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MULTIDISCIPLINARY APPROACH FOR THE INVESTIGATION OF CONTENT-CONTAINER INTERACTIONS

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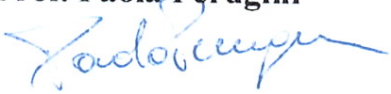

Tesi di Dottorato di

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Multidisciplinary approach for the investigation of content-container interactions

PhD thesis of Benedetta Briasco

This thesis is presented as compendium of publications or works in phase of submission.

SUMMARY

1. GENERAL INTRODUCTION

1.1 What is “packaging”?

Packaging is defined as a technique which allows containment of products from the time of production in a unit till its use. In particular, in pharmaceutical and cosmetic field, the role of packaging is to provide drugs, actives, surgical devices, blood and blood products, nutraceuticals, powders, poultices, liquid and dosage forms, solid and semisolid forms.

Packaging essentially provides containment, drug safety, identity, convenience of handling and delivery. Pharmaceutical packaging has to balance lots of complex considerations. Leaving behind relatively simple issues such as developing good designs and communicating with customers, pharmaceutical packagers are concerned to more pressing concerns which include fighting with counterfeiting, encouraging patient compliance, ensuring drug integrity and balancing child-resistance and accessibility for the elderly. Issue of environment safety is also key concern for both developed and developing countries packaging industry. Pharmaceutical and cosmetic packaging firms are some of the industry's leading innovators evident by the recent advancement in technology. The current trends are result of continuous series of challenges faced by industry. Packaging is a science which is continuously evolving and is a major success contributor for pharmaceutical and cosmetic industries. Packaging is an emerging science, an emerging engineering discipline, and a success contributor to pharmaceutical industries.[1]

Categorically, packaging can be divided as follows:

- *Primary Packaging*. This is the first packaging envelope which is in direct touch with the dosage form or equipment. The primary packaging must provide proper containment and protection of pharmaceuticals. E.g. Blister packages, bottles, jars, tubes, capsules, etc.
- *Secondary Packaging.* This is consecutive covering or package which stores pharmaceuticals packages in it for their grouping. E.g. Cartons, boxes, etc.
- *Tertiary packaging*. This is to provide bulk handling and shipping of pharmaceuticals from one place to another. E.g. Containers, barrels, etc.

Many different materials are used for packaging applications: plastics, glass, metals (aluminum or tinplate), plastic laminates, multi-coupled, paper and cardboard.

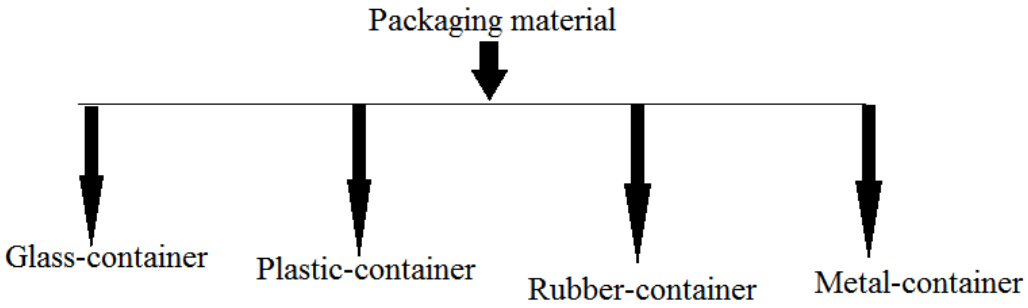

Figure1. Different materials used for packaging applications[2]

The most used types of containers are glass containers and plastic containers. [3]

Glass is commonly used in pharmaceutical packaging because it possesses superior protective qualities. In fact, this material presents a variety of sizes and shapes, impermeability, strength and rigidity, it does not deteriorate with age, it's easy to clean, effective closure and resolves are applicable and colored glass, especially amber, can give protection against light when it is required.

However, it is characterized by fragility, heavy weight and noticeable economical cost. [2]

For this reasons, in the last years plastics became the most used materials for packaging applications.

1.2 Plastics

Plastics are defined as processable materials based on polymers. These materials can be transformed into finished products, such as bottles, containers, films, hoses, coatings, lacquers, etc. They have several advantages compared with glass containers: they are unbreakable, they are collapsible and light in weight. Furthermore, plastic materials are low in cost, durable, pleasant to touch, able to retain their shape throughout their use, odorless and flexible facilitating product dispensing.

As a result of today's multitude of plastic applications there is a corresponding enormous variety of plastic materials. The polymer matrix as well as the incorporated plastic additives can be made to differ in such a variety of ways with respect to their chemical composition and structure that one finds or can develop a tailor made product for every application. [3-5]

1.2.1 Generality on polymers

Polymers are large molecules made up of smaller building blocks, called monomers.[6]

A molecule, to be considered as a monomer, must possess at least two functional groups, which may react and form bonds with other monomers. The corresponding macromolecules may be linear, if the growth takes place always and only in the same direction, branched or crosslinked, if constituent monomers have more than two functional groups. [7-8]

Monomers are either unsaturated, if they have one or more double bonds, or are bifunctional compounds. The corresponding polymer is produced by a technical polymerization reaction of either a free radical chain reaction (unsaturated monomers) or an intermolecular condensation reaction (bifunctional).

Homopolymers consist of a single monomer, while copolymers incorporate two or more monomers. [9]

Polymeric materials are divided in two types, thermoplastic, object of this work, and thermosetting materials.

Thermoplastic polymers are linear or branched polymers which, starting from the granule, are brought to the plastic state by means of a physical heat and transformed into objects which are subsequently cooled to maintain the shape which has been conferred to them. The process is reversible, so they can be melted and transformed into other objects several times. Generally, thermoplastic polymers do not readily crystallize after cooling of the polymer melted in a liquid state, since the polymer chains are very tangled; as they consist of linear chains having a very regular structure, also polymers that crystallize never form perfectly crystalline materials, but semi-crystalline materials characterized by crystalline and amorphous zones.

The crystallinity degree of a solid polymer depends on both the repeating unit and polymeric chain structure and the cooling rate. In fact the crystallization process requires a certain time, during which a molecules fraction loses mobility before being able to be arranged in the crystalline structures. Therefore, with increasing of cooling rate the degree of crystallinity decreases.

Also resulting solid's properties vary with the variation of crystallinity degree. For example, with the increase of crystallinity degree there is a reduction of the resistance to impact and of tear strength, of elongation at break, thermal expansion

coefficient and permeability; in parallel there is an increase of density, yield and break strength, chemical resistance and abrasion resistance.

Thermoplastics are delivered in the form of granules and powders to production sites that are separated from the polymer synthesis. After the addition of the necessary additives, e.g., plasticizers, and after additional processing steps, the final material is referred to as a plastic.

Thermosetting materials, instead, can be heated and processed only once; in fact, when heated, they may become flexible but they do not become liquid. [7-8]

1.2.2 Polymers processing

Beside the classification in thermoplastics and thermosets, plastics can be classified according to whether they are made from converted natural products (e.g. regenerated cellulose) or from completely synthetic products. They can then be further classified according to their manufacturing method in terms of their polymerization reactions, either condensation or addition reactions. The combining of carbon atoms in an unlimited number through covalent bonding leads to the synthesis of macromolecules. Heteroatoms besides carbon such as oxygen, nitrogen, and sulfur can be included.[5]

Addition Polymerization

The most important bulk plastics, e.g., the polyolefins, are produced using addition polymerization processes. The molecules of the starting materials contain double bonds which are broken with the help of initiators or catalysts. The resulting free radicals then undergo a chain reaction to form a macromolecule. The start of chain reactions requires a radical produced as a rule by the disintegration of initiator substances, usually peroxide.

The finished plastic, usually in the form of granules, can contain small amounts of undestroyed residual initiator and/or other disintegration products, residual monomers, and low molecular weight polymerization products (oligomers) as well as residuals of other processing aids. Oxidation reactions resulting from traces of unsaturated compounds, present during the processing of the plastic material, can lead to the formation of sensory active compounds. Some of the necessary additives for further converting to the packaging material may already be added to the plastic

granules.

The monomer can be polymerized either directly, that is undiluted (block or substance polymerization), or in the presence of a not polymerizable solvent (solvent polymerization).

In addition to the use of radical-producing initiators, other catalysts can also be used for ionic addition polymerization reactions.

Another plastic addition polymerization synthesis possibility is ionic polymerization (cationic and anionic). [5]

Condensation Polymerization

Starting materials with two different reactive functional groups can polymerize without any further external assistance with the help of an initiator or a catalyst. Another direct polymerization possibility exists between two different starting materials (monomers), having each two identical functional groups. These reactions are usually subdivided into three groups: polycondensation, polyaddition (not to be confused with radical addition polymerization), and ring opening reactions.

A typical example of a condensation polymerization reaction is the reaction between poly functional alcohol (e.g., glycol) and dicarboxylic acid (e.g., terephthalic acid). Condensation polymerizations are equilibrium reactions, which means they eventually stop reacting when small molecular weight reaction products like water are no longer removed from the system. These characteristics of the condensation polymerization reaction also have an effect on the chemical properties of such plastics. [5]

1.2.3 Plastic Processing

A variety of processing equipment and shaping methods are available to fabricate thermoplastic products. Among these techniques, extrusion is the most popular. Approximately 50% of all commodity thermoplastics are used in extrusion process equipment to produce profiles, pipe and tubing, film, sheet, wire, and cable. Injection molding follows as a preferred processing method, accounting for about 15% of all commodity thermoplastics. Other common methods include blow molding, rotational

molding, thermoforming calendering, and, to some extent, compression molding. (Figure 2)

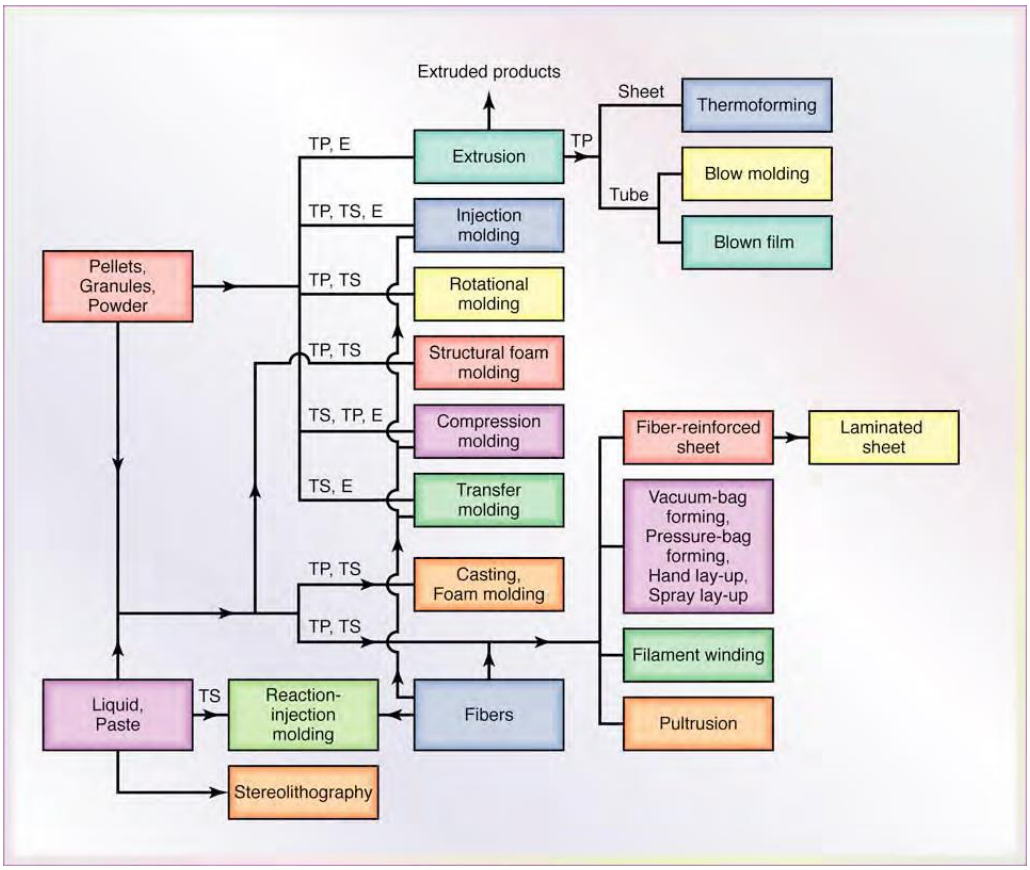

Figure2. Outline of forming and shaping processes for plastics, elastomers, and composite materials. (TP = Thermoplastics; TS = Thermoset; E = Elastomer.) [10]

Extrusion

Extrusion is defined as continuously forcing a molten material through a shaping device. Because the viscosity of most plastic melts is high, extrusion requires the development of pressure in order to force the melt through a die. Manufacturers of plastic resins generally incorporate stabilizers and modifiers and sell the product in the form of cylindrical, spherical, or cubic pellets of about 2–3 mm in diameter. The end-product manufacturers remelt these pellets and extrude specific profiles, such as film, sheet, tubing, wire coating, or as a molten tube of resin (parison) for blow molding or into molds, as in injection molding. To provide a homogeneous product, incorporation of any additives, such as antioxidants, colorants, and fillers, requires mixing them into the plastic when it is in a molten state. This is done primarily in an extruder. The extruder accepts dry solid feed and melts the plastic by a combination

of heat transfer through the barrel and dissipation of work energy from the extruder drive motor. In the act of melting, and in subsequent sections along the barrel, the required amount of mixing is usually achieved. Venting may also be accomplished to remove undesirable volatile components, usually under vacuum through an additional deep-channel section and side vent port. [11]

Injection Molding

In injection molding a molten thermoplastic is injected under high pressure into a steel mold. After the plastic solidifies, the mold is opened and a part in the shape of the mold cavity is removed.

The machine for this kind of process consists of an injection unit and a clamp unit. The injection unit is usually a reciprocating single-screw extruder that melts the plastic and injects it into the mold or tool. The clamp unit opens, closes, and holds the mold closed against the pressure of injection. [11]

Blow Molding

Blow Molding is the most common process for making hollow thermoplastic components. In extrusion blow molding a molten tube of resin called a parison is extruded from a die into an open mold. The mold is closed around the parison, and the bottom of the parison is pinched together by the mold. Air under pressure is fed through the die into the parison, which expands to fill the mold. The part is cooled as it is held under internal air pressure. As the parison is extruded, the melt is free to swell and sag. The process requires a viscous resin with consistent swell and sag melt properties. For a large container the machine is usually equipped with a cylinder and a piston called an accumulator. The accumulator is filled with melt from the extruder and emptied at a much faster rate to form a large parison; this minimizes the sag of the molten tube.

High density polyethylene (HDPE) is the most common blow-molding resin used to produce containers ranging in size from 30 cm^3 to 200 L. In injection blow molding, a parison is injection-molded onto a core pin; the parison is then rapidly transferred via the core pin to a blow mold, where it is blown by air into an article. This process is applied to small and intricate bottles. Soft-drink bottles made from PET are usually made by stretch-blow molding in a two-step process. [11]

Thermoforming

Thermoforming is a process for converting a preform, usually an extruded plastic sheet, into an article such as a thin-wall container or a tray for packaging.

Amorphous resins such as styrenics, acrylics, PVC, and some crystalline resins such as polyolefins, PP, and PET are used for thermoforming. [11]

1.2.4 Plastic additives

Additives are organic or inorganic chemicals enabling processing of plastics, shaping their use, and enhancing end-use performance. The addition of those compounds to plastic products can provide them color, density, opacity, stiffness, flexibility, resistance to heat, light or air, flame retardant, and improve processing properties during pellet creation and final product fabrication.

They are used at levels of 0.05wt.% up to about 20wt.%. Usually additives are classified by function and not chemistry, as shown in Figure3, and are used under stringent legislation and environmental rules. Depending on the additive function they can be stabilizer, modifier or filler. Modifiers additives, such as slip or anti-blocking agents, improve and alter the polymeric properties, while stabilizers additives, such as light stabilizers or antioxidants, preserve the original features of the polymer manufactured. [12-13]

Additive	Polymer							
	HDPE	LDPE	L-LDPE	PP	PS	PA	PET	PVC
Antifogging agent	+	+	+				+	+
Antistatic agent	+	+	+	+	+	+	+	+
Blowing agent				+	+			+
Colorants	+	+	+	+	+	+	+	+
Fillers, reinforcing agents	+	+	+	+	+	+	+	+
Lubricants	(+)	+	+	+	+			+
Nucleating agents	+			+		+	+	
Optical brighteners	+	+	+	+	+	+	+	+
Plasticizers								+
Stabilizers								
Antiacid	+	+	+	+			+	+
Antimicrobials	+	+	+	+			+	+
Benzofuranone (lactone)	+	+	+	+	(+)			
Dehydrating agent							+	
Dialkylhydroxylamine				+				
Heat stabilizers (organotins, metal soaps)							+	+
Hindered amine stabilizers	+	+	+	+	+	+	(+)	
Organic phosphate (not as flame retardants)							+	+
Organic phosphite, phosphonite	+	+	+	+	+		+	+
Phenolic antioxidants	+	+	+	+	+	+	+	+
Thioethers (thiosynergists)				+	+			
UV absorbers	+	+	+	+	+	+	+	

Figure3. Additives used in plastics for contact with food, pharmaceuticals and medical applications. [13]

The most relevant class of additives are here briefly shown.

Antifogging agents: with properties of surface active substances, in order to maintain the optical clarity of packaging materials.

Antistatic agents: static electricity is a considerable problem in highly insulating plastics. Ionogenic and nonionogenic additives reducing the chargeability of plastics are applied either from solutions on the plastic surfaces (external antistatics) or mixed into the plastic masses during processing (migratory internal antistatics decreasing transparency).

Antioxidants: exposing polymers to UV light and air can lead to significant degradation of the materials due to oxidation reactions. Antioxidants can be used to reduce the rate of oxidation and enhance the stabilization of the material of interest, through degrading themselves.

Colorants: medium-soluble dyes having most different structures (derivatives of anthraquinone, quinophthalone, perinone, methine, azine, furanone) and essentially medium-insoluble pigments are used to give plastic articles market appeal or

functional demand (light screening, conductivity). They also change transparency and weathering resistance. Colorants may be either mixed into the polymer mass or applied as printing inks on plastic surfaces. Some processing aids such as dispersants, binding agents (acrylic, alkyd, polyester, or melamine resins), or solvents have to be used together with colorants.

Inorganic pigments, such as titanium dioxide, zinc oxide, or carbon black, also act as efficient light screens and, in this way, protect plastics against photodegradation; iron oxides and structurally very different organic pigments (such as various azo compounds or metal phthalocyanines) act primarily as colorants. Furthermore various effect pigments (powdered metals, fluorescent, or perlescent pigments) are available.

Fillers and reinforcing agents: fillers are mostly powdered inorganic additives, such as calcium carbonate, talc (hydrated magnesium silicate), kaolin (hydrated aluminum silicate), mica (complex potassium/aluminum silicate), or silica (silicon dioxide), used to increase bulk and improve mechanical (impact resistance) and physical (heat and flame resistance) properties of plastics. Glass, carbon, and polyester fibers are used as specific reinforcing additives in the manufacture of large rigid containers. Particular fillers are mostly coated to improve surface properties and compatibility with the polymer matrix.

Lubricants: melt rheology of plastics is affected, and the processing above the glass transition temperature is improved by additives reducing the external friction on plastics/processing equipment interfaces and protecting from sticking to the mold of the machinery (outer lubricants) and internal friction on macromolecule/macromolecule interfaces improving the movement of polymeric chains (inner lubricants added into the polymer mass).

Plasticisers: a plasticiser is defined as a substance which after incorporation into a material (usually a plastic or elastomer) can make it more flexible and facilitate its processing. Most plasticisers belong to the group of esters of phthalic acid (phthalates) and adipic acid.

Stabilizers: all plastics used in packaging materials gradually degrade during their lifetime (processing, storage, application) by combined attacks of chemical deteriogens (oxygen and its active forms, atmospheric pollutants such as NOx or SO_2), harmful physical effects of the environment (tropospheric solar radiation, heat, and mechanical stress), high-energy radiation in sterilization processes, and

microorganisms. The relevant degradation processes are classified as melt (processing) degradation, thermal degradation, long-term heat aging (thermal oxidation), weathering (including photo-oxidation) and biodeterioration.

Antimicrobials: Biodeterioration due to the growth of microorganisms on surfaces contaminated with nutrients or damages in polymers plasticized by esters of fatty acids results in a loss of flexibility and light transparency, development of discoloration, and odor. Antimicrobials (biocides) prevent polymers against growth of microorganisms consuming parts of the material (plasticizers) as a nutrient.

Light Screening Pigments and UV Absorbers: for some applications, white pigments (titanium dioxide in particular), inorganic colored pigments, or carbon black are used to screen harmful solar radiation. [13-14]

1.2.5 Plastic materials

While plastics are definitely superior materials in terms of their production costs and diverse properties, the sustainability of this synthetic material is undoubtedly an issue that needs to be addressed. Due to concerns for the global environment and the increasing difficulty in managing solid wastes, biobased and biodegradable polymeric materials may be among the most suitable alternatives for some applications. In addition, there is also a steadily growing desire to minimize the dependence on petroleum for the material needs of society because of the anticipated depletion of this cheap fossil resource in the near future. The excessive usage of petroleum is also contributing to the increased emission of CO_2 into the atmosphere, which is thought to be among the principal reasons for global warming and climate change. All these issues are providing a strong initiative towards the development of technologies to produce biobased and biodegradable plastics. [15]

Until now petrochemical-based plastics such as polyethylene terephthalate (PET), polyvinylchloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyamide (PA) have been increasingly used as packaging materials because their large availability at relatively low cost and because their good mechanical performance such as tensile and tear strength, good barrier to oxygen, carbon dioxide, anhydride and aroma compound, heat sealability, and so on. But nowadays their use has to be restricted because they are not non-totally recyclable and/or biodegradable so they pose serious ecological problems. [16-17]

Polyethylene

Polyethylene (PE) (Figure4) looks like the simplest of molecules, but the number of ways in which the -CH_2- units can be linked is large.

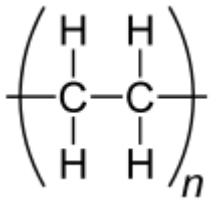

Figure4. Structure of polyethylene

It is the first of the polyolefins, the bulk thermoplastic polymers that account for a dominant fraction of all polymer consumption. Polyethylene is the most widely used mass–produced plastic.

Polyethylene is classified into several categories based mostly on its density and branching:

- Ultrahigh molecular weight polyethylene (UHMWPE)
- Ultralow molecular weight polyethylene (ULMWPE)
- High density polyethylene (HDPE)
- Medium density polyethylene (MDPE)
- Linear low density polyethylene (LLDPE)
- Low density polyethylene (LDPE)
- Very low density polyethylene (VLDPE)

The development of PE began in 1936 with the introduction of the high pressure polymerization process of ethylene to LDPE (0.915–0.94 g cm^3), which produced a relatively low molecular weight polymer. The manufacture of HDPE by low pressure polymerization first began after the discovery of the Ziegler catalysts in 1953. The HDPE produced using this process has a medium density (0.945 g cm^3). The Philips and Standard Oil process was also developed in the 1950s and produces HDPE with the highest density (0.96 g cm^3).

All polyethylenes are semicrystalline. Their densities and melting temperatures decrease with the increase of ramification. The crystalline melting temperatures are about 108°C for LDPE and LLDPE, 135°C for HDPE and 144°C for the ideal crystallites of linear PE.

At present, many hundreds of grades of PE, most of which differ in their properties

in one way or other, are available.

PE possesses good chemical stability. The mechanical properties are dependent on the molecular weight and degree of chain branching. PE can be easily heat sealed, is tough and has high elasticity. It has good cold resistance properties and is a good water vapor barrier. However, LDPE has low barrier properties to gases, aromas, and fats. With increasing density, all the barrier properties increase as well as the stiffness, hardness, and strength, as a result of the higher crystallinity. At the same time, there is a decrease in the impact resistance, toughness, resistance to stress cracking, cold resistance, and transparency.

Materials of similar solubility parameters and low molecular weight will however cause swelling, the more so in low density polymers. LDPE has a gas permeability in the range normally expected with rubbery materials. HDPE has a permeability of about one-fifth that of LDPE.

The chemical stability of PE is comparable to paraffin. Oxidation of polyethylene which leads to structural changes can occur to a measurable extent at temperatures as low as 50°C. Under the influence of ultraviolet (UV) light, the reaction can occur at room temperature.

Polyethylene is cheap, and particularly easy to mold and fabricate. It accepts a wide range of colors, can be transparent, translucent or opaque, has a pleasant, slightly waxy feel, can be textured or metal coated, but is difficult to print on.

LDPE is used mostly in the form of films over thicknesses ranging from 15 to 250 μm. Coextrusions, laminates, shrink films, films for the building industry and for agricultural purposes, shopping bags, trash bags, and household films are all made from LDPE.

Blown containers from LDPE are used as packaging in the pharmaceutical and cosmetic industries as well as for foods, toys, and cleaning agents. The most important application area of HDPE is the production of containers and injection-molded articles. Bottles for detergents, gasoline cans, and heating oil tanks are some examples.

LLDPE is the copolymer of ethylene with about 8% 1-butene, 1-hexene, or 1-octene. opolymers with 10–20% 1-octene are plastomers. LLDPE is denoted as linear, because the ramifications are already contained in the comonomers and not produced as transfer reactions. With these short side chains, LLDPE has a density range from

0.900 g cm^3 for very LDPE (VLDPE) to 0.935 g cm^3 for the octene-ethylene copolymer.

Copolymers of PE with vinyl acetate (EVA), acrylic acid ester, and methyl acrylic acid increase the heat sealability, adhesion to other materials, and seal strength, and they improve the polymers cold resistance and transparency. EVA-copolymers are used as sealants. [5,18]

Polypropylene

Since approximately 1986, polypropylene (PP) has ranked third in the bulk plastic production after PE and polyvinyl chloride (PVC).

PP is composed of linear hydrocarbon chains and therefore its properties quite closely resemble those of PE. (Figure5)

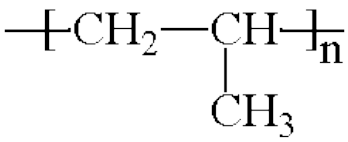

Figure5. Structure of polypropylene

PP and its copolymers can be classified into three categories: monophasic homopolymer (h-PP), monophasic random copolymer (r-PP), and heterophasic copolymer (heco-PP). The h-PP can be either isotactic, syndiotactic, or atactic.

Isotactic PP is obtained with propylene and transition metal catalysts or metallocene catalysts. Syndiotactic PP is also obtained with metallocen catalysts. Atactic PP, which in reality is highly ramificated PP, is an adhesive and not a typical polymer. Large scale commercially produced PP is up to 95% isotactic in nature. Homopolymer PP is one of the lightest thermoplastics, having a density ranging from 0.90 to 0.91 g cm^3. Pure isotactic PP has a melting temperature of 176°C. In general, the melting temperature of commercial materials is around 150–170°C with melting beginning around 140°C, which is much higher than PE.

The chemical compatibility of PP is similar to that of HDPE. PP can be swelled by aromatic and chlorinated hydrocarbons and dissolved in them at higher temperatures. The tertiary C atoms reduce the chemical inertness of PP and make it, above all, more sensitive to oxidation. This sensitivity to oxidation must be compensated for by the addition of antioxidants.

PP possesses good water vapor barrier and fat resistance properties. Normal PP films have limited food packaging applications (e.g., packaging of bread) because of their low cold temperature resistance. Copolymer mixtures with ethylene are used to improve cold resistance and heat sealability as well as material strength and, above all, seal strength.

PP is an excellent material for injection and extrusion processes. New PP packaging developments are multiple-layer bottles and cans with inner barrier layers, which can be hot-filled or sterilized in an autoclave as well as directly steam sterilized. PP packaging can be filled with liquids that are surface active because of its good stress-cracking resistance.

Over 40% of PP produced in Europe is used to make films. [5, 18]

Polyvinyl Chloride

The ability of vinyl chloride to polymerize was first observed over 150 years ago.

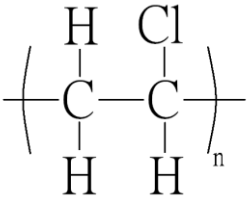

Figure6. Structure of polyvinyl Chloride

Even though pure PVC is fairly unstable, the manifold applications are made possible by the discovery of effective stabilizers and other additives for the polymer. The additional technological effort needed to remove the remaining residual monomer and the decreasing acceptance by consumers of this plastic in the meantime led to a relative decrease in the use of PVC compared to PE, especially for food packaging.

Additionally, PVC can be replaced by PP in various applications. Nevertheless, PVC has still maintained a leading position among the bulk plastics today because of its low price and numerous application possibilities.

PVC is resistant to nonpolar (hydrocarbon) and strongly polar substances (water, inorganic acid). Middle polarity compounds such as cyclohexanone, dimethylformamide, acetone, chlorinated hydrocarbons, tetrahydrofuran, and phenol all either swell PVC or dissolve it. This behavior can easily be attributed to the slightly polar structure of the PVC macromolecule.

When PVC is pyrolyzed, the main decomposition product is hydrochloric acid, along with small amounts of saturated and unsaturated hydrocarbon side products. PVC is easily degraded through the effect of heat, light and mechanical energy. In order to improve the low stability of this plastic, a series of additives are incorporated into the PVC melt. The most important additives for the processing of PVC are the plasticizers, which may be incorporated at elevated temperatures to give mixtures stable at room temperature.

Due to its particularly good polymer characteristics, PVC has an enormously wide spectrum of applications. Blow-molded containers for packaging liquid products (beverages, edible oils, detergents, cosmetics, and pharmaceuticals) receive special consideration, as do dishes for fatty foods (highly stable against low polarity substances) and films (such as soft PVC films with high gas permeability) for fresh meat packaging. Soft PVC is also used as a component in seals.

Because of the increasing amount of criticism from consumer groups due to the formation of hydrochloric acid during burning and because of plasticizer migration from soft PVC films, PVC is continually being replaced by other plastics. [5, 18]

Polyethylene terephthalate

Polyethylene terephthalate (PET) is a semi-crystalline polymer belonging to the family of polyesters.

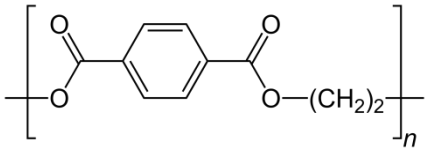

Figure7. Structure of polyethylene terephtalate

It is the most favorable packaging material for beverages.

The prepolymerization of dimethylterephthalate (DMT) or terephthalic acid (TPA) with ethylene glycol (MEG) is the first industrial step in the synthesis of PET. Both reactions generate low-weight oligomers and an intermediate compound named bis(hydroxyethyl)terephthalate (BHET). After this step, a second polycondensation is carried out with an Sb-, Ge- or Ti-based catalyst. [19]

During PET manufacturing, several degradation and decomposition reactions can occur. High temperatures and the presence of oxygen in the PET melt process can promote thermo-mechanical and thermo-oxidative reactions. [20]

Also, PET hydrolysis can be induced by the presence of water during the melt process. [21-22]

PET thermal degradation generates sub-products such as oligomers and diethylene glycol. [19]

It possesses extremely popular physical and aesthetic requirements: high resistance to elongation and dimensional stability, excellent barrier properties to oils and fats, low gas and moisture permeability (especially in the context of plastic films).

PET is not subject to cracking under stress and it has excellent resistance to atmospheric agents.

From an aesthetic point of view, it presents characteristics of high transparency, such as glass.

PET is also inert against the attack of spore-forming bacteria, fungi, molds, and it is not active from a physiological point of view. Compared to other plastic material fibers, PET is the most inactive to human body; for this reason it is commonly used for construction of prosthesis which can remain in the body for long periods without changing their characteristics.

In the flexible packaging industry bi-oriented films of stabilized PET are widely used for the production of multilayer laminates. [18]

Poly(ethyleneterephthalate)-glycol

Poly(ethylene terephthalate)-glycol (PETG), a non-crystallizing amorphous copolymer of PET, does not occupy the same industrial niche as PET, precisely because it lacks the ability to undergo strain-induced crystallization.

The letter G refers to the additional glycol group along the backbone of the copolymerizing agent, poly(1,4-cyclohexylenedimethylene terephthalate) (PCT). Specifically, PETG is a random copolymer consisting of 31 mol% PCT and 69 mol% PET. PET and PETG both exhibit quite similar deformation behavior, have a similar glass transition temperature, and are visually nearly indistinguishable. Yet there is one substantial difference: PET readily undergoes strain-induced crystallization, whereas crystallization is nearly impossible to achieve in PETG at processing temperatures. [23]

This material has a high chemical resistance and, for some types of processing, such as thermoforming, do not require pre-drying of the slab.

On the market there are types of PET-G selected specifically for cosmetics because they are more transparent, easily injectable and with a good chemical resistance; other versions are more tenacious and with improved chemical resistance or with very high transparency, printable up to a thickness of 25 µm.
There are also other kind of PET-G that have excellent toughness and excellent colorability, and variants that have as main feature the biodegradability. Moreover PET-G can be also mixed with natural polymers, like starch or cellulose, and it presents resistance to stress-cracking. [18]

Biobased polymers

In the recent years there is an increased awareness on sustainability, which can in general be achieved on different levels. On the level of raw materials, use of recycled materials or use of renewable resources are two strategies to reduce CO_2 emissions and the dependency on fossil resources.
Biodegradable polymers are polymers that are capable of undergoing decomposition into CO_2, CH_4, H_2O, inorganic compounds or biomass through predominantly the enzymatic action of microorganisms. Some of these polymers can also be compostable, which means decomposition takes place in a compost site at a rate consistent with known compostable materials. [16]
According to the European Bioplastics organization, bioplastics can be defined as plastics based on renewable resources (biobased) or as plastics which are biodegradable and/or compostable. Recently the attention in the packaging industry regarding the use of bioplastics has been renewable resources are not discussed, as their properties do not differ from the crude oil based PE and PET. [24]

Polylactide (PLA)

PLA (polylactide) is a family of biodegradable thermoplastic polyester made from renewable resources which is nowadays seen as one of the most promising polymers for commercial use as a substitute for low-density polyethylene (LDPE) and high-density polyethylene (HDPE), polystyrene (PS) and polyethyleneterephthalate (PET). It is produced by conversion of corn, or other carbohydrate sources, into dextrose, followed by a fermentation into lactic acid. Through direct polycondensation of lactic acid monomers or through ring-opening polymerization of lactide, PLA pellets are obtained. Since lactic acid exist as two optical isomers, L- and D-lactic acid,

three different stereochemical compositions of lactide can be found, i.e. L,L-lactide, D,Dlactide and L,D-lactide. This stereochemical composition determines the final properties of the polymer. The processing possibilities of this transparent material are very wide, ranging from injection molding and extrusion over cast film extrusion to blow molding and thermoforming. [25-29]

Starch

Starch is a widely available and easy biodegradable natural resource. To produce a plastic-like starch-based film, highwater content or plasticizers (glycerol, sorbitol) are necessary. These plasticized materials are called thermoplastic starch (TPS) and constitute an alternative for polystyrene (PS). Starch-based thermoplastic materials (e.g. blends of TPS with synthetic/ biodegradable polymer components, like polycaprolactone, polyethylene-vinyl alcohol or polyvinyl alcohol) have been successfully applied on industrial level for foaming, film blowing, injection molding, blow molding and extrusion applications. [30-33]

PHA

The polyhydroxyalkanoates (PHA) family are biodegradable thermoplastic polymers, produced by a wide range of microorganisms. The polymer is produced in the microbial cells through a fermentation process and then harvested by using solvents such as chloroform, methylene chloride or propylene chloride. More than 100 PHA composites are known, of which polyhydroxybutyrate (PHB) is the most common. The PHAs have potential as a substitute for many conventional polymers, since they possess similar chemical and physical properties. [34-37]

Cellulose

Cellulose is the most widely spread natural polymer and is derived by a delignification from wood pulp or cotton linters. It is a biodegradable polysaccharide which can be dissolved in a mixture of sodium hydroxide and carbon disulphide to obtain cellulose xanthate and then recast into an acid solution (sulfuric acid) to make a cellophane film. Alternatively, cellulose derivatives can be produced by derivatization of cellulose from the solvated state, via esterification or etherification of hydroxyl groups. Cellulose esters like cellulose (di)acetate and cellulose (tri)acetate need addition of additives to produce thermoplastic materials. Most of

these derivatives show excellent film-forming properties, but are too expensive for bulk use. [34,38-39]

Other biobased materials that can be used for packaging purpose are zein, chitosan, soy protein isolate (SPI) and whey protein isolate, (wheat) gluten derived films. [24]

1.3 Regulation of packaging industry

The world of packaging is regulated by different organisms, institutions and legislations, especially regarding all those products that enter in contact with human health (e.g. pharmaceuticals, cosmetics and food).

Specifically, Food and Drug Administration evaluates a drug and the agency must be firmly convinced that the package for a specific drug will preserve the drug's efficacy as well its purity, identity, strength and quality for its entire shelf life. Under the provisions of the Food and Drug Administration Act, however, no specifications or standards for containers or container closures are provided. Under the Act, it is the responsibility of the manufacturer to prove the safety of a packaging material and to get approval before using it for any pharmaceutical product. The Food and Drug Administration does not approve containers as such, but only the materials used in the container are approved. A list of substances considered "Generally Recognized As Safe" (GRAS) has been published by the FDA. In the opinion of the qualified experts they are safe under specified conditions, assuming they are of good commercial quality. A material that is not included under GRAS or prior sanction, and is intended to be used with food, must be tested by the manufacturer, and the data must be submitted to the FDA. The specific FDA regulation states that "containers, closures and other component parts of drug packages, to be suitable for their intended use, must not be reactive, additive or absorptive to an extent that the identity, strength, quality or purity of the drug will be affected." The packaging material must be approved for such use, along with the drug, before going to the market. The drug manufacturer must include data on the container and package components in contact with the pharmaceutical product in its New Drug Application (NDA). If the FDA can determine that the drug is safe and effective, and that the package is suitable, it approves the drug and package. Once approved, however, the package may not be altered in any manner without prior FDA approval. In the case

of plastics, most resin manufacturers maintain Master Files on their resins with the FDA. Upon request from the resin manufacturer, the FDA uses this file as a reference to support a New Drug Application that which a drug manufacturer files. [2]
The Packaging, Storage, and Distribution Expert (PSD) Committee in USP's Council of Experts is responsible for developing and revising USP standards related to packaging. During the 2010- 2015 revision cycle, the Expert Committee has worked to delineate a general chemistry-based approach for establishing the safety and quality of packaging systems and their materials of construction.
In particular, chapter <661> is interesting for my research purpose, because it regards plastic packaging systems and plastic materials of construction to the exclusion of the other materials of construction. Beside this there are other two general chapters on extractables and leachables, specifically <1663> and <1664>. While <661> focuses on plastics, <1663> and the <1664> series are relevant and applicable to all packaging systems, regardless of their materials of construction. Furthermore USP has individual general chapters that deal with the other materials of construction (for example <381> Elastomeric Closures for Injection, <660> Container – Glass, and <662> Containers – Metal (which is under development)).
The purpose of official USP general chapter <661> Containers – Plastics is "to provide standards for plastic materials and components used to package medical articles (pharmaceuticals, biologics, dietary supplements, and devices)." Resin-specific tests for polyethylene, polypropylene, polyethylene terephthalate, and polyethylene terephthalate G are provided in this chapter. Although the chapter has served a useful purpose, it is lacking in its ability to meet the objective of assessing and assuring the safety and quality impact of plastic systems used in the manufacture, packaging, storage, delivery of a pharmaceutical product. Introductory information contained in <661> is augmented with testing protocols and specifications for Plastic Materials of Construction, <661.1>; Plastic Packaging Systems for Pharmaceutical Use, <661.2>; Plastic Systems Used for Manufacturing Pharmaceutical Products, <661.3>; and Plastic Medical Devices Used to Deliver or Administer Pharmaceutical Products, <661.4>. Of these sections, <661.3> and <661.4> are currently under development.
<1663> and <1664> present a framework for the design, justification, and execution of either an extractables assessment for pharmaceutical packaging systems or an assessment for a pharmaceutical product's leachables derived from

packaging/delivery systems. Although these chapters are intended to be informational, helpful and generally applicable, they do not establish specific experimental conditions, specific tests, analytical procedures, or acceptance criteria for particular packaging systems or pharmaceutical products. They also do not delineate every situation in which an extractables or leachables assessment is required. [40-42]

Also in European Pharmacopoeia, this topic is treated. However, the chapters of the European Pharmacopoeia (Ph.Eur.) describing containers and materials for containers do not cover all different types of plastic materials and additives. Therefore, it is not always possible to refer to the specifications of the Ph.Eur. As a consequence, it is not mandatory that all materials comply with the requirements/specifications of the Ph.Eur.

Materials having a different composition and corresponding to a different specification may well be used on the condition that this is justified and agreed upon with the registration authority.

Regarding cosmetic field, instead no specific sections about packaging are present in Regulation. With the complete entry into force Regulation 1223/2009, a specific section in CPSR (Cosmetic Product Safety Report) of every single product was introduced; this point imposes to the safety assessor to report information about "Impurities, traces and information about the packaging material". [43] However, currently no indications by law or guidelines are present in cosmetic scenery. For this reason, the only tests on packaging materials are performed following the food legislation, for analogy. Food sector is regulated by European Regulation No.1935/2004, on materials and articles intended to come into contact with food, and Commission Regulation (EU) No 10/2011, specific for food-contact plastic materials.

European Regulation No.1935/2004 covers 17 groups of different materials. It states that food-contact materials should not transfer its constituents to food in quantities that could incur a human health risk, cause an unacceptable change in the composition of the food or bring about deterioration in the food organoleptic characteristics. This regulation is accompanied by specific measures depending on the type of material. [44]

Food-contact plastic materials are covered by the recent Regulation No. 10/2011. This regulation establishes the list of compounds authorized for use in plastic

formulation on a positive list. The conformity of a plastic material intended to come in contact with food is based on migration tests, performed on food simulants (reported in FigureX). [45]

Table 1

List of food simulants

Food simulant	Abbreviation
Ethanol 10 % (v/v)	Food simulant A
Acetic acid 3 % (w/v)	Food simulant B
Ethanol 20 % (v/v)	Food simulant C
Ethanol 50 % (v/v)	Food simulant D1
Vegetable oil (*)	Food simulant D2
poly(2,6-diphenyl-p-phenylene oxide), particle size 60-80 mesh, pore size 200 nm	Food simulant E

(*) This may be any vegetable oil with a fatty acid distribution of

No of carbon atoms in fatty acid chain: No of unsaturation	6-12	14	16	18:0	18:1	18:2	18:3
Range of fatty acid composition expressed % (w/w) of methyl esters by Gas chromatography	< 1	< 1	1,5-20	< 7	15-85	5-70	< 1,5

Figure8. List of food simulants, reported in Commission Regulation (EU) No. 10/2011

The overall migration limit should not exceed 10 mg of the total constituents released for dm^2 of packaging surface. Specific migration limits (SML) established according to toxicological data is provided for some substances. The main limitation of this regulation concerns impurities and breakdown products generated by authorized initial reactants and additives (NIAS). Furthermore, the new regulation specifies that: "the notion of the risk due to the substance concerns the substance itself, the impurities of this substance and any reaction or degradation products".[19]

1.4 Interactions between content and container

To ensure the efficacy of a product during its total shelf-life, pharmaceuticals and cosmetics must be regarded as a combination of the medicinal product itself and the packaging.

Packaging and the contained product are not two separated entities, but they could interact, especially in presence of particular environmental conditions.

Interactions between the products and/or ingredients and their packaging systems can affect the quality of the product, or less frequently, the quality of the packaging

systems themselves. These interactions are either additive, reductive, or transformative. If the interaction is additive, a constituent of the packaging system is added to the product due to the interaction. If reductive, a product constituent is reduced, in either level or action, due to its uptake into the packaging system. If the interaction is transformative, the product is transformed (for example, loss of stability) due to a physicochemical interaction with the packaging system. An additive, and sometimes transformation, interaction can reflect a single physicochemical process where extractables, organic and/or inorganic chemical entities, migrate out of the packaging system and accumulate as leachables in the product. Leachables in a product can affect safety and/or efficacy. Regulatory guidances on this subject include recommendations for the chemical analysis and the toxicological safety assessment (qualification) of leachables. Nevertheless, consistency in the design and execution of the various chemical assessment studies is sometimes lacking. While the studies are driven by the general principles of good science, it is not clear which principles and practices establish or otherwise reflect good scientific methods, processes, and practices.

Taking these comments into account, an effective and efficient process for establishing a packaging system's suitability for use includes characterization packaging itself, characterization of the entire systems and testing of the packaged product.

Materials of construction undergo considerable stress, such as exposure to high temperatures, while they are being converted into components of and/or finished packaging systems. Furthermore, processing aids and additional additives may be introduced during the manufacturing process for a packaging system. Thus, the extractables profile of a system is likely to be different from, and potentially more complex than, the sum of the extractables profiles of its materials of construction. Therefore, the initial assessment of risk made in material selection is appropriately revisited by testing and qualification of the overall packaging system itself.

Besides the possibility of migration of substances from packaging into the product and vice versa, other interaction phenomena could occur.

The microbiological contamination of products due to contact with inappropriate packaging materials could be an example of microbial interaction.

Other examples of interaction phenomena could be the “intrusion” of micro- and macro-organisms, gases and vapors, water and other compounds of low molecular weight. [14]

Moreover, some packaging materials, for example polyolefin, have a lipophilic nature, so they are able to retain large amounts of nonpolar compounds such as most of the aroma compounds. This food-packaging interaction, known as aroma scalping, causes a loss of aroma content and/or an aroma imbalance, because scalping of the diverse aroma components of a product progresses to different extents and at different rates. Indeed, other materials (e.g. ethylene-vinyl alcohol copolymers, EVOH) of hydrophilic nature promote the sorption of large amounts of water, which results in plasticization of the copolymers and the subsequent loss of mechanical and barrier properties. [46]

1.5 Semisolid formulations: emulsions

An emulsion is a themodinamically instable system, composed by almost two immiscible liquid phases; one of these is dispersed into the other in form of little drops or globules with a diameter in a range of 0.1-100 μm. Emulsions are themodinamically instable because of the excess of surface free energy associated to the surface of dispersed phase. Drops tend to link each other in order to reduce superficial area and this fact could lead to emulsion instability. [47]

In order to reduce the phenomenon a third ingredient is added to the system, the emulsifier agent, that increases stability placing itself at the interphase between two phases.[48] Either the dispersed phase or the continuous phase may vary in consistency from that of a liquid to semisolid .[49] Thus, pharmaceutical and cosmetic emulsions range from lotions (low viscosity) to creams (high viscosity).[50]

This instability could be manifested at different time rates and through a variety of physicochemical destabilizing processes, for example, creaming, sedimentation, flocculation, coalescence or phase inversion.[47,51]

This kind of products should have a storage stability of several months at ambient temperature and under widely varying external influences. Nevertheless, the shelf life assessment of O/W or W/O emulsions remains one of the most difficult issues. The final objective is to save time by predicting whether the emulsion is unstable or not before it breaks and the two phases separate each other.[52]

1.5.1 Types of emulsion: Oil in Water (O/W)

Pharmaceutical and cosmetic emulsions usually consist of mixtures of aqueous phase with a second phase composed by various oils and waxes. If the oil droplets are dispersed throughout the aqueous phase, the emulsion is termed oil-in-water (O/W). They are not greasy and easily removable from the skin surface; furthermore they are used externally to provide cooling effect and internally to also mask the bitter taste of oil. Water soluble actives are more quickly released from O/W emulsion. O/W emulsion give a positive conductivity test as water, that is the external phase, is a good conductor of electricity.[53]

1.5.2 Types of emulsion: Water in Oil (W/O)

A system in which the water is dispersed as globules in the oil continuous phase is termed water-in-oil emulsion (W/O). Water-in-oil emulsions have an occlusive effect hydrating the stratum corneum and inhibiting evaporation of eccrine secretions. It has also an effect on the absorption of actives from W/O emulsions. W/O emulsions are also useful for cleansing the skin of lipidic soluble dirt, although its greasy texture is not always cosmetically acceptable[54] . For these characteristics they are used externally to prevent evaporation of moisture from the skin surface (e.g. cold cream). Oil soluble actives are more quickly released from W/O emulsion. This kind of emulsion is preferred for formulation meant for external use like cream. Furthermore a W/O emulsion does not give a positive conductivity test, because the external phase is oil, which is a poor electricity conductor.[53]

1.5.3 Types of emulsion: Multiple emulsions (W/O/W or O/W/O)

Multiple emulsions are complex systems. They can be considered as emulsions of emulsions, and have been shown to be secured in cosmetic and pharmaceutical applications.[55]. They represent a system in which the oil-in-water or water-in-oil emulsions are dispersed in another liquid medium. In this way an oil-in-water-in-oil (O/W/O) emulsion consists of very small droplets of oil dispersed in the water globules of a water-in-oil emulsion and a water-in-oil-in-water (W/O/W) emulsion consists of droplets of water dispersed in the oil phase of an oil-in water emulsion.[53] Their pharmaceutical applications include taste masking, adjuvant vaccines, an immobilization of enzymes and sorbent reservoir of overdose treatments, and

sometimes for the augmentation of external skin or dermal absorption. Multiple emulsions have been formulated as cosmetics, such as skin moisturizer. Prolonged release can also be obtained by means of multiple emulsions. These systems have some advantages, such as the protection of ensnared substances and the possibilities of incorporating several actives ingredient in the different compartments. However, these systems have limitations because of thermodynamic instability and their complex structure.[55]

1.5.4 Types of emulsion: Water in silicon (W/S)

Another type of emulsion is the one obtained with particular silicone emulsifiers and oils, that is water in silicone emulsions (W/S), also called "light emulsions". The dominant ingredients in this kind of systems are silicones, a category of raw materials with particular sensorial and texturizing effect.[56]

Silicones differ considerably from their organic counterparts due to their weak intermolecular attractive forces. Structurally they are characterized by Si–O–Si bond angles that are larger than C–O–C bond angles, Si–O bond lengths that are longer than C–O–C or C–C bonds, a greater freedom of rotation around the Si–O bond compared to the C–C bond and freely rotating methyl groups which can orient towards interfaces.[57] Silicones are water repellent, heat stable, odorless, and highly resistant to chemical attack, they do not undergo alterations or go rancid. From a nano-scale point of view silicones are not soluble in either hydrophilic or a variety of hydrophobic solvents; instead they often form a third phase.[58,59]

Silicone surfactants show very characteristic properties which makes their use very attractive to the cosmetic industry. One of the advantages of these surfactants is that the molecular weight can easily be controlled. Siloxanes can have a very high molecular weight which is an advantage for forming W/O emulsions. They improve the aesthetic results by eliminating high melting point waxes. Furthermore, they exhibit high surface activity because, in contrast to hydrocarbon-based surfactants, the silicone copolymers have a highly flexible siloxane backbone between the two anchor groups. The siloxane backbone can adapt to the interface geometry very easily, without creating steric hindrance due to this flexibility.[60]

Moreover this emulsions, in addition to silicones, the presence of salts (e.g. NaCl) in aqueous phase provides stability to the system.

For their several properties, silicones have wide range of applications in cosmetics [61], drug delivery[62] , home care products [63,64], printing ink formulations and so on. In particular, for cosmetic applications, silicone oils are considered to be ideal conditioning agents in hair and skin care products to impart smooth and silky feel to hair and skin.[65] The only negative aspect is insolubility of silicone oils in most of hydrophilic and hydrophobic solvents.[66]

1.5.5 Stability of emulsions

Since emulsions are unstable system for definition, as said before, a very important parameter for emulsion products is their stability; however, the evaluation of emulsion stability is not easy.[67] Pharmaceutical and cosmetic emulsion stability is characterized by the absence of coalescence of dispersed phase, absence of creaming and retaining its physical characters like elegance, odor, color and appearance. The instability of emulsion may be classified into four phenomena: flocculation, creaming, coalescence and breaking.[53]

Flocculation

It is the association of small emulsion particles to form large aggregate which is redispersable upon shaking. It is a reversible process in which the droplets remain intact. Flocculation is considered a precursor of coalescence. This is because the presence of excess surfactant in the continuous phase of an emulsion can lead to flocculation of emulsion droplets ("depletion effect"). The depletion mechanism can be explained as, a system containing excess surfactant in the form of micelles, when the dispersed emulsion droplets approach each other to distances closer than the diameter of the surfactant micelles, segregation of micelles from the interparticle space that occurs because of the loss in configurational entropy of the micelles. This phenomenon results in an attractive force between the droplets due to the lowering of osmotic pressure in the region between the droplets, and accordingly, flocculation of droplets occurs.[50,53]

Creaming

Creaming is the phenomenon in which the dispersed phase separates out, forming a layer on the top of the continuous phase. It is notable that in creaming, the dispersed

phase remains in globules state so that it can be redispersed on shaking. Creaming can be minimized if the viscosity of the continuous phase is increased[68] . The rate of creaming is determined by Stokes's law as follows:

$$V = D^2 (\rho s - \rho o) \frac{g}{18\eta}$$

where V is the velocity in cm/s, D is the diameter of particles of dispersed phase in cm, ρs is the dispersed phase density, ρo is the continuous phase density, η is the continuous phase viscosity and g is the gravitational acceleration. O/W emulsions generally face upward creaming when the globules of the dispersed phase are less dense than the continuous phase. In contrast, W/O emulsions face downward creaming when the globules of the dispersed phase are denser than the continuous phase.[53]

Coalescence

Coalescence occurs when the mechanical or electrical barrier is insufficient to prevent the formation of progressively larger droplets. Stabilization against coalescence may be achieved by the addition of high boiling point or high molecular weight components to the continuous phase.[50]

Some experimental studies concluded that W/O emulsions are formed only when the film of emulsifying agent in the interface is uncharged and rigid as a result of complex formation; according to them a W/O emulsion cannot be stabilized against flocculation by charge on the dispersed phase of water droplets, because an electric diffuse layer cannot be built up as oil being a nonionizing medium.[69]

1.6 Ingredients with critical issues

Emulsions are often a vehicle for actives ingredients, both pharmaceutical and cosmetic.

Among the variety of functional substances that can implement a formulation, there are some raw materials with a particular and specific action that are fully employed in semi-solid formulations. However these ingredients could present some critical issues if related with packaging materials.

Specifically, the ingredients that I wanted to deeply investigate in my PhD project were UV filters, essential oils and preservatives.

1.6.1 Sunscreens

"Sunscreen product" means any preparation (such as creams, oils, gels, sprays) intended to be placed in contact with the human skin with a view exclusively or mainly to protecting it from UV radiation by absorbing, scattering or reflecting radiation.[***43***]

The suncare market constitutes approximately 3% of the overall personal care market in terms of retail value. From the three segments (sun protection, after sun, and self-tanning) composing the sun care market, the sun protection is by far the most important one.[70]

In fact, with the rise in the number of skin cancer cases diagnosed annually, negative effects of ultraviolet (UV) radiations are now well recognized and public education programs have been undertaken advocating photoprotection, including the use of sunscreens.

Acute response of human skin to UVB irradiation includes erythema, edema, and pigment darkening followed by delayed tanning, thickening of the epidermis and dermis, and synthesis of vitamin D; chronic UVB effects are photoaging, immunosuppression, and photocarcinogenesis.[71,72]

The sun emits non-ionizing electromagnetic radiation (EMR) composed of UV (100400 nm), visible (400-780 nm) and infrared (780-5000 nm) radiation. With regard to human health, the most relevant and concerning form of EMR is UVR (4-6). Ultraviolet radiation is composed of wavelengths between 100 and 400 nm that are further divided into UVC (100-290 nm), UVB (290-320 nm) and UVA (320400 nm). Wavelengths below 290 nm are absorbed by atmospheric ozone and do not reach the earth's surface.[73]

Ideal sunscreens provide uniform protection against ultraviolet A and B light, having anyway aesthetically pleasing compositions that enhance the user's compliance.

Sunscreen vehicles often determine product efficacy. To maintain the photoprotective properties and photostability of its UV filters, a sunscreen vehicle must minimize interaction of inert and active ingredients. Vehicle type also determines sunscreen durability and water resistance.[74]

The heart of any sunscreen product is the ultraviolet absorber; ultraviolet filters are classified according to their action's mechanism, physical filters (mineral filters), that act like glasses reflecting the light, and chemical filters (organic filters), that absorb the radiation's energy since they contain a suitable cromophore that has

conjugated π-electron systems. Increasing the number of conjugated double bonds in the molecule the absorption maximum shifts to longer wavelengths and also gives rise to a larger absorption cross section and, therefore, stronger absorption.[75]

All countries have a positive list of UV filters, including maximum concentration allowed in sunscreens. In most countries, including Europe and Japan, UV absorbers are regulated as cosmetics, in the United States and Canada as OTC drugs while in Australia as therapeutic drugs. The number of available UV filters differs from region to region; the US sunscreen monograph lists the least number of UV filters.[76]

Regarding the EU legislation, Annex VI indicates 28 authorized substances, of which 26 are organic filters (e.g. Benzophenone-3, Butyl Methoxydibenzoylmethane, Ethylhexyl Methoxycinnamate, Ethylhexyl Triazone, Octocrylene, etc…) and just one is an inorganic filter, that is Titanium Dioxide, with a maximum concentration of 25%, as reported in Figure9.

	INCI (International Nomendature of Cosmetic ingredients)	COLIPA (Cosmetics Europe)	USAN (United States Adopted Names)	Trademark	INCI abbreviation	Form	Concentration limits in sunscreen (%)			
							AUS	EU	JP	USA
Broad-Spectrum and UVAI (340–400 nm)	Bis-ethylhexyloxyphenol methoxyphenyl triazine	S 81	Bemotrizinol	Tinosorb® S	BEMT	p	10	10	3	*
	Butyl methoxydibenzoylmethane	S 66	Avobenzone	Parsol® 1789	BMBM	p	5	5	10	3
	Diethylamino hydroxybenzoyl hexyl benzoate	S 83	–	Uvinul® A Plus	DHHB	p	10	10	10	–
	Disodium phenyl dibenzimidazole tetrasulfonate	S 80	Bisdisulizole Disodium	Neo Heliopan® AP	DPDT	p	10	10	–	–
	Drometrizole trisiloxane	S 73	Drometrizole Trisiloxane	Mexoryl® XL	DTS	p	15	15	–	–
	Menthyl anthranilate	–	Meradimate	–	MA	p	5	–	–	5
	Methylene bis–benzotriazolyl tetramethylbutylphenol	S 79	Bisoctrizole	Tinosorb® M (active)	MBBT	d	10	10	10	*
	Terephthalylidene dicamphor sulfonic acid	S 71	Ecamsule	Mexoryl® SX	TDSA	p	10	10	10	*,†
	Zinc oxide	S 76	Zinc Oxide	Z-Cote® HP1	ZnO	p, d	no limit	‡	no limit	25
UVB (290–320 nm) and UVAII (320–340 nm)	4-Methylbenzylidene camphor	S 60	Enzacamene	Eusolex® 6300	MBC	p	4	4	–	*
	Benzophenone-3	S 38	Oxybenzone	–	BP3	p	10	10	5	6
	Benzophenone-4	S 40	Sulisobenzone	Uvinul® MS40	BP4	p	10	5	10	10
	Polysilicone-15	S 74	–	Parsol® SLX	PS15	l	10	10	10	–
	Diethylhexyl butamido triazone	S 78	Iscotrizinol	Uvasorb® HEB	DBT	p	–	10	–	*
	Ethylhexyl dimethyl PABA	S 08	Padimate O	Eusolex® 6007	EHDP	l	8	8§	10	8
	Ethylhexyl methoxycinnamate	S 28	Octinoxate	Uvinul® MC 80	EHMC	l	10	10	20	7.5
	Ethylhexyl salicylate	S 13	Octisalate	Neo Heliopan® OS	EHS	l	5	5	10	5
	Ethylhexyl triazone	S 69	Octyltriazone	Uvinul® T150	EHT	p	5	5	3	*
	Homomenthyl salicylate	S 12	Homosalate	Eusolex® HMS	HMS	l	15	10	10	15
	Isoamyl p-methoxycinnamate	S 27	Amiloxate	Neo Heliopan® E1000	IMC	l	10	10	–	*
	Octocrylene	S 32	Octocrylene	Uvinul® N539 T	OCR	l	10	10	10	10
	Phenylbenzimidazole sulfonic acid	S 45	Ensulizole	Eusolex® 232	PBSA	p	4	8	3	4
	Titanium dioxide	S 75	Titanium Dioxide	Eusolex® T2000	TiO_2	p, d	25	25	no limit	25
	Tris biphenyl triazine	S 84	–	Tinosorb® A2B	TBPT	d	¶	¶	¶	¶

Figure9. Common UV filters approved in Australia (AUS), Europe (EU), Japan (JP) and United States (USA).

Looking at the previous table, it can be noted that UV filters can be broadly classified into two types: UV absorbers (*chemical* filters) and inorganic particulates (*physical* filters).[77]

The required feature of inorganic sunscreen filters is to screen/block UV light over the whole UVA/UVB range (290-400 nm) through scattering and reflection properties that in turn are determined by the intrinsic refractive index, the size of

particles, dispersion in the emulsion base and by film thickness. This kind of filters absorb considerable UV radiation.

On the other side, chemical organic filters are classified into either UVA (benzophenones, anthranilates and dibenzoylmethanes) or UVB filters (PABA derivates, salycilates, cinnamates and camphor derivates). These filters are almost always used in combination because no single active agent, used at levels currently allowed by Regulations of different countries, provides high enough SPF (sun protection factor) protection or broad-spectrum absorption. Because of the photoinstability and possible unfavorable synergistic interactions between these agents, recent legislative restrictions have limited the choice of suitable combinations of UVB/UVA chemical organic UV filters.[78]

In my PhD project, the attention was particularly focused on two chemical filters, Butyl Methoxydibenzoylmethane and Octocrylene.

Buthyl Methoxydibenzoylmethane

Avobenzone (4-*tert*-butyl-4′-methoxydibenzoylmethane) is one of the most common UVA-filters in sunscreens, and is known to be photounstable.[79]

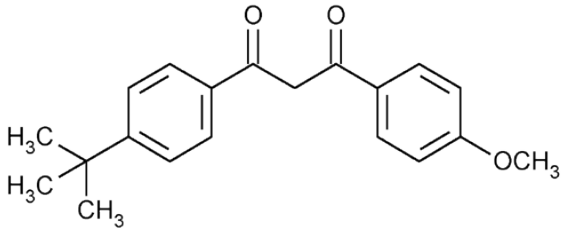

Figure10. Molecule of Avobenzone

Avobenzone has a wavelength of maximum absorption (λ_{max}) ranging from 350 nm to 365 nm depending on the used solvent. It exists in two tautomeric forms: the enol-tautomer (or enol form) and the keto-tautomer (or keto form). The keto form occurs only in one geometric form whereas the enol form has been postulated to occur in many geometric configurations.[80-82] Works in literature report[82]that an equilibrium between these two *cis*-enols exists both in the solid and solution phases of avobenzone. These *cis*-enols are stabilised by an intramolecular hydrogen bond ("chelated" enol). The different photochemical properties of these many tautomers give rise to the complex photochemistry of avobenzone.[79]

The photochemical behaviour of this filter has been extensively studied and it has been found that its photostability is highly dependent on the polarity and proticity of the solvent. [83-85] Photoallergic and cytotoxic reactions have often been associated to avobenzone due to the photodegradation products such as arylglyoxals and benzils.[80]

Several strategies have been explored in order to improve avobenzone-containing sunscreens. For example, it is well known that avobenzone–octocrylene association improves avobenzone photostability. Such as octocrylene, there are many available molecules stabilizing avobenzone but unable to quench reactive species generated due to photofragmentation.[86,87]

Other studies demonstrated that the addition of diethylhexyl 2,6-naphthalate also makes avobenzone photostable.[5] The combination of diethylhexyl 2,6-naphthalatate, avobenzone and oxybenzone is known under the commercial name of Helioplex™ and it is present in commercial products. The addition of Tinosorb S® (Ciba Specialty Chemicals) has also been shown to photostabilize avobenzone.

Octocrylene

Octocrylene is a worldwide approved UV filter that is increasingly used in sunscreens and other personal care products categories.

The Scientific Committee on Consumer Safety (SCCS), reviewed octocrylene in 1994. The SCCS opinion classified it as a nonirritant and a nonsensitizer and it concluded that there was no evidence for phototoxic or photoallergic reactions caused by this filter.[88]

This UV-absorber is an ester formed by the condensation of diphenylcyanoacrylic acid with 2-ethylhexanol, and is considered to belong to the family of cinnamates.

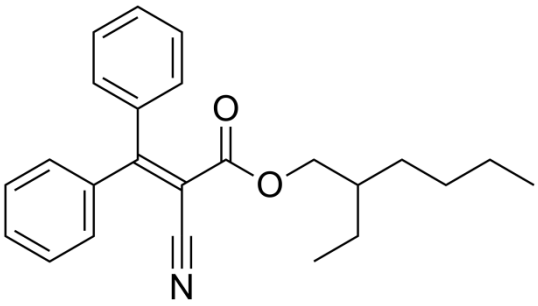

Figure11. Molecule of Octocrylene

The action spectrum of octocrylene (290–360 nm, peak absorption at 303 nm) covers mostly UVB wavelengths, but also short UVA wavelengths (UVAII).[89,90]

However, it is not a very effective filter, and for this reason octocrylene is usually combined with other UVB agents to increase the sun protection factor (SPF) of a sunscreen product, notably other cinnamates.

Octocrylene has excellent photostability [91], and is used as a stabilizer for other photo-unstable UV-filters[90-92] like Avobenzone, and to also improve their overall stability and water resistance.[93]

Octocrylene is miscible with many oils; it is one of the few UV-filters that can easily be incorporated into gel sunscreens.[94]

1.6.2 Preservatives

Many of the materials used in the manufacture of cosmetics, drugs, and toiletries are susceptible to microbial contamination and degradation. An adequate preservation is considered essential to prevent product spoilage and health hazards because of microbial contamination.[95]

In particular, in cosmetic field, according to SCCP's (Scientific Committee on Consumer Products) "Notes of Guidance", cosmetic products are divided into two different categories:

(1) Products specifically intended for children under three years or to be used in the eye area and on mucous membranes;

(2) other products.

Products intended for use on babies and the eye area (category 1) should have not more than 10^2 Cfu/g or ml of aerobic mesophilic microorganisms; other products not more than 10^3 Cfu/g or ml. *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* or *Escherichia coli* must not be detectable in 0.5 g or 0.1 mL of product category 1 and in 0.1 g or 0.1 mL of product category 2.[96]

The specific challenge of developing a multi-dose product is the need for an antimicrobial preservative. These ingredients can inhibit the growth of microorganisms that may be inadvertently introduced into the containers during product withdrawal.[97] Liquid preparations are particularly susceptible to microbial growth because of the nature of their ingredients. Such preparations are protected by the addition of preservatives that prevent the alteration and degradation of the product formulation. Preservatives are mainly effective in controlling mold,

inhibiting yeast growth, and protecting against bacterial proliferation. Their antimicrobial and antifungal properties make them an integral part of the product formulation.[98]

An “ideal” preservative or preservative system should have the following properties:

- Broad-spectrum activity: the preservative should be able to kill all types of microorganisms, that is mold, Gram positive and Gram negative bacteria;
- Effective at low concentrations: low concentration levels reduce the chances of irritation or other toxicity concerns;
- Water-soluble or Oil-insoluble: as the microorganisms grow in the water phase or at the water-oil interface, preservatives must be in the water phase to function;
- Stable: the preservative should be stable under all temperature and pH conditions that it could encounter during product processing and shelf life;
- Colorless and odorless;
- Compatible: preservatives should be compatible with all ingredients and not lose their activity in their presence;
- Shel-life activity: it would function during the manufacturing and throughout the intended life of product;
- Safety: it must be safe to human use;
- Easy to analyze: it should be easy to analyze using popular current methods;
- Easy to handle.[99]

Since the existence of a substance able to satisfy all of these characteristics is not known yet, often mixtures of two or more preservatives are used. Combinations of preservatives can potentially have synergistic or additive effects against microorganisms, and this has several advantages. Firstly, the concentrations needed for sufficient preservation of a product can be lowered. Development of allergic contact dermatitis is dose dependent, so this could potentially lead to fewer allergic reactions. Secondly, the optimal combination of preservatives is also effective against a wider spectrum of microorganisms.[100]

Synthetic preservatives that are added in food items, as antimicrobials and antioxidants, are considered to be without potential adverse effects and are classified as generally recognized as safe.[101]

Frequently used preservatives include benzyl alcohol, boric acid, sorbic acid, chlorhexidine, formaldehyde, parabens, quaternary ammonium compounds, phenol, imidazolidinyl compounds. [102,103]

Phenoxyethanol and benzoic acid derivates are actually the most used preservatives. There is instead a vertical slump of parabens, even if one of the most commonly used preservative systems for cosmetics are standard blends of phenoxyethanol and parabens.[104]

According to U.S. Food and Drug Administration, parabens are among the most commonly used preservatives in cosmetic products. Chemically, parabens are esters of p-hydroxybenzoic acid. The U.S. Food and Drug Administration (FDA) and the Cosmetic Toiletries and Fragrance Association (CTFA) in 2004 proclaimed them safe and effective for use in cosmetic formulations.[102]

However the use of parabens is becoming increasingly controversial, since they have been found in breast cancer tumors (an average of 20 ng/g of tissue). Parabens have also displayed the ability to slightly mimic estrogen (a hormone known to play a role in the development of breast cancer).[105]

Furthermore, there have been problems concerning the safety of some chemicals, including the possibility of allergies from benzoic acid and sulphites, the formation of carcinogenic nitrosamines from nitrites, and the possible rodent carcinogenicity of butylated hydroxyanisole and butylated hydroxytoluene [106,101]

For these reasons, manufacturers are continually interested in new alternative preservation systems. Due to this market demand, preservative suppliers are putting great efforts in developing alternatives for the market[107-109]

Phenoxyethanol

Phenoxyethanol has been widely accepted as an ingredient because of its positive reputation as a relatively gentle preservative that is considered non-irritating; it is also one of the few preservatives that does not release formaldehyde. The CIR (Cosmetic Ingredient Review) approves it for use and it is most often used as a preservative in combination with parabens because of its ability to kill bacteria and stabilize formulations, extending their shelf life and making them safe for use even at low levels.[102]

A Study in turkey reported the use of 0.9% PE and was found to cause contact dermatitis.[110]

Alternatives to traditional preservatives

In cosmetic field, a recent trend in preservation is the replacement of traditional chemical preservatives by antimicrobial agents that are not legislated as preservatives according to Annex V of Regulation CE 1223/2009 but that are safe and effective as preservatives. An approach to achieve preservative-free cosmetics is the selection of natural compounds that have been characterized as safe and effective against microorganisms.[111]

Among the first alternative substances with antimicrobial activity there is Glyceryl Laurate, the lauric acid monoglyceride, with a good activity against Gram +, yeast and mold. The more recent analogous with shorter chain, Glyceryl Caprate and and Glyceryl Caprylate, are characterized by higher efficacy. Ethanol, used in percentage higher than 15-20%, acts like a preservative. Also glycols have a certain antimicrobial activity; the most interesting products are Pentylene Glycol and the most effective are 1,2-Hexanediol and Caprylyl Glycol, even if also Glycerin, Propylene Glycol and Butylene Glycol showed activity.

Actually one of the most used substances is Ethylhexylglycerin, but other ingredients are used with the same purpose: aminoacidic derivates (e.g. Capryloyl Glycine, Sodium Caproyl Prolinate…), aromatic derivates (e.g. Levulinic acid and its salt, Phenethyl Alcohol, p-Anisic acid and its salt…), associations (e.g. Lactoperoxidase in association with Glucose Oxidase and Glucose).[112]

1.6.3 Essential oils

In recent years, consumers have developed an ever-increasing interest in natural products as alternatives for artificial additives or pharmacologically relevant agents. Among them, essential oils have gained great popularity in food, cosmetic, as well as pharmaceutical industries.[113]

As defined by the International Organization for Standardization (ISO), the term “essential oil” is reserved for a “product obtained from vegetable raw material, either by distillation with water or steam, or from the epicarp of citrus fruits by a mechanical process, or by dry distillation” (ISO 9235, 1997), that is, by physical means only. Accordingly, most essential oils available on the market are obtained by hydrodistillation.

Essential oils are composed of lipophilic and highly volatile secondary plant metabolites, reaching a mass below a molecular weight of 300, that can be physically separated from other plant components or membranous tissue.[114-116]

Official opinions and guidelines, such as those from the International Fragrance Association (IFRA), the "Bundesinstitut fur Risikobewertung" (BfR), the Research Institute for Fragrance Materials (RIFM), and the Scientific Committee on Consumer's Safety (SCCS) regulate maximum quantities and uses of certain oils as well as single compounds therein. Furthermore, essential oils for medical purposes need to comply with national or international Pharmacopoeia.

In addition to their widespread use as flavoring material, essential oils represent a "green" alternative in the nutritional, pharmaceutical, and agricultural fields due to reported antimicrobial, antiviral, nematicidal, antifungal, insecticidal, and antioxidant properties [117-125], or even activities on the nervous system.[126,129] These several characteristics result in a broad spectrum of applications: essential oils have been in fact suggested as antioxidants and preservatives in food [130-132] or even incorporated into foodstuff packaging material.[133,134]

Moreover, promising approaches have been reported using essential oils or components thereof in medicinal products for human or veterinary use.[135-137]

Therefore, in recent times, essential oils have gained great popularity as consumers have developed a particular growing awareness toward the use of natural ingredients, especially in food, household, and cosmetic products.[138,101]

A multitude of different, but often structurally closely related, components have been identified in essential oils. Each oil in turn can be composed of only a few up to a complex mixture of far more than 100 single substances, respectively.

Due to their structural relationship within the same chemical group, essential oil components are known to easily convert into each other by oxidation, isomerization, cyclization, or dehydrogenation reactions, triggered either enzymatically or chemically.[113]

Another issue about essential oils is the possibility of their interaction with plastic packaging. In the past, some studies were reported in literature, regarding the interactions between flavor compounds and polymer films was reported.[139,140]

Nowadays, considering the always more extensive use of natural ingredients, in particular essential oil, the absorption of flavor compounds by packaging is becoming an important product-package interaction aspect. Flavor absorption may

alter the aroma and taste of a product [141], or change the mechanical properties of polymers, such as tensile strength [142] and permeability. [143]

Flavor absorption extent is influenced by the properties of the polymer, the flavor molecules, and also external conditions. The chemical composition, chain stiffness, morphology, polarity, and crystallinity of the polymer influences flavor absorption, as does chemical composition, concentration, and polarity of the flavor compounds, as well as the presence of other chemical compounds. External factors such as storage duration, relative humidity, temperature, and the presence of other food components can also affect solubility of aroma compounds in a polymer. [144-147]

Low-molecular-weight compounds (especially apolar compounds, as in most flavor substances) are readily absorbed.[148]

Several investigations have shown that PE and PP can absorb considerable amounts of flavor compounds. However, less information is available in the literature about the amount of flavor absorption by PET, PEN, and PC.[149]

Mandarin essential oil

Mandarin is among the most popular citrus consumed as fresh fruit primarily because of its delicate flavor and it is classified according to Tanaka into more than 30 species, comprising from one to several tens of varieties. Sweet mandarin types have been used for dessert fruit since ancient times, and sour types have been used as rootstocks and for flavorings and medicine.[150]

Bioactive molecules contained in Citrus fruits are phenolic acids, carotenoids, polymetoxyflavones, limonoids, coumarins, furocoumarins and anthocyanines. Among these molecules, polymethoxyflavones (PMFs) have been extensively studied. The most common PMFs in Citrus fruits are nobiletin and tangeretin, present in sweet orange, mandarin, bitter orange, pumelo, and grapefruit. Several studies were carried out to evaluate the therapeutic properties of these two PMFs that have shown to possess lipid-lowering, hypoglycaemic, anti-inflammatory, antioxidant, and anticancer activities; therefore, the interest for these com-pounds is increasing in the last years, due to the potential use as nutraceuticals or therapeutic agents.

Mandarin essential oils, obtained from cold-pressed fruit peels, are widely commercialized all over the world. Italian mandarin oils are considered as the most valuable, because of their organoleptic properties, due to the optimum growth conditions. Mandarin oil is used in different fields, such as food/beverage and

perfumery industries, to enhance the bouquet of flavor and fragrance compositions. Mandarin essential oil is characterized by the presence of: the sesquiterpene aldehyde α-sinensal, its major odorant (also present in orange oil), the aromatic ester methyl-N-methyl anthranilate, the aromatic alcohol thymol, g-terpinene, and b-pinene contribute to the characteristic flavor of mandarin juice and mandarin peel essence oil.[151,152]

Works in literature reported that the major compounds in mandarin essence oil are limonene (77.3%), α-terpinene (14.2%), α-pinene (1.8%), myrcene (1.7%), β-pinene (1.1%) and terpinolene (0.6%).[152]

The quality and the odor of the oil are influenced by the limonene content which may vary in the different agro-climatic conditions.[153]

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2. OBJECTIVES

Packaging is one major field of application for plastic materials.

The most important function of a packaging material is the quality preservation of the packed goods. Among these goods, pharmaceuticals and cosmetics hold a place of special importance for their possible chemical instability due to the presence of active substances.

In order to fulfill the task of safety, quality, and efficacy assurance of the packed product with minimal impact both on the product and on the environment, the packaging must be optimized by taking into consideration various criteria. One of this criteria is the complete knowledge of possible interactions between packaging material and the contained product.

Starting from this consideration, the final goal of my PhD project was to draw up specific protocols and indications for the evaluation of interactions between liquid/semi-solid formulations and plastic packaging.

Specifically, the work was divided into three phases, according to different aims.

The purpose of the first phase was to develop experimental protocols and to set up analytical methods specific for packaging materials.

The second step, instead, focused on the choice of actives, formulation's variables, formulations' set up and characterization.

Finally, the third phase aimed to evaluate specific examples, applying methods and protocols previously set up in order to evaluate the stability and the interactions of semisolid formulations, containing particular actives, with selected packaging materials.

3. RATIONAL OF PROJECT

The materials chosen for the primary and secondary packaging are essential as they must offer to final products all the necessary guarantees to reach consumers in optimum conditions.

Packaging is the most important field of application for plastic materials, according to their several functional properties. In the optimization of packaging from an ecological, waste production, raw materials provision and economical point of view, the quality assurance of the packaged product and therefore the guarantee of consumer's safety must have priority.

According to this assumption it's important to have a complete knowledge of possible interactions between packaging and products during their contact time. Specific classes of polymers, in fact, in particular environmental conditions may be subject to aging, with impairment of both mechanical, chemical and physical properties and their performance respect to the contained formulation. Finally, quality, efficacy and safety of the product may be compromised.

Among the different goods, foods, pharmaceuticals and cosmetics are the most interesting, because of the presence of active substances, which sometimes can have relatively low chemical stability.

In the light of these considerations, the aim of this PhD project was the study of possible interactions between content and container, in order to draw up specific guidelines, now still missing, useful for the evaluation of liquid and semi-solid formulations in relation to final packaging.

This work began with a preliminary scientific research, presented and discussed in my graduation thesis, titled "Experimental protocol for the study of content-container interactions in cosmetic field: investigation on PLA"; this work was focused on a first screening of content-container interactions using polylactide (PLA), a polymer derived from renewable sources, as packaging material. Part of this study was published as a paper titled: "Preliminary Evaluation of Packaging-Content Interactions: Mechanical and Physicochemical Characterization of Polylactide Bottles", reported in Annex I.

Starting from this experience my PhD project was divided in different steps.

After a preliminary screening of polymeric materials used as primary packaging in pharmaceutical and cosmetic field and a study of their characteristics and possible

criticalities, the first phase focused on the set up of protocols in order to study packaging materials' properties and their possible changes after stress conditions. Specifically Chapter I describes an optimized procedure in order to evaluate mechanical properties of commercially available packaging used for semisolid formulations. For this purpose, suitable designed specimens with modified "dog bone" shape (starting from ISO 527, related to polymeric films) were developed in order to obtain repeatable and standardized stress-strain curves by tensile test. Furthermore, some accelerated stability protocols were set up in order to simulate stress conditions that containers could undergo during their shelf life. Polyethylene terephthalate glycol (PETG) containers were used to set up the procedure.

Results highlighted that, starting from mechanical properties of polymer itself, it is possible to characterize, in a reproducibly way, commercially available containers. Thanks to the mechanical parameters obtained by the tensile test it was possible to perform both an assessment of pack stability and the evaluation of interactions between the pack material and the conditions or substances which are in contact with. In fact, we demonstrated that the tensile test is a a suitable method to evaluate the quality of finished packaging and any changes occurring during its time of use.

Once the method for mechanical analysis has been set up, by perfecting a specimen to be usable for any materials and any shapes of final containers, a practical and more complete approach to investigate commercial polymeric containers , in order to perform safety evaluation was proposed (Chapter II). In fact, a variation of mechanical performances could greatly affect cosmetic safety. This work underlined as, first of all, it is essential to consider packaging as a raw material and, for this reason, it is very important to obtain complete information on it from suppliers. Until now suppliers give information about polymeric materials used in the packaging production but not enough technical data about finished package, including mechanical data.

In my work, to the application of an appropriate full factorial experimental design, in order to investigate variables, like polymeric density, treatments, or formulation type involved in changes to packaging properties or in formulation-packaging interaction were proposed. As practical example, containers made of two types of polyethylene with different density, low density polyethylene (LDPE) and high-density polyethylene (HDPE), were investigated.

First results demonstrated that plastic packaging materials were not completely inert respect to the contained formulation but some changes could occur when they undergo simulated stress conditions. Furthermore, as said before, official guidelines to evaluate possible interactions between packaging and contained products are still missing, especially in cosmetic field, so normally the indications followed to perform this kind of studies are the ones provided by food legislation. EU Food Regulation 10/2011 expects the use of some food simulants for reproducing the contact between packaging and contained product. Six simulants are reported, but no alkaline solutions are expected.

However, in cosmetic field also basic products are present on the market, like hair dyes and depilatory cream. From this consideration, the study reported in Chapter III aimed to propose alkaline simulants, to mimic also this kind of products.

The proposed simulants were three, a pH 10 buffer solution and two emulsions, with and without silicones, in order to represent all possible cosmetic products with alkaline pH.

In order to demonstrate the applicability of these simulants, some studies on different polymeric materials (recycled Popyethylene Terephtalate (PET-r), Linear Low Density Polyethylene (LLDPE) and Low Density Polyethylene (LDPE)) were carried out.

These practical study cases confirmed again that some changes in packaging material could occur after the contact with this kind of content.

All these studies showed the importance of analyzing final packaging with respect to the contained product. In fact, the presence of additives, surface treatment, processing, cleaning procedures, contacting media, adhesives may affect the material's properties. In order to deepen this aspect, a study on polyethylene (material extensively used in packaging field) was performed; this work, reported in Chapter IV, considered and analyzed plastic material both as raw material (pellets) or standard samples (ISO specimens) and as final containers. Simultaneously, the characterization of the organic extractables from these materials was performed by controlled extraction studies, via multiple extraction processes and extracting media, in order to establish best practices for performing controlled extraction studies specifically relevant for the plastic materials investigated, usable both for research laboratories and for companies. Extractables, organic and/or inorganic chemical

entities, can migrate out of the packaging system, accumulate as leachables in the product and affect safety and/or efficacy.

The GC/MS was chosen as analytical method.

Comparing results obtained by chromatographic analyses Head Space Solid phase microextraction was selected as test method to perform the successive controlled extraction studies specifically relevant for the plastic materials investigated.

From a migration point of view, the tested packaging showed a possible absorption of some substances of the formulation during their contact time.

Once focused the attention on the set up of experimental protocols and analytical methods for characterizing different packaging materials, the second part of research considered the development of semisolid formulations usable later as content in order to evaluate their stability in relation with plastic packaging and their possible interactions phenomena.

For this purpose, a study of the most critical ingredients in formulations was carried out. A part of this phase was developed through the Erasmus Traineeship program at the Application Laboratory of Merck KGaA, sited in Fontenay-sous-Bois (France). The attention was focused on UV filters, because they could be present both in formulations and in packaging, and these active are very important substances in the prevention of skin cancer and photoageing.. The study concerning the setup of sunscreen formulations is reported in Chapter V.

In order to evaluate the relationship between formulations containing UV filters and plastic packaging (polyethylene), two UV filters were specifically considered, Butyl Methoxydibenzoylmethane (Avobenzone) and Octocrylene, in free or encapsulated form. This study, showed in Chapter VI, aimed to evaluate the behavior of filters in emulsions when undergone to accelerated stress treatments in polymeric containers and the possible differences between free and encapsulated (micro-encapsulation technology that entraps organic sunscreen in sol-gel silica glass) filters.

For this purpose, packaging was characterized before and after treatments, as set up in the previous phase of project, while formulations were evaluated for pH, rheological and organoleptic properties and for content of UV filters in time and after treatments.

Moreover, other two techniques were associated in order to further investigate formulations' stability: NIR (near infrared reflectance) spectroscopy, as non-

destructive characterization of formulations, and the multiple light scattering technique with Turbiscan Tower, as predictive of emulsions' behavior in time.

In order to deepen the use of NIR spectroscopy in cosmetic field, a specific work on the use of this technique as complementary non-destructive analysis to evaluate the stability of a formulation was performed and described in Chapter VII.

Finally, as another applicative example besides the evaluation of sunscreens, other active ingredients were investigated; in particular, a study on the impact of traditional and more natural preservatives on formulation, with and without the presence of a natural essential oil (mandarin E.O.), was performed and illustrated in Chapter VIII.

In fact, in cosmetic field, a recent trend in preservation is the replacement of traditional chemical preservatives by antimicrobial agents that are not legislated as preservatives according to Annex V of Regulation CE 1223/2009. An approach to achieve preservative-free cosmetics is the selection of natural compounds or functional substances that demonstrated also antimicrobial activity.

In addition to methods and techniques already used in the previous works, a microbial count was carried out in order to evaluate the preservatives' efficacy after several treatments and contact with plastic packaging.

Chapter I.

PROTOCOL AND SPECIMEN SET UP FOR MECHANICAL EVALUATION OF COSMETIC PACKAGING

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PROTOCOL AND SPECIMEN SET UP FOR MECHANICAL EVALUATION OF COSMETIC PACKAGING

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PROTOCOL AND SPECIMEN SET UP FOR MECHANICAL EVALUATION OF COSMETIC PACKAGING

ABSTRACT

The main objective and the novelty of this study is to present an optimized procedure in order to evaluate mechanical properties of commercially available packaging used in cosmetic field in order to assure quality and safety of the final product as required from EU Legislation. Specifically, suitable designed specimens with modified dogbone shape are developed in order to obtain repeatable and standardized stress-strain curves. Poly (ethylene terephthalate)-glycol (PETG) containers are used to set up the procedure. Empty and filled bottles containing pH 2 solution are investigated before and after stress treatments performed in according to European Medicines Agency (EMA) guidelines; each sample was subjected to tensile test and
stretched to the breaking point in order to study its stress-strain profile. Results highlighted that, starting from mechanical properties of polymer itself, it is possible to characterize, in a reproducibly way, commercially available PETG containers; after that it is possible to verify if the contact with extreme pH solution or specific treatments (heating or simulating solar irradiation), can lead to packaging modification. This research represents a starting point to study in detail the finished packaging and the possible product-package interactions in the pharmaceutical, cosmetic and nutraceutical fields.

KEYWORDS: mechanical characterization; polymeric packaging; formulation/packaging interactions; polyester; PETG.

Rigid and semi-rigid containers produced from polymeric materials are one of the fastest growing categories of cosmetic packaging. Bottles, jars, tubs, tubes, blister packages and drums are included in this category. Some of their most significant advantages compared to alternative materials are their light weight, resistance to damage, low in cost, pleasant to handle touch and in some cases, characterized by a good transparency grade, recyclable and obtainable from biobased sources.[1-3]

The choice of primary and/or secondary packaging materials depends on the degree of protection required, compatibility with the contents, the filling method, cost, but also on the attractiveness and the convenience for the user (e.g. size, weight, method of opening/reclosing, legibility of printing).[4-6]

The most important function of a packaging material is the quality preservation of the packed goods. It is well known from literature that the interaction with packaging can lead to a degradation of the packed product.[7-9] For these reasons it is absolutely necessary to be able to determine in advance which types of plastics are likely to provide suitable product shelf live and integrity for specific products under a variety of environmental conditions.

Manufacturers often cannot afford to invest time and money in evaluating candidate materials for new products. If there a thorough understanding of the interaction between products, packaging and their storage and distribution environments, then manufacturers could use knowledge about their product to determine, without a substantial investment in testing, whether a particular plastic pack meets their needs.[10]

In the cosmetic field the product makers, designers and plastic packaging formers must cooperate to provide an operational and safe system product-package. Despite the importance of these aspects, there are too little information about the possible chemical-physical interactions between formulation and packaging, because, differing from food packaging, the cosmetic one isn't regulated and nowadays appropriate guidelines are still missing. However, with the full entry in force of EU Cosmetic Regulation 1223/2009, has become mandatory, in the Cosmetic Product Safety Report, a section regarding information about the packaging material and the procedures to evaluate the interactions between content and containers, in order to assure the safety of the product.[11]

Actually, there are no standard procedures for the evaluation of cosmetic product-packaging interactions. An appropriate assessment may be made based on the knowledge of the formulation and primary packaging materials and experienced expert judgment. In other words it is extremely important to verify that there is not incompatibility between materials that could compromise the formulation quality and its maintenance.

Some essential requirements for packaging materials are high tensile strength, ductility, flexibility, sometimes transparency and good barrier properties. In particular, cristallinity and density influence many polymer properties including hardness, tensile strength, stiffness and melting point.

Furthermore, the production process can also affect the distribution of the container wall temperature, thickness, crystallinity and orientation. These distributions are responsible for the final products mechanical, barrier, optical and orientation properties of the polymeric chains.

Finally, these properties could be also influenced from environmental conditions like temperature, humidity and UV-Vis irradiation.

In literature a substantial number of studies related to mechanical properties of polymeric materials have been reported.[7,10,12,13] Most of those studies focus on tension tests and the obtained tensile characteristics help to determine the mechanical properties of polymers. Specifically, official ISO 527 specifies the general principles for determining the tensile properties of plastics and plastic composites under defined conditions (ISO EN UNI 527- 1996). [14] However, the method proposed in this official document are used to investigate mechanical properties of polymeric sheet or film. Plastic finished packaging are not included in this ISO document; in fact, it could be difficult or quite often impossible to obtain the specimens proposed into ISO document due to surface irregularity and manifold design of currently commercially available packaging.

In the last years plastic materials attracted both industries and research institutions for their various properties. In particular polyesters such as poly (ethylene terephthalate) (PET), are thermoplastic polymers easily molded in complex shapes. Polyethylene terephthalate is a condensation polymer typically formed by the reaction of terephthalic acid or dimethyl terephthalate with ethylene glycol in the presence of a catalyst. Polyethylene terephthalate

(PET) since 2000 is the second most used plastic in bottles and other rigid and semi-rigid containers. Historically, the first use of PET bottles was for soft drinks. PET's barrier to carbon dioxide permitted the first successful commercial introduction of plastic soft drink containers for the carbonated beverage industry in about 1977.

The properties of thermoplastics can be controlled by chain length, by degree of crystallinity and by blending and plasticizing.

The large growth in PET use is related to several advantages of PET compared to HDPE. PET has a higher glass transition temperature (78°C) and melting temperature (245±265°C), and it has excellent clarity and sparkle, which allows it to compete with glass. Its impact properties give PET containers a considerable safety advantage over glass and its light weight brings transportation economy.

While PET's water vapor barrier is inferior to HDPE, it is a significantly better oxygen barrier and a much superior carbon dioxide barrier, especially when biaxially oriented. PET is also a better barrier to most odor and flavor compounds and to hydrocarbons. PET is chemically more reactive than HDPE and in particular, must be dry before processing to avoid hydrolysis at elevated temperatures.

A PET cousin is a copolymer, glycol-modified PET (PETG). Specifically, PETG is a random copolymer consisting of about 30 mol% PCT (poly1,4-cyclohexylenedimethylene terephthalate) and 70% PET. The letter G refers to the additional glycol group along the backbone of the copolymerizing agent PCT. Because of its decreased crystallinity and enhanced melt strength, it can be processed in ways that are not possible for most grades of PET, to make highly transparent bottles, blisters and other containers.

Poly (ethylene terephthalate)-glycol (PETG) does not occupy the same industrial niche as PET, precisely because it lacks the ability to undergo strain-induced crystallization. Instead, its uses are directed toward applications involving large, thermoformed parts. [15]

Polyesters in fact are thermally unstable and exhibit rapid loss of molecular weight as the result of thermal treatment. The ester linkages tend to degrade during thermal treatment or under hydrolytic conditions. Several reactions such as hydrolysis, depolymerization, oxidative degradation and inter-intramolecular trans-esterification reactions to monomer and oligomeric esters, are suggested to be involved in the degradation process.

The main goal of this work is to define and to describe a new standard operation procedure to investigate commercially available packaging.

For this purpose PET-g bottles were investigated to verify the suitability and the reproducibility of the procedure herewith described.

The mechanical properties of containers were investigated also by evaluating the effect of chemical, physical and climatic environmental factors (humidity, solar irradiation and heat).

MATERIALS AND METHODS

PETG cosmetic packaging bottles of 150 mL volume, produced by extrusion blow molding, were obtained from an Italian Company.

The raw material is a polyethylene terephthalate glycol (PETG) copolyester 6763, produced by Eastman Easter Chemical Company, USA. It is a clear, amorphous material with glass transition temperature 80°C, average molecular weight (Mn) of about 26,000, density 1,27 g/ml.

Set up of sample for mechanical analysis

All samples were realized from polymeric containers maintained at standard conditions (23°C, 55% R.H.). Specimens were obtained from polymeric flat surfaces paying particular attention not to bend or fold in any way their surfaces cutting out from the flattest portion of the bottles (back end and neck were excluded); only middle area was considered. All the samples chosen are free of twist and free from scratches, pits, sink marks and flash.

Three different shapes and dimensions of test specimens were prepared.

- rectangular shape, with a dimension of 30x100 mm,
- dog bone samples according to ISO specifications
- optimized dog bone shape obtained by punchcutting (Figure 1). This design was developed in order to obtain a localized stress region 3 mm width and thickness depending from the sample.

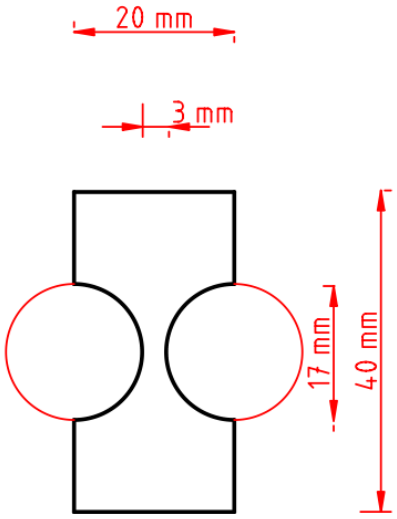


Figure 1. Scheme of dog-bone specimen.

For each PETG container, at least 3 standard samples were obtained.

Wall thickness distribution for each type specimen and sample width were measured at 3 different points using a digital microscope model BW 1008. This thickness control test allowed to determine if the plastic material was regularly distributed on the bottle surface. If not, it could consider that blow-moulding process conditions was not well optimized.

The section of each sample is calculated from thickness and width using a suitable software program (micromeasure vers.1.2).

Measurement of mechanical properties

Uniaxial tensile test was performed using the AGS 500ND tensile machine (Shimadzu corporation, Kyoto-Japan) equipped with a 500 [N] load cell. Rectangular and dogbone strip were cut as described above. The polymeric samples were held between two clamps positioned at a distance of 17 mm, respectively, in according to specimen type. The clamping system is designed to not cause premature fracture at the grips and to avoid any slippage between the grips and the tested specimen.

The test specimen was placed in the grips, taking care to align the longitudinal axis of the sample with the axis of the testing machine. It was centered and aligned using a jig constituted of two steel cylinders of the same punch diameter used for the

preparation of the sample, placed on the jaws (Figure 2).No preliminary tension was applied to the sample during the alignment, centering and clamping phase.

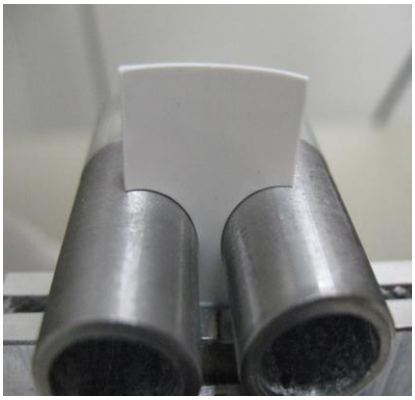


Figure 2. Steel centering and alignment tool.

The tests were performed using a strain rate at 0.5 mm/min. Mechanical measurements were carried out at room temperature (23°C) and 55% R.H.

Of course where an obvious fault has resulted in premature failure, the sample was not included in the analysis.

For each container at least three samples were analyzed.

This procedure permitted to obtain a stress versus strain curve. Data were collected and elaborated by a suitable software (LJStream UD v. 1.14).

The tendency of materials to oppose to deformation until break, and the evaluation of the stress-strain curve profile were investigated.

This kind of analysis permits to determine the tensile properties of plastics and plastic composites, as specified from ISO 527. In particular it is possible to estimate.

- Elastic portion by a linear regression procedure (Et);
- Stress properties: yield stress (σy), tensile strength ((σM) and tensile stress at break (σB);
- Tensile strain expressed as the increase in length: at yield (εy), at tensile strength (εM) and at break (εtB).

With the optimized dog bone shape developed in this work it is possible to obtain the above described parameters concerning the actual behavior of the investigated packaging, as shown in the Figure 3.

Tensile test was performed on all type of test specimens.

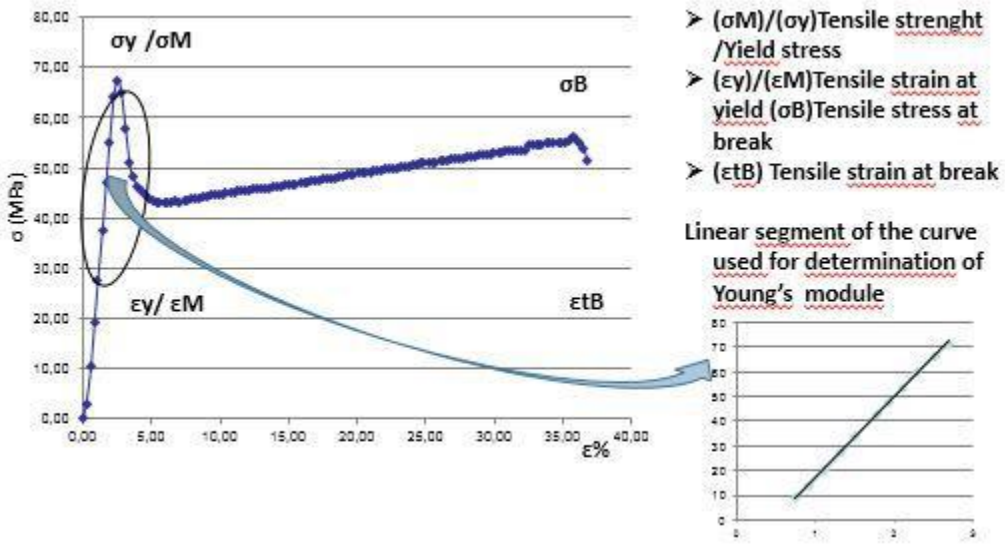


Figure 3. Parameters investigated in stress versus strain curve profile.

In this work PETG is chosen as a representative example for packaging material.

It is possible to validate the procedure proposed in this research by comparison between mechanical properties of raw material (MSDS supplied from the producer) and results obtained experimentally from finished products.

Container test protocol

PETG bottles were numbered, weighted and washed. The washing procedure used at the beginning of the study and at the end of all treatments was the following: all bottles were washed for three times with 1% bicarbonate solution and then rinsed three times with distilled water to remove any residuals.

Empty bottles and bottles filled with pH 2 buffer solution were subjected to an accelerate stability test by incubation for 15 and 30 days into climatic room (Clima Cell 111 MMM) at 40°C with 75% R.H. and through a photostability test by simulating UV-visible ray irradiation using SUNTEST XLS +II (Atlas ®) for 24 and 96 hours. Bottles stored at room temperature were used as controls.

Accelerated stability test was performed in according to cosmetic and EMA guidelines: test parameters (duration, temperature and humidity values) were set up considering accelerated stability testing relative to pharmaceuticals products. [16-18]

5/6 specimens were obtained from each containers at the beginning and at the end of all treatments. Finally, mechanical analysis was performed using only the optimized dog bone shape.

RESULTS AND DISCUSSION

The study here discussed aims to realize an easy and standardizable procedure to characterize mechanical properties of commercially available packaging. This procedure is based on a new simplified designed shape of the test specimen to respect to the International standard guidelines. [14]

Sample set up

Initially, specimens with rectangular shape have been realized. Rectangular sample was not useful for this work. In fact, in this case, ruptures happened randomly and mainly in the tightening area. Data were unreliable since extension behavior of samples were invalidated due to the anchorage. This phenomenon was due to micro-cracks correlated to sample-jaws interactions.

The following choice of dog bone shape to realize standard specimens was derived from the literature (BS EN ISO 527, 1996). Due to the different sort of commercially available packaging it is extremely difficult to obtain samples corresponding to ISO requirements especially for the thickness. Furthermore the variability of pack size and shape greatly affects the capability to obtain dog bone samples with smooth surface and free of buckling. Finally, the available area of the sample doesn't allow quite often to obtain the shape required.

In both cases (rectangular and ISO-designed shapes) random results are obtained and therefore the strain-stress profile curves are not descriptive of material mechanical properties. For these reasons all following analyses were carried out only on optimized dog bone specimens.

The new sample design herewith developed and its dimensions are adequate to the common type of tensile instruments using multipurpose load cells obtaining an high grade of accuracy, precision and sensitivity. Furthermore, the new sample design is compatible with the area extractable from the most part of commercial packaging.

The chosen shape permits a correct and easy placement and clamping of the specimen. In that way sample results quickly aligned and centered with the tensile direction and parallel to its
longitudinal axis, using a suitable jig, as shown in the Figure 2. The simplified design of this dog bone sample minimizes the complexity and the stress of the cutting process.
Anyway it is essential to pay attention in the sample preparation because even the slightest inappropriate deformation can compromise the sample integrity (Figure 4).

Figura4. Compromised sample by improper manipulation.

The dimensional gap between narrow (3 mm width) and clamping portions (20 mm width) permits to exclude the effects happening in the tightening area and to lead to a specific and constant break area. As it is possible to observe from the images (Figure 5 a,b) the section of container specimen reduced and it became matt because of traction. An initial stage of elongation where the sample does not appear to undergo irreversible deformation is present, until the moment in which the point of necking occurs. In this phase the fibers of the polymer begin to fail aligning in the direction of application of force, forming the peak yield strength. Continuing the traction the section of the specimen continues to shrink up to the breaking point. In particular the Figure 5 b shows clearly that specimen rupture occurred exactly in the expected zone, confirming the validity of the procedure.

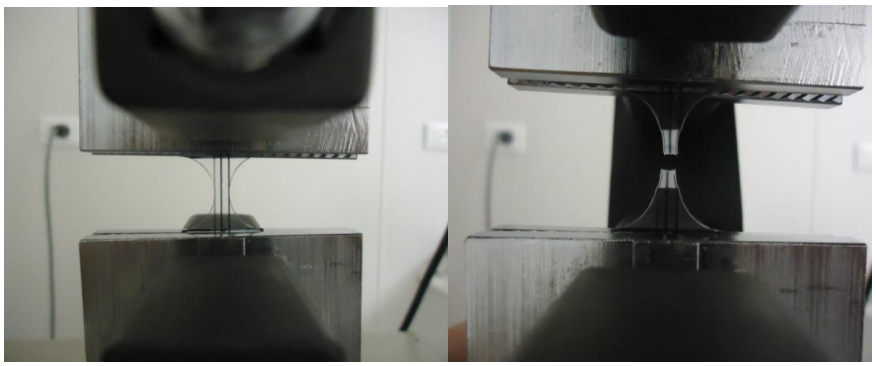


Figure 5. Sample specimen, from left: a) before and b) after stress-strain test.

Mechanical analysis

The tensile test is one of the most important method used to measure the strength of materials. During the tensile test a sample of material is elongated in uniaxial direction at a constant rate. The load necessary to produce the given elongation is measured as a dependent parameter.

Each sample is subjected to tensile test and stretched to the break in order to study its stress-strain profile: the Figure 6 shows a typical stress strain behaviour obtained from PETG bottle samples.

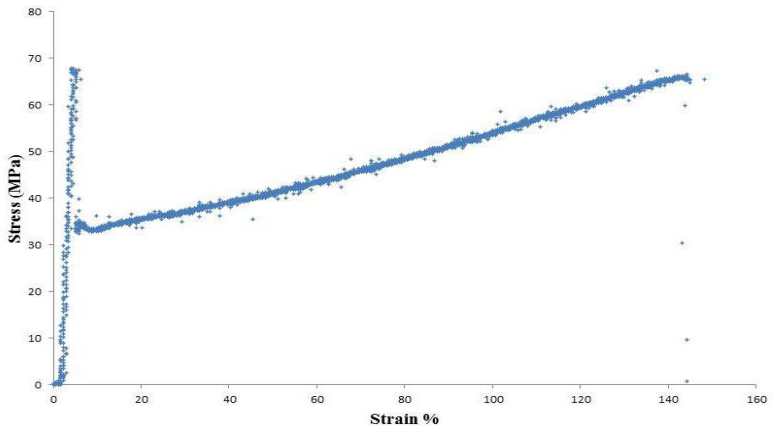


Figure 6.PETg stress-strain profile: mean values of specimens of the same container (S.D. < 5%).

The trend is represented by.

1. Initial linear growth: that is, the elastic phase, in which the specimen keeps its elastic properties and traction does not cause irreversible changes.

2. Yield: fall of the opposing force from the material, which is the peak yield. At that point is the partial rupture of polymer fibers (necking), thus causing a weakening of the structure.
3. Plastic deformation: is the time in which the polymer loses its elastic capacity (reversible deformation) and assumes plastic properties (irreversible deformation). The polymeric fibers, here, tend to align parallel to the zone of elongation; in fact the specimen continues to lengthen until the bonds between the polymer fibers do not become weakened completely.
4. Breaking point of the specimen.

The results highlighted that the mechanical characteristics of tough material with yield point, typical for the PETG raw material, are maintained in the finished packaging.

The Table 1 reports the tensile properties experimentally obtained from empty bottles and as resulting from raw material data sheet.

Table 1. Mean values (±S.D) of mechanical properties experimentally obtained from empty bottles and as resulting from raw material data sheet*.

Mechanical properties	Polymer (raw material)*	PETG empty bottles
Tensile strenght (σM)= Yield stress (σy) (MPa)	50	62 (3.830)
Tensile strain yield (εy)=(εM) (%)	-	5.6 (0.853)
Elastic module (MPa)	2100	1813 (333.04)
Tensile stress at break (σB) (MPa)	28	65 (1.123)
Tensile strain at break (εtB) (%)	100	148 (3.460)

Data highlight that the elastic module obtained from the new dog-bone shape bottle sample is comparable to the value of raw material. These results suggest that the bottle preparation process does not affect material mechanical properties. The results

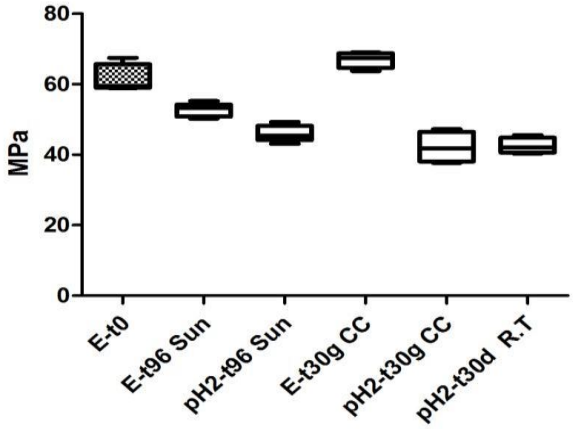
showed clearly that the new sample design fits perfectly to investigate processed materials tensile properties, such as packaging products.

Furthermore it is important to underline that this kind of analysis could not be used to compare results obtained using ISO specimen; the approach described in this work can successfully be employed to evaluate modification of packaging during ageing or during contact with the product packed in order to define the shelf life of the product.

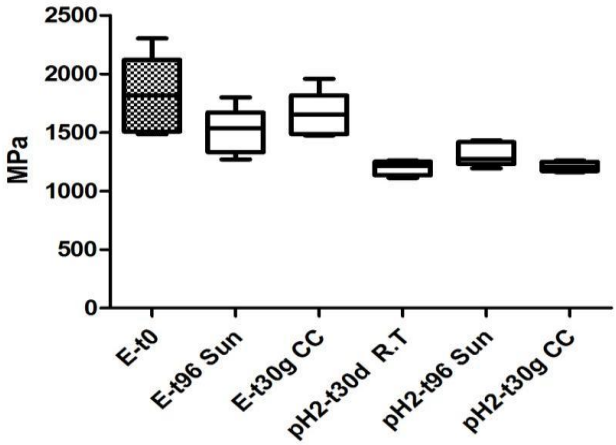
For this purpose mechanical properties of empty and filled bottles, before and after stress testing procedures, were investigated. The stress-strain curve profile is useful to compare specimens subjected to environmental and chemical stress.

Specifically, the mechanical protocol was applied to evaluate how environmental parameters, such as heat, humidity, solar irradiation and chemical exposition to extreme pH, could influence mechanical performances of packaging.

The Figure 7 shows results obtained from mechanical analyses carried out on empty and filled bottles before and after stress test.



a)



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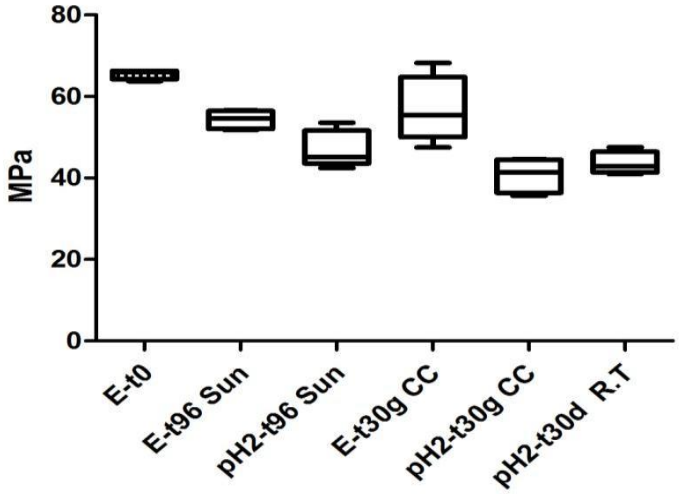

c)

Figure 7. Parameters obtained from Stress-strain profiles of empty and filled PETG bottles before and after stress conditions: a) Yield stress (MPa); b) Elastic module (MPa); c) Tensile stress at break (MPa).

Temperature, water and electrolytes could be considered important factors that influence polymeric structure. In particular, water and electrolytes can penetrate and interact, with polymers chains, decreasing, in the first case, intermolecular forces producing a decrease of polymer chain cohesion.

Instead, heat treatment can determine a modification in mechanical parameters depending from the structure of the polymer tested.

The internal properties of the polymer are very influent on these mechanisms. In particular the amorphous phase greatly affect the polymer behavior already in the early stages of the tensile test in which it starts to align in the direction of traction. [19]

Comparing graphics in Figure 7 with the Table 1, it possible to observe the effects of these all factors. In particular, it is possible to observe a different effect in the empty samples, subjected to climatic room or simulated solar irradiation. Young modulus and tensile stress at break reduce in PETG containers treated in climatic room or solar simulator to respect to baseline, in according to heat effect and water activity.

Observing PETG samples treated with pH2 buffer, curve profiles and values parameters were different respect to corresponded empty sample. Electrolytes destabilize monomer-monomer bond by inducing a degradation of the sample. In Figure 7, it is clear as elastic modulus and yield stress decrease because of

weakening of intermolecular bonds. Moreover, polymeric material becomes more fragile and consequently point break is anticipated.

Finally, all samples, independently from treatment, undergo to structure modifications that determine a dropping of point break.

CONCLUSION

In conclusion, the research, here presented, defined an opportune protocol to examine mechanical properties of polymeric material, used in a cosmetic packaging.

This procedure based on a new designed shape of the test specimen, can be successfully applied to the great part of commercially available packaging.

Starting to PETG bottles with critical shape, specimens with modified dog-bone geometry permitted to execute a qualitative study of polymer and to obtain repeatable stress-strain profiles. Thanks to graphics it was possible to evaluate some characteristic parameters by which it was possible to evaluate as environmental and chemical treatments can determine modifications and consequently, alterations of mechanical performances.

Thanks to this work it observed as PETG could be a critical packaging that could compromise content properties by polymer degradation during ageing. Consequently, PETG could lose its protective function.

This research represents a starting point to study in detail the possible interactions between container and contained in the pharmaceutical, cosmetic and nutraceutical fields.

The tensile test is one of the most important method used to measure the strength of materials and it could be successfully employed to evaluate content-container interaction that causes mechanical alterations.

The new sample design herewith developed and its dimensions are adequate to the common type of tensile instruments using multipurpose load cells obtaining an high grade of accuracy, precision and sensitivity.

In conclusion the optimized procedure presented in this work could successfully employed to evaluate interactions between content and commercially available packaging in order to assure quality and safety of the final product as required from EU Legislation.

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Chapter II.

PACKING EVALUATION APPROACH TO IMPROVE COSMETIC PRODUCT SAFETY

cosmetics

MDPI

Article

Packaging Evaluation Approach to Improve Cosmetic Product Safety

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PACKING EVALUATION APPROACH TO IMPROVE COSMETIC PRODUCT SAFETY

ABSTRACT

In the Regulation 1223/2009, evaluation of packaging has become mandatory to assure cosmetic product safety. In fact, the safety assessment of a cosmetic product can be successfully carried out only if the hazard deriving from the use of the designed packaging for the specific product is correctly evaluated. Despite the law requirement, there is too little information about the chemical-physical characteristics of finished packaging and the possible interactions between formulation and packaging; furthermore, different from food packaging, the cosmetic packaging is not regulated and, to date, appropriate guidelines are still missing. The aim of this work was to propose a practical approach to investigate commercial polymeric containers used in cosmetic field, especially through mechanical properties' evaluation, from a safety point of view. First of all, it is essential to obtain complete information about raw materials. Subsequently, using an appropriate full factorial experimental design, it is possible to investigate the variables, like polymeric

density, treatment, or type of formulation involved in changes to packaging properties or in formulation-packaging interaction. The variation of these properties can greatly affect cosmetic safety. In particular, mechanical properties can be used as an indicator of pack performances and safety. As an example, containers made of two types of polyethylene with different density, low-density polyethylene (LDPE) and high-density polyethylene (HDPE), are investigated. Regarding the substances potentially extractable from the packaging, in this work the headspace solid-phase microextraction method (HSSPME) was used because this technique was reported in the literature as suitable to detect extractables from the polymeric material here employed.

Keywords: safety evaluation; polyethylene; packaging; mechanical properties

INTRODUCTION

Packaging can be defined as an economical means of providing presentation, protection, identification, information, containment, convenience, and compliance for a product during storage, carriage, and appearance until the product is consumed. Packaging must provide protection against climatic conditions and biological, physical, and chemical hazards and must be economical. The package must ensure adequate stability of the product throughout the shelf life. [1]

In recent decades, the interest of research and industry towards plastic packaging, both environmentally friendly and safe for the consumer, has exponentially grown. In the cosmetic and pharmaceutical packaging field, one of the most used plastic materials is polyethylene (PE), a thermoplastic resin obtained by polymerization of ethylene.

As a numerical example, the worldwide production capacity of PE is estimated to be 79,106 metric tons per year. Of this amount about 21,106 tons are low-density PE (LDPE), 22,106 tons linear LDPE (LLDPE), and the remaining 36,106 tons is high-density PE (HDPE).

All types of polyethylene are semi-crystalline polymers. Their densities and melting temperatures decrease with the increase of ramification. Many hundreds of grades of PE, differing in their properties, are actually available. [2]

PE possesses good chemical stability. [3–5] The mechanical properties are dependent on the molecular weight and on the degree of chain branching. With increasing density, the barrier properties increase as well as the stiffness, hardness, and strength, as a result of the higher crystallinity. At the same time, there is a decrease in the impact resistance, toughness, resistance to stress cracking, cold resistance, and transparency. [2]

Furthermore, polyethylene can be produced from renewable resources and it is readily recyclable if it has not been coated with other materials. [6]

Blown containers from LDPE are used as packaging in the pharmaceutical and cosmetic industries as well as for food, toys, and cleaning agents. The most important application area of HDPE is the production of containers and injection-molded articles. [2]

Despite the excellent characteristics of this polymer as packaging material, both plastic and its additives used in the production process can migrate from the packaging to the content over time as a result of an increase in temperature,

mechanical stress, or aging. Like in the food field, the presence of plastic components or additives in cosmetics, if not properly controlled, can affect the organoleptic properties of the product, or its safety, if the levels exceed the legislated or toxicological values. [7]

Furthermore, in contrast to glass or metal packaging materials, polymeric packaging are permeable at different degrees to small molecules like gas, water vapor, and to other low-molecular weight compounds like aromas, flavors, and additives present in the formulation; this is an important point, as contamination from external environment could cause reactions within the contained product (oxidation of lipids, degradation of actives, etc.) or the absorption of ambient vapor or liquid could cause an increase of polymer plasticization, resulting in a decrease in mechanical properties. [8]

In particular, PE it is able to retain large amounts of nonpolar compounds, such as most of the volatile molecules, because of its polyolefin nature: this phenomena, known as aroma scalping, causes a loss of aroma content and or/aroma imbalance. On the other hand, other plastic materials (e.g., ethylene-vinyl alcohol copolymer, EVOH) are medium to poor water barrier plastics and their hydrophilic nature promotes the sorption of large amounts of water, which results in plasticization of the polymers and the subsequent loss of mechanical and barrier properties. [9]

Evidence in literature show that changes in mechanical behavior causes changes on the barrier properties. [10] These kind of modifications in packaging can greatly affect the safety of consumers. In fact, it is well known that some substances can migrate from packaging to the formulation, but it is not well disseminated; yet, the knowledge about the influence of packaging mechanical changes on product safety would be improved. For example, the presence of microcracks can modify oxygen permeability and thus lead to a degradation of substances in the formulation, like preservative, reducing their activity.

For this reason, in the development of a cosmetic product safety assessment, besides the packaging raw materials information issue, other aspects related to packaging functionality should be evaluated, like possible interactions between material and product in relation to primary packaging. [11]

In fact, packaging made from the same starting polymeric material but with different additives or produced by different manufacturing processes, although apparently similar, can interfere differently with the content, causing unwanted reactions on the consumer. [12] Recently, a new preservative ingredient was placed on the market to be

used as an additive in the preparation of “active” packaging composed of glass beads in which silver ions are dispersed. This material received a positive opinion from the Scientific Committee on the Consumer Safety (SCCS). [13] It is clear that any change, also mechanical, of this kind of packaging, will affect in a decisive way the release of the preservative in a cosmetic product and consequently influence the safety of the finished product.

Compatibility tests should be performed on the product, once transferred to the final container. The container-content relationship should be explored for all the packaging materials, as the final quality of the goods is always the result of a delicate balance between these two components.

Despite the importance of these aspects, there is too little information about the possible chemical-physical interactions between formulation and packaging, because, differing from food packaging, the cosmetic one is not regulated and, to date, appropriate guidelines are still missing. However, with Regulation 1223/2009 coming into full entry force, among the voices of the Cosmetic Product Safety Report of the Product Information File (PIF), a section pointing out “Impurities, traces, information about the packaging material” has become obligatory.

This work aims to propose a protocol to characterize final packaging for underlining possible critical issues in order to assure a completely safe product to consumers.

In particular, next to analysis of the extractables, of which a lot of methods and protocols are present in literature [14–16], this work focuses on the mechanical analysis step since, as said before, changes in mechanical properties could provoke alterations of packaging performance, like barrier properties, with a consequent risk for the product’s integrity.

As an example of application, a study conducted on two types of polyethylene with different densities is reported.

A simple experimental design, in order to minimize the number of trials, was employed. [17,18] Polyethylene containers were filled with standard formulations and submitted to different degradation tests (photostability test and accelerated stability test) to mimic stress conditions that products can meet during their shelf life, according to European guidelines for stability tests on cosmetic products.

Standard monophasic formulations (pH 2 and pH 10) were used, in order to carry out the test in extremes conditions.

After this treatment, the samples were analyzed by tensile test, to verify possible changes of mechanical properties. “Bone-shape” specimens, obtained from empty and filled bottles [19], were analyzed with a tensile machine until their break, obtaining stress-strain curve. The comparison between treated and untreated materials permitted the underlining of any mechanical change.

Afterwards, an extraction method was used in order to detect all the potentially extractable substances.

MATERIALS

Packaging materials, the object of this study, were commercial containers of 250 mL capability: HDPE bottles and LDPE tubes obtained from different suppliers. The thicknesses of containers are around 500 μm and 1 mm for LDPE and HDPE, respectively.

The filling solutions were set up with the following substances: potassium chloride, 37% hydrochloric acid, borax, and potassium hydroxide drops, all provided by Carlo Erba reagents (Cornaredo, Italy).

EXPERIMENTAL

The proposed approach foresees different steps.

Provision of Data

The first step is the collection of all data regarding the considered packaging.

Companies operating in the cosmetic industry provide information about packaging for the CPSR (Cosmetic Product Safety Report), for example, the food grade certificate and test reports according to the Regulation (EC) No. 1935/2004 on Food Contact Materials [20]; the declaration/certificate of compliance according to Annex IV of Regulation (EU) No. 10/2011 (plastic materials and articles) [21]; the composition, with the specification/technical data for each raw material, based on knowledge of the process for manufacturing the raw material (origin of substance, production process, synthesis route, extraction process, solvent used, etc.) and with a physical and chemical analysis of possible impurities in raw materials and, if necessary, in the final product (e.g., nitrosamines); and the SVHC (substances of

very high concern) declaration/certificate and test report to comply with REACH regulations (packaging being considered an article under REACH).

The comparison with the requirements of food packaging could be useful because the food grade of packaging is mentioned in several EU cosmetic guidelines; there are migration tests and limits and a positive list of allowed monomers and additives. However, some substances are not included in the Union list, but they may be present in the plastic layers of plastic materials or articles, like non-intentionally added substances and additives for polymerization; furthermore, in food packaging, different from cosmetic field, colorants are not of concern and there are some substances that are allowed in Food, but regulated in EU Cosmetic Regulation (e.g., hydroquinone, phenoxyethanol, etc.).

Experimental Design

In order to maximize the information while reducing the number of analyses, an appropriate experiment design (screening design) has to be developed.

In this study, a simple full factorial design was chosen to investigate the effect of three experimental factors on two response parameters. The results of mechanical tests, such as the variation of stress and the percentage of elongation at break point of containers, compared to non-treated empty ones, were chosen as response parameters. In fact, we have already demonstrated that these parameters can be good indicators of any change occurring in the mechanical behavior of polymeric materials [22]. The three factors of interest were varied on two levels according to the experimental plan showed in the Table 1. The density of polyethylene (low or high density), the pH of contained solutions (2 and 10), and the kind of treatment (accelerated aging and solar simulated irradiation) were chosen as factors, to determine the influence of these parameters on mechanical properties of polyethylene used as packaging material in the pharmaceutical and cosmetic field.

The order of the experiments was randomized to avoid any bias. Statistical calculations were carried out using the software StatGraphics (Statpoint Technologies, Warrenton, VA, USA).

Table 1. Investigated experimental factors and levels experimental design.

Experimental Factors	Level	
	−1	1
Density of polyethylene	LDPE	HDPE
Buffer pH	10	2
Treatment	30 days climatic chamber	24 h solar box

Degradation Testing Procedures

The HDPE and LDPE containers (object of this work) were numbered, weighed, and washed according to a standard washing procedure. [19] Afterwards, 10 bottles for each polymer filled with standard solutions were used for each degradation test:

Photostability test by simulating UV-visible ray irradiation using SUNTEST XLS +II (Atlas®, URAI, Assago, MI, Italy) for 24 h;
Accelerated stability test by incubation in climatic room (ClimaCell 111 MMM) at 40 °C with 75% Relative Humidity (R.H.) for 30 days.

SUNTEST instrument was set up in according to standard European procedures, with the following parameters:

Time: 4 h corresponding to 192 h solar light;
Irradiation control: 300–800 nm;
Irradiation (W/m^2): 750;
Room temperature: 35 °C;
Black standard temperature (BST): 45 °C.

Photostability test was performed in according to Colipa guidelines about cosmetic products. [23] At least three specimens were obtained from each bottle to carry out mechanical and morphological analyses in triplicate.

Mechanical Test

The investigation of the mechanical properties of the bottles was performed using a tensile machine, AGS 500ND (Shimadzu Corporation, Kyoto, Japan) equipped with a 500 N load cell; the test was performed using a strain rate, specific for each material, evaluated by preliminary trials:

LDPE: 5 mm/min

HDPE: 10 mm/min

Five “bone-shape” specimens were obtained from each container; the feature of the specimens followed the principles of the European Standard EN ISO 527 [24], suitably modified for bottle containers. [19] Briefly, an optimized dog bone shape obtained by punchcutting was used. This design was developed in order to obtain a localized stress region 3 mm width and thick. Wall thickness distributions for each sample were measured at 3 different points using a digital microscope Duratool model BW1008-500x (Farnell element14 Trade Counter, Leeds, UK). The section of each sample was calculated from thickness and width using a suitable software program (micromeasure vers. 1.2).

Samples were kept under constant temperature (23 °C) and humidity (52% R.H.) for a week until tension tests started and during the entire test time.

This procedure permitted the obtainment of a stress versus strain curve. From each set of results, it was possible to estimate the tendency of materials to oppose to deformation, and to evaluate the curve profile in elasticity regime, the elongation percentage in elasticity regime, and the absolute elongation elasticity.

A critical analysis and comparison of parameters derived from diagrams allowed a qualitative but also a quantitative assessment of any significant change that occurred in the packaging due to interactions between the material they are made of and the conditions or substances with which they are in contact.

Extractables’ Analysis

The next step aims to obtain and interpret data from a controlled extraction’s study starting from the several methods proposed in the literature.

In this work the headspace solid-phase microextraction (HSSPME, fiber: PDMS 100 micron, Supelco, Sigma-Aldrich, Gallarate, MI, Italy) was the extraction method considered for obtaining information about extractable substances from packaging.

Briefly, 500 mg of polymer was put into a vial and the HSSPME conditions used were the following: fiber: PDMS 100 micron (Supelco); adsorption temperature: 90 °C; extraction time: 60 min; desorption temperature: 250°C; desorption time: 4 min, 30 s.

After extraction, for the identification of compounds a gas chromatography-mass spectrometry (GC-MS, Termo Scientific Trace DSQ II, Fisher Scientific Italia, Rodano, MI, Italy) was used. The GC conditions were the following: column: Restek Rtx-5MS, 30 m × 0.25 mm ID × 0.25 µm; gradient: 60 °C for 4.5 min, 20 °C/min until 280 °C, 280 °C for 5 min; injector: PTV 250 °C, split time 4.5 min, split flux 10 mL/min; gas: He, constant flux 1 mL/min; transfer line: 270 °C.

The MS conditions were: source: 250 °C; ionizing mode: EI 70 eV; ion scan mode: full Scan; ion scan range: 50–650 amu; ion scan rate: 870 amu/s.

After analyses, a search in the spectra library, using databases like NIST/EPA/NIH Mass Spectral Library (Wiley Registry of Mass Spectral Data 8th Edition) with Search Program (MSSP) (Data Version: NIST 2008, *Software* Version 2.0) was performed in order to identify all substances recovered in the sample.

RESULTS AND DISCUSSION

The safety assessment of a cosmetic product can be successfully carried out only if the safety assessor can obtain all information concerning the product, including the specific area of application (face, mucosa, periocular area, etc.), the people for whom the product is intended (baby, elderly people, etc.), and the conditions of use, but it is extremely important also to evaluate the hazard deriving from the use of the designed packaging.

Furthermore, commercial packaging is varies widely and it is very difficult to have complete information about it. For this reason, it is very important to define a general protocol that every manufacturer can apply, modifying it in a suitable way to its own formulation-packaging system for the development of an “in house” stability test.

Following the protocol developed in this study, it is possible to evaluate both the behavior of container itself and the possible interactions between content and container in order to ensure the quality of product and the safety for consumers.

This study case, in particular, focuses on the evaluation of one of the most used plastic packaging materials, polyethylene, to understand which are the most influential factors that could cause variations in their properties, as a starting point to extend the knowledge in this field.

After finding all the information about these packaging materials, the second step aims to evaluate the mechanical properties, designed as behavior to tensile testing, of

final containers. In particular, adapted “bone-shape” specimens [19] were obtained from LDPE and HDPE bottles and then analyzed with a tensile machine.

Here parameters obtained from the tensile test are shown and discussed in order to make a comparison between the different materials.

During the tensile test, the specimen presents five basic stages, resulting in the five areas of a typical stress-strain curve:

Elastic behavior: this corresponds to the first phase of material deformation; deformations that occur during this phase are reversible, so if at this stage the applied stress is stopped there are no residual deformations of the specimen, which restores its initial length. In this phase the elongation is directly proportional to the load (in the stress-strain diagram it is represented by a straight portion);

Continuing the tensile test, it adopts a more linear behavior; this step is called the yield point and it corresponds to a fall of the strength of the material due to the formation of “microcracks” within the material. The yield corresponds to the initial part of the plastic behavior;

Plastic behavior: in this phase there are both elastic (reversible) and plastic (permanent) deformation; this means that if resetting the load during this phase, there will be residual deformations associated with the contribution of plastic deformation, for which the specimen will have a greater length than at the start of the test;

During the test, there is a localized deformation of the specimen, for which a small part of the specimen quickly decreases the area of its cross-section; this is called *necking phase* and it characterizes the descending part of the stress-strain curve;

After necking there is the specimen *break*, which occurs in correspondence with the so-called breaking load, which corresponds to the maximum stress that the specimen can withstand;

The reported graphs in Figures 1 and 2 show, as an example, a different mechanical behavior depending on the considered material, according to the UNI EN ISO 527.[24]

As it can be seen, the mechanical behavior of these two polymers is greatly different, in terms of elongation percentage and stress (MPa); so it is not numerically possible to compare one material with the other. For this reason, every change in mechanical properties has been evaluated, comparing each material untreated with itself after treatment.

Furthermore, it is important to underline that the approach described in this work can be successfully employed to evaluate modification of packaging during aging or during contact with the packed formulation in order to define the shelf life of the product or any interactions between formulation and the packaging.

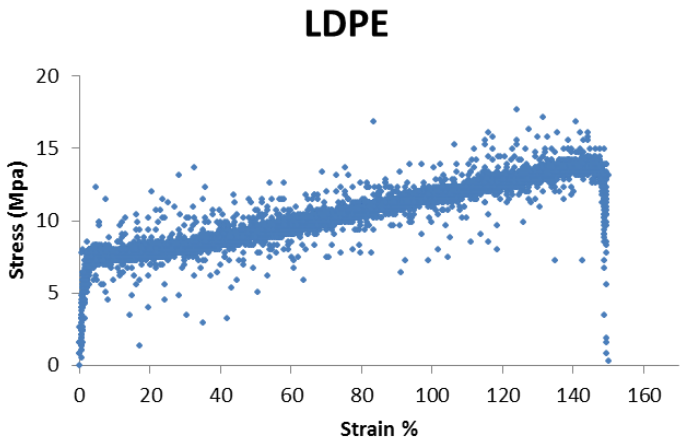

Figure 1. Mechanical behavior of low-density polyethylene (LDPE).

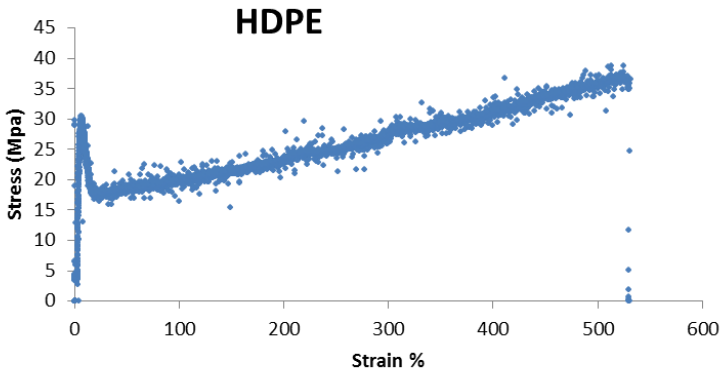

Figure 2. Mechanical behavior of high-density polyethylene (HDPE).

For this purpose, mechanical properties of empty and filled bottles, before and after stress testing procedures, were investigated. The stress-strain curve profile is useful to compare specimens subjected to environmental and chemical stress.

HDPE presents major strength, maybe due its linear structure, that makes the polymer more resistant, while LDPE presents a greater ability to stretch, with a lower stress value.

Results of tensile tests for different materials are reported in Tables 2 and 3, in terms of tensile stress and strain at break.

Table 2. Results obtained by mechanical analyses for low-density polyethylene (LDPE) containers.

LDPE	Tensile Stress at Break (σB) (MPa) *	Tensile Strain at Break (εtB) (%) *	Δ Tensile Stress at Break (%) *	Δ Tensile Strain at Break (%) *
Empty	21.3	150.9	-	-
pH 2 sun 24 h	17.4	122.6	−18.0	−18.7
pH 2 chamber 30 days	22.4	189.0	5.4	25.2
pH 10 sun 24 h	18.4	148.9	−13.5	−1.3
pH 10 chamber 30 days	21.8	189.2	2.3	25.3

* S.D. ≤ 10.0 %.

Table 3. Results obtained by mechanical analyses for HDPE containers.

HDPE	Tensile Stress at Break (σB) (MPa) *	Tensile Strain at Break (εtB) (%) *	Δ Tensile Stress at Break (%) *	Δ Tensile Strain at Break (%) *
Empty	29.6	391.7	-	-
pH 2 sun 24 h	26.4	399.0	−10.7	1.8
pH 2 chamber 30 days	24.8	331.2	−16.1	−15.4
pH 10 sun 24 h	25.3	325.2	−14.6	−16.9
pH 10 chamber 30 days	23.6	289.3	−20.3	−26.1

* S.D. ≤ 10.0 %.

Observing the values, it can be said that for LDPE there is a general reduction of the yield stress at break point. The major reduction is observable for samples treated with irradiation, regardless of the type of solution contained. So, the light has the bigger influence on material changes; this influence is exacerbated by extreme pH.

Also, regarding HDPE, we can observe that there are some changes in stress and elongation at break. The bigger variation can be observed for the samples treated in

climatic chamber. It can be underlined that the container filled with the pH 10 solution has undergone the bigger changes.

Results are very interesting and they agree with literature data. In fact, it is well known that PE polymers are quite stable to degradation depending of their molecular weight, but it is also known that UV irradiation and thermal exposure can increase surface hydrophilicity of these polymer. [25]

Furthermore, in all final PE packaging available in the market, antioxidants and stabilizers, in smaller or bigger amount, are present. The presence of these substances products containing PE become susceptible to degradation and subsequent oxo-biodegradation. They cause initiation and propagation of free radical chain reactions taking place in the presence of atmospheric oxygen, which leads a polymer to gradually reduce its molecular weight. [26,27] These processes cause a change in the hydrophilicity of a polymer surface, that can be more susceptible to extreme pH.

Here the Pareto Charts and the Factor Means Plots of statistical analysis of the mechanical test's results, obtained by the simple screening experimental design descripted above, are reported in Figures 3 and 4.

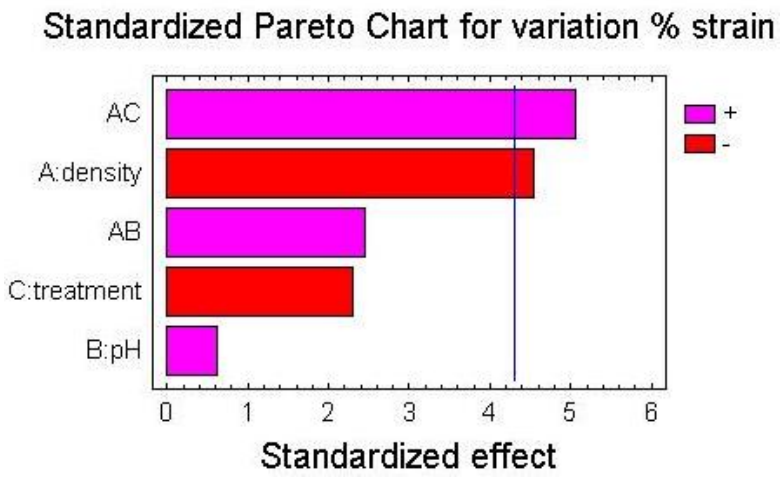

Figure 3. Standardized Pareto Chart for percentage variation of strain.

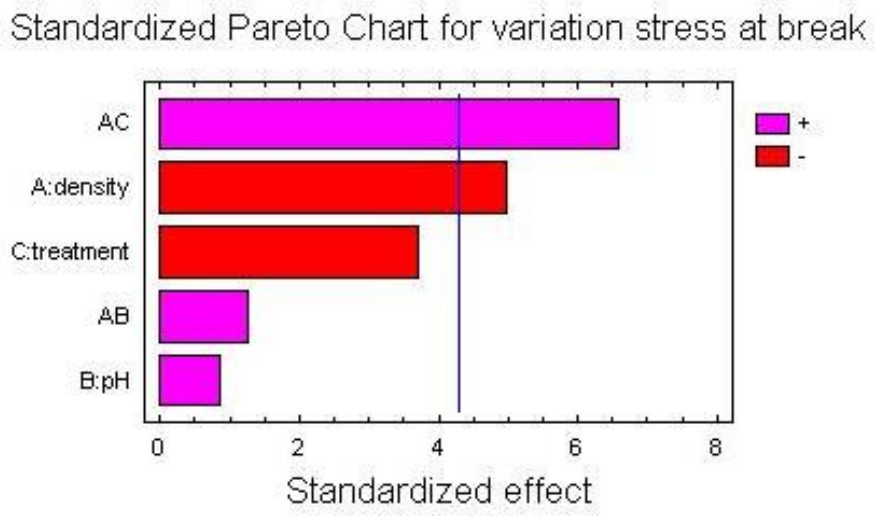

Figure 4. Standardized Pareto Chart for percentage variation of stress at break.

As it is possible to see from the graphs, the only factor that has a significant influence on the mechanical variations after treatment is the density of polyethylene, both regarding the variation of percentage elongation and the variation of the stress at break point.

For both for the variation of percentage elongation and for the variation of the stress at break point, the interactions between two factors—the density of the polymer and the kind of treatment (UV-vis irradiation and climatic chamber)—are significantly influential.

The main effect represents the average result of varying one factor at a time from low to high and keeping the other one constant. The interaction term shows changes in the response when both factors are varied concurrently, as this is possible to observe in the figures 5 and 6 below reported.

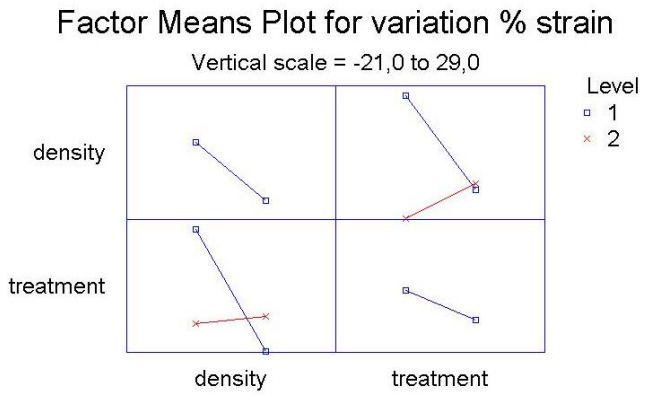

Figure 5. Factors Means Plot for percentage variation of strain.

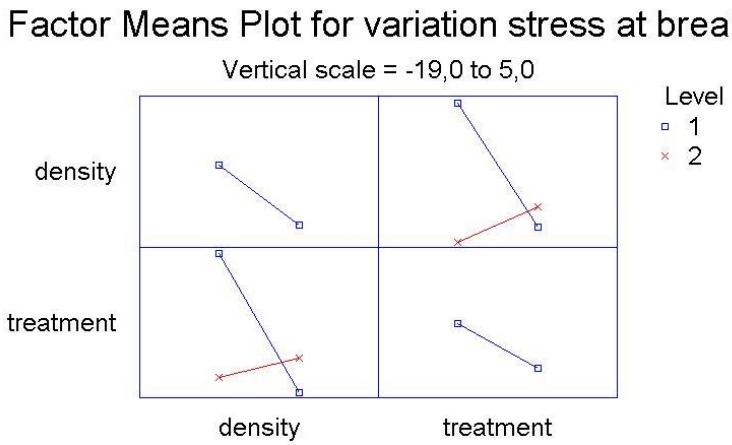

Figure 6. Factors Means Plot for variation of stress at break.

The considered extraction method was headspace solid-phase microextraction (HSSPME). After extraction, for the identification of compounds a gas chromatography–mass spectrometry was used. Figure 7 shows an example of the chromatogram obtained by GC/MS.

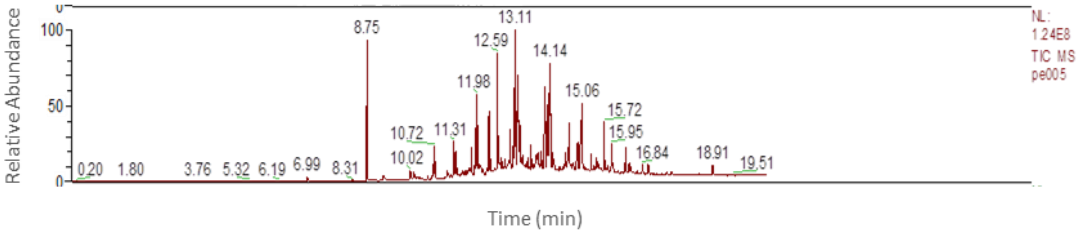

Figure 7. Chromatograms obtained with headspace solid-phase microextraction (HSSPME) on untreated LDPE.

The deconvolution of the chromatographic peaks leads to the identification of more than 100 substances. Many of these substances are linked to the bleeding of stationary phase of the chromatographic column to the SPME fiber coating, and to characteristic analytes also present in blanks used as references. By eliminating the interfering peaks, a list of compounds that can be identified as extractable that were released from the analyzed polymer can be obtained. In this way it is possible to split the substances into several categories, as reported in the Table 4. From the analysis of chromatographic profiles of extraction process and of relative percentage of the different substances present in the packaging it is possible:

To choose the better packaging for the specified cosmetic product;

To define which substance has to be quantitatively evaluated in the final cosmetic product as leachable after stability and interaction studies.

Table 4. Categories of extractable type released from PE polymer.

Extractable Type	**Example**
Initial ingredients	Antioxidants (e.g., Terbutylphenol, Irganox), additives (phtalaths), amides (exadecanammide)
Impurities related to processing	Oligomers, residual solvents, esters (miristyl miristate), siloxane
Degradation products of the polymer	Fragments of saturated and unsaturated hydrocarbons, ketones, acids

During compatibility testing it is also possible to detect products adsorbed by the formulation contained in the packaging material.

The data show that the sample obtained from head space microextraction (HSSPME) is representative, and it also identifies numerous nonpolar organic compounds, even the most significant polar substances.

CONCLUSIONS

This work aims to provide necessary tools and a practical approach to evaluate commercial polymeric containers used in cosmetic packaging in order to assure the safety of the finished product.

In fact, it is well known that packaging can greatly affect the safety of the product by both losing its barrier property and containing substances potentially harmful for the consumer, especially for products for children or containing sunscreens.

Despite the importance of this aspect, there is too little information about the possible chemical-physical modifications of the packaging itself during aging or about the interactions between formulation and packaging.

The correct approach involves the provision of all possible information about the packaging material from suppliers' data sheet and from literature; then, an appropriate design of experiment has to be successfully used in order to obtain relevant indications minimizing the number of trials that must be carried out in order

to perform an effective safety evaluation of the finished packaging used and of the interaction between each couple packaging-formulation.

Actually, the main problem is related to the actual composition of the packaging at the end of the production process. For this reason, it is essential to collect information about the container and not only the polymer raw materials used in the packaging production.

In this work the results of mechanical tests are chosen as predictive system's parameters, but this kind of approach can be used also for describing other system's parameters, for example the viscosity or other characteristics of the contained product.

After mechanical analysis, it is important to perform also an extractables' analysis; in this case the used technique was the headspace solid microextraction (HSSPME), since, compared to other techniques used in preliminary studies, this one allows the definition of almost the total extraction profile of the analyzed material.

The reported study case regards two types of polyethylene containers with different densities, HDPE and LDPE; the commercial containers made of these materials were treated in extreme conditions of pH and accelerated aging, in order to evaluate which factors have the most influence on the mechanical properties of these materials.

This work has shown that the most influential factor is the density of polyethylene, but also that the interaction between the kind of polyethylene and the kind of treatment has significant influence on the mechanical answer of the material in comparison with the same untreated material.

So, these polymers cannot be considered as completely inert and stable. Some particular conditions (for example heat, UV radiation, and humidity) may alter the chemical, physical and mechanical properties of these polymeric materials.

In conclusion, it would be very important to apply this kind of experimental approach in the development phase of a new cosmetic product before its introduction into the market.

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Chapter III.

PROPOSAL OF ALKALINE SIMULANTS FOR COSMETIC-PACKAGING COMPATIBILITY

B. Briasco, P. Capra, P. Perugini

"Proposal of alkaline simulants for cosmetic-packaging compatibility"

under submission to *Journal of Advanced Pharmaceutical Technology and Research*

PROPOSAL OF ALKALINE SIMULANTS FOR COSMETIC-PACKAGING COMPATIBILITY

ABSTRACT

At present in cosmetic field official guidelines to evaluate possible interactions between packaging and contained products are still missing, so the indications followed to perform this kind of studies are the ones provided by food legislation. EU Food Regulation 10/2011 expects the use of some food simulants for reproducing the contact between packaging and contained product; it indicates six simulants, but no alkaline solutions are expected.

However, in cosmetic field also basic products are present on the market, like hair dyes and depilatory cream. So this work aims to propose alkaline simulants, to mimic also this kind of products.

The proposed simulants are two pH 10 emulsions, one with and one without silicones, in order to represent in the best way all possible cosmetic products with alkaline pH.

To demonstrate the applicability of these simulants, two examples of application of these ones are reported; the parameters evaluated after the contact between commercial plastic containers and the basic simulants were the mechanical properties of packaging materials.

These practical study cases show that some changes in packaging material could occur after the contact with this kind of content.

Keywords: alkaline simulants, packaging evaluation, mechanical properties, formulation/packaging interactions

INTRODUCTION

The packaging plays different important roles in commercial products; in fact it provides presentation, protection, identification, information, containment and compliance for a product during storage, carriage, display and until the product is consumed. Overall packaging must provide protection against climatic conditions, biological, physical and chemical hazards and it must ensure adequate stability to a product throughout its shelf life. [1]

All those packaging components which have a direct contact with the product (i.e. bottles, caps, cap liner, etc.) are defined as *primary packaging*. The main functions of primary packaging are to contain and to restrict any chemical, climatic, biological or occasionally mechanical hazards, ensuring that goods reach final consumers in optimal conditions (quality, safety, effectiveness). [2]

The main risk of chemical hazard is due to interaction or incompatibility between the product and package. Interactions between contained products and packaging can be detrimental to quality and/or safety. For example, in food field changes in product flavor due to aroma sorption and the transfer of undesirable flavors from packaging to foods are important mechanisms of deterioration when foods are packaged in polymer-based materials. Instead, in cosmetic field the sorption of some formulation's ingredients by the packaging (e.g. actives or preservatives) could compromise the product's quality or safety.[3]

Compatibility investigations must basically cover any exchange that can occur between the product and the package and vice versa. These may be associated with contamination, covering migration, absorption, adsorption, extraction, corrosion, etc. or some ingredients may either be lost or gained. Such exchange may be identifiable as organoleptic changes, increase in toxicity/irritancy degradation, loss or gain of microbial effectiveness, precipitation, turbidity, color change, pH shift, etc. These external influences may catalyze, induce or even nullify chemical changes.[4]

Regarding food, it's commonly known that a high number of chemical substances can be found in foodstuffs during the different stages of the supply chain, including but not limited to food additives, pesticides, environmentally derived contaminants, mycotoxins, flavorings and micronutrients. There are also occasions when packages and materials that come in direct contact with foods can act as a source of chemicals and elements. This phenomenon is termed "migration". [5, 6]

The same phenomena could occur in other fields; for example a large number of publications have appeared in the literature concerning the migration of antioxidant additives from drug and cosmetic plastic packages to their content [7] because plastic packaging is not completely inert. [8]

Researchers continue to carry out studies on migration of volatile compounds, additives and oligomers from packaging plastic materials to contained products.

Regulatory authorities have introduced several laws to control and regulate these issues. In particular all food-contact materials need to follow European Commission Regulation 1935/2004, which states that substances migrating into food should not be harmful to humans. [9]

Furthermore, Regulation EU 10/2011 is in force within the EU, applying rules for plastic materials and articles intended to come into contact with food; this Regulation covers specific rules for the implementation and is a specific measure for plastics (PIM - plastic implementation measure) as mentioned in the European Framework Regulation EU 1935/2004. Regulation EU 10/2011 states that in order to identify unknown migrants and likely NIAS (not intentionally added substances), analysis can be performed on the packaging material itself or in food simulants that have been in contact with the food packaging material during migration tests. [10]

It indicates an experimental modeling system that makes use of few food simulants with the presumption that they serve as model contact media for all types of foods. [11]

In particular, for demonstration of compliance for plastic materials and articles not yet in contact with food the following food simulants are assigned by the Regulation: ethanol 10 % (v/v) (Food simulant A), acetic acid 3 % (w/v) (Food simulant B), ethanol 20 % (v/v) (food simulant C), ethanol 50 % (v/v) (Food simulant D1), vegetable oil (Food simulant D2; this may be any vegetable oil with a fatty acid distribution of no of carbon atoms in fatty acid chain: No of unsaturation 6-12 14 16 18:0 18:1 18:2 18:3), poly(2,6-diphenyl-p-phenylene oxide), particle size 60-80 mesh, pore size 200 nm (Food simulant E).

Food simulants A, B and C are assigned for foods that have a hydrophilic character and are able to extract hydrophilic substances. Food simulant B shall be used for those foods which have a pH below 4.5, while C shall be used for alcoholic foods with an alcohol content of up to 20 % and those foods which contain a relevant amount of organic ingredients that render the food more lipophilic.

Food simulants D1 and D2 are assigned for foods that have a lipophilic character and are able to extract lipophilic substances. Food simulant D1 shall be used for alcoholic foods with an alcohol content of above 20 % and for oil in water emulsions, while D2 shall be used for foods which contain free fats at the surface.

Food simulant E is assigned for testing specific migration into dry foods. [12]

As it can be seen, no alkaline simulant is expected, because the pH of most food products varies between 3.5 and 7.0. [13]

Since at present in cosmetic field there are still no official guidelines in order to evaluate the relationship between packaging and formulations, most of studies are based on food guidelines. However, in cosmetics, formulations with alkaline pH can be found, for example hair dyes and products for epilation.

In particular, permanent synthetic hair dyes contain up to 6% peroxide and use ammonia as the alkalizing agent. This results in pH values ranging from 9 to 10.5, thus facilitating complete penetration through the hair cortex. [14]

Also depilatory products present high pH, since, although hair removal creams vary between different manufacturers, they use the chemical thioglycolate mixed with sodium hydroxide or calcium hydroxide to melt the hair from skin epidermis. [15]

In order to evaluate this kind of products, this work aims to propose alkaline simulants to mimic the contact between packaging and this type of environment. It can be useful for the safety assessor to obtain data in order to complete the CPSR's section that require information about traces, impurities and packaging material, as expected by EU Regulation 1223/2009. [16]

In particular, two basic formulations are proposed: F1 (pH 10 formulation without silicone) and F1 (pH 10 formulation with silicone).

For demonstrating their applicability, practical examples are shown below.

MATERIAL AND METHODS

For simulants, the following substances have been used: potassium chloride, 37% hydrochloric acid, borax, potassium hydroxide pellet by CARLO ERBA reagents (Cornaredo, Italy), xanthan gum by ACEF spa (Fiorenzuola d'Arda, Italy), Phytosqual hydrogen by Vevy Europe spa (Genova, Italy), Tegosoft DEC and Abil CARE XL 80 by Evonik Industries (Essen, Germany), Progress D5 by Prodotti Gianni Srl (Milano, Italy).

As packaging materials, some commercially available plastic containers by an Italian supplier have been used, specifically tubes made by LDPE and LLDPE.

Preparation of simulants

The aqueous phase for emulsions was prepared following indications of F.U.I. XII Ed. for pH 10 buffer solution. After dissolving all reactive substances in distilled water, the solution was filtered with filtration membranes (mixed esters of cellulose) with 0.22 µm porosity.

Simulants F1 and F1 were prepared by emulsion, slowly adding phase B in phase A, using a Silverson SL2t High Shear Laboratory Stirrer Mixer (Silverson Machines Ltd, England) for 10 minutes, rate 6700 rpm.

The quali-quantitative composition of F1 and F1 is reported in Table 1.

Table1. Composition of F1 and F2 simulants

Composition			
F1	**%**	**F2**	**%**
Xanthan gum	0.8	Xanthan gum	0.8
pH 10 buffer solution (FUI XII Ed.)	59.2	pH 10 buffer solution (FUI XII Ed.)	59.2
Diethylhexyl carbonate	15	Diethylhexyl carbonate	15
Bis-PEG/PPG-20/5 PEG/PPG-20/5 Dimeticone; Methoxy PEG/PPG-25/4 Dimeticone; Caprylic/Capric Triglyceride	5	Bis-PEG/PPG-20/5 PEG/PPG-20/5 Dimeticone; Methoxy PEG/PPG-25/4 Dimeticone; Caprylic/Capric Triglyceride	5
Squalane	20	Cyclopentasiloxane	20

Degradation testing procedures

Following a previous work, bottles filled with simulants were subjected to different degradation tests, in order to simulate credible stress contact conditions that final products could meet during their shelf life. [17] In particular:

- photostability test by simulating UV-visible rays irradiation using SUNTEST XLS +II (Atlas ®, Chicago, USA) for 24 and 96 hours;
- thermal shock cycles (4°C-37°C, two times, 28 days).

SUNTEST instrument was set up in according to standard European procedures, with the following parameters:

- Time: 4 hours corresponding to 192 hours solar light
- Irradiation control: 300-800 nm
- Irradiation [W/m^2]: 750
- Room temperature: 35°C
- Black Standard Temperature (BST): 45°C

Finally, for all samples several specimens were obtained to carry out mechanical analyses.

Mechanical test

The investigation of the mechanical properties of the containers was performed using a tensile machine, AGS 500ND (Shimadzu corporation, Kyoto-Japan) equipped with a 500[N] load cell; the test was performed using a strain rate of 10 mm/min.

"Bone-shape" specimens were obtained from each container; the feature of the specimens followed the principles of the European Standard EN ISO 527 [18], suitably modified for final containers like reported by Perugini et al. in a previous work [17]. Specifically, an optimized dog bone shape obtained by punchcutting was used in order to obtain a localized stress in a region of 3 mm width. Thickness and width for each sample were measured using a digital microscope model BW 1008. The section of each sample was calculated from this two dimensions using a suitable software program (Micro-Measure vers.1.2).

Samples were kept under constant temperature (23°C) and humidity (52% R.H.) for a week until tension test started and during all the test time.

This procedure permitted to obtain a stress versus strain curve. From each set of results was possible to estimate the tendency of materials to oppose to deformation, to evaluate the curve profile in elasticity regime, the elongation percentage in elasticity regime and the absolute elongation elasticity.

A critical analysis and comparison of diagrams made on different specimens allow a first qualitative assessment of any significant changes in the stress-strain diagram due to interactions between the material they are made of and the conditions or substances which are in contact with. [17]

RESULTS AND DISCUSSION

This work aims to propose alkaline simulants in order to evaluate possible interactions between content and container in cosmetic field. At present in cosmetic world official guidelines are still missing, so the indications followed to perform this kind of studies are the ones provided by food legislation, that expects the use of some food simulants for reproducing the contact between packaging and contained product. The food EU Regulation 10/2011 indicates six simulants, but no alkaline solutions are expected.

However, in cosmetic field also basic products are present on the market, so this work wants to propose two alkaline simulants, to mimic also this kind of products.

Here two examples of application of these simulants are reported; the parameters evaluated after contact with the presented simulants and commercial plastic containers are mechanical properties of packaging materials.

Choice of alkaline simulants

The proposed simulants are one pH 10 emulsion with and one pH 10 emulsion without silicone.

The choice to use this kind of system (bi-phasic preparations) is due to the presence on the market of a huge multiplicity of cosmetic products that are biphasic (O/A and A/O emulsions, multiple emulsions, micellar solutions…) and polyphasic (liposomal, nanosomal, microencapsulated emulsions…). [19]

Moreover emulsions without and with silicone represent formulations that are not present in food field; in particular, in the last years, the use of silicones in cosmetics is exponentially grown and their application fields are a lot, thanks to their properties. In fact, they are used in many applications because of their stability, low surface tension and lack of toxicity. [20-21]

Simulant F1: mechanical properties

The first proposed alkaline simulant is a pH 10 emulsion without silicone, that could represent biphasic products, like creams.

To test this simulant, commercial single dose containers made of LDPE and LLDPE were used.

The filled containers underwent accelerated stress treatments; in particular UV-vis irradiation 96 hours, because in first part of work 24 hours of treatment didn't show significant changes in polymers' properties, and thermal shock cycles (4°C-37°C) for 28 days, because it's known from literature that PEs are not permeable to water vapor. [22]

After treatments, ten adapted "bone-shape" specimen were obtained for kind of polymer, like previously described. These specimens were used to perform mechanical analysis. The results are compared with the ones of empty and not treated containers.

Results of tensile test are expressed in terms of strength (MPa) and strain (%) at yield point (where present) and break point.

In table 2, the results obtained from tensile test for LDPE and LLDPE are reported.

Table2. Results of tensile test for LDPE and LLDPE containers filled with F1

F1	Treatment	Tensile strenght (σM)= Yield stress (σy) (MPa)	Tensile strain at yield (εy)= (εM)(%)	Tensile stress at break (σB) (MPa)	Tensile strain at break (εtB) (%)
LLDPE	Empty	10.45	12.17	11.99	272.34
	pH10 sun96h	10.11	10.18	11.61	283.71
	pH10 shock 28d	9.94	11.68	10.64	242.39
LDPE	Empty	6.92	*	6.99	73.95
	pH10 sun96h	7.56	*	9.68	133.04
	pH10 shock 28d	6.92	*	8.16	104.21

According to literature, LLDPE presents major resistance to traction and strain at break than LDPE, with better extensibility and rigidity to flection. [23]

After contact with alkaline simulant F1, LLDPE showed a reduction in strength at break and in % strain at break after thermal shock. [24]

LDPE instead showed an increase in strength at yield and at break after irradiation for 96 hours and also an increase of % strain especially after suntest. It seems that the combination of alkaline pH and treatment affected more LDPE than LLDPE.

Simulant F2: mechanical properties

The second proposed alkaline simulant is a pH 10 emulsion with silicone, that could represent a big part of not claimed "natural" cosmetic biphasic products.

Like for F1 simulant, commercial containers made of LDPE and LLDPE were used to test the effects of this simulant.

The filled containers underwent accelerated stress treatments; in particular UV-vis irradiation 96 hours and thermal shock cycles (4°C-37°C) for 28 days.

After treatments, ten adapted "bone-shape" specimen were obtained for kind of polymer. These specimens were used to perform mechanical analysis. Also in this case, results of tensile test are expressed in terms of strength (MPa) and strain (%) at yield point (where present) and break point.

In table 3, the results obtained from tensile test for LDPE and LLDPE are reported.

Table3. Results of tensile test for LDPE and LLDPE containers filled with F2

F2	Treatment	Tensile strenght (σM)= Yield stress (σy) (MPa)	Tensile strain at yield (εy)= (εM)(%)	Tensile stress at break (σB) (MPa)	Tensile strain at break (εtB) (%)
LLDPE	Empty	10.45	12.17	11.99	272.34
	pH10 sun96h	10.54	10.67	12.92	327.75
	pH10 shock 28d	9.85	12.44	11.85	318.57
LDPE	Empty	6.92	*	6.99	73.95
	pH10 sun96h	7.31	*	8.43	107.95
	pH10 shock 28d	7.38	*	8.79	114.45

For containers in contact with F2 simulant, analogous considerations respect to the ones filled with F1 can be done: LLDPE presents major resistance to traction and strain at break than LDPE.

Differently from F1, after contact with alkaline simulant F2, LLDPE showed no significant changes in strength, but it presented an increase in % strain at break after both treatments. This could be due to the effect of silicone on the structure of the polymer.

Instead LDPE presents a behavior similar to the samples filled with F1, that is an increase in strength at yield and at break after irradiation for 96 hours and also an increase of % strain especially after suntest.

It seems that the presence of silicone don't provoke changes in mechanical properties of LDPE.

CONCLUSION

This work aimed to propose alkaline simulants, useful for performing the evaluation of possible content-container interactions in cosmetic field. In fact, at present official guidelines are still missing; the indications followed to perform this kind of studies are the ones provided by food legislation, but the simulants expected by EU Food Regulation 10/2011 don't include alkaline solutions. However, in cosmetic field also basic products are present on the market.

The proposed simulants are two emulsions, one with and one without silicone.

To demonstrate the applicability of these simulants, examples of application of these ones have been reported; the parameters evaluated after the contact between commercial plastic containers and the basic simulants were the mechanical properties of packaging materials.

From these study cases, it's possible to conclude that after contact with basic simulants and accelerated stability tests, the polymeric materials don't seem to remain completely stable. From a mechanical properties' point of view, some changes occur for tested materials, demonstrating that the combination of alkaline formulations and stress conditions could provoke variations in polymers' structure, causing possible alterations of their performances that could alter the product's safety, quality and effectiveness.

These considerations lead to conclude that also alkaline simulants, representative of a part of cosmetic products on the market, are needed to evaluate the relationship between packaging and formulations.

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Chapter IV.

MECHANICAL ANALYSIS AND EXTRACTABLE TESTING OF LDPE PACKAGING FOR SEMISOLID FORMULATION

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"Mechanical analysis and extractable testing of LDPE packaging used for semisolid formulation"

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MECHANICAL ANALYSIS AND EXTRACTABLE TESTING OF LDPE PACKAGING FOR SEMISOLID FORMULATION

ABSTRACT

Packaging is one major field of application for plastics. To test these materials to assure their good performances lots of standard tests were set up. Almost the totality of these tests is performed on starting material or on polymeric standard films, however there is a great difference with respect to final containers. The presence of additives, surface treatment, processing, cleaning procedures, contacting media, adhesives may affect the material's properties.

For this reasons, this work aims to test the final packaging in terms of mechanical analysis and extractable testing to study the "in use" stability and the possible interactions with the contained product.

Two plastic materials, low density polyethylene and linear low density polyethylene, were considered and analyzed as starting material, as standard samples and as final containers.

An evaluation of mechanical properties by tensile test on standard ISO and specimens obtained from final commercial containers was carried out.

Simultaneously, the characterization of the organic extractables from these materials was performed by means of controlled extraction studies and a first screening to evaluate the interactions between products and packaging was carried out using a semisolid formulation as a simulant.

From a mechanical properties' point of view, some changes occur for these materials, caused by the combination of the type of formulation contained and the stability test used. No extractable substance was released in the formulation in potentially hazardous concentrations. Despite this, the tested packaging showed a tendency to absorb some substances from the formulation packed.

Keywords

Polyethylene, packaging evaluation, mechanical properties, extractables.

INTRODUCTION

Packaging surrounds, enhances and protect goods from processing and manufacturing, through handling and storage, to the final consumer.[1] In fact, it performs a series of fundamental tasks: it protects its content from contamination and spoilage, makes it easier to transport and store, [2-3] and it plays an important role in communication, information and convenience. [4]

Regarding the purpose of preserving and protecting the content from physical, chemical and microbiological hazards, which could affect its safety, quality and effectiveness, [5] it's important to evaluate the relationship and the compatibility between packaging materials and the contained product. In fact, the quality and shelf life of packaged products are mainly determined by the barrier and mechanical properties of the packaging against external agents and the interaction of content's constituents with the packaging material.[6]

For this reason, one of the major functions of packaging is to minimize reactions that could affect the stability of the contained products. [7-8]

Some interactions between these two entities could be the transfer of chemical substances across the package's interface to the content, the absorption of product's components by packaging, the loss of volatile compound due to the change of barrier properties or a microbiological contamination if the packaging is not appropriate.[9-10]

Regarding the development of packaging, especially plastic materials, tests are performed in order to simulate the use's conditions and possible potential risk's situations that could occur during products' shelf life. The aim of these tests is to identify the ones most suitable to certain particular stresses that are supposed to be encountered by products during their way. The more numerous are the situations predicted, the more we will be able to choose the most appropriate materials for each product. [11]

International organisms like ISO and ASTM worked for the definition of technical regulations, definition of materials and their test methods. For example ASTM's plastics standards are instrumental in specifying, testing, and assessing the physical, mechanical, and chemical properties of a wide variety of materials and products that are made of plastic and its polymeric derivatives. These plastic standards allow plastic manufacturers and end-users to examine and evaluate their material or product of concern to ensure quality and acceptability towards safe utilization. [12]

In this perspective a lot of test on materials used in packaging field are performed following these standards, but the majority of them is applied on polymeric films and not on final packaging (e.g. tubes, jars, bottles…). However there is a big difference between the polymeric material before and after transformation in final containers.

Plastics are composed of a mixture of homologous polymers, having a range of molecular weights.

They can contain other substances intentionally added for process, like plasticizers (substances which can make a material more flexible and easier to process; most plasticizers belong to the group of esters of phtalic acid and adipic acid), thermal stabilizers (generally epoxidised seed and vegetable oils), slip additives, light stabilizers (they help to improve the long-term weathering properties of plastics), antioxidants (to reduce the rate of oxidation and to enhance the stabilization of the material), pigments, and lubricants, residual of the process itself (like solvents or monomers/oligomers) or substances derived from process (e.g. substances derived from the composition of additives and monomers), able to alter the material's characteristics. [13-15]

This makes the starting material (pellets) deeply different from processed material.

Furthermore factors like plastic composition, processing and cleaning procedures, surface treatment, contacting media, inks, adhesives, absorption and permeability of preservatives and conditions of storage may affect the suitability of a material for a specific use. [16]

For these reasons it's important to perform stability and compatibility tests with the content on final containers and not only on polymeric film. In this work two plastic materials extensively used in packaging field like low density polyethylene (LDPE) and linear low density polyethylene (LLDPE) were studied.

Polyethylene is the most widely used mass–produced plastic. Blown containers from LDPE are used as packaging in the pharmaceutical and cosmetic industries as well as for foods, toys, and cleaning agents. Injection-molded LDPE is used to make buckets and various household and kitchen containers. The development of PE began in 1936 with the introduction of the high pressure polymerization process of ethylene to LDPE (0.915–0.94gcm3), which produced a relatively low molecular weight polymer; LLDPE is the copolymer of ethylene with about 8% 1-butene, 1-hexene, or 1-octene. [17]

The main aim of this research was to study low density polyethylene (LDPE) and linear low density polyethylene (LLDPE), as starting material as ISO standard samples and as final containers, in order to evaluate their stability and the possible interactions with the content.

For this purpose the evaluation of mechanical properties by tensile test on standard ISO specimens and specimens obtained from final single dose commercial containers made by linear low density polyethylene and low density polyethylene were carried out.

Simultaneously, the characterization of the organic extractables from these materials was performed by means of controlled extraction studies and a first screening to evaluate the interactions between products and packaging system was carried out on the plastic materials in contact with a simulant.

Furthermore, the formulation used as simulant was characterized before and after treatments, in order to evaluate possible changes after the contact with plastic packaging.

As simulant for all these evaluations, a pH 10 emulsion with silicone was used (F3 simulant); it was set up in another work of this research group and it represents a type of formulation not present in food but in cosmetic field, since in the last years the use of silicones in cosmetics is exponentially grown. [18, 19]

MATERIALS

Packaging

Raw materials (pellets) used in this work were:

- Linear low density polyethylene (LLDPE), provided by DEXplastomers (Borealis, Vienna, Austria);
- Low density polyethylene (LDPE), provided by LyondellBasell (Rotterdam, Holland).

Standard ISO specimens were provided by Lameplast SpA (Rovereto s/S, Novi di Modena,

Italy):

- ISO (ISO 527-1:1996) specimens of linear low density polyethylene (LLDPE-ISO);

- ISO (ISO 527-1:1996) specimens of low density polyethylene (LDPE-ISO).

Also final packaging materials, object of this study, were provided by Lameplast SpA (Rovereto s/S, Novi di Modena, Italy):

- 5 ml single-dose containers not recloseable linear low density polyethylene (LLDPE);
- 5 ml single dose containers not recloseable of low density polyethylene (LDPE).

Formulation

For pH 10 buffer solution the following substances were used: potassium chloride, 37% hydrochloric acid, borax, potassium hydroxide pellet by CARLO ERBA reagents (Cornaredo, Italy). The other ingredients for F3 simulant were: xanthan gum by ACEF spa (Fiorenzuola d'Arda, Italy), Phytosqual hydrogen by Vevy Europe spa (Genova, Italy), Tegosoft DEC and Abil CARE XL 80 by Evonik Industries (Essen, Germany), Progress D5 by Prodotti Gianni Srl (Milan, Italy).

METHODS

Preparation of formulation

An alkaline emulsion set up in another work by Perugini et al. was chosen as filling formulation (F3 simulant). [19]

Briefly, F3 simulant was prepared by emulsification, by adding slowly phase B in phase A, using a Silverson SL2T High Shear Laboratory Stirrer Mixer (Silverson Machines Ltd, Chesam, UK) for 10 minutes, rate 6700 rpm, at 50°C.

The quali-quantitative composition of F3 is reported in Table 1.

Table1. Composition of filling formulation

Phase	F3 simulant composition	%
A	Xanthan gum	0.8
	pH 10 buffer solution (FUI XII Ed.)	59.2
B	Diethylhexyl carbonate	15
	Bis-PEG/PPG-20/5 PEG/PPG-20/5 Dimethicone; Methoxy PEG/PPG-25/4 Dimethicone; Caprylic/Capric Triglyceride	5
	Cyclopentasiloxane	20

Degradation testing procedures

Containers filled with simulant were subjected to different degradation tests, in order to simulate the "in use" stress conditions that final product could meet during its life. [20] In particular:

- photostability test by simulating UV-visible rays irradiation using SUNTEST XLS +II (Atlas ®, Chicago, USA) for 96 hours;
- thermal shock cycles (4°C-37°C, two times, 28 days).

Suntest instrument was set up in according to standard European procedures, with the following parameters:

- Time: 4 hours corresponding to 192 hours solar light
- Irradiation control: 300-800 nm
- Irradiation [W/m^2]: 750
- Room temperature: 35°C
- Black Standard Temperature (BST): 45°C

In order to assure that both parts of each single dose container undergoes simulated UV irradiation for the same period and under the same conditions after the 48 hours the containers were turned on the other side. At the end of the irradiation's time the samples were taken, emptied and washed with a standard procedure [20]. For all samples several specimens were obtained to carry out mechanical analyses, as reported in a previous work by this research group. Finally, each obtained specimen was analyzed.

Also a formulation's sample contained in a glass inert packaging underwent the same treatment conditions, as a standard reference.

Mechanical test

The investigation of the mechanical properties of the containers was performed using a tensile machine, AGS 500ND (Shimadzu corporation, Kyoto, Japan) equipped with a 500[N] load cell; the test was performed using a strain rate of 10 mm/min.

Firstly some specimens obtained by molding following ISO 527 standard were prepared for linear low density polyethylene (LLDPE) and low density polyethylene (LDPE) (10 specimens for each material, of which 5 horizontally and 5 vertically), in order to underline differences in results analyzing the standard samples *ad hoc* prepared or specimens obtained from final packaging.

"Bone-shape" specimens were obtained from the central part of each container horizontally; the feature of the specimens followed the principles of the European Standard EN ISO 527 [21], suitably modified, following a previous work by this research group [20]. Specifically, this optimized dog bone shape obtained by punchcutting provides a localized stress region (3 mm width). Each specimen was characterized for the measures of thickness and width of this region using a digital microscope model BW 1008. The section of each sample was calculated from thickness and width using a suitable software program (Micro-Measure vers.1.2).

Samples were kept under constant temperature (23°C) and humidity (52% R.H.) for a week until tension test started and during all the test time.

This procedure permitted to obtain a stress versus strain curve. From each set of results was possible to estimate the tendency of materials to oppose to deformation, to evaluate the curve profile in elasticity regime, the elongation percentage in elasticity regime and the absolute elongation elasticity.

A critical analysis and comparison of diagrams made on different specimens allow a first assessment of any significant changes in the stress-strain diagram due to interactions between the material they are made of and the conditions or substances which are in contact with. [19]

Statistical analysis

The data obtained from the mechanical test on specimens derived from the commercial packaging materials are processed through statistical analysis (Mann-Whitney test) with comparison specific tests for parametric and non-parametric data. It was chosen confidence range of 95%, so the changes were considered statistically significant for $p<0.05$.

Extractables testing

To produce extractables profiles, plastic materials were subjected to different extraction conditions and the resulting samples (i.e., extracts) were analytically characterized via chromatographic means to establish each material's profile of extracted organic compounds. This information was utilized to make generalizations about the appropriateness of the test methods and to establish best practices for performing controlled extraction studies specifically relevant for the plastic materials investigated.

Extraction methods

Plastic materials were first exposed to extreme solvents and conditions to generate every potential extractable. Multiple extraction processes were used (combinations of extraction solvent, extraction method and extraction conditions) to maximize the likelihood that all predominant extractables were detected and appropriately evaluated. Overlap between methods produces corroborating data that demonstrate the validity of the procedures.

According to the recent literature[22], extraction conditions were chosen to be appropriate for the materials investigated. Extraction methods used for this study were Sonication, Sealed Vessel extraction, Soxhlet extraction, Direct Immersion and Head Space Solid phase microextraction (DI-SPME and HS-SPME), like reported in Table 2. Extraction solvents included a low and a high pH water buffer solution (pH =2, pH = 10), a 1/1 isopropanol/water mixture and hexane. All extractions were conducted in duplicate and blanks (negative controls) were prepared for all solvent/method combinations and processed in the same manner as test articles.

Table2. Extraction's methods specification

Sonication 500 mg test article 150 mL of two different water buffer solutions (pH = 2, pH = 10)
Sealed Vessel extraction 500 mg test article 10 mL of 1/1 isopropanol/water (IPA/W) solution 55°C for 3 days
Soxhlet extraction Soxtherm/Multistat Rapid Soxhlet Extraction System (Gerhardt) 500 mg test article, 150 mL hexane Hot extraction (140°C for 30 minutes)
Direct Immersion Solid phase microextraction (DI-SPME) Fiber 65 µm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB), Supelco 500 mg test article, Immersion mode Incubation temperature: 80°C, Extraction time: 15 min
Head Space Solid phase microextraction (HS-SPME) Fiber 100 µm Polydimethylsiloxane (PDMS), Supelco 500 mg test article, Headspace mode Incubation temperature: 90°C, Extraction time: 60 min

Instrumental methods

The resulting extracts were chemically characterized by gas chromatography-mass spectrometry (GC/MS).

Analyses have been carried out on a Thermo Scientific (Thermo Fisher Scientific, Waltham, Massachusetts, USA) GC/MS system (TraceDSQII mass spectrometer, TraceGCUltra gascromatograph, CTC Analytics COMBIPAL autosampler), Xcalibur MS Software Version 2.2. Operating parameters are reported in Table 3. The mass spectra of detected extractable compounds were compared with the databases for GC/MS NIST Mass Spectral Library (NIST 08) and Wiley Registry of Mass Spectral Data 8th Edition. Although the databases were used, some classes of compounds such as alkanes yielded very similar fingerprint patterns or fragments, and thus it was not always possible to make an indisputable identification of every peak (compound) detected.

Table3. Operating parameters of gas chromatography-mass spectrometry

	Organic extracts	Aqueous extracts (DI-SPME)	Headspace (HS-SPME)
Column	Restek capillary column Rtx-5MS 30 m x 0.25 mm ID x 0.25 µm	Restek capillary column Rtx-5MS 30 m x 0.25 mm ID x 0.25 µm	Restek capillary column Rtx-5MS 30 m x 0.25 mm ID x 0.25 µm
Oven Program	Start 50°C, hold for 1 min; ramp 12°C/min to 315°C, hold for 16 min	Start 60°C, hold for 4.5 min; ramp 20°C/min to 280°C, hold for 5 min	Start 60°C, hold for 4.5 min; ramp 20°C/min to 280°C, hold for 5 min
Injector	CT Split/Splitless 300°C Split flow 10 mL/min, Split ratio	PTV Splitless 250°C Splitless time 4.5 min	PTV Splitless 250°C Splitless time 4.5 min
Injection	Split, 1 µL	-	-
Carrier Gas	He, 1 mL/min constant flow	He, 1 mL/min constant flow	He, 1 mL/min constant flow
MS Transfer line	290°C	270°C	270°C
MS Detection details	70 eV (+EI) Ion source 250 °C Mass range 35-650 amu Scan rate 803.7	70 eV (+EI) Ion source 250 °C Mass range 50-650 amu Scan rate 870	70 eV (+EI) Ion source 250 °C Mass range 50-650 amu Scan rate 870

Formulations' characterization

After 24 hours from preparation and after the treatments previously shown (for formulations both in polymeric and glass packaging) a formulation's characterization was performed in terms of pH, organoleptic and rheological properties.

The pH measurement was performed by a pHmeter Jenway 3510 (Jenway, Staffordshire, UK), while viscosity and rheological properties' was performed by rheometer Kinexus Pro+ (Malvern, Worcestershire, UK), equipped with Peltier Plate Cartridge, with cone geometry CP 40/4.

Mechanical analysis

The figure 1 shows, as an example, the stress versus strain typical profile obtained from linar low density polyethylene (LLDPE) and low density polyethylene (LDPE) ISO specimens.

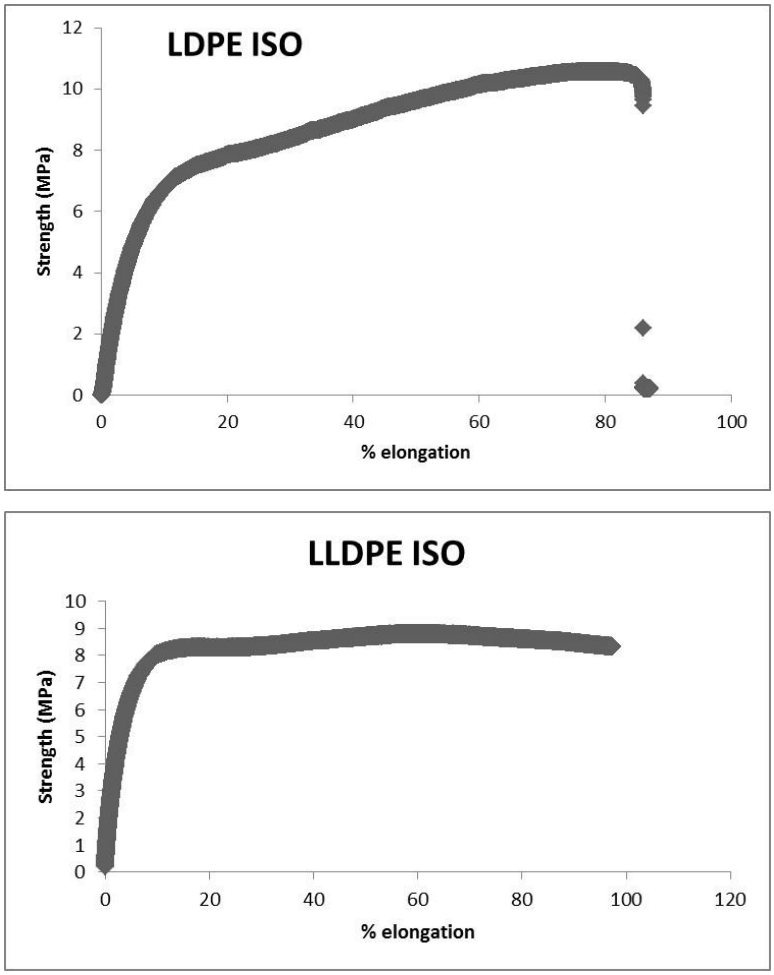

Figure 1. Stress-strain profile obtained by tensile test for LDPE and LLDPE ISO specimens.

Tables 4 summarized the values of width and thickness dimensions measured for ISO specimens for these materials.

Table 4. Measurements of thickness and width obtained for ISO specimens.

		thickness	width	section
LDPE horizontal ISO	**MEAN**	**4.2**	**10.4**	**43.3**
	ST.DEV.	0.03	0.11	0.74
	ST.DEV.%	0.79	1.06	1.71
LDPE vertical ISO	**MEAN**	**4.1**	**10.4**	**42.4**
	ST.DEV.	0.04	0.18	0.67
	ST.DEV.%	0.92	1.77	1.57
LLDPE horizontal ISO	**MEAN**	**4.1**	**10.1**	**41.9**
	ST.DEV.	0.07	0.09	1.06
	ST.DEV.%	1.69	0.86	2.52
LLDPE vertical ISO	**MEAN**	**4.2**	**10.2**	**42.4**
	ST.DEV.	0.04	0.05	0.55
	ST.DEV.%	1.01	0.47	1.29

In Table 5 the values related to stress and strain at break obtained for LDPE are reported. It wasn't possible to obtain the same parameters for LLDPE since, with the used speed, the material's elongation was bigger than the instrument's capability.

Table 5. Stress and strain values at break data obtained for LDPE

ISO specimens			
		Tensile stress at break (σB) (MPa)	**Tensile strain at break (εtB) (%)**
LDPE horizontal ISO	**MEAN**	**10.0**	**83.5**
	ST.DEV.	0.30	2.47
	ST.DEV.%	3.00	2.96
LDPE vertical ISO	**MEAN**	**9.8**	**83.1**
	ST.DEV.	0.43	2.23
	ST.DEV.%	4.41	2.68

In the second part of work, the specimens obtained from LLDPE and LDPE single dose containers have been considered and analyzed by tensile test at different times: not treated, treated with simulated solar irradiation and thermal shock, filled with F3 simulant and treated with simulated solar irradiation and thermal shock as described above.

In Table 6 the global means of width and thickness measures related to all analyzed samples, divided by material, with the relative standard deviations and % standard deviations.

Table 6. Measures of thickness and width obtained from specimens of the different materials

LLDPE	thickness	width (µm)	section
MEAN	**610.4**	**3087.6**	**1.9**
ST.DEV.	10.58	73.36	0.07
ST.DEV.%	1.73	2.38	3.76
LDPE			
MEAN	**565.6**	**3063.2**	**1.7**
ST.DEV.	6.62	33.02	0.04
ST.DEV.%	1.17	1.08	2.07

Once acquired the measures for all the samples (25 specimens for type) the tensile test was carried out. The following tables (Table 7, 8) report the data obtained: illustrative graphs of the stress/stress profile curve for each material were reported (Figure 2, 3).

Table 7. Tensile test's data obtained for LLDPE

LLDPE						
		Tensile strenght (σM)= Yield stress (σy) (MPa)	**Tensile strain at yield (εy)= (εM)(%)**	**Angolar coefficient linear portion**	**Tensile stress at break (σB) (MPa)**	**Tensile strain at break (εtB) (%)**
t0	**MEAN**	**7.514**	**9.391**	**123.635**	**10.825**	**290.057**
	ST.DEV.	0.60	1.60	20.85	0.92	37.14
	ST.DEV.%	7.97	17.02	16.87	8.53	12.81
Formulation suntest	**MEAN**	**7.337**	**12.420**	**122.602**	**10.406**	**285.020**
	ST.DEV.	0.62	3.69	17.07	0.91	32.20
	ST.DEV.%	8.44	29.73	13.93	8.70	11.30
Empty suntest	**MEAN**	**7.389**	**7.843**	**168.164**	**9.911**	**250.023**
	ST.DEV.	0.34	1.46	15.62	0.64	29.18
	ST.DEV.%	4.56	18.64	9.29	6.45	11.67
Formul. thermal shock	**MEAN**	**8.755**	**13.740**	**133.414**	**11.166**	**259.512**
	ST.DEV.	0.20	2.15	17.14	0.63	32.42
	ST.DEV.%	2.30	15.65	12.85	5.62	12.49
Empty thermal shock	**MEAN**	**8.900**	**12.451**	**124.754**	**10.913**	**235.390**
	ST.DEV.	0.26	2.24	15.38	0.86	37.04
	ST.DEV.%	2.88	18.01	12.33	7.86	15.74

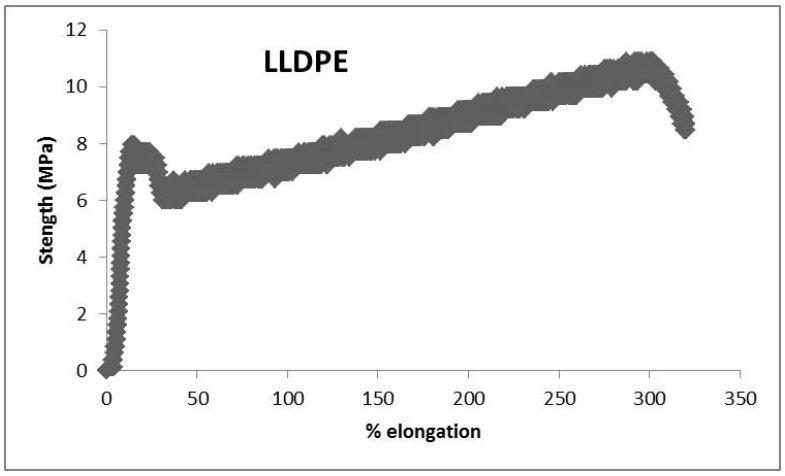

Figure 2. Stress-strain profile obtained by tensile test for LLDPE

Table 8. Tensile test's data obtained for LDPE

LDPE					
		Tensile strenght (σM)= Yield stress (σy) (MPa)	**Angolar coefficient linear portion**	**Tensile stress at break (σB) (MPa)**	**Tensile strain at break (εtB) (%)**
t0	**MEAN**	**6.752**	**110.719**	**7.677**	**86.360**
	ST.DEV.	0.29	10.53	0.70	21.97
	ST.DEV.%	4.29	9.51	9.15	25.44
Formulation suntest	**MEAN**	**6.713**	**116.671**	**7.298**	**73.701**
	ST.DEV.	0.22	8.43	0.60	16.81
	ST.DEV.%	3.28	7.23	8.28	22.81
Empty suntest	**MEAN**	**6.377**	**110.837**	**7.319**	**91.685**
	ST.DEV.	0.24	25.01	0.66	25.49
	ST.DEV.%	3.76	22.56	9.07	27.80
Formulation Thermal Shock	**MEAN**	**6.595**	**111.548**	**7.043**	**64.823**
	ST.DEV.	0.21	7.19	0.47	22.34
	ST.DEV.%	3.22	6.44	6.65	34.46
Empty Thermal shock	**MEAN**	**7.559**	**103.707**	**9.109**	**91.105**
	ST.DEV.	0.33	15.44	0.99	26.92
	ST.DEV.%	4.30	14.89	10.81	29.54

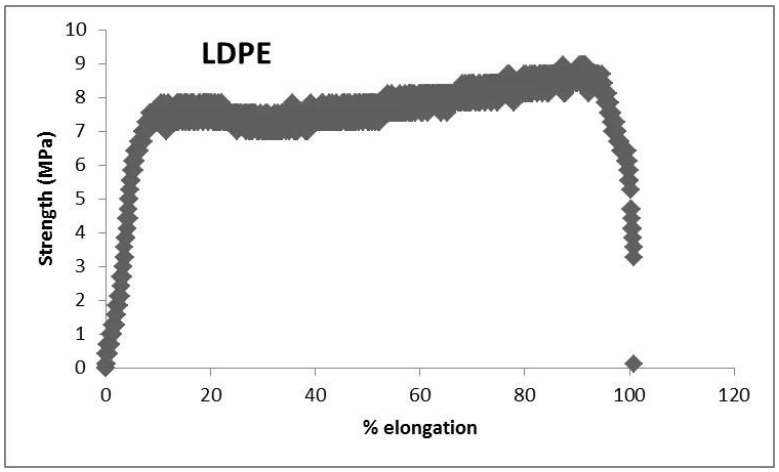

Figure 3. Stress-strain profile obtained by tensile test for LDPE

From the analysis of specimens obtained following the ISO standards, values of stress and percent elongation with standard deviations of less than 5% were obtained. For the single-dose container of LLDPE a graph representing the trend of yield point values (Figure 4) and the summary table (Table 9) of the statistical analysis performed by the Mann-Whitney test with a 95% confidence interval are reported.

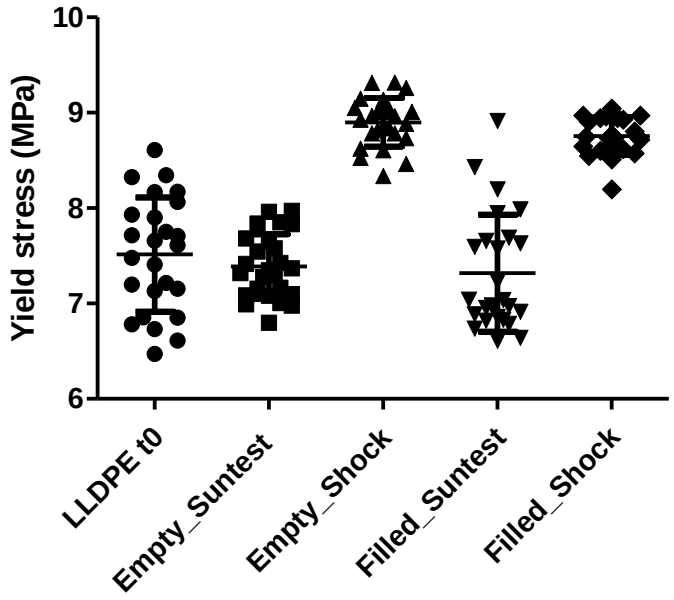

Figure 4. Trend of yield stress values for LLDPE samples

Table 9. Statistical analysis on yield stress data obtained for LLDPE

LLDPE						
	LLDPE-empty t0 vs suntest	**LLDPE-empty t0 vs Shock**	**LLDPE-t0 vs filled suntest**	**LLDPE-t0 vs filled Shock**	**Suntest LLDPE empty vs filled**	**Shock LLDPE empty vs filled**
p	0.3933	<0.0001***	0.2252	<0.0001***	0.1683	0.025*

Here the graphs related to trend of the values of yield stress (YS) and break stress (BS) for LDPE samples (Figure 5) and the summary table (Table 10) of statistical analysis performed by the Mann-Whitney test, with a confidence interval of 95 %, are reported.

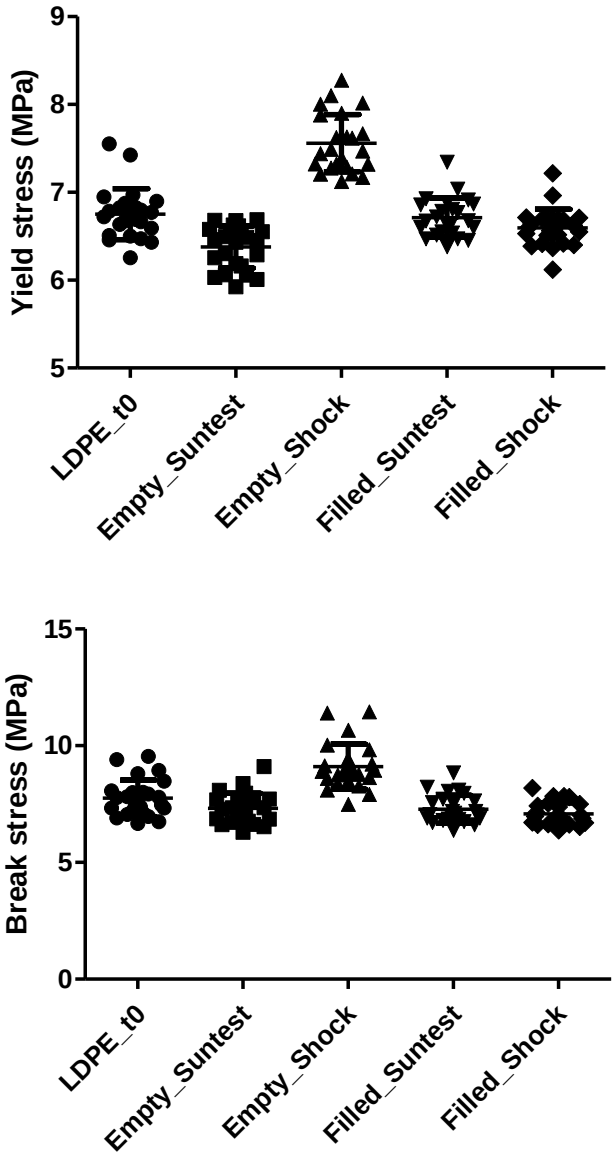

Figure 5. Trend of yield stress (up) and break stress (down) values for LDPE samples

Table10. Statistical analysis on yield stress (YS) and break stress (BS) data obtained for LDPE

LDPE						
	LLDPE-empty t0 vs suntest	**LLDPE-empty t0 vs Shock**	**LLDPE-t0 vs filled suntest**	**LLDPE-t0 vs filled Shock**	**Suntest LLDPE empty vs filled**	**Shock LLDPE empty vs filled**
p(YS)	<0.0001***	<0.0001***	0.7182	0.0253*	<0.0001***	<0.0001***
p(BS)	0.0397*	<0.0001***	0.0263*	0.011**	0.8843	<0.0001***

Extractables characterization

The organic extractables profile of the plastic materials investigated (LLDPE and LDPE containers) was established via multiple extraction processes, multiple extracting media and multiple analysis.

Figure 6 and Figure 9 show the Total Ion Current (TIC) chromatograms related to GC/MS analysis of the various extracts obtained from LLDPE and LDPE containers. These results showed that

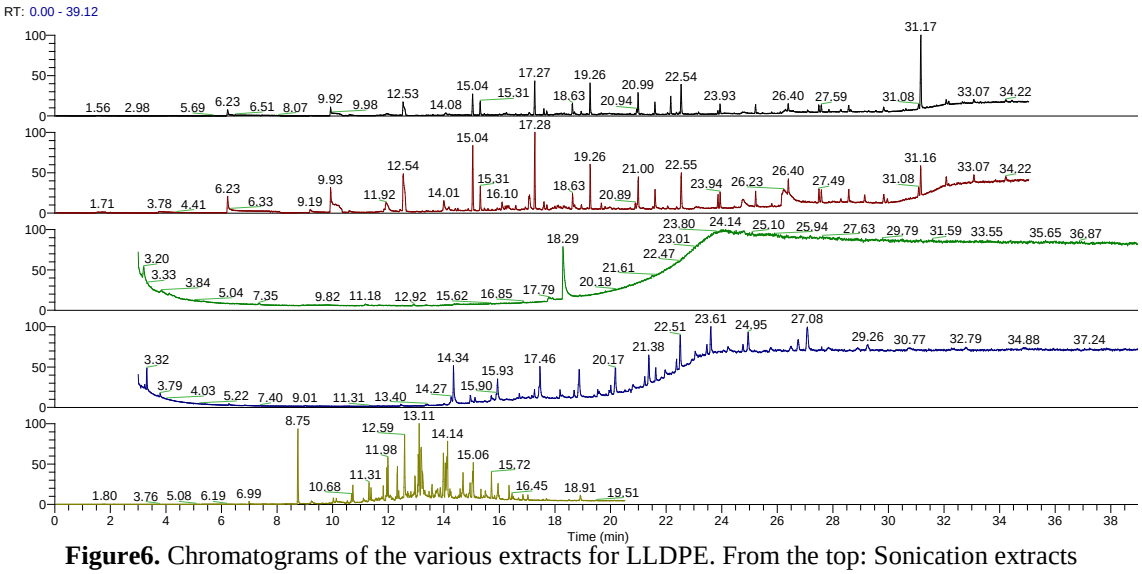

Figure6. Chromatograms of the various extracts for LLDPE. From the top: Sonication extracts pH 2 and pH 10, Sealed Vessel extract, Soxhlet extract, HS-SPME extract

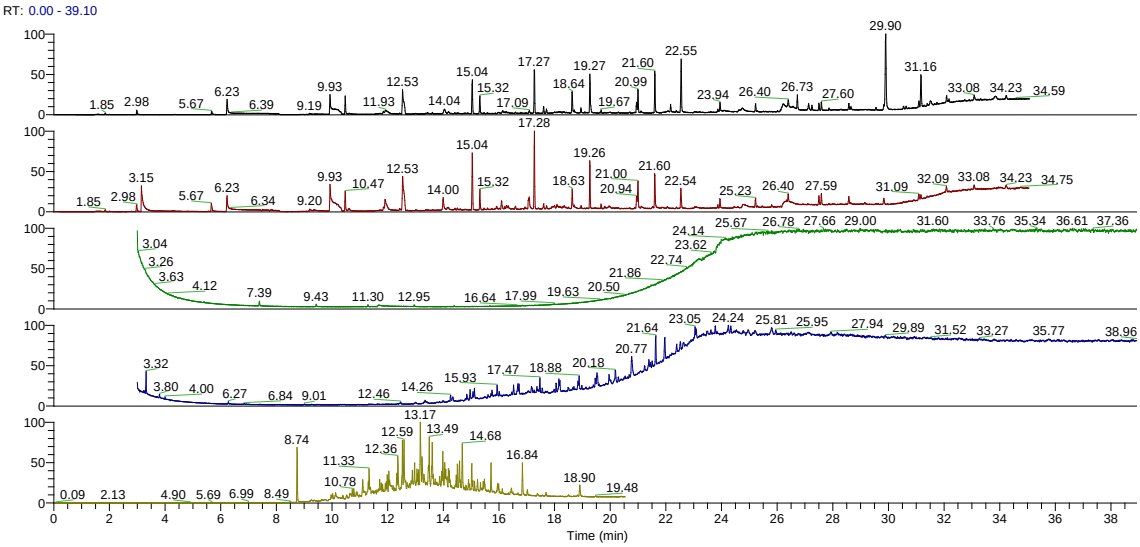

Figure7. Chromatograms of the various extracts for LDPE. From the top: Sonication extracts pH 2 and pH 10, Sealed Vessel extract, Soxhlet extract, HS-SPME extract

After subtraction of the extraction blanks results from the samples results and removal of the interfering peaks associated with bleeding of GC capillary column or SPME fiber coating, a list of compounds released by the analyzed polymers was extracted by GC/MS.

Organic extractables profiles for LLDPE and LDPE containers are summarized in Table 11.

These organic extractables generally fall into classes of compounds linked to the major constituents of the original plastic materials. For example, the profiles included compounds like antioxidants and additives (eg. 2,4-Di-t-butyl phenol, Irganox, phthalates) associated with the initial ingredients, impurities related to processing (eg. esters) and degradation products of the polymers (aliphatic hydrocarbons).

Table 11. Organic extractables profiles of LLDPE and LDPE containers

Identification	CAS NR	Chemical Formula	Molecular Weight	LLDPE % area	LLDPE % area
2,4-Di-t-butyl phenol	96-76-4	$C_{14}H_{22}O$	206	0.11	0.26
Hexadecanamide	629-54-9	$C_{16}H_{33}NO$	255	traces	traces
9-Octadecenamide, (Z)-	301-02-0	$C_{18}H_{35}NO$	281	traces	traces
Hexadecyl 2-ethylhexanoate	59130-69-7	$C_{24}H_{48}O_2$	368	12.39	traces
Diisobutyl phthalate	84-69-5	$C_{16}H_{22}O_4$	278	2.26	5.05
Dibutyl phthalate	84-74-2	$C_{16}H_{22}O_4$	278	2.80	3.81
Aliphatic hydrocarbons*	-	-	-	57.14	61.56
Olefins*	-	-	-	7.39	10.26
Octinoxate	5466-77-3	$C_{18}H_{26}O_3$	290	3.01	1.47
Diisooctyl phthalate	131-20-4	$C_{24}H_{38}O_4$	390	0.32	5.44
Myristyl myristate	3234-85-3	$C_{28}H_{56}O_2$	424	traces	traces
Squalene	111-02-4	$C_{30}H_{50}$	410	2.65	8.59
Irganox 1076	2082-79-3	$C_{35}H_{62}O_3$	530	11.94	3.82

* Class of compounds

Once LLDPE and LDPE containers have been characterized at t0 (not treated), an evaluation of the extractables profiles of the starting materials (LLDPE and LDPE pellets) was performed, in order to make a comparison between the materials before and after process. On these pellets an extraction with the selected test method (HS-SPME) was carried out and the extracts were analyzed by GC/MS.

In Figures 8 and 9 the Total Ion Current (TIC) chromatograms obtained from the analysis of LLDPE and LDPE pellets are shown in comparison to the chromatograms of LLDPE and LDPE containers.

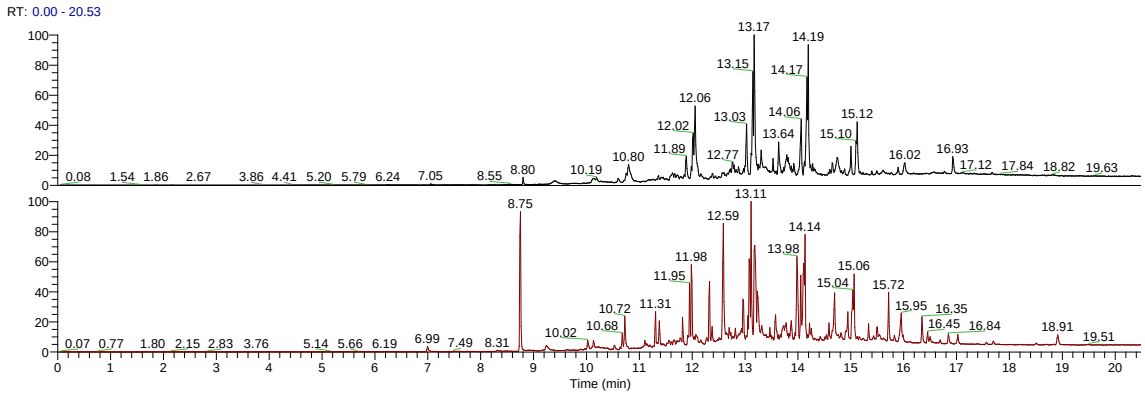

Figure8. Chromatograms for LLDPE. From the top: pellets, containers

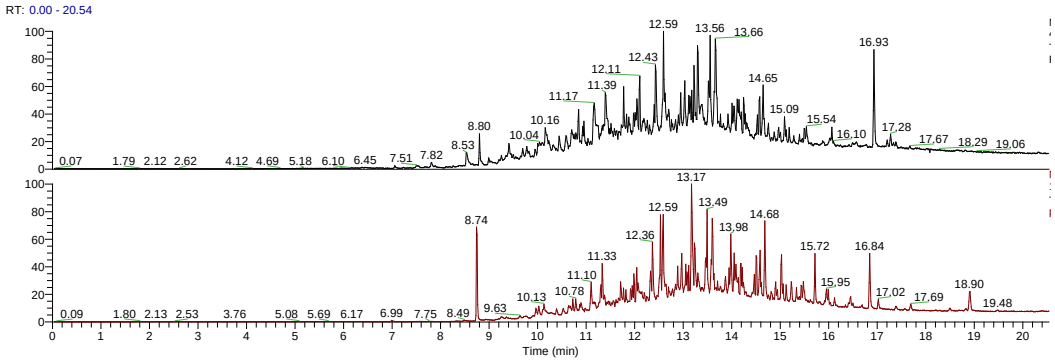

Figure9. Chromatograms for LDPE. From the top: pellets, containers

The next step was the characterization of the substances that could be extracted from LLDPE and LDPE containers after treatment with solar irradiation and thermal shock, empty and filled with the simulant described above.

In Table 12 the percentages of each class of substances extracted for both materials at t0 (not treated) and empty containers after treatments are reported. All samples were extracted with the selected test method (HS-SPME) and the extracts were analyzed by GC/MS.

Table12. % areas of substances extracted from LLDPE and LDPE empty containers at t0 and after treatments

Empty containers	**LLDPE t0**	**LLDPE empty suntest**	**LLDPE empty shock**	**LDPE t0**	**LDPE empty suntest**	**LDPE empty shock**
	% Area	**% Area**	**% Area**	**% Area**	**% Area**	**% Area**
Compounds associated with the initial ingredients	17.43	< 0.01	2.45	18.12	< 0.01	2.10
Compounds related to processing	18.05	1.26	17.55	10.06	0.77	14.74
Degradation products of polymers and/or additives	64.52	98.74	80.00	71.82	99.23	83.16
Compounds absorbed from formulation (simulant)	-	-	-	-	-	-

Instead Table 13 reports the percentages of each class of substances extracted from containers filled with formulation and treated with simulated solar irradiation and thermal shock for both materials.

Table13. % areas of substances extracted from LLDPE and LDPE containers filled with formulation after treatments

Filled containers	LLDPE filled suntest % Area	LLDPE filled shock % Area	LDPE filled suntest % Area	LDPE filled shock % Area
Compounds associated with the initial ingredients	0.04	0.17	< 0.01	0.77
Compounds related to processing	3.38	3.29	4.04	3.10
Degradation products of polymers and/or additives	1.80	1.87	1.14	1.01
Compounds absorbed from formulation (simulant)	94.79	94.67	94.80	95.73

As it can be seen for both materials come into contact with the formulation after UV-vis irradiation and thermal shock cycles, substances closely related to the filling formulation were detected at relatively large levels. These substances were identified as Cyclopentasiloxane (r_t 8.88 min) and Bis(2-ethylhexyl) carbonate (r_t 13.25 min), and they represent nearly 95-96% of the total extracted compounds (see example in Figure 10).

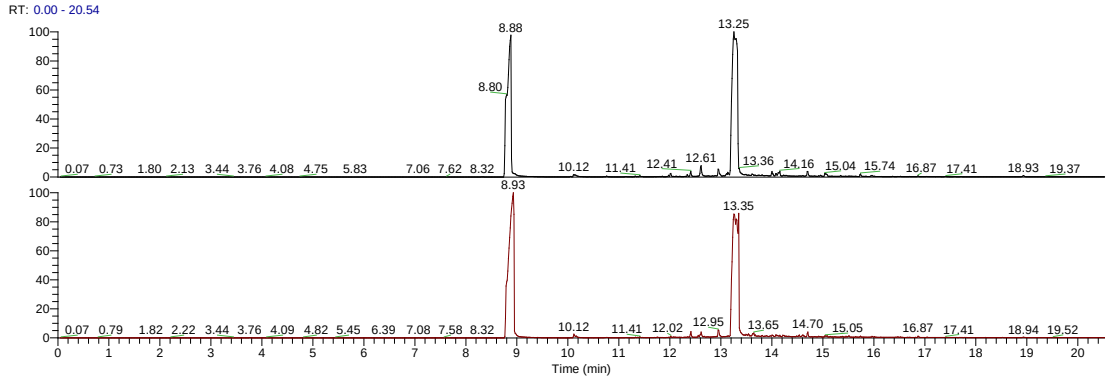

Figure10. From the top: chromatograms for LLDPE and LDPE containers filled with simulant after UV-vis irradiation

In order to exclude the possibility that the identified substances simply remained on the surface of the polymers because of a non-efficient washing system, some samples of the examined plastic materials were put into direct contact with the ingredients of formulation that were detected in the samples of LLDPE and LDPE for 30 minutes.

Then they were cleaned with the washing method, previously mentioned and reported in another work by Perugini et al. [20], and an extraction with the selected test method (HS-SPME) was carried out. After GC/MS analysis, no traces of the substances associated with the formulation were found in the extracts.

In addition to the assessment of the extractables profiles, a first screening to evaluate the interactions between products and packaging system was carried out, by detecting substances eventually migrated as a result of the treatments from the LLDPE and LDPE containers and accumulated as leachables into the formulation above described.

Samples of formulation (300 mg) in contact with LLDPE and LDPE containers and undergone to UV-vis irradiation and thermal shock cycles have been analyzed by HS-SPME/GC-MS. No substances related to the polymeric materials were detected within the formulation, with the exception of very low levels of Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP) and Bis(2-ethylhexyl) phthalate (DEHP), found in the formulation contained in LLDPE after treatment with simulated solar radiation.

Formulations' characterization

From the pH measurements and analysis of the organoleptic properties no difference between formulations at t0 and those contained in glass and in the two types of plastic material were revealed.

The evaluation of the rheological properties of the formulations through the rheometer underlined that the two different types of materials did not change either the viscosity or the rheological behavior of the content.

In fact, the viscosity of the emulsion contained in the two types of plastic material resulted to be unaltered before and after treatment with both solar simulated radiation and with thermal shock with respect to t0 as shown in Figures11-12.

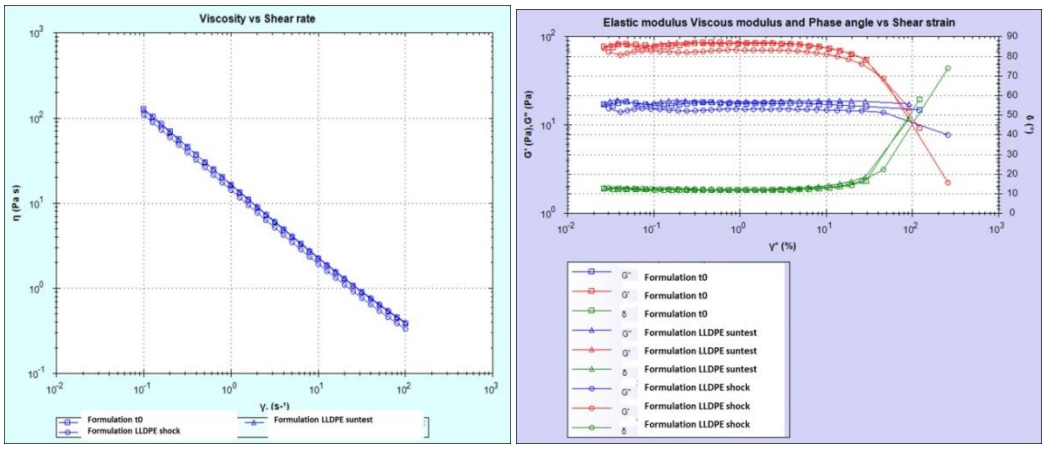

Figure11. On the left: viscosity curve of formulation in LLDPE vs t0. On the right: Elastic and viscous modulus curves of formulation in LLDPE vs t0.

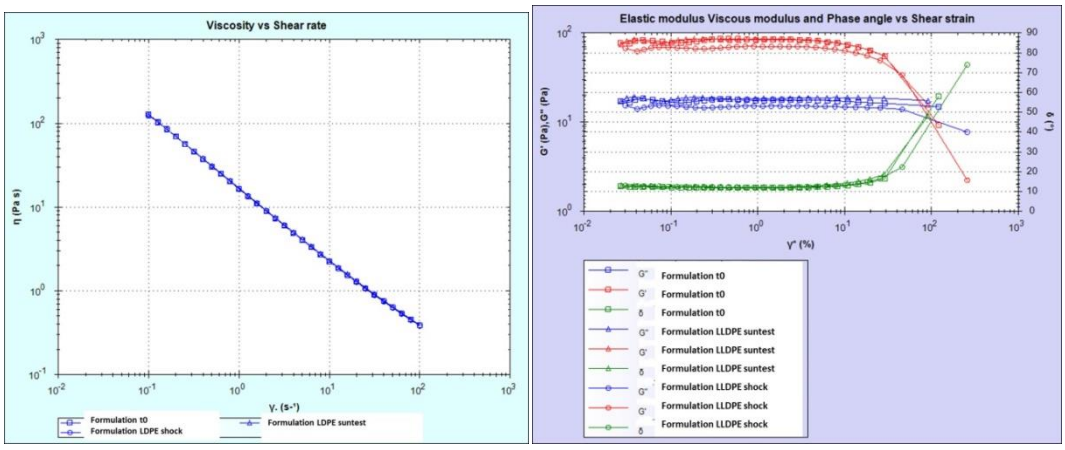

Figure12. On the left: viscosity curve of formulation in LDPE vs t0. On the right: Elastic and viscous modulus curves of formulation in LDPE vs t0.

DISCUSSION

Mechanical analysis

From the analysis of specimens obtained following the ISO standards, as shown in Table 5, values of stress and percent elongation with standard deviations of less than 5% were obtained, underlining the homogeneity of the samples, as expected. Furthermore, the low standard deviation indicates that the used instrument and the applied analytical protocol allow to obtain reliable and reproducible data.

No significant differences can be detected between horizontal and vertical specimens.

In the second part, the specimens obtained from LLDPE and LDPE single dose containers have been considered and analyzed by tensile test at different times: not

treated, treated with simulated solar irradiation and thermal shock, filled with F3 simulant and treated with simulated solar irradiation and thermal shock as described above.

Looking at the results reported in Table 6 an intrinsic difference in the thickness among the different materials can be observed. Observing the value of thickness and width for each specimen, a maximum standard deviation corresponding to 2.38% can be outlined for the measures of both parameters; it reflects a maximum standard deviation of about 3.76% for the section's value.

From these consideration it can be affirmed that the production's method of specimens and measure's method can give reproducible results.

Once acquired the measures for all the samples (25 specimens for type) the tensile test was carried out.

Regarding the assessment of these products, the presence of a yield point for the LLDPE polymer can be observed from Figure 2; the values of stress at yield point and at break reported standard deviations within a range of 10%. For this reason the values of stress at yield point and at break can therefore be considered as significant parameters for possible changes of the material before or after the treatment and/or contact with simulants.

Different considerations can be done, however, in relation to the strain, both at yield point and at breaking point, and to the angular coefficient of the linear portion of the first part of the curve. For these parameters, in fact, found higher standard deviations can be found. This fact doesn't allow, therefore, to detect with certainty possible changes undergone by the material.

In relation to the parameters of percentage elongation and linear coefficient of the linear portion of the first portion of the curve, it could be hypothesized that obtaining standard deviations' values higher than for the ISO specimens is due to the specimen's shape, specifically to the central section (break area), dimensionally very smaller than the ISO standard. This underlines the huge difference between analyze standard specimens, more homogeneous and regular, and specimens obtained by final commercial containers, that represent the real material in contact with the product.

From the analysis of specimens obtained following the ISO standards, values of stress and percent elongation with standard deviations of less than 5% were obtained, also underlining the homogeneity of the samples, as expected . This indicates that the

used instrument and the applied analytical protocol allow to obtain reliable and reproducible data.

Taking into account these considerations, the effects of treatments and contact with the simulant can be evaluated.

From results of the statistical analysis performed by the Mann-Whitney test with a 95% confidence interval (Table 9) we can observe that simulated solar radiation did not induce any changes in the mechanical characteristics of this polymer and it did not induce any interaction between the formulation and the container detectable at the level of alterations of the mechanical properties of this polymer.

The thermal shock treatment instead influenced in a statistically significant way the mechanical behavior of the polymer; the basic formulation interacted significantly with the container when subjected to thermal shock.

Analogous considerations can be done for sample of the single dose containers made by LDPE.

Observing the relative results reported in Table 10, we can conclude that simulated solar radiation induced statistically significant changes in the mechanical characteristics of this polymer and it induced some interactions between the formulation and the container detectable at the level of alterations of the mechanical properties of this polymer.

The thermal shock treatment influenced in a statistically significant way the mechanical behavior of the polymer and also the basic formulation interacted significantly with the container when subjected to this kind of treatment.

Extractables characterization

The organic extractables profile of the plastic materials investigated (LLDPE and LDPE containers) was established via multiple extraction processes, multiple extracting media and multiple analysis. Due to the chemical nature of the solvents and physiochemical nature of the extraction processes, it is expected that the extractables profiles revealed by testing the various extracts would be quite different. It must be noticed that it is thus the combination of the information derived from the analysis of the diverse extracts that establishes extractables profile of the test articles.

After subtraction of the extraction blanks results from the samples results and removal of the interfering peaks associated with bleeding of GC capillary column or

SPME fiber coating, a list of compounds released by the analyzed polymers was extracted by GC/MS.

The chromatographic analyses indicated that HS-SPME and Soxhlet extracts contained more numerous extractables at higher concentrations than did the aqueous extracts. Furthermore, the HS-SPME extracts generally contained the same extractables as did the Soxhlet extracts, providing a complete insight of all the predominant organic extractables for the analyzed materials. This information was utilized to select for further analyses Head Space Solid phase microextraction as test method to perform controlled extraction studies specifically relevant for the plastic materials investigated.

Once LLDPE and LDPE containers have been characterized at t0 (not treated), an evaluation of the extractables profiles of the starting materials (LLDPE and LDPE pellets) was performed, in order to make a comparison between the materials before and after process. On these pellets an extraction with the selected test method (HS-SPME) was carried out and the extracts were analyzed by GC/MS.

Chromatograms revealed a similar compositions of materials in a pellet form and as final packaging.

The next step was the characterization of the substances that could be extracted from LLDPE and LDPE containers after treatment with solar irradiation and thermal shock, empty and filled with the simulant described above.

Data reported in Table 12 indicated that for both polymers, the largest percentage of compounds extracted from containers at t0 and is associated to polymers and/or additives degradation products. Furthermore this percentage becomes higher for empty containers after both treatments, especially after UV irradiation.

Instead from Table 13, as it can be seen for both materials come into contact with the formulation after UV-vis irradiation and thermal shock cycles, substances closely related to the filling formulation were detected at relatively large levels. These substances were identified as Cyclopentasiloxane (r_t 8.88 min) and Bis(2-ethylhexyl) carbonate (r_t 13.25 min), and they represent nearly 95-96% of the total extracted compounds (see example in Figure 10).

The test performed on washing system demonstrated its efficiency, confirming that some substances of the formulation actually could have been adsorbed by the packaging material as a result of the treatments.

In addition to the assessment of the extractables profiles, a first screening of substances eventually migrated as a result of the treatments from the LLDPE and LDPE containers and accumulated as leachables into the formulation above described was carried out.

No substances related to the polymeric materials were detected within the formulation, with the exception of very low levels of Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP) and Bis(2-ethylhexyl) phthalate (DEHP), found in the formulation contained in LLDPE after treatment with simulated solar radiation. The limit of detection, is one of the biggest topics in extractables and leachables analysis. The Safety Concern Threshold (SCT) of below 0.15 µg/day has been defined as the leachables threshold that would present negligible safety concerns from possible carcinogenic to noncarcinogenic toxic effects. [23-24] Results obtained from this study suggest that the phthalate levels would be below the SCT level of 0.15 µg/day. This work was largely qualitative. Future studies will be addressed at quantifying leachables in according to the safety assessment depending on product category and exposure levels in use.

Formulations' characterization

From the pH measurements and analysis of the organoleptic properties no difference between formulations at t0 and those contained in glass and in the two types of plastic material were revealed. Furthermore, the viscosity of the emulsion contained in the two types of plastic material resulted to be unaltered before and after treatment. It can be concluded that polymeric packaging did not influence the rheological behavior and organoleptic properties of formulations.

CONCLUSION

This work aimed to underline the difference that exists analyzing the starting polymer or standard samples ad hoc prepared to respect to the analysis of the final packaging that is in direct contact with a product. Furthermore this research wanted to demonstrate that some interactions between containers and contained formulations could occur in particular conditions and it's necessary to be able to detect them in order to assure the products' quality, efficacy and safety.

The values of stress at the yield and break point have a standard deviation <10% and thus can be considered of significant parameters for any changes in material before and after treatment and/or contact with simulants.

For LLDPE, simulated solar radiation did not induce any changes in the mechanical characteristics and it did not induce any interaction between the formulation and the container detectable as alterations of the mechanical properties of the polymer. Instead, the thermal shock treatment influenced in a statistically significant way the mechanical behavior of the polymer and the basic formulation demonstrated to interact significantly with the container when subjected to thermal shock.

For LDPE, simulated solar radiation induced statistically significant changes in the mechanical characteristics of the polymer and the interaction between the formulation and the container detectable as alterations of the mechanical properties of the polymer.

The thermal shock treatment revealed to influence in a statistically significant way the mechanical behavior of the polymer and the basic formulation showed to interact significantly with the container when subjected to thermal shock.

Simultaneously, the organic extractable profile of the plastic materials investigated was established via multiple extraction processes and extracting media. The chromatographic analyses indicated that the HS-SPME extracts generally contained the same extractables as did the Soxhlet extracts, providing a complete insight of all the predominant organic extractables for the analyzed materials. For this reason Head Space Solid phase microextraction was selected as test method to perform the successive controlled extraction studies specifically relevant for the plastic materials investigated. All extracts obtained were analyzed by GC/MS.

Once LLDPE and LDPE containers have been characterized at t0, the characterization of the substances that could be extracted from LLDPE and LDPE containers after treatments with and without the simulant described above indicated that for both polymers the largest percentage of compounds extracted from containers at t0 and empty containers after both treatments is associated to polymers and/or additives degradation products.

Instead, for both LLDPE and LDPE containers come into contact with the formulation after UV-vis irradiation and thermal shock cycles, substances closely related to the filling formulation were detected at relatively large levels. These substances were identified as Cyclopentasiloxane and Bis(2-ethylhexyl) carbonate.

Furthermore the analysis of the rheological properties carried out on the formulation before and after treatment in glass and plastic showed that no changes occurred as a result of treatment, nor for the formulations contained in the glass nor for those contained in LLDPE and LDPE containers.

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Chapter V.

SET UP OF SUNSCREEN FORMULATIONS

Extract from an internal report of Application Laboratory of Merck Chimie SAS

(Fontenay sous Bois, France)

Work performed during the Erasmus Traineeship project.

SET UP OF SUNSCREEN FORMULATIONS

ABSTRACT

A great number of studies provided evidences that wavelengths present in natural sunlight could provoke harmful clinical consequences on human skin, such as photoaging and skin cancer.

Photoprotection is one of the essential prophylactic and therapeutic factor to avoid these undesired effects. The development of photoprotection has been stimulated by a change in the behavioral habits of human society, including an increased use of sunscreens.

Sunscreen products contain UV absorbers (UV filters), that can be organic or inorganic molecules; EU Regulation 1223/2009 indicate 28 authorized substances. Among these, Avobenzone and Octocrylene are broadly used; however AVO is not photostable.

Furthermore, high requirements in terms of performances and high consumer expectations concerning sensory properties, require raw material suppliers and formulators to be innovative and creative in developing new raw materials and novel formulation types.

In this perspective, this work aims to develop sunscreen formulations responding to market trends (dosage, SPF) and respecting the laws in force. In particular the focal point was to highlight differences in the use of free or encapsulated filters (Avobenzone and Octocrylene) studying their impact in formulations in combination with other organic and inorganic filters in cosmetic form of O/W emulsion spray, highlighting the encapsulated filters' properties.

The final goal is to obtain stable formulations that can be used, in a subsequent step of work, for the evaluation of possible interactions with plastic packaging.

Acknowledgements

This work was carried out during the Erasmus Traineeship period at the Application Laboratory of Merck Chimie SAS, located in Fontenay-sous-Bois, France.

INTRODUCTION

The skin is the most external part of the body and forms a physical barrier to the environment, providing protection against microorganisms, ultraviolet radiation, toxic agents or mechanical insults. The exposure to the solar ultraviolet rays is one of the most important environmental factors affecting skin physiology. [1]

Solar radiation includes the entire electromagnetic spectrum (short, high energy cosmic and gamma rays, longer lower energy UV rays, visible light, infrared radiation, microwaves, radio waves). UV, visible and short IR waves are classified as nonionizing radiation. [2]

Ultraviolet radiation (UVR) from the sun is divided into UVC (270-290 nm), UVB (290- 320 nm), and UVA, which is subdivided into UVA2 (320-340 nm) and UVA1 (340-400 nm). UVC emitted by the sun is filtered by ozone in the stratosphere; therefore, it does not reach the earth's surface. The amount of solar UVB and UVA reaching the earth's surface is affected by latitude, altitude, season, time of the day, cloudiness, and ozone layer. UVA, compared with UVB, can penetrate deeper through the skin, and is not filtered by window glass. [3]

This exposure can lead to short and long term consequences. In particular, the acute effects of UVR include erythema, pigment darkening, delayed tanning, thickening of epidermis, immunosuppression, decreased blood pressure, nitric oxide induction and Vitamin D synthesis. [4] Furthermore, repeated injury may ultimately predispose to chronic effects: photoaging (the development of deep wrinkles, leathery skin, dilatation of blood vessels, dark spots), immunosuppression and photocarcinogenesis (development of skin cancer).[5] In fact, the primary cause of melanoma are ultraviolet radiations. [6] With the rise in the number of skin cancer cases diagnosed annually, significant public education programs have been undertaken advocating photoprotection, including the use of sunscreens. [7]

"Sunscreen product" means any preparation (such as creams, oils, gels, sprays) intended to be placed in contact with the human skin with a view exclusively or mainly to protecting it from UV radiation by absorbing, scattering or reflecting radiation. [8]

Ideal sunscreens provide uniform protection against ultraviolet A and B light, having anyway aesthetically pleasing compositions that enhance the user's compliance.

The heart of any sunscreen product is the ultraviolet absorber; ultraviolet filters are classified according to their action's mechanism, physical filters (mineral filters),

that act like glasses reflecting the light, and chemical filters (organic filters), that absorb the radiation's energy since they contain a suitable cromophore that has conjugated π-electron systems. Increasing the number of conjugated double bonds in the molecule the absorption maximum shifts to longer wavelengths and also gives rise to a larger absorption cross section and, therefore, stronger absorption. [9-10]

All countries have a positive list of UV filters, including maximum concentration allowed in sunscreens. In most countries, including Europe and Japan, UV absorbers are regulated as cosmetics, in the United States and Canada as OTC drugs while in Australia as therapeutic drugs. The number of available UV filters differs from region to region; the US sunscreen monograph lists the least number of UV filters.[11]

Regarding the EU legislation, Annex VI indicates 28 authorized substances, of which 26 are organic filters (e.g. Benzophenone-3, Butyl Methoxydibenzoylmethane, Ethylhexyl Methoxycinnamate, Ethylhexyl Triazone, Octocrylene, etc…) and just one is an inorganic filter, that is Titanium Dioxide, with a maximum concentration of 25%.

From a chemical point of view, organic filters can be divided in two categories, UVA and UVB filters. The most used UVB filter is ethylhexyl methoxycinnamate (λ_{max} in EtOH 311 nm), followed by Octocrylene (λ_{max} in EtOH 303 nm), liquid and fat-soluble, useful for high protection formulations and it can provide an oily touch to finish product. In combination with UVB filters one or more UVA filters have to be used; an example is butyl-methoxydibenzoylmethane (Avobenzone, (λ_{max} in EtOH 358 nm), a yellowish powder that could give crystallization's problems and that is sensible to iron's traces. Avobenzone is the most efficient UVA1 filter, but it is not photostable. It's not approved for combination with TiO_2 because enhanced photodegradation of Avobenzone is observed in the presence of the inorganic filter.[12]

Regarding TiO_2, in order to be efficacious and non-whitening on the skin, the particles of this insoluble substance have to be very small, typically in the size range around 100 nm or below. Furthermore, depending on particle size, this material is semiconductor that absorb photons at different wavelengths. The smaller the primary particles, the shorter its peak absorption spectrum. Because of the photocatalytic effect, TiO_2 for sunscreen applications is coated with aluminum oxide or silica in order to prevent oxygen radical formation. In addition, the rutile crystal form is used in most cases because it has a high refractive index and can absorb UVR. [13]

About the nano form of titanium dioxide the Scientific Committee on Consumer Safety (SCCS) issued its positive opinion (SCCS- Opinion on Titanium Dioxide (nano form) COLIPA n. S75-22nd July 2013), confirming the maximum usable concentration of 25% excepted those cosmetic forms (powder or spray) that can expose to the risk of TiO_2 nanoparticles' inhalation.

For a rational formulations' development, next to the considerations about cosmetic pleasantness, some general requirements have to be kept in mind. A sunscreen would have: smoothing application (easy and homogenous distribution of filters on the skin), efficacy on thin layer, low transdermal absorption (filters have to remain on the skin's surface where radiations arrive), substantivity on the skin (resistance to water and sweat) and no phototoxicity-photosensibilization's potential. [10]

The strategies to formulate broad spectrum sunscreens expect the optimized use of both UVA and UVB filters. Since each filter is characterized by its absorption spectrum of UV radiation with a characteristic maximum peak (λ_{max}), combinations of filters are adopted in order to cover completely and efficaciously the areas of interest of UVA and UVB radiations.

Among the high diversity of formats available on the market, the standard types, lotions, and creams/gel-creams still remain the preferred. However, notable differences appeared around the globe in preferences for different types of products. In Europe, for example, emulsion sprays, consisting of either oil-in-water or water-in-oil emulsions, remain very popular; although, other forms such as sticks, oils, mousses, and powders represent almost 16%of the launched products in contrast to the three other regions where these formats remain marginal. [14]

The high requirements in terms of performance such as high SPF value, UVA protection, water resistance, and photostability, in conjunction with high consumer expectations concerning sensory properties, require raw material suppliers and formulators to be innovative and creative in developing new raw materials and novel formulation types. Innovations in terms of sensory properties or application formats were introduced over last decade to attract consumers and improve compliance. [15]

In this perspective, this work aims to develop sunscreen formulations responding to market trends (dosage, SPF) and respecting the laws in force. In particular the focal point was to highlight differences in the use of free or encapsulated filters (Avobenzone and Octocrylene) studying their impact in formulations in combination with other organic and inorganic filters in cosmetic form of O/W emulsion spray.

The final goal is to obtain stable formulations that can be used, in a subsequent step of work, for the evaluation of possible interactions with plastic packaging.

MATERIALS

For the final formulations, UV filters by Merck KGaA have been used: Eusolex® UV-Pearls™ OB-S X, Eusolex® 232, Eusolex® OCR, Eusolex® 9020, Eusolex® HMS, Eusolex® OS, Eusolex® T-S.

Other two filters were used in order to increase the SPF value, Tinosorb S Aqua and Uvinul A Plus (BASF).

Some actives or functional agent by Merck have been used: Oxynex® ST Liquid, RonaCare®AP, RonaCare® Ectoin, RonaFlair® Soft Sphere.

The other raw materials used for formulations' set up were: Avicel CL 611F (Azelis Germany GmbH), glycerin 85% (Merck KGaA), Titriplex III and Tris(hydroxymethyl)-aminomethan (Merck KGaA), Montanov L and Montanov 82 (Seppic), Tegosoft DC (Evonik), Xiameter® PMX-0245 (Biesterfeld), Euxyl PE 9010 (Schülke & Mayr GmbH).

METHODS

Agitation and homogenization phases

Heating magnetic stirrers, which allow stirring speeds up to 1200 rpm and a maximum heating temperature of 310° C have been used for small quantities (eg. Oil phase of emulsions).

Three RAYNERI Turbotest type 33 / 300P, Type V 2004, type 033, were used for simple stirring or emulsification step. These mixers can achieve a stirring speed of 3300 rpm. They are equipped with two sizes of turbines with radial flow, 35 mm and 80 mm in diameter.

For formulas containing inorganic filters, stronger agitation is often required to ensure a better distribution of powders. In this case, the disperser homogenizer Ultra-Turrax T25 Basic Janke & Kunkel type was used. It allows good distribute loads in the formula, as well as a decrease of the droplet size of the internal phase, allowing to gain stability.

Characterization of formulations

For the characterization of each formulation a check of pH, viscosity and organoleptic properties was performed.

For pH and viscosity's measures, these characterization devices were used:

- The portable pHmeter SevenGo SG2 type, Mettler Toledo
- Viscometer FUNGILAB EXPERT 301107

For the organoleptic evaluation, the observed properties were: color, smell and general appearance.

Stability study

In order to evaluate the stability of emulsions, 250g of the formulated product were divided into four parts : 2 for 100g glass containers, for monitoring room temperature and 45° C (in a conventional oven, Prolabo), and 2 for 15g small glass container for fridge and light.

At room temperature, there is a normal aging of the formula, while at 45 ° C an accelerated aging occurs. At 4 ° C, recrystallization phenomena is observed, while in the light, there could be a possible discoloration.

The four samples were monitored for 3 months; during this time the D + 1, D + 7, D + 14, D + 1 month, 2 months and D + 3 months have been evaluated, in terms of pH, viscosity and organoleptic characteristics.

If, after 3 months, the samples aren't subjected to any change, the formula is considered stable.

RESULTS AND DISCUSSION

The choice of raw materials

For the EU Cosmetic Regulation 1223/2009 Titanium Dioxide cannot be used in sprays or in powders. The aim of this first part was obtaining a spray product (O/W emulsion) with high SPF, with only organic and encapsulated filters complying with Regulation and with a good sensory profile.

For this purpose, a first formula of a spray was set up. It's shown in table 1.

Table 1. First formula for the high protection spray concept: MSAS.815.01.04.15

	Commercial name	INCI	%
A	Water, demineralized	AQUA	32.20
	Keltrol CG-SFT	XANTHAN GUM	0.10
	Glycerin 85%	GLYCERIN, AQUA	3.00
	Simulgel INS 100	HYDROXYETHYL ACRYLATE, SODIUM ACRYLOYLDIMETHYL TAURATE COPOLYMER	0.50
	RonaCare® Ectoin	ECTOIN	0.50
	Titriplex® III	DISODIUM EDTA	0.10
	Eusolex® UV-Pearls® OB-S	AQUA, OCTOCRYLENE, SORBITOL, BUTYL METHOXYDIBENZOYLMETHANE, SILICA, PVP, CHLORPHENESIN, PHENOXYETHANOL, DISODIUM EDTA	30.00
	Tris(hydroxymethyl)-aminomethan	TROMETHAMINE	1.10
A1	Eusolex® 232	PHENYLBENZIMIDAZOLE SULFONIC ACID	4.00
B	Montanov 202	ARACHIDYL ALCOHOL, BEHENYL ALCOHOL, ARACHIDYL GLUCOSIDE	1.00
	Montanov 82	CETEARYL ALCOHOL, COCO-GLUCOSIDE	1.00
	Tegosoft TN	C12-15 ALKYL BENZOATE	8.00
	Eusolex® OCR	OCTOCRYLENE	1.00
	Eusolex® 9020	BUTYL METHOXYDIBENZOYLMETHANE	2.00
	Eusolex® HMS	HOMOSALATE	5.00
	Eusolex® OS	ETHYLHEXYL SALICYLATE	5.00
	RonaCare® AP	BIS-ETHYLHEXYL HYDROXYDIMETHOXY BENZYLMALONATE	1.00
	Oxynex® ST Liquid	DIETHYLHEXYL SYRINGYLIDENE MALONATE, CAPRYLIC/CAPRIC TRIGLYCERIDE	0.50
	Antaron V-220 F	PVP/EICOSENE COPOLYMER	1.00
C	Xiameter® PMX-0245	CYCLOPENTASILOXANE	2.00
D	Euxyl PE 9010	PHENOXYETHANOL, ETHYLHEXYL GLYCERIN	1.00

*Expected SPF: **30** (calculated by sunscreen simulator BASF: 43.9)

The three core components categories required in sunscreens are UV filters, emollients, and emulsifiers; secondary components are photostabilizers, film formers, boosters, and sensory enhancers. [15]

The choice to use a sprayable O/W emulsion derives from the fact that generally O/W systems are preferred for their easier spreading and lighter skin feel.

The external water phase may also provide a fresh and pleasing sensation evaporating during application. Emulsifiers combined with fatty alcohol enable the building of lamellar liquid-crystalline structures that positively impact formulation stability, skin hydration and skin compatibility. Form this consideration derives the choice of the formulation's emulsifiers; in fact, both Montanov 82 and 202 are liquid crystal promoters.

The core active ingredients required to achieve UV protection are inevitably UV filters. A good combination of the UV filters enables the achievement of a high UV protection performance while maintaining pleasant formulation aesthetics. To obtain high SPFs it's necessary a high concentration of UV filters, mainly loaded in the oil phase. So it's a challenge to stabilize the formulations that have such a high oil phase content while simultaneously maintaining the pleasant aesthetics of the product.

To overcome this issue, encapsulated filters, available in aqueous dispersion containing approximately 37% (w/w) of the UV absorber, have been used.

Eusolex® UV-Pearls™ (INCI: Aqua, Octocrylene, Sorbitol, Butyl Methoxydibenzoylmethane, Silica, PVP) - UV filters entrapped in micro-capsules – represent a micro-encapsulation technology that entraps organic sunscreen chemicals in sol-gel silica glass. With the sol-gel process, mineral and amorphous coating materials offer tightness and resistance to extraction forces than polymers or waxes.

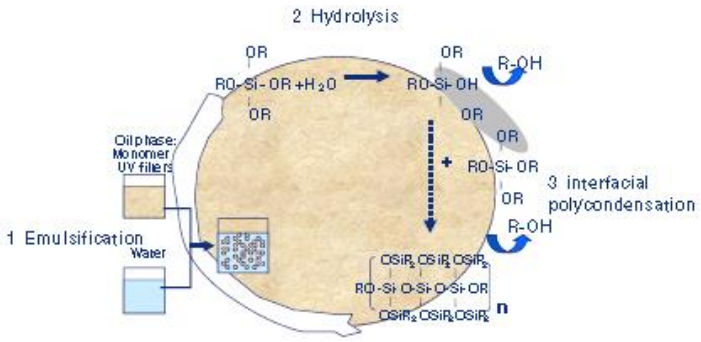


Figure1. Schematic representation of the polycondensation reaction to form the threedimensional shell of Eusolex® UV-Pearls® consisting of SiO2 tetrahedrons.

This type of encapsulation of organic UV filters produces aqueous dispersions; this allows oil-soluble organic sunscreens to be incorporated into the aqueous phase and incompatibilities between cosmetic ingredients can be prevented.

Furthermore Eusolex® UV-Pearls™ make photo-stable UV filters combinations possible. In fact, this product contains about 32 % Octocrylene (OCR) and 10 % Butyl methoxydibenzoylmethane (BMDBM or Avobenzone) in dispersion, with an impact on SPF value of approximatively 1 SPF unit/3% in emulsion.

As the UV filters are encapsulated in glass micro-particles, a protective and homogeneous UV absorbing layer is located on the top of skin's surface and the glass walls prevent interactions between the UV filters and the skin. At the end sol-gel glass-encapsulated UV filters present a low allergy potential because of the inert capsule.

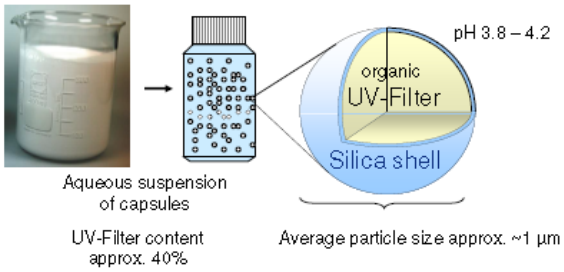


Figure2. Encapsulated filters: Eusolex® UV-Pearls™

Furthermore, the ivory liquids contain Eusolex® UV-Pearls™ of about 1.0 µm diameter on average; this particle are sufficiently small to be transparent when applied to the skin and to give a pleasant skin feeling.

They present also a booster effect on the conventional organic filters: in fact, they increase the protective capacity of the formula with the combination of filters in the two phases (water and oil) in the case of emulsions w/o or o/w. [16]

To increase the presence of UV filters in water phase of emulsion, another water soluble filter was added, Eusolex® 232 (INCI: Phenylbenzimidazole Sulfonic Acid). This UVB filter needs a minimum pH 6.8-7.0 in formulation to avoid recrystallization and it's the reason why the formulation presents a neutral pH.

The incorporation of these UV filters in the aqueous phase prevents overloading of the oil phase and consequently offers more flexibility for sensorial adjustment. Additionally, the presence of UV filters in the two phases of an emulsion leads to an

improved efficacy related to the more homogeneous film left on the skin after application and water evaporation.[17-18]

In oil phase, the used filters were Avobenzone (Eusolex® 9020) and Octocrylene (Eusolex® OCR), because they are two filters broadly used in this field and because they can be compared with the encapsulated ones in terms of SPF value and impact on formulation. The other organic used filters, Homosalate (Eusolex® HMS) and Ethylhexyl salicylate (Eusolex® OS) possess low efficacy but they help to solubilize Avobenzone and they have a boosting effect for the other filters. [19]

Besides UV filters, emollients are key components in sunscreens. They play a triple role, which includes solubilizing, photostabilizing, and sensorial enhancing properties. Firstly, emollients enable solubilization of crystalline UV filters, a prerequisite for their functionality as UV absorbers, and homogeneous distribution of the UV filters in the formulation itself. [20] Examples of effective solubilizers of UV filters are the well-used benzoate esters, that have been used in the starting formulation for this reason. Furthermore emollients also strongly impact the skin feeling in terms of ease of spreading, greasiness, stickiness, etc. For this consideration, emollients such as dibutyl adipate, dicaprylyl carbonate, coco caprylate, propylheptyl caprylate, are particularly suitable for sun care formulations, because they show a good solubilizing power of crystalline UV filters and at the same time provide a light non greasy skin feel.[21] This is the reason why, in the evolution of formulation, the benzoate esters were substituted with other emollients, like decyl cocoate.

Moreover, since synthetic polymers generally show poor compatibility with inorganic UV filters, we decided to use as thickening agent natural raw materials, like xanthan gum and microcrystalline cellulose. Additionally, the introduction of natural gums, such as xanthan gum, mostly has a stabilizing rather than thickening effect.

Additional help in increasing the performance of a sunscreen is provided either by boosting the efficacy of the UV absorber system or by improving the film-forming property on skin.

For this reason in formula some other functional agent were added: RonaCare® AP (INCI: Bis-EthylhexylHydroxydimethoxyBenzylmalonate), a multi-functional antioxidant that protects skin lipids and helps prevent impure skin, protects the skin under IR irradiation and stabilizes UV filters, because it’s a good sulubilizer;

Oxynex®ST Liquid (INCI: Diethylhexyl Syringylidenemalonate (and) Caprylic/Capric Triglyceride), that is able to protect colorants and fragrances from degradation and stabilizes photo-un-stable ingredients, e.g. Tocopherols, and light-sensitive UV filters. Furthermore the antioxidant potential of Oxynex® ST Liquid reduces the risk of UV-induced free-radical damage to the skin. [22-23]

Film formers, in contrast, boost UV performance by improving sunscreen film-forming properties during application, which thus results in a more uniform distribution of the UV actives on skin or by increasing water resistance properties. For this aim, a PVP/eicosene copolymer was added in formulation.

Moreover, sensory enhancers may be added to create an aesthetically appealing formulation. Sensory enhancers mainly are powders or silicones fluids/silicone powders. Depending on their particle size and shape, they could adsorb oil, ease spreadability, provide matte and powdered finish, reduce tackiness, impart velvety touch or provide a soft-focus effect. [24] In this optic, in this formulation a powder filler was added, RonaFlair™ Soft Sphere. This powder is based on synthetic mica platelets (10–40 µm) combined with submicron- sized (250 nm) monodispersed silica spheres. The silica beads act as ball bearings and allow the platelets to slide across each other when applied to the skin. This effect also renders improved application properties to the finished cosmetic products. Its platelet-shaped base structure provides high transparency and subtle luster. [25]

Liquid sensory modifiers are silicones; for example, volatile cyclic or short linear chain silicones (cyclopentasiloxane, ethyltrisiloxane, etc.) are able to enhance spreadability of the formulation.[26] To this aim, in this formulation cyclopentasiloxane was added.

Finally a protective and hydrating active was added, RonaCare® Ectoin (INCI: Ectoin); it offers cellular protection and it can also be used as additional UV protection since it acts protecting cells from harmful effects of UV radiations and controlling the damage induced by these ones. Furthermore, it plays a role in maintaining skin hydration with long-term effects.[27]

Formulation's evolution

The first trial of this formulation resulted too viscous to be a spray; furthermore, after two weeks the formula showed not to be stable at 45°C. For this reason, some

changes have been done in order to obtain a stable emulsion, with an appropriate viscosity and a more pleasant skin feel.

In the table 2, the different changes in the formula are shown.

Table 2. Modifications of formula for the high protection spray concept

N°	Modifications	Conclusion
MSAS.815.02	No Simulgel INS100 because the formula 01 was too viscous; change of one emulsifier: Montanov L instead of Montanov 202.	Not stable formulation a D+7
MSAS.815.03	Same formula of 01, but with Montanov L instead of Montanov 202; higher percentage of Tromethamine for pH	Not stable formulation a D+7
MSAS.815.04	Same formula of 01, but with Simulgel INS100 at 0.3% ; higher percentage of Tromethamine for pH	Too viscous formulation; not stable a D+14
MSAS.815.05	Same formula of 04, but with 2% RonaFlair SoftSphere ; higher percentage of Tromethamine for pH	Too viscous formulation; sticky touch during application; not stable after one month.
MSAS.815.06	No Simulgel INS100, but Xanthan gum at 0.3%	Too viscous formulation; sticky touch during application. STABLE
MSAS.815.07	Same formula of 06, but change in oil : Cetiol CC instead of Cetiol AB	Too viscous formulation; sticky touch during application. STABLE
MSAS.815.08	Same formula of 06, but 0.5% of Antaron V220F instead of 1%	Too viscous formulation; sticky touch during application. STABLE
MSAS.815.09	Change in gelling agent : Avicel PC611 1% instead of Xanthan gum; reduction of oil to 6%	Too viscous formulation; sticky touch during application. STABLE
MSAS.815.10	Same formula of 09, but with Avicel CL 611F (for lab availability)	Like trial 09; STABLE
MSAS.815.11	Avicel 1.5% instead 1% ; no Antaron V220 F	Too viscous formulation; again sticky touch during application. STABLE
MSAS.815.12	Same formula of trial 10, but without Antaron V220 F	Sticky touch during application; STABLE
MSAS.815.13	Same formula of trial 10, but changing Montanov 202 with Montanov L	Sticky touch during application; STABLE
MSAS.815.14	Same formula of trial 13, but without Antaron V220 F	Again sticky touch during application; STABLE
MSAS.815.15	Same formula of trial 14, but changing Montanov L with Sensanov WR for a better skin feel	Too viscous formulation; again sticky touch during application. STABLE
MSAS.815.16	Same formula of trial 14, but with 1% RonaCare SoftSphere instead of 2%	Good viscosity but again sticky touch during application; STABLE

MSAS.815.17	Same formula of trial 16, but changing oil : Cetiol C5 & Dimethicone instead of Cetiol AB for a better skin feel	Good viscosity but again sticky touch during application; STABLE
MSAS.815.18	Same formula of trial 17, but changing oil : DUB VCI 10 instead of Cetiol C5 for a better skin feel	Good viscosity but again sticky touch during application; STABLE
MSAS.815.19	Same formula of trial 16, but changing oil : Tegosoft DC instead of Cetiol AB for a better skin feel	Good viscosity, touch quite good; STABLE
MSAS.815.20	Same formula of trial 19, but changing silicone : Xiameter PMX-200 silicone fluid 5cs (dimethicone) instead of Cyclopentasiloxane for a better skin feel	More viscous formula, touch like previous trials. STABLE
MSAS.815.21	Same formula of trial 19, but with Eusolex UV-pearls OB-SX instead of OB-S	No differences between this formulation and trial 19

For all formulations, an evaluation of organoleptic properties and a stability study have been performed.

After all considerations, the chosen final formula has been MSAS.815.21.

Formulations from MSAS.815.06 to MSAS.815.18 resulted to be stable after three months (room temperature, 45°C, 4°C and light), but they didn't represent the desired characteristics. In fact, some of them were too viscous to be used as sprayable products: they could find other applications, for example as lotions.

Changes in UV filters' system

The second part of the work on this concept consists on the development of different sprays, starting from the same base, changing the filters' system, in order to evaluate the contribution of different types of filters and possibly their boosting effect on the SPF value.

The formula used as a starting point for the creation of this concept is MSAS.815.21.

On the chosen formula some trials in order to investigate the contribution of Eusolex® UV-Pearls and of other kinds of filters to the texture and to SPF value have been done.

Specifically:

- formula with only UV-Pearls (free organic filters replaced by oil): MSAS.815.22;
- percentage of UV-Pearls replaced by only water: MSAS.815.23;

- percentage of filters encapsulated replaced by free filters and water: MSAS.815.24;
- UV-Pearls + titanium dioxide, expecting to have about the same SPF value as with free organic filters: MSAS.815.25;
- Only titanium dioxide (without UV-Pearls, replaced by water): MSAS.815.26;
- Formulations with the half percentage of Eusolex® UV-Pearls: MSAS.815.27-28-29-30;
- Formulations with the addition of other organic filters to obtain a very high SPF: MSAS.815.32-33-34.

In Table 3, the different used filters and their percentage are shown.

Table 3. UV filters' system for the high protection spray with percentage

	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**32**	**33**	**34**
UV-P. OB-SX	30	30			30		15	15		15	30	30	30
TiO_2 (Eus. T-S)					7	7				7			
organic blend	13		13	25			13		19		13	13	13
other											1.2*	3*	1.2* + 3

*percentage of active substance

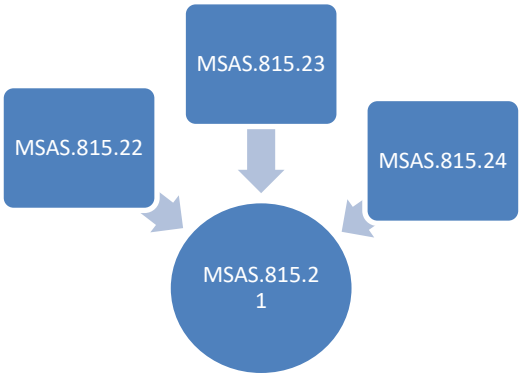
The dark columns indicate that these formulations resulted to be not stable or that emulsion resulted to be not possible during process.

As formulations 26 and 30 were not stable (it's probably due to the high difference in viscosity between the two phases of emulsion, oil phase was much more viscous of aqueous phase) the same formulation than 26 was tried, but with 0.5% of Xanthan gum in order to have a more viscous aqueous phase for investigating if the problem of emulsion really was a physical problem and not a chemical one (MSAS.815.31).

Aim of trials

These trials have been done in order to make comparisons between the different formulations in terms of impact on SPF value and sensorial profile. Particularly:

- Cluster 1 (**Table4**)

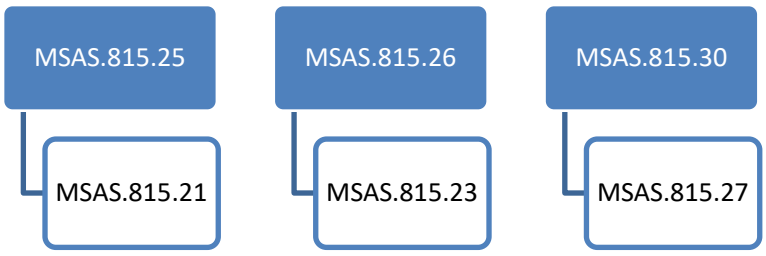


	21	22	23	24
UV-P. OB-SX	30	30		
TiO_2 (Eus. T-S)				
organic blend	13		13	25

For the first cluster of formulations it's possible to compare the starting formulation (MSAS.815.21) with formulation MSAS.815.22 for the evaluation of organic filters' impact, with MSAS.815.23 for the evaluation of UV-Pearls' impact in organic formulation and with MSAS.815.24 for the evaluation of filters' kind impact (encapsulated or not filters).

Sensorial profile: from a sensorial point of view it's clear that the different types of filters produce a different effect in the formulation. The starting formulation presents good spreadability, a light and not sticky touch.
Comparing this one with MSAS.815.22 it's possible to say that the second one presents good spreadability, but a more dry touch and less oily than the other: the organic filters produce a more oily and sticky touch, while Eusolex® UV-Pearls not influence a lot the oily and sticky final effect.
MSAS.815.23 presents instead a final touch that is a little bit less sticky than MSAS.815.21 Formulation MSAS.815.24 is more rich and oily in comparison to the last one, compatibly with a major percentage of free organic filters in oil phase.

- Cluster 2 (**Table5**)



	21	23	27	25	26	30
UV-P. OB-SX	30		15	30		15
TiO_2 (Eus. T-S)				7	7	7
organic blend	13	13	13			

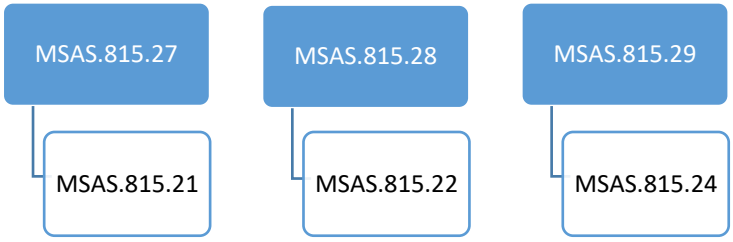
For the second cluster of formulations it's possible to compare the formulation MSAS.815.25 with formulation MSAS.815.21, formulation MSAS.815.26 with MSAS.815.23 and formulation MSAS.815.30 with MSAS.815.27 in order to evaluate the differences on the same formulation with organic or inorganic filters coupled with Eusolex® UV-Pearls and Eusolex 232.

As inorganic filter, Eusolex® T-S (INCI: Titanium dioxide, Alumina, Stearic acid) was chosen. It is a titanium dioxide in rutile form which has the appearance of a slightly yellowish white powder. It is very stable (to temperature, oxygen) thanks to its alumina mineral coating. In formulation, it is highly transparent and exhibits excellent lipodispersibility (conferred by the stearic acid's layer) and there are no pH restrictions on its use.

It has been impossible to prepare formulation MSAS.815.26 because, during the process, the emulsion phase resulted to be impossible, while formulation MSAS.815.30 resulted to be not stable after two weeks.

Sensorial profile: unfortunately, for this second group of formulation there is only one stable product, MSAS.815.25. It's possible to say that this formulation, comparing to MSAS.815.21, is more viscous with a darker yellow color; the spreadability is good but the final skin feel is different from others, it presents a powdery sensation after application, due to the presence of titanium dioxide.

- Cluster 3 (**Table6**)



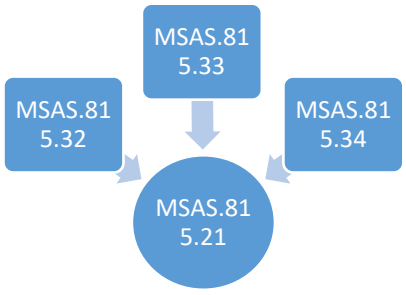
	21	22	24	27	28	29
UV-P. OB-SX	30	30		15	15	
TiO_2 (Eus. T-S)						
organic blend	13		25	13		19

For the third cluster of formulations it's possible to compare the formulation MSAS.815.27 with formulation MSAS.815.21, formulation MSAS.815.28 with MSAS.815.22 and formulation MSAS.815.29 with MSAS.815.24 in order to evaluate the impact of the percentage of Eusolex UV-Pearls on SPF value and on final skin feel.

Sensorial profile: Formulation MSAS.815.27 and formulation MSAS.815.21 present the same behavior from a sensorial point of view, they have the same skin feel; this can suggest that the percentage of encapsulated filters don't have a big influence on the final skin feel. The same consideration can be done for formulation MSAS.815.28 comparing to MSAS.815.22.

Instead, regarding formulation MSAS.815.29 it can be said that this one is less sticky after application comparing to formulation MSAS.815.24, since the percentage of organic filters in oil phase has a bigger impact on formulation's skin feel.

- Cluster 4 (**Table7**)



	21	32	33	34
UV-P. OB-SX	30	30	30	30
TiO_2 (Eus. T-S)				
organic blend	13	13	13	13
other		1.2*	3*	1.2* + 3

For the fourth cluster, these formulations have been prepared in order to obtain a higher SPF value (50/50+). For this goal also UV filters from another supplier (BASF) were used.

*Other:

- Formulation MSAS.815.32: 6% (1.2% active) of Tinosorb S Aqua (BASF); expected SPF: 50
- Formulation MSAS.815.33: 15% (3% active) of Tinosorb S Aqua (BASF); expected SPF: 50+
- Formulation MSAS.815.34: 6% (1.2% active) of Tinosorb S Aqua (BASF) + 3% Uvinul A Plus (BASF); expected SPF: 50+

Evaluation of SPF value

Formulations from MSAS.815.21 to MSAS.815.34 resulted to be stable after three months, so they were evaluated in terms of SPF. However, for company's internal reasons, resulted cannot be reported in this thesis.

CONCLUSIONS

This study, performed during the Erasmus Traineeship project, expected to develop sunscreen formulations responding to market trends (dosage, SPF) and respecting the laws in force. In particular the focal point was to highlight differences in the use of free or encapsulated filters (Avobenzone and Octocrylene) studying their impact in formulations in combination with other organic and inorganic filters in cosmetic form of O/W emulsion spray.

After a series of changes of percentages and ingredients that caused instability phenomena or unpleasant skin feel, a stable formula in form of O/W emulsion spray was produced, containing encapsulated Avobenzone and Octocrylene associated with other organic UV filters in order to obtain high SPF.

On the chosen formula some trials in order to investigate the contribution of encapsulated filters and of other organic filters to the texture and to SPF value have been done. These changes allowed to highlight some instability phenomena due to combination with an inorganic filter (TiO_2).

Finally this set up of different formulations pointed out the capability of encapsulated filters to improve the final skin feel and the texture of emulsions, providing a booster effect on SPF value (even if, for internal reasons of the company, the measured SPF values could not be reported).

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Chapter VI.

COMPARATIVE STABILITY'S STUDY OF SUNSCREENS WITH FREE AND ENCAPSULATED UV FILTERS CONTAINED IN PLASTIC PACKAGING

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"Stability study of sunscreens with free and encapsulated UV filters"

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COMPARATIVE STABILITY'S STUDY OF SUNSCREENS WITH FREE AND ENCAPSULATED UV FILTERS CONTAINED IN PLASTIC PACKAGING

ABSTRACT

Today sunscreens play a fundamental role in skin cancer prevention and in photo-ageing protection. However, some UV filters are photounstable especially in relation with the matrix they are insert in. Furthermore most filters, being lipophilic substances, can interact with plastic packaging leading to a loss of their activity; finally UV filter stability can be strongly affected by the routine use of the product at high temperatures.

This work aims to study the stability of sunscreen formulations, before and after different treatments (simulated solar irradiation and thermal shock cycles), in a mixture of high and low density polyethylene (HDPE/LDPE) packaging.

Butyl Methoxydibenzoylmethane (Avobenzone) and Octocrylene, both in a free form and as encapsulated filters (micro-encapsulation in sol-gel silica glass, commercially available) were chosen as UV filters, in order to investigate the capability of encapsulation to improve formulation's stability and performances, but also to limit interactions with packaging.

Butyl Methoxydibenzoylmethane (Avobenzone) and Octocrylene, both in a free form and as encapsulated filters (micro-encapsulation in sol-gel silica glass, commercially available) were chosen as UV filters.

Stability evaluation of packaging were performed by tensile test, colorimetric assessment and extractables' testing. Formulations were characterized in terms of pH, organoleptic properties, rheological behavior; the amount of filters was monitored using a UV-Vis spectrophotometric method. Moreover, other two non-destructive techniques, Near Infrared Spectroscopy (NIR) and multiple light scattering technique, . were also used in formulation stability evaluation.

Results highlighted that packaging absorbed some ingredients and its external color changed after solar irradiation. Analyses performed on formulations allowed to conclude that, in the chosen packaging, emulsion with encapsulated filters is more stable than the one with free filters.

Keywords: Sunscreens, stability, packaging, NIR, multiple light scattering technique

INTRODUCTION

The growing number of skin cancers diagnosed annually caused mainly by the negative effects of solar exposure, especially to ultraviolet (UV) radiations, are now well recognized. [1] UVB (280-320 nm) typically induces erythema and direct DNA damage via pyrimidine dimer formation, whereas UVA (320-400 nm) is associated with tanning, photoaging and the generation of excess reactive oxygen species which indirectly damage DNA. [2-4] For this reason in order to reduce skin photo-damage and the carcinogenic effects of solar irradiation, the use of sunscreens containing UV filters as an integral part of photoprotection strategy has been extended worldwide. [5]

An ideal sunscreen provides uniform protection against the UVB and UVA wavelength range maintaining sensorial features that enhance the user's experience. Sunscreen efficacy depends on ultraviolet filter type (organic or inorganic), its photostability, the excipients used, the addition of some boosting agents into formulations and the right choice of packaging.

In order to absorb ultraviolet radiation (UVR), an organic ultraviolet (UV) light filter must contain a

suitable chromophore presenting conjugated π-electron systems. [6] The absorption of a UV photon leads the UV absorber molecule to an excited electronic state. If the absorbed energy is not sufficiently and speedily dissipated into heat, the chemical bonds of the molecule may break, resulting in degradation of the UV filter and, sometimes, in a reduction of the final product safety . [7]

Most of the UV absorbers used in sunscreens are photostable in the foreseeable conditions of use. Two exceptions are Butyl methoxydibenzoylmethane (Avobenzone) and Octinoxate.[8]. In particular Avobenzone undergoes rapid photodegradation when used alone; for this reason it is often stabilized by addition of the UV filter Octocrylene. [6]

In literature a lot of works studying the photostability of UV filters report analyses made in diluted solutions; however this could be not relevant because in a complex matrix like water in oil or oil in water emulsions, the photochemistry of filters is very different with respect to a diluted solution. The behavior of sunscreens is not predictable from the photostability of its individual filters, but it's important to evaluate the final formulation. [9-10] In fact, sunscreen vehicles can often determine product efficacy. [11] So, the sunscreen formulation stability is the crucial point for maintaining the product Sun Protection Factor (SPF).

Furthermore, other fact to take into account is the sunscreen nature; in fact they are lipophilic substances and very good solvents, also for packaging material. So the choice of packaging is fundamental for the good quality of the product. For example, the use of low density polyethylene should be avoided since the sunscreen will possibly collapse the package.

Furthermore, polyolefins are lipophilic materials, so they are able to retain large amounts of compounds with the same nature, like suncreens. [12, 13]

Moreover it is also necessary to take into account that often this kind of products are stored in a very warm environment, so the entire system (formulation + package) must remain stable in these conditions.[14]

In view of these considerations, this work aims to study the stability of sunscreen formulations, after two different stress treatments (simulated solar irradiation and thermal shock cycles), simulating the possible stress conditions that solar products could meet during their shelf life, in relation to a plastic packaging commonly used.

In particular, the chosen UV filters were Butyl Methoxydibenzoylmethane (Avobenzone) and Octocrylene. Avobenzone (4-tert-butyl-4′-methoxydibenzoylmethane) is one of the most common UVA-filters in sunscreens.[15] The photochemical behaviour of this filter has been extensively studied and it has been found that its photostability is highly dependent on the polarity and proticity of the solvent. [16-17] Octocrylene is an ester formed by the condensation of diphenylcyanoacrylic acid with 2-ethylhexanol, and is considered to belong to the family of cinnamates. The action spectrum of octocrylene (290–360 nm, peak absorption at 303 nm) covers mostly UVB wavelengths, but also short UVA wavelengths (UVAII). [18]

In this work these filters were used both as free filters and as encapsulated ones.

The micro-encapsulation technology entrapping sunscreens in sol-gel silica glass led to a commercially available aqueous dispersions containing about 32% Octocrylene (OCR) and 10% Avobenzone. [19]

This encapsulation of organic UV filters should permit to prevent both interactions between the substances and the skin and those between filters and external environment (e.g. with package); furthermore, the aqueous dispersion allows to incorporate the oil-soluble organic sunscreens into the aqueous phase.

The characterization and stability of formulations made of these two kind of filters, highlighting possible differences also related to packaging material, were then evaluated in this work.

In order to evaluate system stability, analyses were carried out on packaging and on contained formulations. Containers were analyzed by tensile test, colorimetric assessment, and extractable testing. Sunscreen formulations were characterized in terms of pH, organoleptic properties, rheological behavior and filter content. Moreover, other two non-destructive techniques as near infrared spectroscopy (NIR) and multiple light scattering technique were used in order to highlight the stability of formulations.

MATERIALS

Sucrathix VX by Alfa Chemicals (Berkshire, UK), Glycerin and Disodium EDTA by CARLO ERBA reagents (Cornaredo, Italy), Tegosoft TN by Evonik Industries (Essen, Germany), Montanov L and Montanov 82 by Seppic (Puteaux, France), Eusolex UV Pearls OB S X (Octocrylene and Butyl Methoxydibenzoylmethane encapsulated in silica shell in a white viscous dispersion containing PVP and sorbitol), Eusolex OCR (Octocrylene) and Eusolex 902 (Butyl Methoxydibenzoylmethane) by Merck KGaA (Darmstadt, Germany), Verstatil PC by Dr.Straetmans GmbH (Hamburg, Germany).

Ethanol (96% v/v) and other chemical reagent were obtained by CARLO ERBA reagents (Cornaredo, Italy).

As packaging materials, some commercially available plastic containers were gifted by an Italian supplier . In particular, bottles of 125 ml capacity made by a mixture HDPE/LDPE were used.

METHODS

Formulations' preparation

For this study, three formulations were set up: the first (F1) represents the placebo (without UV filters), the second is the formulation containing the UV filters

Octocrylene and Butyl Methoxydibenzoylmethane (F2) in a free form, the third one contains encapsulated Octocrylene and Butyl Methoxydibenzoylmethane (F3).
The quali-quantitative compositions of these formulations are reported in Table 1.

Table 1. Quali-quantitative compositions of formulations

	Ingredient	INCI	% in F1	% in F2	% in F3
A	Water	Aqua	83.9	71.9	53.9
	Sucrathix VX	Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum	1	1	1
	Glycerin	Glycerin	2	2	2
	Eusolex UV Pearls OB SX	Aqua, Octocrylene, Sorbitol, Butyl Methoxydibenzoylmethane, Silica, PVP	*	*	30
	Disodium EDTA	Disodium EDTA	0.1	0.1	0.1
B	Montanov L	C14-22 Alcohols (and) C12-20 Alkyl Glucoside	1	1	1
	Montanov 82	Cetearyl Alcohol (and) Coco-Glucoside	4	4	4
	Eusolex 9020	Butyl Methoxydibenzoylmethane	*	9	*
	Eusolex OCR	Octocrylene	*	3	*
	Tegosoft TN	C12-15 Alkyl Benzoate	7	7	7
C	Verstatil PC	Phenoxytehanol, Caprylyl Glycol	1	1	1

Each formulation was prepared in triplicate by emulsification, slowly adding phase B in phase A, using a Silverson SL2T High Shear Laboratory Stirrer Mixer (Silverson Machines Ltd, Chesam, UK) at 7000 rpm for 10 minutes, at 75°C. The O/W emulsion was then cooled and phase C was added when the temperature was below 40°C. Then formulations were divided into LDPE/HDPE bottles. Some samples were maintained in glass as controls.

Stress testing procedures

All formulations maintained in LDPE/HDPE mixture bottles were subjected to two degradation tests, in order to simulate foreseeable stress conditions that final products could meet during their use. In particular:

- Photostability test by simulating solar irradiation using SUNTEST XLS +II (Atlas ®, Chicago, USA) for 96 hours. Suntest instrument was set up in according to standard European procedures [20], with the following parameters:

- Time: 4 hours corresponding to 192 hours solar light
- Irradiation control: 300-800 nm
- Irradiation [W/m^2]: 750
- Room temperature: 35°C
- Black Standard Temperature (BST): 45°C

In order to assure that both parts of each single dose container underwent simulated UV irradiation for the same period and under the same conditions, after the 48 hours containers were turned on the other side.

- Thermal cycles (20°C for 36 hours - 45°C for 12 hours, three times) were performed using an incubator BRE60 BioExpert 56L (Froilabo, Meyzieu, France).

At the end of both treatments the samples were taken, emptied out and the bottles were washed with a standard procedure. Briefly, all bottles were washed for three times with 1% bicarbonate solution and then rinsed three times with distilled water to remove any residuals.

The packaging were then characterized by mechanical, colorimetric analysis and extractable profile, as below reported.

All formulations were then kept in glass at room temperature for 3 months and evaluated over time by organoleptic and rheological characterization, an evaluation of UV filters' content, an evaluation by near infrared spectroscopy (NIR) and a multiple light scattering in order to highlight possible changes due to the contact with packaging and/or the undergone treatments.

Mechanical test

The investigation of the mechanical properties of the containers was performed on each bottle, as reported in a previous work by Perugini et al. [21], using a tensile machine, AGS 500ND (Shimadzu corporation, Kyoto, Japan) equipped with a 500[N] load cell; using a strain rate of 10 mm/min.

Briefly, "bone-shape" specimens were obtained from the central part of each container horizontally; the feature of the specimens followed the principles of the European Standard EN ISO 527 [22], suitably modified for bottle, following a previous work by this research group. Specifically, this optimized dog bone shape obtained by punchcutting provides a localized stress region (3 mm width). Each

specimen was characterized for the measures of thickness and width of this region using a digital microscope model BW 1008. The section of each sample was calculated from thickness and width using a suitable software (micromeasure vers.1.2).

Samples were kept under constant temperature (23°C) and humidity (52% R.H.) for a week until tension test started and during all the test time.

This procedure permitted to obtain a stress versus strain curve. From each set of results the tendency of materials to oppose to deformation has been estimated, and the curve profile in elasticity regime, the elongation percentage in elasticity regime and the absolute elongation elasticity have been evaluated.

A critical analysis and comparison of diagrams made on different specimens allows a first assessment of any significant changes in the stress-strain diagram due to interactions between the material they are made of and the conditions or substances which are in contact with. [21]

The data obtained from the mechanical test are processed through statistical analysis (Mann-Whitney test). It was chosen confidence range of 95%, so the changes were considered statistically significant for $p<0.05$.

Colorimetric analysis

For the colorimetric evaluation of packaging, the measurement of Yellowness Index (YI), following ASTM E313, was performed. [23]

Yellowness Index describes the color change of a test sample from clear or white to yellow. This test is most commonly used to evaluate color changes in a material caused by real or simulated light exposure.

For this purpose the instrumental assessment of packaging color was performed with a Colorimeter CL400 (Cutometer MPA580, CK electronic GmbH, Cologne, Germany). Technical data of probe are length: 126 mm, illumination: Ø 24 mm, measuring area: Ø 8 mm, weight: 85 g, illuminated area approx. 17 mm Ø; units: xyz, and RGB, L 3 a 3 b; light: 8 white LEDs arranged circularly, range of emitted wavelengths: 440–670 nm; and accuracy: 65%.

The color acquisition was done by specifying the three tristimulus values X, Y, and Z, of a color in according to the CIE system, where X is the tristimulus value of red,

Y is the green value, and Z the blue value. With a mathematical equation it was possible to use these parameters in order to calculate YI value.

Extractables testing

This step aims to evaluate the possible migration of substances from packaging or the absorption of some content's ingredients after the contact between plastic containers and the prepared formulations after stress treatments.

Packaging materials were exposed to extreme solvents and conditions to generate every potential extractable. The extraction's technique selected for this purposed was Head Space Solid phase microextraction (HS-SPME), since HS-SPME extracts generally contained the same extractables as did the Soxhlet extracts, providing a complete insight of all the predominant organic extractables for the analyzed materials. [24]

HS-SPME conditions:

Fiber 100 µm Polydimethylsiloxane (PDMS), Supelco

500 mg sample, Headspace mode

Incubation temperature: 90°C, Extraction time: 60 min

The resulting extracts were chemically characterized by gas chromatography-mass spectrometry (GC/MS). In Table 2 parameters of GC/MS system were reported.

Analyses have been carried out on a Thermo Scientific (Thermo Fisher Scientific, Waltham, Massachusetts, USA) GC/MS system (TraceDSQII mass spectrometer, TraceGCUltra gascromatograph, CTC Analytics COMBIPAL autosampler), Xcalibur MS Software Version 2.2. The mass spectra of detected extractable compounds were compared with the databases for GC/MS NIST Mass Spectral Library (NIST 08) and Wiley Registry of Mass Spectral Data 8th Edition. Although the databases were used, some classes of compounds such as alkanes yielded very similar fingerprint patterns or fragments, and thus it was not always possible to make an indisputable identification of every peak (compound) detected.

Table 2. Operating parameters for GC/MS analysis

Column	Restek capillary column Rtx-5MS 30 m x 0.25 mm ID x 0.25 µm
Oven Program	Start 60°C, hold for 4.5 min; ramp 20°C/min to 280°C, hold for 5 min
Injector	PTV Splitless 250°C Splitless time 4.5 min
Carrier Gas	He, 1 mL/min constant flow
MS Transfer line temperature	270°C
MS Detection details	70 eV (+EI) Ion source 250 °C Mass range 50-650 amu Scan rate 870 amu/sec

Formulations' characterization

All formulations were characterized in terms of pH, organoleptic and rheological properties both after 24 hours from preparation and after stressing tests.

The pH measurement was performed by a pHmeter Jenway 3510 (Jenway, Staffordshire, UK); pH 4 and 7 standard buffers were employed to calibrate the instrument before use.

Rheological properties were evaluated using a Brookfield Viscometer, model RVT (Brookfield AMETEK, Middleboro, Massachusetts, USA). The sample (125 g) was placed in a glass pot and left to equilibrate for 15 minutes before measuring with a RV 5 –spindle at different speed (0.5, 1, 2.5, 5, 10, 20, 50, 100 rpm). The measurements were carried out in controlled conditions of temperature and humidity (23°C- 52% RH). Viscosity's values were expressed in mPa*s.

Assessment of UV filter content

The amount of free and encapsulated UV filters in formulations was monitored by UV analysis at 303 (λ_{max} of Octocrylene) and 361 nm (λ_{max} of Avobenzone) using a spectrophotometer UV-Vis, model AGI-UV 8453 (Agilent, Santa Clara, CA, USA).

The calibration curves of Octocrylene and Butyl Methoxydibenzoylmethane (Avobenzone) were constructed by reading the absorbance of four levels of diluted stock solutions prepared in the concentration range of 5-30 and 2-15 µg/ml for

Octocrylene and Avobenzone, respectively, starting from stock solutions of 2 mg/ml in ethanol. All samples were prepared in triplicate.

For the analysis of filters' content in formulations, stock solutions (500 mg of formulation in 100 ml of ethanol) were prepared and left under agitation on magnetic stirrer overnight to allow the complete extraction of filters from the formulation. The next day they were filtered using PTFE syringe filters (0.45 μm porosity) and different dilutions were prepared in triplicate. Then samples were analyzed at the spectrophotometer.

Near InfraRed (NIR) analysis

The formulations were also analyzed using the NIR spectroscopy, for a further non-destructive characterization in order to reveal possible changes before and after treatments in formulations themselves. In NIR spectroscopy, formulations' samples were scanned in a range of near-infrared region (950-1650 nm) by use of a monochromator. In this region, each constituent of a complex organic mixture has unique absorption properties due to stretching and bending vibrations in molecular bonds. [25]

For this kind of analysis the MicroNIR™ Pro Spectrometer, (JDSU, Milpitas, California, USA) was used. In Table 3 MicroNIR parameters are reported . This technique presents some advantages with respect to other analytical techniques, for example its ability to record spectra of solid and liquid samples without prior manipulation; furthermore it's simple, fast and cost-effective. [26]

For this kind of analysis formulations samples were put on a non-reflective support, with a fixed and constant distance (3 mm) from acquisition's window. Three replicates for each sample were always performed.

Table 3. MicroNIR instruments' parameters

Illumination source	Two integrated vacuum tungsten lamps
Illumination geometry	Flood illumination/0° observer
Input aperture dimensions	2.5 x 3.0 mm
Sample working plane	0 – 15 mm from window, 3 mm optimal distance
Detector	128 pixel InGaAs photodiode array
Pixel Size / Pitch	30 µm x 250 µm / 50 µm
Wavelength range	950 – 1650 nm
Pixel to pixel interval	6.2 nm
Spectral bandwidth (FWHM)	<1.25% of center wavelength (1% typical)
Spectral in-band	LVF blocking >4 OD Average

Data were elaborated using the Unscrambler® X software, version 10.4 (Camo software AS, Oslo, Norway).

Principal components analysis (PCA) on pretreated spectra was performed on the obtained spectra in order to evidence the capability of NIR measurement system to discriminate different samples and possible changes in formulations due to treatments and/or aging. The spectra were pretreated

by using Standard Normal Variate (SNV) followed by first derivative with Savitzy-Golay smoothing.

Multiple Light Scattering analysis

The multiple light scattering technique was used for studying and predicting the stability of formulations by their investigation after three months of storage. This technique is an optical method to characterize concentrated liquid dispersions without dilution. Specifically, for this purpose Turbiscan MA 100 (Formulaction SA, L'Union, France), was used. The main advantage of Turbiscan is the ability to detect particle size change and/or local concentration change, which results in variation of the backscattering (BS) and transmission (T) signals, while the sample is destabilising, in a more accurately and faster way than human eye can do.

The function principle consists to send photons (NIR light source, 880 nm) into the sample. The photons, after being scattered many times by the particles (or droplets) in the dispersion emerge from the sample and are detected by the 2 detectors of

Turbiscan reading head: transmission for not opaque samples (0° from light source) and backscattering for opaque samples (135° from the light source).

Backscattering is directly related to the photon transport mean free path, so backscattering intensity depends on particle size and concentration.

After different storage times the variation of the ΔT and ΔBS is measured as a function of the test tube height and compared to the initial transmission and backscattering profiles. A plot is produced of these results with ΔT or ΔBS on the y-axis and the sample height (h, mm) on the x axis. A sample height of 0 mm corresponds to the bottom of the measurement cell. A backscattering increasing indicates droplet size changes due to aggregation and coalescence as well as sedimentation.

Finally, formulations with UV filters were compared using the Turbiscan Stability Index (TSI), that represents an absolute value related to the general behavior of the formulation; TSI summarizes all the variations detected in the samples in terms of size and/or concentration. The higher is the TSI, higher is the instability rate of the sample. In this work, TSI values at different heights of the sample and at different times were considered.

Formulations were mixed and then transferred in test tube (10 cm height) and stored at 20°C for 3 days. Samples were scanned (number of total scans: 216) for at least 3 days.

RESULTS AND DISCUSSION

Packaging evaluation

Mechanical analysis

For the evaluation of possible changes in packaging due to treatments and/or contact with filling formulations, a tensile test was performed on packaging in order to investigate the mechanical behavior of plastic containers after stress conditions.

For this purpose 20 specimens for each type of containers (empty and not treated, filled respectively with the two formulations and treated with simulated solar irradiation and thermal shock as described above) were obtained. Each specimen was then characterized measuring thickness and width of central section (break point).

Once acquired the measures for all the samples the tensile test was carried out. The following table (Table 4) reports the mean data obtained, with an illustrative graph of the stress/stress profile curve of the packaging at the beginning.

Table 4. Tensile test's mean data obtained for packaging

	Tensile strenght (σM)= Yield stress (σy) (MPa)	Elongation at yield/ section (mm^{-1})	Tensile stress at break (σB) (MPa)	Elongation at break/ section (mm^{-1})
T0 empty	23.954	2.099E-01	39.228	37.079
F1 shock	20.960	2.870E-01	38.418	38.568
F1 sun	21.268	2.827E-01	39.074	39.184
F2 shock	23.415	2.587E-01	41.783	36.387
F2 sun	21.524	2.635E-01	39.490	35.625
F3 shock	24.249	2.918E-01	41.958	35.396
F3 sun	21.941	2.661E-01	38.879	33.853

S.d. < 10%

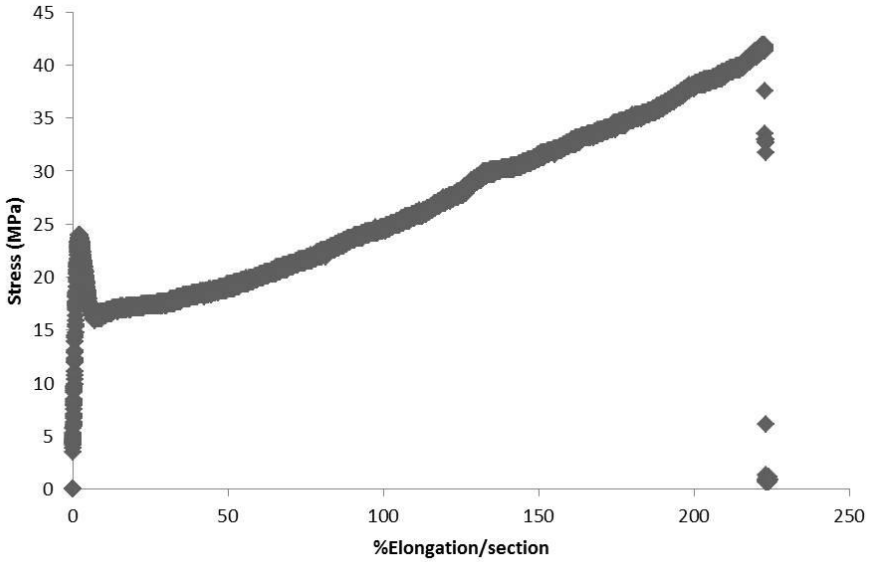


Figure 1. Graph obtained from tensile test for packaging

Empty containers (t0 samples) presented the lowest value of tensile strain at yield respect to treated samples.

Here below the trend of all values obtained from mechanical analysis (Figure 2) and the summary table (Table 5) reporting the significance values (p) of the statistical analysis performed by the Mann-Whitney test with a 95% confidence interval are reported. As observable from the table, significant changes occurred in the yield region of the curve after both treatments, but especially after sun irradiation

simulating test . In this first part of the curve polymer behavior is changing from elastic to plastic one. If the treatments did something to the polymer structure at this point, they acts in particular on the ability of the material to stretch and then return to its initial state. The effect of the treatments on polymer structure is not so evident in the break region maybe because this phenomenon occurs after a much longer time (almost 10 minutes of testing) and in this time the material fibers have time to regroup and act as the untreated material. These results explain essentially as the treatments do something, but the change is not so great as to change their ability to resist breakage.

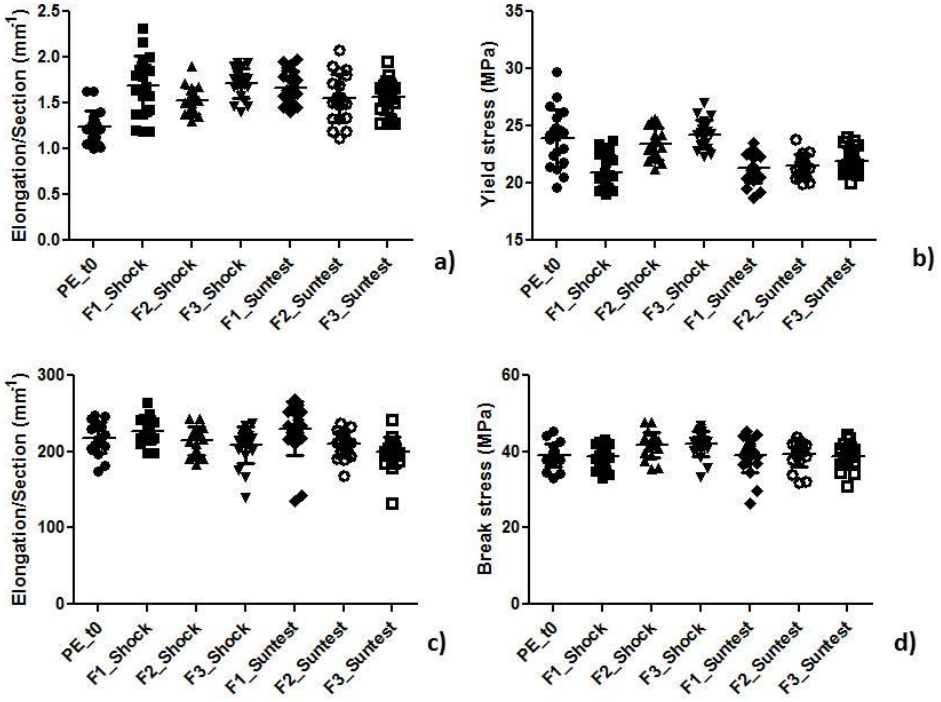


Figure 2. Trend of : a) yield strain; b) yield stress; c) break strain; d) break stress values obtained from all samples

Table 5. Statistical significance p values obtained from packaging analysis

	SUNTEST				SHOCK			
	t0 vs F1	**t0 vs F2**	**t0 vs F3**	**F2 vs F3**	**t0 vs F1**	**t0 vs F2**	**t0 vs F3**	**F2 vs F3**
Yield stress	0.0002***	0.0007***	0.0040**	0.3104	0.0001***	0.4735	0.5609	0.0658
Yield strain	<0.0001***	0.0003***	<0.0001***	0.7972	<0.0001***	<0.0001***	<0.0001***	0.0005***
Break stress	0.4094	0.2287	0.7972	0.2792	0.8604	0.0071**	0.0023**	0.7353
Break strain	0.0193*	0.1264	0.0031**	0.0601	0.1896	0.5075	0.2853	0.4735

Colorimetric analysis

Bottles before and after treatments were also analyzed from a colorimetric point of view, in order to found possible changes in color of external part of containers due to treatments.

For the colorimetric evaluation of packaging, the measurement of Yellowness Index (YI) was performed. For this purpose, the colorimeter was used in order to obtain the three tristimulus values X, Y, and Z, of a color in according to the CIE system; using the equation reported in ASTM 313, the Yellowness Index was calculated.

Table 6 and Figure 3 report the Yellowness Index (YI) values with and the related difference between t0 and treated samples (ΔYI).

Table 6. YI and ΔYI values of not treated and treated packaging samples.

	YI	**ΔYI**
t0	8.966	
empty shock	5.886	-3.080
empty sun	4.400	-4.566
F1 shock	7.442	-1.524
F1 sun	4.709	-4.257
F2 shock	5.402	-3.564
F2 sun	2.712	-6.255
F3 shock	4.120	-4.846
F3 sun	2.666	-6.301

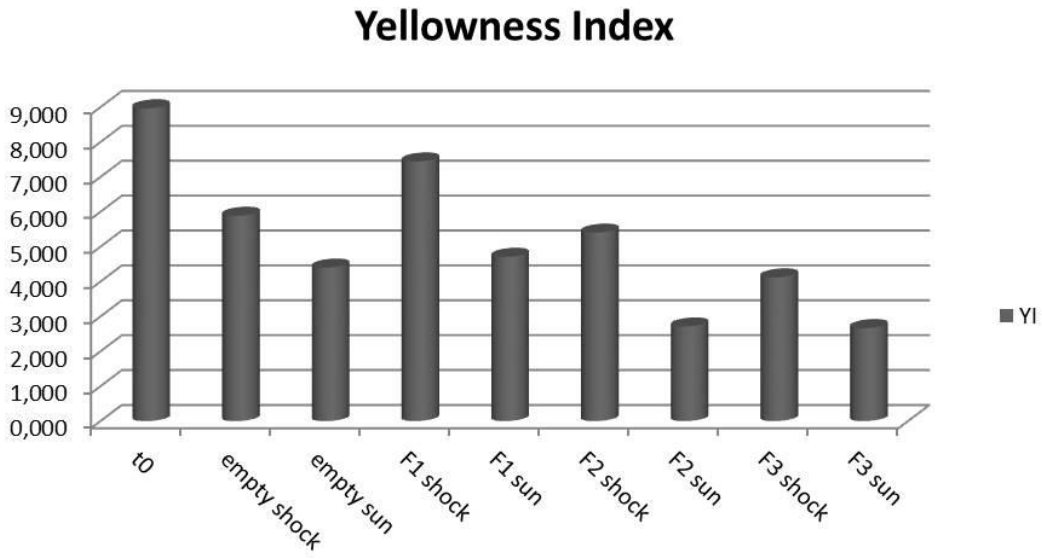


Figure 3. YI and ΔYI values of not treated and treated packaging samples

Observing the Yellowness Index calculated values, it's possible to note that all treated samples reported a decrease in Yellowness Index respect to not treated and empty sample (t0). The YI is a number that indicates the degree of departure of an object color from colorless or from a preferred white toward yellow. By this calculation, positive (+) ΔYI indicates increased yellowness and negative (-) ΔYI indicates decreased yellowness or increased blueness. [20] From this consideration it's possible to conclude that all treated samples presented a shift toward blue, so a decrease in yellowness. In particular, the most influenced samples from treatments were the containers that underwent the solar simulated irradiation, as radiations provoke the biggest degradative effect. However, for both treatments and especially for irradiation, the biggest variations in color were observed for containers filled with formulations containing UV filters respect to placebo and the empty bottles (t0), showing that the presence of filters in content could mainly influence changes in color.

Extractables testing

The organic extractables profile of the packaging material investigated (LDPE/HDPE mixture containers) was established via HS-SPME extraction processes, following a method set up in a previous work on polyethylene, because it revealed to provide a complete insight of all the predominant organic extractables for the analyzed material. [24]

After subtraction of the extraction blank results from the samples results and removal of the interfering peaks associated with bleeding of GC capillary column or SPME fiber coating, a list of compounds released by the analyzed polymer was extracted by GC/MS.

Organic extractables profile of not treated containers (t0) is summarized in Table 7 and the Total Ion Current (TIC) chromatogram related to GC/MS analysis of the obtained extract is shown in Figure 4.

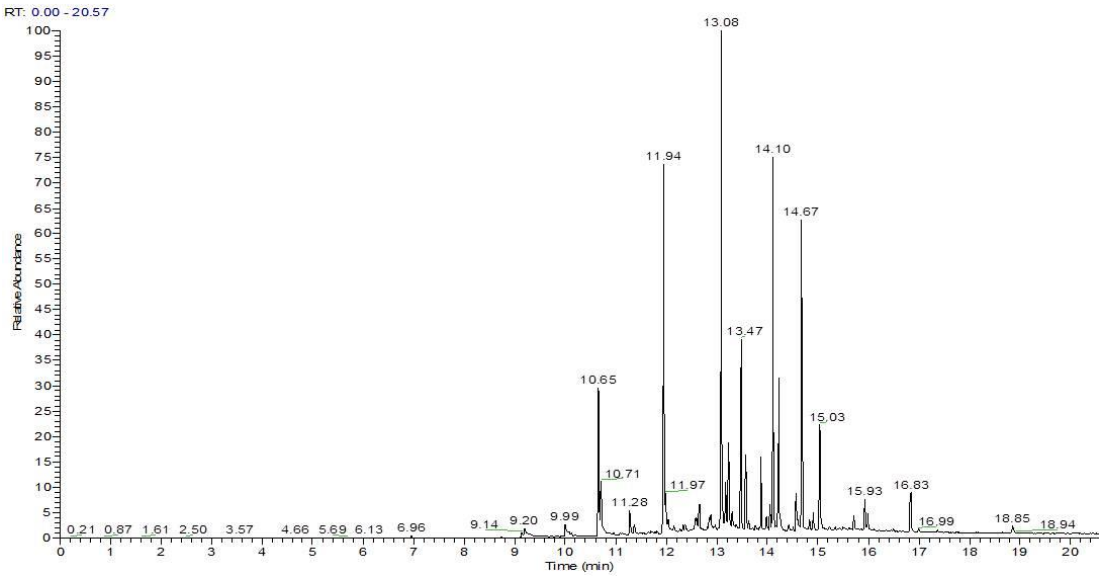


Figure 4. Chromatogram of the HS-SPME extract for HDPE containers.

Table 7. Organic extractables profile of LDPE/HDPE mixture containers

Identification	CAS NR	Chemical formula	Molecular weight	HDPE % area
2,6-di-tert-butyl-benzoquinone	719-22-2	C14H20O2	220	1.59
Diisopropylnaphtalene	-	C16H20	212	1.71
Phosphoric acid tris(2-chloro-1-methylethyl) ester	13674-84-5	C9H18Cl3O4P	326	5.76
Diisobutyl phthalate	84-69-5	C16H22O4	278	2.61
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	82304-66-3	C17H24O3	276	2.24
Octinoxate	5466-77-3	C18H26O3	290	1.00
Diisooctyl phthalate	131-20-4	C24H38O4	390	1.57
Squalene	111-02-4	C30H50	410	0.71
Siloxanes	/	/	/	18.65
Aliphatic hydrocarbons	/	/	/	34.29
Olefins	/	/	/	29.87

These organic extractables generally fall into classes of compounds linked to the major constituents of the original plastic materials. For example, the profiles included compounds like antioxidants and additives (e.g. 2,4-Di-t-butyl phenol, phthalates) associated with the initial ingredients, impurities related to processing (e.g. esters) and degradation products of the polymers (aliphatic hydrocarbons).

Once containers have been characterized at t0 (not treated), the next step was the characterization of the substances that could be extracted from containers filled with the formulations described above after treatment with solar irradiation and thermal shock.

Figure 5 represents graphs reporting the percentages of each class of substances extracted from containers filled with formulations (placebo, F2 and F3) and treated in comparison with sample at t0. All samples were extracted with the selected test method (HS-SPME) and the extracts were analyzed by GC/MS.

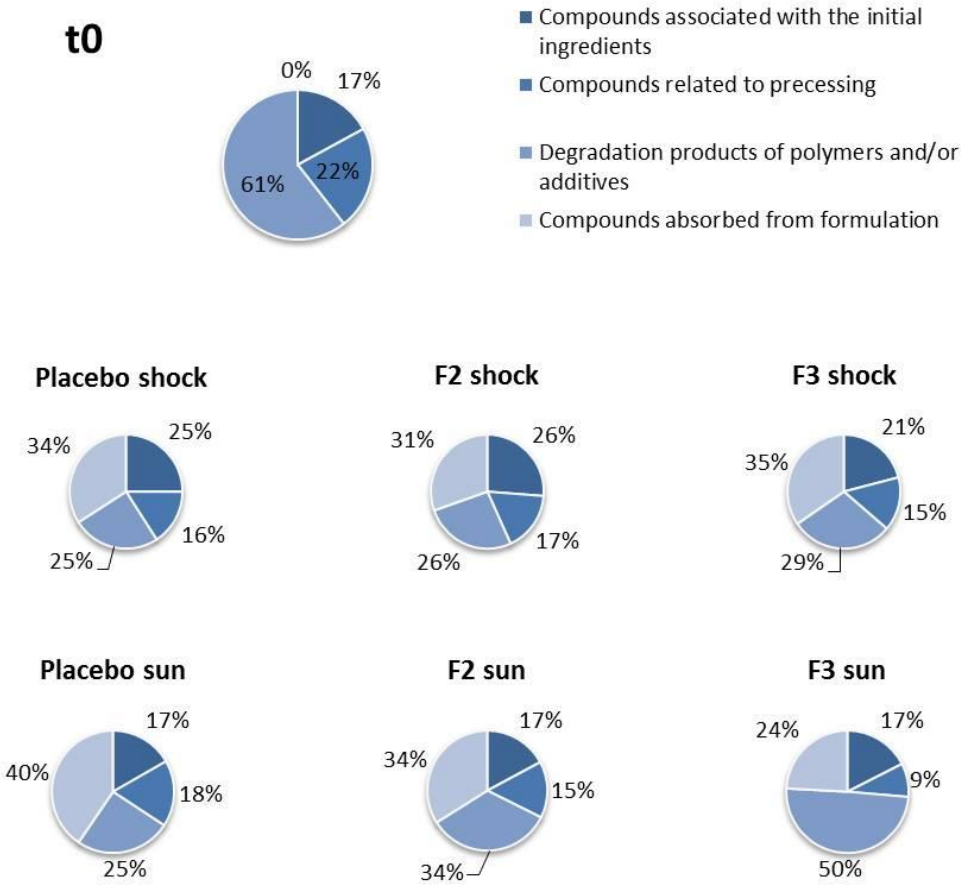


Figure 5. % areas of substances extracted from containers filled with formulations after treatments.

Data indicated that the largest percentage of compounds extracted from containers at t0 is associated to polymers and/or additives degradation products.

As it can be seen for the material in contact with the formulations after UV-vis irradiation and thermal shock cycles, substances closely related to the filling formulation were detected at relatively large levels. These substances were identified as residual of C12-15 Alkyl benzoate, 2-Phenoxyethanol for all three formulations and Octocrylene only for F2 and F3 samples. They represent an interval of percentage included between 24 and 40% of the total extracted compounds.

Samples treated with simulated solar irradiation presented the biggest variations in relative percentages of extracted compounds' class.

In addition to the assessment of the extractable profiles, a first screening of substances eventually migrated as a result of the treatments from the containers and accumulated as leachables into the formulations was performed.

Samples of each formulation (300 mg) in contact with package and undergone to UV-vis irradiation and thermal shock cycles have been analyzed by HS-SPME/GC-MS. No substances related to the polymeric materials were detected within the formulations.

Formulations evaluation

Formulation characterization

24 hours after preparation, 24 hours after the end of treatments and after three months from treatments, all formulations were characterized from an organoleptic and rheological point of view.

Regarding the organoleptic characterization of formulations (color, odor, general aspect) no changes were observable after treatments and after three months aging.

Also regarding pH measurements, no significant changes were revealed for the analyzed samples, as shown in Table 8.

Table 8. pH values of formulations before and after treatments and after two months

	t0	***suntest***	***shock***	***t3 months***	***suntest 3months***	***shock 3 months***
Placebo	5.65	5.66	5.51	5.58	5.55	5.57
F2	5.49	5.70	5.47	5.52	5.48	5.50
F3	4.61	4.65	4.64	4.62	4.62	4.63

F3 formulation showed a lower pH than placebo and F2 because of the presence of encapsulated filters' suspension, that has a pH of 3.8-4.2.

Regarding the evaluation of viscosity, as index of rheological behavior of formulations', Table 9 reports viscosity values expressed in mPa*s corresponding to a shear rate value of 10 rpm as representative of the entire curve obtained.

Table 9. Viscosity values of formulations before and after treatments and after three months

Shear rate: 10 rpm					
Placebo	**η (mPa*s)**	**F2**	**η (mPa*s)**	**F3**	**η (mPa*s)**
t0	5600	**t0**	9200	**t0**	9000
suntest	5800	**suntest**	8800	**suntest**	9200
shock	6800	**shock**	9000	**shock**	11800
t3months	5600	**t3months**	7800	**t3months**	9600
suntest t3months	5000	**suntest t3months**	7600	**suntest t3months**	9000
shock t3months	5600	**shock t3months**	7800	**shock t3months**	12400

As the previous table shows, placebo formulation presents lower viscosity values; this leads to conclude that the presence of UV filters, both free and encapsulated ones, improve the viscosity of formulations. For placebo, no significant changes were found after treatments neither after three months of storage.

Formulation with free UV filters (F2) showed no changes after treatments, but a decrease in viscosity for all samples after a natural aging (three months). Instead, formulation with encapsulated filters (F3) presented an increase in viscosity after thermal shock cycles treatment, that remained the same after three months of storage. This result could be caused by the presence of the rheological agent PVP in the dispersion of encapsulated filters.

Assessment of UV filter content

In order to monitor the content of UV filters in time and after treatments, a spectrophotometric analysis was performed.

The calibration curves for Avobenzone and Octocrylene presented the following equations:

For Avobenzone: $y = 0.0644x + 0.1071$ ($R^2 = 0.9919$)

For Octocrylene: $y = 0.0262x + 0.0484$ ($R^2 = 0.9963$)

Using these equations, the amount of UV filters in formulation was evaluated. The matrix influence was evaluated analyzing the placebo absorbance at 303 and 361 nm (λ_{max} of Octocrylene and Avobenzone); the results demonstrated that no ingredient of formulation absorbs at the same wavelengths of filters.

Subsequently the capability of filter extraction method was investigated by using different dispersions of formulations in solvent (ethanol) in a wide range of concentration of the two filters. The method demonstrated being able to extract almost the entire amount of filters present in formulations, included in a range of 95-105%.

For monitoring the content of UV filters in formulations after stress treatments stock solutions were prepared in triplicate.

Formulations were analyzed after treatments and after three months of stability (treated and untreated), withdrawing formulations' samples from two different part of containers, the upper part and the bottom, in order to reveal possible changes in filters' amount due to separation and/or stratification phenomena.

Table 10 reports the results of spectrophotometric analysis, expressed as a percentage with respect to the content of filters in not treated formulation (t0 = 100%).

Table 10. % of filters in formulations with respect to not treated formulations (t0)

					F2				
	t0	**Sun**	**shock**	**t3m up**	**t3m sun up**	**t3m shock up**	**t3m bottom**	**t3m sun bottom**	**t3m shock bottom**
OCR	100.00	99.64	101.25	113.32	110.59	102.88	102.26	103.14	103.77
AVO	100.00	98.92	99.39	109.57	107.29	101.31	98.50	101.94	102.04
					F3				
	t0	**Sun**	**shock**	**t3m up**	**t3m sun up**	**t3m shock up**	**t3m bottom**	**t3m sun bottom**	**t3m shock bottom**
OCR	100.00	94.81	92.37	102.27	107.06	101.88	100.59	99.85	99.37
AVO	100.00	94.68	95.42	100.05	103.96	99.97	97.91	96.57	97.39

Considering an acceptability range for filters' recovery of 90-110%, as observable from the table there are no significant changes for filters' amount in formulations F3, neither after treatments nor after three months of storage.

Regarding formulation F2 there is a trend towards higher percentages of Octocrylene recovery for the withdrawals in the upper part of container after three months; this could be due to a water evaporation over time, or to migration of filters or separation

phenomena, indicating the beginning of instability behavior of formulations containing free UV filters.

Near InfraRed (NIR) analysis

The NIR (Near Infrared) spectroscopy technique was used for a further non-destructive characterization of formulations in order to reveal possible instability phenomenon before and after treatments.

All samples were analyzed in triplicate by MicroNIR and data were evaluated using principle component analysis (PCA) on pretreated spectra, as previously explained. This technique was able to confirm the results obtained by spectrophotometer analysis.

Here the results of PCA are reported.

First of all, as observable from Figure 6, this technique demonstrated to be able to distinguish the different formulations (placebo, F2 and F3).

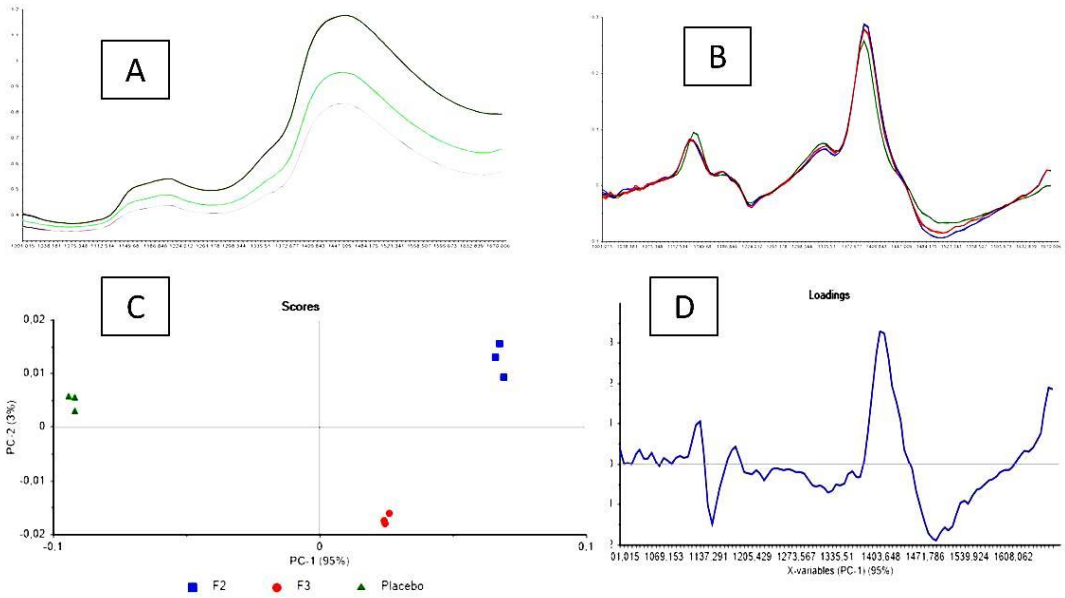


Figure 6. A: spectra, B:pretreated spectra, C: PCA plot, D: loadings plot, for placebo, F2 and F3 samples.

In fact the samples are distinct and separated from each other; in particular placebo is well separated along the PC-1, while the two formulations that contain UV filters are closer together, but anyway differentiated along the PC-2.

The second step expected the comparison between the not treated and the treated formulations in order to reveal possible changes due to treatments in plastic packaging. Figure 7 shows the results of PCA performed on F2 and F3 formulations before and after UV irradiation (suntest) and thermal shock cycles (shock).

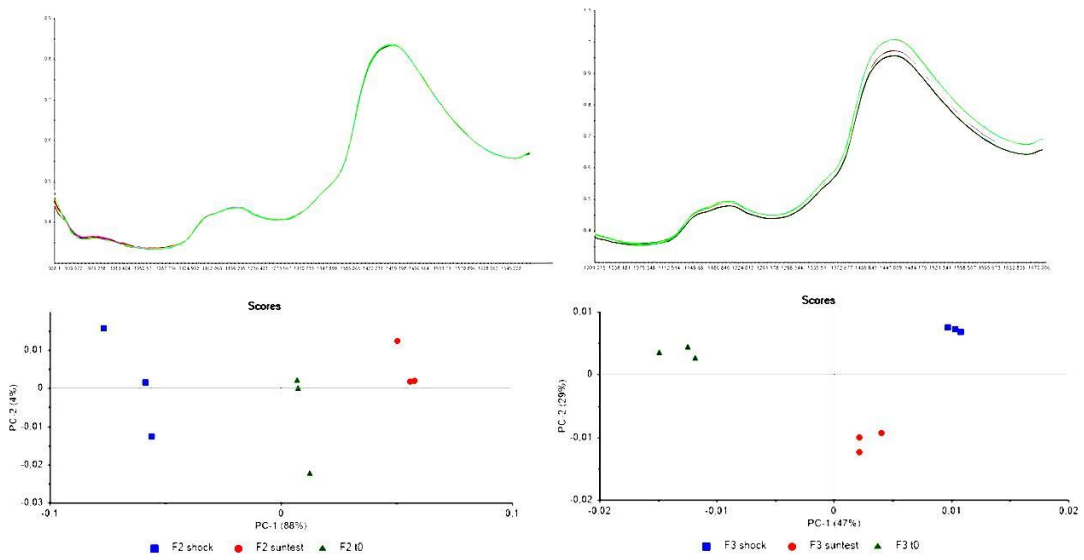


Figure 7. On the left: Spectra and PCA for treated and untreated F2. On the right: Spectra and PCA for treated and untreated F3.

As observable from figure 7, along PC-1, the samples are distinct from each other, to indicate a difference between the sample at t0 and the samples which underwent the two treatments. This consideration can be done both for F2 and F3 formulations. It can be concluded than the two treatments caused some changes in both formulations that allow to reveal the samples as different from each other.

The last step was comparing each formulation at t0 (not treated) with the same after three months of natural aging; the samples after three months were withdrawn from two different position of containers, the top and the bottom, in order to take over possible changes in formulations' structure, due to stratification, creaming or sedimentation phenomena, as shown in Figure 8.

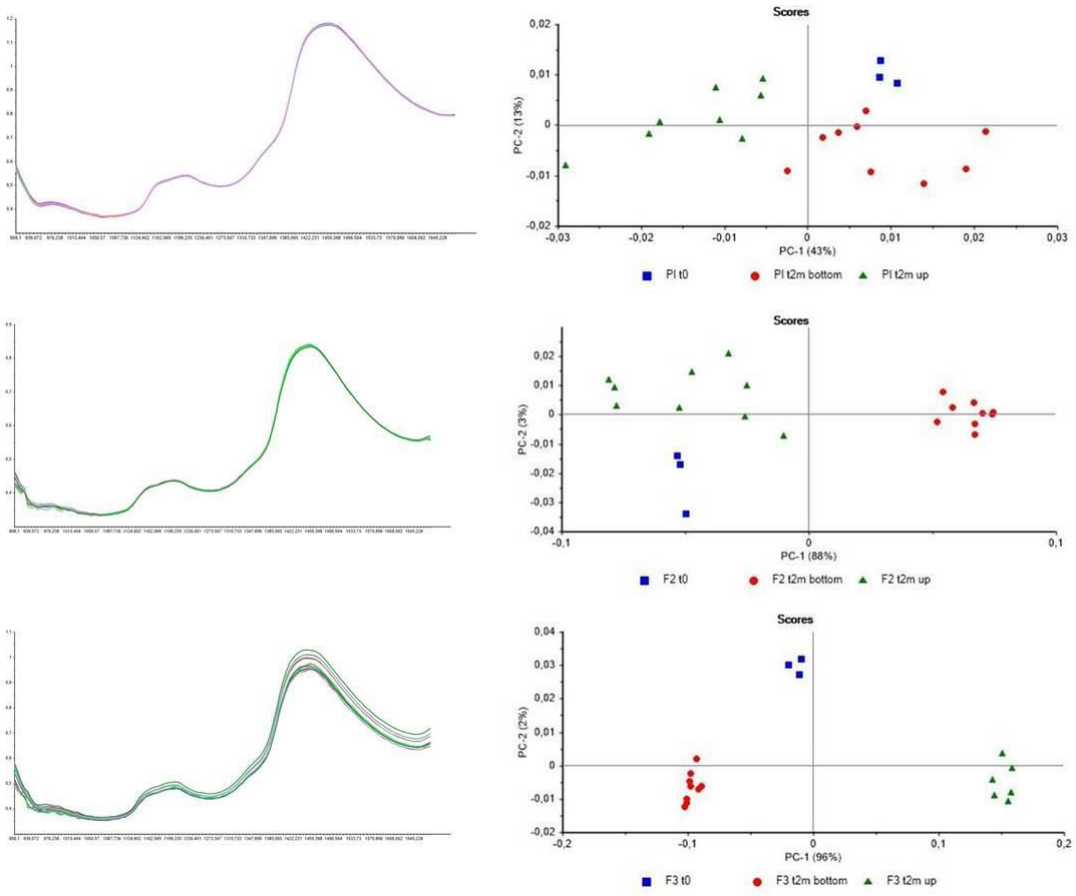


Figure 8. From the top: PCA of placebo t0 and after two months, up and bottom; PCA of F2 t0 and after three months, up and bottom; PCA of F3 t0 and after three months, up and bottom.

As it can be observed from these graphs, the placebo does not present differences between the sample at t0 and after three months and withdrawn in both positions of container. Different considerations can be done instead for samples of F2 and F3 formulations.

In fact, for F2 the NIR analysis revealed a difference between the sample deriving from the bottom and the one deriving from the top of container after three months since preparation. Sample at t0 is instead comparable and not distinguished from the sample withdrawn from the top after three months.

For formulation F3, this analysis revealed a difference between all samples, as the PCA sharply separated the three withdrawals.

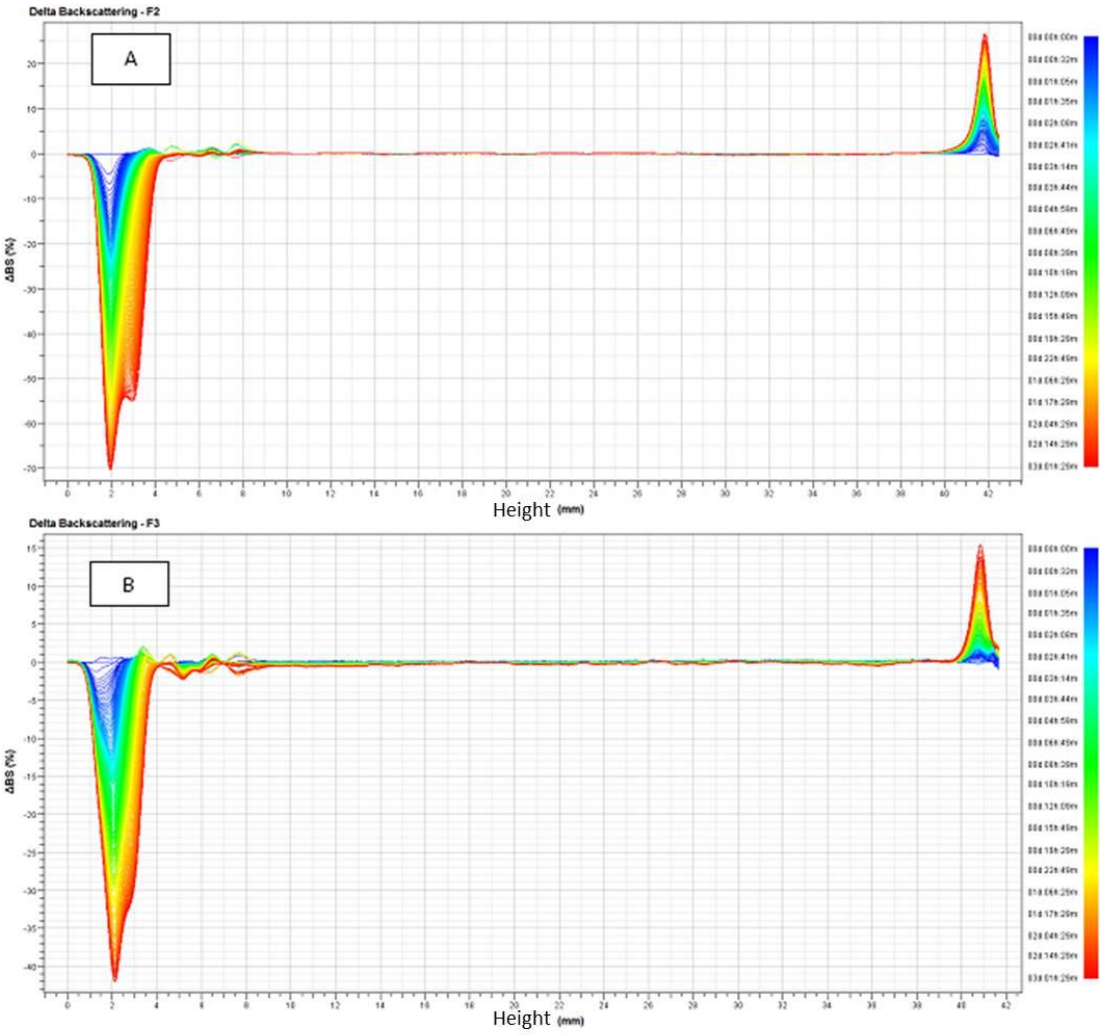
These results confirmed the analyses performed by spectrophotometer on filter content; some changes in the formulations occur over time.

Multiple Light Scattering analysis

The multiple light scattering technique was used in order to evaluate the stability of formulations for confirming the results obtained with other techniques. In particular, NIR analysis and spectrophotometric evaluation on F2 and F3 revealed a difference between samples withdrawn on the top and the ones on the bottom of containers, as there were some instability phenomena (creaming, stratification, etc.) after three months stability.

The ΔBS graphs (%) are reported in Figure 9.

ΔT values are not shown because the samples are milky and therefore there are no variations in transmission.



. **Figure 9 .** ΔBS (%) of F2 (A) and F3 (B) samples

As seen in Figure 9, F2 sample showed a clear and significant clarification on the vial's bottom (70% ΔBS). Simultaneously on the upper portion of the vial, there was instead a moderate creaming phenomenon (+25% ΔBS). Observed clarification and

creaming phenomena are indicative of an instability of the sample, since values are outside the range of ± 10% ΔBS.

Contrary to sample F2, for F3 sample, although it exhibited an instability profile similar to the previous one, variations of ΔBS (%) on the bottom and on the top portion are markedly different: -40% vs. -70% (clarification) and + 15% vs. 25% (creaming), respectively.

The found significance of changes must be considered as referred to samples held at room temperature for three months.

In Figure 10, the kinetic profile of the creaming phenomenon for both formulations is shown.

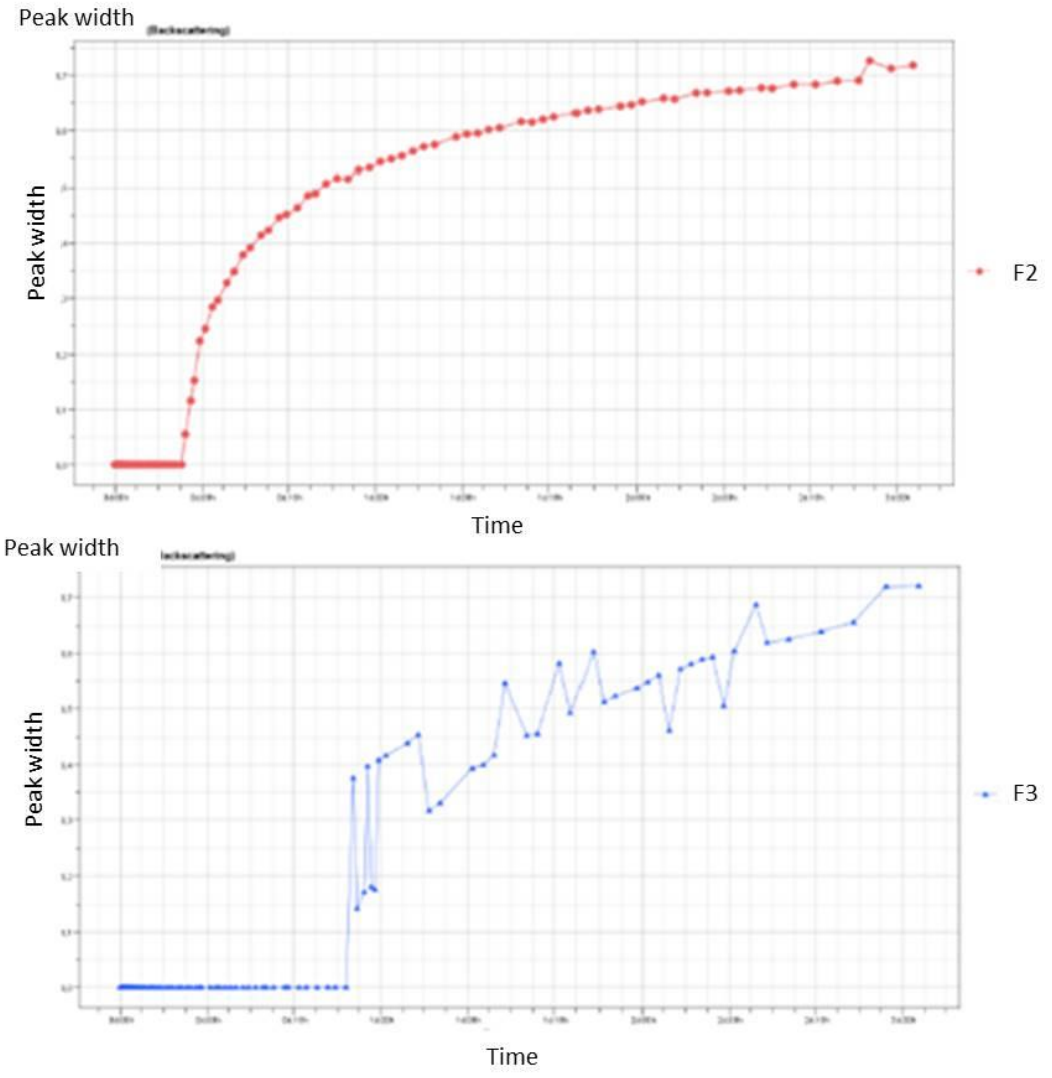


Figure 10. Peak's width (BS) of F2 (up) and F3 (down) samples.

For each curve, the slope was calculated by software for three time intervals: from 6 to 12 hours, from 12 to 48 hours, from 48 to 72 hours. The first segment of curve F2 showed a high kinetic rate of creaming, with a slope value of 1.67 mm/d; the formulation F3 showed no instability phenomena (slope = 0) until about 20 hours. So this formulation demonstrated to be more stable than F2 for the first part of the study. The second portion of curve presented a slope for F2 of 0.16 mm/d, while F3 started to show creaming phenomenon, with a slope = 0.32 mm/d.

For both curves, the third portion seemed to reach nearly the plateau, with a slope for F2 equal t0 0.06 and for F3 equal t0 0.19 mm/d.

Here the graph related to l* parameter in the central part of vial is also reported (Figure 11). It represents the photon mean free path in µm.

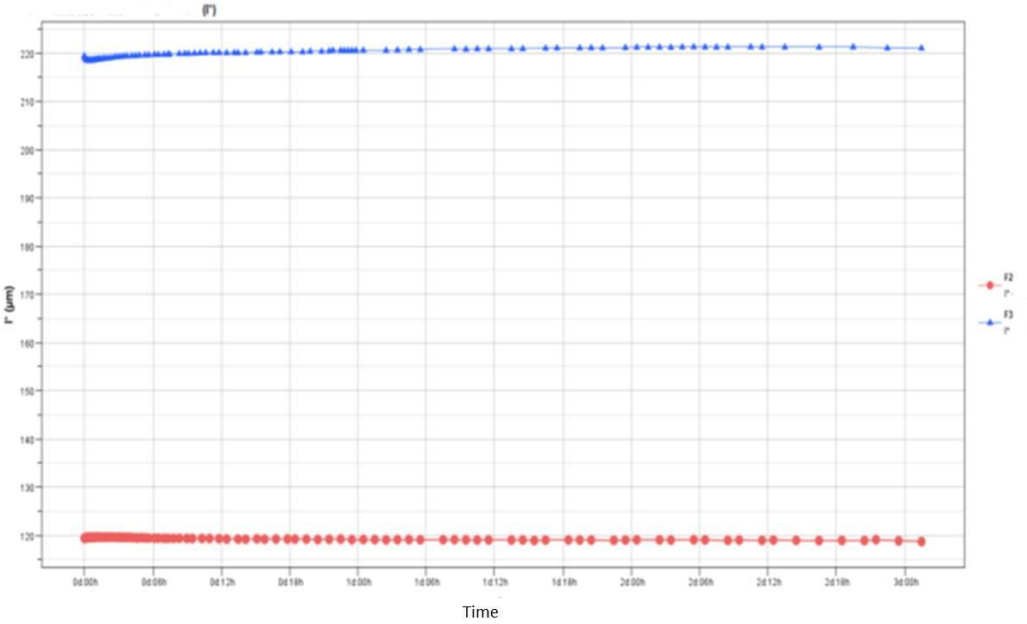


Figure11. l* parameter for F2 and F3 in the central part of vial.

This graph confirms the different structure of the two emulsions, due to the presence of encapsulated filters in F3. In fact, l* value is higher for F3 formulation because of the presence of the filters suspension and the capsules of filters themselves; this more complex structure makes the optical path longer.

Finally, the TSI values are reported for both formulations. The TSI at one day for the three part of container (bottom, centre, top) is reported in Figure 12, while Table 11 reports the values of global TSI for both formulations at day one, two and three.

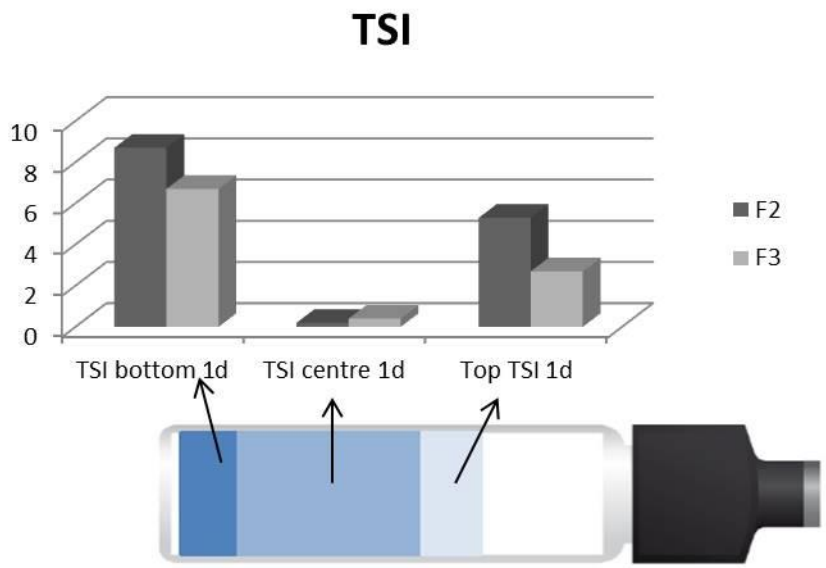


Figure12. TSI for F2 and F3 at day one for bottom, centre and top of vial.

Table 11. Global TSI for F2 and F3, day one, two and three.

Measure	Global TSI 1d	Global TSI 2d	Global TSI 3d
F2	3.3	4.3	5.1
F3	2.3	3.1	3.5

The TSI for the different positions of vial indicates that the bottom is the zone that presents the highest instability (clarification phenomenon) because of the highest value of TSI. The central part does not present instability phenomena (lowest values of TSI). This confirms the absence of aggregation phenomena.

Formulation F2 revealed to be more instable than F3 both for top and for bottom zone, in fact clarification and creaming phenomena are more pronounced.

The global TSI profiles, top and bottom, only reconfirm what has already emerged from the ΔBS profiles (%): formulation F3 is more stable than F2 sample, proving to separate more slowly during the three days of analysis. This confirms the different stratification previously found by the other techniques.

CONCLUSIONS

This work aims to study the stability of sunscreen formulations, before and after two different treatments (solar irradiation simulating test and thermal shock cycles), wanting to mimic the possible stress conditions solar products could meet during

their “in use” life, in relation to a plastic packaging (made by a mixture LDPE/ HDPE), commonly used for this kind of products.

In particular, the chosen UV filters were Butyl Methoxydibenzoylmethane (Avobenzone) and Octocrylene, in free and encapsulated (sol-gel silica glass; commercially available product) form.

Data obtained from analyses on packaging materials revealed that plastic containers underwent significant changes in their elastic/plastic behavior especially after solar irradiation simulating test. Furthermore, treated containers presented also some changes in external color (Yellowness Index variations), more accentuated for samples treated with simulated solar irradiation. Finally, from the evaluation of extractables’ profile of not treated and treated packaging material a possible absorption of some ingredients of formulations were shown. In particular, substances closely related to the filling formulation were detected; these substances were identified as residual of C12-15 Alkyl benzoate, 2-Phenoxyethanol for all three formulations and Octocrylene only for F2 and F3 samples.

Regarding the characterization of formulations, no changes in pH or organoleptic properties were revealed and no significant differences were obtained by rheological analysis.

The simultaneous use of NIR and Multiple Light Scattering permit to reveal the instability of formulation F2. A difference between samples withdrawn from the top or from the bottom of containers was revealed also by NIR spectroscopy, confirming possible instability phenomena.

In fact, observing data obtained by Turbiscan analysis, it can be found as clarification and creaming phenomena are more pronounced in F2 than in F3, so the formulation containing encapsulated filters is more stable than formulation with free UV filters.

The evaluation of UV filter amount indicates that there are no significant changes for filter amount in formulations F3, neither after treatments nor over time.

Regarding formulation F2 there is a trend towards a more concentrated filter in the upper part of container after three months of storage.

Results obtained from this work permitted to conclude that the LDPE/HDPE mixture chosen as packaging is not suitable for solar products; the solar simulator irradiation is the essential test to evaluate the stability of this kind of products. Furthermore the use of NIR and Multiple Light Scattering techniques can be promising to assess

short-term changes hardly detectable with other techniques commonly used in cosmetic.

In conclusions this study confirms the importance to study all aspects related to a final product; in fact the evaluation of formulation is important but it is essential to verify the use of a suitable packaging in order to assure the quality, the efficacy and also the safety of the final product.

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Chapter VII.

APPLICATION OF NIR SPECTROSCOPY AS COMPLEMEMTARY TECHNIQUE FOR THE CHARACTERIZATION OF SOLAR PRODUCTS

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"Application of NIR spectroscopy for stability evaluation of sunscreen formulations"

under submission to *International Journal of Cosmetic Science*

APPLICATION OF NIR SPECTROSCOPY AS COMPLEMEMTARY TECHNIQUE FOR THE CHARACTERIZATION OF SOLAR PRODUCTS

ABSTRACT

Near-Infrared (NIR) spectroscopy is a simple, rapid and accurate method for non-destructive measurement of solid and liquid samples. In recent years, this technique has been used for different applications for quality control and process control in several fields, including food, agriculture, chemical, pharmaceuticals, textiles, polymers, cosmetics and medical. In particular, in cosmetic area of interest this spectrometric method is used for quality control of both raw materials and finished products.

With this work a new application of NIR spectroscopy wants to be proposed; specifically it was used for stability evaluation of emulsions as a complementary analysis, in support of more traditional techniques. For this purpose sunscreen formulations were characterized. Three O/W emulsions was prepared: a placebo, a formulation containing UV filters (Butyl Methoxydibenzoilmethane and Octocrylene) in free form and a formulation containing the same filters in encapsulated form (commercially available raw materials). These formulations were analysed using NIR spectroscopy after preparation, after two different stress treatments and after two months in order to reveal possible change in stability of emulsions over time.

From the study carried out it is evident that the NIR spectroscopy is a technique capable of discriminating an aged formulation from the same emulsion freshly prepared, even at a distance of only two months. The interactions with the surrounding and with the packaging and the filters' influence make changes that, although minimal, can be highlighted with this spectroscopic technique.

NIR spectroscopy can be successfully used as a complementary technique to a comprehensive study of the stability of the semi-solid emulsions.

Keywords: emulsions, spectroscopy, formulation/stability, NIR, sunscreens

INTRODUCTION

In recent years, Near-Infrared (NIR) spectroscopy has been used in a large number of instances for quality control and process control purposes in several fields[1], including food, agriculture, chemical, pharmaceuticals, textiles, polymers, cosmetics and medical.

This spectrometric technique is a simple, rapid and accurate method for non-destructive measurement of solid and liquid samples. More importantly, it has the potential to measure multiple quality attributes simultaneously.[2-11]

In NIR spectroscopy, samples are scanned in a range of near-infrared region (950-1650 nm) by use of a monochromator. In this region, each constituent of a complex organic mixture has unique absorption properties due to stretching and bending vibrations in molecular bonds. [12]

This technique presents some advantages respect to other analytical techniques, for example its ability to record spectra of solid and liquid samples without prior manipulation; furthermore it's simple, fast and cost-effective. [13]

In cosmetics field, NIR spectroscopy technique is used for several applications, as reported in literature.

First of all, this technique can be used in quality control or raw materials. In fact, since manufacturers of cosmetics products have to follow GMP guidelines, they are required to supply extensive product information, comprehensive and exact production guidelines, analytical controls and detailed raw material data. In order to meet legal requirements each container must be checked to see if the content corresponds to the description on the packaging material. NIR technology plays an increasingly important role in this application. In fact, by controlling the identity of every raw material container, rather than just a small number of samples, a significantly processing benefit is assured. [14]

Secondly, NIR spectroscopy was used in order to evaluate skin's properties. In particular this technique was applied as a method to directly determine water content in skin as well as the nature of water binding. In fact the method revealed to be able to quantitate water on a relative basis and to distinguish free, bulk, and bound water. In addition, scattering of near-infrared radiation off the skin surface can be used as a measure of skin smoothness. [15]

Another application in cosmetic field was for the evaluation of skin compatibility testing of colored cosmetics. In this case, the technique was used to quantify

reddening of human skin in vivo below colored cosmetics (e.g. hair dye, lipstick, makeup). The skin compatibility of non-transparent cosmetic products was determined by detection of a remission band in the near-infrared spectral region. [16]
Moreover, it is used also for the quality control of cosmetic finished products. In particular, many components of cosmetic emulsions are natural products consisting of complex mixtures of chemically very similar substances (isomers, oligomers). This makes their analysis rather complicated and often requires the determination of global indices for the mixture rather than individual values for specific components.
NIR spectroscopy and multivariate spectral processing chemometric techniques are useful for establishing the composition of complex samples with acceptable levels of analytical properties, such as accuracy, precision and throughput.
This technique is especially useful in this context as it allows spectra to be recorded in a noninvasive, nondestructive manner at any point in the industrial production process without the need for any reagents. Also, the use of multivariate chemometric techniques facilitates the development of analytical methods for the simultaneous determination of both physical and chemical parameters from the NIR spectrum of the sample.[17]
In this work we wanted to propose a further application of NIR spectroscopy for cosmetic end-products. In particular this study aimed to highlighted the possibility to use this technique for stability evaluation of emulsions as a complementary analysis, in support of more traditional techniques. The NIR spectroscopy, in fact, constitutes a very fast and simple technique that requires no sample preparation. It could then be easily inserted during or at the end of a production process as an additional step to ensure the conformity of the product, or after some time to evaluate changes that occurred.
For this purpose, NIR spectroscopy has been used to characterize sunscreen formulations. Three O/W emulsions was prepared: a placebo, a formulation containing UV filters (Butyl Methoxydibenzoilmethane and Octocrylene) in free form and a formulation containing the same filters in encapsulated form (commercially available raw materials). These formulations were analysed using NIR spectroscopy after preparation, after two different stress treatments and after two months in order to reveal possible change in stability of emulsions over time.

MATERIALS

For formulations' preparation the following substances were used: Sucrathix VX by Alfa Chemicals (Berkshire, UK), Glycerin and Disodium EDTA by CARLO ERBA reagents (Cornaredo, Italy), Tegosoft TN by Evonik Industries (Essen, Germany), Montanov L and Montanov 82 by Seppic (Puteaux, France), Eusolex UV Pearls OB SX, Eusolex OCR and Eusolex 9020 by Merck KGaA (Darmstadt, Germany), Verstatil PC by Dr.Straetmans GmbH (Hamburg, Germany).

METHODS

Formulations preparation

For this study, a basic formulation of a cream was made. The resulting semi-solid emulsion was then available in three versions, referred to as F1, F2 and F3. The qualitative and quantitative compositions of formulations used in this work is reported in Table I.

TableI. Quali-quantitative compositions of formulations

	Ingredient	INCI	% in F1	% in F2	% in F3
A	Water	Aqua	83.9	71.9	53.9
	Sucrarhix VX	Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum	1	1	1
	Glycerin	Glycerin	2	2	2
	Eusolex UV Pearls OB SX	Aqua, Octocrylene, Sorbitol, Butyl Methoxydibenzoylmethane, Silica, PVP	*	*	30
	Disodium EDTA	Disodium EDTA	0.1	0.1	0.1
B	Montanov L	C14-22 Alcohols (and) C12-20 Alkyl Glucoside	1	1	1
	Montanov 82	Cetearyl Alcohol (and) Coco-Glucoside	4	4	4
	Eusolex 9020	Butyl Methoxydibenzoylmethane	*	9	*
	Eusolex OCR	Octocrylene	*	3	*
	Tegosoft TN	C12-15 Alkyl Benzoate	7	7	7
C	Verstatil PC	Phenoxytehanol, Caprylyl Glycol	1	1	1

F1 corresponds to the placebo cream. F2 corresponds to the basic formulation to which two sunscreens were added, avobenzone and octocrylene, in the ratio 1: 3. F3

corresponds finally to the formulation in which the filters were inserted conveyed in inclusion complexes, in a final concentration equal to the previous and always in the ratio 1: 3.

The formulations were prepared by emulsion, slowly adding phase B in phase A, using a Silverson SL2t High Shear Laboratory Stirrer Mixer (Silverson Machines Ltd, Chesam, UK) for 10 minutes, rate 7000 rpm at 75°C. After emulsion phase, when the temperature was below 40°C, phase C were added.

The formulations, after a preliminary spectroscopic analysis at time zero, were divided in three different shares which were subjected to two forced aging treatments, simulated solar irradiation and thermal shock cycles, while one portion was kept as control at room temperature.

Stress treatments

Simulated solar irradiation

A portion was placed in Suntest for a simulated solar irrradiation. Suntest instrument was set-up according to functional standards in European procedures, with the following parameters:

- Time: 4 hours to 192 hours Corresponding solar light
- Irradiation control: 300-800 nm
- Irradiation [W / m2]: 750
- Room temperature: 35 ° C
- Black Standard Temperature (BST): 45 ° C

Thermal shock cycles

Another portion was subjected to a heat shock treatment. The thermal cycles were performed in the refrigerated incubator BRE60 BioExpert 56L (Froilabo, Meyzieu, France).

After preparation (t0), at the end of treatments and after two months formulations were evaluated by NIR spectroscopy to highlight any differences.

NIR Spectroscopy

The NIR probe used in the study is the MicroNIR ™ Pro Spectrometer (JDSU, Milpitas, California, USA). The source and the other specifications of the probe are shown in Table II. The measurements are performed in reflectance. For the analysis a specific holder was used that maintains steady the distance between the source and the sample to be analysed. The support, cylindrical, and is provided with a guide with a fixed thickness placed along the edge, which allows to obtain a uniform layer of formulation 3 mm thick. The support is then covered with a transparent glass slide, on which rests the probe in order to take the measurement. Each analysis is performed in triplicate.

For analysis of formulations, samples were withdrawn from three different positions of container (top, centre and bottom), in order to evaluate the homogeneity of emulsions and the presence of possible separation phenomena (stratification, creaming, sedimentation, etc.).

TableII. MicroNIR instruments' parameters

Illumination source	Two integrated vacuum tungsten lamps
Illumination geometry	Flood illumination/0° observer
Input aperture dimensions	2.5 x 3.0 mm
Sample working plane	0 – 15 mm from window, 3 mm optimal distance
Detector	128 pixel InGaAs photodiode array
Pixel Size / Pitch	30 µm x 250 µm / 50 µm
Wavelength range	950 – 1650 nm
Pixel to pixel interval	6.2 nm
Spectral bandwidth (FWHM)	<1.25% of center wavelength (1% typical)
Spectral in-band	LVF blocking >4 OD Average

The spectroscopic data obtained from the analysis is exported and processed with Unscrambler® X software, version 10.4 (Camo AS, software Oslo, Norway). In particular, the spectra are subjected to a SNV and to a Savitzky-Golay smoothing. On the data thus treated, a PCA is performed, that suggests, even visually, the common meeting points between the analysed formulations.

The first element taken into consideration is the stability over time of the placebo formulation. This is verified by the PCA performed on the spectra of the placebo formulation at t0 and t2, considering three different sampling locations for a complete analysis. As indicated by the PC-1 and PC-2 values, the spectra obtained after 2 months from the placebo formulation were not significantly different. Any differences found in F2 and F3 are thus attributable to the presence of the filter and its influence on the formulation.

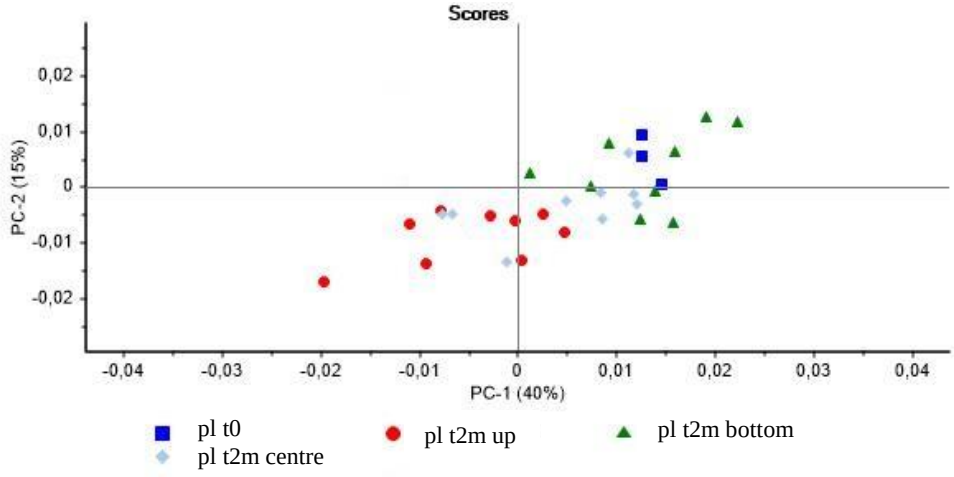

Figure 1. PCA of placebo t0 and after two months, up, centre and bottom.

Using increasing concentrations of free and encapsulated filters added to different rates of placebo we see that the obtained formulations are distinguishable from placebo as such by NIR spectroscopy. (Figure 2 and 3)

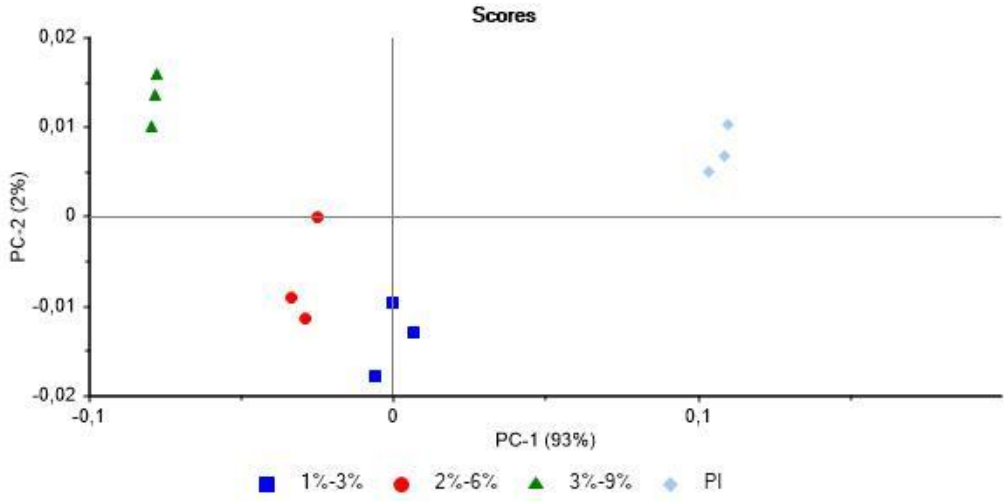

Figure 2. PCA of placebo compared with placebo charged with different concentrations of free filters (ratio 1:3)

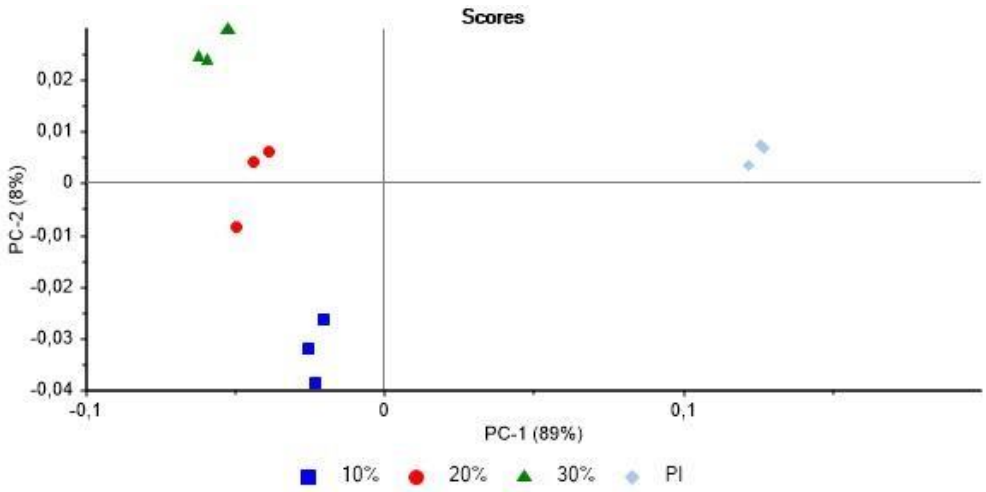

Figure 3. PCA of placebo compared with placebo charged with different concentrations of encapsulated filters' suspension

The different concentrations of active are distinguished on the basis of PC-2 (2% and 8% respectively), but not sufficiently to ensure a quantitative distinction. It can be said that a change is recognized in the overall composition of the formulation, probably due not only to the presence of the filter but also to the variation of the quantity of water and of the secondary components of the formulation.

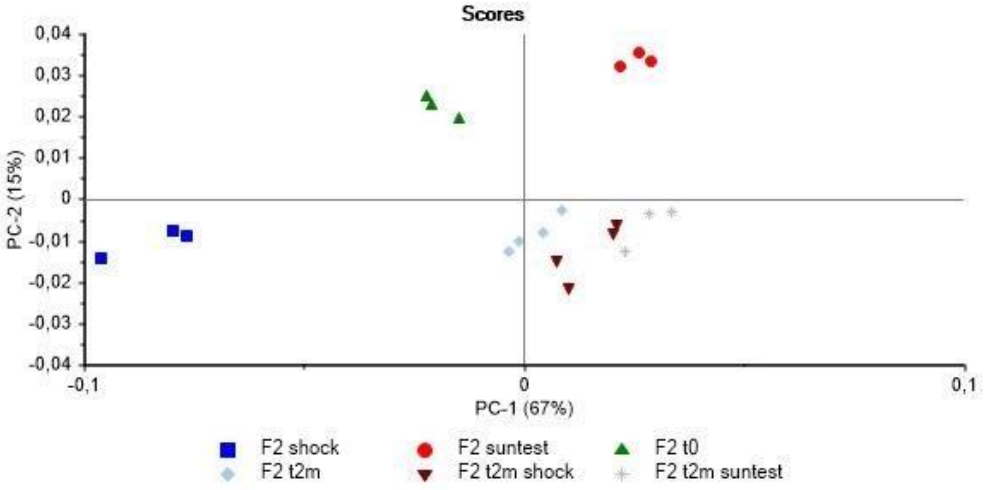

Figure 4. PCA of F2 t0 and treated compared the same after two months

The spectra obtained analyzing F2 before and after the treatments both at t0 and t2 are compared in a single PCA. From the graph it is evident that the natural aging of the formulation makes the substantial changes, so that the treated formulations'

spectra are closed to the untreated ones. At t0, however, the samples are distinct from each other on the basis of the treatments to which they are subjected.

The same analysis is replicated for the F3 formulation containing the encapsulated filters. (Figure 5)

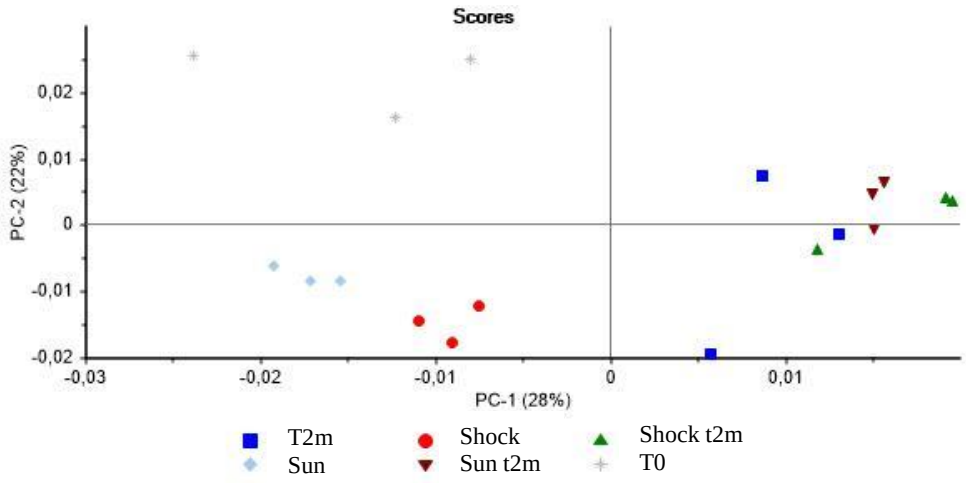

Figure 5. PCA of F3 t0 and treated compared the same after two months

In this case we observe lower values of PC-1 and PC-2. This indicates that the spectra obtained do not show a marked variability with a specific trend. Clearly, however, the samples F3 at t0, treated and untreated, are separated from the F3 t2 months samples, treated and untreated. Again, the natural aging is confirmed as a much more influential factor on the nature of the emulsion with respect to accelerated aging treatments. In two months, the samples tend to collapse towards a common point regardless of their origin, countering the effect of suntest or heat shock.

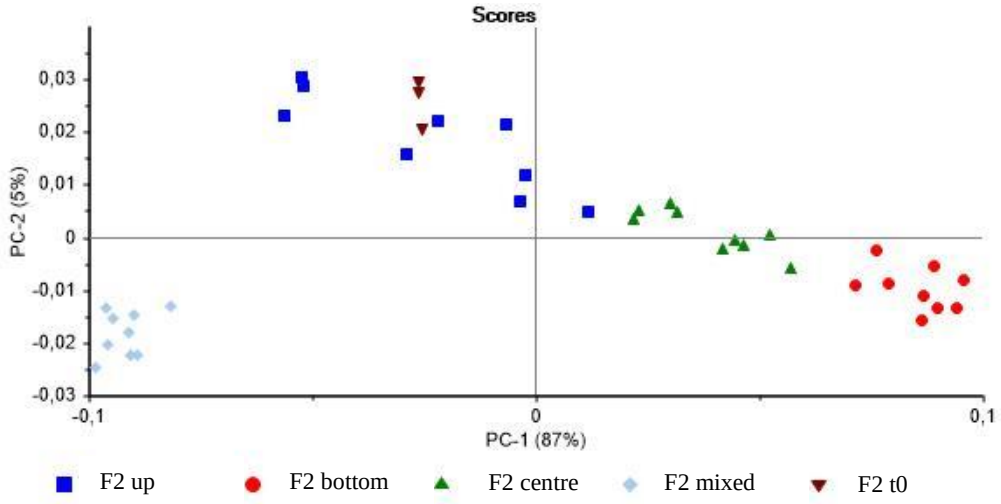

Figure 6. PCA of F2 t0 and after two months stability, withdrawn from different positions

The F2 formulation is analyzed by taking samples at three different levels to check the homogeneity of the formulation after two months of its preparation. The layers are defined, starting from the bottom, "bottom", "medium" and "high". As reference are placed in the PCA the spectra of the same formulation at t0 and of the formulation F2 at t2 months, mixed. Clearly it shows that the mixed formulation is the most homogeneous of all those analyzed, so that the portions withdraw on top or on the bottom are not distinguished from each other absolutely. The reference to t0 is more similar to the high portion of F2 probably because in this section are found the best conditions to replicate the initial composition of the formulation. As for the three levels of F2 t2 months analyzed, it is observed a stratification which is probably due to a migration of some components to the bottom and to the evaporation of water from the surface portion. This changes the relative concentrations of the ingredients in each level, generating the distinction highlighted the PCA on the PC-1.

The same analysis is performed on F3. (Figure 7)

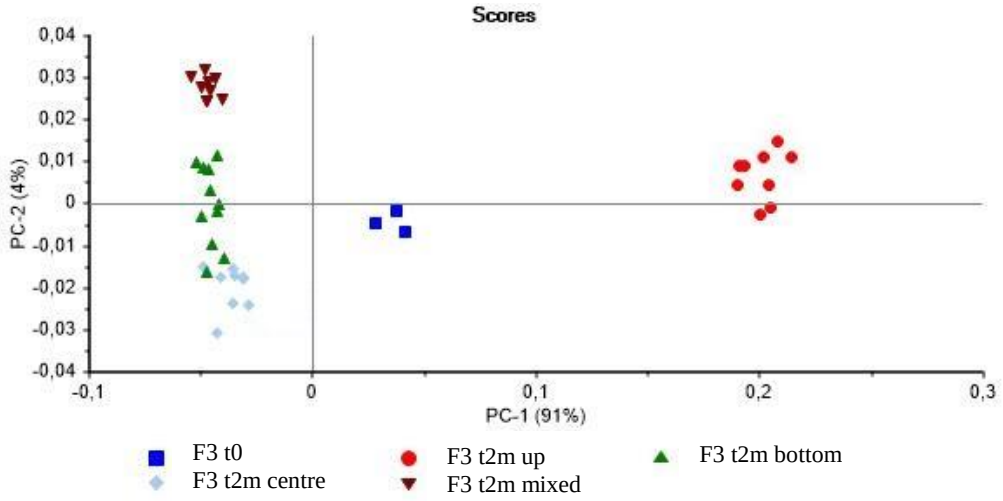

Figure 7. PCA of F3 t0 and after two months stability, withdrawn from different positions

Also in this case it is observed the stratification in the medium and low levels of F3 t2 months, the homogeneous group of F3 mixed aligned with the analysis of the 3 positions along the PC-1 and the comparison with the formulation at t0. The group of spectra of the F3 t2 months formulation taken at the top appears very detached from other samples. This is probably due to a greater interaction with the environment that significantly changed the composition in this portion.

CONCLUSIONS

This study aimed to propose a new application of NIR spectroscopy for cosmetic field; we wanted to demonstrate its applicability for stability evaluation of sunscreen formulations.

The PCA performed on the spectra of the placebo formulation indicate no significant different in sample after 2 months Any differences found in F2 and F3 are thus attributable to the presence of the filter and its influence on the formulation.

Using increasing concentrations of free and encapsulated filters added to different rates of placebo the technique was able to distinguish them from placebo and from each other.

Natural aging of the formulation revealed to be a much more influential factor on the nature of the emulsion with respect to accelerated aging treatments, provoking substantial changes in formulations.

Furthermore, analysing samples withdrawn from different height, it is observed a stratification which is probably due to a migration of some components to the bottom and to the evaporation of water from the surface portion.

From the study carried out it is evident that the NIR spectroscopy is a technique capable of discriminating an aged formulation from the same emulsion freshly prepared, even at a distance of only two months. The interactions with the surrounding and with the packaging and the filters' influence make changes that, although minimal, can be highlighted with this spectroscopic technique.

NIR spectroscopy can be successfully used as a complementary technique to a comprehensive study of the stability of the semi-solid emulsions.

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Chapter VIII.

PERFORMANCE OF DIFFERENT PRESERVATIVES IN FORMULATIONS STABILITY AND EVALUATION OF THEIR INTERACTION WITH PACKAGING

B. Briasco, P. Capra B. Mannucci,, P. Perugini

"Performance of different preservatives in formulations stability and evaluation of their interaction with packaging"

under submission to *Journal of Cosmetic Science*

PERFORMANCE OF DIFFERENT PRESERVATIVES IN FORMULATIONS STABILITY AND EVALUATION OF THEIR INTERACTION WITH PACKAGING

ABSTRACT

A preservative is a natural or synthetic chemical that is added to products such as foods, pharmaceuticals, cosmetics, biological samples, etc. to prevent decomposition by microbial growth or by undesirable chemical changes. The addition of preservatives to such products, especially to those that have higher water content, is essential for avoiding alteration and degradation by microorganisms during storage.

Since consumers are more conscious about the health problems caused by several synthetic preservatives, manufacturers are continually interested in new alternative preservation systems.

This work aimed to study the stability of O/W emulsions with different preservatives, a traditional system (Phenoxyethanol, Caprylyl Glycol) and a more innovative mixture (Triethyl Citrate, Glyceryl Caprylate, Benzoic Acid), in relation with two different plastic packaging (LDPE/HDPE mixture and PET) in order to evaluate possible interaction phenomena. Furthermore, the influence of the presence of mandarin essential oil in the formulation stability was also investigated.

Results highlight that different preservatives can have influence on formulation stability, independently from the presence of essential oil. No negative results were reported about preservative efficacy, but some interactions were found in relation with packaging: some substances from formulations were absorbed to the packaging and solar simulating irradiation determined variations in formulation containing the essential oil.

Keywords: preservatives, mandarin essential oil, plastic packaging, stability, interactions

INTRODUCTION

Cosmetics, pharmaceutics and food need preservation to prevent microbial growth and spoilage since water, oils, peptides, and carbohydrates acts as good medium for growth of microbes. [1] Such labile goods are exposed to numerous spoilage possibilities and one of the most important factors leading to longer shelf-lives is their packaging. [2]

If a product is contaminated by microorganisms either during manufacture or use, they may directly or indirectly affect the human health. [3] Microbes can cause infection, discoloration, may produce gas and odour. Various preservatives are employed to prevent contamination by pathogens or spoilage microorganisms. In doing so the preservatives extend the shelf life of the products. [4]

A preservative is a natural or synthetic chemical that is added to products such as foods, pharmaceuticals, paints, biological samples, wood, etc. to prevent decomposition by microbial growth or by undesirable chemical changes. The addition of preservatives to such products, especially to those that have higher water content, is essential for avoiding alteration and degradation by microorganisms during storage. Chemical preservation cannot totally keep products from spoiling, but they slow the spoiling process caused by microorganisms. [5]

A perfect preservative is a "colourless, odourless, water soluble, nontoxic, non-allergenic, non-irritating chemical capable of inhibiting the growth of a broad spectrum bacteria and fungi". [6]

Frequently used preservatives include parabens, benzyl alcohol, boric acid, sorbic acid, chlorhexidine, formaldehyde, quaternary ammonium compounds, phenol, imidazolidinyl compounds. [4, 7]

The choice of the preservative depends not only on the characteristics of the product and the nature of the ingredients used, but it is also subjected to legislative restrictions (1223/2009 Regulation, Annex V). Specifically, Annex V includes a list of permitted substances and their using concentrations. [8]

Among the most traditional preservatives, phenoxyethanol (PE) is widely used because of its positive reputation as a relatively gentle preservative that is considered non-irritating; it is also one of the few preservatives that does not release formaldehyde. It presents good activity against Gram+, Gram $^{-}$ and fungi. [4] The Cosmetic Ingredient Review (CIR) expert panel approved it for use; actually phenoxyethanol is most often used as a preservative in combination with parabens

because of its ability to kill bacteria and stabilize formulations, extending their shelf life and making them safe for use even at low concentrations.[4] Nevertheless, a study in literature reported that the use of phenoxyethanol at 0.9% w/v was found to cause contact dermatitis.[9]

However, recently more and more restrictions are imposed for certain classes of preservatives; in literature a lot of studies demonstrating negative effect of some traditionally used chemical preservatives are reported. For example the use of parabens is becoming increasingly controversial, since they have been found in breast cancer tumors (an average of 20 ng/g of tissue). Furthermore they also displayed the ability to slightly mimic estrogen (a hormone known to play a role in the development of breast cancer).[4, 10] Furthermore, there have been problems concerning the safety of some chemicals, including the possibility of allergies from benzoic acid and sulphites, the formation of carcinogenic nitrosamines from nitrites, and the possible rodent carcinogenicity of butylated hydroxyanisole and butylated hydroxytoluene. [11]

For these reasons, manufacturers are continually interested in new alternative preservation systems. Due to this market demand, preservative suppliers are putting great efforts in developing alternatives for the market. [12]

Beside the trend towards the use of preservative "not preservative" (antimicrobial agents that are not legislated as preservatives according to Annex V of Regulation CE 1223/2009, like Glyceryl Laurate, Glyceryl Caprate, Glyceryl Caprylate, Ethanol, Pentylene Glycol, Ethylhexylglycerin, 1,2-Hexanediol, Capryloyl Glycine, Sodium Caproyl Prolinate, Caprylyl Glycol… [13]), the use of essential oils is becoming popular to increase the shelf-life of food products. Additionally, essential oils have therapeutic uses in human medicine due to its anticancer, antinociceptive, antiphlogistic, antiviral, antibacterial and antioxidant properties. [14-21] However, essential oils are complex mixtures of volatile compounds, so absorption of flavor compounds by packaging is becoming an important product-package interaction aspect. [22-24] Flavor absorption may alter the aroma and taste of a product, or change the mechanical properties of polymers, such as tensile strength and permeability. [25-28]

Starting from these evidences, this work aimed to study the stability of O/W emulsions with different preservatives, a traditional system (Phenoxyethanol, Caprylyl Glycol) and a more innovative mixture (Triethyl Citrate, Glyceryl

Caprylate, Benzoic Acid), in relation with two different plastic packaging (LDPE/HDPE mixture and PET) in order to evaluate possible interaction phenomena. Furthermore, the presence of an essential oil into formulation was also investigated.

For system's stability evaluation, analyses were carried out both on packaging materials and on contained formulations. Containers were analyzed by tensile test, in order to evaluate their mechanical properties, by colorimetric assessment, and by extractable testing. Formulations were characterized in terms of pH, organoleptic properties, rheological behavior and microbial contamination. Moreover, a further investigation of formulation behavior by using near infrared spectroscopy (NIR) and multiple light scattering technique was carried out.

MATERIALS

For formulations' preparation the following substances have been used: Sucrathix VX (Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum) by Alfa Chemicals (Berkshire, UK), Glycerin and Disodium EDTA by CARLO ERBA reagents (Cornaredo, Italy), Tegosoft TN (C12-15 Alkyl Benzoate) by Evonik Industries (Essen, Germany), Montanov L (C14-22 Alcohols (and) C12-20 Alkyl Glucoside) and Montanov 82 (Cetearyl Alcohol (and) Coco-Glucoside) by Seppic (Puteaux, France), Verstatil PC (Phenoxytehanol, Caprylyl Glycol) and Verstatil TBG (Triethyl Citrate (and) Glyceryl Caprylate (and) Benzoic Acid) by Dr.Straetmans GmbH (Hamburg, Germany), Mandarin essential oil (Mandarin e.o.) by CreaSens (Gattico, Italy).

As packaging materials, some commercially available plastic containers by an Italian supplier have been used. In particular, bottles of 150 ml capacity made by a mixture HDPE/LDPE and bottles of 100 ml capacity made by PET were used.

For microbiological analysis the following products were used: Eugon LT 100 broth, Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar by Scharlau Science SL (Sentmenat, Spain).

METHODS

Formulations' preparation

For this study, four formulations were set up: a formulation with traditional preservative (INCI: Phenoxytehanol, Caprylyl Glycol) (F1); a formulation containing an innovative blend for reliable preservation (INCI: Triethyl Citrate (and) Glyceryl Caprylate (and) Benzoic Acid) (F4), a formulation with composition like F1 but containing also the mandarin essential oil (INCI: Citrus Nobilis peel oil) (F5), and a formulation like F4 but containing the essential oil (F6).

The quali-quantitative compositions of the set up formulations is reported in Table 1.

Table1. Quali-quantitative % compositions of formulations

Phase	INCI	F1	F4	F5	F6
A	Aqua	83.9	71.9	83.4	71.4
	Microcrystalline Cellulose & Cellulose Gum & Xanthan Gum	1	1	1	1
	Glycerin	2	2	2	2
	Disodium EDTA	0.1	0.1	0.1	0.1
B	C14-22 Alcohols (and) C12-20 Alkyl Glucoside	1	1	1	1
	Cetearyl Alcohol (and) Coco-Glucoside	4	4	4	4
	C12-15 Alkyl Benzoate	7	7	7	7
C	Citrus Nobilis peel oil	*	*	0.5	0.5
	Triethyl Citrate (and) Glyceryl Caprylate (and) Benzoic Acid	*	1	*	1
	Phenoxyethanol, Caprylyl Glycol	1	*	1	*

Each formulation was prepared in triplicate by emulsification, slowly adding phase B in phase A, using a Silverson SL2T High Shear Laboratory Stirrer Mixer (Silverson Machines Ltd, Chesam, UK) at 7000 rpm for 10 minutes, at 75°C. The O/W emulsion was then cooled and phase C was added when the temperature was below 40°C. F1, F4 and F5 were divided into both LDPE/HDPE and PET bottles while F6

formulation was divided in PET containers. Some samples were maintained in glass as controls.

Degradation testing procedures

All formulations maintained in bottles were subjected to degradation tests, in order to simulate foreseeable stress conditions that final products could meet during their use. [29] In particular:

- Photostability test by simulating solar irradiation using SUNTEST XLS +II (Atlas ®, Chicago, USA) for 96 hours. Suntest instrument was set up in according to standard European procedures [30], with the following parameters:

  Time: 4 hours corresponding to 192 hours solar light

  Irradiation control: 300-800 nm

  Irradiation [W/m^2]: 750

  Room temperature: 35°C

  Black Standard Temperature (BST): 45°C

In order to assure that both parts of each single dose container underwent simulated solar irradiation for the same period and under the same conditions, containers were turned on the other side after 48 hours.

- Thermal cycles (20°C for 36 hours - 45°C for 12 hours, three times) were performed using an incubator BRE60 BioExpert 56L (Froilabo, Meyzieu, France).

F1, F4 and F5 formulations kept both in the HDPE/LDPE mixture bottles and in PET bottles were subjected to both degradation procedures instead of F6 formulation stored in PET containers underwent only the thermal cycle treatment.

At the end of the treatment the samples were taken, emptied out and washed with a standard procedure. For all packaging samples several specimens were obtained to carry out mechanical analyses, as reported by Perugini et al. in a previous work [29]. Furthermore a colorimetric analysis and an evaluation of the extractable profile was performed on packaging.

Regarding formulations, an organoleptic and rheological characterization, a total microbiological count, an evaluation by near infrared spectroscopy (NIR) and a short

term stability study were carried out in order to highlight possible changes due to the contact with packaging and/or the undergone treatments.

Mechanical test

The investigation of the mechanical properties of the containers was performed using a tensile machine, AGS 500ND (Shimadzu corporation, Kyoto, Japan) equipped with a 500[N] load cell; the test was performed using a strain rate of 10 mm/min for HDPE and 3 mm/min for PET.

"Bone-shape" specimens were obtained from the central part of each container horizontally; the feature of the specimens followed the principles of the European Standard EN ISO 527 [31], suitably modified for bottle, following a previous work by this research group. Specifically, this optimized dog bone shape obtained by punchcutting provides a localized stress region (3 mm width). Each specimen was characterized for the measures of thickness and width of this region using a digital microscope model BW 1008. The section of each sample was calculated from thickness and width using a suitable software program (Micromeasure vers.1.2).

Samples were kept under constant temperature (23°C) and humidity (52% R.H.) for a week until tension test started and during all the test time.

This procedure permitted to obtain a stress versus strain curve. From each set of results was possible to estimate the tendency of materials to oppose to deformation, to evaluate the curve profile in elasticity regime, the elongation percentage in elasticity regime and the absolute elongation elasticity.

A critical and comparative analysis on different specimens allowed a first assessment of any significant changes in the stress-strain profile due to interactions among the polymeric material, the stress to which it was subjected and substances in contact with. [29]

Colorimetric analysis of packaging

The colorimetric evaluation of packaging was performed by Yellowness Index measurement (YI), following ASTM E313. [32]

Yellowness Index is a number calculated from spectrophotometric data that describes the change in color of a test sample from clear or white to yellow. This test is most

commonly used to evaluate color changes in a material caused by real or simulated light exposure.

For this purpose the instrumental assessment of both external and internal side of packaging color was performed by the Colorimeter CL400 (Cutometer MPA580, CK electronic GmbH, Cologne, Germany). Technical data of probe are length: 126 mm, illumination: Ø 24 mm, measuring area: Ø 8 mm, weight: 85 g, illuminated area approx. 17 mm Ø; units: xyz, and RGB, L 3 a 3 b; light: 8 white LEDs arranged circularly, range of emitted wavelengths: 440–670 nm; and accuracy: 65%.

The color acquisition is done by specifying the three tristimulus values X, Y, and Z, of a color in according to the CIE system, where X is the tristimulus value of red, Y is the green value, and Z the blue value. With the mathematical equation below reported it is possible to use these parameters in order to calculate YI value:

$YI = [100(1.28X_{CIE} - 1.06 Z_{CIE})] / Y_{CIE}$

The change in yellowness index (ΔYI) represents the difference between an initial value, YI_0, and YI detect at the end of a specify treatment.

$\Delta YI = YI - YI_0$

By this calculation, positive (+)ΔYI indicates increased yellowness and negative (-) ΔYI indicates decreased yellowness.

Extractable testing

This step aims to evaluate the possible migration of substances from packaging or the absorption of some content ingredients after the contact between plastic containers and formulations after stress treatments.

According to a previous work packaging materials were exposed to conditions to generate every potential extractable. For this purpose Head Space Solid phase microextraction (HS-SPME) was selected as the extraction technique. [33]

Briefly, 500 mg of polymer was put into a vial and the HS-SPME conditions used were the following: fiber: PDMS 100 µm (Supelco); adsorption temperature: 90 °C; extraction time: 60 min; desorption temperature: 250°C; desorption time: 4 min, 30 s. After extraction, for the identification of compounds a gas chromatography-mass spectrometry (GC-MS, Termo Scientific Trace DSQ II, Fisher Scientific Italia, Rodano, MI, Italy)equipped with Xcalibur MS Software Version 2.2 was used. The GC conditions were the following: column: Restek Rtx-5MS, 30 m × 0.25 mm ID ×

0.25 µm; gradient: 60 °C for 4.5 min, 20 °C/min until 280 °C, 280 °C for 5 min; injector: PTV 250 °C, split time 4.5 min, split flux 10 mL/min; gas: He, constant flux 1 mL/min; transfer line: 270 °C.

The MS conditions were: source: 250 °C; ionizing mode: EI 70 eV; ion scan mode: full Scan; ion scan range: 50–650 amu; ion scan rate: 870 amu/s.

The mass spectra of detected extractable compounds were compared with the databases for GC/MS NIST Mass Spectral Library (NIST 08) and Wiley Registry of Mass Spectral Data 8th Edition. Although the databases were used, some classes of compounds such as alkanes yielded very similar fingerprint patterns or fragments, and thus it was not always possible to make an indisputable identification of every peak (compound) detected.

Formulation characterization

24 hours after preparation and immediately after stability treatments the formulation was characterized in terms of pH, organoleptic and rheological properties.

The pH measurement was performed by a pHmeter Jenway 3510 (Jenway, Staffordshire, UK); pH 4 and 7 standard buffers were employed to calibrate the instrument before use.

Rheological properties were evaluated using a Dial Reading Brookfield Viscometer, model RVT (Brookfield AMETEK, Middleboro, Massachusetts, USA). The sample (100 g) was placed in a glass pot and left to equilibrate for 15 minutes before measuring the dial reading with a RV 5 –spindle at different speed (0.5, 1, 2.5, 5, 10, 20, 50, 100 rpm). The measurements were carried out in controlled conditions of temperature and humidity (23°C- 52% RH).

Colorimetric analysis of formulation

The colorimetric evaluation of formulations was performed by the Colorimeter CL400 (Cutometer MPA580, CK electronic GmbH, Cologne, Germany) describe above.

In particular, for the analysis of formulation color, an amount of each sample was transfer in a specific small bin, filling until the edge. On the surface, a glass slide was put in direct contact with formulation; on the top of this one, the Colorimeter was laid and the acquisition of color parameters was carried out.

The color difference of formulation after stress treatment is calculated as the numerical comparison of the sample's color to the standard, identify as the formulation color detected 24 h after preparation. It indicates the differences in absolute color coordinates measured in the CIE L*a*b color space and it is referred to a Delta (ΔE*).

The ΔE* was calculated using the measured values of L*, a* and b*, where dimension L* represents lightness, a* (with green at negative a* values and red at positive a* values) and b* (with blue at negative b* values and yellow at positive b* values) the color-opponent dimensions. ΔE* indicates the difference or distance between two colors.

Using L*a*b* system, ΔE* is calculated as:

$$\Delta E^* = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

Generally, the chromatic difference perceivable by human eye is about ΔE*= 1.

NIR analysis

The formulations were also analyzed using the NIR spectroscopy, for a further non-destructive characterization in order to reveal possible changes before and after treatments in formulations themselves. In NIR spectroscopy, formulation samples are scanned in a range of near-infrared region (950-1650 nm) by use of a monochromator. In this region, each constituent of a complex organic mixture has unique absorption properties due to stretching and bending vibrations in molecular bonds. [34]

For this kind of analysis the MicroNIR™ Pro Spectrometer, (JDSU, Milpitas, California, USA) working in the wavelength range between 950 and 1650 nm, was used. This technique presents some advantages respect to other analytical techniques, for example its ability to record spectra of solid and liquid samples without prior manipulation; furthermore it's simple, fast and cost-effective. [35]

For this kind of analysis formulations samples were put on a non-reflective support, with a fixed and constant distance (3 mm) from acquisition's window (2.5X 3.0 mm input aperture dimensions). Three replicates for each sample were always performed. Data were elaborated using the Unscrambler® X software, version 10.4 (Camo software AS, Oslo, Norway).

Principal components analysis (PCA) on pretreated spectra was performed on the obtained spectra in order to evidence the capability of NIR measurement system to discriminate different samples and possible changes in formulations due to treatments and/or aging. The spectra were pretreated
by using Standard Normal Variate (SNV) followed by first derivative with Savitzy-Golay smoothing.

Multiple Light Scattering

In order to further investigate some changes found with the previous analysis in formulations after two months, a multiple light scattering technique was used for studying and predicting the stability of formulations. This technique is an optical method to characterize concentrated liquid dispersions without dilution. Specifically, for this purpose Turbiscan MA 100 (Formulaction SA, L'Union, France), was used. The main advantage of Turbiscan is the ability to detect particle size change and/or local concentration change, which results in variation of the backscattering (BS) and transmission (T) signals, while the sample is destabilising, more accurately and faster than human eye.

The principle consists of sending photons (NIR light source, 880 nm) into the sample. These photons, after being scattered many times by the particles (or droplets) in the dispersion emerge from the sample and are detected by the 2 detectors of Turbiscan reading head: transmission for not opaque samples (0° from light source) and backscattering for opaque samples (135° from the light source).

Backscattering is directly related to the photon transport mean free path, so backscattering intensity depends on particle size and concentration.

After different storage times the variation of the ΔT and ΔBS is measured as a function of the test tube height and compared to the initial transmission and reflectance profiles. A plot is produced of these results with ΔT or ΔBS on the y-axis and the sample height (h, mm) on the x axis. Change in reflectance indicate droplet size changes due to aggregation and coalescence as well as sedimentation.

Finally, formulations with UV filters were compared using the Turbiscan Stability Index (TSI), that represents an absolute number related to the general behavior of the formulation; TSI sums all the variations detected in the samples in terms of size

and/or concentration. The higher is the TSI, the worse is the stability. In this work, TSI values at different heights of the sample and at different times were considered.
Formulations were mixed and then transferred in test tube (10 cm height). Samples were scanned (number of total scans: 216) for 3 days at 20°C.

Evaluation of microbiological contamination

Microbial contamination of formulations was determined by a microbial count. For this purpose, 10 ml of a non-selective broth (Eugon LT100) were added to 1g of each formulation and stirred at room temperature by vortex mixer.
Afterwards a selective medium was used; specifically Triptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) culture mediums were used to search for bacteria and yeast/mold, respectively. 1ml of formulation diluted with non-selective broth was withdrawn and seeded in an empty Petri plate. Finally, the media were added in a suitable amount to the neutralized product solution and mixed up to homogeneity.
Once the medium was solidified, the plates were turned upside down and placed in incubation: the samples intended for search of bacteria were incubated at 36°C ± 1 ° C for 24-48 hours. In parallel, samples for research of mold and yeast were incubated at 20°C ± 1 ° C for 48-72 hours.
Emergent colonies were counted after the necessary incubation. All operations were carried out in triplicates.
All materials (water for culture mediums, culture mediums, broth, tips) were sterilized by autoclaving [1 atm, 121°C for 20 minutes] before use. The analytical procedure was in according to UNI EN ISO 22718. [36]

RESULTS AND DISCUSSION

Mechanical test

For the evaluation of possible changes in packaging due to treatments and/or contact with filling formulations, a tensile test was performed on packaging in order to investigate the mechanical behavior of plastic containers after stress conditions.
For this purpose 20 specimens for each type of containers (empty and not treated, filled with formulations and treated with simulated solar irradiation and thermal

shock as described above) were obtained. Each specimen was then characterized measuring thickness and width of central section (break point).

Once acquired the measures for all the samples the tensile test was carried out. The following tables (Table 2, 3) report the data obtained, with an illustrative graph of the stress/stress profile curve for both polymers (Figure 1).

Table2. Tensile test's data obtained for HDPE packaging

HDPE	Tensile stress (σM)= Yield stress (σy) (MPa)	Elongation at yield/ section (mm^{-1})	Tensile stress at break (σB) (MPa)	Elongation at break/ section (mm^{-1})
T0 empty	23.954	2.099E-01	39.228	3.708E+01
F1 shock	20.960	2.870E-01	38.418	3.857E+01
F1 sun	21.268	2.827E-01	39.074	3.918E+01
F4 shock	20.050	3.353E-01	39.107	4.339E+01
F4 sun	20.889	2.986E-01	38.842	3.973E+01
F5 shock	22.481	2.684E-01	39.671	4.062E+01
F5 sun	23.213	2.511E-01	38.371	3.854E+01

S.d. < 10%

Table3. Tensile test's data obtained for PET packaging

PET	Tensile stress (σM)= Yield stress (σy) (MPa)	Elongation at yield/ section (mm^{-1})	Tensile stress at break (σB) (MPa)	Elongation at break/ section (mm^{-1})
T0 empty	58.513	4.306E-01	112.975	1.921E+01
F1 shock	62.639	5.902E-01	115.831	1.969E+01
F4 shock	55.707	5.736E-01	108.089	1.838E+01
F5 shock	57.355	5.216E-01	107.208	1.823E+01
F6 shock	56.764	5.700E-01	108.652	1.847E+01

S.d. < 10%

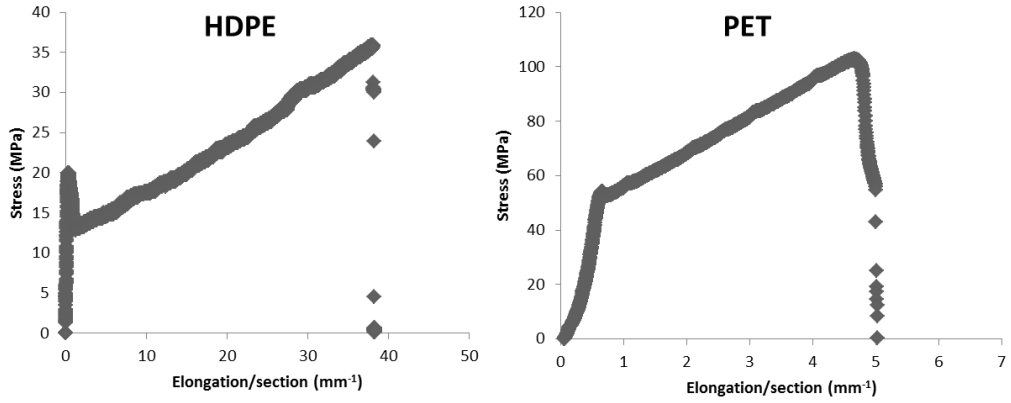

Figure1. Graphs obtained from tensile test for HDPE (on the left) and PET (on the right) packaging

Empty containers (t0 samples), both for HDPE and PET, presented the lowest value of tensile strain at yield respect to treated samples. As observable from Table 6, containers filled with F4 formulation and treated with thermal shock cycles presented a bigger increase in values of elongation at yield and at break.

For PET containers, stress conditions seem to provoke changes in mechanical parameters in the presence of different preservatives and/or essential oil for treatments and contact times considered.

Colorimetric analysis of packaging

Bottles of HDPE before and after treatments were also analyzed from a colorimetric point of view, in order to find possible changes in color of external and internal part of containers due to treatments. The same measurement could not be done for PET containers because of they were transparent and translucent.

For the colorimetric evaluation of packaging external side, the measurement of Yellowness Index (YI) was performed. For this purpose, the colorimeter was used and with the equation reported in ASTM 313, the Yellowness Index was calculated.

Table 4 report the Yellowness Index (YI) values and the related difference between t0 and treated samples (ΔYI), for HDPE polymer (external side of containers).

Table4. Yellowness Index (YI) and ΔYI values of not treated and treated packaging samples evaluated in the external and internal side.

	External side		Internal side	
	YI	**ΔYI**	**YI**	**ΔYI**
t0	8.966		6.840	
empty shock	5.886	-3.080	4.861	-1.979
empty sun	4.400	-4.566	7.351	0.511
F1 shock	7.442	-1.524	5.584	-1.256
F1 sun	4.709	-4.257	7.549	0.709
F4 shock	6.846	-2.120	5.474	-1.366
F4 sun	4.247	-4.719	7.565	0.725
F5 shock	6.227	-2.739	5.377	-1.463
F5 sun	3.994	-4.972	7.811	0.971

Observing the Yellowness Index calculated values, it's possible to note that all treated samples reported a decrease in Yellowness Index respect to not treated and empty sample (t0). The YI is a number that indicates the degree of departure of an object color from colorless or from a preferred white toward yellow. By this calculation, positive (+) ΔYI indicates increased yellowness and negative (-) ΔYI indicates decreased yellowness or increased blueness. [29] From this consideration it's possible to conclude that all treated samples presented a shift toward blue, so a decrease in yellowness. In particular, the most influenced samples from treatments were the containers that underwent the solar simulated irradiation, as radiations provoke the biggest degradative effect. No formulations demonstrated to have a bigger influence than the others on external color of packaging.

Extractable testing

The organic extractable profile of the packaging material investigated (HDPE and PET containers) was established via HS-SPME extraction processes, following a method set up in a previous work on polyethylene, because it revealed to provide a complete insight of all the predominant organic extractables for the analyzed material. [30] HDPE was already characterized regarding extractables profile in a previous work by this research team (in phase of submission), but for completeness data and chromatogram are equally reported.

After subtraction of the extraction blanks results from the samples results and removal of the interfering peaks associated with bleeding of GC capillary column or SPME fiber coating, a list of compounds released by the analyzed polymer was extracted by GC/MS.

Organic extractables profiles of not treated containers (t0) for both materials are summarized in Table 5 and 6.

Table 5. Organic extractables profile of HDPE containers

Identification	CAS NR	Chemical formula	Molecular weight	HDPE % area
2,6-di-tert-butyl-benzoquinone	719-22-2	C14H20O2	220	1.59
Diisopropylnaphtalene	-	C16H20	212	1.71
Phosphoric acid tris(2-chloro-1-methylethyl) ester	13674-84-5	C9H18Cl3O4P	326	5.76
Diisobutyl phthalate	84-69-5	C16H22O4	278	2.61
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	82304-66-3	C17H24O3	276	2.24
Octinoxate	5466-77-3	C18H26O3	290	1.00
Diisooctyl phthalate	131-20-4	C24H38O4	390	1.57
Squalene	111-02-4	C30H50	410	0.71
Siloxanes	/	/	/	18.65
Aliphatic hydrocarbons	/	/	/	34.29
Olefins	/	/	/	29.87

Table6. Organic extractables profile of PET containers

Identification	CAS NR	Chemical formula	Molecular weight	PET % area
O-Acetylcitric acid triethyl ester	77-89-4	C14H22O8	318	0.41
2-Propanol, 1-chlorophosphate	13674-84-5	C9H18Cl3O4P	326	5.94
Diisobutyl phthalate	84-69-5	C16H22O4	278	3.22
Dibutyl phthalate	84-74-2	C16H22O4	278	1.31
1-Octadecanol da AMDIS	112-92-5	C18H38O	270	38.57
Octinoxate	5466-77-3	C18H26O3	290	6.73
2-Ethylhexyl trans-4-methoxycinnamate (isomer of Octinoxate)	83834-59-7	C18H26O3	290	8.6
Diisooctyl phthalate or Bis(2-ethylhexyl) phthalate	131-20-4	C24H38O4	390	3.74
Terephthalic acid, bis(2-ethylhexyl)ester or isomer	6422-86-2	C24H38O4	390	0.29
Squalene or isomer	111-02-4	C30H50	410	3.24
Aliphatic hydrocarbons	/	/	/	14.59
Siloxanes	/	/	/	13.36

These organic extractables generally fall into classes of compounds linked to the major constituents of the original plastic materials. For example, the profiles included compounds like antioxidants and additives (e.g. 2,4-Di-t-butyl phenol, phthalates) associated with the initial ingredients, impurities related to processing (e.g. esters) and degradation products of the polymers (aliphatic hydrocarbons).

Data indicated that the largest percentage of compounds extracted from containers at t0 is associated to polymers and/or additives degradation products.

Once containers have been characterized at t0 (not treated), the next step was the characterization of the substances that could be extracted from containers filled with the formulations described above after treatment with solar irradiation and thermal shock.

Results obtained from this analysis revealed that, for both materials in contact with the formulations after UV-vis irradiation and thermal shock cycles, substances closely related to the filling formulation were detected at relatively large levels. These substances were identified as residual of C12-15 Alkyl benzoate and C14-22 Alcohols (in all formulations samples), 2-Phenoxyethanol (in samples of F1 and F5)

and components of mandarin essential oil, specifically o-Cymene, d-Limonene, γ-Terpinene (for F5 and F6). They represent an interval of percentage included between 25 and 58% of the total extracted compounds.

Samples containing the essential oil presented the biggest relative percentage of substances related to formulations; this could be due to the composition of essential oil, that is a mixture of volatile substances and so easily lost by emulsion.

Figure 2-3-4 report Total Ion Current (TIC) chromatograms related to GC/MS analysis of both packaging materials before and after treatments and of formulations containing the essential oil (F5 for HDPE and F6 for HDPE and PET) before and after treatments in plastic bottles.

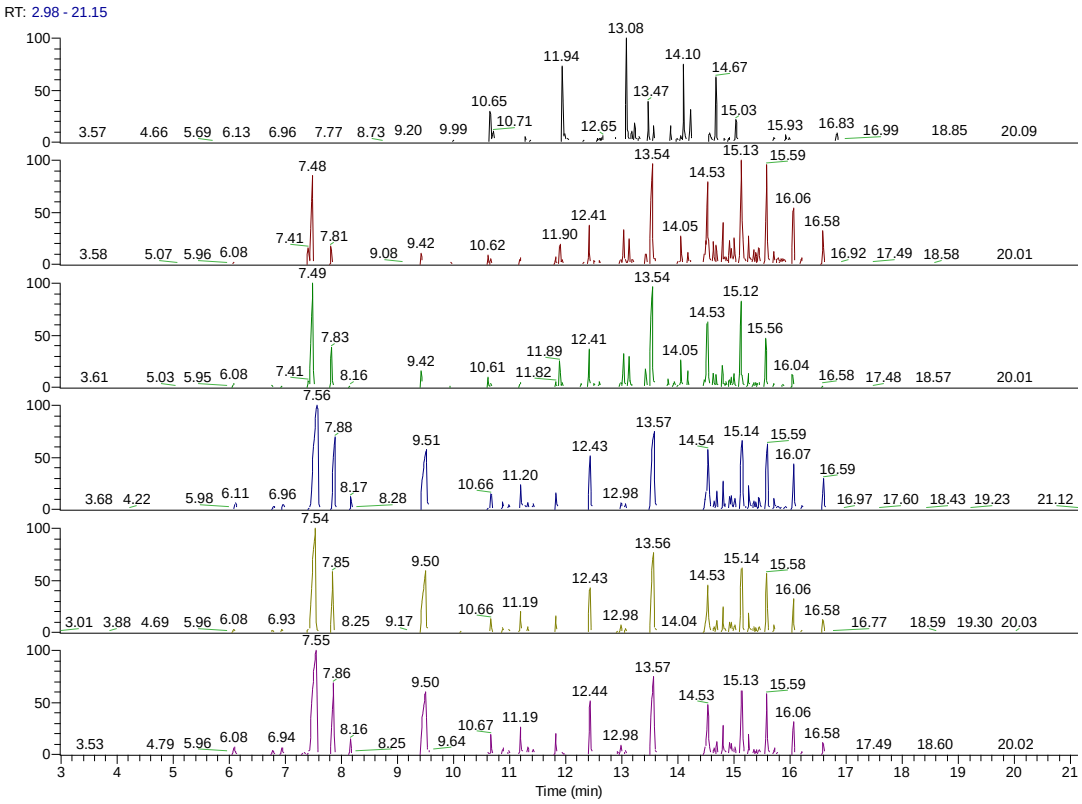

Figure2. Chromatograms of the HS-SPME extracts for HDPE. From the top: containers at t0, after suntest and after thermal shock with F5 formulations, formulation F5 at t0, after suntest and after thermal shock.

As observable from the figure, HDPE presents a characteristic extraction profile; after contact with formulation and subjected to treatments, the profiles of packaging materials' samples became more similar to formulations' profiles. This demonstrates an absorption of substances of formulations by packaging material.

Furthermore, analyzing formulations with the same method, it was observed that samples of formulation F5 containing the essential oil present in their profile a peak (RT 8.16 min) corresponding to a molecule contained in essential oil, p-Mentha-1,4(8)-diene. This substance is present in emulsion at t0 (not treated) and after thermal shock, but it no more appears in samples treated with UV-vis irradiation. This could be index of a partial degradation/loss of components of by essential oil, confirming the results obtained from colorimetric evaluation.

Analogous comparison can be done for samples of F5 and F6 formulations contained in PET bottles. (Figure 5-6)

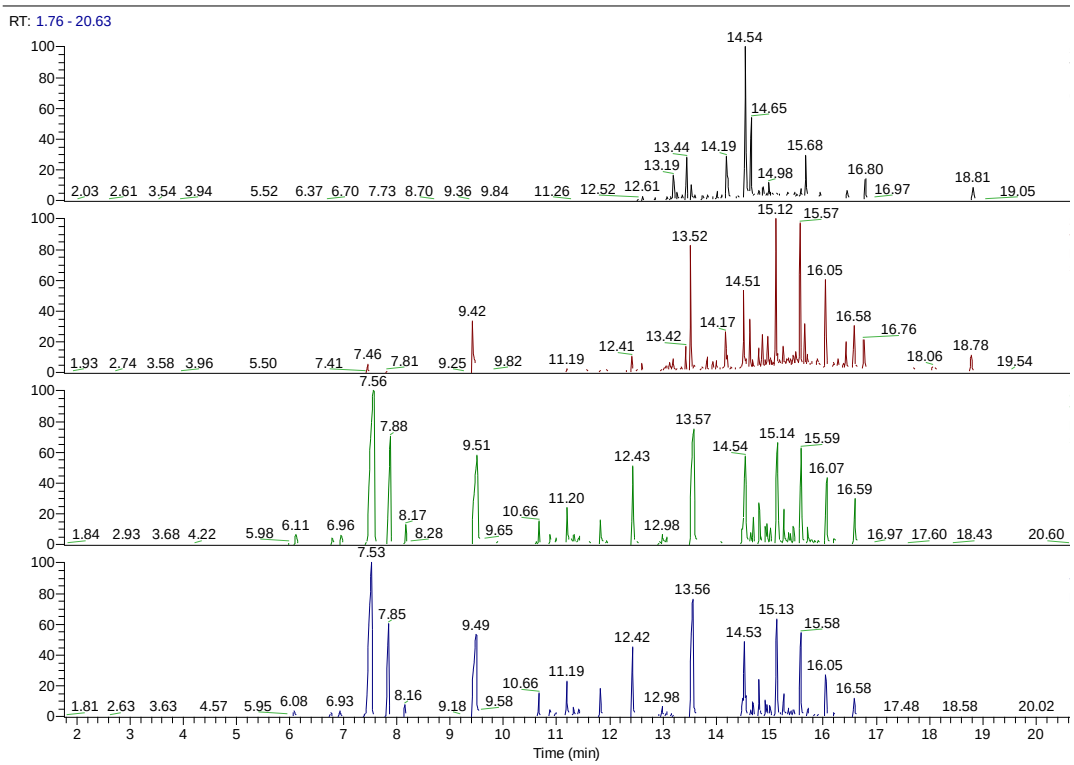

Figure3. Chromatograms of the HS-SPME extracts for PET. From the top: containers at t0, after suntest and after thermal shock with F5 formulations, formulation F5 at t0, after suntest and after thermal shock.

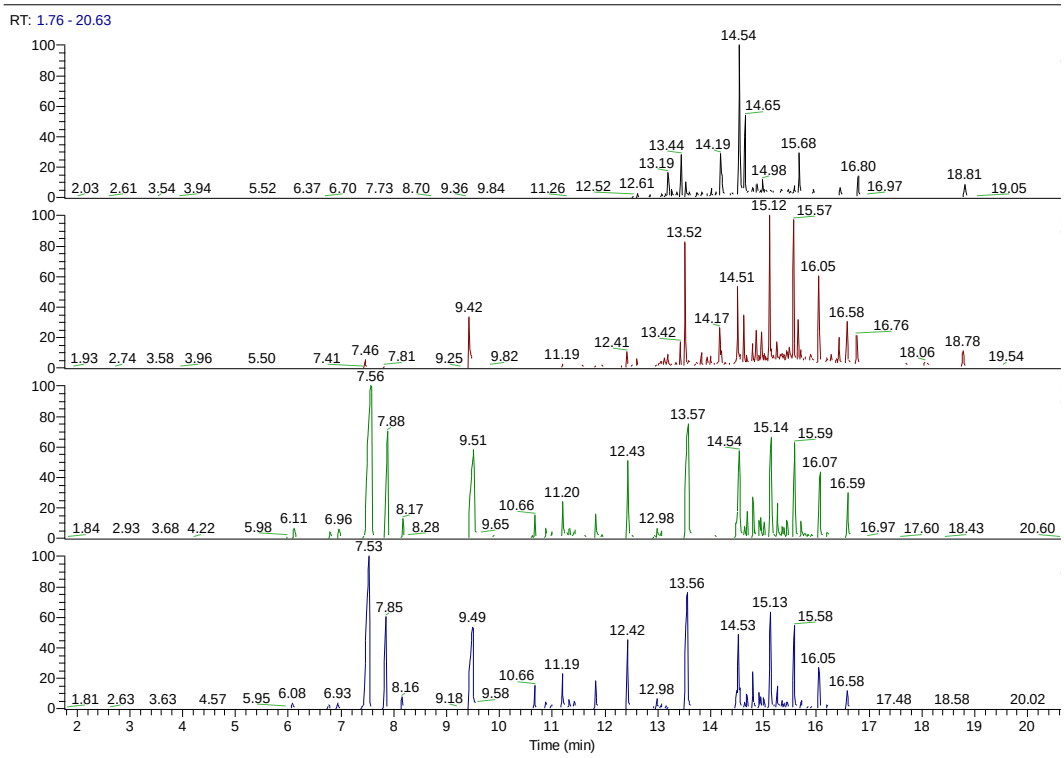

Figure4. Chromatograms of the HS-SPME extracts for PET. From the top: containers at t0, after suntest and after thermal shock with F6 formulations, formulation F6 at t0, after suntest and after thermal shock.

Observing chromatograms, it's possible to notice that also PET packaging absorbs substances from formulations, but this phenomenon has lower entity than HDPE. In particular, it can be observed as PET samples presented a lower absorption of essential oil's components than HDPE containers respect to other substances of formulation.

Furthermore, in addition to the assessment of the extractables profiles, a first screening of substances eventually migrated as a result of the treatments from the containers and accumulated as leachables into the formulations was performed.

Samples of each formulation (300 mg) in contact with package and undergone to UV-vis irradiation and thermal shock cycles have been analyzed by HS-SPME/GC-MS. No substances related to the polymeric materials were detected within the formulations.

24 hours after preparation, 24 hours after the end of treatments and after two months from treatments formulations were characterized from an organoleptic and rheological point of view.

Regarding the organoleptic characterization of formulations (color, odor, general aspect) no changes were observable after treatments and after two months aging, except for formulation F5, containing the mandarin essential, oil after treatment with UV-vis irradiation. In fact, this sample, contained in HDPE bottles, revealed a change in color, with a great discoloration respect to not treated sample, as shown in Figure 5.

Figure5. Discoloration for F5 sample treated with UV-vis irradiation (on the left) respect to t0 sample (on the right).

This evident variation was confirmed and quantified also by colorimetric analysis.

Regarding pH measurements, no significant changes were revealed for the analyzed samples, as shown in Table 7.

Table7. pH values of formulations before and after treatments and after two months

	t0	***HDPE suntest***	***HDPE shock***	***PET shock***
F1	5.65	5.66	5.51	5.55
F4	4.3	4.31	4.24	4.23
F5	5.53	5.57	5.63	5.61
F6	4.32	-	-	4.35

F4 and F6 formulations showed a lower pH than F1 and F5 because of the presence of the more innovative and natural preservative, that contains Triethyl Citrate, Glyceryl Caprylate and Benzoic acid.

Regarding the evaluation of viscosity, as index of rheological behavior of formulations', Table 8 reports viscosity's values expressed in mPa*s corresponding to a shear rate value of 10 rpm as representative of the entire curve obtained.

Table8. Viscosity values of formulations before and after treatments

Shear rate: 10 rpm							
F1	**η (mPa*s)**	**F4**	**η (mPa*s)**	**F5**	**η (mPa*s)**	**F6**	**η (mPa*s)**
t0	5600	**t0**	6000	**t0**	5200	**t0**	6600
HDPE suntest	5800	**HDPE suntest**	6200	**HDPE suntest**	4800	**HDPE suntest**	-
HDPE shock	6800	**HDPE shock**	6200	**HDPE shock**	6000	**HDPE shock**	-
PET shock	5800	**PET shock**	6400	**PET shock**	5800	**PET shock**	7000

As the table shows, for F1 formulation no significant changes were found after treatments. Only the sample treated with thermal shock cycles reported a little higher viscosity values. The same behavior was found for sample F5.

From reported viscosity values it can be concluded that in this case the different preservatives do not influence rheological properties of analyzed formulations; also the presence of the essential oil has not impact on products viscosity.

No differences were revealed in terms of rheological properties between formulations contained in HDPE or PET bottles.

Colorimetric evaluation

With the same technique, an evaluation of internal side of containers was performed, in order to investigate the effect of formulations on the packaging after contact times. For this purpose ΔE* values were calculated; this parameter indicates the difference or distance between two colors. L1, a1 and b1 represent the parameters' value for not treated sample (t0), taken as reference.

The same evaluation was performed for formulations themselves. Table 9 reports all ΔE* values related to formulations after treatments in HDPE and PET packaging; each sample is compared with not treated formulation (t0).

Table9. ΔE* values of formulations.

	ΔE*
F1 HDPE shock	0.612
F1 HDPE sun	1.020
F1 PET shock	0.998
F4 HDPE shock	0.159
F4 HDPE sun	1.682
F4 PET shock	0.843
F5 HDPE shock	0.394
F5 HDPE sun	7.595
F5 PET shock	0.528
F6 PET shock	1.363

Looking at the table, formulations' samples do not show important changes after treatment in both packaging materials. The only sample that reported a great variation in color is the formulation F5, containing the essential oil, treated with simulated solar irradiation. This instrumental evaluation confirms the observation already made during the organoleptic evaluation; in fact, the entity of color change is enough high to be observed by human eye.

NIR analysis

The NIR (near infrared reflectance) spectroscopy technique was used for a further non-destructive characterization of formulations in order to reveal possible changes before and after treatments.

All samples were analyzed in triplicate by MicroNIR and data were evaluated using principle component analysis (PCA) on pretreated spectra, as previously explained. This technique was able to confirm the results obtained by spectrophotometer analysis.

Here the results of PCA are reported.

First of all, as observable from Figure 6, formulations that differ each other for an ingredient (preservative or essential oil) were compared. In order to further investigate the stability of not treated formulations, each sample were analyzed in two different position of container (top and bottom), in order to evaluate possible stratification or separation phenomena.

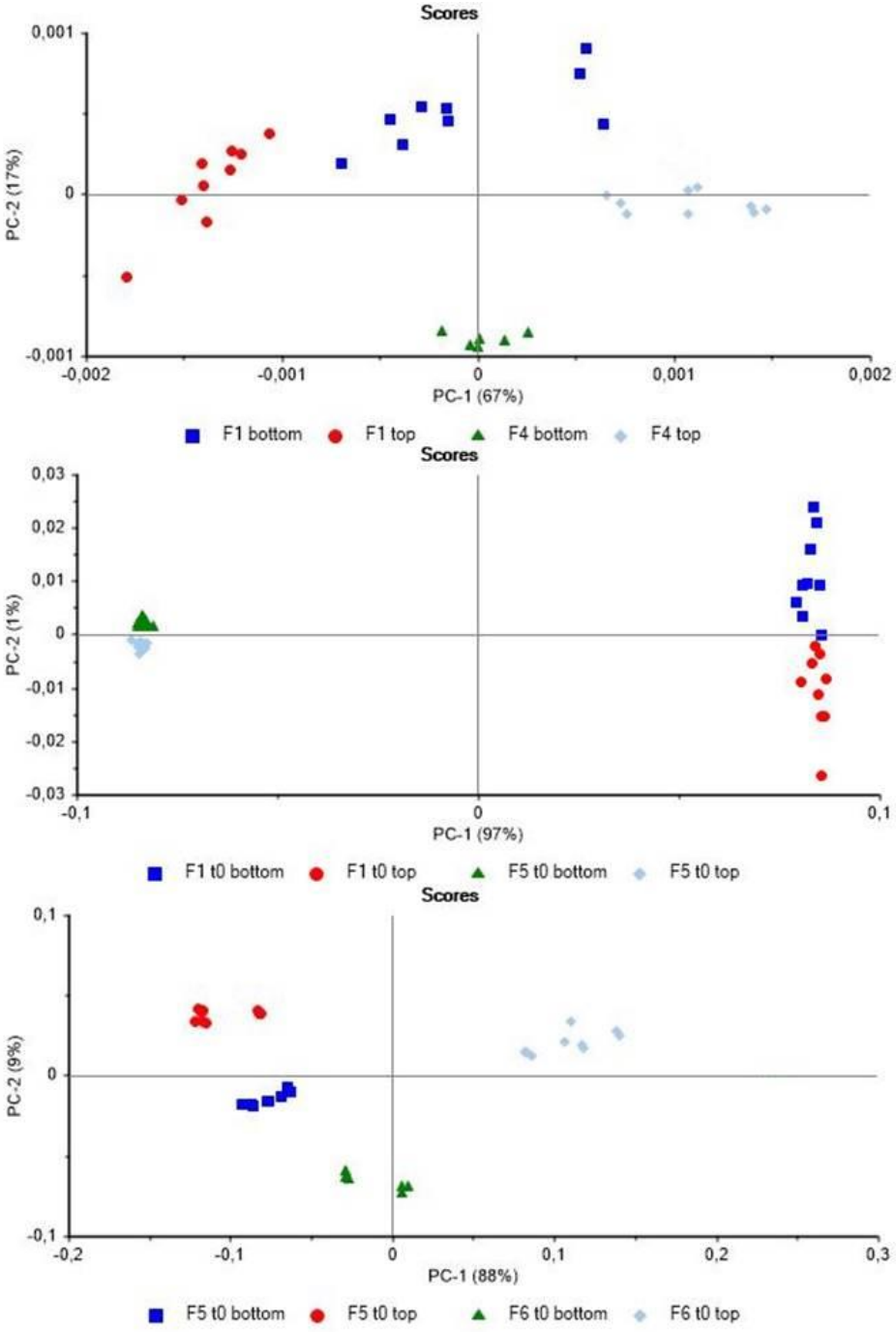

Figure 6. From the top: PCA with comparison between F1 and F4, up and bottom; PCA with comparison between F1 and F5, up and bottom; PCA with comparison between F5 and F6, up and bottom.

As observable from figure, for the comparison between F1 and F4, PC-1 is able to discriminate the two samples for preservative, but the concentration of this ingredients is too low to have a netter distinction. No evident difference was noted between samples withdrawn from the top or from the bottom of container. Regarding the second comparison, it's evident that the discriminant represented by PC-1 is the presence of essential oil. Finally, from the third comparison it's possible to note that samples are distributed more homogeneously because of the presence of essential oil in both formulations; however, for both samples, the withdrawal from the top and from the bottom are distinguished for both formulations, as there was a stratification phenomenon. Furthermore, the different preservative system is highlighted on PC-1.

From these considerations, an analysis on formulations F1 and F5, at t0 and treated with simulated solar irradiation and thermal shock cycles (in HDPE and PET containers), were performed, always sampling from top and bottom of container. Figure 7 reports the resulted PCA.

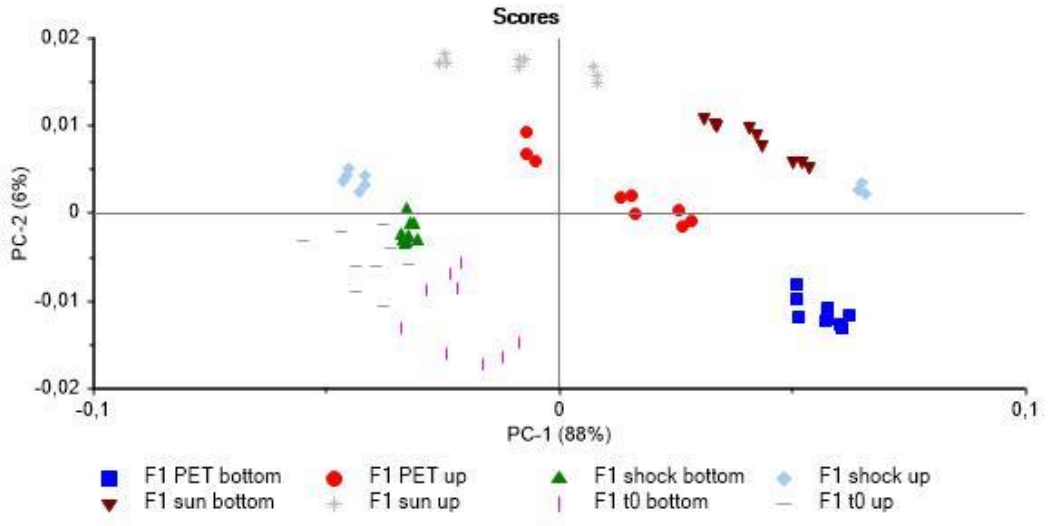

Figure 7. PCA with comparison between F1 t0 up and bottom with F1 treated in suntest (up and bottom) and with thermal shock cycles (up and bottom), in PET and HDPE.

Observing the figure it emerged that thermal shock cycles seemed not to have effect on sample, there are no distinction between top and bottom of container, but the sample seems to be homogeneous. Instead, the simulated solar irradiation in HDPE bottles and thermal shock in PET bottles result as different, with a netter difference between top and bottom withdrawal. This indicates that sample is less homogeneous. Finally, sample F5 containing the essential oil was analyzed in the same way. Resulted PCA is reported in Figure 8.

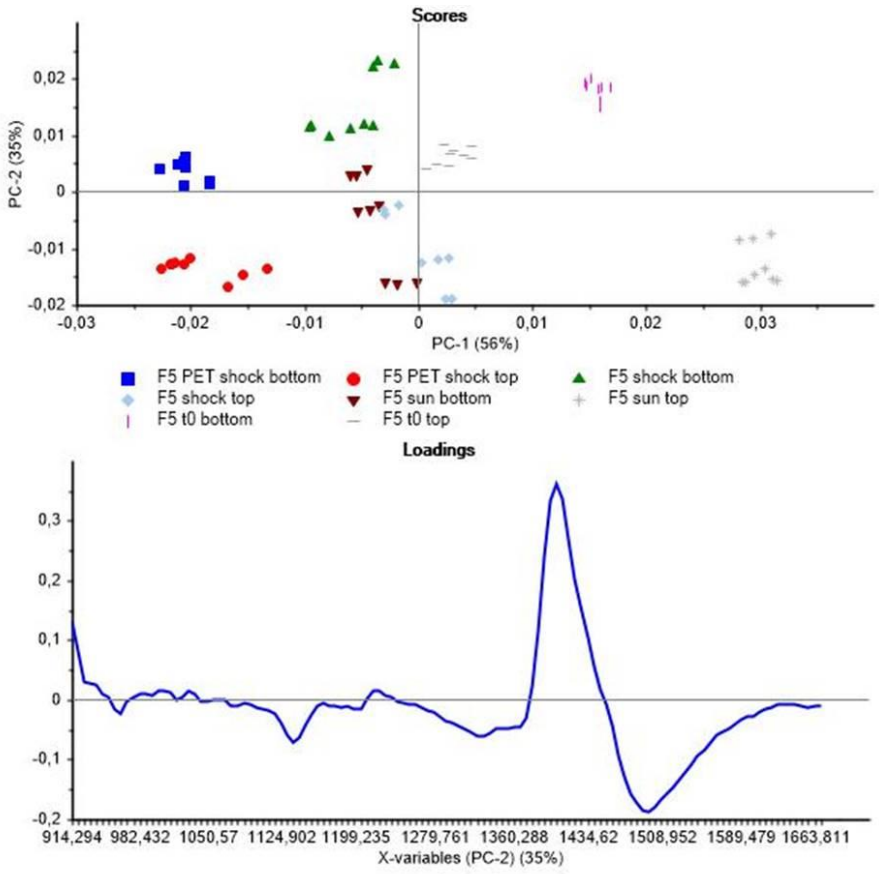

Figure 8. On the top: PCA with comparison between F5 t0 up and bottom with F5 treated in suntest (up and bottom) and with thermal shock cycles (up and bottom), in PET and HDPE. On the bottom: loadings chart for PC-2.

From this image, it can be observed that sample in PET is differentiated from sample in HDPE bottles on PC-1. In this case thermal shock treatment have a lower influence because sample is more homogeneous and nearer to the t0. Sample at t0 id homogeneous respect to the withdrawal positions, while for sample treated in suntest, there is a separation between top and bottom.

Finally it can be considered that the contribute on PC-2 is given by water variation, index of possible evaporation phenomenon, as illustrated by the loadings chart, where the peak corresponding to water is evident.

Multiple light scattering

The multiple light scattering technique with Turbiscan Tower was used as further technique in order to evaluate the stability of formulations, after three months since formulations' preparation. In the previous part of work, NIR spectroscopy has already revealed a difference between samples withdrawn on the top and the ones on

the bottom of containers, as there were some instability phenomena (creaming, stratification, etc.) after three months since preparation.

Samples were scanned (number of total scans: 216) for at least 3 days, stored at 20°C.

Here the ΔBS graphs (%) are reported in Figure 9.

ΔT values are not shown because the samples are milky and therefore there are no variations in transmission. Also the TSI value of the central zone is not reported because from the ΔBS graph (%) no significant changes have been observed (<1%); this also confirms the absence of coalescence phenomena in the central portion of samples examined.

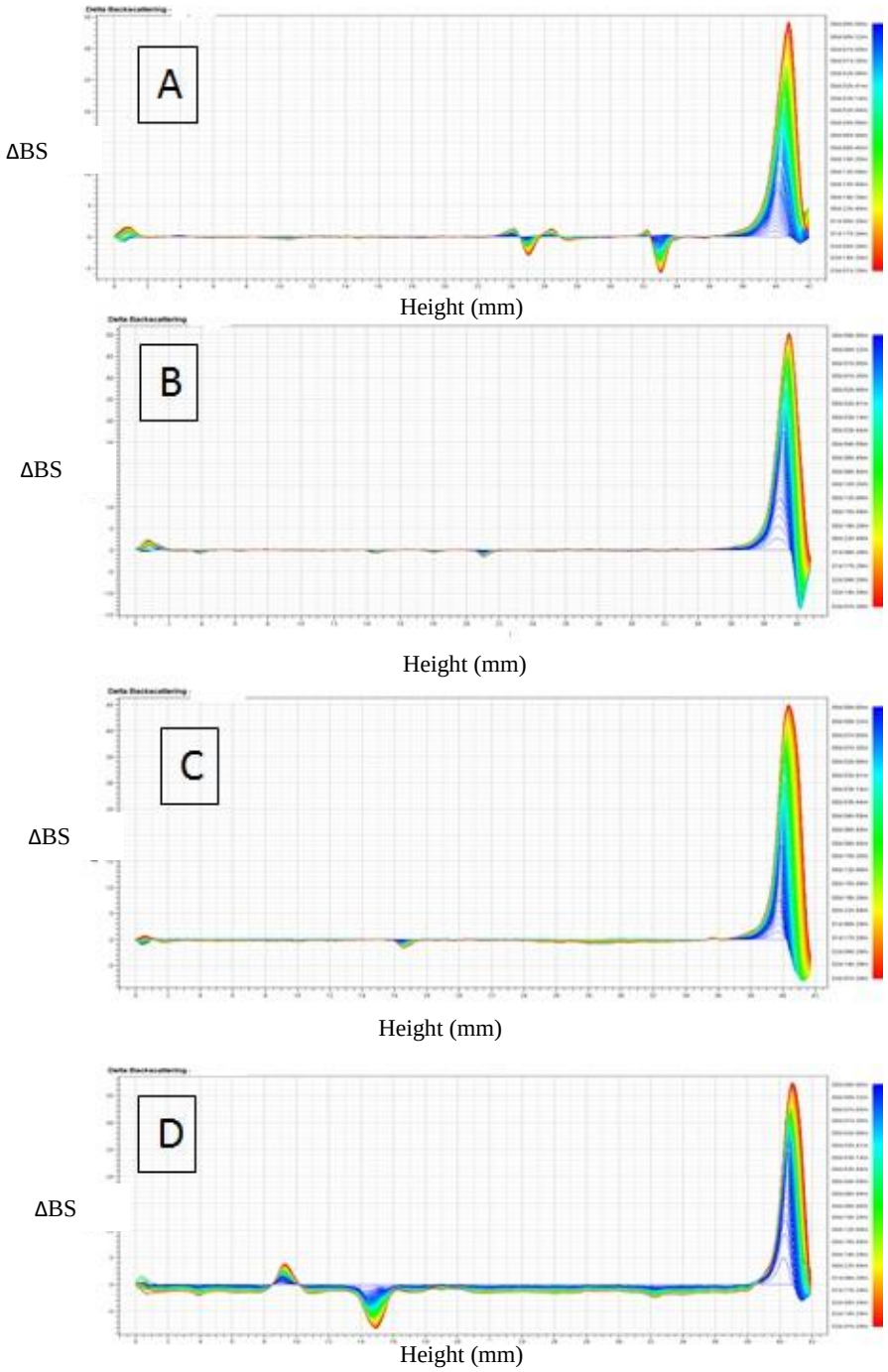

Figure9. ΔBS (%) of F1 (A), F4 (B), F5 (C) and F6 (D) samples.

For all samples kept in stability for three months a creaming phenomenon can be observed in the upper part of vial.

Observing the kinetic profiles, it can be noted that formulation F1 presented an increase of creaming phenomenon of about 5.30 mm/day. On the other side, formulations F4, F5 and F6 presented an increase of creaming phenomenon in a range of 3.50-3.90 mm/day.

However all four formulations reached a plateau phase after about 6 hours since the test begin, keeping a profile almost parallel to abscissas axis (kinetic profiles of 0.20 mm/day).

Coupled of formulations F1-F5 and F4-F6 have a comparable profile, coherently with the presence of the same preservative, that is the mixtures Phenoxytehanol, Caprylyl Glycol and Triethyl Citrate, Glyceryl Caprylate, Benzoic Acid respectively.

Finally, the TSI values are reported for all formulations. Table 10 reports the values of global TSI for both formulations at one hour, 12 hours, day one, two and three.

Table10. Global TSI for all formulations, 1h, 12h, day one, two and three.

Measure	Global TSI 1h	Global TSI 12h	Global TSI 1d	Global TSI 2d	Global TSI 3d
F1	0.7	1.6	1.9	2.2	2.3
F4	1.2	2.5	2.8	3.1	3.3
F5	0.6	1.7	2.1	2.4	2.6
F6	1.3	2.6	3.1	3.4	3.6

Samples kept in stability for three months and added with the innovative preservative demonstrated to be more unstable than the others. From this consideration, it's possible to infer that addition of the mixture Triethyl Citrate, Glyceryl Caprylate, Benzoic Acid could cause an higher emulsion instability (higher TSI values) and this phenomenon is independent from the presence of essential oil; the essential oil has no influence on system's stability.

Evaluation of microbiological contamination

Microbial contamination of formulations was determined by a microbial count, in order to evaluate if the different preservative systems underwent some variations in terms of activity caused by treatments and/or contact with packaging.

For this purpose, the microbial count was performed after formulations preparation and one month since the end of treatments (UV vis irradiations and thermal shock cycles).

In Table 11, the results of count (expressed in Colony Forming Unit, CFU) are reported for each analyzed sample.

Table11. Results of microbial count for all samples before and after treatments in both packaging materials, expressed as CFU/g

Sample	t0	HDPE sun	HDPE shock	PET shock
F1	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
F4	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
F5	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
F6	<10 CFU/g	/	/	<10 CFU/g

All samples resulted to be complied with the acceptability limits imposed by the law in force, that is 10^2 CFU/g for this king of product.

These results show that there are no difference in terms of efficacy between the two type of preservatives, but both product were able to inhibit the microbial growth inside O/W emulsions.

Furthermore, no treatments or contact with packaging resulted to be able to decrease the preservatives' systems activity.

CONCLUSIONS

This work aims to study the stability of topical formulations, before and after two different treatments (solar irradiation simulating test and thermal shock cycles), wanting to mimic the possible stress conditions that cosmetic products could meet during their "in use" life, in relation to a plastic packaging (made by a mixture LDPE/ HDPE or PET), commonly used for this kind of products.

Results of mechanical analysis showed that empty containers (t0 samples), both for HDPE and PET, presented the lowest value of tensile strain at yield respect to treated samples; moreover, for PET containers, stress conditions seem to provoke changes in mechanical parameters.

About the evaluation of packaging color, it's possible to conclude that all treated samples presented a shift toward blue, so a decrease in yellowness. In particular, the most influenced samples from treatments were the containers that underwent the solar simulated irradiation.

The extractables testing led to note that substances closely related to the filling formulation were detected at relatively large levels in packaging after contact with

formulations, especially for emulsion containing the essential oil. Furthermore, a partial degradation/loss of components of by essential oil, especially for products contained in HDPE bottles, was conjectured after analysis of formulations with the same method.

A colorimetric evaluation of formulations allowed to highlight a great variation in color in the formulation F5, containing the essential oil, treated with simulated solar irradiation.

Finally, multiple light scattering technique showed that samples added with the innovative preservative were more unstable than the other, independently from the presence of the essential oil.

In conclusion, results obtained from this work permitted to conclude that the choice of preservative can affect emulsion stability; furthermore, the packaging chosen can greatly interfere with component stability of a cosmetic product.

Therefore, this study confirms the importance to study all aspects related to a final product; in fact the evaluation of formulation is important but it is essential to verify the use of a suitable packaging in order to assure the quality, the efficacy and also the safety of the final product.

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CONCLUSIONS

These three years of work on this project represented a systematic approach to the problem of packaging-content interaction's studies.

In particular this work, reported in different research articles, focused on the safety of the end-product, with an evaluation of the risk associated to the packaging seen as an ingredient in relation to the specific product it is intended for. It can be defined as a rationalization of a complex problem, specifically a semi-solid matrix, selecting critical issues and parameters, like pH, oils typology, actives, etc.

Starting from traditional and mostly used materials this PhD project represents an approach for the future evaluation of innovative packaging materials, that are becoming increasingly important, in relation to a semi-solid contained product.

The work in its completeness involved the use of several packaging materials, with different characteristics (e.g. molecular weight, density, chemical reactivity, shape, capacity…). The setup of the different methods was performed using a single material, but subsequently these protocols were applied to other plastics in order to demonstrate their usability and reproducibility.

First of all this work underlined the importance of acquisition of necessary information in order to perform a complete safety assessment that expects the aspects of final packaging as raw material (ingredient); in particular these features regard the mechanical characterization and the extractables profile.

The obtained results have to be seen in function of what is contained in the product and which is the intended use; according to this, specific study protocols for the interactions' evaluation have to defined applying a design of experiment.

Looking at the results reported it can be noted that it's necessary identifying test conditions and parameters that can be indicative of a product shelf life and stability.

During this project regarding the mechanical characterization a sampling optimization was obtained, in order to have reproducible results, applying to every shape and dimension of containers.

Relying on evaluation of extractable substances, it can be concluded that packaging suppliers should provide the extractable profile (divided into classes of substances) for a specific end-product, associated to its mechanical characteristics. On the other side, the company of finished product has to define the substances that can be hazardous and to perform a quantitative analysis during stability period, using parameters defined by specific protocols based on kind of packaging and use.

Furthermore new simulants usable for studying of interactions were proposed. In particular one alkaline solution and two alkaline emulsions were set up in order to define different kinds of alkaline simulants, missing in the actual Regulations and guidelines.

Moreover, this work wanted to propose the use of NIR spectroscopy technique associated to a classic quality and stability evaluation (e.g. rheological measurements, etc.), as a non-destructive method.

The importance of a colorimetric evaluation was pointed out, both for packaging and colored formulations, because almost all products can be exposed to sunlight or artificial light during their shelf life, from production to final use by consumer. Linked to this aspect the utility of use UV-vis irradiation and thermal shock cycles as stress treatments in stability protocols was highlighted. In fact, observing results of the different work phases, it emerges how these kind of protocols are more realistic and predictive than traditional test conditions for stability evaluation.

Finally, an alternative non-destructive technique was proposed as further stability evaluation, that is multiple light scattering. It allowed to have responses about the system's behavior in short times, confirming the results obtained by other traditional techniques.

This work represent just a start point for enlarging the knowledge in this unexplored field. With this awareness the future perspective are a lot.

First of all, the next step of this research should regard the study and the set up of a smart method for barrier properties of final plastic containers, in order to evaluate the capability of packaging to assure a complete protection from external environment, avoiding exchanges of substances from and to the outside.

The second focus should be the validation of a method for extractables and leachables evaluation, usable not only for polyethylene, but also for other plastic materials used in packaging field.

Finally, another possible point to develop could be the study of interactions problems related to the closures of packages and all those small parts that constitute them.

Annex I

Journal of Applied Polymer Science

Preliminary Evaluation of Packaging-Content Interactions: Mechanical and Physicochemical Characterization of Polylactide Bottles

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ABSTRACT: Biodegradable materials as polylactide (PLA) are very interesting for cosmetic packaging application. However, these polymers, under environmental conditions or/and chemical treatments, can undergo "aging," compromising their performances such as container. The aim of this study was the evaluation of mechanical, physicochemical, and organoleptic properties of PLA bottles present in the cosmetic market. In particular, mechanical tests and thermal analyses were applied to study the PLA container degradation under stressed physicochemical conditions. Calorimetric and morphological analyses were applied to evaluate differences between internal and external surface of containers. Results highlighted that the heating process together with chemical treatment determined a significant modification on polymer, leading to a more resistant and fragile material, whereas the only physical or chemical treatment alone showed a plasticizing effect. In conclusion, this study represents a start point to evaluate content–packaging interactions to optimize the choice of PLA polymer as cosmetic packaging. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40067.

KEYWORDS: biodegradable; packaging; properties and characterization; biopolymers and renewable polymers; biomedical applications

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INTRODUCTION

The packaging plays a very important role in the quality of cosmetic products. It must carry the correct information and identification of the product and it must protect the formulation against all adverse external influences, e.g., moisture, light, oxygen, and temperature variations, that could alter its properties.

It also must protect the content against biological contamination and physical damage. The complexity of packaging materials and the highly technological nature of cosmetic products is such that manufacturers have to deal with significant problems. The stability of the product and its compatibility with the containing material are distinct concepts, separate and complementary, that must be evaluated before that the product is commercialized. In fact, polymeric packaging can interact heavily with all components such as active ingredients, excipients, and solvents, used in a variety of cosmetic formula, and it is not said that protective layers, present onto contact area, are really efficient.

In some cases, this interaction determines color and thickness alteration of the cosmetic formula. In other cases, substance present into formulation could be absorbed or could be attacked from substances migrated from packaging (Figure 1).[1] For this reason, it is very important to reveal these effects by setting up of protocols and specific tests.

Testing about migration of material additives into cosmetic products is a crucial point of safety assessment of packaging material. However, New Regulation 1223/2009 establishes clearly that packaging–product interactions must be studied but it does not explicate them in details.[2] In fact, unlike the food and pharmaceutical products, in cosmetic field there are not yet specific guidelines concerning packaging/content interaction studies. At the present moment, the environmental problem from the waste of plastic packaging increases every year due to its light weight, easy process, and good properties for various application. However, these packaging, obtained from petrochemical-based polymers, such as polyethylene (PE), polypropylene, and polystyrene, are not biodegradable and can lie around for 500–1000 years without degrading. To solve this problem, the biobased polymers, made from renewable natural resources, biodegradable in 0.5–2 years, such as polylactide (PLA), polyhydroxyalkanoates, and polycaprolactone, are selected as packaging material.[3] Replacing the use of petrochemical plastic with bioplastic with comparable properties can reduce the use of fossil fuel such as crude oil, gas, and coal, which increase the CO_2 level in the air.[4]

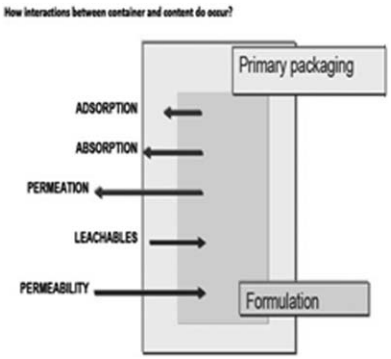

Figure 1. Scheme of possible interactions between packaging and contents.[1]

In particular, among bioplastic materials, PLA attracts both industries and research institutions. PLA is a member of the polyester family and it plays a predominant role as sustainable alternative to petrochemical-derived products. PLA is the linear thermoplastic aliphatic polyester produced by either ring-opening polymerization of lactide or condensation polymerization of lactic acid monomers that are produced from renewable resources such as corn by a fermentation process.[5–7]

The production of PLA presents numerous advantages: it can be obtained from a renewable agricultural source (corn); its production consumes small quantities of carbon dioxide; it provides significant energy savings; it is recyclable and compostable; its physical and mechanical properties can be manipulated by polymer architecture.[6] PLA exhibits good properties such as biodegradability, heat resistance, transparency, good mechanical properties, and processability, leading it to be used in many packaging applications.[8–10] The important requirement for packaging materials is high tensile strength, ductility, flexibility, transparency, and good barrier properties. However, PLA is still limited for its application because of its price, brittleness, rigidity, and low crystallization rate.[11–13] Due to its higher cost, the initial use of PLA as a packaging material has been in high value films, rigid thermoforms, food, and beverage containers and coated papers. In the last 5 years, the use of PLA as a packaging material has increased all across Europe, Japan, and the United States, mainly in the area of fresh products where PLA is being used as a food packaging polymer for short shelf life products. Currently available, PLA is used in compostable yard bags to encourage recycling and composting programs.

The main objective of this study was to investigate product–polymeric packaging relationship into cosmetic field, because low information have been found in this field. In fact, even more and more information are present in the literature relatively to characteristics and properties of polyethylene terephthalate and PE, a very few studies are related to PLA behavior used as packaging. In fact, about PLA, it is known from literaturethat degradation of this kind of polymer, when processed as plates or blends, is mainly due to intramolecular transesterification reactions leading to cyclic oligomers of lactic acid and lactide.[14–16]

It has been found that PLA degradation is dependent on a range of factors, such as molecular weight, crystallinity, purity, temperature, pH, presence of terminal carboxyl or hydroxyl groups, water permeability, and additives acting catalytically that may include enzymes, bacteria, or inorganic fillers.[3,17,18] Polymer degradation determines changes in the properties—tensile strength, color, shape, etc., of a polymer or polymer-based product under the influence of one or more environmental factors suchas heat, light, or chemicals.[19] In our knowledge, only one paper reports the degradation of PLA commercial film asfinished cosmetic packaging.[20] For this reason in this work, PLA commercial bottles were studied and characterized. PLA bottles, both empties and filled with monophasic standard solution of pH 2, were studied under stressed conditions (ultraviolet [UV]–vis exposition and temperature/humidity treatment) to evaluate the possible degradation both of packaging and contained formulation. These conditions are meant to simulate what will happen to the product during its life cycle and they were selected for the degradation experiment according to the recommendations of stability tests for cosmetic products.[21] In the case of PLA, the contact with cosmetic formulation can have an influence on crystallinity, leading to thermal and barrier property changes. Mechanical and thermal analyses techniques were employed to characterize physicochemical properties of containers. In particular, in this study, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and Fourier Transform Infrared Spectroscopy (FT-IR) were successfully applied to monitor and control the degradation of packaging due to photo-oxidation and/or environmental conditions by analyzing both internal and external surfaces of bottles. Finally, the ultimate goal of this work was to identify and understand the possible degradation phenomena that the PLA container may sustained during the life of the cosmetic product, to choose the best use of this polymer as cosmetic packaging depending on the type of product that must be marketed. In according to literature, parameters such as humidity, temperature, pH, salinity, presence or absence of oxygen have important effects onto degradation process.[3] For these reasons, in this work, PLA containers, in climatic room stored and UV–vis photoexposed were tested. Furthermore, it was very important to study simultaneously the internal and the external sides of the container to better understand what is the main factor that influences packaging stability and content–container interactions.

EXPERIMENTAL

Materials

Packaging materials, object of this study, were commercial bottles of 150 mL made of the resin Ingeo biopolymer produced by Nature Works, Minnetonka. The resin is a PLA biopolymer, PLA7001D, semicrystalline material, specific gravity 1.24, glass transition temperature 55–60°C, and crystalline melting temperature 145–160°C.

Degradation Testing Procedures

PLA bottles were numbered, weighted, and washed according to a washing procedure described below. Afterward, bottles filled with standard solution of pH 2 and empty bottles were subjected to different degradation tests:

- photostability test by simulating UV–visible ray irradiation using SUNTEST XLS +II (Atlas®) for 24 and 96 h;
- accelerated stability test by incubation into climatic room (ClimaCell 111 MMM) at 40°C with 75% RH for 30 days.

SUN TEST instrument was set up according to standard European procedures[22] and precisely to the following parameters:

- Time: 4 h corresponding to 192 h solar light
- Irradiation control: 300–800 nm
- Irradiation (W/m^2): 750
- Room temperature: 35°C
- Black standard temperature: 45°C.

In particular, solar ID65 filter has been used. This type of filter simulated solar radiation behind 6 mm window glass; it meets CIEID65 according to ICH Guideline.

Photostability test was performed according to Colipa guidelines about cosmetic products.[21]

Accelerated stability test was performed according to EMA: test parameters (duration, temperature, and humidity values) were set up by considering accelerated stability testing relative to pharmaceuticals products.[22,23]

The washing procedure, used at the beginning of the study and at the end of all treatments, was the following: all bottles were washed for three times with 1% bicarbonate solution and then for another three times with distilled water to remove bicarbonate residuals. The bicarbonate solution was considered appropriate to immediately buffer a possible release of additional acidic monomers from the polymeric chain, subsequent to treatment. The subsequent washing with water served, in turn, to remove any possible residual bicarbonate.

Finally, for all samples, several specimens were obtained to carry out mechanical, physicochemical, and morphological analyses.

Evaluation of Organoleptic Properties and pH Values of Contained Solutions

Organoleptic control of pH 2 standard solution, at the end of described treatments, was examined. In particular, color alterations, unpleasant odor, and particulate precipitation in aqueous solution were considered. Finally, pH of buffer solution was controlled after each treatments to verify a possible degradation of PLA. Values were compared with pH ones obtained considering PLA containers filled with distilled water and treated in the same conditions of photostability test after 24 and 96 h.

Mechanical Testing

The investigation of the mechanical properties of the bottles was performed using a tensile machine, AGS 500ND (Shimadzu corporation, Kyoto-Japan) equipped with a 500-N load cell; the test was performed using a strain rate of 2.0 mm/min. Five "bone-shape" specimens were obtained from each bottles sample; the feature of the specimens followed the principles of the European Standard EN ISO 527,[24] suitably modified for bottle containers. Samples were kept under constant temperature (23°C) and humidity (52% RH) for a week until tension test started. It has been retained opportune to maintain a temperature of 23°C and a relative humidity of 52% also during mechanical characterization at tensile tester. This procedure permitted to obtain a stress versus strain curve. From each set of results was possible to estimate the tendency of materials to oppose to deformation, to evaluate the curve profile in elasticity regime, the elongation percentage in elasticity regime and the absolute elongation elasticity.

Colorimetric Analysis

The instrumental assessment of packaging color was performed with Cutometer MPA580 (CK electronic GmbH, Germany) equipped with the Colorimeter CL400 probe. Technical data of probe are length: 126 mm, illumination: Ø 24 mm, measuring area: Ø 8 mm, weight: 85 g, illuminated area approx. 17 mm Ø; units: *xyz*, and RGB, $L \times a \times b$; light: 8 white LEDs arranged circularly, range of emitted wavelengths: 440–670 nm; and accuracy: ±5%.

The color acquisition is done by specifying the three tristimulus values *X*, *Y*, and *Z*, of a color in according to the CIE system, where *X* is a tristimulus value of red, *Y* to a green value, and *Z* to a blue value. Results are expressed by using the chromatic coordinates, which are set with *x*, *y*, and *z*, not to be confused with thefunctions *X*, *Y*, and *Z*; only two of these "chromatic coordinates" are independent, because the third is determined by the relationship: $x + y + z = 1$. So, since the chromatic characteristics of a color, such is the tone and the saturation, are easy specified by two coordinates, it is possible to use a planar representation of simple construction and easy to understand.

Differential Scanning Calorimetry

Temperature and enthalpy values were measured with a Mettler STARe system (Mettler Toledo, Novate Milanese, MI, Italy) equipped with a DSC821^e Module and an Intracooler device for subambient temperature analysis (Julabo FT 900) on 6–7 mg (Mettler M3 Microbalance) samples in sealed aluminum pans with pierced lid. Each sample was cut in a flat piece of suitable size to completely cover the bottom surface of the crucible. The piece was placed in the crucible in such a way that its internal or external side was in closely contact whit the bottom to evaluate possible differences in the polymer glass transition temperature due to the acid solution and/or the UV irradiation effect, respectively. To better characterize the solid state of PLA, thermal cycling, i.e., heating from 0 to 75°C, cooling to 0°C and reheating to 200°C [$\beta = 10$ K min^{-1}, nitrogen air atmosphere flux (50 mL min^{-1})], were performed. The instrument was preventively calibrated with Indium as standard reference. Measurements were carried out at least in triplicate.

Simultaneous Thermogravimetric Analysis (TG/DSC1)

Mass losses were recorded with a Mettler STARe system (Mettler Toledo) TG simultaneously DSC (TG/DSC1) on 6–8 mg samples in open alumina crucibles [$\beta = 10$ K min^{-1}, nitrogen air atmosphere (flux 60 mL min^{-1}), and 30–500°C temperature range]. The instrument was preventively calibrated with Indium as standard reference. Measurements were carried out at least in triplicate.

Fourier Transform Infrared Spectroscopy

Mid-IR (650–4000 cm^{-1}) spectra were recorded on samples using a Spectrum One Perkin-Elmer FT-IR spectrophotometer

(resolution 4 cm^{-1}, 16 scansions; Perkin Elmer, Wellesley, MA) equipped with a MIRacle™ ATR device (Pike Technologies, Madison, WI). Internal and external surfaces were considered. To confirm physicochemical analyses and to evaluate differences between internal and external surfaces, bottles were macroscopically examined also by visual evaluation during the degradation tests. Morphological characterization of both bottles surface was performed by scanning electron microscopy (SEM, Zeiss EVO MA10, Germany). Samples were immobilized on aluminum stubs and gold sputtered.

RESULTS AND DISCUSSION

Evaluation of Organoleptic Properties, pH, and Color

Buffer solutions were opportunely characterized to identify possible organoleptic alterations as a consequence of thermal and UV–vis treatments. Buffer solutions did not show color alterations and precipitate was not observed into bulk. Similarly, alterations of container shape and color of surface packaging were evaluated. Figure 2 shows results about colorimetric analyses carried out on PLA bottles before and after stability tests. In detail, color of PLA container as change in the chromatic coordinates *x*, *y*, and *z*, was observed. At the beginning of the study,

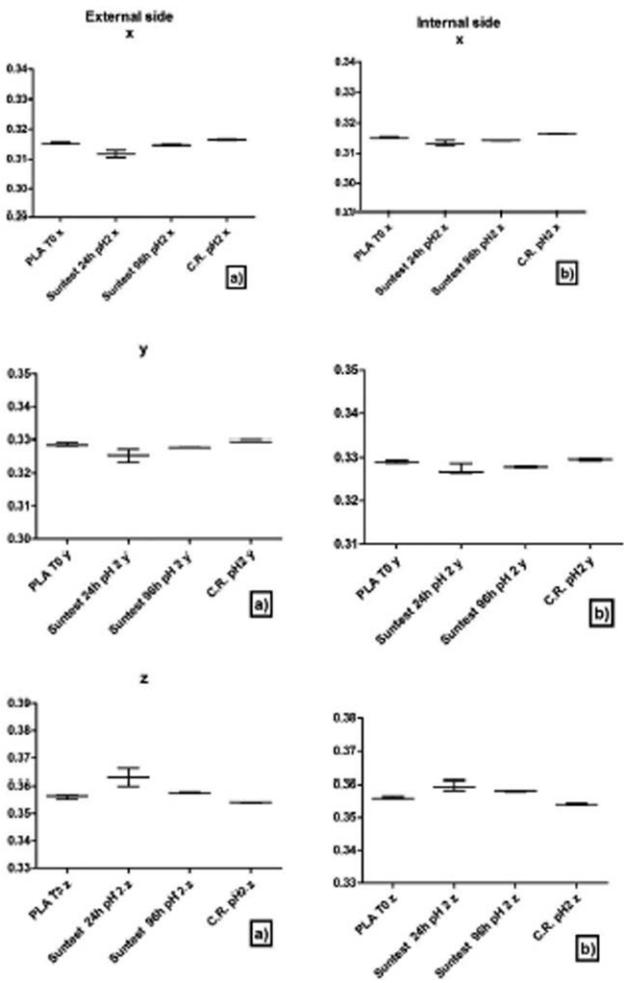

Figure 2. Chromatic coordinates *x*, *y*, and *z* values measured on PLA bottles during stability tests.

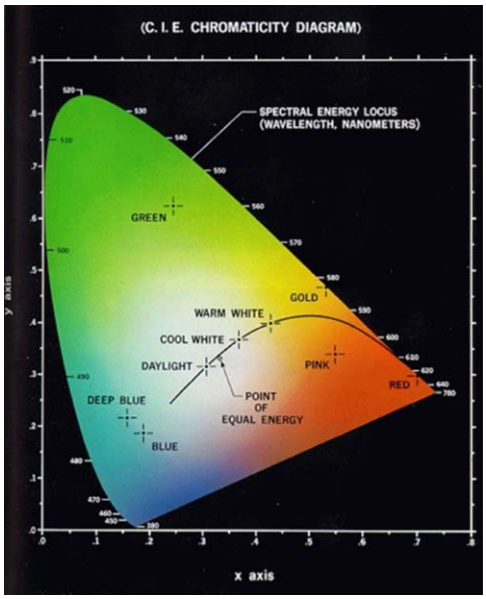

Figure 3. C.I.E. chromaticity diagram.[26] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

containers showed the same color both in the external and in the internal sides with coordinates values in the white color gradation of the CIE chromaticity diagram (Figure 3).[25] However, irradiated containers lost this gradation and seem clear.

This alteration was confirmed also from colorimeter analysis. In fact, after 24 h in suntest, all coordinates were different form the standard, both in the external and in the internal side (Figure 2). Instead, visible color changes were not revealed for containers maintained at room temperature and into climatic room. Table I shows the pH decrease percentage of buffer solution and distilled water, after photo and heat treatment. A significant pH reduction was observed after 24 h in suntest and after 30 days in climatic room both for buffer and for water. However, after 96 h UV–vis exposition, a considerable pH reduction was not noticed. We explain these results considering that, according to the literature, PLA degrades more quickly if subjected to high humidity percentage and high temperature and that degradation involves high molecular weight polyester chains that could hydrolyze into oligomers of lower molecular weight. In this way, lactic acids residuals could be released into solution determining a possible pH reducing and supporting an hydrolytic process. It is known that hydrolysis can be accelerated from acids and basis or simply from water, and affected by temperature and moisture levels.[26,27] Thus, we claim that pH reductions after 24 h of UV–vis irradiation and after 30 days in the climatic room could be due to respectively a release of some acid interchain monomers release and a surface erosion mechanism with consequent scission of acid lactic monomers. On the contrary, after 96 h of photoirradiation, the buffer effect of standard solution justified the insignificant difference of the pH value in respect to the initial one. In the samples containing water was not possible to speak about buffer effect. Indeed, the pH decrease was bigger than the one of samples containing buffer solution. In conclusion, results reported in Table I show that the solar radiation has a degradative effect greater, in terms

Table I. Percentage of pH Reduction in Respect to the Initial pH Value During Stability Test

Sample	pH decrease (%)
PLA suntest (24 h) H_2O	27 ± 1.2
PLA suntest (96 h) H_2O	8.2 ± 0.1
PLA suntest (24 h) pH 2	23 ± 0.17
PLA suntest (96 h) pH 2	1 ± 0.09
PLA climatic room (30 days) pH 2	15 ± 0.3

of pH, compared to the combined effect of humidity and temperature, characteristic of the climatic chamber. The lowering of pH, characteristic of buffer and water, resulted more evident when the samples have been treated in suntest for 24 h. It has been observed a drop of almost double compared to the value recorded in a climatic chamber at 30 days. However, the data recorded for irradiated samples at 96 h go against this trend and appear to be characterized by a lower degradation. An explanation for this phenomenon could be correlated to a possible tendency of the system to achieve a balance polymer–water/buffer more or less stable, depending on the ionic strength. In this sense, one could explain the greater lowering of pH immediately after 96 h in the case of polymer–buffer system, compared to the polymer–water system: the higher ionic strength of buffer system determines a bigger competitions between charges and this slows down the achievement of a balance.

Mechanical Properties

Starting from the principles of the European Standard EN ISO 527 concerning the evaluation of tensile properties of plastic materials,[21] in a previous not yet published work, Capra et al. set up an experimental protocol to obtain reproducible and standardized measurement of mechanical properties of commercial bottles thanks to a specimen realization with a suitable geometry. The same protocol was used to obtain mechanical results reported in this study. Figure 4 shows some stress/strain curves obtained in this work. From these curves, it is possible to calculate, for all samples, angular coefficients, as representative values of Young's modulus, % elongation in elasticity regimen and % absolute elongation.

Table II reports these parameters for the analyzed samples. It is possible to observe a correspondence between empty and filled

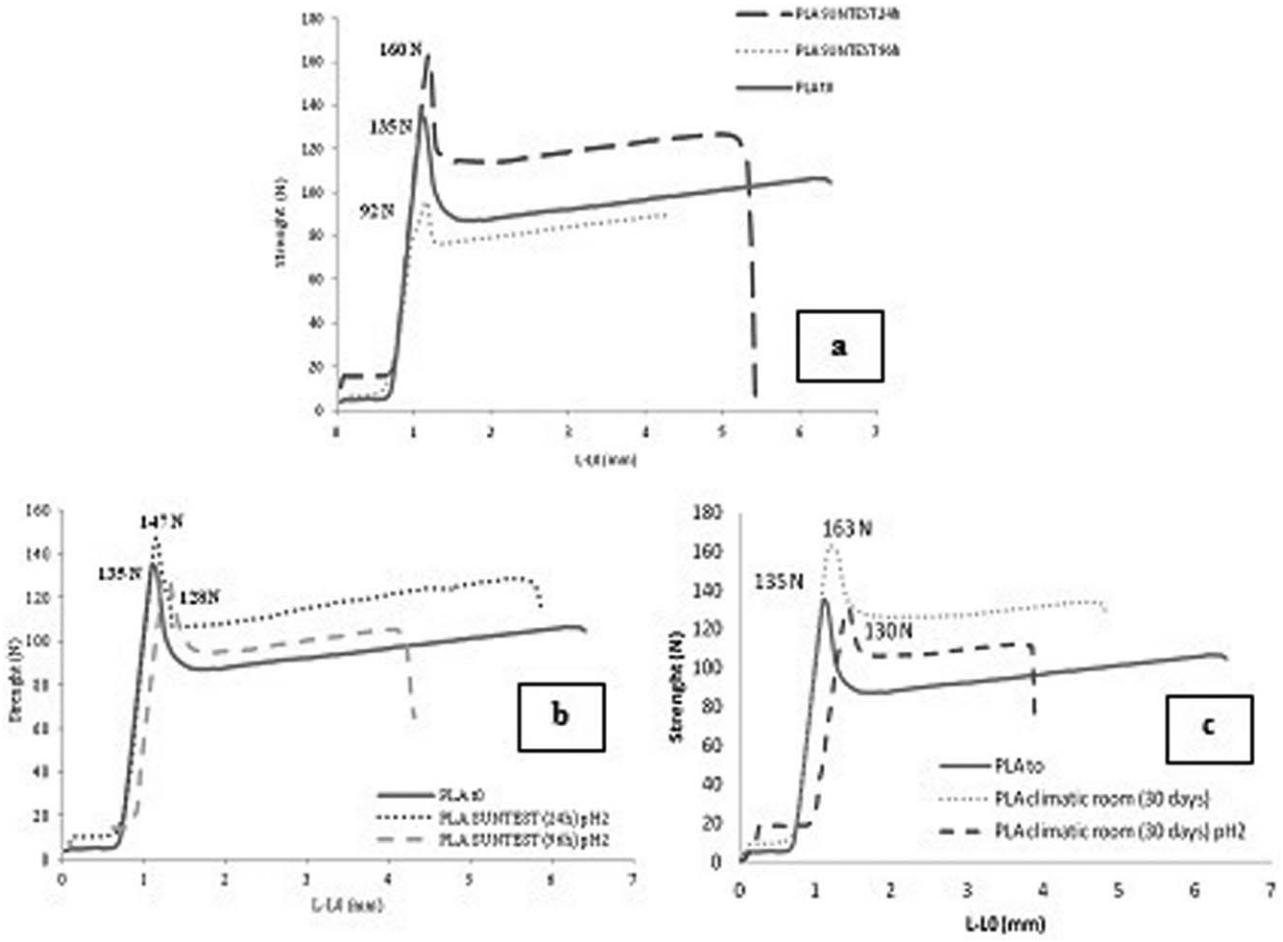

Figure 4. Stress–strain profiles of: (a) empty PLA bottles after photostability test; (b) PLA filled bottles (pH2) after photostability test; and (c) PLA filled bottles (pH2) after accelerated test, compared with the standard.

Table II. Mechanical Parameters of PLA Bottles, Treated With Standard Solution at pH 2 and to Different Simulated Environmental Conditions

Sample	Angular coefficient[a]	Elongation in elasticity regime (%)	Absolute elongation (%)
PLA0 (standard)	317.81 ± 7.1	6.4	79.4
PLA suntest (24 h)	321.28 ± 5.7	7	23.6
PLA suntest (96 h)	286.53 ± 3.2	6.9	25.5
PLA suntest (24 h) pH 2	330.32 ± 8.2	6.6	32.9
PLA suntest (96 h) pH 2	252.40 ± 1.3	7.1	23.8
PLA climatic room (30 days) pH 2	269.02 ± 3.2	8.1	21.2
PLA climatic room (30 days)	361.32 ± 3.3	7.1	26.4

[a] Angolar coefficient represents the tendency of material to oppose to deformation in the portion of elastic regime.

irradiated samples: after 24 h of exposition, elasticity profiles increase compared to the standard sample (PLA t_0). However, after 96 h of treatment an opposite tendency was observed: the angular coefficient decreases relative to standard. Comparing values of angular coefficient and elongation in elasticity regime with graphs reported in Figure 4(a,b), it is evident that PLA t_0 and PLA irradiated 24 h do not show significant differences in elasticity regime, because curve profiles are overlapped. However, strength at necking of irradiated sample (24 h) is higher than standard sample and this phenomenon can be explained with polymer plasticization during photoirradiation, but also, in the case of filled samples [Figure 4(b)], with plasticizing effect due to water contact. Otherwise, prolonged UV–vis exposition led to a significant reduction of strength at necking. These results highlight that, with exacerbate expositions, PLA degrades by polymeric chain break down. In particular, samples filled with acid solution show lower values: in this case, degradation must be attributed to the association irradiation pH 2. Moreover, water content could have to also hydrolytic effect by inducing depolymerization of PLA chains. Also where results obtained by photoirradiation are compared to those from samples stored in climatic room for 30 days [Figure 4(c)], it is worthwhile to speak about degradation process due to humidity, heat and buffer contact. In particular, from Table II, it is evident as filled sample is more fragile, with an higher tendency to break. This result can be explained by the fact that packaging material, in stressed environmental conditions of climatic room, become more permeable because of polymeric chain mobility and consequently more susceptible to structure degradation. Finally, in all treated samples, percentage of absolute elongation was obviously lower than that of standard containers. Consequently, environmental and chemical factors made polymer more fragile and more easily subjected to fracture.

Physicochemical and Morphological Characterization

Thermal analysis techniques were applied to control the degradation effects on the PLA packaging, assuch or containing pH 2 solution, caused by UV–vis irradiation, temperature and humidity. Every PLA packaging was cut in pieces weighing approximately 6–7 mg and analyzed both on internal and external side.

In DSC thermal cycling, the first heating up to 75°C had the aim to eliminate the thermal history of the sample. The second heating, for all samples, revealed similar thermal events, in particular the glass transition, the cold crystallization and the melting processes. In Figure 5 is shown, for example, the thermal cycling of internal (Curve a) and external (Curve b) sides of empty PLA packaging at room temperature. In the first heating, an endothermic effect, due to the structural relaxation, superimposed to the glass-transition temperature (T_g) is observed at 65.7 ± 0.6 and 66.6 ± 0.3°C for internal and external side, respectively. As expected, during the second heating, the enthalpy relaxation disappeared, and the T_g was precisely measured as the temperature at the inflection point of the effect, $T_{midpoint}$ = 60.5 ± 0.2 and 62.3 ± 0.1°C for internal and external sides, respectively. The difference in the T_g between the two sides can be correlated to a non homogeneity of the starting sample as confirmed by SEM measurements. Indeed, as evident in the microphotograph of Figure 6, internal and external surfaces are different: the first one [Figure 6(b)] is smooth while the second one shows high surface roughness [Figure 6(a)].

After the T_g, the polymer chains acquire some translation mobility which allow the sample to freely crystallize at 86 ± 3°C with an exothermic effect, in both cases. The following endothermic peak is dueto melting process and is characterized by thermal and calorimetric parameters very similar for internal

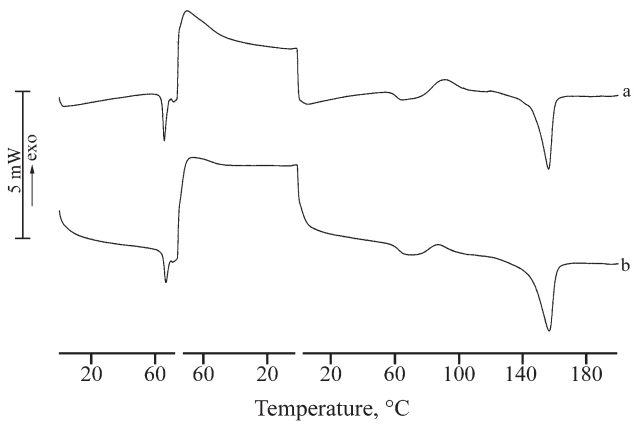

Figure 5. DSC cycling of internal (Curve a) and external (Curve b) side of empty PLA packaging.

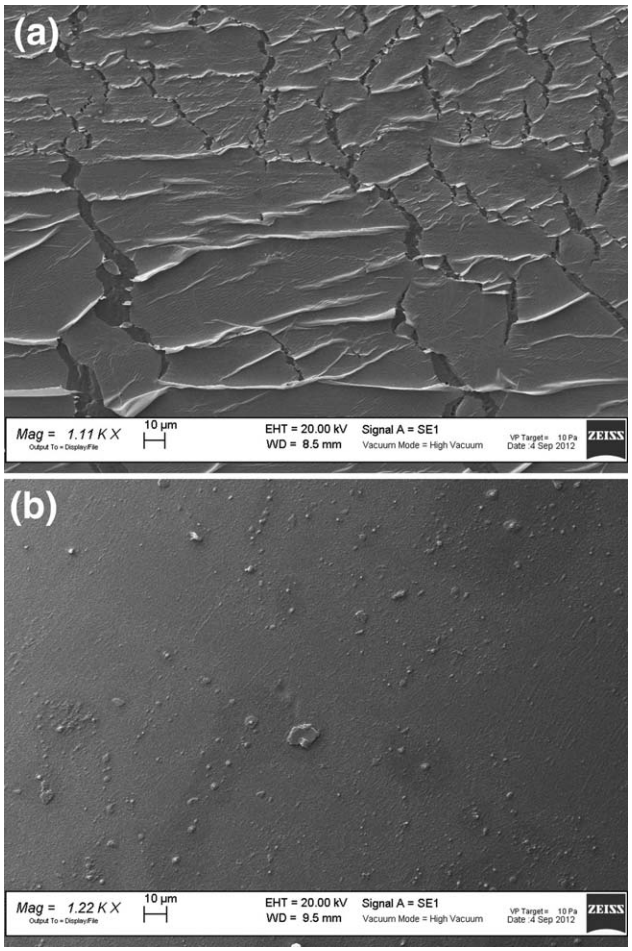

Figure 6. External (a) and internal surface (b) PLA filled bottles, at zero time.

and external sides ($T_m = 156 \pm 1$°C; $H_m = 27 \pm 2$ J g^{-1}). In Table III and IV are listed the DSC parameters (second heating) of all samples, recorded for internal and external sides, respectively.

The absence of significant differences in the thermal data of the internal side of all PLA packaging suggested that the polymer solid state of this side is not influenced by the different storage conditions. The presence of a shoulder at around 147°C in the melting endotherm of the original PLA crystallites, recorded after UV irradiation for 96 h and storage in controlled climatic conditions, is probably due to a rearrangement of chains during crystallization, as reported in the literature.[28] This effect is probably correlated to the formation of a more fragile crystalline packaging, confirming the different mechanical behavior of these samples described above. For the external side, the thermal cycling performed on empty PLA shows a progressive decrease in T_g with the increasing of the irradiation time ($T_{g\,t=0} = 62.3 \pm 0.1$, $T_{g\,t=24\,h} = 61.7 \pm 0.5$ e $T_{g\,t=96\ h} = 59.4 \pm 0.7$, respectively) probably due to polymer plasticization. The same effect on T_g is observed also for the samples stored in controlled climatic conditions probably as a consequence of the combined action of temperature and humidity. The same behavior recorded for internal side of empty PLA, is recorded also for the internal side of PLA packaging containing pH 2 solution to confirm that for this side the storage conditions not influenced the polymer solid state. In the external side of PLA containing pH 2 solution the T_g decreasing, after UV irradiation and storage in controlled climatic conditions, is concomitant with the increasing of the cold crystallization and melting temperatures. The presence of acid solution together with storage conditions cause a lower chainsmobility in the amorphous region responsible also for the advance breaking recorded in mechanical analysis. For all samples, the melting enthalpy of original samples is unchanged after treatment ($H_m = 27 \pm 2$ J g^{-1}). It is not possible to calculate the crystallinity degree of samples because of the difficulty in assessing the enthalpy of the cold crystallization effect due to low data reproducibility.

All the samples were also characterized by TGA to evaluate the thermal decomposition after melting. In all cases, the thermal weight loss (around 97–98%) takes place in a single step. The-corresponding decomposition onset, peak and endset temperatures (T_{onset}, T_{peak}, and T_{endset}, respectively) are extrapolated by the first derivate of the TGA curves (DTG) as shown in Figure 7 for empty PLA at the beginning (Curve a) and after UV irradiation for 24 and 96 h (Curves b and c). All decomposition temperatures recorded are listed in Table V.

For the empty PLA after suntest, the decomposition temperatures progressively increase with the irradiation time. These results suggest modifications in the chains arrangement of the polymer; in particular the hydrolytic reactions caused by UV irradiation can generate smaller chains able to reorganize causing a higher resistance to thermal decomposition.[15] A similar behavior is observed for empty PLA stored for 30 days in controlled climatic conditions. In the PLA packaging containing pH 2 solution, an increasing of the thermal decomposition temperatures is observed, higher after 24 h in suntest, probably as a

Table III. Temperatures Recorded in the DSC Second Heating for Internal and External Sides of Empty PLA (Standard Deviation in Parentheses)

	Internal side				External side			
Empty PLA	t_0	24 h suntest	96 h suntest	30 days C.R.	t_0	24 h suntest	96 h suntest	30 days C.R.
$T_{midpoint}$ (°C)	60.5 (2)	61.3 (1)	60.2 (1)	60.2 (6)	62.3 (1)	61.7 (5)	59.4 (7)	61.1 (2)
T_{exo} (°C)	86 (3)	86.6 (1)	86.5 (1)	86.0 (9)	86 (3)	86 (1)	87 (1)	87 (1)
T_m (°C)	156 (1)	155 (1)	156 (1)[a]	157 (1)[a]	156 (1)	156 (1)	157 (1)	157 (1)

C.R. = climatic room.
[a] Presence of a shoulder at 147 (2)°C.

Table IV. Temperatures Recorded in the DSC Second Heating for Internal and External Sides of PLA Packaging Containing pH 2 Solution (Standard Deviation in Parentheses)

	Internal side				External side			
PLA pH 2	t_0	24 h suntest	96 h suntest	30 days C.R.	t_0	24 h suntest	96 h suntest	30 days C.R.
$T_{midpoint}$ (°C)	60.5 (2)	59.3 (9)	60.3 (4)	59.8 (9)	62.3 (1)	61.6 (6)	60.5 (1)	60.8 (9)
T_{exo} (°C)	86 (3)	86 (2)	86 (1)	89 (2)	86 (3)	86 (1)	88 (1)	92 (1)
T_m (°C)	156 (1)	156 (1)	155 (1)	157 (1)	156 (1)	156 (1)	158 (1)	159 (1)

C.R. = climatic room.

consequence of the initial hydrolytic decomposition effect of the acid solution. The following buffer solution effect determine an increasing of decomposition temperatures, lower than that recorded at 24 h in suntest, probably associated with the different chains mobility in the amorphous region, correlated to the higher cold crystallization and melting temperatures described above in the DSC. To better characterize PLA solid state, all the samples were analyzed by FT-IR spectroscopy, on internal and external sides. In Figure 8, the FT-IR spectra recorded on the internal side at $t = 0$ and after 24 and 96 h of UV irradiation of empty PLA and on PLA containing pH 2 solution after 24 h at room temperature are compared (the same results were obtained for external side). UV irradiation causes the appearance of two peaks at 1638 and 1546 cm^{-1} which become more intense with increasing the time of irradiation and in the presence of the acid solution just after 24 h. These vibrational effects are due to the —C═O carboxyl stretching generated by hydrolytic reactions, confirming the thermal decomposition results obtained by thermal analysis. The unchanged —C═O carbonyl stretching band at 1744 cm^{-1} indicates that no phase transitions occurs in the crystalline regionof these samples. The effect of the acid solution is confirmed by SEM and from the evaluation of shape container. In particular, Figure 9 shows the external and internal surfaces of a sample of PLA bottle treated withacid solution and irradiated for 24 h; it is evident that the external side [Figure 9(a)] shows a surface homogeneous and more resistant to high vacuum treatment, during picture collection. However, onto internal side, the effect of irradiation in addition to acidic solution make the surface more susceptible to high vacuum effect, emphasized by the breakage [Figure 9(b)] and this confirms the brittleness of irradiated sample with pH2 solution. Finally, visual evaluation of container shape showed a swelling of the bottom of the 24 h irradiated bottles treated with acid solution (Figure 10), confirming, one more time, degradation process due to contact with low pH.

CONCLUSIONS

In conclusion, the research presented in this study investigated the effects of acid buffer solution onto mechanical,

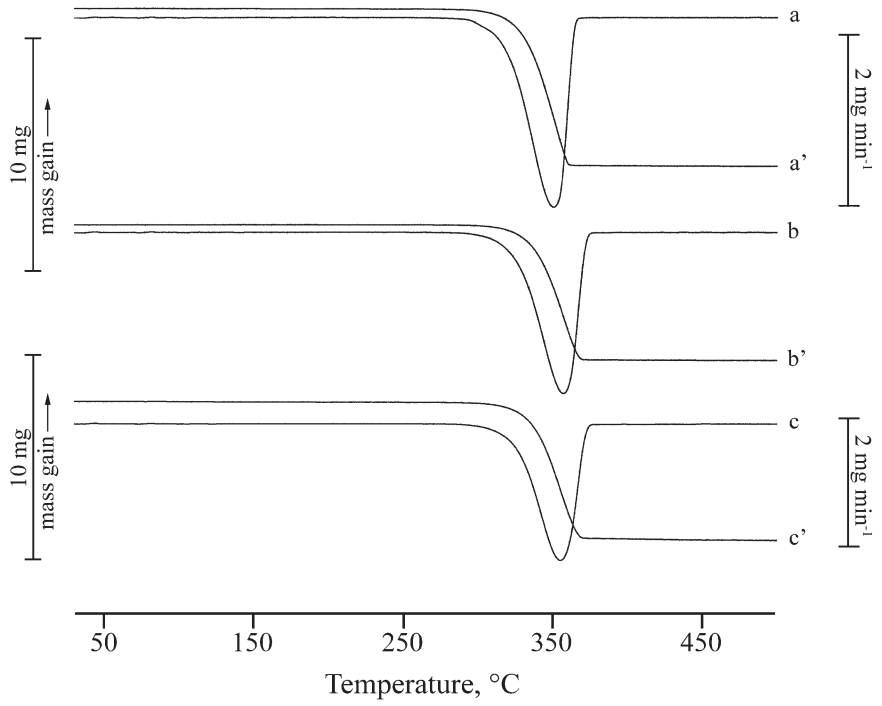

Figure 7. TGA and DTG curves of empty PLA as such (Curve a), after UV irradiation for 24 h (Curve b), and 96 h (Curve c).

Table V. Decomposition Temperatures From DTG Curves (Standard Deviation in Parentheses)

	t_0	24 h suntest	96 h suntest	30 days C.R.
Empty PLA				
T_{onset} (°C)	324.6 (1)	329.4 (9)	331.3 (3)	330.6 (9)
T_{peak} (°C)	356.8 (2)	359.9 (8)	360.6 (9)	359.3 (9)
T_{endset} (°C)	371.7 (2)	374.9 (8)	377.1 (9)	373.6 (8)
PLA pH 2				
T_{onset} (°C)	324.6 (1)	330.2 (9)	328.3 (5)	329.2 (9)
T_{peak} (°C)	356.8 (2)	361.2 (1)	358.4 (9)	360.1 (9)
T_{endset} (°C)	371.7 (2)	377.9 (3)	374.2 (9)	375.9 (4)

C.R. = climatic room.

physicochemical, and morphological properties of PLA bottles exposed to stressing environmental conditions (heat, humidity, and UV–vis irradiation). The experimental techniques put into evidence that UV–vis irradiation and acid medium can affect stability of PLA used as packaging in cosmetic products. Furthermore, internal and external surfaces can be influenced very differently depending on the type of the stress. In particular, thanks to this study, it was possible to set up a protocol to study packaging–content interaction sustained during the life of the cosmetic product to choose the best use of the PLA polymer as packaging depending on the type of product that must be marketed.

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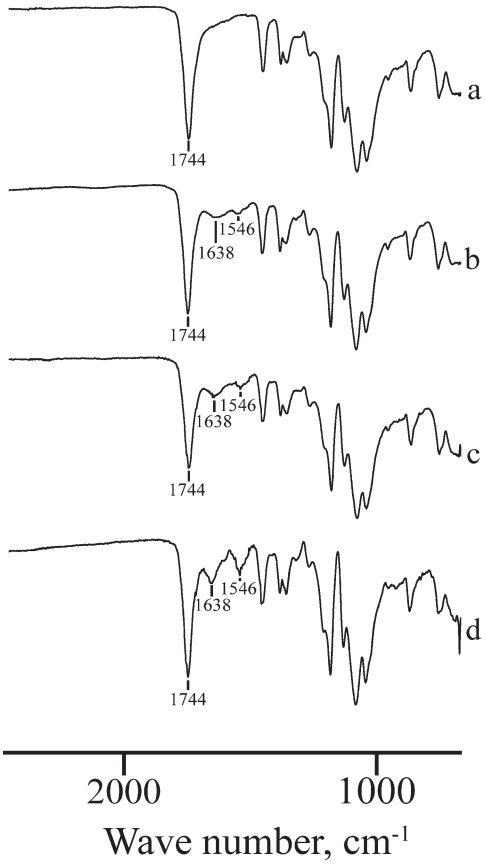

Figure 8. FT-IR spectra of empty PLA internal side as such (Spectrum a) after 24 h (Spectrum b), and 96 h (Spectrum c) of UV irradiation, and of PLA containing pH 2 solution after 24 h at ambient temperature (Spectrum d).

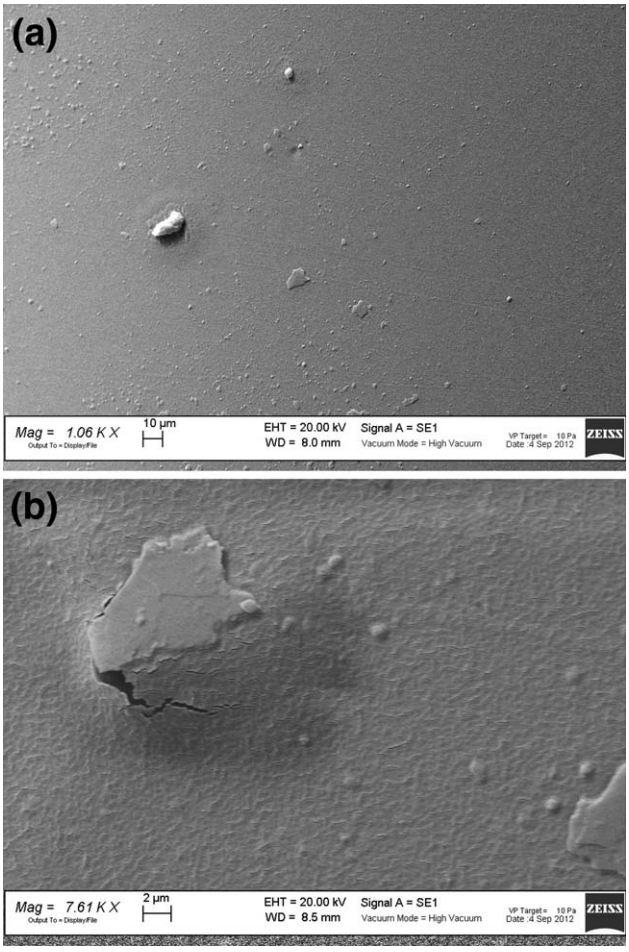

Figure 9. External (a) and internal (b) surface of PLA samples containing buffer solution (pH 2), after UV–vis irradiation for 24 h.

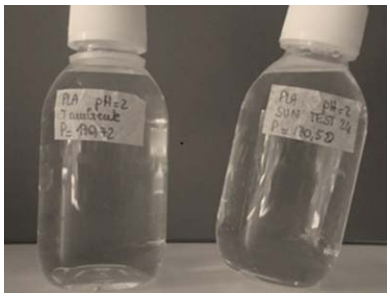

Figure 10. Digital photograph of container alteration after UV–vis irradiation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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