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Ambre Issart

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THÈSE

UNIVERSITE DE PAU ET DES PAYS DE L'ADOUR

École doctorale Sciences Exactes et leurs Applications (ED 211)

Présentée et soutenue le 18 octobre 2019

par **Ambre ISSART**

pour obtenir le grade de docteur

de l'Université de Pau et des Pays de l'Adour

Spécialité : Chimie des polymères

POTENTIEL D'ANTIOXYDANTS NATURELS POUR LA STABILISATION DE POLYMERES POUR EMBALLAGES ALIMENTAIRES ET LE DEVELOPPEMENT DE METHODES POUR EVALUER LEUR MIGRATION.

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'It always seems impossible, until it's done'

Nelson Mandela

ABBREVIATION LIST

- BFR: Brominated Flame Retardant
- BHA: Butyl Hydroxy Anisole
- BHT: Butyl Hydroxy Toluene
- CHCl₃: Chloroform
- CID: Collision Induced Dissociation
- D₂O: Deuterated water
- Da: Dalton
- DART: Direct Analysis In Real Time
- DESI : Desorption ElectroSpray Ionisation
- DMSO: DiMethylSulfOxyde
- DOSY: Diffusion Ordered Spectroscopy
- DPPH: 2,2-DiPhenyl-1-PicrylHydrazyl
- DSC: Differential Scanning Calorimetry
- EtOH: Ethanol
- EFSA: European Food Safety Authority
- ESI: ElectroSpray Ionisation
- EU : European Union
- GT: Green Tea
- HALS: Hindered Amine Light Stabilizers
- HDPE: High Density PolyEthylene
- HT-SEC: High Temperature Size Exclusion Chromatography
- HSP: Hansen Solubility Parameters
- HSPiP: Hansen Solubility Parameters in Practice
- Ip: Polydispersity Index
- LDPE: Low Density PolyEthylene
- LESA: Liquid Extraction Surface Analysis
- LSO: LinSeed Oil
- MALDI: Matrix Assisted Laser Desorption Ionization
- MeOH: Methanol
- MFI: Melt Flow Index
- Mn: number average molar mass
- MPa: MegaPascal
- MS: Mass Spectrometry
- Mw: mass average molar mass
- Mz : z average molar mass
- NMR : Nuclear Magnetic Resonance
- o-PEW : oxidised-PolyEthylene Wax
- OIT: Oxidation Induction Time
- OOT: Onset Oxidation Temperature
- PEG: PolyEthylene Glycol
- PEO: PolyEthylene Oxyde
- PET: PolyEthylene Terephthalate
- PLA: PolyLactic Acid
- PP: PolyPropylene / iPP: isotactic PolyPropylene
- PVC: PolyVinyl Chloride
- RPM: Rotation Per Minutes
- TAN: Tannins
- TBBP-A: TetraBromoBisphenol-A
- TGA: ThermoGravimetric Analysis
- TTS: Time-Temperature Superposition
- UHPLC: Ultra High Performance Liquid Chromatography
- VC: vitamin C
- VE: vitamin E

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GENERAL INTRODUCTION

The use of plastics in our daily life is huge. Actually, due to their biological, physical and chemical properties, our society lean on the use of plastics. In the food industry, plastic packaging plays a fundamental role as it protects the products from the environment. The food packaging can be separated into three categories: physical, chemical and biological [1]. When using a “physical type” of packaging it involves a protection toward temperatures or pressure for example whereas “chemical types” bring a protection against water or oxygen. Finally, the “biological type” of food packaging provide protection against living organisms (microorganisms, insects...). Hence, plastic packaging is used not only to avoid microbial and chemical contamination but it also has a broad range of other functions: it helps for the conservation (hence helping the diminution of the use of food additives), gives information (nutritional values, expiry date) and practicality. Hence, it plays a role in the food supply chain by enhancing shelf-life of the products it protects and is omnipresent in our daily lives. However, so far, traditional plastic packaging is mainly an inert material with mechanical function toward food: making them practical to carry and protected from atmospheric conditions. As a result of this passive use of plastics, consumers usually don't pay attention to their composition and have in mind that it doesn't represent an important part of the product. Actually, it was proven that plastic packaging could be harmful in many different ways. Opinions about their safety is very diverse and in spite of 50 years of studies, safety assessments remain elusive.

The migration of contaminants from the packaging to the foodstuff is more and more reported [2-4]. Those contaminants can have many different origins: they can be degradation products due to high temperatures (or irradiations) used during polymer manufacturing creating molecules with lower molecular weight (i.e. higher diffusion capacity) but also additives which are also very sensitive to temperatures or presence of impurities from the raw material or finally they can come from contaminants often brought by recycled materials' former life [5]. To a higher extent, the contaminants can also be Non-Intentionally Added Species (NIAS) created during plastic life through chemical reaction with its environment [5]. Their identification is quite difficult due to plastics the various compositions, various ways to manufacture and various environment in which they can be involved in. It is thus almost impossible to predict the final composition of a plastic material.

The risks on human health arising from migrating plastics components are more and more known. One of the main concern is endocrine disrupting molecules such as bisphenol A or di(2-ethylhexyl) phthalate (DEHP). However, consumers have to deal with a very hard exercise when trying to minimize the food exposure to plastics. The only information that the consumer can have on the packaging comes from the labelling which remains limited to the type of polymer that is used (thanks to the recycling symbols) but which doesn't give

information about the potential toxicity or on its persistence in the environment. Furthermore, 89 million tons of foods are wasted every year. The European Commission believe that almost 25 million tons could be avoided thanks to more responsible food handling [6]. Thus, the European parliament stated that food packaging should enhance food conservation. To avoid these contaminants migration, it is necessary to find a way to characterise them and to know their origin. However, diffusion is almost impossible to stop. Hence, for lack of being able to stop molecule diffusion from plastic to food, there is a possibility to take advantage of it by adding active compound to the plastic formulation and thus improving shelf-life and food quality. This type of packaging are called active packaging [7]. They represents a good alternative to all these issues brought by traditional plastic packaging as a system able to provide deliberate interactions with the food or the headspace in order to extend the shelf-life and the maintenance of food safety, thus decreasing the addition of preservatives directly to the food [7, 8]. Active packaging role toward food wastes then becomes obvious. The problematic is thus to maintain good mechanical and thermal properties of the polymer without the actual controversial additives. Inevitably, many regulations came out for such new materials in food contact applications [6]. Those regulations have increased the attention of food industries towards new products and considering that this field is approximatively 10 years old, the innovation in active packaging becomes a strategic mean to have competitive advantages. Moreover, despite a large number of patents in the last 10 years, only few active packaging were actually commercialized [6].

Although, it is clear that active packaging is a promising way to fix food packaging's contaminants issues, it appears that it is essential to find ways to analyse additives but also their degradation products. The determination and characterisation of those additives is major in a broad range of fields from pharmaceuticals to photovoltaic modules [9]. Mass spectrometry (MS) techniques provide answers to the full range of questions coming from stabilizers analysis in plastics. Different methods for mapping additives within polymers have been described, often requiring extraction and fractionation of the initial products, followed by chromatographic analysis coupled with mass spectrometry [2, 10-12]. Also, mass spectrometry has become an increasingly valuable tool in the characterisation of synthetic polymers, providing information regarding molecular weight, monomer sequence, and polymer chain terminal groups, among others. However, so far, the analyses of contaminants were done after extraction of the analyte, but these processes gave time-consuming incomplete extraction and can even downgrade the additive. A solution would be to find a way to analyse the additive directly from pieces of polymers.

Also, plastic wastes has become a huge issue all over the world as it represents 15 to 20% solid waste and in Europe 40% of the plastics are used for packaging [13]. Actually, more than one-third of the production of plastics is for single-use items leading to such an amount of wastes. Most of the plastics are petroleum

based and represents 8% of the global oil consumption. Moreover, due to all the stabilizing additives that are added to polymers during the manufacture process, their decomposition is very long. Hence, mixing natural antioxidant would be another way to use natural additives in polymer matrices. In fact, partially biodegradable plastics could be obtained by adding an organic material which is consumable by microorganisms in the plastic matrix: adding some natural additives seems to initiate a biodegradation process [14]. Nowadays, waste incineration is the only effective way to definitely get rid of nonbiodegradable plastics. 15% of the wastes are treated this way, however, this process releases huge amount of carcinogenic species and persistent organohalogens in the atmosphere. Recycling would seem to be a good alternative to incineration. However, it remains challenging nowadays due to, on one hand, the complexity of the sorting of the wastes leading to contamination jeopardizing the recycling process. On the other hand, the very complex and various compositions of plastics and their sensitivity to being reprocessed several times (leading to poor mechanical properties of recycled materials) make it, as well, very hard to reprocess as new materials. As a consequence, nowadays only 9% of the wastes are recycled.

The European union starts to act in order to limit the plastic impact on the environment with laws such as the prohibition project of disposable items (Q-tips, straws, plates...) which represents a huge part of marine wastes. In France, since 2017 single-use plastics bags are prohibited and by 2025 plastic containers intended to be used for cooking in public facilities (from day-care to universities) will also be banned.

As plastics are essential in our actual society, human health impact as well as the ecological issues brought by this material lead to the necessity to develop smarter plastic materials. This PhD was created in the frame of the FOODYPLAST project which is a cross-border European project in the POCTEFA program. The project is conducted by six partners located around the Franco Spanish border: Leartiker (Bizkaia), University of Zaragoza, University of Perpignan, IMT Mines Ales, University of Pau and Pyragena (Arzacq). The aim of the project was to produce a material which is environmental and human friendly. Actually, the Aquitaine region is one of the richest areas in terms of labelled products (AOC, IGP, Bio, Label Rouge...). However, those products are protected by traditional food packaging which, as said before, doesn't fit with the quality of the products they protect. We thus want to create a fully innovative and recyclable food packaging stabilised by natural and healthy additives. The migration of this healthy antioxidant will be fully controlled and analysed thanks to the development of a mass spectrometry method.

This thesis manuscript is divided into four chapters. In the first part, a state-of-the-art was carried out in order to better understand the current problems concerning food plastics packaging. In a second part, the methods used and developed for this study will be detailed as well as the raw materials. In the third chapter,

the results obtained from the formulations of our materials will be detailed and the mechanisms occurring within the materials will be discussed. Finally, in the last chapter, the phenomena of migration from packaging to the food have been evaluated and will be detailed as well as the application of a new method of mass spectrometry developed during this thesis.

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I. FOOD PACKAGING

Food packaging includes a broad range of materials among which plastics prevail. The pre-eminence of plastic in packaging compared to other materials comes from different aspects. First of all, it is very cheap and very light making it the best candidate for industrial scale transportation. Then, plastic offers a unique convenience as many of their intrinsic properties can be chemically or physically modified: from very flexible to stiff, from transparent to cloudy, from very thin to thick ... However, those materials are petroleum-based thus not biodegradable, hence leading to global wastes concerns and the associated environmental issues. In the recent years, fully biodegradable plastics made their appearance [15-17]. Two types of biodegradability have to be considered:


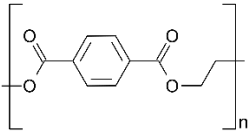

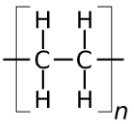

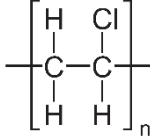

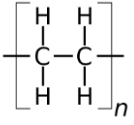

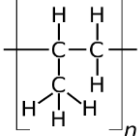

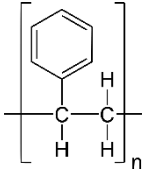

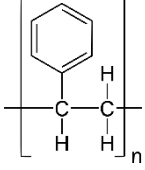

- In industrial conditions: polymers like Poly Lactic Acid (PLA) are considered biodegradable but only under specific industrial conditions (temperature between 55 and 70°C and humidity higher than 50% [18]). The problem of such a degradation system is that PLA is thrown in the “normal” plastic bin and nowadays’ waste sorting system doesn’t allow to separate PLA and Polyethylene Terephthalate (PET). As they are sorted out by gravimetry, plastics with close densities can’t be properly separated. As a consequence, PLA ($d=1.21-1.43 \text{ g.cm}^{-3}$) is a contamination of PET ($d=1.37-1.45 \text{ g.cm}^{-3}$) in the recycling process [19].
- In natural conditions: all the polymers that can be biodegraded in a domestic composter (starch-based polymers for example). In spite of their poor mechanical properties, those plastics seem to be a promising alternative to the traditional plastics. However, as they degrade by microbial attack, they can be contaminated by fungi, mould, or other microbes (which is not the case of PLA which degrades by hydrolysis [20]). More research needs to be done in order to use them as packaging (mechanical aspect) and safety assessments should be done to evaluate which molecules are created during degradation (to avoid contamination in the case of food packaging).

Above their environmental value, bio-based polymers have bad dispersion stability and limited mechanical properties [21]. Moreover, most of bio sourced polymers also need additives to be shaped and stable enough [22] and their conversion rate is quite low compared to polymers, such as polyolefin, which involves remaining monomers in the plastic film (i.e. more toxic molecules able to migrate to the food). The migration of contaminants from the packaging to the foodstuff highly depends on the type of food, the type of polymers and the type of additives used to stabilise it [23, 24].

I.1. Traditional plastic packaging

An international classification exists for packaging used in the food packaging. It uses numbers to indicate to consumers, the type of plastic they are using. This classification is detailed in **table I-1**.

Table I-1. Plastic classification and some of their applications.

Pictogram	Monomer structure	Polymer	Example of applications
		Polyethylene terephthalate (PET) - ($d=1.4 \text{ g.cm}^{-3}$)	Water and soft drinks, salad containers, biscuit trays ...
		High Density Polyethylene (HDPE) - ($d=0.96 \text{ g.cm}^{-3}$)	Milk bottles, freezer bags, shampoo, detergent bottles ...
		Polyvinyl Chloride (PVC) - ($d=1.49 \text{ g.cm}^{-3}$)	Cosmetics, cling films, vegetable oil bottle, plumbing, insulation of electrical wires...
		Low Density Polyethylene (LDPE) - ($d=0.92 \text{ g.cm}^{-3}$)	Rubbish bags, flexible wrap, squeezable bottles...
		Polypropylene (PP) - ($d=0.905 \text{ g.cm}^{-3}$)	Microwave dishes, lunch boxes, bottle caps, disposable syringe...
		Polystyrene (PS) ($d=1.05 \text{ g.cm}^{-3}$)	Water disposable cups, trays, packing materials...
		Expanded Polystyrene (EPS) ($d=1.05 \text{ g.cm}^{-3}$)	Foamed polystyrene, hot drinks cup, take away clamshells...
	-	-	Multi material packaging

Among those polymers, the most used are polyolefin (including HDPE, LDPE and PP) [25]. **Figure I-1** shows the worldwide production of different polymers in 2015.

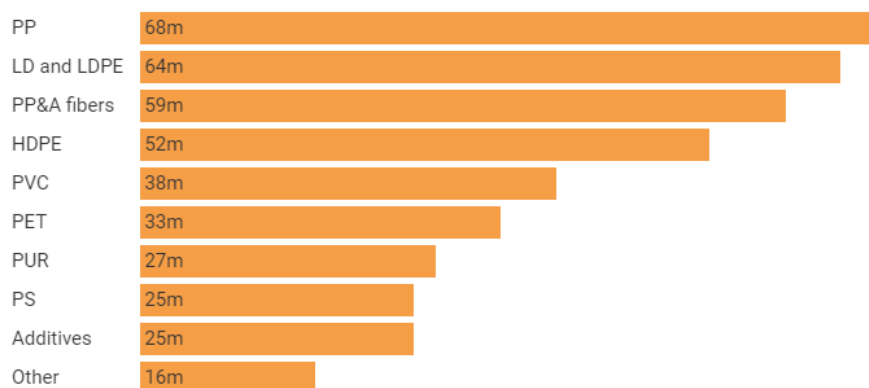


Figure I-1. Global production of different polymers in 2015 [26].

Several reasons can explain the dominance of polyolefins. First of all, they are the lightest of all the synthetic polymers depicted here (see densities in **Table I-1**). Then, polyolefins are hydrophobic macromolecules and highly resistant to water, air, solvents... Things that they can meet during their processing and lifetime. Finally, they are easy to shape and very resistant as packaging. Nevertheless, the recycling of those polymers is not that straightforward as polyolefins are very sensitive to oxygen and heat and as they can be easily contaminated by food for example. Also, due to their properties, polyolefins are often used with other materials in multi-layers packaging which are impossible to recycle [27].

PVC and PET are as well produced a lot, even though PET is more used in the food packaging field. In terms of migration, the polyethylene terephthalate is more and more controversial as it is often related to the presence of phthalates coming from its polymerization process [28, 29]. Phthalates are usually used as plasticizers (especially in PVC) but can also be chemical derivatives from esters (like PET). Some phthalates have shown to be endocrine-disruptors and toxic for the male reproductive system in rodents [30]. Moreover, PET is often used as bottle for drinking water, however it hydrolysis very easily leading to the easy migration of contaminant in water [25]. In the case of plastic made of polystyrene, the frequent risk is the release styrene oligomers or monomers that are known to be endocrine disruptors [31]. Like PET, PVC can be formulated with phthalates which release is very dangerous and controlled by legislation [32]. In the case of polyolefins, the main migrating species are additives: due to their very high conversion rate (conversion of monomers to polymers during polymerization) almost no monomer residues can be found in the final polymer. Also, the polymerization process is very well known (it was invented in the 1930's) resulting in no catalysts traces remaining in the polymer. For HDPE for example, the potential migrating species are antioxidants including nonylphenol, an endocrine-disrupting compound used as a plasticizer [33]. However, migration phenomenon from HDPE remains limited and are not really considered as a potential health risk. The same observation can be done for LDPE and PP for which the migration rates are quite low as compared to the legislation.

I.2. Wastes management

I.2.a. Landfill

During the last 60 years, landfill has been the traditional way to manage plastic wastes. However, being non-degradable, the accumulation of millions of tons of plastics through the years made landfill saturated. Landfill site, if well-managed, isn't a harm on a short-time scale but for very long periods soils and water contamination can occur .

I.2.b. Incineration

Incineration of plastic wastes remains the only way to completely get rid of them. However, hazardous substances (furans, dioxins, hydrocarbons...) are released during the process which makes it riskier than landfill deposition. Exceptions can be made for some countries which possess specific incinerators infrastructures (Japan, Denmark or Sweden) [34]. Also, the energy released during incineration can be exploited and allow to deal with very complex plastic formulations (which is a challenge with other methods).

I.2.c. Reusable plastics

With the increase of plastic production, single-use products have little by little replaced the re-usable glass containers that were once common. In Germany, the deposit system is very wide-spread leading to a lower amount of wastes per inhabitants (see data in **fig I-2**). As a consequence, legislation starts to be created to avoid those disposable plastics and creating products with longer lifetimes would definitely be a suitable way to manage wastes.

I.2.d. Recycling

Recycling is a very complex process that can be divided into 4 categories:

- Primary recycling which consists in reprocessing a material into an equivalent one with close properties;
- Secondary recycling which means reprocessing a material into one with different properties (generally less stringent);
- Tertiary recycling also called "depolymerisation" and which intend to recover chemical components;
- Quaternary recycling which is the energy recovery described above.

In theory, primary recycling could be possible for the polymers described in part I.2.a. However, the fact that they are blended with different materials (whether they are other polymers, same polymer but with different grade, several additives, metals...) makes it very challenging and often downgraded to secondary recycling. To successfully achieve a primary recycling, all sorts of contaminations should be avoided and the polymer being re-processed should be efficiently stabilised to avoid degradations. Also, when products are called "recycled"

(whatever the type of recycling) less than 10% average is actually from recycled source. The 90% rest are virgin polymer. The EU stated that by 2025, at least 25% and 30% by 2030 of recycled materials should be found in plastic bottles and 50% of domestic wastes should be managed [35]. Most of the Europeans countries are still very far from this value as shown by the diagram from **figure I-2**.

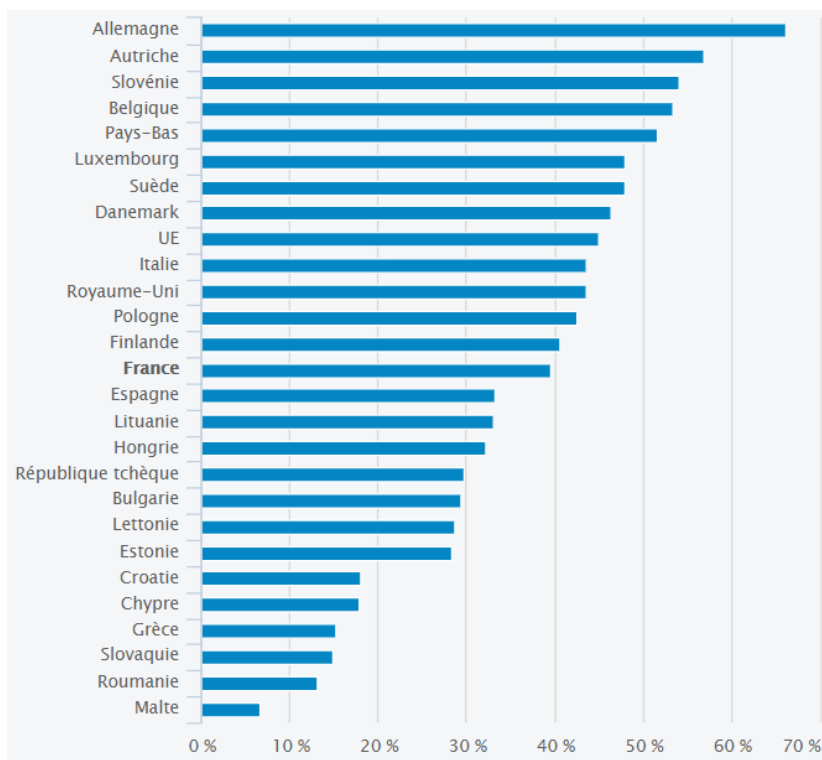


Figure I-2. Recycling rates of domestic wastes in 2015 (source: Eurostat).

Moreover, recycling is not just about the mechanical process, it also involves the whole systems of wastes collection: it is called an open-loop recycling. Actually, when domestic wastes are properly sorted out, it allows a relatively easy recycling whereas when plastic a badly sorted out, they act as contaminant disrupting the whole process [36]. **Figure I-3** shows the journey of our wastes from our homes to a new product.



Figure I-3. Plastics recycling process.

Once collected (step 1 and 2), wastes are taken to a facility where they will be sorted out according to their type by flotation (see the polymers densities in **Table I-1**). The sorted plastics, now considered as a raw material, are packed together and sent to plastics processors (step 3). There, plastics are pelletized and washed to remove big residues remaining from their former lives (adhesives, food, ink...) (step 4). The shredded polymers are then ready to be melted all together through the critical extrusion step (step 5). This step is the challenging one because even though plastics were sorted out according to their structure, they were not sorted out according to their formulation (i.e. their original function). For example, polypropylene coming from electronic devices contain high amount of flame-retardant additives which usually contain brominated species. Those species are prohibited in toys, medical devices or food packaging due to their toxicity. However, those polypropylenes will be blended with polypropylene coming from food packaging, medical devices, cars... and turned into a new product (step 6). First of all, those polypropylenes are very likely to be different grades (from injection grades with high viscosities to extrusion grades with very low viscosities), different structures (isotactic, atactic or syndiotactic, see part II) and different composition (additives, solid fillers, multilayers...) which makes their compatibility very uncertain. If the melted-state blend can be successfully achieved, other issues appear. The newly extruded material will end up being contaminated by brominated flame retardants and all the other components presents in all the polypropylenes [37]. The previous example is applicable to all the polymers from **Table I-1**, even to a higher extent for some of them. Hence, with the increase of the use of recycled materials, contamination can reach almost any domains [38, 39].

1.3. Active packaging

One of the main functions of food packaging, whatever its type, is to protect the food it contains and to extend its shelf life. Actually, food is made of very sensitive compounds that easily suffer from physical, chemical and biological deterioration which will influence the quality of food. Food can be affected by almost every variable in the environment: change in temperatures, light, oxygen, radicals, change in the humidity, living organisms... [40]. The broad range of degradation pathways that the food can undergo is thus very wide. Even though food degradation is a complex process involving lots of different reaction, oxidation remains the main source of food deterioration. The most important oxidation concerns lipids which spontaneously react with oxygen (auto-oxidation). To delay this auto-oxidation reaction, additives (antioxidants, conservatives...) are often added directly to the food. However, the absence of preservatives is preferred to satisfy consumers requests and to respect the traditional food formulation. To avoid the extensive use of food additives, novel types of packaging appeared during the last few years [41].

To retard oxidation, for example, deoxygenation can be realised. However, the simple reduction of oxygen content only has partial effect on the control of oxidation reactions. Almost total control of these reactions can be achieved thanks to antioxidants. The so-called active packaging aim at stopping the passive use of packaging by providing deliberate interactions between polymer and food in order to extend their shelf-life [7]. In other words, it consists in incorporating an active agent into the packaging rather than in the foodstuff. Several types of active packaging exist among which antimicrobial packaging, moisture absorbers or antioxidant packaging.

Antimicrobial packaging was the first type of successful active packaging and aim at stopping the growth of microorganisms [42]. The antimicrobial agent must be in direct contact with the food (vacuum packaging for example but no headspace should be present) and is effective if the microorganisms are located on the food surface [43]. Another type of antimicrobial package is carbon emitters-based systems [44]. Actually, CO₂ is known to reduce the microbial growth resulting in better conservation of foodstuff. This technology enters in the family of the modified atmosphere systems (MAP) but remains quite complex, hence targeting specific applications.

Then, we can also find moisture absorbers' packaging. For food with elevated content of water, bacterial and mould growth are eased leading to a reduced shelf life. To control the water content in food packaging, moisture absorber systems involves absorbent polymers or porous systems for example [45].

Finally, antioxidant packaging acts by counteracting the oxidation of the food. The main pathways to effectively avoid oxidation is the incorporation of antioxidant or free radical scavengers' additives directly in the packaging. Many successful examples have been reported even when the antioxidant is not in direct

contact with the food [46]. This type of packaging can be applied to a broad range of food and application compared to the previously described packaging types which all have specificities.

Nowadays produced antioxidant polymeric films are mainly based on the use of commercial polymers such as polyolefins and/or polyester. The interactions between the polymer and antioxidants are a key point to estimate their affinity and thus their stability but also the number of antioxidants released to the food product. Some natural additives were incorporated by different ways in the plastic. Firstly, during the melting process allowing having antioxidant in the material from the beginning of its lifetime. In fact, it appears that the introduction of additives during the melt-process is the best way to disperse fillers in polymers. However, it requires high-temperatures which can degrade both additives and polymers generally causing the loss of huge amount of additives due to low heat resistance. Then, it can be incorporated as a coating at the package surface or by the introduction of the additive in an internal layer. Such active packaging system allow to concentrate their actions directly on the food surface and through the headspace around the food [47]. Finally, natural additives can also be incorporated in the polymer in the form of capsules: the natural additive is trapped into inorganic capsule which are mixed with the polymer during its extrusion (melt state) [48].

Until now, we've spoken about the very beneficiary effect that natural additives could have on food. However, this beneficiary effect is not limited to food conservation but also benefit the polymer. In fact, polymers can't be used without additives as they are all sensitive to UV light, temperature variations, humidity, among others. Hence, active packaging allows to formulate a safe packaging thanks to the use of natural safe additives that will benefit both the food and the polymer. The challenge is thus multiple: find natural molecules that handle high temperatures, formulate a material with enough good properties to be recyclable and not to obtain a single-use packaging and control the release of the additives at the food surface without changing the organoleptic properties of the food.

II. POLYOLEFINS

The low toxicity of component migrating from polyolefins (part I.1) as well as their intrinsic properties led us to focus on polyolefin polymers for our study.

Polyolefins, as indicated in their name, are olefin-based materials which means that they have alkene monomers C_nH_{2n} (ethylene, propylene, butylene). They are petroleum-based molecules. Most polyolefins are metallocene catalysed polymers which results in very pure and consistent resins with specific properties [49]. Polypropylene (PP) and Low-Density Polyethylene (LDPE) are the most known polyolefins and are commonly

used in packaging applications involving contact with food. Polyethylene is produced by polymerising ethylene (fig I-4.A) molecules and polypropylene by polymerising propylene molecule (fig I-4.B).

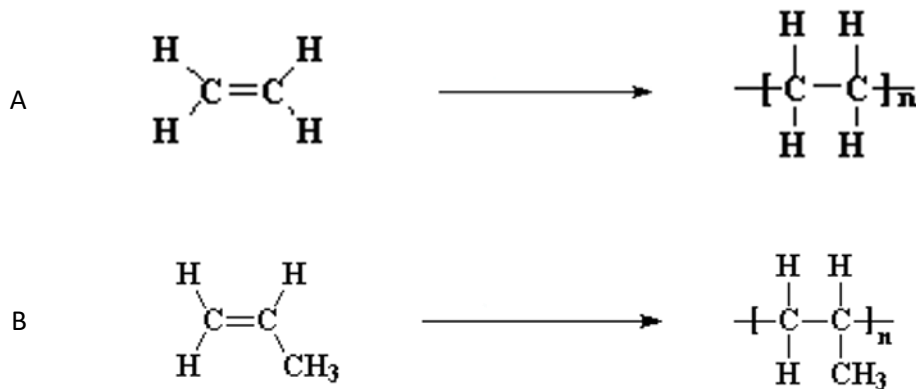


Figure I-4. A. From ethylene to polyethylene; B. From propylene to polypropylene.

They are semi-crystalline polymers (even though they can have amorphous versions). In semi crystalline polymers like polyolefins, during a cooling process the molecules pack together in ordered regions called crystallites. Linear polymers, having a very regular structure (PP or HDPE), are more likely to be semi crystalline. The ability of polymers to crystallize depends on their molecular structure. Hence, polyethylene can be crystalline or amorphous: linear PE can reach almost 100% crystallinity whereas the branched LDPE will have a much higher amorphous rate (usually around 30% crystallinity). Crystalline regions of the polymer are linked by the amorphous regions hence preventing the amorphous and crystalline phases from separating under any load as schematized on figure I-5 [50].

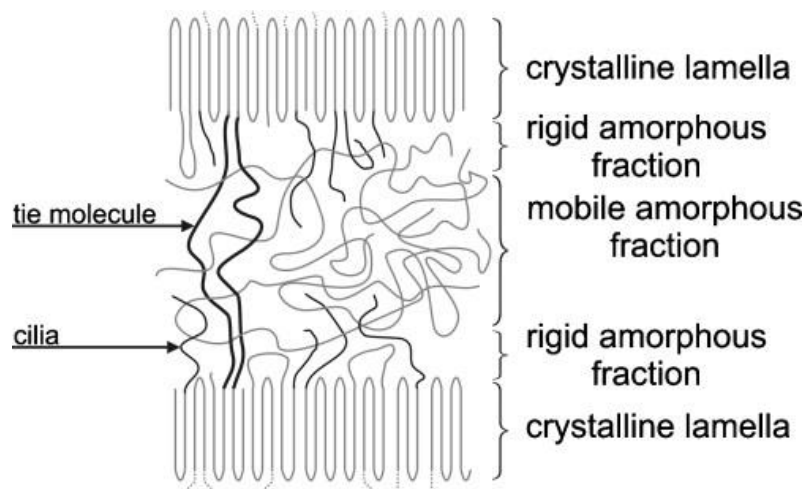


Figure I-5. Lamellar structure of a semi-crystalline polymer [51].

As a consequence, polymers like LDPE with high rates of amorphous areas have a higher flexibility than PP or HDPE. The crystallinity rate is thus central in the understanding of physico-chemical behaviours of polymers. Also, crystallinity is an important aspect of active packaging as the crystalline regions are less permeable to additives; they tend to go preferentially in the amorphous area. However, when migrating the crystalline regions act like obstacles that the additives have to face in their movement.

Some of the main physical characteristics of polyolefins are detailed in **Table I-2** and compared to those of PS and PET.

Table I-2. LDPE and iPP physico-chemical properties (average values) [52].

	Density (g.cm ⁻³)	Glass transition T _g (°C)	Melt temp. T _m (°C)	Crystallisation temp. T _c (°C)	Crystallinity χ (%)	Tensile strength (MPa)	Elongation at break (%)
LDPE	0.92	-100	120	95	15 to 85	10	400
PP	0.905	-5	165	110	0 to 80	30	80
PET	1.4	75	245	130	0 to 40	55	300
PS	1.05	92	240	210	0 to 50	35	2

What makes those polymers different from the other depicted above is there very low glass transition T_g (around -100 and around -10°C for PE and PP respectively). Glass transition is one of the most characteristic transition for polymers as it corresponds to the temperature at which macromolecules start to acquire movement. Below this temperature the polymer is brittle because the whole system is motionless whereas above this temperature the polymer is relatively flexible as chains can move from one another. Semi crystalline polymers' main transitions (versus amorphous' ones) are schematized in **figure I-6**.

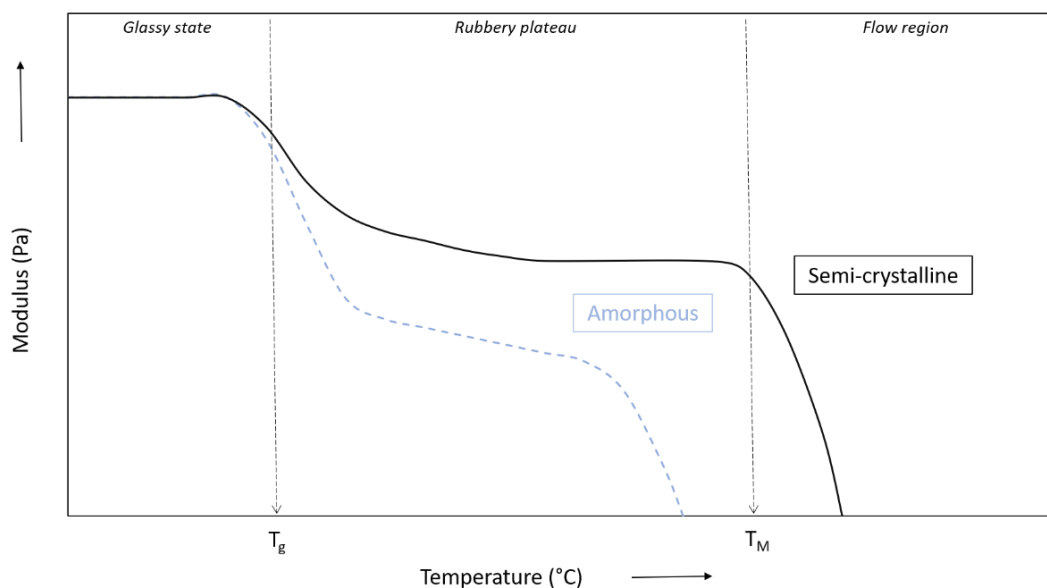


Figure I-6. Idealized modulus–temperature behaviour of amorphous and semi crystalline polymers.

Hence, polyolefins are very malleable at room temperature at the opposite of the other polymers from **Table I-1** which T_g are above 70°C . Also, polyolefin's melting temperatures T_M (110°C and 165°C for PE and PP respectively) are high enough for the polymers to be at a solid state at room temperature (the differences between polyolefin's T_g and T_M are huge compared to those of PET or PS due to polyolefins low chains relaxation, see **Table I-2**).

Both polymers are theoretically recyclable and can be reprocessed into many other products for many different applications, however they are not biodegradable.

II.1. Polyethylene

Polyethylene possesses one of the simplest molecular structure and is the largest volume commodity thermoplastic and cheapest packaging film. It was accidently discovered in 1933 and was first commercialized in 1939 for electric insulation. Since then, it has spread over a large number of applications such as food packaging, electronics, household items, industrial transportation... Its annual global production is around 80 million tonnes according to *PlasticEurope*.

Two synthesis processes can be distinguished: the “high pressure” one (1000 to 3000 atm between 80 and 300°C) and the “low pressure” one (1 to 50 bar around 70°C). The first process involves free radical initiators from ethene monomer. This pathway is the one used to produce Low Density Polyethylene and the high-pressure process involves the creation of numerous short or long-chain branching [53]. The second process (“low pressure”) is used for HDPE synthesis industrially known as Ziegler-Natta catalysis [54]. The structures of HDPE, LDPE and LLDPE (Linear Low-density Polyethylene) are described in **figure I-7**.

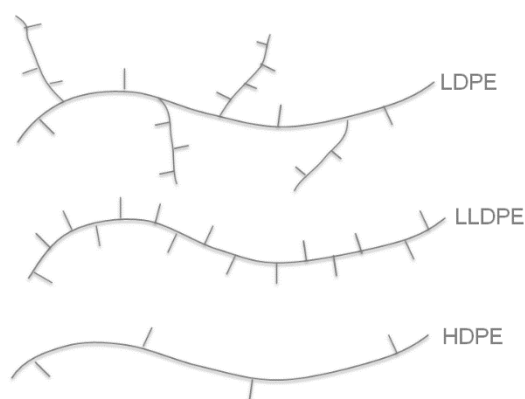


Figure I-7. Different structures of polyethylene.

The properties of polyethylene are highly dependent on the structure and the number of chain branches: higher crystallinity increases density, mechanical and chemical stability. The less the polymer chains are branched, and the smaller the molecular weight, the higher the crystallinity of polyethylene. The regularity of HDPE is directly related to its high crystallinity (as explained above). At the opposite, LDPE has a high degree of short and long-chain branching, which means that the chains have more difficulties to pack together into the crystal structure. Actually, crystallinity is around 35% for LDPE and 80% for HDPE. Within the crystallites, polyethylene has a density of $1.0 \text{ g}\cdot\text{cm}^{-3}$, in the amorphous regions of $0.86 \text{ g}\cdot\text{cm}^{-3}$. Thus, according to the crystallinity rate, there is an almost linear relationship between density and crystallinity [55].

II.2. Polypropylene

Polypropylene is another widely used polymer especially for packaging, stamping, textiles, reusable containers, medical devices, automotive... About 60 million tons of PP is produced every year according to *PlasticEurope*. It was commercialized shortly after Natta discoveries on catalysis: after having shown that it was possible to prepare polymers from ethylene groups, he showed that the same process could be applied to propylene in 1954. PP is chemically equivalent as LDPE but present a methyl group instead of the hydrogen of LDPE. The location of this methyl group gives PP different tacticities (**figure I-8**).

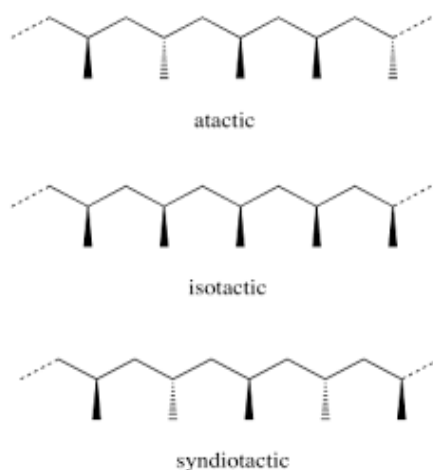


Figure I-8. Polypropylene different tacticities.

In the case of atactic polypropylene (aPP), the methyl groups are located randomly along the polymer chain. For the isotactic type (iPP) and syndiotactic (sPP), the stereochemical distribution is more regular as the CH₃ are all on the same side or alternatively located on one side or another. As explained before, crystallisation occurs more easily for regular structures, hence polypropylene crystallinity varies as follow:

$$\chi(\text{isotactic PP}) > \chi(\text{syndiotactic PP}) > \chi(\text{atactic PP})$$

Hence, aPP is almost amorphous showing wax-like properties whereas sPP and iPP are semi-crystalline (up to 40% and 80% crystallinities respectively). The isotactic version is the most widely used (>95% of the market) thanks to its rigidity and hardness.

Also, PP has a far better fat resistance than LDPE and is less prone to swelling, characteristics which make PP suitable for packaging of fatty food products. It has a higher melting point than PE, which makes it suitable for applications that require sterilization at high temperatures for example [56]. However, PP remains more sensitive to oxidation than PE.

II.3. Mechanisms of degradation

When exposed to oxygen, temperatures or other aggressive conditions, organic materials suffer from chemical modifications leading to a loss of their initial properties. This aging or degradation reactions can arrive throughout the polymer's life starting with their processing. Actually, during this first step, polymers has to face the combined effect from oxygen, elevated temperatures and important shear. Then during its service life, oxygen is always omnipresent as well as, depending on the application, temperatures (ex: cooking items), UV (ex: outdoor items) or humidity (ex: aeronautics). Those harsh conditions promote materials aging.

11.3.a. Thermo-oxidation

As indicated in its name, thermo-oxidation affects polyolefins when elevated temperatures are applied. The reaction takes place in three steps: initiation, propagation and termination (**figure I-9**).

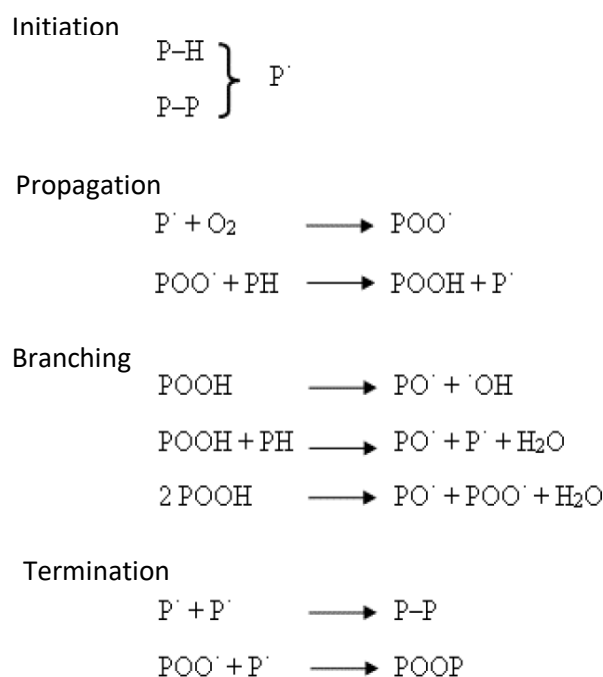


Figure I-9. Simplified thermo-oxidation mechanism of polyolefins [48] (P represents a polymer chain).

The degradation mechanism is initiated by the dissociation of a C-H bond followed by the intramolecular loss of a random atom of hydrogen. The exact origin of P^{\cdot} radical is still elusive and is often attributed to the presence of impurities or to defaults caused by the temperatures or mechanical shear [57].

The propagation step is composed by two reactions. First, the P^{\cdot} radical reacts with oxygen to give rise to an intermediate unstable peroxy: POO^{\cdot} . This step is extremely quick and the reaction between oxygen and the alkyl radical almost doesn't require any energy; it is thus extremely. Then, this reactive POO^{\cdot} forms a more stable species by the abstraction of a labile hydrogen from the polymer chain. The latter step is much slower than the previous one as it requires energy corresponding to the activation energy for C-H bond break. In turn, this step is the one determining one for the auto-oxidation reactions. The speed of the step diminishes as follow: hydrogen in alpha position of a $\text{C}=\text{C}$ > benzyl hydrogen or tertiary hydrogen > secondary hydrogen > primary hydrogen.

During the branching step, hydroperoxide decomposes in alkoxy radicals PO^{\cdot} and hydroxyls $\cdot\text{OH}$. Those radicals are very reactive and will engender new radical chain reactions. The decomposition speed is function of the temperature and can be accelerated by the presence of metals.

Finally, the creation of non-reactive species will end up the whole process. These species can be obtained by recombination of either two P• radicals or P• with peroxy radicals depending of the concentration of each species.

When polyolefins are melted and processed under elevated shear rates, C-C bond breaking occur leading to the creation of alkyl radicals. The mechanisms of creation of those radicals are detailed in **figure I-10** [58].

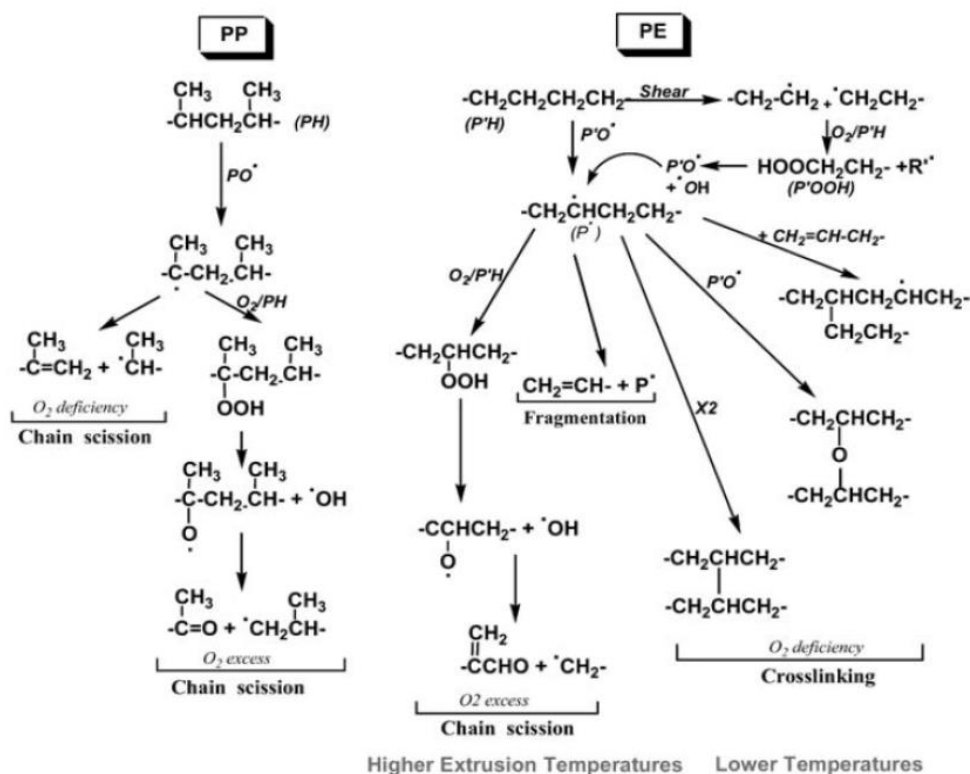


Figure I-10. Thermo-oxidation mechanisms of polyolefins.

Polypropylene degradations’ mechanisms are generally characterized by the creation of carbonyls groups, the creation of double bonds and chains scission leading to a loss of viscosity [59]. For polyethylene, the mechanism is more complex as it can lead either to chains scission or to chains ramifications [60].

II.3.b. Photo-oxidation

In most applications, polymers are exposed to UV lights which they are not naturally protected against. These types of reactions are initiated by the effect of photons on polymers. However, light absorption is necessary to engender photo-oxidation and polyolefins are non-absorbers as they don’t absorb radiations above 300 nm [61]. For polyolefins, light absorption is thus attributed to the presence of already oxidised species or impurities which play the role of chromophores. Generally, hydroperoxides and ketones are at the

origin of photo-chemical reactions [62]. Simplified mechanisms of polypropylene and polyethylene photo-oxidations are shown in **figure I-11** and **I-12** respectively.

The very first step of photooxidation is the hydroperoxide photolysis.



The created radicals are then involved in several chemical reactions such as hydrogen abstraction or reaction with oxygen leading to the formation of the **PO**[·] radicals. The latter will then be able to follow several pathways:

- Formation of hydroxyl groups (without chain scissions) by the abstraction of a hydrogen;
- Formation of aldehydes or alkyl radicals by fragmentation in β scission;
- Formation of ketones by reaction with a $\cdot\text{OH}$ radical.

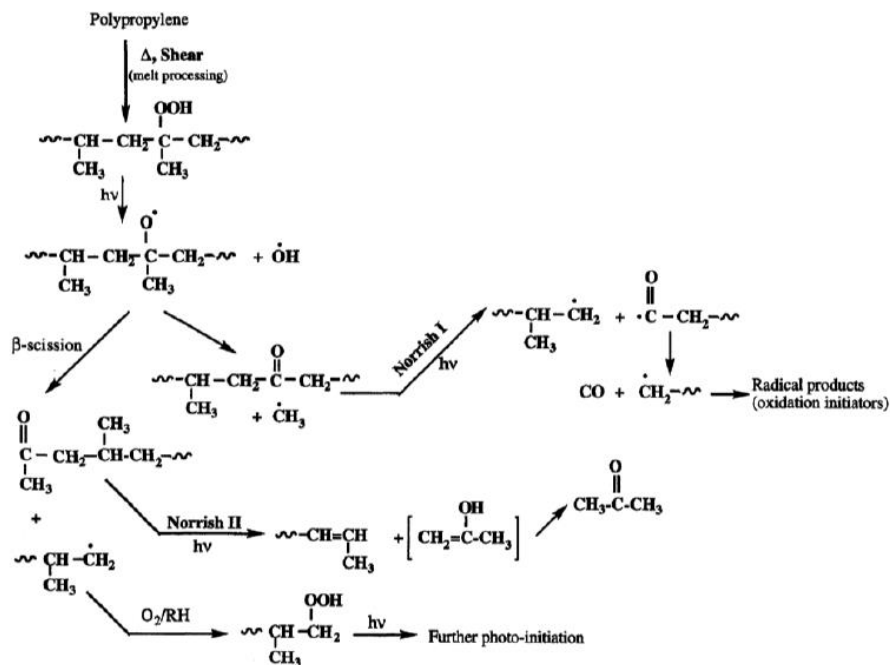


Figure I-11. Simplified photo-oxidation of polypropylene.

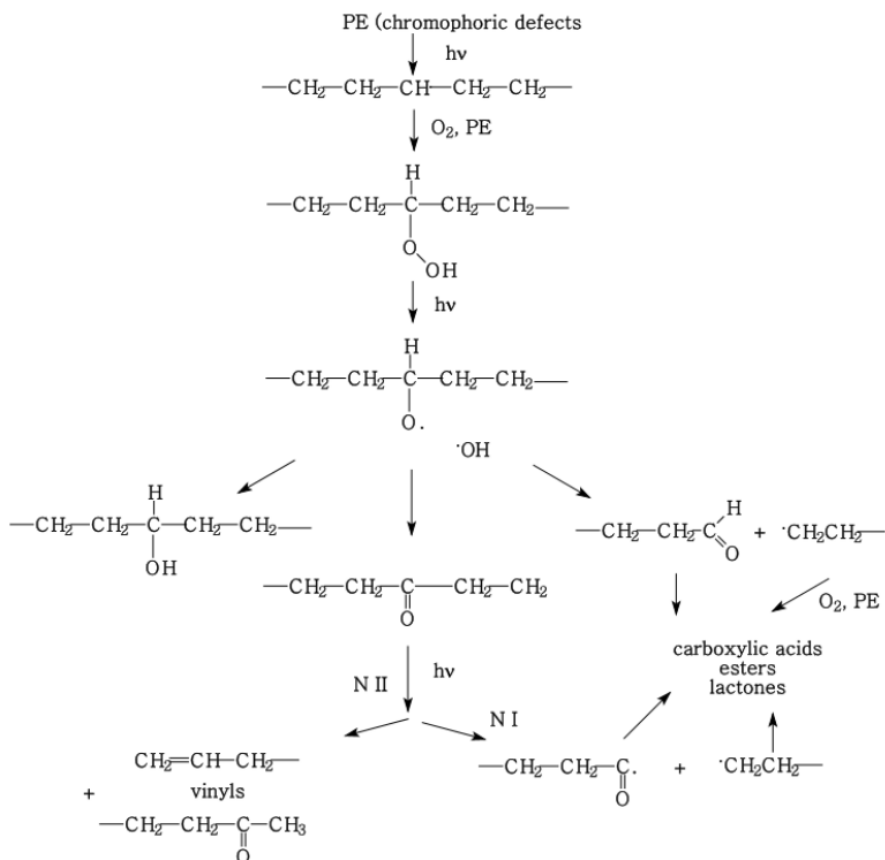


Figure I-12. Simplified photo-oxidation mechanism of polyethylene [57].

Ketones can further react through Norrish (NI or NII) reactions (described in **Annexe 1**) which specifically occur in photo-oxidation conditions. Those photo-induced reactions lead to the scission of polymers skeleton giving rise to new free radicals or rearranged sub-products (NI) or to vinylic-type unsaturation (NII) [63].

It is thus clear why additives are inevitable in polymers.

III. ADDITIVES

Additives are used to stabilise polymers from the previously described degradations. They are usually added in low amount (below 5%) and can scavenge radicals or the intermediate reactive products such as hydroperoxides.

III.1. Traditional synthetic additives

III.1.a. Additives used to prevent from thermo-oxidation

Several types of additives can be used in order to avoid thermo-oxidation reactions in polyolefins: hindered amine stabilizers (HAS), hydroxylamine antioxidants, alkyls radical scavengers, organosulfur [64, 65]... They all have their own stabilisation mechanisms. However, the most common stabilisers currently used are free radical scavengers and peroxide scavengers.

- Free radical scavengers (primary antioxidants)

Free-radical scavengers are usually hindered phenolic antioxidants and secondary aromatic amines which are used a lot in polyolefins [66]. They are very effective radical scavengers whether it is for processing stabilisation or service life (long-term) thermal aging. Free-radical scavengers, also called primary antioxidants, react with chain-propagating radicals (peroxy, alkoxy, and hydroxy radicals) and neutralize them thanks to a chain terminating step [67]. Their mechanism of action is described in **figure I-13**.

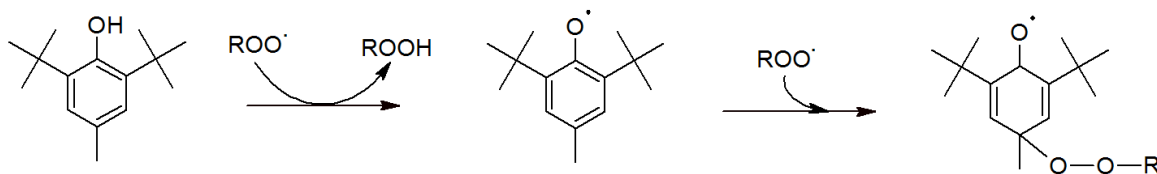


Figure I-13. Mechanism of scavenging oxy radicals [68].

Different phenolic groups with steric hindrance can be in position 2,4 or 6 of the aromatic cycle. The presence or not of those groups influences the reaction: for example, it was shown large substituents tend to prevent from the phenoxyl radical to react with the polymer [69].

These antioxidants generally act by giving hydrogen to the alkoxy and hydroxy radicals turning it into non-reactive alcohols or water. However, two sub-categories can be distinguished: first, the hindered phenols which reaction consists in the reaction between the phenol and the peroxy radical; then, the partially hindered phenols comprising a hydrogen close to the phenyl group which will participate to inter and intramolecular recombination.

Free radical scavengers are known under the commercial name of IRGANOX®. Also, BHT (Butyl Hydroxy Toluene) or BHA (Butyl Hydroxy Anisole) are very well-known primary antioxidants.

- Peroxide scavengers (secondary antioxidants)

Secondary antioxidants are usually organophosphorus compounds decomposing hydroperoxides (ROOH) into products before decomposing themselves into radicals [67]. They are thus often used in with another antioxidants (usually primary antioxidants) to achieve a synergistic inhibition effect. Their high reactivity makes them very good stabilisers for polymers in contact with high temperatures. However, their contribution is limited in time [70].

The most popular secondary antioxidants are phosphites (trivalent phosphorus compounds) which reduce hydroperoxides into alcohols and are themselves transformed into phosphates as shown in **figure I-14**.

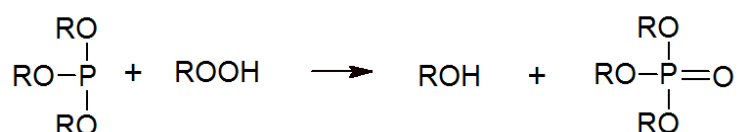


Figure I-14. Mechanism of peroxide decomposition.

III.1.b. Additives used to prevent from the photo-oxidation

The first visible effects of the previously described photo-oxidation reactions are usually located at the polymer surface with cracks leading to the quick degradation of the material properties. As explained before, polyolefins' C-C and C-H bonds do not absorb UV radiation. Nevertheless, pigments, impurities or degradation residues are usually present in the materials and act as an initiator for photodegradation. Among the stabilisers used to prevent from photooxidation, there are UV absorbers, quenchers, hydrogen donors or hydroxides decomposers [71]. The most commonly used are Hindered Amines Light Stabilisers (HALS) and UV absorbers.

- Hindered Amines Light Stabilisers (HALS)

Hindered amine light stabilizers (HALS) are one of the most used family of antioxidant for long-term heat protection of polymers. They were one of the first stabilisers as they were discovered in the 1960's and played an important role in the plastic development. They are efficient against both photo-oxidation and thermo-oxidation in most polymers, particularly for olefins [72]. HALS are derivative from 2,2,6,6-tetramethylpiperidine (**figure I-15**) and are commercially known as TINUVIN® (almost 30 different HALS structures exist).

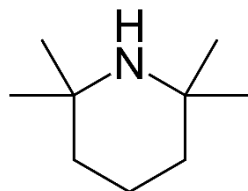


Figure I-15. Structure of the 2,2,6,6-tetramethylpiperidine.

Their mechanism of action is quite complex and still subject to researches [73]. This mechanism is based on a complex series of free-radical scavenging reactions, initiated by the HALS oxidation by reaction with peroxides which is a relatively slow step, and which is temperature-dependent reaction. This first step leads to the creation of a nitroxyl radical (RNO \cdot) and corresponds to the “activation” of the additive, thus responsible for its efficiency. The nitroxyl radicals will then react with alkyls and peroxy radicals yielding hydroxylamine ethers that can react with a broad range of components.

Nevertheless, they are not very efficient at elevated temperatures (above 80°C), as a consequence they are not very good processing stabilizers. Hence, HALS are often used in combination with primary and secondary antioxidants which combination can show synergistic or antagonistic effects. Due to unpredictable effects and lack of knowledge, complex mixtures of additives are often used.

- UV absorbers

UV absorbers are chemical compounds having intramolecular hydrogen bonds such as hydroxy-benzophenones or hydroxy-phenyl benzotriazole (figure I-16) [70, 74].

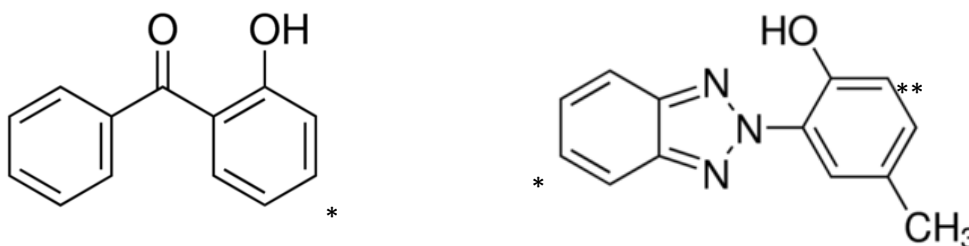


Figure I-16. Structure of hydroxy benzophenones (*=H or CH₃ up to C₁₂H₂₅) and hydroxyphenyl benzotriazole (*=H or C_nH_{2n+1} and **=CH₃ up to C₈H₁₇).

UV absorbers have a strong extinction coefficient (how strongly a substance absorbs light at a given wavelength) from 300 to 400 nm. When dispersed in the polymer matrix, the additive absorbs UV light and reaches an excited state. By intramolecular reactions, the additive goes back to its initial state without initiating unwanted side reactions. The main limitation of such additives is that they should be in the bulk to properly stabilize a polymer but UV radiation first attacks materials' surface. It is thus limited by the thickness of the sample.

They are commercially available under the name of CHIMASSORB[®] among others.

III.2. Actual additives controversies

Despite how essential all those additives are for polyolefins, their unavoidable diffusion to any medium they are in contact with leads to growing concern. Whether it is un soil, air or food, additives are potential contaminants. Hahladakis et al. [75], built a scheme of the impact of plastics and its additives throughout its lifetime that is reported in **figure I-17**.

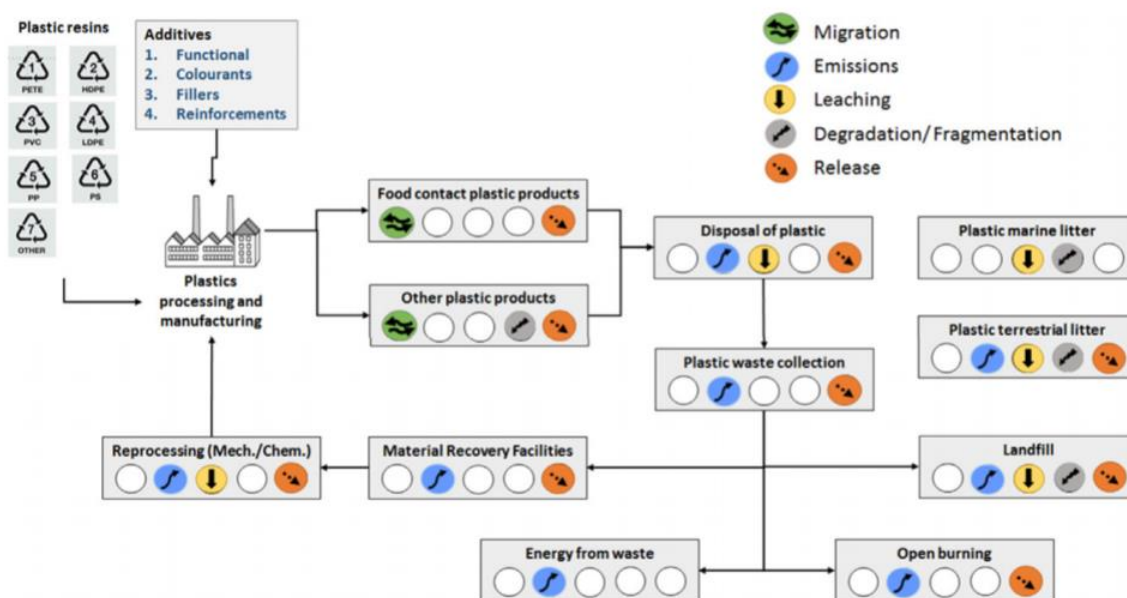


Figure I-17. Migration, release, fate and environmental impact of plastic additives during their use, disposal and recycling.

It is thus undeniable that additives can be a source of health risks. Of principal concern are, of course, endocrine disrupting molecules such as bisphenol A or di(2-ethylhexyl) phthalate (DEHP). Despite worldwide regulations and almost 60 years of research polymers' additives safety remains ambiguous.

In the case of bisphenol A (BPA) for example (a chemical used in the production of polycarbonate plastics and coatings/resins used in some food packaging materials), it was first thought to be easily eliminated by humans but it was proven that it actually accumulates in the body, in foetuses and new-borns and identified as an endocrine disrupter, immunotoxic, carcinogenic additive [76, 77].

Aside from the negatively famous BPA, other additives happen to show human health and ecological concerns. First, as most of the polymers are petroleum-based, they are flammable thus requiring the addition of flame retardants (such as plastics used in electronic devices). The most used flame retardants are brominated or fluorinated-based additives [78]. These compounds are not only controversial due to their repeated detection in the environment but also show similar toxicities as BPA [79, 80].

Phthalates (esters of phthalic acid) are also very monitored as they have shown to have a negative effect on the reproduction system of animals especially males [30]. Particular attention is given to diethylhexyl phthalate (DEHP) used as plasticizer in food packaging [81].

Other additives intended to be in contact with food showing uncertainties on their possible hazardousness are butylhydroxy toluene (BHT) and butylhydroxy anisole (BHA). They are among the most used additives in polyolefins and were reported in a variety of experimental studies. For example, when used in plastic materials, BHT and BHA are known to provoke contact urticaria and eczema. When orally administrated, acute flare of eczema was also shown to appear [82]. Also, studies mice which were fed with BHT (dose of 750 mg/kg bw/day) for 12 months developed lesion which were not observed on the control mice. In the same study, higher amount of BHT were given to mice (1000 mg BHT/kg and at week 4, one group was given 2500 mg BHT/kg feed; at week 8, another group 5000 mg BHT/kg) until 100 weeks. This resulted in an increased number of lungs tumours development [83]. Subsequently, studies on BHA's toxicity led the International Agency for Research on Cancer (IARC) conclude that there are evidences of carcinogenicity in experimental animals, but no data for humans [84]. As a result of these studies, BHT and BHA rates allowed to be in contact with food diminished all along the years but was never forbidden [83, 85].

However, phthalates, flame retardants or butylhydroxy species are only substantial portion of the additives allowed to be in contact with food that fails regulations as many chemicals haven't been fully characterized. Printing inks are a good example as there are no regulations (neither in the US nor in the EU) about their use in food packaging despite their suspected toxicities. Actually, the presence of 2-isopropyl thioxanthone or 4-methylbenzophenone (initiators for paint curing) were found in liquid milk for babies or in cereals [86]. Therefore, in 2005 the European Food Service Authority (EFSA) declared that the lack of toxicity data about those components no regulations could be set up (EFSA, 2005b). Two years later, the EFSA re-examined both ink derivatives and stated that their suspected toxicities should led to health risks assessment and strict regulations (EFSA, 2007).

Finally, even though, some of those hazardous substances were (or are being) prohibited, they remain persistent due to plastics recycling. Plastics from every field arrive to the recycling plants; they are sorted out according to the polymer type (polypropylene, polyethylene, polystyrene...) but not according to their use. Consequently, plastic e-wastes containing a lot of flame retardants for example can be blended with food packaging, toys, medical materials etc and reprocessed as new materials. As a consequence, only few is known about the composition of recycled material raising concerns about the diffusion of unknown species into food [87].

III.3. Natural additives

Accordingly with consumer safety and market trends researches to replace synthetic controversial additives by natural ones are more and more reported [41, 88, 89]. The incorporated of antioxidants additives have two main functions: avoid polymer degradation during processing and reduce food oxidation by migrating at their surface. The interactions between the polymer and antioxidants are a key point to estimate their affinity and thus their stability but also the amount of antioxidants released to the food product. Also, the problematic is to maintain good mechanical properties of the polymer.

Other drawbacks of natural additives are their limited resistance to high temperatures and long-term use. Therefore, they are rarely suited for extrusion which can lead to degradations and loss of properties. Solutions to counteract natural molecules fragility were investigated. For example, the encapsulation of natural molecules was extensively studied before their introduction in plastics. In this way active compound can extruded without compromising its quality as it often happens in the case of traditional extrusion. Also, it allows to have a controlled migration of the additive in the plastic and the food [12]. However, it requires more steps which represent additional costs and is incompatible with recycling [90]. Also, some natural molecules have strong organoleptic properties (essential oils for example) and could interfere with food sensory properties. However, the conservation of food sensory properties is essential as the decrease of sensorial characteristics causes the loss of food commercial value.

Among the natural antioxidants most (phenols, polyphenols, carotenoids, vitamins...) find their origin in plants and are highly present in spices, herbs, essential oils, fruits and vegetables. These natural compounds are usually, effective in the prevention and/or treatment of diseases caused by free radicals [6]. Subsequently, the use of natural antioxidants such as tocopherols, ascorbic acid or natural extracts with high levels of polyphenols (catechin, epicatechin, and resveratrol) are increasingly used as antioxidants directly added to food.

III.3.a. Vitamins

- **Alpha-tocopherol**

Tocopherol, also known as vitamin E, is a lipid-soluble antioxidant extracted from sunflower, wheat, palm oil and soybeans It is one of most effective radical-chain breakers in unsaturated fatty food. It is recognized as a safe food additive and known as E346. Vitamin E consists of a chromanol ring with an aliphatic side chain.

Vitamin E is made of two classes of compounds: tocopherols and tocotrienols, consisting of a saturated side chain or an unsaturated one, respectively. Each of these two classes are also divided in four subgroups of compounds: alpha (α), beta (β), gamma (γ) and delta (δ). Due to the three chiral centres of vitamin E, these alpha (α), beta (β), gamma (γ) and delta (δ) species can have up to eight stereoisomers (details are shown in **annexe 2**). All of these molecules show great antioxidant activities but α -tocopherol remains the most active chemically and biologically. The “synthetic” forms of vitamin E consists of mixtures of alpha-tocopherol’s eight stereoisomers, also called dl-vitamin E (**figure I-18**) [91].

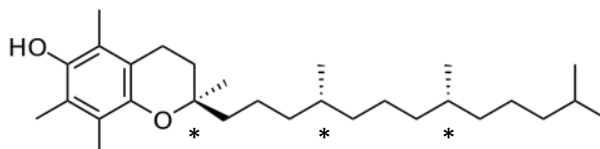


Figure I-18. Structure of alpha-tocopherol.

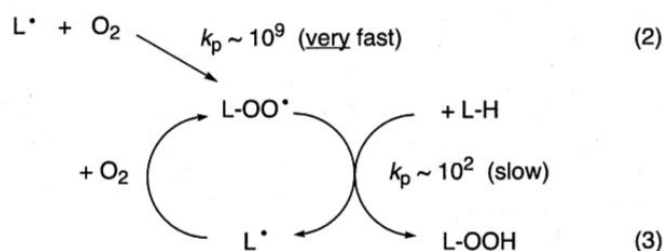
As an oil, alpha tocopherol is prone to oxidative degradation and is thus chemically modified by esterification. The addition of acetate ester group greatly improves the stability of alpha-tocopherol and prevents it from losing its antioxidant properties. Alpha-tocopherol can also be stabilised by being encapsulated [12].

The strong antioxidant properties of alpha-tocopherol are related to inhibition of radical chain propagation in lipids by its own conversion into an oxidized product, α -tocopheroxyl free radical. This radical is very stable due to the pair of electrons of the heterocycle and the π electrons of the aromatic cycle. The antioxidant reaction of α -tocopherol on lipids is shown in **figure I-19** [92]. The first step is the lipid auto-oxidation reaction which starts with the formation of the fatty acid radical (L^{\cdot}) (its hydrogen can be abstracted

(A) Initiation (hydrogen abstraction):



(B) Propagation (autoxidation chain reaction):



(C) Termination (antioxidant reactions):

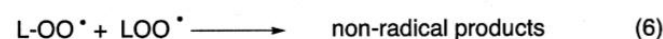


Figure I-19. Mechanism of action of α -tocopherol on lipids auto-oxidation reaction [92].

due to different agents (X) like metal ions, UV...) (1). The fatty acid radical immediately reacts with oxygen leading to the formation of a peroxy radical (2). This peroxy radical will be able to abstract hydrogens from other fatty acids (L-H): this is propagation of the chain (3). The reaction of the fatty acid radical with oxygen is very fast (it is only dependant on the diffusion of oxygen toward the fatty acid radical) and the abstraction of the hydrogen from another fatty acid is the slowest. Hence, the peroxy radical is the main target of the antioxidant. When in contact with α -tocopherol (TH), the peroxy radical abstracts a hydrogen forming a fatty acid hydroperoxide and a tocopheroxyl radical (T') (4). In fact, the higher the ability of the antioxidant to donate hydrogen to peroxy radicals, the better its antioxidant capacity. Other reactions leading to the termination of lipid auto-oxidation are the reaction of the tocopheroxyl radical with a fatty acid peroxy radical (5) or the dimerization of two peroxy radicals (6) [92].

RRR- α -tocopherol intake is considered safe up to 1000 mg daily for adults. Also, several studies have shown the beneficial effect of α -tocopherol on human disease. For example, several preclinical and clinical studies showed the preventive and therapeutic role for vitamin E in Alzheimer Disease [93]. Also, thanks to its ability to synergistically interact with other molecules, α -tocopherol helps maintaining a healthy cardiovascular system. Finally, beside its antioxidant properties, α -tocopherol has also shown to have effects on the immune system which makes it a potential anti-cancer candidate [91].

Thanks to those properties, alpha tocopherol can be used in both polymers and in food. Its use in polyolefins was extensively studied. In the case of LDPE, the use of alpha-tocopherol was compared to the use of commercial synthetic additive. Also, alpha-tocopherol's migration from LDPE into different food was studied and showed to be like the behaviour of commercial hindered phenol. It showed that after several extrusion passes PE/ α -tocopherol film has largely higher melt stability than the other films. However, several degradation products appeared during the process; seven different degradation products appeared from α -tocopherol (dimeric, trimeric and aldehydic structures). These degraded species could have a role in the stabilisation process [94].

Other studies displayed the efficiency of α -tocopherol as an antioxidant in LDPE through a controlled release from the package to the food. α -tocopherol properties appeared to be even more effective when it is complexed with a β -cyclodextrin (which is a host-guest complex with a hydrophobic cavity able to trap hydrophobic molecules) [12].

Also, vitamin E was compared to Irganox 1076 or to BHT which are commercial antioxidant currently used in polyolefins. The studies showed that the antioxidant capacities and thermal stability of packaging containing alpha-tocopherol is better than those containing Irganox or BHT for compressed-moulded samples [95]. However, for long-term stabilization of thin films the efficiency of α -tocopherol was less good than that of Irganox 1076 [96].

For PP, the first effect of alpha-tocopherol is a very effective melt especially at low concentration (it can be used at ¼ of the current concentration of use of common synthetic additive) [97]. Furthermore, studies investigated the differences between alpha-tocopherol-LDPE and alpha-tocopherol-PP films and showed differences between both materials [98]. It was shown that the retention of alpha-tocopherol in PP film is higher than that of LDPE films when in contact with food. The nature of the package influences the occurring interactions. Consequently, alpha-tocopherol mixed with LDPE offers active transfer of high concentrations of antioxidant whereas alpha-tocopherol mixed with PP leads to strong scavenging activity in the polymer material.

- **Ascorbic acid**

Vitamin C is a water-soluble compound that is essential for all humans and some mammals. Ascorbic acid, also known as vitamin C, is found in various foods like citrus fruits, tomatoes, kiwifruit, Brussels sprouts, strawberries... Prolonged storage or cooking may reduce vitamin C content in foods.

It was the first vitamin to be chemically produced and is one of the most effective and safe medicines needed in a health system [99]. Human body can't synthesize vitamin C itself, so it is an essential dietary component: it is essential for the protein metabolism, the biosynthesis of collagen, L-carnitine or certain neurotransmitters. Vitamin C is also a strong antioxidant and was proven to regenerate other antioxidants in the body, especially alpha-tocopherol [100].

Vitamin C refers to a mix of ascorbic acid (structure shown in **figure I-20**) and dehydroascorbic acid (DHA). Ascorbic acid is the in vivo form of the vitamin .

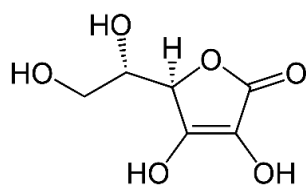


Figure I-20. Structure of ascorbic acid.

Ascorbic acid contains an asymmetric carbon atom that allows two enantiomeric forms. Among these enantiomers, the L-form is the natural one. Both enolic hydrogens gives ascorbic acid its acidic properties and allow to provide electrons for its reductant and antioxidant functions. **Figure I-21** details the possible chemical structures of vitamin C.

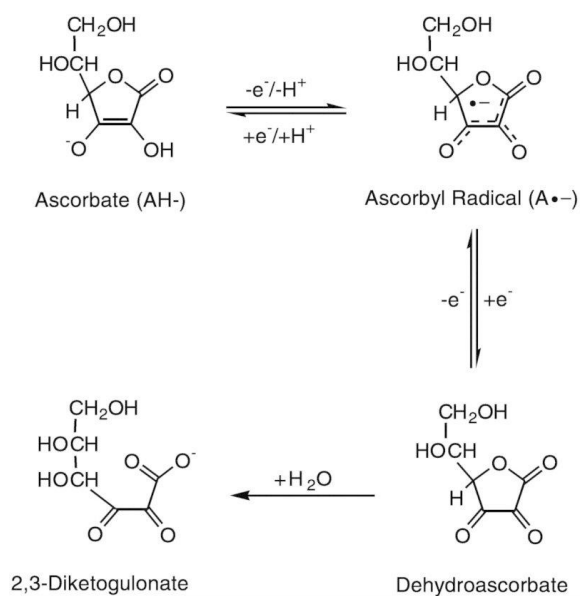


Figure I-21. Structures of vitamin C [102].

Ascorbate, by losing one electron, forms the ascorbyl radical which can dismutate into DHA (or into ascorbate) which also correspond to a two-electron oxidation product. The biological functions of ascorbic acid are based on its ability to provide reducing equivalents for a variety of biochemical reactions.

Ascorbic acid intake is considered safe up to 2000 mg daily for adults. Higher intakes can cause gastrointestinal discomfort, headache, trouble sleeping, and flushing of the skin. Beside its biosynthetic and antioxidant properties, it is very important for the immune system and enhance the absorption of nonheme iron (iron from plants) . Low vitamin C intake can scurvy (characterized by fatigue or lassitude, widespread connective tissue weakness, and capillary fragility) [101].

Due to its sensitivity to temperature, ascorbic acid was only rarely used in polymers. Few data can be found about ascorbic acid blend in Polyethylene glycol (PEG) which temperature of fusion is around 50°C . However, whatever its application, most of the time, encapsulation of ascorbic acid is achieved to prevent its degradation [102].

III.3. b. Phenolic compounds

Phenolic compounds can be found in plants and more often in their leaves, such as green tea leaves or olive leaves. Different spices and herbs also contain phenolic compounds, such as basil, cinnamon, ginger, oregano, rosemary... In this case, the phenolic species are mainly phenolic acids (gallic acid, caffeic acid). The phenolic compounds encompass the phenolic diterpenes, the flavonoids (catechin, quercetin...) and the

volatile oils (eugenol, thymol...) [103]. Generally, phenolic compounds are strong antioxidants thanks to their ability to provide hydrogens and electrons.

Researches showed that their use in plastic packaging could provide antioxidant and antimicrobial protection. For example, LDPE was blended with phenolic additives such as vanillin, turmeric and curcumin for their antimicrobial properties showing that curcumin and turmeric has efficient antimicrobial activity against all the tested bacteria. Moreover, as the selection of the additive is limited by its compatibility with the polymer matrix or by its heat instability, turmeric appeared to be a great candidate (rather high melting point) [42]. In PP, it was also shown that polyphenols (tannin esters) are good additives as they have minimal effect on PP mechanical properties and help prevent from PP oxidative degradation. Also, tannic acid, as tannic esters, delays the thermal degradation of the polymer and improves its processability. Hence, bio-based tannins could potentially substitute synthetic additives and lower plastic toxicity for human and hearth [22, 104].

- **Tannins**

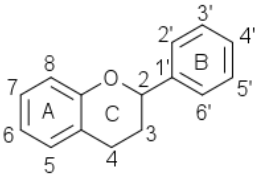
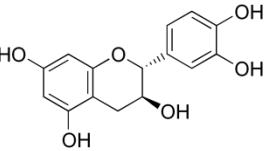
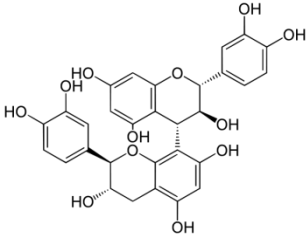
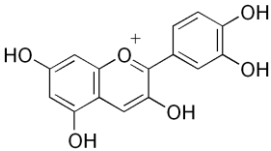
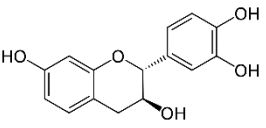
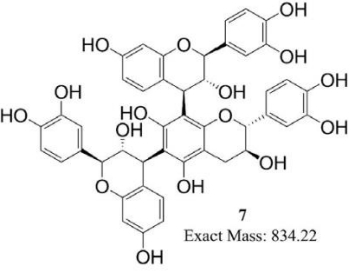
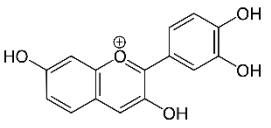
Tannins are a category of high molecular weight polyphenols that can bind and precipitate proteins, amino acids or alkaloids and to create inter and intra-molecular hydrogen bonds. Their role in plants is to protect them against their environment (UV radiations, oxidation and pathogens. Tannins are known for their antimicrobial and antioxidant properties but also for their nutritional value, for being anti-cancer... .

Two main families of tannins can be distinguished: the hydrolysable tannins and the condensed tannins. The hydrolysable tannins which hydrolyses when in contact with weak acids and decomposes at elevated temperatures to form pyrogallol (an irritating hepatotoxic compound). Within this family of tannin an additional distinction must be done between gallotannins (which hydrolyses into sugar and gallic acid) and andellagitannins (which hydrolyses into sugar, gallic acid but also ellagic acid).

The condensed tannins (also called proanthocyanidins) which is the most abundant source of plant-based polyphenols. They are oligomers of flavan-3-ol (catechin monomer) and/or flavan-3,4-diol as described in **table I-3**. Those monomers are bonded together by carbon-carbon bonds (C4 with C8 or C6 with C8) or C-O-C (more rarely). Those tannins are very hard to hydrolyse but can decompose into acid alcoholic solutions (this is the extraction process of the red pigments, called phlobaphene, from tannins) or depolymerized into anthocyanidols. For example, if the monomer is catechin (see line 2 in **Table I-3**), the corresponding proanthocyanidin will be catechol-(4 α →8)-catechol (also called Procyanidol B-3) as two catechin monomers are bonded the carbon 4 and the carbon 8, respectively. The latter can depolymerize to form the cyanidol. Another example following the exact same reasoning is illustrated in the 3rd line of **table I-3** for a fisetinidol

monomer leading to the formation of a trimer fisetinidol – catechin - fisetinidol. These tannins mainly exist as dimers, trimers, tetramers or oligomers with polymerization degrees from 5 to 11.

Table I-3. Description of condensed tannins.

Monomer units	Corresponding dimeric oligomer (condensed tannin)	Degradation products
<p><u>Flavan-3-ol</u></p> 	<p><u>Proanthocyanidol</u></p>	<p><u>Anthocyanidol</u></p>
<p>(+)-Catechin</p> 	<p>Catechol (4α→8) catechol (Procyanidol B-3)</p> 	<p>Cyanidol</p> 
<p>Fisetinidol</p> 	<p>Fisetinidol-(4b→8)-catechin-(6→4b)-fisetinidol</p>  <p>7 Exact Mass: 834.22</p>	<p>Fisetinidin</p> 

However, the chemistry of those proanthocyanidins is still elusive. Actually, in spite of their natural abundance (and their abundance in our diet) they are not extensively studied due to their complexity and their huge dispersity. Nevertheless, recent trends show otherwise as more and more studies deal with proanthocyanidins and their monomers in order to better understand their effect on humans, especially their immunomodulatory, anti-inflammatory, anti-cancer, antioxidant, cardioprotective and antithrombotic properties .

Condensed tannins can be naturally found in fruits, vegetables, nuts or beverages such as beans, almond, strawberries or red wine. From one species to another, the number of dimers, trimers, tetramers or oligomers changes as illustrated in **table I-4** [105].

Table I-4. Condensed tannins content in some food and beverages.

Quantity of proanthocyanidins in mg/100 g or mg/100 mL of product						
Product	Dimers	Trimers	4-6mers	7-10mers	Polymers (>10mers)	Total
Green beans	25.3	25.2	107.9	105.5	325.3	589.2
Almonds	9.5	8.8	40	37.7	80.3	176.3
Strawberries	6.5	6.5	28.1	23.9	75.8	140.8
Red wine	4	2.7	6.7	5	11	31.4

Another major source of condensed tannins is quebracho tree as it is constituted of 95% proanthocyanidins (mostly of profisetinidins as those described in **table I-3**) and 5% water soluble sugars. Quebracho (*Schinopsis lorentzii*) are very dense (density 0.9–1.3) trees native from south America. They produce either ordinary or warm soluble tannins (also called insoluble Quebracho) which are very rich in high molecular weight condensed tannins and barely soluble.

It was shown that the composition of quebracho sapwood and heartwood were different. The main difference is related to flavan-3,4-diol which are highly present at sapwood/heartwood interface and disappear when going in the centre of mature trees. Also, the increase of molecular weight from the edge to the central heartwood proanthocyanins revealed that the sapwood constitutes the raw material for the biosynthesis of the condensed tannins found in the heartwood [106]. The quebracho tannins have a close structure to that of grape tannins.

Nowadays, quebracho condensed tannins are mainly used for the wine industry, the production of tanned leather, adhesives with nucleophilic aromatic cycles, to replace formaldehyde in foams or in wood panels or as flocculant agent to remove surfactant in water treatment.

Condensed tannins are primary antioxidants as those described in III.1. a. Their mechanism of action in the human body is still elusive but researches proved that (contrary to hydrolysable tannins) condensed tannins cannot be degraded by micro-organisms possibly due to the structural stability of profisetinidins in quebracho. Subsequently, they cannot be found in organs such as liver and act indirectly by improving the antioxidant properties of tissues (leading to improvement of tissue antioxidant properties or by participating

in the regeneration of other antioxidant compounds through synergistic reactions). This way, antioxidant effect were found in different animal tissues and meat colour stability was proven to be enhanced [107].

The use of condensed tannins in polyolefins was, to our knowledge, never achieved. Nevertheless, other forms of tannins were used. For example, tannins esters shown great UV and oxidation inhibition with minimal effects on its mechanical properties [104]. Beside polyolefins, tannins ester was also used to stabilize polyhydroxyalkanoates (PHA) and provided effective thermal stability and improved the processability [22].

- **Green tea**

Tea, after water, is the most consumed beverage all over the world. Its composition is highly function of the climate, the season, the culture technics, the type/origin of the tea and its age. The most occurring component of green tea are catechins. A general catechin composition of green tea could be described as: (+)-catechin (1-2%), (-)-epicatechin (1-3%), (-)-epicatechin gallate (3-6%), (+)-gallocatechin (1-3%), (-)-epigallocatechin (3-6%) and (-)-epigallocatechin gallate (7-13%). Catechins are in vitro and in vivo strong antioxidants and is mixed with certain minerals and vitamins in green tea composition, thus increasing its antioxidant potential (see details in **Table I-5**).

Table I-5. Green tea beverage average composition.

Components of green tea beverage	Quantities (w%)
Catechins	30-42
Flavonols and other flavonoids	7-14
Theogallin	2-3
Ascorbic acid, gallic acid, quinic acid and other organic acids	5-9
Theanine and other amino acids	8-12
Methylxanthines	7-9
Carbohydrates	10-15
Minerals	6-8
Volatiles	0.02

The catechin content in green tea leaves is function of the age of the plant: catechins are the most abundant in young leaves. The catechins are essential for tea as they play a major role in the oxidation that the tea must face during its production. They are easily oxidizable which justifies their use as food antioxidant. They delay rancidity in lipidic foodstuffs by quenching free radical peroxide activity and proved to be more effective than BHA. In terms of scavenging effects (of some active oxygen radicals), green tea is more efficient than ascorbic acid or alpha-tocopherol; nevertheless, it is less strong in terms of hydroxyl free radical scavenging (antioxidant effect depends on the studied free radical system) [108].

In antic china, green tea was already considered as wholesome by traditional Chinese medicine. Green tea extract is a great source of flavonoids with the status of food additive, presenting considerable interest due to their potential benefits on human health as antiviral, antiallergic, anti-inflammatory, weight control, UV protection, antitumor, and antioxidant activities . All those beneficial properties allowed green tea to be in the list of beverages with functional properties [109].

The use of green tea in plastics is showing growing interest but its thermal properties remain limited: its stability during processing and storage can be dramatically reduced as it is readily oxidizable and sensitive to thermal decomposition. Green tea was used in maleic anhydride grafted polypropylene. The antioxidant was immobilized in the matrix thanks to interaction with maleic anhydride. The thermal stability improvement brought by green tea was relatively low but the oxidative stability was enhanced (however, they couldn't discriminate green tea and maleic anhydride to evaluate which one brought those properties) [110]. However, polyethylene with encapsulated green tea proved to provide effective stability to meat thanks to a controlled migration of non-degraded green tea [48]. Also, researches were done by directly adding flavonoids, catechin or quercetin for their antioxidant properties; nevertheless, they don't have the food additives status. On this way, green tea (official food additive) is a source of flavonoid and its antioxidant activity is well known. Its food protection activity was proven with no colour or odour effect even at high quantity, it even brings good stabilisation properties in polyolefin. Beside polyolefins, green tea was also mixed with other polymers such as ethylene vinyl alcohol polymer (EVOH) by extrusion giving rise to a brown and transparent film (sugar caramelization). The thermal process affected green tea's composition but didn't affect its antioxidant activity. Green tea led to an increase in the polymer glass transition, increased film rigidity and increased the crystallinity (act like a nucleating agent). Finally, the release of green tea extracts is function of the type of food: catechin have low compatibility with water, hence they are less released in aqueous simulant (diminishing antioxidant activity) than in ethanol. Also, the two main compounds released were gallic acid and caffeine (among 4 identified) following monitored kinetics [111].

III. 3. c. Vegetable oils

Vegetable oils activity against human pathogenic microorganisms and microorganisms that cause food spoilage have been studied a lot. Actually, vegetable oils contain polyunsaturated fatty acids, terpenoids and phenolic compounds which are known for their antimicrobial and antioxidant activities [112]. Subsequently, oils such as linseed oil, fish oil, olive oil are used as food additives. For example, lower lipid oxidation occurred in meat stabilised by linseed oil compared to fish oil.

Linseed oil and flaxseed oil are mainly constituted by triglycerides but distinctive for their unusual large amount of α -linolenic acid (omega-3), which has a distinctive reaction with oxygen in air. Both have the same origins: they are extracted from the flax plant (*Linum usitatissimum*). Linseed oil is extracted under a heating process whereas flaxseed oil is the cold extracted equivalent. Having a high content of di- and triunsaturated esters, these oils are particularly susceptible to polymerization reactions upon exposure to oxygen in air due to the chemical reactivity of the double bonds of the fatty acids: the double bonds (especially from α -linoleic acid) react with oxygen of the atmosphere. Subsequently, linseed/flaxseed oil can be used alone or mixed with other oils, with solvents, resins, as a plasticizer, in coatings, as a pigment in oily paints or in the manufacture of linoleum [113].

Linseed oil and flaxseed oil are complex mixture which compositions can vary easily according to several factors. A general average composition is given here:

- α -linolenic acid (51.9-55.2%), triply unsaturated;
- palmitic acid (about 7%) and stearic acid (3.4-4.6%), saturated acids;
- Oleic acid (18.5-22.6%), monounsaturated
- Linoleic acid (14.2-17%) doubly unsaturated.

Like green tea, linseed oils were already considered as healthful by traditional Chinese medicine. The polyunsaturated fatty acids like linoleic acid plays an important role in diminishing cholesterol and maintaining serum lipids. However, excess consumption of linoleic acid can have the opposite effect: increase the vulnerability of lipids to peroxidation. Also, linseed oils are very fragile and can be almost toxic when it is too degraded (release of a strong unpleasant smell). For these reasons, the mixing of other antioxidants with linseed oils is much recommended to counteract the oxidative degradations: alpha-tocopherols or ascorbic acid have shown to stabilise linseed oils .

To our knowledge, no food plastic packaging was realized using linseed or flaxseed oil as antioxidant. However, linseed oil was used as a coating in an active paper intended to pack fruits. The use of such oil offered

strong antimicrobial properties [112]. Other applications of linseed oil for polymeric materials is the use of the latter as raw materials to prepare thermoset resins and polymers.

III.3.d. Synergistic effects

As said before, linseed oils should be blended with other antioxidant for better stability. This doesn't correspond to a synergistic effect. Synergistic effects can be defined as the interaction of two or more entities to produce a combined effect greater than the sum of their separate effects.

For example, secondary antioxidants (part III.1.a) decompose themselves in radicals after having reduced hydroperoxides. In turn, they are often combined with primary antioxidants to achieve an enhance synergistic inhibition.

A famous example of such a synergetic effect is the *in vivo* reaction between α -tocopherol and ascorbic acid . As described earlier, α -Tocopherol is one of the strongest antioxidants employed in our cells providing protection against lipid peroxidation. On the other hand, ascorbic acid is one of the main water-soluble antioxidants. *In vivo*, the reaction occurs at the surface of the membrane where the two incompatible antioxidants can meet. **Figure I-22** schematize the cyclic reaction between α -tocopherol and ascorbic acid [114].

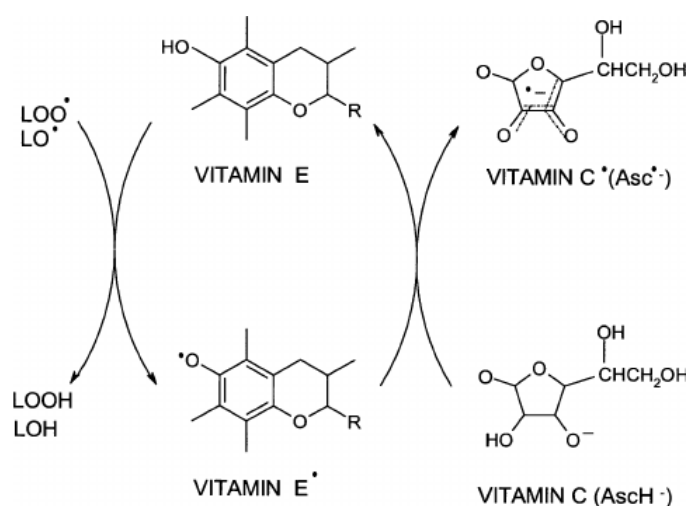


Figure I-22. Regeneration of vitamin E through one-electron oxidation of vitamin C.

The reaction starts with the creation of α -tocopherol radical by giving its labile hydrogen to a lipid species ($\text{LOO}\cdot$ or $\text{LO}\cdot$): the tocopheroxyl radical (vitamin E \cdot). The oxidised vitamin E is then regenerated into its initial form thanks to the reaction with ascorbic acid (which oxidizes by giving one of its labile hydrogen).

α -tocopherol also proved to synergistically react with green tea and more specifically green tea's polyphenols (see part III.3. b). Also, green tea contains ascorbic acid, hence it is likely that the synergism between α -tocopherol and green tea is a three-component reaction. It was found that the mechanism is very close from the one previously described as green tea reduce the α -tocopheroxyl radical and regenerate α -tocopherol when mixed in in sodium dodecyl sulphate micelles. The reaction proved to inhibit linoleic acid peroxidation and reduce the loss of α -tocopherol [115].

Nowadays polymer industrial processing employs cocktails of commercial additives such as hindered phenols with phosphites or phosphonites. Studies showed that by replacing hindered phenols by α -tocopherol, synergism was obtained. The Synergistic mixtures between α -tocopherol and the commercial phosphite showed increasing melt stabilisation. Moreover, presence of phosphite lowered the α -tocopherol loss during the extrusion process. Then, in terms of colour stability, α -tocopherol is not as efficient as the synthetic additive at high concentrations, hence the synergetic reaction with phosphites helped maintaining the colour stability [56].

The use of antioxidants is thus an effective and essential way to prevent or minimize the oxidation phenomenon in food products, delaying the formation of toxic oxidation products maintaining the nutritional quality and prolonging their shelf life. To choose the most effective stabilizer package, it is important to know what temperature range the polymer will be exposed to. A good stabilizer mix should protect the plastic during both processing and during lifetime when exposed to its upper service temperature. However, the release of any species from the plastic to the food must be monitored.

IV. MIGRATION

Due to their growing presence, the concerns about polymer-related risks are more and more studied [116, 117]. At the same time, the development of new polymer-based materials requires their characterization and safety control. Medical applications, food contact materials, children's toys, plastic wastes, among others, are fields that are particularly monitored and, as explained before, with the increase of the use of recycled materials, contamination can reach almost any fields [38, 39]. More and more attention is focused on those additives as they can easily diffuse throughout the polymer and be in direct contact with human beings [118, 119]. However, beside additives, other (so called non-intentionally added species) can represent hazards, coming from many different sources. Polymers and additives are sensitive to high temperature and thus degrade when they are processed: as polymers are manufactured at very high temperature severe degradations may occur, producing smaller molecules and creating residues, pollution and odour. Also, some of these pollutants can be post polymerization residues (by-products, catalysts, monomers...) or can come

from the different processes that the polymer as to undergo in its life such as recycling or dishwashing (reactions with chemicals). Moreover, even if some additives are not harmful in their initial state, their degraded version or metabolites could be an additional danger. These various origins of contaminants make plastic final composition hard to predict. Therefore, polymeric materials should be carefully analysed to assess the presence of species able to show any toxic effects.

IV.1. Diffusion principle in food packaging

Migration can be defined as the mass transfer (desorption) from the packaging material to the packed food under given environmental conditions[120]. Migration results in interaction between food and plastic leading to food composition modifications. Two types of migration can be distinguished: the global migration and the specific migration. The global migration represents the total quantity of substances that migrate (known or not). It allows to reveal whether the packaging is inert or not. On the other hand, specific migration corresponds to the amount of a targeted migrating substance.

Migration depends on many parameters. In polymeric food packaging, migration is a consequence of the need to reach an equilibrium common to all the chemicals controlled by two mechanisms: diffusion and sorption. It follows several stages like the diffusion of the migrants in the polymer matrix (as they are small molecules, they easily move within the matrix), the dissolution of the migrant at the food-plastic surface and the dispersion of the migrant within the food (**figure I-23**) [121].

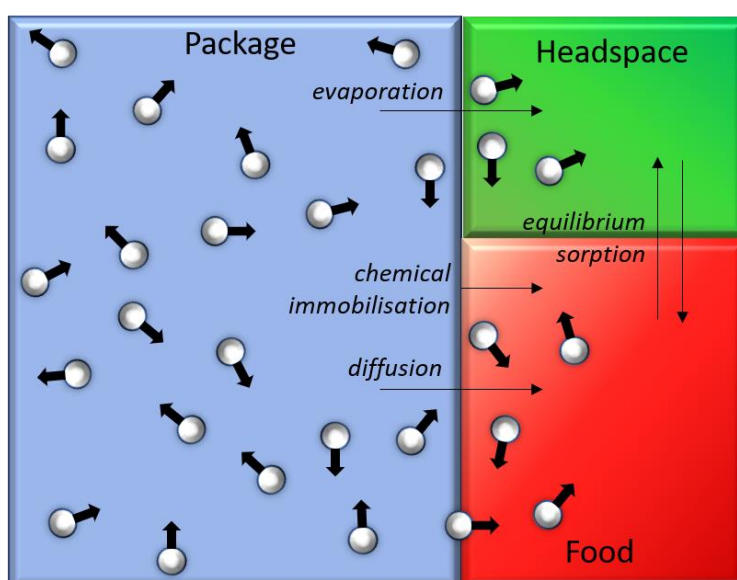


Figure I-23. Food packaging systems.

As shown on **figure I-23**, food packaging is either a package/food (diffusion) system or a package/headspace/food system (evaporation and equilibrated diffusion of volatiles compounds) [9].

The diffusion of migrant from the package to the food is controlled by the diffusion coefficient (D , cm^2/s) and depends on the molecular structure, the weight, the affinities between the packaging material and the migrant and the affinities between the food and the migrant [122].

The partition coefficient ($K_{P,S}$) corresponds to the quantity which migrated from the packaging to the food at the equilibrium. It depends on the solubility and the affinities between the polymer and the food (or food simulant). It can be mathematically described as the equation (1) where $C_{P,\infty}$ ($\mu\text{g}/\text{cm}^3$) is the ratio of the concentration of the migrating species in the film and the simulant and $C_{S,\infty}$ ($\mu\text{g}/\text{cm}^3$) at equilibrium.

$$K_{P,S} = \frac{C_{P,\infty}}{C_{S,\infty}} \quad (1)$$

The partition coefficient can be considered constant at low concentrations.

The diffusion phenomena is described Fick's laws of diffusion equation (2) [3].

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(-\frac{Dq_n^2}{l^2} t\right) \quad (2)$$

where $\frac{M_t}{M_\infty}$ is the ratio between the concentration of any substances at a time t and the concentration at the equilibrium; l is the thickness of the polymeric film; q_n is the non-zero positive roots of $\tan q_n = -\alpha q_n$ and α is the concentration mass ratio as expressed by equation (3).

$$\alpha = \frac{V_S}{V_P \cdot K_{P,S}} \quad (3)$$

where V_S and V_P are the volume of the simulant and the polymer respectively.

The equation (2) can be simplified under some circumstances to give equation (4): if the diffusion occurs in one dimension, for a limited volume of polymer and in an infinite volume of solvent ($\alpha \gg 1$ since $V_S \gg V_P$ and /or $K_{P,S} < 1$).

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{m=1}^{\infty} \frac{1}{(2m+1)^2} \exp\left(-\frac{D(2m+1)^2 \pi^2 t}{l^2}\right) \quad (4)$$

This equation involves that there are no strong interactions between the migrant and the polymer (the substances are dispersed in the matrix and not chemically bonded to the polymer).

Temperature is one of the parameters governing diffusion as it readily affects the migrant mobility within the polymer matrix and through the interfaces. As explained in part II, when the temperature is above the glass temperature (T_g) of the polymer (rubbery state), the polymer chains can move: the diffusion is eased. At the

opposite, when the temperature is lower than the polymer T_g , motion of the chains is strongly diminished (chains restrictions), the diffusion of migrant will then be longer. The temperature parameter doesn't appear in the previous equations but the diffusion (D) depends on it. Actually, the diffusion coefficient also follows an Arrhenius law (equation (5)).

$$D = D_0 \exp\left(-\frac{Ea}{RT}\right) \quad (5)$$

With D_0 the diffusion of a compound free of any obstacles, Ea the activation energy ($\text{J}\cdot\text{mol}^{-1}$), R the gas constant ($8,31 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$) and T the temperature (K).

Finally, for a Fickian diffusion of $\frac{M_t}{M_\infty} < 0.6$ under the same conditions as equation (4) and for a single substance homogeneously dispersed in the polymer film, the migration can be described by equation (6).

$$\frac{M_t}{M_\infty} = \frac{4}{l} \sqrt{\frac{Dt}{\pi}} \quad (6)$$

IV.2. Migration evaluation

IV.2.a. EU regulations for migration testing

Migration testing is based on specific regulation in order to ensure materials safety. Those regulations are provided by bodies such as the Food and Drug Administration in the US (FDA), the European Commission, the Mercado Común del Sur (MERCOSUR), the Japan legislations... Materials intended to be in contact with food cannot, under any circumstances, induce unacceptable changes in the food product (changes in the food composition, taste, odour), nor release substances to levels that could be dangerous for human health. In Europe, food packaging must respect the commission regulation (EU) No 10/2011 of 14 January 2011 on "Plastic materials and articles intended to come into contact with food". This guidance is based on the fact that no material is inert: when in contact with foodstuffs, materials always release substances. It thus provides a positive list of substances that are allowed in food packaging. However, many parameters can be involved when it comes to migration. Also, researched on the possible toxicities of some compounds is still ongoing as explained in part III.2. leading to regular update in the legislation.

Those regulation organizations give guidance and recommendations toward migration from food packaging, and more specifically guidance on how to simulate the migration process as close as a real case as possible. Beside the experimental way to evaluate migration, organisation like the EU commission allows the migration to be assessed in a theoretical way (thanks to the previously described equations). In terms of experimentation, the simulation of migration is realized according to the future use of plastic (type of food, storage time and temperature conditions).

- Migration cells

Different migration cells exist. They are all made of glass or unreactive materials. Among those migration cells, the first one is the two-sided migration cell also called “immersion migration cell”. It allows both sides of the material to be in contact with the food simulant (solvent) and to put one or several plastic sample in the food simulant (one sample can be risky in terms of reproducibility but too many samples can quickly saturate the food simulant). The other type of migration cells is the one-sided as it will only analyse one side of the plastic (usually the one in contact with the food). It is made of stainless-steel plates, one cylindrical ring which contains the solvent and two access ports (one for filling and another for air exhaust).

Aside from the type of cells, the plastics samples themselves are also concerned by the regulations. It is considered that 1kg of food is consumed daily by a person of 60 kg and that the food is packed in a cubic container of 6 dm² surface area. The real surface area of packaged food changes a lot: their surface area must be normalized before comparing testing results with migration limits. The value of migration is expressed in mg/kg applying a surface to volume ratio of 6 dm² per kg of food. The piece of plastic should be 6cm² per faces (the thickness must be taken into account, if not negligible), as schematized in **figure I-24**.

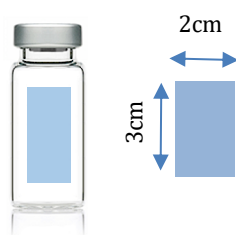


Figure I-24. Technical specifications for migration analysis.

Under those circumstances, the EU state that the overall migration must not exceed 60mg/kg of food (or 10mg/dm²) in the case of non-toxic species and specific levels are fixed for the other substances with specific migration limit (SML) which are established based on the available toxicity data of each specific substance.

The information about migration cells serves as recommendation and other testing methods exist but they are more specific.

- Food simulants

The solvents used in the migration cells are called food simulants. Although migration testing in real food prevails, for practical reasons it is, most of the time, tested using simulants. They are chosen according to the type of food that is going to be packed. The food simulant will mimic real food as described in **table I-6**. The letters RF assigned to some of the food stand for “Readjustment Factor”. The migration induced by some of the simulants can be too strong compared to the reality, in this case the migration obtained with the food

simulant should be divided by RF. Also, vegetable oil is a great simulant of fatty food, however, polyolefins have a high affinity with these oils and thus absorb them; for this reason, it is replaced by 95% Ethanol.

Table I-6. Specific assignments of food simulants as a function of the type of foodstuff.

Food simulant	Abbreviation	Examples of food assignments
Ethanol 10 % (v/v)	Food simulant A	<ul style="list-style-type: none"> - Sugar syrups, honey, - Fresh vegetables, peeled or cut, - Processed meat products (such as ham, salami, bacon, sausages, and other) or in the form of paste, creams, - Marinated meat products in an oily medium, - Mustard.
Acetic acid 3 % (w/v)	Food simulant B	<ul style="list-style-type: none"> - Non-alcoholic beverages, - Fruit in the form of purée, - Preserved meat in an aqueous medium, fermented milk, cream, processed cheese, vinegar...
Ethanol 20 % (v/v)	Food simulant C	<ul style="list-style-type: none"> - Clear drinks (Water, ciders, clear fruit ...), - Alcoholic beverages of an alcoholic strength of between 6 %vol and 20 %, - Fruit in the form of purée, Sauces With aqueous character, - Ice-creams.
Ethanol 50 % (v/v)	Food simulant D1	<ul style="list-style-type: none"> - Alcoholic beverages of an alcoholic strength above 20 % and all cream liquors, - Preserved meat in aqueous media, - Milk and milk-based drinks whole, - Processed cheese.
Vegetable oil (*)	Food simulant D2	<ul style="list-style-type: none"> - Pastry, biscuits, cakes, bread, and other bakers' wares (RF: 3), - Chocolate, and products coated with substitutes (RF: 3), - Animals and vegetable fats and oils (no RF), - Margarine, butter and other fats and oils (RF:2), - Processed meat products (such as ham, salami, bacon, sausages, and other) or in the form of paste, creams (RF:4), - Marinated meat products in an oily medium (no RF), - Preserved meat (RF:3), - Natural cheese without rind or with edible rind (RF:3), - Sandwiches, pizza ... (RF: 5).

▪ Migration conditions

Here again, the migration simulation experiments depend on the foodstuff application. For example, for ambient temperature use, the recommended test temperature is 40°C for 10 days. For cold use, the test temperature is 20°C. For accelerated testing, higher temperatures can also be applied. **Table I-7** details the conditions that should be applied for experimental migration testing.

Table I-7. Specific conditions of migration analysis assessments.

Conditions of contact in actual use	Test conditions
<u>Contact Time</u>	<u>Test Time</u>
t ≤ 0,5 hours	0,5 hours
0,5 hours ≤ t ≤ 1 hour	1 hour
1,0 hours ≤ t ≤ 2 hours	2 hours
2,0 hours ≤ t ≤ 24 hours	24 hours
t ≥ 24 hours	10 days
<u>Contact Temperature</u>	<u>Test Temperature</u>
T ≤ 5 °C	5 °C
5°C ≤ T ≤ 20°C	20°C
20°C ≤ T ≤ 40°C	40°C
40°C ≤ T ≤ 60°C	60°C
60°C ≤ T ≤ 100°C	100°C (or reflux temperature)
100°C ≤ T ≤ 121°C	121°C
121°C ≤ T ≤ 130°C	130°C
130°C ≤ T ≤ 150°C	150°C
T ≥ 150°C	175°C

Once extracted under specific conditions in the appropriate food simulant, the migrating substances must be analysed. Several methods can be employed to do so.

IV.2.b. Mass Spectrometry techniques for packaging characterizations

Liquid chromatography (LC), especially high-performance liquid chromatography (HPLC) is commonly applied to identify and quantify specific migrants in migration studies. The chemical compounds extracted from a polymeric film in the food simulant can be injected into an HPLC system for the quantification analysis. According to the chosen food simulant, additional dilution steps can be needed. The separation of the studied antioxidant from other chemical compounds in the extract is carried out a column which is installed into the HPLC equipment and designed to identify these compounds. Different types of columns are used as stationary phase and different mix of solvents are used as carried or mobile phases. The elution of the mobile phase in the HPLC system can be either isocratic or gradient depending on the composition of the sample and the detector used in the HPLC system. Various detectors are used for an HPLC, such as ultraviolet (UV) detector, fluorescence (FL) detection and mass spectrometry (MS) detection. This allows an optimal sensitivity for a wide range of analytes. MS detection is carried out by using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), and it provides more reliable identification compared to UV and fluorescence detectors.

IV.2.c. Ambient ionization methods for the direct analysis of polymer materials

So far and as explained above, the migration analyses were done after extraction of the analyte, but these processes gave incomplete extraction or downgrade the additive. An alternative would be to analyse the contaminants directly from pieces of polymers. All the mass spectrometry (MS) techniques provide answers to a full range of questions coming from stabilizers analysis in plastics. The progress in the soft ionisation techniques, such as Electrospray Ionisation (ESI) [123], matrix-assisted laser desorption/ionization (MALDI), Desorption Electrospray Ionisation (DESI) and Direct Analysis in Real Time (DART)[124], and large molecular mass range detection (time-of-flight MS) opened new possibilities to the polymer analysis offering not only complementary information to conventional methods but also new possibilities (surface mapping, on-site analysis...).

Actually, ESI uses high voltages to assist the transfer of ions from the initial solution into the gaseous phase by creating a liquid aerosol. The transport of ionic species from the initial solution to the mass analyser follows three steps: creation of the aerosol, solvent evaporation and ejection of the charged droplets (**figure I-25**). It is very useful, for example, for the study of macromolecules as it helps preventing from their tendency to fragment when they are ionized. For this reason, ESI is considered as a soft-ionization process. It differs from other ionization source as it is able to create multiple-charged ions hence broadening the mass range of the analyser (very useful for proteins analysis for example) [125].

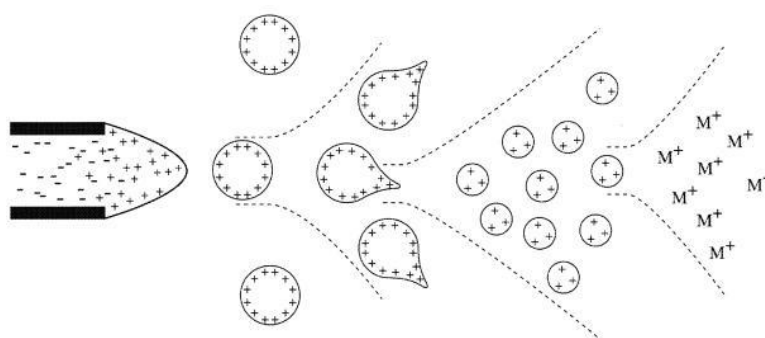


Figure I-25. Mechanism of electrospray ionisation (ESI).

Moreover, considering the increasing complexity of synthetic polymer-based materials and the huge diversity of additives used in polymer formulations, it is necessary to develop adapted ways to characterize those complex materials and more specifically their surface. Actually, the contaminants always have lower viscosities than the polymer they are contained in; hence they tend to be located at the surface of the material (viscosity segregation) [126]. Also, another type of segregation within the polymer matrix is based on architectural differences. For example, in the case of two polymers of the same chemical unit but with different

chain architecture (linear versus cyclic), the surface enrichment occurs as a function of the architectural differences [127]. Moreover, surface contamination of any kind could modify the surface properties; for example, if large amounts of lubricant migrate at the surface of a coating it will change its adhesion with the substrate or the migration of UV sensitive components at the surface can cause colour changes... [128]. Consequently, surface analysis of polymeric materials appears to be essential as it can provide information about the chemical structure of the polymer, the additives but also any possible contaminants. Furthermore, many polymers are hard to solubilize: some polyolefin are insoluble at room temperature (without degrading the polymer) limiting their analysis to the migrating stable contaminants and making their analysis very complicated with conventional methods (like liquid chromatography, NMR, UV-VIS, GPC...). Hence, their direct analysis and surface chemical mapping offer a promising solution.

New techniques of analysis are more and more reported in publications answering the problem of the characterisations of food additives which involves exhaustive extractions and polymer extract sample preparation. It appears that ambient ionisation mass spectrometry is a huge family of techniques allowing fast analysis of samples under atmosphere conditions. Within this family, Direct Analysis in Real Time Mass Spectrometry (DART MS) and Desorption Electrospray Ionisation Mass Spectrometry (DESI MS) are very adapted. The first paper talking about DART-MS was published in 2005 a few months after DESI which is derived from electrospray ionisation (ESI) [129]. They both can be considered as the first atmospheric pressure desorption/ionization techniques. They wipe off analyte molecules from samples by ionizing their surfaces with ionizing gas or aerosol. The sample is presented in the open atmosphere to the ionizing medium without pre-treatment allowing maintaining sample integrity.

- **Desorption Electrospray Ionization (DESI)**

Within the ambient ionisation family, DESI-MS is really studied in the late years. DESI desorbs analytes thanks a pneumatically assisted electrospray directed at the sample surface. This process leads to the creation of charged microdroplets which wet the sample surface creating a solid-liquid interface. The analytes are extracted at this interface and then goes into the MS where solvent evaporation occurs breaking the droplets and thus creating a gaseous ionic analyte. For example, DESI-MS allowed analysing the distribution of component at the material surface showing that a common additive specific to PP (phthalate) was found all over the surface of studied materials. This experiment shows the usefulness of DESI-MS for the study of the distribution of target components of non-volatile on the polymer [130].

- Direct Analysis in Real Time (DART)

DART, at the opposite of DESI, uses dry (solvent-free) heated gas stream, usually helium, which carry ionizing atoms. The DART apparatus is described in **figure I-26**. Helium is oriented through a tube and in the first part of this tube (I in **figure I-26**), a discharge between two needle electrodes produces ions, electrons and excited atoms. The produced ions, electrons and atoms then go through the tube's second part (II of **figure I-26**) where cations are separated from the gas stream by going through another electrode. Then, helium goes through the third part of the tube (III of **figure I-26**) and is heated to eliminate anions and electrons. As a result, the exited helium gas only contains electronically excited neutral species. These species are emitted and ionize the surrounding room atmosphere. The close ions of the analyte are then ionized by chemical ionisation at atmospheric pressure and are transferred to the mass analyser [131].

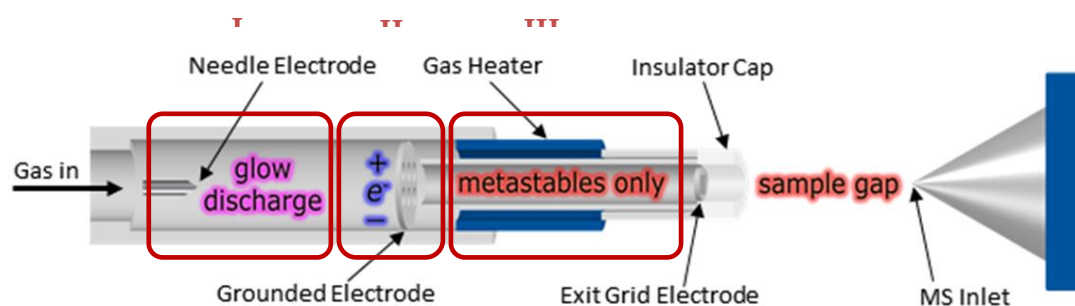


Figure I-26. The DART ion source.

DART allows the formation of both positive-ion and negative-ion. Moreover, it has shown to be a complementary tool for ESI ionization described above [132]. Some analyses were done on LDPE, HDPE and PP polymers or on PET bottle or PVC food packaging. Characteristic ions and optimal conditions of analysis were established for each additive taken separately and the method was successfully to the rapid screening of those packaging materials and their additives. The combination of DART with an orbitrap analyser with a high mass accuracy and accurate isotopic abundances makes it more efficient and accurate thus allowing the analysis of relatively low molecular weight synthetic and natural homopolymers (the principle of the Orbitrap is detailed in **Annexe 3**).

As we said, the major issue about analysing additives in polymers matrix with ambient ionisation techniques is the inability to extract them with no damage as well as their limited ability to provide accurate quantitative information. DESI-MS and DART-MS desorb analyte molecules from samples by ionizing their surfaces with ionizing gas or aerosol. Both DESI and DART require high temperatures and may lead to polymer degradation; degradation products can thus be detected falsifying the analysis of the materials' intrinsic

contaminants. Thus, a fully automated technique allowing efficient extraction and highly reproducible results is Liquid extraction surface analysis mass spectrometry (LESA-MS) analysis [133].

- Liquid Extraction Surface Analysis (LESA)

Liquid Extraction Surface Analysis Mass Spectrometry (LESA-MS) is a relatively new ambient direct analysis method, developed by the Van Berkel group [134]. Similarly, to other ambient MS techniques, it can be performed under atmospheric conditions; but it differs in that no sample pre-treatment is needed other than cutting the (solid) sample. This technique is commercially available thanks to a robotic pipette tip system (under the commercial name: Triversa Nanomate®). The principle is described in **figure I-27**. The pipette tip creates a microjunction between the sample and the pipette tip allowing extracting the analyte in a small volume of solvent. The solvent is, then, aspirated back and analysed by the mass analyser.

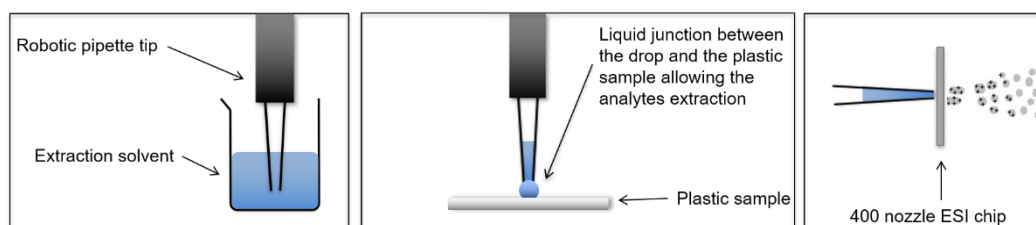


Figure I-27. LESA-MS principle.

The compatibility between the LESA apparatus and the mass spectrometer relies on the nano-ESI chip that is shown on **figure I-28**. This microfluidic silicon chip is made of 400 nano-electrospray nozzles. Those nozzles engender an electrical field leading to an efficient ionization, and a stable spray. The chip thus allows to obtain long lasting spray from very low amounts of sample at a rate of approximately 100-200nL/min (3 μ L of sample can be sprayed during 15 min).

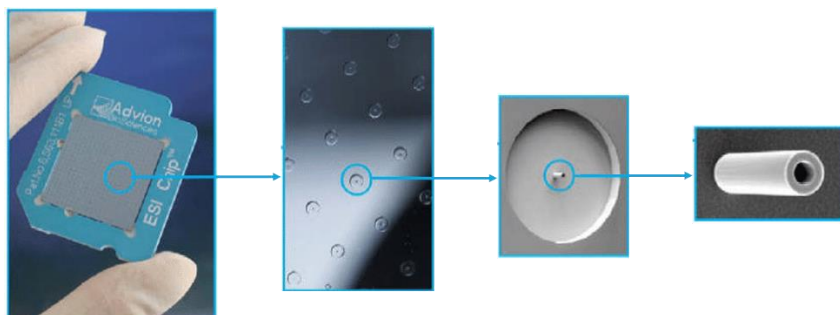


Figure I-28. Nano-ESI-chip nozzles.

The technique separates the desorption and ionisation steps. The extraction of the analytes requires only a small volume of solvent (unlike MALDI or DESI-MS which require an addition of the matrix or a sample swelling before the analysis, respectively). Hence, it gives a lot of possibilities such as modifying the solvent to extract specifically some species or analysing changes in the chemistry. On this way, it gives much cleaner spectrum and gives information about mechanism causing degradations [135]. The main limitation of this method is the creation of the microjunction: it can be difficult on rough, wettable or absorbent surfaces. It works best on homogeneous hydrophobic surfaces making it highly compatible for many synthetic polymers [133]. The method has a wide range of applications in metabolite, lipid, protein, and carbohydrate analysis [136, 137], and it is starting to be applied in polymer sciences [37, 73, 138-140].

For example, the HALS described in part III.1.b were analysed by LESA-MS directly from the polymer it was stabilising. Two separate studies reported their study: one following the chemical changes of two commercial HALS within cross-linked polyester or polyacrylate [139] and the other one which focused on the analysis of commercial HALS in polyester-based thermoset coil coating [73]. So far, mass spectrometric analysis of HALS were realized by MALDI MS, DESI MS [141], and Electron Spin Resonance (ESR) [142] but they were unable to give information about the possible modification of the additives structure. The study of the structural changes was of utmost importance as it is the key to understand the HALS stabilisation mechanisms. The use of LESA-MS was successful as it allows to draw important conclusion about HALS such a better understanding of their mechanism of action in coatings or the monitoring of their degraded metabolites, leading to polymer blooming. Also, excellent reproducibility of LESA-MS method under difficult conditions (very low quantities, hard localisation...) was demonstrated. This study could take advantage of particular features of LESA MS such as a possibility to carry out the analysis of low quantities hardly accessible material.

Another example of successful LESA MS applications is the analysis for brominated flame retardant (BFRs) from plastic e-wastes intended to be recycled [37]. The study published by Paine et al by LESA MS is the first one reporting the successful use of ESI-MS for the detection of BFRs. Nanoelectrospray ionisation coupled to LESA-MS allowed the simultaneous analysis for BFRs over a large range of polarities. Moreover, the e-wastes' pellets were sorted out according to their colour and analysed. The analysis was done 8 times for each colour confirming the presence of TBBP-A in different amounts and took only few minutes to be realised thanks to the robotic system [37].

Finally, LESA-MS was also applied to polymers used in other specific applications such as the analysis of worn contact lenses and, more specifically, the extraction and determination of the biological deposits on the polymeric material [138]. The deposition of biological molecules on contact lenses can cause discomfort to the people wearing them. Hence, the nature of the deposited molecules must be assessed as well as how the composition of the contact lens affects this deposition. So far, the analysis of lipid deposits on contact lenses was done through a bulk extraction of the soluble part of the lens. Therefore, no discrimination could be done

between the airside and the eyeside. LESA allows such discrimination. The obtained results were compared to human tear and meibum samples used as standards and revealed that most of the lipids contained in the tears and the meibum were also found in both worn contact lenses.

The still unexplored potential of the LESA-MS methods is related to the possibility of its coupling to tandem mass spectrometry which offers the possibility of the formal identification of the studied species. In the case of contact lenses, LESA-MS/MS gave access to the exact structure of the extracted species (wax, cholesterol esters and polar lipids). Moreover, when LESA was coupled to a sensitive tandem mass spectrometer, extraction and mass spectrometric analysis could be achieved in a very short period, still producing accurate data. These data highlight the efficiency of LESA-MS/MS for the extraction and identification of bio-residues on polymeric medical materials with limited degradation of the material. The use of MS/MS on coil coatings samples was also reported for which the use of LESA-MS/MS allowed the correlation of the blooming apparition with the presence of melamine in degraded samples [139].

Also, as said before, the weak point of ambient ionization methods is their limited ability to give quantitative results. However, thanks to its extraction process, LESA offers the possibility of semi-quantitative analysis. If we still consider the example of the lenses, 25 different worn lenses were characterized for which the authors managed to put forward variations in concentration without using a standard. While studying e-wastes and the possible presence of BFR's, the authors demonstrated semi-quantitative reproducible analysis for TBBP-A.

Liquid Extraction Surface Analysis Mass Spectrometry (LESA-ESI-MS) is a direct analysis method suited for the analysis of polymers. In comparison with batch methods, it is a "green" method using negligible volumes of organic solvents and is cost-effective avoiding lengthy sample preparation procedures. It can be used for the detection of known molecules (targeted analysis), identification of unknown species (exploratory analysis requiring MS/MS) and semi-quantitative analysis, if standards are available.

CHAPTER 2: MATERIALS AND METHODS

Abstract: This chapter aims at describing the different characterization methods and materials that we used during this PhD project. Among the characterization methods we can distinguish two main families: the physico-chemical methods and the analytical tools. The so-called physico-chemical methods are relatively known and were adapted to our study. The analytical tools that were used are less traditional. A novel direct mass spectrometry method based on liquid extraction surface analysis nanoelectrospray mass spectrometry (LESA-nanoESI-MS) was developed for the analysis of the migrating species from a polymer film. The development of this method is detailed within this chapter and was validated by a comparison with a standard method. It offers considerable advantages in terms of reduced analysis time and solvent consumption. Also, LESA-nanoESI-MS produced simpler spectra (limited to possible migrating compounds with the migration potential into food) than Direct Analysis in Real Time (DART). Finally, the raw materials (virgin polymers and virgin additives) of our study were described and characterized.

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I. MATERIALS PROCESSING

I.1. Twin-screw extruder

The twin-screw extrusion process is the most commonly used for the mix of additives and polymers. The extruder used during our study is a LabTech co-rotating twin-screw extruder (L/D 40) and is described in **figure II-1**.

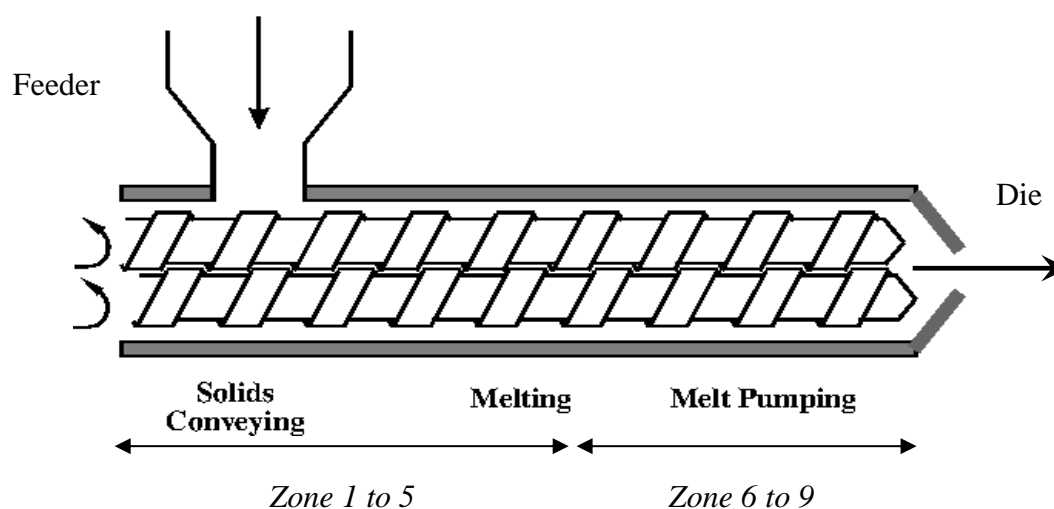


Figure II-1. Detailed scheme of an extruder.

Our extruder presents 10 distinct zones. The polymers' pellets are placed in a feeder which will deliver the pellets in the hopper in a controlled flow rate. The pellets are then brought in the compression section and the melting section by the rotating screws (zone 1 to 5). Once melted, the pellets form a homogeneous filament that goes (zone 6 to 9) out the extruder through the die (10th zone). This filament is cooled in a water bath and then pelletized.

Additives as powders can be directly mixed with the polymers' pellets prior to the extrusion. In the case of oils, like α -tocopherol, the additive cannot be directly mixed with the pellets, hence a pump is used. In

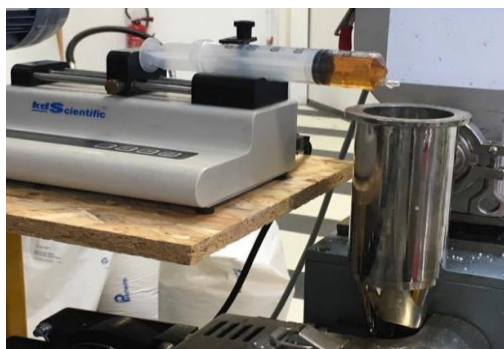


Figure II-2. Syringe allowing the introduction of oils in the extruder.

our case, instead of a pump a syringe was placed on top of the hopper and controlled by a syringe holder which allows to control the flow rate (see **figure II-2**).

Once the additives are introduced in the extruder, they are mixed with the polymers at the melted state to obtain a homogeneous blend.

I.2. Blow moulding extrusion

This type of extrusion is used to produce films. It is composed by a single-screw extruder that melts pellets (just like explained before) creating a hollow tube thanks to a specific die (and not a filament). Air is then blown into this tube, inflating the melted polymer into a big bubble (see picture in the **figure II-3**). The bubble is stabilized thanks to a cooling ring. It is then caught and flattened by rolls. Those rolls allow to have a continuous process. The blown air and the rolls speed can be regulated to control the thickness of films.

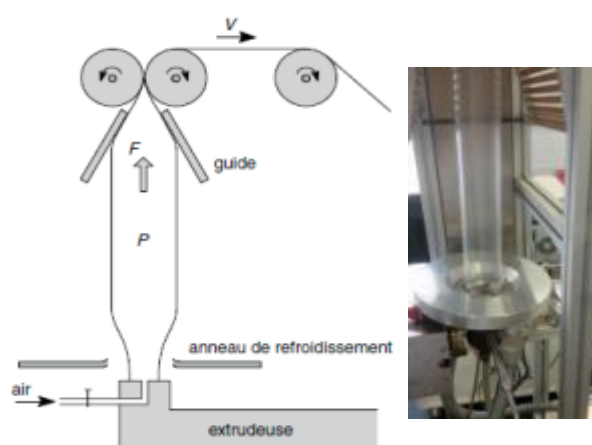


Figure II-3. Blow-moulding extrusion principle.

II. PHYSICO-CHEMICAL CHARACTERIZATIONS METHODS

II.1. Thermal characterizations

II.1.a. Thermo-Gravimetric Analysis (TGA)

This thermal characterization technic consists in measuring the mass loss of a given sample according to the temperature. Hence it gives data about the thermal behaviour, degradation characteristics and the volatile content of any material.

The experiments were done on a TGA Q50 from TA instruments. The samples (between 5 and 12mg) are placed in an aluminium pan (that was previously tared) and introduced in the TGA oven thanks to a nacelle. This nacelle is suspended to a precise balance that evaluates the sample's mass loss under different thermal conditions.

In our case, a 10°C/min temperature ramp is applied from the ambient temperature to 600°C under nitrogen atmosphere or under air.

The obtained curves are shown in **figure II-4** and present the temperature as x-axes and the percentage of mass loss (or the derivative weight loss for red curve) in the y-axes.

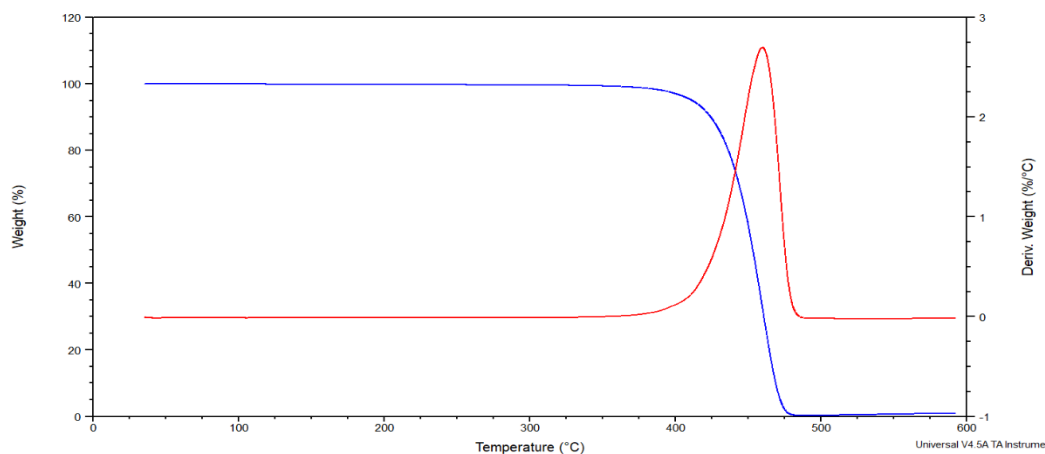


Figure II-4. Mass loss and derivative curves obtained by the application of a temperature ramp.

The shape of the curves usually shows similarities: first it shows a plateau (stable mass), then, from a certain temperature, appears the first negative slope (first mass loss) or the first peak (in the case of the derivative curve) and at the end another plateau is reached when almost all the sample was consumed. For some complex substances such as mixtures, several negative slopes or peaks can appear corresponding to the loss of different compounds within the mixture. Due to the general aspect of this method, it is often used as a first characterisation: it gives general information on the sample we are working with.

This method allows to evaluate the sample's thermal behaviour/resistance but also to quantify the ratio corresponding to each component within the sample. As the quantity of additives in the polymers is rather low, the degradation peak from the polymer tend to flatten the rest of the signal (**figure II-5 (a)**). However, when zooming on the degradation peak area of the additive we wanted to quantify (**figure II-5 (b)**), this peak tends to overlay with the polymer's one. Hence, a MATLAB program was done in order to separate both peaks to be able to integrate them more precisely. Hence, precise quantitation can be achieved thanks to this program.

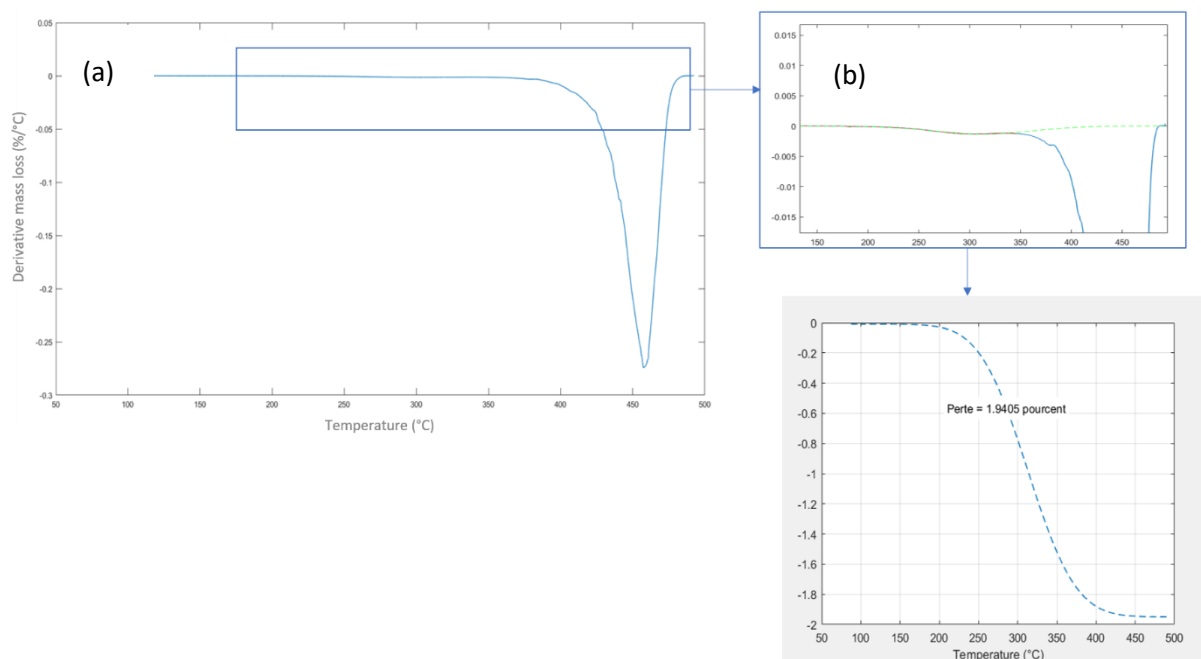


Figure II-5. Illustration of the quantitation of additives in polymers by TGA.

II.1.b. Differential Scanning Calorimetry (DSC)

The Differential Scanning Calorimetry consists in measuring the differences in thermal energy between a sample and a reference, exposed to the same conditions.

After having acquired the thermal degradation temperatures by TGA, DSC can be achieved. The degradation temperature must not be exceeded in DSC as it leads to false data. The DSC method allows to evaluate material's characteristic transitions as they generate endo- or exothermic calorific phenomenon. For polymers, the observed transitions are generally the glass transition (T_g), the melting temperature (T_m) and the crystallisation temperature (T_c) (see **figure II-6**). Moreover, the DSC technic provides the enthalpies associated with each transition. Those enthalpies result from the quantity of energy provided (fusion) or released (crystallisation) to/by a specific quantity of substance to change its state from a solid to a liquid or to crystals, at constant pressure.

The apparatus that was used is a DSC Q100 from TA instruments.

- **Method 1: Fusion-crystallisation cycles**

For polyolefins, the crystallisation peak will appear during the cooling process and is highly function of the temperature ramp (fast cooling gives lower crystallinities as the crystallites don't have enough time to grow). Hence for the study of those polymers, 3 cycles of heat/cooling will be performed under nitrogen:

- Cycle 1: 20°C/min from the ambient temperature until 190°C for iPP and 130°C for LDPE (this cycle is often used to erase the thermal history of the studied polymer);
- Cycle 2: 10°C/min cooling from 190°C to -10°C;
- Cycle 3: 10°C/min from the ambient temperature until 190°C for iPP and 130°C for LDPE (this cycle can be different from the 1st one if the thermal history is very important).

Cycle 1 and cycle 3 reveal the melt peak and cycle 2 the crystallization, as schematized in **figure II-6**.

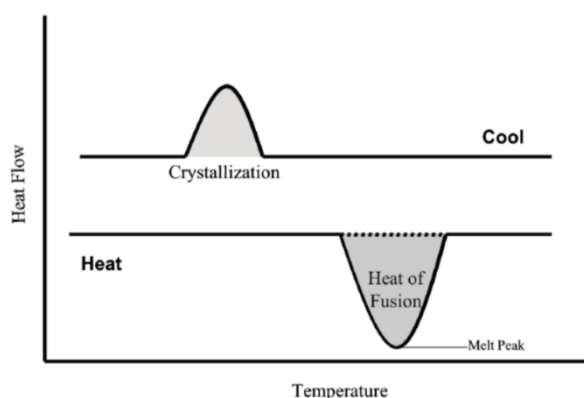


Figure II-6. Schematic DSC data obtained for semi-crystalline polymers.

The DSC programs must be adapted to each polymer as the temperature must not exceed the polymer's degradation temperature. If the degradation temperature is exceeded during the first cycle, it will completely impact the crystallisation and give rise to false data.

▪ **Method 2: Onset Oxidation Temperature (OOT)**

Other specific data can be obtained by DSC, such as oxidation kinetics, by applying adapted programs. Onset Oxidation Temperatures (OOT) is obtained by applying a 10°C/min ramp to a sample placed in an open pan (to be in contact with oxygen) from the ambient to 350°C under air. It corresponds to the norm ISO 11357-6. The curves obtained during this kind of method usually shows the melting peak followed by a plateau. This plateau ends when the oxidation starts as shown on **figure II-7**.

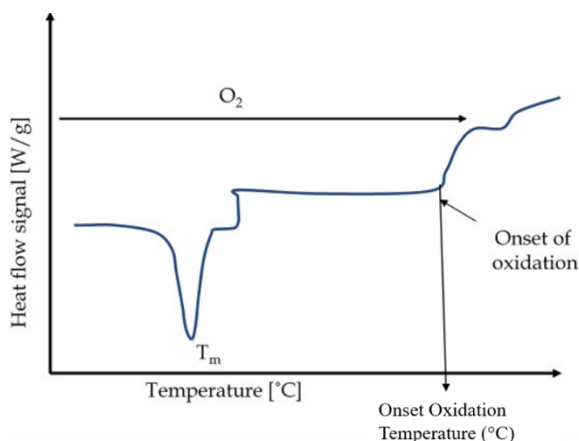


Figure II-7. Schematic results of the onset oxidation temperature (OOT) method.

Oxidation is an exothermic phenomenon that is characterized by an increase in the slope. This slope gives information about the oxidation kinetic. Finally, the slope decline indicates the beginning of the polymer thermal degradation, but it is not systematically visible.

- **Method 3: Oxidative Induction Time (OIT)**

Oxidative Induction Time (isothermal OIT), like the previously described OOT, is a measure of the resistance of a material to oxidative decomposition. It is determined thanks to a DSC by applying the samples to a fix temperature: the sample is heated under nitrogen, when the defined temperatures (210°C for iPP and 200°C for LDPE) are reached the gas is switched to air for 90 minutes. The OIT is the time needed for the oxidation peak to appear. The method is described in numerous technical standards such as DIN EN ISO 11357-6.

II.2. Rheology

Rheology corresponds to the study of deformation and flow of materials under a given constraint. Polymer materials are characterized by a law connecting the stress history to the strain (**equation 7**).

$$\sigma = f(\gamma) \quad (7)$$

It can be applied to materials with complex behaviours under a mechanical stress such as complex fluids or polymers. Actually, polymers behaviour at the melted state is an intermediate between a viscous Newtonian liquid behaviour and an elastic solid behaviour. Elasticity can be easily described as the capacity for a material to go back to its initial state after deformation.

The behaviour of the so-called Newtonian viscous liquid can be represented by the Newton law in which σ is proportional to the strain speed gradient $\dot{\gamma}$ (**equation 8**).

$$\sigma = \eta \dot{\gamma} \quad (8)$$

with η the Newtonian viscosity.

The behaviour of an elastic solid can be represented by laws of solid mechanics and more specifically Hook's law in which σ is proportional to the strain γ (**equation 9**).

$$\sigma = G\gamma \quad (9)$$

with G the elastic modulus.

Polymers present both elastic and viscous properties: they have a viscoelastic behaviour. The linear viscoelasticity is characterized by this behaviour at low strain.

Melted state rheology is very sensitive to structural changes in a polymer matrix. Hence, in dynamic rheology, thanks to the polymer's chain movement, the chain scissions or the reticulations phenomenon can be visualized.

In dynamic rheology, a sinusoidal strain γ^* is applied to a viscoelastic material with: γ_0 in amplitude, ω as pulsation. We measure the material response σ^* , which is a sinusoid with: σ_0 in amplitude phase-shifted of δ (the loss angle) (**equation 10 and 11**). The opposite can also be done: application of a constraint on a viscoelastic material and measurement of the resulting strain. The strain and constraint are expressed as follow:

$$\sigma^* = \sigma_0 \cdot e^{i\omega t + \delta} \quad (10)$$

$$\gamma^* = \gamma_0 \cdot e^{i\omega t} \quad (11)$$

Polymer's viscoelastic behaviours are characterized by the complex modulus G^* which represents the overall resistance of a material to strain (**equation 12**).

$$G^* = \frac{\sigma^*}{\gamma^*} = G'(\omega) + iG''(\omega) \quad (12)$$

With $G'(\omega)$ elastic modulus and $G''(\omega)$ imaginary part, viscous modulus (or loss modulus).

II.2.a. Spectro-mechanical characterizations

The description of G' and G'' as a function of the solicitation frequency is the way to describe polymer's viscoelastic properties but also to inform about the molecular structure of the studied material. G^* depends on the entanglements between the polymer chains, the density of the junction areas but also on the chains motion.

G' and G'' were measured at the melted state by a rotary rheometer in parallel plate configuration (**figure II-8**). The apparatus used is an Ares rheometer. In this method, the solid plastic sample is placed in between parallel plates of 25mm diameter geometries. The geometries are then locked in a heating oven under nitrogen or air. Once the whole system has reached the imposed temperature and that the polymer is soft

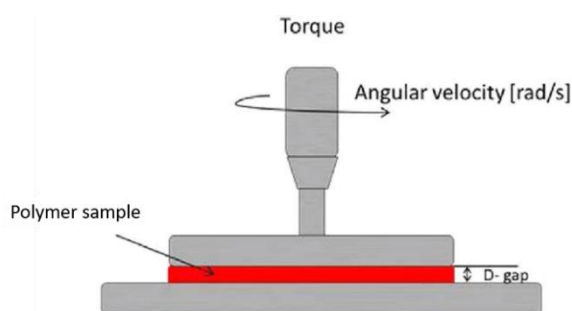


Figure II-8. Scheme of a rheometer in parallel plate configuration.

enough, the gap between the parallel plates can be diminished in order to be fully in contact with the polymer sample.

The soft material will then be exposed to shear, temperature or frequencies to obtain the material response under stimulations thanks to a detector placed in the upper plate (torque).

- **Determination of the linear domain**

All the measurements have to be achieved in the viscoelastic linearity domain. In this zone, the observed systems don't evolve under the application of a strain (it involves that the macromolecular chain's movements are negligible). It is of major importance for the following experiments as in this linear domain the viscoelastic properties are independent of deformation and the only variations that we are interested in must be due to the frequency. Hence, in this domain, we get rid of the fluctuations due to the strain.

To determine this linear domain, G' and G'' are measured as a function of a strain sweep (from 0.1% to 10%), under the same conditions that will be applied for the other experiments (same temperature, same geometries...). The linear domain is function of the frequency and should thus be measured at all the frequencies used for the study. However, the lower the frequency, the longer the linear domain: it can thus be determined at high frequencies and still be correct for the lower ones (see **figure II-9**).

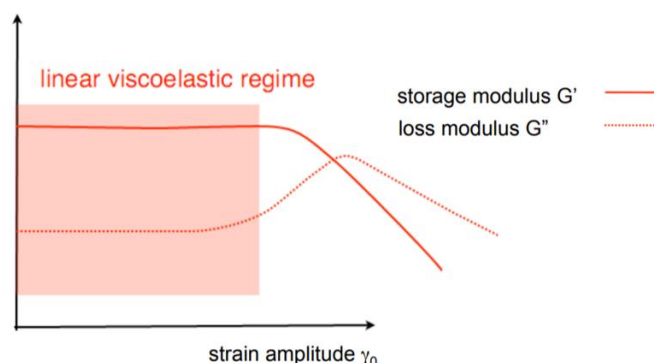


Figure II-9. Illustration of the linear domain regime for semi-crystalline polymers.

The diminution of the modulus reveals the end of the linear domain.

- Frequency sweep

After having determined the linear domain, frequency sweep can be realized. The frequency sweep consists in applying a range of frequencies (from 0.1 to 100 rad/s) to a polymeric material under fixed temperature and strain rate (chosen in the linear domain, usually around 2% in our case). The frequency curve obtained for a given sample at different temperatures are schematized in **figure II-10**.

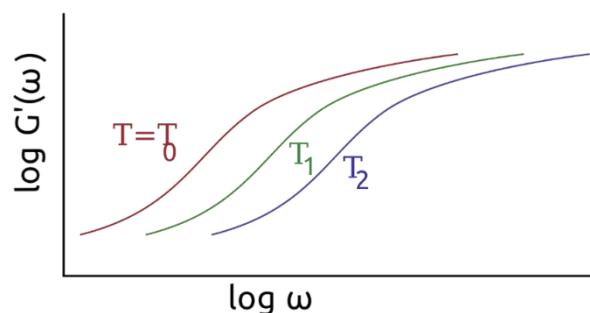


Figure II-10. Illustration of curves obtained at different temperatures thanks to frequency sweeps.

Subsequently, those pieces of curves can be shifted to overlap (as long as the data sets did not suffer from ageing effects during the test time) thanks to a translation along the x and the y axis relative to an arbitrary reference: the curve at temperature $T=T_0$ (the curve at that temperature undergoes no shift). The translation factors following those axes are $a(T)$ and $b(T)$ respectively such as:

$T > T_0$, $a(T) < 1$ (translation toward the frequency lower values (left));

$T < T_0$, $a(T) > 1$ (translation toward the frequency higher values (right));

$T = T_0$, $a(T) = 1$ (no translation, this is the curve of reference).

In the frequency range, if the temperature increases from T_0 , the complex modulus decreases. Hence, increasing the temperature leads to the exploration of low frequencies. At the opposite, lowering the temperature corresponds to the exploration of the part of the curve corresponding to high frequencies. In other words, low temperatures correspond to elevated frequencies and high temperatures to low frequencies. Based on this fact, it is clear that the Time-Temperature superposition aims at determining the temperature-dependent properties of viscoelastic materials at a reference temperature. Moreover, frequency sweeps only offer small curves giving narrow information (in a narrow frequency range) whereas thanks to the overlap of several frequency sweeps, the curve became much broader (extrapolation beyond the field of experimental frequencies) as shown in **figure II-11**. Actually, it shows the master curve obtained after the time-temperature superposition (**figure II-11** is a more detailed version of **figure I-6**).

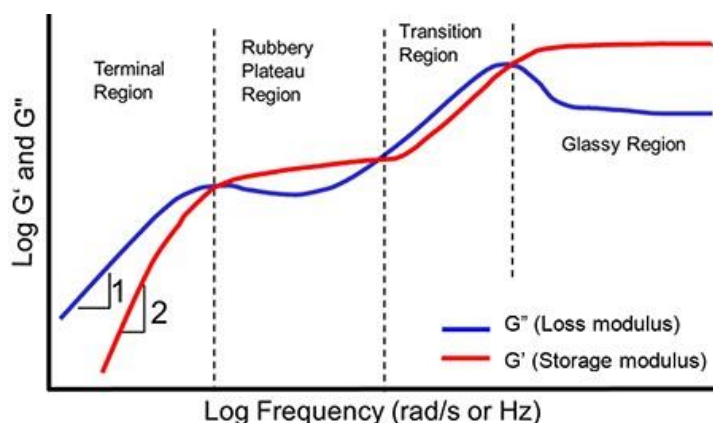


Figure II-11. Master curve of a non-crystallising polymer.

Also, the shift factor can be defined, below T_g , using an Arrhenius law (**equation 13**).

$$\log(a(T)) = \frac{-E_a}{2.303R} \left(\frac{1}{T} - \frac{1}{T_0} \right) \quad (13)$$

Where E_a is the activation energy (the energy that is required to make the polymers' chains move), R is the gas constant and T_0 the reference temperature (K).

When plotting the modulus of a non-crystallizing crosslinked polymer as a function of its frequency (and in turn as a function of temperatures), several regions of physical behaviour appear.

- At high frequencies (low temperatures) the polymer act like a glass with elevated modulus: the polymer chain can barely move;
- When increasing the temperature (lower frequencies), the polymer will go from glassy to rubbery (beginning of the chain motions corresponding to the T_g);
- Finally, at elevated temperatures (close to the T_M), the "terminal region" of "flow region" is reached. The material shows very low modulus due to its low viscosity.

The crossovers between G' and G'' that can be observed reveal changes in the state: at very low frequencies, G'' is much larger than G' and, hence, a liquid-like behaviour predominates. However, at higher frequencies, G' takes over and solid-like behaviour prevails. In other words, when $G' > G''$ the material behaves as a solid (glassy region and rubbery plateau) and inversely when $G'' > G'$ the material behaves like a liquid (flow region).

In the case of polyolefins, as they crystallise at a temperature comprised between the T_g and the T_M (rubbery plateau region), the area of the transition and the glassy region cannot be observed at the melt state.

Actually, parallel-plate rheology must be realized at the melted state, but crystallites solidify the samples, making it impossible to reach low temperatures (or high frequencies). The parameters used for frequency sweeps in our study are the following ones:

- Strain: 2.5%;
- Reference temperatures: 150°C for iPP, 130°C for LDPE;
- Other temperatures for frequency sweeps: 250°C and 190°C for both polymers;
- Frequency sweep: from 100 rad.s⁻¹ to 0.1 rad.s⁻¹ for both polymers.

▪ Time sweep

The time sweep analysis corresponds to a kinetic study at the melted state. The samples are placed in-between parallel plate geometries as explained above and exposed to constant parameters for 1 hour:

- Frequency: 10Hz;
- Strain: 2.5%;
- Temperature: 250°C;
- Under air or nitrogen.

The obtained curves put forward the evolution of the system through the time.

II.2.b. Melt Flow Index

The MFI is a measure of the ease of flow for a melted thermoplastic polymer. It is defined as the mass of polymer flowing in ten minutes (unit: g/10minutes) through a capillary of a specific diameter and length by a pressure applied with weights under a given temperature. The apparatus that was used is a Meltfliker^{MT} from ThermoHaake.

The method follows the standards ISO 1133 which gives the pressure and temperature to apply as a function of the polymer. For PP the temperature is 230°C and the pressure must be applied with a 2.16kg weight; for PE the pressure is the same but the temperature is 190°C. Melt flow rate is an indirect measure of molecular weight, with high melt flow rate corresponding to low molecular weight (the apparatus is described in **figure II-12**). At the same time, melt flow rate is a measure of the ability of the material's melt to flow under pressure. Melt flow rate is inversely proportional to viscosity of the melt at the conditions of the test.

The procedure for determining MFI is as follows:

- A small amount of the polymer (4 to 5 grams) is taken in the MFI apparatus. A die with an opening of 2mm diameter is at the exit the apparatus.
- The material is putted inside the barrel to avoid formation of air pockets.
- A piston is introduced which acts as the medium that causes extrusion of the molten polymer.
- The sample is preheated for a specified time: 5 min at 190 °C for PE and 6 min at 230 °C for PP.
- After the preheating the 2.16kg weight is introduced onto the piston.
- The weight exerts a force on the molten polymer and it immediately starts flowing through the die.
- A sample of the melt is taken after few minutes and is weighed accurately (unit: g/10min)

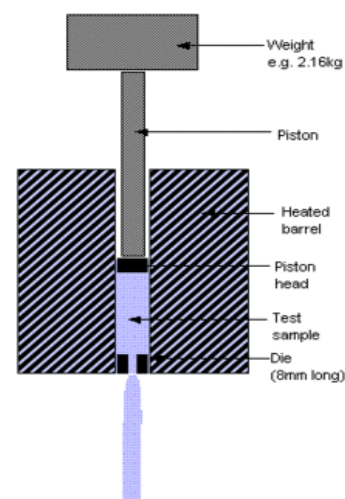


Figure II-12. Melt flow index apparatus.

II.2.c. Cole-cole representation

The study of the properties at the melted state allow to have access to data such as the mass distributions, the rate of branching, the viscosities... Moreover, it allows to evaluate the modifications of the polymers chains length which are characteristic from the thermo- or photochemical degradations of polyolefins. Chain scission processes lead to higher mobility of the chains and, as a consequence, it also leads to a higher viscosity. Branching, at the opposite, corresponds to the creation of nods involving the formation of three-dimensional network. The latter reinforces the elastic character of the material.

The cole-cole representation was initially used for dielectric relaxation in polymers [143]. Once applied to polymers, it allows to put forward the elastic or viscous aspects induced by chains scissions or reticulations. It also has the advantage of zooming on the data from the lower frequencies.

The general resistance of a material to flowing is expressed by the following expression:

$$\eta^* = \frac{G^*(\omega)}{i\omega} = \eta'(\omega) - i\eta''(\omega) \quad (14)$$

where η^* is the dynamic complex viscosity.

This results in the following expressions:

$$\eta' = \frac{G''(\omega)}{\omega} \quad (15)$$

$$\eta'' = \frac{G'(\omega)}{\omega} \quad (16)$$

where η' is the real component (viscosity) and η'' the imaginary one (elasticity) of the complex dynamic viscosity.

The Cole-cole diagram represents $\eta''(\omega)$ as a function of $\eta'(\omega)$, as shown in **figure II-13** and is obtained thanks to the time-temperature equivalence described above.

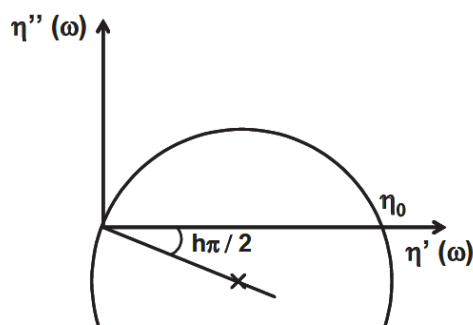


Figure II- 13. Cole-cole representation.

The curve is a semi-circle which centre is below the abscise axis and that crosses the origin of coordinate system. Three main parameters are obtained thanks to this representation:

- the intersection between the semi-circle and the abscise axe corresponds to the Newtonian viscosity η_0 . The Newtonian viscosity of linear polymers (or limit viscosity) is related to the molar mass with the expression:

$$\eta_0 = K. (M_W)^\alpha \quad (17)$$

Where K is a constant depending on the temperature, M_W the average molar mass and $\alpha=3,4$ for high molecular masses polymers.

- relaxation times;

- the angle $(h\pi/2)$ called α between the abscise and the circle radius represents the width of the mass distribution h.

II.3. Hansen Solubility Parameters

The initial concepts of the solubility parameters were developed by Scatchard and extended by Hildebrand [144]. Hansen proposed an extension of the single Hildebrand solubility parameter to three components solubility parameters: the Hansen Solubility Parameters, HSP (nonpolar δ_D , polar δ_P and hydrogen-bonding

parameters δ_H) [145]. This is based on the fact that all types of physical bonds are broken when they are evaporated including those three kinds of bonds. Hence total solubility is represented by equation 18.

$$\delta_t^2 = \delta_D^2 + \delta_P^2 + \delta_H^2 \quad (18)$$

Hansen solubility (cohesion) parameters (HSP) are widely used for predicting compatibility between two materials [146, 147]. Materials with similar HSP will show physical affinities. Both theoretical and experimental methods can be used to estimate the HSP of a material. The experimental method to determine HSP for a polymer is to evaluate whether or not it dissolves in selected solvents. Experimental data is based on the observation of the interaction between studied materials and well-known solvents. The solvents that have close HSP to the studied material will interact with the material. These different interactions are used to divide the chosen solvents into two groups, one which is considered “good” (score 1) and the other which is considered “bad” (score 0). These scores can then be processed to determine the HSP parameters thanks to the HSPiP software (Hansen Solubility Parameters in Practice): these scores will allow the software to draw a 3-dimensioned sphere with “Good” solvents inside and “Bad” solvents outside (**figure II-14**).

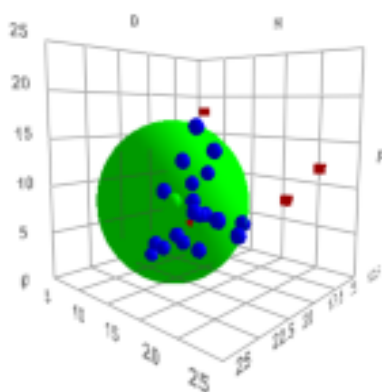


Figure II-14. Hansen solubility sphere of a given compound (green sphere) and representation of the good (blue dots) and bad solvents (red squares).

The axes of the 3D space are δ_D , δ_P and δ_H . The centre of the sphere represents the HSP of the studied material. The blue dots are the “Good” solvents of the studied material and the red ones are the “bad”. Then, the closer a solvent is from the centre of the sphere, the more affinity the components will have. The HSP distance is the distance between a component 1 and a component 2 can be calculated thanks to their HSP by the software or thanks to the following equation:

$$\text{HSP distance} = \sqrt{4(\delta_{D1} - \delta_{D2})^2 + (\delta_{H1} - \delta_{H2})^2 + (\delta_{P1} - \delta_{P2})^2} \quad (19)$$

Also, the software allows to calculate theoretical HSP by the “Group contribution” method: this method consists in cutting a molecule into pieces and to calculate its HSP based on these small pieces.

Hence, we got interested in having our polymers and our additives solubility spheres. However, our polymers aren't soluble in any solvents at room temperature. Going at high temperature would involve other parameters making the study not representative. For this reason, we decided to build the Hansen Solubility Sphere by two methods:

- For the additives, we mixed them into different solvents and measured the time it took to solubilize;
- For polymers, we chose to use the surface tension method described below.

To draw a precise sphere, the software requires to have as many good and bad solvents. Hence, a median was chosen, and marks were given according to it. Also, in order to have a better distribution of the marks, there is a possibility to score the solvent from 1 to n:

- Grade 1: score 1 to good solvents and 0 to bad ones;
- Grade 2: score 1 to good solvents, 2 to intermediate ones and 3 to bad ones;
- Grade 3: score from 1 to 4;
- Grade 4: score from 1 to 5;
- Grade 5: score from 1 to 6.

▪ Surface tension

To assess the affinity of polymers with solvents, surface tension tests were realised thanks to a Digidrop Fast/60 from GBX device as shown on the **figure II-15**.

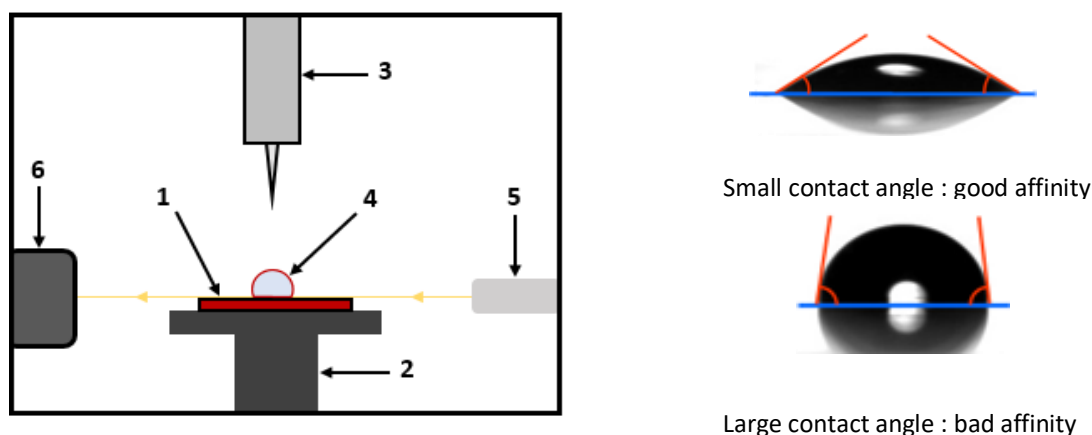


Figure II-15. Surface tension measurement principle.

The polymer to be tested (1) is shaped as a thick film and is positioned on the support (2). On top of the film, a syringe (3) will deposit a drop (4) of a selected solvent and is enlightened by a white lamp (5). The surface of the drop will be observed thanks to a precise camera (6). A picture is then taken and sent to a software that allow calculating the surface contact angle between the drop and the plastic as shown on the

figure II-15. This way, we can observe different wettability: perfect contact angle ($\alpha = 0^\circ$), partial ($\alpha < 90^\circ$), weak ($\alpha > 90^\circ$) or bad ($\alpha = 180^\circ$). For example, for a hydrophilic compound the angle will be close to perfect with a drop of water. At the opposite, if a compound is hydrophobic the angle will be close to 180° . Hence, the lower the angle, better are the affinities.

II.4. Spectroscopy

The UV-Vis spectrometer that was used is a Shimadzu UV2450.

II.4.a. DPPH method for antioxidant capacity assessments

According to the UE regulation CE-1333-2008, antioxidants are substances that enhance food shelf-life by protecting them from degradation caused by oxidation (lipids rancidity, colour modifications...). In order to respect this, our antioxidants have to be stable through the extrusion process so that they keep their antioxidant efficiency when migrating to the food surface. To assess the antioxidant efficiency of the migrating species the DPPH (2,2-diphenyl-1-picrylhydrazyl) method was applied [148]. This test allows to evaluate the anti-radical power of a sample by measuring its capacity in reducing the DPPH radical (**figure II-16**).

DPPH is a stable free radical thanks to the delocalization of the spare electron over the whole molecule, hence the molecules do not dimerize, at the opposite of other free radicals. The delocalization gives rise to the deep violet colour, characterized by an absorption band in methanol around 515 nm (**figure II-16**). When a solution of DPPH is mixed with a substance that can donate a hydrogen atom (antioxidant), the reduced form is created with the loss of the violet colour.

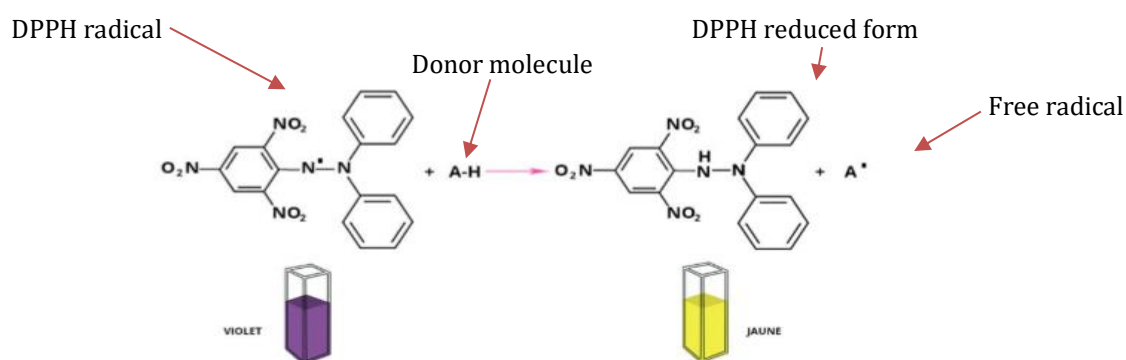


Figure II-16. DPPH radical principle.

The absorbance of this coloured solution will allow to calculate the rate of radicals that was consumed by the analysed sample thanks to equation (20).

$$I\% = \left(\frac{A_0 - A}{A_0} \right) \times 100 \quad (20)$$

where A_0 is the virgin DPPH absorbance and A is the sample absorbance.

This colour loss must not be too quick. The incubation time (time during which the DPPH[°] solution and the sample are in contact) is 15 minutes. If the colour change is too fast, all the radicals will be consumed quickly and thus the solution will turn fully yellow immediately making the analysis impossible (see details in **figure II-17**).

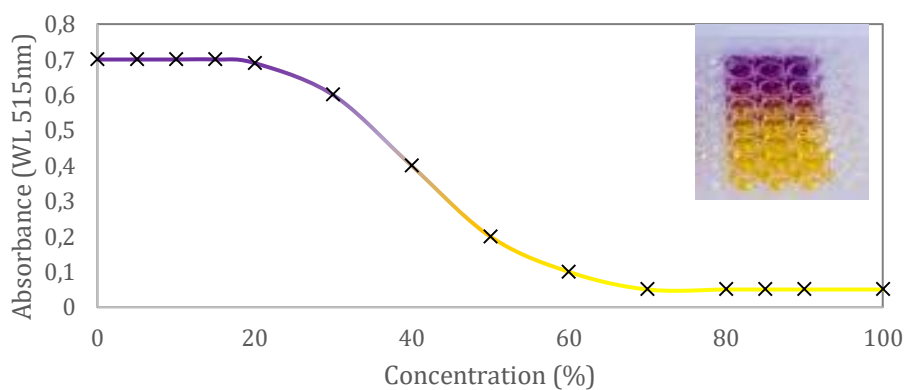


Figure II-17. Illustration of the need to have the appropriate dilutions for an efficient analyse by the DPPH[°] method.

As illustrated in **figure II-17**, at the extremities the absorbance reaches a plateau: if the antioxidant capacity is very strong all the results will be on the “yellow plateau” and the values cannot be discriminated. For this reason, the appropriate concentration of the sample solution must be found in order to have enough differences in absorbance.

Moreover, several dilutions of the same sample must be done to calculate what is called the IC50. It corresponds to the concentration of antioxidant needed to reduce 50% of the DPPH[°] radicals and is calculated by plotting the inhibition rate (equation 20) as a function of the concentration.

The first step for the antioxidant capacity assessment is to set up a calibration curve of DPPH. Different concentrations of the radical were chosen and mixed with methanol. Their absorbances were measured at 515nm thanks to a UV-VIS spectrometer. The results are summarized the **figure II-18**.

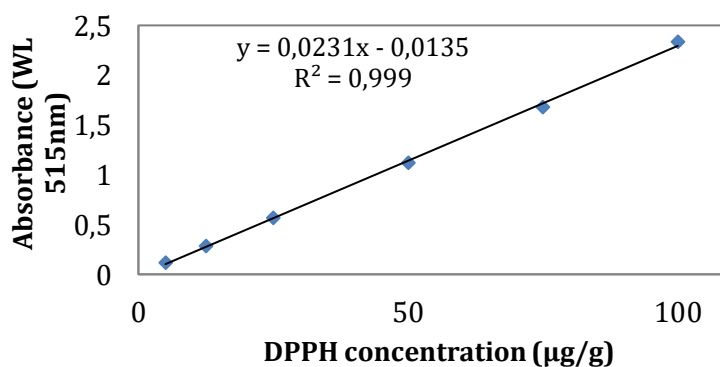


Figure II-18. Evolution of the absorbance as a function of the concentration in DPPH.

Once the calibration curve is obtained, a stock solution at 1000ppm of DPPH° in methanol must be prepared. This solution will then be diluted at 30 ppm which will be the experiment solution.

Then, 3,5mL of the 30ppm solution of DPPH are mixed with 100 μ L of the studied solutions and incubated for 15min in darkness. The same experiment is repeated for the diluted solutions and the I% values are plotted as a function of the concentration.

II.4.c. 2D-NMR

The use of two-dimensional Nuclear Magnetic Resonance (NMR) allows to measure the diffusion of molecules in solution and, in turn, interactions between molecules (if two molecules interact in a given media, they will diffuse slower than if they don't have any interactions). The diffusion NMR technique is often referred to as Self-Diffusion (SD)-NMR or Diffusion Ordered Spectroscopy (DOSY). Diffusion spectra are usually presented as a 2D plot with chemical shift on the horizontal axis and $\log(\text{Diffusion constant})$ on the vertical axis (**figure II-19** which is an example of a DOSY spectrum of norcamphor and β -cyclodextrin in DMSO- d_6 [149]).

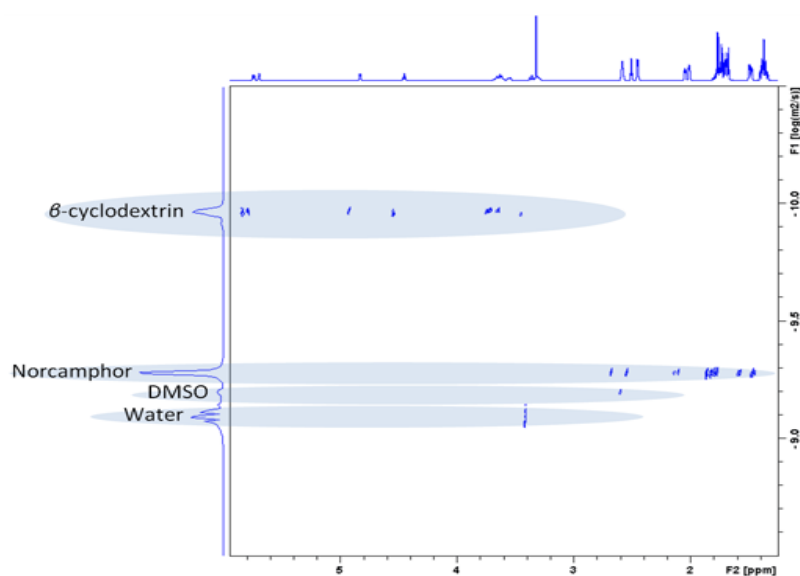


Figure II-19. DOSY spectrum of norcamphor and β -cyclodextrin in DMSO- d_6 .

The spectra obtained thus have two dimensions; one dimension presenting the chemical displacements and the other dimension comprising the coefficients of diffusion of the species present in solution. DOSY maps represent the classical NMR ^1H spectrum in abscissa and the $\log D$ in ordinate. These are "pseudo-2D" because we make a series of 1D spectrum by varying a parameter.

The self-diffusion constant is measured in $\text{m}^2 \text{s}^{-1}$ and is larger for smaller molecules and less viscous solvents than it is for large molecules and viscous media. The diffusion coefficient being inversely proportional to the

hydrodynamic radius of the molecules (considered spherical), a large molecule will have a lower diffusion and therefore less signal attenuation will be observed than for a smaller molecule.

II.5. Mechanical characterizations

Mechanical characterisations were achieved via tensile tests thanks to a traction machine called Alliance RT/50 from MTS comprising a 10kN sensor. Tensile tests are widely used to characterize plastic materials. The tensile test measures the resistance of a material to a static or slowly applied deformation. The data obtained from the tensile tests describes many mechanical features of the polymer: tensile strength, breaking strength, maximum elongation and reduction in area (see detailed description in **table II-1**).

Table II-1. Definition of terms on a stress-strain curve.

Definition	Description
Yield Point (strength)	Yield point is the first point (load) at which the specimen yields. This is when the specimen's cross-sectional area begins to decrease. This is called necking
Modulus of Elasticity	The ratio of stress to strain within the elastic region of the stress-strain curve
Yield Elongation	The strain at the yield point
Elastic Region	The portion of the curve before the yield point
Plastic Region	The portion of the curve after the yield point
Ultimate (tensile) strength	Ultimate tensile strength is the maximum stress a material can withstand before failing
Ultimate elongation	The total elongation just before fracture

There are two main deformation regions in a stress strain curve: elastic and plastic deformation (see **figure II-20**).

Characteristic stress-strain curve for a semi-crystalline polymer is characterized by a linear elastic region; a yielding followed by a drop-in stress, a formation of a neck, an increase in stress due to straightening of polymer chain, and finally fracture.

The elastic deformation in semi-crystalline polymers is due to several mechanisms. Actually, applied stress causes the covalent bonds to stretch, allowing the chains to elongate elastically. When the stress is stopped, recovery from this distortion is almost instantaneous.

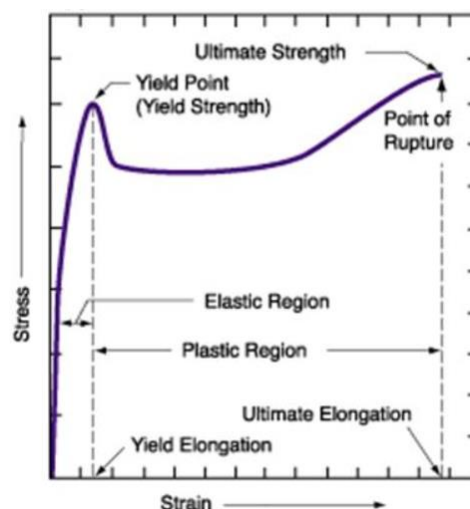


Figure II-20. Typical Stress-Strain curve.

Once the yield strength is exceeded the polymer deforms plastically. Plastic deformation is the result of chains sliding, stretching, rotating, and disentangling under load. This causes permanent deformation. This is because the initially tangled chains become straight and untangled. Once the chains are straightened, additional stress causes necking, in which there is the continued sliding and deformation of the chains. Also, additives are often small molecules which occupy positions between the larger polymer chains. This increases the inter-chain distance with a reduction in the secondary intermolecular bonding.

For our uniaxial traction tests, we use films made by blow-moulding extrusion (around 100 μ m thick). The analysis was done following the norm D882-02, "Tensile Properties of Thin Plastic Sheeting". The film is tightened at both ends in jaws: in order to prevent slippage and to have homogeneous analysis marks were made on the samples to position the jaws 2 cm from the edge. Among those jaws, one of them is fixed, the other is mobile and connected to the training system which moves at a constant speed. The speed was fixed at 10mm/min. For each elongation of the sample (Δl), the force (F) is measured giving a "raw curve". These raw data are processed to obtain intrinsic data of the studied material:

- the nominal stress σ (Pa) is calculated via the expression $\sigma=F/S_0$ where S_0 is the initial section of the sample;
- the deformation ϵ is calculated by dividing the elongation Δl by the initial length l_0 : $\epsilon= \Delta l/ l_0$.

The stress-strain $\sigma =f(\epsilon)$ curve can thus be drawn like in **figure II-20**.

II.6. Imaging: Scanning Electron Microscope (SEM)

Scanning electron microscopy (SEM) is a microscopy technique based on electron-matter interactions. It allows to obtain images of the observed surface in high resolution. The principle of this technique is to scan the surface of the sample with a beam of electrons. In response, the surface emits electrons of different nature that pass through different detectors and allow building an image of the surface. To facilitate the return of these electrons, the samples surfaces are metallized (having a conductive surface allow the excess electrons to flow, preventing the sample from loading when exposed to the electronic probe in the SEM). To do so, samples are introduced into the chamber of the metallizer in which the vacuum is made. A powerful electric field is applied between a cathode (gold target) and an anode that carries the sample. Ar^+ ions are created and accelerated to the cathode where they pull out gold atoms that fall on the surface of the samples, forming a thin conductive layer. The metallized samples are then introduced into the SEM (the apparatus that was used is an Hirox SH-3000), vacuum is realized, and image acquired.

III. ANALYTICAL CHARACTERIZATIONS

The high contact surface between the food and its package can lead the contamination of the packed food: migration phenomenon [3]. The plastics' additives, as well as post-polymerisation residues or degradation products, can migrate into food. Actually, polymers and additives are sensitive to high temperature and thus degrade when they are processed, producing smaller molecules and creating residues, pollution and odor. Some of these pollutants can be post-polymerization residues (by-products, catalysts, monomers, etc.) or come from polymer recycling or reactions with chemicals (e.g., dishwashing). Possible contamination of recycled materials needs to be scrutinized. Also, even if some additives are not harmful in their initial state, their degraded version or metabolites can be. Particular attention is focused on additives as they can easily diffuse throughout the polymer and are in direct contact with consumers [118, 119]. They are a risk for the consumers but, at the same time, "intelligent" packaging releasing natural antioxidants improve food shelf life [150]. In order to evaluate the safety and functionalization of polymers in direct contact with food, they have to be monitored for the presence of species able to migrate into food. The analysis and the characterisation of the mobile polymer fraction are necessary to evaluate the risk they can represent for food consumers.

Also, as explained before, most of the contaminants have lower viscosities or different structures compared to the polymer they are stabilizing and can thus be located at the surface of the material (viscosity segregation). Then, surface contamination can modify surface properties. Subsequently, surface analysis of polymeric materials can provide information about the chemical structure of the polymer, the additives, and also any possible contaminants.

Different methods for the mapping of polymer additives have been described, usually requiring extraction followed by chromatographic analysis coupled with mass spectrometry [2, 11, 151-153]. Mass spectrometry is increasingly used as an efficient tool for the characterization of synthetic polymers, in terms of molecular weight, monomer sequence, terminal groups, additives, etc. [154-156]. The progress in soft ionization techniques, such as electrospray ionization (ESI) [123] and large molecular mass range detection opened new possibilities to polymer analysis, offering not only complementary information to conventional methods but also new possibilities (e.g., surface mapping, on-site analysis, etc.) [124, 129, 157]. Indeed, many polymers are hard to solubilize. Hence, their direct analysis and surface chemical mapping offer a promising solution.

III.1. UHPLC qOrbitrap MS

Liquid chromatography (LC), especially high-performance liquid chromatography (HPLC) is commonly applied to identify and quantify specific migrants in migration studies. Sample preparation is the first step for an HPLC analysis. Solvent extraction is a commonly used for the antioxidant extraction from polymers. The

chemical compounds from polymeric films are extracted in a solvent at specific temperature and for a certain time as explained in chapter I, part IV. The extracts can then be injected into an HPLC system (see **figure II-21**) for the quantification analysis. The separation of the studied antioxidant from other chemical compounds in the extract is carried out in a column which is installed into the HPLC equipment and designed to identify these compounds. Different types of columns are used as stationary phase and different mix of solvents are used as carried or mobile phases. Various detectors are used for an HPLC, such as ultraviolet (UV) detector, fluorescence (FL) detection and mass spectrometry (MS) detection. This allows an optimal sensitivity for a wide range of analytes. MS detection is carried out by using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), and it provides more reliable identification compared to UV and fluorescence detectors.

In our case, the specific species quantifications were done thanks to a UHPLC- QOrbitrap- MS (Thermo Scientific Dionex Ultimate 3000 UHPLC system coupled to a Q Exactive hybrid quadrupole-Orbitrap mass spectrometer equipped with electrospray ion source).

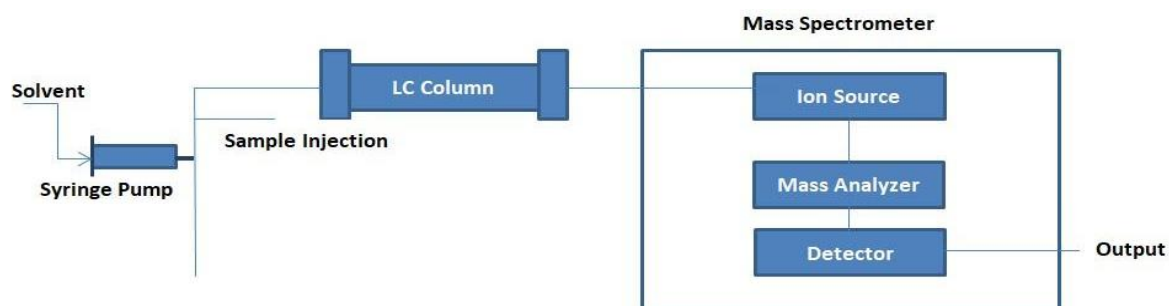


Figure II-21. Scheme of a LC-MS system.

The HPLC conditions that were applied are the following ones:

- flow rate: 0.4 mL min⁻¹,
- column type: Acquity UPLC BEH C18 column of 3.0x0.75mm and 1.7µm particle size from Waters;
- column temperature: 40°C,
- injection volume of sample: 20 µL,
- mobile phase: Water + 0.1% formic acid (φA) and Ethanol (φB) in gradient elution mode (**figure II-22**):

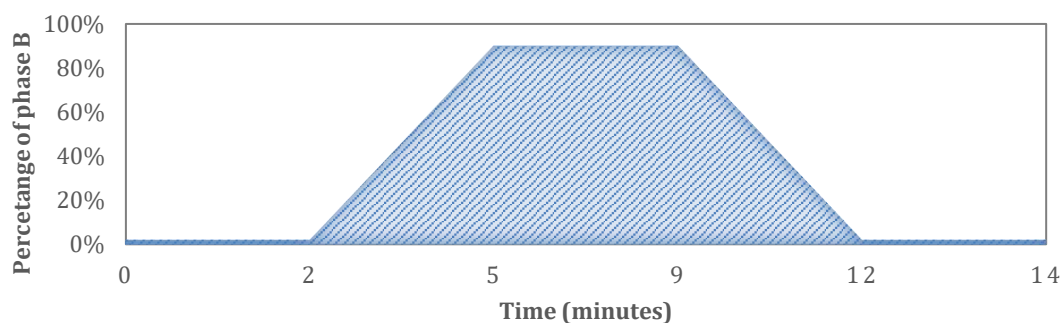


Figure II-22. HPLC parameters applied for the mobile phase injection.

initial time—2 min 98% A and 2% B;

2 min—98% A and 2% B; 5 min—10% A and 90% B;

5 min—9 min—10% A and 90% B;

9 min—10% A and 90% B; 14 min—98% A and 2% B.

The mass spectrometer was working simultaneously in positive and negative ionization mode, to detect all of our molecules. The resolution was fixed at 35,000 at m/z 200. The automatic gain control (AGC) was set to 1.0×10^5 ions and a maximum injection time of 150 ms. The RF lens was fixed at 30% and the capillary temperature at 350°C.

III.2. Ambient ionisation method

III.2.a. DART

DART MS principle was described in chapter I, part IV. The analyses were performed using a VELOS Orbitrap (Thermo) Electrospray Ionization (ESI) mass spectrometer equipped with an IonSense DART source (Danvers, MA). Helium was used as the reagent gas at flow rate of $3 \text{ mL} \cdot \text{min}^{-1}$, between 200 and 300°C. Positive ion mode was used for a mass range between 150 and 1000 m/z with helium for the analysis and nitrogen gas in standby mode. The grid electrode voltage used value was 350 V in both positive and negative mode. The detector voltage was 1.8 kV with a maximum value of 2.3 kV. The mass spectra were recorded every second with a resolution of 60,000 at m/z 400.

III.2.b. Adaptation and optimisation of a new type of ambient ionisation method: LESA-MS

As described in the bibliography from chapter I, part IV, liquid extraction surface analysis (LESA) [37, 158] was recently introduced for microvolume metabolomics, lipid analysis, protein analysis, carbohydrate analysis and enzyme monitoring [136, 137, 159, 160]. LESA requires a minimum amount of solvent applied

directly to the sample surface and is rapid and eco-friendly. The choice of extraction conditions can be optimized to focus on mobile species which results in simpler mass spectra [135]. LESA works well on homogeneous hydrophobic surfaces making it highly compatible for many synthetic polymers used for food packaging [133]. The applications of LESA in polymer science are scarce [37, 73, 135, 138, 161]; to date, it has never been used for the quality control of food contact materials.

Our first objective was thus, the development of a method based on LESA for automated extraction of mobile polymer additives and contaminants and to combine it with nanoESI-MS analysis for the extracted compounds identification and quantification.

The LESA-MS system is commercially available under the name Triversa Nanomate from Advion (Ithaca, NY, USA) and works using a robotic system that allows a nanoelectrospray infusion of samples dissolved in an aqueous organic solvent. The sample is placed on a plate and any part of its surface can be analysed by a two-step procedure involving (i) the extraction of analytes into a hanging solvent drop by the creation of a micro-junction between the solvent drop and the sample and (ii) spraying the solvent (containing the extracted analytes) through a nanoESI silicon-based chip. The use of a nano-ESI chip allows the creation of a long-lasting spray from very low amounts of sample (at a rate of approximately 100–200 nL min⁻¹ (see **figure I-28**). The optimization of LESA-MS for polymer analysis involves several parameters:

- the composition of the solvent and its volume (aspired and dispensed),
- the tip height above the sample,
- the contact time between the solvent and the surface,
- the aspiration speed,
- the number of repetitions of the extraction operation,
- the pressure and voltage applied during the spray.

As a consequence, many different combinations of parameters have to be tested in order to achieve the most efficient extraction.

▪ In-bulk extraction

A sample of candidate packaging material has been investigated to evaluate possible migration of its components into food, and more specifically into ham.

The extraction of analytes from ham consisted of 8 different steps: first about 0.25 g of the film was cut in small pieces, the sample was extracted 3 times with 2 mL of hexane, the latter extraction being carried out in the presence of 0.5 mL of 10% NaCl. Then, pooled extracts were salted out with NaCl. The organic layer was

taken and dried. After drying, 1 mL of acetonitrile was added, and the solution was warmed up to 37° and centrifuged. The supernatant was separated and dried again. Finally, the obtained residue was re-dissolved in 0.1 mL of acetonitrile and analysed by ESI-MS. Those analyses revealed the presence of molecules from one of the plastic packaging candidates in the ham (as shown in **Figure II-23**): polyethylene terephthalate (PET) sample.

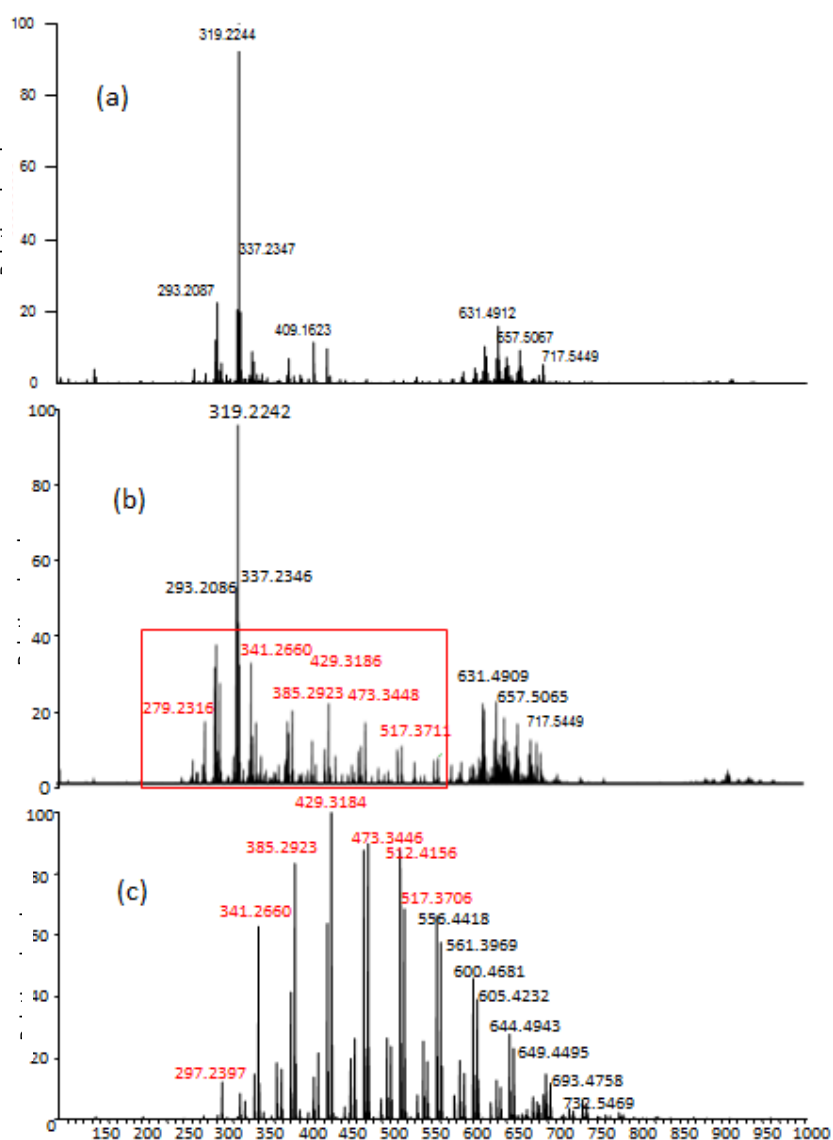


Figure II-23. In-bulk MS of ham and its packaging. (a) Clean ham; (b) Ham in contact with the PET plastic packaging; (c) PET packaging.

Each mass spectrum shows molecular ions of several hydrocarbons' subunits (m/z values) as well as their fragment ions; their distinctive fragmentations patterns helped identify the composition of the polymer (**Figure II-23.c**). The pattern of a polymer is very specific: it is composed of peaks spaced-apart by a regular mass: the monomer mass comes off during the ionisation lowering the polymer chain mass regularly. When

analysing the ham that was in contact with the PET sample (**Figure II-23.b**) it revealed the presence of peaks coming from the plastic packaging in the ham (regular peaks spaced apart by 44Da).

For the LESA's parameters' optimization, this commercial PET film sample was used. The polymer samples were analysed directly after having been cut to the appropriate size and put into the instrument holder. The robotic system allowed a nanoESI infusion of the extract. The measurements were done in the positive ionization mode with a scan range from m/z 100 to 2000 at a resolution of 500,000 (FWHM at m/z 200). The automatic gain control (AGC) was set to 1.0e05 ions and a maximum injection time of 100 ms. Care was taken not to touch and contaminate the surface to be analysed. The instrument was calibrated daily and optimized for sensitivity by infusing the manufacturer's standard calibration solution employing TriVersa NanoMate chip-based nanoESI ion source.

- **Time**

The first step in the optimization was to evaluate the contact time between the solvent drop and the studied sample. The key point of a successful extraction by LESA-MS is to obtain (and to keep) a good microjunction. Hence, a compromise has to be found between a good enough extraction and a microjunction that doesn't break. The maximum time allowed by the software is 1 minute and the minimum is 1 second.

The tests realized with a 1 minute-extraction revealed large standard deviations, probably due to a composition modification: the solvent drop extracts a huge amount of analytes thus revealing other species located underneath.

The tests realized during a 1 second-extraction showed smaller standards deviation but a strong increase of the background noise due to the insufficient amount of analyte extracted.

Time thus seems to have a huge impact on the results and so does all the other parameters, so they all need to be optimized. In the case of our plastic packaging, the best compromise seems to be between 5 and 8 seconds of extraction: great extraction (no background noise), no composition modification and a great microjunction.

- **Homogeneity and reproducibility**

The second step in the optimization was to evaluate the homogeneity of our PET plastic sample. To do so, five points were chosen all over the plastic sample and analysed by LESA-MS under the exact same conditions, shown in **Table II-2**.

Table II-2. Main LESA-MS parameters used for the homogeneity and reproducibility study.

	Solvent	Solvent aspired	Air aspired	Solvent dispensed	Dispention height	Aspiration height	Time	Spray Voltage	Pressure
Parameters	1:1 H ₂ O-ACN+ 0,1% formic acid	2 µL	0,5 µL	1,8 µL	1,6mm	1,4mm	5s	1,4kV	0,35psi

This step is major as the size of the drop is tiny as compared to the size of plastic samples. The study of the spectrums that were obtained consisted in comparing the number of common peaks on the five spectrums and to compare their intensity (and relative intensity). Thanks to a statistical analysis, it came out that 76.4% peaks are common between the five analyses. There is however a slight deviation in terms of intensities. This percentage is thus qualitative but allows us to consider the plastic sample as homogeneous.

Also, the reproducibility of the analyses was studied and revealed that the intensities obtained for the most intense peaks presents a very low standard deviation: below 9%. At the opposite, when looking at low intensities' peaks, it comes out that the differences in terms of intensities are much more important due to a lower abundance: up to 29%.

These analyses showed that the studied sample is homogeneous and can thus be used for the method optimisation. Moreover, the method proved to be reproducible even if the standard deviation increases with the least intense species.

- Consecutive extractions

A "concentration effect study" was done with the LESA-MS by realizing several consecutive extractions (up to 5) on the same point of our PET film. The applied conditions remained the same as those described in **table II-2**. It was repeated twice for reproducibility reasons and two tendencies appeared:

- A diminution of the common peaks through the extraction can be observed:

2nd extraction: 88% common peak with the 1st extraction,

3rd extraction: 84% common peak with the 1st extraction,

4th extraction: 82% common peak with the 1st extraction,

5th extraction: 72% common peak with the 1st extraction.

- Diminution and stabilisation of the intensities of the most intense peaks.

It shows the diminution of the availability of migrating species from the surface and the apparition of new ones. It looks like there are kind of layers that are removed by the solvent drops' extractions.

Species identifications

As said before, most of the detected signals are separated by a regular space of 44Da. Among these signals we can distinguish several regular series of organic compounds. In total, and thanks to the development of an informatics program, 29 different series were identified. Among them, 4 are very intense and present in all the different analysis (even in the multi-step extraction in **Figure II-23**), they are summarized below in **Figure II-24**.

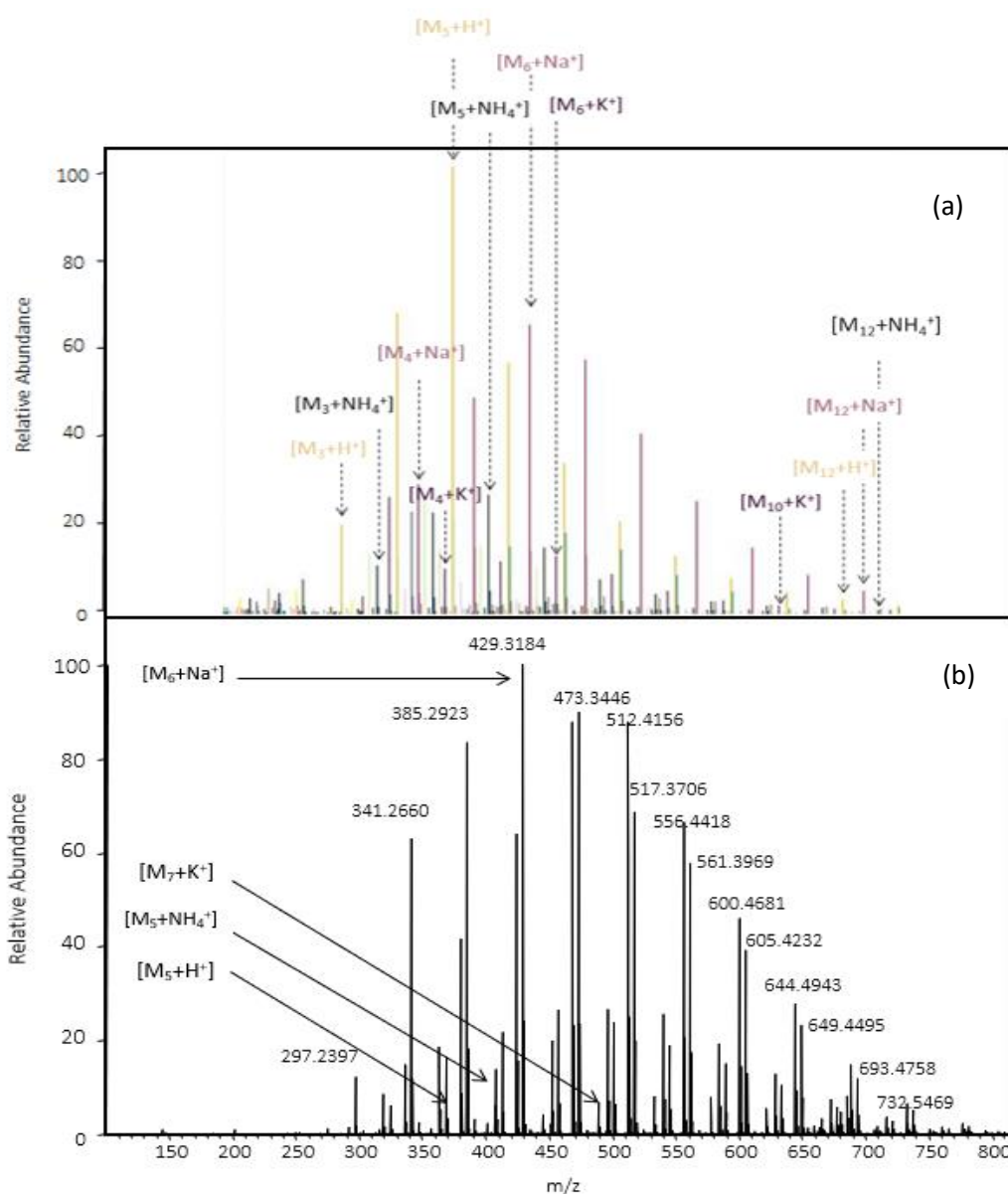


Figure II-24. Mass spectra obtained for the analysis of a candidate packing materials (a) by LESA MS accompanied by the identification of the signals and (b) after a classical batch sample preparation method.

As explained before, mass spectrometry of polymers allows the identification of the polymer thanks to the difference in weight between each regular peak: it is the monomer weight. Here 44Da, corresponds to a residual Polyethylene Oxyde (also called polyethylene glycol). This PEO is used for PET synthesis or PET recycling, it can thus come from polymerization residues or polymer degradation residues. Also, PEO is often used as an additive for its plasticizing properties.

In total, $[M_n+H^+]$ series were identified (with variable n) as well as different adducts: $[M_n+Na^+]$, $[M_n+NH_4^+]$ and $[M_n+K^+]$. This can be due to the fact that LESA is an ambient ionisation source, allowing ambient molecule to graft on the polymer chain during ionisation, also adducts are common when using an electrospray ionisation. Finally, these $[M_n+H^+]$ molecules and their adducts also have ^{13}C equivalent (up to three ^{13}C were found in formulas).

- Solvent optimisation

The main limitation of the method is the necessity to create a micro-junction: it can be difficult on rough, wettable or absorbent surfaces. It works best on homogeneous hydrophobic surfaces, making the method highly compatible for many synthetic polymers [133]. The solvent composition was optimized for the analysis of polymer films used for food packaging to achieve a stable liquid micro-junction between the solvent droplet and the polyester surface, ensuring sufficient swelling of the polymer substrate to extract the analytes and generate a stable ion current from the electrospray source [140]. Solvent mixtures reported in the literature (detailed in **Table II-3**) were tested and compared with (iv) 0.1% formic acid in (3:1) acetonitrile – water: 0.1% formic acid in (i) (4:1) methanol – water, (ii) (2:1) methanol – chloroform, (iii) (4:2:1) propanol – methanol – chloroform.

Table II-3. LESA-MS parameters used for polymer analyses.

Polymer Matrix	Analyte	Solvent Mixture	Contact Time (s)	Repetitions	Ref
Polyethylene, terephthalate (PET),	Polyethylene oxide (PEO)	1:1 H ₂ O:ACN + 0.1% formic acid	8	-	[140]
Worn contact lenses	Lipids	(2:1 v/v) MeOH:CHCl ₃ + 8 mm CH ₃ COONH ₄ (negative ion mode)	5	-	[138]
Worn contact lenses	Lipids	isoPrOH:MeOH:CHCl ₃ (4:2:1 v/v/v) + 20 mm CH ₃ COONH ₄ (positive-ion mode)	5	-	[138]
Polyester-based coil coatings	HALS (additive)	MeOH:CHCl ₃ (2:1) + 0.1% formic acid (v/v)	1	3	[139]
Polyacrylate	HALS	MeOH:CHCl ₃ (2:1) + 0.1% formic acid (v/v)	1	2	[73]
Plastic e-wastes	Brominated flame-retardant (additives)	MeOH:CHCl ₃ (2:1) + CH ₃ COONH ₄ (20 mm)	1	3	[37]

Our commercial plastic sample was analysed under the same conditions as above (**table II-2**) with all these solvents to evaluate whether there is an effect on the analyte's extraction. The previously described series were separated from the resulting spectrum and compared in terms of intensities as shown in **Figure II-25**.

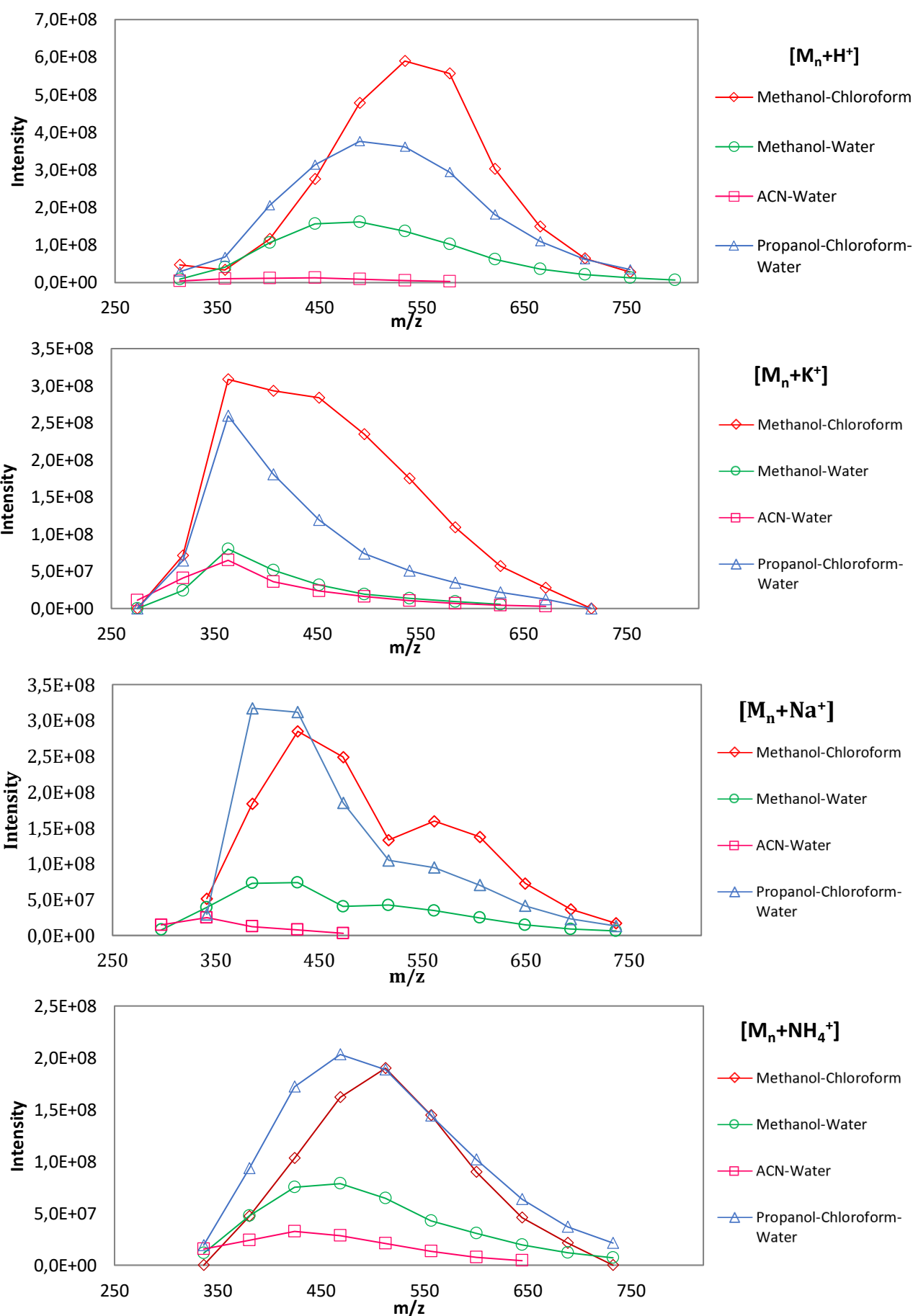


Figure II-25. Evolution of the intensities of each series of peaks as a function of the extraction solvent.

These results show the evolution of the intensity of the $[M+H^+]$ and its adducts as a function of the solvent that was used. Whatever the solvent an extraction can be seen, but of course its intensity is significantly function of the chosen solvent: the most efficient one is (2:1) Methanol – Chloroform + 0,1% formic acid for all the series. The MeOH provides a stable electrospray, chloroform responsible for swelling of the polymer and facilitating extraction and formic acid (for positive-ion mode ESI-MS) helping protonation of the analytes (increasing the abundance of ions for detection) [140]. In the case of polymers, analyte desorption involves simultaneous swelling and extraction. The possibility to change the solvent allows targeting the extraction of a chosen component rather than another.

This tendency is the same for the other series of peak.

The previously described optimisation led to the obtention of optimum parameters for our specific application (see details in **Table II-4**).

Table II-4. LESA-MS optimum parameters for the polymer surface analysis.

Solvent	Solvent aspired	Air aspired	Solvent dispensed	Dispention height	Aspiration height	Time	Repeat	Spray Voltage	Pressure
(2:1) Methanol – Chloroform + 0,1% formic acid	2 μ L	0,8 μ L	1,8 μ L	3mm	2.8mm	8s	0	1,4kV	0,35psi

However, a huge number of parameters can be changed according to the application. For example, the dispention and aspiration height depends on the width and the hardness of the sample but also on its composition of the surface as the microjunction creation depends on its wettability. The number of repeat and the time of the extraction were optimized accordingly with these concentration phenomena explained above. In fact, as there are some composition variations due to the use of a solvent drop, we chose to avoid repetitions and optimized the time at 8 seconds (compromise between a good enough extraction and a microjunction that doesn't brake). The spray voltage and the pressure where chosen based on the supplier advices: each solvent has its own values.

- **Comparison with DART-MS**

The method was compared with another ambient ionisation method: DART (Direct Analysis in Real Time) MS proving its suitability for rapid screening for the presence of post-polymerization residues in food packaging materials. The results obtained with the same commercial packaging as above showed a spectrum where the polymer pattern is less clear than the ones obtained with the LESA (**figure II-26**).

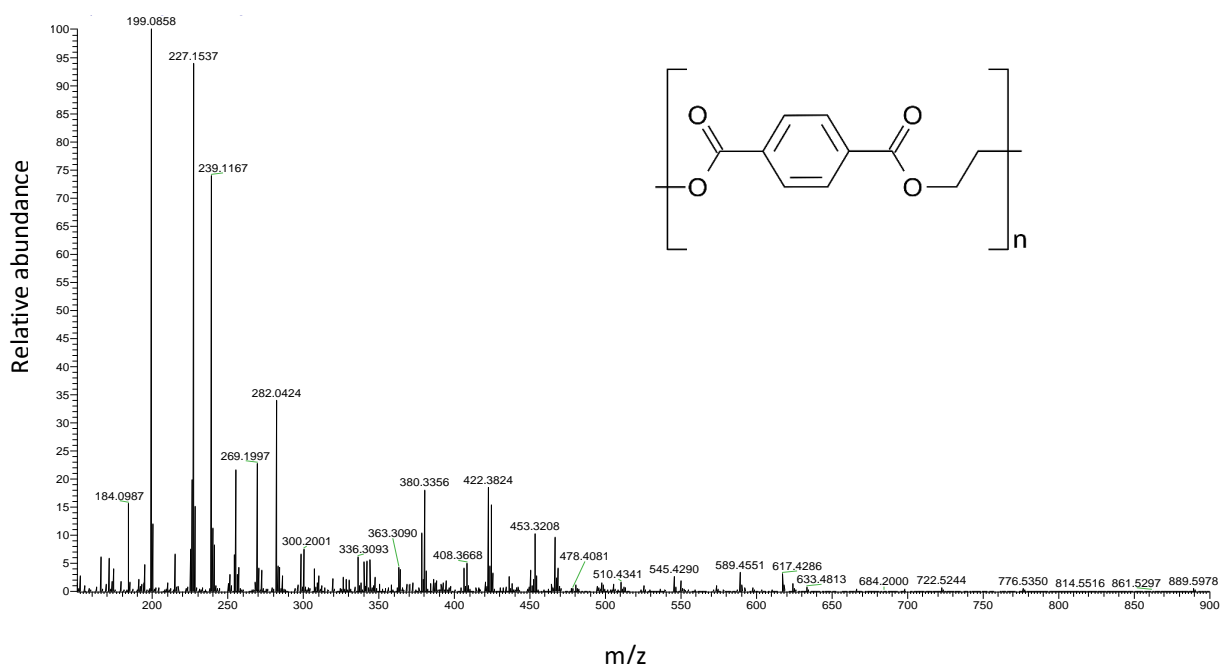


Figure II-26. DART-MS spectrum of the commercial PET film.

In fact, the polymer that can be seen is the PET itself (schematized in **figure II-26**) and not the migrating residual PEO. The principle of this ambient method involves only a heated gas stream (and no solvents); it creates degradations of the plastic samples. As our study deals with the identification of the molecules that can migrate, by degrading the polymer the DART will force the migration of other molecules that wouldn't have migrated in a "normal" case. LESA-MS is a softer extraction method and thus appears to be more adapted for polymers surface analysis and migrations analysis than DART-MS. However, DART-MS offers other possibilities for polymer analysis allowing to have access to different data as compared to LESA-MS.

As a conclusion we can say that LESA combined with nanoESI ionisation - MS demonstrated to be a convenient method for the direct analysis of polymer packing materials. The method was validated by the currently used method based on the extraction from the bulk followed by ESI-MS. LESA-nanoSI MS does not require sample preparation, is fast, eco-friendly and highly reproducible. It also compares favourably with ambient ionisation DART-MS in terms of selectivity producing simpler spectra limited to the mobile compounds of interest. It has to be mentioned that the sensitivity of LESA MS is inversely size dependent due to higher solubility and thus easier extraction from the surface of smaller species. At the same time, the potential migration into food is also facilitated in the case of small molecules.

IV. MATERIALS OF THE STUDY

IV.1. Polymers

Low Density Polyethylene is commercially available under the name ALCUDIA® PE015 (see datasheet in **annexe 4**). The Polypropylene is isotactic and is an experimental one as it is very difficult for industrial facilities to produce an additive-free PP, thus not commercially available.

The polypropylene and the polyethylene used in our study was provided by REPSOL (Madrid, Spain): they were both extrusion grade (rather low viscosities) and both free of any additives.

IV.1.a. Isotactic polypropylene

- Thermal properties

Thermal characterisation of the virgin polymer was achieved by thermogravimetric analysis (TGA) under air atmosphere and under nitrogen as described earlier in part II-1. The applied procedure was a 10°C/min ramp from the ambient temperature to 600°C nitrogen and air. The results are shown in **figure III-27**.

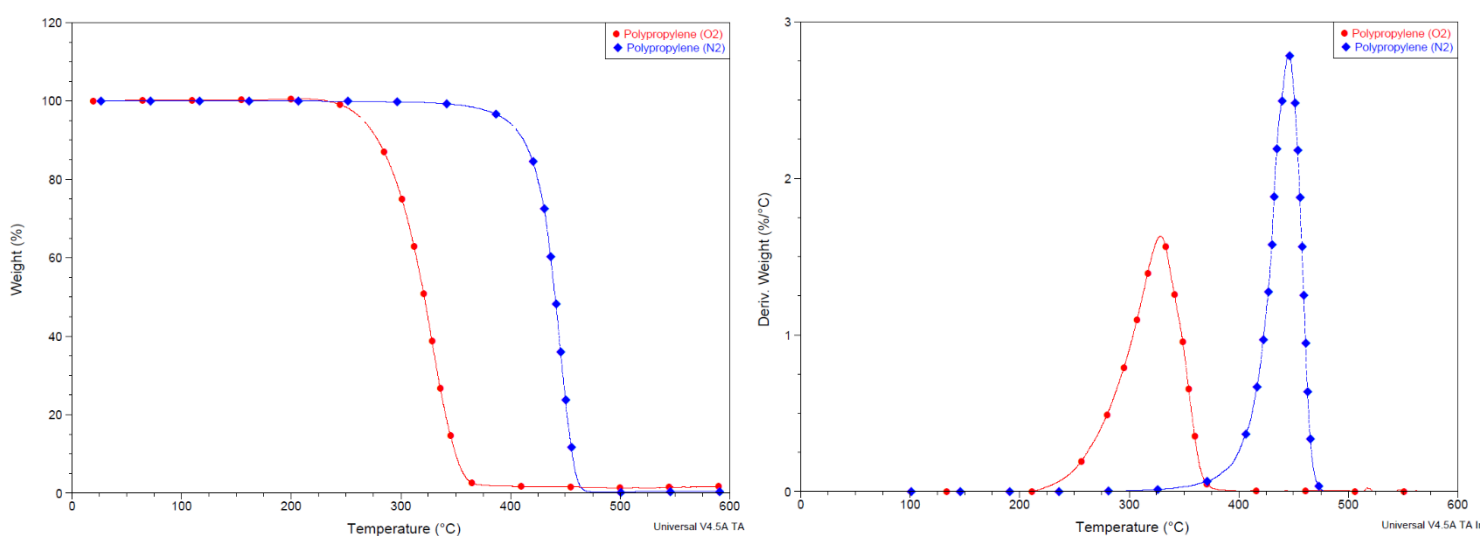


Figure II-27. TGA of virgin isotactic polypropylene under air and nitrogen.

First, the differences that can be observed according to the atmosphere that was used can be related to the polymer's sensitivity to oxidation. Actually, under nitrogen, the polypropylene degradation is completely delayed showing that most of the degradation occurring during thermal analysis under air are due to oxidation. The onset of degradation (very beginning) is around 210°C and 296°C under air and nitrogen respectively. Moreover, the maximum of degradation (maximum of the derivative curve) is logically also delayed (around 100°C delay). However, the kinetic of degradation is higher under nitrogen than under air: the derivative curve

of iPP analysed under nitrogen presents a thinner peak representative of a faster degradation (or the degradation slope of the blue curve in normal mode is higher).

Other important information was acquired by Differential Scanning Calorimetry (DSC) following the method 1 described earlier in part II-1. The applied procedure consisted in three cycles: the first one to erase the thermal past (data not shown); a second one for the cooling process and a last one to melt the polymer. The results are shown in **figure II-28**.

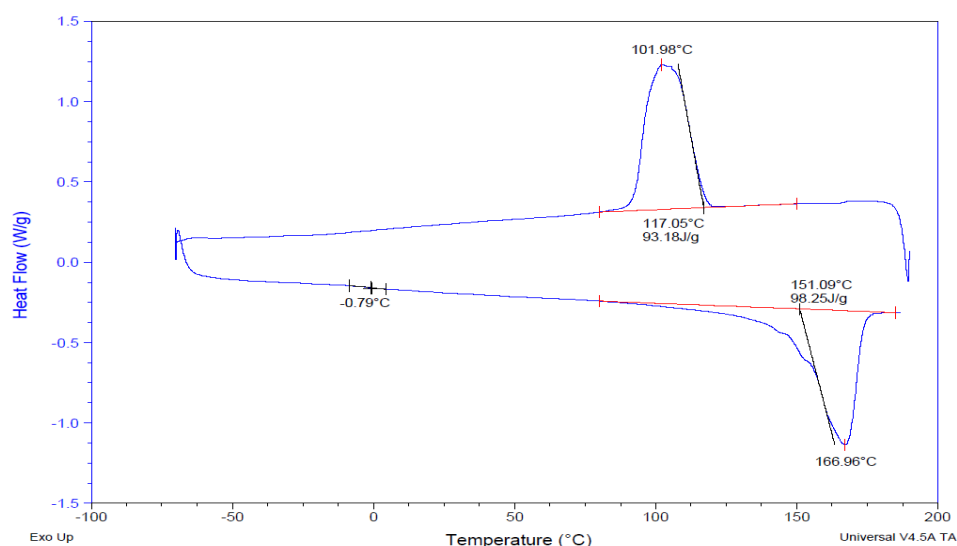


Figure II-28. DSC analysis of virgin isotactic polypropylene.

Polypropylene's glass transition (T_g) is around -1°C , the fusion temperature is around 169°C and the crystallisation temperature around 118°C . The enthalpy values obtained for those characteristic transition allow to calculate the crystallinity rate thanks to **equation 21**).

$$\chi = \frac{\Delta H_f}{\Delta H_f^0} * 100 \quad (21)$$

Where χ is the crystallinity rate (%), ΔH_f is the experimental fusion enthalpy (J/g) and ΔH_f^0 the standard fusion enthalpy of the studied polymer (J/g). iPP's standard enthalpy is 207 J/g. The crystallinity rate is highly function of the cooling ramp, so the crystallinity for a 10°C ramp is around 40%. When applying a 5°C ramp, the crystallinity reaches 55%.

All the obtained values correspond to the crystallinity of classic polypropylenes in the literature.

The thermal analysis thus gives a lot of information toward the processability of our iPP: the processing temperature must be above 167°C . Nevertheless, those results are not sufficient: the viscosity is a critical parameter for the extrusion process.

All those characteristic values are summarized in **table A-1** in **annexe 5**.

- Viscoelastic properties

The method used to determine the viscoelastic behaviour of our polymers was described in part II-2. These analyses were achieved thanks to a rotational rheometer in parallel-plate configuration with a 25mm diameter geometries under nitrogen. The gap between the plates is usually around 1.8 mm but can slightly vary according to the sample.

According to the data previously obtained, we know that no degradation will occur until 290°C under nitrogen. However, we will apply a certain shear that can cause degradations that were not present with TGA; hence the maximum temperature was fixed at 250°C. To confirm the choice of this temperature value, a time-sweep analysis was realized under nitrogen at 250°C (10hz, 2.5% shear strain for 1 hour) and no variations of the modulus were obtained: the sample is stable up to 11 minutes under nitrogen. Also, thanks to the DSC analysis, we know that iPP crystallises around 118°C and we also know that crystallisation brings solidity to the sample and could thus damage the rheometer (as it is a melted state analysis). In turn, the lowest temperature is fixed at 130°C. To summarize, in order to achieve the Time-Temperature superposition (TTS), the analysis was done at three different temperatures: 250°C, 190°C and 130°C. The chosen reference temperature was the lowest one (130°C).

After having evaluated the linear domain of our polypropylene and realized the frequency sweep at each of those temperatures, the TTS was realized (**figure II-29**).

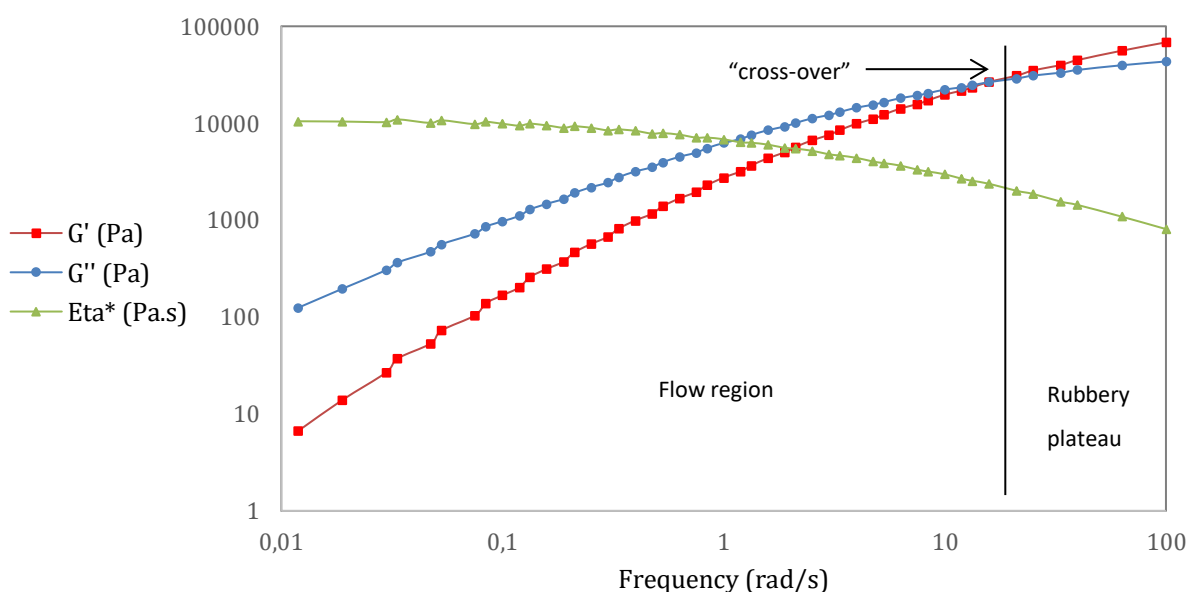


Figure II-29. Virgin isotactic polypropylene's master curve.

The obtained curve shows the evolution of the modulus (curves blue and red) and of the complex viscosity (grey curve) as a function of the frequency. The complex viscosity evolution puts forward the shear thinning aspect of our polymer as the viscosity diminishes with increasing frequencies.

This TTS (also called master curve) gives access to a lot of data. The main values that can be extracted from this master curve are:

- the limit viscosity which is also called the Newtonian viscosity (because it is taken at low frequency when the viscosity isn't a function of time according to the Newton model). It can be evaluated than to the grey curve η^* which is the complex viscosity of the polymer. At low frequencies, it reaches a plateau: this is the limit viscosity η .
- the maximum time of relaxation τ_w corresponds to the relaxation of the longest chains (their ability to go back to their initial state). It is found at the intersection of G' and G'' tangent;
- the local or the average relaxation time τ_n which corresponds to the G' and G'' curves intersections (the cross over);
- the activation energy E_a (KJ/mol) of the polymers which described the amount of energy needed for the polymer chains to move.

Finally, information is given by the slope of each curves at low frequencies: G'' slope is 1, but G' slope is close from 2 following the Maxwell model. When those slopes are obtained, the polymer have reached its flow terminal area (it is a liquid which relaxation time corresponds to that of the reputation).

All those characteristic values are summarized in **table A-1** in **annexe 5**.

IV.1.b. Low Density Polyethylene

The previous characterisations were also applied to Low Density Polyethylene (LDPE).

- **Thermal properties**

Thermal characterisation of LDPE was achieved by TGA (10°C/min ramp from the ambient temperature to 600° nitrogen and air). The results are shown in **figure II-30**.

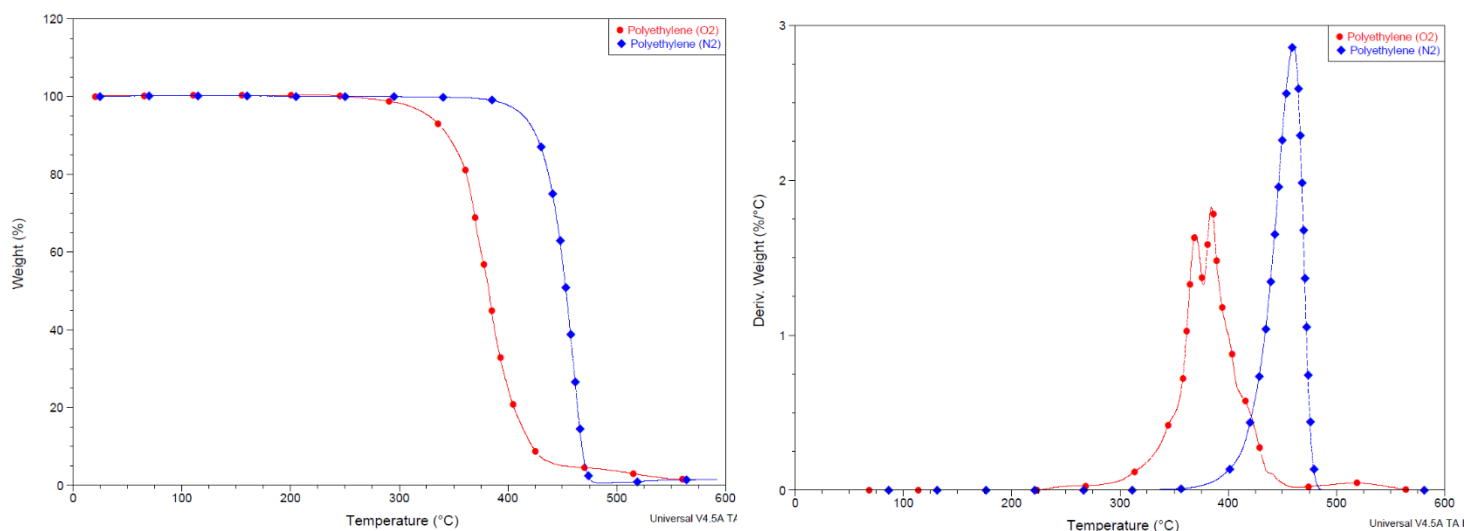


Figure II-30. TGA of virgin isotactic polyethylene under air and nitrogen.

The differences between the thermogram obtained under air or under nitrogen are smaller for LDPE than for iPP. There is around 60°C different between both onset of degradation (for around 80°C for iPP) and around 70°C difference between the maximum of degradations (for over than 100°C for iPP). Polypropylene is more prone to oxidation than LDPE (mainly because of the methyl ramifications of the iPP that are more reactive than the hydrogen of LDPE).

Also, the degradation of LDPE under air gives rise to a non-homogeneous peak (see red derivative curve). It could show the presence of different populations of polymer chains within the polymer.

In terms of kinetic, when evaluating the slope of the mass loss, the degradation appears to be faster under nitrogen (the same observation was done for iPP).

All those characteristic values are summarized in **table A-1** in **annexe 5**.

Differential Scanning Calorimetry (DSC) was also realized but the applied temperatures are different than for iPP: the first one was done with a 20°C/min ramp from the ambient temperature to 140°C, (data not shown); the second one from 140°C to -10°C with a 10°C/min ramp and the last one from -10°C to 140°C with a 10°C/min ramp. The results are shown in **figure II-31**.

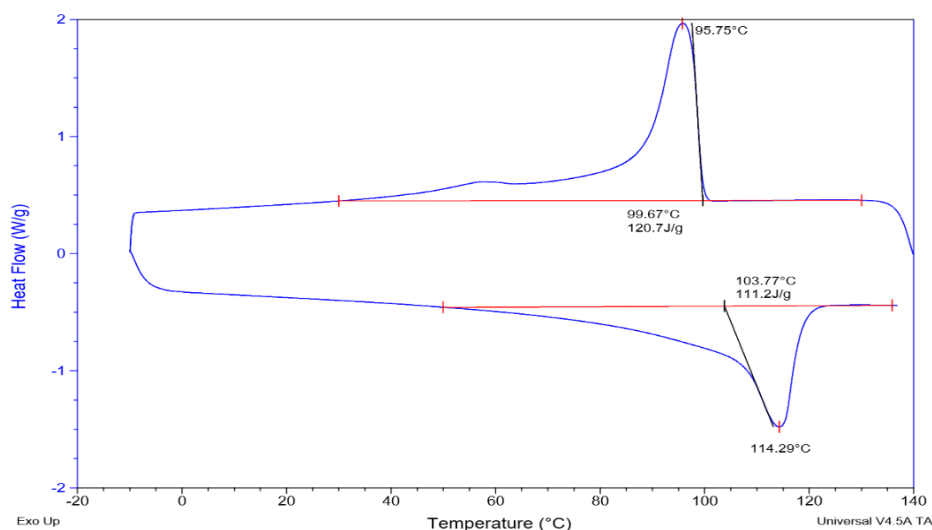


Figure II-31. DSC analysis of virgin isotactic polypropylene.

The fusion and crystallisation peaks are not well defined. It seems to correlate with the non-homogeneous peak observed by TGA (**figure II-30**) and show a rather high polydispersity. Actually, there seems to be a crystallisation of another polymer population around 60°C. When this peak is integrated alone, it represents around 5% of the crystallising polymer. Nevertheless, in further analysis the possible modification of this “secondary” population could lead to elevated standard deviations. If we only consider the main population (apex of the peaks), the fusion temperature is around 114°C and the crystallisation temperature around 96°C. LDPE’s standard enthalpy is 293 J/g and the calculated crystallinity for a 10°C/min ramp is 38%. When applying a 5°C ramp, the crystallinity reaches 47%.

Also, the glass transition of polyethylene cannot be observed as LDPE’s T_g is around -100°C and our DSC only goes down to -70°C. In turn, at the opposite of the polypropylene, LDPE’s glass transition is very hard to observe as it requires technics able to go down to very low temperatures.

- **Viscoelastic properties**

The LDPE’s master curve was obtained using the same parameters as those detailed above (**figure II-32**). The thermal stability was also assessed using a time-sweep analysis realized under nitrogen at 250°C (10hz, 2.5% shear rate for 1 hour): the virgin LDPE is stable during to 35 minutes under nitrogen.

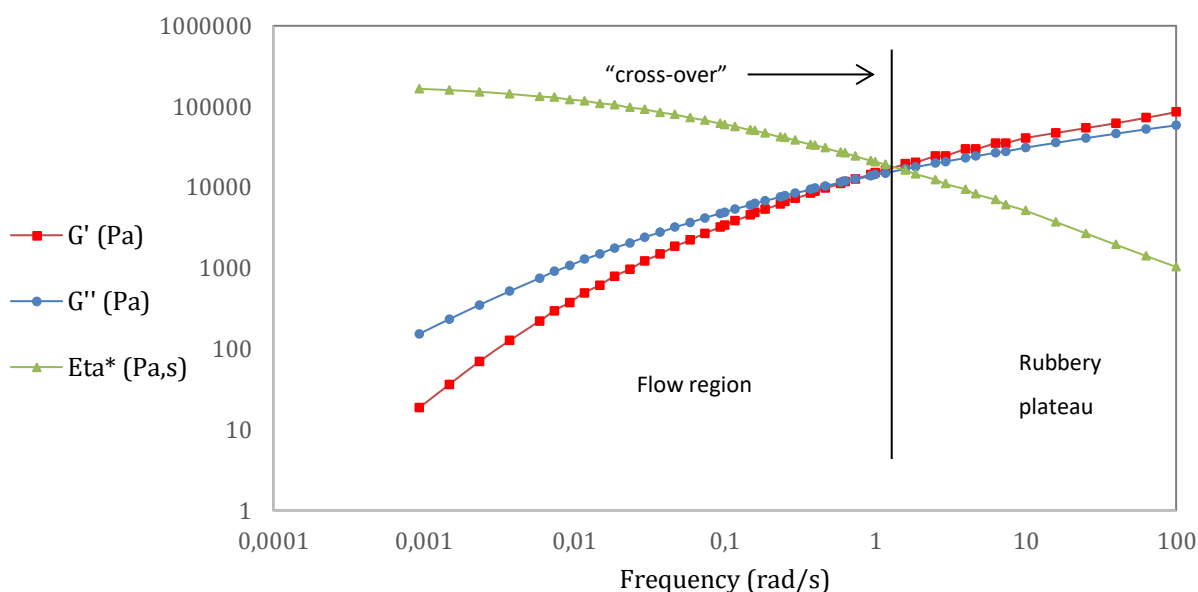


Figure II-32. Virgin low-density polyethylene's master curve.

Both polymers' master curves cannot be compared to each other. Actually, even though they were realized under the exact same conditions, they have completely different thermal transitions. To be comparable, the experimental temperatures should have been chosen at iso-energies of activation. However, results from **table A-1** from the **annexe 5** shows that, under those experimental conditions, LDPE's E_a is almost twice as big as iPP's one. Also, the slope 1 and 2 corresponding to Maxwell's model are present showing that the terminal zone was reached (the same analysis was done at 230°C and didn't allow to reach this terminal zone).

IV.1.c. Polymers recyclability

To answer to the Foodyplast project, the synthesized material must be fully recyclable. To evaluate our virgin polymers' recyclability, they were submitted to consecutive extrusions (up to 9) and analysis were realized for each recycling cycles. The evolution of the polymer properties with recycling is assessed by the determination of the polymer melt flow index (MFI) along the 9 extrusion passes, by thermal and rheological analysis.

- Melt Flow Index evaluation

The Melt Flow Index (MFI) method is described earlier in part II-2. The parameters specified by the norm for the MFI evaluation of LDPE are: 190°C under 2.16kg weight. For iPP, the parameters are: 230°C, 2.16

kg weight. This index was measured for all the recycling cycles five times and the extreme values were deleted. The results are shown in **figure II-33 and II-34**.

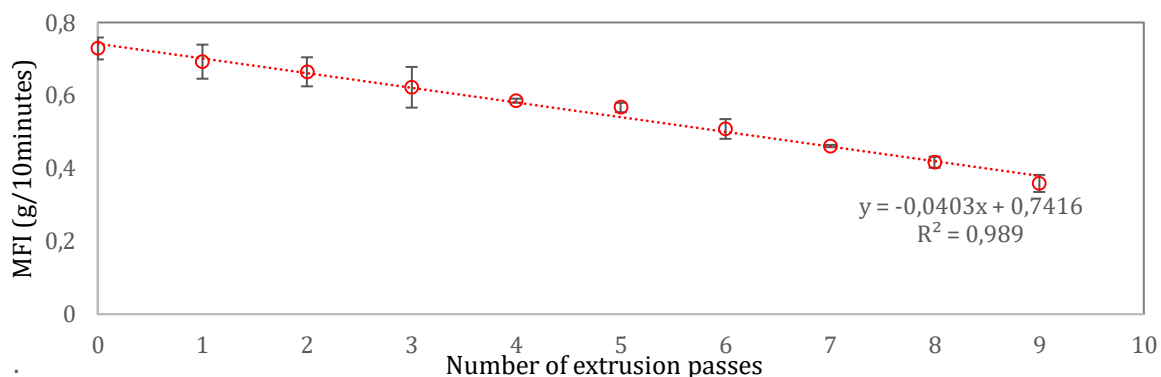


Figure II-33. LDPE MFI values through extrusion cycles.

In the case of LDPE (curve from **figure II-33**), the MFI value specified by the supplier is 1 g/10minutes. The experimental obtained value for the cycle 0 (before any thermal processes) is 0.74 ± 0.08 g/10minutes. Through the recycling cycles, the MFI (which is inversely proportional to the viscosity) diminishes, revealing a branching of the polymer chains. LDPE is known to easily reticulate due to its numerous ramifications before suffering from chain's scissions (the degradation process is not necessarily a reticulation followed by chain scission, it can be more random). The delta in MFI between the cycle 0 and the cycle 9 is a loss of around 0.36 g/10minutes.

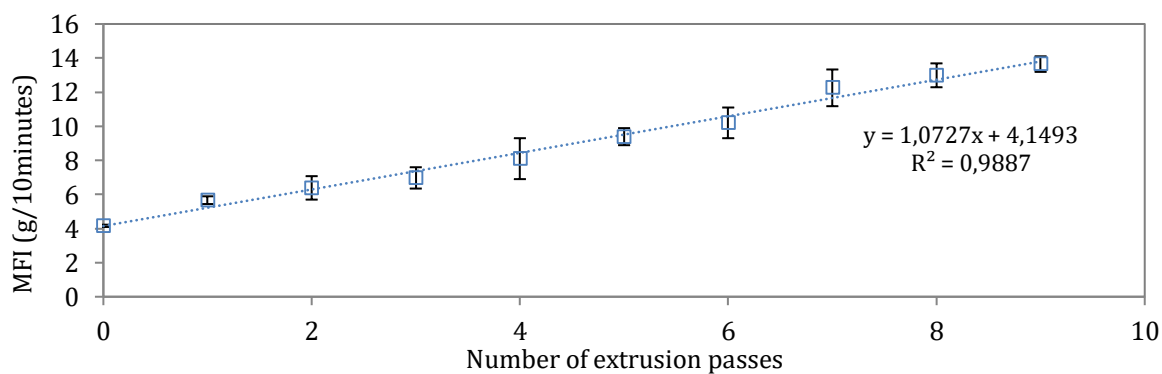


Figure II-34. iPP MFI values through extrusion cycles.

For iPP, the curve presents a positive slope corresponding to a diminishing viscosity. iPP degrades by chain scissions and cannot undergo any branching as it is linear. Subsequently, this chain breaking degradation involves an increase in the MFI from 4g/10 minutes to almost 14g/10minutes.

- Molecular evolution by Cole-cole representation

The several extrusion cycles were analysed by rheology showing a strong and regular deviation throughout the cycles. This deviation is more visible in Cole-cole curve and so does the polymer degradation process. In fact, the Cole–Cole equation is a relaxation model described in part II-2. When drawn, the cole-cole diagram forms a semicircle allowing having access to information about any changes in polymer’s chains length (characteristic from the thermo- or photochemical degradations of polyolefins). This diagram is drawn from the polymer’s master curve by plotting η'' as a function of η' . **Figure II-35** shows the Cole-cole diagram of each polymer for the some of the recycling cycles (cycle 0, 1, 3, 6 and 9). In order to easily extract all the data from those diagrams, a MATLAB program was set up. This program allows to draw a fitting circle based on the experimental data and, when needed, allowed to complete the semi-circle (as explained later, the experimental data don’t always form the entire circle).

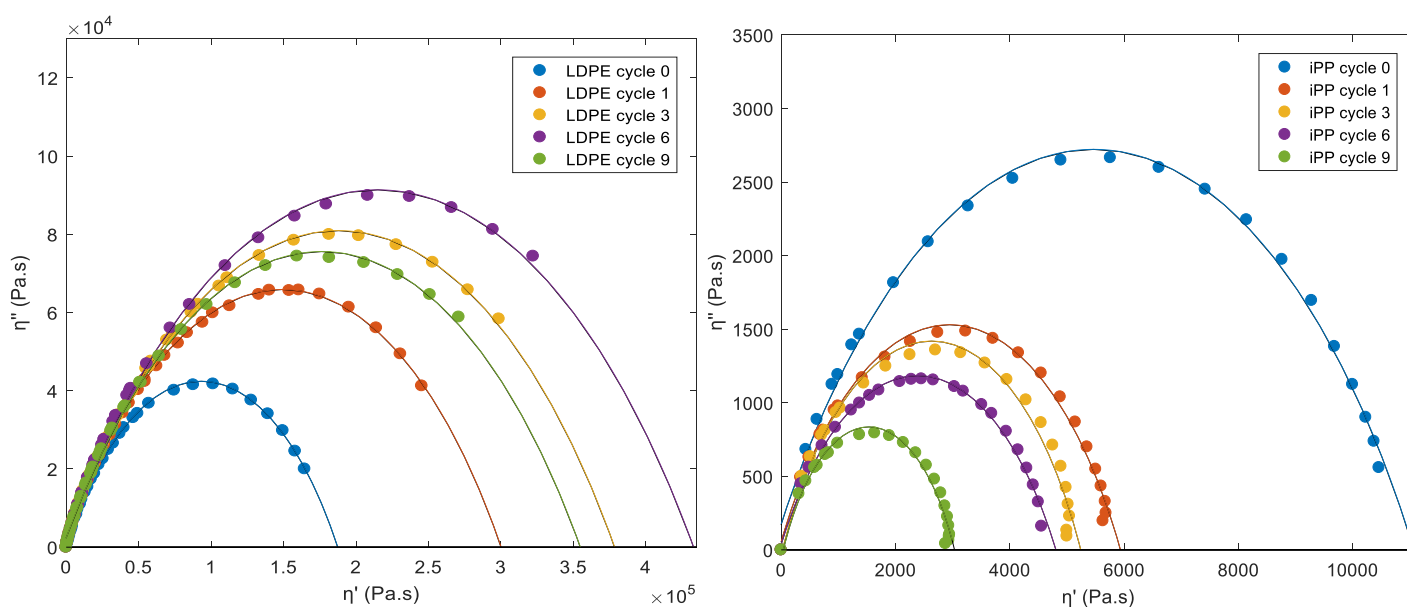


Figure II-35. Cole-cole diagram of virgin LDPE and virgin iPP.

Those diagrams clearly put forward the effect of recycling on the polymer chains. In the case of LDPE, with increasing number of extrusions, the relaxation time (corresponding to the frequency of the apex of the circle) and viscosity (corresponding to the cross section between the circle and the x axis) increase between the cycle 1 and 6. The increasing of the relaxation time is a consequence of the increase in viscosity: when chain reticulation occurs, new nodes are created making chains movement more difficult. After the 6th cycle, we can see the diminution of circle radius, synonymous of viscosity and relaxation time diminution. As said before, LDPE’s degradation process is very complex as the polymer can undergo both branching and chain scissions. What we observe here reveals that LDPE first degrades by creating branching and then, at some point, degrades by chains scissions.

Due to LDPE very elevated viscosities, the semi-circle cannot be entirely shaped: the MATLAB program thus allowed to fulfil the cole-cole diagram and have access to all the information. At the opposite, in the case of iPP which degradations processes are less complex, the viscosity and the relaxation time keep decreasing due to chain scissions reactions. Also, we can notice that, at the end of the iPP's semi-circle, the curve seems to deviate from their trajectory. This phenomenon could be due to a viscosity drop in the slope 1 and 2 (at long times, or elevated temperatures). These artefacts could be due to the inhomogeneous degradation occurring during parallel-plate rheology: the degradation at the outer of the polymer is more important than the degradation in the core leading to the creation of deviations in the master curves.

Moreover, the curves allowed to have access to the energy of activation as shown in **table II-5**. The activation energy is the least amount of energy needed for a chemical reaction to take place; more specifically, here it describes the chains movement.

Table II-5. Activation energies of both polymers through the extrusion passes.

Number of extrusions passes	Average Energy of activation of LDPE	Average Energy of activation of iPP
0	68,8 ± 0.6	38.9 ± 1.7
1	70.8 ± 0.3	38.3 ± 2.1
3	72.3 ± 3.1	38.6 ± 1.1
6	73.6 ± 2.4	38.2 ± 0.7
9	71.8 ± 1.6	39,3 ± 0.7

LDPE's activation energy increase before reaching a plateau: this shows that polymers chains need more and more energy to move due to a stronger and stronger branching. However, a slight decrease can be observed for the 9th cycle due to possible chain scission reactions, as explained above (nevertheless, this decrease can't really be considered as significative due to the standards deviations). On the other hand, iPP's activation energy remains constant throughout the recycling cycles. Subsequently, it seems that the chains scission doesn't have any significative effects on the polymer chains motion: the chains are smaller, which should normally involve easier chain motion (lower E_a) but with chain scissions, they are more numerous which can counterbalance this previous phenomenon.

- **Thermal analyses**

Recycled virgin polymers were also analysed by DSC following the method described above. The obtained transitions (enthalpies and temperatures) are summarized in **table II-6**, as well as the calculated crystallinities.

Table II-6. DSC analysis of recycled virgin polymers.

	Crystallization enthalpy (J/g)	Fusion enthalpy (J/g)	Crystallization Temp (°C)	Fusion Temp (°C)	χ_f (%)
LDPE cycle 0	115 ± 8	114 ± 4	96 ± 1	113 ± 1	38,9 ± 1.4
LDPE cycle 9	106 ± 1 ↘	115 ± 2 =	98 ± 0.9 =	112 ± 0.6 =	39,3 ± 0.8 =
iPP cycle 0	95 ± 2	81 ± 0.7	118 ± 0.4	168 ± 3.4	39,4 ± 0.3
iPP cycle 9	97 ± 8 =	98 ± 8 ↗	118 ± 0.2 =	165 ± 1.1 =	47.2 ± 4.9 ↗

For LDPE, the overall values are relatively constant except for the enthalpy of crystallization which diminishes slightly (but this variation remains in the standard deviation). For iPP, more significative modifications can be observed with increased fusion enthalpy and, in turn, crystallinity. In fact, the chains scissions that iPP undergoes during recycling lead to a wider distribution of the crystal sizes which is synonymous from a broad melting range (loss of homogeneity).

Another DSC method was also applied to recycled virgin polymers: onset oxidation temperature method (OOT, described in part II-1). It consists in evaluating the oxidation kinetic thanks to a 10°C/min ramp under air. At the opposite of traditional DSC method, in the OOT assessment the samples are placed in an open pan to be fully in contact with oxygen. The DSC apparatus then evaluates the exothermic and endothermic transitions that the sample undergoes: oxidation is an exothermic process.

The results are shown in **annexe 6**.

As no additives giving antioxidants properties are present in those polymers, the OOT value remains relatively constant through the recycling cycles. However, the kinetic of degradation characterized by the slope of the curve at elevated temperatures increase: brutally at the 6th cycle for LDPE, gradually with increasing cycles for iPP.

- **High Temperature Size Exclusion Chromatography**

High Temperature Size Exclusion Chromatography experiments were realized in partnership with the C2P2 laboratory of Lyon.

Traditional size exclusion chromatography (SEC) is used mainly for the analysis of large molecules. It works by trapping molecules according to their size in the pores of a stationary phase contained in a chromatography column. The solution containing the analytes goes through this column: smaller molecules will enter the pores

while the biggest won't. At the end of the column, different types of detector can be found to measure the size of the molecules such as spectrometers (UV, IR, mass), viscosimeters, light diffusion detectors (LALLS, MALLS) ... However, polyolefins are not soluble at room temperature and thus not compatible with traditional SEC. The C2P2 laboratory possesses a heating SEC allowing to analyse polyolefins at temperatures at which they are soluble. Hence, both polymers were solubilized in 1,2,4-trichlorobenzene and maintained at 150°C. The columns that were used are PLGEL Olexis ones from Agilent. Three of those were putted in series.

Several aspects were evaluated at the end of the column: refractive index, right angle light scattering, viscosimeter, gyration radius, weight fraction... Different average values can be defined.

- M_n : number average molar mass, characteristic from the small masses. It is the ordinary way of evaluating the molecular masses of the individual macromolecules.

$$M_n = \sum \frac{M_i N_i}{N_i} \quad (22)$$

- M_w : mass average molar mass, also commonly called weight average. It is another way of describing the molar mass of a polymer but here a larger molecule will have a larger contribution than a smaller molecule.

$$M_w = \sum \frac{M_i^2 N_i}{M_i N_i} \quad (23)$$

- M_z : average molar mass, characteristic from the biggest masses;

$$M_z = \sum \frac{M_i^3 N_i}{M_i^2 N_i} \quad (24)$$

- I_p : polydispersity index.

$$I_p = \frac{M_w}{M_n} \quad (25)$$

The figures are reported in **annexe 7** and detailed in **table II-7**.

Table II-7. Results obtained on virgin polymers from high temperature SEC.

	Mn (Da)	Mw (Da)	Mz (Da)	Ip
LDPE	21242 ± 1854	159359 ± 16120	512588 ± 49826	7,5
LDPE cycle 9	15396 ± 998	134617 ± 11265	427510 ± 40235	8,7
iPP	71454 ± 7259	296817 ± 25123	723051 ± 73265	4,2
iPP cycle 9	92860 ± 8612	198288 ± 17892	341000 ± 33956	2,1

In the case of LDPE, after 9 cycles of extrusions all the masses (Mn, Mw and Mz) have diminished. The lowering of Mw and Mz confirms the presence of chain scissions reactions; however, the diminution of the number of smaller chains (Mn) also indicates that those chains reticulates leading to a low variation of the Ip. In other words, the Gaussian representing the distribution of the masses should move towards the large masses but instead we observe an enlargement of this Gaussian due to the increase of Ip. It means that there is a shift of the Gaussian towards the lower masses by keeping the Mw value constant: at the 9th cycle the chains scissions take over the branching process, confirming the results of the cole-cole.

For iPP, Mn increases due to an augmentation of the number of chains: creation of new smaller chains which also leads to Mz and Mw decline. The diminution of Mz perfectly illustrates the iPP degradation as the loss almost reach 50%. It shows the creation of a low molecular weight tale: the Gaussian is shifted towards the lower masses and get thinner (lower Ip) which confirms the scission of the chains in the middle of the polypropylene chain.

Now that the virgin polymers were fully described and before speaking about polymer blends, the raw additives properties will be detailed

IV.2. Stabilizers

α -tocopherol, ascorbic acid and linseed oil can be either natural or synthetic. For reproducibility reasons they were purchased from Sigma-Aldrich (Merck), known under the commercial references T3251 (\pm)- α -Tocopherol, A5960 L-Ascorbic acid and 47559-U linseed (flaxseed) oil, respectively. Quebracho tannins were provided by Unitan under the commercial name Colatan GTH[®]. Matcha green tea powder was bought from a local supermarket.

IV.1.a. Characterizations of the antioxidants of the study

▪ Thermal stability

α -tocopherol, ascorbic acid, linseed/flaxseed oil, green tea and quebracho tannin were analysed by TGA (10°C/min ramp from the ambient to 600°C under nitrogen) to evaluate their thermal resistance, essential to handle the extrusion process. The results are shown in **figure II-36** and **figure II-37**.

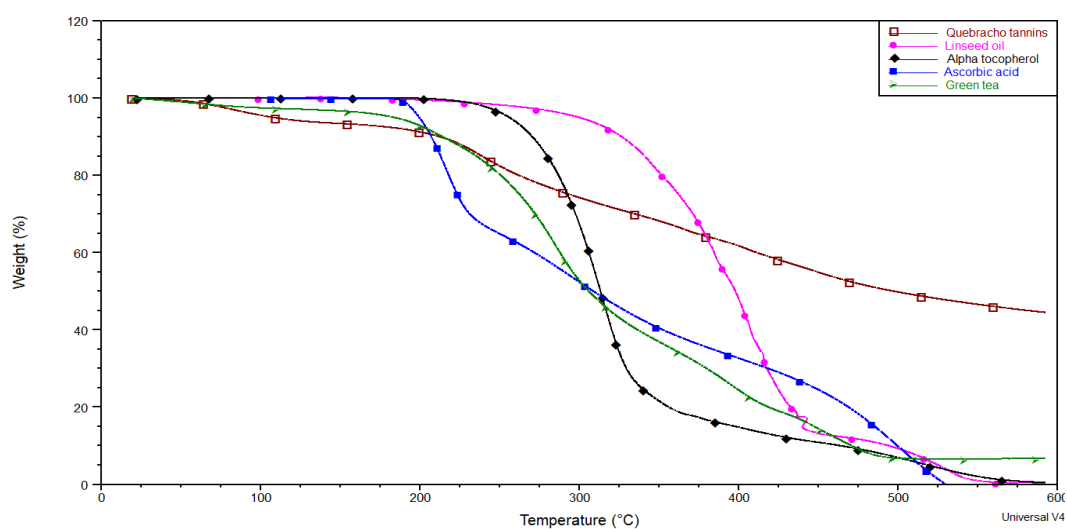


Figure II-36. Mass loss versus temperature for the different antioxidants.

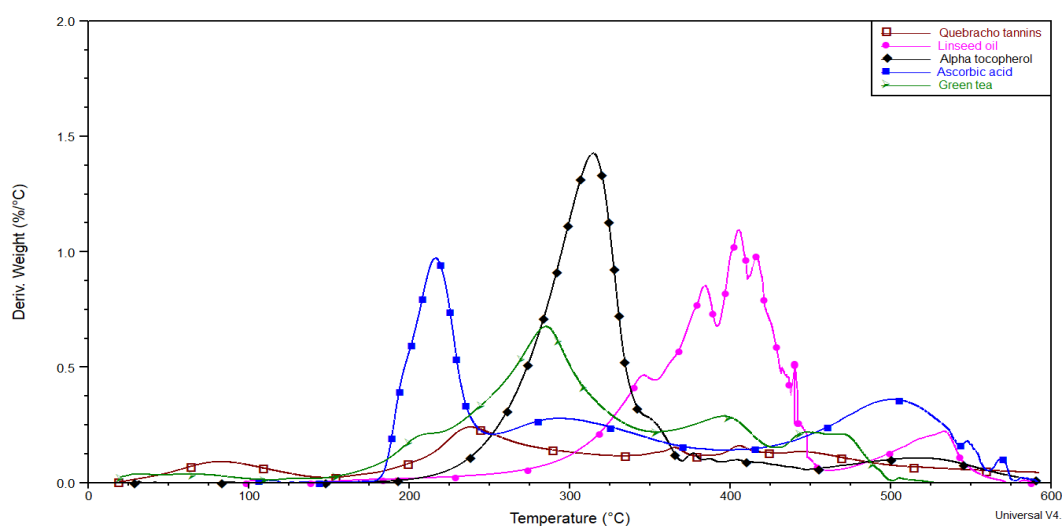


Figure II-37. Derivative weight loss of the different antioxidants.

Linseed oil (pink curve) is a mix of fatty acids; thus, its thermogram shows different decomposition steps. At low temperature, the oil absorbs oxygen to form peroxidised components. Above 100°C, the “peroxy”

groups decompose to form radicals [162]. On the derivative curve of **figure II-37**, we can see three different stages in the decomposition of linseed oil (black curve):

- Between 150 and 250°C: first phase corresponding to fatty acids oxidation;
- Between 250 and 400°C: decomposition due to the oxidation;
- Beyond 400°C: combustion/ volatilisation.

Another antioxidant which decomposes in three stages is the ascorbic acid (blue curve). Its thermogram presents a very specific shape: the first mass loss occurs around 170°C closely followed by a maximum of decomposition around 220°C (very quick degradation characterized by an elevated slope: $-0.75\%/^{\circ}\text{C}$); the second step is characterized by a maximum decomposition around 300°C (smoother slope: $-0.21\%/^{\circ}\text{C}$). Both steps correspond to the vitamin C degradation. Finally the third stage carbonisation and the combustion of the formed coal [163].

Alpha-tocopherol (black curve) presents two steps in its degradation, a first one which corresponding to a loss of 70% of its total mass starting around 190°C and presenting a maximum of degradation around 315°C. The 30% left are degraded in the second phase which has its maximum of degradation around 500°C.

Green tea (green curve) presents a first degradation around 40°C (with a maximum around 70°C) which corresponds to the loss of the absorbed water. Then, the degradation pattern is rather complicated due to the complex composition of green tea: a little maximum of degradation appears around 210°C followed by the main peak of decomposition around 285°C. Those two peaks account for almost 75% of the total weight. The degradation of organic compounds such as ferulic acid or quercetin, could be the cause of this weight loss. The remaining 25% are divided into two peaks which might be attributed to the combustion of carbohydrates and the less condensed structures of the lignin-like molecules. Also, the degradation (glycosylation) of the catechins might contribute to the weight loss. Finally, the losses observed at high temperatures are probably due to the decomposition of resistant aromatic structures like flavonoids (also observed for quebracho tannins) [110].

The degradation of green tea shows common points with that of tannins (maroon curve) even though the latter display a very slow degradation. The first degradation step corresponds to the loss of absorbed water just like for green tea. Then, the first degradation maxima occur around 240°C followed by several other maximum of degradations: around 365°C, around 405°C and 450°C. The observed degradations can be attributed to the same species as for green tea. However, the low amount of degrading species (at 600°C, almost 45% of the tannin sample remains undegraded) reveals that quebracho tannins components are much more robust than that of green tea.

These temperatures are very important for the application, but another value must be considered: the kinetic of degradation. Even if one of the antioxidants starts degrading at low temperature it doesn't mean that it will handle extrusion process. As we can see on the thermograms, linseed oil starts degrading approximatively at the same temperature as the other antioxidants, but its mass loss is really slower: at 300°C, it has only 5% loss when vitamin C and green tea lost almost half of their weight.

All those antioxidants were also analysed under DSC to check if they have any transitions that could interfere with those of the polymer, but nothing was seen with these analyses.

- **Antioxidant capacity**

The antioxidant capacity of each additive was determined thanks to the DPPH method: radical 2,2-diphenyl-1-picrylhydrazyl. All of our compounds are known to have great antioxidant capacity and were chosen specifically for that; however, working with natural antioxidant involves taking precaution in terms of thermal degradation as many of them don't lose mass at high temperature but lose their antioxidant capacities. The antioxidant capacity assessment method is described in part II-4 and requires the use of the DPPH radical to measure the radical scavenging capacity. DPPH is purple in solution at its initial state due to a strong absorption band around 520 nm and becomes yellow when neutralized by an antioxidant species. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm [148].

This method was thus applied to alpha tocopherol and ascorbic acid with ethanol as a media for the radical scavenging reaction. A different method was applied to linseed oil as it is a blend of different compounds: methanolic and lipidic fraction were separated and analysed separately in ethyl acetate [164].

Ascorbic acid is very well known for its excellent antioxidant capacities. We thus wanted to know how these capacities behave when the antioxidant is exposed to high temperature. Ascorbic acid was thus exposed to 5 minutes and 30 minutes isotherms at 70°C, 140°C and 180°C, either under air or under nitrogen. However, with increasing the temperature, a colour appeared (picture in **figure II-38**) and even a huge swelling at 180°C, causing problems toward the DPPH which is a colorimetric indicator.

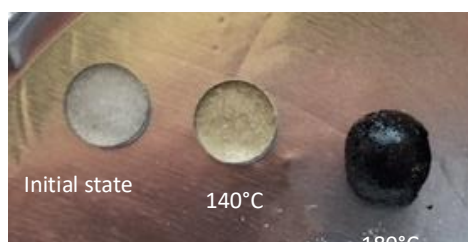


Figure II-38. Ascorbic acid physical evolution after a 5 minutes exposure to different temperatures.

The results (shown in **annexe 8**) show that the scavenging capacity of ascorbic acid is strong: no antioxidant capacity variations could be seen even at high temperature (almost 80% of the DPPH radicals were scavenged). These results show that the ascorbic acid antioxidant activity should resist to the extrusion process below 180°C (above, the antioxidant burns completely). Nevertheless, the extrusion is a very aggressive process as high shear rates are applied leading to self-heating phenomena (internal energy). Hence, precautions should be taken with ascorbic acid as it seems to be very sensitive. Also, it is known that when used at high amount vitamin C can be a pro-oxidative component.

The same analysis was done for alpha-tocopherol. Whatever the process, whether it is under nitrogen or under air, the radical scavenging is always significant (more than 80% of DPPH radical scavenging) even through long thermal exposure (results are detailed in **annexe 8**).

Vitamin E and vitamin C thus seem to be excellent candidate for the protection of our polymers toward oxidative degradation.

For linseed oil, only the phenolic compounds were analysed after separation of the methanolic and the lipidic phase.

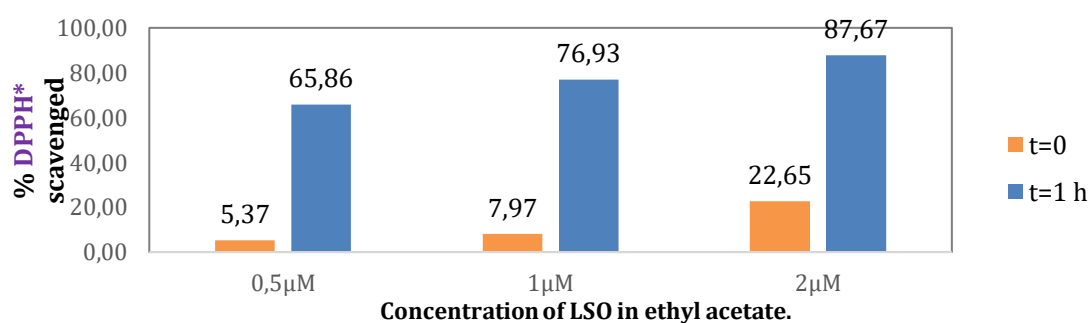


Figure II-39. Linseed oil consumption of DPPH radicals.

It came out that, at the opposite of vitamins, the scavenging activity is very poor at short times of exposure between the radical and linseed oil (LSO). After one hour of incubation, the antioxidant activity increased reaching the levels of vitamins. Nevertheless, this data doesn't really tell us if linseed oil will handle the extrusion process and if it will protect the polymer from oxidation. The attack of free radicals during the oxidation reactions is very fast and linseed oil might, in turn, not be efficient enough.

In the case of tannins, their limited solubility in solvents made the antioxidant capacities assessments trickier. They were thus solubilised in water and ethanol and filtered. The amount of filtrate was weighted and removed in the concentration calculation. Based on the percentage of scavenged DPPH radicals, the IC_{50} was calculated. It represents the concentration of antioxidant required to consume 50% of the DPPH radicals. The values are summarized in **table II-8**.

Table II-8. IC₅₀ values for vitamin E, vitamin C, tannins and green tea.

Antioxidant	Solvent of the study	IC ₅₀ (µg/mL)
Alpha-tocopherol	Ethanol	39.2 ± 6.4
Ascorbic acid	Water	3.6 ± 1.3
	Ethanol	31.4 ± 8.1
Tannin	Water	167 ± 16
	Ethanol	247 ± 34
Green tea	Ethanol	48.9 ± 6.1

The lower the IC₅₀ the better the antioxidant properties. Hence, ascorbic acid in water presents the best antioxidant capacity as only 3.6 µg/mL are needed to consume 50% of the DPPH radicals. In ethanol the value is 10 times higher due to a less good solubility, but it remains impressive. Alpha-tocopherol in ethanol also shows remarkable antioxidant properties with an IC₅₀ of 39 µg/mL. For tannins, the values are higher due to their low solubilities which engendered elevated standards deviations. However, the values remain very good. Also, it was shown that the granulometry of the particle size had an influence on the antioxidant properties which explains the high IC₅₀ obtained for tannins [165]. In the case of green tea (which is a very thin powder), polyphenols account for 25–35 % and for more than 80% in the case of quebracho tannins. Green tea's antioxidant activity is mainly attributed to catechins. The obtained value in ethanol is close from that of vitamin E: 48.9 µg/mL.

These IC₅₀ fit with those found in the literature even though the limitation of the DPPH method is its lack of reproducibility (many parameters govern this method and no norm exist; hence anyone can adapt the parameters making the results hard to compare).

- Solubilities

The idea here is to highlight the fact that the antioxidant transfer through a polymer depends on the its interaction with the packaging materials as well as the solubility and affinity of the additives with the food product [166]. If there is a high affinity between the polymer and the additives, it is hard for them to migrate out from the polymer. On the other side, when the affinity is weak, the additives migrate to the food very easily, which is also not good for a long-term improvement of the product's shelf life. To predict migration behaviour, it is necessary to estimate the compatibility and affinity between the polymer and the additives. There is not very much research conducted and reported to estimate the produce compatibilities and

affinities. The Hansen solubility parameters (HSP) assessment method is described in part II-3. It consists in evaluating whether a compound dissolves or interact with the selected solvents. The solvents that have close HSP to the studied material will interact with the material.

These different interactions are used to divide the chosen solvents into groups of “good” solvents (score 1) or “bad” solvents (score 0). These scores are then entered into the HSPiP software (Hansen Solubility Parameters in Practice) which will set up a 3-dimensioned sphere with “Good” solvents inside and “Bad” solvents outside. The axes of the 3D space are δ_D , δ_P and δ_H . The centre of the sphere represents the HSP of the studied material. **Annexe 9** shows the results obtained for the two polymers and for the additives. Hence, thanks to those experimental data, the solubility spheres were drawn (**figure II-40**) and the HSP were determined.

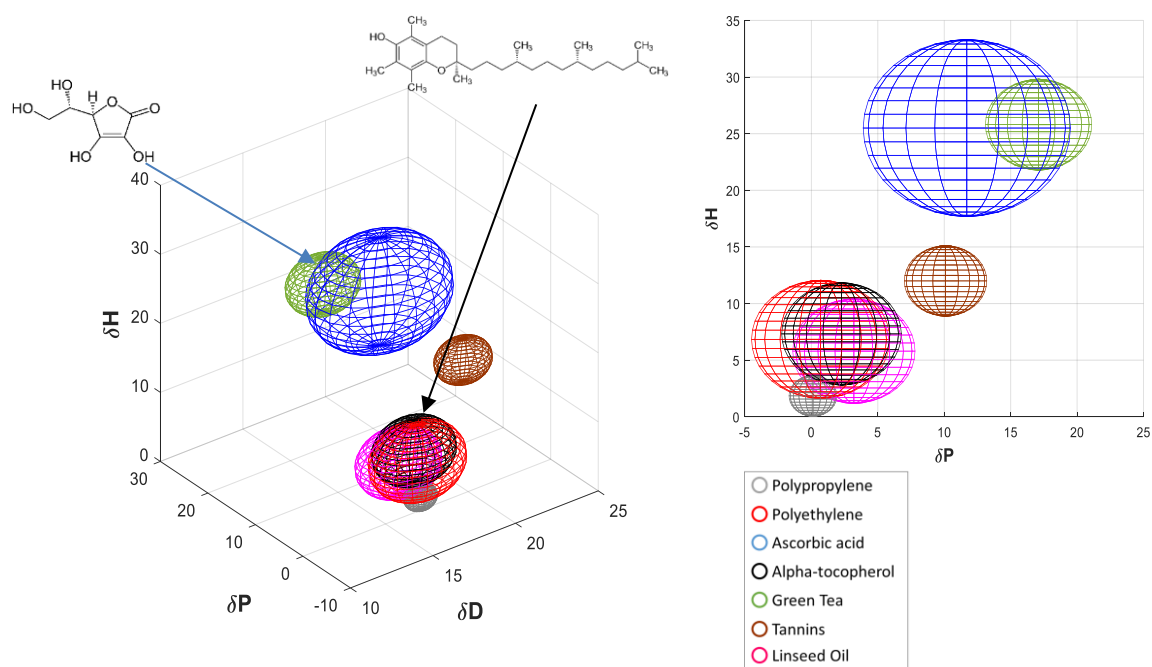


Figure II-40. Hansen solubility spheres of the different compounds of the study.

In the case of polymers, due to their lack of solubility, the HSP were calculated by evaluating the surface tension of 15 different solvents at the polymer surfaces. For additives, the HSP were assessed by evaluating the time needed to get solubilized the same 15 solvents.

The blue and the green spheres represent vitamin C and green tea’s solubilities, respectively. They are the most polar (high δ_P and δ_H) compounds of the study and thus relatively distant from the other spheres (i.e. low affinities) as detailed with the HSP distances in **table II-9**. Another detail that can be putted forward is the radius of the spheres. This parameter shows the capacity for a component to solubilize in different media (the more a product is soluble the bigger the radius). The radius of vitamin C’s sphere is elevated (7.8) showing its

ability to be solubilized in a large number of solvents; that of green tea is smaller (4) showing a limited solubility due to its complexity. The HSP of ascorbic acid are: $\delta_D=18$; $\delta_P=11.7$; $\delta_H=25.5$ and green tea's ones are: $\delta_D=18.2$; $\delta_P=16$; $\delta_H=25$.

Located between the very hydrophobic and the hydrophilic species, tannins shows an elevated polarity and an intermediate ability to create hydrogen bonds; its HSP are: $\delta_D=22.6$; $\delta_P=10.1$; $\delta_H=12.5$. The very low solubility of tannins in any solvents made this assessment very difficult and the amount of filtrate was very important due to the elevated size of the molecules it is composed of. The biggest molecules (main components of tannins) are thus not represented in this figure. The photo in **figure II-41** shows the solutions of tannins in different solvents (detailed in **Annexe 9**). We can see that the powder deposits in most of the solvents, except for the 3, 4, 12, 14 and 15 (Chloroform, dichloromethane, ethanol, water and diethylene glycol).

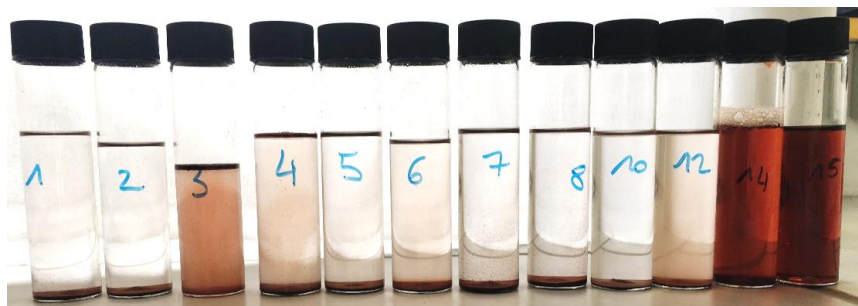


Figure II-41. Tannins' solubility tests in different solvents.

Linseed oil and alpha-tocopherol show very close HSP as they are both completely hydrophobic. Linseed oil's HSP are: $\delta_D=16.7$; $\delta_P=3.2$; $\delta_H=5.8$ and alpha tocopherol ones are: $\delta_D=17.4$; $\delta_P=2.3$; $\delta_H=7.3$. Moreover, their proximity with the polymers' spheres (which are hydrophobic polymers) shows their high affinity.

The HSP distance is the distance between a component 1 and a component 2 can be calculated thanks to their HSP by the software or thanks to the **equation (19)**.

Table II-9. HSP distance between polymers and additives.

HSP distance	LDPE	iPP
Green Tea	23.8	28.2
Ascorbic acid	21.7	26.4
Tannins	15.5	18.3
Linseed Oil	2.8	5.1
Alpha-tocopherol	1.8	5.9

To conclude with this part, a scheme (**figure II-42**) was set up summarizing the thermal properties of both polymers and the additives. It schematizes the temperatures window in which they should not degrade. The processing temperatures of both polymers were also added to this scheme.

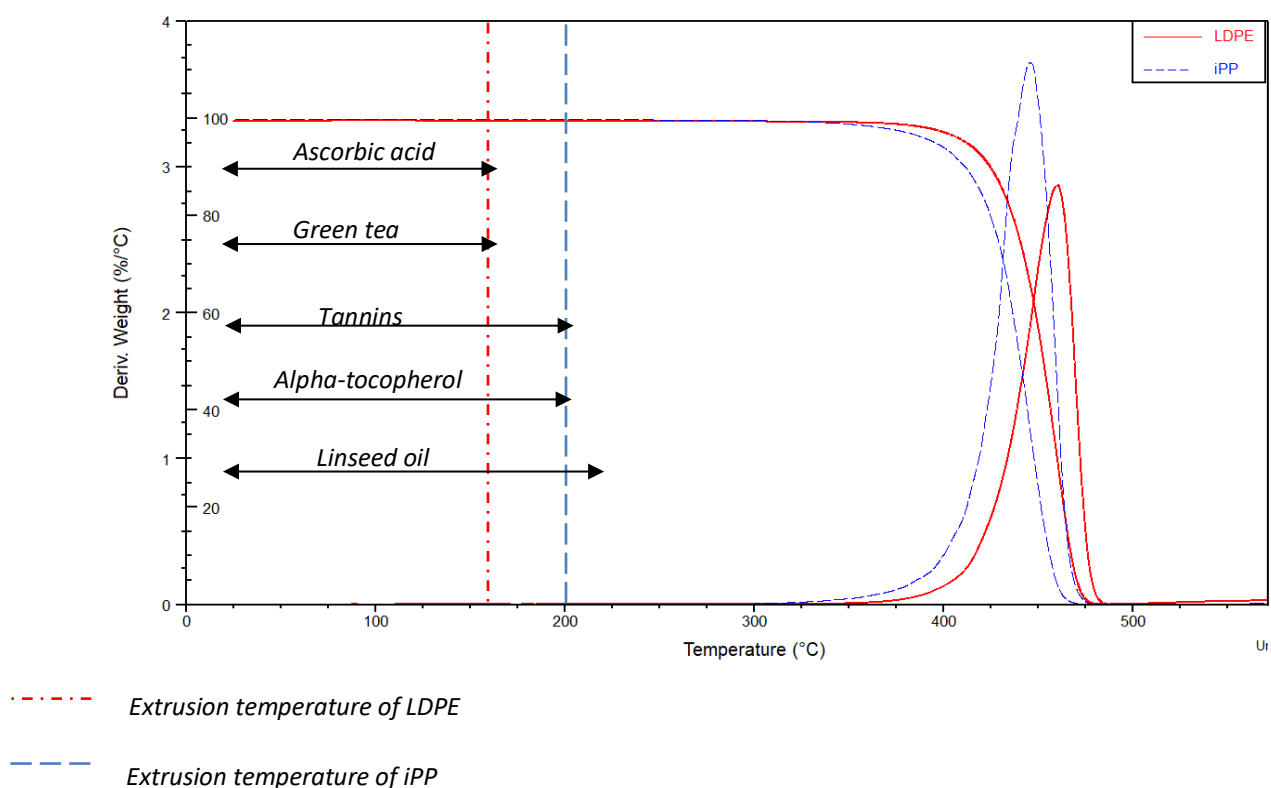


Figure II-42. Scheme summarizing the thermal properties of the studied antioxidants.

IV.1.b. Mixtures of antioxidants

As said before, blending vitamin E and vitamin C as proven to show strong synergistic effects. Nevertheless, the use of both compounds was never applied to the polymer field. Several antioxidants blends were evaluated as those synergistic effects could be very effective for our application. In the following part, only vitamin/vitamin C and vitamin E/tannin blends are detailed because they are the blends in which we are the most interested. The other studied additive blends are detailed in **annexe 10**.

- Vitamin E, vitamin C mixture

It seemed obvious to explore the famous synergetic effect of vitamin E (VE) and vitamin C (VC). However, vitamin E is lipophilic and vitamin C hydrophilic, it is then complicated to mix them properly. The **figure II-43** shows the results obtained after TGA analysis for different mass ratios of vitamin E and vitamin C (85/15; 90/10 and 95/5). The procedure that was used is the same as previously: ramp of 10°C/min from the ambient temperature to 600°C under nitrogen.

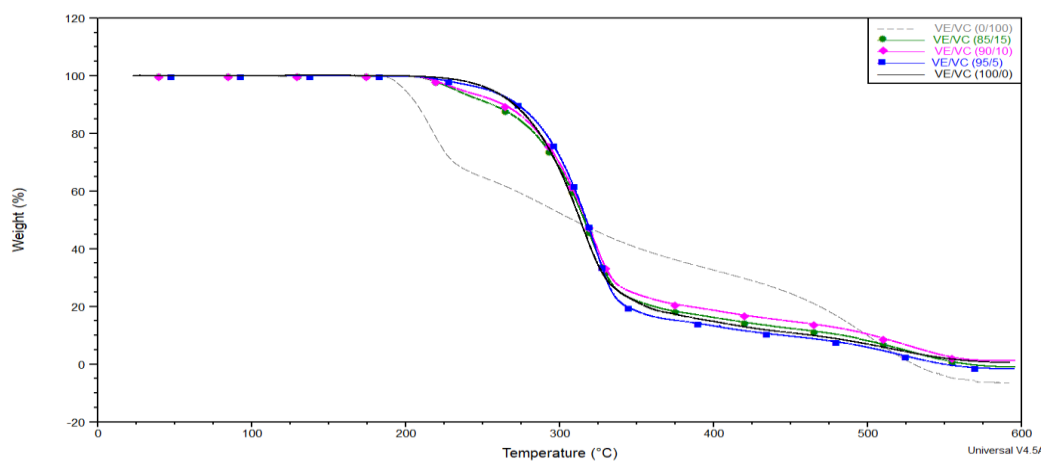


Figure II-43. Mass loss of vitamin E/vitamin C different ratios.

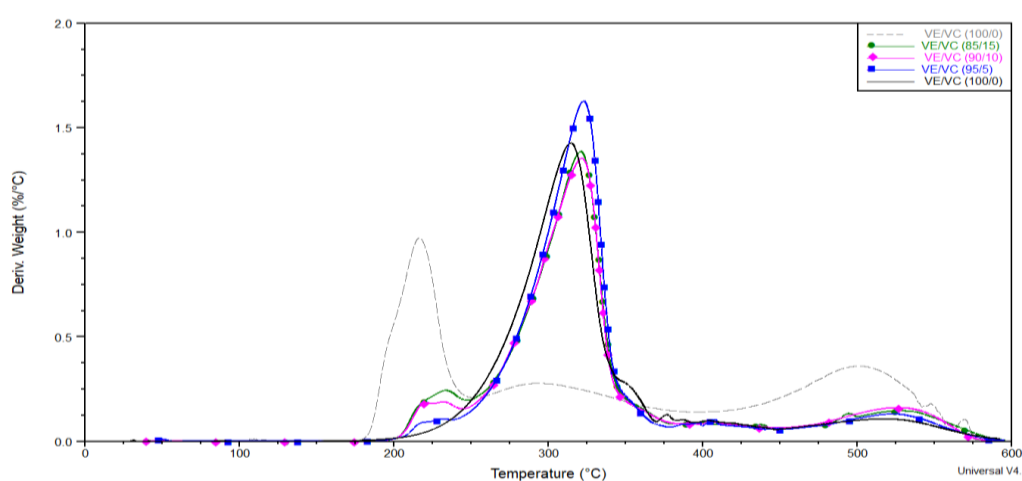


Figure II-44. Derivative weight loss of the different vitamin E/vitamin C ratios.

An optimum seems to appear from those thermal analysis: the VE/VC (95/5) ratio. Its onset degradation is delayed compared to the other blends and compared to raw additives (alpha-tocopherol (black curve) and ascorbic acid (grey curve)). Of course, the TGA was repeated three times for each mix to verify that this delay was not comprised in the standard deviation or in the machine error.

On the derivative curve shown in **figure II-44**, a slight shift of the VE/VC (95/5) curve can be observed showing that this formula is better than alpha-tocopherol alone. However, those differences remain small and are, therefore, hard to consider as significant: there are no clear evidence of any synergism mechanism. Also, this

method is relatively quantitative and by integrating the degradation peaks, the exact composition of our blends was verified (values below are average on the three experiments):

- VE/VC (95/5%_m) is (95.4 ± 0.9/4.6%_m);
- VE/VC (90/10%_m) is (92.3 ± 1.2/7.7%_m);
- VE/VC (85/15%_m) is (87.4 ± 0.5/12.6%_m).

▪ Vitamin E, tannins mixture

Vitamin E and quebracho tannins (Tan) were also mixed. Here again solubility problems were encountered as tannins presents water-soluble and elevated molar masses fractions. TGA were realised on different ratios of both compounds in which vitamin E is always predominant: from 5 to 15% tannin (we didn't go further in quantities because tannins have a very low density: 0,4g/ml for 0.95g/mL for vitamin E). The results are shown in **figure II-45** and **figure II-46**.

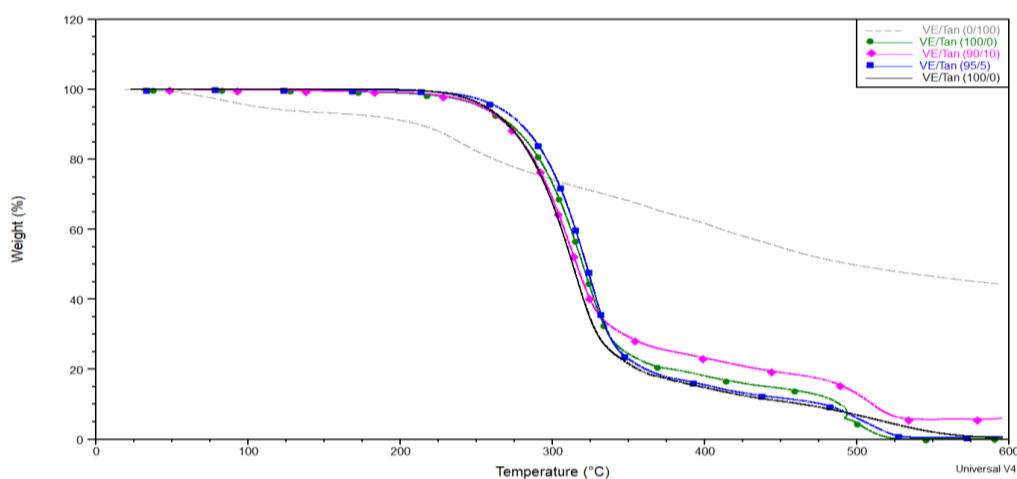


Figure II-45. Mass loss of vitamin E/tannins different ratios.

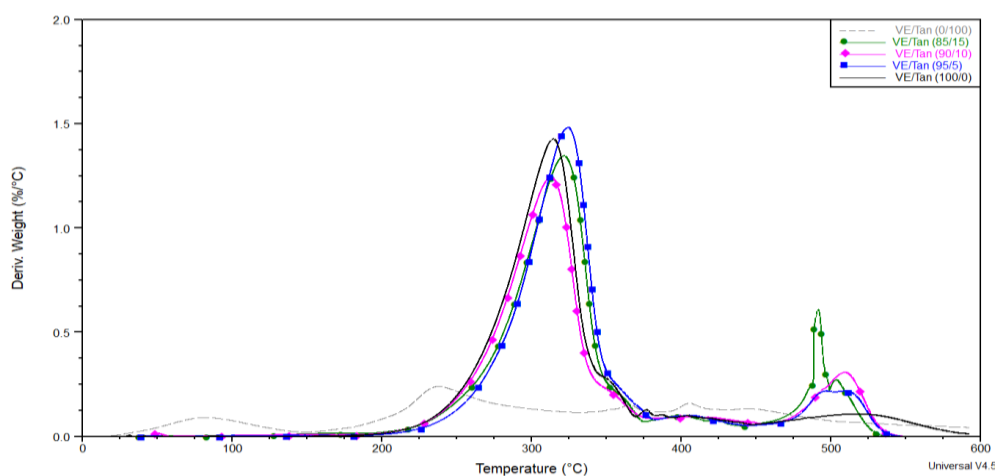


Figure II-46. Derivative weight loss of the different vitamin E/tannins ratios.

Here as well, an optimum seems to appear for the (95/5) ratio (blue curve from **figure II-45**) with a delayed onset degradation is delayed to raw alpha-tocopherol (black curve) and raw tannins (grey curve). This delay remains little but is more easily visible than it was for VE/VC blends.

On the derivative curve shown in **figure II-46**, this shift is also visible putting forward a possible synergism between tannins and alpha-tocopherol. The exact compositions of those blends were impossible to determine because both compounds degrade in the same temperature ranges.

The rate chosen for each mixture was based on the heat resistance of the mixture. The optimum rate is the one for which the degradation begins at the highest temperature and presenting the slowest kinetics of degradation. These blends thus seem to be promising for our application and will be introduced in the polymer: their aim will be to stabilize the polymer mainly by inhibiting oxidation reactions, which are the main source of polymer degradation.

V. CONCLUSION

The characterisation methods were carefully detailed in this chapter. Among those characterisations, one had a particular attention as it represented a huge part of this PhD research: LESA-MS. LESA combined with nanoESI ionisation - MS was demonstrated to be a convenient method for the direct analysis of polymer packing materials. It was optimized to monitor the release of residual polymerisation or polymer degradation residues. The method was validated by the currently used method based on the extraction from the bulk followed by ESI-MS. LESA-MS does not require sample preparation, is fast, eco-friendly and highly reproducible. It also compares favourably with ambient ionisation DART-MS in terms of selectivity producing simpler spectra limited to the mobile compounds of interest.

Also, the materials of the study were investigated. Virgin polymers were characterized by thermogravimetric analysis, differential scanning calorimetry, rheology and high temperature size exclusion chromatography. The raw additives were also characterized, and particular attention was paid to their thermal resistivity in preparation for implementation processes. It appeared that vitamin C is relatively sensitive too temperature and may degrade during extrusion. The other additive showed good thermal resistivity. Finally, the thermal properties of combination of additives were tried, following the synergism detailed in the literature chapter. A protection effect from one additive to another seems to appear but should be monitored within the polymer matrix. Those characterisations represent the basis of our study and will be used as references along this thesis report.

CHAPTER 3: POTENTIAL USE OF NATURAL ADDITIVES FOR POLYOLEFINS STABILISATION

Abstract: Natural antioxidants were introduced within polyolefins. They were characterized thermally, rheologically and proved to have an outstanding potential as thermoplastic stabilizer. This stabilizing effect is the most pronounced when combination of additives is used. The blends containing a single additive were firstly evaluated for thermal, structural and morphological properties by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and rotational rheology. Those analysis putted forward the different effects provided by those natural antioxidants on polypropylene and polyethylene matrices. The analysis of the effect of the combination of additives on the matrices was realized using the same methods. The combination of vitamin E and vitamin C or vitamin E and quebracho tannins proved to drastically increase the stability of the matrices. The synergism between the additives was evaluated by DOSY-NMR and by rheology. A proposition of stabilisation mechanism based on those analysis was set up.

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I. INTRODUCTION

This chapter deals with the food packaging materials' processing and characterizations. As explained before, one of the main constraints of natural compounds are their thermal stability and the maintain of their antioxidant capacities. After having carefully characterize the thermal behaviour of each component of the study, blends were realized.

This chapter thus deals with formulation. It is made of a first part dealing with the introduction of single antioxidants within the polymer matrices as well as their characterisation. At this stage, only the most efficient additives will be chosen. Finally, combination of additives will be introduced within our polymers and characterized. This chapter will be concluded by the choice of the most promising formulas which will be further characterized in the following chapters.

II. CHARACTERISATION OF THE STABILISING EFFECT OF NATURAL ADDITIVES

Each of the additives described previously were introduced into polyethylene and polypropylene and characterized. At first, 2.5% of the additives were introduced; this value was chosen as a compromise to first evaluate the effect of antioxidants on the polymer (above would be too much and lower would involve rather high deviations). The following part will detail the formulation of the different mix as well as their characterisations.

II.1. Processing and formulation

Polymers were extruded with a LabTech double screw extruder as explained in chapter 2, part I. The temperature profiles that were used for LDPE and iPP are described in **table III-1**.

Table III-1. Temperatures (°C) profiles for the extrusion of LDPE and iPP.

Zones	1 to 5	6 to 9	Die
LDPE	80/130/140/150/150	160	160
iPP	80/130/150/150/180	190	190

In order to minimize the residence time of the polymers within the extruder to avoid degradations, elevated screw speeds were applied (the same speed was applied to both polymers as they have close MFI). However,

with high screw speeds the risk is to have a non-homogeneous blend. A compromise had to be found: the feeder’s speed was thus fixed at 15 rpm and the screw speed at 400 rpm.

The introduction of the antioxidants in the polymers is achieved either by mixing the antioxidant powders with polymers pellets or by introducing directly the oils in the double screws. In the case of oils, in order to have a quantitative addition, calibrations of the feeder and of the syringe pump had to be done.

- Calibration of the feeder

This calibration will allow knowing the throughput of the feeder according to the speed. The feeder throughput is function of the polymer pellets’ shape: LDPE pellets have a diameter of around 0.5mm whereas iPP pellet’s is close to that of a powder. The calibration curves of the feeder are shown in **figure III-1**.

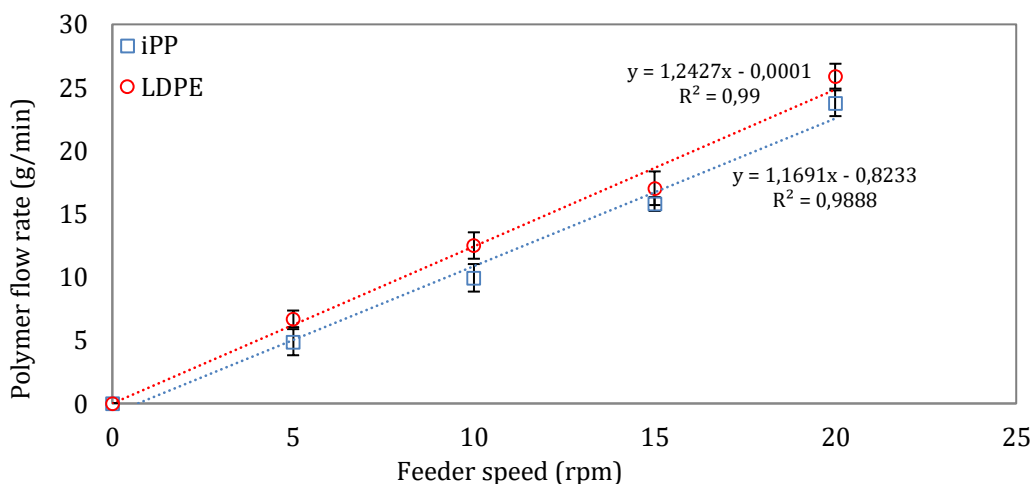


Figure III-1. Calibration curves of the feeder for LDPE and iPP.

The same calibration was realized with the syringe pump for all the oils of the study (i.e. alpha tocopherol and linseed oil) and is shown in **figure III-2**.

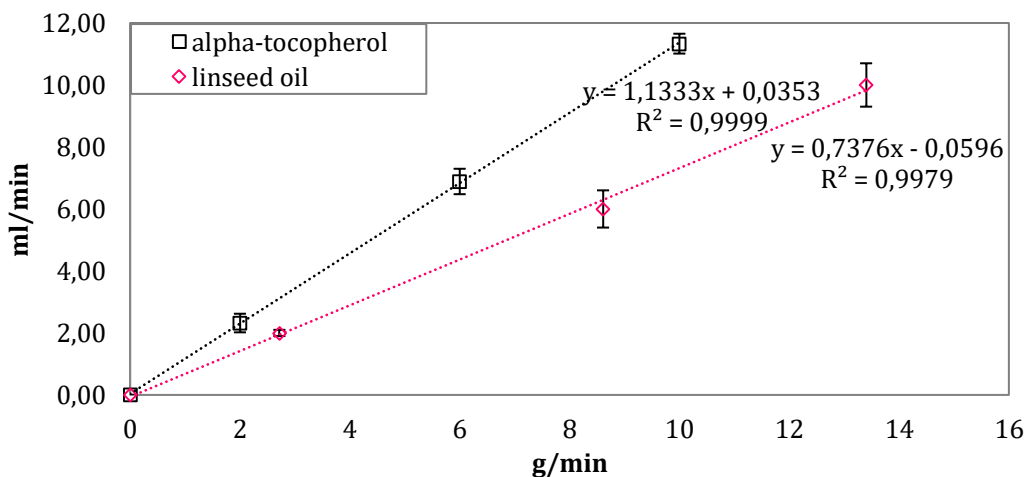


Figure III-2. Calibration curves of the syringe pump for linseed oil and alpha tocopherol.

These curves put forward the differences in viscosities between the two oils: alpha tocopherol is very viscous with a texture close to that of honey whereas linseed oil, which is an edible oil, is much more liquid. Based on those calibrations, the parameters of the syringe were set up.

For example, for an addition of 2.5% of additives in polyethylene at a feeder's speed of 15 rpm, the calculation was:

$$Volume_{additive} = \frac{2,5}{100} \times 18.6 = 0,46 \text{ g/min}$$

Where 18.6 is the mass of polymer delivered by the feeder during 1 minute with a 15rpm speed. The syringe flow rate to obtain 0.46 g/min for alpha-tocopherol is then calculated thanks to the equations of **figure III-2**:

$$Syringe \text{ flow rate} = 1.13 * 0.46 + 0.0353 = 0,55 \text{ ml/min}$$

Table III-2 summarizes the extrusion parameters.

Table III-2. Extrusion parameters for polymers and their additives.

		iPP	LDPE
Extruder	Temperature (at the die, °C)	190	160
	Screws speed (rpm)	400	400
	Feeder speed (rpm)	15	15
Syringe pump flow rate (mL/min)	Alpha-tocopherol	0.33	0.37
	Linseed oil	0.24	0.28

The extrusion of ascorbic acid (even at 160°C in LDPE) turned out to be impossible: the additive completely burned within the extruder resulting in a very dark, inhomogeneous matter that clogged the apparatus. This puts forward the previously described phenomenon of self-heating occurring during extrusion. In turn, vitamin C cannot be used on its own in our polymers. The following pictures show the physical aspects of the blends that were successfully extruded (**figure III-3**).

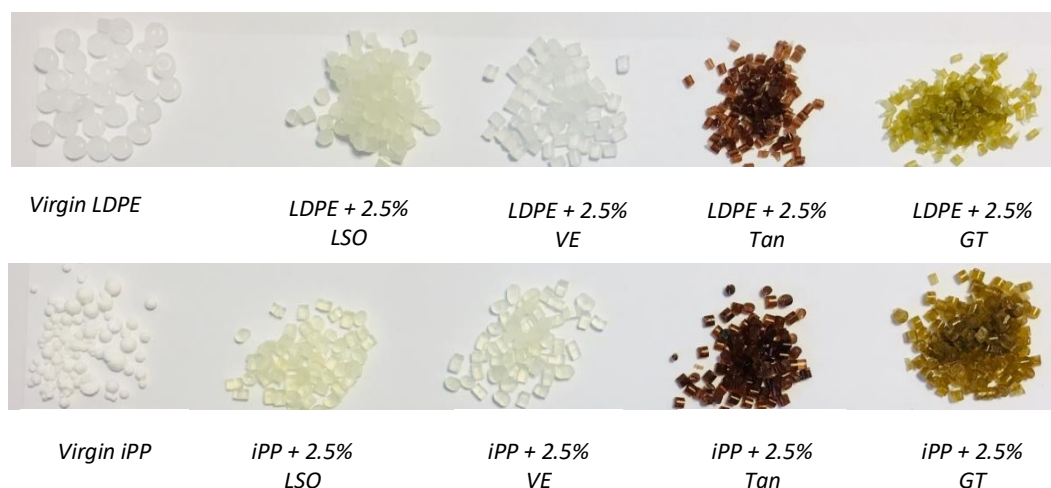


Figure III- 3. Pellets of the different polymer-additive blends.

The addition of linseed oil in both polymers made them turn yellowish as well as the addition of vitamin E in iPP (vitamin E doesn't seem to have an influence on LDPE's colour). When adding tannins, a brown colour appears. Finally, green tea brings a green colour to LDPE and a brown/green one to iPP (this difference is probably due to the more elevated extrusion temperature of iPP leading to a stronger carbonisation of the additives).

II.2. Thermal analysis

II.2.a. Thermogravimetric Analysis (TGA)

Figure III-4 and III-5 show the TGA of virgin polymers versus polymers containing single additives. Those thermal properties were assessed following the same procedure as that described in chapter 2: 10°C/min from the ambient temperature to 600°C under nitrogen.

In the case of LDPE's blends (**figure III-4**), the thermal analysis reveals that the addition of vitamin E (black curve) shows a similar behaviour of that of virgin LDPE. The addition of linseed oil (pink curve), of green tea (green curve) and of tannin (maroon curve) lower the thermal resistivity of the polymer, especially green tea which thermogram shows a 50°C shift at 400°C.

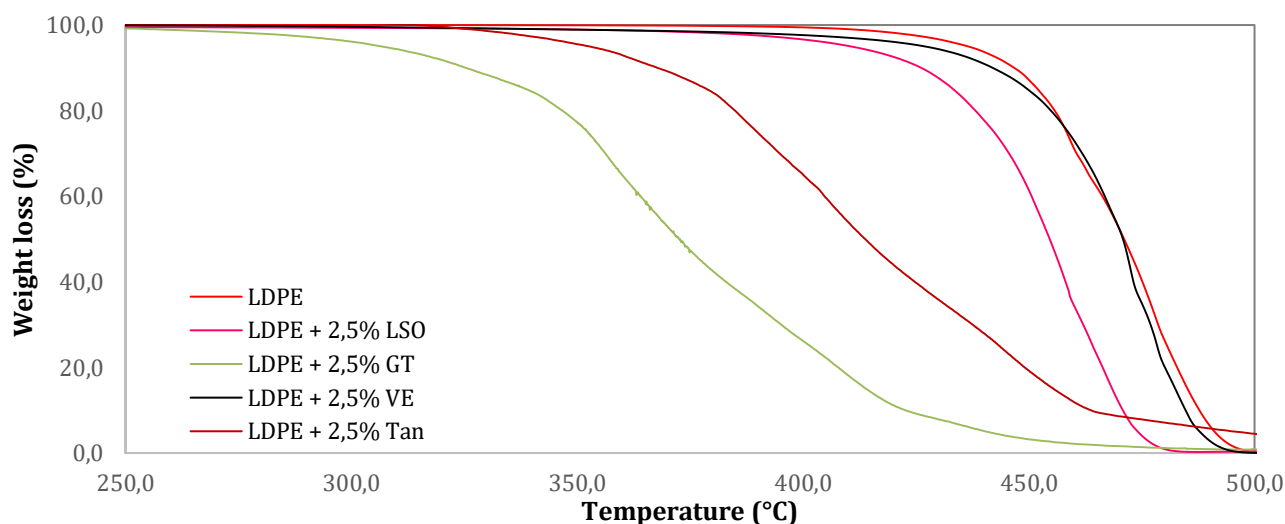


Figure III-4. Thermograms obtained by TGA for LDPE's based formulations.

For iPP (**figure III-5**), the blend containing vitamin E also shows the same thermal properties as virgin polymer. The addition of linseed oil induces earlier thermal degradations but shows greater thermal resistance than the virgin matrix at high temperature (above 420°C). When green tea and tannins are introduced (green and maroon curves), drastic decrease of the thermal resistance can be observed.

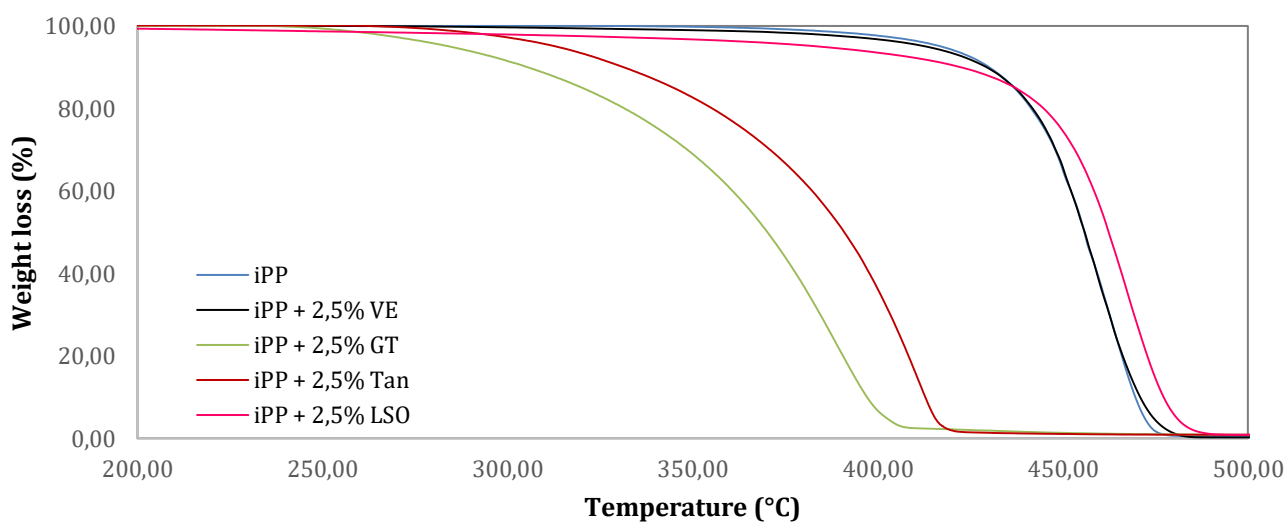


Figure III-5. Thermograms obtained by TGA for iPP's based formulations.

The TGA values are summarized in **Table III-3**.

II.2.b. Differential Scanning Calorimetry (DSC)

In the case of DSC analyses, three different procedures were applied:

- the “normal” DSC analysis with the three cycles of heat and cooling (called “method 1” in chapter II part X);
- Onset Oxidation Temperature (OOT) (“method 2” from chapter II, part X);
- Oxidation Induction Time (OIT): ramp from 70°C to 210°C for PP and 200°C for PE under nitrogen followed by an isotherm (210°C or 200°C) for 90 minutes under air atmosphere (“method 3” chapter 2, part II-1).

The “normal” DSC characterization results don’t really show any significant variations (probably due to the low amount of antioxidant in the polymer) except for tannins which shows nucleating effects (higher enthalpy of crystallisation). It is known that nucleation starts with nanometer-sized areas where, as a result of heat motion, some chains become parallel. Apart from the thermal mechanism, nucleation is strongly affected by impurities, dyes, plasticizers, fillers and other additives in the polymer: also called heterogeneous nucleation [167].

The DSC values are summarized in **Table III-3**.

The OOT assessment method (as well as the OIT) helps evaluating the effect of the addition of the additives on polymer’s oxidation. The oxidative retardant effect of our additives was evaluated using this type of thermal analysis: 10°C/min ramp from the ambient to 350°C under oxygen (**figure III-6 and III-7**).

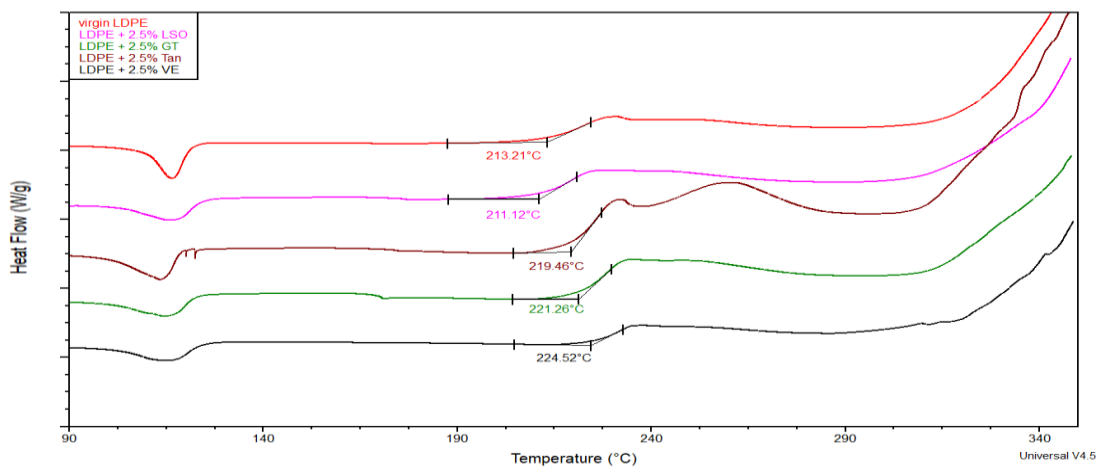


Figure III-6. OOT determination of LDPE and LDPE with additives.

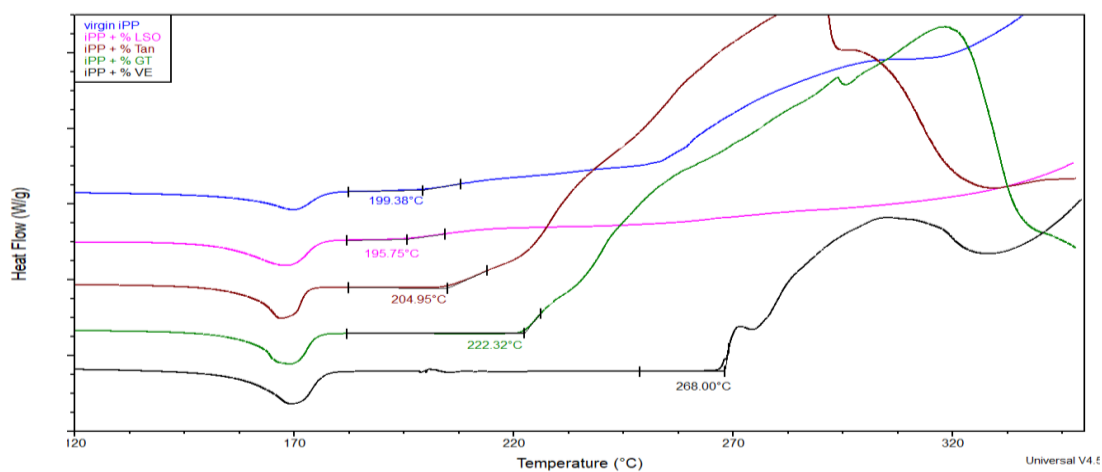


Figure III-7. OOT determination of iPP and iPP with additives.

First, the endothermic melting peaks of our polymers can be seen around 120°C and 170°C for LDPE for the iPP respectively. Moreover, an exothermic process can also be observed which corresponds to the beginning of the thermo-oxidative degradation.

In the case of virgin polyethylene, thermo-oxidation starts around 213°C. The oxidative degradation of LDPE blended with linseed oil (pink curve) occurs at a similar temperature which indicates that this antioxidant has a low oxidative retardant effect on the polymer. At the opposite, for PE containing vitamin E (black curve), a delay in the thermo-oxidative degradation temperature appears (a gain of around 10°C). Green tea and tannins both provide little improvements by delaying slightly the oxidation peaks. Moreover, we can observe that the oxidation happens in two times for green tea and tannins. This reveals the presence of a secondary oxidation probably related to the complexity of those components. Tannins and green tea are very complex blends.

Those improvements are very similar to those observed for iPP. Also, in the case of LDPE, VE's effect is negligible whereas it is very notable for iPP: the oxidative degradation of iPP occurs at 200°C and is shifted of almost 70°C for the blend containing vitamin E. For the formulations containing linseed oil and tannins, the thermo-oxidative degradation is equal to that of the virgin polymer: no protection toward oxidation is provided by linseed oil.

Figure III-8 shows isothermal calorimetric curves giving access to the OIT: the time at which the oxidation starts. The differences in terms of antioxidant protection are even clearer than those obtained with the OOT method.

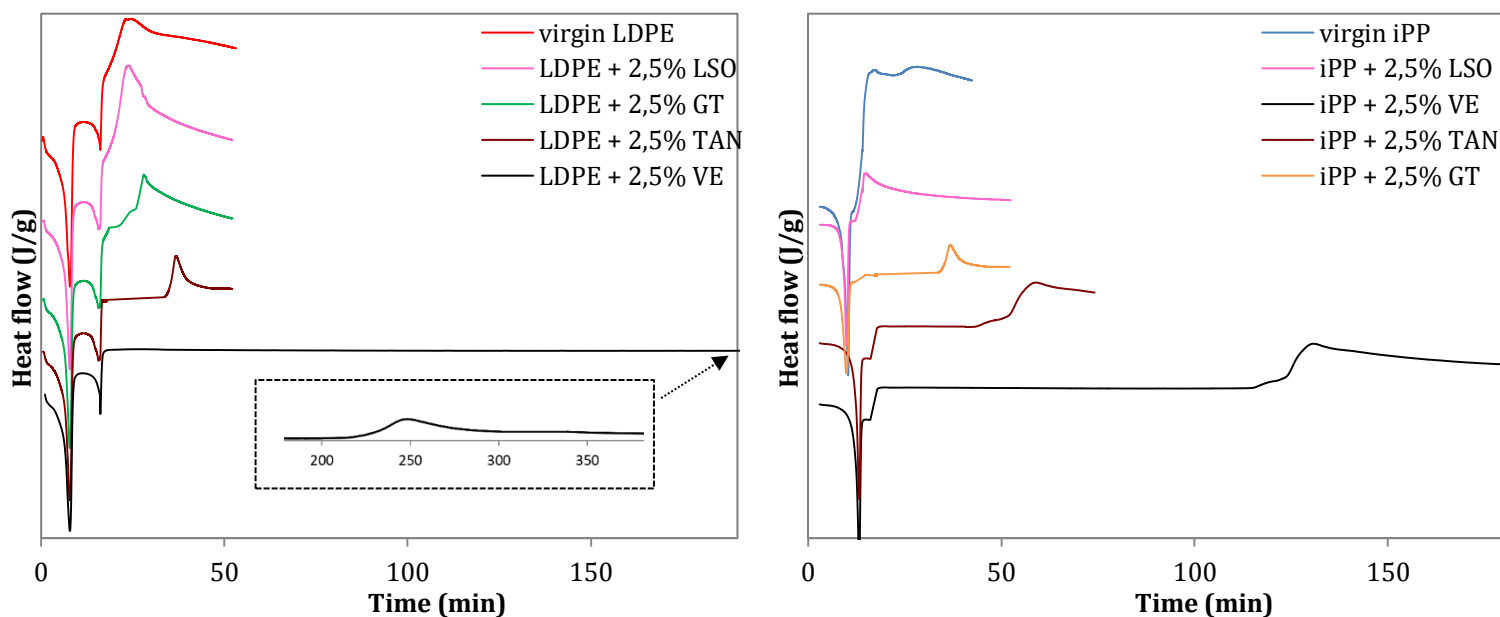


Figure III-8. OIT determination for LDPE and iPP with natural additives.

Non-stabilized polymers have an oxidation induction time (OIT) around 20 minutes (details data are summarized in **table III-3**) and so does the polymers which contain linseed oil (due to the weak thermo-oxidative protection of LSO). The addition of vitamin E within both polymers leads to a significant increase in OIT values: an increase of 310 minutes and 100 minutes respectively for LDPE and iPP. Green tea and tannins both provide little improvements by delaying slightly the oxidation peaks. The effect of vitamin E, meanwhile, is far more substantial: the protection toward thermo-oxidation provided by VE allows the OIT to be delayed of 95 and 212 minutes for iPP and LDPE respectively. The difference in OIT between both vitamin E-based formulation is likely to be due to the difference in extrusion temperatures (190°C for iPP, 160°C for LDPE) probably degrading a little bit the antioxidant.

II.3. Molecular evolution by Cole-cole representation

Cole-Cole curve were chosen to illustrate the viscoelastic properties rather than traditional master curve for visual reasons (even though they correspond to the same raw data). **Figure III-9** illustrates the cole-cole representation obtained for the different polymer-additive blends.

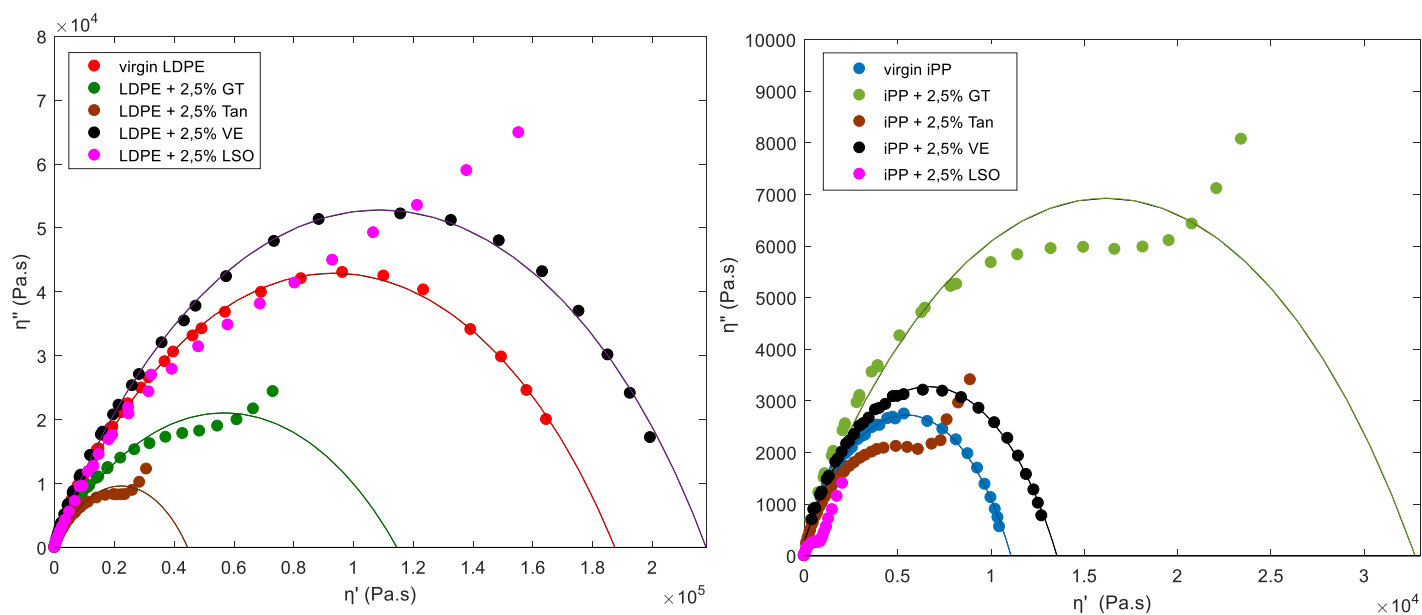


Figure III-9. Cole-cole representation of LDPE and iPP containing different additives.

In the case of polyethylene containing linseed oil (pink curve), an almost straight curve is obtained. This reveals the impossibility of the chains to relax and the gel-like behaviour induced by linseed oil (creation of a network within the polymer matrix): the G' and G'' curves are parallel at low frequencies. Actually, linseed oil is known to polymerise due to the chemical reactivity of the double bonds of the fatty acids it is made of: the double bonds react with oxygen of the atmosphere to form a polymer network. Therefore, the polymerisation of linseed oil within the polymers matrices is responsible for the shape of the cole-cole representations. Indeed, in the terminal zone in which only the longest relaxation time contributes to the viscoelastic behaviour, G' and G'' are supposed to present 1 and 2 slopes ($G' \sim \omega^2$ and $G'' \sim \omega^1$). This deviations from the semi-circle shape reveal a “non-terminal” behaviour probably due to a change in the relaxation time. When tannins and green tea are involved the deviation from the semi-circle is very visible due to the presence of solid particules of tannins and green tea restricting the chains' motion. G' and G'' also appears to be almost parallel (the slope 1 in the flowing area is obtained but not the slope 2 showing the “gel-like” or solid-like behaviours of the blends), confirming the previous hypotheses. Also, when those polyphenols are involved the viscosity decreases in comparison with the virgin polymer. This is due to the introduction of solid incompatible particles creating voids in the polymer matrix (this is illustrated by the SEM picture present in **figure III-10**). The obtained materials are thus not homogeneous and porous.

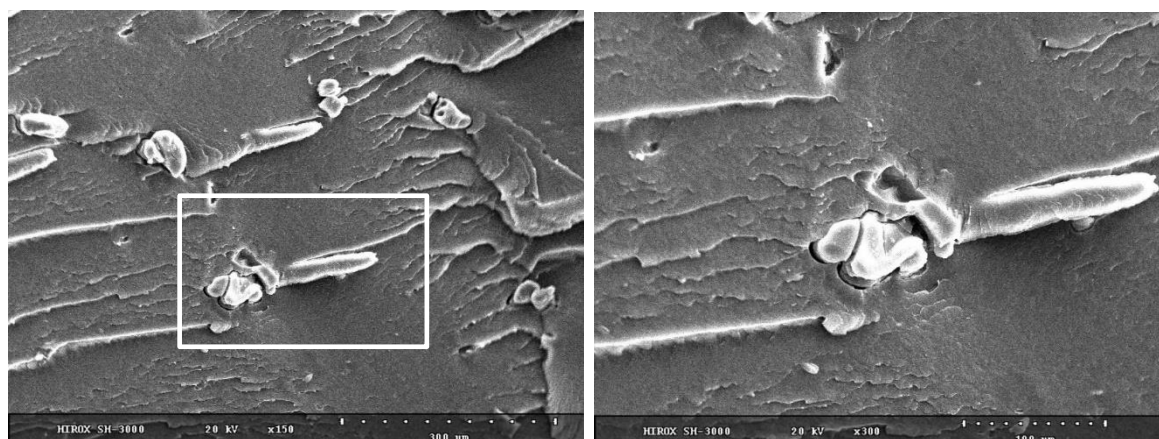


Figure III-10. SEM images of a tannin particle in a polyethylene matrix.

Finally, when vitamin E is added the effect is very low and seems to remain in the standard deviation.

In the case of polypropylene, the addition of linseed oil prevent from any chain relaxation but also induce degradations as the viscosity decreased dramatically. Green tea and tannins have similar effects on polypropylene as they had on polyethylene: no polymer relaxations and creation of voids. However, here it seems that green tea interacts with the polymer as the viscosity is increased. When vitamin E is used as an additive in polypropylene the observed effect is relatively low (like for polyethylene) and seems not to be significant.

II.4. Recycling

The previous analyses were realized after one of extrusion pass. Nevertheless, recyclability is a major point in our study, thus several passes were done in extrusion to evaluate the blends' ability to be recycled. The resulting pellets were characterized.

For the polyphenols (green tea and tannins) when evaluating the OOT values as a function of the recycling, no degradation seems to occur during the recycling: when drawing the OOT values as a function of the number of consecutive recycling, a constant curve was obtained.

In the case of vitamin E, a slight degradation can be observed when analysing recycled vitamin E-based blends but the thermo-oxidative protection it provides to our polymers remains correct.

Finally, linseed oil presents the biggest degradations with a loss of OOT of around 7°C between cycle 0 and cycle 9 for LDPE and 12°C for iPP. This loss is not, itself, dramatic but this loss makes LSO-based blends having lower OOT values than that of virgin polymers. In turn, it reveals that the additive itself seems to degrade the

polymer matrix. Moreover, stronger and stronger odours are released from the product with increasing recycling.

Characteristic values' summary

Table III-3. Summary of the characteristic values obtained after characterisation of the polymer-additive systems.

Polymer		LDPE				iPP			
Additives (2.5%)		LSO	VE	Tan	GT	LSO	VE	Tan	GT
TGA	Onset of degradation (°C)	371 ± 9	392 ± 6	328 ± 13	259 ± 9	343 ± 5	383 ± 9	278 ± 11	285 ± 6
	Offset of degradation (°C)	475 ± 6	489 ± 8	495 ± 9	477 ± 13	490 ± 11	483 ± 7	419 ± 9	423 ± 8
	Slope (%/°C)	-2.4±0.1	-2.1±0.2	-0.8 ± 0.1	-0.7±0.2	-2.3±0.1	-2.4±0.2	-1.4 ± 0.6	-1.6±0.3
DSC	Fusion temperature T_f (°C)	112 ± 1	112 ± 0.1	118 ± 4	112 ± 4	164 ± 3	166 ± 1	165 ± 0.6	166 ± 1
	Fusion enthalpy (J/g)	106 ± 2	118 ± 6	117 ± 4	117 ± 3	99 ± 6	108 ± 5	119 ± 2	95 ± 6
	Crystallisation temperature T_c (°C)	97 ± 0.6	97 ± 1	97 ± 0.4	98 ± 1	113 ± 1	111 ± 1	114 ± 0.3	113 ± 3
	Crystallisation enthalpie (J/g)	103 ± 4	110 ± 6	121 ± 0.6	117 ± 6	94 ± 3	110 ± 1	118 ± 2	91 ± 5
	Crystallinity rate χ (% at 10°C/min)	36 ± 2.5	40 ± 2.2	40 ± 1.4	40 ± 3	48 ± 3	52 ± 2.6	57 ± 1.3	46 ± 3
	OOT (°C)	212 ± 4	224 ± 2	217 ± 1	218 ± 1	196 ± 2	269 ± 6	207 ± 6	222 ± 8
	OIT (min)	23 ± 3	233 ± 11	34 ± 6	26 ± 2	18 ± 0.9	115 ± 1	43 ± 6	33 ± 4
Rheology	Limit viscosity η (Pa.s)	-	237189 ± 19874	51888 ± 4252	114530 ± 8964	-	15024 ± 19874	12899 ± 1033	31926 ± 2334
	Activation energy (kJ/mol)	64.4	65.5	65.4	65.8	39.1	36.8	38.6	39.6
	Slope 1	X	✓	✓	✓	X	✓	✓	✓
	Slope 2	X	✓	X	X	X	✓	X	X

II.5. The case of linseed oil

As said before, linseed oil is a mix of fatty acids known for its ability to dry due to the chemical reactivity of the double bonds of the fatty acids: the double bonds react with oxygen of the atmosphere to form a polymer network. Actually, the high levels of linolenic acid in linseed oil affects the drying property of the oil, making it particularly suitable in formulations of drying paints. Actually, the oil can form a film with good optical and mechanical properties. This process results from an auto-oxidation followed by a cross-linking polymerisation. The polymerisation arises from intermolecular coupling of radicals coming from degradation of peroxides. In fact, linseed oil has shown to naturally contain rather elevated amount of hydroperoxides probably created during the extraction process. Light and heat (among others) accelerate this phenomena, especially for linseed oil [168].

Despite its excellent thermal resistance, linseed oil appears to be incompatible with our application. Moreover, after extrusion (i.e. once oxidized), it has a strong odour which is not compatible with food packaging applications. Then, when degrading, the oil released CO₂, hence creating bubbles within the polymer material. Moreover, the volatile compounds arising from linseed oil were characterized by the laboratory “Les Mines d’Ales”. The results showed that the release of aldehydes from the oil are very important compared to the other additives (see details in **Annexe 11**).

Rheological analysis showed that the relaxation of the chains never occurs when linseed oil is involved in a formulation (see Cole-Cole diagram in **figure III-9**). The absence of chain relaxation can be due to a severe degradation or to the reticulation of the oil involving serious constraint. The addition of vitamin E in the polymer-linseed oil formulation didn’t improved the properties of this formula. Also, thermal analysis confirmed that the antioxidant capacities of linseed oil were not efficient enough to protect the polymer (very poor thermo-oxidative protection).

As a replacement for linseed oil, another oil was tried: Flaxseed oil. Both oils have the same origins, but flaxseed oil is extracted under cold processes, thus limiting the creation of peroxides. Better properties were obtained but still not as good as we expected. Moreover, the same smell appeared, especially when the product was recycled.

For all the reasons mentioned above, we will not go further in the study of linseed oil or flaxseed oil.

Furthermore, in order to avoid dealing with too many formulations choices were made despite its incompatibility with the extrusion process we will go further with ascorbic acid. Actually, we were motivated by the synergism it can have with alpha tocopherol. Then, green tea showed rather great properties, but they remain close from that of tannins which has a far better thermal resistance. Hence, green tea will be set aside for the moment.

III. CHARACTERIZATIONS OF TERTIARY BLENDS (POLYMER-ADDITIVE-ADDITIVE)

Following the previous results and observations, tertiary blends (which consists of the addition of two additives in a polymers) were realized and characterized. The blends that caught our attention and that will be developed in this part are alpha-tocopherol/ascorbic acid (95/5) %m (VE/VC) and alpha-tocopherol/tannins (95/5) %m (VE/Tan).

III.1. Processing and formulation

The introduction of the two additives in the extruder was realised thanks to the syringe device described earlier. Both vitamin E and the second antioxidant (either tannin or vitamin C) were mixed prior to extrusion and introduced together in the extruder. Another method could have been done consisting in extruding the polymer with each additive separately (master batches). These blends would have, then, be mixed together. However, we wanted to avoid as many thermal constraints as possible.

A new calibration of the syringe pump was necessary as the viscosity of the additive mix is lower than that of vitamin E alone (**figure III-11**). The extrusion parameters are summarized in **table III-4**.

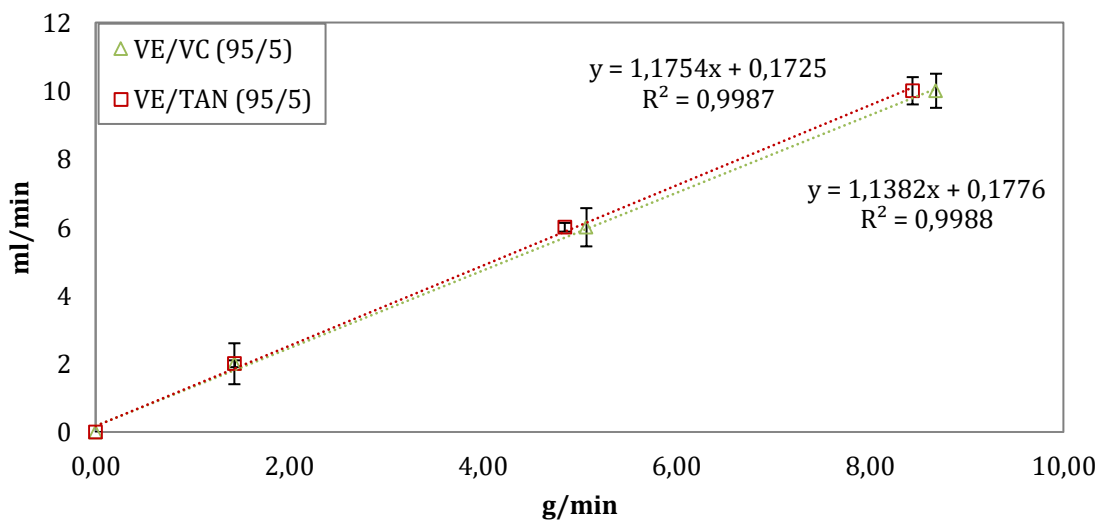


Figure III-11. Calibration curves of the syringe pump for mix of additives.

Table III-4. Extrusion parameters for polymers and their additives.

		iPP	LDPE	
Extruder		Temperature (at the die, °C)	190	160
		Screws speed (rpm)	400	400
		Feeder speed (rpm)	15	15
Syringe pump flow rate (mL/min)	VE/VC (95/5) %m	5% (VE/VC) (95/5)	1.11	1.22
		2.5% VE/VC (95/5)	0.65	0.71
		1% VE/VC (95/5)	0.35	0.38
		0.5% VE/VC (95/5)	0.26	0.28
		0.25% VE/VC (95/5)	0.21	0.22
	VE/Tan (95/5) %m	5% (VE/Tan) (95/5)	1.08	1.19
		2.5% VE/Tan (95/5)	0.64	0.70
		1% VE/Tan (95/5)	0.36	0.39
		0.5% VE/Tan (95/5)	0.26	0.28
		0.25% VE/Tan (95/5)	0.21	0.22

Following those parameters, both polymers were mixed with different loads of additives (5%, 2.5%, 1%, 0.5%, 0.25%), recycled and characterized.

The presence of vitamin C in those blends didn't cause any problems during the extrusion process. The vitamin E acts as a vehicle for its introduction in polymers. However, to avoid any carbonisation only small amounts of vitamin C were added.

III.2. Thermal analysis

III.2.a. Onset Oxidation Temperature assessment (OOT)

The OOT (Onset Oxidation Temperature) assessment procedure previously putted forward the effect of the addition of single additives on the polymer's oxidation. The same procedure was applied to polymer-additive-additive systems as shown in **figure III-13** for LDPE and **figure III-14** for iPP. **Figure III-12** shows the evolution of the OOT value for polymer-vitamin E systems.

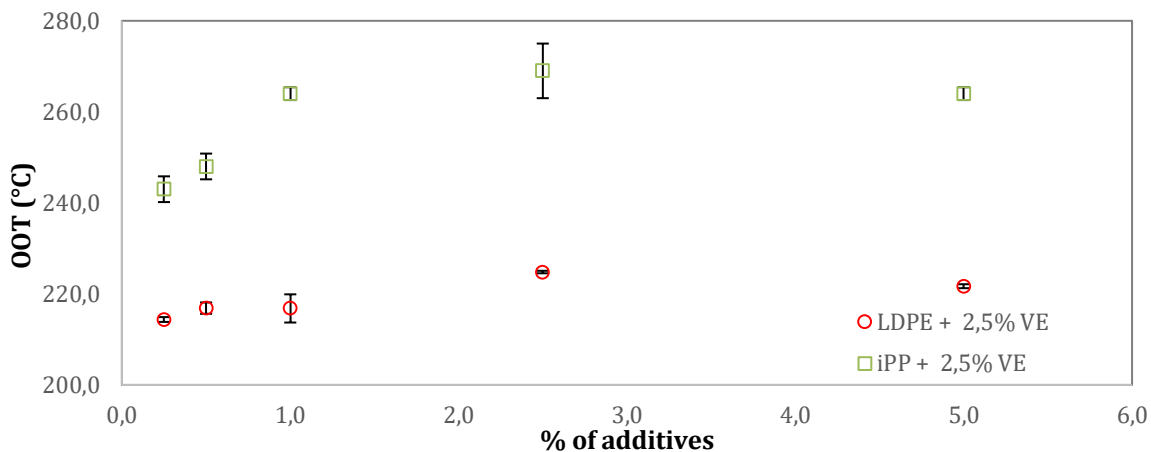


Figure III-12. Evolution of the OOT value as a function of the percentage of vitamin E (VE) in LDPE and iPP.

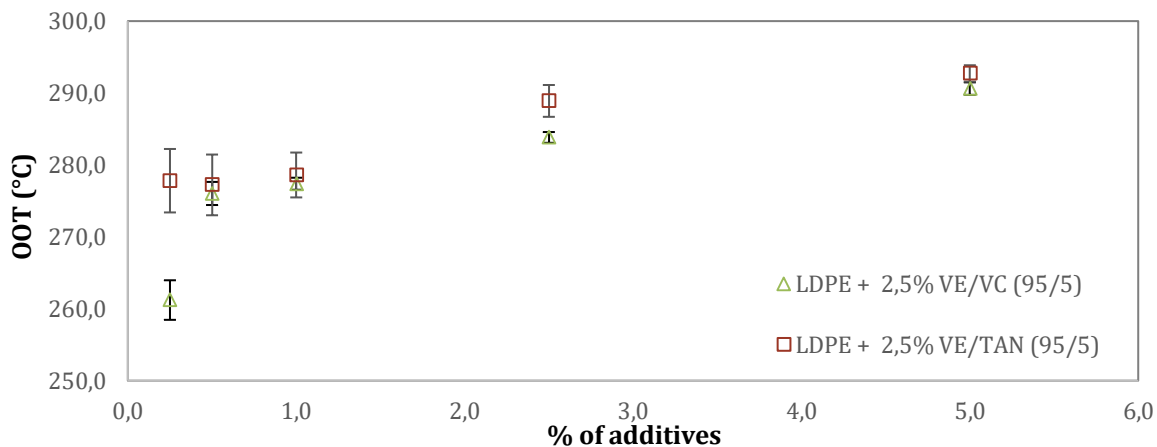


Figure III-13. Evolution of the OOT value as a function of the percentage of VE/VC and VE/Tan in LDPE.

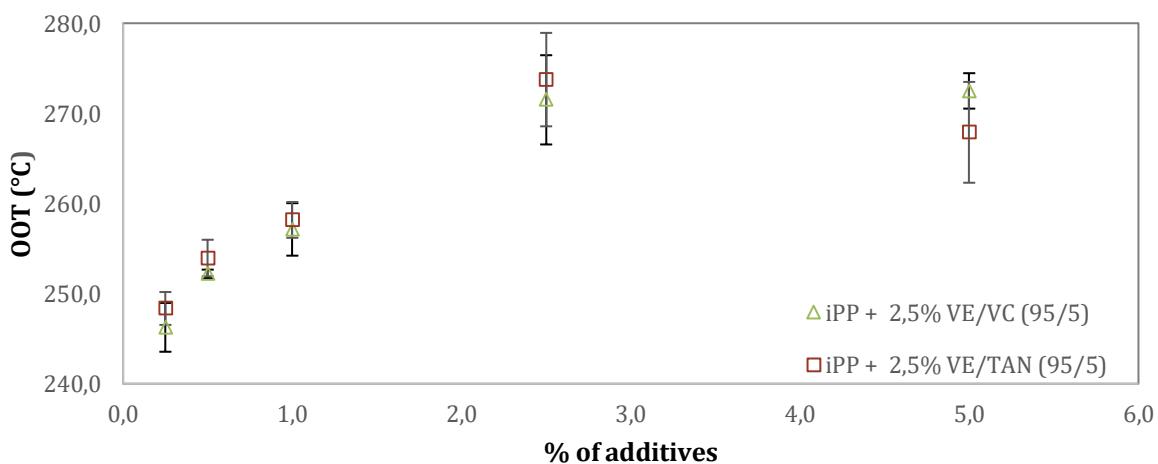


Figure III-14. Evolution of the OOT value as a function of the percentage of VE/VC and VE/Tan in iPP.

First, the previous curves reveal that when vitamin E alone is used in our polymers, a maximum is reached for 2.5% load showing that vitamin E is more effective at relatively low rates and can even promotes oxidation at very high loads. This suspected “pro-oxidant effect” can no longer be seen when vitamin C or tannins are added to the vitamin E. In both cases and for both polymers, the onset oxidation temperature reaches a plateau at high quantities. Also, the OOT values for a given polymer are comparable, except for LDPE + 2.5% VE/Tan (95/5) which shows a better thermo-oxidative resistance at low additives’ loads (**figure III-13**). This could reveal a very good protection of vitamin E and tannins on each other. At higher amount than 2.5% the physical aspect of the polymer is very oily, as if the plastic was saturated. Hence, the following studies were realised at 2.5% loadings. Values lower than 2.5% are as well very efficient but in order to avoid elevated standard deviations 2.5% seems to be the best compromise for the study. Picture from **figure III-15** shows the pellets obtained for the 2.5% loaded blends.

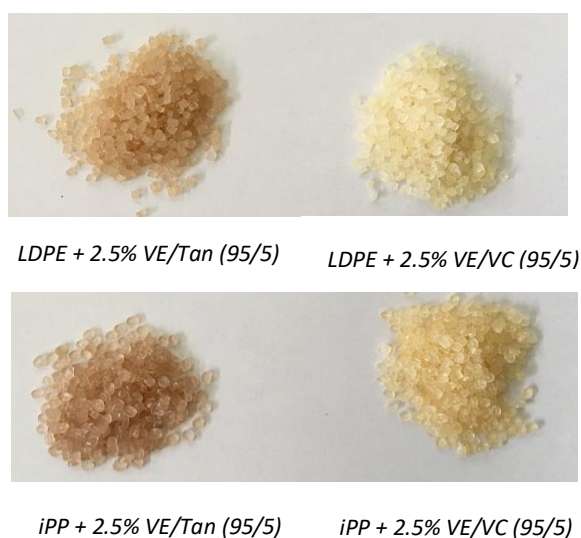


Figure III-15. Pellets of the different polymer-additive/additive blends.

When comparing the tertiary blends with their equivalent single blends, the stabilising effect provided by the combination of two additives appears evident. The comparison of the single-additive blends with the two-additives blends are shown in **figure III-16** and **figure III-17**.

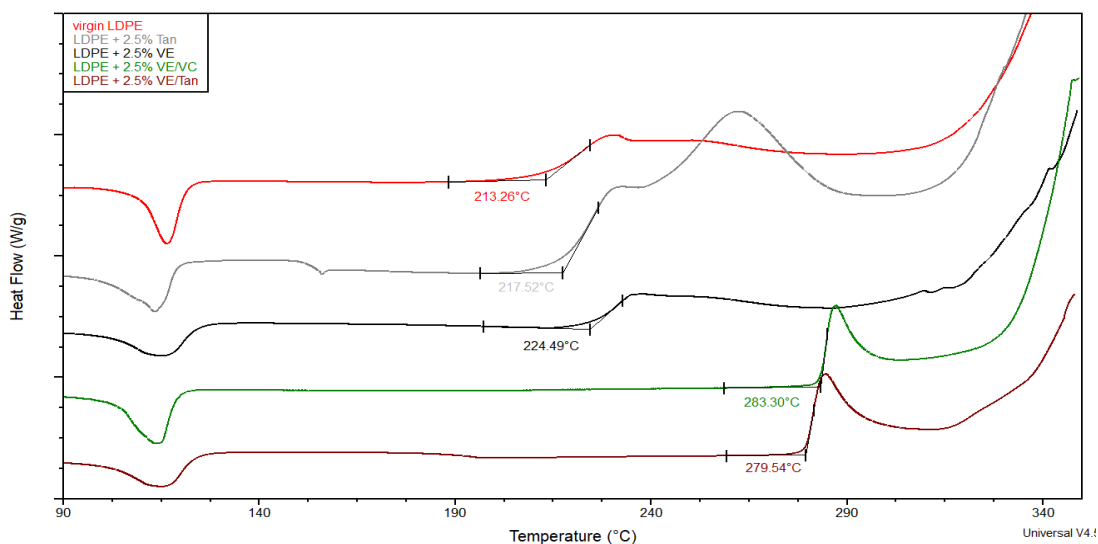


Figure III-16. OOT values comparison as a function of the formulations for LDPE.

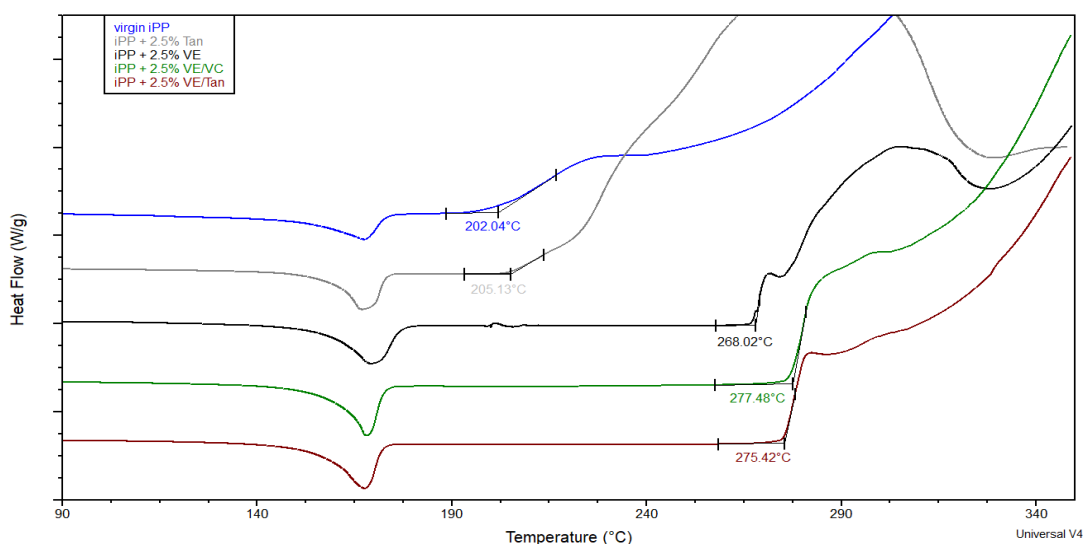


Figure III-17. OOT values comparison as a function of the formulations for iPP.

Polymers blended with vitamin E/Tannins or vitamin E/vitamin C show a remarkable increase in the polymer stability toward thermo-oxidation. These results were confirmed by the OIT value assessment which values are summarized below (table III-5).

Table III-5. OIT values obtained for LDPE and iPP mixed with two additives.

Sample	LDPE	iPP
Virgin polymer	18.6 ± 1.6	20.3 ± 2.1
VE/VC (95/5)	326 ± 3	301 ± 2
VE/Tan (95/5)	925 ± 11	542 ± 9

As said before, the stabilisation provided by the combination of additives is obvious. Nevertheless, the OIT assessment method showed its limitation as more than 15 hours (925 minutes) were necessary to obtain the oxidation peak of LDPE + 2.5% VE/TAN.

III.2.b. Differential Scanning Calorimetry (DSC)

DSC analysis was achieved to evaluate the possible structural changes in the polymer. In the case of LDPE, no significant modification of the fusion and crystallization peaks could be observed by DSC. At the opposite, for polypropylene, the characteristic transitions are impacted by the addition of our additives. The fusion peaks of the different iPP's formulation are illustrated in **figure III-18**.

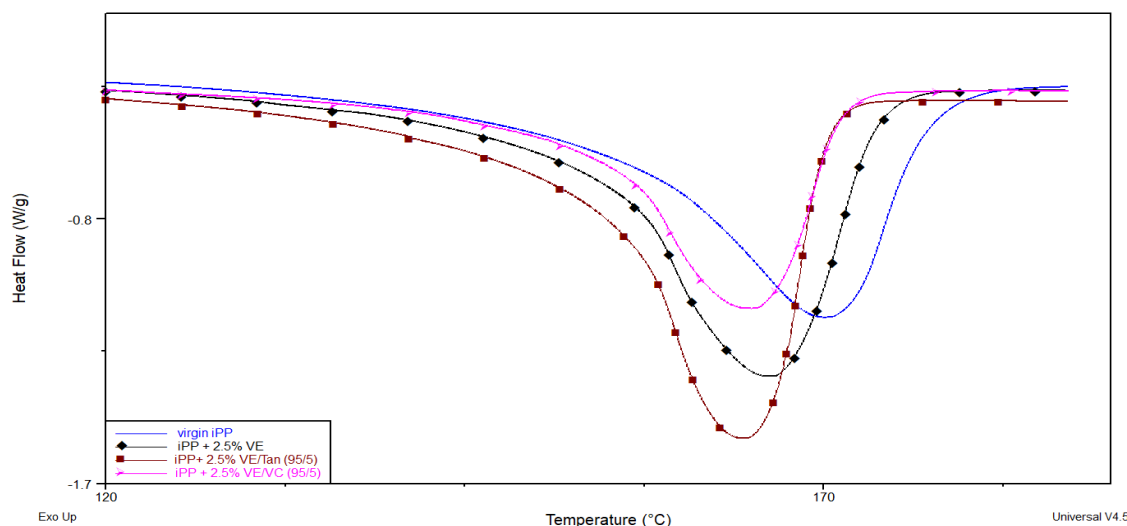


Figure III-18. Fusion peak of iPP and iPP with additives obtained by DSC.

The fusion temperature of virgin polypropylene is around 170°C and slightly shifted toward lower temperatures for formulations containing vitamin E. This shift could be a consequence of the suspected plasticizing effect of vitamin E [169].

III.3. Rheology

III.3.a. Molecular evolution by Cole-cole representation

The effect of the additives combination on the polymer's stabilisation was also assessed using the cole-cole representation as shown in **figure III-19**.

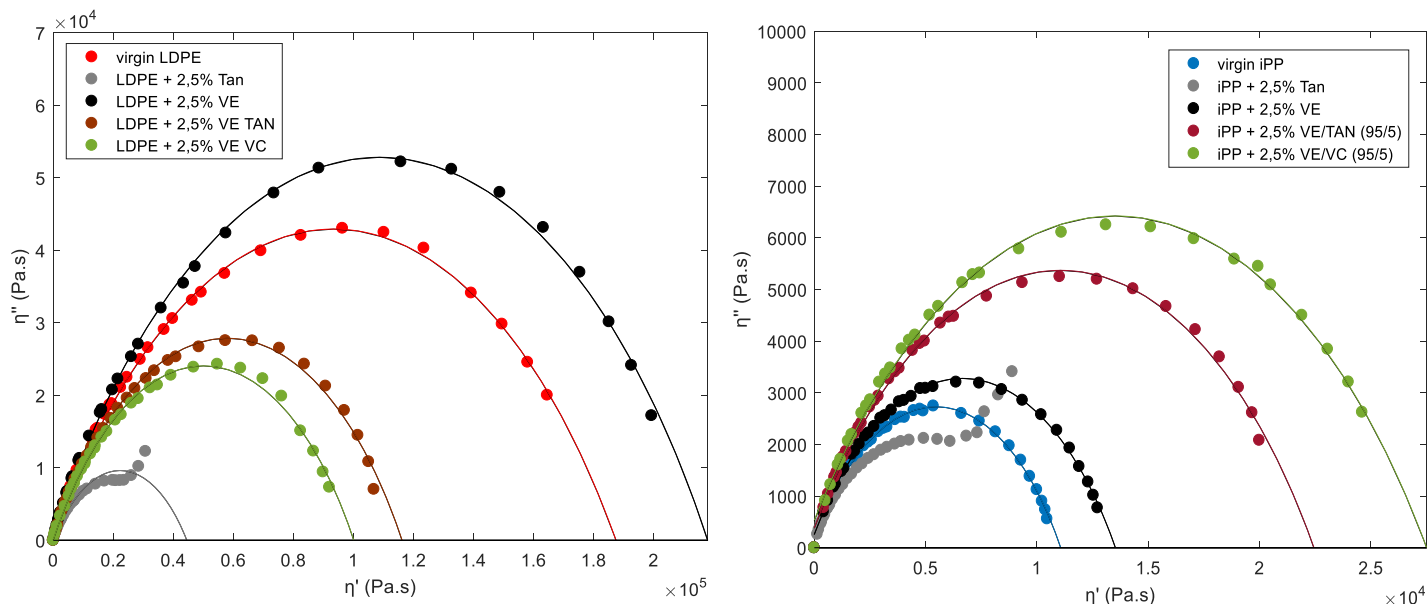


Figure III-19. Cole-cole representation of LDPE and iPP containing different additives.

In the case of polypropylene, all the formulations presents higher viscosities (and relaxation times) than the virgin polypropylene except for iPP + 2.5% tannins (grey curve). The cole-cole representation of the latter doesn't represent a semi-circle: the curve derivates from the circle shape due to the impossibility of the chains to relax. This is due to the presence of solid particules of tannins within the polymer matrix restricting the chains' motion. This aspect could also be related to a degradation of the polypropylene due to the increase of the shear rate induced within the extruder by the tannins solid particles. The increase of the viscosity of the other formulations versus that of the virgin polymer can be attributed to several aspects. It shows that no degradation of the matrix occurred and it is likely that the introduction of solid particles (vitamin C and tannins) also lead to an increase of viscosities.

For polyethylene, the same observations can be done about the formulation containing tannin alone: the semi-circle shape is not obtained showing the impossibility for the polymer chains to relax relaxation (this could be due to a strong relaxation and/or the presence of solid tnnin particules blocking the chains during the relaxation process). However, the evolution of viscosities and of relaxation times is very different from that of polypropylenes' formulation. It seems that the additives just act by preventing the chains' branching phenomena (i.e. degradation) leading to lower viscosities.

The additives thus play their stabilizing function in both polymers, but their mechanism of action is very different according to the matrix they are in.

II.3.b. Time sweep

Kinetics studies were realized on our formulations by realizing time sweep analyses at 250°C, 10Hz and 2.5% deformation under oxygen atmosphere. This test allows to evaluate how long the studied samples can handle such conditions. **Figure III-20** and **figure III-21** show the ratios of the obtained G' and the initial $G'(t=0)$. This normalization allows to better visualize the effects of the additives.

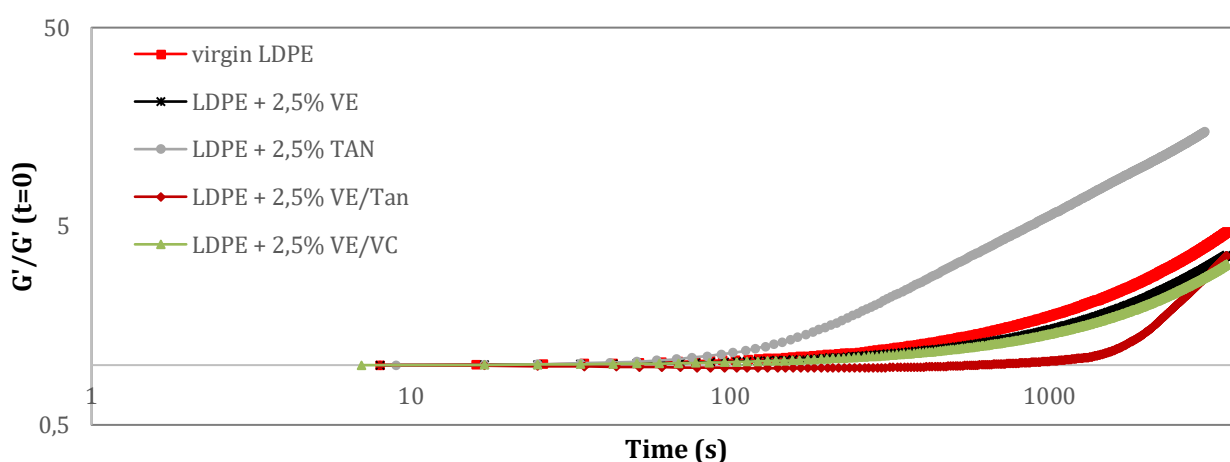


Figure III-20. Time sweep analysis for LDPE's formulations.

First of all, all of the LDPE's G' curves increase due to the polymer's degradation mechanism. The formulation showing the quickest degradation is LDPE + 2.5% Tan (grey curve) starting increasing from around 60 seconds. Then, LDPE + 2.5% VE and LDPE + 2.5% VE/VC show similar degradation kinetics, slightly better than the virgin polymer (red curve). For LDPE+2.5% VE/TAN, the system's degradation is significantly delayed showing the great stabilising effect of the combination of vitamin E and tannins.

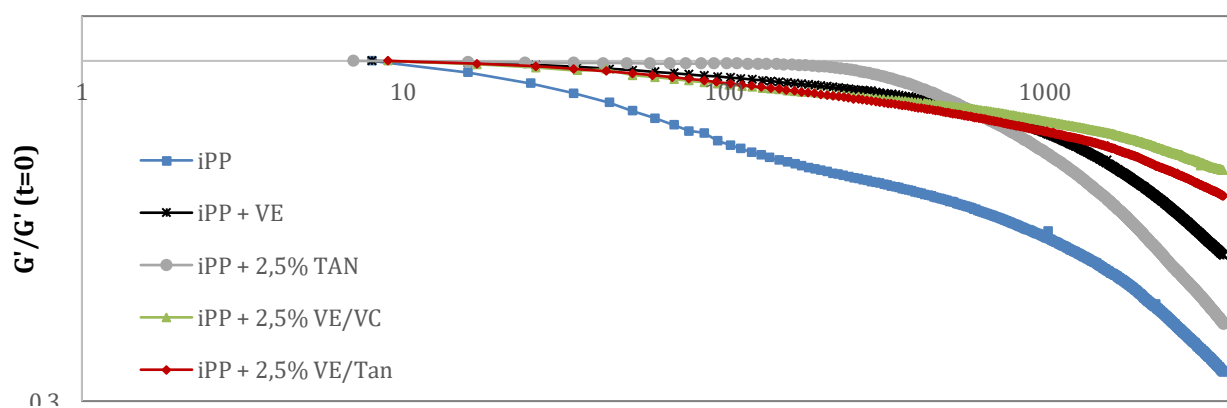


Figure III-21. Time sweep analysis for iPP's formulations.

In the case of isotactic PP, the time sweep curves show diminishing G' values due to lowering of the viscosities. Virgin polymer degrades almost from the very beginning of the test and shows two different slopes. When tannins alone are added a slight delay can be observed showing that they bring some stabilisation to the system. However, the slope of the iPP+2.5% Tan curve above 300 seconds is relatively important showing the quick degradation of this blend. The addition of vitamin E seems to degrade relatively early but, at the opposite of iPP+2.5% Tan, the kinetic slope at elevated times is slower. Similarly, iPP + 2.5% VE/VC and iPP + 2.5% VE/Tan seems to degrade quite early but their degradation kinetics are significantly lowered due to their great stabilisation effects.

III.4. Mechanical analysis

Mechanical characterisations were achieved via tensile tests. Tensile tests are widely used to evaluate plastic materials. The tensile test measures the resistance of a material to a static or slowly applied force as described in chapter 2, part II-5. In our case, both polymers show very different behaviours, as one is an elastomer-like polymer (LDPE) and the other one is much more rigid (iPP). Those differences can be attributed to different aspect. Relatively strong intermolecular forces in semi crystalline polymers prevent softening even above the glass transition temperature. It also depends on the degree of crystallinity: higher crystallinity results in a harder and more thermally stable, but also more brittle material, whereas the amorphous regions provide certain elasticity and impact resistance. Mechanical properties depend on polymers chemical nature (rigidity of the bond, possible connections between chains), chain length, chains ramifications, connection between chains (Van Der Waals, hydrogen bond) and crystallinity. Those tensile tests were achieved in thin films. Results are shown in **figure III-22**.

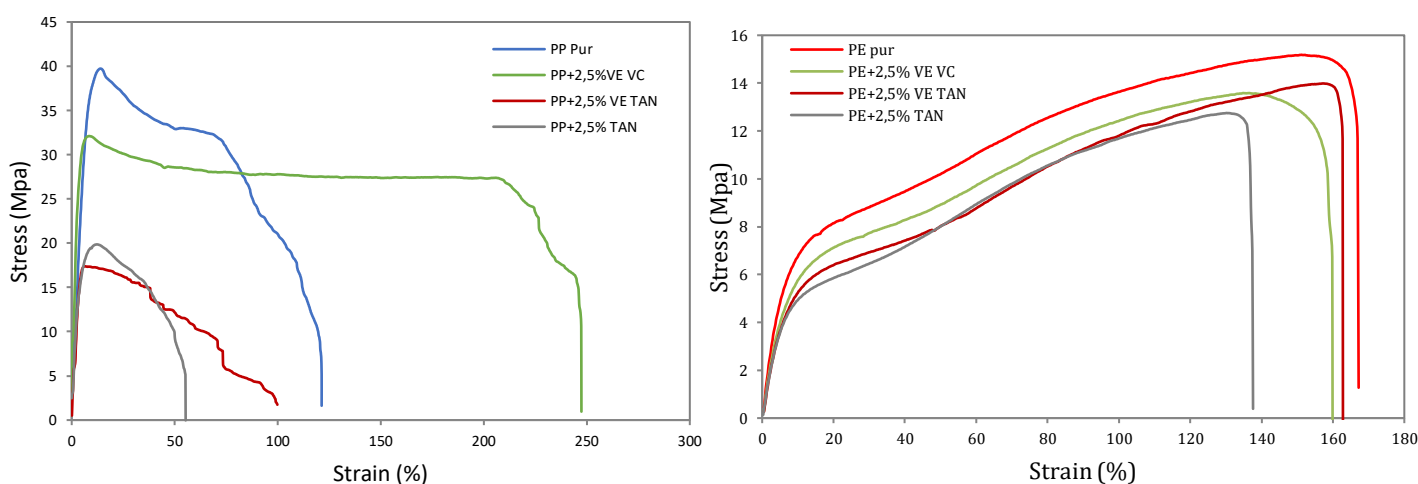


Figure III-22. Stress-strain curves for polypropylene formations (PP) and polyethylene formulations (PE).

The obtained results are in accordance with the bibliography concerning the Young modulus of PP and PE films. Actually, the Young modulus of the PE is about 100 MPa, that of PP films is in the range of 600 MPa. Overall, the Young modulus is not influenced by the addition of 2.5% additives. Then, the yield point reaches 40MPa for the virgin polypropylene, whereas it only reaches 8MPa for polyethylene (which shows the stronger plastic properties of polypropylene). This value, at the opposite of the Young modulus, is affected by the addition of additives. Actually, when tannins are involved (polymer + 2.5% VE/Tan, polymer + 2.5% Tan) the yield point is almost divided by 2 in the case of polypropylene and by around 1.6 in the case of polyethylene. It shows that the compositions containing tannins tend to weaken the film due to the solid inclusion formed by the tannin's particles. In other words, the solid tannin particles in the polymer are cracking initiators. This aspect is also visible when evaluating the total elongation before fracture. Virgin iPP lengthens up to around 110% whereas the formulations containing tannins only reach 46% and 75% elongation for iPP + 2.5% Tan and iPP + 2.5% VE/Tan respectively. At the opposite, the addition of VE/VC increase the percentage of elongation up to 200%. For virgin LDPE the ultimate elongation goes up to 160% whereas LDPE+ 2.5% Tan and LDPE + 2.5% VE/Tan reach 135% and 155% respectively.

III.5. Recycling

Both polymers containing the previously described additives were re-extruded up to 9 times and characterized in order to anticipate the effects of recycling on the formulations. The recycled blends were analysed by tensile tests, by DSC (OOT method) and by rheology.

The tensile tests didn't show any modification of the mechanical properties of the materials even after 9 cycles of extrusion.

Then, the OOT plot as a function of the recycling cycles (**figure III-23**) revealed that the thermo-oxidative properties of our blends are very stable as no loss was observed between cycle 0 and cycle 9.

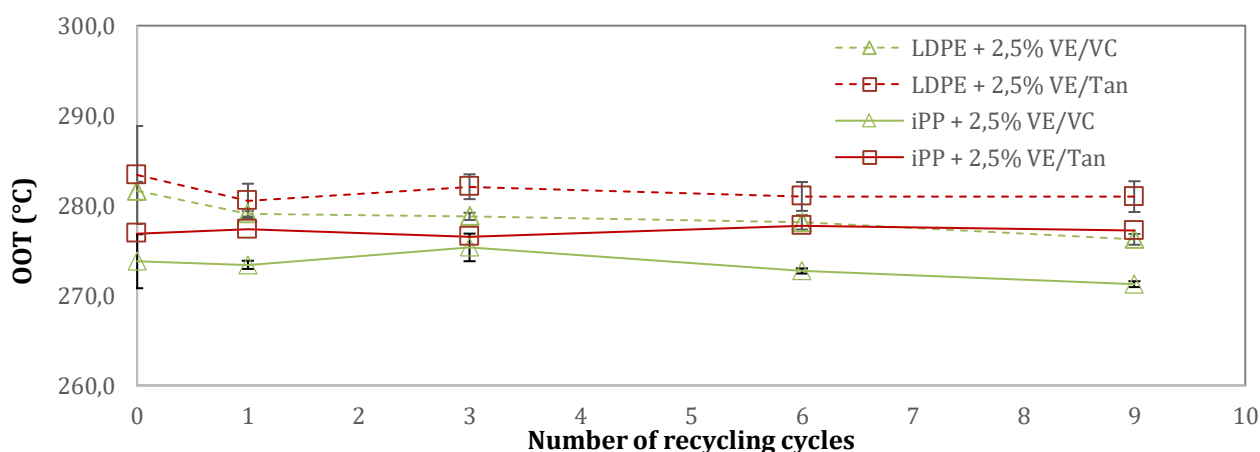


Figure III-23. OOT values as a function of the recycling numbers for our main formulations.

The same conclusion can be drawn from the MFI assessment as no loss in MFI values can be observed through the recycling numbers. However, this shows the limitation of the MFI: the absence of variation in MFI doesn't mean there are no variations in viscosity as proved by the cole-cole representation below. Actually, when setting up the cole-cole representation of the blends through recycling, variations appear (see **figure III-24** for LDPE and **figure III-25** for iPP).

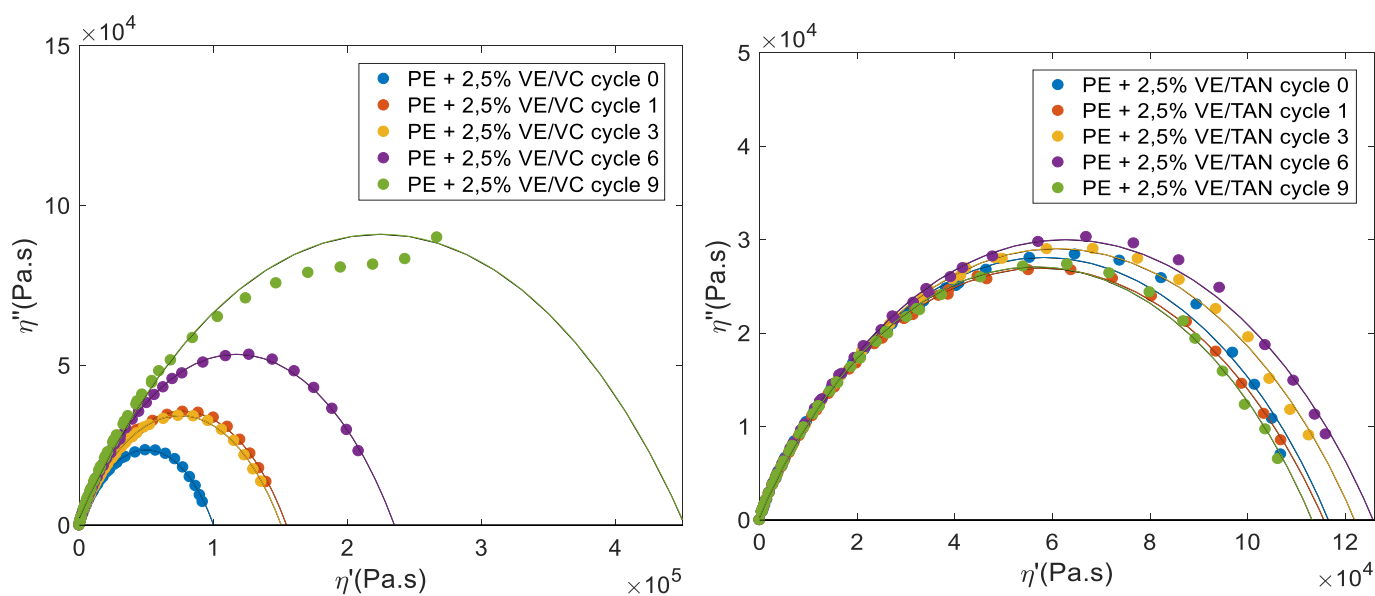


Figure III-24. Cole-cole representation of polypropylene compounded with different mix of additives.

Those variations are very small for LDPE + 2.5% VE/Tan showing the very good stability of that blend. For LDPE + 2.5% VE/VC, in contrast, there is a clear increase in viscosity characteristic due to LDPE's chain branching (i.e. degradation). A first increase in the viscosity and the relaxation times can be observed for the cycle 1 and 3 but remains slight. More consequent degradations start at the 6th cycle with a viscosity multiplied by almost 2.5. Moreover, for the 9th cycle (green curve) a deviation from the semi-circle shape appears showing the impossibility for the polymer chains to properly relax. This is probably due to the degradation of the material, leading to strong reticulations within the polyethylene matrix. Also, the chain scission phenomenon that was observed for the 9th recycling cycle of virgin LDPE in chapter 2, part IV-1 (**figure II-35**) isn't present here (only chains' branching occurs) confirming that the polyethylene's degradation process is softer when antioxidants are involved.

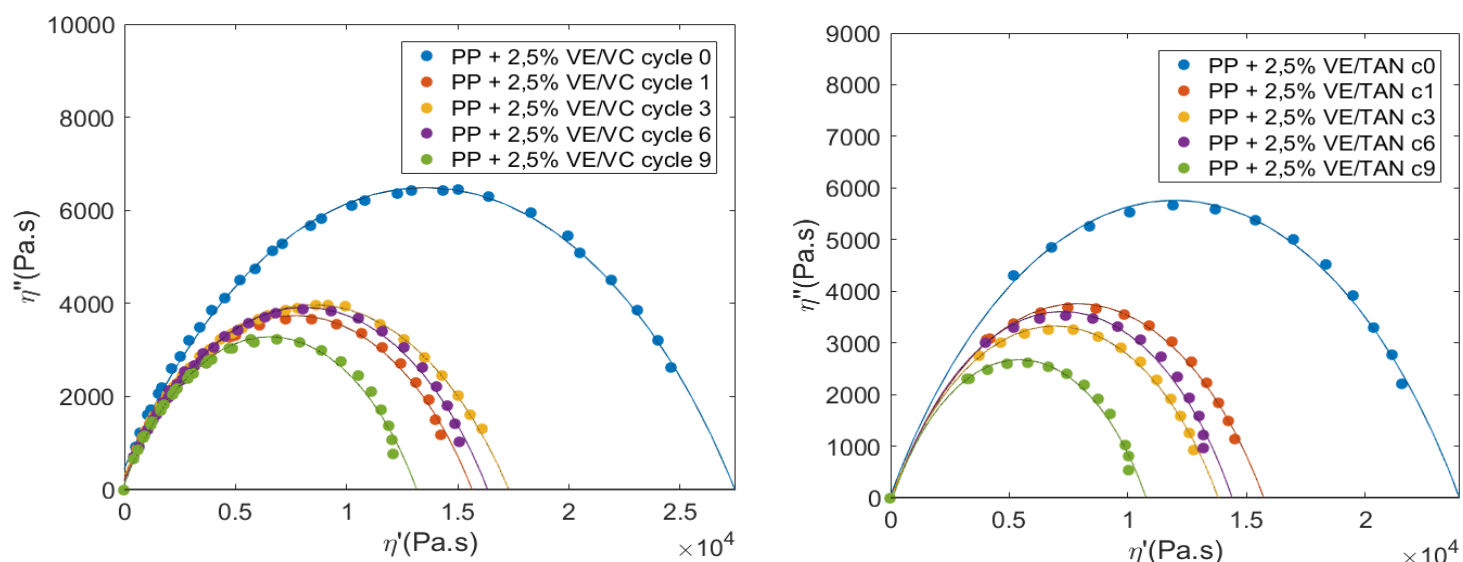


Figure III-25. Cole-cole representation of polypropylene compounded with different mix of additives.

In the case of iPP, as the degradation occurs by chains scission, the viscosity diminishes a lot through the extrusion cycles. Hence, the relaxation of chains still happens but as they are smaller and smaller, it happens at lower and lower viscosities. Whatever the additives, it seems that cycle 1,3 and 6 (yellow, red and purple curves) have very close behaviours. However, after 9 recycling cycles (green curves), the circle is still well defined but tend to lose its shape at the very end of the circle. When looking in deep, this small deviation can also be seen for the cycle 9. This reveals the beginning of a degradation from the sixth cycle.

Also, the mechanical properties of recycled materials were assessed and showed that the ageing of polymeric films has only little impact on the young's modulus. At the opposite, the maximum of elongation is affected by the ageing of the films (see diagram from **figure III-26**).

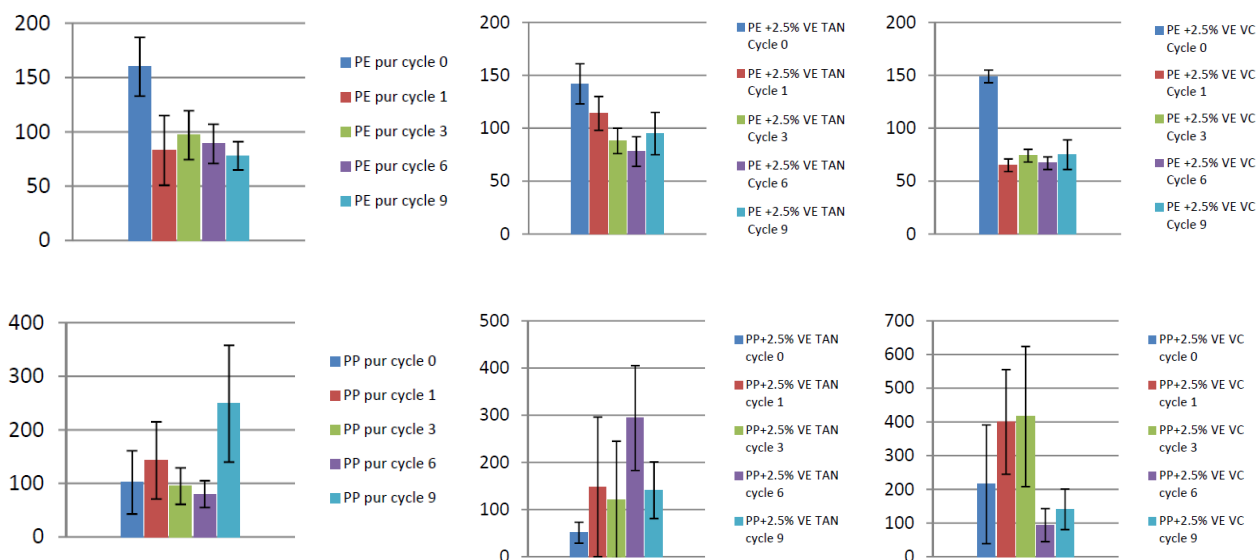


Figure III-26. Ultimate elongation rate of the recycled formulas.

In the case of LDPE and LDPE + 2.5% VE/VC, a strong decrease (values divided by around 2.3) can be observed between the cycle 0 and the cycle 1. The values are, then, stable between TAN cycle 1 and cycle 9. For LDPE+ 2.5% VE/Tan this decrease is smoother and gradual. In the case of iPP, very large standard deviations were obtained due to the large variation in film thicknesses. It is thus complicated to conclude on the impact of recycling on the polypropylene’s mechanical properties.

Those formulations were also characterized by HT-SEC, the results are expressed in **table III-6**.

Table III-6. Values of the different molar masses obtained for all the formulations.

	Mn (g/mol)	Mw (g/mol)	Mz (g/mol)	Ip
Virgin LDPE	21242	159359	512588	7,5
Virgin LDPE Cycle 9	15396	134617	427510	8,7
LDPE + 2.5% VE/Tan	20684	158084	505464	7,6
LDPE + 2.5% VE/Tan cycle 9	18648	153504	485222	8,2
LDPE + 2.5% VE/VC	22658	143227	436170	6,3
LDPE + 2.5% VE/VC cycle 9	15167	148045	485505	9,8
Virgin IPP	71454	296817	723051	4,2

Virgin IPP Cycle 9	92860	198288	341000	2,1
IPP + 2.5% VE/Tan	94468	308162	671106	3,3
IPP + 2.5% VE/Tan cycle 9	89113	273500	594202	3,7
IPP + 2.5% VE/VC	67065	286276	711127	4.3
IPP + 2.5% VE/VC cycle 9	85593	193829	327311	2,3

The molar mass distribution is quantified by the Mw/Mn ratio. This ratio is relatively high for LDPE compared to iPP due to the very complex structure of LDPE. For LDPE, the Mn value (representing the smaller chains) diminishes between cycle 0 and cycle 9 due to the branching processes. Mw remains relatively constant, except for virgin LDPE which, as said before, also suffers from chain scissions. In the case of polypropylene, the opposite phenomenon occurs: Mn values increase between cycle 0 and cycle 9 due to the increase of smaller chains (chain scissions) except for IPP + 2.5% VE/Tan which Mn is constant. Consequently, Mw and Mz values decrease as well. Whatever the polymer, the changes in morphology are lowered when the additives are involved.

Also, evaluating the rate of long branching thanks to the factor g' can give information. It is the ratio of the intrinsic viscosity of the molecule connected to the intrinsic viscosity of the linear molecule of the same mass. For a linear material, the term g' is therefore equal to 1 and g' decreases for a material with an increasing connection rate. Zimm and Stockmayer defined another parameter (equation 26) as the ratio of the radius of gyration of the connected molecule on that of the linear molecule of the same mass [170].

$$g' = g^{1.2} = \frac{Rg_{branched}^2}{Rg_{linear}^2} \quad (26)$$

Zimm and Stockmayer also proposed a relationship linking this ratio to the average number of connection points per molecule, N (equation 27).

$$g = \left[\sqrt{1 + \frac{N}{7} + \frac{4N}{9\pi}} \right]^{-0.5} \quad (27)$$

This relation applies to isomolecular systems with trifunctional ramifications (like LDPE). The gyration radius was obtained thanks to a multiangle light scattering measurements (MALLS) located at the end of a high temperature SEC (experiments realized by the C2P2 laboratory in Lyon). Another way to express the branching rate is the number of branches per 1000 carbon atoms which illustrates the degradation of LDPE.

Table III-7 gives the values of g' , as well as the number of branching points per molecule, N_c , and the number of branches per 1000 C, N_b . They were calculated using equations (26) and (27).

Table III-7. g' values and rate of long branching for LDPE's formulations.

	g'	N_c	N_b
Virgin LDPE	0.47	18.3	1.6
Virgin LDPE cycle 9	0.40	17.9	1.9
LDPE + 2.5% VE/Tan	0.48	12.1	1.0
LDPE + 2.5% VE/Tan cycle 9	0.36	25.6	2.3
LDPE + 2.5% VE/VC	0.46	13.8	1.3
LDPE + 2.5% VE/VC cycle 9	0.35	24.6	2.3

The higher N_c or N_b the longer the chains 'branching. In turn, recycled polymers show longer chain branching. This shows that some degradations occur leading to chain branching. The number of chains branching for virgin LDPE is smaller due to the combination of both branching and chain scissions.

IV. PROPOSAL OF STABILISATION MECHANISMS

IV.1. Additives interactions by DOSY-NMR

IV.1.a. Interactions between vitamin E and tannins

First, tannins alone were characterized by $^1\text{H-NMR}$ and DOSY-NMR. For proton NMR, tannins were mixed with two different solvents (and filtered): DMSO and water (see **figure III-27**).

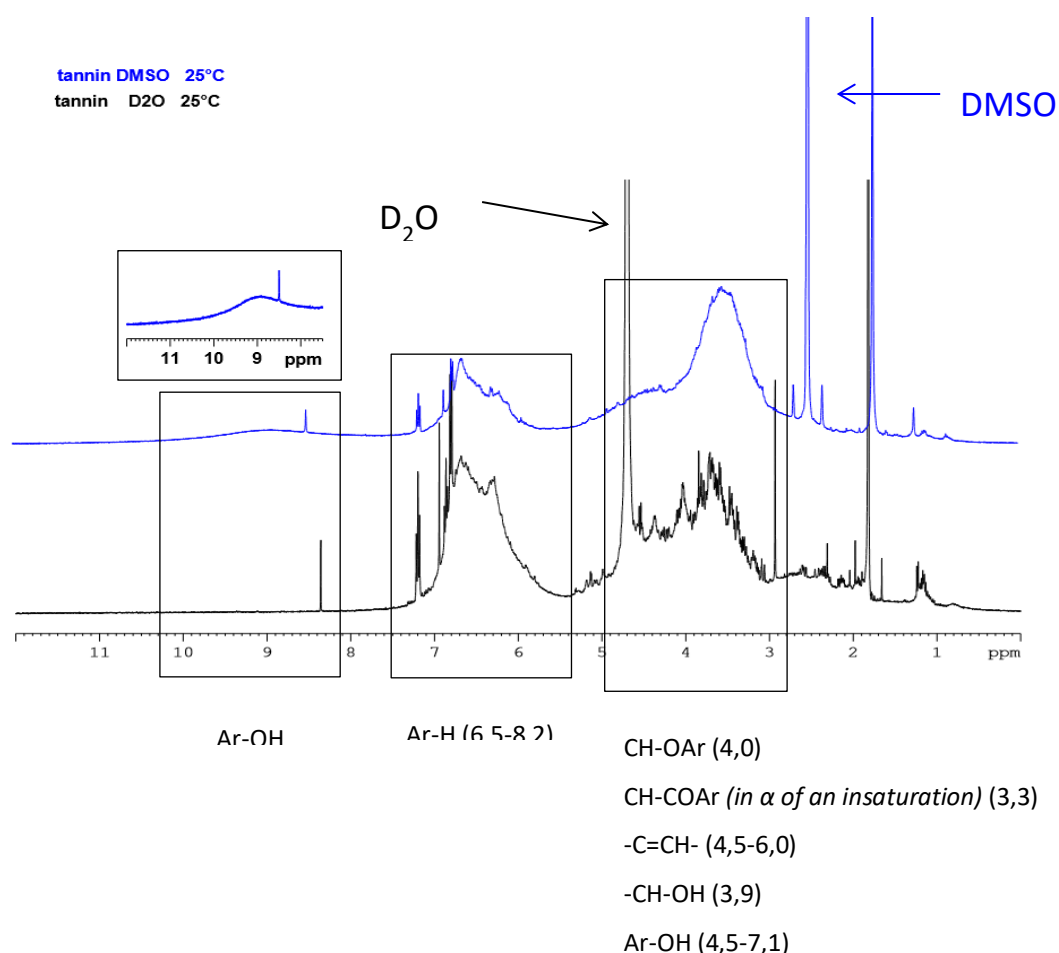


Figure III-27. ¹H-NMR of quebracho tannins in D₂O versus in DMSO.

The problem with water is that it can interfere with the alcohol functions which are omnipresent in tannins. It can be seen on the peaks around 10 ppm as the aromatic alcohols are visible on the blue curve obtained with DMSO but not on the black curve. This proton NMR spectra confirms the information detailed in chapter I about the structures of the tannins, namely that tannins' monomer unit is fisetin, as detailed in the **figure III-28**.

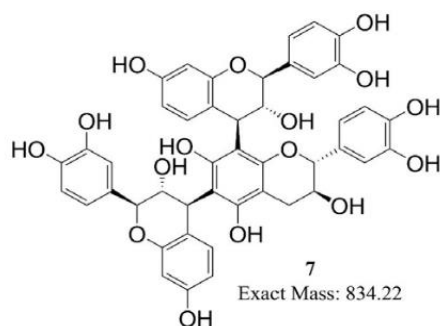


Figure III-28. Trimer of two fisetinidol unit and a catechin unit, characteristic from quebracho tannins.

Also, DOSY-NMR was also realized on tannins solubilized in DMSO (**figure III-29**).

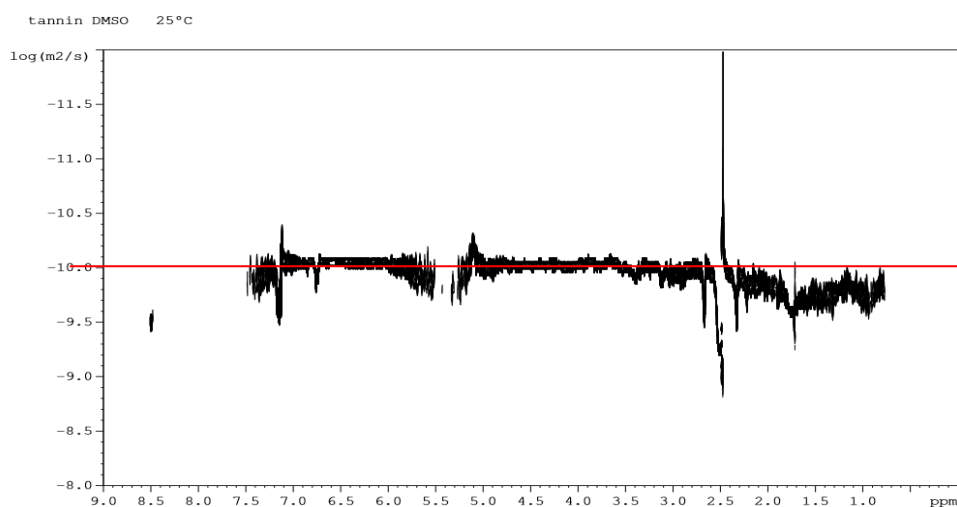


Figure III-29. DOSY-NMR of quebracho tannins in DMSO.

The obtained DOSY-NMR shows that most of the signal is on the same diffusion line (red line). This reveals that the studied molecules are made of only one common structure, the structure shown in **figure III-28**.

In order to evaluate the possible synergisms between vitamin E and tannins, several blends were realized: (50/50) %w VE/Tan, (75/25) %w VE/Tan, (90/10) %w VE/Tan, (95/5) %w VE/Tan and (98/2) %w VE/Tan. Those blends were heated at 190°C and stirred in order to mimic the extrusion conditions and solubilized (as much as possible) within deuterated methanol. Those solutions were then analysed by NMR (H^1 and DOSY). The DOSY- H^1 NMR of the VE/Tan (100/0), VE/Tan (50/50) and VE/Tan (95/5) blends are illustrated in **figure III-30**.

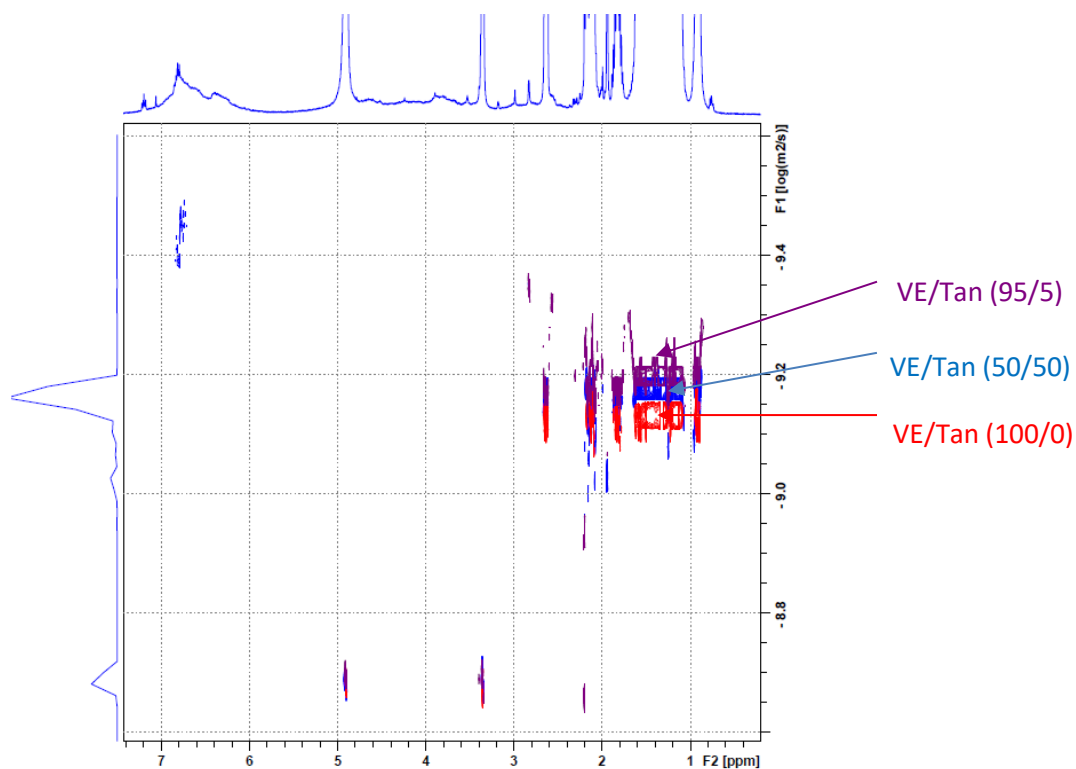


Figure III-30. DOSY-NMR of vitamin E-tannins' blends.

For visibility reasons, only three DOSY-NMR spectra are shown on this figure. We can observe that the lower diffusion is obtained for the VE/Tan (95/5) blends, which shows that the strongest interactions between the two compounds occur in this mixture. Actually, when studying interactions, the higher the DOSY, the stronger the interactions. This is due to a lowering of the diffusion coefficient when the interactions between two compounds increase. The obtained diffusion coefficients were drawn as a function of the composition of the blends (figure III-31).

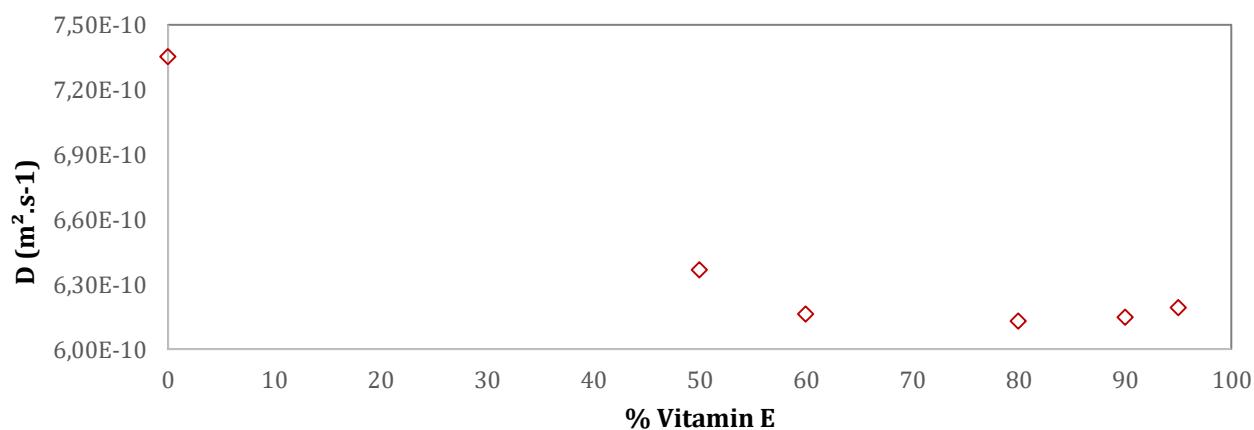


Figure III-31. Diffusion coefficient of alpha-tocopherol as a function of the blend composition.

A tendency can be observed. The diffusion coefficient of the vitamin E-tannin system diminishes regularly until reaching a plateau at around 40% tannins loading. It shows that the diffusion coefficient of the blend is completely affected by its composition putting forward the interactions between those two components. Also, the interactions seem to be equivalent at low tannins loading rate than at 40% loading.

IV.1.b. Interactions between vitamin E and vitamin C

The exact same work was realized for vitamin E-vitamin C system: (50/50) %w VE/VC, (75/25) %w VE/VC, (90/10) %w VE/VC, (95/5) %w VE/VC and (98/2) %w VE/VC. Those blends were heated at 190°C, stirred and solubilized (as much as possible) within deuterated methanol. The $^1\text{H-NMR}$ of the different blends are illustrated in **figure III-32** (they were not drawn for vitamin E-tannin because results were less visually representative).

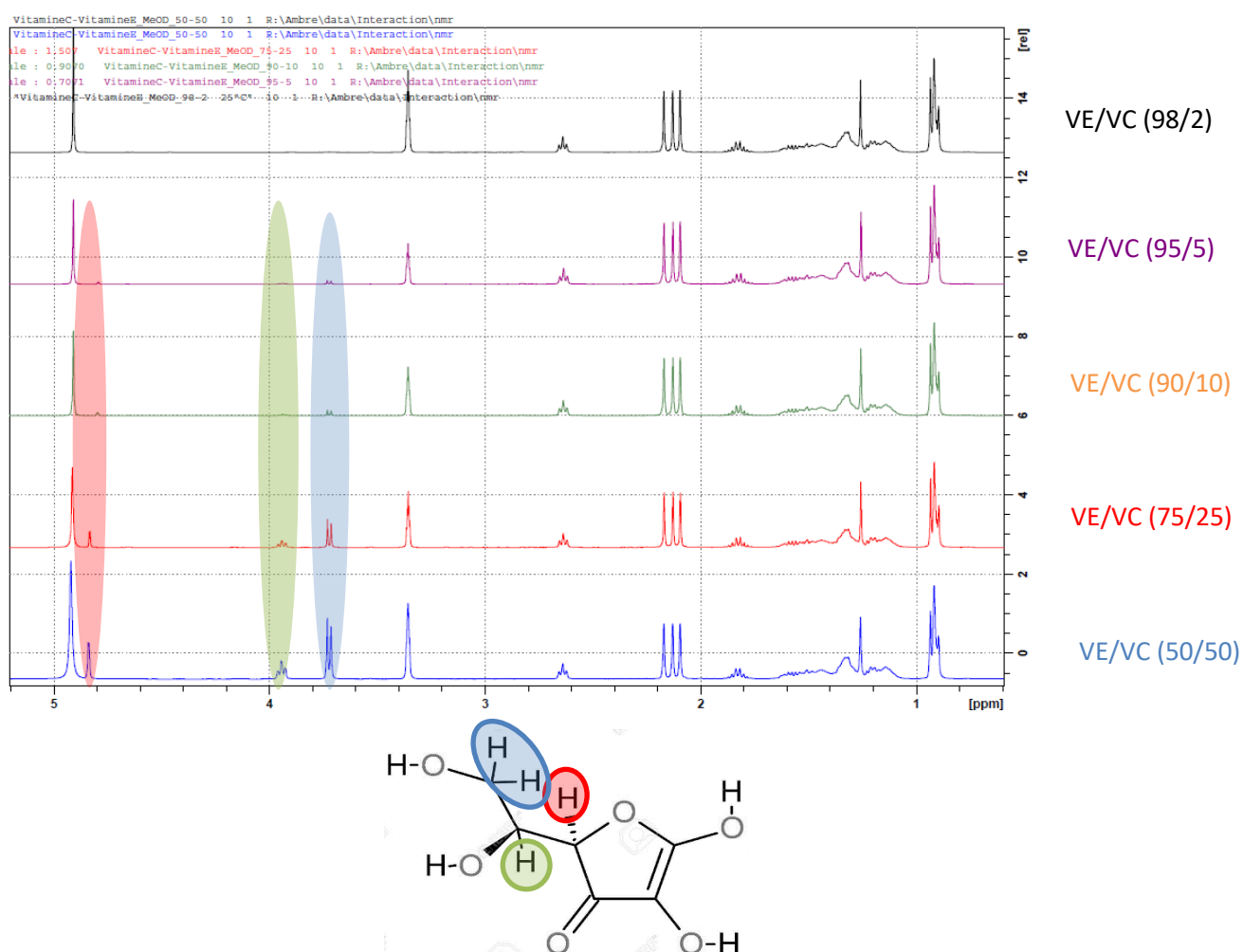


Figure III-32. $^1\text{H-NMR}$ of the different vitamin E-vitamin C blends.

The peaks corresponding to ascorbic acid are highlighted (the peaks at 4.9 and 3.4ppm correspond to the deuterated methanol). Three main groups of peaks can be observed and were highlighted in red, green and blue. When zooming on those peaks (**figure III-33** below), a shift can be observed from one formula to another.

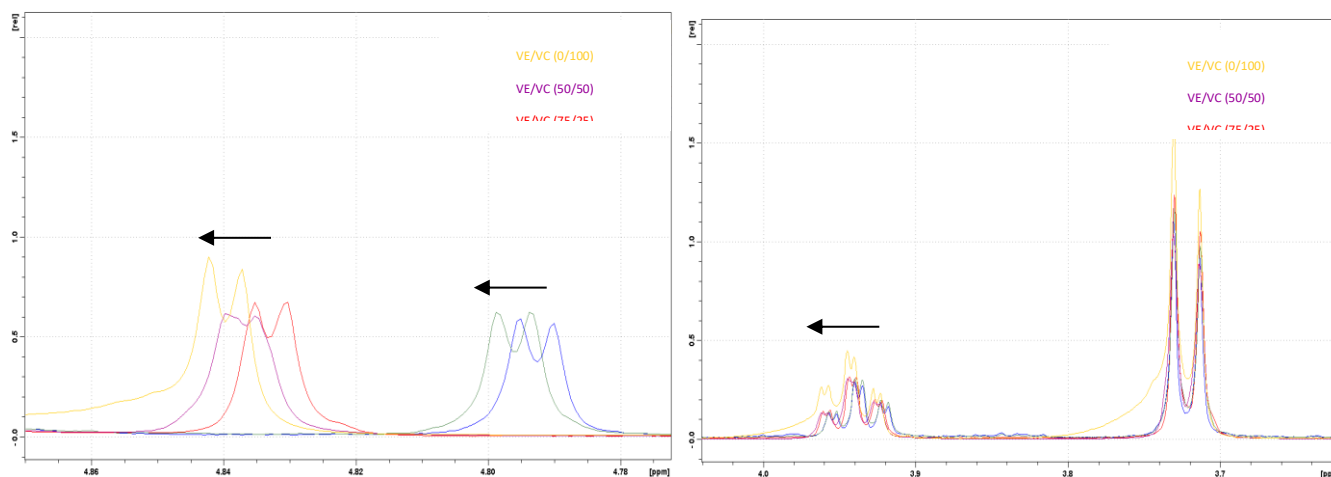


Figure III-33. Shift from the vitamin C's peaks according to the composition of the formulation.

When plotting the differences between the chemical shifts (the blend VE/VC (0/100) was taken as a reference) as a function of the composition, a clear tendency appears as detailed in **figure III-34**. The chemical shifts were drawn for two peaks: $\delta = 3.9$ ppm (proton highlighted in green) and $\delta = 4.8$ ppm (proton highlighted in red).

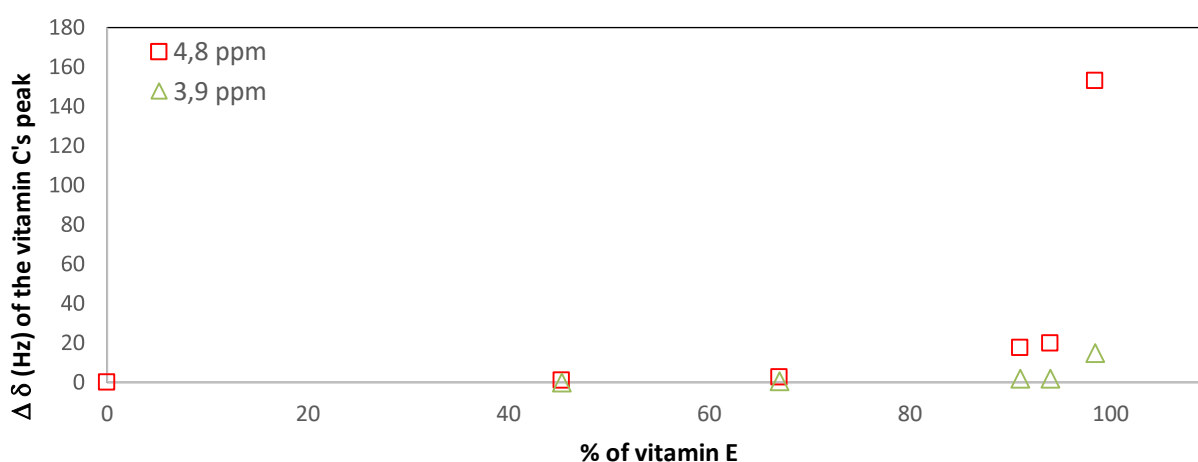


Figure III-34. Evolution of the chemical shift of two protons from ascorbic acid according to the amount of vitamin E in the blend.

The peaks shift is thus function of the composition of the blends, showing possible interactions between the two compounds. This also shows that the interactions between vitamin E and vitamin C are due to hydrogen

bonding. In order to verify the presence of those interactions, DOSY-NMR was realized. The following figure (Figure III-35) is an overlay of the DOSY-NMR obtained for vitamin E alone, vitamin C alone and VE/VC (95/5) which appeared to be an optimum (like explained for vitamin E-tannins before).

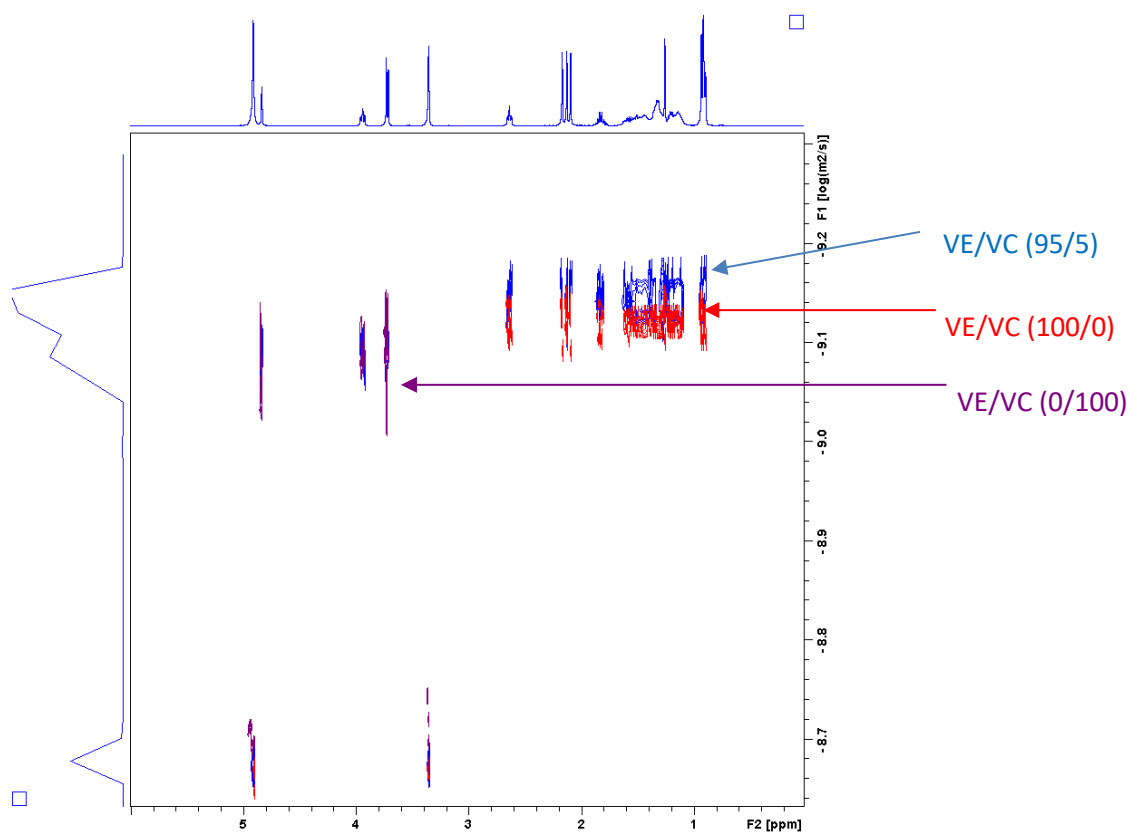


Figure III-35. DOSY-NMR of vitamin E-vitamin C blends.

The obtained diffusion coefficients were drawn as a function of the composition of the blends (figure III-36).

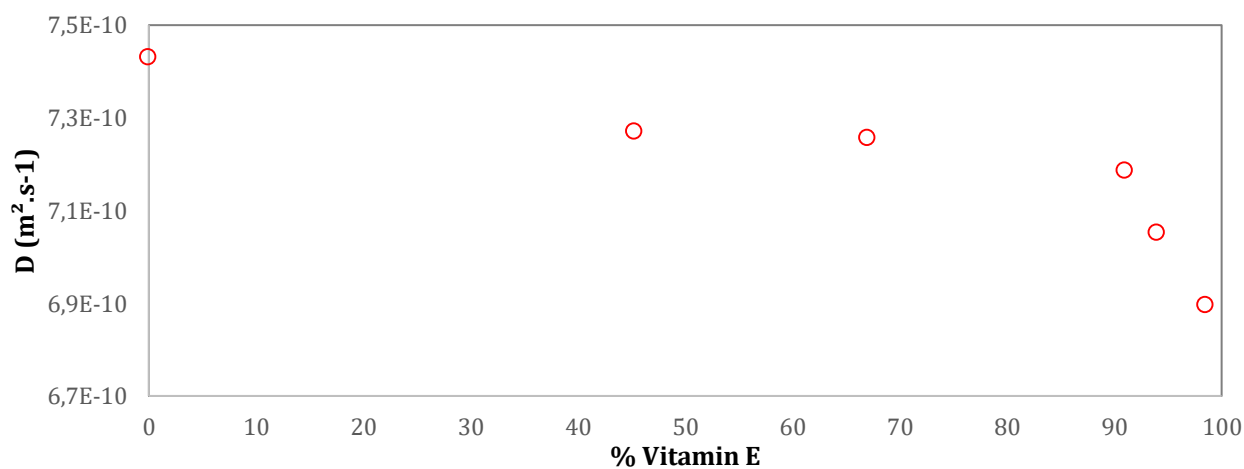


Figure III-36. Evolution of the diffusion coefficient of vitamin E as a function of the composition of the blend.

Here as well, a tendency can be observed. The diffusion coefficient of the blend diminishes from the VE/VC (90/10) formula. This puts forward the interactions between vitamin E and vitamin C which seem to be stronger at low amounts of vitamin C. The evolution of the diffusion coefficient of the vitamin E-vitamin C blend is different from that observed for vitamin E-tannin blend.

The obtained results brought us many precious information and it would have been very beneficial to apply the same work to polymer blends. However, the method could not be applied to the polymers' blends due to the lack of solubility. Some recent work was tried with low molar mass PE and PP which would eventually be more soluble (i.e. easier to analyse).

IV.2. Polypropylene's stabilisation

In general, for homopolymers, the flow behaviour depends on the flow geometry and on the processing conditions. When solid particles are introduced within a polymer matrix, the flow behaviour becomes more complex. The rheological behaviour of polymers containing tannins and vitamin C were investigated at the melt state, through oscillatory rheological tests, in the linear domain of viscoelasticity.

IV.2.a. Polypropylene stabilized with vitamin E and tannins

Figure III-37 shows the double logarithmic plots of η^* as a function of ω for polypropylene blends.

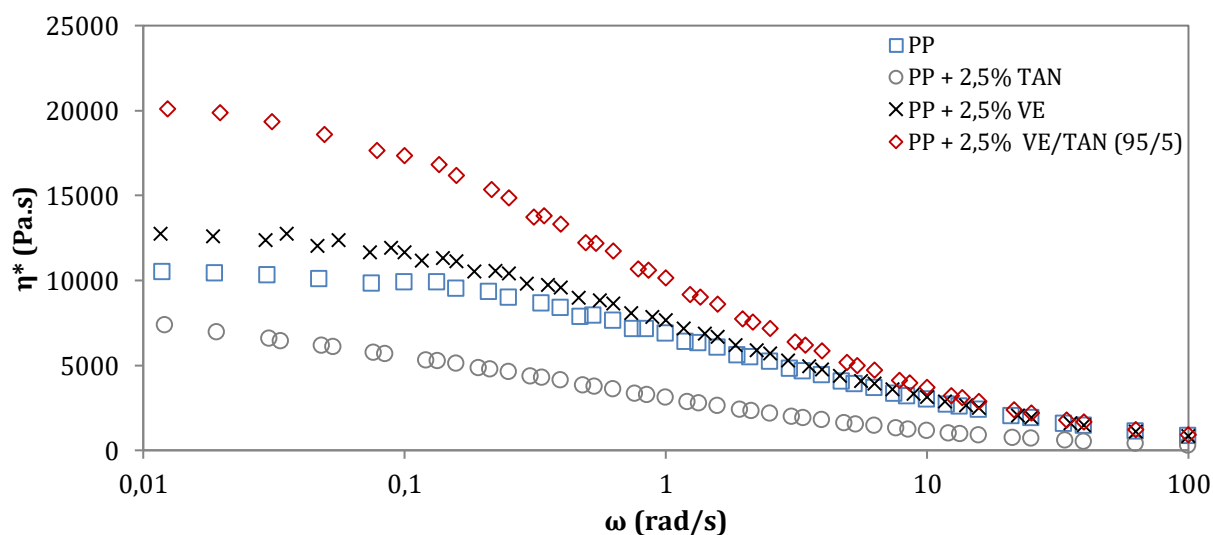


Figure III-37. Evolution of the limit viscosity η^* as a function of the frequency of different iPP-tannins blends

First, all the curves show shear-thinning behaviour for all the blends. The virgin iPP and the iPP containing vitamin E show similar behaviours. At the opposite, the addition of tannins alone and tannins combined to vitamin E present very different behaviours. The viscosity of iPP + 2.5% Tan (grey curve) is very lowered compared to all the other blends. Those changes can be attributed to the formation of a two-phase structure of immiscible components. However, the addition of vitamin E involves huge differences: the complex viscosity is largely increased probably putting forward interfacial interactions between vitamin E, tannins and iPP. Those interactions seem to be absent from the blend iPP + 2.5% Tan as a drop in the complex viscosity appears revealing a very poor miscibility. Actually, tannins being relatively hydrophilic and polypropylene being hydrophobic, the introduction of tannins alone within the iPP matrix involves the creation of voids leading to the loss of the complex viscosity and the modulus.

The increase in the viscosity upon the compatibilization of immiscible compounds was already reported [171].

Moreover, extra information can be obtained from the $\tan(\delta)$ curve which corresponds to the ratio between G'' and G' . This value, obtained by a rotational rheometer, is a very sensitive and effective value for expressing the contribution of a specific structure within a viscoelastic system. **Figure III-38** shows the evolution of $\tan(\delta)$ as a function of the frequency ω .

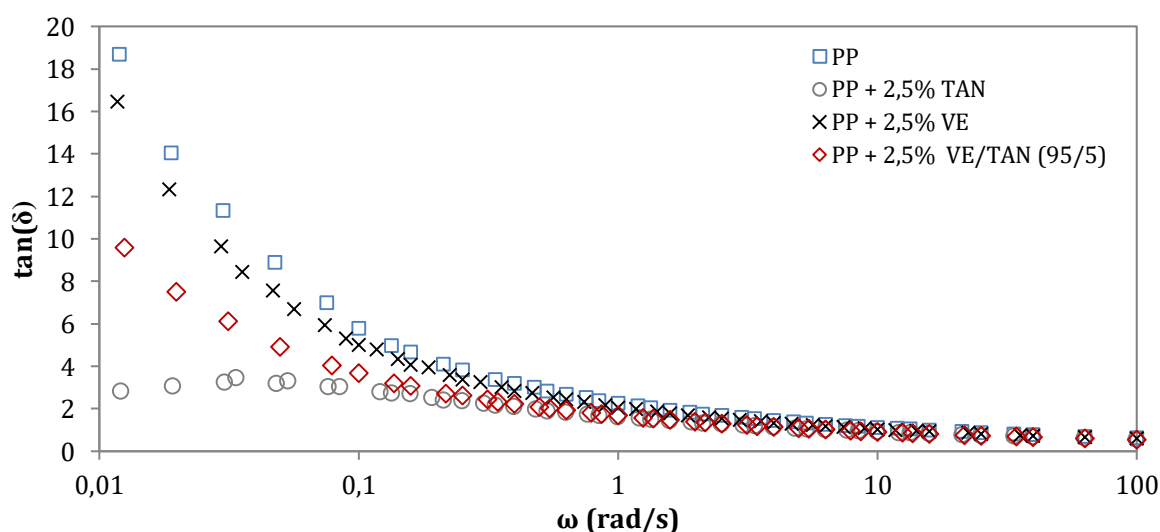


Figure III-38. Evolution of the $\tan(\delta)$ as a function of the frequency of different iPP-tannins blends

First of all, at high frequencies, all the blends show similar $\tan(\delta)$. However, significant differences occur at lower frequencies. The virgin iPP, iPP + 2.5% VE and iPP + 2.5% VE/Tan present negative slopes in $\tan(\delta)$ on the entire tested frequency range. However, in the case of iPP + 2.5% Tan, the slope of $\tan(\delta)$ at low frequencies is positive, then reaches a plateau (slope equals to zero) and finally becomes negative. This phenomena indicates that indicates that this material has a solid-like behaviour (or gel-like) at low frequencies whereas all the others present melt-like behaviours. Actually, it was shown that at the “gel point” of such

systems, $\tan(\delta)$ is not dependant from the frequency anymore resulting in the zero-slope curve [172]. Then, the slopes in the $\tan(\delta)$ curves are negative in the case of liquids/melts and positive in the case of solids/gels systems.

This confirms the hypotheses made before, especially those expressed with the cole-cole representation. Actually, the deviations observed for iPP + 2.5% Tan revealed the nonhomogeneous dispersion and phase separation within the blend, due to immiscibility leading to the creation of voids at the interface between tannins and polypropylene. This deviation could no longer be observed in the case of iPP + 2.5% VE/Tan. It means that the latter is a compatible blend or at least well dispersed. Vitamin E thus acts like a compatibilizer. Having in mind the molecular structure of vitamin E it seems logical that it can behave like a surfactant at the interface between tannins particles and polypropylene.

The $\tan(\delta)$ value being the ratio between G'' and G' , a lower value is synonymous from an increase in the relaxation time (which fits with the Cole-cole observations made earlier).

Also, the size of the dispersed particles is of major importance. Actually, tannins present a very large scatter in the size distribution of its particles: between 50 to 150 μm . Also, the ratio of vitamin-tannins is (95/5) %m in weight but tannins density is very low: $>0.4\text{g}\cdot\text{cm}^{-3}$ (for 0.95 for alpha-tocopherol and 0.92 for iPP). In turn, the volumic ratio of the blend is (88/12) %v which means that the amount of tannins is much more significative than that of vitamin C which density is $1.65\text{g}\cdot\text{cm}^{-3}$ (volumic ratio of (97/3) %v).

The scheme in **figure III-39** represents the hypothetic interactions occurring within the binary (polypropylene-tannins) and the tertiary system (polypropylene-vitamin E-tannin).

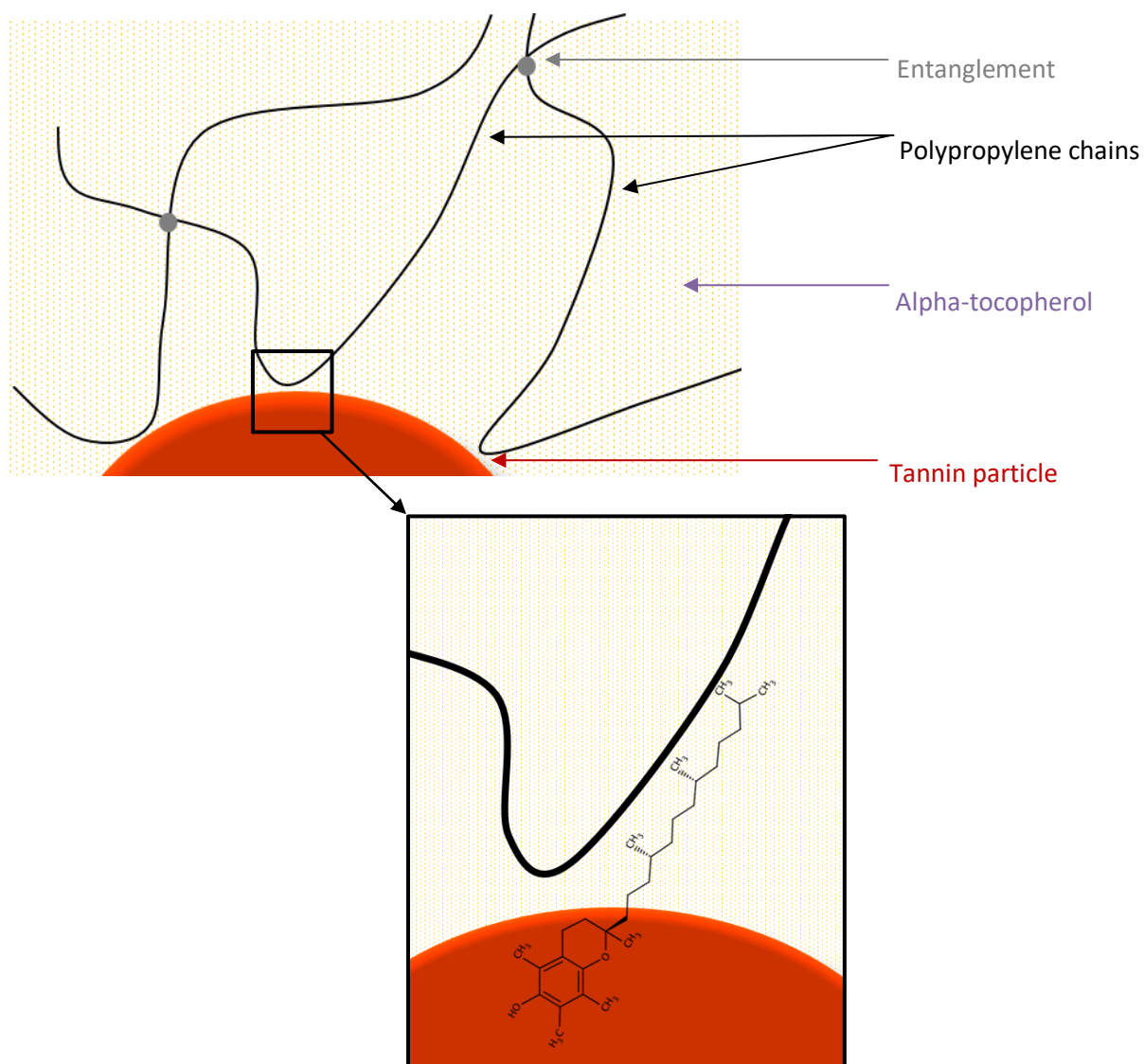


Figure III-39. Illustration of the hypothetical interaction occurring within the polypropylene-vitamin E-tannin system.

IV.2.b. Polypropylene stabilized with vitamin E and vitamin C

First, as explained above, the blends involving vitamin C alone couldn't be realized (the vitamin C alone completely burnt during the extrusion, degrading the polymer completely). It can be assumed that vitamin C (if it could have been extruded) would have acted as a solid charge involving poor interfaces with the polymer due to its hydrophilicity. Subsequently, only three curves are presented on **figure III-40** and **figure III-41**: virgin iPP, iPP + 2.5% VE and iPP + 2.5% VE/VC (95/5). Those curves show the evolution of the complex viscosity and the $\tan(\delta)$ as a function of the frequency.

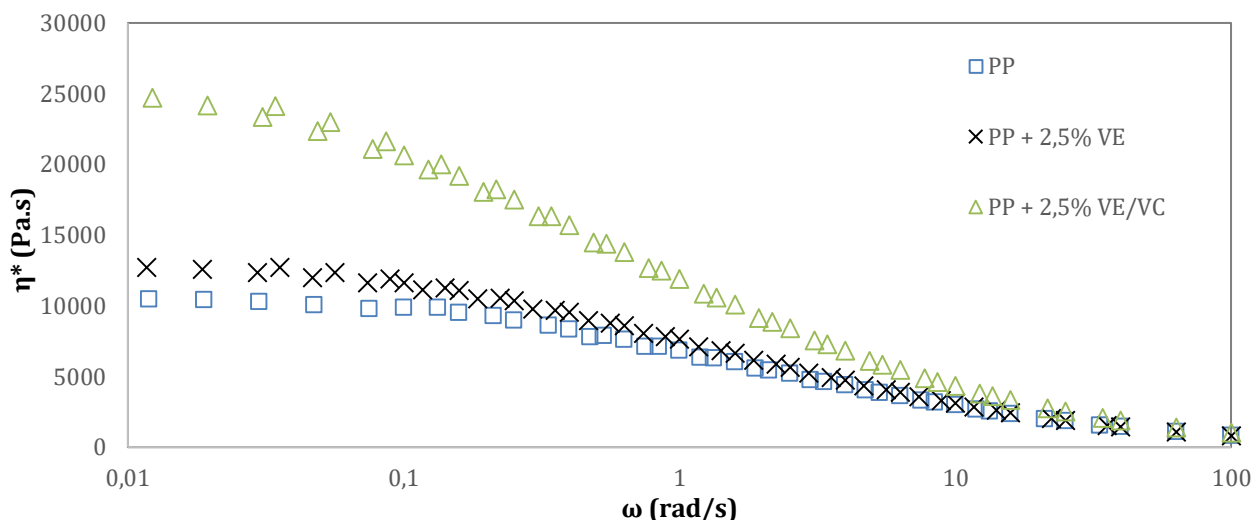


Figure III-40. Evolution of the limit viscosity η^* as a function of the frequency of different iPP-vitamin C blends.

Similarly to tannins based formulation, the addition of vitamin C (green curve) present a very different behaviour than that of virgin iPP or iPP containing vitamin E alone. Actually, the complex viscosity is largely increased probably also putting forward an interfacial interaction between the two additives and the polymer matrix.

Tannins and vitamin C combined to vitamin E seem to have the same impact on the polypropylene matrix when studying the complex viscosities. The $\tan(\delta)$ curve was also drawn to confirm our observations and is illustrated in **figure III-41**.

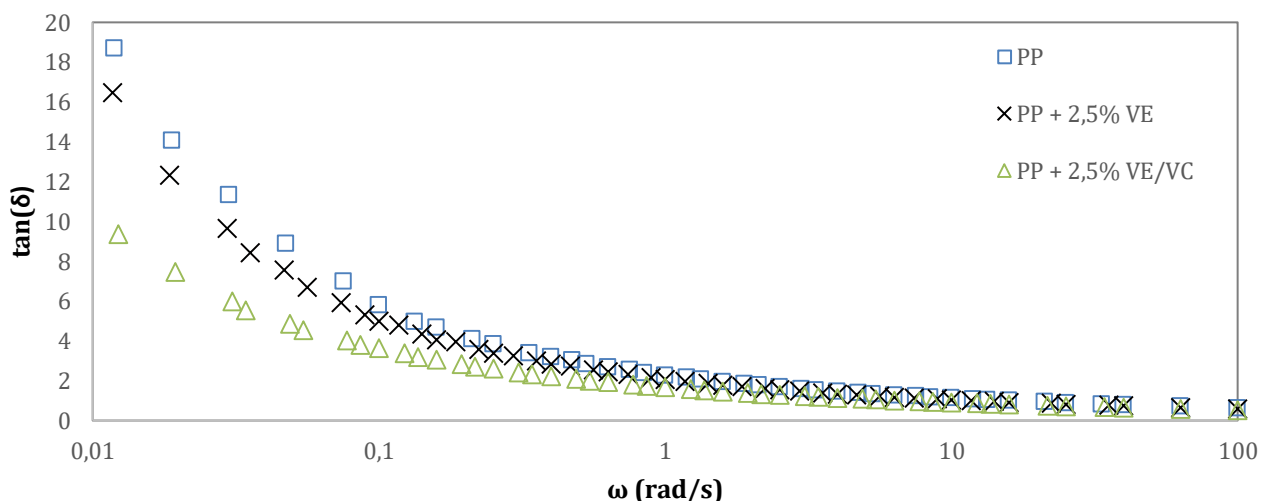


Figure III-41. Evolution of the $\tan(\delta)$ as a function of the frequency of different iPP-vitamin C blends.

First of all, here again, at high frequencies, all the blends show similar $\tan(\delta)$ but show differences at lower frequencies. All the curves present negative slopes in $\tan(\delta)$ on the entire tested frequency range as the

iPP + 2.5% VC blend wasn't realized. This shows that, again, vitamin E thus like a compatibilizer between the hydrophilic vitamin C and the hydrophobic polypropylene matrix.

Moreover, as we said before, the size of the dispersed particles is of major: we explained that tannins present a broad range of molecule sizes and a very low density. This is not the case for vitamin C which present a narrow size dispersion and a density of 1.65 g.cm^{-3} leading to a volumic ratio of (97/3) %v. In turn, vitamin C should be less present all over the matrix than tannins, but more homogeneously dispersed.

It seems, with those results, that the interactions occurring within this system are very close from that described before for iPP-tannins based materials.

IV.3. Polyethylene's stabilisation

Now that hypotheses were done in order to better understand the stabilisation mechanisms of the combination of our additives on polypropylene, let's move on with polyethylene.

VI.3.a. Polyethylene stabilized with vitamin E and tannins

The double logarithmic plots of η^* as a function of ω for polyethylene stabilised with vitamin E and tannins is shown in **figure III-42**.

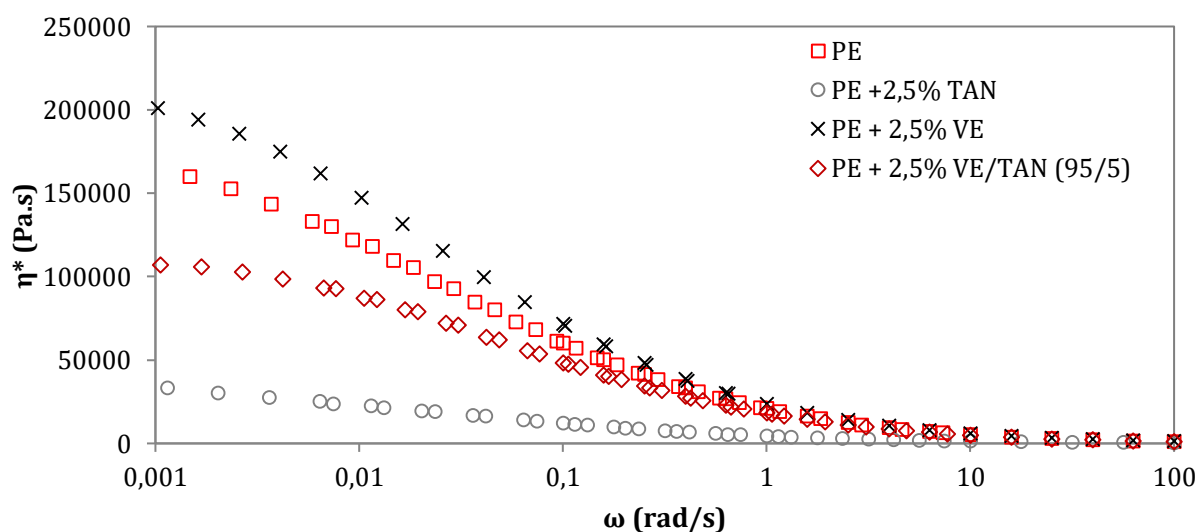


Figure III-42. Evolution of the limit viscosity η^* as a function of the frequency of different LDPE-tannins blends.

First of all, the shear-thinning behaviour is still present for all the blends.

The highest viscosity is obtained for the LDPE containing vitamin E, but the curve remains close to that of the virgin polymer. At the opposite, the addition of tannins alone and tannins combined to vitamin E present significantly lower the viscosity. This is a major difference when comparing these curves to that of polypropylene containing the same additives. Actually, in the case of PP, the viscosity of the blend containing

vitamin E and tannin shows strongly enhanced values, characteristic from a composite with stabilised interfaces. Here, the polyethylene containing vitamin E and tannins is half-way between the blend containing only vitamin E and that containing only tannins. It can be attributed to a simple logarithmic mixing law following an equation close to that shown in equation (28).

$$\log(\eta_{mix}^*) = \phi_1 \log(\eta_1) + \phi_2 \log(\eta_2) \quad (28)$$

Also, the study of the $\tan(\delta)$ curve (**figure III-43**) brings extra information.

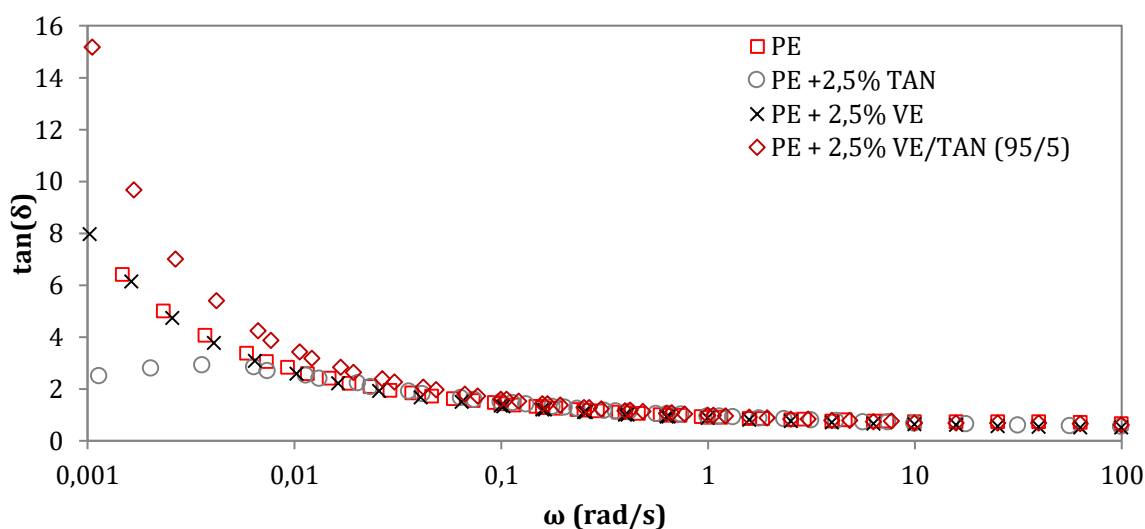


Figure III-43. Evolution of $\tan(\delta)$ as a function of the frequency for different LDPE-tannins blends.

First of all, like for polypropylene, all the blends show similar $\tan(\delta)$ at high frequencies but significant differences appear at lower frequencies. Moreover, still like in the case of polypropylene, the virgin PE, PE + 2.5% VE and PE + 2.5% VE/Tan present negative slopes on the whole tested frequency range. PE + 2.5% Tan presents a different behaviour synonymous from a gel-like behaviour: at low frequencies the slope of $\tan(\delta)$ is positive, then reaches a plateau and becomes negative. In turn, the introduction of tannins solid particles have the same effect on both matrices.

In the case of PE + 2.5% VE/Tan, the $\tan(\delta)$ curve is higher than that of virgin PE (or PE + 2.5% VE). The $\tan(\delta)$ value being the ratio between G'' and G' , a higher value is synonymous from shorter relaxation times: acceleration of the phenomena occurring within the matrix.

The interactions of the additives within polyethylene are very different from those observed within polypropylene. This could be due to the very complex structure of LDPE which is a highly branched polymer (and polypropylene is linear). As a consequence, we can imagine that the vitamin E will be stabilising the interface between tannin and the ramifications of LDPE and not the polymer skeleton itself due to the steric hindrance brought by the ramifications. Scheme in **figure III-44** is an illustration of that hypotheses.

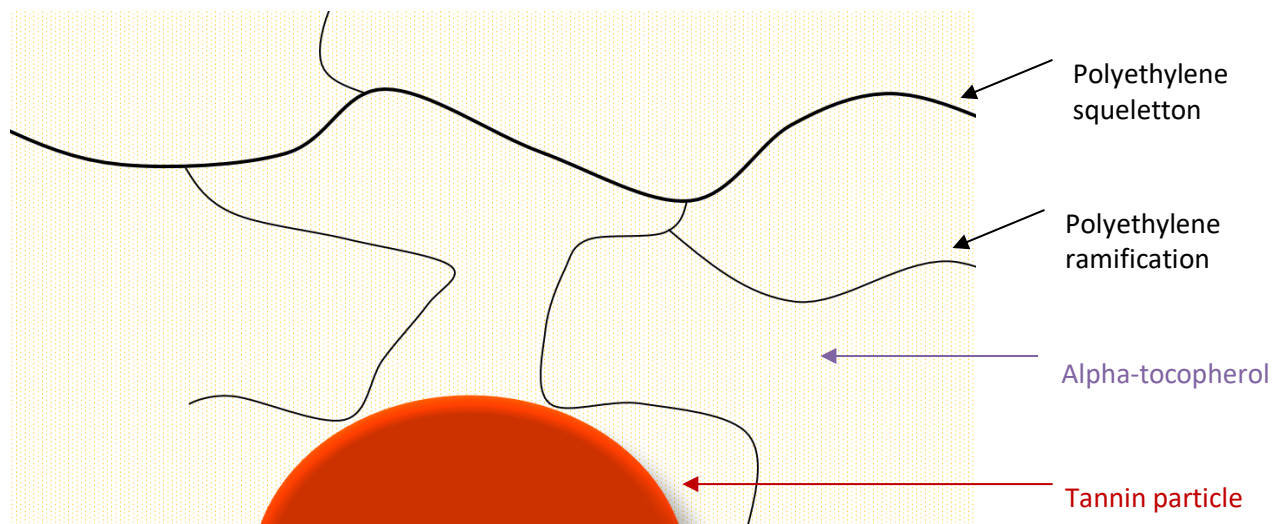


Figure III-44. Illustration of the hypothetical interaction occurring within the polyethylene-vitamin E-tannin system.

IV.3.b. Polyethylene stabilized with vitamin E and vitamin C

Finally, LDPE-vitamin C blends were studied, keeping in mind that the “reference” sample LDPE + 2.5% VC couldn’t be achieved. The double logarithmic plots of η^* as a function of ω for polyethylene stabilised with vitamin E and vitamin C is shown in **figure III-45**.

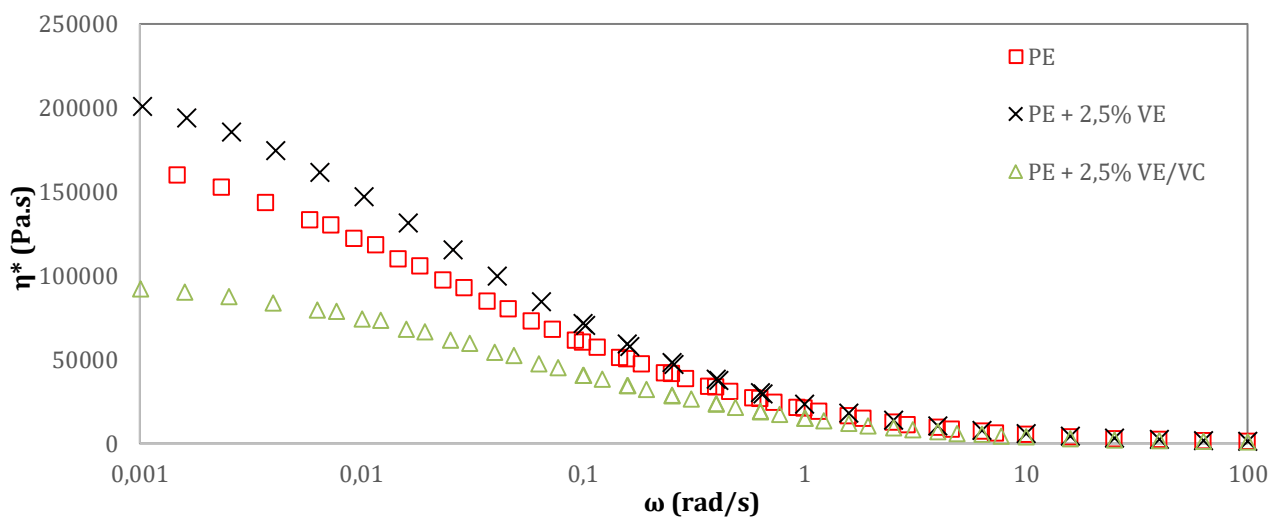


Figure III-45. Evolution of the limit viscosity η^* as a function of the frequency of different LDPE-vitamin C blends.

Like in the case of LDPE-tannins formulation, the addition of vitamin C (green curve) shows a decreased viscosity compared to virgin LDPE or LDPE + 2.5% VE. Here again, the impact of our additives on LDPE is different to their impact on iPP.

The $\tan(\delta)$ curve is shown on **figure III-46** and confirms the differences with iPP.

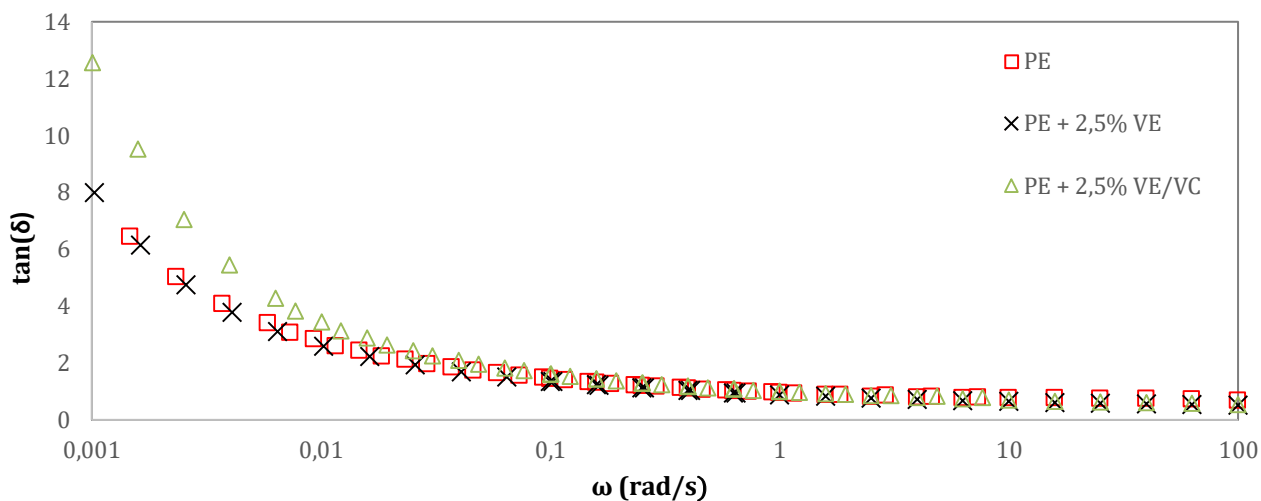


Figure III-46. Evolution of $\tan(\delta)$ as a function of the frequency for different LDPE-vitamin C blends.

Virgin polyethylene and LDPE containing vitamin E show similar $\tan(\delta)$ curves meaning that the evolution of G' and G'' with viscosity is very close for those formulation. The interactions occurring within this system are believed to be the same as that explained before for LDPE-tannins based materials.

V. CONCLUSION

This chapter aimed at evaluating the effects of additives on polypropylene and polyethylene matrices. First of all, the effect of single additives on the polymer matrices was studied. It first appeared that vitamin E is very efficient for the thermo-oxidative stabilisation of the polymer matrices. Solid additives showed interesting properties but prevented the polymers from relaxing by inducing “gel-like” behaviours. Linseed oil, despite its very good thermal properties, didn't stabilised the polymer matrices at all and brought strong odours to the material.

The use of combinations of additives (VE/VC and VE/Tan) to stabilise polyethylene and polypropylene was also studied. Actually, keeping in mind the synergism that could occur between vitamin E and vitamin C and between vitamin E and polyphenols such as tannins, combinations of additives were added within our polymers. It revealed the very effective stabilising properties of the vitamin E-vitamin C and vitamin E-tannins combination of polyethylene and polypropylene.

The synergism between those additives was demonstrated by DOSY-NMR but could not be assessed in the case of polymer blends. The study of the stabilization mechanisms showed that, according to the additives, very different systems were shaped. Actually, when polypropylene is involved it seems that we are in the case of as composites with stabilized interfaces whereas for polyethylene stabilised with additives follows a mixing law. In turn, we showed that vitamin E acts like a vehicle for the introduction of sensitive compounds within polyolefins matrices. Green tea was not precisely described here but the obtained results are detailed in **annexe 12**.

Diagrams summarizing the studied formulations (marks were given to the formulation according to their properties, in comparison with that of the virgin matrix) are presented in **annexe 13**.

The numerous analyses that were done on those blends showed that natural antioxidants are able to stabilize polymers and prevent chain-scissions or cross-linking for polypropylene and polyethylene respectively. The formulations generally meet the criteria laid down by the Foodplast project, namely recyclability and oxidation resistance. In addition, these additives' migration was tested, and the results are detailed in the following chapter.

CHAPTER 4: MIGRATION CHARACTERISATION

Abstract: The migration of the antioxidant additives (mainly alpha tocopherol) from polyolefin material into oily food simulant (95% ethanol) was assessed. The study of the antioxidant release process was done under different conditions: different temperatures, different times, different thicknesses. Both polyolefins, containing either vitamin E alone, vitamin E combined with vitamin C or vitamin E combined with tannins, were characterized following the European legislation on migration assessments but also thanks to the newly developed LESA-MS method. Both methods showed complementary results: the EU regulation method is a time-consuming method but shows very precise results whereas LESA-MS remains semi-quantitative but shows very practical, fast and easy to set up experiments. Also, the antioxidant capacity of the migrating species was evaluated and showed a very strong scavenging capacity of our antioxidants especially when vitamin E is combined to tannins. The obtained data helped us to confirm the previously described effect of our antioxidants on each other and on polymer matrices.

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I. INTRODUCTION

Polymers, at the opposite of glass, interact with small molecules, which can be sorb and move through their structure. Active packaging uses this principle to release active compounds from the polymers and to protect sensitive food products. In our case, active compounds are antioxidant molecules which can serve dual functions: (i) avoid polymer degradation during processing, and (ii) reduce food oxidation. Alpha-tocopherol (vitamin E), ascorbic acid (vitamin C) and condensed tannins were investigated as potential substituent for common synthetic additives. They are delivered to the food thanks to the diffusion which corresponds to the so-called “active packaging” [173]. The migration of such a component may lead to a prolonged shelf life and preservation of the quality of the product [7, 8, 150, 174, 175]. Due to the increasing concerns about health, the importance of the migration of substances from food packaging to food attracts attention.

The analytical control of these processes is not straightforward due to the variety of plastics compositions. As a result, it is complicated to predict the target analytes to be followed even though this information is essential. The analysis and the characterisation of the labile polymer fraction in food contact materials is a real challenge in order to evaluate the risk they can represent for food consumers. It is also of interest to evaluate polymer performance and control technological processes.

In this chapter, we detail the migration assessment of our newly formulated polymers following the European legislation. The migration of natural compounds from active packaging was extensively studied. However, to our knowledge, only few to no papers deal with packaging containing combinations of additives.

Also, the recently introduced LESA-MS methodology combined with a sensitive nanoelectrospray ionisation (nano-ESI) was evaluated for the characterisation of our packaging; results are detailed in the chapter.

II. SAMPLES PREPARATION ACCORDING TO THE EUROPEAN REGULATIONS

As explained in chapter 1, part IV, migration can be considered as the desorption process of chemical compounds from the packaging to the foodstuff. In Europe, food packaging must respect the commission regulation (EU) No 10/2011 of 14 January 2011 on “Plastic materials and articles intended to come into contact with food”.

II.1. Samples preparation

II.1.a. Polymer samples preparation

In order to properly assess migration, our polymers were shaped as films. Thin films were formed thanks to a blow moulding extruder and thick films thanks to a single screw extruder with a flat die. It is described in chapter 2, part I-2. Blow-moulding extrusion allows the formation of films of around 60µm thick and flat-die extrusion of films of around 1 mm thick. In turn, our polymer blends suffer from 2 extrusion passes to be shaped as films. This can lead to degradations or to the loss of some additives. Hence the theoretical rate of 2.5% appears uncertain. However, the precise initial amount of additives within the polymer matrices is an essential parameter. The exact amount of additives present in our films was thus assessed thanks to a TGA analysis and a MATLAB program as described in chapter 2, part II-1. The exact thicknesses were also measured thanks to a precise calliper. Results are shown in **table IV-1** and **figure IV-1**.

Table IV-1. Exact amount of additives present in the polymer films after extrusion.

		iPP			LDPE		
		+ 2.5% VE	+ 2.5% VE/TAN	+ 2.5% VE/VC	+ 2.5% VE	+ 2.5% VE/TAN	+ 2.5% VE/VC
Thick samples	Thickness (mm)	0,58 ± 0.17	0,81 ± 0.09	0,91 ± 0.06	0,59 ± 0.09	0,99 ± 0.10	0,75 ± 0.14
	Additives quantity (%)	1,77 ± 0.08	1,91 ± 0.17	2,10 ± 0.12	1,24 ± 0.02	2,00 ± 0.15	1,58 ± 0.09
Thin samples	Thickness (mm)	0,086 ± 0.03	0,058 ± 0.016	0,056 ± 0.015	0,056 ± 0.011	0,067 ± 0.021	0,086 ± 0.013
	Additives quantity (%)	1,16 ± 0.15	2,28 ± 0.09	2,35 ± 0.01	1,42 ± 0.03	2,32 ± 0.14	1,73 ± 0.03

The average quantity of additives remaining in the thick polymer films is of 1.76% and 1.87% in the thin polymer films, which corresponds to a loss of almost 30% and 25% respectively. This loss is not very surprising knowing how the blends are realized and especially knowing how the antioxidants are introduced within the extruder. The same quantitation was realized on the recycled materials (after 9 cycles) and compared with their non-recycled equivalent (**figure IV-1**, only thin films).

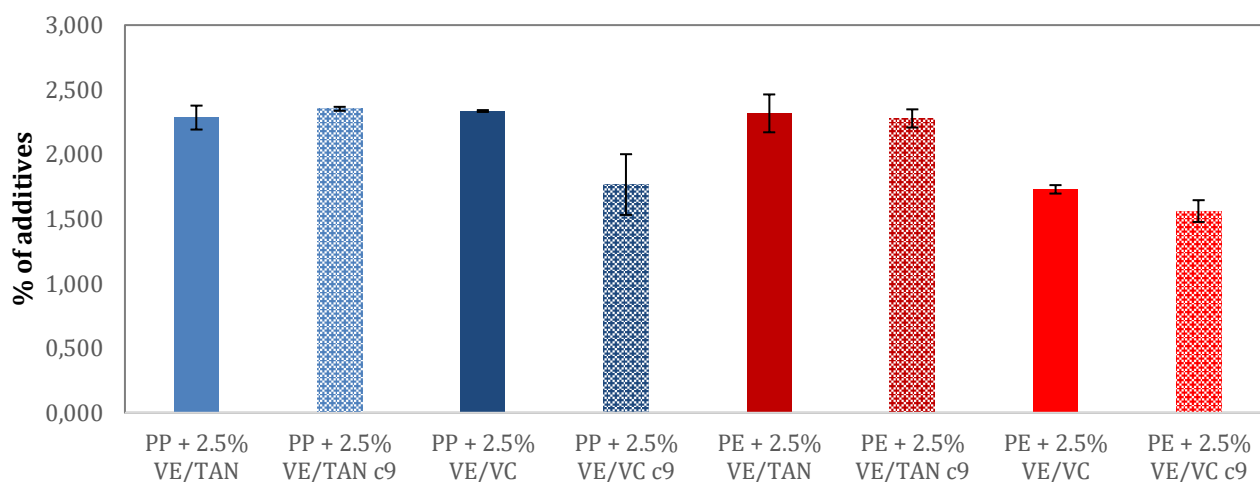


Figure IV-1. Evolution of the amount of additives present within the polymer films after 9 recycling (the printed columns correspond to recycled materials).

For polymer + VE/Tan blends, there are no variations of the quantity of additives present in the matrix even after 9 recycles. However, when vitamin C is involved, whatever the polymer, the amount of additives in the recycled matrix diminished of around 24% for iPP and of 10% for LDPE. This could be attributed to the thermal sensitivity of vitamin C.

II.1.b. Migration cells preparation

Those films will be introduced into migration cells in order to evaluate the quantity of additives migrating, as well as their state after migration. Their release depends on :

- the type of material (diffusion coefficient, molecular weight of migrants);
- the type of foodstuffs (partition factor)
- the contact conditions (continuous/discontinuous, time, temperature).

The experimental conditions of extraction of the antioxidants from our plastics were thus fixed based on this norm. Actually, in the Foodoplast project, the migration analyses must be carried out for ham. Then 95% ethanol was chosen as a food simulant (see **Table I-6** explaining the food simulant choices).

Also, temperature is a major factor, as it affects the mobility of migrants in polymeric materials and influences the diffusion process. **Table I-7** from chapter I, detailed the conditions of temperature and time to apply according to the actual use of the studied foodstuff. In turn, our samples were kept in 95% ethanol for 10 days (analysis were realized regularly during those 10 days: 30 minutes, day 1, day 2, day 3, day 7 and day 10) at 20 °C. The migration analyses were also done at other temperatures (5°C and 60°C).

The pieces of plastic introduced in vials should be 6cm² per face (the thickness must be taken into account, if not negligible) such as we have: 6dm²/kg of simulant.

Obviously, all the experiments were repeated three times. **Table IV-2** summarizes the parameters under which the migration tests were realized.

Table IV-2. Migration testing conditions.

Temperature	Time	Solvents	Sample dimensions	Conditions
From 4 to 60°C	From 30 min to 10 days.	(20g) 95% ethanol/5% water	3cm x 2 cm x variable thicknesses	Vials are used as "immersion cells" and kept in the dark

II.2. Additives characterisation

Legally, polymers for packaging are regulated through global or specific migration levels [4]. Global migration measures the total amount of compounds migrated to a food simulant under the designed temperature and time conditions. Specific migration corresponds to the migration of a specific chemical component. In our case, the analyses of our packaging were done by following the specific migration of each additives (i.e. alpha-tocopherol, ascorbic acid and tannins). Before analysing our migration solutions, the virgin additives were characterized. Nevertheless, tannins are very complex blends of different molecules, different size and different solubilities. Tannins was thus strongly filtered making us lose a lot of information. To avoid losing more data, it was injected directly within the mass spectrometer without going through any column. The relevant peaks that were obtained are detailed in **Table IV-3**.

Table IV-3. Main phenolic species identified in quebracho tannins samples.

m/z [M-H] ⁻	Formula	Identification
285.0400	C ₁₅ H ₁₀ O ₆	Fisetin
561.1393	C ₃₀ H ₂₆ O ₁₁	Dimer of 1 fisetinidiol unit and a catechin unit
833.2077	C ₄₅ H ₃₈ O ₁₆	Trimer of 2 fisetinidiol unit and a catechin unit
1105.2755	C ₆₀ H ₅₀ O ₂₁	Tetramer of 3 fisetinidiol unit and a catechin unit
1377.3012	C ₇₅ H ₇₀ O ₂₆	Pentamer of 4 fisetinidiol unit and a catechin unit

These compounds correspond to the combination of a fisetinidiol and a catechin. The mass range of the study was from m/z 150 to 1500; however, higher m/z values could have been obtained at higher m/z ratios. Those results fit with the tannins description made in the chapter 1.

In the case of ascorbic acid and alpha tocopherol, no solubility problems were encountered making them very easy to analyse. They were solubilized into MeOH/H₂O (1:1) and sent into the HPLC-MS through a C18 column (Acquity UPLC BEH C18 column of 3.0x0.75mm and 1.7µm particle size from Waters, as described in chapter 2, part III-1) the chromatogram gave a well-defined peak for VC but a very wide peak for VE. The obtained chromatograms are shown in **figure IV-2**.

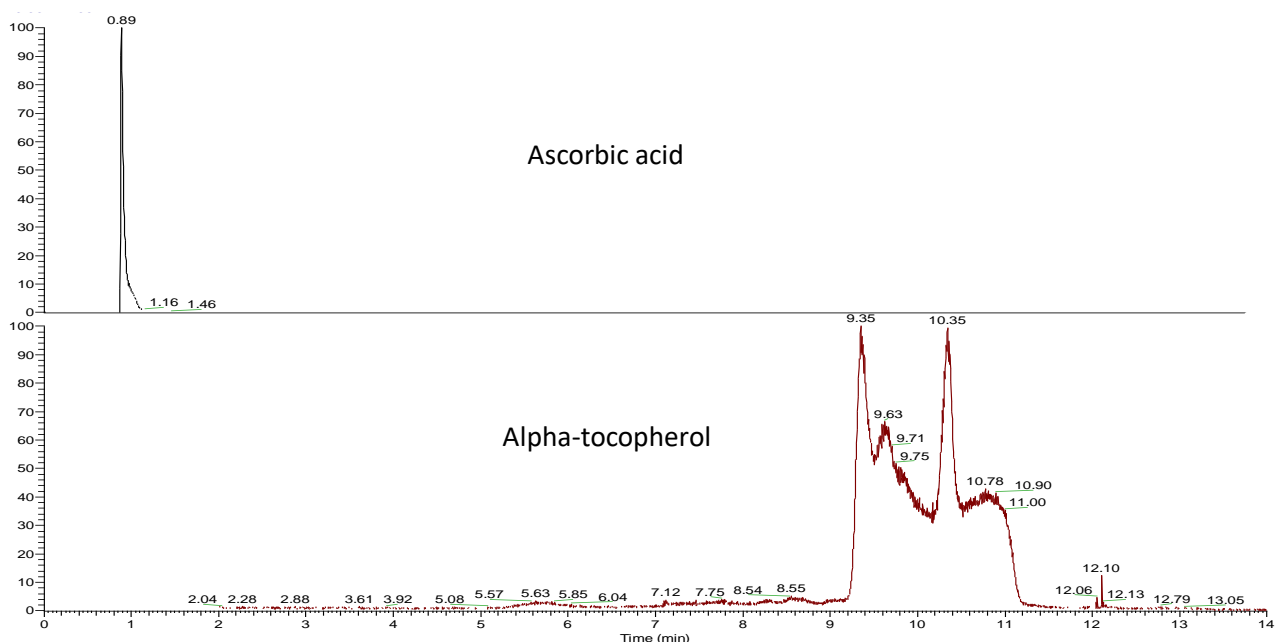


Figure IV-2. Chromatograms of ascorbic acid and alpha tocopherol.

Alpha-tocopherol (which molar mass is $430,7061 \pm 0,0273$ g/mol) have a specific mass spectra pattern (**figure IV-3**). In positive ionization mode, in MeOH/H₂O (1:1), the major peak of alpha-tocopherol is m/z 429,3730.

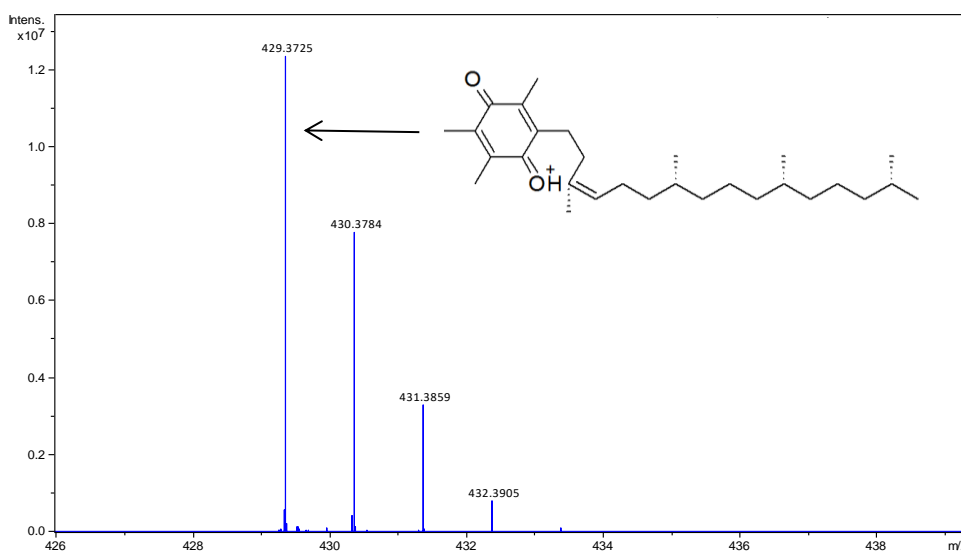
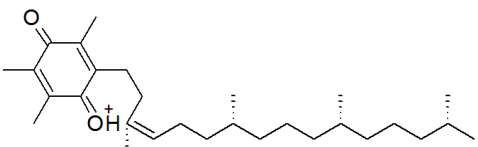
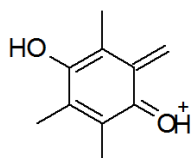
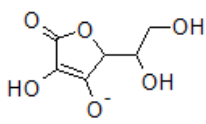
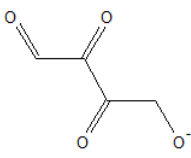


Figure IV-3. Mass spectra of alpha tocopherol in MeOH/H₂O (1:1).

Ascorbic acid's mass spectrum (which molar mass is $176,1241 \pm 0,0072$ g/mol) is obtained in negative ion mode and shows a single peak: $[M-H]^- = 175.0248$.

To avoid any contamination that could false our quantitation (our migration samples are not set up in a clean environment as compared to the MS sensitivity), we chose to use tandem mass spectrometry (also called MS^n). In a tandem mass spectrometry, ions are formed in the ion source and separated by mass-to-charge ratio (m/z) in the first stage of mass spectrometry (called MS^1). Ions of a particular mass (the precursor ions) are selected (in our case, the vitamins are the precursor ions) and isolated. This isolated precursor ion is re-fragmented. Specific fragment ions are created from the precursor by collision-induced dissociation (CID): this is the MS^2 . Those fragment ions are thus specific for their precursor and allow to get rid of any mistake in the identification and quantification. The fragment obtained with a CID (collision-induced dissociation) of 30 for vitamin E and vitamin C are shown in **table IV-4**.

Table IV-4. Fragment ions obtained by tandem mass spectrometry.

Precursor ion	Fragment ion
Alpha-tocopherol – m/z 429,3730 	m/z 161,0910 
Ascorbic acid – m/z 175.0248 	 m/z 115.0037

In order to achieve exact quantitation, calibration curves were achieved for alpha-tocopherol and ascorbic acid's fragments, following the procedure described in chapter 2, part III-1. The obtained curves are illustrated in **figure IV-4**. It was not done for tannins because of the complexity of the blend.

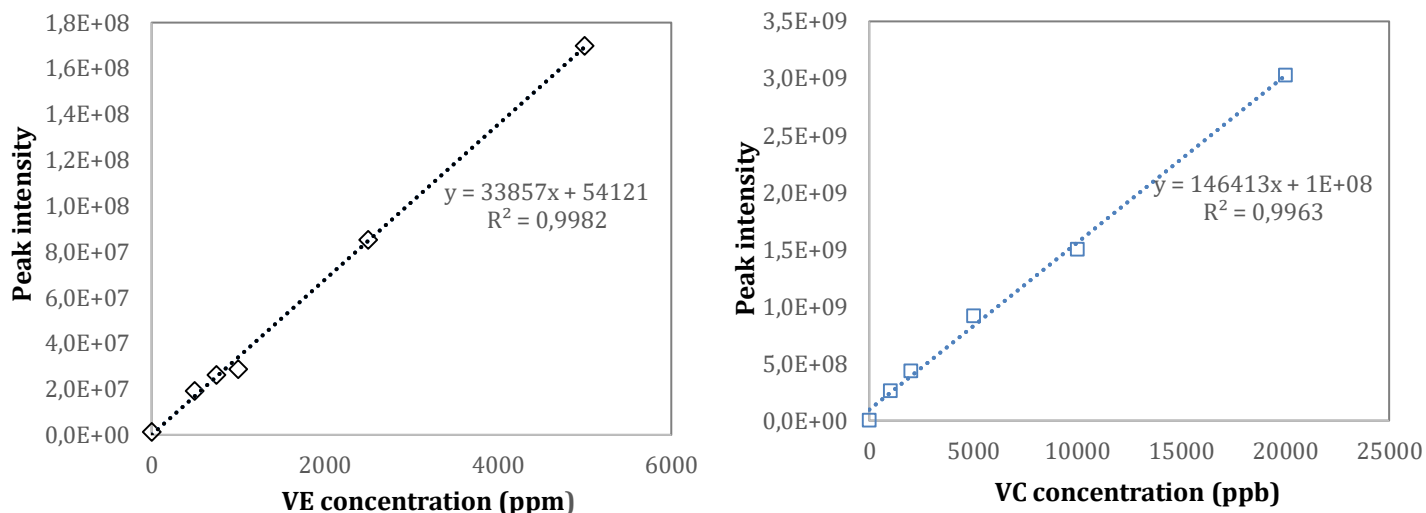


Figure IV-4. Vitamins' calibration curves.

Once the calibration curves are set up easy quantitation can be achieved. To do so, after 10 days in the migration cells at the chosen temperature, the plastic samples are removed, and the food simulant is injected directly into the HPLC. The following table (table IV-5) summarizes the polymer's samples for which migration assessments were realized as well as the conditions.

Table IV-5. Plastic formulations subjected to migration testing.

Polymer type	Additives	Sample thickness	Temperature (°C)	Time (days)
LDPE	-	~ 1 mm	5, 20 and 60	1,2,3,7 and 10
	+ 2.5% VE	~ 1 mm ~ 0.06mm	5, 20 and 60	1,2,3,7 and 10 + 30 min, 2hours, 5hours, 8hours
	+ 2.5% VE/VC (95/5)	~ 1 mm ~ 0.06mm	5, 20 and 60	1,2,3,7 and 10 + 30 min, 2hours, 5hours, 8hours
	+ 2.5% VE/Tan (95/5)	~ 1 mm ~ 0.06mm	5, 20 and 60	1,2,3,7 and 10 + 30 min, 2hours, 5hours, 8hours
iPP	-	~ 1 mm	5, 20 and 60	1,2,3,7 and 10
	+ 2.5% VE	~ 1 mm ~ 0.06mm	5, 20 and 60	1,2,3,7 and 10 + 30 min, 2hours, 5hours, 8hours
	+ 2.5% VE/VC (95/5)	~ 1 mm ~ 0.06mm	5, 20 and 60	1,2,3,7 and 10 + 30 min, 2hours, 5hours, 8hours
	+ 2.5% VE/Tan (95/5)	~ 1 mm ~ 0.06mm	5, 20 and 60	1,2,3,7 and 10 + 30 min, 2hours, 5hours, 8hours

All the experiments were replicated three times (in total 1062 migration cells were realized).

III. MIGRATION QUANTITATION

III.1. Experimental migration assessment

III.1.a. Migration of tannins and ascorbic acid

Being a very complex blend, tannin cannot be used as a standard for quantitation. Eventually, few characteristic molecules of tannins could have been identified and used as reference for the tannin quantifications. However, tannins are introduced in our material in very low amounts (5%_m) and a major fraction of those is too big to migrate (particles easily can be seen by the naked eye in the polymer matrix). Moreover, the European norm stated that molecules above 1000 Da should not be monitored as they are too big to be assimilated by the human body and will thus be eliminated easily. In order to be as rigorous as possible, the chromatograms and the mass spectra obtained migration solutions of polymers containing VE/Tan (95/5) and tannins (and their three replicates) were compared to migration solutions of virgin polymers. This was done thanks to a software (Compound Discoverer) which evaluates the common peaks between several chromatograms, it thus gives a lot of results. In our case, matching peaks are considered significant if they are not present in the migration solutions of the virgin polymers and if they are present in all the samples containing tannins. **Figure IV-5 (a)** below is an example of a matching result: the underlined line shows the presence of common compounds for all the polymers containing tannins (absent from the blanks). This line corresponds to the peak illustrated in **Figure IV-5 (b)** appearing at a retention time of around 10.2 minutes with a $[M-H^+]$ m/z of 181.0459 in negative ionization mode. The software proposed $C_9H_{10}O_4$ as a possible empirical formula. No other peak was found to significantly fit.

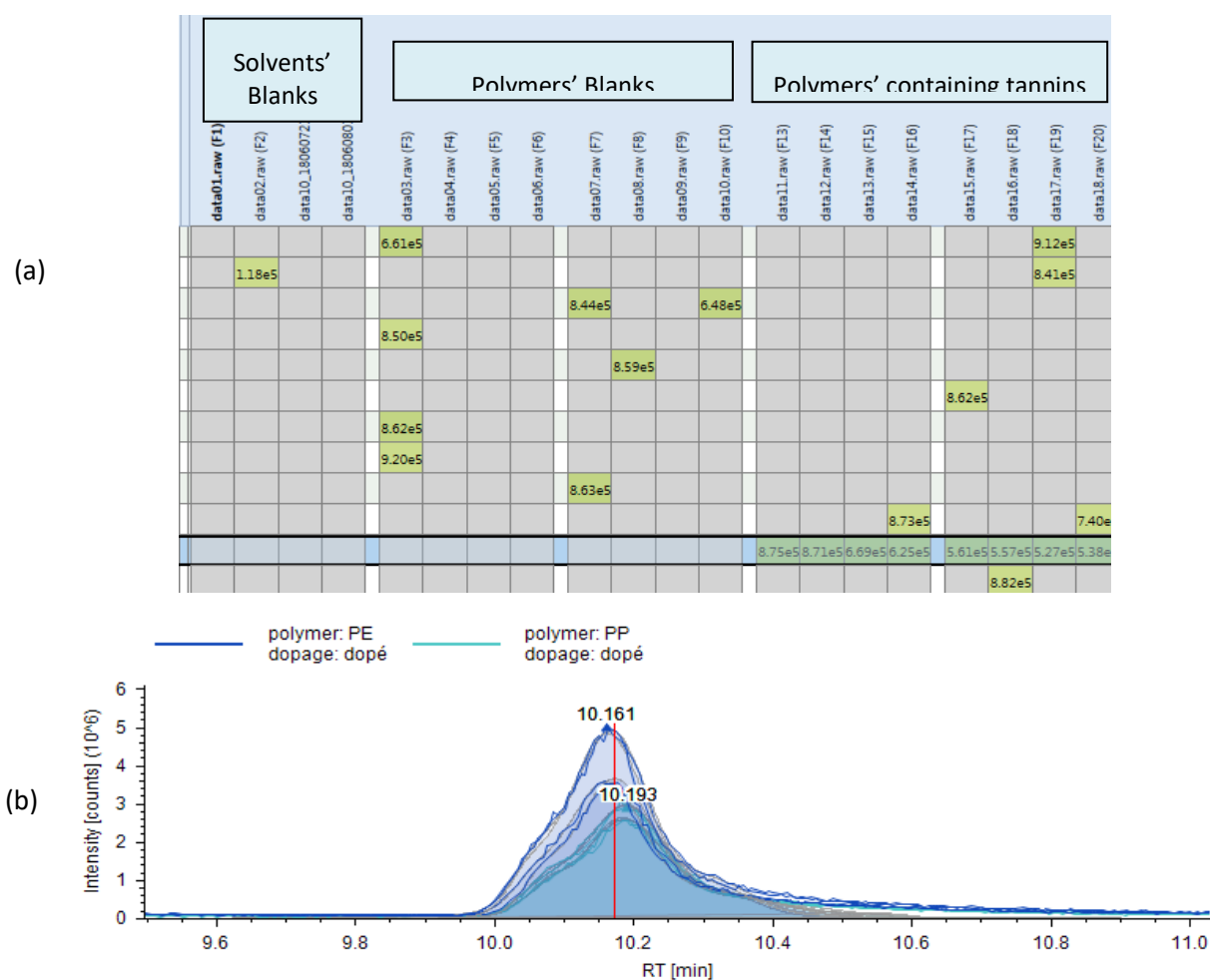


Figure IV-5. Print screen of the results obtained for the migration solutions of polymers containing tannins.

This peak could correspond to several molecules. The possible matching molecules we found are related to polyphenols, gallic acids or/and terpenes. First of all, syringaldehyde is a phenolic acid deriving from gallic acid found in trees [176]. Syringaldehyde is a derivative from 3,4-dihydroxybenzoic acid which is a characteristic compound from quebracho tannin [177].

Then, the other matching molecule is from the ubiquinone family which are molecules belonging to a family of compounds distinguished by their n number of terpene units (n typically between 6 and 10). Tannins being a terpene compound, finding the monomer unit called ubiquinone-0 is not unreasonable. Its empirical formula and m/z ratios also fit the obtained results. Those two molecules are illustrated in **figure IV-6**.

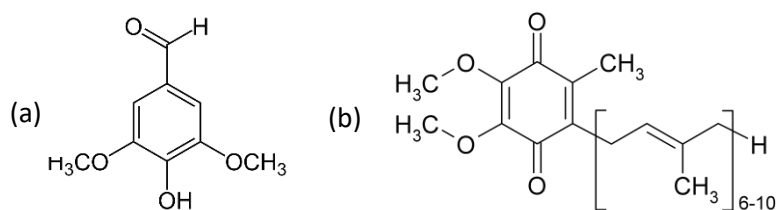


Figure IV-6. Possible molecules migrating from quebracho tannins. (a) Syringaldehyde; (b) Ubiquinon-0.

In the case of ascorbic acid, only traces were found in our food simulant (>0.1% of the initial amount and decreasing through the time). This could show that ascorbic acid degrades in 95% ethanol. Also, even if tannins and ascorbic acid compounds had been used in higher amount, their migration profiles would have been different than that of alpha-tocopherol. Due to their different chemical structures (tocopherol has a long alkyl chain, which facilitates its movement in the hydrophobic polymers, whereas tannins are bulkier, which hinders its movement and ascorbic acid is fully lipophobic) and different shape (powders versus oil) these antioxidants would have released at different rates. The migration of alpha-tocopherol is detailed in the following parts.

III.1.b. Migration of alpha-tocopherol

- Migration results obtained at 60°C up to 10 days

First of all, the thick samples' migration was evaluated (~ 1 mm) after 10 days at 60°C. Analysis were done at short (30 minutes, 2 hours, 5 hours and 8 hours) and long times (1 day, 2 days, 3 days, 7 days and 10 days). The amount of migrating alpha-tocopherol was evaluated as explained before giving a result in mg/L. This result has been normalized to the initial value of vitamin E calculated by TGA. **Figure IV-7** and **figure IV-8** show the evolution of the migrating alpha-tocopherol for iPP and LDPE respectively, as a function of the time.

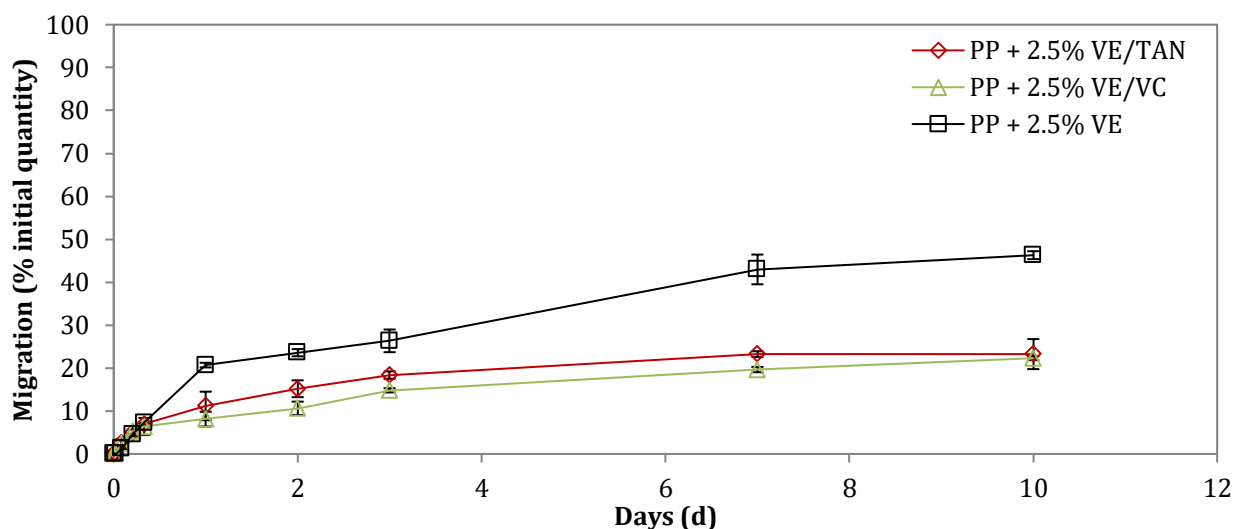


Figure IV-7. Migration of alpha-tocopherol from the different iPP's thick films.

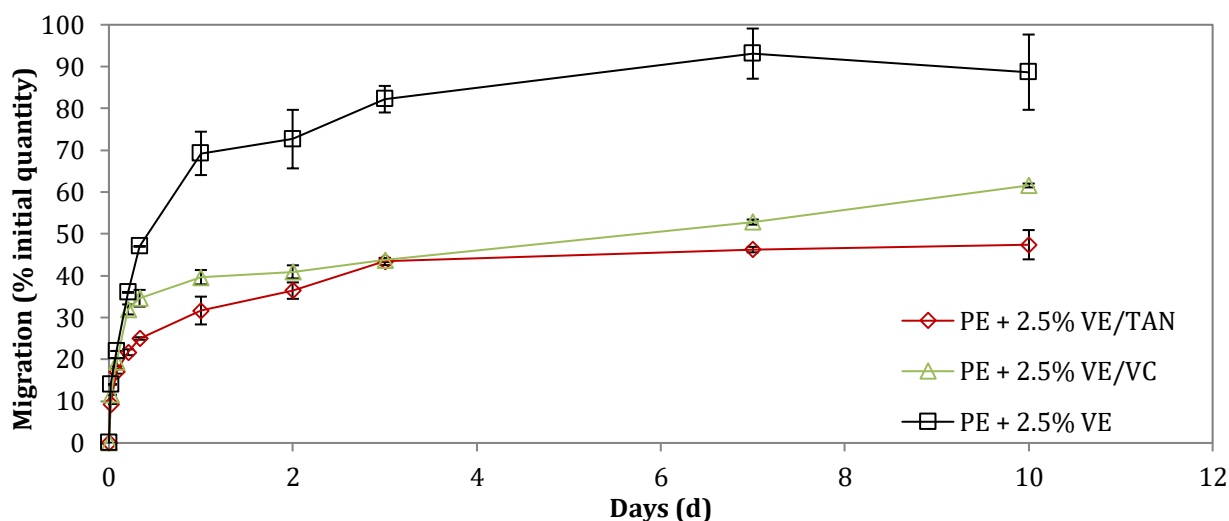


Figure IV-8. Migration of vitamin E from the different LDPE's thick films.

First, we can observe that similar migration mechanisms are obtained whatever the polymer or the additives: the migration follows a Fick's law. Then, we can also see that the migration from iPP is twice as low as LDPE's. These differences could be due to the differences between the glass transition temperatures (T_g) of the polymers, as well as their crystallinities. Actually, LDPE's T_g is around -100°C and iPP's one is around -1°C , hence the storage temperature employed (60°C) is much closer from iPP's T_g than that of LDPE. In turn, LDPE's chains are more mobile at the test temperature, making the diffusion of the compounds easier. Also, the crystalline regions are less permeable to additives. In turn, additives tend to go preferentially in the amorphous area: when migrating, the crystalline regions act like barriers, explaining why there is less migration from the iPP than from the LDPE (higher crystallinity).

Then, another major difference can be observed: it appears that alpha-tocopherol (black curves) alone migrates more than alpha-tocopherol combined with tannins or ascorbic acid. The migration of LDPE + 2.5% VE can be considered total (considering the uncertainties) whereas the migration of LDPE + 2.5% VE/Tan and LDPE + 2.5% VE/VC reach 49 and 61% respectively. In the case of iPP, the migration of iPP + 2.5% VE reach an equilibrium at 46% whereas iPP + 2.5% VE/Tan and iPP + 2.5% VE/VC at around 22%. Whatever the polymer, the difference between the migration percentage of alpha-tocopherol from polymer + 2.5% VE and polymer + 2.5% VE/X is the same: around 2. This diminution could show the presence of stronger interactions within the tertiary blends in comparison with the binary blend.

Finally, the limitation imposed by the European regulation state that the migration must not exceed 60mg/kg of food simulant. For our thick samples, this value is exceeded of around 4 times for LDPE + 2.5% VE and of around 3 times for LDPE + 2.5% VE/Tan and LDPE + 2.5% VE/VC. For iPP's formulations, this value is exceeded 2.7 times and around 1.3 times for VE alone and VE/X respectively. Of course, those elevated values are due to the fact that the experiments were realized at 60°C and that the readjustment factor of 95% ethanol must be taken into account according to the food intended to be packed (see **table I-6**): for ham, the migration values obtained with 95% ethanol must be divided by 4 (our formula thus fit the European legislation for ham kept between 40 and 60°C).

Our thin samples ($\sim 0.06\text{mm}$) were also studied under the exact same conditions and the results are shown in **figure IV-9** for LDPE's formulations and in **figure IV-10** for iPP's.

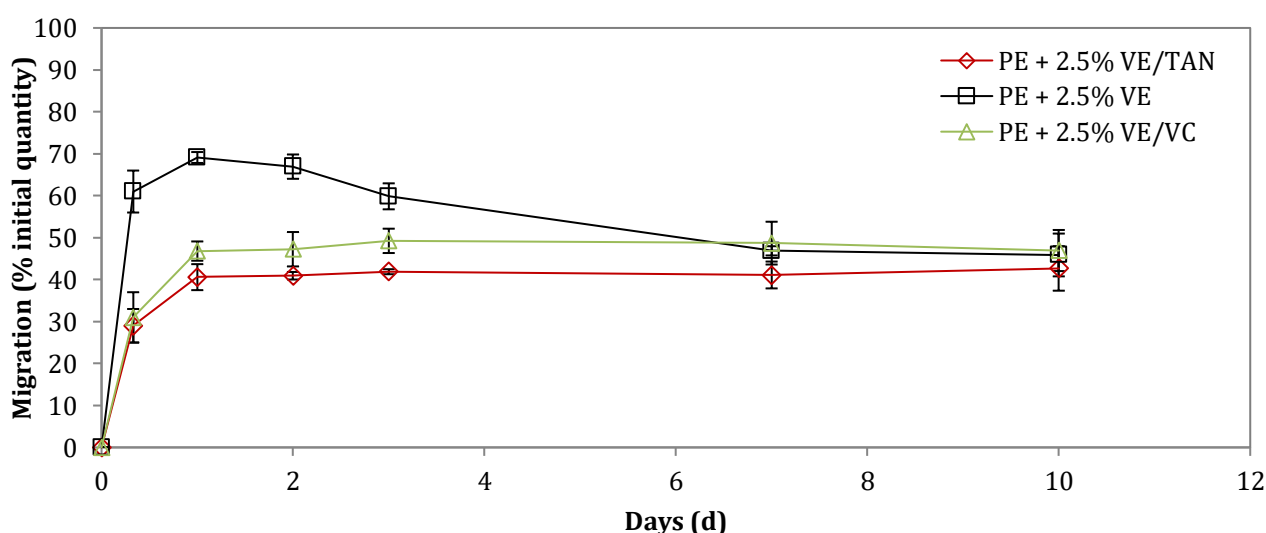


Figure IV-9. Migration of vitamin E from the different LDPE's thin films.

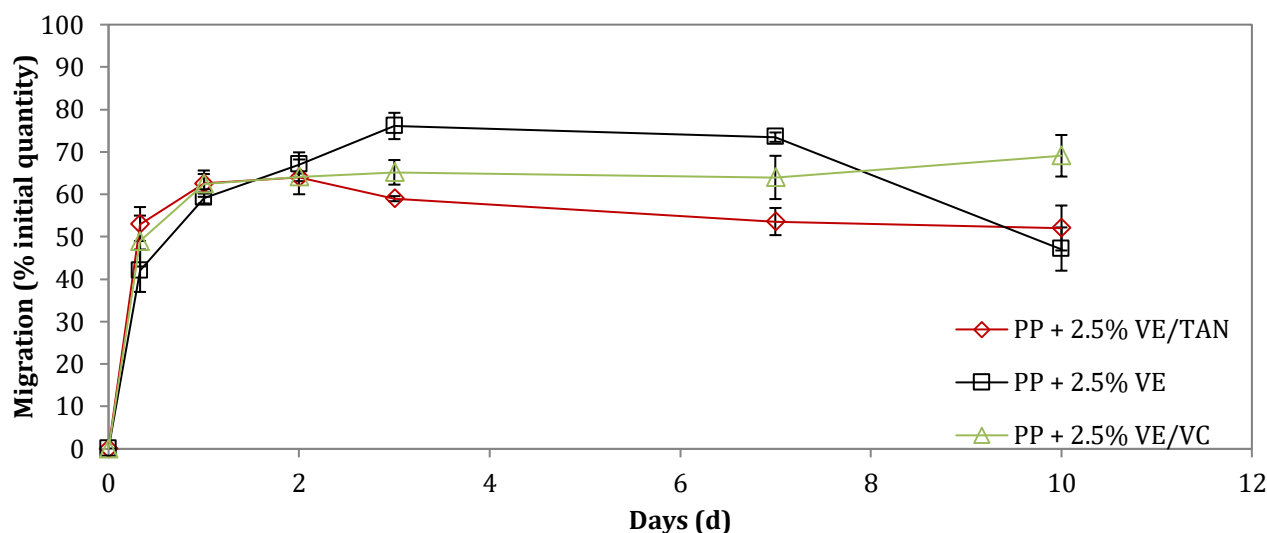


Figure IV-10. Migration of vitamin E from the different iPP's thin films.

The first observation that can be done is that the kinetic of migration is much faster for thin films than for thick films. Actually, the plateau is reached much faster (between the day 1 and the day 2). This can be explained by the fact that the additives are much closer from the surface for a 60 μ m thick sample than for a 1mm one. The path the additives have to travel to reach the food simulant is thus longer in the case of thick plastics and the additives can encounter more obstacles slowing their diffusion (chains, crystallites, impurities...). Also, it is much easier for the solvent to penetrate within thin samples (even though the polymers don't really have any affinities with ethanol) than within thick ones.

Then, we can notice that the polymer + 2.5% VE (black curves) tend to lose alpha-tocopherol through the time which could be characteristic from a slight degradation of the antioxidant. Polymer + 2.5% VE/X do not show this diminution probably related to the protection of each additives on each other.

Finally, the migrating values at equilibrium are comprised between 21.1 and 34.1 mg/kg of food simulant and thus below the norm specifications.

- The influence of the temperature

The previous migration tests were realized at 60°C in order to accelerate the diffusion phenomenon. In the following part, two other temperatures were studied: 20°C and 5°C. The results are shown in **figure IV-11** for LDPE and in **figure IV-12** for iPP.

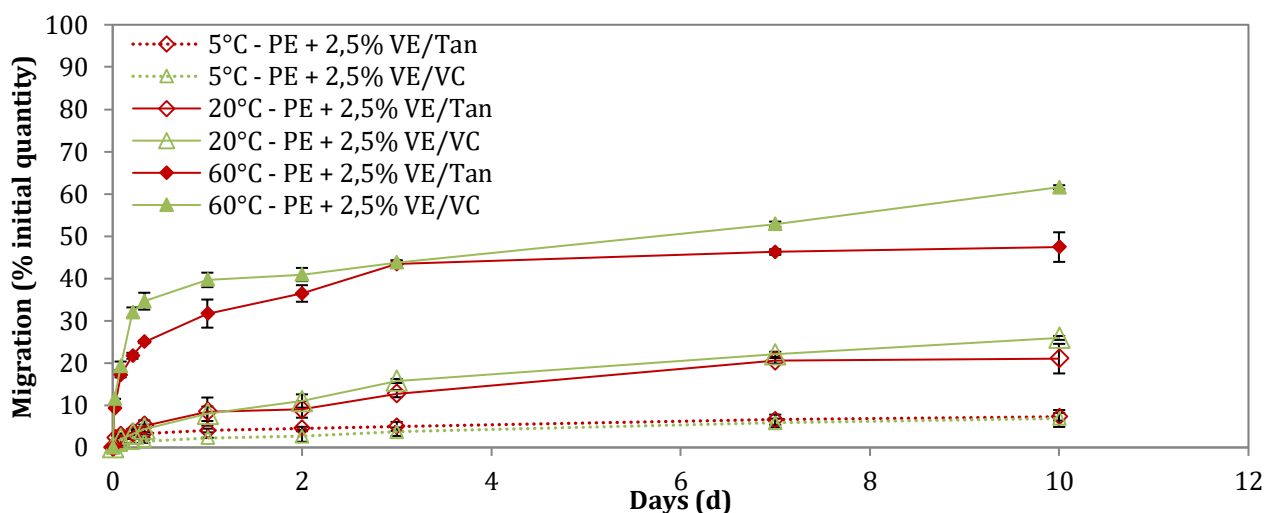


Figure IV-11. Migration of vitamin E from LDPE + 2.5% VE/VC (green curves) and LDPE + 2.5% VE/Tan (maroon curves) at 60°C, 20°C and 5°C.

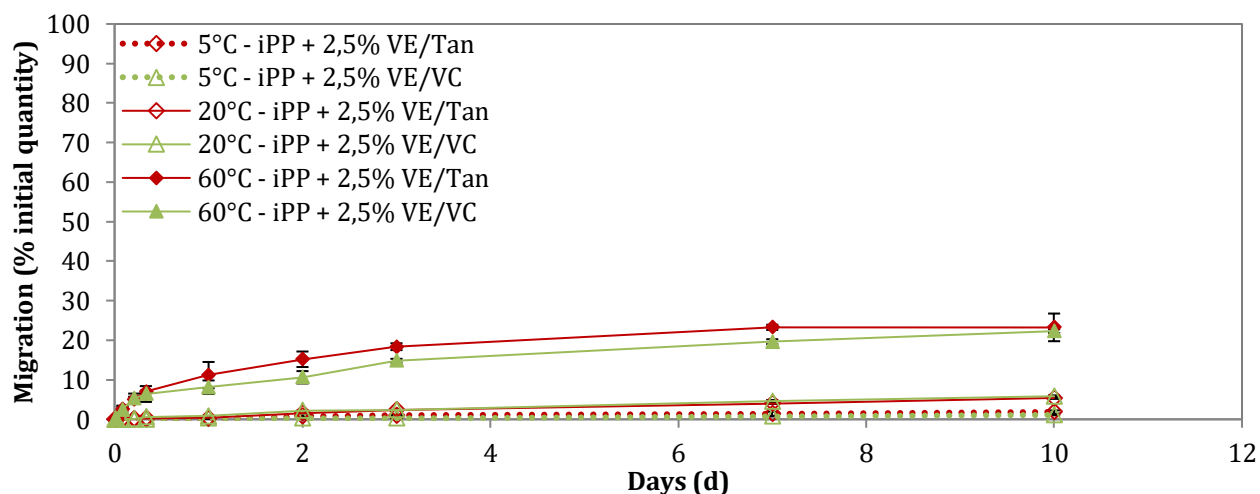


Figure IV-12. Migration of vitamin E from iPP + 2.5% VE/VC (green curves) and iPP + 2.5% VE/Tan (maroon curves) at 60°C, 20°C and 5°C.

The values at the 10th day for each temperature were extracted from the previous curves and plotted in the curve from **figure IV-13**. The obtained curve of the evolution of migration of alpha-tocopherol with the temperature is fully linear.

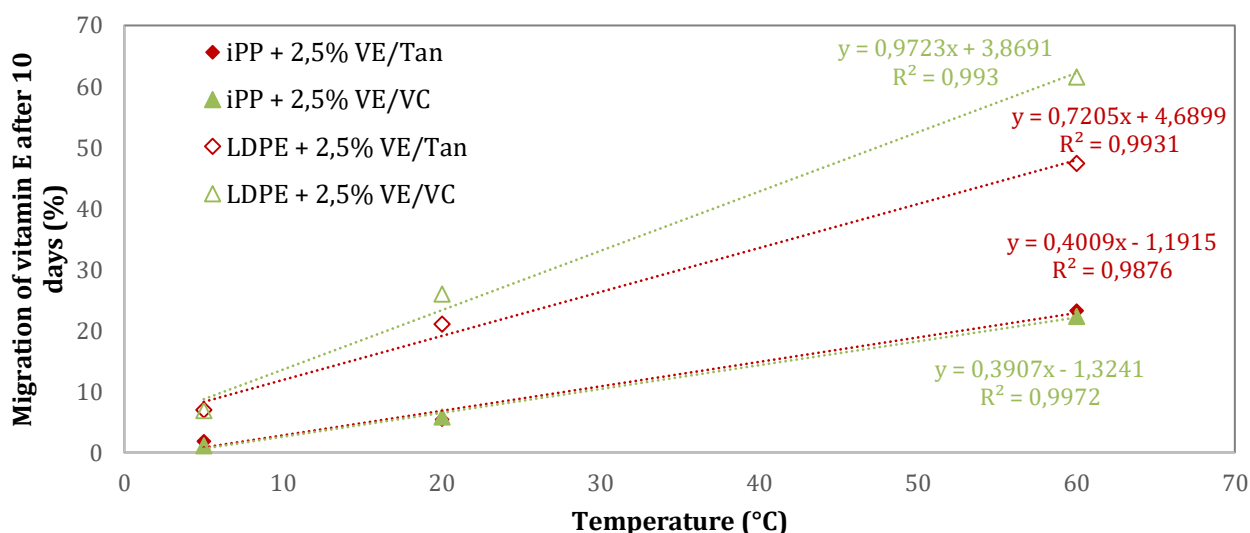


Figure IV-13. Evolution of the migration of alpha-tocopherol (VE) as a function of the temperature.

The obtained migration values are relatively low:

- For LDPE's formulation, around 20% of alpha-tocopherol migrated after 10 days at 20°C (approximately twice higher than the norms specification) and around 7% at 5°C (lower than the norms' specification);
- For iPP's formulation, a little bit less than 6% of alpha-tocopherol migrated at 20 °C and around 1% at 5°C which corresponds to values below the 60mg/kg of food simulant asked by the european's legislations.

Subsequently, temperature is one of the parameters governing diffusion as it readily affects the migrant mobility within the polymer matrix and through the interfaces. The temperature parameter doesn't appear in the diffusion equations but the diffusion (D , $\text{m}^2 \cdot \text{s}^{-1}$) depends on it. Actually, the diffusion coefficient also follows an Arrhenius law (**equation (29)**).

$$D = D_0 \exp\left(-\frac{E_a}{RT}\right) \quad (29)$$

With D_0 the diffusion of a compound free of any obstacles, E_a the activation energy ($\text{J} \cdot \text{mol}^{-1}$), R the gas constant ($8,31 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$) and T the temperature (K).

The diffusion coefficient D of alpha-tocopherol according to the formulation was evaluated. Actually, for a Fickian diffusion, when $\frac{M_t}{M_\infty} < 0.6$ with M_t the migration at a time t and M_∞ the migration at equilibrium, the migration can be described by **equation (30)**.

$$\frac{M_t}{M_\infty} = \frac{4}{l} \sqrt{\frac{Dt}{\pi}} \tag{30}$$

(l represents the thickness of the sample and t the time).

Hence, $\frac{M_t}{M_\infty}$ at 60, 20 and 5°C was drawn as a function of the square root of the time for thick samples (the kinetic of thin samples being too quick, only one or two points are present below 0,6) as shown in **annexe 14**.

These curves allowed used to calculate the diffusion coefficient D: the curves' slope correspond to $\frac{4}{l} * \sqrt{\frac{D}{\pi}}$.

The obtained values of D can now be used to calculate the energy of activation of the migration thanks to equation X: $\log(D)$ was plotted as a function of $\frac{1}{T}$ (K⁻¹) as shown on **figure IV-14**. **Table IV-6** summarises the calculated values. Some literature diffusivities are also summarized in the data from this work are within the literature values.

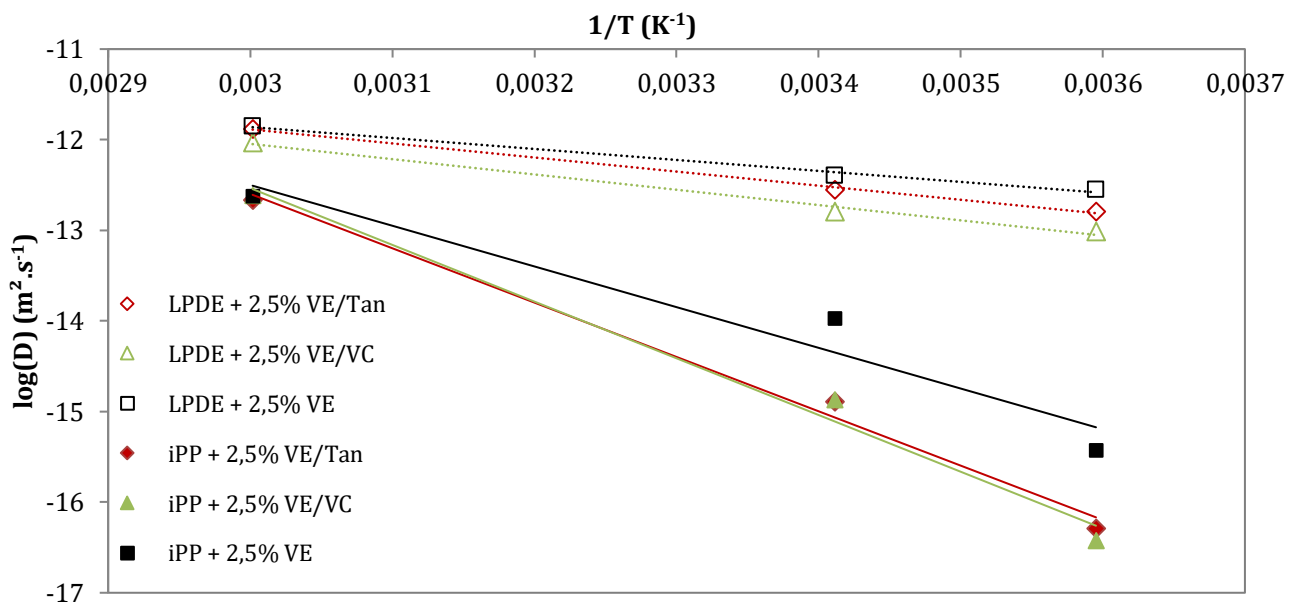


Figure IV-14. Evolution of the diffusion coefficient D as a function of the temperature T.

Table IV-6. Values of diffusion and activation energies of the different formulations.

	T (°C)	D (m ² .s ⁻¹)	Ea (kJ/mol)
Experimental			
LPDE + 2,5% VE/Tan	60	1,32E-12	12,7

	20	2,81E-13	
	5	1,61E-13	
LPDE + 2,5% VE/VC	60	9,28E-13	13,7
	20	1,61E-13	
	5	9,67E-14	
LPDE + 2,5% VE	60	1,41E-12	9,8
	20	4,01E-13	
	5	2,78E-13	
iPP + 2,5% VE/Tan	60	2,17E-13	54,2
	20	1,28E-15	
	5	5,15E-17	
iPP + 2,5% VE/VC	60	2,40E-13	54,3
	20	1,35E-15	
	5	3,75E-17	
iPP + 2,5% VE	60	2,38E-13	41,1
	20	1,05E-14	
	5	3,71E-16	
Literature [12, 178]			
LPDE + VE (from 0.5 to 2%)	40	4,6E-14	10,9
	30	3,2E-14	
	25	2,85E-14	

The migration of additives from the polypropylene matrix requires much more energy than from LDPE. This fits with the previous explanations stating that the lower Tg and higher crystallinity rates involved lower migration rates (i.e. higher energy of activation). Also, unlike LDPE, PP does not show any interactive tendencies towards any of the foods (or food simulant) limiting the migration when compared to LDPE. If the aim is to avoid the migration of a plastics additive from the packaging material into the food, PP containing α -tocopherol as antioxidant seems to offer the perfect choice. Also, the migration of α -tocopherol alone requires less energy than when two additives are involved. The affinity between additives can lead to the increase of the energy of activation as well as the presence of solid particle within the matrix which can block physically the migration. Finally, several values of migration can be found for LDPE containing α tocopherol alone but none were found for polypropylene.

III.3. Antioxidant capacities

The antioxidant capacity of the food simulant was assessed thanks to the DPPH method. It was realized on the food simulant resulting from the thick samples migration kept at 60°C. The DPPH radical presenting a concentration limit above which it is not efficient, thin samples couldn't be characterized as the migration coming from our thin samples was not sufficient to give satisfying data.

The DPPH method procedure is described in chapter 2, part II-4. The measured value (which was obtained thanks to a UV-Vis spectrophotometer) is the IC_{50} which corresponds to the concentration of antioxidant needed to consume 50% of the DPPH radicals. Hence, the lower the IC_{50} the better the antioxidant capacities. Moreover, in order to obtain this IC_{50} , several measurements were done on the same sample at different dilution rate. Subsequently, the IC_{50} unit from **figure IV-15** is in percentage corresponding to the dilution that was realized. The IC_{50} will then be normalized to the exact amount of migrating species (**Figure IV-16**).

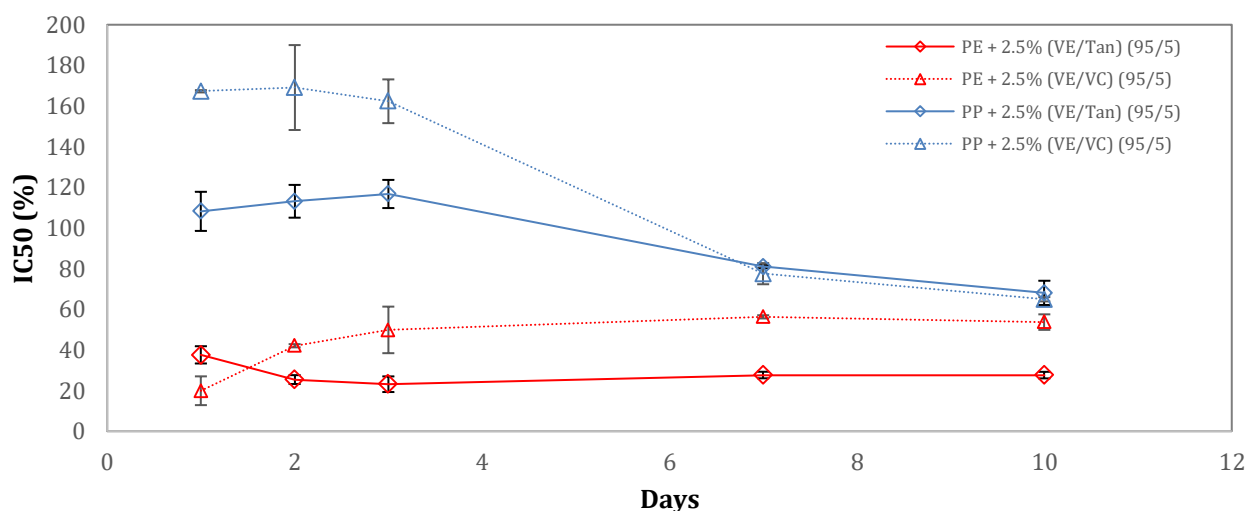


Figure IV-15. Antioxidant capacity (%) of the migration solution (95% EtOH) of different thick plastic formulations through the time.

In the case of polyethylene, the antioxidant capacity of the migration solutions evolves from the 1st day to the 3rd and then stabilizes until the 10th day which fits with the HPLC-MS results. However, two different tendencies appear according to the additives:

- For LDPE + 2,5% VE/TAN (95/5), between day 1 and 3 a slow decrease of the IC₅₀ is observed before reaching the plateau. The overall values are very low, revealing a very efficient antioxidant capacity (after 10 days of migration, around 27% alpha-tocopherol and tannins are enough to consume 50% of the radicals).
- At the opposite, for LDPE + 2,5% VE/VC (95/5), between day 1 and day 3 an increase of the IC₅₀ can be observed revealing a loss in the antioxidant properties. The value obtained after 10 days of migration is 53,6%, which still reveals a very good antioxidant capacity of alpha-tocopherol and ascorbic acid.

Researches were realized to explain the loss of antioxidant capacity of PE + 2,5% VE/VC (95/5). Each additive (i.e. alpha-tocopherol and ascorbic acid) were taken separately, solubilized in 95% ethanol and kept at 60°C. The antioxidant capacity of those solution was then evaluated at day 1, 2, 3, 7 and 10 and revealed that ascorbic acid's antioxidant capacity degrades through the time. This was explained by Hsin-Yun Hsu et al, who showed that, in aqueous systems, ascorbic acid is very unstable and easily degraded [179]. Hence, tannins seem to provide a stronger protection of alpha-tocopherol than ascorbic acid. In fact, as ascorbic acid's antioxidant capacity is one of the strongest among all antioxidants, we should obtain better IC₅₀ for the VC-based formulation. These results confirm the point that tannins play a fundamental role in the formulation stabilisation.

In the case of polypropylene, IC_{50} remains constant during the 3 first days and then starts to diminish. It reaches around 65% for both formulations after 10 days of migration. Here, ascorbic acid seems to suffer less from being in ethanol probably due to the slower migration from polypropylene: the vitamin is less quickly exposed to the solvent. This slower migration rates also explains the differences in antioxidant properties between the two polymers.

To conclude, it seems appropriate to say that LDPE formulations present better antioxidant capacities than PP-based formulations. However, the 3 days delay offered by polypropylene would be very interesting for a food-contact application. In fact, having such a delay in the delivery of additives allows a longer contact between antioxidants and foodstuff (even though the concentration in additives remains low) and it allows to start delivering additives to the food when it starts aging (and not at the beginning of its shelf life).

However, those values are expressed in percentage of the initial solution. But we know that the migration is very different from one polymer to another. Hence, these percentages were replaced by the real concentration of migrating species (**figure IV-16**).

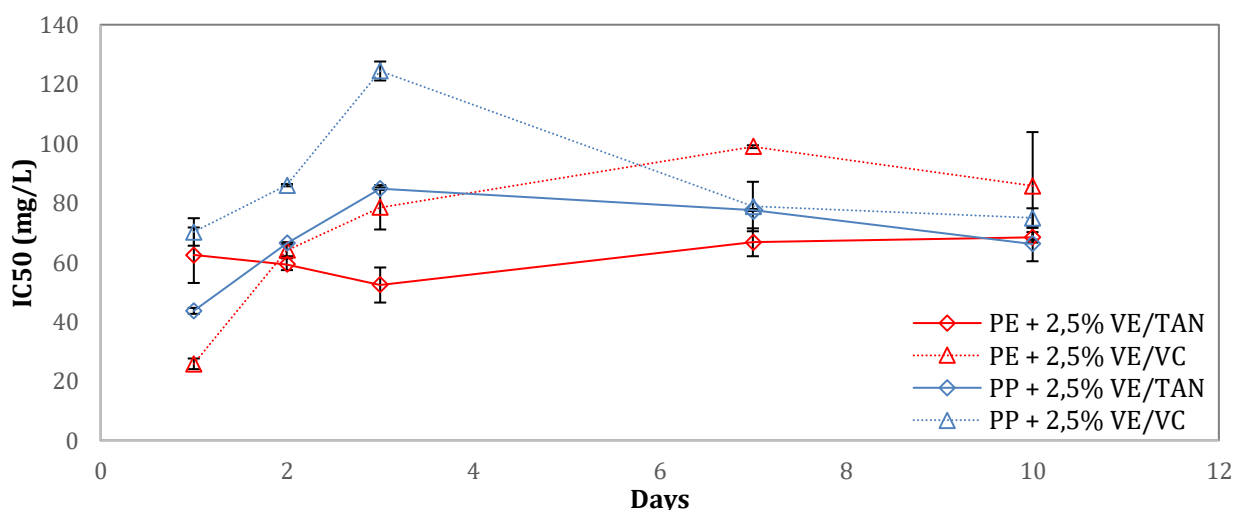


Figure IV-16. Antioxidant capacity (mg/L) of the migration solution (95% EtOH) of different thick plastic formulations through the time.

First of all, **figure IV-16** shows that LDPE + 2.5% VE/Tan presents relatively constant antioxidant capacities. Actually, the fact that a flat curve is obtained reveals that the antioxidant capacity of the solution and the amount of additives migrating are completely correlated: when the antioxidant capacity increases (i.e. diminution of the IC_{50}), the amount of species migration increase as well, giving rise to a constant curve.

At the opposite, for the three other curves, we can observe an increase in the IC_{50} followed by a plateau:

- For both iPP's formulations, the increase of the IC_{50} value between day 1 and 3 corresponds to the 3-days delay from **figure IV-15**: the antioxidant capacities don't evolve whereas the amount of additives migrating increase. After day 3, the IC_{50} from figure IV-x decreases a lot whereas the

amount of migrating species continues to go on: this corresponds to the plateau showing a good correlation between the antioxidant capacity and the quantity of additives.

- For LDPE + 2.5% VE/VC, the behaviour observed in **figure IV-15** (increase of IC₅₀ due to ascorbic acid's degradation) is made worse by the normalisation as migration and IC₅₀ evolve the same way

The study of the migration from our newly formulated plastics into 95% ethanol led us to several conclusions. First of all, the migration follows a Fickian law and is always higher from LDPE than from iPP. Also, when alpha tocopherol is added alone within the polymer matrix, it tends to migrate more than when it is combined to another additive (i.e. vitamin C or tannins). This could be due to interactions within the system. Moreover, migration highly depends on temperature. The maximum level of migration (60mg/kg of food simulatant) was respected by our packaging in case they are containing ham. Also, our packaging proved to have efficient antioxidant activity by releasing active molecules. The combination of alpha-tocopherol and tannins tend to be a little bit more effective than the combination of alpha-tocopherol and ascorbic acid. The lipophobicity of ascorbic acid could play a role here, as it involves that ascorbic acid cannot (or very few) interact with lipids.

IV. POTENTIAL OF THE LESA-MS METHOD FOR FOOD PACKAGING CHARACTERIZATIONS

As described in chapter 2, a novel rapid and ecological method has been developed for the direct analysis of polymer packaging. In few words, the method works thanks to a robotic pipette tip that delivers a drop of a chosen solvent at the sample surface. Then, a micro junction is created between the solvent drop and the plastic resulting in the diffusion of plastic impurities into the drop. Then, the drop is sprayed using a nanoESI silicon-based chip. A precise optimization of all the parameters was realized in order to use the method for plastics analysis. The polymer samples were analysed directly after having been cut to the appropriate size and put into the instrument holder.

IV.1. Direct analysis of the migrating compounds from polyolefin by LESA-MS

The virgin polymer samples were analysed directly after having been cut to the appropriate size and put into the instrument holder. A care was taken not to touch and contaminate the surface to be analysed.

IV.1.a. Characterisations of virgin polymer matrices

Our virgin polymers were recycled up to 9 times and directly analysed using LESA-MS [140]. The aim of this study is to evaluate the possible migration of polymeric residues. Actually, as recycling requires the

polymer treatment at high temperature, the polymer is prone to oxidation leading to the possible formation of smaller species able to migrate. The parameters that were used are detailed below and the results are shown in **figure IV-17**. This figure illustrates the results obtained for virgin LDPE but the same results were obtained for iPP.

- Solvent volume: 3.0 μ L
- Dispense: 2.0 μ L
- Delay post-dispense: 5sec
- Aspirate: 3.0 μ L
- Repeat: 0 times
- Delay post aspirate: 0sec
- Aspirate air after sample: yes
- Volume of air to aspirate after sample: 1.0 μ L
- Pressure: 0.15psi
- Spray voltage: 1.35kV

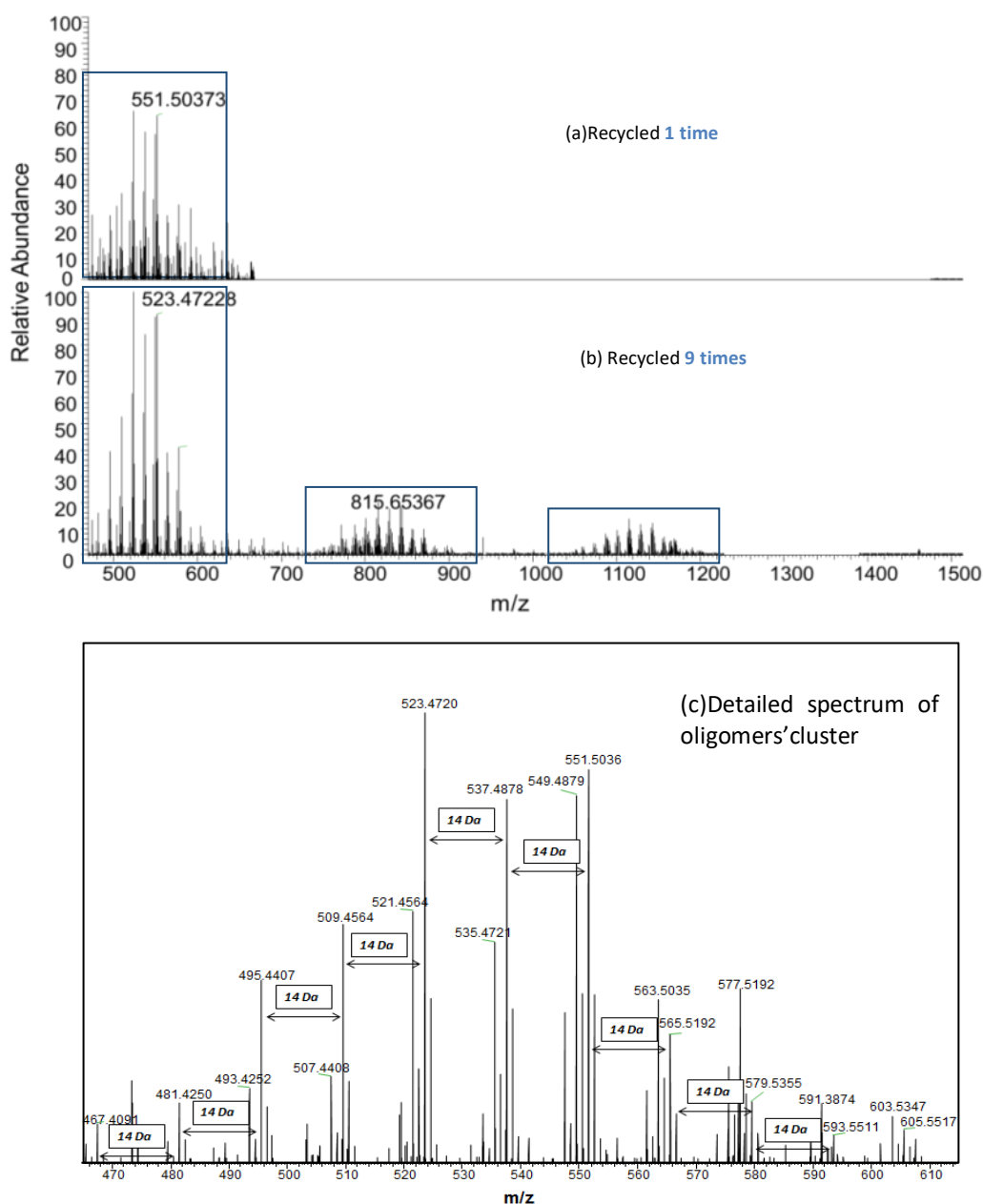


Figure IV-17. LESA-MS analysis on recycled bare polyethylene: (a) one cycle, (b) nine cycles and (c) zoom on one of the oligomers' cluster.

Figure IV-17 reveals the formation of low molecular species during recycling. Actually, clusters of oligomers appeared with the increasing number of recycling cycles: for the polymer recycled 9 times there are more of them and with higher intensities as compared to the 1 time recycled one. It clearly shows polymer degradation as a result of the extrusion and the creation of small molecules potentially able to migrate into food. Here, the polymer patterns are composed of peaks separated by 14 Da (see details in **figure IV-17 (c)**), corresponding to the methyl group of which polyethylene and polypropylene are made. This phenomenon is even more visible when analysing polypropylene as it degrades by chain-scissions (whereas polyethylene degrades by chains reticulation), creating those clusters of oligomers. Polyolefin thermal degradation is known to engender mainly volatile toxic compounds [180, 181]. However, the LESA-MS analyses of non-stabilized polyolefin surfaces

showed that they also generated non-volatile molecules: smaller pieces of polymers. These pieces of polymers correspond to n-alkanes/alkenes up to 1200 Da. We have shown in chapter 3 that the recycling of our polymers leads to the formation of a low-molecular weight tail. Moreover, during the extrusion process a segregation of small masses towards the surface occurs (see scheme from **figure IV-18**) explaining the easy analysis of those oligomers thanks to a surface analysis. Actually, the viscosity of the created oligomers is probably very low compared to that of the polymer. This results in a phase separation: under shear the components with the lower viscosities tend to segregate to go to the high shear rate areas (usually the surface) [126].

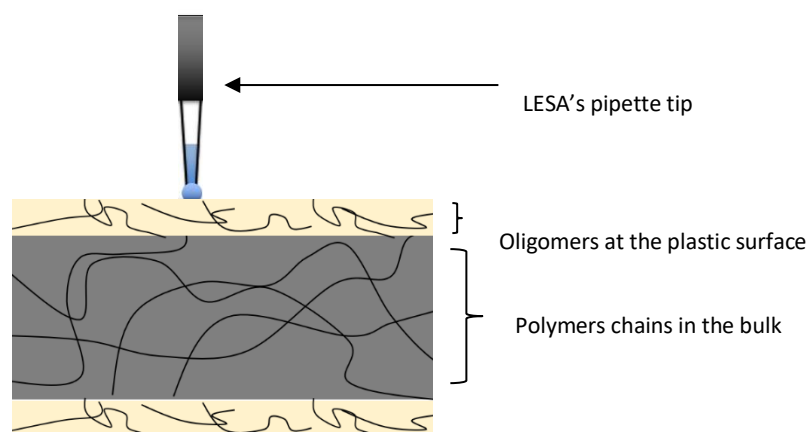


Figure IV-18. Scheme of the size segregation occurring within polyolefin samples after extrusion.

Indeed, when sheared, in contact with oxygen and with elevated temperatures, polyolefin chains tend to break to form those oligomers [182]. Hence, LESA-MS helped us to state that the more we recycle, the more we create molecules able to migrate into food.

Beside thermo-oxidative degradations, polyolefins also suffer from exposure to UV: photo-oxidative degradation as detailed in chapter 1, part II-3. After exposure to UV light, the creation of a carboxylic group is very common. Hence, additive-free LDPE and iPP were exposed to natural light at room temperature for 1 month and analysed by LESA-MS under the conditions detailed above. The obtained spectrum is shown in **Figure IV-19**.

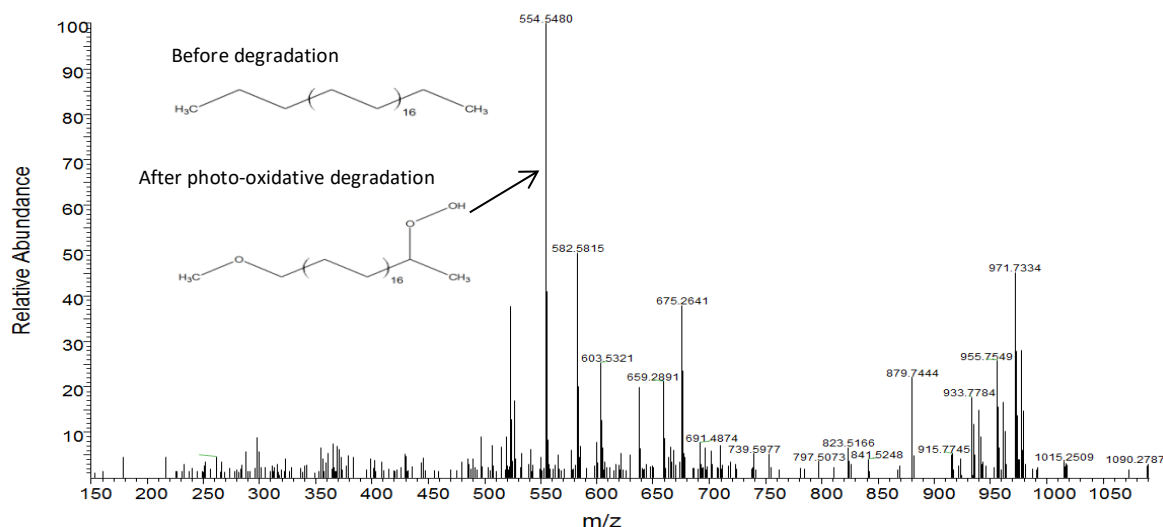


Figure IV-19. LESA-MS spectrum acquired by analysing an oxidized non-stabilised low-density polyethylene (LDPE).

The mass spectrum from **figure IV-19** revealed the presence of different degradation products such as alcohols or esters but the presence of a carboxylic group on the polymer chain was the most systematic. This stage of degradation is the one following oligomers formation described above (**Figure IV-17** and **IV-18**). Actually, the oligomers can undergo further degradations due to heat, UV and humidity, which seems to be the case here. As a consequence, from those further degradations, the regularity of the oligomer chains observed in **Figure IV-17** is lost due to chemical reactions, giving rise to new species (alcohols, ester, carboxylic acid, etc.). For example, the major peak from **Figure IV-19** (m/z 554.5506 Da) is an oxidized molecule with a carboxylic function. Oxidized species are known to be more toxic than their non-oxidized equivalents. It has to be noted that different adducts can also be identified.

In order to compare the analysis of olefins oligomers by LESA-MS and by the traditional “batch method”, standards were analysed. Oxidized polyethylene wax (o-PEW), synthesized by Radecka et al. [183], was analysed in infusion mode and compared with direct analysis by LESA-MS. Normally, those samples are used as carbon source for the bacterial synthesis of polyhydroxyalkanoates (PHA). They were solubilized in 0.1% formic acid in MeOH/CHCl₃ (2:1 v/v) and infused directly (**Figure IV-20 (a)**). The same solvent was used for LESA-MS (**Figure IV-20 (b)**).

The LESA extraction parameters were:

- Solvent volume: 3.0μL
- Dispense: 2.0 μL
- Delay post-dispense: 5sec (or 2sec) *
- Aspirate: 3.0 μL
- Repeat: 0 times

- Delay post aspirate: 0sec
- Aspirate air after sample: yes
- Volume of air to aspirate after sample: 1.0 μL
- Pressure: 0.15psi
- Spray voltage: 1.35kV

*several delays were tried in order to have the best extraction. First of all, 8seconds of delay was applied but the high affinity between the solvent and the o-PEW lead to a loss of the micro-junction. The time was thus diminished to 5 seconds and 2 seconds (the spectra obtained with 2sec extraction showed that with lower extraction times, only the smaller molecules are extracted).

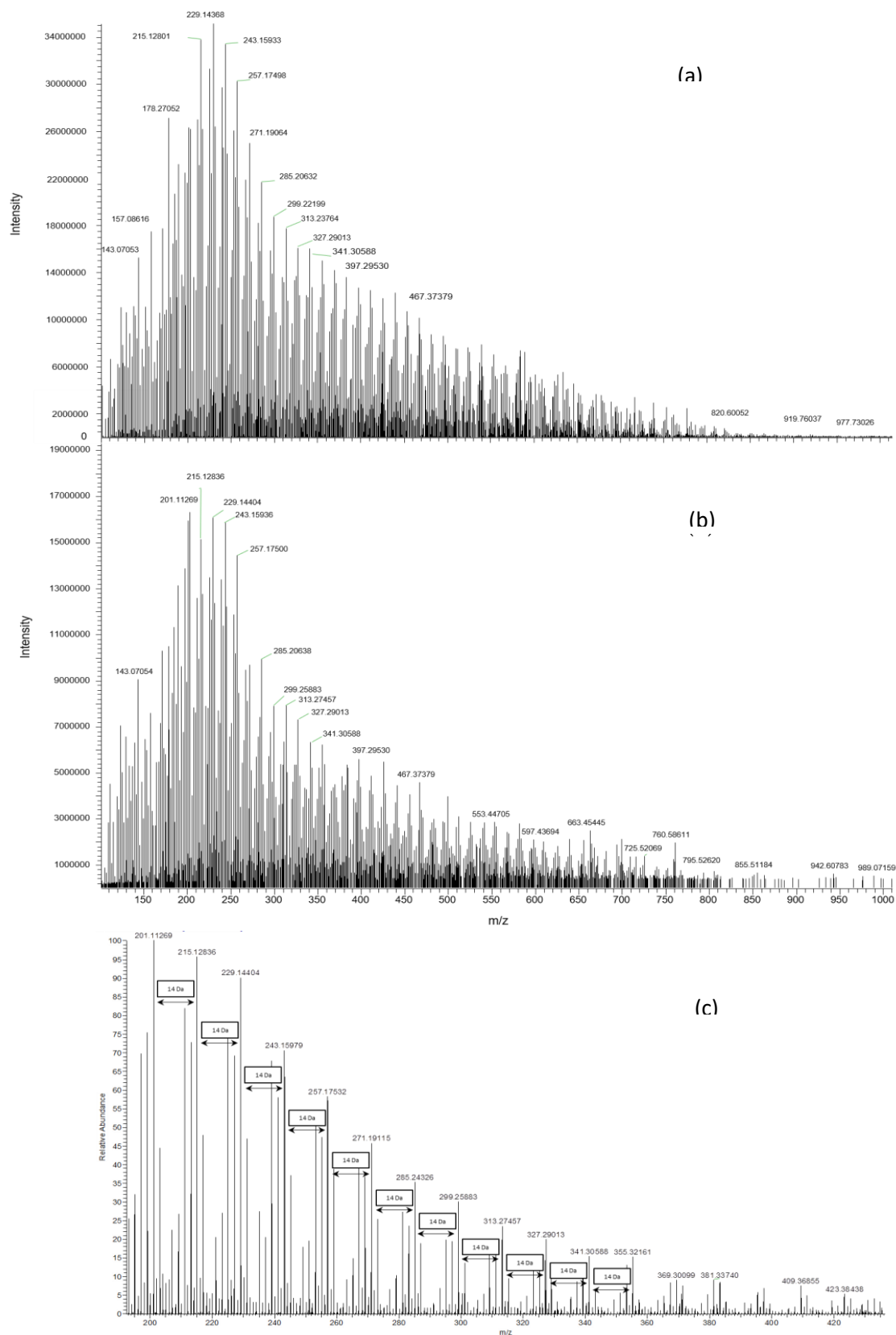


Figure IV-20. Direct infusion nano ESI-MS (a) vs. LESA-MS (b) of oxidized PE wax in positive ionization mode. (c) zoom detailing the oligomer pattern.

Figure IV-20 reveals that the same pattern was obtained by LESA-MS and by batch method (the spectrum obtained by LESA-MS is clearer). The series of peaks $[M + H^+]$ with a separation of 14 Da is the same for both methods and the adducts $[M + Na^+]$ and $[M + K^+]$ were also identified in both spectra.

LESA-MS is a powerful tool in the quality control of polymers able to detect post-polymerization residues and products of polymer degradation. Oligomers migrating from non-stabilized polyolefins food film packaging were detected [140]. The presence of easily migrating products of polymer degradation such as those resulting from UV exposure or those created during high temperature processing (e.g., manufacturing or recycling) was also demonstrated [184]. The release of small molecules able to migrate and be released from the polymer surface as a result of any process that the polymer can undergo in its lifetime can be easily detected by this technique. Also, the information provided by LESA-MS is the same as that obtained by using traditional batch methods with solvent extraction (and usually subsequent evaporation preconcentration).

IV.1.b. Characterisations of the additives and their effects on the polymers

Additives are commonly used to improve the polymer's mechanical properties and stability. The natural additives of our study proved to be able to effectively stabilise the polymers, especially toward thermo-oxidation. Also, they proved (particularly alpha tocopherol) to migrate in food simulant. The characterization of those components is major in polymer and food quality assessment. However, the European legislation's method for migration assessment is time-consuming, requires several repetitions (leading to the handling of a lot of migration cells) and proved to degrade the analytes (in our case, ascorbic acid was degraded in 95% ethanol).

Hence, the efficiency of our natural antioxidants to stabilize our polymers matrices was assessed by direct analysis. Our additives-polymer blends were analysed by LESA-MS under the same conditions as virgin polymers. The idea is to monitor the apparition of oligomers, characteristic from our polymer's degradation. Results of the surface analysis of polymers mixed with one single additive and with our best formulations (VE/VC and VE/Tan) are shown in **figure IV-21**. Those diagrams were obtained by summing the relative intensities of the 1000 most intense peaks corresponding to the previously described oligomers for each formulation. The peaks representing the additives were obviously removed to avoid biased results. The raw spectra are shown in **annexe 17**.

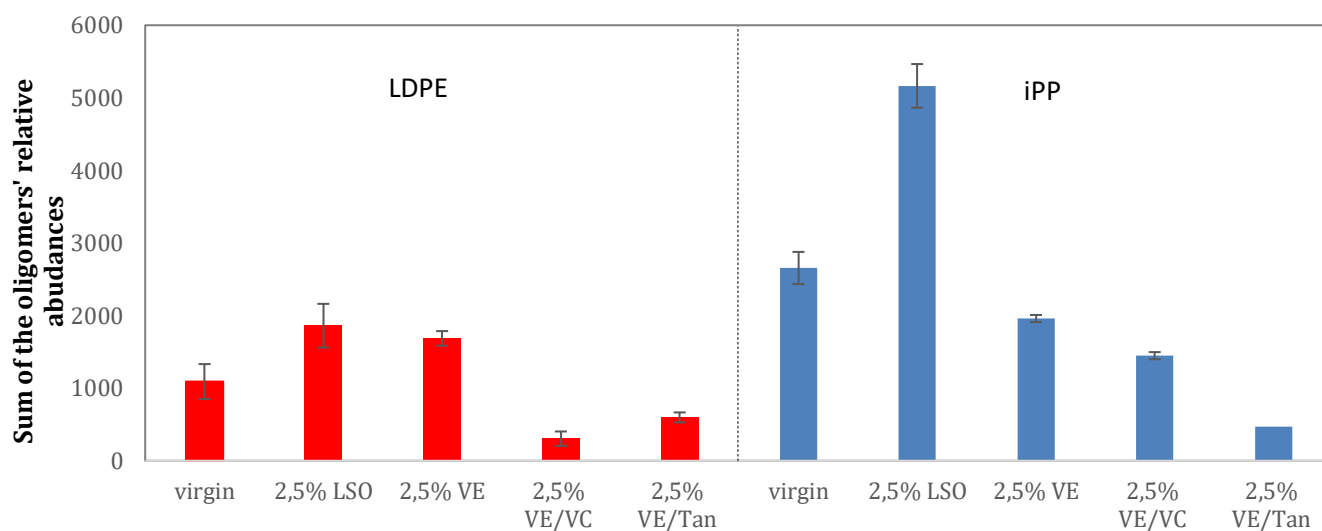


Figure IV-21. Evolution of the apparition of oligomers according to the type of additive.

Figure IV-21 clearly confirms the results detailed in the previous chapter: the amount of oligomers created diminishes as follow: virgin polymers > polymer + 2.5% linseed oil (LSO) > polymer + 2.5% vitamin E (VE) > polymer + 2.5% vitamin E/vitamin C (VE/VC) or polymer + 2.5% vitamin E/tannins (VE/Tan). In other words, the best stabilization (i.e. the lower amount of created oligomers) is provided by the additives combinations. In the case of polyethylene (red bars), extruded at 160°C, the combination of vitamin E and vitamin C seemed to provide better stabilization than vitamin E-tannins' combination. The opposite effect can be observed for polypropylene (blue bars) as the presence of tannins involves lower amount of oligomers created. Actually, as vitamin C is fragile, it probably degrades more when it is incorporated within iPP than within LDPE, its protection effect is thus less effective for iPP. This aspect is even more visible when studying the recycled blends by LESA-MS, as shown in the following diagram (**figure IV-22**).

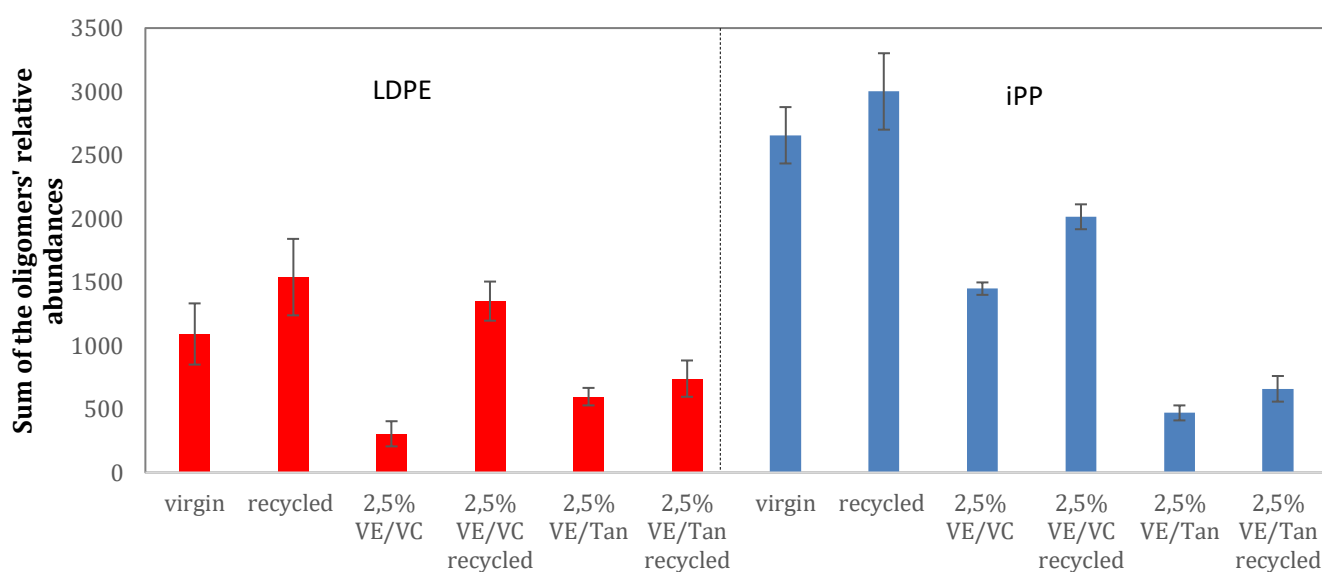


Figure IV-22. Evolution of the apparition of oligomers with the recycling ("Recycled" means recycled 9 times).

As explained in part IV-1-a, the more we recycle virgin polymers, the more we create oligomers able to migrate to the foodstuff. In the case of stabilized polymers (i.e. polymers containing either VE/VC or VE/Tan), this phenomenon is diminished. In order to know which formula is the most efficient in preventing the formation of those oligomers, the ratio between the number of oligomers of non-recycled and the recycled was calculated. The higher this ratio, the higher the number of oligomers created.

- In the case of LDPE (red bars), the ratio for the virgin polymer reaches 0.8 whereas that of VE/VC and VE/Tan are 0.64 and 0.48 respectively.
- In the case of iPP (blue bars), the ratio for the virgin polymer reaches 0.73 whereas that of VE/VC and VE/Tan are 0.5 and 0.58 respectively.

Relatively close ratios are obtained for the stabilised polymers; their values are largely lowered compared to that of virgin polymers. Even though the combination of vitamin E and vitamin C was shown to be less efficient than VE/Tan (**figure IV-22**), it appears that toward recycling this formulation is as effective as VE/Tan.

LESA-MS was optimized to study the release of low molecular species from polymer food packaging films and the monitoring of degradation products during recycling of additive-free polymers and migration of antioxidants from those polymer matrices. The LESA-MS screening of our blends confirms that the synergistic effects between vitamin E and vitamin C and vitamin E and tannins provided a very good stabilization of the polymer: greatly lowering the number of clusters of oligomers (created after degradation of polymers chains). Hence, in the formulation process, LESA-MS allowed choosing the best components for the stabilization of polymers to be found.

IV.1.c. Semi-quantitative aspect: targeted quantitative analysis of antioxidant in polymer films.

The main drawback of the surface analysis like LESA-MS is their inability to provide quantitative results. However, we showed that, in addition to its potential for qualitative analysis, the LESA – MS procedure can be useful for the quantitative determination of additives in the polymers and in food. To achieve a quantitative analysis, calibrations curves obtained thanks to standards must be drawn. To do so, polyethylene doped with different amounts of vitamin E (from 0.25% to 5%) were produced. The polyethylene containing vitamin E were analysed by LESA-MS following the same parameters as those describe above. The peaks of alpha-tocopherol (m/z 429.373) were targeted and their intensities were used to construct a calibration curve. That calibration curve showed a linear response ($R^2=0.9948$), as shown in **figure IV-23**.

The presence of clear signal from well-defined antioxidant additives allows their quantitative in-bulk determination provided suitable solid phase standards of polymers doped with the analyte are available. However, due to the nature of the extrusion process the solid standards may show a certain bias between the theoretical and experimental additive content, however they present a very attractive option for the calibration of the system in view of the semi-quantitative analysis. This observation is a proof-of-principle of the possibility of direct quantitative analysis of polymer films by LESA – nanoESI MS.

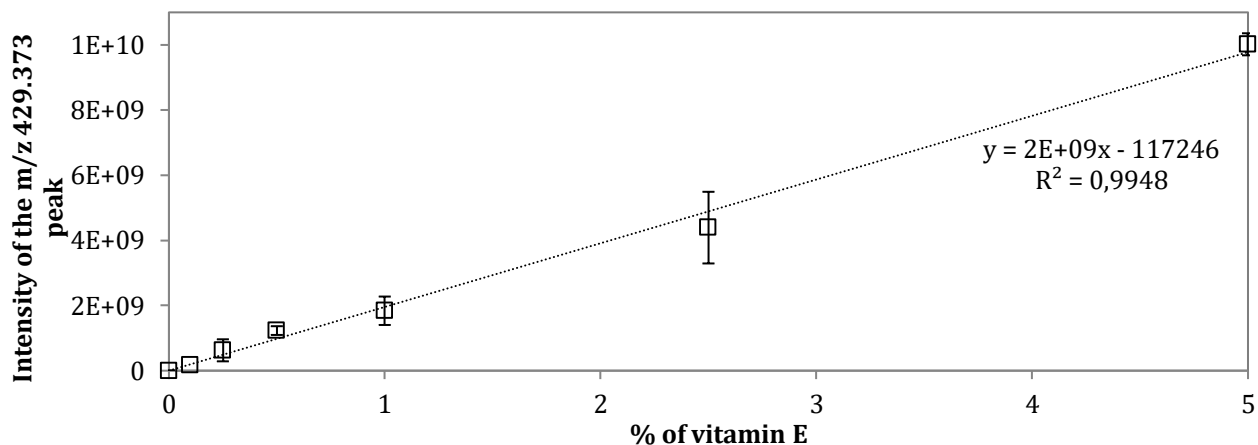


Figure IV-23. Calibration curve of vitamin E within LDPE obtained by LESA-MS.

The potential of the LESA-MS procedure for the semi-quantitation was already studied. For example, Brown et al.[138] characterized 25 worn lenses and managed to identify, by LESA-MS and LESA-MS/MS, 42 cholesterol esters with esterified acyl chains and demonstrated their variation in concentration without using a standard. The evaluation of the 20 most abundant cholesterol esters in two types of lenses was assessed and thanks to the cholesterol esters ions' absolute abundances, concentration profiles were set up. It allowed them to conclude that, in spite of the similarities in the compositions of both lenses, the differences in abundances could show that lipid deposition on lenses depend on the lens itself.

Another example of the potential of LESA-MS for semi-quantitation analysis is the study realized by Paine et al. Actually, while studying e-wastes and the possible presence of brominated flame retardant, Paine et al. demonstrated semi-quantitative reproducible analysis for tetrabromobisphenol A (TBBP-A) by reporting TBBP-A absolute intensity (ion counts) according to the colour of the plastic e-wastes [185]. The obtained result is illustrated in **figure IV-24**.

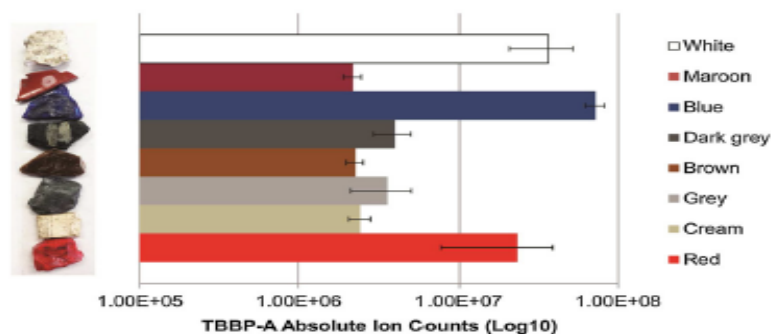


Figure IV-24. Histogram of the intensities obtained after the analyses of plastic e-wastes as a function of their colour. (Reprinted from [32] with permission of John Wiley and Sons.)

The demonstrated semi-quantitation potential of LESA-MS was applied to the migration assessments of vitamin E from our polymers and compared with the results obtained following the official method prescribed by EU regulations in part III [186]. According to the official EU method, the food simulant (95% ethanol which simulates fatty food) must be analysed by LC-MS after contact with the plastic sample. Our polymers blends were analysed by LESA-MS after having been in contact with 95% ethanol for several time: from 30 minutes to 10 days. The obtain vitamin E peak (m/z 429.373) was targeted and the obtained value thus corresponds to the vitamin E remaining in the polymer matrix. This value was thus subtracted to the initial value (i.e. the intensity of the vitamin E peak before the polymer is immersed in ethanol) and converted in percentage. The study revealed a good correlation between the EU method and LESA methods shown in **figure IV-25** for LDPE's formulation and **figure IV-26** for iPP's.

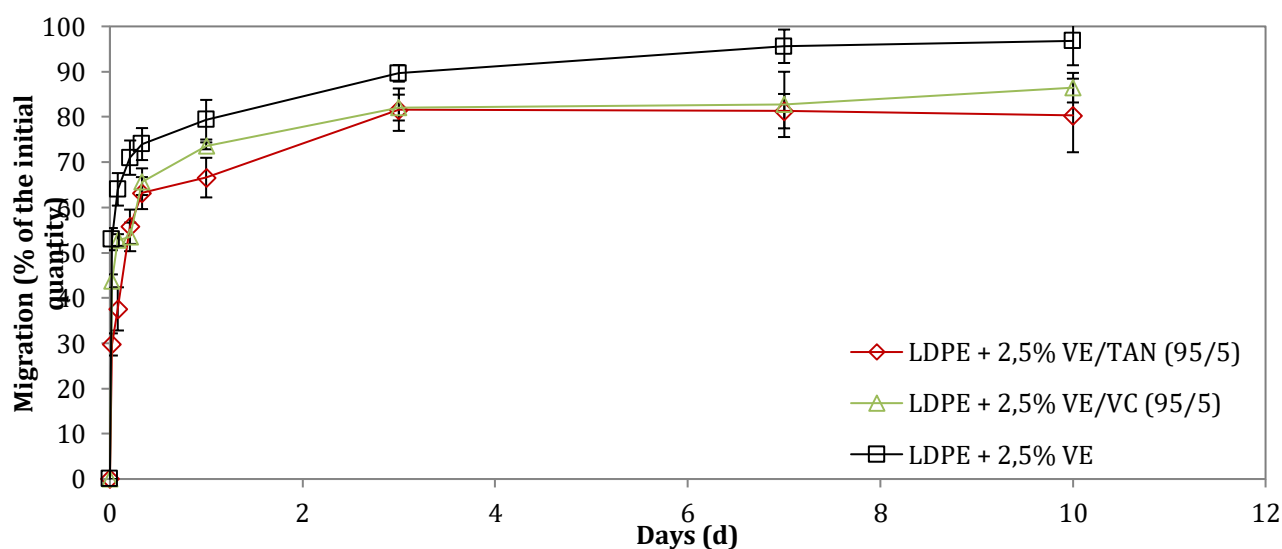


Figure IV-25. Migration of alpha-tocopherol from the different LDPE's thick films obtained by LESA-MS.

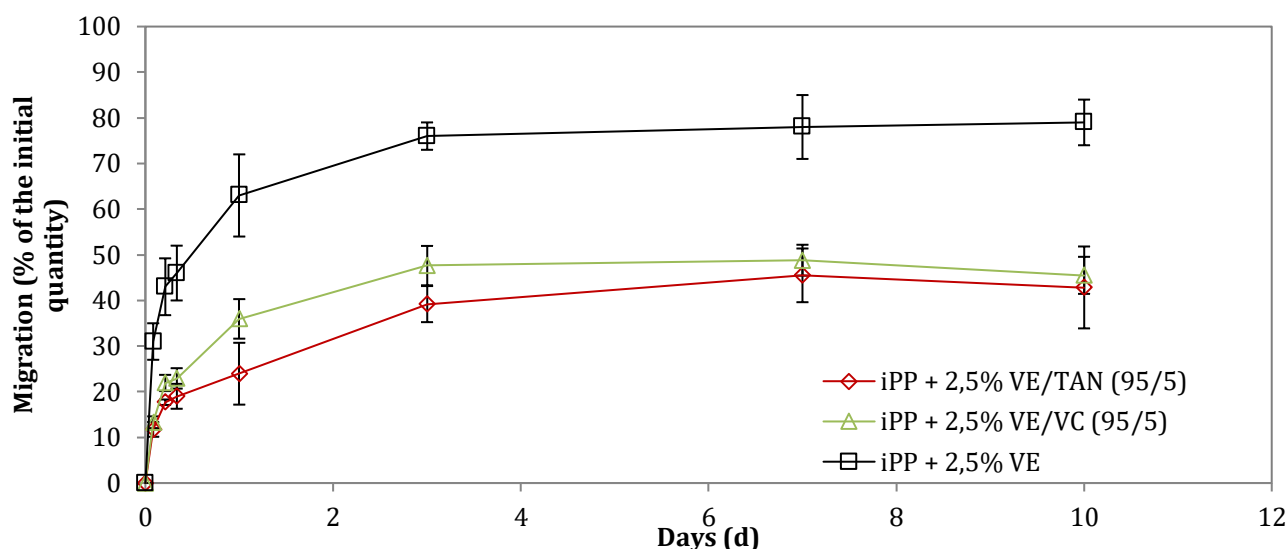


Figure IV-26. Migration of alpha-tocopherol from the different iPP's thick films obtained by LESA-MS.

First of all, a Fickian behaviour is obtained which is similar to the migration profile obtained by UHPLC-MS. Also, even though it is less clear than in part III, the migration of alpha-tocopherol from the polymer + 2.5% VE is still more important than when combinations of additives are involved. Moreover, the migration of alpha-tocopherol from the polypropylene matrix is lower than that from LDPE; which is also an observation that was done earlier.

As explained in part III-1.b., the diffusion coefficient D of alpha-tocopherol according to the formulation can

be evaluated thanks to equation 30: $\frac{M_t}{M_\infty} = \frac{4}{l} \sqrt{\frac{Dt}{\pi}}$ when $\frac{M_t}{M_\infty} < 0.6$ with M_t the migration at a time t , M_∞ the migration at equilibrium, l the thickness of the sample and t the time. The calculated diffusion coefficients are

detailed in **figure IV-27** and **table IV-7** and compared to those obtained by UHPLC-MS method (part III).

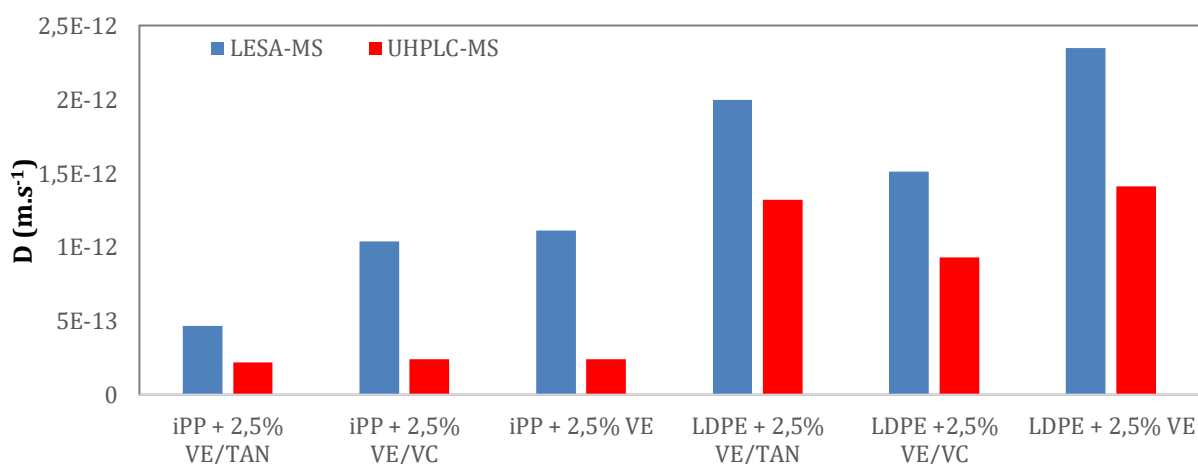


Figure IV-27. Diffusion coefficient of alpha-tocopherol from the different formulations according to the method that was used: LESA-MS versus UHPLC-MS.

Table IV-7. Diffusion coefficient of alpha-tocopherol from the different formulations according to the method that was used.

	D by LESA-MS (m ² .s ⁻¹)	D by UHPLC-MS (m ² .s ⁻¹)
iPP + 2,5% VE/TAN	4,64E-13	2,17E-13
iPP + 2,5% VE/VC	1,31E-12	2,40E-13
iPP + 2,5% VE	8,08E-13	2,38E-13
LDPE + 2,5% VE/TAN	1,99E-12	1,31E-12
LDPE + 2,5% VE/VC	1,51E-12	9,28E-13
LDPE + 2,5% VE	2,34E-12	1,41E-12

The values of diffusion obtained by LESA-MS for the migration of alpha-tocopherol from LDPE and iPP are always higher than that obtained by UHPLC-MS. This can be due to the way the values from **figure IV-25** and **figure IV-26** were calculated. Actually, the values measured by LESA-MS are the amount of alpha-tocopherol left within the polymer matrix whereas the values calculated by UHPLC-MS are the amount of alpha-tocopherol that migrated within 95% ethanol. Even though almost all the alpha-tocopherol migrates in 95% ethanol, there is still some alpha-tocopherol remaining in the matrix which can then be extracted by LESA-MS. Also, in order to keep the best microjunction and the best extraction by LESA-MS, the solvent that was used is MeOH/ChCl₃ (2:1) + 0.1% formic acid (see optimisation details in chapter 2, part III-2). This solvent (especially chloroform) may have enhanced the migration.

Despite these shifted values, the tendencies obtained by UHPLC-MS can also be observed with LESA-MS.

The use of solid-state standards thus made it possible to achieve semi-quantitation of the antioxidant release and thus quality control of “intelligent” packaging.

IV.2. Direct analysis of ham

IV.2.a. Colorimetric analysis

Lipids, proteins and polysaccharides oxidation leads to foodstuff degradation. For example, Luciano et al. reported that quebracho tannins in lamb diets can improve meat colour stability by delaying myoglobin oxidation during refrigerated storage [187]. We thus got interested in the effect of our packaging on enhancing food shelf life, the colour change of ham that was packed was checked through the time. Actually, meat or ham lose its red colour when getting aged. A *Lab* colourimeter was thus used to evaluate the loss of the red

colour of ham. The colourimeter works as shown in the following **figure IV-28** and **table IV-8**. Colours are characterised by cartesian/cylindric coordinates.

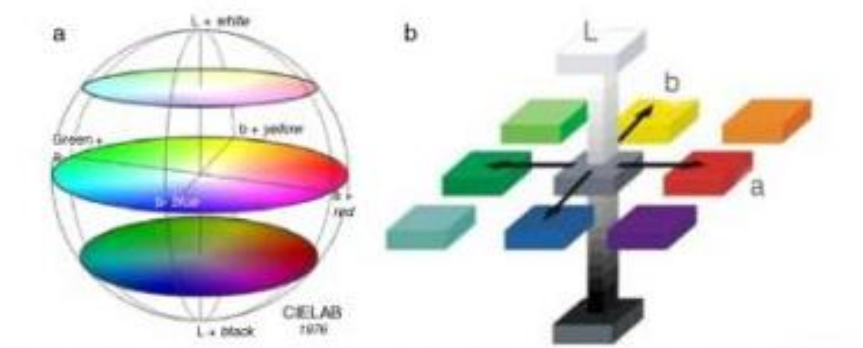


Figure IV-28. Coordinates representing the colour distribution used by the CIELAB colorimeter.

Table IV-8. Description of the evolution of the colorimeter's parameters.

	Positive	Negative
ΔL^*	The sample is clearer	The sample is darker
Δa^*	The sample is redder (or less green)	The sample is greener (or less red)
Δb^*	The sample is yellower (or less blue)	The sample is bluer (or less yellow)

Ham samples were cut (3x3cm) and placed into films of our different formulations:

- Virgin iPP and virgin LDPE
- Polymer + 2.5% VE/Tan
- Polymer + 2.5% VE/VC

The packed samples were kept in the fridge and the colour change was analysed regularly through the days. The parameter **a^*** was evaluated as it corresponds to the red colour: a loss in **a^*** means a loss of red or an apparition of the green colour. The results are described below in **figure IV-29**.

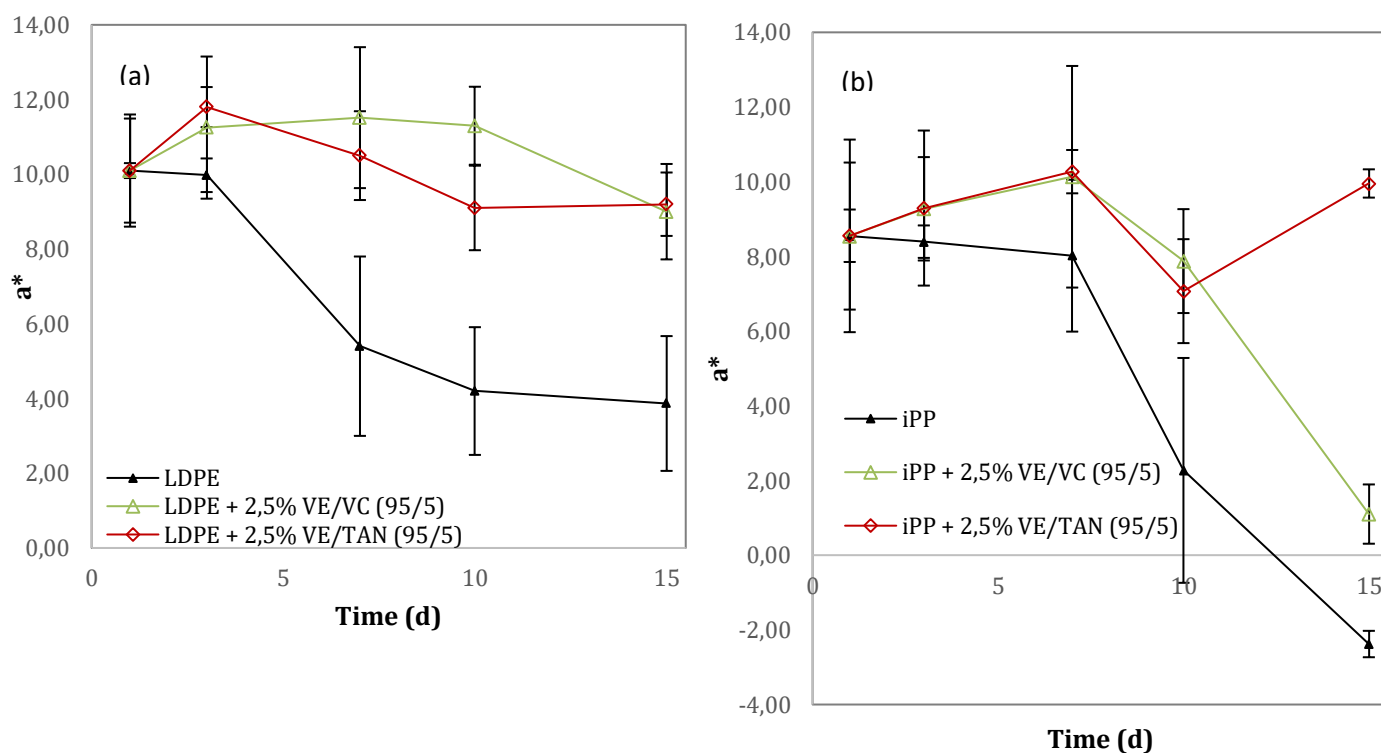


Figure IV-29. a^* evolution of ham packed in active LDPE packaging (a) or active PP packaging (b).

The common point both polymer's influence on the loss of the red colour through the days is that it is clearly lowered by the presence of natural additives. It puts forward the stabilisation provided by the additives present in the packaging. In the case of polyethylene (**figure IV-29 (a)**) both formulations seem to act equivalently in the redness stabilisation. However, in the case of PP (**figure IV-29 (b)**), both formulations seem equivalent until day 10 after which VE/VC based packaging (green curve) seems not to protect the ham as much as VE/TAN based- packaging does. Vitamin C and tannins are relatively hydrophilic. They thus do not have affinity with the ham; hence they act by protecting alpha-tocopherol which is lipophilic (i.e. protects the ham). Vitamin C is more hydrophobic than tannins (see the HSP detailed in chapter 3), it can thus not interact with the ham. The lower efficiency of VE/VC blend could be related to this lower hydrophobicity or to a less good protection of alpha-tocopherol.

IV.2.b. Detection of the antioxidant migration in ham by LESA-MS

5 mm-thick ham was packed in LDPE + 2.5% VE/Tan packaging. After 10 days exposure, ham slices were prepared thanks to a cryo-microtome apparatus. The samples were frozen in liquid nitrogen and placed in the cryo-microtome (maintained at -25°C). Different thicknesses were realized from the surface of the ham until deeper in the heart. The slices were deposited on glass slides as shown on the picture from **figure IV-30**.

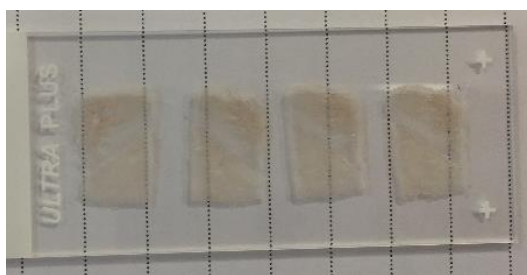


Figure IV-30. Slices of ham cut thanks to a cryo-microtome deposited on a glass slide.

LESA-MS was applied to the direct analysis of the ham slides. The idea is to evaluate if alpha-tocopherol can be detected within the ham after exposure with our packaging. As explained in chapter 2, part III-2, the main limitation of this method is the creation of the micro junction: it can be difficult to apply to rough, wettable or absorbent surfaces. Subsequently, the analysis of thick ham samples was very difficult as the microjunction was lost: the solvent drop was absorbed by the ham and could not be aspired back. Hence, working with thin slices deposited on a glass slide made it easier to maintain the microjunction. Alpha-tocopherol was identified in ham slices that were in contact with the packaging, no alpha-tocopherol could be found in ham blanks.

Alpha-tocopherol was found in ham whatever the thicknesses. In order to visualize the dispersion of alpha-tocopherol within the ham matrix a curve of the normalized intensity of the m/z 429.373 as a function of the depth (z) of the cut was drawn (**figure IV-31**). The intensity of alpha-tocopherol was normalized to the thickness of the slice that was studied.

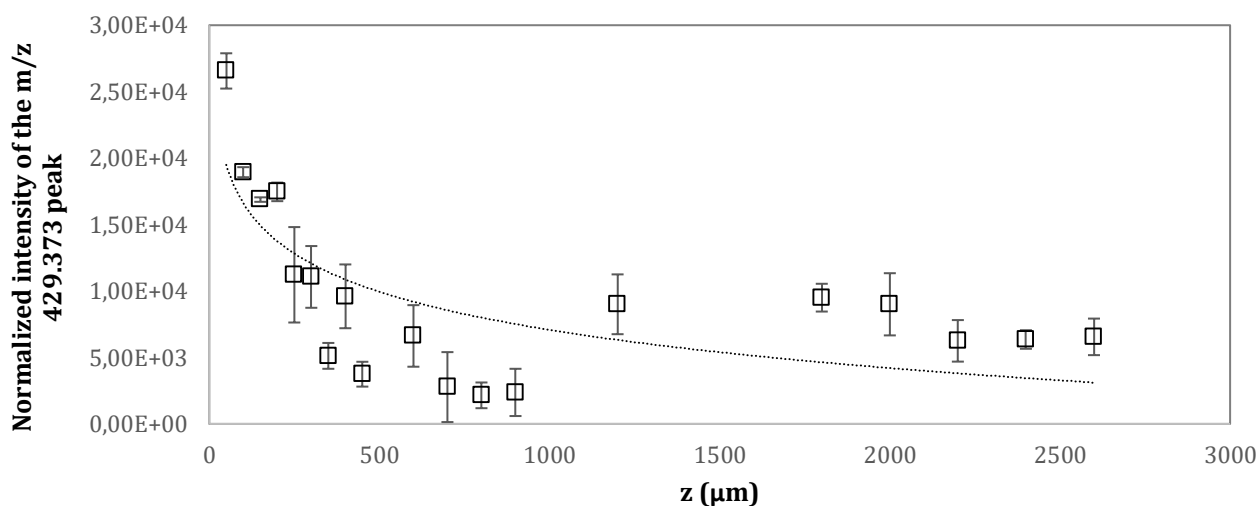


Figure IV-31. Evolution of the concentration of alpha-tocopherol in ham.

First of all, those results confirm that alpha-tocopherol isn't only located at the ham surface but is dispersed all over the ham. The obtained tendency is symmetrical: 2500 μm corresponds to the middle of the ham sample (as shown in **annexe 15**). In turn, concentration of alpha-tocopherol at the surface are high and diminishes before stabilizing when going deeper within the ham: as shown on **figure IV-31**, the curve follows a logarithmic

law. In other words, alpha tocopherol is relatively easily going from the packaging to the ham surface and then diffuses within the ham until the core. If the **figure IV-31** was also drawn as a function of z^2 (data not shown) and not z , two straight lines are obtained:

- one at low z^2 which slope represents the diffusion coefficient of vitamin E in ham;
- another one equals to zero, showing that the vitamin E has a single diffusion coefficient ($\text{m}^2 \cdot \text{s}^{-1}$) in ham.

V. CONCLUSION

After having been characterized following the European norm procedure (using UHPLC-MS), our newly formulated plastics were directly analysed by LESA-MS. Liquid extraction surface analysis mass spectrometry (LESA-MS) is a direct analysis method suitable for the analysis of polymers.

The migration profiles that were obtained by UHPLC-MS and by LESA-MS were compared and showed to be similar corresponding to Fickian laws. Both methods showed that the migration of alpha-tocopherol is more important from LDPE than from iPP. Also, both methods showed that alpha tocopherol tends to migrate more than when it is combined to another additive (i.e. vitamin C or tannins). In terms of diffusion coefficient, higher values were obtained by LESA-MS than by UHPLC-MS due to the differences in method set up, but the same tendencies could be observed.

In comparison with batch methods, LESA-MS is a “green” method, using small volumes of organic solvents, and is cost-effective, avoiding lengthy sample preparation procedures. It can be used for the detection of known molecules (targeted analysis), identification of unknown species (exploratory analysis requiring MS/MS) and semi-quantitative analysis. Thanks to the possibility to use of different solvents LESA-MS gives a lot of possibilities such as modifying the solvent to extract specifically some species or analysing changes in the chemistry. In this way, it gives much simpler spectra and may give additional information about mechanisms causing degradations [135]. After having been optimized for the polymer analysis, LESA/MS was applied to the detection of polymeric degradation products and polymer additives was tested. The method had been already used in applications such as metabolomics, lipid analysis, protein analysis, carbohydrate analysis and enzyme monitoring [136, 137, 159, 160, 188]. However, its applications in the polymer field are scarce; it and was only applied few times to polymer and additives analysis [37, 73, 135, 138, 161]. The method proved to be very efficient in the analysis of polymers’ and food quality.

Also, even if it does not allow exact quantification, its proven reproducibility offers the possibility to have a relative quantitation. The still unexplored potential of the LESA-MS methods is related to the possibility of

its coupling to tandem mass spectrometry which offers the possibility of the formal identification of the studied species. For food packaging degradation studies, LESA-MS/MS allowed the confirmation of the chemical structure of the migrating alkanes and of the UV oxidized species.

Another specific potential use of LESA-MS is the evaluation of food packaging after use and before being cleaned, recycled or reused; LESA-MS allows rapid assessment of the presence of fatty residues on the plastic surface.

These advantages open possibilities for future use where a fast evaluation of solid polymer samples is needed in the context of polymer quality control, aging and degradation, specific uses (medical, food-related, everyday consumer products) or recycling.

GENERAL CONCLUSION

This thesis work fits into the identified context of the need to develop new healthy polymer materials for consumers, packed foods and for the environment. Much research has focused on different aspects of the packaging life cycle, proposing the formulation of both biodegradable and non-biodegradable polymers. The main concern is to obtain an excellent material both in terms of mechanical properties, and throughout its product life; the aim being to extend the life expectancy of materials and to be able to reuse them without loss of properties. The growing problem of the migration of additives from plastic packaging to food is another central subject of this thesis. Since additives (essential to the life of plastics) are small molecules, they are able to migrate into food. The problem of food waste is also at the heart of this thesis work. We truly believe that plastic packaging can no longer be used passively but must interact with the food to reduce food wastes. From this point of view, it seemed obvious to us to use natural stabilizers. These natural stabilizers will have two distinct functions: stabilisation of the Polymer matrix towards thermo-oxidation and stabilisation of the food toward lipid-oxidation.

Bibliographic researches, initially carried out, allowed making a general presentation of the current problems related to plastic food packaging. Those literature researches, associated with suggestions from members of the Foodyplast project, naturally led to the choice of antioxidant molecules that could eventually stabilize our polymers without "negative" migration to foodstuff. Those molecules are linseed oil, alpha-tocopherol, ascorbic acid, tannins from quebracho trees and green tea. Alpha-tocopherol was already extensively used as a stabiliser for polyolefins and so does green tea. However, green tea being very sensitive to temperature, it required encapsulation process prior to its introduction within the polymer matrix. The other additives were not used for the stabilisation of polyolefins but showed promising properties thanks to their known antioxidant capacities.

The chapter 2 aimed at describing all the characterisation methods used within this PhD in order for this thesis manuscript to be as clear as possible for the reader. Among those characterisations' methods, most are already known except one: the LESA-MS method. Indeed, this method is still unknown for the characterisation of polymers and has therefore been optimised as part of this thesis work. The LESA-MS method is a direct method based on liquid extraction surface analysis nanoelectrospray mass spectrometry (LESA-nanoESI-MS). The idea was to optimise this method for the analysis of the migrating species from a polymer film. All the parameters offered by the LESA were tested and optimized for the analysis of different types of molecules post-polymerization residues, degradation products (oligomers resulting from polymer

recycling, products of polymer oxidative degradation). The method was validated by a comparison a standard method based on with bulk extraction mass spectrometry or based on Direct Analysis in Real Time (DART).

The chapter 3 detailed the formulation work carried out in order to evaluate which of our natural additives is effective for the stabilization of polyethylene and polypropylene. Initially, additives alone were introduced into our polymers via extrusion. All the processed mixtures have been characterized using thermal analysis (thermogravimetric analysis, and differential scanning calorimetry), rotational rheology, scanning electron microscope and mechanical characterisations. Those characterisations helped us drawing conclusions about the effect of the additives on the polymer matrices. Ascorbic acid couldn't be used on its own due to its sensitivity toward the extrusion process. Alpha-tocopherol appeared to be very efficient for the thermo-oxidative stabilisation of the polymer matrices. Solid additives (green tea and tannins) showed interesting properties but prevented the polymers from relaxing by inducing "gel-like" behaviours. Linseed oil, in spite of its very good thermal properties, didn't stabilised the polymer matrices at all and brought strong odours to the material. From those conclusions and based on the literature study, combination of additives was tried. The blends were very easily processed by extrusion, showing that vitamin E acts like a vehicle for the introduction of temperature-sensitive compounds like vitamin C. Then, the already known positive effect that vitamin E and vitamin C have on each other seems to occur within the polymer: the stabilisation provided by their combination on polypropylene and polyethylene is very significant. Another combination that was tried is vitamin E-quebracho tannins. No researches were found dealing with this type of blends. However, the stabilisation effect provided by this combination is obvious. For both combinations, the physico-chemical characterisations showed enhanced stability toward oxidation (assessed by the OOT method) compared to the virgin matrices or the single additives blends, and almost constant properties even after 9 cycles of recycling were observed. Those synergisms were evaluated by diffusional-NMR (DOSY) and proved to correspond to hydrogen bonding between the two molecules. However, due to the lack of solubility of our polymers, the synergisms occurring within the polymers could not be assessed. Nevertheless, the interactions occurring within those tertiary systems were hypothesized thanks to rheological analysis and showed different behaviours from one polymer to another: polypropylene seems to show composite-like behaviour with stabilised interfaces (alpha-tocopherol plays the role of a surfactant between the polymer and the solid incompatible particle) whereas polyethylene seems to follow a mixing law.

Finally, chapter 4 is devoted to the study of the migration phenomena from our new plastics to food simulants and to food. The food simulant used for those migration studies was chosen according to the Foodypast project which aims at packing ham, hence fatty food simulant was chosen: 95% ethanol. The migration assessments were realized following the European regulation and compared to the newly developed LESA-MS method. The only identified species migrating from tannins are terpene-like molecules (syringaldehyde or ubiquinone-0). For ascorbic acid, only traces could be identified in the migration solutions.

The migration profile was obtained by following the migration of alpha-tocopherol. The migration profiles that were obtained by UHPLC-MS and by LESA-MS were compared and showed to be similar corresponding to Fickian laws. Both methods showed that alpha-tocopherol migrates more when it is used alone in the polymer than when it is combined with another compound (i.e. tannin or ascorbic acid). This diminished migration could be due to the interactions between the additives. Also, the migration of alpha-tocopherol was always higher from LDPE than from iPP (which fits the literature data). In terms of diffusion coefficient, higher values and higher standard deviations were obtained by LESA-MS than by UHPLC-MS due to the differences in method set up and to the lower sensitivity of LESA-MS (which is just a surface analysis) but the same tendencies could be observed. Also, LESA-MS allowed detecting degradation molecules (pieces of polymer chains or strongly oxidised species) from our polymers and thus allowed to evaluate the quality of the plastic itself. Another specific potential use of LESA-MS is the evaluation of food packaging after use and before being cleaned, recycled or reused. As LESA-MS allows rapid assessment of the presence of fatty residues on the plastic surface, it could be valuable for the plastic sorting out. These advantages open possibilities for future use where a fast evaluation of solid polymer samples is needed in the context of polymer quality control, aging and degradation, specific uses (medical, food-related, everyday consumer products) or recycling.

PERSPECTIVES

The main scientific perspective concerns the confirmation of the hypotheses on the polymer-additive-additive interactions. Actually, as explained in chapter 3, DOSY-NMR couldn't be realized for the evaluation of interactions within the polymer due to solubility and concentration problems. The idea would be to shape a "model" mixture with lower molecular mass polyolefin (more easily soluble) and higher concentrations in additives (the 2.5% used in our materials is too low to be able to evaluate interactions). This model would help confirming the stabilisation mechanisms provided by the combinations of alpha-tocopherol-ascorbic acid and alpha-tocopherol-quebracho tannins. Also, our results led to other use of alpha-tocopherol: studies are being carried out using alpha-tocopherol as a stabilizer for recycled polyethylene terephthalate- recycled polypropylene blends.

In terms of "industrial" perspectives, this research work will be promoted through the recently deposited patent (« *Matériau pour emballage alimentaire et procédé de préparation* » FR1907060) which will be exploited by the recently created company NOOSTRIM. An industrial demonstrator was realized by producing 250kg of each of the four formulations (LDPE + 2.5% VE/VC (95/5), LDPE + 2.5% VE/Tan (95/5), iPP + 2.5% VE/VC (95/5) and iPP + 2.5% VE/Tan (95/5)). The blends were achieved in CELTIPAK (Saint-Thuriau, France). However, issues are still to be solved such as the availability of additive-free polyolefins. Actually, this whole research project was realized on additive-free polymers but the latter are not commercially available.

Another industrial issue is the price of the product: above 4€/kg. This price can definitely not fit a single-use plastic but can be justified pour a reusable product (which fits with the objectives of the project).

SCIENTIFIC PRODUCTIONS

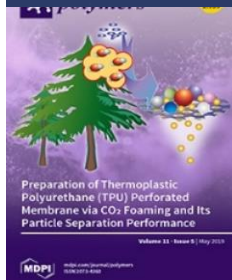
▪ PUBLISHED ARTICLES



“Direct screening of food packaging materials for post-polymerization residues, degradation products and additives by liquid extraction surface analysis nanoelectrospray mass spectrometry (LESA-nESI-MS).”

Ambre Issart, Simon Godin, Hugues Preud'homme, Katarzyna Bierla, Ahmed Allal, Joanna Szpunar

Analytica Chimica acta 2019, vol 1058, doi :10.1016/j.aca.2019.01.028



“Potential of Liquid Extraction Surface Analysis Mass Spectrometry (LESA—MS) for the Characterization of Polymer-Based Materials.”

Ambre Issart and Joanna Szpunar

Polymers 2019, 11, 802; doi:10.3390/polym11050802

▪ PATENT

« **Matériau pour emballage alimentaire et procédé de préparation** » deposited on the 27th of July, 2019 number : FR1907060.

INTERNATIONAL CONFERENCES

International Conference on Instrumental Methods of Analysis (2017)



Oral presentation

“Development of liquid extraction surface analysis mass spectrometry for rapid screening for the presence of post-polymerization residues in food packaging materials.”

Ambre Issart, Simon Godin, Hugues Preudhomme, Katarzyna Bierla, Ahmed Allal and Joanna Szpunar

International Mass Spectrometry Conference (2018)



Poster

“Potential of liquid extraction surface analysis mass spectrometry in the evaluation of safety of polymer food packaging materials.”

Ambre Issart, Simon Godin, Ahmed Allal and Joanna Szpunar

Annexes

CHAPTER 1 – LITERATURE REVIEW

Annexe 1: Norrish reactions

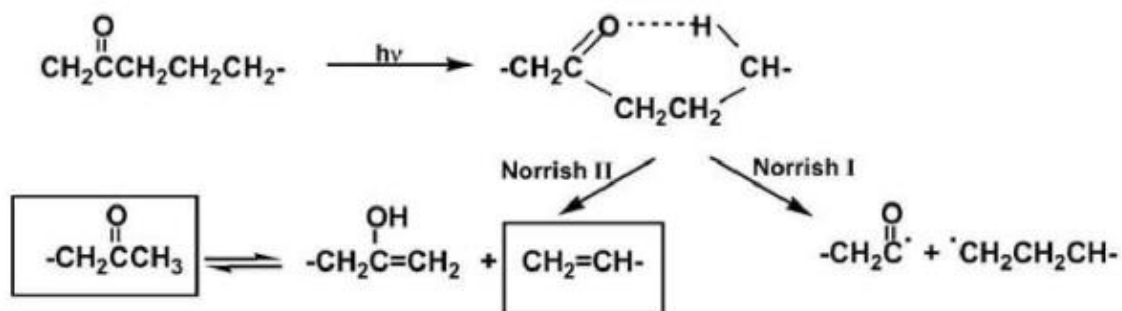


Figure A-1. Norrish reaction occurring during polyolefins' degradations.

Figure A-1 described the Norrish reactions I and II (NI and NII) that ketones can undergo. They specifically occur in photo-oxidation conditions and lead to the scission of polymers skeleton creating free radicals, rearranged sub-products (NI) or to vinylic-type unsaturation (NII).

Annexe 2: Vitamin E

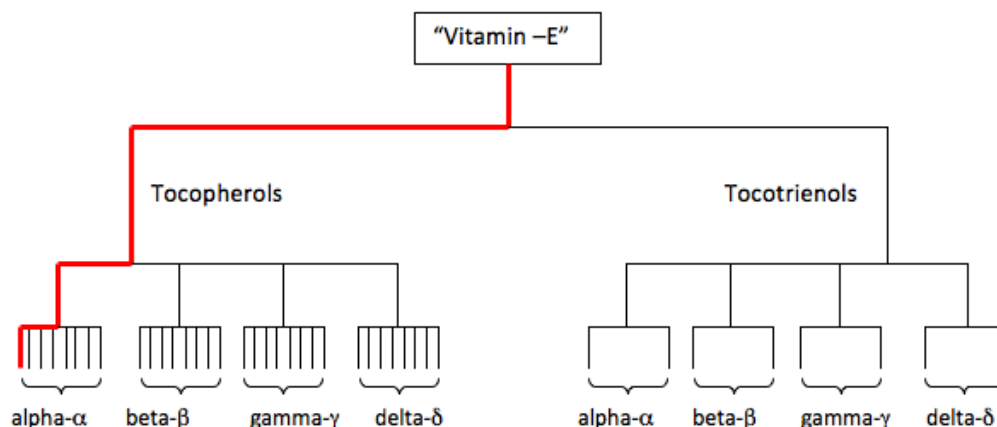


Figure A-2. Vitamin E classification.

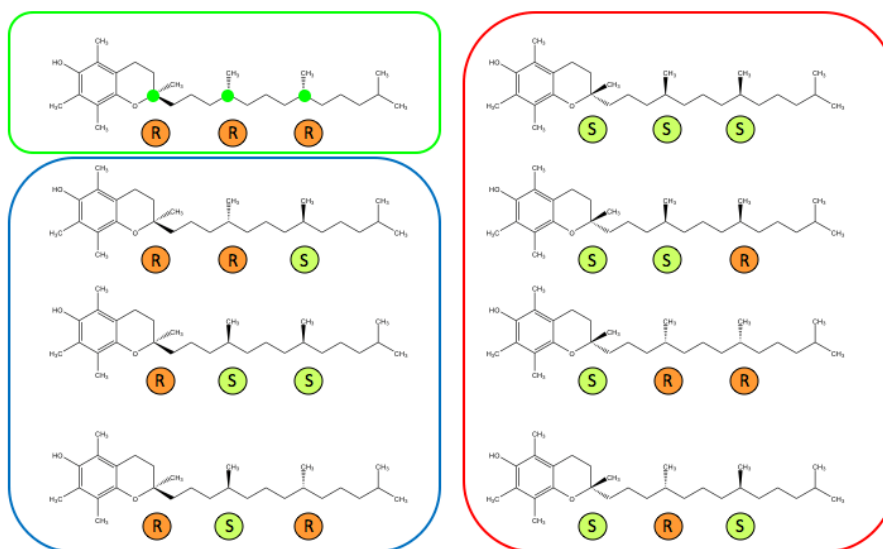


Figure A-3. Stereoisomers of vitamin E.

Figure A-2 et **A-3** details the different forms that vitamin E can take. It can be differentiated into two main families: tocopherols and tocotrienols (a saturated side chain or an unsaturated one, respectively). Those families are also divided in four subgroups of compounds: alpha (α), beta (β), gamma (γ) and delta (δ). As shown in **figure A-3**, the three chiral centres of vitamin E lead to the possible formation of up to eight stereoisomers.

Annexe 3: The principle of Orbitrap

In the late years, the orbitrap has proven to be a very powerful mass analyser with high resolving power and mass accuracy: total possible maximum resolution (FWHM) of 1,000,000 at m/z 200 and a sub-1 ppm mass accuracy. Contrary to traditional ion traps, it uses only electrostatic fields to confine and to analyse ions. A scheme of the orbitrap is represented in **figure A-4**. It is composed by two external and a central electrode. The central electrode has an alternative tension with a polarity opposed to that of the ions. Ions arrive in the orbitrap tangentially to the field and then turn around the central electrode. Different ions oscillate at different frequencies, resulting in their separation. The frequency of this trajectory is converted into m/z spectrum by Fourier transformation.

Also, as ions are produced continuously backward, so they need to be stocked before entering the orbitrap. Subsequently, all the commercial orbitraps use a C-trap (curved linear trap) for the injection of the ions. When

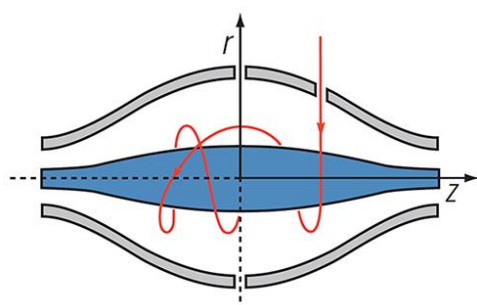


Figure A-4. Scheme of the principle of the Orbitrap.

the ions arrive in the C-trap, the ions collide with the gas and lose a little bit of their kinetic energy. Ion clouds are then sent and accelerated into the orbitrap (the loss of some kinetic energy is beneficial so that the ions don't collide with the upper electrodes).

Thanks to its resolution, mass accuracy and effectiveness toward tandem MS, Orbitrap has been increasingly used in a broad range of applications requiring high selectivity and sensitivity.

CHAPTER 2 – MATERIALS AND METHOD

Annexe 4: ALCUDIA® PE015 data sheet

Alcudia® Polietileno baja densidad

Química

Nota técnica



PEBD ALCUDIA® PE015

DESCRIPCIÓN

El grado ALCUDIA® PE015 se fabrica en reactor autoclave. Está indicado para la producción de películas por extrusión tubular y plana. Asimismo, se utiliza en la fabricación de envases pequeños por extrusión-soplado. Combina una notable facilidad de procesado con buenas propiedades mecánicas y ópticas. No contiene aditivos.

APLICACIONES

- Películas para envasado y embalaje y película retráctil de gran resistencia mecánica.
- Sacos de mediana capacidad.
- Extrusión-soplado de cuerpos huecos de baja capacidad, hasta 2 litros y para uso alimentario.

Se recomienda trabajar con temperaturas de fundido entre 170-190°C. Las condiciones óptimas de transformación se deben ajustar para cada línea de producción.

PROPIEDADES	VALOR	UNIDAD	MÉTODO
General			
Índice de fluidez (190°C, 2.16kg)	1.00	g/10 min	ISO 1133
Densidad a 23°C	921	kg/m ³	ISO 1183
Película ⁽¹⁾			
Resistencia al impacto (F ₅₀)	150	g	ISO 7785-1
Resistencia al rasgado (Elmendorf) (DM/DT)	175 / 150	cN	ISO 6383-2
Resistencia a la tracción en el punto de rotura (DM/DT)	24 / 22	MPa	ISO 527-3
Resistencia a la tracción en el punto de fluencia (DM/DT)	11 / 10	MPa	ISO 527-3
Alargamiento en el punto de rotura (DM/DT)	300 / 550	%	ISO 527-3
Coefficiente de fricción (Dinámico)	0.9	-	ISO 8295
Brillo (45°)	60	-	ISO 2813
Turbidez	11	%	ASTM D-1003
Otras			
Temperatura de reblandecimiento Vicat (carga 10 N)	92	°C	ISO 308

(1) Película de 50 µm de espesor, relación de soplado 2.25:1, altura de la línea de enfriamiento 50 cm.

El grado ALCUDIA® PE015 cumple con la normativa europea de materiales para uso en contacto con alimentos. Para información más detallada, contacten con el Laboratorio de Asistencia Técnica y Desarrollo o con el Servicio de Atención Comercial.

ALMACENAMIENTO

El grado ALCUDIA® PE015 debe almacenarse en ambiente seco, zona pavimentada y con buen drenaje para evitar la posibilidad de acumulación de agua, temperatura inferior a 60°C y protegido de la radiación UV. El almacenamiento en condiciones no adecuadas puede iniciar procesos de degradación que influyen negativamente en la procesabilidad y en las propiedades del producto transformado.

Junio 2013

Esta información se da solamente a título orientativo. En cada caso el transformador será responsable de las condiciones de transformación, del uso final del producto y deberá tener en cuenta la posible existencia de patentes y derechos de la propiedad industrial.

Asistencia Técnica y Desarrollo
Centro de Tecnología Repsol
Ctra. de Entremadura AS, Km 20
28521 Pórticoles
Tel. +34 91 751 86 00
atd_polinefines@repsol.com

Sede Central:
Méndez Álvaro, 44
28045 Madrid
España
www.quimica.repsol.com

Atención Comercial:
sacrq@repsol.com

Figure A-5. Data sheet of the LDPE of the study.

Annexe 5: Characteristic properties of polyethylene and polypropylene

Table A-1 summarizes the values obtained after the characterisation of virgin polymers.

Table A-1. Summary of iPP and LDPE characteristic transitions.

Type of analysis		iPP under oxygen	iPP under nitrogen	LDPE under oxygen	LDPE under nitrogen
TGA	Onset of degradation (°C)	210 ± 12	296 ± 9	247 ± 7	309 ± 5
	Maximum of degradation (°C)	328 ± 1	446 ± 2	384 ± 6 and 370 ± 8	461 ± 1
	Offset of degradation (°C)	389 ± 6	479 ± 10	461 ± 26	484 ± 12
	Slope (%/°C)	-1.2 ± 0.3	-2.3 ± 0.4	-1.3 ± 0.6	-2.06 ± 0.8
DSC	Fusion temperature T_f (°C)		167 ± 3		113 ± 1
	Fusion enthalpy (J/g)		95 ± 4		114 ± 4
	Crystallisation temperature T_c (°C)		118 ± 3		96 ± 1
	Crystallisation enthalpy (J/g)		95 ± 2		115 ± 8
	Crystallinity rate χ (% at 10°C/min)		48 ± 4		39 ± 2
Rheology	Limit viscosity η (Pa.s)		12129 ± 1500		165758 ± 14165
	Activation energy (kJ/mol)		38.9 ± 0.7		68.8 ± 0.6
	Maximum time of relaxation τ_w (s)		1.58 ± 0.15		54.3 ± 9
	Slope 1		✓		✓
	Slope 2		✓		✓

All the experiments were replicated three times.

Onset of degradation (°C): beginning of the degradation;

Maximum of degradation (°C): maximum of the peak when the thermogram is in derivative mode;

Offset of degradation (°C): when the weight percentage reaches 0;

Slope (%/°C): slope at the maximum of degradation when the thermogram is in normal mode.

Annexe 6: OOT assessment of virgin polyethylene and polypropylene

Figure A-6 and A-7 shows the OOT values obtained for virgin recycled polymers. Different parameters can be observed:

- the OOT ($^{\circ}\text{C}$) which corresponds to the onset of oxidation;
- the enthalpy of oxidation ΔH_{ox} (J/g) which calculated by integrating the oxidation peak when visible;
- the slope of curve (W/g $^{\circ}\text{C}$) after the main oxidation peak which corresponds to the polymer's thermal degradation.

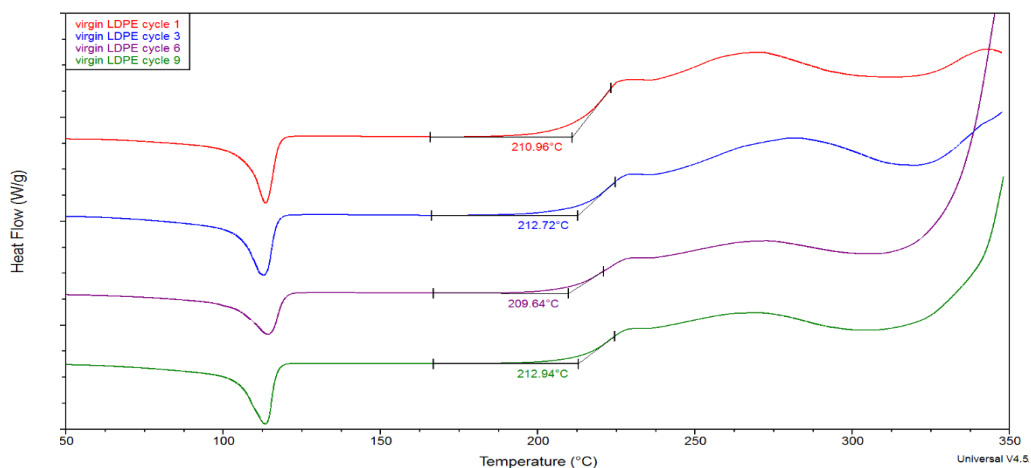


Figure A-6. Onset Oxidation Temperature (OOT) method applied to recycled virgin polyethylene.

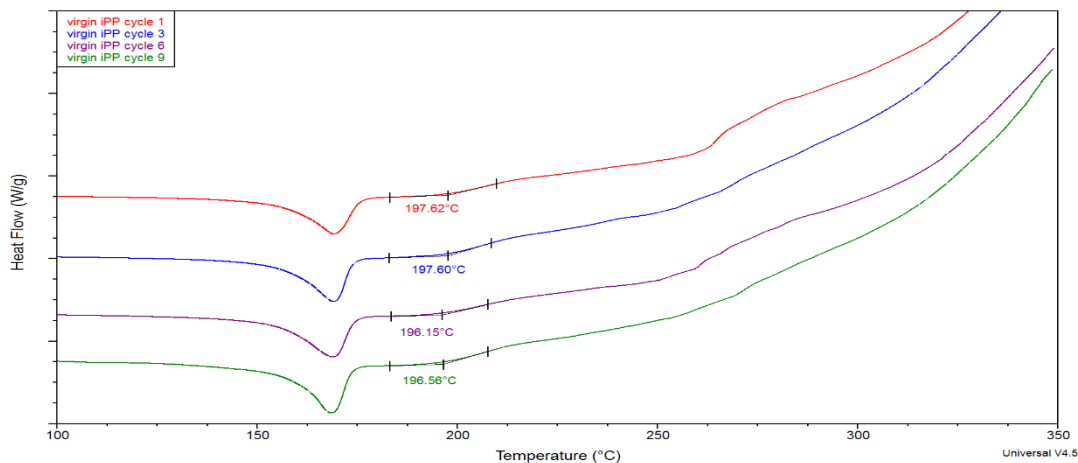


Figure A-7. Onset Oxidation Temperature (OOT) method applied to recycled virgin polypropylene.

Annexe 7: High Temperatures Size Exclusion Chromatography (HT-SEC)

LDPE



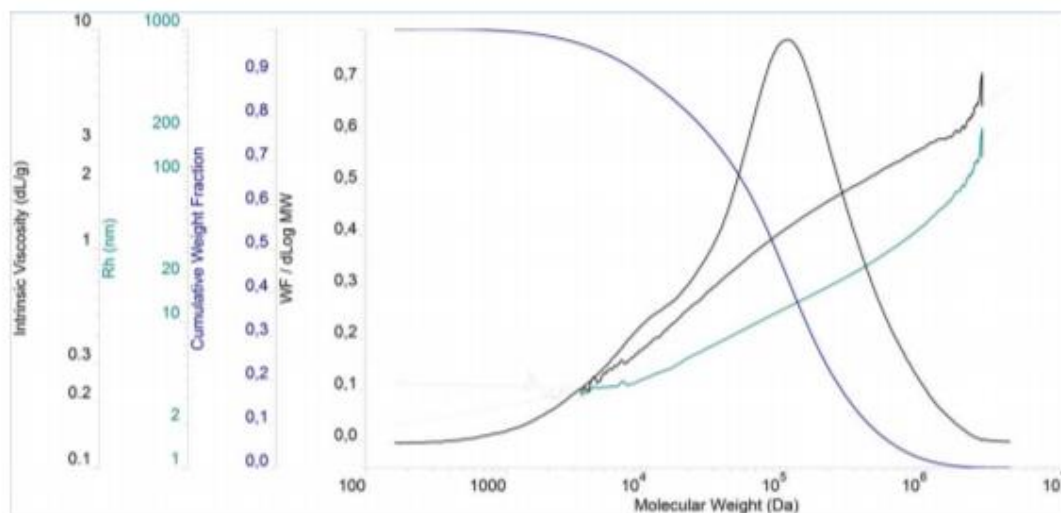
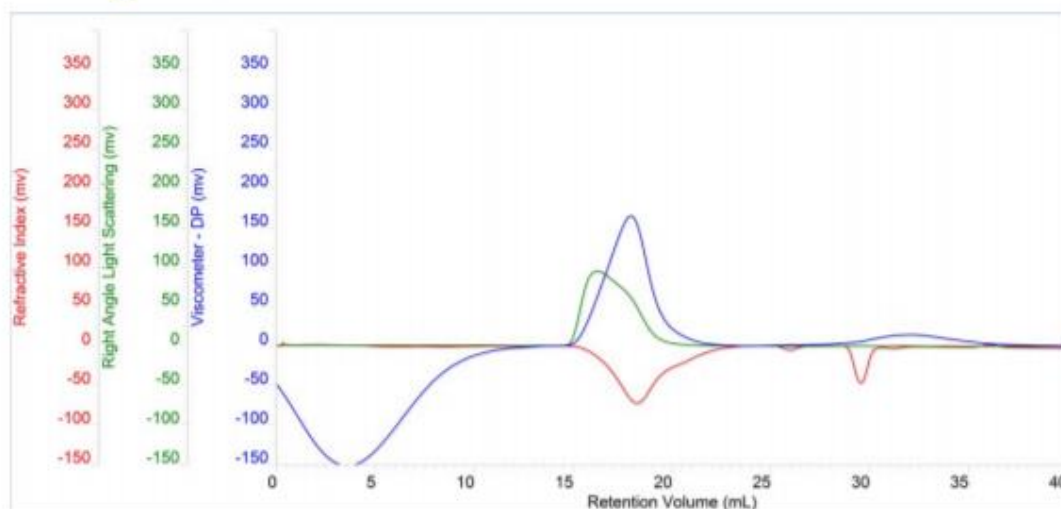
Rapport d'analyse

Chromatographie d'Exclusion Stérique Multi-Détection

Conditions analytiques

Acq. Date	Method	1-LDPE Vierge					
Nov 05, 2018 - 18:30:02	CT-12-11-2018-0001.vcm						
System	Column	Col Temp	Solvent	Flow Rate	Inj Vol	Conc	dn/dc
GPC HT	PSS	150.00	1,2,4 TCB	1.0000	100.0	3.2558	0.1050

Chromatogrammes



Résultats

Peak	Ret Vol (mL)	Mp (Da)	Mn (Da)	Mw (Da)	Mz (Da)	Mw/Mn
1	18.217	100 453	21 242	159 359	512 588	7.502

Figure A-8. High temperature SEC analysis of virgin LDPE.

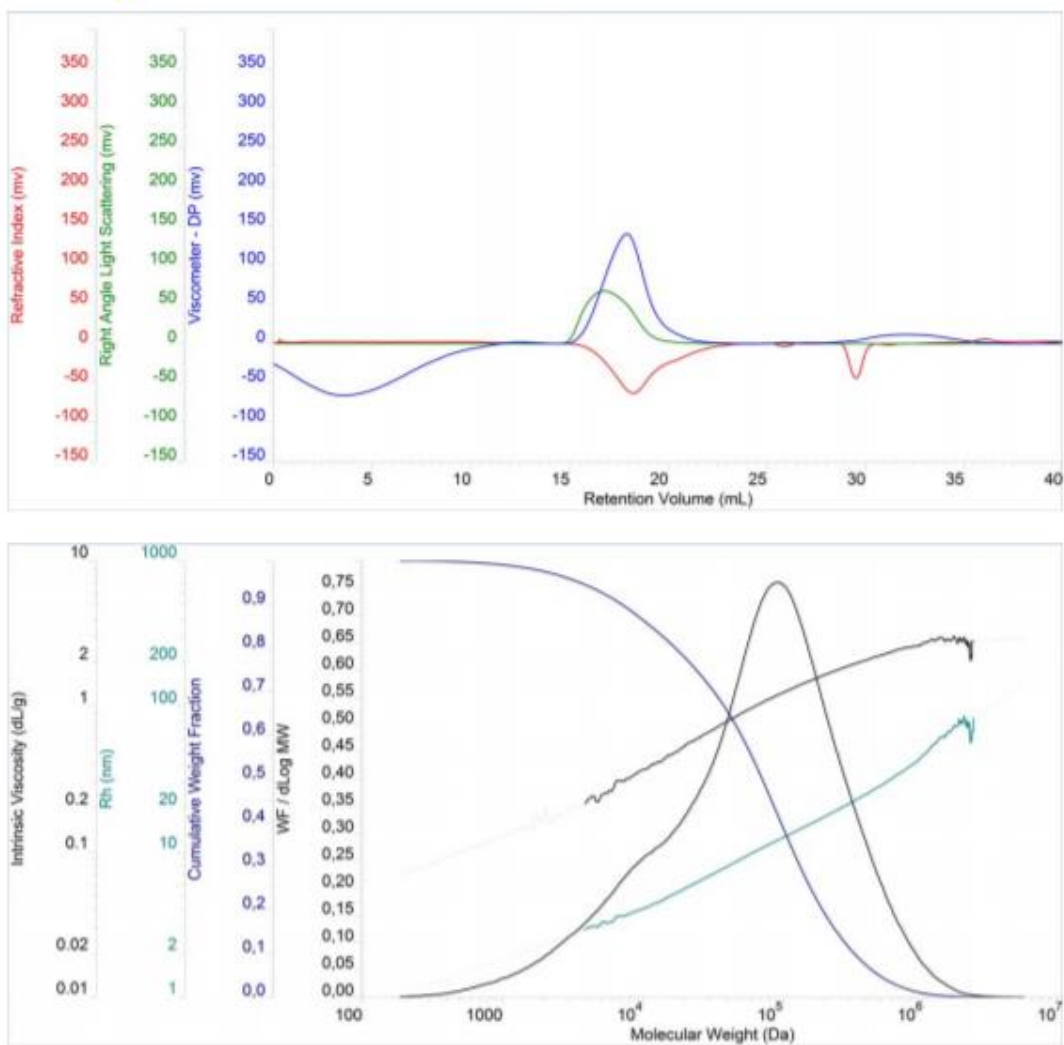
Rapport d'analyse

Chromatographie d'Exclusion Stérique Multi-Détection

Conditions analytiques

Acq. Date		Method		2-LDPE Vierge recycl			
Nov 05, 2018 - 19:13:50		CT-12-11-2018-0002.vcm					
System	Column	Col Temp	Solvent	Flow Rate	Inj Vol	Conc	dn/dc
GPC HT	PSS	150.00	1,2,4 TCB	1.0000	100.0	2.8546	0.1050

Chromatogrammes



Résultats

Peak	Ret Vol (mL)	Mp (Da)	Mn (Da)	Mw (Da)	Mz (Da)	Mw/Mn
1	18.213	91 867	15 396	134 617	427 510	8.743

Figure A-9. High temperature SEC analysis of 9 times-recycled virgin LDPE.

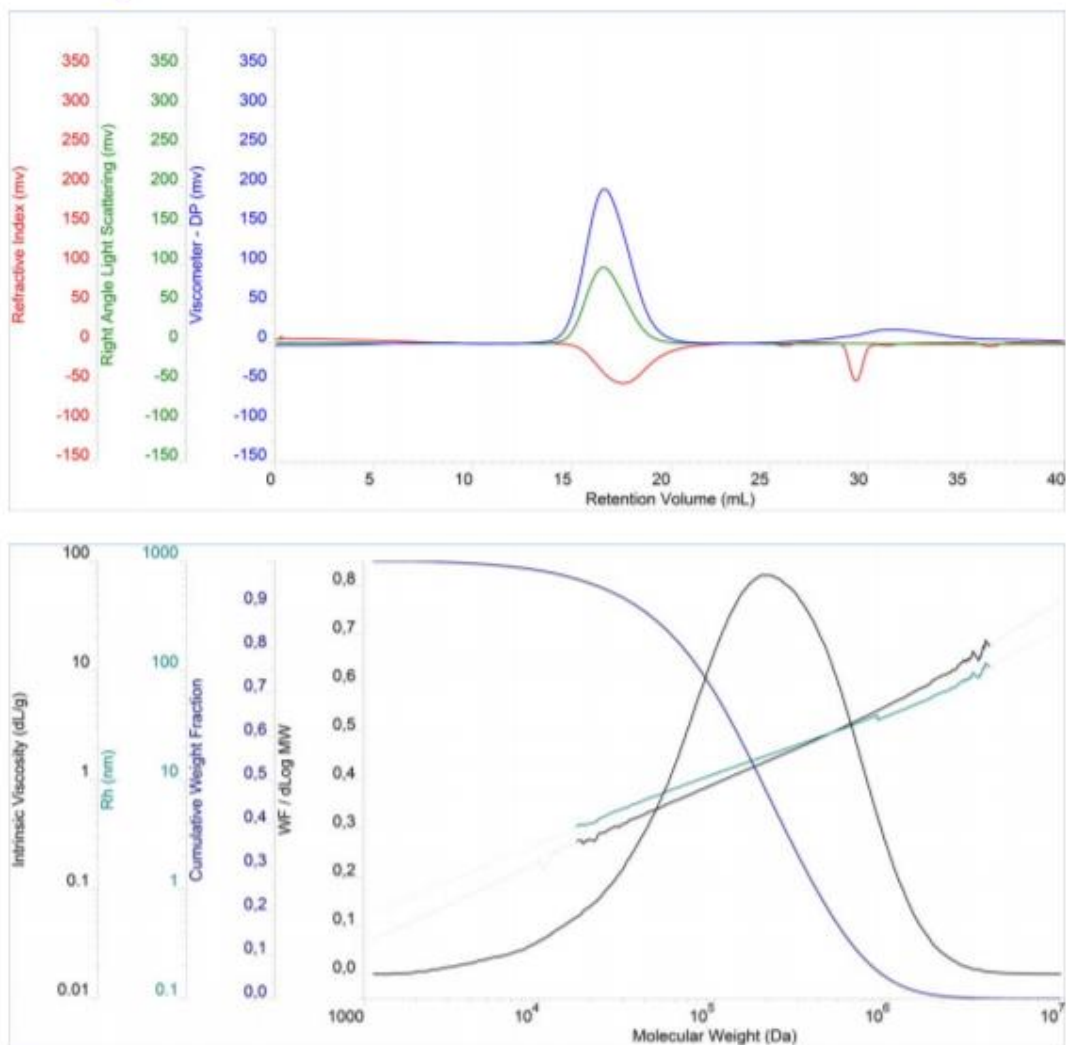
Rapport d'analyse

Chromatographie d'Exclusion Stérique Multi-Détection

Conditions analytiques

Acq. Date	Method	7- PP Vierge					
Nov 09, 2018 - 12:02:07	CT-12-11-2018-0001.vcm						
System	Column	Col Temp	Solvent	Flow Rate	Inj Vol	Conc	dn/dc
GPC HT	PSS	150.00	1,2,4 TCB	1.0000	100.0	2.5822	0.0960

Chromatogrammes



Résultats

Peak	Ret Vol (mL)	Mp (Da)	Mn (Da)	Mw (Da)	Mz (Da)	Mw/Mn
1	17.677	191 927	71 454	296 817	723 051	4.154

Figure A-10. High temperature SEC analysis of virgin iPP.

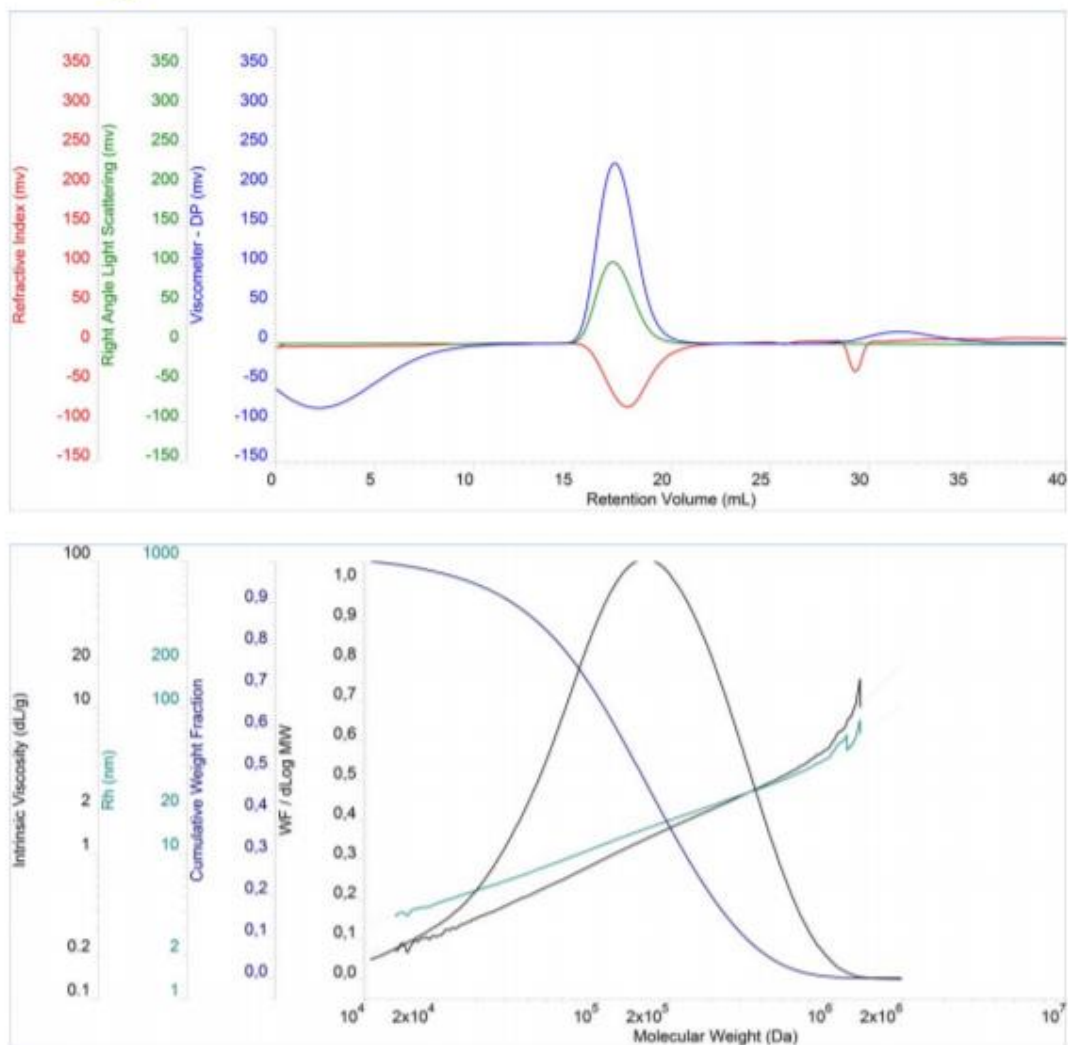
Rapport d'analyse

Chromatographie d'Exclusion Stérique Multi-Détection

Conditions analytiques

Acq. Date	Method	8-PP vierge recycl					
Nov 09, 2018 - 12:45:54	CT-12-11-2018-0002.vcm						
System	Column	Col Temp	Solvent	Flow Rate	Inj Vol	Conc	dn/dc
GPC HT	PSS	150.00	1,2,4 TCB	1.0000	100.0	3.4140	0.0960

Chromatogrammes



Résultats

Peak	Ret Vol (mL)	Mp (Da)	Mn (Da)	Mw (Da)	Mz (Da)	Mw/Mn
1	17.783	161 587	92 860	198 288	341 000	2.135

Figure A-11. High temperature SEC analysis of 9 times-recycled virgin iPP.

Annexe 8: Antioxidants capacities of vitamin E and vitamin C (DPPH method)

Figure A-12 and A-13 show the results obtained on alpha-tocopherol and ascorbic acid y the DPPH method. The results show that for both antioxidants, more than 80% of DPPH radicals are scavenged even through long thermal exposure.

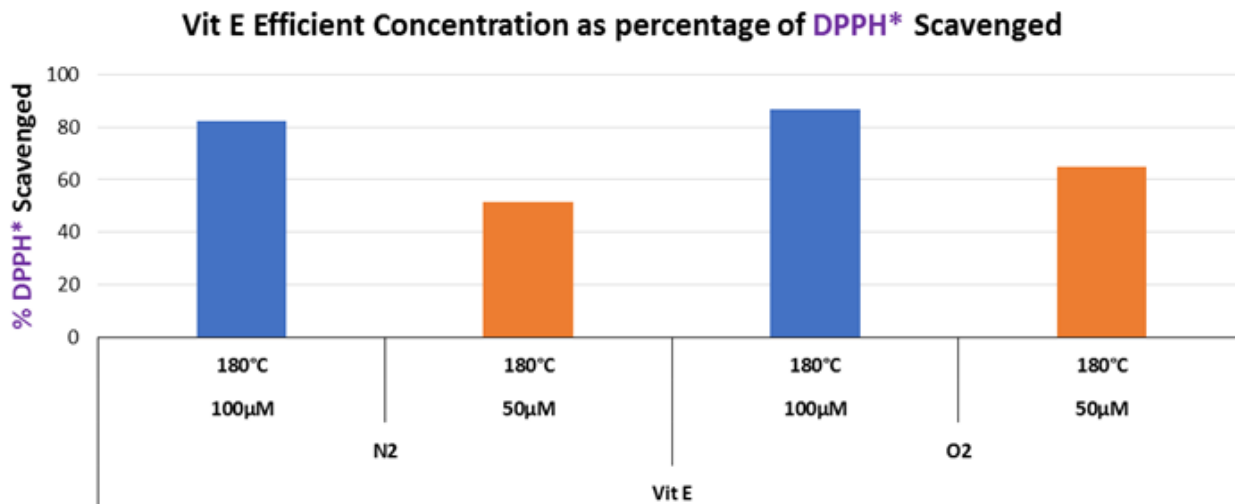


Figure A-12. Alpha tocopherol consumption of DPPH radicals under different thermal conditions.

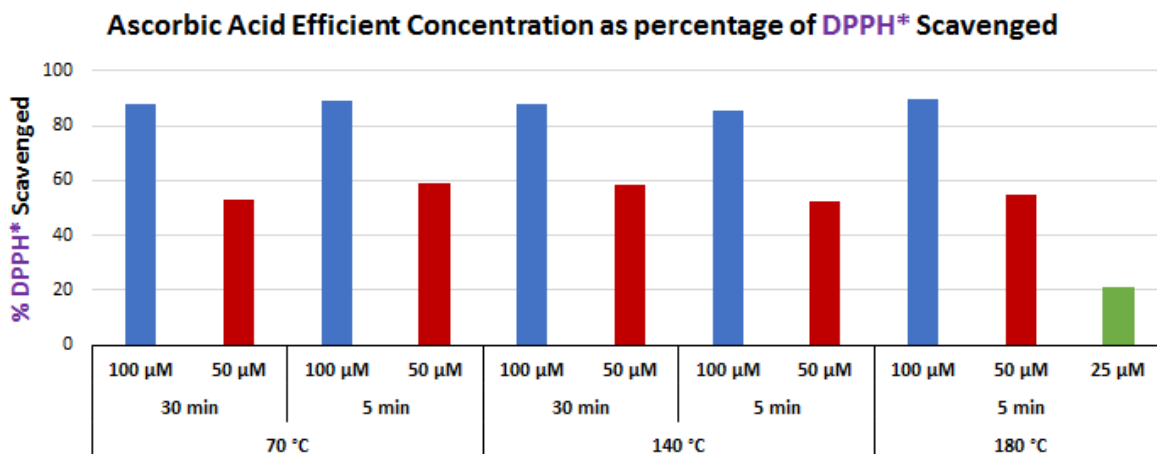


Figure A-13. Ascorbic acid consumption of DPPH radicals under different conditions.

Annexe 9: Hansen Solubility Parameters calculated thanks to surface tension and the HSPiP software.

The following tables show the results obtained for the contact angle evaluation (polymer's HSP assessment), the time to solubilize vitamin E and vitamin C and the obtained HSP.

Table A-2. Contact angle and HSP scores obtained after determining surface tension of different solvents on LDPE and iPP surfaces.

		HSP			Contact angle (°)		Score	
		δ_D	δ_P	δ_H	LDPE	iPP	LDPE	iPP
1	Ethyl acetate	15,8	5,3	7,2	4,20 ± 0,8	12,30 ± 1,01	2	3
2	Tetrahydrofuran (THF)	16,8	5,7	8,0	1,13 ± 6,70	7,60 ± 0,00	1	2
3	Chloroform	17,6	5,8	6,3	6,60 ± 0,38	11,50 ± 0,69	3	3
4	Dichloromethane	17,3	6,5	9,0	6,30 ± 0,97	17,00 ± 0,92	3	4
5	Xylene	17,6	1,0	3,1	3,40 ± 0,28	7,10 ± 0,15	1	1
6	Acetone	15,5	10,4	7,0	32,37 ± 1,50	25,73 ± 0,35	5	5
7	Toluene	18,0	1,4	2,0	1,00 ± 0,00	13,53 ± 0,06	1	3
8	Cyclohexane	16,8	0,0	0,2	5,43 ± 0,29	6,80 ± 0,50	2	1
9	N,N-Diméthyle Formamide (DMF)	17,4	13,7	11,3	42,20 ± 0,56	33,80 ± 0,98	6	5
10	Acetonitrile	15,3	18,0	6,1	42,37 ± 0,35	36,43 ± 0,85	6	5
11	Dimethyl Sulfoxide (DMSO)	18,4	16,4	10,2	44,87 ± 0,93	46,00 ± 0,30	6	6
12	Ethanol	15,8	8,8	19,4	18,90 ± 0,14	20,40 ± 0,40	5	4
13	Methanol	14,7	12,3	22,3	29,03 ± 1,06	20,27 ± 1,06	5	4
14	Water	15,5	16,0	42,3	75,57 ± 2,47	80,97 ± 0,15	6	6
15	Diethylene Glycol	16,6	12,0	20,7	56,17 ± 0,65	63,37 ± 0,50	6	6

Table A-3. HSP scores obtained after solubility tests of vitamin E (VE) and vitamin C (VC) in different solvents.

		HSP			Time to solubilize		Score	
		δ_D	δ_P	δ_H	VE	VC	VE	VC
1	Ethyl acetate	15,8	5,3	7,2	33''	2'42''	1	4
2	Tetrahydrofuran (THF)	16,8	5,7	8,0	45''	1'20''	2	3
3	Chloroform	17,6	5,8	6,3	4''	2'35''	1	4
4	Dichloromethane	17,3	6,5	9,0	4''	2'27''	1	4
5	Xylene	17,6	1,0	3,1	34''	∞	1	4
6	Acetone	15,5	10,4	7,0	1'14''	32''	2	2
7	Toluene	18,0	1,4	2,0	55'	1'52''	2	3
8	Cyclohexane	16,8	0,0	0,2	1'44''	1'59''	2	3
9	N,N-Diméthyle Formamide (DMF)	17,4	13,7	11,3	54''	4''	2	1
10	Acetonitrile	15,3	18,0	6,1	2'54''	25''	3	2
11	Dimethyl Sulfoxide (DMSO)	18,4	16,4	10,2	15''	6''	2	1
12	Ethanol	15,8	8,8	19,4	1'53''	4''	2	1
13	Methanol	14,7	12,3	22,3	2'13''	3''	3	1
14	Water	15,5	16,0	42,3	∞	2''	3	1
15	Diethylene Glycol	16,6	12,0	20,7	∞	3''	3	1

Table A-4. Hansen Solubility Parameters of LDPE and iPP.

	δ_D	δ_P	δ_H
Polyethylene			
Theoretical data *	17,6	0,0	0,0
Experimental data	17,1 ± 0,7	0,7 ± 0,3	6,8 ± 0,9
Polypropylene			
Theoretical data *	18,0	0,0	0,0
Experimental data	17,1 ± 0,3	0,1 ± 0,8	1,8 ± 0,5

*From Hansen Solubility data base

Table A-5. Hansen Solubility Parameters of VE and VC.

	δ_D	δ_P	δ_H
<i>α-tocopherol (VE)</i>			
Theoretical data *	17,0	1,7	4,6
Group contribution data	16,9	1,5	3,6
Experimental data	17,3 \pm 0,6	2,2 \pm 1,8	7,3 \pm 1,7
<i>Ascorbic acid (VC)</i>			
Theoretical data *	18,0	11,7	25,5
Group contribution data	20,3	14,2	25,8
Experimental data	17,6 \pm 0,7	15,6 \pm 1,0	19,0 \pm 0,8
<i>Tannins (tan)</i>			
Theoretical data *			
Group contribution data			
Experimental data	22.6 \pm 0.4	10.1 \pm 0.9	12.5 \pm 1.1

*From Hansen Solubility data base

Annexe 10: Additives blends.

Vitamin E, green tea mixture

Green tea (GT) is well known for its excellent antioxidant capacity; nonetheless it is very poor in terms of temperature resistance, as seen before. However, it can be protected by other compounds such as vitamin E or linseed oil. Vitamin E and green tea were thus mixed in several amounts from 5 to 50% green tea (just like tannins, green tea have a very low density: 0,43g/ml). The mixture that was obtained is a green paste which is thicker and thicker with growing amounts of green tea. As for the other mixes, TGA analyses were done (**figure A-14** and **figure A-15**) showing improvements in terms of temperature resistance.

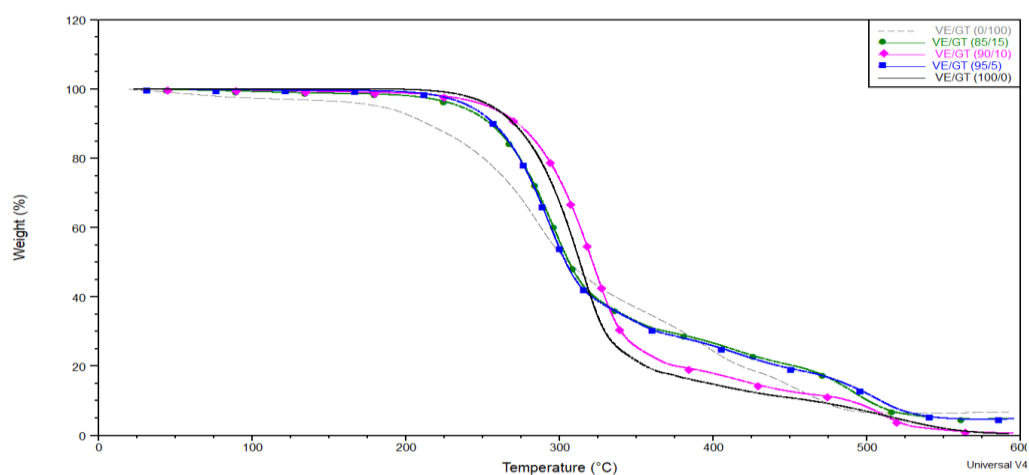


Figure A-14 Mass loss of vitamin E/green tea different ratios.

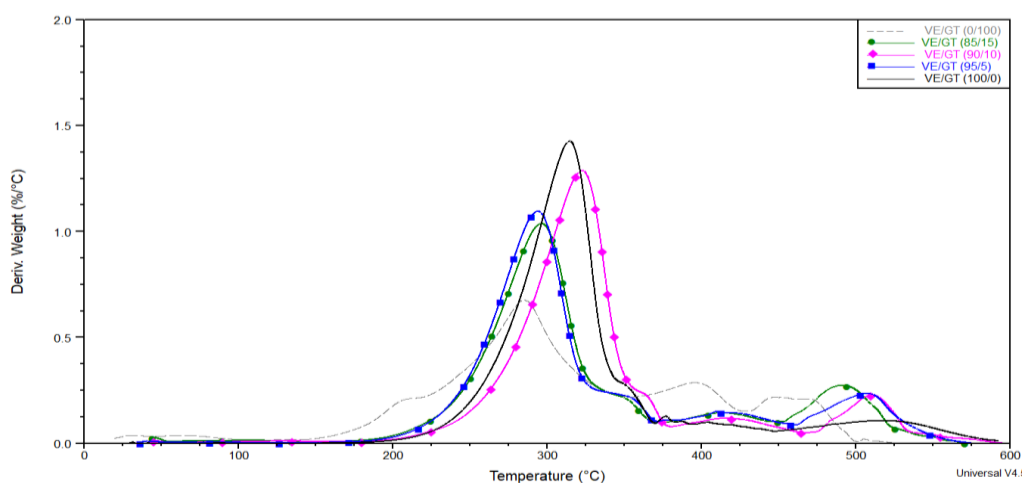


Figure A-15. Derivative weight loss of the different vitamin E/green tea ratios.

An optimum appears: the VE/GT (90/10) ratio (pink curve) shows a thermogram with an improve resistance to high temperature. At the opposite of the previous blends (VE/VC and VE/Tan), the onset degradation temperature isn't delayed in comparison with VE alone, but its thermal resistivity in the temperature range going from 280-500°C is improved. This is confirmed by the derivative mass loss curve in **figure A-15** which shows a shift of the maximum of degradation for the 90/10 ratio. A synergism can here also be suspected.

These thermograms looks a lot like tannins' ones, especially due to the very late degradation of a portion of the samples. Actually, whether it is for tannins or for green tea, some species degrades around 500°C letting us think that those compounds could be some of the polyphenols presents in both additives.

Vitamin E, linseed oil mixture

For compatibility reasons, the previous mixes were both problematic. We thus decided to try to analyse a mixture of linseed oil (LSO) and alpha-tocopherol (VE) which are two compatible oils soluble. Many formulations were tried to evaluate which was the optimum quantity of each oils: LSO/VE (50/50), LSO/VE (75/25); LSO/VE (60/40); LSO/VE (85/15); LSO/VE (95/5); LSO/VE (97.5/2.5); LSO/VE (99/1) and LSO/VE (99.5/0.5). All these formulations were analysed by TGA.

Figure A-16 and **A-17** show the mass loss evolution at 250°C and 300°C according to the amount of vitamin E within linseed oil. The formulation that were tried are: LSO/VE (50/50), LSO/VE (75/25); LSO/VE (60/40); LSO/VE (85/15); LSO/VE (95/5); LSO/VE (97.5/2.5); LSO/VE (99/1) and LSO/VE (99.5/0.5). A tendency appeared when evaluating the mass loss as a function of the proportion of each oil in the mix.

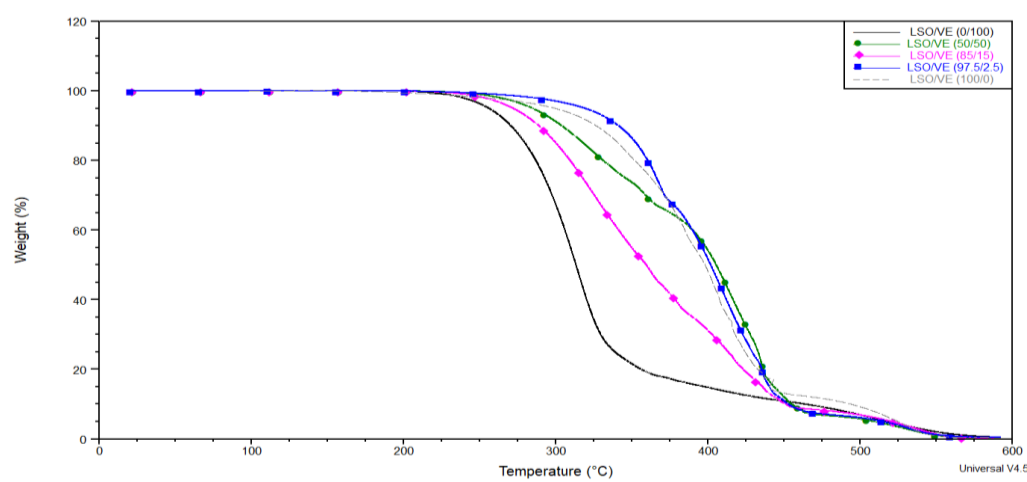


Figure A-16. Mass loss of linseed oil/vitamin E different ratios.

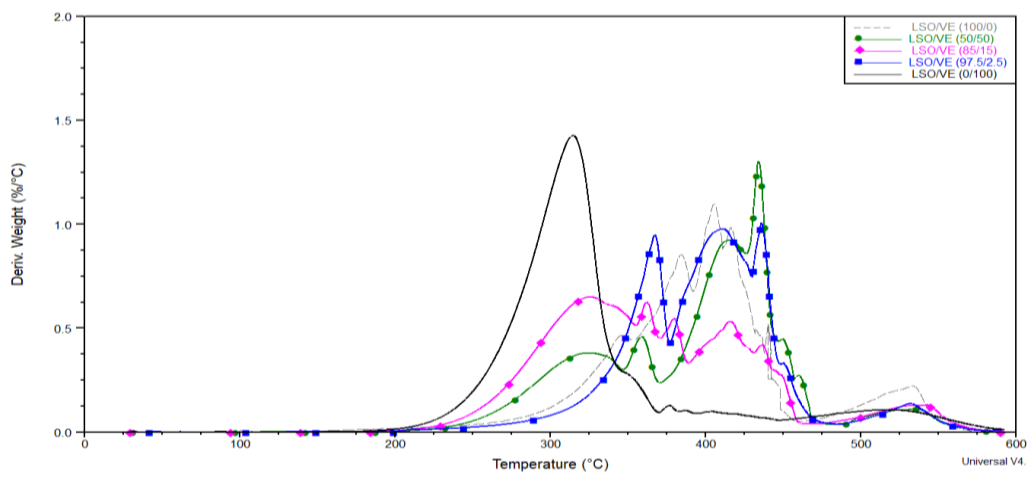


Figure A-17. Derivative weight loss of the different linseed oil/vitamin E ratios.

The effect of both oils on each other is relatively visible. The blends 50/50 and 85/15 presents thermograms remaining in-between the raw additives curves, showing no specific mechanism. However, the ratio 97.5/2.5 seems to have a better thermal protection as the curve goes beyond the raw linseed oil’s one. The maximums of degradation shown on **figure A-17** also seem to be shifted toward higher temperatures.

A tendency appeared when evaluating the mass loss as a function of the proportion of each oil in the mix. Curves illustrating this tendency are shown below.

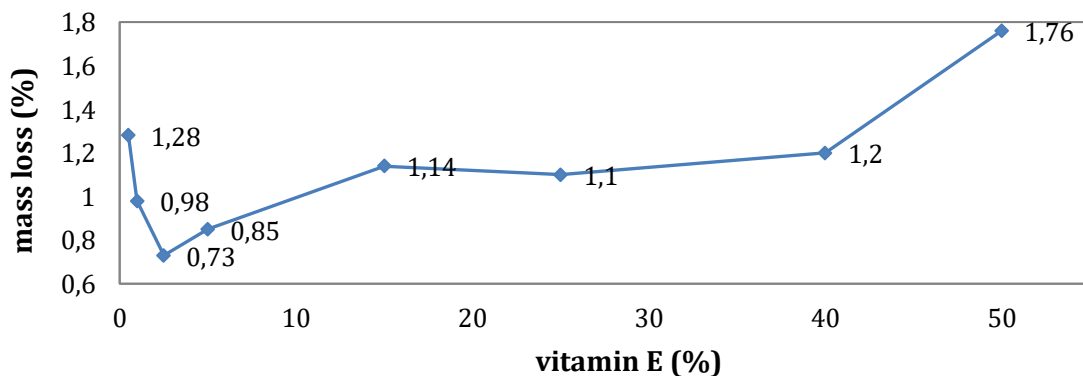


Figure A-18. Mass loss at 250°C of the linseed oil/vitamin E blend as a function of the vitamin E amount.

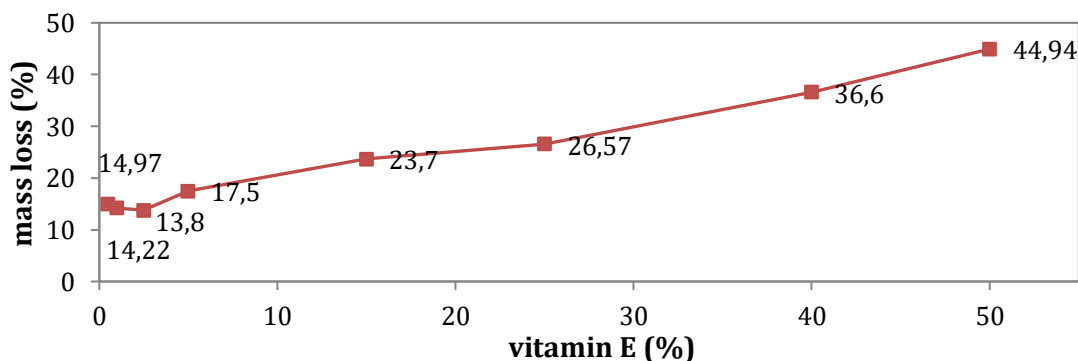


Figure A-19. Mass loss at 300°C of the linseed oil/vitamin E blend as a function of the vitamin E amount.

This tendency is very clear at 250°C: from 50% to 2.5% of vitamin E, the mass loss diminishes with diminishing amounts of vitamin E; below 2.5%, the mass loss increases again. The optimum mix seems to be the LSO/VE (97.5/2.5) which offers the best thermal resistance.

Linseed oil, green tea mixture

For the same reasons as vitamin E/green tea, the mix linseed oil/green tea seems promising: LSO/GT (10/90), LSO/GT (50/50) and LSO/GT (90/10). The different formulations were analysed by TGA and also showed impressive results for the protection of green tea (**figure A-20**).

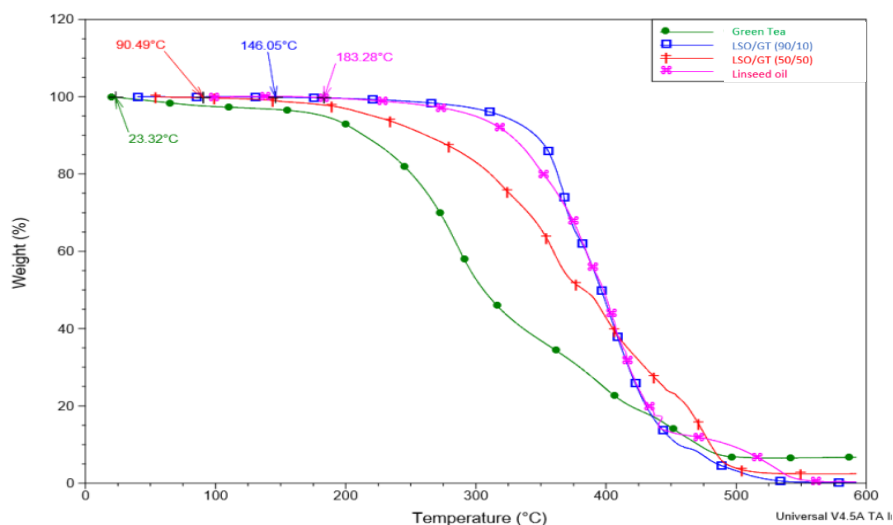


Figure A-20. TGA analysis of the different formulations for the linseed oil/green tea mix.

There is a gain of almost 120°C on the onset temperature and a lowering of the mass loss at 300°C from 47% to 3,2%. Also, the “best” curve goes beyond pure antioxidants curves showing here again a clear protection effect. This effect of protection could be attributed to a protection of antioxidant on each other.

Tertiary mixture of antioxidants

Ternary blend involving linseed oil, vitamin E and green tea or linseed oil, vitamin E and vitamin C were tried. To avoid having to many formulations to try, we relied on the results obtained for the linseed oil/vitamin E mix. The basis of this study is thus made on a mix of 97.5%v linseed oil and 2.5%v vitamin E with various amount of green tea as explained in the **table A-6**.

Table A-6. Different formulations for the linseed oil/vitamin E/green tea mix.

Formulation	Quantities (%m)
α -tocopherol	2,4
Linseed oil	96,6
Green tea	1
α -tocopherol	5
Linseed oil	90
Green tea	5
α -tocopherol	2,3
Linseed oil	87,7
Green tea	10

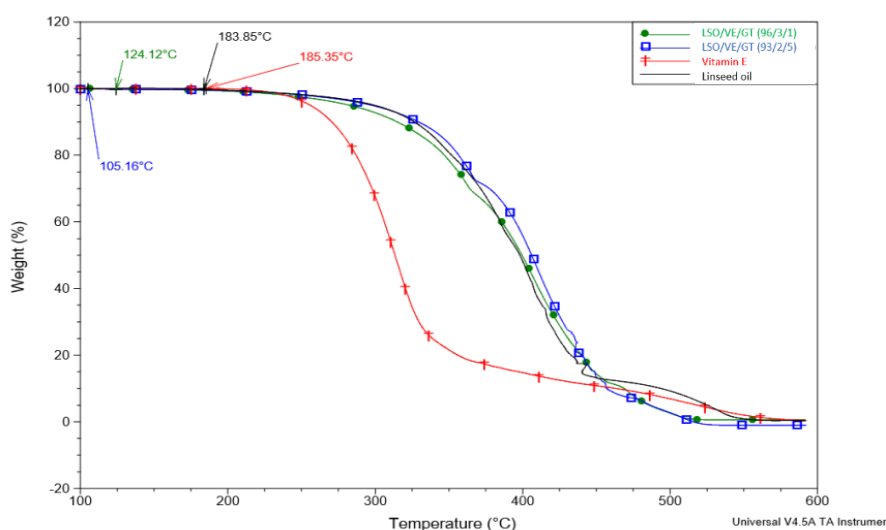


Figure A-21. TGA analysis of the different formulations for the linseed oil/vitamin E/green tea mix (a) normal mode (b) derivative mode.

The protection effect is visible as the curve of the mix goes ahead of the curves of pure antioxidants. The gain in onset temperature is lower than for binary blends but still almost 80°C are added and the mass loss at 300°C is ten times lower than for green tea alone (5,4% mass loss at 300°C for Aim091; 51,34% mass loss at 300°C for green tea).

The same analyses were done on a tertiary mix: Linseed oil, vitamin C and vitamin E. The two formulations are:

- Aim10: Linseed oil (88,72 %m); α -tocopherol (2,28% m) and ascorbic acid (9% m).

- Aim101: Linseed oil (78 %m); α -tocopherol (2%m) and ascorbic acid (20%m).

The mixes seem efficient in terms of thermal protection when compared to the vitamin C alone. In fact, it is visible that the kinetic of degradation is improved compared to the vitamin C alone: at 300°C the mixture Aim10 as a mass loss of 13,3% for 31,51% for Aim101 and 47,7% for vitamin C. The onset temperature is also slightly improved. Even if the mixes don't equal the linseed oil degradation kinetic, they tend to get closer to it. We can thus say that vitamin C is protected by the two oils. Nevertheless, the Aim10 remains interesting even if the mixing process needs to be optimized. About the peak's distribution, it is clear that vitamin C and linseed oil are present (except the peak at 385°C from linseed oil), but it is less obvious for vitamin E. Also, the maximum degradation for Aim101 is reached by a characteristic peak of vitamin C (around 216°C) meaning that vitamin C causes the bigger degradation in this blend whereas the maximum degradation for Aim10 is reached by a characteristic peak of linseed oil (the ones around 400°C).

CHAPTER 3- FORMULATION

Annexe 11: Linseed oil degradation

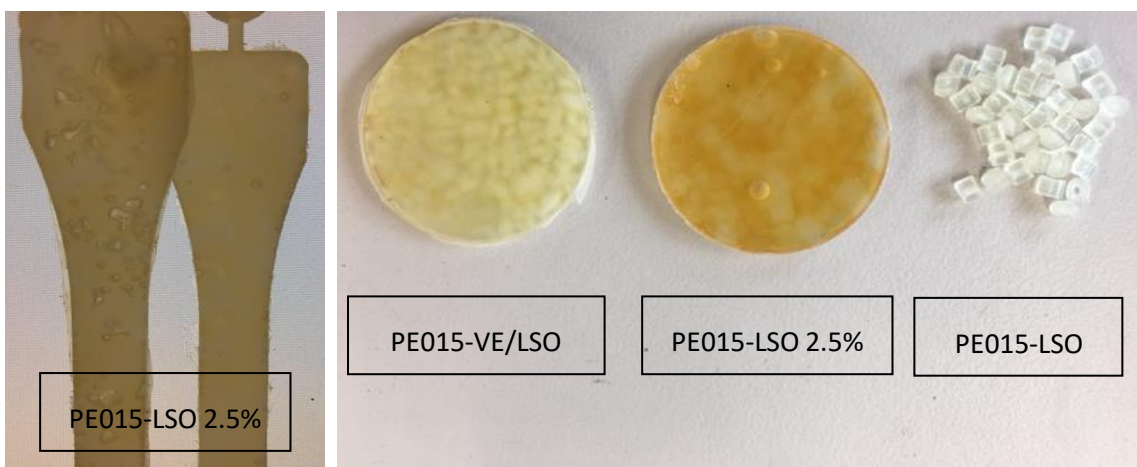


Figure A-22. Physical aspect of polymers containing linseed oil.

Figure A-22 shows the physical aspect of the blends containing linseed oil. It puts forward the presence of degradation (the oil releases CO₂ hence creating bubbles).

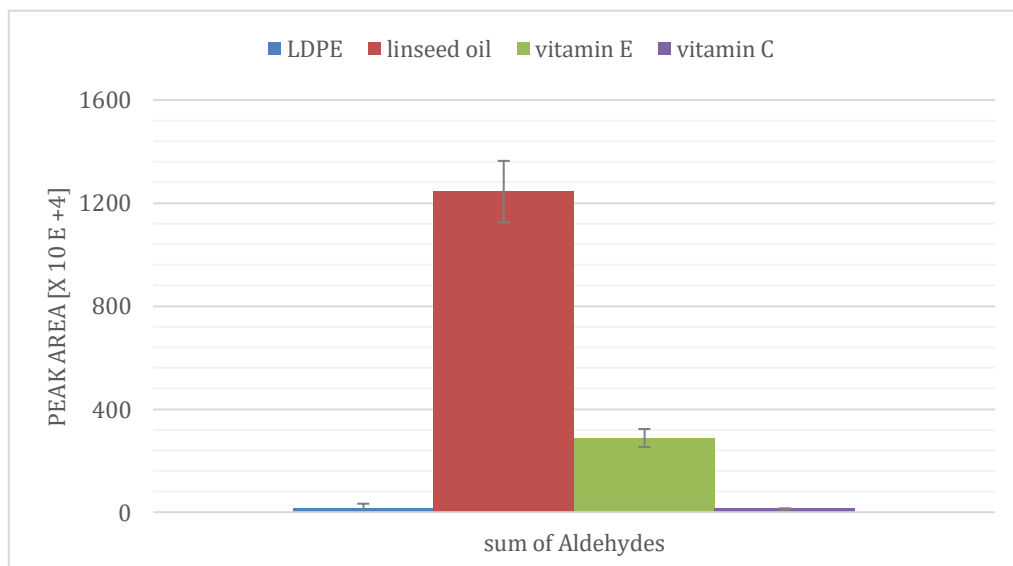


Figure A-23. Amount of aldehydes released from the product of our study.

Annexe 12: Blends involving green tea

Table A-7. Summary of the new formulations.

	Polymers	Additives	Rate of additives	Recycling
Single additives blends	LDPE - iPP	Green tea	2.5%	√
		Tannins		√
Binary blends		Vitamin E / Green tea (90/10)		√
Tertiary blends		Vitamin E / Vitamin C/ Green tea (90/5/5)		√
		Vitamin E / Vitamin C/ Tanins (90/5/5)		√

The previously described blends were characterised.

Single additives blends (binary blends)

Green tea was dried and introduced in PE and iPP. Different rates were tried: 0.5%, 1% and 1.5%.

In all the cases, the compounds' thermal behaviours are always lower than those of the bare polymers. It is even stronger in the case of the PP, probably due to the high extrusion temperature. Moreover, OOT were achieved on these composites and compared with reference blends (**figure A-24**).

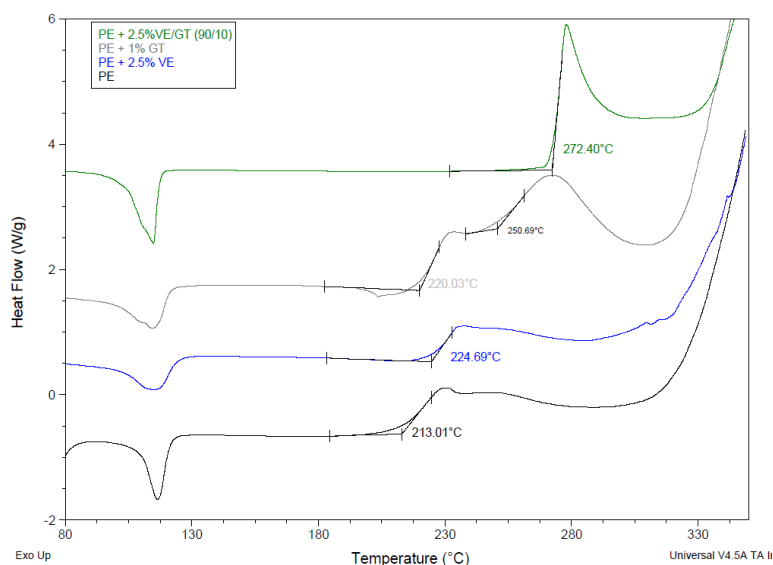


Figure A-24. OOT of PE compounded with green tea.

The Onset Oxidation Temperature shows that the stabilisation provided by the single components (green tea alone) is much weaker than the one provided by the VE's equivalent blend. Moreover, we can observe that the oxidation happens in two times for green tea. This reveals the presence of a secondary oxidation probably

related to the complexity of this compound. Actually, green tea is made of 20 et 36% of polyphenols, hence it contains several groups of flavonoids.

Moreover, the compounds were recycled up to 9 times, not showing big deviations in the values. The obtained OOT values are summarized in **table A-8** below.

Table A-8. OOT results for the different blends involving tannins and green tea.

	Additives (%)	Onset Oxidation Temperature (OOT) (°C)	Difference in OOT with the bare polymer matrix (°C)
PE $T_{ox} = 214,14 \pm 0,13$	Green tea 0,5	$219,98 \pm 0,49$	$5,85 \pm 0,49$
	1,0	$220,61 \pm 1,29$	$6,47 \pm 1,29$
	1,5	$222,13 \pm 0,06$	$7,99 \pm 0,06$
PP $T_{ox} = 197,52 \pm 0,22$	Green tea 0,5	$209,21 \pm 1,39$	$11,69 \pm 1,39$
	1,0	$211,91 \pm 0,15$	$14,39 \pm 1,77$
	1,5	$222,16 \pm 0,15$	$24,64 \pm 0,15$

Tertiary blends

Thermal tests were achieved to assess the efficiency of green tea blended with vitamin E. The thermal spectra show that the optimum blend is 90% vitamin E and 10% green tea: it degrades later than the other ratios. Hence, this blend was also introduced in LDPE and in iPP. The OOT reveal much better results (**figure A-25**), putting forward a possible synergetic effect.

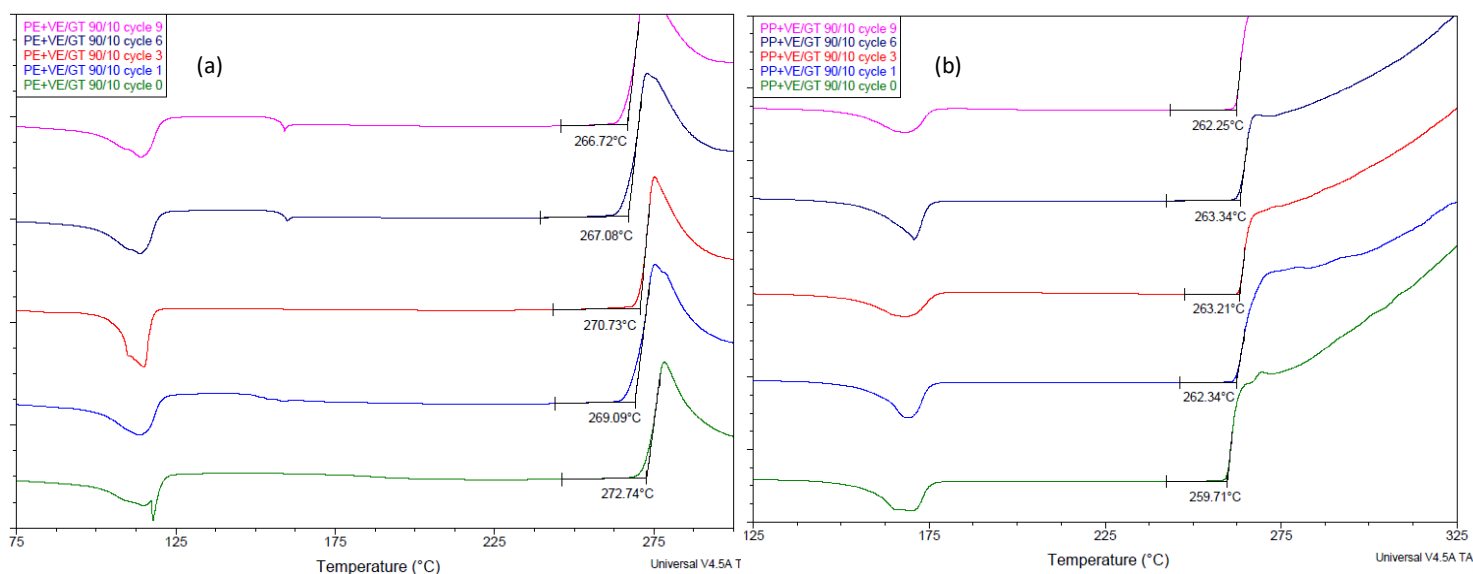


Figure A-25. Binary blends of vitamin E and green tea in PE (a) and in iPP (b).

Those results open the door to new research about green tea's synergy with alpha-tocopherol, or more specifically polyphenols and alpha tocopherol. Phenolic acids such as rosmarinic acid showed synergetic interactions with beta-tocopherol. Moreover, the synergism between green tea and alpha tocopherol was studied showing synergistic and antagonist effect. Actually, according to the medium in which the effect happens the synergy can be either positive or negative: strong synergistic effect was found for the combination of alpha-tocopherol and green tea in an oil-in-water emulsion and in pure bulk oil. On the contrary, combinations of alpha-tocopherol with either epicatechin or catechin showed clear synergistic effects in both heterogeneous systems, but antagonistic or additive effects in bulk oil.

Quaternary blends

Finally, tertiary blends were achieved to check if it could bring something to associates 3 strong antioxidants. The three additives were blended on different ways:

- A pre-prepared master batch of Polymer + 2.5% VE/VC (95/5) was used and blended with the green tea powder (bag mixing);
- The three components are putted directly in a syringe in the appropriate quantities and blended to the polymer drop by drop.

The second process always showed better properties than the first one, mainly related to a much better homogeneity.

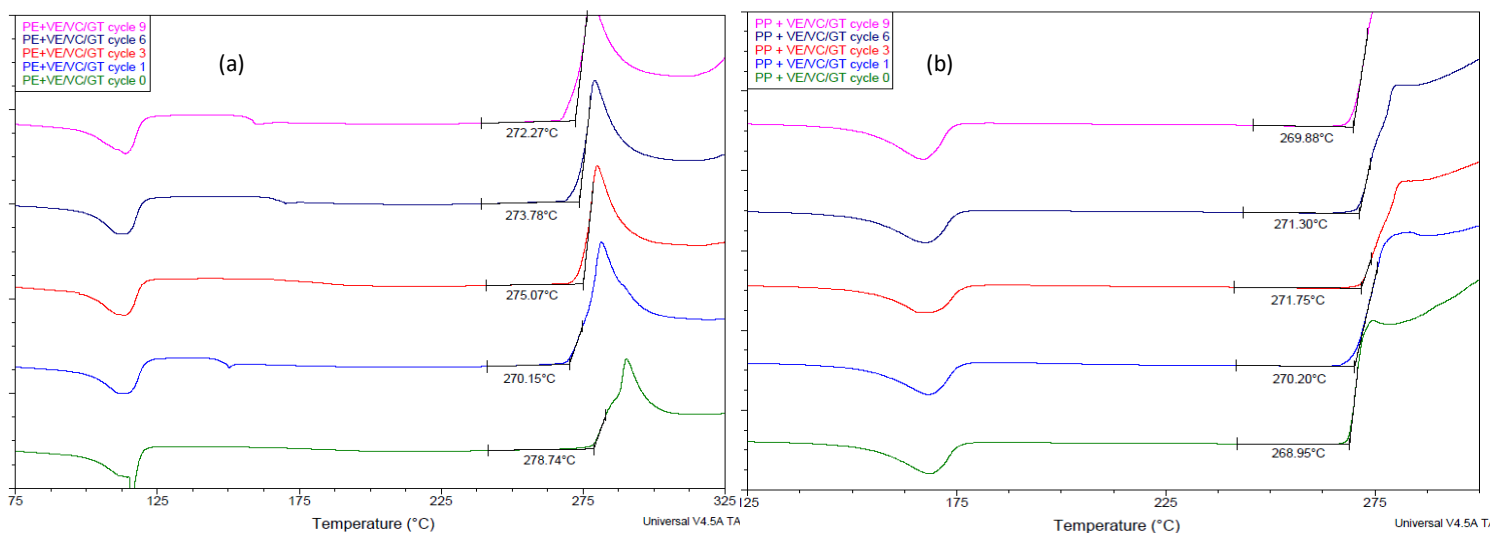
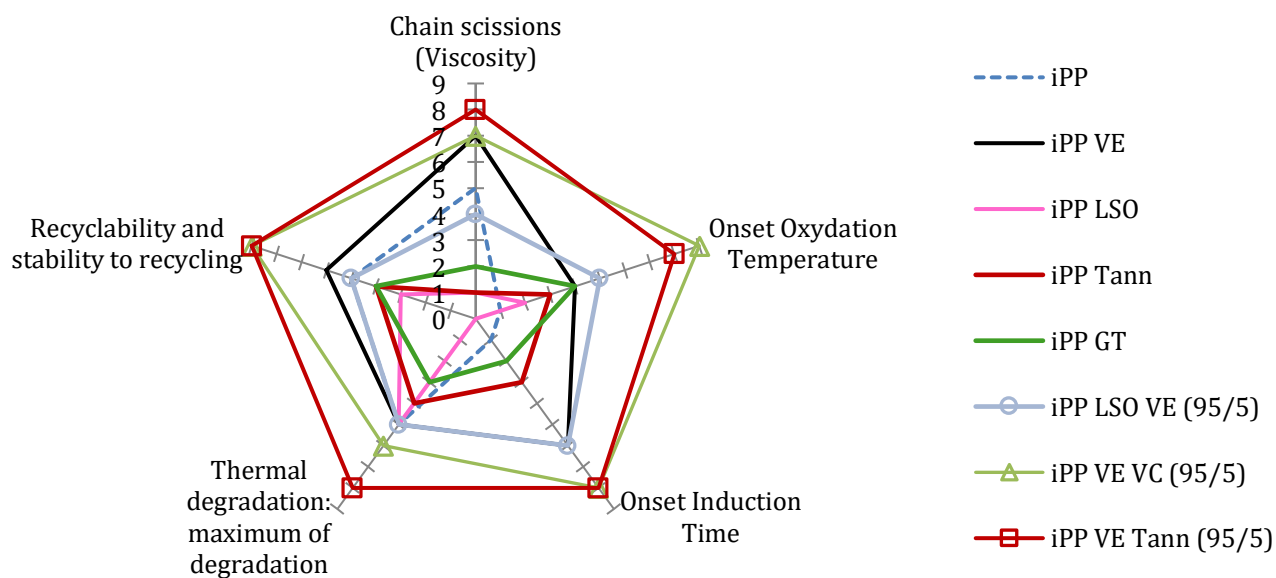
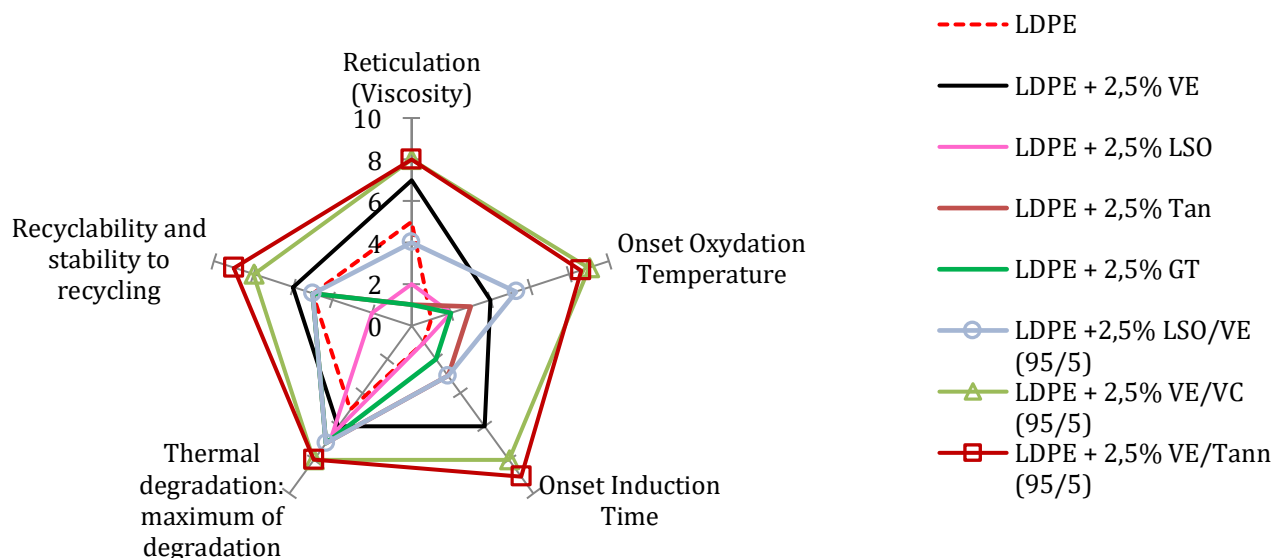


Figure A-26. Tertiary blends of vitamin E, C and tannins in PE (a) and in IPP (b).

Green tea based-ternary blends show slightly better results (let's say equivalent) to their binary equivalent. However, this effect is almost negligible. At the opposite of green tea, ternary blends involving tannins are less good than their binary equivalent showing poorer onset oxidation temperature.

Annexe 13: Summary of the studied formulations



- Viscosity (Branching or chain scissions) was evaluated based on the viscosity evolution by rotational rheology, compared to the virgin polymers;
- Onset Oxidation Temperature marks were given according to the delay in oxidation temperature (DSC) provided by the additives used compared to virgin polymers;
- Onset Induction Time marks were given according to the delay in oxidation temperature (DSC) provided by the additives used compared to virgin polymers;
- Thermal degradation maximum degradation marks were given according to the delay in the apex of the derivative curve of the thermograms obtained by TGA provided by the additives used compared to virgin polymers;
- Recyclability and the stability to recycling was evaluated by combining all the analysis realised on the recycled samples (TGA, DSC, rheology, migration).

CHAPTER 4 – MIGRATION ASSESSMENT

Annexe 14: Migration of alpha-tocopherol drawn as a function of $t^{1/2}$ in order to evaluate the diffusion coefficients

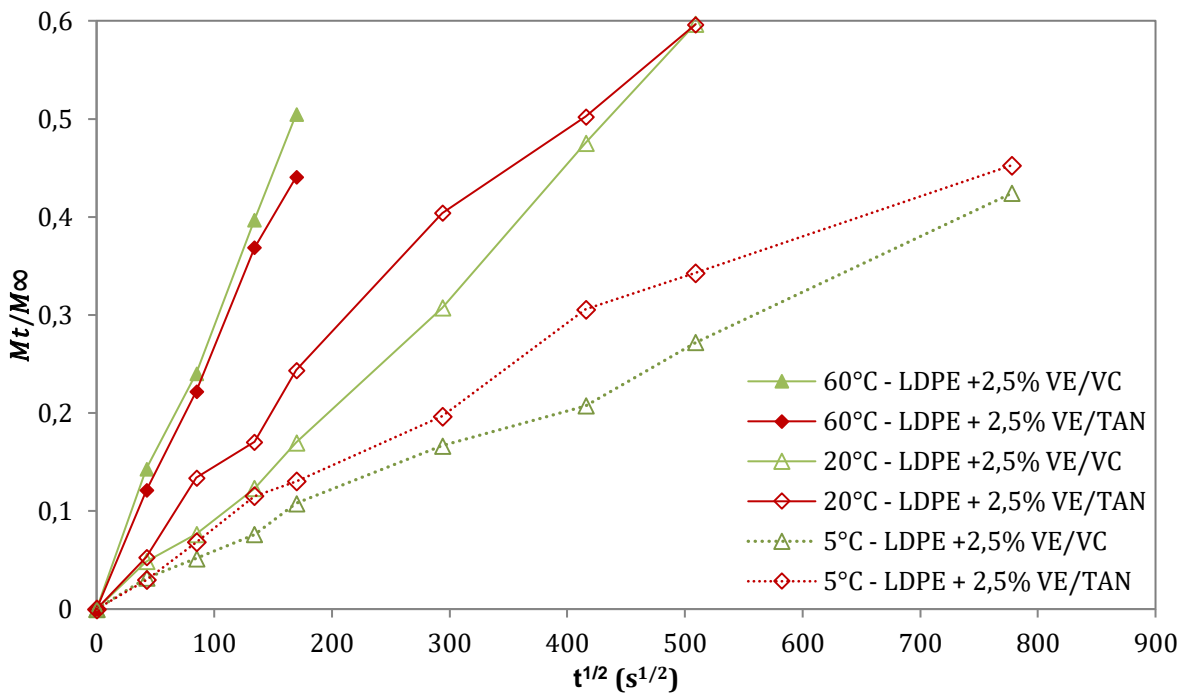


Figure A-27. Evolution of the migration of alpha-tocopherol for LDPE's formulations.

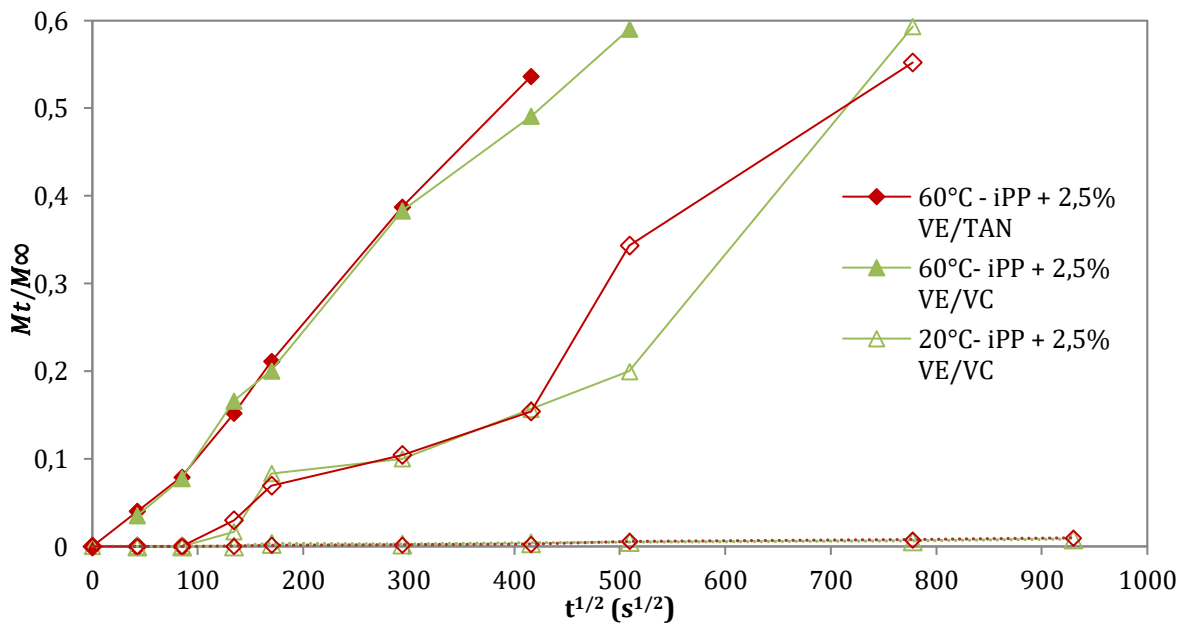


Figure A-28. Evolution of the migration of alpha-tocopherol for iPP's formulations.

Annexe 15: LESA-MS analysis of the different formulations.

Polypropylene-based formulations

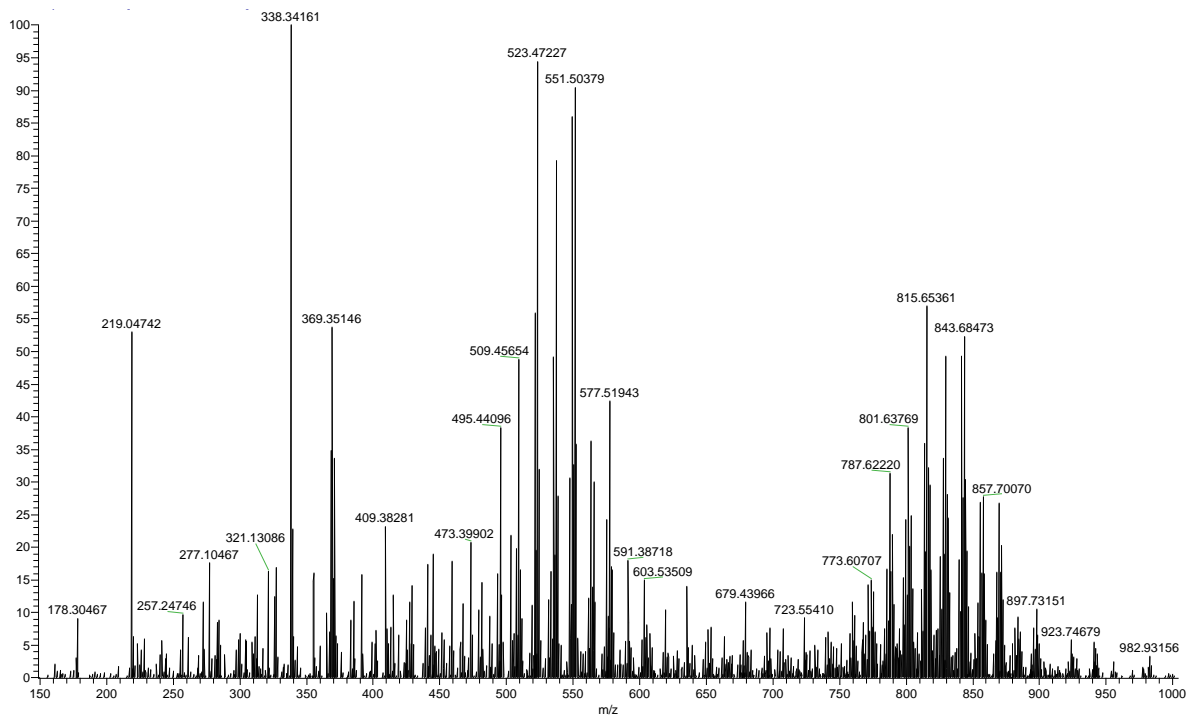


Figure A-29. LESA-MS spectra of iPP + 2.5% LSO

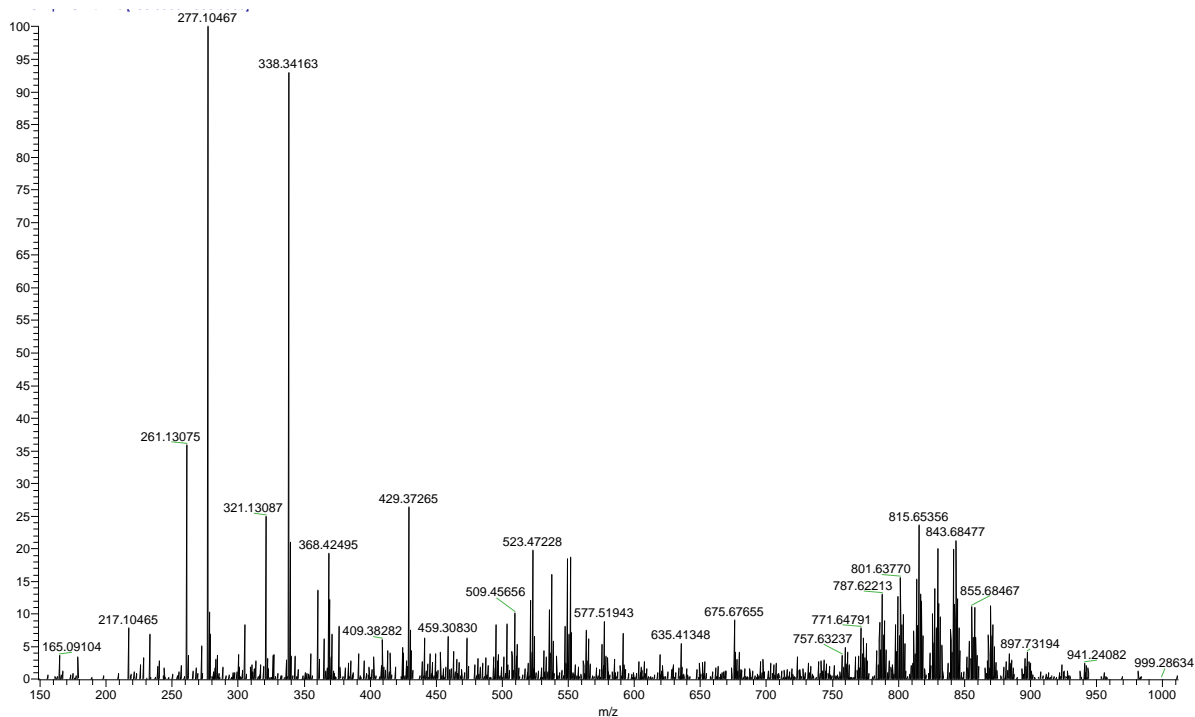


Figure A-30. LESA-MS spectra of iPP + 2.5% VE

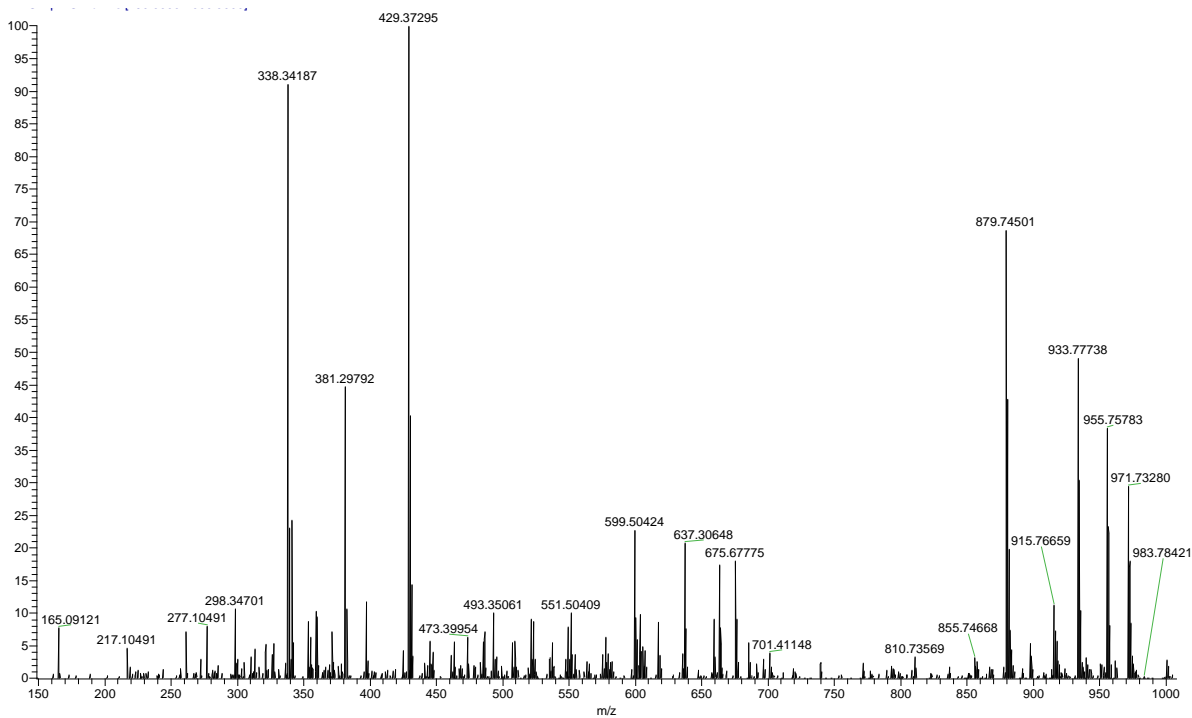


Figure A-31. LESA-MS spectra of iPP + 2.5% VE/VC (95/5).

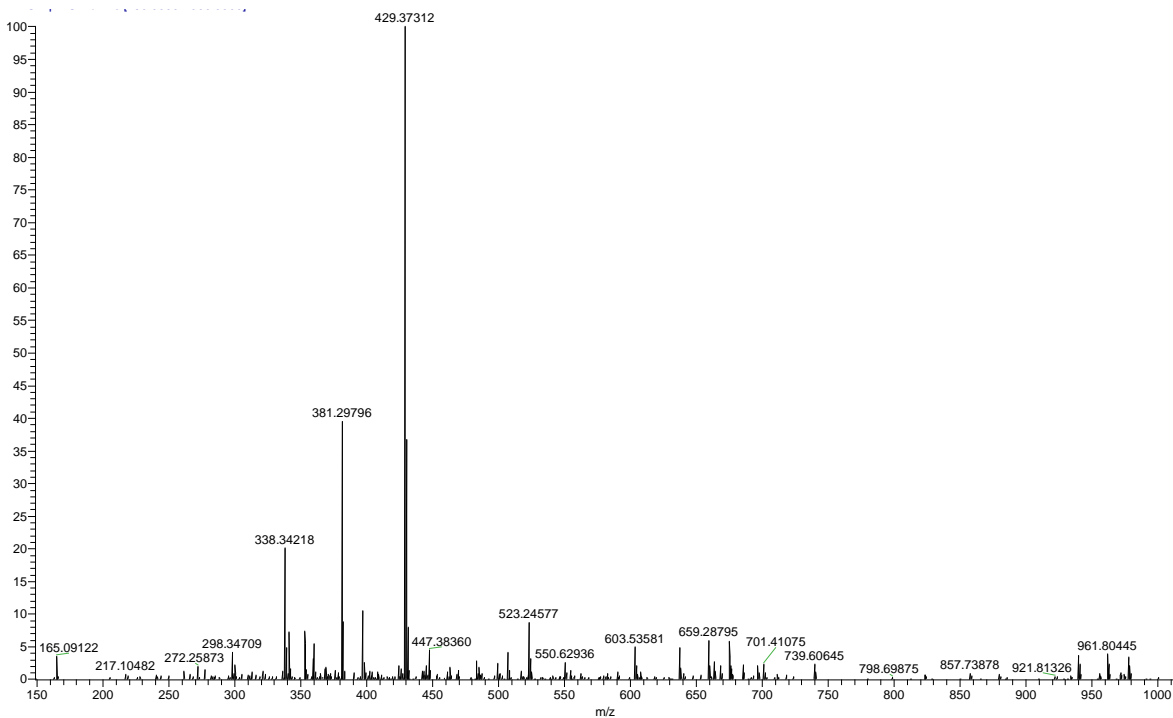


Figure A-32. LESA-MS spectra of iPP + 2.5% VE/Tan (95/5).

Polyethylene-based formulation

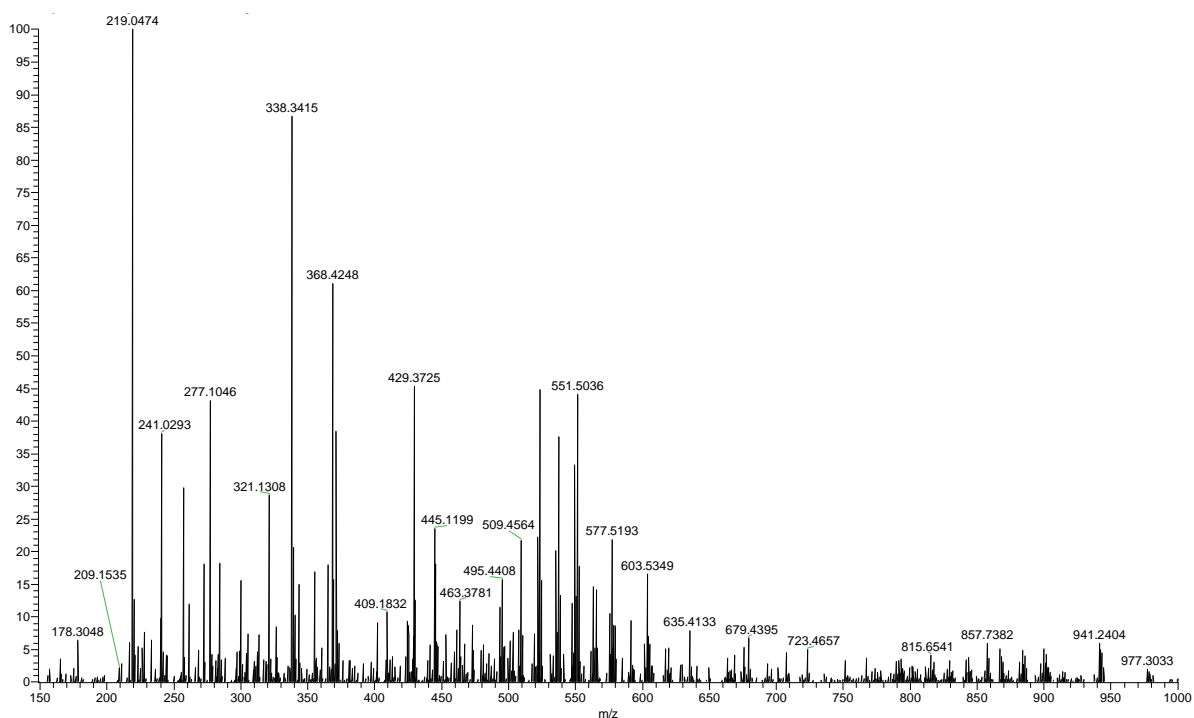


Figure A-33. LESA-MS spectra of LDPE + 2.5% LSO

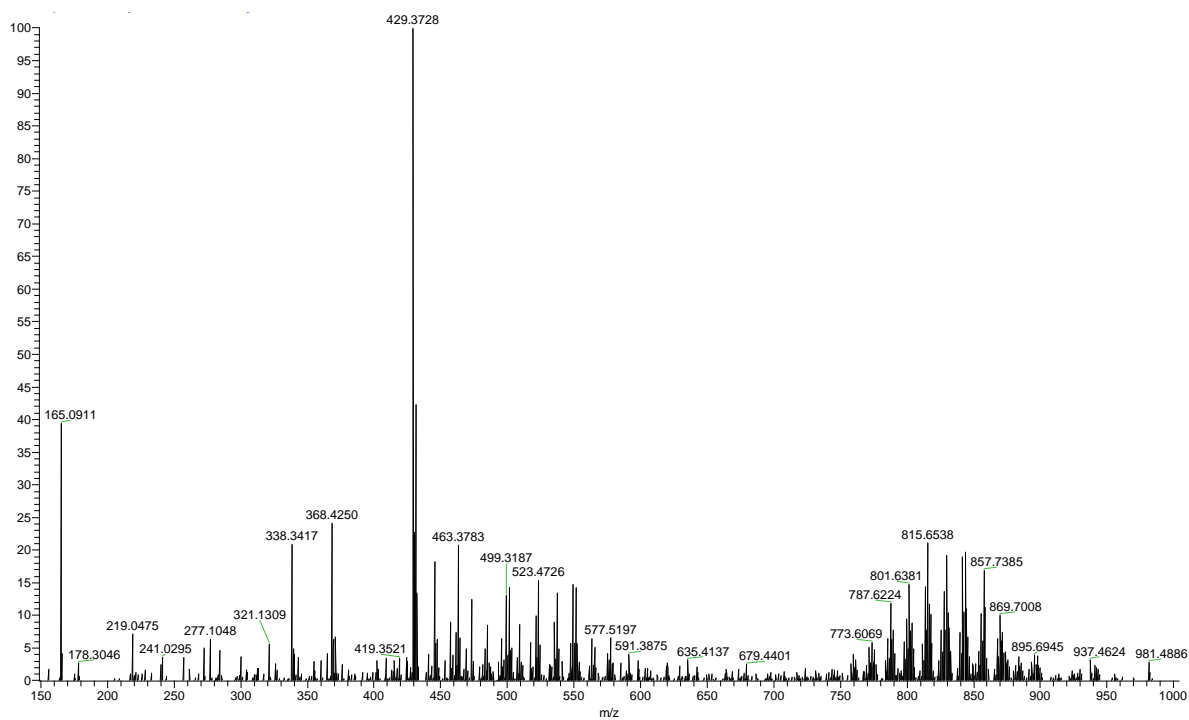


Figure A-34. LESA-MS spectra of LDPE + 2.5% VE.

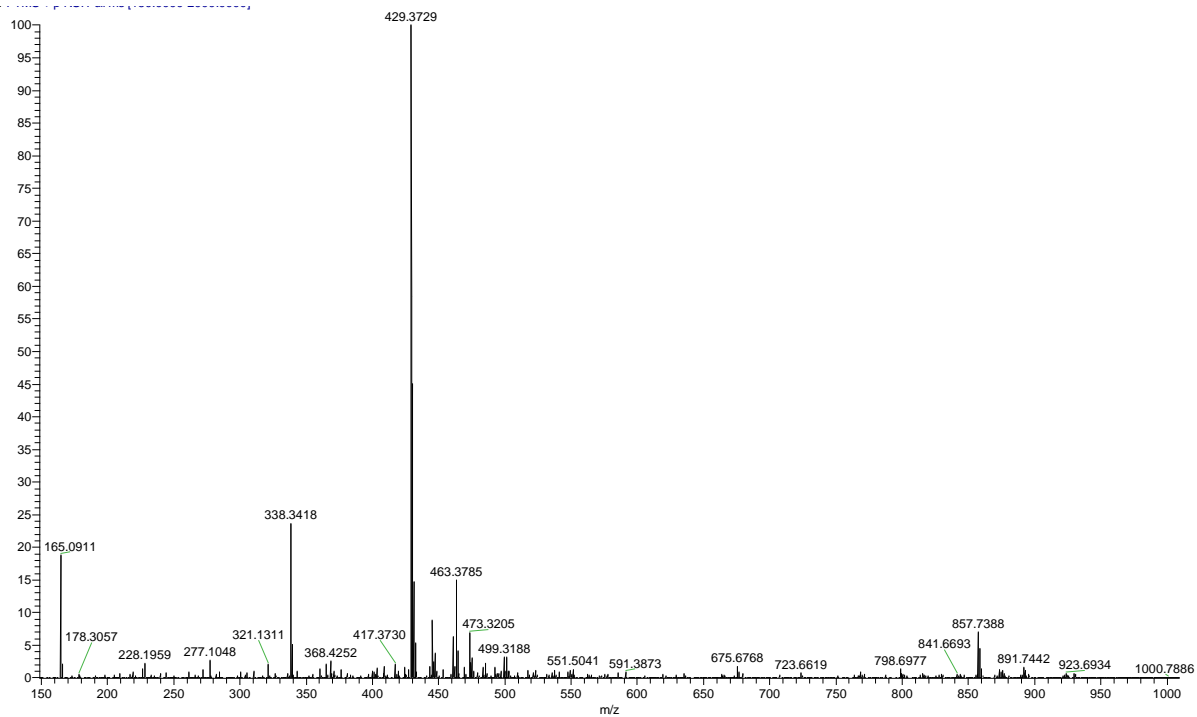


Figure A-35. LESA-MS spectra of LDPE + 2.5% VE/VC (95/5).

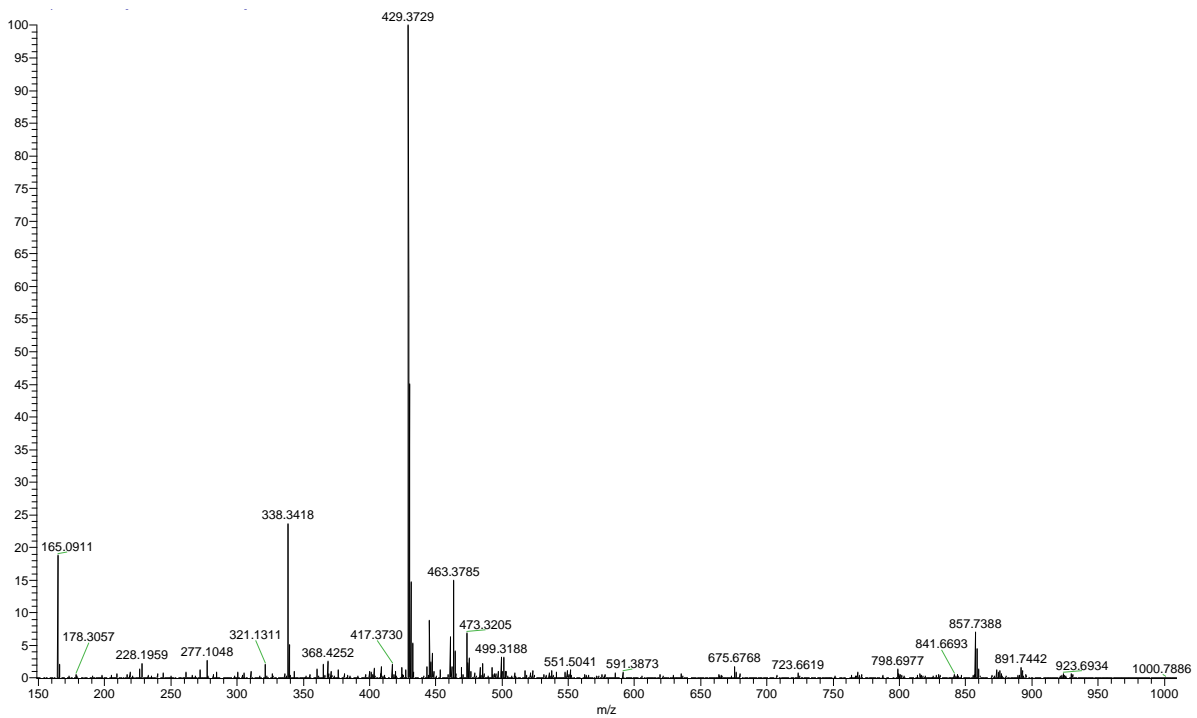


Figure A-36. LESA-MS spectra of LDPE + 2.5% VE/Tan (95/5).

Recycled blends

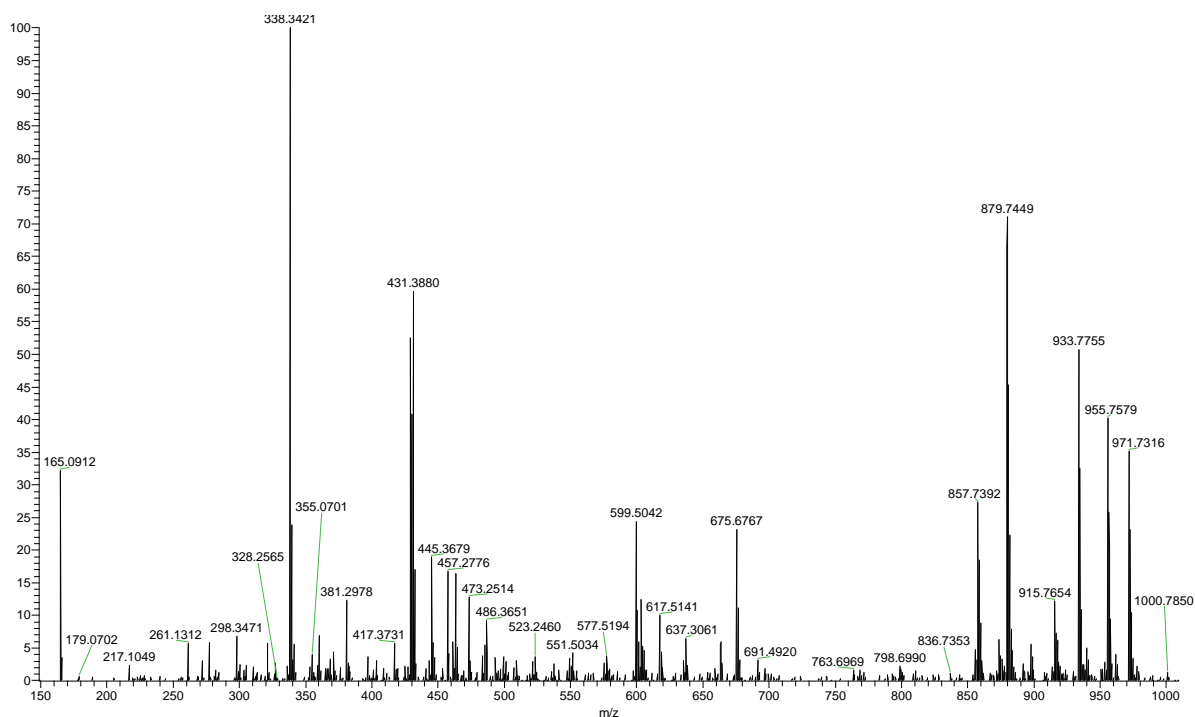


Figure A-37. LESEA-MS spectra of recycled LDPE + 2.5% VE/VC (95/5).

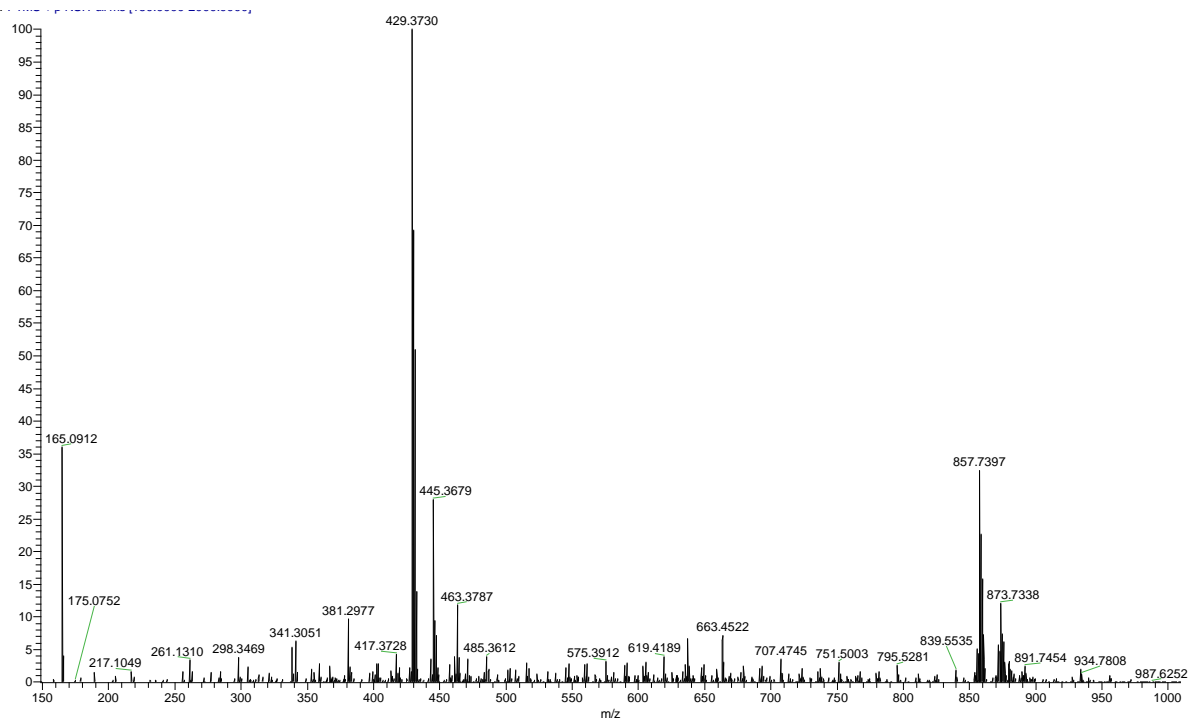


Figure A-38. LESEA-MS spectra of recycled LDPE + 2.5% VE/Tan (95/5).

Annexe 16: Evaluation of the concentration of alpha-tocopherol which migrated in ham as a function of the thickness of the ham sample

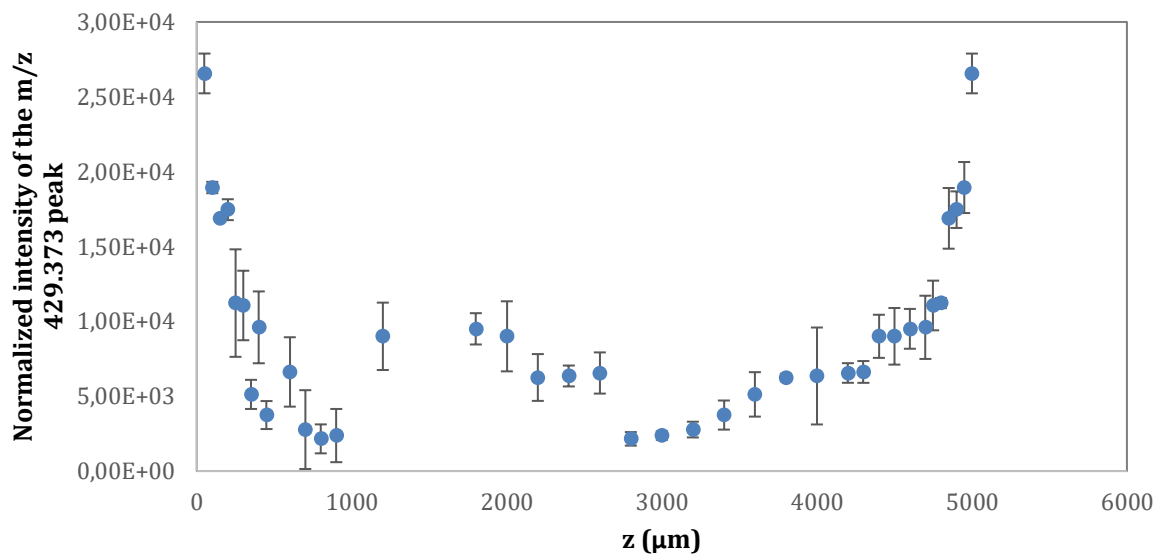


Figure A-39. Evolution of the alpha-tocopherol concentration as a function of the ham depth

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