodynamic conditions at the surface of the system). This static method consists in a microbalance which measures the weight change of the sample and allows an accurate automatic record of data with the time during transient and steady state sorption. Microbalances have sensitivities around 0.2 μg (Figure 1.10).

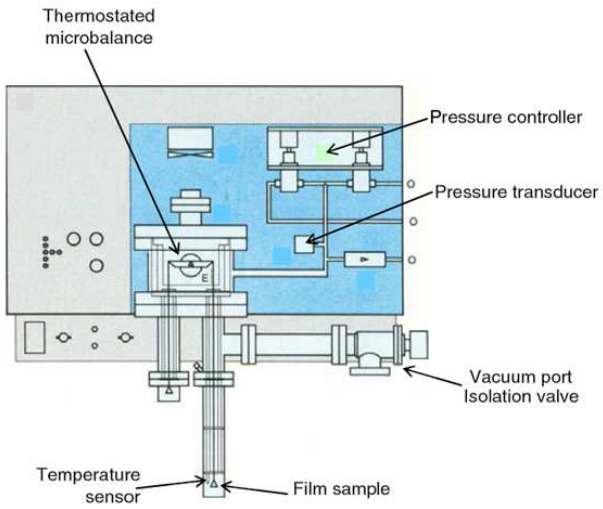


Figure 1.13. Automated microbalance IGA 002 (Hiden Isochema Ltd, Warrington, UK), from (Dury-Brun et al. 2008).

The vapor activity conditions are generated and constantly maintained by a vaporizer or by controlling the partial pressure of the molecule by a pressure transducer. Commercial apparatus based on gravimetric techniques are available to follow the sorption kinetics of aroma compounds and water in polymers (Intelligent Gravimetric Analyser, Hiden Isochema Ltd., Warrington, UK; Dynamic Vapor Sorption, Surface Measurement Systems Ltd., Middlesex, UK) (Dury-Brun et al. 2008). The use of this method in the aroma sorption studies in polymer packaging has been reported in literature. Thus, the temperature effect on the solubility of ethyl acetate and ethyl hexanoate in polyethylene films was evaluated using an electronic microbalance, IGA-002, supplied by Hiden Analytical, Warrington (UK) (Hirata

and Ducruet 2006). The sorption and diffusion behaviors of benzaldehyde in PP were studied using a SGA-100R gravimetric analyzer (VTI, Hialeah, FL) (Qin et al. 2008). Ethyl acetate sorption was investigated as function of the crystallinity of the PLA film, using an electronic microbalance (Intelligent Gravimetric Analyzer 002, Hiden Isochema Ltd, Warrington, UK) with a sensitivity of 0.2 µg (Colomines et al. 2010).

This methodology allows the measurement of sorption coefficients (*S*) of organic compounds in a polymer, determining also the diffusion coefficient (*D*) from the transient part of the sorption-desorption kinetic curve (Figure 1.14). The permeability coefficient (*P*) is then obtained by deduction, using the relation $P = S \times D$.

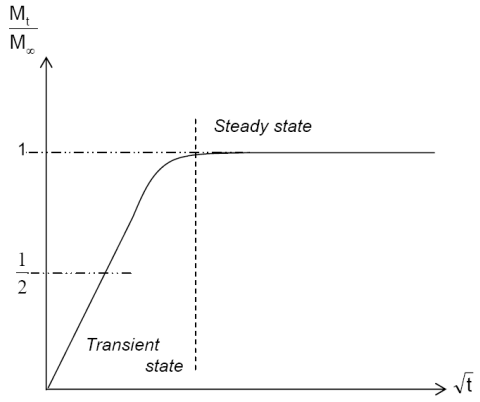


Figure 1.14. Theoretical curve of sorption experiment through a flat membrane, from (Dury-Brun et al. 2007).

The solubility S ($Kg.m^{-3}.Pa^{-1}$) can be obtained at equilibrium according to (Equation 1.9:

$$S = \frac{m_\infty}{m_0} \times \frac{d}{p}$$

(Equation 1.9)

Where m_∞ is the equilibrium sorption obtained theoretically after infinite time ($Kg.Kg^{-1}$), m_0 is the initial sample mass, d is the film density ($Kg.m^{-3}$) and p is the compound vapor partial pressure (Pa).

The diffusion coefficient can be calculated from the (Equation 1.10:

$$\frac{m_t}{m_\infty} = \left(\frac{16 \times D}{\pi \times l^2}\right)^{1/2} \times \sqrt{t}$$

(Equation 1.10)

Where m_t is the mass (Kg) at time t (s), m_∞ is the mass uptake at infinite time (Kg), l is the thickness of the film (m) and D, the diffusion coefficient ($m^2.s^{-1}$).

Commercial apparatus based on gravimetric techniques show particularly some limitations, for example the lower limit in the regulation of the pressure, the establishment of pseudo-equilibrium for high activities and long experiment time, and the time delay in the establishment of the concentration equilibrium at the surface of the system (Dury-Brun et al. 2008, Hirata and Ducruet 2006).

1.3. PLA

Polylactide is an aliphatic polyester produced from the monomer of which is produced from renewable resources by fermentation, it is easily biodegradable/compostable and it is processable using standard equipment. PLA is produced by ring-opening polymerization of lactide, which is obtained from lactic acid through the fermentation of sugar feed stocks (Auras et al. 2004b, Avérous 2008, Garlotta 2001, Inkinen et al. 2011).

Until the last decade, due to its high cost, the PLA uses were limited to medical applications. New techniques of economical production of high molecular weight PLA polymer have expanded its uses (Auras et al. 2004b, Lim et al. 2008). Nowadays, PLA have the third position in terms of bio-based plastics volume production in Europe, after starch plastics and cellulose (Shen et al. 2009). The major PLA resins supplier is Cargill (in the USA known as Ingeo™) with a production capacity in 2006 of 140 kT per year at 2-5 euros per Kg (Avérous

2008). Other PLA suppliers are Mitsui Chemicals, Inc. (in Japan known as LACEA™), Purac (in Netherlands), and Teijin Limited (in Japan known as Biofront®).

1.3.1. Structure

Commercial PLA are copolymers of poly(L-lactic acid) (PLLA) and poly(D,L-lactic acid) (PDLA), which are made from L,L-lactides and D,L-lactides, respectively (Figure 1.15) (Auras et al. 2004b).

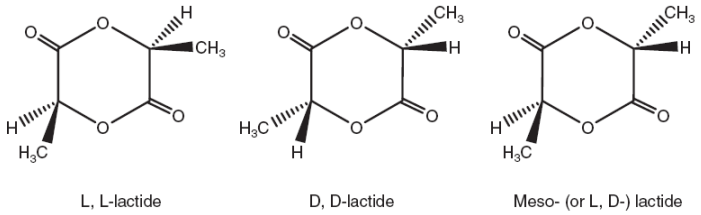


Figure 1.15. Chemical structures of lactides (Domenek et al. 2011).

There are three main routes of synthesis of PLA from lactic acid (Figure 1.16) direct condensation polymerization, azeotropic dehydration condensation and ring-opening polymerization (Auras et al. 2004b, Avérous 2008, Garlotta 2001, Lim et al. 2008).

The ring opening polymerization route for PLA is generally the most applied route of PLA industrial production. This is the synthesis route used by NatureWorks, which is the PLA supplier used in the present work. It involves three separate steps: The first one is the polycondensation of lactic acid to produce a polymer of low molar mass (Mw between 1000 and 5000 g/mol). From this intermediate polymer, the second step is the lactide manufacturing in presence of a catalyst. The result is a mixture of lactide stereo-isomers which are then purified by distillation at reduced pressure. Finally, the third step is the ring opening polymerization (cationic or anionic), which allows produce high molecular weight PLA (100000 g/mol) (Auras et al. 2004b, Garlotta 2001).

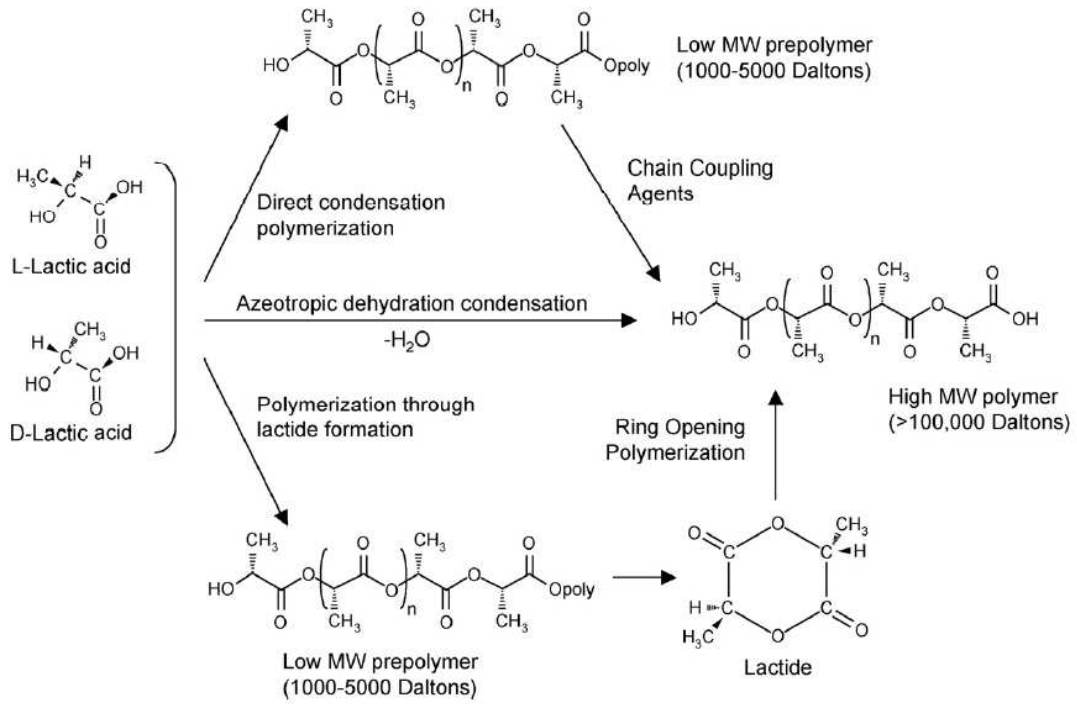


Figure 1.16. Synthesis of PLA from L- and D-lactic acids (Lim et al. 2008).

It is possible to obtain different types of PLA in function of stereochemistry of polymer chain, as showen in Table 1.4.

Table 1.4. Stereoisomers of PLA*.

PLA form	Structure
Isotactic poly(L-lactide)	PLLA LLLLLLLL
Isotactic poly(D-lactide)	PDLA DDDDDDDD
Random optical copolymers	Random level of meso or D-lactide in L-lactide or D-lactic acid in L-lactic acid
Stereocomplex PLLA/PDLA	LLLLLLLL mixed with DDDDDDDD
PLLA/PDLA stereoblock complexes	LLLLLLLLDDDDDDDD
Syndiotactic poly(meso-lactide)	DLDLDLDL
Heterotactic poly(meso-lactide)	LLDDLLDDLLDD
Atactic poly(meso-lactide)	No stereocontrol
Atactic poly(lactide)	PDLLA No stereocontrol

* from (Domenek et al. 2011).

1.3.2. PLA Properties

The stereochemistry of PLA, being the ratio of L,L- and D,L- enantiomers, established during the synthesis of PLA, influence its physical and mechanical properties. Compared with conventional polymers, PLA presents interesting properties such as high yield strength and modulus, but it is brittle with low impact strength and has low thermal resistance. Table 1.5 gives a summary of some literature data from a recent review (Carrasco et al. 2010).

Table 1.5. Physical properties of PLA, Polystyrene (PS), Polypropylene (PP) and Polyethylene terephthalate (PET)*.

	PLA	PS	PP	PET
Relative density	1.24	1.04-1.06	0.91	1.37
Clarity	Transparent	Transparent	Translucent	Transparent
Mechanical properties				
Tensile Yield Strength (MPa)	48-110	34-46	21-37	47
Tensile Modulus (GPa)	3.5-3.8	2.9-3.5	1.1-1.5	3.1
Tensile Elongation (%)	2.5-100	3-4	20-800	50-300
Notched Izod impact, 23° C (J/m)	13	-	72	79
Thermal properties				
Glass Transition Temperature (°C)	60	95	0	75
Melting Temperature (°C)	153	-	163	250
Vicat Temperature (°C)	55-60	84-106	80-140	74-200
Processing Temperature (°C)	210	230	225	255

*from (Carrasco et al. 2010).

1.3.3. Solubility

The solubility of PLA is highly dependent on the crystallinity degree and the molar weight of polymer. Thus, enantiopure PLA is soluble in chloroform and other chlorinated organic solvents, furan, dioxane, dioxolane and pyridine, whereas non enantiopure PLA is soluble in those solvents and also in ethyl acetate, dimethylsulfoxide, tetrahydrofuran, acetone, xylene, methyl ethyl ketone, ethyl lactate and dimethylformamide. In contrast, PLA is non soluble in water, alcohol (e.g. ethanol, methanol) and unsubstituted hydrocarbons (e.g. cyclohexane,

heptane) (Södergård and Stolt 2002). Moreover, literature reported that lactide is miscible with benzene, toluene, xylene, methylenechloride, chloroform, tetrahydrofuran, ethyl acetate, methanol, isopropanol, acetone, and butanone. The solubility in those solvents increases with increasing temperature (Jin et al. 2010).

1.3.4. Barrier properties

The barrier properties of a packaging polymer against the transfer of small molecules, such as O_2, CO_2 or H_2O, are important for preservation of food safety and quality during shelf life. Currently, the gas barrier properties of PLA are being studied; however no comprehensive review has been reported to the date. For that, Table 1.6 shows only some data of mass transfer coefficients of PLA and some conventional packaging polymers. Table 1.6 shows that the oxygen and carbon dioxide permeability coefficients of PLA are lower than those of low density poly(ethylene) (LDPE) and they are intermediate between poly(ethylene terephthalate) (PET) and polystyrene (PS).

Table 1.6. Oxygen (O_2) and Carbon dioxide (CO_2) permeability, O_2 diffusion and solubility coefficients of PLA and some conventional polymers.

	O_2			CO_2
	$P \times 10^{-18}$ (Kg.m/m^2.s.Pa)	$D \times 10^{-14}$ (m^2/s)	$S \times 10^{-6}$ (Kg.m^{-3}.Pa^{-1})	$P \times 10^{-17}$ (Kg.m/m^2.s.Pa)
PLA	4.948 [a]	-	-	1.52 [a]
	3.289 [b]	137 [b]	2.431 [b]	1.76 [d]
	5.0 [c]	2.7 [c]	225 [c]	-
PET	0.3 [a]	-	-	0.02 [a]
	0.27 [c]	5.4 [c]	5.25 [c]	-
LDPE	82.498 [a]	-	-	52.82 [a]
PS	16.4 [a]	-	-	13.2 [a]

[a] at 30 ºC, from (Almenar and Auras 2010); [b] at 23 ◦C and 0% RH, from (Courgneau C. et al. 2012); [c] at 23ºC 0 %RH, from (Auras et al. 2004a); [d] from (Auras et al. 2004b).

Concerning the water vapor transfer rate (WVTR), different values have been reported in literature, which can be explained from different methodologies and PLA grades used (Coltelli et al. 2008, Courgneau et al. 2011, Haugaard et al. 2002, Holm et al. 2006). Thus, at

38°C and 90 % RH, Holm et al. (2006) studied the WVTR in PLA trays (thickness of 500 µm) following ASTM F 1249, 1990 and reported a WVTR value of 30.6 g/m^2.day, while Courgneau et al. 2011, studying PLA films at same conditions of temperature and RH, reported a value of WVTR of 61.9 ± 6.7 g/m^2.day following the norm NFH00-30. The latter authors studied also the same PLA film at 25°C and 50 %RH, reporting a WVTR value of 12.1 ± 5.7 g/m^2.day.

Thermoformed PLA cups presented a WVTR (calculated using the reported data) of 6 g/m^2.day at 23°C and 50% RH following the norm ASTM F 1249 (Haugaard Vibeke et al. 2002), whereas other study reported a value of 80 g/m^2.day at 23°C and 85% RH, following the norm DIM 53122 in compression molded films of PLA2002D (Coltelli et al. 2008).

PLLA films (high molecular weight) showed a WVTR value (normalized to a thickness of 25 µm) of 228 g/m^2.day at 25°C and 90% RH following the norm JIS Z 0208 (Tsuji et al. 2006). The authors reported that the changes in the number average molecular mass (Mn) of PLLA films in the range of 9 x 10^4 – 5 x 10^5 g mol^{-1} and D-lactide unit content of PLA films, in the range of 0–50%, had insignificant effects on their WVTR values. The WVTR of PLLA films decreased monotonically with increasing Xc from 0 to 20%, while leveled off for Xc exceeding 30%, which was probably due to the higher resistance of restricted amorphous regions to water vapor permeation compared with that of the free amorphous regions (Tsuji et al. 2006).

When compared with literature data reported on commodity materials, water vapor permeability (WVP) of PLA is between 1.8 and 2.3 × 10^{-14} Kg.m/m^2.s.Pa at 25°C (Rhim et al. 2009, Sanchez-Garcia et al. 2008, Tsuji et al. 2006), which is one order of magnitude higher than PS and PET (Auras et al. 2004a) and two orders than HDPE (Morillon et al. 2002).

The transport of water molecules in the PLA matrices was studied by Siparsky et al (1997). They explained by the water cluster model instead the solution diffusion model. The water

cluster model proposes that the water molecules sorbed in the polymer matrix form clusters through preferential binding to each other by hydrogen bonding. The water molecules are not necessarily soluble in the polymer, but they permeate through it in the cluster form (Siparsky et al. 1997).

Few studies were reported on sorption, diffusion and permeability of aroma compounds in PLA and those mainly for ethyl acetate and D-limonene at high levels of activity (Auras et al. 2006, Colomines et al. 2010, Courgneau et al. 2012). In the case of ethyl acetate, which is part of many fruity aroma formulations, it was shown than it had a plasticizing effect and induced crystallization in PLA, changing its properties during storage (Colomines et al. 2010, Courgneau et al. 2012). Auras et al. (2006) studied the sorption of ethyl acetate and D-limonene, and showed that PLA had more affinity to more hydrophilic molecule, ethyl acetate, compared to hydrophobic one, limonene.

Auras et al. (2006) reported the solubility coefficient of PLA films containing 98% L-lactide to ethyl acetate (EA) as 6.17 $\times 10^{-3}$ $kg.m^{-3}.Pa^{-1}$ at 30 °C and 0.2 activity, using a gravimetric method. The authors compared their results in PLA with literature data and found that S was slightly higher than those reported in PET, PP and LDPE. They also reported the values of P and D as 5.34 $\times 10^{-19}$ $Kg.m.m^{-2}.s^{-1}.Pa^{-1}$ and 8.66 $\times 10^{-17}$ m^2s^{-1}, respectively. P of PLA was similar to that one of PET and lower than in PP and LDPE.

Colomines et al. (2010) reported *S* values of EA for an amorphous PLA extruded film (2% Xc) with 99% L-lactide as 0.1 $\times 10^{-3}$ $kg.m^{-3}.Pa^{-1}$ at 25 °C and 0.2 activity, using an electronic microbalance. Additionally, the authors showed that, at higher levels of activity as 0.5 and 0.9, the increasing the crystallinity of PDLLA produces a decrease of the ethyl acetate solubility coefficient.

Thermo-compressed PLA films (3% Xc and 300 μm thickness) with 92% L-lactide were studied by Courgneau et al. (2012), who reported the ethyl acetate solubility coefficient as

0.156×10^{-3} kg.m^{-3}.Pa^{-1} at 25 °C and 0.2 activity, using an electronic microbalance. They also reported the ethyl acetate diffusion coefficient *D* as 2.4×10^{-17} m^{2}s^{-1} in PLA.

In the case of D-limonene, its permeability and solubility in PLA was estimated by Auras et al. (2006) in a value lower than 9.96×10^{-21} kg.m.m^{-2}.s^{-1}.Pa^{-1} and lower than 4.88×10^{-6} kg.m^{-3}.Pa^{-1} at 45 °C and a partial pressure of 258 Pa. They also showed that *P* and *S* were lower than those for PET, PP and LDPE. So, they concluded that PLA seems to be a good barrier to this hydrophobic molecule.

The scalping of four aroma compounds at high pressure thermal treatments was evaluated for low density polyethylene (LDPE) and polylactide (PLA). The results showed that aroma uptake of the more polar molecules (2-hexanone and ethyl butanoate) in the more polar polymer matrix, PLA, were higher than in LDPE, whereas the aroma uptake of less polar compounds (ethyl hexanoate and limonene) in the more apolar matrix, LDPE, was much higher than in PLA (Mauricio-Iglesias et al. 2011).

1.3.5. Microstructure

The crystalline or amorphous structure of PLA can strongly influence on the conditions of processing and its final properties. PLLA and PDLA are semi-crystalline polymers must likely due the enantiomeric purity of the monomers and the stereoregularity of the polymer chain. The PDLLA or poly(meso-lactide) is, in contrast, completely amorphous due to its irregular structure.

The temperature at which the amorphous phase of the polymer changes from the glassy to the rubbery states is known as the glass transition temperature (Tg). The Tg of PLA is a function of the molecular weight and the stereochemistry. The Tg increases with molecular weight to maximum values, at infinite molecular weight, of 60.2, 56.4 and 54.6 ºC for PLA with contents of 100, 80, and 50% L-Lactide, respectively (Lim et al. 2008). A typical

thermogramm of PLA, obtained by differential scanning calorimetry (DSC) is given in Figure 1.17.

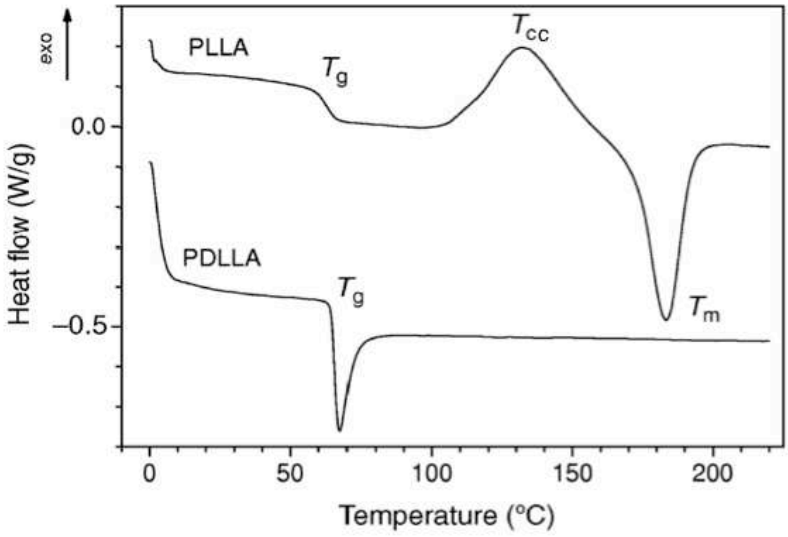


Figure 1.17. Differential scanning calorimetry (DSC) thermograms of amorphous PLLA and PDLLA (heating rate 10 ºC/min), from (Fambri and Migliaresi 2010).

The melting temperature (Tm) of PLA ranges from 130°C to 180°C, as summarized by Avérous (2008). Above Tg, PDLLA exhibits an endothermic peak, in the interval 65 – 90 ºC. This endothermic relaxation is due to a secondary molecular relaxation in the amorphous phase of semi-crystalline polymers during aging. For amorphous PLA, the glass transition temperature is one of the most important parameters since dramatic changes in polymer chain mobility take place at and above Tg. For semicrystalline PLA, both the Tg and melting temperature (Tm) are important physical parameters for predicting PLA behavior during service (Auras et al. 2004b).

PLLA crystallizes usually between 85 and 150 °C (Tc) but its fastest rate of crystallization is between 95 and 115 °C (Di Lorenzo 2005). The crystallinity degree of PLLA is strongly dependent on the crystallization time and temperature. Under adequate conditions, the crystallinity degree of PLLA can reach 45-60% (Day et al. 2006).The crystallinity of a polymer is expressed as a percentage of the theoretical maximum crystallinity of high molar

mass polymer. The most common value adopted for the melt enthalpy of a enantiopure PLA of 100% crystallinity is 93 J/g (Fischer et al. 1973), although higher values also have been reported. So, the degree of crystallinity (X_c) of PLA can be calculated using DSC, according to Equation 1.11:

$$X_C = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0} \qquad \text{(Equation 1.11)}$$

where ΔH_m is the enthalpy of fusion, ΔH_{cc} is the enthalpy of cold crystallization and ΔH^0_m is the heat of melting crystalline PLA (93 J/g).

1.3.6. PLA thermal degradation

Thermal degradation of PLA is the most important degradation pathway during the forming process due to the residual moisture contained in the pellets, high temperatures of processing and shear induced by the extrusion screw. Thermodegradation of PLA is a complex phenomenon and is observed above 200 ºC (Kopinke et al. 1996) leading to the appearance of low molecular weight molecules and oligomers with different molecular weight.

Thermal degradation of PLLA has been studied by pyrolysis-gas chromatography-mass spectrometry (Py–GC/MS) analysis, involving different reactions (Figure 1.18) that are summarized below (Carrasco et al. 2010, Kopinke et al. 1996, McNeill and Leiper 1985, Nishida 2010, Södergård and Stolt 2002)

- Hydrolysis by trace amounts of water, leading to acid and alcohol.
- Intermolecular transesterification producing monomer and oligomeric esters.
- Intramolecular transesterification (backbiting ester interchange), resulting in formation of monomer and oligomer lactides of low Mw.
- Cis-elimination, leading to acrylic acid and acyclic oligomers.

- Radical and concerted non radical reactions, producing acetaldehyde, carbon monoxide and methylketene
- Unzipping depolymerisation, leading to lactides.
- oxidative, random main-chain scission, leading to lactides.

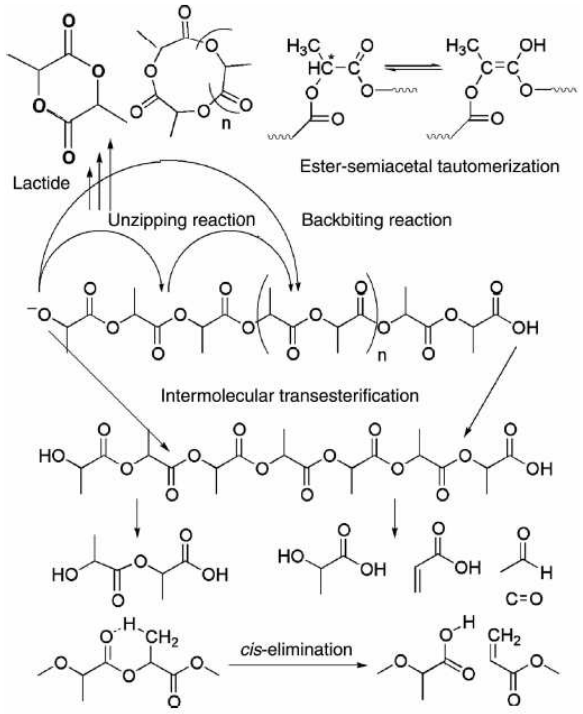


Figure 1.18. Thermal degradation of PLA (Nishida 2010).

Depolymerization by back-bitting (intramolecular transesterification) is considering that is the dominant degradation pathway at the temperature range of 270 °C - 360 °C (Kopinke et al. 1996). Nevertheless degradation occurs by different coupled mechanisms leading to the formation of different molecules which depend on the temperature. They are primary degradation products such as acetaldehyde, lactides and ring-formed oligomers of different sizes then secondary degradation products are formed due to oxidation, hydrolysis and cross reaction between primary products, such as carbon dioxide, carbon monoxide, methane, 2,3-

butanedione, 2,3-pentanedione, acrylic acid, acyclic oligomers, methylketene (fragmentation product), ethylene and propylene (Kopinke et al. 1996, McNeill and Leiper 1985).

Acetaldehyde is a right representative of these cross routes of degradation. The proportion of acetaldehyde is highest at 230 °C, and then decreases with increasing temperature to 440 ºC, leading to concomitant formation of methane and carbon monoxide (McNeill and Leiper 1985).

$$CH_3CHO \rightarrow CH_4 + CO$$

But acetaldehyde can be also produced at temperature higher than 350°C by radical pathway. The pyrolysis of acetaldehyde is a complex chain reaction, which may be initiated at moderate temperatures (230 ºC) also by the presence of free radical species. The initiation and propagation steps are (McNeill and Leiper 1985):

$$CH_3CHO \rightarrow CH_3^- + CHO^-$$

$$CH_3^- + CH_3CHO \rightarrow CH_4 + CH_3CO^-$$

$$CH_3CO^- \rightarrow CH_3^- + CO$$

McNeill and Leiper (1985) reported that during polylactide decomposition, radical combination involving a pair of acetyl radicals would lead to produce 2,3-butanedione:

$$2CH_3CO^- \rightarrow CH_3COCOCH_3$$

Ethylene and propylene were found at degradation temperatures of 362 °C and 440 ºC and their presences could be explained from decomposition of methylketene or its dimer.

1.3.7. Additives in PLA

Literature reported the use of several types of additives and processing aids in PLA (Table 1.7) to prevent blocking of blown film, slip agents which reduce friction, and additives which prevent the build-up of static electricity and improve printability.

Erucamide is a primary fatty acid amide used as a slip agent in blown films. This additive can migrate quickly to the surface of the product and lubricates it (Renstad et al. 1998). PLA blended with triacetin plasticizer (glycerol triacetate) has been used to reduce the adhesion between PLA films. Slip additives (oleamide, stearamide, N,N-ethylene bisstearamide, oleylpalmitamide) have also been added to reduce the coefficient of friction between overlapping films. In general, slip additive is used at low concentration (0.5 – 1.0% w/w) as higher amounts will affect the ability of print inks and stickers to adhere to the film surface (Lim et al. 2008).

In order to improve the mechanical properties of PLA while reducing its stiffness and brittleness, several plasticizers have been used such as citrate esters in particular Acetyl Tributyl Citrate or Maleates (Courgneau et al. 2011, Renstad et al. 1998) and oligomeric lactic acid (OLA). For example, Tg and modulus decreased 20 °C and 63 %, respectively, when 20% w/w of OLA was added into poly(92 % L-lactide, 8% meso-lactide). Additionally, low molecular weight polyethylene glycol (PEG), polypropylene glycol and fatty acid can act as plasticizers of PLA (Avérous 2008).

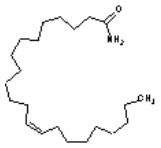
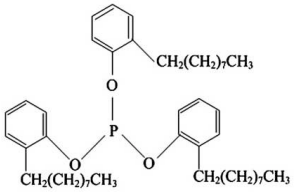
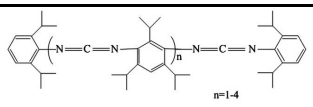
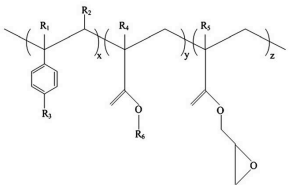
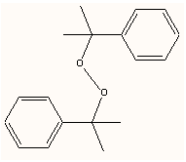
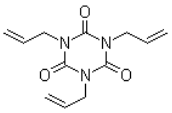
McNeill and Leiper (1985) and De Jong et al. (2001) showed the importance of non radical backbiting ester exchange involving terminal hydroxyl groups. These works showed that the end-groups play an important role on the thermal and hydrolytic stability of PLA (de Jong et al. 2001). The active OH groups of PLA are usually capped with appropriate reagents, including bis-functional couplers, as anhydrides and diisocyanates. Some thermal degradation stabilizers such as tris(nony1-phenyl) phosphite (TNPP) have been evaluated in PLA. The research group of Dorgan (Cicero et al. 2002) has shown the interest of using TNPP (tris(nonylphenyl)phosphite) as a stabilizer for PLA. According to the reaction mechanisms presented by Cicero et al. (2002), the phosphate groups of TNPP react with the hydroxyl end groups of the PLA chains to produce a chain with a phosphited end group and nonylphenol.

However, TNPP will stabilize PLA only if it is used in the right amount. However Najafi et al. (2012) have shown that if TNPP is added at a too low concentration, it results in the scission of polymer chains by hydrolysis, while if the concentration is higher (0.50% wt) TNPP can reconnect cleaved chains leading to increasing the molecular weight. 0.35% wt seems the best concentration for high molecular weight PLLA (Najafi et al. 2012). Al Itry et al. (2012) showed that the more efficient chain extender against thermal degradation of PLA is Joncryl ® ADR 4368, a chain extension/branching agent, containing nine Glycidyl methacrylate (GMA) functions and in particular when PLA is processed with organically modified clays which intensified the rate of PLA degradation (Al-Itry et al. 2012).

Polycarbodiimide (PCDI) (trade name Stabaxol P) was added into NatureWorks PLA pellets in an amount of 0.1 – 0.7 % (w/w) as an "acidic scavenger", showing significant improvement in the thermal stability of PLA during post-treatment of polymerization or before processing. The carbodiimide (-N=C=N-) groups are very reactive and react with residual moisture, with –COOH end group of PLA but also block –COOH group of acidic compounds as residual or newly formed lactic acid monomer and acetic acid thus limiting their actions in the self catalyzed degradation of PLA. So, the addition of PDCI would be necessary and effective (Yang Lixin et al. 2008a).

Literature reported that crosslinks agents were added in aliphatic polyesters to improve properties such as mechanical strength and elasticity. Yang et al. (2008b) reported the chemical crosslinking of a commercial PLA (NatureWorks, PLA3001D) in presence of small amounts of triallylisocyanurate (TAIC, 0.5 % to 3% w/w) and dicumyl peroxide (DCP, 0.5% to 1.5% w/w), improving the thermal stability and mechanical properties of PLA, but increasing its brittleness.

Table 1.7. Additives used in PLA.

Function	Additives		Structure	Reference
Plasticizer	Citrate esters oligomeric lactic acid Polyethylene glycol Polypropylene glycol			(Renstad et al. 1998) (Avérous 2008)
Slip agent	Erucamide	Mw = 337.58 CAS No.: 93050-58-9		(Renstad et al. 1998)
thermal degradation stabilizer	TNPP	Mw = 689.00 CAS No.: 26523-78-4		(Yang Lixin et al. 2008a); (Najafi et al. 2012)
OH, COOH, lactic acid scavengers,	Polycarbodiimide (PCDI) Trade name: Stabaxol P	Mw = 42.03 CAS No.: 151-51-9		(Yang Lixin et al. 2008a); (Najafi et al. 2012)
chain extender	Joncryl ® ADR4368 From BASF	x, y and z are all between 1 and 20.		(Najafi et al. 2012)
Crosslinking agent	dicumyl peroxide (DCP)	MW=270.37 CASNo.: 80-43-3		(Yang Sen-lin et al. 2008b)
	triallylisocyanurate (TAIC)	Mw = 249.27 CAS No. : 1025-15-6		(Yang Sen-lin et al. 2008b)

1.3.8. Food packaging applications of PLA

The hydrolytic and thermal degradations of PLA are a main drawback for food packaging applications in particular when food must be conditioned at high temperature. However PLA

meets many requirements as a packaging thermoplastic and is suggested as a commodity resin for general packaging applications (Auras et al. 2004b). Nevertheless barrier properties, impact strength or flexibility, stiffness, barrier properties, thermal stability, are to be improved. PLA like others biopolymers has lower oxygen barrier performances than petrochemical polymers coupled to its moisture susceptibility. A particular attention was developed on the barrier properties against water vapor and oxygen and reviewed by Mensitieri et al. (2011) in particular on the development of PLA-claynanocomposites (Mensitieri et al. 2011).

Major limitations in term of oxygen and water vapor transfer of PLA relative to traditional packaging materials were assessed as well as the impact of light transmission for food containing nutritional markers.

Food products such as unpasteurized orange juices, orange juice simulants, dressings, yoghurts and cheeses have been evaluated during storage in PLA packaging materials (Frederiksen et al. 2003, Haugaard Vibeke et al. 2002, Haugaard V. K. et al. 2003, Holm et al. 2006). Thus, Haugaard et al. (2002) studied an unpasteurized orange juice stored in PLA, HDPE and PS at 4 °C for 14 days. They showed a significantly greater loss of ascorbic acid in orange juice stored in HDPE and PS compared to PLA, significantly less colour changes of orange juice stored in PLA and PS than in HDPE, and no detectable limonene scalping into PLA and PS, as opposed to high degree of scalping into HDPE. Haugaard et al. (2003) showed that cups based on PLA and on poly(hydroxybutyrate) (PHB) were as effective as HDPE cups in protecting an orange juice simulant and a dressing from quality changes during storage under fluorescent light or in darkness (10 weeks at 4 °C).

Likewise, quality changes of plain yoghurt (3.5% fat) were studied during 5 weeks of storage in PLA or polystyrene (PS) cups under fluorescent light (3500 lux) (Frederiksen et al. 2003). With that purpose, Frederiksen et al. (2003) analyzed the color stability, formation of lipid

hydroperoxides, release of styrene and lactate from the packaging materials, and degradation of β-carotene and riboflavin. They showed that PLA was at least as effective in preventing color changes and formation of lipid hydroperoxides as PS. Moreover, they found that losses of riboflavin and β-carotene were less in light-exposed yoghurt packed in PLA than in PS. Finally, these authors showed that the amount of styrene in yoghurt stored in PS cups increased during storage, whereas lactate was not found in yoghurts stored in PLA. Thus, they concluded that PLA provided a better protection against photo-degradation processes than PS.

Holm et al. (2006) evaluated the influence of barrier properties against water vapor and oxygen of PLA relative to an amorphous poly(ethylene terephthalate)/polyethylene (APET/PE) packaging material on quality of Danbo cheese during storage under light exposure and in the dark. They showed that PLA packages can be used for storage of Danbo cheese for a period of 56 days where moisture loss and lipid oxidation were limited. However, when light was excluded, the PLA packages provided a sufficient protection against lipid oxidation during the entire storage period (84 days).

Although PLA shows a poorer barrier properties to oxygen than PET (Colomines et al. 2010), PLA could be a right candidate to replace PET in the conditioning of "oxygen tolerant" food products as salad dressings, oils and shortening, peanut butter (Domenek et al. 2011) and for short storage time.

Chapter 2

Materials and Methods

2 Materials and Methods

2.1 Materials

2.1.1 Polymers

Polylactides studied in this thesis were provided by NatureWorks® LLC (USA) in pellet form and by Treofan in film form. Four types of PLA samples were analyzed in this work:

- a PDLLA BiophanTM 121 (Treofan) film of 40 micrometers thickness. This film is composed of three layers, a core layer and two sealable outer layers.
- a PDLLA INGEO 4032D (NatureWorks LLC ®) in pellet form for forming biaxially oriented films. The films were produced by the "Ecole des Mines de Douai" as a partner of SENSOPLAST project. The film was made by single screw extrusion from pellets previously dried in an oven under vacuum (104 Pa) at 80 °C for 20 h. The final moisture content in pellets after drying was close to 250 ppm. The pellets were converted into PLA4032D film using a Collin single screw extruder with a screw of 30 mm rotating at 60 revolutions per minute. The temperature profile of the five zones of the screw was 190-190-190-200-200 ° C and the die temperature was 200 ° C. A flat die having a width of 200 mm was used to produce a film of 100 mm in wide and about 235μm in thickness and another film of 190 mm in wide and about 55 micrometers thickness. The film was cooled by passing between two rollers at 45 °C and 4.8 m/s.
- a PDLLA 2002D (NatureWorks ® LLC) grade for extrusion and thermoforming. The production of films was made at Conservatoire Nationale d'Arts et Métiers. The pellets were dried at 80 °C for 8 h in a SOMOS® dryer (MANN+HUMMEL ProTec). The residual moisture in pellets after drying was 700 ppm. Then they were extruded using a single screw extruder (MAPRE) of 30 mm diameter, 33 L/D (length to diameter) and composed of 3 screw sections without mixing elements. The temperature profile of the six zones of the screw was 180-185-190-195-200-200 °C and the die temperature was 200 °C. A flat die of 200 mm wide

and a chill roll equipment were used to obtain a film of 100 mm wide and about 65 micrometers thickness. The temperature of the cylinders was regulated with a water container at 25 ºC and the speed of the rollers was set at 10 m/min.

• a PDLLA 2003D (NatureWorks ® LLC) grade for extrusion and thermoforming. The films were made at Ecole Nationale Supérieur d'Arts et Métiers. The pellets were dried at 60 °C for 14 h in a SOMOS® dryer (MANN+HUMMEL ProTec), and then they were extruded using the MAPRE extruder before described. The extrusion conditions were the same as those used for PLA2002D. In this case, we obtained a film of 120 mm wide and about 60 micrometers thick.

Two types of PLA trays were analyzed in this work:

• a tray made at the Ecole de Mines de Douai using a PDLLA4032D film of 235 µm thick. The tray was made by thermoforming (ILLIG Maschinenbau GmbH, Germany) using a positive aluminium mold. Thermoforming was done at 180°C for 24s.

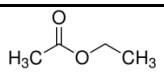
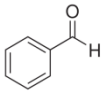
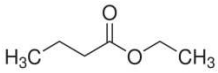
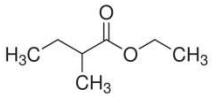
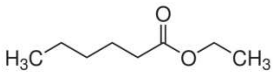
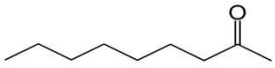
• a tray made at Ecole Nationale Supérieur d'Arts et Métiers from PDLLA 2003D film of 60µm thick using a thermo-forming machine (Formech 660, UK)and a semi-hemispheric mold of 0.5 H/D. Thermoforming was done at 90 ° C for 15 seconds.

A NATIVIA^{TM} NZSS (Aluminium/PLA) lid of 35µm of thickness was obtained graciously from Taghleef Industries and was used to seal trays. The PLA face of lids was in contact with the headspace inside the tray.

2.1.2 Aroma compounds

Aroma compounds used in this work are presented in Table 2.1. Four of these compounds are a homologous series of ethyl ester with an increasing number of carbon and decreasing polarity. Ethyl acetate (>99.5%, EA), ethyl butanoate (99%, EB), ethyl 2-methylbutanoate (99%, E2MB), ethyl hexanoate (>99%, EH) and 2-nonanone (>99%, 2NNE) were purchased from Sigma-Aldrich and benzaldehyde (>99%, BZA) was purchased from Fluka.

Table 2.1 Physicochemical properties of the aroma compounds.

Aroma compounds	Mw (g/mol)	BP (°C)	P_{vs} at 25 °C (kPa)	log P	Chemical structure
Ethyl acetate	88.11	77.91	12.43	0.86[a]	
Benzaldehyde	106.12	16.90	0.12	1.71[a]	
Ethyl butanoate	116.16	121.79	2.07	1.85[a]	
Ethyl 2-methylbutanoate	130.18	133.00	1.10	2.12[b]	
Ethyl hexanoate	144.21	170.05	1.71	2.83[a]	
2-Nonanone	142.24	192.00	0.86	3.03[c]	

MW: molecular weight; BP: boiling point; P_{vs}: saturated vapour pressure at 25 °C; log P: octanol-water partition coefficient; [a] from Pozo-Bayon et al. (2006); [b] from Goodner (2008); [c] from Syracuse Research Corporation, available: http://www.syrres.com/what-we-do/databaseforms.aspx?id=386

2.1.3 Reagents

Sodium caseinate (Lactalis, France), Tween 80 (Merck, Germany), xanthan gum (Keltrol®, Monsanto, UK) were used in this work. Potassium sorbate, triheptadecanoin standard, sodium sulphate anhydrous, potassium hydroxide, β-carotene (>95%) and boron trifluoride methanol complex were purchase from Sigma-Aldrich. Rapeseed oil of food grade (Lesieur, France) was used throughout this work. The characteristics of reagents used in this work are shown in Annexe 1.

2.1.4 Solvents

Methanol, ethanol and n-heptane (99%) were purchased from Carlo Erba Reagents. Dichloromethane, pentane (98%), hexadecane (99%) and butyl acetate (≥99%) were purchased from Sigma-Aldrich. The characteristics of the solvents used are shown in Annexe 2.

2.1.5 Sponge cakes

Sponge cake dough was made with wheat flour - type 55 (25% w/w), sucrose (25% w/w), pasteurized whole eggs (liquid) (45% w/w), palm oil (4% w/w) and salt (1% w/w). All these ingredients were purchased in a local supermarket. Additionally, potassium sorbate was added to dough at 0.1 % w/w. Flavoring was carried out using 6 aroma compounds (Table 2.1) solubilized in palm oil. So, each aroma compound was added to the sponge cake dough at 1000 ppm.

To prepare the sponge cake dough, eggs, sucrose, salt and potassium sorbate were mixed together using a household electric mixer (Kenwood Chelf) for 10 min at high speed to obtain a foam. Then, the electric mixer was set at low speed and flour was added little by little mixing for 2 min. Finally, palm oil containing the aroma compounds was added and mixed for 1 min at low speed.

The dough (50 g) was placed in an aluminum mold (5 cm diameter × 3 cm height) and baked for 30 minutes in an electric oven (REACTIAL Bongard F-67 Wolfisheim) pre-conditioned at 170°C.After baking, the cakes were immediately removed from the molds and left to cool at room temperature during 30 minutes.

2.1.6 Food emulsions

Rapeseed oil of food grade (Lesieur, France) was used throughout this work. Five types of emulsions were prepared (**Table 2.2**) with xantahm gum (Keltrol®, Monsanto, UK) and with an emulsifier, Tween 80 (Merck, Germany) or sodium caseinate (Lactalis, France). To prepare the emulsions, xanthan gum was dissolved in warm water (60 °C) inside a hermetic flask by stirring with a magnetic mixer during 15 min. The emulsifiers (Tween 80 or sodium caseinate) were added subsequently to the cooled solution by stirring for 15 min. Then, potassium sorbate was added as microbial stabilizer. Rapeseed oil and β-carotene (Sigma Aldrich, >95%) were added to the cooled water solution under vigorous stirring using a Polytron® PT3000 homogenizer (Kineatica AG) at 10000 rpm during 10 min. The flasks

were placed in an ice bath to prevent heating during the homogenizing phase. Finally, the emulsion was treated by ultrasound (Ultrasonic Processor, Bioblock Scientific, France) during 20 min in pulses of 10 s and placed in an ice bath to prevent temperature rise.

Table 2.2. Emulsion formulations (% in weight proportions).

Emulsion	Code	Rapeseed Oil	Distilled water	Sodium caseinate	Tween 80	Xanthan gum[a]	Potassium sorbate	β-carotene[b]
Emulsion caseinate	ECas	40	57.6	2		0.3	0.1	
Emul caseinate + β-carotene	ECas-β	40	57.6	2		0.3	0.1	0.01
Emulsion Tween80	ETW	40	57.6		2	0.3	0.1	
Emul Tween80 + β-carotene	ETW-β	40	57.6		2	0.3	0.1	0.01
Flavoured emulsion Tween 80	ETW-F	40	57.6		2	0.3	0.1	

[a] being 0.5 % in the aqueous phase; [b] % of rapeseed oil.

The emulsion ETW-F was flavoured with the six aroma compounds above mentioned. For that, a solution of six aroma compounds in ethanol (5000 ppm of each aroma compound) was prepared. Then, a volume of 2 mL was added to 100 mL of emulsion to have a final concentration of 100 ppm of each aroma compound in the emulsion and stirred by a magnetic mixer during 15 min.

2.2 Methods

2.2.1 Procedures of PLA conditioning

2.2.1.1 Conditioning of PLA samples in aroma atmosphere

The experimental system used to conditioning the PLA samples is shown in Figure 2.1. It consisted in an airtight flask equipped with a Mininert® valve which was placed in a climatic chamber for temperature control. The conditioning was carried out for 20 days at 25 ± 0.3°C. Three samples of each PLA material were conditioned separately. Specific partial pressure of the aroma compounds in the atmosphere was generated by a solution of a mixture of aroma

compounds in hexadecane. The concentration of the different molecules in the hexadecane solution is given in Table 2.3.

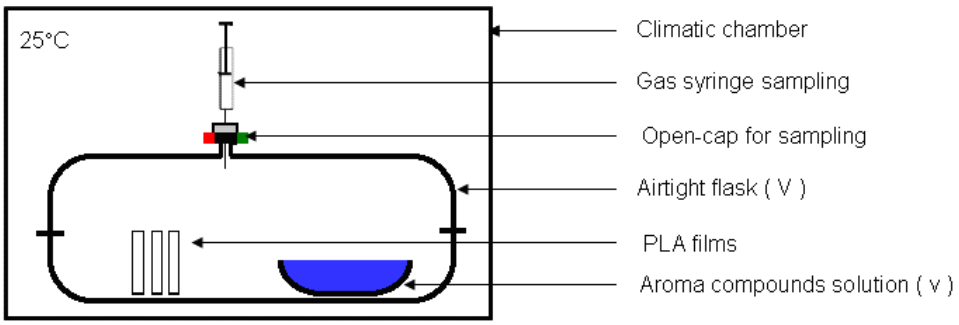


Figure 2.1 System used to conditioning of PLA samples

Film samples were cut into rectangles with mass of 0.3 g for PLA4032D (thickness 235 μm), 0.08 g for PLA2002D (thickness 65 μm) and 0.04 g for PLA Biophan (thickness 40 μm). At low levels of aroma compound concentration (10^{-4}mol/L) analysis of the sorbed quantities in PLA was done by MHE and at very low levels of aroma compound concentration (10^{-2} mol/L), by MHS-SPME.

Table 2.3 Concentrations of aroma compounds solution in hexadecane

Aroma compound	Aroma solution in hexadecane (mol/L)	Method
Ethyl acetate	2.0E-04	MHS-SPME
	2.0E-02	MHE-GC
Ethyl butanoate	1.5E-02	MHE-GC
Ethyl 2-methylbutanoate	2.0E-04	MHS-SPME
	2.0E-02	MHE-GC
Benzaldehyde	3.0E-04	MHS-SPME
	3.0E-02	MHE-GC
Ethyl hexanoate	1.8E-04	MHS-SPME
	1.8E-02	MHE-GC
2-nonanone	1.7E-04	MHS-SPME
	1.7E-02	MHE-GC

In order to reach the equilibrium of sorption, the thickness of the PLA samples (PLA4032D and PLA2002D) were reduced to 10 μm using a microtome (LKB Bromma Historange

microtome 2218). Three microtomed samples (30 ± 1 mg) of each PLA were then studied following the procedure of conditioning.

2.2.1.1.1 Determination of the aroma partial pressure by gas chromatography

The aroma compounds concentration in the atmosphere of the airtight flask was measured after 20 days using a gas syringe. For that, 0.5 ml of the headspace of the hermetic flask were collected through the Mininert® valve installed on the flask and injected into a gas chromatograph (GC) Agilent Technologies, Model 6890GC/FID equipped with a DB5-MS capillary column (30 m length x 0.32 mm, 0.5 µm film thickness, J&W Scientific).

The aroma compound concentration was determined using an external calibration curve measured on the same gas chromatograph. It was carried out by injecting 2 µL of a solution of a mix of aroma compounds in pentane (10, 25, 50, 100, 500, 1000 and 5000 µg/g). The temperature of the split/splitless injector was 250 ºC with a purge time of 0.5 min. The flame ionisation detector was set at 250 ºC with hydrogen as carrier gas (45 mL/min). The oven temperature was programmed to increase from 30 to 230°C at 5 ºC/min.

Partial pressure (Pp) was calculated using ideal gas law. The activity of each compound was calculated as Pp/Psv, where Psv is the saturated vapour pressure of aroma compound at 25°C.

2.2.1.2 Conditioning of sponge cakes in PLA pouches

Flavored sponge cakes were packed in PLA pouches (12 cm x 15 cm, made by thermosealing from PLA4032D film of 50 µm thickness), one cake per pouch. Each pouch was put into the conditioning system (Figure 2.2a) during 10 days. Additionally, a second way to conditioning the cakes with the same PLA4032D film was used (Figure 2.2b). With the purpose of compare the flavored cake with a model solution, we prepared 10 mL of an aroma solution in hexadecane which contained 12.5 mg of each aroma compound. This solution was then conditioned according the Figure 2.2b. The quantities of aroma compounds used to prepare those solution was chosen with based on the hypothesis that about 25% of the aroma

compounds present in the dough (1000 ppm of each aroma compound) are retained after baking (Pozo-Bayon et al. 2006).

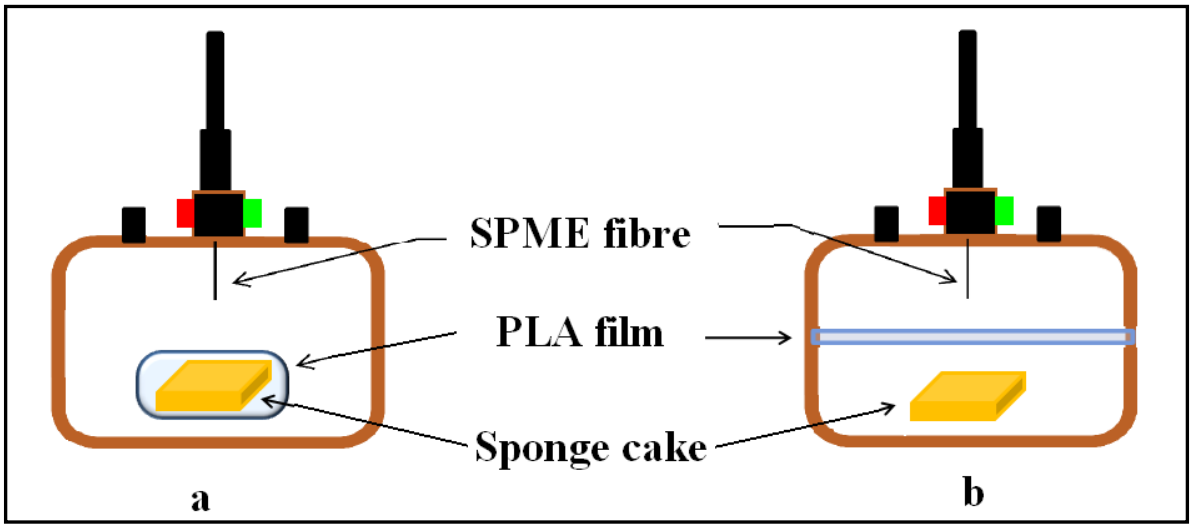


Figure 2.2 Systems used to conditioning of sponge cakes in PLA4032D pouch a) or film b).

2.2.1.3 Conditioning of PLA samples in rapeseed oil

The conditions used in this study were adapted from the norm NF-EN-1186-2 of Materials and articles in contact with foodstuffs – Plastics, which describes the test of overall migration by total immersion in olive oil. So, PLA4032D film (thickness 55 µm) was cut into rectangular pieces measuring 50 mm by 20 mm using a paper-cutter. Ten strips (1 dm^2) were immersed in 100 mL of rapeseed oil with or without β-carotene in a 250 mL flask then closed with a stopper and put in a chamber for 28 days at 40 °C. Five flasks were prepared in this way.

2.2.1.4 Conditioning of emulsion in PLA trays

100 mL of each type of emulsion were placed in three PLA trays and then they were sealed with a NATIVIA™ NZSS (Aluminium/PLA) lid. Trays were placed into an oven at 40 °C and left for 30 days.

2.2.1.4.1 Characterization of emulsions

Emulsion flow curves were measured at 20 ± 1 °C over a shear rate range of 0.001 –10 s^{-1} using a dynamic rheometer (MCR301, Anton Paar) equipped with cone-plate geometry (CP 50/4°). All measurements were performed within 24 h after emulsion preparation. The flow curves were modeled with the law of Herschel-Bulkley for liquids showing yield stress:

$$\sigma = \sigma_0 + K \cdot \dot{\gamma}^n \qquad \text{(Equation 2.1)}$$

Where σ is shear stress, γ shear rate, *n* flow behavior index, σ_0 yield stress and *K* consistency index. Experiments were carried out in duplicate.

The oil droplet size was characterized in water dispersion by multi-angle light scattering measurement with the help of a Mastersizer 2000 (Malvern, France) equipment. Experiments were carried out in duplicate.

The stability of the different emulsions was studied by multi-angle light scattering measurement using Turbiscan MA 2000 (Formulaction, France) as previously described by Relkin and Shukat (Relkin and Shukat 2012). The reading head of Turbiscan is composed of a pulsed near-infrared light source (850 nm) and two synchronous detectors. The transmission detector allows collecting the light which goes through the sample (0°), while the backscattering detector receives the light backscattered by the sample (135°). The sample to be analyzed is placed in a cylindrical glass measurement cell, then the detection head scans the entire length of the sample, acquiring transmission and backscattering data every 40 µm. The scan of a same sample at different time intervals (zero, 6 or 17 days of storage at 40 ºC) was carried out and light transmission and backscattering profiles were used to evaluate the physical stability of emulsions.

2.2.2 Moisture loss and water vapor transfer rate (WVTR)

The moisture loss of the model foods packaged in PLA trays was measured following the weight loss during storage which implied the hypothesis that the moisture loss was the

principal cause of the weight loss of the foodstuff. Different storage conditions were used for each model food. Thus, five sponge cakes packaged in PLA bags (12 cm x 15 cm) were stored at 23ºC and 50 %RH (Bioblock scientific Vötsch Industrietechnik) for 30 days, while three trays containing the food emulsions were stored at 40 °C for 20 days in an oven without RH control. Weight loss was measured using an analytical balance (10^{-3}g). The water vapour transfer rate (WVTR) of PLA trays was calculated from the slope of the plot of weight loss over time. Given values are averages of experiments.

2.2.3 Partition coefficients

The partition coefficient is defined as the mass concentration relation of a molecule between two phases. The partition coefficients calculated in this work were:

$$K_{polym/hs} = \frac{C_{polymer}}{C_{headspace}}, \qquad \text{(Equation 2.2)}$$

$$K_{polym/liq} = \frac{C_{polymer}}{C_{liquid}}, \qquad \text{(Equation 2.3)}$$

$$K_{hs/liq} = \frac{C_{headspace}}{C_{liquid}}, \qquad \text{(Equation 2.4)}$$

Where *Cpolymer* is the concentration of the compound in the polymer (µg/g), *Cheadspace* is the concentration of the compound in the headspace (µg/g) and *Cliquid* is the concentration of the compound in the liquid or emulsioned matrix (µg/g).

2.2.4 Solubility coefficient

The apparent solubility coefficient (S) of each aroma compound was calculated according to:

$$S = \frac{M_\infty}{M_0} \times \frac{d}{P_p}, \qquad \text{(Equation 2.5)}$$

Where M_∞ is the mass sorption after conditioning (kg), M_0 is the initial mass of PLA sample (kg), d is the film density (kg/m^3) and P_p is the partial pressure of each aroma compound.

2.2.5 Extraction methods

2.2.5.1 Headspace extraction methods

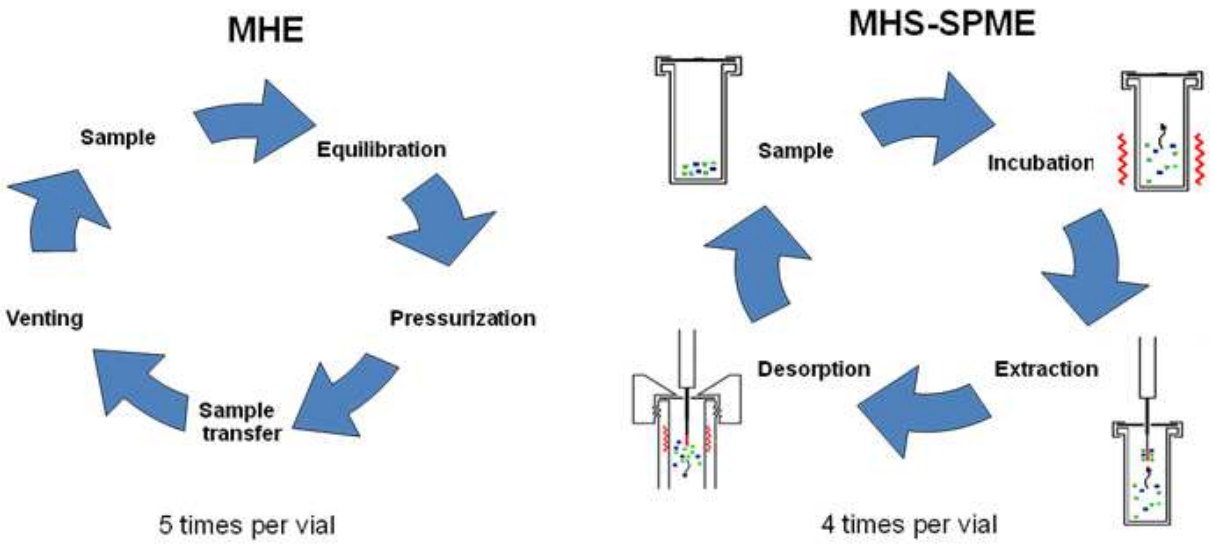


Figure 2.3 Principle of MHE and MHS-SPME methods.

MHE and MHS-SPME techniques are dynamic gas extractions carried out stepwise on a same sample (Figure 2.3 and Figure 2.4). At each extraction step, the overall equilibrium of the analyte in the system must be first established then analyte present in the headspace volume was removed and analyzed as GC area. Various extractions of the same sample are performed in order to reach an exponential decay of the analyte present in the sample and then to calculate the total area of the analyte, according to geometric progression showed in Equation 2.6. It means that from a restricted number of successive measurements (ideally N=5), the sum of GC areas would be calculated if carrying out the successive measurements ad infinitum (Ezquerro et al. 2003, Kolb and Ettre 1991).

$$A_T = \sum_{i=1}^{N} A_i = \frac{A_1}{1-\beta}, \qquad \text{(Equation 2.6)}$$

In Equation 2.6, A_T is the theoretical total GC area, A_i is the GC peak area relative to the i-th extraction, N is the total number of extractions, A_1 is the GC peak area relative to the first extraction, and β is a constant which describes the exponential decay of peak areas during the stepwise multiple headspace extraction procedure. The constant β can be obtained from the slope of the plot resulting from the Equation 2.7:

$$\ln A_i = (i-1)\ln\beta + \ln A_1, \qquad \text{(Equation 2.7)}$$

So, the accuracy of the measurement depends on the right linearity (depending of R^2) of the N successive extractions and not on the total extraction of the compound. Therefore, parameters like temperature and time of equilibration require to be optimized.

Then, the quantity of analyte in the sample can be calculated from the total peak area A_T using a calibration by external standard.

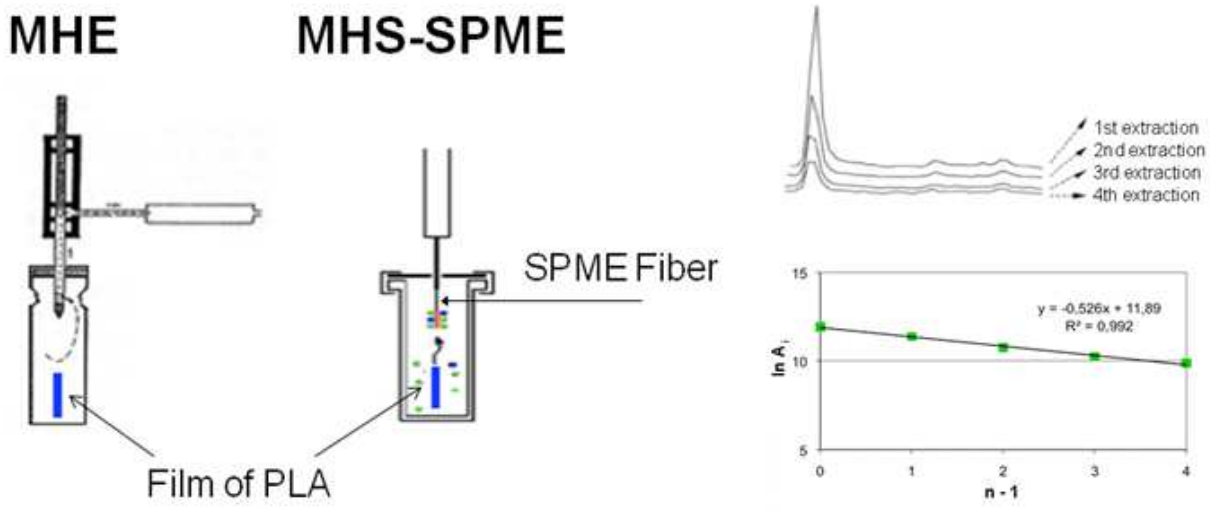


Figure 2.4 Principle of MHE and MHS-SPME sampling. Adapted from Kolb and Ettre (1991).

2.2.5.1.1 MHE: Quantification of aroma compounds

After conditioning time, PLA samples were removed from airtight flasks and sealed in 20 ml headspace glass vials with silicon/PTFE septa and an aluminium cap. An automatic headspace sampler coupled with GC/FID (Perkin Elmer HS40 XL) was used to monitor the extraction and analysis of the released aroma compounds. The specific headspace sampler operation conditions were: head pressure (Helium) 200 kPa, equilibration temperature 60 °C, equilibration time 55 min, pressurization time 2 min, injection time 0.05 min, hold up time 0.2 min, needle temperature 110 °C, transfer line temperature 130 °C. The oven temperature was programmed at 35°C for 2 min, 6 °C/min up to 230 °C. Injector temperature was 250 °C and its split was 15.6 ml/min. Detector temperature was 250 °C and it was set at range 1. The column was a SupelcoWax-10 capillary column (30 m length x 0.32 mm, 0.5 μm film thickness). The carrier gas was He at 1.8 ml/min. Three vials were prepared for each type of PLA and each vial was sampled five times at equal time intervals (55 min) at 60 °C. Total peak area of each aroma compound sorbed was calculated using equation 1. Calibration of each aroma compound was performed by external standards. For that, a solution of a mix of aroma compounds was prepared in hexadecane. Dilutions of 100 to 10000 μg/g in hexadecane were used. Ten μL of the diluted solution was introduced in a 20 ml glass vial sealed with silicon/PTFE septa and an aluminium cap. Three vials were prepared for each dilution and then each vial was sampled five times following the method already described.

2.2.5.1.2 Multiple headspacesolid-phase micro extraction (MHS-SPME)

2.2.5.1.2.1 Quantification of aroma compounds

After conditioning time, samples were removed from airtight flasks and sealed in 20 ml glass vials with silicon/PTFE septa. The sample vials were incubated at 60 °C for 30 min and agitated at 250 rpm. MHS-SPME was performed for 15 min at 60 °C using a 75 μm carboxen–polydimethylsiloxane (CAR-PDMS) fibre providing best results in terms of amount

of compound extracted for low molecular mass compounds (Ezquerro et al. 2002). Desorption time was 5 min into the injector port of chromatograph. For MHS-SPME injections, three vials were prepared for each type of PLA and each vial was sampled four times. Total peak area of each aroma compound sorbed was calculated using Eq3.6. The calibration was carried out by external standard in hexadecane. For that, a solution of a mix of aroma compounds was prepared in hexadecane. Dilutions of 0.5 to 25 µg/g in hexadecane were used for ethyl butanoate, ethyl 2-methylbutanoate, ethyl hexanoate and 2-nonanone, whereas dilutions of 25 to 500 µg/g in hexadecane were used for ethyl acetate and benzaldehyde. Twenty µl of the diluted solution were introduced in a 20 ml glass vial sealed with silicon/PTFE septa and a steel cap. Three vials were prepared for each dilution and each vial was sampled four times as described for PLA samples.

2.2.5.1.2.2 Sampling procedure for VOCs quantification

Immediately after extrusion and thermoforming, 50 mg, 200 mg or 1000 mg of PLA2003D samples were sealed in 20 mL glass vials with silicon/PTFE septa. With the purpose to evaluate the extraction temperature, the sample vials were incubated at 30 °C or 60 °C for 30 min and agitated at 250 rpm. Then, the VOCs were extracted for 15 min at 30 °C or 60 °C using a 75 µm carboxen–polydimethylsiloxane (CAR-PDMS). Desorption time was 5 min into the injector port of the chromatograph.

For MHS-SPME injections, three vials of each sample were prepared and each vial was sampled four times. Total peak area of each compound was calculated using Equation 2.6.

After identification of volatile organic compounds, the calibration was carried out by external standard in hexadecane. For that, a solution of a mix of four organic compounds previously identified was prepared in hexadecane (10000 ppm each). Dilutions of 1 to 50 µg/g in hexadecane were used for acetaldehyde, 2-methyl 2-Propanol, 2,3-Pentanedione and 2-

Pentanol,2,4-dimethyl. Twenty µL of the diluted solution were introduced in a 20 ml glass vial sealed with silicon/PTFE septa and a steel cap. Three vials were prepared for each dilution and each vial was sampled four times as described for PLA samples.

2.2.5.1.2.3 Sampling procedure for lactide quantification

PLA samples were sealed in 20 mL glass vials with silicon/PTFE septa. Different parameters such as amount of PLA and extraction temperature were tested with the purpose of obtaining an exponential decrease of peak areas of Lactide. The sample vials were incubated at 30 °C, 60 °C and 80 °C for 30 min and agitated at 250 rpm. Then, the compounds present in the headspace of the vial were extracted for 15 min at 30 °C or 60 °C using a 75 µm carboxen–polydimethylsiloxane (CAR-PDMS). Desorption time was 5 min into the injector port of the chromatograph.

With the aim of facilitating the diffusion of lactides from the polymer matrix and promote its volatilization, some samples were pre-incubated in an oven (Yamato DP32) at 80 °C for 4h, 8h, 12h, 18h or 24h and then analyzed by MHS-SPME.

For MHS-SPME injections, each vial was sampled four times. Total peak area of lactide was calculated using Equation 2.6.

The calibration was carried out by external standards in butyl acetate. For that, a solution of 1000 ppm of L-lactide (CAS 4511-42-6, Aldrich) was prepared in butyl acetate. Dilutions of 50, 100, 250 and 500 µg/g in butyl acetate were used. Twenty µL of the diluted solution were introduced in a 20 ml glass vial sealed with silicon/PTFE septa and a steel cap. Three vials were prepared for each dilution and each vial was sampled four times as described for PLA samples.

2.2.5.1.2.4 Identification by GC-MS

An Agilent Technologies 6890 GC/FID coupled with a mass spectrometer 5975 INSERT and an Autosampler MPS2 GERSTEL, which allows automated HS-SPME injections, were used. The oven temperature programme began with an initial temperature of 30 °C for 5 min, and then temperature increased at a rate of 5 °C/min up to 230 °C, and then this temperature was maintained for 5 min. Split/splitless injector and detector temperatures were 250 °C. The column was a J&W Scientific DB5-MS capillary column (30 m length x 0.32 mm inner diameter x 0.5 μm film thickness). The carrier gas was He at 1.4 ml/min.

Identification of volatile organic compounds was achieved using a mass spectrometer 5975 INSERT. The parameters of mass spectrometer were: electron impact ionization; electron energy, 70 eV; ion source, 230 °C; electron multiplier voltage, 1470eV; transfer line, 270 °C; scanning, between 29 and 400 amu. The data were recorded by MSD ChemStation software and the data base used was Wiley7NIST05. Kovats indices were determined to confirm identification. For that, a standard mixture of alcanes C6 to C19 in pentane was analyzed under the same conditions as the samples.

2.2.5.1.2.5 Limit of detection and quantification

The limit of detection (LOD) was calculate as the quantity of compound producing a signal exceeding the average background signal by three standard deviation of the signal-to-noise ratio in the lowest concentration standard, divided by the slope of the corresponding analytical curve (Miller and Miller 1993). The limit of quantification (LOQ) was obtained as the quantity of compound that is 10 standard deviation above the average background signal, divided by the slope of the corresponding calibration curve (Gibbons and Coleman 2001).

2.2.5.2 Liquid extraction methods

2.2.5.2.1 Quantification of oil sorbed in PLA

The rapeseed oil sorbed in PLA samples was extracted and determined by gas chromatography after conversion to methyl esters according to the method described in the norm NF EN 1186-2 (January 2003). After conditioning time, PLA strips were removed from the rapeseed oil and rinsed with ethanol in order to remove the rest of oil. The same procedure was carried out in PLA trays after removal of emulsion. Then, strips and trays (1 dm^2 each) were wiped using KimTech precision wipes, cut in small squares and placed in a 25 mL vial. The extraction of oil was carried out by adding 10 mL of n-pentane into vials under magnetic stirring at 50 °C for 30 min. The operation was renewed two times with fresh solvent. 5 mL of a solution of 2000 ppm of triheptadecanoin in heptane were added into vial as internal standard prior to the first extraction.

The resulting extracts were collected and evaporated to dryness at 40°C and 105 mbars using a rotary evaporator (Büchi R-114, Suisse) then dissolved in 10 mL n-pentane. The flask containing the extract, a few carbon beads and 10 mL of a solution of potassium hydroxide (11 g/L) in methanol was placed under reflux for 10min until that oil droplets disappear. Subsequently, 5 mL of boron trifluoride/methanol complex (BF3) in methanol were added to the flask and once again placed under reflux for 2 min. The resulting solution was cooled at room temperature until to obtain two phases. 20 mL of a solution of saturated sodium sulphate in methanol were added and shaked vigorously. Then, further solution was added until the liquid level reached the neck of the flask. Finally, the flask containing the mixture was placed in the freezer for up to 24 hours, to favour the separation of phases. Then, the upper phase containing heptane and the fatty acids were recovered in 1 mL vials for GC analysis.

Finally, samples (2 µL) were injected into a gas chromatograph (GC) CE Instrument, Model GC8000Top (GC/FID) equipped with a DB-FFAP capillary column (30 m length x 0.32 mm, 1 µm film thickness, JW Scientific). The temperature of the split/splitless injector was 250 ºC

with a purge time of 0.5 min. The flame ionisation detector was set at 250 ºC with hydrogen as carrier gas (50 kPa). The oven temperature was programmed to increase from 60 to 250°C at 7 ºC/min. Calibration was carried out using different quantities of rapeseed oil (5, 10, 20, 50 and 150 mg) which were subjected to same procedure of samples. Following the norm already cited, for each quantity of oil, the ratios of the combined area of C16, C18:1; C18:2 and C18:3 peaks to area of internal standard (C17) were calculated and then ratios were plotted against the oil quantities.

2.2.5.2.2 Quantification of residual lactide

Residual L,L-lactide present in PLA samples was quantified by gas chromatography according to NatureWorks LLC procedure (number PLA_GC_13_4) (NatureWorksLLC 2010). This procedure is based on dissolution – precipitation extraction method (methylene chloride – cyclohexane) and addition of 2,6 dimethyl-γ-pyrone (Aldrich) as an internal standard. So, 1 g of PLA sample, 18 mL of methylene chloride and 1 mL of a solution of 10000 ppm of 2,6 dimethyl-γ-pyrone in methylene chloride were placed in a 25 mL scintillation vial with sealed cap. The vial was agitated using a magnetic stirring until the polymer was dissolved. Then, 1 mL of this solution was placed in other 25 mL scintillation vial with 3 mL of acetone and 16 mL of cyclohexane. The resulting solution was filtered into a GC auto sampler vial using a disposable syringe equipped with a 25 mm, 0.45 μm PTFE filter. Three extractions were carried out for each type of PLA. Finally, 2 μL of each extract was sampled using a GC/FID 8000 Top Instruments equipped with split/splitless injector which was set at 200 °C. The oven temperature programme began with an initial temperature of 50°C for 1 min, and then temperature increased at a rate of 10 °C/min up to 250 °C, and then this temperature was kept for 5 min. Detector temperature was 270 °C. The column was an Agilent J&W Scientific DBFFAP capillary column (30 m length x 0.32 mm inner diameter x 0.5 μm film thickness). Hydrogen was used as a carrier gas at 50 kPa. Relative response

factor for L,L-lactide standard ((3S)-cis-3,6-Dimethyl-1,4-dioxane-2,5-dione, Aldrich) and the internal standard were calculated following the gas chromatography method already described. L,L and D,D-lactide co-elute using the DBFFAP column for separation. The weight of lactide enantiomers (g) in samples were calculated using the Equation 2.8:

$$\text{L, L and D, D} - \text{Lactide (g)} = \frac{\text{Peak area of L, L and D, D} - \text{Lactide in sample} \times \text{amount (g) of internal standard}}{\text{Relative response factor} \times \text{Peak area of Internal standard}}$$

(Equation 2.8)

2.2.5.2.3 Solvent extraction of additives in PLA

Some extraction techniques were used to determine the presence of additives in PLA. Additives were extracted by two methods (Figure 2.5):

- Dissolution of PLA by dichloromethane with heating or not then using ethanol as a precipitating solvent of PLA and extracting solvent of additives
- Direct extraction of additives using ethanol

2.2.5.2.3.1 Dissolution by reflux – Precipitation

10 g of PLA pellets were dissolved in 200 ml of dichloromethane under reflux for 90 minutes. Then, using a rotary evaporatorat 700 mbar and 40 ºC (Buchi), the solvent was evaporated until 100 ml of solution remains. This concentrated solution was precipitated in 1L of ethanol and after the precipitated polymer was separated by filtration at vacuum with filter paper and then evaporated using a rotary evaporator (140 mbar and 40 ºC) until 25 ml of extract. This extract was then filtered at vacuum using a membrane with a pore size of 0.45µm (Sartorius Stedim Biotech, Germany) and finally it was stored in a freezer at -5 ºC.

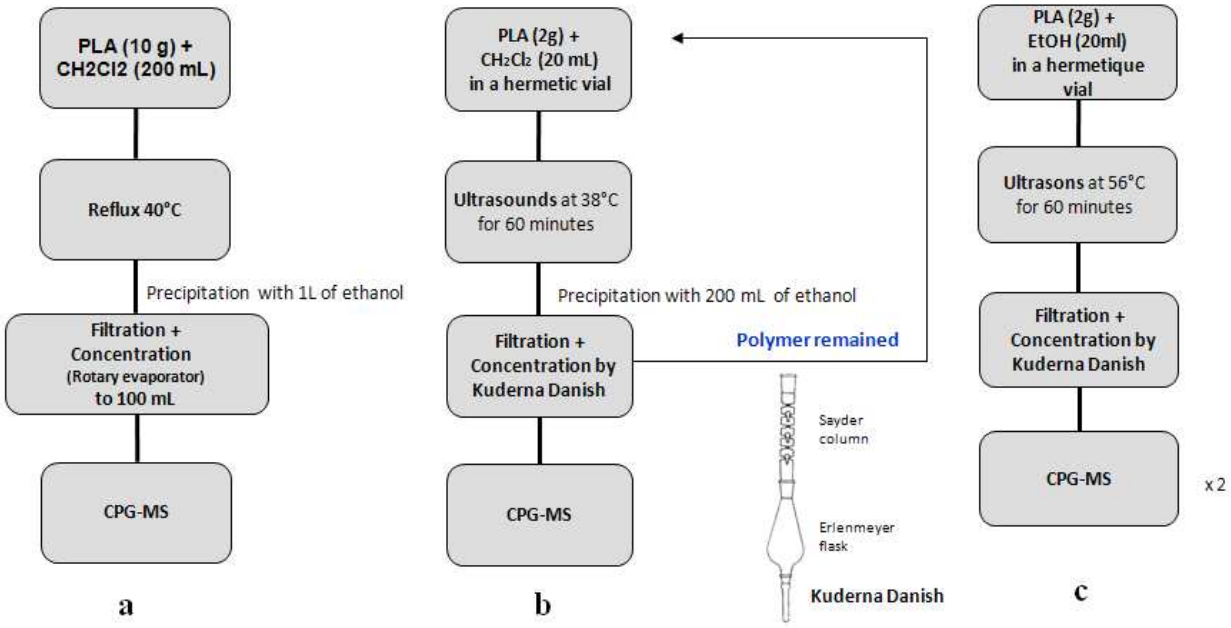


Figure 2.5. Solvent extraction methods: a) Dissolution by reflux – precipitation; b) Dissolution by ultrasound– precipitation; c) Ethanol extraction by ultrasounds.

2.2.5.2.3.2 Dissolution by ultrasound– Precipitation

In a glass bottle with sealed cap we put 2g PLA7000D, 20 mL of dichloromethane (bp 39.8 °C) and about 0.5 g of molecular sieve A3. The closed flask was placed in an ultrasound system at 38 °C for 60 minutes. Then, the solution was precipitated in 200 mL of ethanol and the polymer was separated by filtration at vacuum on filter paper and then on a membrane with pore size of 0.45 mm (Sartorius Stedim Biotech, Germany). After, using a Kuderna-Danish evaporation column and a water bath at 95 °C, the solution was concentrated to about 5 mL. Finally, the concentrated solution was stored in a freezer at -5 ºC. The polymer remained which was precipitated by ethanol was extracted a second time following the same method.

2.2.5.2.3.3 Ethanol extraction by ultrasounds

2g of PLA7000D pellets, 20 ml of ethanol (bp 78.32 °C) and about 0.5 g of molecular sieve A3 were put in a glass bottle with sealed cap. The closed flask was placed in an ultrasound system at 56 °C for 60 minutes. Then, the solution was filtered on filter paper and membrane pore size of 0.45 mm (Sartorius Stedim Biotech, Germany). The recovered polymer pellets

were used to carry out a second extraction. The first and second filtered solutions were mixed getting about 40 mL of solution. Then, using a Kuderna-Dannish evaporation column and a water bath at 95 °C, the solution was evaporated until about 5mL. Finally, the concentrated solution was stored in a freezer at -5 ºC.

2.2.5.2.3.4 Analysis by GC-MS

2 µL of PLA7000D extracts were injected in "splitless" mode in an equipment of gas chromatography (Agilent Technologies, Model 6890GC), coupled with a mass spectrometer 5975 INSERT and an Autosampler MPS2 GERSTEL. The temperature of FID detector was 305 °C. The analysis conditions were as follows: column DB5-MS (J&W Scientific, 30 m × 0.32 mm id, 0.5 µm film), He as carrier gas at a pressure of 0.6 bar, injector temperature at 250 °C. The oven program started at 60 °C for 5 min, the temperature was then increased at a rate of 5 °C/min to 300 °C, and then this temperature was maintained for 10 min.

2.2.6 PLA characterization techniques

2.2.6.1 Size Exclusion Chromatography (SEC)

The average molecular weight and the dispersity of PLA samples were measured by SEC using a Gilson 305 apparatus equipped with a RID-101 (Shodex) refractive index detector. PLA samples were dissolved in chloroform (5 mg/mL) and analyzed at 25 °C and 1.0 mL/min in THF stabilized with BHT on a PL Gel Mixed-C column (5µm, 100 Å, 600 x 7.5 mm). The calibration was performed with PS standards (EasiCal) from 580 to 377400 g/mol.

2.2.6.2 Differential scanning calorimetry (DSC)

Before and after conditioning with aroma compounds, the thermal properties of film samples were determined in triplicate using a differential scanning calorimeter QSC 100 instrument (TA Instruments, France). The samples (between 2 and 5 mg) were put into hermetic

aluminium pans (TZero, TA Instruments) to avoid the loss of aroma compounds upon heating. For the calculations of Tg, extrapolation of baselines was done subtracting the enthalpy relaxation peak. In this work our samples cannot be "update" by heating to keep the sorbed aroma compounds.

For the measurement of glass transition and crystallinity degree the equipment was operated in standard mode or in modulated mode. The standard program used consisted in an isotherm at -30 ºC for 1 minute, then a single temperature ramp at 10 °C/min from -30 °C to 190 °C, and a isotherm at 190 ºC for 1 min. The modulated temperature program consisted in a isotherm at 10 ºC for 10 minutes, then heating from 10 °C to 190 °C, with an oscillation amplitude of 0.32 °C, an oscillation period of 60 s and with a heating rate of 2 °C/min. Crystallinity (Xc) of materials was calculated according to Equation 2.9:

$$X_C = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0} \qquad \text{(Equation 2.9)}$$

Where ΔH_m is the enthalpy of fusion, ΔH_{cc} is the enthalpy of cold crystallization and ΔH_m^0 is the heat of melting crystalline PLA (93 J/g) (Fischer et al. 1973). The glass transition temperature was taken at the midpoint of heat capacity step.

2.2.7 Statistical analysis

The statistical analysis of data was performed through one-way analysis of variance (ANOVA) using XLSTAT-Pro 7.0 software (Addinsoft, Paris, France). When differences were significant ($p < 0.05$), Duncan's test was used to evaluate the differences between pairs of groups.

Chapter 3

Plasticization of poly(lactide) by sorption of volatile organic compounds at low concentration

Few studies concerning vapor aroma sorption in PLA have been reported and all of them were performed at high levels of aroma compound activity (Auras et al. 2006, Colomines et al. 2010, Courgneau et al. 2012). Indeed, some aroma compounds present in food showed a plasticization effect at high concentrations. However, no data were reported at low levels of activity corresponding to real service conditions, which means that there is a void of knowledge in literature at realistic conditions in which PLA could be used as food packaging.

Therefore, the goal of this chapter is the investigation of the sorption of aroma compounds at low activities in PLA and the effects of this sorption of the polymer properties of PLA. Therefore a model aroma compound solution giving rise to low activities of each compound in the gas phase was constructed and brought into contact with PLA samples with the help of a sorption cell developed for this purpose.

An important part of this study was developing and optimizing an original methodology that allows measuring the sorption of volatile compounds in PLA samples, at low concentrations. For that, multiple headspace extraction methods were selected, as they are suitable for solvent free trace analysis of volatile compounds.

The work has brought forth a publication in the journal Polymer Degradation and Stablity, which is presented hereafter.

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Plasticization of poly(lactide) by sorption of volatile organic compounds at low concentration

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ABSTRACT

Polylactide (PLA) is one of the most promising bio-based polyester for wide applications and in particular for food packaging. However aroma compounds can interact with packaging in contact leading to modification of its thermal properties during storage. Up to date, no study has focused on aroma sorption in PLA at realistic food packaging conditions implying low vapor activities, and thus using dedicated methodology ranging over concentrations below 200 μg of aroma compound per gram of polymer. In the present study the sorption of four ethyl esters (C4, C6, C7 and C8), benzaldehyde and 2-nonanone in three different types of poly(D,L-lactide) (PDLLA) at 25 °C was investigated in order to determine their solubility coefficients and their impacts on the thermal properties of the tested PDLLA. The sorption of aroma compounds at low vapor activities was studied using two methods of headspace extraction (MHE and MHS-SPME) after 20 days of conditioning in a hermetic flask. The results showed that PLA films studied have a very high affinity for benzaldehyde. Ethyl acetate solubility coefficient in PLA was comparatively similar to poly(ethylene terephthalate), polypropylene (PP) and low-density polyethylene (LDPE). Differential Scanning Calorimetry results showed a decrease in glass transition temperature (Tg) of PDLLA films and an increase in the crystallinity degree that depend on the concentration of the sorbed aroma compounds. It could be clearly demonstrated that plasticization of PDLLA by aroma compounds occurred at very low vapor activities (0.01) and that the importance of the effect depended on the chemical nature of the molecule.

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1. Introduction

Polylactide (PLA) is one of the most promising bio-based polyester for food packaging [1,2] because of its good mechanical and barrier properties and ease of processing. However, polymeric packaging materials are not inert and mass transfer occurs between packaging polymer and foodstuff [3] leading to quality issues.

Indeed, plastic packaging materials can absorb a significant quantity of aroma compounds from food which can involve modifications of the flavour composition, decrease of intensity, or unbalance flavour [4–7].

Furthermore, a number of studies showed that the sorption of flavour molecules can lead to modifications of the properties of the packaging material induced by swelling and/or plasticization. Such phenomena can favour for example oxygen permeation which in turn can reduce the shelf-life of the product [8–10].

Most of the literature concerned with interactions between flavour molecules and polymers deals with polyolefins, polypropylene and polyethylene [11] and very few studies show results on glassy polymers such as polyesters [5,12–14] and polystyrene [15].

One of the principal difficulties in dealing with sorption studies in glassy polymers is the low mass transfer rate which challenges analytical methods. Analysis of sorbed quantities is eased when excessive aroma concentrations are employed. The drawback of this strategy is that at aroma activities sorption isotherms usually do not follow the Henry model because of modification of the polymer morphology (plasticizing, swelling…). In order to avoid this phenomenon, experiments should be conducted with low concentration of aroma compounds, ideally at infinite dilution.

Tedious methods need often to be employed to quantify the amount of flavour absorbed by the plastic materials, such like solvent extraction or supercritical solvent extraction [12,16]

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followed by a careful solvent concentration [17]. Presence of oligomers and additives coming from polymer is a limiting factor in using this extraction procedure as sorbed aroma compounds can be co-extracted with these molecules leading to a complex gas chromatography profile [18,19].

For these reasons, headspace techniques such as Multiple Headspace Extraction (MHE) and Multiple HeadSpace – Solid Phase Micro Extraction (MHS-SPME) coupled to gas chromatography (GC) are promising tools for analysing flavour compound sorption in low quantity in polymeric structures without interference of non-volatile compounds. Multiple Headspace Extraction Gas Chromatography (MHE-GC) technique and Multiple headspace-solid phase micro extraction (MHS-SPME) were usually used to quantify the concentration of volatile organic compounds [17,20,21] into solid matrix. They allow avoiding matrix effect, use of solvent and concentration. The multiple headspace extraction (MHE) is an absolute quantitative method by dynamic gas extraction carried out stepwise. At each step, equilibrium conditions are established in the vial between the sample and its headspace (gas phase of the vial). Several extractions are made on one sample by automatic headspace injector. Quantities of sorbed analytes exponentially decay with the number of extractions and total quantity corresponds to the total peak area, calculated as the sum of peak areas for each extraction until the complete desorption of the volatiles. By a suitable calibration procedure, the peak area versus amount relationship can be established [22]. MHS-SPME provides more sensitivity and selectivity than MHE-GC due to the concentration step of the volatile molecules on the solid phase of a properly chosen fibre, where low concentrations can be detected and thus it is useful to extract volatile organic compounds sorbed into packaging materials [23]. It is, however, more challenging with respect to calibration issues, in particular when mixtures of volatile compounds are to be analyzed. The amount of analyte extracted by the fibre is proportional to the initial amount but also subjected to a partition coefficient [23,24].

To date, only a few studies were conducted on the sorption of aroma compounds in PLA, which is glassy at room temperature, and those mainly for ethyl acetate and D-limonene [25–27]. In the case of ethyl acetate, which is part of many fruity aroma formulations, it was shown than it had a plasticizing effect and induced crystallization in PLA, changing its properties during storage [26,27]. Auras et al. [25] studied the sorption of different volatile compounds, and showed that PLA had more affinity to more hydrophilic molecules, such as ethyl acetate, compared to hydrophobic one such as limonene. They concluded that PLA had therefore promising properties for the conservation of aroma profiles, as most of the aroma compounds are hydrophobic. The main drawback of those studies was however the high aroma compound activity. No study has focused on aroma sorption in PLA at realistic food packaging conditions where vapour activities are very low, and where the use of specific analytical methodology capable to detect concentrations even below 200 μg of aroma compound per gram of polymer is required. Under such conditions, one can assume that the aroma compounds are present in an infinite dilution. Therefore Henry's law should apply and the solubility coefficients should be independent of the partial pressure.

The present work aims to study the sorption of common aroma compounds in PLA, alone and in mixture at activities usually encountered in the headspace of food products. For that, four commercial types of poly (D,L-lactide) were put in vapor contact with six aroma compounds. Their solubility coefficients at low activities were determined and the impact of aroma sorption on the thermal properties of materials was investigated.

2. Materials and methods

2.1. Materials and processing

2.1.1. Aroma compounds

Ethyl acetate (Sigma Aldrich, >99.5%), ethyl butanoate (Aldrich, 99%), ethyl 2-methylbutanoate (Aldrich, 99%), benzaldehyde (Fluka, >99%), ethyl hexanoate (Aldrich, >99%) and 2-nonanone (Aldrich, >99%) were used. The physicochemical properties are given in Table 1. Hexadecane (Sigma Aldrich, 99%) and pentane (Aldrich, 98%) were used as solvents for the aroma solution and for the preparation of standards.

2.1.2. Poly(D,L-lactide) films samples

Extruded P(D,L-L)A Biomer L9000 (Biomer, Germany), PLA4032D (NatureWorks LLC), PLA2002D (NatureWorks LLC) and PLA Biophan 121 film (Treophan, Germany) with thickness of 120 μm, 235 μm, 65 μm and 40 μm, respectively, were studied. The content of D-lactic acid present in materials was about 1% for P(D,L-L)A Biomer L9000 [26], approximately 2% for PLA4032D [28], and between 4% and 4.5% for PLA2002D [29,30]. In the case of PLA Biophan 121, the exact proportion of D, L monomer was not specified in the manufacturer's datasheet.

Extrusion conditions of PLA Biomer L9000 were already reported by Colomines et al. [26]. In the case of PLA2002D, the films were prepared using a MAPRE single-screw extruder of 30 mm in diameter with a 33 L/D (Length on Diameter) barrel and a three section screw without mixing elements and turning at 35 rpm. The temperature profile of the six zones of the screw was 180–185–190–195–200–200 °C and the temperature of the die was 200 °C. A flat die of 200 mm in width and chill roll equipment were used to manufacture a film of 100 mm in width and approximately 65 μm in thickness. The roll temperature and roll speed were set at 25 °C and 10 m/min, respectively. The PLA4032D granulates were converted into a film using a Collin single-screw extruder with a 30 mm screw turning at 60 rpm. The temperature profile of the five zones of the screw was 190–190–190–200–200 °C and the temperature of the die was 200 °C. A flat film die with a width of 200 mm was used to produce a film of 100 mm in width and approximately 300 μm in thickness. The film was cooled by passing 2 rolls at 25 °C and 4.8 m/s.

In order to obtain very thin films three samples (30 ± 1 mg each one) of PLA4032D and PLA2002D with a thickness of 10 μm were prepared using a microtome (LKB Bromma Historange microtome 2218).

2.2. Conditioning of PLA films in aroma atmosphere

The experimental system used to conditioning of PLA samples is shown in Fig. 1. It consisted in an airtight flask equipped with a Mininert® valve which was placed in a climatic chamber for temperature control. The conditioning was carried out for 14 days (at high activities) or 20 days (at low activities) at 25 ± 0.3 °C. Three

Table 1
Physicochemical properties of the aroma compounds.

Aroma compounds	MW (g/mol)	BP (°C)	P_{vs} at 25 °C (kPa)	log P
Ethyl acetate	88.11	77.91	12.43	0.86
Benzaldehyde	106.12	16.90	0.12	1.71
Ethyl butanoate	116.16	121.79	2.07	1.85
Ethyl 2-methylbutanoate	130.18	133.00	1.10	2.12
Ethyl hexanoate	144.21	170.05	1.71	2.83
2-Nonanone	142.24	192.00	0.86	3.03

MW: molecular weight; BP: boiling point; P_{vs}: saturated vapour pressure at 25 °C; log P: octanol-water partition coefficient.

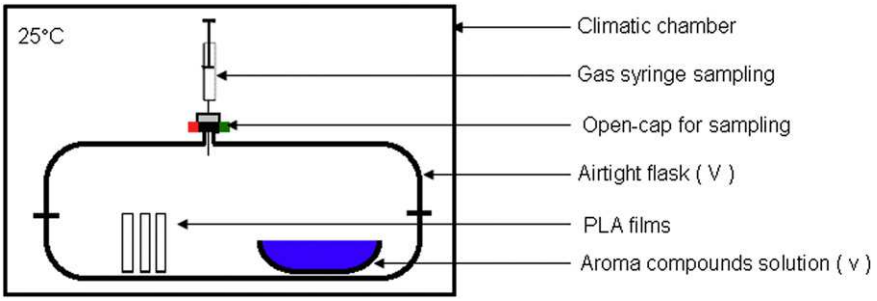


Fig. 1. System used to conditioning of PLA samples.

samples of each material were conditioned separately. Specific partial pressure of the aroma compounds in the atmosphere was generated by a solution of a mix of aroma compounds in hexadecane.

In order to measure the tightness of the system, preliminary experiments were conducted to establish the partition coefficient between headspace and aroma solution. The solutions of a mix of six aroma compounds to be used in MHE and MHS-SPME methods were put into a headspace vial of 20 ml and then sealed with silicon/PTFE septa and an aluminium cap. Three vials were prepared by each solution and they were conditioned at 25 °C. After 20 days, 0.5 ml of headspace volume was sampled using a gas syringe and injected into the gas chromatograph. One headspace injection was made per vial. The partition coefficient was calculated and the results are presented in Table 2. The same procedure was carried with the sorption system (Fig. 1) without PLA films (blank). The headspace/aroma solution partition coefficients measured (Table 2) (k) were compared to the preliminary experiments. No significant differences between the measured partition coefficients were observed. Therefore it was concluded that experimental system was tight during the 20 days of experimentation.

The concentration of the different molecules in the hexadecane solution is given in Table 3. Partial pressure (P_p) was calculated using ideal gas law. The activity of each compound was calculated as P_p/Psv, where Psv is the saturated vapour pressure of aroma compound at 25 °C. P_p and Activities of each aroma compound after 20 days are presented in Table 3.

For experiments at low aroma compound activities (3.4×10^{-5}–9.06×10^{-2}), film samples were cut into rectangles with mass of 0.3 g for PLA4032D, 0.08 g for PLA2002D and 0.04 g for PLA Biophan. Three samples of each material were conditioned in a separate flask. At activities between 2.9×10^{-3} and 9.06×10^{-2} analysis of sorbed quantities in PLA was done by MHE and at vapor activities between 3.4×10^{-5} and 1.5×10^{-3} by MHS-SPME.

For the analysis of aroma compound sorption at high vapor activity between 0.1, 0.2, 0.5 and 0.9, PLA Biomer L9000 films were cut in rectangles with total mass of 1 g.

2.3. Determination of the aroma partial pressure by gas chromatography

The aroma compounds concentration in the atmosphere of the airtight flask was measured after 20 days using a gas syringe. For that, 0.5 ml of the headspace of the hermetic flask were collected through the Mininert® valve installed on the flask and injected into a gas chromatograph (GC) Agilent Technologies, Model 6890GC/FID equipped with a DB5-MS capillary column (30 m length × 0.32 mm, 0.5 μm film thickness, JW Scientific).

The aroma compound concentration was determined using an external calibration curve measured on the same gas chromatograph. It was carried out by injecting 2 μl of a solution of a mix of aroma compounds in pentane (10, 25, 50, 100, 500, 1000 and 5000 μg/g). The temperature of the split/splitless injector was 250 °C with a purge time of 0.5 min. The flame ionisation detector was set at 305 °C with hydrogen as carrier gas (45 ml/min). The oven temperature was programmed to increase from 30 to 230 °C at 5 °C/min.

2.4. Sorbed aroma compounds quantification

2.4.1. Quantification by headspace methods

MHE and MHS-SPME techniques are dynamic gas extractions carried out stepwise on a same sample. At each extraction step, the overall equilibrium of the analyte in the system must be first established then analyte present in the headspace volume was removed and analyzed as GC area. Various extractions of the same sample are performed in order to reach an exponential decay of the analyte present in the sample and then to calculate the total area of the analyte, according to geometric progression showed in Eq (1). It means that from a restricted number of successive measurements (ideally $N = 5$), the sum of GC areas would be calculated if carrying out the successive measurements ad infinitum [22,23].

$$A_T = \sum_{i=1}^{N} A_i = \frac{A_1}{1-\beta}, \quad (1)$$

Table 2
Headspace/aroma solution partition coefficient (k) at 25 °C after 20 days of conditioning in a flask of 20 ml and in the sorption system without PLA films with the aroma solution used in MHE and MHS-SPME methods.

	In 20 ml flask		In sorption system	
	MHE method	MHS-SPME method	MHE method	MHS-SPME method
Aroma compound	k	k	k	k
Ethyl acetate	6.63 ± 0.62 E−03[b]	3.42 ± 0.88 E−03[a]	8.79 ± 0.78 E−03[b]	6.12 ± 2.90 E−03[a,b]
Ethyl butanoate	1.52 ± 0.32 E−03[a]	1.72 ± 0.46 E−03[a]	1.32 ± 0.14 E−03[a]	nd
Ethyl 2-methylbutanoate	7.32 ± 1.61 E−04[a]	4.87 ± 1.81 E−04[a]	6.94 ± 0.19 E−04[a]	4.50 ± 2.24 E−04[a]
Benzaldehyde	2.44 ± 0.79 E−04[a]	3.76 ± 1.59 E−04[a]	1.90 ± 0.32 E−04[a]	2.90 ± 2.18 E−04[a]
Ethyl hexanoate	1.19 ± 0.49 E−04[a]	1.08 ± 0.64 E−04[a]	9.69 ± 3.06 E−05[a]	1.10 ± 0.69 E−04[a]
2-nonanone	6.77 ± 3.23 E−05[a]	8.24 ± 4.12 E−05[a]	8.13 ± 4.76 E−05[a]	9.40 ± 5.19 E−05[a]

k = concentration in headspace (mg/L)/concentration in solution (mg/L) nd = not determinate because it was not included in aroma solution.
[a, b] Different letters indicate significant differences at $p < 0.05$ (Duncan). Statistical analysis was carried out separately for each aroma compounds.

Table 3
Conditioning of the PLA samples for the three methods: Concentrations of aroma compound in solution and in headspace expressed as partial pressure and activity.

Aroma compound	Aroma solution in hexadecane (mol/L)	Concentration of aroma in headspace (mol/L)	P_p (Pa)	Activity	Method
Ethyl acetate	2.0E-04	1.6E-06	3.0	2.4E-04	MHS-SPME
	2.0E-02	1.7E-04	429.0	3.5E-02	MHE-GC
	4.2E-01	5.2E-04	1243.0	1.0E-01	Gravimetry
	8.4E-01	1.0E-03	2486.0	2.0E-01	Gravimetry
	2.3E+00	2.6E-03	6215.0	5.0E-01	Gravimetry
	5.2E+00	5.2E-03	11187.0	9.0E-01	Gravimetry
Ethyl butanoate	1.5E-02	1.9E-05	47.6	2.3E-02	MHE-GC
	1.1E-01	6.8E-05	207.0	1.0E-01	Gravimetry
	2.7E-01	1.4E-04	414.0	2.0E-01	Gravimetry
	6.6E-01	3.4E-04	1035.0	5.0E-01	Gravimetry
	1.2E+00	6.1E-04	1863.0	9.0E-01	Gravimetry
Ethyl 2-methylbutanoate	2.0E-04	8.8E-08	0.2	2.0E-04	MHS-SPME
	2.0E-02	1.3E-05	33.0	3.0E-02	MHE-GC
Benzaldehyde	3.0E-04	7.0E-08	0.2	1.5E-03	MHS-SPME
	3.0E-02	4.5E-06	11.1	9.6E-02	MHE-GC
Ethyl hexanoate	1.8E-04	2.3E-08	0.1	3.4E-05	MHS-SPME
	1.8E-02	2.0E-06	5.0	2.9E-03	MHE-GC
	8.1E-01	4.5E-05	855.0	5.0E-01	Gravimetry
	1.6E+00	8.1E-05	1539.0	9.0E-01	Gravimetry
2-nonanone	1.7E-04	1.6E-08	0.0	4.6E-05	MHS-SPME
	1.7E-02	1.4E-06	3.4	3.9E-03	MHE-GC

In previous equation, A_T is the theoretical total GC area, A_i is the GC peak area relative to the i-th extraction, N is the total number of extractions, A_1 is the GC peak area relative to the first extraction, and β is a constant which describes the exponential decay of peak areas during the stepwise multiple headspace extraction procedure. The constant β can be obtained from the slope of the plot resulting from the next equation:

$$\ln A_i = (i-1)\ln\beta + \ln A_1, \quad (2)$$

So, the accuracy of the measurement depends on the right linearity (depending of R^2) of the N successive extractions and not on the total extraction of the compound. Therefore, parameters like temperature and time of equilibration require to be optimized.

Then, the quantity of analyte in the sample can be calculated from the total peak area A_T using a calibration by external standard.

2.4.1.1. Multiple headspace extraction (MHE). After conditioning time, PLA samples were removed from airtight flasks and sealed in 20 ml headspace glass vials with silicon/PTFE septa and an aluminium cap. An automatic headspace sampler coupled with GC/FID (Perkin Elmer HS40 XL) was used to monitor the extraction and analysis of the released aroma compounds. The specific headspace sampler operation conditions were: head pressure (Helium) 200 kPa, equilibration temperature 60 °C, equilibration time 55 min, pressurization time 2 min, injection time 0.05 min, hold up time 0.2 min, needle temperature 110 °C, transfer line temperature 130 °C. The oven temperature was programmed at 35 °C for 2 min, 6 °C/min up to 230 °C. Injector temperature was 250 °C and its split was 15.6 ml/min. Detector temperature was 250 °C and it was set at range 1. The column was a SupelcoWax-10 capillary column (30 m length × 0.32 mm, 0.5 μm film thickness). The carrier gas was He at 1.8 ml/min. Three vials were prepared for each type of PLA and each vial was sampled five times at equal time intervals (55 min) at 60 °C. Total peak area of each aroma compound sorbed was calculated using Eq. (1). Calibration of each aroma compound was performed by external standards. For that, a solution of a mix of aroma compounds was prepared in hexadecane. Dilutions of 100–10,000 μg/g in hexadecane were used. Ten μl of the diluted solution was introduced in a 20 ml glass vial sealed with silicon/PTFE septa and an aluminium cap. Three vials were prepared for each dilution and then each vial was sampled five times following the method already described.

2.4.1.2. Multiple headspace solid-phase micro extraction (MHS-SPME). After conditioning time, samples were removed from airtight flasks and sealed in 20 ml glass vials with silicon/PTFE septa. An Agilent Technologies 6890 GC/FID coupled with a mass spectrometer 5975 INSERT and an Autosampler MPS2 GERSTEL, which allows automated MHS-SPME injections, were used. The oven temperature programme began with an initial temperature of 30 °C for 5 min, and then temperature increased at a rate of 5 °C/min up to 230 °C, and then this temperature was maintained for 5 min. Split/splitless injector and detector temperatures were 250 °C. The column was a JW Scientific DB5-MS capillary column (30 m length × 0.32 mm inner diameter × 0.5 μm film thickness). The carrier gas was He at 1.4 ml/min. For MHS-SPME injections, three vials were prepared for each type of PLA and each vial was sampled four times. The sample vials were incubated at 60 °C for 30 min and agitated at 250 rpm. MHS-SPME was performed for 15 min at 60 °C using a 75 μm carboxen–polydimethylsiloxane (CAR-PDMS) fibre providing best results in terms of amount of compound extracted for low molecular mass compounds [31]. Desorption time was 5 min into the injector port. Total peak area of each aroma compound sorbed was calculated using Eq (1). The calibration was carried out by external standard in hexadecane. For that, a solution of a mix of aroma compounds was prepared in hexadecane. Dilutions of 0.5–25 μg/g in hexadecane were used for ethyl butanoate, ethyl 2-methylbutanoate, ethyl hexanoate and 2-nonanone, whereas dilutions of 25–500 μg/g in hexadecane were used for ethyl acetate and benzaldehyde. Twenty μl of the diluted solution were introduced in a 20 ml glass vial sealed with silicon/PTFE septa and a steel cap. Three vials were prepared for each dilution and each vial was sampled four times as described for PLA samples.

2.4.2. Gravimetric method

Once the samples were conditioned at high activity (14 days at 25 °C), they were weighed by an analytical balance (Mettler AE160, 10^{-4} g) to calculate the mass uptake and the solubility coefficients.

2.5. Solubility coefficient

The apparent solubility coefficient (S) of each aroma compound was calculated according to:

$$S = \frac{M_\infty}{M_0} \times \frac{d}{P_p}, \quad (3)$$

where M_∞ is the mass sorption after 14 or 20 days of conditioning (kg), M_0 is the initial mass of PLA sample (kg), d is the film density (kg/m^3) and P_p is the partial pressure of each aroma compound.

2.6. Thermal properties: differential scanning calorimetry (DSC)

Before and after conditioning, the thermal properties of film samples were determined in triplicate using a differential scanning calorimeter QSC 100 instrument (TA Instruments, France). Films samples were analyzed at 10 °C/min from −30 °C to 190 °C and the degree of crystallinity (X_c) of materials was calculated according to:

$$X_C = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0}, \quad (4)$$

where ΔH_m is the enthalpy of fusion, ΔH_{cc} is the enthalpy of cold crystallization and ΔH_m^0 is the heat of melting crystalline PLA (93 J/g) [32].

2.7. Statistical analysis

The statistical analysis of data was performed through one-way analysis of variance (ANOVA) using XLSTAT-Pro 7.0 software (Addinsoft, Paris, France). When differences were significant ($p < 0.05$), Duncan's test was used to evaluate the differences between pairs of groups.

3. Results and discussion

3.1. Optimization of the headspace extraction methods

To apply the MHE technique for the extraction of aroma compounds the temperature and time of equilibration before injection have to be optimized. The temperature of equilibrium was set at 60 °C, in the region of the glass transition of PDLLA in order to facilitate the extraction of all sorbed aroma compounds. For each compound the equilibration time producing stabilized and reproducible chromatographic peak areas was tested using conditioned samples of Biophan film. Furthermore, the quality of the linear decrease of peak areas during multiple headspace extraction was checked. The effect of time on the area of peaks depended on each compound, for example while the peaks of 2-nonanone still increased at 55 min, the other compounds achieved a plateau at 30 min. The incubation time of 55 min was retained as a good compromise for all aroma compounds and produced a very good linear decrease ($R^2 \geq 0.99$) during multiple extraction. Furthermore, it was in agreement with the duration of chromatographic cycle.

In order to obtain an efficient extraction of aroma compounds, 5 extraction cycles were chosen. Kolb et al., 1991 [22] showed the possible use of only 2 extraction cycles, but only for very volatile compounds. Furthermore the behaviour of compounds with a large difference in their volatilities as it is the case here (Table 1) could vary during the extraction cycles. To keep low cycle numbers, in some case authors add solvents or liquid displacers to improve extraction [33], but this strategy would have included further analytical difficulty in our case.

In the case of MHS-SPME the incubation time of the CAR–PDMS 75 μm fibre was optimized. For that, three incubation times were studied (15, 30 and 45 min) using conditioned samples of Biophan film which were placed in 20 ml headspace glass sealed vials, heated at 60 °C. The fibre extraction time was set to 15 min. The peak areas showed that all compounds started to reach a plateau at 15 min. Therefore 30 min incubation time was selected as a condition for further experiments. Finally, the linearity of the relationship between the logarithm of peak area and extraction number was confirmed by finding good correlation coefficients ($R^2 \geq 0.99$). When the amount of compound in the vial is too large, exponential decay of peak area is not observed and low correlation coefficients are obtained [34]. In our study, the sample mass in the vial was different for each material due their different thickness. It did not present any effect on the correlation coefficients when we used thick PLA films; however, microtomed PLA which sorbed a higher amount of aroma compounds than thick PLA samples presented linearity but lower correlation coefficients ($R^2 \geq 0.98$) in accordance to the effects observed by Carrillo and Tena [34].

3.2. Aroma compounds sorption

The amount (μg/g) of aroma compounds sorbed in PLA samples after conditioning for 20 days and the solubility coefficients are shown in Fig. 2A and B by MHE and MHS-SPME determinations respectively. The sorption profiles are similar between the two methods, but MHE-SPME allowed for determining concentrations one hundred times lower.

The sorption of ethyl acetate in PLA is high, as already observed in literature [25,26]. The main explanation for this affinity can be sought in the quite similar molecular structure of ethyl acetate being the most hydrophilic of a homologous series of ethyl esters. The more surprising result was however the high affinity of the aromatic structure of benzaldehyde for PLA. However, those results were done on PLA samples with high thickness, and therefore the sorption equilibrium might not be reached after 20 days of conditioning. In this case, the rate of diffusion of the considered molecule into the structure might importantly change the picture, as larger molecules are known to diffuse slower. The dependence of the diffusion coefficient (D) on molecular weight is indeed very steep in the case of glassy materials as was shown by Pennarun et al. [35] for PET and probably the same for PLA under our measurement conditions.

The values of D of ethyl acetate in PLA were reported between 10^{-14} m^2/s [36] and 10^{-17} m^2/s [25,27]. In the hypothesis of the lower D, 20 days of conditioning of the thick PLA samples are not enough to reach thermodynamic equilibrium. For this reason, we did an experiment on microtomed samples, where the characteristic film thickness was reduced to 10 μm and sorption equilibrium could be reached in 20 days under the hypothesis of a diffusion coefficient in the order of 10^{-17} m^2/s.

The results of the adsorbed amount are given in Fig. 2C and D. The microtomed samples sorbed a higher quantity of aroma compound whatever the method compared with thick PLA films samples. For example, in the case of ethyl acetate and MHE method (Fig. 2C, activity 10^{-2}), concentration sorbed in microtomed samples is 10 times and 5 times higher than in thick samples of PLA2002D and PLA4032D, respectively (Fig. 2A). However, one observes a similar sorption pattern, still with a high affinity of benzaldehyde. There seemed to be a difference of the sorption of the aroma compounds for both PLA grades, but this difference depended on the activity.

To get a clearer picture on the affinity of the aroma compounds for both PLA grades, the data were normalized by the calculation of the solubility coefficient after Eq. (3). The results are given in Fig. 2E and F for both levels of activity differing by a factor of 100. The first conclusion is that S is equivalent for both activities, within a reasonable variation linked to the different experimental determination methods. Therefore, in the case of such low vapour activities, one can conclude that the sorption isotherm follows Henry's law.

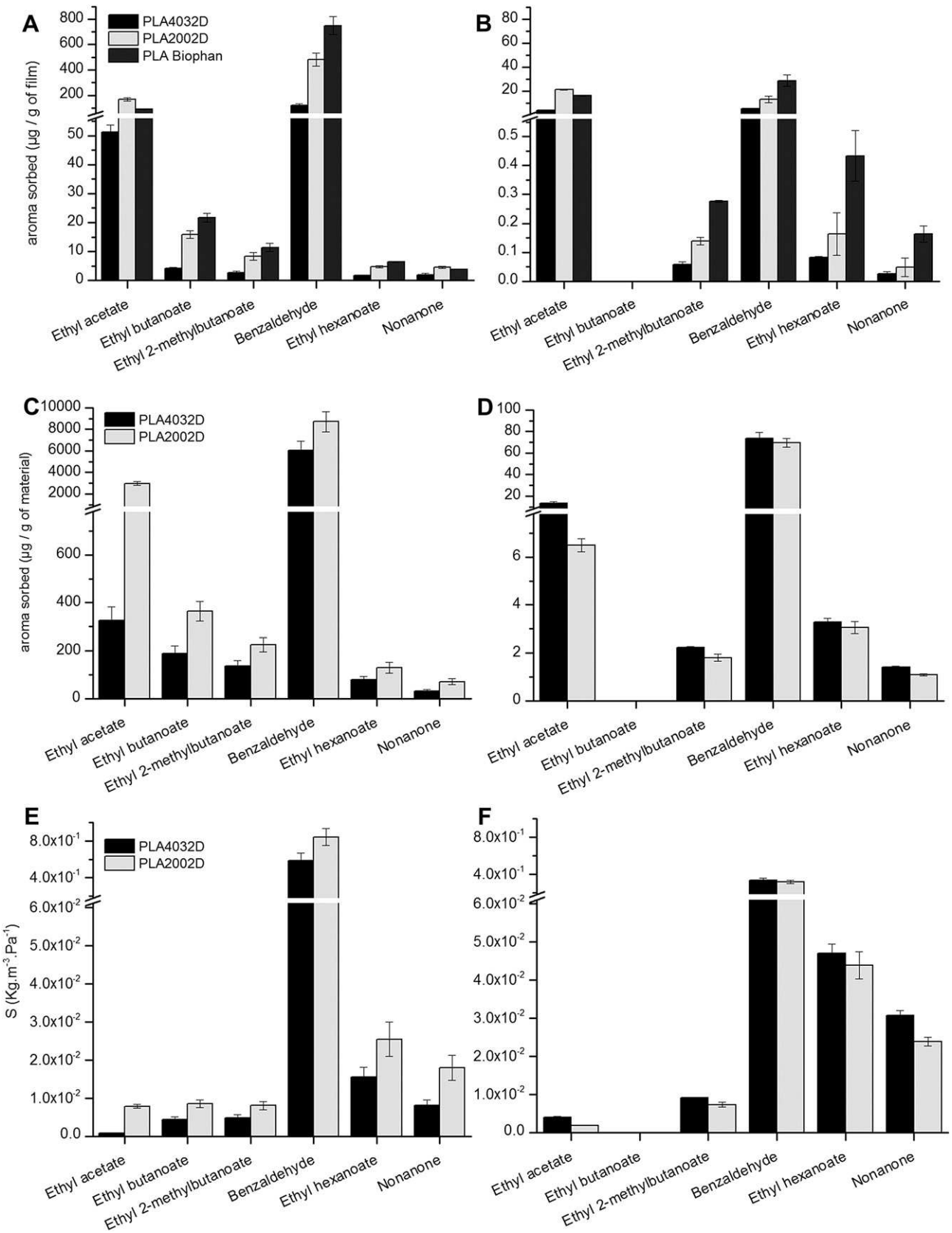


Fig. 2. Apparent sorption of aroma compounds in PLA films samples determined by (A) MHE and (B) MHS-SPME. Aroma sorption in microtomed PLA samples (thickness = 10 µm) determined by (C) MHE and (D) MHS-SPME. Solubility coefficients (S) of aroma compounds in microtomed PLA samples (thickness = 10 µm) determined by (E) MHE and (F) MHS-SPME. The ordinate is discontinuous as indicated to present all small values, $n = 3$. Partial pressures used for each method were done in Table 2.

Table 4
Values of Hansen solubility parameters $(J/cm^3)^{1/2}$ for PLA and aroma compounds.

Compound	V (cm^3/mol)	δ_D	δ_P	δ_H	δ_T [a]	$\Delta\delta$ [b]	Reference
PLA	47.1	18.6	9.9	6.0	21.9	–	[39]
Ethyl acetate	98.5	15.8	5.3	7.2	18.2	5.5	[38]
Ethyl butanoate	132.9	15.5	5.6	5.0	17.2	5.4	[38]
Ethyl 2-methylbutanoate		n.d.	n.d.	n.d.			
Ethyl hexanoate	149.6	15.5	3.2	5.9	16.9	7.4	[38]
Benzaldehyde	101.5	19.4	7.4	5.3	21.4	2.7	[38]
2-nonanone		n.d.	n.d.	n.d.			
PP	49.5	16.1	0.0	0.0	16.1	–	[25]
LDPE	32.8	17.9	0.0	0.0	17.9	–	[25]

n.d. No data literature available.
[a] Values were calculating according to Eq (5).
[b] Values were calculating according to Eq (6) between PLA and aroma compounds.

The results of the homologous series of ethyl esters show that the most hydrophobic component ethyl hexanoate had the highest solubility coefficient, most likely due to its structure similar to the PLA chain. In the case of benzaldehyde which presented the highest *S*, the high affinity may be explained by the well-known miscibility of PLA with aromatic solvents such as toluene and benzene [37]. Ethyl-2-methylbutanoate had comparable *S* compared to that of ethyl butyrate, which hints that the additional methyl group does not change the behaviour of the molecule in the polymer structure. 2-Nonanone had comparatively higher *S*, which could be compared to that of ethyl hexanoate.

The calculation of the Hansen solubility parameters is a semi-quantitative method from which the affinity of a polymer for a substance can be obtained. This affinity can be estimated from Hildebrand's theory by calculating the total solubility parameter, according Eq. (5):

$$\delta_T^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \quad (5)$$

were δ_T $(J/cm^3)^{1/2}$ corresponds to the overall Hildebrand solubility parameter and δ_D, δ_P, and δ_H are the Hansen solubility parameters that represent contributions from the nonpolar interaction, polar interaction and hydrogen bonding, respectively [38].

When the distance $\Delta\delta = 0$ (Eq. (6)), the solvent and polymer are thermodynamically similar that the polymer will be highly soluble. As the distance gets greater, the solubility declines [39]. A good solubility level is assigned when distance $\Delta\delta$ is smaller than 5 $(J/cm^3)^{1/2}$ [25].

$$\Delta\delta = \left[(\delta_{DP} - \delta_{DS})^2 + (\delta_{PP} - \delta_{PS})^2 + (\delta_{HP} - \delta_{HS})^2\right]^{1/2} \quad (6)$$

δ_T and $\Delta\delta$ were calculated from literature values of δ_D, δ_P, and δ_H. Results are given in Table 4.

So, a small distance was found between PLA and benzaldehyde, which confirmed the experimental results shown in Fig. 2. Moreover, this experimental result is in accordance with theoretical studies that reported that the obvious place to look for a rational plasticizer is in the aromatics compounds because PLA presents a high nonpolar interaction value δ_D [39].

Few papers in literature reported data on sorption of organic compounds in PLA. Fig. 3 represents a comparison of the solubility coefficients of aroma compounds in PLA and other polymers derived from literature. Obviously physical and chemical properties of a same kind of polymer like crystallinity, morphology, and chemical composition were not identical in these papers and could influence the value of *S* [40–42].

These data show that for a same couple polymer/solute a large discrepancy appears for the value of *S*. The main difference in the values of *S* comes from first on the methodology and the activity of the aroma compound used and secondly if *S* is measured directly from the polymer weight uptake or indirectly from the coefficient of permeability. For example, Colomines et al. [26] studied vapor phase sorption (% mass uptake) of ethyl acetate in PLA samples at different activities (0.2, 0.5 and 0.9) using a microbalance by 20 h.

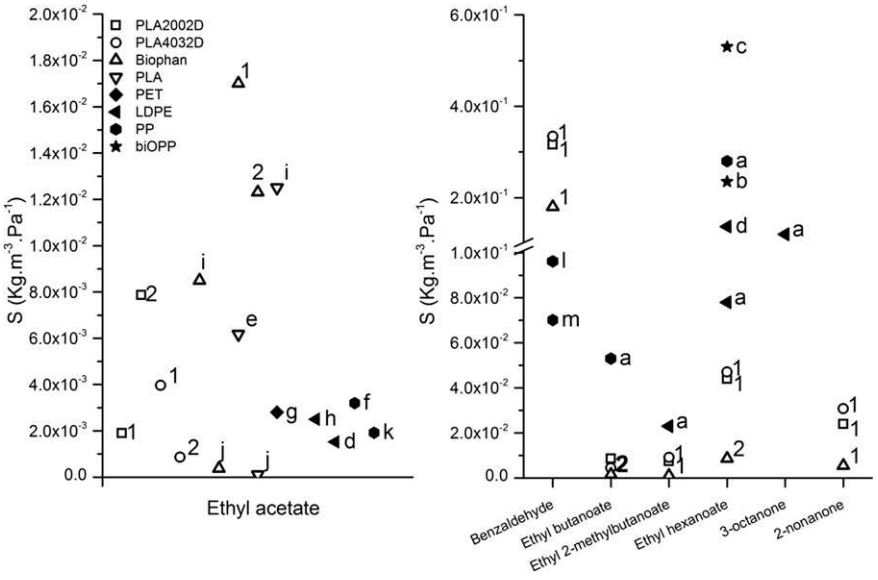


Fig. 3. Solubility coefficients of aroma compounds in plastic packaging materials. **1** this work between 0.34–14.93 × 10^{-4} vapor activities (P_p between 0.039 and 3.04 Pa). **2** this work between 0.29–9.59 × 10^{-2} vapor activities (P_p between 3.4 and 429 Pa) **a** at 25 °C, vapor activity not detailed, by permeation kinetic [43] **b** 0.5 vapor activity (P_p = 855 Pa), 25 °C and 0% RH by Micro-atmosphere-derived method [44] **c** 0.5 vapor activity(P_p = 855 Pa), 25 °C, 0% RH by microgravimetric method [44] **d** 0.5 vapor activity(P_p = 855 Pa), 25 °C, 0% RH by gravimetric method [45] **e** 0.2 vapor activity (P_p = 3030 Pa), 30 °C by gravimetric method − digital electrobalance [25] **f** 0.56 vapor activity (P_p = 6088 Pa), 22 °C by gravimetric method [16] **g** 0.6 vapor activity (P_p = 9435 Pa), 30 °C by gravimetric method [46] **h** 0.4 vapor activity (P_p = 4348 Pa), 22 °C by gravimetric method [47] **i** 0.5 vapor activity (P_p = 6215 Pa), 25 °C, 0% RH by static method − electronic microbalance [26] **j** 0.2 vapor activity (P_p = 2486 Pa), 25 °C, 0% RH by static method − electronic microbalance [26] **k** 0.9 vapor activity (P_p = 11,187 Pa), 25 °C, 0% RH by gravimetric method [45] **l** 0.5 vapor activity (P_p = 58 Pa), 25 °C, 0% RH by microgravimetric method, adapted from [48] **m** 0.3 vapor activity (P_p = 34.8 Pa), 25 °C, 0% RH by microgravimetric method, adapted from [48].

Table 5
Masse uptake, glass transition temperature (Tg) and degree of crystallinity (χ) of PLA films using DSC before and after sorption of aroma compound.

Material	Conditioning	Aroma compound	P_p (Pa)	Masse uptake (μg/g)	Tg (°C)	χ
PLA Biomer L9000	Before	–	–	–	58.4 ± 0.3^{a}	2 ± 1^{a}
	After	Ethyl acetate	1243.0	7000	46.5 ± 1.6^{b}	20 ± 2^{b}
			2486.0	43,000	43.5 ± 5.4^{b}	37 ± 2^{c}
			6215.0	98,500	9.5 ± 0.9^{c}	$39 \pm 5^{c,d}$
			11187.0	178,000	0.9 ± 1.6^{d}	42 ± 2^{d}
PLA Biomer L9000	Before	–	–	–	58.4 ± 0.3^{a}	2 ± 1^{a}
	After	Ethyl butanoate	207	2000	$57.3 \pm 1.1^{a,b}$	$14 \pm 3^{b,c}$
			414	3500	$57.3 \pm 0.3^{a,b}$	12 ± 1^{b}
			1035	4000	56.9 ± 0.5^{b}	16 ± 2^{c}
			1863	7000	28.7 ± 0.5^{c}	34 ± 1^{d}
PLA Biomer L9000	Before	–	–	–	58.4 ± 0.3^{a}	2 ± 1^{a}
	After	Ethyl hexanoate	855	1000	57.3 ± 0.3^{a}	10 ± 1^{b}
			1539	6000	56.6 ± 1.7^{a}	12 ± 3^{b}
PLA Biophan	Before	–	–	–	57.7 ± 0.2^{a}	20.9 ± 0.1^{a}
	After	Mixed aroma solution	Between 3.4 and 429	883 ± 78	54.8 ± 0.7^{b}	20.1 ± 0.2^{b}
PLA2002D	Before	–	–	–	59.9 ± 0.1^{a}	1.3 ± 0.3^{a}
	After	Mixed aroma solution	Between 3.4 and 429	685 ± 67	58.6 ± 0.3^{b}	2.6 ± 0.4^{b}
PLA4032D	Before	–	–	–	64.3 ± 0.5^{a}	4.2 ± 1.0^{a}
	After	Mixed aroma solution	Between 3.4 and 429	184 ± 18	62.6 ± 0.1^{b}	2.4 ± 1.5^{a}

[a, b, c, d] Different letters indicate significant differences at $p < 0.05$ (Duncan). Statistical analysis was carried out separately for each PLA type and its aroma compounds.

They showed that at an activity of 0.2, Biophan (30 μm in thickness) sorbed 700 μg of ethyl acetate per g of polymer whereas PLA Biomer L9000 (190 μm in thickness) sorbed only 190 μg of ethyl acetate per g of polymer. In our study, activities of ethyl acetate were 0.035 and 0.00024 and we found that PLA Biophan sorbed 91.8 and 16.17 μg per g of polymer, respectively. Our results for Biophan (between 12.3 and 17.0×10^{-3} kg m^{-3} Pa^{-1}) are thus one order of magnitude higher than the results of Colomines et al. [26]. They showed that at 25 °C the apparent solubility coefficient was 0.35×10^{-3} kg m^{-3} Pa^{-1} at an activity of 0.2. The values of Colomines et al. [26] were therefore highly underestimated, because the sorption equilibrium was not reached as also mentioned by the authors.

Nevertheless, independently of the method, for a same couple polymer/solute when low activities were used and equilibrium was reached, solubility coefficients were close. That can be observed in the case of ethyl acetate sorbed in PLA, where S founded in this work is on the same order of magnitude than the results of Auras et al. [25] who studied PLA 98% L-isomers and presented a solubility coefficient of 6.17×10^{-3} kg m^{-3} Pa^{-1}at 30 °C and an activity of 0.2. These results showed that at low activities, S was independent of partial pressure following Henry's law.

Fig. 3 also shows values for a same solute and different polymers, for example, the sorption of ethyl acetate in the PLA types studied at low activity in this work are similar to values reported for poly(ethylene terephthalate), low-density polyethylene (LDPE) and polypropylene (PP). In the case of ethyl hexanoate, its solubility coefficient in PLA is lower than in bi-axially oriented polypropylene (biOPP), PP and LDPE. Benzaldehyde presented a solubility coefficient in PLA higher than in PP. From the total solubility parameters calculated for PP and PE, 16.1 and 17.9, respectively, it could be predicted that ethyl hexanoate should have a slighter affinity for PP ($\Delta\delta = 6.7$) and PE ($\Delta\delta = 7.1$) compared with PLA ($\Delta\delta = 7.4$). In the case of benzaldehyde, its affinity for PLA ($\Delta\delta = 2.7$) is higher than for PP ($\Delta\delta = 9.7$) and PE ($\Delta\delta = 9.2$). So, experimental sorption values showed a good agreement with literature and theoretical affinity parameters.

3.3. Effect of aroma compounds sorption on PLA samples

Colomines et al. [26] and Courgneau et al. [27] showed that the sorption of ethyl acetate into PLA induced morphological changes in the polymer structure. Both authors evidenced plasticizing and induced crystallization. However, the ethyl acetate activities used were high, 0.5 in Colomines's paper and 0.2 in Courgneau's paper. For example, Colomines et al. [26] found that Tg decreased from 50.9 ± 0.1 °C to 16.9 ± 1.9 °C, after 3 days of contact at 0.5 activity. Such high activities are at least one order of magnitude higher than expected activities upon application of PLA for example as food packaging film. In order to test the effect of aroma compound sorption at low activities (10^{-2}) on the thermo-mechanical properties of PLA samples, they were tested before and after conditioning. The results are given in Table 5 and typical raw DSC data in Fig. 4 and Fig. 5.

For each PLA type and the aroma compounds sorbed, statistical analysis showed significant differences ($p < 0.05$) in Tg and X_c (Table 5).

From Table 5 and Fig. 4 it becomes clear that ethyl acetate has a high plasticizing effect on PLA and induces crystallization although the sorption equilibrium was not reached in the case of the presented experiment after 14 days of conditioning. The effect of ethyl acetate was already observed by Colomines et al. [26] and Courgneau et al. [27]. After contact with the two homologous molecules of the ethyl esters serie, DSC measurements were carried

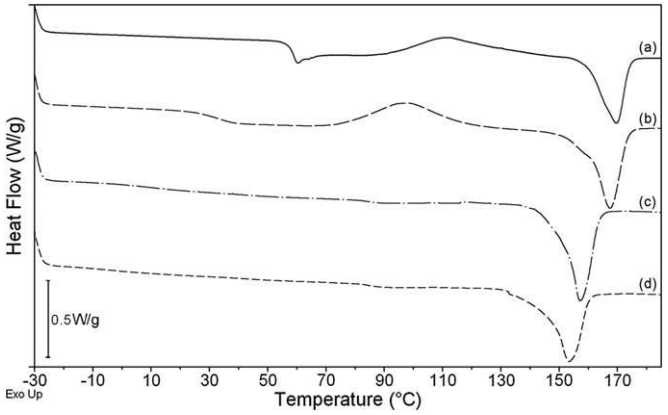


Fig. 4. DSC thermograms for PLA Biomer L9000 before conditioning (a) and after conditioning with ethyl acetate at a vapor activity of 0.2 (b) 0.5 (c) and 0.9 (d).

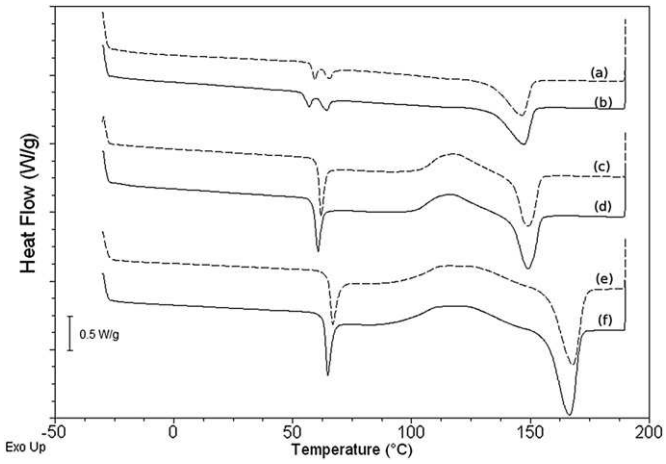


Fig. 5. DSC thermograms for PLA Biophan before conditioning (a) and after conditioning (b), for PLA2002D films before conditioning (c) and after conditioning (d) and PLA4032D films before conditioning (e) and after conditioning (f) in mixed aroma compound headspace at a partial pressure between 3.4 and 429 Pa.

out on the thick PLA samples. Comparison of DSC data after contact with ethyl acetate and with ethyl butanoate shows that the effect on the PLA microstructure was higher with ethyl butanaoate as the decrease of Tg is larger. Ethyl hexanoate showed smaller effects, but the sorbed quantity was also very small. However, it was still able to induce significant crystallization of the polymer.

Furthermore, Table 5 shows that, when mass uptake increased, the glass transition temperatures of PLA Biomer L9000 clearly decreased and its crystallinity increased. However, for ethyl butanoate, a sorption of 7000 μg/g was necessary to shift the Tg to 30 °C and to observe a cristallinity multiplied by 2. For the ethyl esters series, crystallinity gradually increased with the activity until to reach an average maximum of 35%.

What is even more interesting is that at very low activities of the six aroma compounds in mixture, such as 3.45×10^{-2} for the ethyl acetate (Table 3, Fig. 5) a slight decrease in Tg was observed in all types of PLA (Table 5, Fig. 5). Small plasticization of PLAs by aroma compounds at very low concentration (<200 μg/g) is thus found. However, at very low activities, no solvent induced crystallization of the samples was observed.

4. Conclusions

Sorption of aroma compound was determined by MHE and MHS-SPME at low vapour activities comparable to conditions which can be found in the headspace of packed foodstuff. The analysis of the polymer thermal properties after conditioning showed a slight decrease in Tg of PLA films, which means that a plasticization effect of aroma compounds occur already at vapor activities in the order of 10^{-2}. An effect of solvent induced crystallization was observed from 10^{-1} vapor activities. The results showed that PLA films studied have a high affinity for common aroma compounds and in particular for benzaldehyde, that could have a plasticizer effect.

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Chapter 4

Sorption of oil and aroma compounds from model foods in poly(lactide)

4 Sorption of oil and aroma compounds from model foods in poly(lactide)

After having accomplished the study of the interactions between PLA and model aroma compounds, the findings were transposed to food models in order to have applicative validation. Two different food models were envisaged, a dry food model being sponge cake and a food emulsion containing lipids and water.

In the previous chapter, the solubility coefficients determined for ethyl hexanoate and 2-nonanone were higher, although their high hydrophobicity. In the case of ethyl hexanoate, the value of S was not in accordance with the distance value ($\Delta\delta$) calculated from Hansen solubility parameters. This fact could be explained by a synergy effect between the aroma compounds sorbed in PLA. However, no data about this phenomenon was obtained in that study. For that, with the purpose of verifying this hypothesis, we carried out a study about the mixture effect of aroma compounds on the sorption in PLA.

So, in this chapter, firstly the results obtained in the sponge cake model are presented. Then, an article about the results obtained with the food emulsion model and the mixture effect of aroma compounds on the sorption in PLA samples is presented.

4.1 Sorption of aroma compounds from flavored sponge cakes in poly(lactide)

One of the most studied food models inside the research unit is sponge cake. It exists therefore large literature on the reaction schemes of aroma creation in such kind of product. Furthermore, dry products, such as cakes are less sensitive to moisture loss. The high water vapor permeability of PLA might therefore not interdict PLA packaging. In the aim to investigate the permeability of different chemical structures of aroma compounds through PLA, a model sponge cake produced and flavored or not by an aroma solution, containing three ethyl esters, benzaldehyde and 2-nonanone.

The fabrication procedure of the sponge cake is given in the materials and methods chapter. The sponge cake was cooled after baking for 30 min at ambient temperature and then conditioned in PLA packaging. The conditioning of the sponge cakes was carried out with different systems that were showed in Figure 2.2:

1) A PLA pouch was fabricated by hot-sealing of two PLA films and placed inside the climatic chamber during 10 days at 25 ºC and 50 % RH. A second series of pouches was placed inside a permeation cell in the thermo-regulated oven at 25 °C and ambient RH for 10 days (Figure 2.2a).
2) The sponge cake was placed inside a permeability cell and sealed with a PLA film and placed inside a thermo-regulated oven at 25 °C and ambient RH for 10 days (Figure 2.2b).
3) An aroma compounds solution in hexadecane of equivalent concentration was put in the permeability cell, sealed with PLA and placed inside a thermo-regulated oven at 25 °C and ambient RH for 10 days.

During conditioning in the second system (permeability cell), progressive extractions of the headspace system were made using a spme fibre (CAR/PDMS). The extraction time was 15 minutes at 25 ºC, then injected for 5 min in a GC-FID. After each extraction, the headspace of

the system was vented with air during 2 minutes with the purpose of removing the aroma compounds presents in the headspace and thus to produce the flux of aroma compounds. The pouches in the climatic chamber were weighed regularly to follow weight loss.

This system should allow the measure of aroma compound flux through the films or pouch, and thus, the permeation. However, the heat-sealed pouches were very sensitive to leakage and no reproducible results were obtained. Most probably, the PLA was not adapted to the household heat-sealing system used. Also the permeation cells showed leakages due to the very thin PLA films (55 µm) used which makes it difficult to tighten the system.

For that, we present only two parts of the methodological approach which interesting results: the measure of WVTR and the quantification of aroma compounds in the PLA samples after 10 days of permeation.

4.1.1 Moisture loss and water vapour transfer rate (WVTR)

The moisture loss of the sponge cakes packaged in PLA pouches was measured following the weight loss during storage at 23 °C and 50% RH for 30 days, which implied the hypothesis that water loss was the principal cause of the weight loss of the cakes. Weight loss was measured using an analytical balance (10^{-3} g). The water vapour transfer rate (WVTR) of PLA pouches was calculated from the slope of the plot of weight loss over time. Given values are averages of five experiments.

We found in PLA4032D pouches (thickness 55 µm) a WVTR of 33.9 ± 3.5 g/m^2.day which is about three times higher than the WVTR value (12.1 ± 5.7 g/m^2.day) reported by others authors (Courgneau et al. 2011), that studied the WVTR in PLA thermo-molded films (thickness 100 µm) at 25 °C and 50% RH, according to the norm NF H 00–030. Differences

in the values of WVTR could be linked to the uncertainty of the determination of the exchange surface in the case of the pouch and also showed that there might have been some leakages in the packaging.

4.1.2 Quantification of sorbed aroma compounds in pouches and films of PLA by MHS-SPME.

To quantify the aroma compounds sorption in PLA pouches and films after conditioning with flavored sponge cakes or with an aroma solution in hexadecane (AC) (Figure 2.2), we used the parameters of MHS-SPME optimized in chapter 3. So, after 10 days of conditioning time, the aroma compound solution and the flavored cakes were removed from the permeability cells and PLA pouches. Then, about 25 mg of PLA pouch and film were sealed separately in 20 ml glass vials with silicon/PTFE septa. For MHS-SPME injections, three vials were prepared and each vial was sampled four times, according the sampling procedure described in chapter 3.

The results of the aroma compound sorption for the three different systems are given in Figure 4.0. This figure clearly shows a difference in the sorption values between PLA samples, which could be related to conditioning. In the case of PLA films conditioned with an aroma solution in hexadecane (PLA4032D film with AC), the sorption was related to molecular weight of compounds. Sorption was clearly far from its equilibrium value, but the general pattern was coherent with the findings on the model aroma compounds in chapter 3. So, the compounds with high Mw and more hydrophobicity as ethyl hexanoate and 2-nonanone were the less sorbed in PLA. Ethyl acetate and benzaldehyde presented the higher sorption values, as already showed in literature (Colomines et al. 2010) and in our previous chapter.

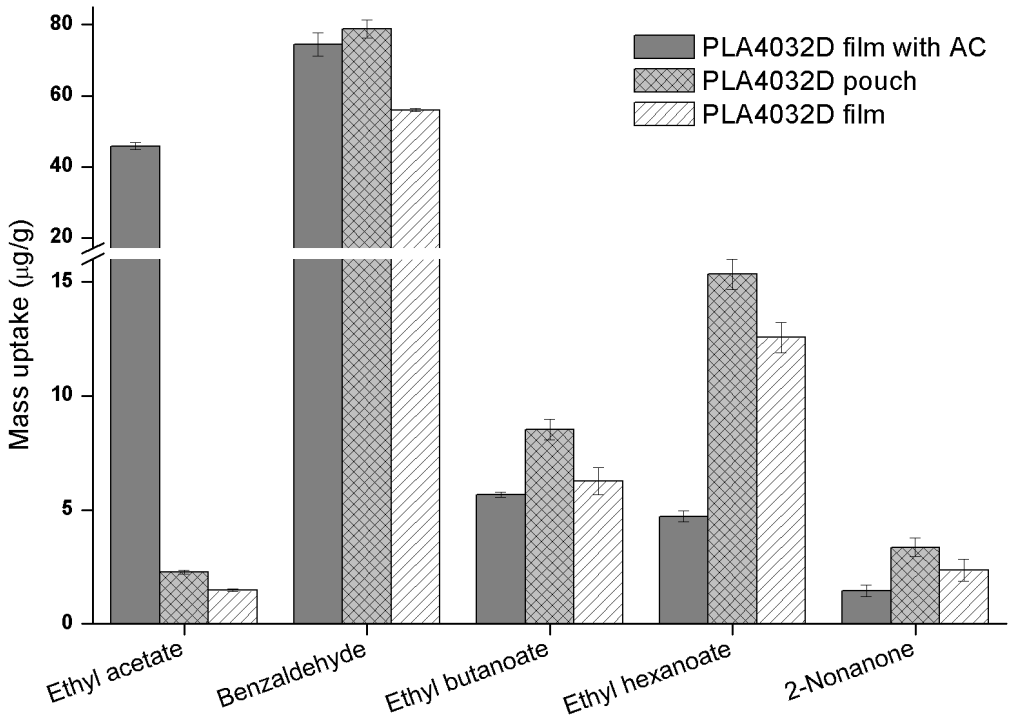


Figure 4.0. Mass uptake of aroma compounds in PLA4032D samples (thickness = 55 μm) after 10 days of permeation at 25 ºC. The ordinate is discontinuous as indicated to present all small values, n=3.

In the case of pouches and films after contact with flavored sponge cakes, the sorption values were slightly higher in pouches than films, whatever the aroma compound. The picture becomes difficult to explain due that no data about the aroma concentration in sponge cakes after baking were obtained. However, according to literature, the percentages of retention of ethyl butanoate, ethyl hexanoate and benzaldehyde in a sponge cake after baking were 30%, 55.78% and 143.72%, respectively (Pozo-Bayon et al. 2006). So, in our case, the aroma compounds could have been retained in different percentages in the matrix and therefore the sorption could be related to quantity available in the same. For example, the most volatile compound, ethyl acetate, could have been the most lost during baking and for that reason presented the lower sorption, compared with the other ethyl esters. In contrast, benzaldehyde was retained or formed during baking and although its low partition coefficient, Khs/sponge cake matrix, of 0.28 at 38ºC (Dury-Brun et al. 2007), it presented the higher sorption in PLA samples, in the same order of magnitude of values determined in films with the aroma

solution, must likely due to its high affinity, as already described in the previous chapter. The interaction between the food matrix and the volatile compounds influenced therefore most likely strongly the sorption in PLA. The investigation the influence of the aroma retention inside the food matrix on the permeability through the packaging film is therefore of high interest. However, facing the high experimental difficulty to obtain reproducible values on permeation for the sponge cake system and the high complexity of this foodstuff, we decided to pursue the study with a liquid, emulsified system. The advantage of the emulsified system was that the lipid content could be easier controlled, no heat treatment was involved, during which aroma compounds could be evaporated and the packaging inside trays was easier from a practical point of view.

4.2 Sorption of oil and aroma compounds from model food emulsions in poly(lactide)

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4.2.1 Abstract

Eco-design is one of the drivers for innovation in packaging. Polylactide (PLA), being a biobased polymer, might contribute to decrease ecological footprint if it proves to maintain food quality. A food model emulsion was formulated with ethyl esters, 2-nonanone, benzaldehyde and lipids then stored in a PLA pack for 30 days at 40 °C. Aroma compound concentrations were close to concentrations in foodstuff (100 ppm). To test the composition of aroma mixture, a model study was carried out where sorption equilibrium was reached. A low oil sorption was determined. The sorption of aroma compounds was low and occurred preferentially into the lid through the headspace of the emulsion. The model approach proved that benzaldehyde and ethyl acetate are the most interacting compounds with PLA and a synergy effect was detected with benzaldehyde. Glass transition decreased slightly with sorption of aroma compounds. Cristallinity increased when benzaldehyde was present. Synergy effect of ethyl acetate and benzaldehyde was shown in PLA which must be anticipated during the formulation of food products intended to be packed in PLA.

Keywords: polylactide, PLA, emulsion, aroma compound, sorption, packaging.

4.2.2 Introduction

Food packaging is one of the main actors in preservation of food safety and quality during shelf life. This justifies the large research effort undertaken by industry and academia in proposing novel strategies, such as modified atmosphere packaging, active packaging, nanocomposites, etc. Eco-design of the system packaging/food is now also a driver for innovation and within this framework novel polymers based on renewable resources have been proposed. Poly(lactide) (PLA) forms part of this new generation of polymers, being one of the possible solutions for reducing ecological footprint (Hermann, Blok, & Patel, 2010).

However, all polymers interact with the packed foodstuff during storage time. During this possibly long period (up to several years in function of the considered food) a large number of molecules are exchanged between food and packaging. Additives, polymer stabilizer, volatile compounds from degradation products of additives or polymers may migrate into food and conversely constituents of food could be sorbed by the packaging (Gillet, Vitrac, & Desobry, 2010). Besides food safety issues due to contamination coming from the packaging, migration and sorption have also impact on the sensorial quality of the foodstuff. In particular, sorption of volatile compounds in packaging polymers is of interest as it might cause imbalance of flavor profiles (Dury-Brun, Chalier, Desobry, & Voilley, 2007; Sajilata, Savitha, Singhal, & Kanetkar, 2007). Most of the studies in this subject are concerned with polyolefins and were recently reviewed (Caner, 2011). Up to date, there is little literature on this topic for new biopolymers, and most of them concern polysaccharides and proteins (Kurek, Descours, Galic, Voilley, & Debeaufort, 2012; Pau Balaguer, Gavara, & Hernández-Muñoz, 2012).

Polyolefins are hydrophobic materials and rubbery at room temperature, both properties being *a priori* favourable for sorption of hydrophobic aroma compounds. The biopolymers films are on the contrary more hydrophilic. For example, films based on gliadins or chitosan presented low sorption capacities for aroma compounds (Pau Balaguer et al., 2012). PLA is a synthetic polymer but also more polar than polyolefins and glassy at room (service) temperature. Much

less knowledge exists on glassy polymers, though. Literature data on ethyl vinyl alcohol (Gavara, Catalá, Aucejo, Cabedo, & Hernandez, 1996), poly(ethylene terephthalate) (PET) and poly vinyl chloride (PVC)(Ducruet, Vitrac, Saillard, Guichard, Feigenbaum, & Fournier, 2007) showed indeed that interactions of foodstuff and glassy polymers are reduced. Main explanations are the higher polarity of the polymers reducing partitioning of hydrophobic compounds and lower diffusion coefficients in glassy matrices. As an example, the comparatively low sorption of hydrophobic compounds such as limonene in PLA has already been shown (Auras, Harte, & Selke, 2006), but came with the downside of high sorption of more hydrophilic molecules sharing structure similarities with PLA, such as ethyl acetate (Auras et al., 2006; Colomines, Ducruet, Courgneau, Guinault, & Domenek, 2010; Courgneau, Domenek, Lebosse, Guinault, Averous, & Ducruet, 2012).

Due to methodological constraints, single aroma compound was tested at high concentration or high partial pressure, which is far from service conditions of the considered polymers. Moreover, aroma compounds are usually present in a mixture and at low concentration, being typically in the range of 100 ppm in the foodstuff and of 1 to 10 ppm in the headspace. Foodstuff is however a complex system including interacting compounds with aroma compounds, the most important of which being lipids and proteins (Caner, 2011). Furthermore polysaccharides, such as pectin or carboxymethylcellulose change sorption behavior by increasing viscosity of foodstuff and slowing down diffusion of flavor compounds from the food matrix to packaging (van Willige, Linssen, & Voragen, 2000b).

The approach undertaken in the present work aims to address this issue in using both, model food systems and model aroma compound mixtures at low concentrations for the investigation of interactions with glassy PLA. For that a model food emulsion was formulated and flavored with a mixture of molecules being representative of different structure classes (esters with increasing aliphatic chain length, ketones and aromatic molecules). It was packed in a global PLA pack, tray and lid. In parallel, PLA samples were conditioned in the headspace of the

same aroma compounds mixture and sorption and mixture effects were studied. The polymer morphology was controlled for providing structural explanations of observed behavior.

4.2.3 Materials and methods

4.2.3.1 Materials

4.2.3.1.1 Chemicals

Sodium caseinate (Lactalis, France), Tween 80 (Merck, Germany), xanthan gum (Keltrol®, Monsanto, UK) were used in this study. Potassium sorbate, triheptadecanoin standard, sodium sulphate anhydrous, potassium hydroxide, β-carotene (>95%), boron trifluoride methanol complex, pentane (98 %), hexadecane (99%) were purchased from Sigma-Aldrich. Methanol and n-heptane (99%) were purchased from Carlo Erba Reagents. Rapeseed oil of food grade (Lesieur, France) was used throughout this work.

Ethyl acetate (>99.5%, EA), ethyl butanoate (99%, EB), ethyl 2-methylbutanoate (99%, E2MB), ethyl hexanoate (>99%, EH) and 2-nonanone (>99%, 2NNE) were purchased from Sigma-Aldrich and benzaldehyde (>99%, BZA) was purchased from Fluka. The physicochemical properties are given in Table 4.1.

Table 4.1. Physicochemical properties of the aroma compounds.

Aroma compounds	MW (g/mol)	BP (°C)	P_{vs} at 25 °C (kPa)	log P
Ethyl acetate (EA)	88.11	77.91	12.43	0.86
Benzaldehyde (BZA)	106.12	16.90	0.12	1.71
Ethyl butanoate (EB)	116.16	121.79	2.07	1.85
Ethyl 2-methylbutanoate (E2MB)	130.18	133.00	1.10	2.12
Ethyl hexanoate (EH)	144.21	170.05	1.71	2.83
2-Nonanone (2NNE)	142.24	192.00	0.86	3.03

MW: molecular weight; BP: boiling point; P_{vs}: saturated vapour pressure at 25 °C; log P: octanol-water partition coefficient

4.2.3.1.2 Preparation of food emulsions

Five types of emulsions were prepared: two emulsions based on sodium caseinate as emulsifier, one without β-carotene (ECas) and another with β-carotene (ECas-β); three emulsions based on Tween 80 as emulsifier, one of them without β-carotene (ETW), another

with β-carotene (ETW-β) and the last without β-carotene but flavored with model aroma compounds (ETW-F). The emulsions consisted (in weight proportions) in 40% rapeseed oil containing or not 0.01 % β-carotene, 2% emulsifier (sodium caseinate or Tween 80), 0.1% potassium sorbate, 0.3% of xanthan gum (being 0.5 % in the aqueous phase), and 57.6% distilled water. To prepare the emulsion, xanthan gum was dissolved in warm water (60 °C) inside a hermetic flask by stirring with a magnetic mixer during 15 min. The emulsifiers (Tween 80 of sodium caseinate) were added subsequently to the cooled solution by stirring for 15 min. Then, potassium sorbate was added. Rapeseed oil and β-carotene were added to the water solution under vigorous stirring using a Polytron® PT3000 homogenizer (Kineatica AG) at 10000 rpm during 10 min. The flasks were place in an ice bath to prevent heating during the homogenizing phase. Finally, the emulsion was treated by ultrasound (Ultrasonic Processor, Bioblock Scientific, France) during 20 min in pulses of 10 s also placed in an ice bath to prevent temperature rise.

The emulsion ETW-F was flavored with the six aroma compounds mentioned above. For that, a solution of six aroma compounds in ethanol (5000 ppm of each aroma compound) was prepared. Then, a volume of 2 mL was added to 100 mL of emulsion to have a final concentration of 100 ppm of each aroma compound in the emulsion and stirred by a magnetic mixer during 15 min.

4.2.3.1.3 Polylactide films and trays

Two extruded PLA4032D (NatureWorks LLC) and one PLA2002D (NatureWorks LLC) with thickness of 235 µm, 55 µm and 65 µm, respectively, were studied. The extrusion conditions of PLAs such as the content of D-lactic acid present in materials were described in our previous work (2% for PLA4032D and between 4% and 4.5% for PLA2002D) (Salazar, Domenek, Courgneau, & Ducruet, 2012). PLA4032D trays for conditioning of emulsions were produced by thermoforming at 180 °C for 24 seconds (ILLIG Maschinenbau GmbH, Germany) and had a thickness of 150 µm. Trays were heat sealed with a NATIVIA^{TM} NZSS

(Aluminium/PLA) lid of 35µm of thickness, where the PLA face was in contact with the headspace inside the tray.

In order to obtain very thin films, three samples (30 ± 1 mg each one) of PLA4032D and PLA2002D with a thickness of 10 µm were prepared using a microtome (LKB Bromma Historange microtome 2218).

4.2.3.2 Methods

4.2.3.2.1 Characterization of emulsions

Emulsion flow curves were measured at 20 ± 1 °C over a shear rate range of 0.001 –10 s^{-1} using a dynamic rheometer (MCR301, Anton Paar) equipped with cone-plate geometry (CP 50/4°). All measurements were performed within 24 h after emulsion preparation. The flow curves were modeled with the law of Herschel-Bulkley for liquids showing yield stress:

$$\sigma = \sigma_0 + K \cdot \dot{\gamma}^n \qquad \text{(Equation 4.1)}$$

where σ is shear stress, $\dot{\gamma}$ shear rate, *n* flow behavior index, σ_0 yield stress and *K* consistency index.

The oil droplet size was characterized with the help of a Mastersizer 2000 (Malvern, France) equipment. The stability of the different emulsions was studied by multi-angle light scattering measurement using Turbiscan MA 2000 (Formulaction, France) as previously described by Relkin and Shukat (2012). The scan of a same sample at different time intervals was carried out and light transmission and back scattering profiles were used to evaluate the physical stability of emulsions.

4.2.3.2.2 Conditioning of PLA samples in oil and emulsions

For immersion tests, PLA4032D film (thickness 55 µm) was cut into rectangular pieces measuring 50 by 20 mm using a paper-cutter. Ten strips (1 dm^2) were immersed in 100 mL of rapeseed oil with or without β-carotene in a 250 mL flask with stopper for 28 days at 40 °C.

The conditions were adapted according to the norm NF-EN-1186-2 of overall migration into olive oil by total immersion. Five flasks were prepared in this way.

For storage tests of emulsions in PLA 100 mL of each type of emulsion were filled in three PLA trays and heat sealed with a NATIVIA^{TM} NZSS (Aluminium/PLA) lid. Trays were stored in an oven at 40 °C for 30 days.

4.2.3.2.3 Quantification of oil sorbed in PLA samples

The rapeseed oil sorbed in PLA samples was extracted and determined by gas chromatography after conversion to methyl esters according to the method described in the norm NF EN 1186-2 (January 2003). After conditioning time, PLA strips were removed from the rapeseed oil and rinsed with ethanol in order to remove the rest of oil. The same procedure was carried out for PLA trays after removal of emulsion. Then, strips and trays (1 dm^2 each) were wiped using KimTech precision wipes, cut in small squares and placed in a 25 mL vial. The extraction of oil was carried out by adding 10 mL of n-pentane into vials under magnetic stirring at 50 °C for 30 min. The operation was renewed two times with fresh solvent. 5 mL of a solution of 2000 ppm of triheptadecanoin in heptane were added into each vial as internal standard prior to the first extraction.

The resulting extracts were collected and evaporated to dryness using a rotary evaporator (Büchi R-114, Suisse) and then dissolved in 10 mL n-pentane. Fatty acids were formed by hydrolysing the extracts with 10 mL of a solution of potassium hydroxide (11 g/L) in methanol under reflux. Then, methylation was carried out by reacting the extracts with 5 mL of boron trifluoride/methanol. Finally, the upper phase of extracts was injected into a gas chromatograph (CE Instrument, Model GC8000Top, GC/FID) equipped with a DB-FFAP capillary column (30 m length x 0.32 mm, 1 μm film thickness, JW Scientific). The temperature of the split/splitless injector was 250 ºC with a purge time of 0.5 min. The flame ionisation detector was set at 250 ºC with hydrogen as carrier gas (50 kPa). The oven temperature was programmed to increase from 60 to 250°C at 7 ºC/min. Calibration was

carried out using different quantities of rapeseed oil (5, 10, 20, 50 and 150 mg) which were subjected to same procedure of samples. For each quantity of oil, the ratio of the combined area of C16 and C18 peaks to area of internal standard was calculated and then ratios were plotted against the oil quantities.

4.2.3.2.4 Moisture loss and water vapour transfer rate (WVTR)

The moisture loss of the emulsions packaged in PLA trays was measured in following the weight loss during storage at 40 °C for 20 days, which implied the hypothesis that water loss was the principal cause of the weight loss of the emulsion. Weight loss was measured using an analytical balance (Santorius E5500S,10^{-2} g). The water vapour transfer rate (WVTR) of PLA trays was calculated from the slope of the plot of weight loss over time. Given values are averages of three experiments.

4.2.3.2.5 Conditioning of PLA films in aroma atmosphere

The experimental system used to condition microtomed PLA samples has already been described in our previous work (Salazar et al., 2012). It consists in a hermetic flask equipped with a Mininert® valve which is placed in a climatic chamber for temperature control. The conditioning was carried out for 20 days at 25 ± 0.3°C. Three microtomed samples (30 mg each one) of PLA4032D and PLA2002D were conditioned separately. Specific partial pressure of the aroma compounds in the atmosphere was generated by a solution of aroma compounds in hexadecane. Four aroma compound solutions were tested individually and in mixture. The compositions are presented in Table 4.2.

4.2.3.2.6 Quantification of aroma compounds in PLA samples by headspace methods

After contact time, PLA samples from trays, lids and microtomed PLA sample were analysed using Multiple headspace extraction (MHE) or Multiple headspace solid-phase micro extraction (MHS-SPME). The two applied procedures were described in Salazar et al. (2012). The apparent solubility coefficient (S) of each aroma compound was calculated according to:

$$S = \frac{M_\infty}{M_0} \times \frac{d}{P_p}, \quad \text{(Equation 4.2)}$$

where M_∞ is the mass sorption after 20 days of conditioning (Kg), M_0 is the initial mass of PLA sample (Kg), d is the film density (Kg/m^3) and P_p is the partial pressure of each aroma compound. Pp was determined by gas chromatography following the procedure previously described in Salazar et al. (2012) and given in Table 4.2.

The partition coefficient is defined as the mass concentration relation of a molecule between two phases. The partition coefficients calculated in this study were:

$$K_{polym/hs} = \frac{C_{polymer}}{C_{headspace}}, \quad \text{(Equation 4.3)}$$

$$K_{polym/liq} = \frac{C_{polymer}}{C_{liquid}}, \quad \text{(Equation 4.4)}$$

$$K_{hs/liq} = \frac{C_{headspace}}{C_{liquid}}, \quad \text{(Equation 4.5)}$$

where *Cpolymer* is the concentration of the compound in the polymer (µg/g), *Cheadspace* is the concentration of the compound in the headspace (µg/g) and *Cliquid* is the concentration of the compound in the liquid or emulsioned matrix (µg/g).

Table 4.2. Conditioning of the PLA samples: concentrations of aroma compound in solution and in headspace, partition coefficient headspace/aroma solution ($k_{hs/liq}$), partial pressure (P_p) and activity.

Aroma compound		Aroma solution in hexadecane (mol/L) $\times 10^{-2}$	Concentration of aroma in headspace (mol/L)	$K_{hs/liq}$	P_p (Pa)	Activity
Ethyl acetate		2.0	1.7×10^{-4}	8.50×10^{-3}	455.36	0.037
Benzaldehyde		3.0	4.5×10^{-6}	1.50×10^{-4}	12.45	0.104
Aroma compound mixture 1 (M1)	EB	1.5	1.9×10^{-5}	1.27×10^{-3}	51.08	0.025
	E2MB	2.0	1.3×10^{-5}	6.50×10^{-4}	33.61	0.031
	BZA	3.0	4.5×10^{-6}	1.50×10^{-4}	12.45	0.104
	EH	1.8	2.0×10^{-6}	1.11×10^{-4}	6.13	0.004
	2NNE	1.7	1.4×10^{-6}	8.24×10^{-5}	4.74	0.006
Aroma compound mixture 2 (M2)	EB	1.5	1.9×10^{-5}	1.27×10^{-3}	51.08	0.025
	E2MB	2.0	1.3×10^{-5}	6.50×10^{-4}	33.61	0.031
	EH	1.8	2.0×10^{-6}	1.11×10^{-4}	6.13	0.004
	2NNE	1.7	1.4×10^{-6}	8.24×10^{-5}	4.74	0.006

K = concentration in headspace (mol/L)/ concentration in solution (mol/L) at 25 °C after 20 days of conditioning

4.2.3.2.7 Quantification of sorbed aroma compounds in trays and lids by MHS-SPME

To quantify the aroma compounds sorption in PLA trays after conditioning with a flavored emulsion, we used the parameters of MHS-SPME optimized by Salazar et al. (2012). So, after conditioning time, the flavored emulsion was removed from PLA trays. Then, trays and lids were rinsed with ethanol and wiped. Approximately 80 mg of PLA tray and 40 mg of lid were sealed separately in 20 ml glass vials with silicon/PTFE septa. For MHS-SPME injections, three vials were prepared and each vial was sampled four times using an Agilent Technologies 6890 GC/FID coupled with a mass spectrometer 5975 INSERT and an Autosampler MPS2 GERSTEL. The oven temperature programme began with an initial temperature of 30 °C for 5 min, and then temperature increased at a rate of 5 °C/min up to 230 °C, temperature which was maintained for 5 min. Split/splitless injector and detector temperatures were 250 °C. The column was a J&W Scientific DB5-MS capillary column (30 m length x 0.32 mm inner diameter x 0.5 μm film thickness). The carrier gas was Helium at 1.4 mL/min. The sample vials were incubated at 60 °C for 30 min and agitated at 250 rpm. MHS-SPME was performed for 15 min at 60 °C using a 75 μm carboxen–polydimethylsiloxane (CAR-PDMS) fibre. Desorption time was 5 min into the injector port. Total peak area of each aroma compound sorbed was calculated according Salazar et al. (2012). The calibration was carried out by external standard using 20 μL of aroma compound solutions in hexadecane with different concentrations sampled in the same way.

4.2.3.2.8 Quantification of aroma compounds in microtomed PLA samples by MHE

To quantify aroma sorption in PLA samples after conditioning in an aroma atmosphere, MHE method was used following the methodology and optimized parameters described in our previous work (Salazar et al., 2012). In brief, after conditioning time, microtomed PLA samples (30 mg) were removed from airtight flasks and sealed in 20 ml headspace glass vials with silicon/PTFE septa and an aluminium cap. Each vial was sampled five times at equal

time intervals (55 min) at 60°C using an automatic headspace sampler (Perkin Elmer HS40 XL) coupled with GC/FID. The oven temperature was programmed at 35°C for 2 min, 6 °C/min up to 230 °C. The column was a SupelcoWax-10 capillary column (30 m length x 0.32 mm, 0.5 µm film thickness). Injector temperature was 250 °C and its split was 15.6 mL/min. Detector temperature was 250 °C and it was set at range 1. The carrier gas was Helium at 1.8 mL/min. Total peak area of each aroma compound sorbed was calculated according Salazar et al (2012). Calibration was performed by external standard using 10 µL of aroma compound standard solutions in hexadecane with different concentrations.

The limit of detection (LOD) was calculated as the quantity of compound producing a signal exceeding the average background signal by three standard deviations of the signal-to-noise ratio in the lowest concentration standard, divided by the slope of the corresponding analytical curve (Miller & Miller, 1993). The limit of quantification (LOQ) was obtained as the quantity of compound that is 10 standard deviation above the average background signal, divided by the slope of the corresponding calibration curve (Gibbons & Coleman, 2001).

4.2.3.2.9 Size Exclusion Chromatography (SEC)

The average molecular weight and the dispersity of PLA samples were measured by SEC using a Gilson 305 apparatus equipped with a RID-101 (Shodex) refractive index detector. PLA samples were dissolved in chloroform (5 mg/mL) and analyzed at 25 °C and 1.0 mL/min in THF stabilized with BHT on a PL Gel Mixed-C column (5µm, 100 Å, 600 x 7.5 mm). The calibration was performed with PS standards (EasiCal) from 580 to 377400 g/mol.

4.2.3.2.10 Thermal properties: Differential scanning calorimetry (DSC)

Before and after conditioning, the thermal properties of PLA samples were determined in triplicate using a differential scanning calorimeter QSC 100 instrument (TA Instruments, France). PLA samples were analyzed following the modulated temperature programme used by Delpouve, Saiter and Dargent (2011). Samples were heated from 10 °C to 190 °C, with

oscillation amplitude of 0.318 °C, an oscillation period of 60 s and a heating rate of 2 °C/min. The degree of crystallinity (X_c) of materials was calculated according to Equation 4.6:

$$X_C = \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0} \quad \text{(Equation 4.6)}$$

where ΔH_m is the enthalpy of fusion, ΔH_{cc} is the enthalpy of cold crystallization and ΔH^0_m is the heat of melting crystalline PLA (93 J/g) (Courgeau et al., 2012).

4.2.3.2.11 Statistical analysis

The statistical analysis of data was performed through one-way analysis of variance (ANOVA) using XLSTAT-Pro 7.0 software (Addinsoft, Paris, France). When differences were significant ($p < 0.05$), Duncan's test was used to evaluate the differences between pairs of groups.

4.2.4 Results

4.2.4.1 Characterization of emulsions

In the aim of investigating the interaction of a food emulsion and the packaging polymer PLA, the stability of the emulsion needed to be guaranteed during the storage. Storage conditions were adapted from the protocol proposed for migration studies of food contact materials by the norm NF-EN-1186-2 and represented conservation for 28 days at 40 °C. Emulsions are, however, instable systems where coalescence and phase separation due to creaming (or sedimentation in other cases than ours) are the main factors of destabilisation. Creaming is induced by gravity due to the density difference between the aqueous and the oil phase. Eventually, in emulsions that cream, oil droplets move to the top, a movement which is governed by viscous flow. Coalescence comes from the rupture of the interfacial film surrounding two different droplets. Due to the high importance of emulsified systems in many applications, strategies to overcome creaming and coalescence have been extensively investigated. These strategies were applied in the aim of formulating a stable food emulsion

coloured or not with β-carotene for contact testing at 40 °C for 30 days. Tween 80 is a largely used emulsifier in food systems for providing stability against coalescence by decrease the interfacial tension between the aqueous and the oil phase, an example being given in Krstonosic, Dokic, Dokic and Dapcevic (2009). Similarly, sodium caseinate as polymeric emulsifier has received large interest (Bos & van Vliet, 2001; Sun, Gunasekaran, & Richards, 2007). For stabilization against creaming, xanthan gum was used, as the rate of creaming is linked to viscous flow. The use of hydrocolloids increases stability due to higher viscosity of the continuous phase (Dickinson, 2003), and xanthan gum features excellent thickening properties (Becker, Katzen, Puhler, & Ielpi, 1998). The concentrations chosen of emulsifier and hydrocolloid were in the upper level of concentrations usually used in literature (Krstonosic et al., 2009; Sun et al., 2007). Samples of different solutions are photographed in Fig. 4.1, where 4.1a is the emulsion stabilized by sodium caseinate without β-carotene and emulsion 4.1b is stabilized with Tween 80 and contains β-carotene.

The droplet size of the emulsions containing sodium caseinate ECas (1.5 ± 0.1 μm) was slightly higher than the size of the ones containing Tween 80 ETW (1.2 ± 0.2 μm). The addition of β-carotene to the emulsions did not change droplet size, being for ECas 1.8 ± 0.5 μm and for ETW 1.3 ± 0.3 μm.

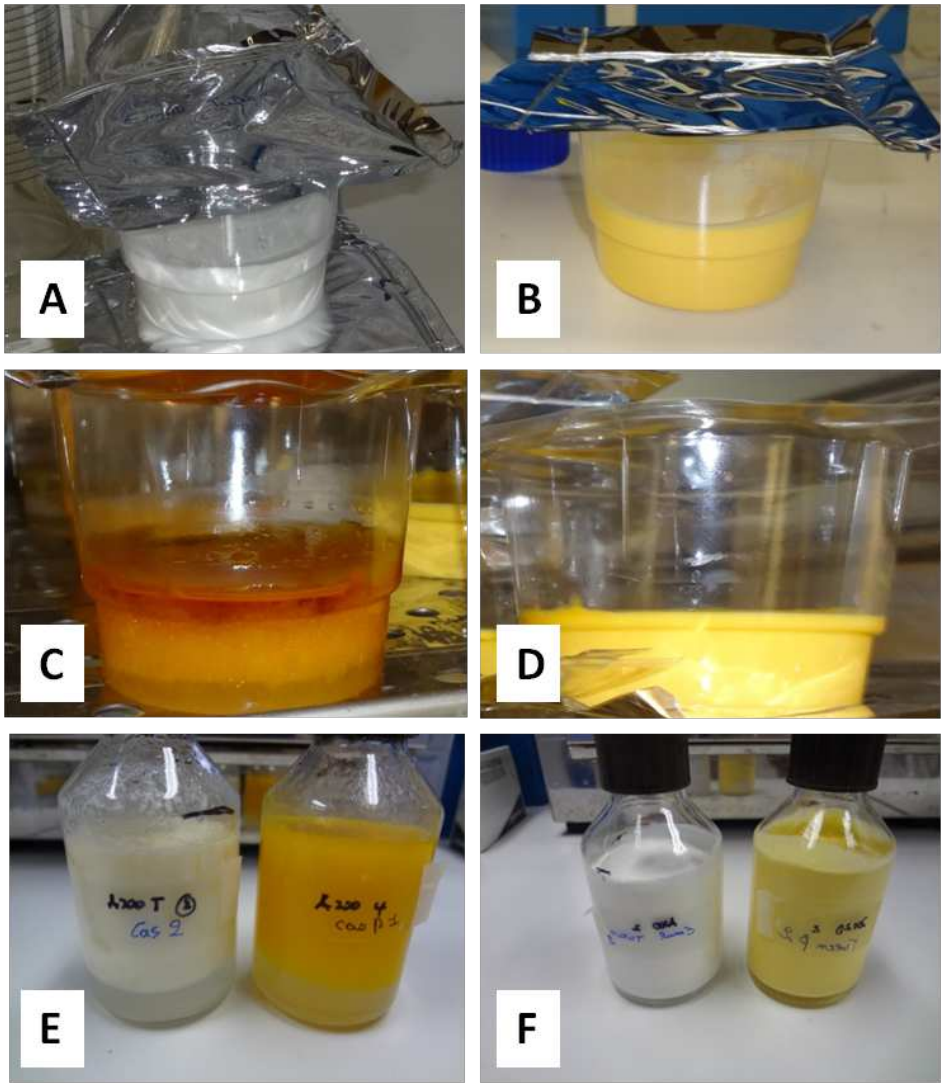


Figure 4.1. Stability of emulsions containing 0.3% xanthan gum during storage at 40 °C. Emulsion stabilized with sodium caseinate before storage in trays (A), after storage in trays (C) and after storage in flask (E); Emulsion stabilized with Tween 80 + β-carotene before storage in trays (B), after storage in trays (D) and after storage in flask (F).

The flow curves of ETW and ECas were recorded (Fig. 4.2) and modeled with the help of the Herschel-Bulkley model following the approach of others studies (Sun et al., 2007; Krstonosic et al., 2009). It can be observed in Fig. 4.2 that emulsions showed shear-thinning, which is the expected behavior for a system thickened with xanthan gum. The Herschel-Bulkley model predicted a small yield stress, which is consistent with others (Aben, Holtze, Tadros, & Schurtenberger, 2012; Krstonosic et al., 2009; Sun et al., 2007). The occurrence of a yield stress can be explained by depletion flocculation in the solution induced by non adsorbing polymers, such as xanthan gum. In fact, when the adsorption of the polymer to the droplets is unfavorable, the center of mass of the polymer becomes displaced from the droplet

surface, leading to a locally polymer depleted zone. This can induce reversible droplet aggregation. Very recently, the creation of a particle network due to depletion flocculation has been evidenced (Aben et al., 2012), using an oil in water emulsion stabilized with a nonionic emulsifier and 0.5 wt% xanthan gum.

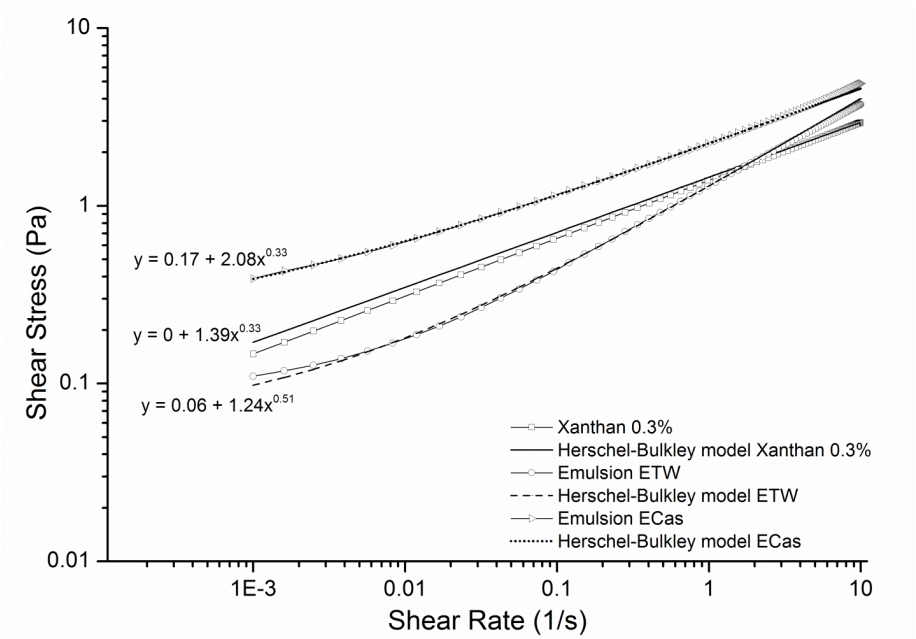


Figure 4.2. Flow upward curves of pure xanthan (0.3%), emulsion ETW (stabilized with Tween 80 and containing 0.3% xanthan) and emulsion ECas (stabilized with sodium caseinate containing 0.3% xanthan).

Emulsion stability with storage time was analyzed by changes in light backscattering and transmission profiles of tubes containing the emulsions after their preparation (time zero) and after 6 days or 17 days of storage at 40 °C. Unfortunately, the ECas emulsion showed microbial growth after 6 days of storage although an antimicrobial agent was added; therefore, the experiment could was not prolonged further. Transmission profiles (Fig. 4.3a and 4.3b) showed no clearing zone inside the sample tube. Backscattering profiles (Fig. 4.3c and 4.3d) showed that ECas seemed to have smaller droplets after 6 days, compared to the initial emulsion. We attribute this effect to the microbial growth, which in fact increased the number of particles inside the emulsion. The ETW was, however, stable for 17 days of storage.

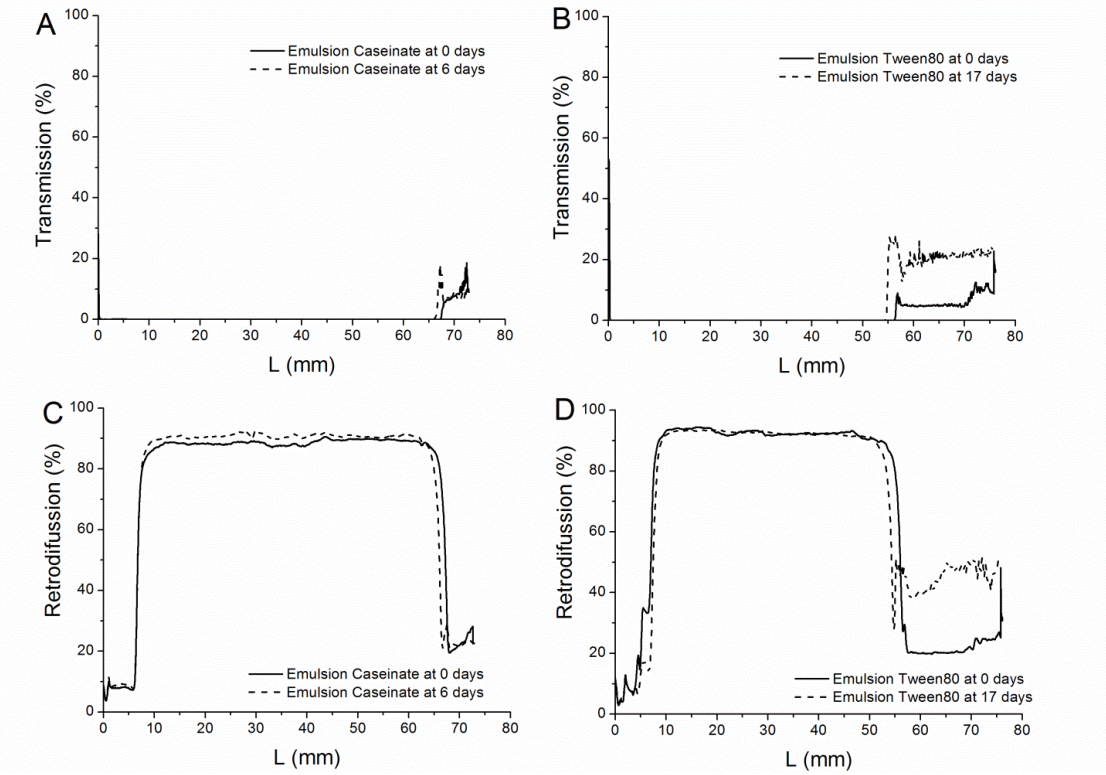


Figure 4.3. Stability of emulsions by turbidimetry at time zero, and after 6 or 17 days of storage at 40 ºC. Transmission profiles of emulsion stabilized with sodium caseinate (A), Transmission profiles of emulsion stabilized with Tween 80 (B), Backscattering profiles of emulsion stabilized with sodium caseinate (C), Backscattering profiles of emulsion stabilized with Tween 80 (D).

The emulsions packed in PLA were observed visually. Changes in emulsion during the 30 days of storage at 40 ºC in PLA4032D trays and in glass bottles are shown in Fig. 4.1. The emulsion containing sodium caseinate, ECas, was not stable due to microbial growth in whatever the package material (Fig. 4.1c and 4.1e). ETW showed no destabilization in PLA (Fig. 4.1d) and in the glass bottles (Fig. 4.1e) during the storage time. It could be concluded that PLA had no destabilizing effect on the model food emulsion.

However, emulsion volume decreased. In order to assess moisture loss of emulsions packaged in PLA trays and stored at 40 °C, the trays were weighted continuously during 30 days and the WVTR was calculated. We found in PLA trays (thickness 150 µm) a WVTR of 73.73 ± 0.92 g/m^2.day which is two times higher than the WVTR value (30.6 g/m^2.day) reported by Holm, Mortensen and Risbo (2006), who studied the WVTR in PLA trays (thickness of 500

μm) at 38 °C and 90% RH. However, our result is in accordance with Courgneau, Domenek, Guinault, Avérous and Ducruet (2011) who reported a WVTR of 61.9 ± 6.7 g/m^2.day at 38°C and 90% RH in PLA films. Differences in the values of WVTR were attributed to the different film thickness. Domenek, Courgneau and Ducruet (2011) recently reviewed data on water vapor permeability (WVP) of PLA between 1.8 and 2.3 × 10^{-14} Kg.m/m^2.s.Pa at 25°C, which is one order of magnitude higher than PS and PET (Auras, Harte, & Selke, 2004) and two orders than HDPE (0.024 × 10^{-14}Kg.m/m^2.s.Pa, at 38°C) (Morillon, Debeaufort, Blond, Capelle, & Voilley, 2002). The high WVTR of PLA is one of its drawbacks in packaging of aqueous goods. Furthermore, PLA is a semicrystalline polymer and crystallinity of the studied PLA might have an impact on WVTR. For example Tsuji, Okino, Daimon, and Fujie (2006) reported that WVTR of PLLA films decreased rapidly with increasing of Xc. The WVP of PLA is however not only dependent on the degree of crystallinity but also on the crystalline structure of PLA (Cocca, Di Lorenzo, Malinconico, & Frezza, 2011), wherefore it needs to be finely controlled.

4.2.4.2 Oil sorbed in PLA

In order to get information on the oil stability of PLA, the quantity of rapeseed oil sorbed in PLA4032D tray samples after immersion in rapeseed oil or storage of the model emulsions was measured. A first observation was that the β-carotene used as color tracer in the oil and in the solution did not penetrate the polymer and no coloration of PLA strips and trays was observed. This is an advantage over polyolefins, where this coloration occurs (Nielsen & Olafsson, 1995). The oil uptake of PLA is given in Table 4.3. To the best of our knowledge, there are no studies dealing with oil sorption in PLA and only few results exist on interactions between oil and conventional polymer packaging materials. Literature reported values of rapeseed oil sorption in films of HDPE, APET and PP after 40 days of contact at room temperature. The amount of oil sorbed by the polymer films was about 27.5 mg/dm^2 for HDPE, 14.5 mg/dm^2 for APET and 5 mg/dm^2 for PP (Johansson & Leufvén, 1994). Arora and

Halek (1994) studied the fat sorption in PP and PET after 10 days at 23 ºC. The authors reported that sorption of triglycerides was higher in PP than in PET. In the PP case, sorption was 4.4 mg/dm^2 for tripalmitin, 2.93 mg/dm^2 for triolein and 1.3 mg/dm^2 for trilinolein, while in PET it was 1.2 mg/dm^2, 0.6 mg/dm^{2}and 0.3 mg/dm^2, respectively. Kassouf (personal communication, 2012) mesured values of olive oil sorption in PET samples after 10 days of contact by total immersion at 40 ºC with a equivalent protocol than the ours. He found 33 ± 2 mg/dm^2 of olive oil sorbed by PET. In conclusion, a large discrepancy exists among the quantitative results depending on the experimental procedure but the order of the oil sorption propensity of polymers seems to be explainable by simple physicochemical considerations. Glassy and more polar polymers sorb generally less oil than hydrophobic and rubbery polyolefins. When we compared our result with the results of Kassouf who applied the same experimental approach, PLA showed an oil sorption lower than PET.

It is interesting to look also on the oil sorption of PLA from the model emulsions. From the results given in Table 4.3, it appeared that the amount of oil sorbed in PLA4032D was correlated to the oil content of the contact medium. It was approximately two to three times higher in plain oil compared to the emulsions with 40 % oil content. Sorption of lipids contained in different types of foodstuff in polyolefins has been measured in literature (Arora & Halek, 1994; Nielsen & Olafsson, 1995). Correlations between fat content and sorption where rather poor, though. One of the reasons might lie in the very different food products used, being often not in the same physical state (solid or liquid) and the presence of other food components (proteins, hydrocolloids…). Vom Bruck, Figge and Rudolph (1981) did a systematic study of interactions of fat-containing food with plastics packaging. After 5 days of contact, fat sorptions were 1760 µg/g, 960 µg/g, 255 µg/g and 20 µg/g, respectively, when LDPE samples were put in contact with food such as olive oil, mayonnaise, coffee cream and yogurt, having a fat content of 100%, 80%, 10% and 3.7%. Thus they concluded that fat sorption in LDPE is related to the global fat content of foodstuffs whatever the properties of

the oil/water emulsion in the foodstuff.

Table 4.3. Oil sorbed, glass transition temperature (Tg), degree of crystallinity (X_c), number and weight average molecular mass (Mn and Mw) and polydispersity index (I) of PLA samples before and after oil sorption.

Packaging		medium in contact	Oil sorbed (mg of oil / dm^2 of material)	Tg (°C)	X_c	Mn (g/mol)	Mw (g/mol)	I
PLA4032D film	before contact			56.8 ± 0.9^a	12 ± 1^a	75165	160928	2.1
	after contact	rapeseed oil	$10 \pm 1_a$	56.0 ± 0.5^a	11 ± 1^a	nd	nd	
		rapeseed oil + ß carotene	$15 \pm 1_b$	55.2 ± 0.1^b	10 ± 1^a	nd	nd	
PLA4032D tray	before contact			55.8 ± 0.9^a	10 ± 2^a	91355	173161	1.9
	after contact	Emulsion caseinate (ECas)	<5	55.5 ± 0.3^a	14 ± 1^a	86230	143239	1.7
		Emul caseinate + β-carotene (ECas-β)	<5	59.1 ± 0.7^b	36 ± 1^c	81719	137898	1.7
		Emulsion Tween80 (ETW)	<5	61.3 ± 0.4^c	36 ± 1^c	83813	149197	1.8
		Emul Tween80+β-carotene (ETW-β)	<5	59.6 ± 1.1^b	33 ± 4^b	74226	140761	1.9
		Flavored emulsion tween 80 (ETW-F)	<5	55.8 ± 0.9^a	28 ± 2^b	72684	138156	1.9

[a, b, c] Different letters indicate significant differences at $p < 0.05$ (Duncan). Statistical analysis was carried out separately for each PLA type.

In order to study changes in the packaging polymers itself, PLA characteristics were determined before and after conditioning with oil and emulsions (Table 4.3). PLA undergoes hydrolytic degradation, wherefore the molar weight average was measured before and after contact with oil/water emulsions for 30 days. As expected, both characteristic quantities (Mn and Mw) decreased which was attributed to hydrolysis.

Glass transition temperature (Tg) and crystallinity degree (X_c) of PLA films before and after oil contact showed no significant differences, even though oil was sorbed in the structure. In the case of emulsions where water was present, X_c and Tg increased. Exceptions were the caseinate containing (Ecas) and the flavored emulsion (ETW-F), which also showed the smallest X_c. Tg and X_c are correlated in polymers, because the growing crystalline phase gradually restricts the mobility of the amorphous phase which yields an increase in Tg (Delpouve et al., 2011). The reason for the difference between emulsion types could not be determined; a hypothesis is linked to the methodology. The effects of time, temperature and

humidity on physical properties of PLA were studied by Auras, Harte and Selke (2004). They showed that humidity had a significant effect on Tg of PLA (98% L-lactide) after 7 days of contact at 40°C and 0.9 activity, but after 30 days of contact, no significant effect was found. Although PLA shows a poorer barrier properties to oxygen and to water than PET, some food products as salad dressings, oils and shortening, peanut butter are "oxygen tolerant" (Domenek, Courgneau, & Ducruet, 2011) or need moderate to high water vapor transmission rates like fruits, vegetables or cheeses (Pantaleao, Pintado, & Poças, 2007) and our results show that PLA could be a an alternative candidate to PET in the conditioning of these products.

4.2.4.3 Sorbed aroma compounds in PLA after conditioning with a flavored emulsion

PLA is generally claimed to provide good aroma barrier and some literature works exist on the purpose (Auras et al., 2006; Salazar et al., 2012). The change of flavor profiles of packed food is a recognized issue of plastic food packaging. Indeed, the specific sorption of given aroma compounds can modulate significantly the sensorial attributes and thereby decrease food quality. The sorption of aroma compounds in the packaging polymer depends on the chemical nature of the polymer and the considered molecule, but also on the composition of the foodstuff. The effects of these factors on the aroma sorption into packaging materials have already been reported in literature (Dury-Brun et al., 2007;Sajilata et al., 2007) and the relation can be expressed as an apparent partition coefficient (*Kapp*).

Table 4.4. Sorption of aroma compounds in trays and lids, apparent partition coefficients (*Kapp*), and partition coefficients from literature.

Aroma compound	Clid (µg/g)	$Kapp_{lid/hs}$	Ctray (µg/g)	$Kapp_{tray/liq}$	Literature data			
					K (polymer/hs)	*K (PLA/hs)*[g]	*K (polymer/liquid)*	*K (LDPE/liquid)*
EA	6.638 ± 1.056	3.46	1.682 ± 0.082	0.0142		197	1.9 PS[d]	
BZA	8.374 ± 0.610		0.142 ± 0.023	0.0012		18750		
EB	2.476 ± 0.224	1.11	0.252 ± 0.009	0.0021	562.8 PP[a]	157	0.9 PP[a]	3.0[b]; 10.5[c]
E2MB	3.33 ± 0.427		0.196 ± 0.023	0.0017		116	0.65×10^{-4} PET[h]	9.4[b]; 17.1[c]
EH	1.914 ± 0.203	0.54	0.073 ± 0.009	0.0006		414	0.8 m-PET[e]; 0.15 m-PET[f] 2.2×10^{-4} PET[h]	0.45[f] ; 0.55[f]
2NNE	0.265 ± 0.054		0.015 ± 0.001	0.0001		447		

[a] from a mixture of 6 aroma compounds (50 ug/cm3 each) in ethanol/water (40%) at 23°C for 14 days, in Johansson and Leufven (1997)
[b] from a mixture of 10 aroma compounds (1mg/Kg each) in water at 25°C for 7 days, in Nielsen, Jagerstad, Oste and Wesslen (1992)
[c] from a mixture of 10 aroma compounds (1mg/Kg each) in oil at 25°C for 7 days, in Nielsen et al. (1992)
[d] from an aroma compound (400 mg/L) in water at 25°C for 60 days, in Gavara, Hernandez and Giacin (1996)
[e] from an aroma compound (500 ppm) in ethanol at 25°C for 30 days for LLDPE or 90 days for m-PET, in Hernandez-Muñoz, Catalá and Gavara (2001)
[f] from an aroma compound (500 ppm) in oil at 25°C for 30 days for LLDPE or 90 days for m-PET, in Hernandez-Muñoz et al. (2001)
[g] from a mixture of 6 aroma compounds at low concentration in hexadecane at 25 ºC after 20 days for PLA2002D, calculated from Salazar et al. (2012)
[h] estimated from a pasteurized strawberry syrup (surcrose syrup + strawberry, elder and lemon juices) at 20 ºC after 90 days, in Ducruet et al. (2007

In the aim to investigate interactions between different chemical structures of aroma compounds and PLA, the emulsion has been flavored by a model aroma solution (ETW-F). The physicochemical data of the studied molecules are given in Table 4.1. After 30 days storage of the flavored emulsion, aroma sorption in PLA trays (thickness = 150 µm) and lids (thickness = 35 µm) was determined by MHS-SPME. PLA trays were in liquid contact with a flavored emulsion whereas PLA lids were in contact with the vapor atmosphere generated by the emulsions.

The results, presented in Table 4.4, show that the aroma compound uptake of PLA trays was in any case lower than in PLA lids. The difference between uptake of tray and lid was smallest in the case of ethyl acetate and highest in the case of 2-nonanone. Going more into detail, Table 4.4 revealed that in the tray, the aroma compound uptake decreased with the increase of its molecular weight with exception of benzaldehyde. Only few diffusion coefficients of aroma compounds in PLA are available today. Courgneau et al. (2012) gave a diffusion coefficient of ethyl acetate in the order of 10^{-17} m^2/s. The diffusion coefficient being negatively correlated to molecular mass (Berens, 1981; Fang, Domenek, Ducruet, Refregiers, & Vitrac, 2013) even lower values might be expected for the other aroma compounds. The sorption equilibrium between the PLA tray and the emulsion can therefore not be reached within the timeframe of the experiment especially as the food matrix contained xanthan gum which plays a double role. Xanthan as a thickener increases the viscosity of the food matrix and could slow down the diffusion of aroma compounds to PLA tray and as a weak interaction of xanthan with flavor compounds could exist retention into the matrix of the more hydrophobic compounds (Bylaite, Adler-Nissen, & Meyer, 2005; Jouquand, Aguni, Malhiac, & Grisel, 2008). For that aroma uptake seems to be correlated to molecular mass. An apparent partition coefficient was then calculated and values are given in Table 4.4. They are low due to the fact of being taken far from thermodynamic equilibrium. Literature values of aroma

compound sorption in PET and polyolefins from different solutions are printed for comparison reasons in Table 4.4. The composition of the emulsion has also influence on the partitioning. For example, literature shows the influence of proteins, carbohydrates, oil and real food products on the sorption of flavors compounds by linear low-density polyethylene (LLDPE) (van Willige, Linssen, & Voragen, 2000a, 2000b). These authors showed that the aroma sorption in LLDPE of limonene, decanal, linalool and 2EMB in water solutions decreased significantly with the increase of oil concentration in the emulsion after 14 days of contact at 4°C. Moreover, they reported that casein retains some aroma compounds in the food matrix and decreases slightly the partition coefficients in LLDPE.

The quantity of aroma compounds sorbed from the headspace in the lid showed a different picture (Table 4.4). The sorbed quantities were higher and no longer correlated to the molecular mass. This was coherent with values measured for vapour contact between PLA and different aroma compounds (Salazar et al., 2012), which brought up the conclusion that sorption might be closer to the equilibrium value. The apparent sorption coefficient can be calculated. For that, the partitioning between emulsion and headspace must be known. It was, however, not accessible in our experimental setup, therefore literature values were used. Table 4.5 gives a literature overview of partition coefficients between vapour and model liquid foods. It becomes clear that partitioning is very much dependent on the composition of the liquid and on the hydrophobic/hydrophilic character of the observed aroma compound. On the one hand xanthan could interact weakly with hydrophobic compounds and presence of oil into the emulsion emphasizes the retention of these compounds into the matrix and on the other hand, xanthan could lead to a release of the more polar compounds into the headspace here ethyl acetate and benzaldehyde as Bylaite et al. (2005) shown for alcohols or unsaturated aldehydes. Table 4.5 also shows the influence of carbohydrates and surfactants inducing

aroma compound retentions of the more hydrophobic molecules. This effect is major in the case of triglycerides (Philippe, Seuvre, Colas, Langendorff, Schippa, & Voilley, 2003).

Table 4.5. Headspace/matrix partition coefficients ($K_{hs/liq}$) from different liquid matrix.

Descriptor	Liquid matrix	T (°C)	EA	EB	EH	EHP[b]	References
Water	aroma solution in water + NaCl 0.01M	35		3.00×10^{-2}	6.90×10^{-2}		Jouquand et al (2008)
Water	aroma solution in water	37		2.6×10^{-2}		9.33×10^{-2}	Bylaite et al (2005)
Water	aroma solution (50 ppm) in water after 48 h	37	10	25	41		Philippe et al (2003)
Xanthan gum	aroma solution in water with xanthan gum (0.1% w/w) + NaCl 0.01M	35		2.82×10^{-2}	4.76×10^{-2}		Jouquand et al (2008)
Xanthan gum	aroma solution in water + 0.4% of xanthan gum	37		2.52×10^{-2}		6.42×10^{-2}	Bylaite et al (2005)
Carbohydrates[a] with triolein	aroma solution (50 ppm) in complex matrix of carbohydrates with 3.5% of triolein + 0.4% soybean lecithin after 48h	37	9.1	8.5	1.64		Philippe et al (2003)
Triolein	aroma solution (50 ppm) in water emulsion 3.5% triolein (w/w) + 0.4% soybean lecithin after 48h	37	9.1	9.25	1.64		Philippe et al (2003)
Triolein	aroma solution (50 ppm) in purified triolein after 48 h	37	3.2	0.5	0.41		Philippe et al (2003)

[a] 2.4% waxy corn starch, 1.3% pectin, 1.8% D-glucose + 0.15% citric acid + 0.05% trisodiumcitrate (w/w).[b] Ethyl heptanoate.

For the sake of comparison, we used for the calculation of the apparent partition coefficient between lid and headspace the value of the aqueous solution. This value is therefore overestimated compared to the model emulsion. In conclusion, the apparent partition coefficient given in Table 4.4 is underestimated. What was interesting in the comparison between K_{app}tray/emulsion and K_{app}lid/headspace, was the fact that the K_{app}(lid/headspace) was 10 to 100 times higher than the K_{app}(tray/emulsion). Scarce literature observations of partitioning between a liquid or a vapour phase and a polymer show a qualitatively similar result (Johansson & Leufven, 1997). These authors observed that the partition coefficient of ethyl butanoate and polypropylene in the headspace amounted to 562, while it was only 0.9 in the case of a solution of ethyl butanoate in ethanol/water.

The practical implication of those data was that only 0.072% of EA, 0.015% of EB, 0.017% of E2MB, 0.027 % of BZA, 0.008% of EH and 0.001% of 2NNE present in the emulsion were sorbed by the whole PLA packaging (tray and lid). This shows the good aroma barrier properties of PLA in the present case. On the other hand, the higher partition coefficient and faster sorption kinetics of the PLA lid showed the preponderant importance of transfers coming from the headspace into the polymer. The technique of lidding and the properties of the lid are therefore of primary importance in the design of a packaging container.

4.2.4.4 Aroma compounds sorption in PLA after aroma vapor conditioning

The drawback of our investigation of flavor sorption phenomena with the model emulsion was that the thermodynamic sorption equilibrium was probably not installed between the headspace and the lid and that the partitioning between emulsion and headspace was no known.

In order to install thermodynamic equilibrium between headspace and PLA samples, a model system of the flavored emulsion was used. The characteristic film thickness of PLA was reduced to 10 μm for being able to reach the sorption equilibrium in 20 days under the hypothesis of a diffusion coefficient in the order of 10^{-17} m^2/s (Auras et al., 2006; Courgneau et al., 2012). The microtomed samples were conditioned in a sorption cell optimized in previous work (Salazar et al., 2012). For having a low but precise concentration of aroma compounds in the headspace a calibrated hexadecane solution was used. The influence of the composition of the headspace on the sorption of compounds in PLA and the effect of sorption on thermal properties was investigated. This enabled to draw conclusions not only on single molecules, but also on the synergistic or antagonistic effects between molecules.

The measured data of sorption were normalized by the calculation of the solubility coefficient (S) after Equation 2 and the results are given in Fig. 4.4. The sorption profiles were

qualitatively similar between both commercial PLA grades tested, although PLA2002D showed a tendency to higher sorption. This difference was however small and not significant in all cases.

As expected according to literature (Auras et al., 2006; Colomines et al., 2010; Salazar et al., 2012), the sorption of ethyl acetate and benzaldehyde in PLA was high. In the case of ethyl acetate, this affinity can be sought in its quite similar molecular structure to PLA being the most hydrophilic of a homologous series of ethyl esters. The benzaldehyde case may be explained by the miscibility of PLA with aromatic solvents such as toluene and benzene (Jin, Tian, & Wang, 2010).

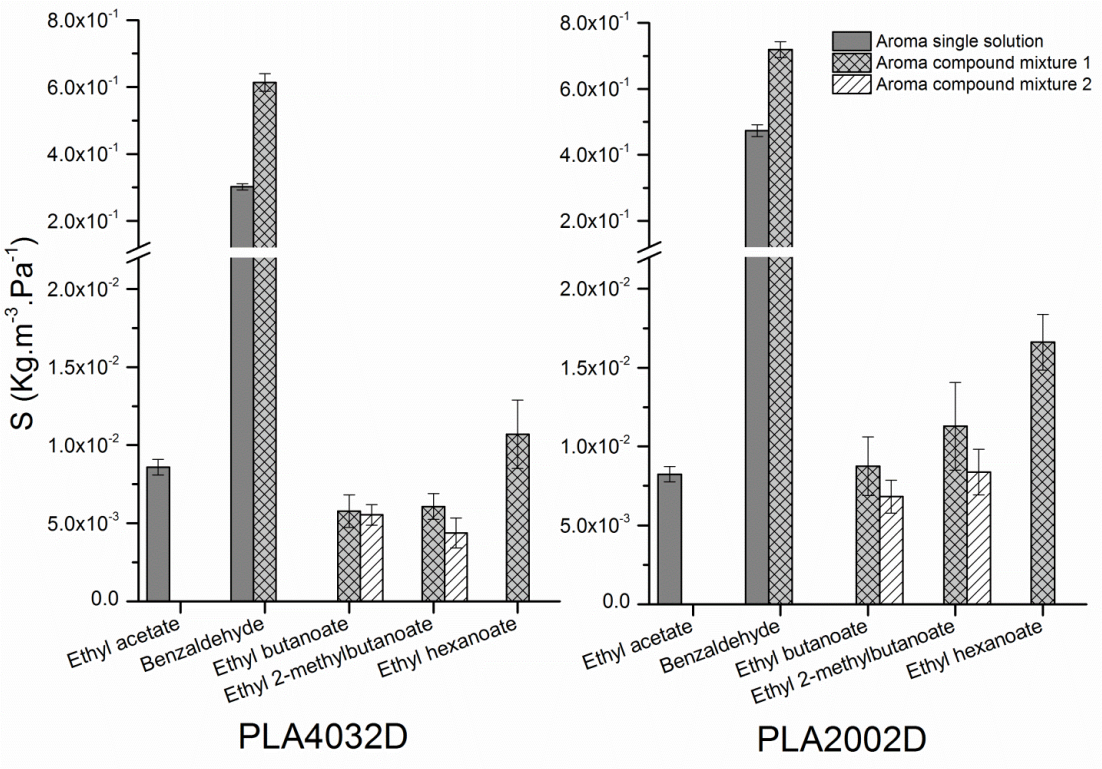


Figure 4.4. Solubility coefficients (S) of aroma compounds in PLA samples (thickness = 10 µm).The ordinate is discontinuous as indicated to present all small values, n=3. Partial pressures used were done in Table 4.2.

In the aim of comparing to the model food emulsion, the partition coefficients were calculated and are given in Table 4.6. They are several orders of magnitude higher than the apparent

partition coefficient of the headspace of the model solution. This shows that K_{app} was clearly underestimated and that the system headspace of emulsion/lid was still far from thermodynamic equilibrium.

Table 4.6. Sorption (C_p) of aroma compounds in PLA samples (thickness = 10 µm) determined by MHE and partition coefficient ($K_{polym/hs}$) polymer/headspace.

		LOD	LOQ	PLA4032D			PLA2002D		
		(µg)	(µg)	C_p (µg/g)	C_{hs} (µg/g)	$K_{polym/hs}$	C_p (µg/g)	C_{hs} (µg/g)	$K_{polym/hs}$
Ethyl acetate		0.010	0.069	3263.27 ± 189.63	15.23	214	3125.46 ± 184.99	15.23	205
Benzaldehyde		0.028	0.220	3133.82 ± 97.59	0.48	6156	4913.15 ± 181.01	0.48	10299
Aroma compound mixture 1	EB	0.007	0.051	245.83 ± 44.45	2.23	110	372.36 ± 79.42	2.23	167
	E2MB	0.009	0.072	170.29 ± 22.78	1.73	98	315.93 ± 78.11	1.73	183
	BZA	0.028	0.220	6368.0 ± 273.12	0.48	8829	7457.75 ± 249.74	0.48	13605
	EH	0.011	0.085	54.67 ± 11.17	0.29	187	84.87 ± 9.05	0.29	291
	2NNE	0.038	0.328	<LOD	0.19		<LOD	0.19	
Aroma compound mixture 2	EB	0.007	0.051	235.80 ± 27.67	2.23	106	290.38 ± 44.59	2.23	130
	E2MB	0.009	0.072	122.95 ± 26.88	1.73	71	234.41 ± 40.51	1.73	135
	EH	0.011	0.085	<LOD	0.29		<LOD	0.29	
	2NNE	0.038	0.328	<LOD	0.19		<LOD	0.19	

The synergy effect between aroma compounds was tested with the help of different aroma compound mixtures and in comparison to values reported in our previous work (Salazar et al., 2012) using a mixture of ethyl acetate, ethyl butanoate, ethyl-2-methyl butanoate, benzaldehyde, ethyl hexanoate, and 2-nonanone at same concentrations that those used in the present work. In the previous work, the sorption of 2-nonanone in PLA was evidenced, while in the present case it was lower than the MHE detection limit. The presence of ethyl acetate in the mixture of the previous work had thus most probably a synergistic effect in facilitating the sorption of other aroma compounds. Furthermore, S values of ethyl hexanoate here were

smaller that previous ones ($1.66 \pm 0.18 \times 10^{-2}$ Kg m^{-3} Pa^{-1} in this work for PLA2002D and $2.55 \pm 0.45 \times 10^{-2}$ Kg m^{-3} Pa^{-1}in the previous one, e.g) (Salazar et al., 2012).

Benzaldehyde showed also a synergy effect, if mixtures M1 and M2 are compared, where M2 is solely composed of ethyl butanoate, ethyl-2-methyl butanoate, ethyl hexanoate, and 2-nonanone. In the case of M2 only ethyl butanoate, ethyl-2-methyl butanoate can be detected after sorption on PLA by MHE. The synergy was therefore of importance primarily for the aroma compounds being present in the headspace at very low activity. Furthermore, Table 4.6 showed that synergy was also true for benzaldehyde. At the same activity in headspace, benzaldehyde sorption is higher when it is in presence of other compounds (M1) than when it is alone, independently of PLA type.

From a general point of view, sorption of volatile organic compounds follows Flory-Huggins law, where the solubility coefficient increases strongly with the activity. The sorption isotherm of volatile organic compounds in the low activity (concentration) region can be approximated by Henry's law, though. Synergy of sorption between aroma compounds is expected in the higher concentration range (approx. > 1ppm) where sorption causes swelling of the polymer while antagonistic effects can be expected in the low concentration range where polymer morphology remains unchanged. In the latter case the permeation is predicted to follow the laws of permanent gases, which are based on competitive sorption and diffusion (Story & Koros, 1989). Table 4.7 presents a literature review over different studies carried out on the sorption of molecules in polymers from aroma compound mixtures. The method most often used was permeation, which only allows for an indirect determination of solubility coefficients and the most prominent polymer LDPE. The overview shows that the hypothesis of antagonistic effects in the low concentration range and synergy in the high concentration range holds for many cases, but there are exceptions. Exceptions are found in particular, when limonene, a very strongly interacting molecule with LDPE is present. This is comparable to

our case, where synergy is observed when the very strongly interacting molecule benzaldehyde is part of the mixture. Benzaldehyde sorption is high and the fact that benzaldehyde favours sorption of other aroma compounds in the packaging needs to be anticipated in the creation of aroma of foodstuff intented to be packed in PLA.

Table 4.7. Literature review of synergy and antagonistic effects of aroma compounds mixtures in sorption.

Reference	Polymer	Molecules	Method*	Concentration range	Effect
Theodorou and Paik (1992)	LDPE	Linalool Citral Ethyl butanoate Limonene Octanal	Permeation	Low (1 ppm)	Antagonist
Nielsen et al. (1992)	LDPE	Ethyl butanoate Hexyl acetate Hexanal Isopentanol	Permeation	Low (1 ppm)	Antagonist for Ethyl acetate and hexyl acetate, no effect for hexanal and isopentanol
Johansson and Leufven (1997)	LDPE	Ethyl butanoate Hexyl acetate	Sorption in liquid contact	Low (50 ppm)	Antagonist
DeLassus, Tou, Babinec, Rulf, Karp and Howell (1988)	LDPE	Trans-2-hexanal Hexanal Ethyl-2-methylbutanoate	Permeation	High (144 ppm)	Synergy
Johansson and Leufven (1997)	PP	Hexyl acetate in mixture with Ethyl butyrate	Sorption in liquid contact	High (1000 ppm)	Synergy for Hexyl acetate. No effect for ethyl butanoate.
Dury-Brun et al., (2007)	HDPE	Limonene (21 ppm) in mixture with ethyl butanoate (100 ppm)	Permeation	Low	Synergy
Dury-Brun et al., (2007)	BOPP	Ethyl acetate in mixture with limonene	Permeation	High (activity ≥ 0.3)	Antagonist for ethyl acetate. Synergy for limonene.

*Permeation refers to studies were solubility was calculated from the permeation experiment with the time lag method. Sorption refers to studies were sorption was measured directly by mass uptake.

One of the main drawbacks of many studies is that the thermal properties were not measured in line with aroma sorption and therefore eventual changes in the polymer due to plasticization or induced crystallization were not detected. Solvent induced crystallization is however a known phenomenon. For example, PET crystallizes when brought into contact

with acetone (Ouyang, Lee, Ouyang, Shiue, & Wu, 2004). Colomines et al. (2010) and Courgneau et al. (2012) evidenced plasticizing and induced crystallization due the sorption of ethyl acetate into PLA at high activity, 0.5 in Colomines's and 0.2 in Courgneau's paper. Salazar et al. (2012) reported that at very low activities of six aroma compounds in mixture, such as 3.45×10^{-2} for the ethyl acetate, a slight decrease in Tg was observed in PLA2002D and PLA4032D. These authors reported a small plasticization of PLAs films samples by aroma compounds at very low concentration (<200 μg/g) but they did not observe any solvent induced crystallization of the samples. The effect of aroma compound sorption on the glass transition temperature and the crystallinity degree of both PLA grades is plotted in Fig. 4.5 including statistical evaluation of significant differences. It is shown that PLA4032D was more crystalline than PLA2002D, which might explain the small difference in sorption of aroma compounds observed in Fig. 4.4. Crystallites are excluded volume where sorption cannot take place, therefore higher crystallinity leads to smaller sorption, which was verified in Fig. 4.4. Moreover, solvent induced crystallization was evidenced for both PLA types when benzaldehyde was present, but effect was stronger for the initially less crystalline material (PLA2002D).

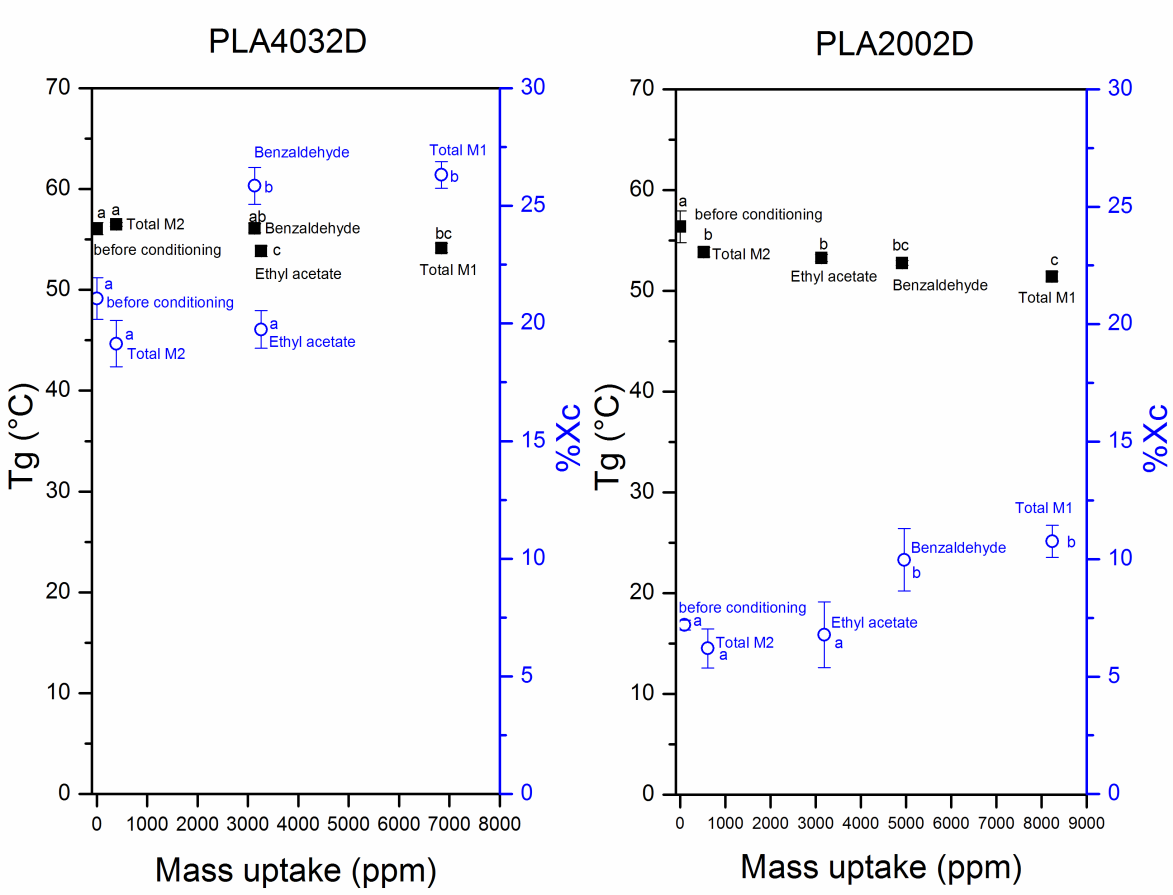


Figure 4.5. DSC results and total mass uptake for PLA4032D and PLA2002D before conditioning and after conditioning with different aroma solutions. Tg (■). Xc (○). [M1] Aroma compound mixture 1, [M2] Aroma compound mixture 2. [a, b, c] Different letters indicate significant differences at $p < 0.05$ (Duncan). Statistical analysis was carried out separately for each PLA type and the aroma compounds.

Glass transition of PLA4032D was almost not affected by the sorption of aroma compounds, whereas PLA2002D showed a slight decrease. The temperature of the glass transition seemed to be correlated to the total quantity of sorbed aroma compound, and it was thus smaller for mixture 1 compared to mixture 2 and benzaldehyde alone. The synergy of aroma compound sorption in our case might therefore be explained by small but measurable changes in the PLA morphology when benzaldehyde was present.

4.2.5 Conclusion

The interaction between food constituents and PLA has been tested by conditioning a flavored model food emulsion in tray and lid using rather severe conditions for the polymer, more

precisely storage for 30 days at 40 °C, a temperature near the PLA glass transition temperature. The aroma compounds chosen for flavoring represented different chemical structure classes *a priori* interacting with PLA, namely esters, ketones and aromatic rings. PLA showed good compatibility with the chosen model food. Polymer hydrolysis was moderate and crystallinity degree increased but oil and aroma compound sorption was low. The colour marker contained in the food emulsions did not stain the polymer. The apparent partition coefficients of the different aroma compounds were evaluated and showed that sorption passed preferentially by the headspace of the food emulsion. In order to investigate the sorption behavior of the aroma compounds and the effects of mixtures, a model study of gaseous contact was carried out in parallel, ensuring the reaching of the thermodynamic equilibrium. It showed that the mixture of aroma compounds had a synergistic effect if the highly interacting molecule benzaldehyde was present. The main conclusion on interactions between flavored foodstuff and PLA is that aromatic molecules seem to favour interaction and sorption of aroma compounds, where interactions occur preferentially through the headspace. This has to be anticipated in the aroma formulation of the concerned food product and in the design of the packaging container.

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Chapter 5

Identification and quantification of additives, lactides and volatile organic compounds (VOCs) in PLA

5 Identification and quantification of additives, lactides and volatile organic compounds (VOCs) in PLA.

After having performed the study of the interactions between PLA and food models, we carried out a study about the compounds present in PLA that may migrate into foodstuffs causing an impact on its quality.

Particular attention was put in additives, monomers and volatile compounds from degradation products of additives or polymers. In the case of monomers, we present an alternative methodology for quantification of lactides. In the latter case, the study of VOCs in PLA was associated to SENSOPLAST project, where we evaluated if the VOCs present in PLA could be quantified by the methodology developed in this thesis, particularly the VOCs that could be produced during PLA extrusion and thermoforming.

5.1 Deformulation of PLA.

Part of the results of this study were published in a special edition of the Italian Journal of Food Science dedicated to the Shelf-life International Meeting 2010, realized in Zaragoza, Spain (Lalanne et al. 2010).

This work aimed to evaluate the additives present in PLA, and for that an extraction method based on dissolution-precipitation technique was carried out using firstly dichloromethane as solvent and secondly ethanol as non-solvent of PLA for the precipitation of the polymer. The extraction was performed on five different grades of PLA pellets and the extracts were analyzed by gas chromatography (GC) coupled with mass spectrometry (MS), according to the procedure already described in chapter 2. The results presented in Table 5.1 showed the presence of compounds as ethyl lactate and lactides but also some additives as plasticizers (tri-ethyleneglycol and phthalate by-product) and slip agents (oleamide and erucamide).

Table 5.1. Compounds identified in extracts of PLA pellets obtained by the dissolution-precipitation method (Lalanne et al. 2010).

Compounds	Mw (g/mol)	CAS	%*	IKc (DB5)	PLA 7000D	PLA 2002D	PLA 4042D	PLA 3251D
Ethyl Lactate	118	97-64-3	78	814	+	+	+	+
Meso-Lactide	144	4511-42-6	78	1129	+	+	+	+
L,L and D,D-Lactide	144	4511-42-6	83	1169	+	+	+	+
Triethyleneglycol	150	112-27-6	47	1184	+	+	+	+
Isopropyl myristate	270	110-27-0	93	1825	-	-	+	-
Dibutyl phthalate	278	84-74-2	96	1970	-	-	+	-
Diisooctyl adipate	370	1330-86-5	94	2390	-	+	-	-
Bis-(2-ethylhexyl) phthalate	390	117-81-17	83	2538	+	-	+	+
Oleamide	281	301-02-0	90	2579	-	+	+	-
Erucamide	337	112-84-5	98	2788	+	+	+	-

*Correspondence percent with the database Wiley7Nist05.

Ethyl lactate could be likely formed during the extraction procedure from lactic acid, and ethanol used as non-solvent. Lactides found in PLA are residual cyclic monomers or products of PLA degradation. Our analytical method uses a DB5 column allowing the separation of the meso-lactide and a mix of L,L and D,D-Lactide, which are co-eluted.

Esters of fatty acids with low molecular weight can be used as plasticizers to improve the mechanical properties of polymers and also as viscosity depressants for polymers pastes. This is the case of the isopropyl myristate found in PLA4042D, the di-isooctyl adipate found in PLA2002D and Bis-(2-ethylhexyl) phthalate found in PLA7000D, PLA4042D and PLA3251D.

Slip agents as primary amides provide surface lubrication during the processing of thermoplastics. These additives act as internal lubricants that gradually migrate to the surface once the film has been produced and reduce friction coefficients in post-processing operations. Erucamide was found in all the PLA grades with the exception of the PLA3251D whereas oleamide was found in PLA2002D and PLA4042D.

In conclusion, the method of extraction used allowed identifying some compounds present in PLA, however this method has some disadvantages like tedious procedures and high solvents requirements. For that, we tested in PLA7000D pellets additionnaly two different methods of

deformulation to identify additives presents in PLA. The first one method was based in the dissolution of polymer with ultrasounds and then its precipitation in ethanol. The second one method was based on extraction with a non-solvent (ethanol) under ultrasounds. The procedures were described in detail in chapter 2.

Table 5.2. Compounds identified in PLA7000D pellets using different methods of deformulation.

Compounds	Mw	CAS	IK	D-P ultrasounds[1]	E with no solvent[2]
Ethyl Lactate	118	97-64-3	820	+	+
Meso-Lactide	144	4511-42-6	1131	+	-
L,L and D,D-Lactide	144	4511-42-6	1170	+	-
Triethyleneglycol	150	112-27-6	1185	+	+
Bis-(2-ethylhexyl) phthalate	390	117-81-17	2538	+	-
Erucamide	337	112-84-5	2786	+	+

[1] dissolution under ultrasounds and precipitation method; [2]extraction with non solvent under ultrasound.

The results presented in Table 5.2 showed that, under the conditions studied, the procedure of dissolution with ultrasounds and precipitation allowed identifying more compounds than the procedure of extraction with non-solvent. In particular lactides were not extracted by ethanol extraction. In the case of the first one, the compounds identified were the same as those determined by the method of dissolution under reflux and ethanol precipitation (Table 5.1). So, the use of ultrasounds to carry out the dissolution of PLA was a good alternative to the method of dissolution by reflux due its equivalent results and easy processing.

5.2 Quantification of lactides in PLA

The lactide content in PLA can have an effect on the properties of polymer. For example, the residual monomer can increase the hydrolytic degradation rate of PLA and deteriorate thus the performance of the end product (Feng et al. 2012). Also, the lactides present as residual monomers in PLA can serve as plasticizer, changing its mechanic properties. Moreover, lactide remaining in the final packaging material can have a high migration to the foodstuff due to its small molecular size (Avérous 2008). Additionally, during forming, large amounts

of lactide as residual monomer can change the viscosity and rheological behavior, as well as fuming (Braun et al. 2006).

For that, analytical methods for quantifying lactides in PLA are of high interest. In this work, two methods were used to determine the quantity of lactides present in pellets and films of PLA. The first one method consisted in a solvent extraction procedure, whereas the second one was a free solvent headspace extraction.

5.2.1 Quantification of lactides in PLA by the method of NatureWorks

The main supplier of PLA, Natureworks has developed a methodology of quantification of lactides (procedure number PLA_GC_13_4), which is based on solvent extraction and analysis by GC-FID. This method uses a FFAP column allowing the separation of the lactides in two peaks, the first one is meso-lactide and the second one is a mix of L,L and D,D-lactide, which are co-eluted. In our study, three samples of 1g of PLA2002D film, PLA4032D pellets, PLA4032D films and PLA7000D pellets were analyzed following the method described in chapter 2. The results are presented in Table 5.3.

The amount of lactides determined in PLA samples showed no significant differences, excluding PLA2002D film which presented a higher value. Comparing the film samples, the difference in amount of lactides could be explained by the PLA grade. In the case of PLA4032D samples, it seems that extrusion and thermoforming conditions used did not affect significantly the amount of lactides in PLA.

Table 5.3. Lactide quantified in PLA samples using the NatureWorks method.

sample		Wt %	ppm
PLA2002D	extruded film	2.11 ± 0.18^{a}	21050± 1754
PLA4032D	pellet	1.49 ± 0.12^{b}	14887 ± 1240
PLA4032D	extruded film	1.51 ± 0.10^{b}	15088 ± 1044
PLA4032D	thermoformed tray	1.70 ± 0.04^{b}	16956 ± 430
PLA7000D	pellet	1.31 ± 0.12^{b}	13105 ± 1191

[a, b] different letters indicate significant differences at $p < 0.05$ (Duncan).

Natureworks LLC reported in the procedure here used, that PLAs could have about 3 %wt of lactides and this value could decrease by devolatization to <0.3 %wt. So, our results showed a good agreement with values reported by Natureworks LLC.

5.2.2 Quantification of lactides by MHS-SPME

After having quantified the lactides by a solvent extraction method, we aimed to find a solvent-free method in order to automating the process and requiring less time of sample preparation.

To use the MHS-SPME method on semi-volatiles compounds as lactides, the parameters of the methodology must to be optimized. As already mentioned, our analytical method uses a DB5 column allowing the separation of the meso-lactide and a mix of L,L and D,D-lactide, which are co-eluted. Also, as meso-lactide standard was not obtained from chemical suppliers, it was no quantified and the optimization of this method was about to the quantification of the mix of L,L and D,D-lactide.

5.2.2.1 Optimization of MHS-SPME

One of the most important steps in dealing with MHS-SPME studies is the optimization phase of some variables, as type of SPME fibre, amount of packaging sample and the incubation temperature. However, in the case of quantification of semi-volatiles compounds as L,L and D,D-Lactide from glassy polymer matrix, the optimization phase becomes more complex. Indeed, those compounds are not detected at room temperature and therefore samples need to be heated with the aim of favor its volatilization. Consequently, after selecting the SPME fibre, we focused our attention on incubation temperature and time variables. Then we optimized the amount of packaging sample.

So, preliminary experiments in PLA pellets were performed using CAR/PDMS and DVB/CAR/PDMS fibres. A temperature as 70 ºC was chosen because it is higher than the

glass transition temperature of PLA (between 55 and 65 °C) and therefore the volatilization of lactides from PLA pellets can be promoted. Samples of 1000 mg of PLA2002D pellets were incubated for 25 min at 70 ºC with the purpose of selecting the appropriate SPME fibre.

The results obtained showed that both types of fibre allowed identifying meso-Lactide and the mixture of L,L- and D,D-Lactide, however the DVB/CAR/PDMS fibre presented GC peak areas slightly higher than CAR/PDMS fibre. Since both types of fibre showed very high peak areas of lactides, both could be used to next phases of our study; however the CAR/PDMS fibre was selected because, according to literature (de Oliveira et al. 2012, Ezquerro et al. 2003), it can provide simultaneously more information about the organic volatile compounds with low molecular weight present in PLA, which are also interesting in our work.

Then, to optimize the temperature of incubation, samples of 1000 mg of PLA2002D film were tested for 30 minutes at 50, 60, 70 and 80°C before analysis by MHS-SPME. The GC areas of lactides increased with temperature and the peak area at 80 °C was about 20 times higher than that at 60 °C (Figure 5.1a). However, the incubation of samples using 60, 70 and 80 ºC for 30 minutes showed no exponential decrease of peak areas during four progressive determinations of the same sample (Figure 5.1b). So, ours results showed that the thermodynamic equilibrium was not reached and consequently the quantification of lactides is not possible under these parameters of incubation.

This effect could be explained either by the increase of diffusion of lactides with temperature as well as by the production of lactides with temperature. The first hypothesis seems the most probable as (Jamshidi et al. 1988) showed that PLA only undergoes thermal degradation at temperatures above 190ºC. So, temperatures higher than the glass transition temperature of PLA i.e. between 55 and 65 °C would only favor firstly, the diffusion of lactides into the rubbery matrix and, secondly, their volatilizations into the headspace of the vial, as it was concluded in literature by others (Hakkarainen 2010).

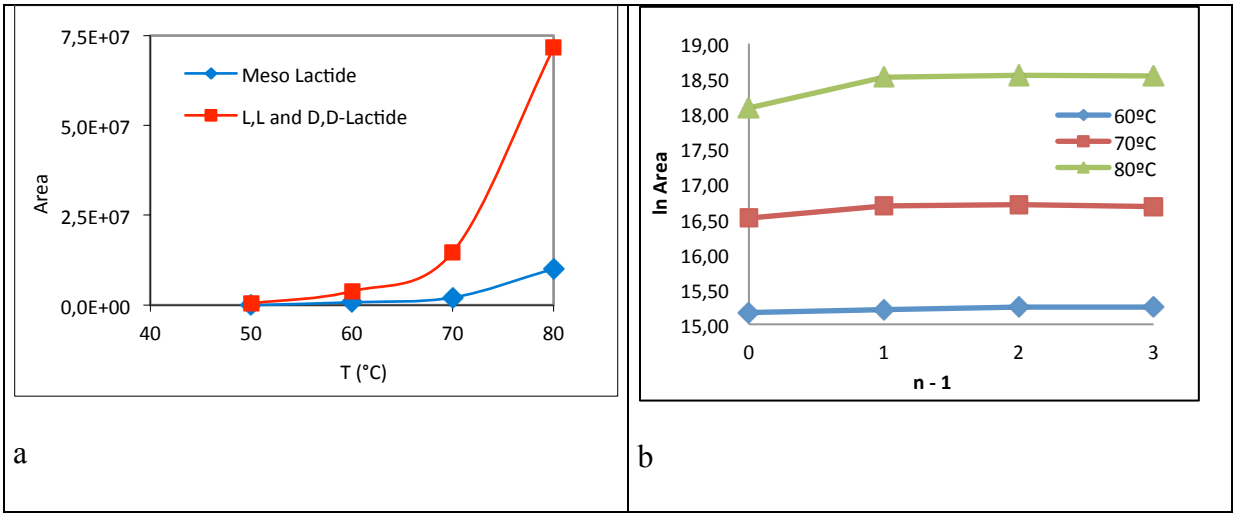


Figure 5.1. GC area of lactides during first MHS-SPME CAR/PDMS extractions of 1000 mg of PLA2002D films analyzed for 30 min at different incubation temperatures (A); Plot of ln GC areas of lactide versus n-1 determinations (B).

With the purpose of increasing the release of lactides from the PLA matrix to the headspace of the vial, we tested two treatments of pre-incubation of samples before MHS-SPME analysis. For that, PLA samples were placed in an oven at 60 °C or 80 °C during 4 or 8 hours, and then analyzed at 60 °C for 30 min of incubation. The results presented in Figure 5.2 showed that the GC area of L,L and D,D-lactide of samples pre-incubated at 80 ºC were higher than those pre-incubated at 60 ºC. Moreover, at 80 ºC, the results showed that the peak area increased slightly with the increase of time of pre-incubation. This means that the thermodynamic equilibrium could be reach after 8 hours of pre-incubation at 80 ºC. Therefore, this treatment of pre-incubation was selected for later experiments.

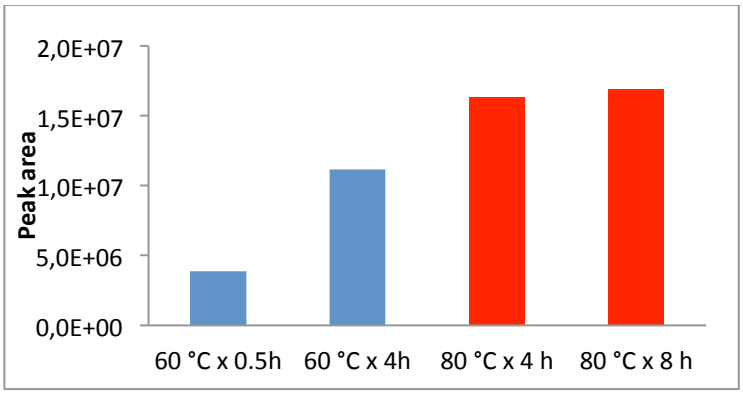


Figure 5.2. GC areas of L,L and D,D-Lactide of the first MHS-SPME extraction of a sample of 1000 mg of PLA2002D films pre-incubated by different treatments in an oven and then analyzed at 60 ºC for 30 min using a CAR/PDMS fibre.

In order to evaluate the amount of PLA sample that allow a good exponential decay of peak area, we carried out experiments using 1000 mg or 50 mg of PLA2002D film and the parameters already selected. The results showed only an exponential decay of GC peak area and good correlation coefficients ($R^2 \geq 0.99$) with 50mg amount of PLA, as is presented in Figure 5.3. We selected thus this amount of sample for later experiments. This result agrees with the results of Carrillo and Tena (2006) who reported that if the amount of sample in the vial is too large, no exponential decay of peak area is observed and bad correlation coefficients are obtained.

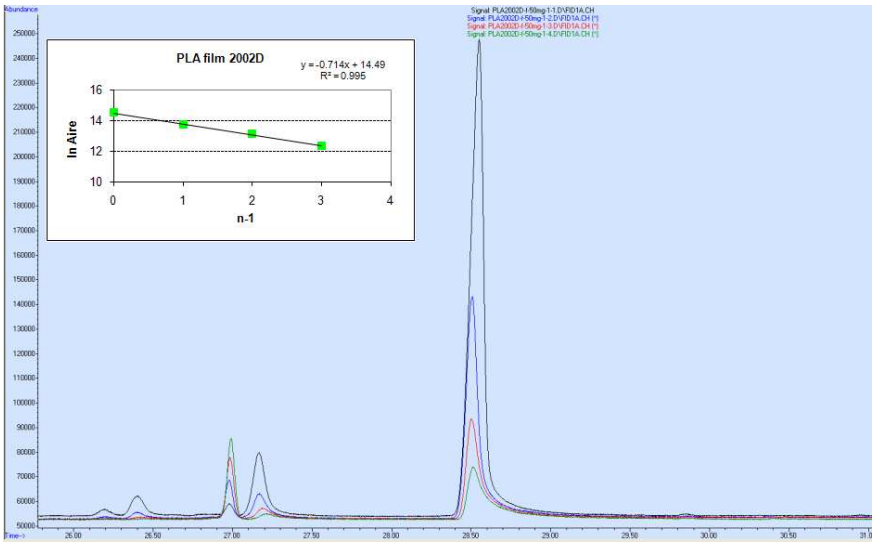


Figure 5.3. Illustrative GC-FID chromatograms for L,L and D,D-lactide (retention time = 28.5 min) corresponding to four MHS-SPME extractions of a pre-incubated (80ºC for 8 h) PLA2002D film sample, analyzed at 60ºC for 30 minutes of incubation, using CAR/PDMS fibre.

5.2.2.2 Lactide quantification

With the aim of quantifying the L,L and D,D-lactide by MHS-SPME, three samples of 50 mg of PLA2002D film (65 µm thickness), PLA4032D film (235 µm thickness) and PLA4032D tray (190 µm thickness) were analyzed using a CAR-PDMS fibre and the optimized procedure. The samples were pre-incubated in an oven at 80 ºC for 8 hours and then analyzed

by MHS-SPME at 60 ºC for 30 minutes of incubation, 5 minutes of extraction and 5 minutes of desorption in the injector of the GC.

Three solvents were tested as lactide solvents to prepare the standard solutions for calibration: hexadecane, dimethyl sulfoxide (DMSO) and butyl acetate. After preliminary essays, the first one was immediately discarded because lactide was not miscible, whereas DMSO and butyl acetate presented good affinity with lactide and allowed dissolve it. The preliminary GC results showed chromatograms with good lactide peak resolution with both solvents, however we selected butyl acetate due that it was already used in literature (Hakkarainen 2010) and it is not toxic as DMSO. So, calibrations curves of lactide standard in butyl acetate were carried out according the testing conditions selected previously. Calibration curve presented a linear range between 0.49 and 9.92 μg of L,L-lactide (Annexe 6) and a good correlation coefficient ($R^2 = 0.99$). The results of quantification are shown in Table 5.4.

Table 5.4. L,L and D,D-lactide quantification using MHS-SPME.

sample		Lactides (ppm)
PLA2002D	extruded film	32.01 ± 3.27^{a}
PLA4032D	extruded film	14.25 ± 2.57^{b}
PLA4032D	thermoformed tray	13.34 ± 0.82^{b}

[a, b] different letters indicate significant differences at $p < 0.05$ (Duncan).

Table 5.4 shows that the concentration of L,L and D,D-Lactide in the PLA2002D film samples was higher than PLA4032D films and trays. This could be explained by differences in PLA grades as well as by differences in samples thickness or by differences in drying of PLA pellets before extrusion. In the case of the first hypothesis, the PLA grades would be formulated with a different content of Lactide by the supplier (NatureWorks LLC). Moreover, the difference in thickness of the samples could have affected in a different way the diffusion of lactides from samples to headpace of vials. Finally, the drying phase of pellets could have impacted on Lactide content after extrusion. Lim et al. (2008) reported that PLA

pellets must be dried sufficiently, typically to less than 100 ppm (0.01%, w/w), to prevent excessive hydrolysis during extrusion. However, Natureworks LLC, one of the main suppliers of PLA polymers, recommend that resins should be dried up to a minimum or even below 250 ppm moisture content (0.025%, w/w) before extrusion. In our case, PLA2002D was dried to 700 ppm whereas PLA4032D was dried close to 250 ppm. So, the higher water content in PLA2002D probably contributed to PLA degradation during extrusion, producing a higher content of lactide than in PLA4032D.

However, lactides determined by NatureWorks (Table 5.3) method were three orders of magnitude higher than the results obtained by MHS-SPME (Table 5.4). This large difference led us to think that the use of the headspace microextraction method doesn't allow quantifying the total residual amount of lactides present in PLA samples, however it allows determining the lactide amount that is not retained inside the polymer matrix.

To get a clearer picture, we investigated the literature data on the quantification of lactides in PLA. To our best knowledge, the quantification of lactides in PLA materials by a headspace extraction method was reported only by Hakkarainen (2010). The author quantified the lactide amount in PLA5200D (Natureworks LLC) cast samples after thermo-oxidation at 140 °C, using a multiple headspace single-drop micro-extraction method. The author reported values of 20, 65, 80 and 130 µg of lactide/g of PLA after 2 hours, 1, 3 and 7 days of thermo-oxidation, respectively. The blank value of Hakkarainen (2010) was thus comparable to the value shown in Table 5.4.

Literature reported also the quantification of lactides by other methods. The lactide content can be measured by isocratic reverse phase HPLC assay with UV detection at 210 nm (Rothen-Weinhold et al. 2000), by quantitative HPLC using hydrolysis kinetics (Feng et al. 2009), by infrared spectroscopy (Braun et al. 2006) or thermogravimetric analysis (Feng et al. 2012). The key problem of quantitative analysis of lactide by HPLC lies in its easy hydrolysis, thus a little water can decompose lactide into lactic acid, but non aqueous organic

mobile phases cannot separate lactide from other compounds. For that, Feng et al. (2009) developed a method to quantify lactides in PLA samples (Zhejiang Hisun Biomaterials Co., Ltd) applying hydrolysis kinetics to a HPLC system, which most important advance is its practical approach to the quantification of analytes that are unstable in the HPLC mobile phase, as lactide. These authors studied the kinetics of lactide hydrolysis and treated the lactide degradation as a pseudo-first-order reaction, calculating thus the lactide content, under their given HPLC conditions. Apart from seem a laborious method, its drawback is that the hydrolytic degradation of lactide during the analysis cannot be controlled. These authors reported values of 3120 ppm of lactide in PLA samples. Additionally, Feng et al. (2012) used thermogravimetric analysis to determine quantitatively lactide in PLA. This method was established by comparison with results obtained by HPLC. However, the principal problem of this method is that all the volatile mass loss during heating in the TGA is considered as lactide loss. In this sense, MHS-SPME is more interesting because it allow quantifying the lactide directly.

We concluded that the MHS-SPME method was most probably not quantitative for the extraction of lactides present inside the bulk material, but could be used for estimating the amount of residual lactide in PLA. In fact, the heaspace/polymer matrix partition coefficient (*Khs/polym)* of lactide can be calculated from the results obtained by MHS-SPME and NatureWorks methods. Thus, the average of *Khs/polym* is $1.08 \pm 0.39 \times 10^{-3}$. So, this value could be used as a rapid method to determine lactide in PLA, from MHS-SPME results obtained under the conditions of our work. Obviously, before that, a validation phase of this value is necessary.

5.3 Identification and quantification of VOCs in PLA by HS-SPME

In the context of the SENSOPLAST project, we evaluated the VOCs present in PLA using the methodology of quantification developed in this thesis. For that, the HS-SPME method

coupled to gas chromatography (DB5 column) was firstly used to identify the VOCs presents in PLA and then a optimization phase for quantification was performed.

5.3.1 Identification of VOCs

With the purpose of testing the HS-SPME method, preliminary experiments were carried out with different samples of PLA (pellets and films), at different temperatures (30°C or 70°C) and times of incubation (5, 15 or 25 min) using the CAR/PDMS or DVB/CAR/PDMS fibers. The results obtained in PLA pellets are shown in Table 5.5 whereas the results in PLA films are presented in Table 5.6.

Preliminary results (Tables 5.5 and 5.6) mainly showed the presence of lactides which were separated by GC using a DB5 capillary column in two peaks, the first one is meso-lactide and the second one is a mix of L,L and D,D-Lactide, which are co-eluted. Meso-lactide was found only in samples heated at high temperature (70°C) which favored its volatilization in the headspace of the vial. Meso-lactide, L,L- and D,D-lactide can be produced by the reactions of degradation of PLA such as the simple depolymerization reaction by intra molecular ester exchange (Kopinke et al. 1996, McNeill and Leiper 1985).

Several organic volatile compounds whatever the PLA sample and analysis conditions used, thus some aldehydes, ethanol, acetone, acetic acid were identified. The hydroxyl end group in PLA mainly participates to the degradation of the polymer leading to molecular and radical reactions (Kopinke et al. 1996, McNeill and Leiper 1985). In our study by HS-SPME, the most volatile compound acetaldehyde was identified. Acetaldehyde can be produced by different pathway including cis-elimination reaction during PLA processing and also by radical reactions (McNeill and Leiper 1985). The residual acetaldehyde in PLA could be an important marker for the industry in the selection of PLA grades as shown in the PET industry. Removing acetaldehyde from PLA is important as this aroma compound can migrate from PLA leading to sensorial deterioration to foods or beverages in contact. Acetaldehyde has a distinct odor and taste and has a low sensory detection threshold level especially in

mineral water where its migration is detectable at low concentrations of 10–20 ppb (Ozlem 2008). 2,3-pentanedione is a compound that may be produced by the radical reaction between acetaldehyde and propen-1-one (McNeill and Leiper 1985).

Pentanoic, hexanoic, octanoic and nonanoic acids are present in pellets only for some PLA grades (PLA2002D) and mainly observed with a DVB/CAR/PDMS fibre at 70°C for 25min. They could be secondary reaction products by cis-elimination reactions on short chains oligomers of the PLA. The aldehydes as hexanal, heptanal, octanal, nonanal and decanal could be produced from the reactions of cis-elimination or trans-esterification. Unsaturated acid such as 2-Butenoic acid, (E)-; 2-Pentenoic acid could be secondary degradation products through hydrolysis of primary acrylic oligomers as described by Kopinke, Remmler et al. (1996). These two compounds increase in PLA2002D after processing (Figure 5.6). Moreover 2-butenoic acid (Figure 5.5) is the major compound that allows discrimination between 2002D and 4032D grades. Hydrolysis of ester groups could explain the presence of alcohols such as ethyl alcohol, 2-ethyl 1-hexanol, 1-(2-methoxypropoxy) 2-Propanol, and 1-Butanol.

Some compounds identified in our study remain unexplained and it is difficult to suggest a predominant pathway of production among cis-elimination and trans-esterification reactions. These compounds are: Diethoxyethane; 4-hydroxy-4-methyl-2-Pentanone; 2,2,4,6,6-pentamethyl-Heptane; 2-methyl propanoic acid,2,2-dimethyl-1-(2-hydroxy-1-methylethyl) propyl ester; 2-methyl propanoic acid,3-hydroxy-2,4,4-trimethylpentyl ester; 2-methyl propanoic acid-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester.

Table 5.5. GC Peak areas of compounds identified by HS-SPME extraction[a] from 1000 mg of PLA pellets analyzed at different temperatures and times of incubation using two types of SPME fibre.

Compound	IKcalc (DB5) *	IK (DB5) ref	CAR/PDMS 70°C x 25 min PLA 2002D*	CAR/PDMS 70°C x 25 min PLA 3152D*	CAR/PDMS 70°C x 25 min PLA 4042D*	CAR/PDMS 70°C x 25 min PLA 7000D*	DVB/CAR/PDMS 70°Cx 25 min PLA 2002D*	DVB/CAR/PDMS 70°C x 5 min PLA 2002D**	DVB/CAR/PDMS 70°C x 5 min PLA 3152D**	DVB/CAR/PDMS 70°C x 5 min PLA 4042D**	DVB/CAR/PDMS 70°C x 5 min PLA 7000D**	DVB/CAR/PDMS 30°Cx2 5min PLA 7000D**	DVB/CAR/PDMS 30°C x 15 min PLA 2002D**	DVB/CAR/PDMS 30°C x 15 min PLA 3152D**	DVB/CAR/PDMS 30°C x 15 min PLA 4042D**	DVB/CAR/PDMS 30°C x 15 min PLA 7000D**
Acetaldehyde	nd	427b	327367	318522	353860	262008	114965	249650	129322	126489	75647	-	203315	182889	188525	246346
Ethyl alcohol	nd	445b	-	-	-	-	-	8019079	3571853	3756483	1430259	+	1072241	3705751	1620427	270841
Acetone	500	500b	416057	338522	480621	673458	-	2580715			1086688	+	1190562	606919	1703656	1510172
Acetic acid	601	600b	1913396	5803189	1558791	2627541	488089	1224806	516080	497278	146624	+	-	-	-	-
2,3-Pentanedione	702	715c	316130	47955	281046	268699	-	-	-	-	-	-	-	-	-	-
Hexanal	803	801b	-	-	-	329180	-	204122	-	104017	100336	+	-	-	-	-
2-Pentanone, 4-hydroxy-4-methyl-	847	840b	-	-	-	-	-	138716	-	-	-	-	-	-	-	-
Pentanoic acid	878	875b 852c	-	-	448787	-	-	-	-	-	-	-	-	-	-	-
Heptanal	903	900c	-	-	255023	243973	-	-	-	106360	112139	-	-	-	-	-
Hexanoic acid	982	989b 970c	-	-	712362	498528	994785	-	-	-	-	-	-	-	-	-
Heptane, 2,2,4,6,6-pentamethyl-	1005	997b	-	-	-	-	1046923	-	-	-	-	-	-	-	-	-
Octanal	1017	1006b 1004c	27524	195182	492804	619217	316827	345665	192596	281400	271123	-	-	-	-	-
2-Propanol, 1-(2-methoxypropoxy)	1022	-	-	-	373636	-	-	-	-	-	-	-	-	-	-	-
1-hexanol, 2-ethyl-	1053	1028c	-	-	-	-	-	1266762	-	-	-	-	-	-	-	-
Nonanal	1108	1104c	262510	1084938	1127402	1538423	1141672	843831	652230	1150288	789281	+	112522	116203	172517	104256
Meso-Lactide	1133	-	1442085	-	1186157	2578773	1614666	2820698	700676	2530913	1890266	-	-	-	-	-
Octanoic acid	1170	1179c	-	-	-	-	738158	-	-	-	-	-	-	-	-	-
L,L and D,D-Lactide	1177	-	14124145	4920995	20201961	26152932	18642656	25720839	31678723	57221852	21121603	+	353086	154060	368136	371844
Decanal	1217	1204c	37198	-	-	-	282837	387466	478936	276555	254940	-	-	-	-	-
Nonanoic acid	1263	1275b	-	-	678306	-	-	340154	118531	-	-	-	-	-	-	-
2-methyl Propanoic acid, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	1487	-	69116	-	710320	620969	1379031	394004	1314708	739156	562159	-	-	-	-	-
2-methyl Propanoic acid, 3-hydroxy-2,4,4-trimethylpentyl ester	1549	-	41808	-	433887	340895	2906064	642130	1884962	878585	595895	-	-	-	-	-
,2-methyl Propanoic acid, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	1670	-	-	-	258884	-	-	-	-	331426	379693	-	256694	388403	327188	304728

a spme extraction time = 15 min, desorption time = 5 min; b from NIST available in http://webbook.nist.gov/chemistry/cas-ser.html; c INRAmass; + Compound indentified, FID signal no recorded; nd = not determined.

Table 5.6. Peak areas of compounds identified by HS-SPME extraction[a] from 1000 mg of PLA films analyzed at different temperatures and times of incubation using two types of SPME fibers.

						70°C x 25 min		30°C x 15 min	
						CAR/PDMS		DVB/CAR/PDMS	
Compound	Mw	CAS	IKcalc (DB5)	IK (DB5) ref[b]	IK (DB5) ref[c]	PLA2002D film	PLA4032D film	PLA2002D film	PLA4032D film
Acetaldehyde	44	75-07-0	nd	427	-	1979314	1837042	-	1574696
Ethanol	46	64-17-5	nd	445	-	437787	328688	-	527692
Acetone	58	67-64-1	500	-	-	4573364	2002831	+	2685101
Butanal	72	78-84-2	559	596	552	512433	-	-	-
Acetic acid	60	64-19-7	612	600	628	5397626	3423740	+	-
1-Butanol	74	71-36-3	670	675	-	4918940	537840	-	-
2,3-Pentanedione	100	600-14-6	701	-	715	3936084	2411155	-	-
Diethoxyethane	116	73506-93-1	791	730	-	-	787558	-	-
Hexanal	100	66-25-1	803	801	799	1222900	817223	-	-
No identified (m/z: 29, 39, 41, 42, 43*, 44, 55, 57, 58, 59, 72)			846	-	-	-	191592	-	-
2-Butenoic acid, (E)-	86	107-93-7	850	-	-	9378424	-	-	-
Heptanal	114	111-71-7	903	903	900	434522	434877	-	-
2-Pentenoic acid	100	626-98-2	921	911	-	720418	-	-	-
Octanal	128	124-13-0	1017	1006	1004	295641	444598	-	115665
Nonanal	142	124-19-6	1108	1104	1104	407885	763621	+	344801
Meso-Lactide	144	4511-42-6	1133	-	-	3310874	1107307	-	-
L,L and D,D-Lactide	144	4511-42-6	1177	-	-	23901958	16051954	+	917274
Decanal	156	112-31-2	1217	1209	1204	114276	300378	+	248912
,2-methyl Propanoic acid, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	286	74381-40-1	1670	-	-	-	-	+	367718

[a] spme extraction time = 15 min, desorption time = 5 min; [b] NIST http://webbook.nist.gov/chemistry/cas-ser.html; [c] INRAmass; + Compound indentified, FID signal no recorded; *m/z more intense; nd = not determine

Acetone and acetic acid have been already reported in literature about PLA degradation (Kopinke et al. 1996). However, concerning the other molecules, no studies about PLA degradation reported their presences, thus in some cases, they could be degradation products from additives.

Finally, one compound present in PLA4032D film (Table 5.6) was not identified. Its mass spectrum is showed in Figure 5.4. This molecule shows a major fragment (m/z= 43) that can come from propylene ($C3H5^-$). A second fragment (m/z = 57) can be derived from a fragment butyl ($C4H9^-$). The molecular ion (m/z = 72) can be an alkene or may be lactic acid (m/z = 90) minus water molecule (C5H12). So, this molecule could be a lactic acid oligomer.

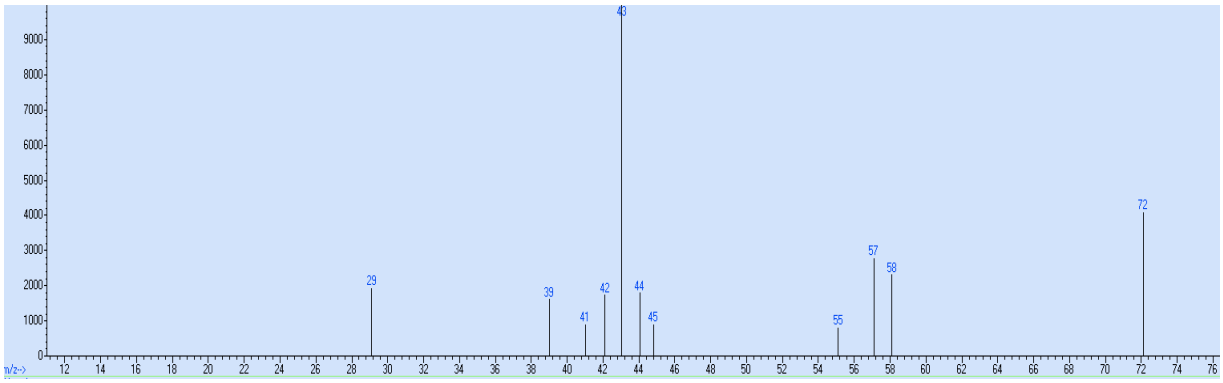


Figure 5.4. Mass spectrum of no identified compound from PLA4032D film (see Table 5.6).

Literature reported the formation of volatile compounds in PLA samples exposed to outdoor soil environment during two years (Gallet et al. 2000). The authors used solid phase microextraction (SPME) coupled to gas chromatography and mass spectrometry to monitor the volatile compounds. They reported the presence of Lactic acid, lactide and lactoyl lactic acid in unaged and aged films.

Khabbaz et al. (2000) studied the biotic and abiotic degradation of polylactide (PLLA) using fractionated Py-GC-MS at 400 and 500°C. At these temperatures, they identified acetaldehyde, acrylic acid, lactoyl acrylic acid, two lactide isomers and cyclic oligomers up to

the pentamer as thermal decomposition products of PLA, as well as some other not completely identified products (Khabbaz et al. 2000).

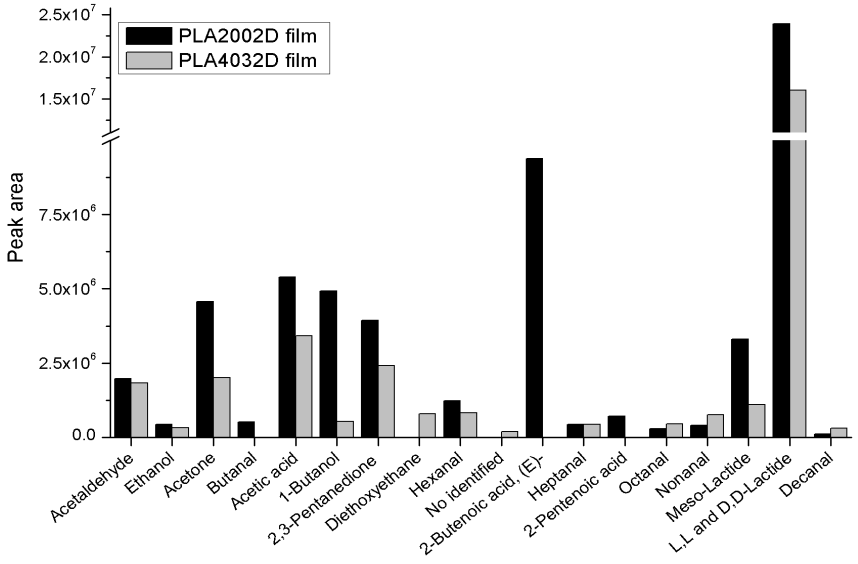


Figure 5.5. Comparison of compounds identified in PLA2002D and PLA4032D films during the first HS-SPME extraction from 1000 mg of sample incubated at 70 ºC for 25 min using a CAR/PDMS fibre.

The results of preliminary experiments allow also compare the different grades of PLA analyzed by HS-SPME. Figure 5.5 shows the peak areas of compounds identified in PLA films of two different grades. The samples were analyzed by SPME using a CAR/PDMS fiber at 70 ºC for 25 minutes of incubation, 15 minutes of extraction and 5 minutes of desorption in the GC injector. Comparing the two PLA films, it is clear that the peak areas of PLA2002D were higher than those of PLA4032D for all the compounds identified, with the exception of octanal, nonanal and decanal. However, the most interesting is that three components are present only in the PLA2002D: butanal, 2-butenoic acid, 2-pentenoic acid. These differences could be explained by the PLA grade or by the extrusion process. The latter hypothesis seems the most logical. Indeed, the molecules identified in the PLA2002D film are two acids and an aldehyde. Among the degradation pathways of the PLA, the only one that explains the production of acids is hydrolytic degradation. So, since that before extrusion the two PLA

grades were dried under different conditions, the content of water after drying was also different, being about 250 ppm in the case of PLA4032D and 700 ppm in the case of PLA2002D. The higher amount of water present in the PLA2002D would thus lead to hydrolytic degradation of PLA2002D during extrusion, which would be the origin of these molecules in the film. Thereby the impact of drying the PLA in the production of VOCs during extrusion was shown.

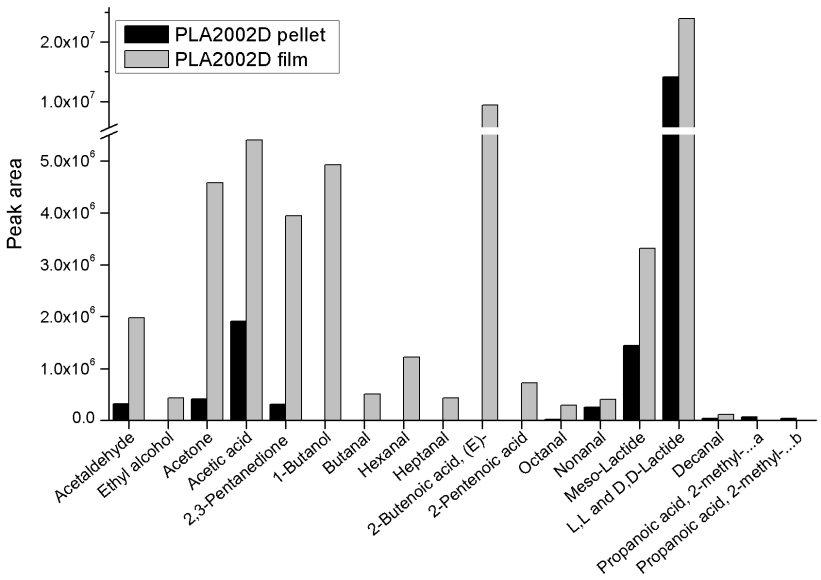


Figure 5.6. Comparison of compounds identified in PLA2002D pellet and PLA2002D film during the first HS-SPME extraction from 1000 mg of sample incubated at 70 ºC for 25 min using a CAR/PDMS fibre. [a] 2-methyl propanoic acid, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester; [b] 2-methyl propanoic acid, 3-hydroxy-2,4,4-trimethylpentyl ester.

Figure 5.6 shows the peak areas of compounds identified in pellets and films of a same PLA grade (PLA2002D), using a CAR/PDMS fiber at 70 ºC for 25 minutes of incubation. This figure allows observed the production or increasing of VOCs in PLA after processing. All the compounds identified in PLA2002D pellets increased after extrusion, with the exception of 2-methyl- propanoic acid 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)-propyl ester and 2-methyl-propanoic acid 3-hydroxy-2,4,4-trimethylpentyl ester, which were no present. That means that these compounds could be additives that were degraded during processing.

5.3.2 Optimization of MHS-SPME

After preliminary experiments and with the purpose of quantifying the VOCs present in PLA, experiments were carried out to optimize the MHS-SPME method.

5.3.2.1 SPME fibre

The type of coating to be used depends on the chemical nature of the target analytes. In this work the VOCs have to be identified, which require a fibre with good sensitivity for small volatile compounds. With the purpose of selecting the appropriate SPME fibre, we compared the results obtained in preliminary experiments using CAR/PDMS and DVB/CAR/PDMS fibres on samples of 1000 mg of PLA2002D pellets. As it was mentioned, the samples were incubated for 25 min at 70°C, a temperature higher than the glass temperature of PLA (between 55 and 65 °C), to promote the volatilization of organic compounds from pellets. The results are presented in Figure 5.7.

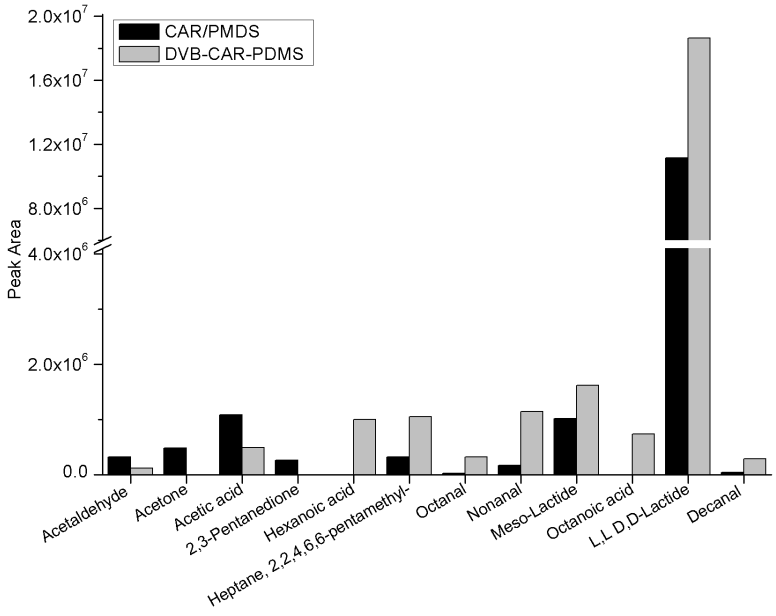


Figure 5.7. Peak areas of compounds identified in the first MHS-SPME extraction from 1000 mg of PLA2002D pellets incubated at 70 ºC for 25 min using two types of SPME fibre.

As expected according to literature (de Oliveira et al. 2012, Ezquerro et al. 2003), the CAR/PDMS fibre provided better results for compounds with low molecular weight, while

DVB-CAR-PDMS was better for the compounds with high molecular mass (Figure 5.7) and also polar compounds such as carboxylic acids. The CAR/PDMS fibre allowed identifying also some semi-volatile compounds such as meso-Lactide and the mixture of L,L- and D,D-Lactide. Van Aardt et al. (2001) reported the use of CAR/PDMS solid phase microextraction in static headspace at 45 °C for 15 min as an effective method for the recovery of acetaldehyde in milk and water media with detection levels as low as 200 and 20 ppb, respectively (van Aardt et al. 2001). So, as our principal aim is quantifying the VOCs, the CAR/PDMS fibre was selected for the next phases of our study.

5.3.2.2 PLA amount and incubation temperature

In this part of our work, the purpose was to identify and quantify the VOCs presents in PLA just after extrusion. The films used were obtained by extrusion of PLA2003D pellets according to the process already described in chapter 2 and then sampled immediately after extrusion in glass vials. All samples were stored at room temperature before to be analyzed during the next seven days.

MHS-SPME method was used to quantify the VOCs present in the PLA samples. As it was explained in previous chapter, MHS-SPME can be used only if an exponential decay of peak areas is obtained that is when a significant amount of organic compound is removed from the matrix at each extraction step. When the amount of sample in the vial is too large, exponential decay of peak area is not observed and low correlation coefficients are obtained (Carrillo and Tena 2006). According to MHS-SPME literature, the volatile compounds can be extracted at room temperature from polymers; however, the extraction of semi-volatile compounds is lower and could be enhanced by increasing the extraction temperature (Ezquerro et al. 2002). However the choice of the temperature must be tested carefully as a too elevated temperature causes a saturation into the headspace of the MHE-SPME vial and therefore affects the extraction rate (Ezquerro et al. 2002, Kataoka et al. 2000).

Table 5.7. Effect of the PLA amount and the incubation temperature on the peak areas of the first HS-SPME of VOCs identified in PLA2003D films using a CAR/PDMS fibre.

Compound						60°C		30°C	
	IKc (DB5)	IKref[a] (DB5)	IKref[b] (DB5)	Mw	CAS	1000 mg	50mg	1000 mg	50 mg
Acetaldehyde	nd	~427	-	44	75-07-0	7883928	1127136	12386336	835235
Acetone	500	500	-	58	67-64-1	1591456	1431174	1099132	3122296
2-methyl-2-Propanol,	524	526	-	74	75-65-0	4374424	2866235	5522590	3747835
Acetic acid	603	~600	628	60	64-19-7	1764628	771177	416755	-
3-Buten-2-ol, 2-methyl-	617	~614	609	86	115-18-4	-	-	-	387574
2,3-Pentanedione	703	697	715	100	600-14-6	11678387	875583	2110002	245652
2-Pentanol, 2,4-dimethyl-	804	-	-	116	625-06-9	1256210	882316	851957	736434
2,3,4-trimethyl- Hexane,	870	~850	-	128	921-47-1	261350	-	281710	-
Heptanal	903	896	900	114	111-71-7	-	-	-	1026264
6-methyl-5-hepten-2-one	999	994	987	126	110-93-0	-	-	-	135547
2,2,4,6,6-pentamethyl- Heptane,	1005	997	1034	170	13475-82-6	785053	688642	919540	643269
Octanal	1017	1006	1004	128	124-13-0	-	-	-	497340
Nonanal	1108	1102	1104	142	124-19-6	-	-	-	541164
Meso-Lactide	1133	-	-	144	4511-42-6	1265571	452546	-	-
L,L and D,D-Lactide	1177	-	-	144	4511-42-6	9424269	5365719	-	-

[a] NIST http://webbook.nist.gov/chemistry/cas-ser.html; [b] INRAmass; nd = not determined

To optimize these two variables, 50 and 1000 mg of PLA2003D film were incubated at 30 ºC (room temperature) and 60 ºC for 30 min and then analyzed by MHS-SPME, each vial was sampled four times. The extraction time was 15 minutes and desorption time in the injector of the chromatograph was 5 minutes. The results of VOCs thus identified in PLA2003D films are shown in the Table 5.7 and one example of chromatograms obtained is presented in Annexe 8.

Table 5.7 clearly shows that samples of 1000 mg provided higher peak areas than samples of 50 mg, whatever the temperature used. Moreover, these results showed that an increase of temperature (60 °C) caused an increase in the peak areas except for acetaldehyde and 2-2-methyl propanol, which presented the highest peak areas at 30 °C and 1000 mg of sample. This fact agreed with SPME literature which reported that the compounds with high volatility, as acetaldehyde, are sorbed easily by the SPME fibre but they are quickly desorbed when the temperature increases (Kataoka, Lord et al. 2000).

The most of VOCs presented in Table 5.7 were already found in our previous experiments, however, surprisingly, six new compounds were identified. So, three alcohols i.e. 2-methyl-2-Propanol, 2-methyl-3-Buten-2-ol, 2,4-dimethyl-2-Pentanol, two alkanes i.e. 2,3,4-trimethyl hexane, 2,2,4,6,6-pentamethylheptane, and one unsaturated compound 6-methyl-5-hepten-2-one were identified. These compounds were not found in PLA2002D pellets and therefore they have been produced during the extrusion process, however, to the best of our knowledge, these molecules were not reported in literature about PLA degradation In the case of alcohols, the hydrolysis of ester groups could explain their presence. In the case of the other compounds identified, they remain unexplained.

To verify the relationship between the logarithm of peak area and the extraction number, we used the results of PLA2003D film vials that were sampled four times. For the sake of comparison, we plotted in Figure 5.8 only the chromatograms of acetaldehyde and 2,3-

Pentanedione during four successive extractions by MHS-SPME at 60 °C of incubation for 30 min in samples of 1000 mg. The responses obtained for these two compounds were different. In the case of acetaldehyde, no exponential decrease was found while in the case of 2,3-Pentanedione the linearity of decrease was satisfying. This behavior was also found in PLA samples of 50 mg. So, these conditions cannot be used to quantifying acetaldehyde.

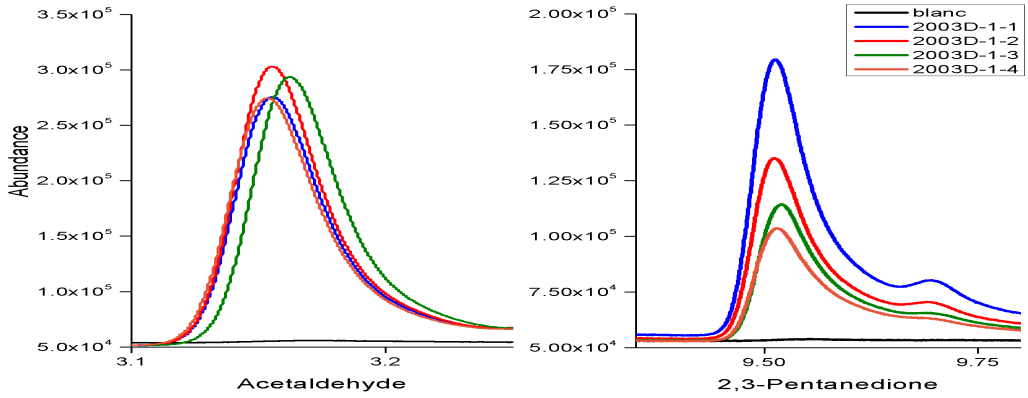


Figure 5.8. Chromatograms of four successive MHS-SPME extractions (CAR/PDMS) from 1000 mg of PLA2003D films analyzed at 60 ºC for 30 min.

However, the samples of 1000 mg of PLA2003D film incubated at 30 °C for 30 min allowed a linear response with good correlation coefficients for acetaldehyde (R^2 = 0.9983), 2-methyl 2-Propanol, (R^2 = 0.9921), 2,3-Pentanedione (R^2 = 0.9937) and 2,4-dimethyl 2-Pentanol, (R^2 = 0.9860) during four successive extractions by MHS-SPME. So, these results showed that the CAR/PDMS fibre, the incubation temperature of 30 °C and the samples of 1000 mg were the best conditions to quantify the VOCs with low molecular mass, under testing conditions studied in this work.

5.3.3 Quantification of VOCs in PLA

To quantify the VOCs present into PLA 2003D by MHS-SPME, we have firstly demonstrated that an exponential decrease of GC peak areas is achieved with a good correlation coefficients ($R^2 \geq 0.99$) for some volatile organic compounds and secondly it needs to obtain calibration curves of the VOCs with the same procedure. After optimization phase, four compounds were

selected as markers because they presented good correlation coefficients of the exponential decay during optimization phase: acetaldehyde; 2-methyl-2-Propanol; 2,3-Pentanedione and 2,4-dimethyl-2-Pentanol; however, the standard of the last one is not commercialized and needed to be dropped. In the case of the three other compounds, calibrations curves of VOC standards in hexadecane were carried out according to the testing conditions selected previously.

Table 5.8 shows the characteristics of method using standard solutions. We originally assumed that all compounds selected would present larger linear ranges in the calibration curves, however only acetaldehyde and 2-methyl2-Propanol, presented a linear response to ratio between total peak area and compound mass whereas 2,3-Pentanedione was co-eluted with a minor compound present in hexadecane. For that, new solutions of 2,3-Pentanedione in propyleneglycol (>99.5%, Fluka) were carried out and a good linearity in decay for 2,3-Pentanedione was obtained under chromatographic conditions already used.

Table 5.8. Features of MHS-SPME method using standard solutions.

Compounds	Studied range (µg)	Linear range (µg)	LOQ (µg)	Calibration curve equation	R^2
Acetaldehyde*	0.016 - 1.524	0.077 - 0.757	0.077	y = 68437527.92x	0.98
2-methyl-2-Propanol*	0.016 - 1.524	0.016 - 0.762	0.016	y = 92508061.21x	0.98
2,3-Pentanedione**	0.007 - 0.589	0.031 – 0.243	0.031	y = 45791165.03x - 2013665.82	0.99

*in hexadecane; ** in propyleneglycol.

Since the calibration curves were obtained, their quantity in extruded films and thermoformed samples of PLA2003D was obtained. The analyses were performed in triplicate using samples of 1000 mg each one. Results showed that, in the case of acetaldehyde, total peak areas obtained using 1000 mg of sample were above the linear range of the calibration curve of this compound. Consequently, with the aim of quantifying acetaldehyde, we used PLA samples of 200 mg each one during MHS-SPME. Additionally, the change in VOCs of PLA2003D as

film or thermoformed samples was assessed after six months of storage at room temperature in capped vials. The VOCs identified in these samples are shown in Table 5.9 and the concentrations of three of them are presented in Table 5.10. The results of VOCs identified in PLA2003D samples after storage (Table 5.9) showed the presence of eight compounds, all of them already identified in samples before storage (see Table 5.7, 30ºC), with exception of 3-methyl 2,4-Pentanedione. The later molecule would be produced during storage at room temperature; however it is difficult to suggest a pathway of production among primary or secondary reactions of PLA degradation. Also, the peak areas of the VOCs identified after storage were lower than the peak areas determined in samples before storage. In contrast, the results found in thermoformed samples after storage showed the presence of only three compounds (ethanol, acetone and 2-methyl 2-Propanol) of eight compounds identified in extruded films. So, on the basis of these results, it is clear that the storage of samples decreased the presence of VOCs in PLA samples.

Table 5.9. Peak area of VOCs identified in PLA2003D samples (200 mg) after 6 months of storage in capped vials using MHS-SPME.

Compounds	KIc* (DB5)	KI ref[a] (DB5)	Mw	CAS	Extruded film	Thermoformed
Acetaldehyde	nd	~427	44	75-07-0	2675215	no present
Ethanol	nd	-	46	64-17-5	357882	304495
Acetone	nd	500	58	67-64-1	2080827	715924
2-methyl-2-Propanol	525	526	74	75-65-0	1979250	540379
2-methyl-3-Buten-2-ol	617	614	86	115-18-4	223863	no present
2,4-dimethyl 2-Pentanol-	804	-	116	625-06-9	212373	no present
3-methyl 2,4-Pentanedione -	902	903	114	815-57-6	448309	no present
2,2,4,6,6-pentamethyl-Heptane	1005	997	170	13475-82-6	172611	no present

[*] Kovats index calculated; [a] from NIST available in http://webbook.nist.gov/chemistry/cas-ser.html; nd = no determined.

For freshly new thermoformed PLA samples (Table 5.10), the concentration of acetaldehyde decreased and the 2,3-Pentanedione was no present in comparison with extruded film. The process of thermoforming is carried out at 90 ºC during 15 seconds that could cause the loss

of these two compounds which cannot contribute to produce further degradation of PLA. After storage, the acetaldehyde concentration was lower than LOQ in extruded films and was not at all found in thermoformed samples. 2,3-Pentanedione was no present in all PLA samples after storage. Hence, it seems that these molecules were degraded during storage at ambient conditions.

In the case of 2-methyl-2-Propanol, before storage, no significant difference was found between the concentration determined in films and thermoformed samples (Table 5.10). After 6 months of storage in capped vials, the concentrations were lower than the LOQ of the method. Under our experimental conditions it is however difficult to propose a PLA degradation pathway in extrusion and thermoforming that explains the production of this molecule or that allows knowing if this molecule is produced from the secondary reactions of primary products.

From a commercial perspective these results are of interest because they show that there is not VOCs production in PLA samples during storage at room temperature, but during the forming at higher temperatures.

Table 5.10. Concentrations of VOCs (µg of compound/g of sample) in PLA2003D samples (200 mg) obtained by MHS-SPME before and after storage at room temperature in capped vials.

Compounds	LOQ*	Before storage		After 6 months	
		extruded film	thermoformed	extruded film	thermoformed
Acetaldehyde	0.385	1.40 ± 0.18	< LOQ	< LOQ	not present
2-methyl 2-Propanol	0.080	0.39 ± 0.08[a]	0.48 ± 0.04[a]	< LOQ	< LOQ
2,3-Pentanedione	0.069	0.074 ± 0.003**	Not present	Not present	Not present

* LOQ = limit of quantification (µg of compound/g of sample); ** determinated in a PLA samples of 1000 mg; [a] different letters indicate significant differences at p< 0.05 (Duncan).

Concerning the acetaldehyde content in PLA, to the best of our knowledge, no data were reported about it. However, large literature reported values of acetaldehyde content in PET (Mutsuga et al. 2006, Redzepovic et al. 2012). Mutsuga et al. (2006) reported acetaldehyde

values in PET bottles from Europe, North America and Japan, in ranging from 5.0 to 13.1 mg/kg, from 9.1 to 18.7 mg/kg and from 8.4 to 25.7 mg/kg, respectively. Redzepovic et al. (2012) found acetaldehyde levels in mineral water after filling in PET bottles from 1.62 to 2.22 ppm and from 13.94 to 17.68 ppm, after 1 day and 90 days of storage at room temperature, respectively. PLA samples studied in our work showed acetaldehyde concentrations lower than PET. So, from the residual acetaldehyde content determined in this work, it seems that PLA could present minor problems of migration related to this molecule and VOCs than PET.

Conclusions

and Perspectives

This thesis was developed in the context of the aim of investigating the performance of a new bio-based polymer, Polylactide, as food packaging material. For that, special attention was payed to mass transfer between foodstuff and PLA which can impact sensorial quality of foodstuffs and morphology of the polymer.

Up to date, only few studies reported on food-PLA interaction were focused to food quality evaluation during storage. Moreover, most of those studies were performed on aroma sorption into PLA at high levels of activity, which is far from real service conditions of PLA as food packaging. To study the interaction of aroma compound with PLA at concentrations usually present in packed foodstuffs and to take into account the effects on the thermal properties of the PLA a dedicated methodology using the volatility of the aroma compounds was developed and assess in a quantification point of view.

Aroma compounds in mixture or separately were put in vapor contact at low concentrations (between 1.6×10^{-8} and 1.7×10^{-4} mol/L), with PLA samples. The aroma compounds chosen are frequently found in bakery and "viennoiserie" products and represented different chemical structure classes *a priori* interacting with PLA, namely esters, ketones and aromatic rings. Two headspace methods to quantifying the aroma sorbed in polymer samples were used: MHE and MHS-SPME. These methods allowed the study of two low levels of vapor activity of aroma compounds. Thus, MHE and MHS-SPME allowed calculating S at low levels of activity as an alternative to the gravimetric methods by microbalance, but presenting as advantage that a mixture of aroma compounds can be studied. For ethyl acetate, from the two values of solubility obtained by MHE and MHS-SPME and the value obtained by the gravimetric method at 0.1 of activity (6.76×10^{-3} Kg/m^3.Pa, Chapter 3), we showed that the solubility coefficient was independent of the partial pressure and that the sorption followed Henry's law until 0.1 of vapor activity (5.2×10^{-4} mol/L). The sorption results showed that PLA films studied have a high affinity for more hydrophilic molecules sharing structure similarities with it, such as ethyl acetate. In the case of ethyl acetate, the results of S were in

agreement with results reported in literature. The more surprising result was however the very high affinity for the aromatic structure of benzaldehyde. To the best of our knowledge, for the other aroma compounds, this study gives the first values of S to literature about PLA. For instance, the ethyl hexanoate solubility coefficient measured in this work was 10 times lower than the values reported in literature for polypropylene (PP) and low-density polyethylene (LDPE), measured by microbalance. Additionally, the thermal properties of the PLA films after contact with aroma vapor showed a slight decrease in Tg, which means that a plasticization effect of aroma compounds occurred at vapor activities in the order of 10^{-2} (between 1.4×10^{-6} and 1.7×10^{-4} mol/L). However no solvent induced crystallization of film samples was observed at this low vapor activities. The degree of crystallinity only increased when PLA was put in contact with ethyl acetate, ethyl butanoate or ethyl hexanoate at vapor activities from 0.1 (5.2×10^{-4} mol/L), 0.1 (6.8×10^{-5} mol/L) or 0.5 4.5×10^{-5} mol/L), respectively.

The coefficients of solubility in PLA were surprisingly high for ethyl hexanoate and 2-nonanone in comparison with ethyl acetate and ethyl butanoate, although their high hydrophobicity. This result was not in accordance with the distance value ($\Delta\delta$) calculated from Hansen solubility parameters. For that, the sorption behavior of the aroma compounds and the effects of mixtures were studied by a vapor contact in model system using PLA microtomed samples. This study showed that the mixture of aroma compounds had a synergistic effect when the highly interacting molecule, benzaldehyde, was present. So, the main conclusion is that aromatic molecule seems to favor interaction and sorption of aroma compounds in PLA. Moreover, contrasting the results with those obtained in the chapter 3, it becomes clear that ethyl acetate had also a synergistic effect on the sorption of the more hydrophobic compounds. The synergic effect of the aromatic compound on the sorption of the others compounds might be explained by small but measurable changes in the PLA morphology when benzaldehyde was present. The temperature of the glass transition of PLA

samples seemed to be correlated to the total quantity of sorbed aroma compound and the solvent induced crystallization to the sorption of benzaldehyde.

The importance of the constituents of the food matrix on the aroma sorption in PLA was confirmed in the study of interactions between flavoured food models and PLA. In these cases, the presence of water, carbohydrates, oil, emulsifiers and stabilizers induced aroma compound retentions by the matrix, decreasing consequently the sorption in PLA. The study of a food emulsion conditioned in a PLA pack was carried out using rather severe conditions for the polymer, more precisely storage for 30 days at 40 °C, a temperature near the PLA glass transition temperature. Under these conditions, the polymer hydrolysis was moderate and crystallinity degree increased but oil and aroma compound sorptions were low. The apparent partition coefficients of the different aroma compounds were evaluated and showed that sorption passed preferentially by the headspace of the food emulsion, which should be anticipated in the aroma formulation of the concerned food product and in the design of the packaging container.

This work provides quantitative data on the coefficient of solubility of aroma compounds in controlled conditions that are important features for academic approach to understand and predict the mass transfer of organic compound in glassy polymers and finally to confirm data calculated by modeling. Moreover these data help food industries that would like to develop food packed in PLA and would like to preserve organoleptic properties throughout shelf life. The specific interaction of some aroma compounds leads to reinterpret the aroma formulation of food when PLA is considered to replace traditional petro based packagings.

In the context of food-packaging interactions, a second aim of this work was to evaluate the organic compounds that potentially could migrate to food from PLA. These compounds can be the additives of the polymer or even volatile organic compounds coming from the degradation of the polymer or additives during processing. The evaluation of additives in PLA only revealed the presence of compounds as ethyl lactate and lactides but also some

additives as plasticizers (tri-ethyleneglycol and phthalate by-product) and slip agents (oleamide and erucamide). The lactide content present in PLA can have an effect on the properties of polymer as well as can migrate easily due its low molecular mass. For that, in the chapter 5, the lactide content was evaluated in PLA samples using a solvent extraction method and an alternative methodology based on MHS-SPME. However, from the results obtained, the latter method cannot be used directly to quantifying the lactide content in PLA samples due to the high concentration of this compound in the samples (between 1.3 and 2.1 %w/w) which exceeds the limits of the MHS-SPME method. Finally, although that this work used a food-grade PLA, it is necessary to verify by global or specific migration test if the compounds that were here identified may migrate to foodstuff.

Finally, the VOCs present in PLA samples after extrusion and thermoforming were evaluated by HS-SPME and discussed according to the known degradation reactions. Several organic volatile compounds were identified by HS-SPME whatever the PLA sample and analysis conditions used in this work, being consequently a powerful tool for the screening of VOCs. The volatile organic compounds quantified in PLA samples as acetaldehyde and 2,3-Pentanedione increased after the extrusion and then decreased or disappeared after thermoforming, which can be explained by evaporation due to process (90 ºC). Although thermoforming conditions are not so severe to promote the degradation of the PLA, they allow a loss of the most volatile molecules, positively influencing the sensorial quality of the packaging. Additionally, after 6 months of PLA storage at ambient conditions, the concentrations of these compounds decreases and were lower than the LOQ of the method or even absent. So, from a commercial point of view, these results are of interest for storage of PLA because they show that there is no VOCs production in PLA samples during storage at room temperature, but only during the forming at higher temperatures.

Indeed, this study brings a particular result on one step of the PLA process. The work reported here used PLA films of different PLA grades, which were dried and then extruded

using different procedures. Differences in lactide content and VOCs generated by extrusion were shown. Even the processes were different for each grade, we could think that the hydrolytic degradation of PLA during extrusion have been favoured in one PLA grade with higher water content after drying. So the importance of efficient drying of pellets on the PLA stability before extrusion was also confirmed in the study.

Perspectives

The perspectives of this work are several with the purpose of better understanding the transfers of volatile molecules in PLA packaging at real service conditions. These perspectives will go through the development of new methodological approaches to measure mass transfer in dense polymer.

Methodology

The assessment of mass transfer kinetics and sorption equilibria in glassy materials is still a methodological challenge, although a performing off line tool by HS-SPME was developed here. One of the drawbacks of this method is the difficulty of quantification. To complete our study, the determination of the lactide content in PLA samples by MHE could be of interest as a methodological perspective to quantifying these important molecules.

The next step would be to develop an on line tool with automated measurement of permeation kinetics. One way is exploring the development of a new set-up coupling of different permeation cells with trapping and thermodesorption of the adsorbent.

Methodology should also be further developed in the aim of better understanding of PLA degradation pathways and the impact of present additives on the degradation pathways. Using TGA coupled with GC-MS is certainly a performing tool for identifying *on line* the formed products, and could contribute to trace the appearance of volatile organic compounds with potentially negative impact on the sensorial qualities of food.

Interactions PLA – VOCs and mixtures with water vapor

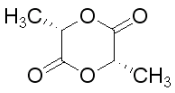
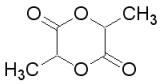
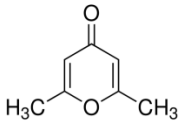
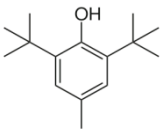
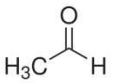
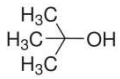
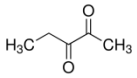
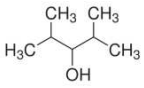
The comparison of the sorption of different aroma compounds in different PLA grades showed significant differences. The study of those grades did however not show notable differences in additive formulation. In this study there is therefore a part of results remaining unexplained. One of the hypothesis is that the stereochemistry might influence sorption behavior. The ratio of L-lactide to D-lactide is known to affect the PLA properties, such as melting temperature and the degree of crystallinity. It might be very interesting to study the effects of this ratio on the permeability, diffusion and solubility of aroma compounds in PLA. This could lead to better understanding of the PLA microstructure and the influence of stereochemistry on available voids inside the polymer.

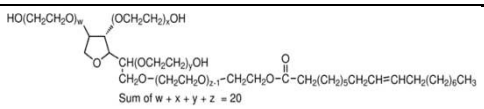
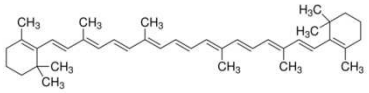
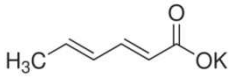
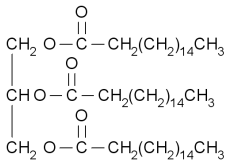
The main component of food is water and PLA possesses a great affinity for water. So the role of water in the transfer of organic compounds through PLA, as aroma compounds from food models, should be of interest because the water can affect the interactions between aroma compounds and packaging and can change properties of PLA packaging.

From a practical point of view, such knowledge would allow for better anticipation of aroma formulation in function of the PLA grade used.

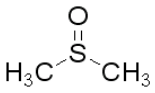
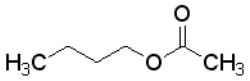
Annexes

Annexe 1. Characteristics of reagents used in this study.

Name	Chemical name	References	Chemical structure
L-Lactide	(3S)-cis-3,6-Dimethyl-1,4-dioxane-2,5-dione	Aldrich L-Lactide 98% CAS 4511-42-6 Mw =144.13 Reference du produit: 367044	
Lactide	3,6-Dimethyl-1,4-dioxane-2,5-dione	Aldrich Lactide CAS 95-96-5 $C_6H_8O_4$ Mw =144.13 Reference du produit: 303143	
2,6 dimethyl-γ-pyrone	2,6-Dimethyl-4H-pyran-4-one	Aldrich CAS 1004-36-0 $C_7H_8O_2$ Mw 124.14	
BHT	2,6 Di-tert-butyl-4-methylphenol	Aldrich (Germany) EC: 204-881-43 CAS: 128-37-0 Mw = 220.36 $[(CH_3)_3C]_2C_6H_2(CH_3)OH$	
nonadecane	Nonadecane	Aldrich (USA) CAS: 629-92-5 Fw: 268.52 $CH_3(CH_2)17CH_3$	
Acetaldehyde		Aldrich >99.5% Mw = 44.05 CAS Number 75-07-0 CH_3CHO	
ter butanol	2-Propanol, 2-methyl	Aldrich CAS 75-65-0 Mw 74.12 $(CH_3)_3COH$	
2,3-Pentanedione	2,3-Pentanedione	Adrich CAS Number 600-14-6 Mw 100.12 $CH_3CH_2COCOCH_3$	
2,4-dimethyl-3-pentanol	2,4-dimethyl-3-pentanol	Aldrich CAS 600-36-2 Mw 116.20 $(CH_3)_2CHCH(OH)CH(CH_3)_2$	
Sodium caseinate		Lactalis CAS 9005-46-3	

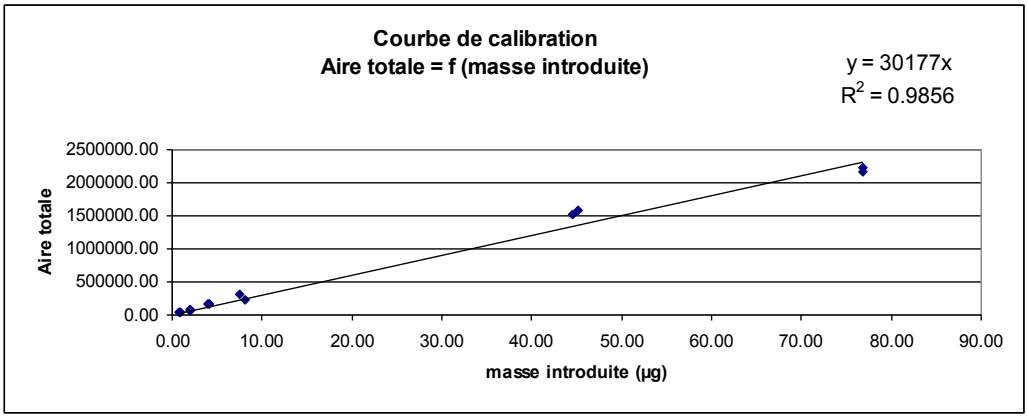
Tween 80	Polyethylene glycol sorbitan monooleate	Merck CAS 9005-65-6	
xanthan gum		Keltrol®, Monsanto	
β-carotene		Sigma Aldrich >95% CAS 7235-40-7 C40H56 Mw 536.87	
potassium sorbate	Potassium 2,4-hexadienoate	Sigma-Aldrich CAS 24634-61-5 Mw 150.22 $CH_3CH=CHCH=CHCOOK$	
triheptadecanoin	Glyceryl triheptadecanoate	Sigma CAS 2438-40-6 Mw 849.40 $[CH_3(CH2)_{15}COOCH_2]_2CHO$ $CO(CH_2)_{15}CH_3$	
sodium sulphate anhydrous		Sigma-Aldrich CAS 7757-82-6 Na_2SO_4 Mw 142.04	
potassium hydroxide		Sigma-Aldrich CAS 1310-58-3 KOH Mw 56.11	
boron trifluoride methanol complex		Sigma-Aldrich CAS 2802-68-8 BF_3 $2CH_3OH$ Mw 131.89	

Annexe 2. Characteristics of solvents used in this work.

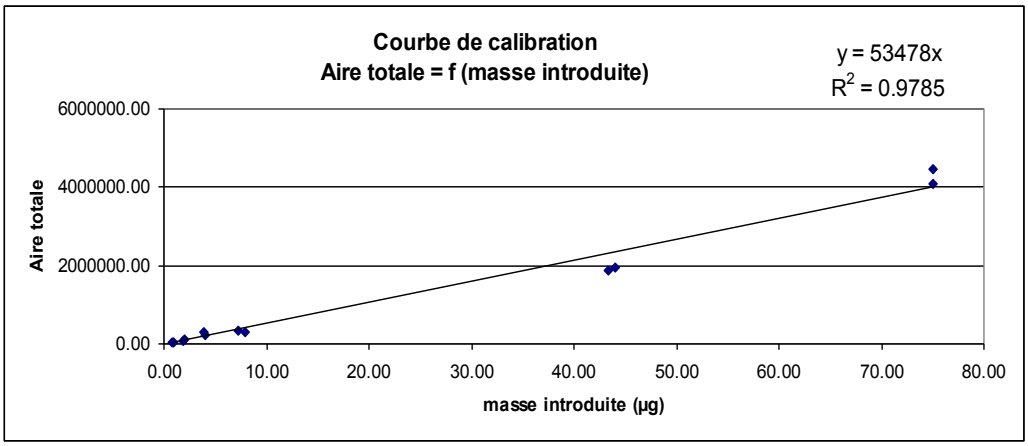
Name	Chemical name	References	Chemical structure
DMSO	Diméthylsulfoxyde	Aldrich Mw = 78,133 ± 0,007 g/mol CAS: 67-68-5 C_2H_6OS	
Butyl acetate	Butyl acetate	Aldrich anhydrous, ≥99% CAS 123-86-4 $CH_3COO(CH_2)_3CH_3$ Mw = 116.16	
Hexadecane		Sigma - Aldrich CAS: 544-76-3 Mw = 226.4 g/mol $C_{16}H_{34}$	$CH_3(CH_2)_{14}CH_3$
dichloromethane		Sigma - Aldrich CAS 75-09-2 Mw 84.93 CH_2Cl_2 b.p. 39.8°C	CH_2Cl_2
Ethanol		Carlo Erba Reagents CAS 64-17-5 Mw 46.07 CH_3CH_2OH b.p. 78.32°C	CH_3CH_2OH
Methanol		Carlo Erba Reagents CAS 67-56-1 Mw 32.04 CH_3OH	
n-heptane (99%)		Carlo Erba Reagents CAS 142-82-5 Mw 100.20 $CH_3(CH_2)_5CH_3$	
Pentane (98%)		Sigma-Aldrich CAS 109-66-0 Mw 72.15 $CH_3(CH_2)_3CH_3$	

Annexe 3. MHE calibration curves of aroma compounds

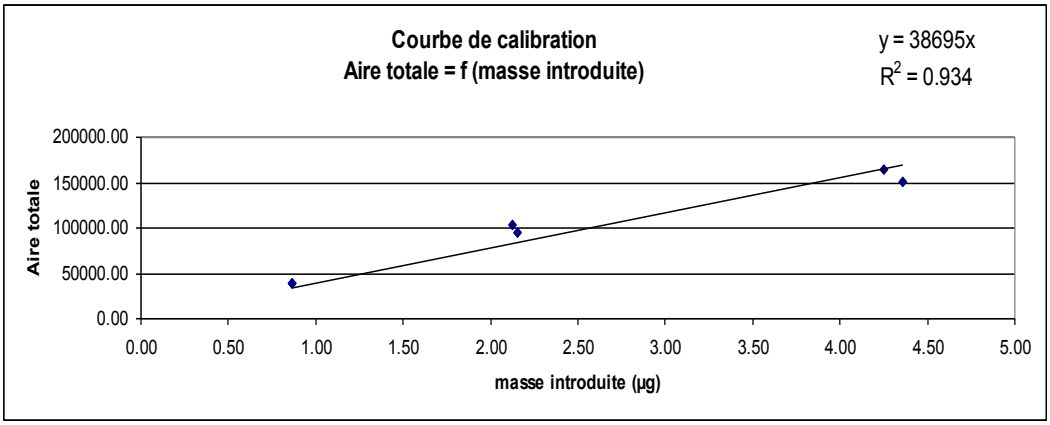
Ethyl acetate:



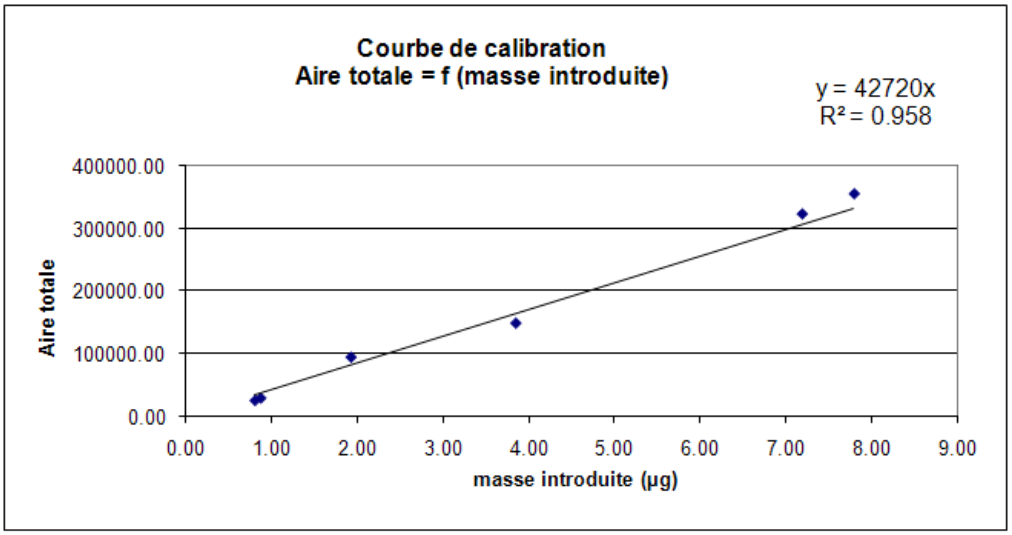
Ethyl butanoate :



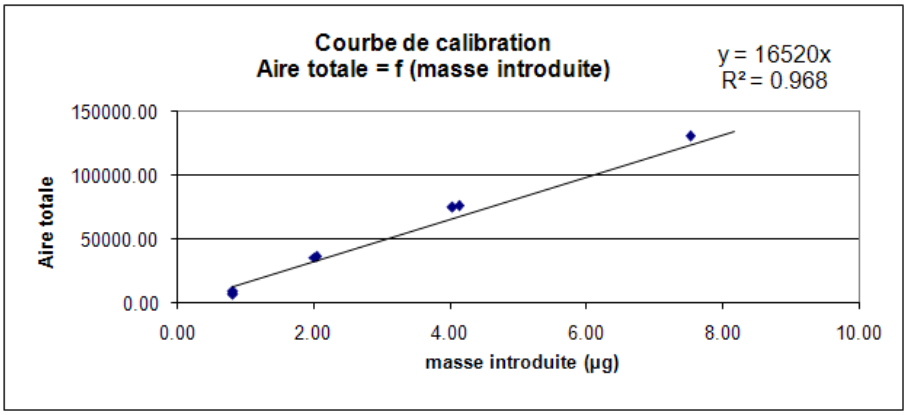
Ethyl 2-methylbutanoate :



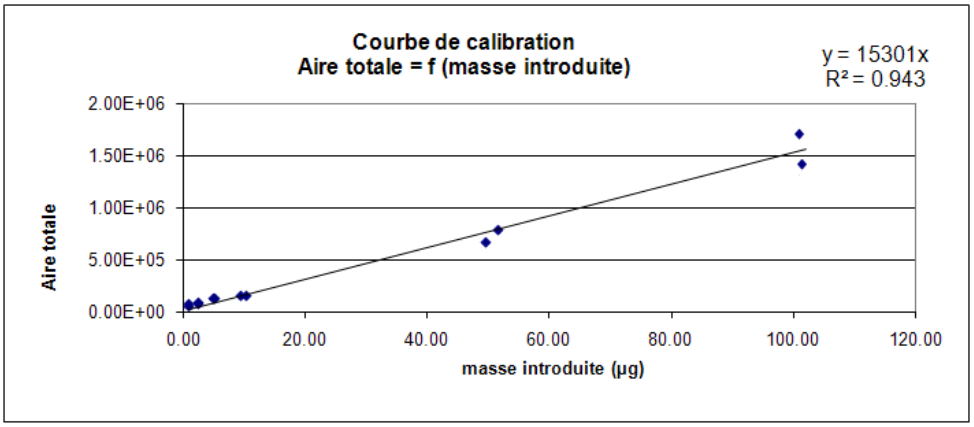
Ethyl Hexanoate:



2-nonanone :

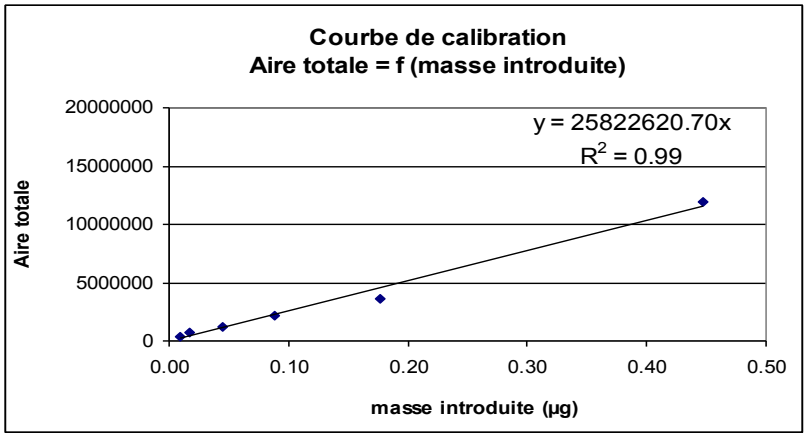


Benzaldehyde :

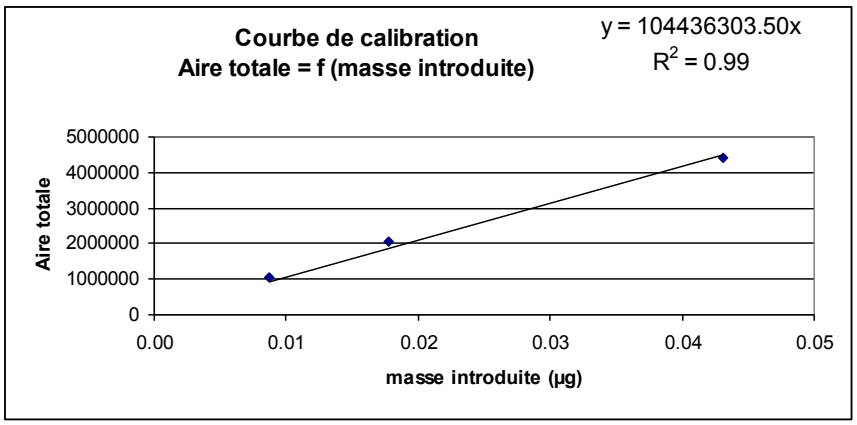


Annexe 4. MHS-SPME calibration curves of aroma compounds

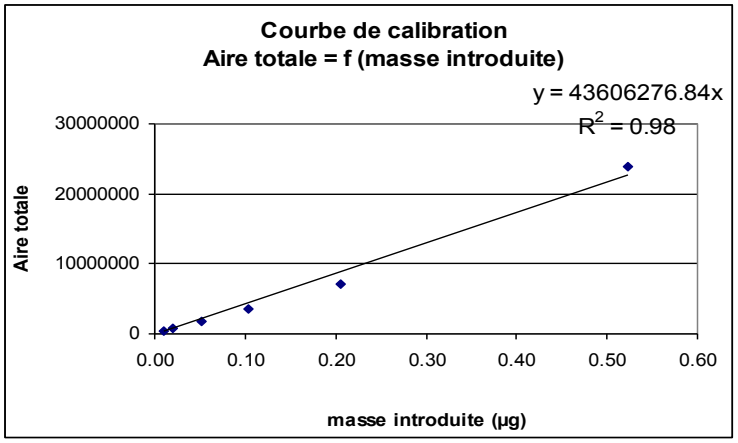
Ethyl acetate:



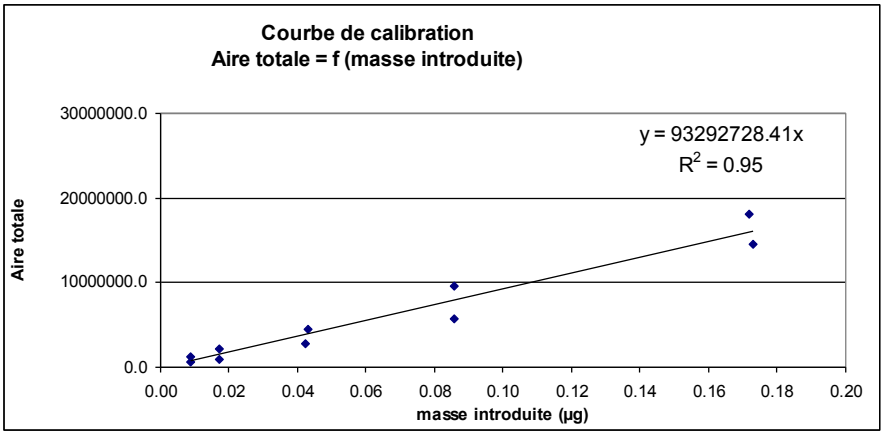
Ethyl 2-methylbutanoate:



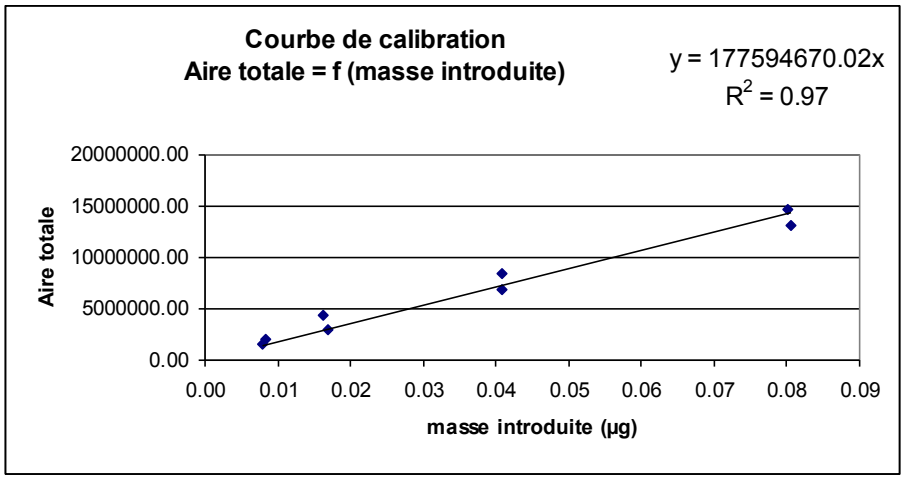
Benzaldehyde :



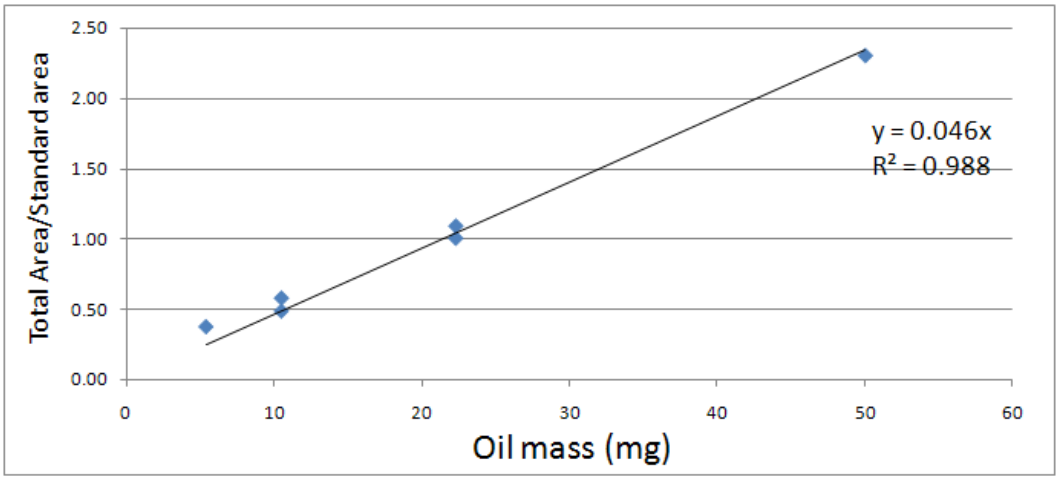
Ethyl Hexanoate:



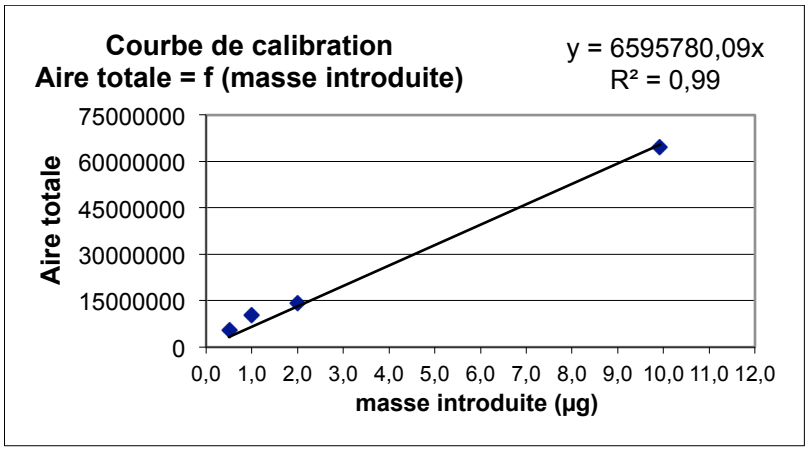
2-nonanone :



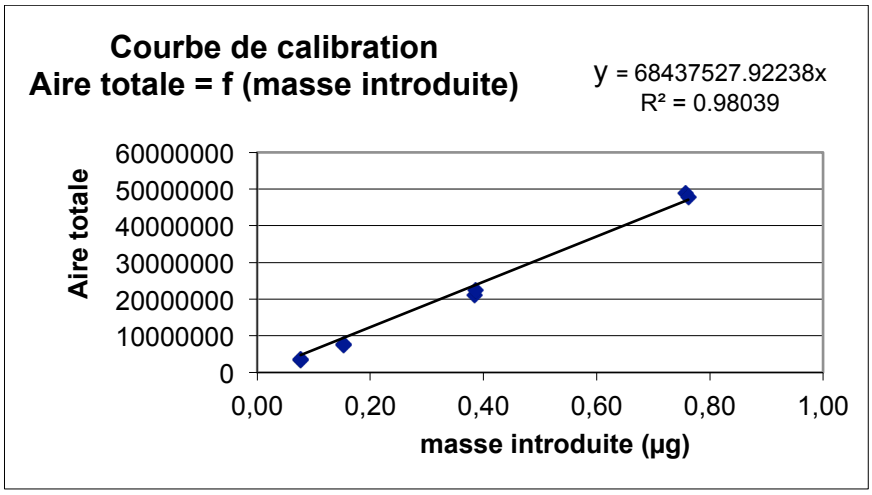
Annexe 5. Calibration curves of rapeseed oil.



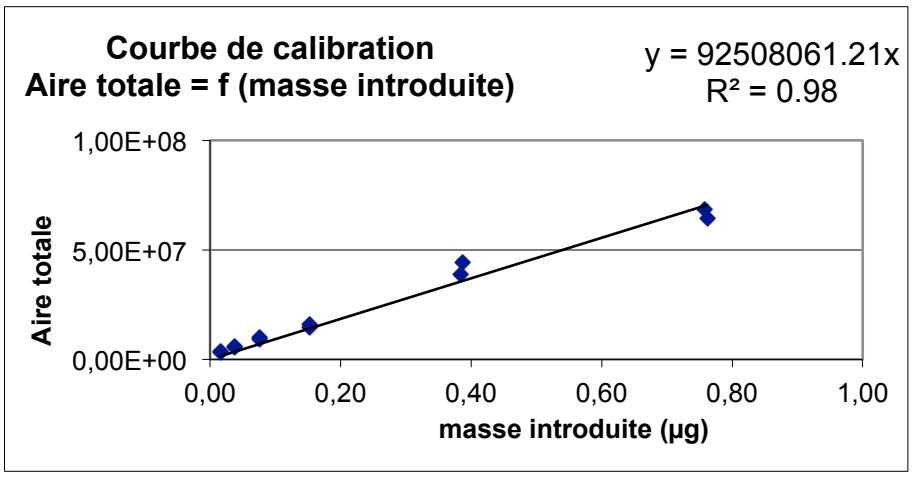
Annexe 6. MHS-SPME calibration curve of L,L-Lactide



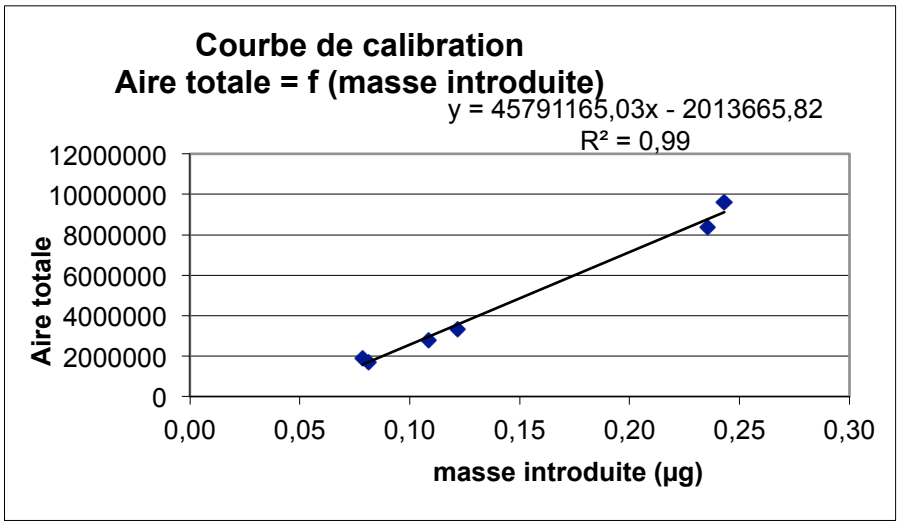
Annexe 7. MHS-SPME calibration curve of acetaldehyde



MHS-SPME calibration curve of 2-Propanol, 2-methyl-



MHS-SPME calibration curve of 2,3-Pentanedione



Annexe 8.

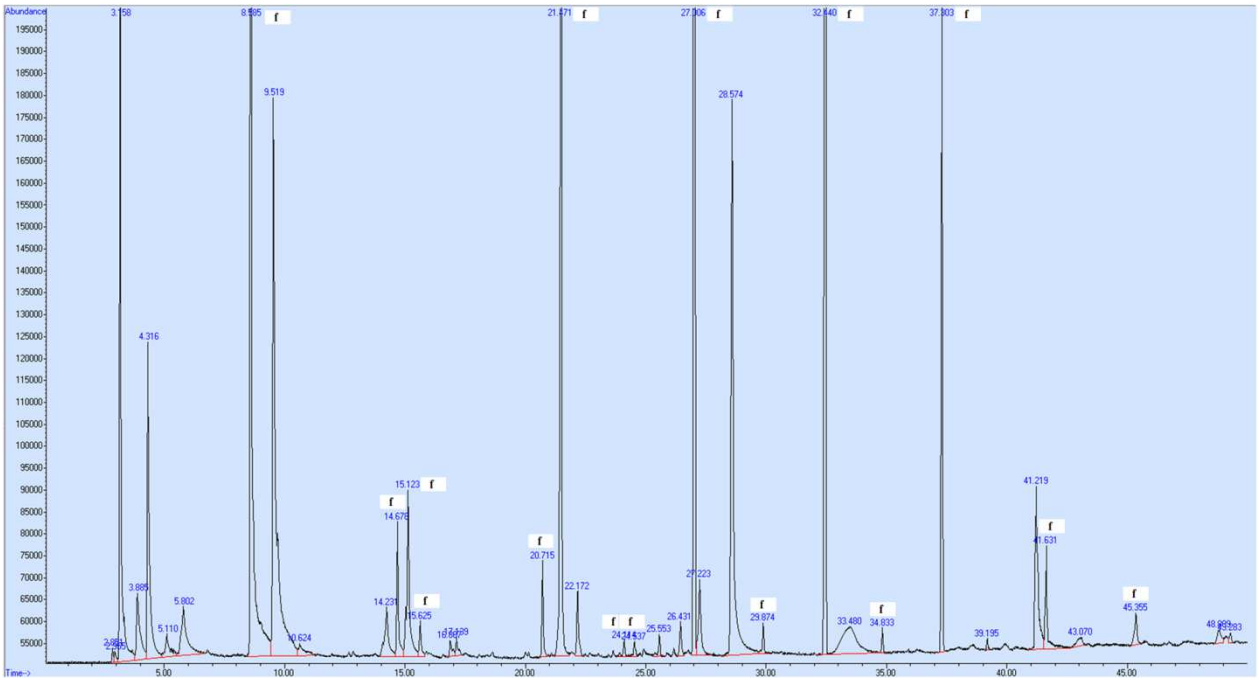


Figure Annexe 8. Chromatogram of the first HS-SPME extraction from 1000 mg of PLA2003D films analized at 60 ºC for 30 min. [f] peak of siloxane based compounds from spme fibre.

References

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Impact du Polylactide (PLA) sur la qualité des bioproduits au contact

L'emballage alimentaire est l'un des principaux acteurs de la préservation de la sécurité alimentaire et de la qualité au cours du stockage. Toutefois, de transferts de masse se produisent entre l'emballage polymère et les denrées alimentaires. Polylactide (PLA) est un nouveau polymère biosourcé utilisé dans les applications d'emballage. Pour cela son interaction avec des denrées alimentaires a été étudiée dans des conditions réelles d'utilisation. Une méthodologie originale basée sur l'extraction multiple de l'espace de tête (MHE) et l'extraction multiple de l'espace de tête couplée à la microextraction sur phase solide (MHS-SPME) pour la mesure de sorption de composés organiques volatils (COVs) dans le PLA a été développé à des activités faibles et dans le domaine vitreux du polymère. Les COVs ayant une structure chimique modérément hydrophobe et des similarités de structure avec le PLA montrent une forte affinité pour le polymère. Plus surprenant, la forte sorption de composés aromatiques, notamment du benzaldéhyde, a été montrée. En mélange avec d'autres COVs le benzaldéhyde favorise leur sorption par un effet synergique. De plus, même à des activités faibles (0,01) et en présence de benzaldéhyde la sorption de COVs induit une cristallisation du PLA. En contact avec un aliment aqueux, le PLA se montre résistant à l'hydrolyse et la sorption des matières grasses est faible, même en utilisant des conditions de stockage proches de sa transition vitreuse. L'apparition de COVs pendant le procédé de transformation a été étudiée par MHS-SPME. Des lactides, l'acétaldéhyde et le 2,3-pentanedione étaient détectés après extrusion et thermoformage. Les quantités des deux derniers composés diminuaient cependant pendant le procédé de thermoformage.
Mots-clés: polylactide, composé d'arôme, MHE, MHS-SPME, sorption, composés organiques volatils.

Impact of polylactide (PLA) on the quality of bio-products in contact

Packaging plays a major role in the preservation of food but mass transfer between the packaging material and foodstuff occurs during shelf life leading to the quality deterioration. Polylactide (PLA) is a novel packaging material; therefore its interaction with food was investigated at service conditions. The sorption of ethyl esters, benzaldehyde and 2-nonanone at low concentrations and in mixture and the effects on the thermal properties of PLA were studied. Multiple Headspace Extraction (MHE) and Multiple Headspace coupled to Solid Phase MicroExtraction (MHS-SPME) developed here allow to show a high affinity for moderately hydrophilic molecules sharing structure similarities with PLA such as ethyl acetate. However, a very high affinity for the aromatic structure of benzaldehyde was revealed which seems to favor interaction and sorption of the aroma compounds present in the mixture. At 0.01 activities, a plasticization by aroma compounds was observed and a solvent induced crystallization seemed to be correlated to the sorption of benzaldehyde. The contact between a flavored food emulsion and PLA, using conditions near the PLA glass transition showed that PLA hydrolysis was moderate and oil and aroma compound sorption was low. The formation of VOC during the PLA manufacturing process was assessed by MHS-SPME. Lactides, acetaldehyde and 2,3-pentanedione are VOCs present in PLA after extrusion and thermoforming. The two latter compounds increased after extrusion and then decreased or disappeared after thermoforming.
Keywords: Polylactide, aroma compound, solubility, MHE, MHS-SPME, volatile organic compound.