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FOOD SAFETY AND MINERAL OIL CONTAMINATED PAPERBOARD PACKAGING: AN ANALYTICAL CHALLENGE AND A MIGRATION STUDY

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ABSTRACT

Food packaging plays an important role in protecting food, extending its shelf life and giving relevant information to consumers. Paper based materials (e.g. paper, paperboard and corrugated board) are within the most used to produce food packaging, being light, cheap and renewable materials. Furthermore, they are perceived by consumers as “natural” and safe. Safety of food packaging materials is of paramount importance in order to guarantee food safety, due to the capacity of some substances to migrate from packaging to food content. Paper based materials represent no exception, but little has been done regarding migration studies and legislation, compared e.g. to plastic materials. The main food safety issues related to paper and paperboard lay not much in their base ingredients (timber, minerals), but in contaminants coming from inks, solvents, glues, varnishes, additives and other process substances. Recycled paper, often used to produce food packaging, contains high amounts of contaminants coming from those substances, which accumulate at every recycling cycle. Among them are phthalates and other plasticizers used for glues, ink photoinitiators and other ink additives, mineral oil hydrocarbons from printing inks and recycled fibers, di-isopropyl naphthalenes from carbonless paper, etc. All these contaminants, if sufficiently volatile, tend to migrate from paper based food packaging into food through the gas phase. Even dry food, which was considered not prone to problems from packaging migration, is often contaminated.

Mineral oil is a product of petroleum with various industrial applications. It is composed by thousands of hydrocarbons, many of which are isomers; they can be distinguished in two main groups: saturated mineral oil hydrocarbons (MOSH) and aromatic mineral oil

hydrocarbons (MOAH). Mineral oil is widely used as solvent for offset printing inks, and this is way newspaper can bear a contamination of thousands of mg kg^{-1} of mineral oil. Being newspapers the base of recycled paper, their contamination is passed onto paperboard food packaging made from recycling. The paperboard is then often offset printed itself, with an additional mineral oil contamination. The toxicity of mineral oil at present is not fully evaluated, but a JECFA (Joint FAO/WHO Expert Committee on Food Additives) temporary ADI (Acceptable Daily Intake) of 0.6 mg kg^{-1} has been established for MOSH. MOAH are probably more toxic. The presence of a barrier between paperboard and food is not always able to avoid food contamination, depending on barrier chemical nature and thickness.

Extraction and analysis of MOSH and MOAH is difficult due to the complexity of molecules mixture. In this study, a dedicated extraction method has been optimized for paperboard, plastic and food, respectively. Instrumental analyses were performed using online LC-GC/FID. Only hydrocarbons up to 24 carbon atoms ($<C_{24}$) were quantified, because they have sufficient volatility to easily migrate to food content. Furthermore, high molecular weight hydrocarbons show a lower toxicity due to their scarce capacity of being absorbed through gastrointestinal tract and skin. Applying the optimized methods, a survey on over 100 Italian and Swiss market food products has been conducted. Even though some of the most volatile hydrocarbons had already evaporated from paperboard, the average concentration of MOSH $<C_{24}$ was 626 mg kg^{-1} . Nearly 15% of packs contained more than 1000 mg kg^{-1} (maximum, 3500 mg kg^{-1}). Many had the potential of contaminating the food at a level exceeding JECFA ADI hundreds of times. Food contamination was particularly high in case of direct contact with recycled paperboard.

Deeper understanding of mineral oil hydrocarbons migration speed and kinetics is necessary to help food and packaging producers tackle the problem. Only few studies are available due to the complexity and variability of contaminants mixture. A long term migration study was designed in order to understand the influence of main factors influencing migration, such as temperature, time, storage position and food packaging structure. Egg pasta and müesli were chosen as representative foods due to high surface to weight ratio (worst case scenario), and stored at different temperatures and conditions for up to 1 year (products end of shelf life). Release of MOSH and MOAH from paperboard, along with their increase in plastic barrier (when present) and food, have been measured at specific intervals of time. Tested temperatures were 4, 20, 30, 40 and 60°C , to represent refrigeration, room temperature, storage during warm months and accelerated migrations.

Tested storage conditions were free, shelved and boxed packs, to represent domestic, supermarket and warehouse storage. About 200 samples have been analyzed. Kinetics curves show that migration is an extremely fast process, mostly influenced by temperature: in egg pasta model (where food is in direct contact with paperboard), half of MOSH <C24 is transferred to food in a week at 40°C and in 8 months at 20°C. The internal plastic bag present in müsli slowed down the startup of migration: mineral oil is accumulated in plastic and then released towards food, creating a “lag time” in the curves. Packs stored in corrugated board boxes show the long term highest contamination. At 40 and 60°C (accelerated migration), full migration is rapidly reached, but at these temperatures high molecular weight hydrocarbons, poorly volatile at normal storage conditions, move towards food misrepresenting the real migration pattern. Therefore, using high temperatures to accelerate migration to obtain quick laboratory results is not easily applicable.

Keywords: food safety; migration of packaging contaminants; paper-based food packaging; recycled paper; printing inks; mineral oil hydrocarbons.

LIST OF ABBREVIATIONS

C6	hexane
d	day/s
DIPN	di-isopropyl naphthalenes
EtOH	ethanol
FID	flame ionization detector
GC	gas chromatography
h	hour/s
IS	internal standard
LC	liquid chromatography
MeOH	methanol
min	minute/s
MOAH	mineral oil aromatic hydrocarbons
MOSH	mineral oil saturated hydrocarbons
MS	mass spectrometry detector
MW	molecular weight
POSH	polyolefin oligomeric saturated hydrocarbons
RT	room temperature
s	second/s
T	temperature

1. INTRODUCTION

1.1 FOOD PACKAGING

Packaging has both technical functions and marketing functions. In fact, its primary roles are to contain, protect (especially during transportation) and preserve food, or beverage. Preservation is intended not only from microorganisms (mainly bacteria and moulds), but sometimes also from heat (or cold), light, dust, humidity, and atmospheric gases: the increasing success of modified atmosphere packaging, where air is removed to a special mixture of nitrogen and/or carbon dioxide and/or oxygen, demonstrates that it is possible, through a tailored packaging project, to maintain nutritional and organoleptic value of food for a long time without chemical preservatives. More recently, highly engineered product such as active and intelligent packagings entered the market: the first are able to interact with food content in order to give and or preserve favorable characteristics (e.g. flavor) and/or eliminate unwanted chemicals (e.g. oxygen or humidity sorbents); the latter are able to signal critical food conditions e.g. breaking of cold chain during transportation. Packaging also measure food, “selling” to customers a precise amount of it, and sometimes helps to dispense food, to serve it, to cook it (either in conventional ovens or microwave) and so on.

There are then other packaging functions, not so tangible but nevertheless very important for the product success on the market: packaging has the duty to display, inform, sell, promote, motivate, communicate to customers both through wording and images. This unusual skill range makes the packaging industry, and especially the food packaging one, a very challenging sector, where engineering and marketing skills have to combine.

Packaging development is closely associated with society evolution: its features at every stage of a society's growth represented people's needs, culture, material availability and technological advances at that very moment. In this sense, a study of packaging's changing roles and characteristics over the centuries could be a study of civilization¹. Early humans were nomadic hunters and gatherers, so they used to follow their food sources with little occasions, possibilities for food storing. Nevertheless, food was very likely kept in wraps made of leaves, animal skin or nut shells. Around 5000 BC some plant and animals domestication begun, along with small tribal villages establishments: storage and transport containers for milk, honey, grains, nuts and meat were used, such as fabricated sacks and baskets, or wood and clay jars. Later on glass was discovered and used (already used by Egyptians), then metal packaging. Some "food packaging legislation" was already enforced during the Greek city-state period (about 250 BC), when olive oil packaged in clay amphoras was marked with a stamp identifying production city-state, time of production and person responsible for it². Paper materials introduction is credited to China back in 105 AD or even earlier, but it became popular in Europe only centuries later. The real industrialized production of packaging came after the industrial revolution started in England in the XXVIII century, "when rural agricultural workers migrated into cities to be employed in factories, and low cost mass-produced goods became available to large segments of population: the consumer society was born. Factory workers needed commodities and food that were previously produced at home in a self-sufficient way, so many new shops opened in urban area, requiring food to be transported from producing areas to cities. Initially, shops simply adapted the bulk delivery system (often in wood barrels) to consumer selling, measuring goods out into a container provided by the purchaser, later on shopkeepers started to create individual packages in the amounts that people preferred to purchase. Medicines, cosmetics, teas, liquors and other expensive products were the first products to be prepackaged. Most packages that existed in the mid-1800s were for higher cost goods, and the evolving printing and decorating arts were applied to these early upscale packages". The most common packaging material for food was paper: "it was realized that the papers used to wrap products for sale were easily imprinted with a brand mark, with some message of instruction or with a description of the product's virtue. In 1907, phenol formaldehyde plastic, later known as Bakelite, was discovered. A few years later, in 1911, a machine was built to manufacture continuous

¹ Soroka, 2009.

² *Ibid.*

cellulose film: DuPont chemists perfected the cellulose casting process in 1927 and called their product Cellophane, which dominated the clear film market until the advent of polyethylene and polypropylene. When later on the self serve concept was widely introduced, the shopkeeper was not there to aid or influence the consumer's purchase. The consumer was face to face with the package, so its motivational and informational roles became critical: the package had to inform the purchaser and to sell the product"³. Since those years, industrial packaging design and technical performances have continuously been evolving to better meet consumers needs, being this the best way to ensure high product sales.

Worldwide packaging production in 2010 has been valued 443 billions of Euros, with the following shares: Asia 27% (on the rise), North America 26.5%, Western Europe 27.5%, Eastern Europe 9.9%, South and Central America 5.2%, Africa 2.3% and Oceania 1.6%. Italy has a packaging production worth 25.8 billions €, and represents the 5.8% of worldwide production, placed within the ten major packaging producers⁴. In Italy, over 70% of packaging is destined to food and beverage (Figure 1).

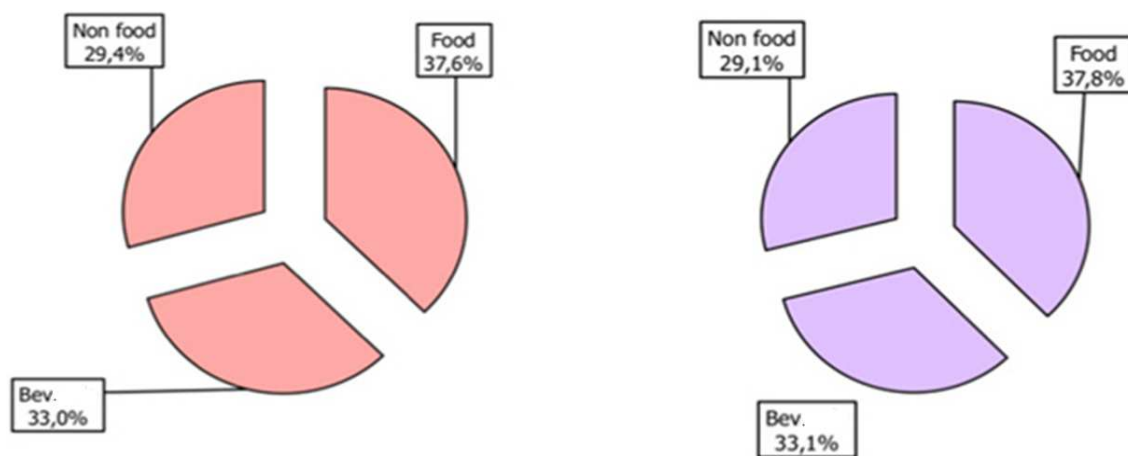


Figure 1. Packaging final use destinations (non food, food and beverage) in Italy. Modified from Istituto Italiano Imballaggio, 2010 report on 2009 data.

Paper is by far the most representative food packaging material with over 5 millions tons produced every year in Italy (Table 1). Glass is second (but its high weight is somehow influencing the ranking), and plastic third. For every material, just small fluctuations can be noticed along the years, except for some few cases: e.g. significant decrease in wood

³ Soroka, 2009.

⁴ Istituto Italiano Imballaggio, www.istitutoimballaggio.it/dati-di-mercato.

use due to wood crates for fresh agricultural produce transportation nowadays often substituted by plastic.

Table 1. Trends of different packaging materials production in Italy from 2001 to 2010 (values: thousands of tons). Modified from Istituto Italiano Imballaggio.

	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Steel	662	658	680	710	680	660	684	686	645	677
Steel tanks	110	105	103	104	100	103	105	100	86	95
Aluminium	85	84	92	94	97	99	102	102,2	100,3	102,1
Cellulosic materials	5.041	5.140	5.160	5.169	5.163	5.270	5.303	5.080	4.708	5.010
Multimaterials	122	122	124	124	125	129	132	132	131	135
Timber	3.170	2.999	2.887	2.914	2.951	2.943	3.169	3.111	2.343	2.433
Plastic	3.174	3.237	3.295	3.344	3.340	3.383	3.469	3.179	2.875	2.996
Flexible packaging	227	235	250	259	270	286	300	297	287	304
Glass	3.519	3.540	3.561	3.602	3.561	3.568	3.641	3.695	3.352	3.528
Others	74	30	30	30	30	30	30	30	29	28
Total	16.184	16.150	16.182	16.350	16.317	16.471	16.935	16.413	14.556	15.308

1.1.1 SUSTAINABILITY AND SAFETY ISSUES

In recent years, consumers feeling about packaging have started to change: from an undesirable piece of waste to get rid of, its functions and abilities are now more clear, as it is the concern about its end of life destiny and environmental impact. The importance of a correct and sustainable waste management (e.g. recycling or composting) is becoming more relevant to consumers, whom start to take into account this aspect when shopping.

A popular perceiving suggests that a simpler and lighter packaging is always better, because this implies using less resources to produce it and, consequently, cost reduction. However, the fundamental function of packaging is to protect an even more precious resource: food. Thus, a delicate balance must be struck between the amount of resources invested in packaging materials, technologies and related activities, and the amount of food resources saved through the efficient protection that packaging provides. This is known as the “packaging paradox”⁵: when investing in packaging, we are using resources in order to achieve the product protection, and afterwards we might be able to reuse, recycle or recover in some other way (e.g. energy production or composting) such materials. If we

⁵ Flexible Packaging Europe data (www.flexpack-europe.org), 2011.

underinvest in packaging, we are at risk of wasting resources through the spoilage and wastage of the very contents we are trying to protect. Steph Carter (Packaging Sustainability and Functional Capability Director at Unilever) stated: “I suspect that the understanding of sustainability will change in all sectors, including retail, as everyone learns more and understands it better. If there is one issue that is affecting retailer policies at the moment it is lightweighting, which favors flexible packaging. I am not convinced, however, that it is as black and white as using less material. For instance, do you choose a very lightweight packaging plastic that can’t be recycled, or a heavier plastic that can be? There is a place for flexible packaging, but we need to change our view on recycling. Across the industry there is ignorance about packaging when it becomes waste. The whole sector is guilty of looking at things in terms that are too simple.” Packaging must therefore be seen in the context of the packaged product and its use in order to find the optimum environmental balance. Sometimes a vast proportion of food production in emerging economies is lost due to poor preservation and deterioration. Also the industrialized world faces challenges: food wastage along the supply chain and at household level is a critical issue for Europe and USA, and it is responsible for significant economic and environmental impacts both directly and indirectly. Underperforming packaging can lead to much larger negative environmental impacts than “over packaging”.

A common framework for more sustainable packaging, identified within the Global Protocol on Packaging Sustainability, is that packaging should increasingly be:

- designed holistically together with the product;
- made from responsibly sourced materials;
- efficiently recoverable after use;
- manufactured using clean production technologies;

Packaging will at the same time need to:

- meet market criteria for performance and cost;
- meet consumer needs and expectations;
- be safe for human health at the designed conditions (food contact, any in-pack heat treatments, etc.).

There are then a number of design improvements that can add extra value to the packaging, also from the sustainability point of view:

- compact and “cube efficient” packs that minimize impact of transport space consumption and storage energy;

- optimized packaging, where the materials, the amount used and the related processes to obtain it are optimized to the required functionality and the available end of life infrastructures;
- portion packs that offer the right amount of food at its top conservation quality to the target consumer, avoiding food wastage;
- packs that eliminate the need for refrigeration in the supply chain and/or extend shelf life (e.g. vacuum or modified atmosphere, active packaging), thus saving the associated energy;
- packs that optimize product use (e.g. less energy needed for food preparation);
- reclosable packs, that allow unused content to be preserved;
- easy to open and/or easy to empty packs (to minimize residual product left in packaging);
- packaging with adequate barrier effect: this optimizes shelf life and minimizes deterioration of food caused e.g. by penetration of oxygen and water vapor from outside; barrier effect can also be applied to protect food from contamination deriving from a contaminated outer packaging layer (e.g. materials of recycling origin);
- on pack information for correct use and end of life disposal: giving instruction on how to reuse or recycle packaging materials can also help companies to improve their “green image”⁶.

To summarize the principles for designing environmentally responsible packaging, the “4 Rs” rule/formula developed in the early 1990s is still valid today:

- Reduce: packaging designs should use the minimum amount of material necessary to achieve its technical functions.
- Reuse: when possible, practical and safe for consumers health.
- Recycle: in order to recycle as much packaging as possible, collection and treatment plants for the different materials must be widespread and effective; furthermore, especially in the case of plastic, recyclability must be taken into account since the very beginning of packaging design: multilayer multimaterial packaging have high technical qualities but are not ideal for this purpose.
- Recover: finally, before consigning packaging to a landfill, other disposal ways should be considered to recover at least a part of packaging value; many plastic polymers and paper-based materials have excellent calorific properties when burned in dedicated furnaces.

⁶ Mahalik and Nambiar, 2010.

If sustainability can be considered a very desirable but “optional” packaging feature, its safety for consumers health is an infeasible prerequisite. Food packaging is by all means a part of the food system, and food safety and quality also depend on its packaging safety and quality: several undesired substances (either off-flavors or toxic chemicals) can migrate from packaging to food, by direct contact, or through the gas phase if sufficiently volatile at the storage conditions.

The European Rapid Alert System for Food and Feed continuously monitors food and feed safety issues related to goods circulating in Europe and also imported from extra European countries. Alerts regarding migration from food contact materials are on the rise every year: they were 61 in 2005, 133 in 2006, 172 in 2007, 229 in 2010 and 310 in 2011. This five folds increase in only 6 years is partly due to higher awareness of migration risks⁷ and thus more intense control activity from Authorities on these materials. A significant proportion of alerts regard migration of contaminants such as chromium, nickel, cadmium, lead, aromatic amines, formaldehyde, bisphenol A and derivatives, benzophenone and derivatives, etc. from packaging and kitchenware produced outside Europe, often from China (in 2011, 220 out of 310 alerts).

European food packaging legislation has been rapidly evolving in the last few years, with the publication of milestones as the general regulations Reg. 1935/04 and Reg. 2023/06. These apply to every food contact material and establish a new and more safety oriented approach for all food packaging production companies to their product, regardless of their collocation inside the production chain.

Reg. 1935/04⁸ has been introduced with the purpose of ensuring a high level of protection of human health and consumers interests. This Reg. crucial point is Art. 3, which leaves little room to misunderstanding. It reads: “1. Materials and articles, including active and intelligent materials and articles, shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could: a) endanger human health; or b) bring about an unacceptable change in the composition of the food; or c) bring about a deterioration in the organoleptic characteristics thereof.” It is clear that also food organoleptic modifications caused by packaging, even without toxicological implications, are considered unacceptable.

⁷ Grob *et al.*, 2006.

⁸ Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC ([http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:338:0004:0017:en: PDF](http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:338:0004:0017:en:PDF)).

Reg. 2023/06⁹ is a very concise text that introduces the Good Manufacturing Practices (GMP) as compulsory for food packaging production as they already were for food production, in order to assure food packaging quality during every production step and avoid any uncontrolled problem to arise. Its introduction follows the “ITX scandal”, when a packaging production unforeseeable problem caused the contamination of baby food (formula milk): the problem was caused by a non efficient printing ink UV fixation system, which let the photoinitiator ITX (2-isopropylthioxanthone) free to migrate from the printed surface of a paperboard reel for brick packaging production to the inside (food contact) part of following reel coil. This incident made it clear that, during food packaging production operations, the same care has to be taken as for food production, in order to avoid any health safety issue.

This new and more responsible approach, brought about by these important regulations, will take time to be fully enforced in all food packaging producing companies, especially if they are small or far apart from final product delivery (e.g. companies producing plastic polymers either for food and non food applications). Some voluntary standards are already available, specific for the food packaging sector, e.g. the BRC-IoP (British Retail Consortium – Institute of Packaging) standard. Some European Countries, among which is Italy, also have a national register of qualified Business Operator – Food Contact Expert, figure introduced by Reg. 1935/04 as the reference professional able to deal with every aspect related to food contact materials applications and safety.

1.1.2 GLASS

Glass is one of the most ancient packaging materials: its use started about 3000-3500 years ago. It is an amorphous nonmetallic solid, produced by high temperature fusion of silicates and their cooling without crystallization. Along with 70-74% of SiO₂ (e.g. from siliceous sand), glass usually contains other ingredients as vitrifiers (Na₂B₄O₇, H₃BO₃), melting aids (Na₂CO₃, K₂CO₃), stabilizers (CaO, MgO, BaO, Al₂O₃), refining agents (As₂O₅, Sb₂O₃) and dyes (Fe₂O₃ for green glass, Cu₂O or AuCl₃ for red, Cr₂O₃ for yellow, CoO for blue, etc.)¹⁰.

⁹ Commission Regulation (EC) No 2023/2006 of 22 December 2006 on good manufacturing practice for materials and articles intended to come into contact with food (http://www.eurofins.ie/media/1456878/reg%202023_2006%20gmp.pdf).

¹⁰ Piergiovanni and Limbo, 2010.

The advantages of glass as a packaging material are: transparency, inertness, impermeability, rigidity, thermal resistance (when properly tempered) and general consumer appeal. Its disadvantages are fragility and weight.

Glass containers are standardized to a much lesser degree compared to other packagings, e.g. metal cans. In fact, most bottles and jars are tailor-made specifically for one product or one manufacturer. On the other hand, closures for glass containers are somehow more standardized. Glass containers can be reused (rare nowadays) or recycled. Adding some recycled glass to the melting sand is not only desirable from an environmental point of view, but it is necessary in order to obtain a fast and good melting; it also allows some energy saving: glass furnaces burn petroleum fuels to reach very high temperatures, above 1200°C. Up to 80% glass from recycling can be added in the production of dark coloured glass, but only 10% in the production of white glass, unless a separate collecting system is in place to separately recover green, brown and white glass (e.g. in Switzerland and USA). Glass use as food packaging is still widespread, despite its high weight and fragility compared to plastics, thanks to inertia towards foods and beverages (no off-odours or off-flavours are passed onto them), stability during high temperature treatments as hot fills and sterilization, and recyclability. Main glass containers produced in Italy are bottles (ca. 88%), jars (ca. 8%) and flasks (ca. 3%)¹¹.

CASE STUDY: MIGRATION FROM JAR LIDS

A part for possible lead migration from crystal glass kind, glass has virtually little migration problems when in contact with food, even at high temperatures.

Nevertheless, in the case of jars, migration can occur from twist-off lids¹². To have proper sealability and to maintain it over time after many opening and closing operations, metal twist-off lids need a “plastisol” gasket ring. Plastisols usually consist of heavily plasticized PVC, containing on average the 35% by weight of additives, most of which are plasticizers¹³. These substances can easily migrate to jar food content, especially if oily¹⁴ and frequently “shaken” during operations such as transportation and supermarket/household handling.

¹¹ Piergiovanni and Limbo, 2010.

¹² Fankhauser-Noti and Grob, 2006.

¹³ Biedermann-Brem *et al.*, 2005.

¹⁴ Grob, 2006.

More toxic plasticizers, as phthalates, have been replaced over time with less toxic substances as epoxidized soy bean oil (ESBO)¹⁵, polyadipates¹⁶ and polycaprolactone¹⁷. However, migration has to be kept under control for all of them and often legal limits of migration into foods are present.

1.1.3 METAL

Metals have some characteristics that make them ideal for many food packaging applications. They have a compact molecular structure that makes it impossible any light, gas or liquid passage, even through a thin metal layer. They are quite easily molded into any shape. They are resistant to mechanical stress (e.g. food protection during conveying in the food industry and during transportation). They have high thermal conductivity, allowing fast and effecting post packaging sanitizing treatments as pasteurization or sterilization. Finally, they can be recycled virtually without any performance loss. Unfortunately, their extraction cost is quite high, and this is one of the reasons that makes important to recycle them as much as possible. Main applications are the production of cans for beverages or long shelf life food, the production of lids for glass jars and the production of kitchenware as cutlery, stainless steel surfaces and machinery for the food industry, etc. Another important application of metal, in particular aluminum, is the production of thin layers to be applied as a functional barrier against gases and water, alone or together with other materials as plastics and paper, in multilayer multimaterial flexible packaging (e.g. TetraPak[®] bricks).

Metals used for food applications are often combined into alloys to obtain the desired performances. The most represented alloys used in food contact are aluminum alloys (both for kitchenware and packaging), stainless steel (for kitchenware, cutlery and food industry surfaces) and iron alloys covered by tin oxides or, less frequently, chromium oxides.

Metal cans used for food and beverage packaging are mainly manufactured using:

- Tinplate (“three-pieces cans”). The first material used to make metal cans and canisters consists of a thin sheet of steel, coated with tin. The purpose of the tin coat is to reduce the risk of corrosion. This is because ordinary steel, on the contrary of stainless steel (containing at least 10% chromium and much more expensive), rusts readily when exposed to air and moisture. The traditional method for coating the steel

¹⁵ Fankhauser-Noti *et al.*, 2005.

¹⁶ Biedermann *et al.*, 2008.

¹⁷ Shi *et al.*, 2011.

plates with tin, the “hot dip” method, has now been replaced by a process of electrolytic deposition, which forms a more uniform tin coat with much less tin per unit area.

- Aluminum (“two-pieces cans”). Unlike steel, aluminum does not require the application of an anti rust protective coat, because the thin film of aluminum oxide formed on the surface protects the metal against further corrosion. Aluminum is much lighter and more ductile than tinfoil, but it is more expensive.

Both materials have nowadays an internal non metallic coating to further protect the food content from any metal migration. In fact, in some cases the protection provided by tin or aluminum is not sufficient for the prevention of internal (or external corrosion) of the can. Sometimes the can is to face particularly severe corrosive conditions, e.g when filled with acidic food as tomato preserves. Therefore, a protective layer of polymeric lacquer or enamel is applied (see next case study).

Metal migration is quite common from low quality kitchenware, i.e. cutlery, oven dishes, teapots, etc. made of poor quality steel can lead to the migration of iron, chromium, nickel and other toxic metals. These findings are quite common in the European Rapid Alert System for Food and Feed (RASFF) report, and usually regard kitchenware imported from outside Europe, mainly from China. On the opposite, migration of metal (tin or aluminum) from cans into canned food is nowadays rare, thanks to the special internal coatings that protect the metal and vary depending on the food characteristics (e.g. a food with high acidity will need a more resistant coating). These coatings are the real material “in contact with food”, and migration can occur from them, as treated in the next case-study.

CASE STUDY: MIGRATION FROM CAN COATINGS

Food and beverage cans usually have an internal thin coating layer in order to avoid direct contact between food and metal. Such coatings can be based on vinyllic or phenolic lacquers or epoxy or epoxyphenolic resins¹⁸. Epoxies are thermoset polymers obtained by reaction within epichlorohydrin and bisphenol A (BPA) and/or derivatives such as bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE) and Novolac glycidyl ethers (NOGE, which is a complex mixture of isomers and oligomers obtained by reaction of phenol with formaldehyde under acidic conditions). They form particularly strong bonds with many materials, thus they are used both as protective coatings and as adhesives. Some

¹⁸ Piergiovanni and Limbo, 2010.

monomers (e.g. BPA and its derivatives) can remain in the coating and later migrate to food, with a speed and extent depending on food characteristics. BPA is also used for other food packaging applications, e.g. for the production of polycarbonate (PC) plastic. The use of PC to produce baby bottles has recently been banned in many countries worldwide, including the European Union¹⁹. This decision is based on the increasing scientific evidence against BPA safety^{20,21,22}, especially for its role as an endocrine-disrupting agent, dangerous for newborns, infants and children.

The migration of BPA, BADGE and derivatives from can coatings is higher in oily food due to the high extractive power of oil and its chemical affinity towards BPA and derivatives²³. Furthermore, foods rich in proteins, such as canned fish, can bind a significant proportion of these contaminants, giving misleadingly low contamination results²⁴. This reaction with food components probably occurs during post-packaging high-temperature treatments of cans²⁵ in order to sterilize the content and thus obtain a prolonged shelf life. The importance of reaction products between packaging contaminants and food, and their toxicological profile, is probably underestimated at present²⁶.

1.1.4 PLASTICS

Under the definition of plastic, there is a vast group of materials with very different chemical compositions, mechanical behaviours and appearances. The only thing they have in common is that they are made up of polymers. Monomers for plastic production are usually sourced by petroleum refining, but this is not always the case (see next case study on bioplastics). A concise description of the most important plastic polymers, and their applications in the food packaging sector, is now given^{27,28}.

- **Polyethylene (PE)**. It is a vinyl polymer, from the monomer ethylene obtained from the gaseous fraction of petroleum. It is probably the most popular plastic polymer worldwide, used for food and non-food soft (“squeezable”) and semi-rigid bottles and jars, toys, shopping bags, etc. In Italy it accounts for nearly half of plastic used for

¹⁹ Commission Directive 2011/8/EU of 28 January 2011, amending Directive 2002/72/EC as regards the restriction of use of Bisphenol A in plastic infant feeding bottles.

²⁰ MacLusky *et al.*, 2005.

²¹ vom Saal and Hughes, 2005.

²² Viberg *et al.*, 2011.

²³ Biedermann *et al.*, 1997.

²⁴ Petersen *et al.*, 2008.

²⁵ Richard *et al.*, 1999.

²⁶ Grob *et al.*, 2010.

²⁷ Soroka, 2009.

²⁸ The Macrogalleria, <http://pslc.ws/macrog.htm>.

packaging²⁹. It has the simplest structure among plastic polymers, being made of long chains of covalently linked carbon atoms completely saturated with hydrogen. These long chains might be branched, with side alkylations of variable length: the originating PE has a low density (LDPE), because the chains cannot closely pile together due to the branching. If side branches are absent, the originating PE is called “linear” and has higher density (HDPE). HDPE is harder and stronger, but LDPE is cheaper because it is easier to produce. In fact these two PE behave like they were two very different polymers: HDPE is used to produce rigid bottles or jars, whereas LDPE is used to produce flexible packaging, stretch films and internal and/or welding layers in multilayer multimaterial packaging. PE is usually considered a plastic with medium or low technical and mechanical performances, but if polymerized using the new metallocene catalysis it is possible to obtain ultra high molecular weight PE, which has incredible mechanical performances (e.g. used to produce bullet proof vests).

- **Polypropylene (PP)**. As PE, it is a vinyl polymer, obtained by polymerization of propylene from petroleum. It differs from PE because it has a methyl group attached to every other carbon of the polymeric chain. It provides higher performances compared to PE, e.g. it can be used for dishwasher proof containers because its melting point is over 160°C. It is also used to produce moisture proof textile fibres. It is widely used for food packaging applications, e.g. various bottles and containers and flexible packaging, usually combined with other materials because PP is a good barrier against moisture but not against oxygen.
- **Polystyrene (PS)**. It is a vinyl polymer produced from the monomer styrene, obtaining a chain that has a phenyl group attached to every other carbon atom. It is a hard and resistant plastic widely used both for non food objects (computers and telephone casing, molded parts inside cars, toys, etc.) and for food packaging such as drinking cups, containers for dairy products, white trays for meat and cheese, etc. For this latter applications, polystyrene is expanded with a particular production technique, trapping gas bubbles that give the structure its typical lightness.
- **Polyethyleneterephthalate (PET)**. It is the most important representative of the polyesters family, obtained by the condensation of ethylene groups with dicarboxylic terephthalic acid. It gives a plastic with excellent clarity and impact strength, ideal to produce shatter proof packaging: it is widely used for the production of beverage

²⁹ Source: Istituto Italiano Imballaggio.

bottles (absorbing up to 70% of PET production). Thanks to its high resistance to gas and moisture permeation, it is ideal also for carbonated drinks. Its use in flexible packaging is limited by the lack of heat sealability, difficult package openability and poor machine performance. Careful control of production processing temperatures is necessary to reduce thermal degradation leading to the formation of acetaldehyde, with a typical off-flavour that can be passed to foods and beverages. PET is probably the more recycled plastic worldwide due to its value. With the methanolysis process the polymer can also be reverted back to the original monomers, which are then repolymerized.

- **Polyvinylchloride (PVC).** It is a vinyl polymer with structure similar to PE, but one of the hydrogens attached to every other carbon atom is substituted with a chlorine. It is mostly used to produce pipes (both in food and non food industry). One of its main assets is to be fire resistant, thanks to the presence of chlorine, but on the other hand, such presence makes this polymer not environmentally friendly. Besides, when used for food contact, PVC can release its monomer vinyl chloride, which has high toxicity compared to other plastic monomers: this is why PVC cling films have been phased out in most Countries. Also plasticizers, essential to soften PVC, can migrate to food. These reasons have caused a decline in PVC use over time, especially for food applications. At present one packaging application that is still PVC domain is the production of gaskets for jar lids (see case study on migration from jar lids).
- **Polyamides (PA).** It is a family of polymers obtained by the condensation of diacid chlorides and diamines. Due to the presence of amide groups, these polymers are polar and sensitive to humidity, but in turn they offer a good barrier against gas permeation. Their backbone chain is regular and symmetrical so they are often in crystalline form, and make very good fibres (e.g. Nylon). In food packaging applications, PA is often used in the manufacture of kitchen utensils and to produce gas proof layers for flexible packaging, in alternative to ethylvinylalcohol (EVOH).

In several packaging designs, plastics are coupled to paper based materials: Tetrapak[®] bricks for milk, juices and vegetable products are one of the most famous applications, with a printed paperboard layer on the outside and a plastic layer in the inside (food contact), sometimes with an aluminum foil between them. This plastic coating of paper allows its use in contact with moist or liquid foods/beverages. Looking for more simple and basic application of paper and plastic together in food packaging, there are many foods packaged in a paperboard box which has inside a plastic bag, thinner or thicker, of various

polymers depending on the barrier needed towards oxygen and moisture. The chemical nature and thickness of this plastic bag or layer can have a protective function not only against the penetration of oxygen and moisture, but also against the migration of volatile contaminants from paperboard towards food. This role of plastics will be deeper discussed under paragraph 1.3.4.1.

Since the vast majority of plastics are not simply constituted by a polymer, but also contain many additives (plasticizers, antioxidants, antifog, gliding agents, etc.), potential migrating substances from plastics to foods form a long list³⁰:

- Monomers (vinyl chloride, acrylonitrile, phthalic acids, glycols, acetaldehyde, 1-hexene, 1-octene and other olefins, formaldehyde, melamine, primary aromatic amines, bisphenol A, etc.). Also low molecular weight oligomers must be taken into account, being one of the primary migrants to food from a polymeric food contact substance from the quantitative point of view³¹.
- Plasticizers (phthalates, maleates, adipates, sebacates, epoxidized soy bean oil - ESBO, acetylated tributyl citrate - ATBC, trimethyl pentadiol diisobutyrate - TXIB, di-isononyl cyclohexane dicarboxylate - DINCH, etc.).
- Other additives (antioxidants e.g. Irganox[®] range, antistatics e.g. etoxylated amines, antifog agents, etc.).
- Dyes and printing inks and their additives, including photoinitiators for UV printing (benzophenone and derivatives, 2-isopropylthioxanthone - ITX, etc.) and solvents (ethyl acetate, acetyl acetone, 2-butoxyethanol, etc.). If solvent is not fully evaporated during drying time (e.g. before bobbin coil winding), the plastic will have an off-odour and possibly transfer it to food content. These production problems can be detected looking for volatile organic compounds (VOCs) with a specific laboratory test on packaging.
- Adhesives, including solvent and poor reticulation residues: adhesion between packaging parts or layers can be achieved in many ways. Sometimes a softer plastic layer (e.g. LDPE) can be used to glue together layers of harder plastic. Also polyurethanes can be used to join together different layers in a flexible multilayer packaging, but if their reticulation is incomplete, contaminants such as primary aromatic amines can migrate from the polyurethane layer to food content. Another way to glue together plastic layers or packaging parts is to use adhesives dispersed in

³⁰ Lau and Wong, 2000.

³¹ Nelson *et al.*, 2011.

solvent and let them dry and fix, with possible VOCs problems as seen for printing application.

- Catalysts (metals, metallocenes, etc.).

Recently, a specific regulation for plastic material has been issued: Reg. 10/2011³², also referred as PIM (Plastic Implementation Measure). This is a complex regulation, with detailed annexes, which has the merit of harmonizing the plastic legislation among European Countries³³.

CASE STUDY: BIOPLASTICS

The terms “bioplastic”, “biopolymer” and “biopackaging” are somehow synonymous but there is no univocal definition of their meaning. The basic concept behind them is the renewability of the source they are produced from, so they are perceived as “green” and good for the environment. Paper based materials and bioplastics are the most common kind of biopackaging. On the environmental benefit of using bioplastics many packaging experts are skeptical, because often the bio-sources are food crops as corn, with potential rise in their international price. At present bioplastics produced from cereals as corn (e.g. polylactic acid - PLA) are not widely used, and consume about 0.05%³⁴ of corn worldwide, but this percentage will probably be on the rise. The Food and Agriculture Organization (FAO) is worried about food crops destination to non-food processes³⁵, based on forecast of future consumption growth trends. Another aspect criticized by many is the use of OGM cultivars for the production of bioplastics. The main PLA producing company ensures that no genetic material is passed from corn to bioplastic, and anyway an OGM-free guaranteed production line is available for customers committed to OGM-free policy. Another important point, which is not clear to the majority of customers, is that “bioplastic” does not necessarily mean “biodegradable”: e.g. bio-PE is produced from corn instead of petroleum, but has exactly the same environmental impact as petroleum-sourced PE.

European Bioplastics³⁶ association assessed that in 2007 bioplastics represented about the 0.2% of total plastic production, but in future this percentage could reach 5-10%, because

³² Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:012:0001:0089:EN:PDF>.

³³ Lorenzini R. Plastiche a contatto con alimenti: anteprima sul nuovo regolamento 10/2011 – PIM. *Macchine Alimentari (Tecniche Nuove)*, May 2011.

³⁴ “Food versus Fuelling the United States – Can Both Win in the Era of Ethanol?” *Institute for Agriculture and Trade Policy*, September 2007.

³⁵ “The State of Food and Agriculture 2008” *Annual report*, 2008.

³⁶ European bioplastics association, www.european-bioplastics.org.

annual growth is above 20% (Figure 2). About ¼ of bioplastics is at present represented by PLA.

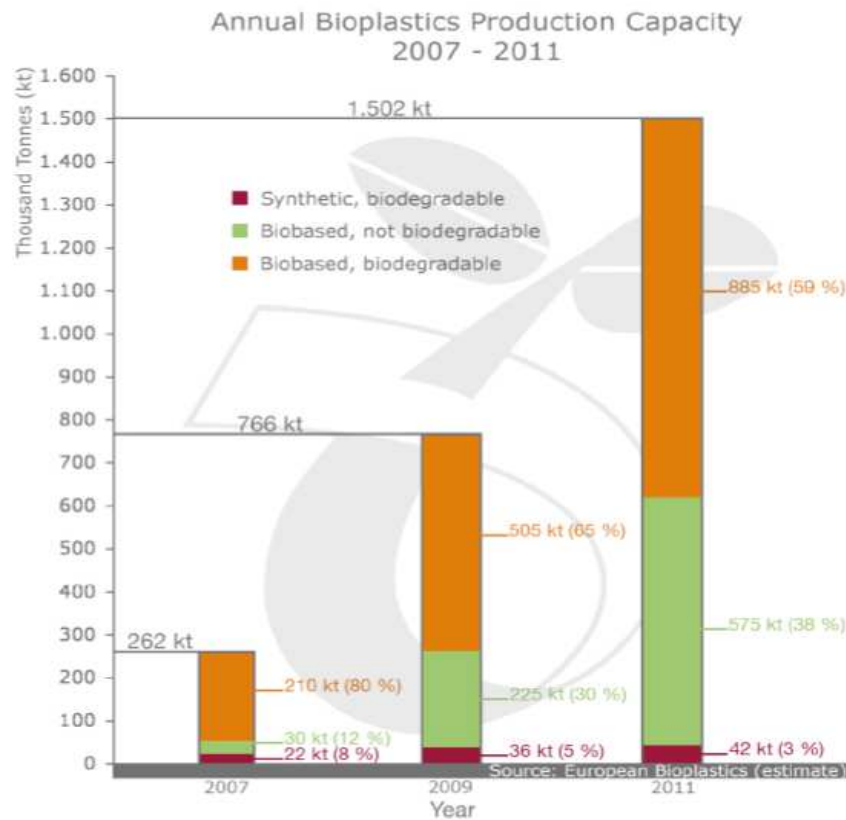


Figure 2. European Bioplastic estimate of bioplastics and biodegradable plastics growth trend.

Bioplastics production growth is hindered many factors, such as technological knowhow needed for their production, bureaucracy needed for production approval, availability of packaging converters to change their lines or adapt them to biopolymers, and most of all by cost of raw materials, being cereal kernels in many cases more expensive than petroleum. The real turning point will be reached when food industry byproducts, either of vegetal or animal origin (i.e. straw, potato and tomato peels, oily seeds remainders after oil extraction, milk whey, etc.), will be used as raw materials for biopolymers production instead of food valuable sources. These biopolymers, defined as “second generation bioplastics”, will allow a real production cost lowering and environmental benefit, but they are still some time away from the technical point of view. In fact, the main fibre present in vegetal byproducts is cellulose, not as easy as starch (from cereal kernels) to transform into glucose by microbial or enzymatic action, which is the first step in bioplastics production. Another environmentally friendly approach consists in the use of non-food crops for

bioplastic production, e.g. switch grass grown in marginal areas not suitable for food crops cultivation.

At present the more representative biopolymers on the market are the following:

- A) **NATURAL BIOPOLYMERS:** these polymers are used “as they are” to produce films or objects, without a previous depolymerization followed by chemical transformations and repolymerization. They are all characterized by not excellent mechanical properties, and the final product may vary slightly depending on different raw material batches, but they have many assets: they do not require long and costly chemical transformations, with a low environmental impact and low final cost.
- a. **CELLULOSE AND DERIVATES.** Paper is probably the most important cellulose based packaging material, and will be discussed thoroughly from paragraph 1.2. Also Cellophane[®], one of the first films produced by packaging industry, is cellulose based. Cellulose acetate and nitrate are within the first cellulose derivatives with many applications (e.g. first movie film supports). Unfortunately some cellulose derivatives lose the biodegradability typical of cellulose. An innovative cellulose derivative is NatureFlex[®], by Innovia: it is a film with a cellulose core obtained from managed forestry, between two thin printable plastic layers, which do not compromise the material biodegradability. The final film is resistant, transparent and with low permeability to gas and humidity, but it is not stretchable nor thermo-sealable.
 - b. **STARCH AND DERIVATES.** It has been used for a while, alone or mixed with other components, to produce kitchenware and other objects. A brand product is Mater-Bi[®], by Novamont: famous representatives of this family of products are the Mater-Bi[®] biodegradable shoppers, which use is now compulsory in Italy in substitution of traditional plastic shoppers. This material resistance to mechanical stresses, to gases and humidity can be improved adding mineral nanoparticles.
 - c. **PROTEINS:** proteins from legumes peels can be used to produce a biodegradable film resistant to gas and lipids. Also gluten, whey proteins, ovalbumin, gelatin and collagen can be used to produce films.
 - d. **FIBRES:** fibres from tomato peels and other undesired food industry byproducts can be used, mixed with other substances as jellifying agents, to produce thick films and trays with agronomic (e.g. mulching, plant nursery) and packaging (e.g. trays for fruit and vegetables) applications. Agronomic applications are particularly suitable for this kind of film, which is readily biodegradable and can be added

with fertilizers and natural antimicrobials. Also pectin from apples or citrus fruits byproducts can be used.

- e. CHITOSAN: this polysaccharide extracted from crustacean exoskeleton can be used to produce films, with the asset of having a natural antibacterial effect.
- B) SYNTHETIC BIOPOLYMERS: these polymers are obtained from a natural raw material, which is depolymerized, transformed and then repolymerized, using strong chemical processes and/or enzymatic and microbial action (bioreactors). Typically, starch from a food source (e.g. cereals) is depolymerized into glucose by engineered bacteria; glucose is then transformed in another suitable molecule, which can be then polymerized to the final desired biopolymer.
- a. POLYLACTIC ACID (PLA): it is an aliphatic polyester, first synthesized in 1932 and then developed by DuPont. Glucose from cereals starch is fermented to lactic acid, which is then transformed into cyclic lactide and eventually polymerized into PLA. At present the bigger producer is NatureWorks, with the product brand name Ingeo™: this is probably the more representative and abundant food packaging biopolymer present in the market. It is very transparent, thermoplastic, quite rigid and less “machinable” compared to conventional plastics. Its weak points are the low resistance to temperature and humidity, but in recent year awesome improvements have been obtained in engineering this material (often using nanoparticles), and now PLA water bottles are marketed, but with shorter shelf life compared to PET water bottles. At end life PLA can be mechanically recycled (being quite well separated from PET), composted or incinerated.
 - b. POLYHYDROXYALKANOATES (PHA): they are biodegradable polyesters obtained by microbial metabolism on sugars or starches, with high cost, e.g. Mirel™ by Metabolix.

The end life destiny should be, along with the renewability of their production source, the strong point of bioplastics: they should be biodegradable, or even compostable. Unfortunately, waste collection organization in many areas is still unable to fully exploit bioplastic potential: they are often disposed off together with conventional plastic, or even worse in the undifferentiated waste. If bioplastics end life is not properly managed, a great part of their “raison d’être” is lost. If the bioplastic is compostable, it should be disposed off as compost waste in the dedicated bin, but is often impossible or very difficult for average customers recognize the different plastic kinds (e.g. PLA appearance is similar to PET or PS). PLA thrown into the recycled plastic circuit can pose a series of troubles: if an

automatic separation system is not in place, PLA will be melted with other plastics, “contaminating” them and lowering quality of renewed plastic. The more suitable end life disposal for PLA would be the industrial composting, even if some authoritative institutions pose doubt on the fast biodegradability of this material³⁷. It much depend on the additives used to confer to PLA higher resistance (e.g. to heat and to humidity) to improve packaging performances. Unfortunately a lower interaction with water and food also means a lower biodegradability. In fact, not all polymers of natural origin are biodegradable (e.g. natural rubber is not), whereas some polymers of synthetic origin are (e.g. polycaprolactone)³⁸. Biodegradation and composting are not synonymous. A material is biodegradable when, in a certain % and in a certain time, is degraded under the action of different microorganism³⁹. A material is compostable when, at certain controlled conditions, is completely degraded to CO₂, H₂O and inorganic compounds within the established time, without releasing any phytotoxic substance (this would render the compost not suitable for soil fertilization). Time and controlled conditions are established by specific standards, e.g. UNI EN 13432, UNI EN 14855, ASTM D6400-04, D7081-05, D6868-03, D5511, D5526. Sometimes the same material results compostable according to one standard but not to another. In Italy, compostable plastics receive a distinguishing mark by Consorzio Italiano Compostatori (CIC)⁴⁰, but many materials are still under test, and anyway consumers are largely unaware of the way to distinguish compostable materials, e.g. MaterBi[®] compostable shoppers (recently introduced by law instead of conventional plastic shoppers) should be used to collect compostable waste and thrown into bins going to composting, but many consumers continue them for undifferentiated waste, with many waste collection problem due to the fast deteriorating of MaterBi[®] when in contact with garbage. About the legislation covering bioplastics, they are subject to the same legislation as conventional plastics, unless they are not considered as such despite being plastic look-alike (i.e. biopolymers made from cellulose, starch, fibres, etc.): for these peculiar materials, a case to case evaluation is needed. Bioplastics are often less inert to food contact compared to conventional plastics, being this the same chemical base of their better biodegradability, so even if they are perceived as more “natural” and thus “safe”, thorough evaluation of additives, monomers and reaction/degradation product potentially migrating to food content is needed.

³⁷ “PLA: arriva la plastica biodegradabile?”. WWF Italia.

³⁸ Siracusa *et al.*, 2008.

³⁹ Bioplastic Council, www.plasticsindustry.org/BPC.

⁴⁰ www.compost.it.

1.2 PAPER BASED FOOD PACKAGING

Paper has a long history, beginning with the ancient Chinese: pulp was made from old rags, peels and nets reduced to mush from which paper was manufactured. While handmade methods dominated for thousands of years, paper production became industrialized during the 19th century: the first machine to continuously manufacture paper was invented by the Frenchman Louis-Nicolas Robert in 1799⁴¹.

Paper based materials are the most used for food packaging in Italy, with over 5 million tons produced every year⁴². Their main assets are lightness, low cost and appeal to customers compared to less “natural felt” materials as plastics.

Paper is a material usually made of plant fibres, in the past from papyrus, linen, sugar cane, cotton, straw, etc. Nowadays, paper is almost exclusively made from timber cellulose and from recovered material, of lower quality, obtained from paper recycling. Chemically pure cellulose consists of long, ribbon-like molecules made up of glucose monomers. These molecules are held together side-to-side by hydrogen bonds to form “sheets”, which in turn are stacked together in tightly packed layers to form “microfibrils”. The microfibrils group themselves in bundles, and groups of these bundles form the paper fibre.

Paper and board can be used in contact with food in very different ways, either directly or indirectly, and either alone or laminated with other materials such as plastic or metal foil. In the latter case, so-called “functional barriers” are aimed at suppressing any mass transfer between food, paper and external environment. The subject of functional barriers will be separately treated in a dedicated paragraph.

Some definitions are needed to clarify the meaning of the most used paper based materials in food packaging manufacturing:

- **PAPER:** this term in food packaging industry is commonly used to identify sheets under 300 µm of thickness. This material has no mechanical resilience and no barrier effect so it is mostly used as a component of composite packaging: paper bags inside boxes, chocolate wraps together with aluminium foils, etc. Paper is still widely used also as fast wrapping sheets and sachets for non pre-packaged foods in butcheries, bakeries, groceries, farmers markets etc. Paper can be white (bleached), coloured or printed, can have a glossy finish on the outside and can be “greased” or “plasticized”

⁴¹ ILSI Europe Report Series. Packaging materials. 6: Paper and board for food packaging applications. 2004.

⁴² Source: Istituto Italiano Imballaggio.

(e.g. PE coated paper) in the food contact side, to gain resistance to humidity and fat and thus to better preserve food content: this coating is fundamental if paper is meant to come in contact with fresh food (meat, fish, etc.) or in bags used to pack crispy food (biscuits, crisps, etc.). Coated paper is better defined as a multilayer multimaterial packaging: the material coming in contact with food (and thus the relevant legislation) is not paper but plastic.

- **PAPERBOARD:** also known as “folding box board”, it is characterized by a higher thickness compared to paper. The International Standards Organization (ISO) indicates that material weighing more than 250 g m^2 shall be defined “paperboard”. General industrial practice defines “paperboard” those materials thicker than $300 \mu\text{m}$ ⁴³. Paperboard is probably the most widely used paper based food packaging material, thanks to its low cost, lightness, quite good resilience to mechanical stresses, good printability, recyclability, and appeal on customers thanks to its “natural” look compared to plastic materials. Figure 3 shows the typical composition of a paperboard section; inner coating is needed if paperboard is intended for contact with humid food, like frozen fish, to avoid paperboard swelling during defrosting.

Components of board based food packages

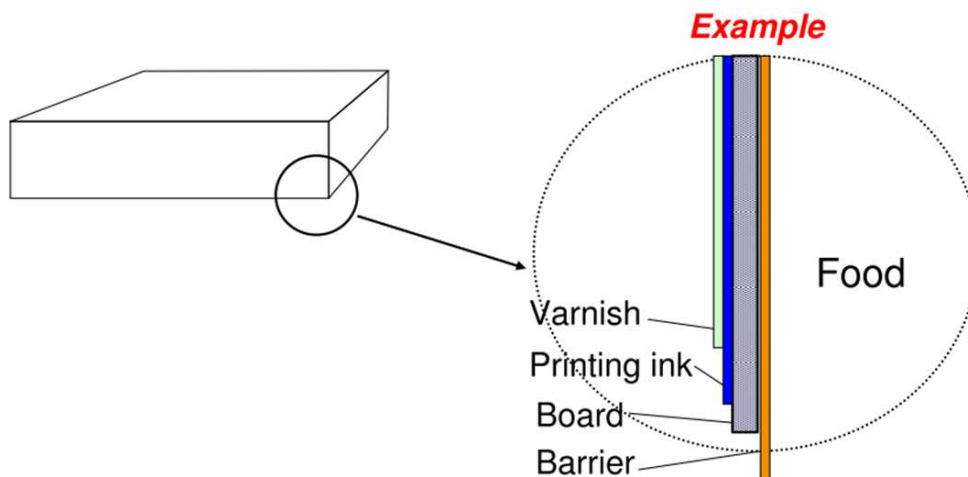


Figure 3. Paperboard layers composition. Modified from Iggesund Paperboard, Sweden.

- **CORRUGATED BOARD:** also known as “carton board”, “cardboard”, “fibreboard” or “shipping board”, is a thick material, often of brown colour and unprinted, characterized by a core of undulated paperboard between two layers of straight

⁴³ Soroka, 2009.

paperboard; this manufacture provides to its content (food or non food) mechanical protection and also some transient temperature insulation during transportation. There are even thicker boards with a “double wave” core⁴⁴.

Figure 4 shows the different destinations of corrugated board and paperboard packaging materials in Italy: considering all foods and beverages, the 46.7% of total corrugated board is used for the shipping of food. When talking of paperboard, this percentage goes up to 61.5%, confirming the fact that paperboard is the first choice for many foods: pasta, rice, breakfast cereals, sweet and savoury snacks, teas and herbal teas, eggs, frozen foods, etc.

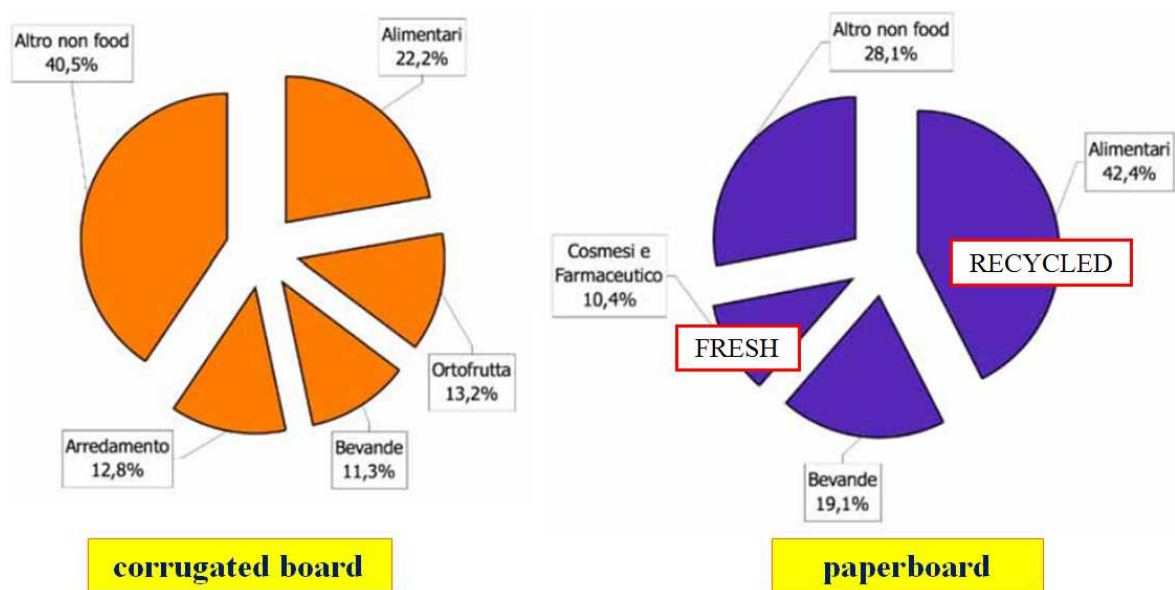


Figure 4. Corrugated board (left) and paperboard (right) packaging destination in Italy. While nearly all paperboard for the cosmetic and pharmaceutical sectors is made of fresh fibres, the majority of paperboard destined to become food packaging is made of recycled paper. Modified from Istituto Italiano Imballaggio, report 2010 on 2009 data.

The abundant use of paperboard for beverages (nearly 20%) is not surprising either: of course paper materials are not suitable for direct contact with beverages, but are present in the vast majority of multilayer multimaterial bricks (e.g. Tetrapack[®] widely used for milk and fruit juices packaging) where they constitute the bulk of the packaging, and are separated from liquid food by an aluminum thin layer (optional) and a plastic inner layer in contact with beverage.

⁴⁴ Piergiovanni and Limbo, 2010.

Is interesting to note that fresh fibres paperboard (white “high quality” appearance) is used for cosmetics and pharmaceutical products, whereas recycled fibres paperboard is very often used for food products, despite the migration risk, confirming that appearance expectations and food safety assurance at the moment travel in opposite directions. The presence of recycled material inside paperboard gives it a grey unpleasant appearance, and it is also linked with contaminants migration (see paragraph 1.2.3).

1.2.1 PAPER MANUFACTURING TECHNIQUES

Paper and board is manufactured from natural fibres of unbleached or bleached cellulose obtained from plants (“virgin” or “fresh” fibres) or obtained from recovered paper-based materials such as newspapers, magazines, books, packaging, leaflets, etc. (recycled fibres). For the manufacture of paper and board, different mixtures of fresh and recycled fibres may be used, depending on end use, and ranging from 100% virgin pulp to 100% recycled material. Quality and characteristics of paper and paperboard depend on several variables: fibre source, how they were obtained and prepared, papermaking technique, paper mill machinery, further substances added during papermaking, final finish treatments, etc. Many plants can give cellulose fibres suitable for paper production. Fibre length is the most important variable determining paper quality and strength (either tensile, burst and tear strength, and fold endurance): the longer the fibre, the better the fibre entanglement and the stronger the final product. E.g. hardwood as maple, aspen and poplar gives short fibres (about 2 mm), whereas softwood as hemlock, spruce and pine gives longer fibres (about 4 mm)⁴⁵. Recycled fibres length depends on the source and on how many recycling cycles the material has undergone: at every cycle fibres quality and length is deteriorated, and this makes it necessary to add a proportion of fresh fibres during paper-based product manufacture from recycled materials. On the other hand, shorter fibres produce a paper with a smoother surface, a finer aspect and an even density, which is easier to print and with better final results.

About 50% of wood is represented by cellulose; the other major components of wood are lignins and carbohydrates (sugars and starches), both unsuitable for papermaking because they are not fibrous and not as stable as cellulose. While carbohydrates are washed away during pulping, lignins cannot be so easily removed by water. Fibres of cellulose can be separated from the wood mass in different ways⁴⁶, with different quality outcomes:

⁴⁵ Soroka, 2009.

⁴⁶ *Ibid.*

- Mechanical pulping: is the fast and more economic method, and it consists in mechanically abrade or cut the wood; nowadays this method is applied to wood chips resulting as cheap byproduct of the timber industry (e.g. furniture making, etc.). The mechanical action breaks fibres, thus mechanical short fibres are used for low-quality papers (e.g. newspapers) or to be blended with more expensive pulps.
- Chemical pulping: chemicals (usually alkali or acids) are used to dissolve lignins, leaving undamaged bundles of cellulose fibres. The alkali chemical pulping is the one that produces the highest quality paper (also referred to as “kraft”), used for quality paperboard production.
- Semichemical pulping: has an intermediate cost between the two previous method, and also the final quality of paper is intermediate. The wood is partly digested by chemical before mechanical treatment.
- Thermomechanical pulping: the wood is softened at high temperature before mechanical treatment.

Once the cellulose pulp is obtained with one of above methods, it is refined by beating in paper mills, to release away smaller fibres. Low refining gives paper with high tear strength and high absorbency, but low burst and tensile strength. High refining gives more shiny and humidity resistant paper, with high burst and tensile strength. Additional technical demands (mechanical strength, optical properties) often placed on the paper and board are normally obtained through the use of chemical additives which are combined with the fibrous raw materials. Some of these additives can also be applied after paper production, e.g. onto the paper surface, with a process called “coating”. The amount of most additives required to achieve the technical effect is very small, i.e. less than 1% by weight of the paper. This is not true for some of them, required in higher amounts, such as mineral fillers. The basic chemistry of the chemical additives is broad, some additives are made from synthetic chemicals while others are made from natural products but nevertheless have a toxicity potential (e.g. colophony components). Some are polymeric while others are small molecules. The chemical additives are either soluble or readily dispersible in water. This property is important because the papermaking process is an aqueous process that allows the chemical additives to be added directly to the papermaking process without further modification. The chemical additives used by the paper industry fall into the following general categories⁴⁷:

⁴⁷ ILSI Europe Report Series. Packaging materials. 6: Paper and board for food packaging applications. 2004.

FUNCTIONAL ADDITIVES. These are used to either improve or change the properties of the paper and they are designed so that they are retained in the paper. Typical examples are sizing agents, wet and dry strength resins, softeners, dyes and pigments. The use of these additives is not universal and depends on the required type of paper or board.

- **SIZING AGENTS:** they are a group of substances added to generate hydrophobicity, as cellulose untreated fibres are highly absorbent and blotting, leading to excess water and ink penetration; “hard-sized” papers show high water resistance and are printed more effectively. If the final paper needs an extra wet-strength (e.g. high humidity or damp conditions), some resins can be added along with sizing agents. For paperboard production, sizing agents are added both to pulp (in order to obtain a stronger and more rigid board) and to the surface to improve printability. Originally they were called engine sizes because they were added to the paper before it was formed but now surface sizing agents are deposited on the surface of the paper after it has been formed. Typically the “engine” sizes used are based on rosin, alkyl ketene dimer or alkenyl succinic anhydride while those added to the surface are polymeric materials based on either styrene or polyurethane. The rosin based sizing agents are primarily based on tall oil rosin (also called colophony), which is a by-product of the pulp industry. Alkyl ketene dimer is made from fatty acids of animal or plant origin. Alkenyl succinic anhydride is a synthetic material derived from the oil industry. The styrene and polyurethane based surface sizing agents are also made from synthetic materials derived from the oil industry. Normally, the rosin based sizing agents are used under mildly acid conditions while the other products are used under neutral or mildly alkaline conditions.
- **FILLERS:** they usually consist of clay, kaolin, calcium carbonate or even titanium dioxide (more expensive) and are added to modify the optical properties (in particular opacity) of the paper and board or as a partial fibre substitute. Also talc and gypsum can be used, in particular for paperboard production, in order to give extra strength and stiffness to final product. Also starches and gums can be added, to improve burst and tensile strength. Retention aids can be added to help fillers retention.
- **WET and DRY STRENGTH AGENTS:** they often are resins that are used to make the paper strong while it is wet. Wet strength resins are polymers based on urea-formaldehyde, melamine-formaldehyde or polyamide resins crosslinked with epichlorohydrin. The formaldehyde-based resins are most effective under mildly acid conditions while the epichlorohydrin-based resins are normally used under neutral or

mildly alkaline conditions. The development of the neutral/alkaline based wet strength resins was critical to the production of soft, absorbent grades of paper. Dry strength resins are not only based on natural products such as starch and carboxymethylcellulose but also synthetic materials, such as polyacrylamide. The use of polyacrylamide is not restricted to functional additives such as the dry strength resins.

- COLOURANTS, BLEACHING and WHITENING AGENTS: they are usually of synthetic origin. They are seldom used in paper and board for food contact, and if such is the case, are subject to particular requirements. Natural pulp color varies from light to dark brown, so often it is whitened bleaching it with chlorine-based chemicals or with hydrogen peroxide; unfortunately these substances reduce to some extent the strength of fibres. Fluorescent whitening agents (FWAs) are also synthetic chemicals with an optical brightening effect: their function is to absorb ultra-violet rays in daylight and restore it into visible, blue light, thus increasing the brightness of paper and board. Only certain FWAs are permitted in paper and board for food contact.

PROCESSING AIDS. These are used to improve the efficiency of the paper making process and they are designed so that they are not intended to be retained in the paper. Therefore the potential for migration to the food is minimal, if the paper production process is under control. Typical examples are antifoamers and defoamers, biocides, felt cleaners and deposit control agents.

- DEFOAMERS: paper and board production involves high sheering and steering; furthermore, large volumes of water are used favouring the occurrence of foam in chests and circuits. Foam is detrimental to production efficiency and air bubbles may cause defects in the finished products; defoamers are used to prevent its formation.
- BIOCIDES: circuit closure involves soluble material concentration in process water. Microbiological growth is avoided by biocides and precipitation on felts, and in circuit walls by felt cleaners and deposit control agents. Biocides are essential especially if recycled material is present in the pulp.

There is also an intermediate group of products that are retained in the paper but are designed to improve the efficiency of the paper-making process. Typical examples are retention aids and drainage aids.

- RETENTION and DRAINAGE AIDS (DEWATERING ACCELERATORS). sheet formation involves swift dewatering of low consistency pulp suspension. Retention aids are meant to assist in retaining fines and fillers in the wet web, while drainage

aids increase dewatering speed; they are often plastic polymers or resins. The main challenge facing the papermaker is retaining the chemical additives in the paper so that they can perform their intended technical function. This challenge is the main reason why the contaminants and by-products present in the additives supplied to the paper industry do not end up in the paper. They are mostly soluble in water and therefore remain in the process water during filtration. They can also be evaporated with the steam in the dryer section of the paper machine. There are often limitations placed on the amount of the chemical additives as well as limitations on the by-products and residual monomers present in the polymeric products that can be found in the paper.

- OTHER SUBSTANCES: dispersion and flotation agents, precipitating and fixing agents, slimicides (often enzymes or antimicrobial agents), refining agents, humectants, etc.

Now pulp treated with additives is ready for papermaking machines, which can be distinguished in three main kind⁴⁸:

- FOURDRINIER MACHINES: pulp is fed on a wire screen belt through which the water is continuously drained. Finally, paper is passed around a series of heated drying drums taking moisture content down to final product specifications. It is mostly used to produce paper, seldom for paperboard.
- TWIN-WIRE MACHINES: as the name suggests, pulp is fed between two wire screen belts, with the advantage of draining water faster, from both surfaces. These machines usually produce single or multilayer paper identical on both sides.
- CYLINDER MACHINES: screens are onto rotating drums; on the surface of each of them a thin layer of fibres is formed and then transferred onto a moving felt belt which receives all forming paper layers. These machines can be used to produce paperboard, with the advantage of combining layers of different paper kind and quality (e.g. quality bleached short-fiber on the outside for ideal printability, several low quality recycle layers in the middle, unbleached fiber in the inside for a “natural” look). In Figure 5 some examples of different paperboard layer combinations can be seen.

Finally, paper or paperboard undergo the “calendering” operation to improve caliper consistency and to smooth out the surface of paper. This is obtained passing the formed dried paper between several heavy rolls. Paperboard can also have some surface sizing agents (starch, clay, calcium carbonate, etc.) and/or coatings to further improve surface

⁴⁸ Soroka, 2009.

brightness, printability and damp-resistance. Often coated boards have uncoated margin areas to permit adhesives to hold together paperboard boxes once formed.

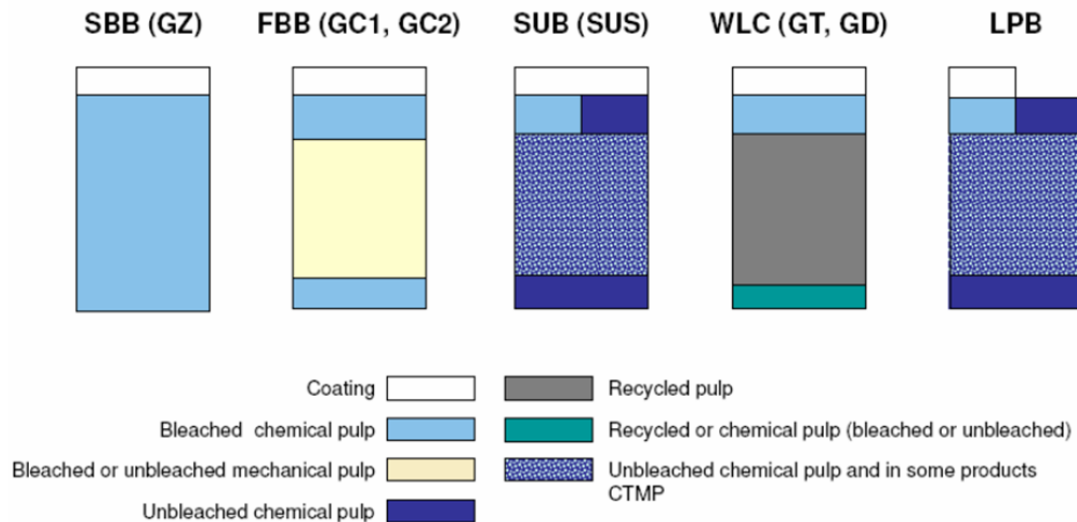


Figure 5. Different paperboard layer combinations, with their commercial abbreviations. Coating often consists in a simple white colour mineral finish (e.g. clay). Modified from Iggesund Paperboard, Sweden.

1.2.2 PAPER PRINGING TECHNIQUES

Only printing techniques applicable to paper based materials will be discussed in this paragraph. These materials, in fact, are highly porous and thus tend to absorb inks and their carriers, requesting a specific formulation in order to obtain a good final result. Paperboard needs a special finishing prior to printing, at least a coating with mineral materials such as kaolin or calcium carbonate, in order to offer an even and smooth surface to inks. The main printing techniques applicable to paper and paperboard are the following.

- **OFFSET SOLVENT BASED PRINTING.** In this technique the pigments are dispersed in a solvent that has to be oily and thick, e.g. mineral oil. This is because there is no need for a quick evaporation of solvent (as in the case of printing of plastics): in the case of paperboard the ink is rather soaked into the fibres and fixed after a certain amount of hours. Mineral oil can be substituted with other solvents, e.g. vegetal oil, which unfortunately are not as stable as mineral oil and tend to oxidize releasing off odours like aldehydes. More recent applications use special triglycerides as solvents, in order to have molecules both stable and not tending to migrate, but

mineral oil remains the cheapest solvent for offset printing. It is available in different quality grades: “white” mineral oil is refined in order to contain only saturated hydrocarbons, whereas “technical grade” mineral oil also contain aromatic hydrocarbons, up to 30% of total; the latter should not be used for paper based food packaging printing. Also water based inks are available, offering better food safety approach and environmental impact, but the finished result on paper-based materials is still unsatisfactory.

- UV PRINTING. This kind of inks are solventless, but contain special molecules, called photoinitiators or photosensitizers, along with pigments and monomers and/or oligomers. After printing, the paper-based material is exposed to UV light, which converts the photoinitiators into active radical species, starting a polymerization procedure on the monomers/oligomers which transforms the liquid ink into a solid layer; fixing the monomers/oligomers into a tridimensional net, also fixes the pigments. The absence of solvents and the instantaneous exsiccation allows to reach high printing quality, but some migration issues have arose in the past if the ink was not perfectly dry (e.g. because of UV lamps malfunctioning). Besides, the most volatile photoinitiator can migrate to food content through the gas phase, even if the printing technique is correctly performed: this is why in recent years higher molecular weight photoinitiators are preferred.
- ELECTRON BEAM. Electron beam curing avoids both the migration issues typical of solvent printing and UV printing. The electron beam imaging process features a dielectric cylinder which is selectively charged by a stream of electrons to attract the ink in the patterns to be printed, similarly of what happens in laser printing. It was initially relegated to niche applications due to machinery of large size, complex integration and frequent maintenance, with an overall high capital expense. In the last years it is slowing gaining market shares, especially in USA.

1.2.3 RECYCLED PAPER SAFETY ISSUES

Paper represent the most abundant (by weight) packaging material in residential solid waste, followed by glass⁴⁹. Recovered paper is an important raw material in terms of volume and utilization for the paper industry in many Countries. The recycling of paper is an example of sustainable use of resources⁵⁰.

⁴⁹ Source: Environment Protection Agency (USA).

⁵⁰ Laurijssen *et al.*, 2010.

Paper products vary considerably in their fibre and other ingredients composition, depending on quality and intended use. Despite this poor homogeneity, reasonably efficient post consumer collection and sorting systems are in place, and anyway some cross-contamination within different paper products type does not represent a big problem, or at least not as much as for mixed plastics. The main problem for paper-based materials recycling, aside the recycling contaminants issue, is the fact that paper fibre quality (and in particular fibre length) deteriorates at every recycling cycle, making it impossible to recycle paper indefinitely. In fact, very often a proportion of fresh cellulose fibres from timber or other sources is added at every recycling cycle in order to obtain sufficient final product resilience.

Although recycling is both economically and ecologically sound, recovered paper cannot be used in all paper grades for its lower fiber quality; furthermore, there are health safety issues connected to its use in contact with food, which are object of the present work. Various contaminants can be present in recycled paper used to produce food packaging: mineral oil used as solvent for offset printing inks (newspapers, magazines, leaflets, packaging, etc.), phthalates and other additives present in the glues used to shape up the paperboard boxes, plastic additives present in the plastic windows or in plasticized paper (all ends up into post consumer recycled paper), etc. Because of all this, it is important to monitor the presence of contaminants able to migrate from paper based food packaging to food content.

Broadly speaking, the production process for recycled paper is similar to the process used for paper made from primary fibres. The main difference is that recovered paper fibres have already been used, so that non fibrous material, originating from previous uses, will have to be removed. The major steps in the recycling process are⁵¹:

- Collection and Transportation: recovered paper is sorted, graded, formed into bales and delivered to a paper mill.
- Repulping and Screening: having reached the paper mill, recovered paper is mixed with water and chemicals, which separates the paper into individual fibres.
- Cleaning: following pulping, the pulp mix is diluted with water (roughly 1:10) and passes through a system of centrifugal cleaning equipment and screens: the pulp is filtered and screened through a number of cycles to make it more suitable for papermaking. This is done to remove large contaminants like wood, plastic, stones,

⁵¹ ILSI Europe Report Series. Packaging materials. 6: Paper and board for food packaging applications. 2004.

glass and paper clips, along with small contaminants like string, glue and other sticky materials: pulp is cleaned in a large spinning cylinder and the heavy contaminants move to the outside of the cylinder and are removed.

- De-inking: for certain uses (e.g. for the production of graphic, sanitary and domestic papers but rarely for manufacture of packaging materials) and for certain types of recovered papers (e.g. newspapers and magazines), the fibres have to be de-inked. It is an additional cleaning step that can be performed if needed: it is a costly operation so it is performed only if the added product value justifies it. Besides, only a few paper mills have the additional equipments to perform it. The deinking process can be carried out by flotation, with or without washing, with or without kneading, with or without bleaching. Flotation involves the pulp being fed into a large vat called a flotation cell. Soapy chemicals are added to help the ink separate from the pulp. Air bubbles are blown into the mixture. The ink attaches to the bubbles and rises to the top. The inky bubbles are then skimmed off, leaving the pulp ink-free. During kneading the pulp fibres are rubbed against each other, further loosening the inks, while chemicals are added to begin the bleaching process. Bleaching the pulp counters any yellowing effect sometimes seen in paper containing wood fibres like those used for newspaper. The fibres are soaked in chemicals for about three hours in a storage chest. The pulp that went into the bleaching process grey and dirty in appearance comes out much whiter and cleaner. Optionally, more de-inking, washing, kneading or bleaching loops are implemented. If coloured paper is present in the recovered paper furnish, colour stripping may have to be carried out. The pulp is then washed, pressed, kneaded and placed in the decolourization chest. A chemical is added to remove any colours that might tint the pulp. Subsequently, the pulp is washed again to remove any remaining ink particles, fillers or other contaminants.

The finished recycled pulp is now ready to be made into paper and is either sent on a mile-long conveyor to the mill for papermaking, or is formed into sheets of pulp for shipment and sale. Depending on the grade of paper being produced, quantities of virgin pulp from sustainable sources may be added. Some papers, such as newsprint and corrugated materials, can be made from almost 100% recycled paper. Once the paper is used, it can be recycled and the process starts again. Individual fibres will gradually be degraded in the process so a continuous addition of new fibres is necessary to sustain the recycling cycle.

There are different grades of recovered paper and board to satisfy the needs of different producers according to strict specifications. More than 50 grades of recovered paper and

board are defined in the European List of Standard. The standard EN 643, “Grades of Recovered Paper and Board”, describes the following:

- Low grades (mixed papers, old corrugated containers, board, etc.) constitute the main part of the recovered paper consumed. These are used to produce secondary packaging papers and boards, and are not intended to be in direct contact with food
- De-inking grades (newspapers and magazines, graphic papers, etc.) are usually also considered as low grades because they need extensive recycling treatments. These are for graphic and sanitary papers.
- High grades (scraps, sheets, print off-cuts, etc.) require little or no cleaning. They can be used for the production of any paper product as pulp substitute. They may therefore be suitable for food contact packaging.

While the first two grades derive from post-consumer waste, the third derives from post-industrial, cleaner and less printed waste.

Over the past decades, recovery and utilization of recycled paper have increased all over the world due to economic, environmental and social issues; however, extended recovered paper collection is detrimental to its quality, either by the exploitation of lower quality sources such as households, or the spreading of commingles systems instead of selective collection systems⁵². Need for toxicological evaluations and European harmonized regulation was already perceived a decade ago⁵³ but no conclusive official steps have been taken since.

Both paperboard and corrugated board often contain a high portion of recycled material. It has been found that volatile contaminants are not only able to migrate from paperboard to food, but also from corrugated board to food thus passing paperboard and plastic layers to reach the food content: contaminants from corrugated board are able to migrate, through the gas phase, into the food content even if this is protected by a multilayered packaging⁵⁴. Postconsumer waste contains many extraneous bodies and contaminants, and only some of them can be removed during recycled paper repulping, because they are not water soluble. The more abundant contaminants are adhesives (either hot-melt or liquid), plastic debris (especially from plastic coated packaging), printing inks and their solvents and additives, varnishes, etc. As a result, a long series of contaminants can be found in recycled paper:

⁵² Miranda *et al.*, 2011.

⁵³ Escabasse and Ottenio, 2002.

⁵⁴ Biedermann *et al.*, 2011b.

- **MINERAL OIL:** the main source for the introduction of mineral oil into the recycling system are the inks used for the offset printing of newspapers, magazines and leaflets, which contain 20-30% mineral oil as solvent. Mineral oils are also introduced from other sources (e.g. adhesives and solvents used in the formulation of paper additives). This contaminant will be discussed in deep detail in the next paragraph.
- **PHOTOINITIATORS** from UV printing: benzophenone, 4-methylbenzophenone, 2-isopropylthioxantone (ITX), etc. Benzophenones final content in the printing ink ranges from 5 to 10%. They are fairly small molecules, not chemically bound to the ink and thus can easily migrate to food through the gas phase, both from the printing (if UV) and from the recycled paper (if food packaged in paper-based materials from recycling). Benzophenones residues can be detected in vast majority of population because they are also present in other products (e.g. some cosmetics). Their toxicity, both acute and chronic, is not particularly high, but many studies point out at their endocrine disrupting effect⁵⁵.
- **PHTHALATES** (e.g. dibutyl phthalate - DBP, diethyl hexyl phthalate - DEHP, etc.). Phthalates are widely used additives for plastics (e.g. PVC) and other materials (e.g. building materials, clothing, toys, medical devices), primarily to make them soft and flexible. Since phthalates are not chemically bound to plastics, they can be easily released into the environment. Thus, phthalates present in packaging materials may be released into beverages and foods (especially if rich in lipids). They are also used in solvents, lubricating oils, fixatives, detergents and in products such as cosmetics and wood finishes. In addition, they are released directly into the environment during phthalate-containing goods production, their use and after disposal. This family of substances has been used for decades, and being persistent in the environment, the level of contamination is still high despite the decline in their production and use in the last decade. Phthalates bioaccumulate in invertebrates, fish, and plants but do not biomagnify, because higher animals efficiently metabolize and excrete them. They have become ubiquitous contaminants in food, indoor air, soils, and sediments. In the general population, the major exposure source is food contaminated during growth, production, processing, or packaging. Food surveys have documented the highest levels in fatty foods, such as dairy (including infant formulas), fish, meat, and oils⁵⁶. Each phthalate has a different toxicity profile and potency, but some of them show

⁵⁵ Muncke, 2011.

⁵⁶ Staples *et al.*, 1997.

carcinogenic effect in rodents⁵⁷, and have ascertained endocrine disrupting activity which is particularly worrying in case of foetuses, infants and children exposure^{58,59,60}. Where limits lower than EU for phthalates have been established (e.g. in Germany), and efforts have been put in place to reduce usage of these substances in most industrial sectors, a slow decreasing trend of their presence in recycled paper can be noticed.

- MALEATES: e.g. di-(2-ethylhexyl)maleate (DEHM)⁶¹.
- Paper additives for carbonless copy paper, thermal and pressure sensitive inks: di-isopropyl naphthalenes (DIPN).
- Other plasticizers and additives (from adhesives, coatings, inks, plastic residues, etc.): adipates, sebacates, epoxidized soy bean oil (ESBO), acetylated tributyl citrate (ATBC), trimethyl pentadiol diisobutyrate (TXIB), di-isononyl cyclohexane dicarboxylate (DINCH), antioxidants of the Irganox[®] range, etc.
- Inks, dyes, and their additives.
- Ink solvents: mineral oil hydrocarbons, ethyl acetate, etc.
- Additives from glues and adhesives used to close up the paperboard boxes: e.g. aromatic amines from polyurethanes.
- Other volatile organic compounds (VOCs), e.g. formaldehyde.
- Rosin components: also called colophony, is a solid form of resin contained in timber. Abietic acid and dehydroabietic acid are both found in rosin. Despite being natural substances, they show some toxicity⁶² and are amongst the major toxicants of paper mill effluents, causing water pollution and damages to aquatic organisms⁶³. They can often be detected as migrants from both fresh and recycled paper fibres used for food packaging.

None of the possible solutions to the problem of contamination from recycled paper materials for food packaging is readily and easily applicable. Some packaging producers and food producers are already considering or using some of them:

- 1) Completely eliminate the use of recycled fibres for packaging production, and only use fresh fibres. This radical approach is not environmentally friendly, and will cause

⁵⁷ Caldwell, 1999.

⁵⁸ Howdeshell *et al.*, 2008.

⁵⁹ Huang *et al.*, 2009.

⁶⁰ Cirillo *et al.*, 2011.

⁶¹ Fiselier *et al.*, 2010.

⁶² Ozaky *et al.*, 2005.

⁶³ Ozaki *et al.*, 2006.

a sharp rise in costs (fresh fibres cost ca. 200 € more per ton compared to recycled fibres). A more reasonable approach would be to pursue a progressive change of destination for fresh and recycled fibres: the first ones should be used preferentially for food packaging manufacture, while recycled fibres should be used to package all non-food products (including pharmaceuticals and cosmetics that at present always use fresh fibres, for appearance reasons). A portion of recycled paper could also be burned as an efficient fuel, instead of timber.

- 2) Use mineral oil free inks for food packaging. This measure is already requested by some high quality food producers, but it eliminates just a part of the problem: if a paperboard from recycling is used, the inks present in the recycled material will be mineral oil based (newspapers print). Some newspaper printers do not use mineral oil based inks (which is probably the cheapest printing technique), especially in some Countries (e.g. Japan⁶⁴). Using different techniques is not necessarily a better approach: e.g. water-based inks used from some newspapers are very difficult to de-ink during papermaking.
- 3) Always use an efficient protective barrier between paperboard and food. Such barrier can be made of aluminium or of special plastics able to avoid volatile contaminant passage. The barrier can be either present as a wrapping bag for the food, or as an internal coating of paperboard: this latter approach is at present developed by some leader food packaging producers. In any case, additional packaging layers will mean more costs and more waste.
- 4) Implement or improve cleanup procedures in paperboard production plants using recycled materials. Some cleanup procedures are already in place in a few paper mills using recycled materials, e.g. de-inking steps, which also allow for some mineral oil and other contaminants elimination. These procedures are time consuming and costly, furthermore they provoke loss of a part of paper fibres, with decreasing production yield. These reasons make de-inking, and recycled materials cleanup procedures in general, not worthy from an economical point of view: the price difference between de-inked recycled paper and paper made from fresh fibres is often considered too little to stimulate research (with the consequent time and resources needed) and applications in this field. Nevertheless, from an environmental point of view the European Union is keen to increment the use of recycled materials as much as possible, possibly investing

⁶⁴ Biedermann and Grob, 2010.

some funds to stimulate the necessary research to assure the safety of these materials when used for food applications.

To conclude, none of the four points illustrated can be considered the ultimate solution for all food packaging recycled materials applications. An approach based on risk assessment is probably the best way to choose the ideal paper-based packaging for every food application: very sensitive foods (e.g. with high surface to weight ratio and high lipid content) have to be packed in fresh fibres paperboard or alternatively an efficient protective barrier should be used. Less sensitive foods could be packed in higher quality recycled paperboard (e.g. from post-industrial waste, less contaminated, instead of post consumer waste), and possibly the product shelf life could be reduced to avoid reaching high contamination levels. Finally, some foods (e.g. salt, sugar), which do not tend to adsorb mineral oil hydrocarbons due to chemical nature, and which are consumed in small amounts, could may be still be packed in recycled paperboard⁶⁵.

1.3 MINERAL OIL CONTAMINANTS

Petroleum, meaning literally “rock oil”, is the term used to describe a hydrocarbon rich fluid that have accumulated in the subterranean reservoirs. Petroleum, also called crude oil, varies dramatically in colour, odour, and flow properties that reflect the diversity of its origin⁶⁶. Petroleum derivatives are any petroleum based products that can be obtained by refining and comprise refinery gas, ethane, liquefied petroleum gas, naphtha, gasoline, aviation and marine fuels, kerosene, diesel fuel, distillate fuel oil, residual fuel oil, gas oil, lubricants, white oil, grease, wax, asphalt, as well as coke (see Figure 6).

The use of petroleum products is widespread in human activities and go from fuels to various products for industry (lubricants, pneumatic, etc.) to pharmaceutical products and cosmetics⁶⁷. Often petroleum derivatives are highly complex chemicals, and considerable effort is required to characterize their chemical and physical properties, which determine their use. Mineral hydrocarbons may be straight chain (*n*-paraffins), branched chain (e.g. iso-paraffins) or cyclic (naphthenics). The oils are generally described according to the predominant type of material present, as either paraffinic or naphthenic, but paraffinic oils

⁶⁵ Lorenzini R. Contaminazione da olio minerale: come minimizzarla? Soluzioni al problema con approccio risk assessment. *Macchine Alimentari (Tecnica Nuova)*, November 2011.

⁶⁶ Speight, 2002.

⁶⁷ Gary *et al.*, 2007

may contain some cyclic structures, and similarly naphthenic oils may contain some straight and branched chain paraffins. Mineral waxes contain mainly paraffinic hydrocarbons, with only very low levels of saturated cyclic naphthenic structures. It is the ratio of straight chain to branched chain paraffinic hydrocarbons, and to a certain extent molecular weight, which determines whether the wax is classified as a paraffin, intermediate or microcrystalline wax. Paraffin waxes contain mainly straight chain components with the proportion of branched chain components as low as 5%; the proportion of branched chain components increases as average molecular weight increases. Intermediate waxes have higher average molecular weights than paraffin waxes and consist of approximately equal proportions of straight chain and branched chain alkanes. Microcrystalline waxes have the highest average molecular weight and contain mainly branched chain components with less than 30% straight chain alkanes. Hydrocarbon waxes which are completely synthetic will also be mixtures of components with varying chain length, but comprise mostly straight chain components only.

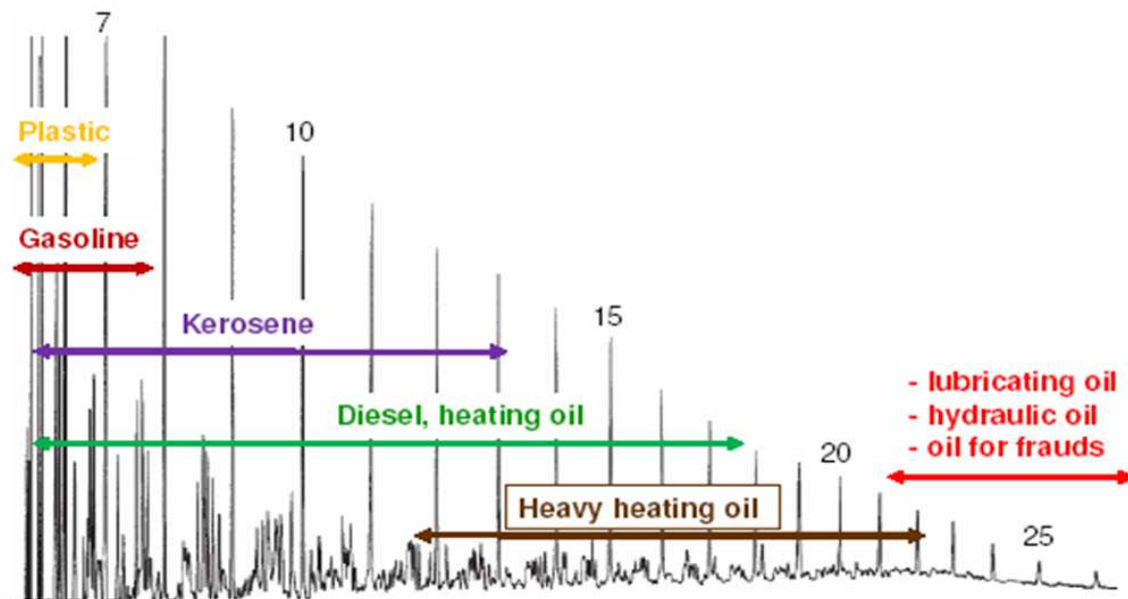


Figure 6. GC chromatogram of petroleum (crude Arabian light) and molecular weight range of some of its products. Chromatogram from Restek Corp.

Mineral oil is an oily liquid ranging from transparent to yellowish color, widely used for many industry sectors, among which as cheap solvent for several applications, including printing inks. Mineral oil is mainly composed of short chain paraffinic hydrocarbons containing an abundant aromatic fraction (10-25%). Mineral oil average molecular weight,

expressed in carbon number, usually ranges from C10 to C35. Chain length does not only affect physicochemical properties like viscosity, but also substantially influences physiological (absorption by skin or gastrointestinal tract, accumulation in fat tissue) and toxicological character. Mineral oil in newspapers is about 3000 mg kg⁻¹; content in unprinted recycled board ranges from 300-1000 mg kg⁻¹, and of course increases after board printing⁶⁸.

Contamination of food mainly occurs through gas phase transfer. Dry foods having a large specific surface, containing fat and with long shelf life are of special concern, as some of them can reach contaminations of various tens of mg kg⁻¹⁶⁹. Migration is roughly limited to volatile components up to about C24^{70,71}.

Mineral oil saturated hydrocarbons (MOSH, see Figure 7) are paraffinic (open chain, mostly branched) and naphthenic (cyclic) hydrocarbons, with molecular weight distribution of chain length centred below C24, corresponding to volatility which enables transfer into dry food at ambient temperature. Mineral oil aromatic hydrocarbons (MOAH, see Figure 6) can have one (benzenes), two (naphthalenes), three (anthracenes and phenanthrenes) or four (chrysenes, pyrenes, fluoranthenes, benzanthracenes) aromatic rings, with different degrees of alkylation (alkyl side chains differ in length and branching): this alkylations make them differ, both chemically and toxicologically, from the polycyclic aromatic hydrocarbons (PAH). Actually, the absence of PAH is often a purity requirement for mineral oil.

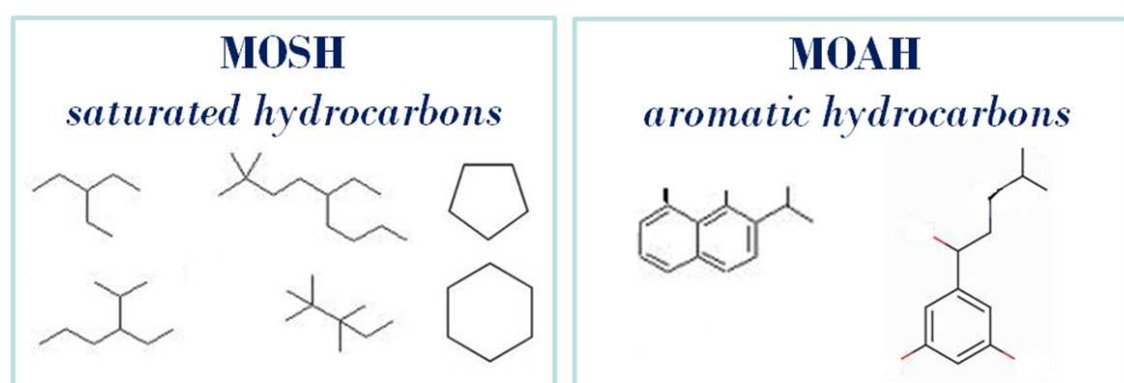


Figure 7. Examples of Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH) structure.

⁶⁸ Vollmer *et al.*, 2011.

⁶⁹ *Ibid.*

⁷⁰ Droz and Grob, 1997.

⁷¹ Lorenzini *et al.*, 2010.

Di-isopropyl naphthalenes (DIPN) are additives widely employed in the paper industry for carbonless copy paper manufacture and for thermal sensitive and pressure sensitive paper manufacture. They are also used in other industrial sectors, e.g. for the production of dielectric fluid and thermal oil substituting polychlorinated biphenyls⁷². They are chemically related to MOAH (see figure 8), and are therefore extracted and eluted along with them during analysis. They differ from MOAH because they are toxicologically better characterised, and considered of low toxicity, without carcinogen or mutagenic effects nor toxic for the reproduction.

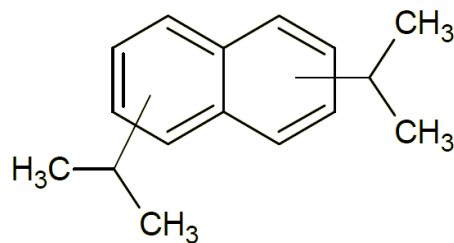


Figure 8. Di-isopropyl naphthalenes general chemical structure.

1.3.1 MINERAL OIL IN ENVIRONMENT AND FOOD CHAIN

Known sources of mineral oil hydrocarbons in food are many. Only in the last few years, the European Rapid Alert System for Food and Feed (RASFF) received various notifications of foods contaminated by mineral oil, among which butter, palm oil, noodles contaminated by packaging, dried raisins, rapeseed oil, sunflower oil, maize oil, walnut oil, red wine, biscuits, fresh egg pasta and sauces⁷³. The most important sources of mineral oil in food and environment are now described^{74,75}.

Jute bags. They are big carrier bags made with strong fibres from plants of the *Corchorus* gender. They are widely used in Countries producing food raw materials as coffee, cocoa, tree nuts, tea leaves, dry fruits, etc. Because such vegetal fibres are very hard to batch, they are sprinkled with mineral oil (“batching oil”) in order to make them slide easily. Mineral oil is then easily transferred to food content during storage and transportation^{76,77},

⁷² Boccacci Mariani *et al.*, 1999.

⁷³ <https://webgate.ec.europa.eu/rasff-window/portal/index.cfm?event=searchResultList>.

⁷⁴ Heimbach *et al.*, 2002.

⁷⁵ Lorenzini R. Contaminazione da olio minerale: come e perché è presente nei nostri alimenti. *Macchine Alimentari (Tecniche Nuove)*, December 2010.

⁷⁶ Grob *et al.*, 1991a.

⁷⁷ Grob *et al.*, 1991b.

especially in the case of foods rich in lipids, for which mineral oil has high chemical affinity. In this way, many products are already contaminated at the raw materials stage.

Use as antisticking and release agent. Thanks to its chemical properties similar to a vegetal oil, mineral oil was often sprinkled on oven trays for bakery products in order to facilitate the release of the finished products⁷⁸. Other similar food applications were found in the sweets and candies production industry. Since the introduction of antisticking materials for ovenware, this use of mineral oil is not widespread anymore.

Use as dust binder. Another unhealthy food industry practice consists in sprinkling mineral oil on cereal kernels (or other seeds) masses, when stored in warehouses, prior to their mechanical movement, to avoid dust formation. Of course such mineral oil can be adsorbed and partly penetrate into the seeds, contaminating all derived food products (e.g. flours). Also feed is sometime treated with mineral oil in this way⁷⁹. Furthermore, mineral oil is used as pelletizing aid for some feeds: hens fed with this kind of pellets transfer part of mineral oil to their eggs and meat; in chicken, pork and bovine meat a mineral oil contamination up to hundreds of mg kg⁻¹ has been found⁸⁰. Dust binding is still allowed in USA and in other extra-EU Countries.

Polishing of fruit and dried fruit. In some Countries, fruits as apples and citrus fruits are polished with waxes to improve their appearance. Some exotic fruit, as pineapple, are also waxed, in order to slow down their ripening. Dried fruit as plums, apricots and raisins can contain a significant contamination⁸¹: they are sometimes sprayed with mineral oil to give them a shining appearance.

Cheese waxing/glazing. Some cheeses have a wax coating to protect them from moisture loss and molding. Wax hydrocarbons can migrate into the cheese mass for a few mm, depending on cheese composition (fat and water content), wax composition, ageing time and temperature, etc.

Pesticide formulations. Some pesticides are dispersed in oily-bases formulation instead of water-based formulations, which are more common but not always possible or desirable. Besides, mineral oil has an insecticide effect *per se*, by a suffocating mechanism. Mineral oil is accumulated especially in fruit with high fat content (e.g. olives and consequently olive oil).

⁷⁸ Grob *et al.*, 1991c.

⁷⁹ Grob *et al.*, 2001.

⁸⁰ European Commission - DG for Health & Consumers - and Kantonales Labor Zurich, Workshop on mineral oil material in foods: analytical methods, occurrence and evaluation, 17-18 September 2008.

⁸¹ Fiorini *et al.*, 2010.

Contamination/adulteration of edible oil and fat. Mineral oil contamination has been found at some stage on the majority of vegetal oils and fats⁸²: cocoa butter, olive oil and olive-pomace oil⁸³, peanut oil, sunflower oil, soy seeds oil, flaxseed oil, grape seed oil, etc. The latter are almost always contaminated in the range of 30 to 200 mg kg⁻¹: contamination probably derives from grape skin (atmospheric pollution, treatment with mineral oil-based pesticides), then is concentrated in the little oil present in the seeds⁸⁴. In fact vegetal oil represents an ideal medium for mineral oil accumulation, regardless of its multisource origin. Also fat of animal origin is not immune from contamination: at the beginning of 2012, over 1000 mg kg⁻¹ of mineral oil have been found in butter from France⁸⁵. Mineral oil can be present in vegetal oil also as a fraud (being cheaper than the adulterated oil): the case of Ukrainian sunflower oil contaminated at 7000 mg kg⁻¹ in 2008 is probably the most resounding one, but not the only one.

Use as laxative. Liquid paraffin has been used for decades as mild oral laxative (e.g. for the elderly), but nowadays this application is declining. The use of liquid paraffin as condiment instead of vegetal oil has been reported in some disputable low-calories diet, thanks to the fact that hydrocarbons are not energetically metabolized by human body.

Petroleum accidental spillages during extraction, storage and sea transportation. With the 2010 Mexico Gulf explosion at the Deepwater Horizon platform (owned by British Petroleum), the serious petroleum accident from '50 rises to 75 worldwide. They acknowledge tanker ships collisions, petroleum wells explosions, deepwater platform accidents, leakages from tanks and mains, etc., with a total environmental pouring of over 5 million tons of raw petroleum, very often in the sea. In Italy, the more serious recent accident regards river Lambro, with 10 million litres of diesel oil leaked from an ex refinery plant. Petroleum is immiscible with water, but the fate of petroleum fraction in water depends on many factors, among which the molecules chemical and physical characteristics: the lighter molecular weight fraction distribute on the water surface⁸⁶, acting as a barrier for light and oxygen penetration, thus damaging many aquatic species (both vegetal and animal). The remaining fractions are partly dispersed in water, and partly sediment, over time. It is inevitable that a part of this sediment enters the marine food chain, contaminating many food products as shellfish, crustaceans and fish⁸⁷.

⁸² Wagner *et al.*, 2001.

⁸³ Moret *et al.*, 2001.

⁸⁴ Fiorini *et al.*, 2008.

⁸⁵ RASFF portal, <https://webgate.ec.europa.eu/rasff-window/portal/>.

⁸⁶ Anderson *et al.*, 1974.

⁸⁷ Gin *et al.*, 2001.

Leakages of lubricating and hydraulic oils from industrial plants. With the automation of food and beverages production and packaging at industrial level, it is not uncommon to incur in contamination caused by lubricating and hydraulic oils or fuels⁸⁸. These contaminations are usually caused by accidental spillages from machinery as conveyor belts, moving organs as stirring devices, pistons, kneaders, measuring devices, cutters, pipes, etc.

Atmospheric pollution from industries and traffic. Besides the many problems caused by this kind of pollution (e.g. respiratory tract diseases), there is also the contamination of food⁸⁹: plants and animals can be contaminated especially if near to sources of pollution, as industries and heavy-traffic roads. Plants are particularly at risk if with high surface (green leafed plants such as lettuce and similar) and cultivated in open field. Some plants can also adsorb hydrocarbon fractions from contaminated soil.

Food packaging. Many food packaging materials can be source of hydrocarbons contamination in food⁹⁰. In the case of metal packaging (i.e. cans for beverages, fruit, legumes, tomato products, tuna, etc.), mineral oil can be sprayed on machinery tools which cut and shape the cans, in order to avoid friction and excessive heating: the mineral oil left in the can will contaminate its food or beverage content⁹¹. In the case of fish products as tuna, the contamination can be double: from marine sea during the fish predator life and from packaging. Sometimes also the outside of glass bottles and jars is sprayed with mineral oil or other gliding agents to avoid ruptures during these containers conveying. Also plastic can release hydrocarbons when in contact with food, especially if not well polymerized: in fact, plastic oligomers are hydrocarbons. Paper based product are probably the most common source of mineral oil when used for food contact: in the past, a grease-proof paper was used in contact with meat and cheese, obtained with a paraffinic layer on the paper; nowadays a plastic film is usually coupled to plastic for this purpose. A food safety issue that has been known for decades, but it is drawing attention in the last few years, is the use of recycled paperboard in contact with many foods such as pasta, rice, breakfast cereals, sweet and savoury snacks and other bakery products, cocoa powder, teas and herbal teas, frozen food, eggs, etc. Paper-based products are perceived as safe and “natural” by consumers, compared to other materials such as plastic. On the contrary, many contaminants are present such as printing inks solvents and additives, and

⁸⁸ Grob *et al.*, 1991c.

⁸⁹ Neukom *et al.*, 2002.

⁹⁰ Grob *et al.*, 1991d.

⁹¹ Grob *et al.*, 1997.

contaminants from recycling (if used in the paperboard manufacture). Post-consumer recycled paper materials contain high proportion of newspapers and leaflets, which in Europe are printed with mineral oil based inks: therefore, these materials represent the main source of mineral oil in paperboard obtained from recycled fibres, whereas office paper, books and corrugated board were the starting materials of lowest mineral oil content. On average, European newspapers produced by offset printing contained 4100 mg kg⁻¹ <C24 mineral oil with 21% aromatic hydrocarbons. One out of four Japanese newspapers only contained 430 mg kg⁻¹ <C24 saturated and less than 15 mg kg⁻¹ aromatic hydrocarbons⁹². Contaminants from recycling and from paperboard printing are partly overlapping and all contributing to the final contamination level, being both represented by substances like mineral oils, phthalates and other plasticizers, photoinitiators, etc. If these contaminants have sufficient volatility, they can pass from paperboard to food content through the gas phase and be adsorbed on the food surface, particularly if the food is in direct contact with paperboard⁹³. In the case of food destined to water boiling before consumption (e.g. pasta and rice), some mineral oil is lost in the process⁹⁴, but the majority of foods packed in paperboard do not undergo this treatment. Being the safety of paper based materials used in contact with food the object of the present work, a deeper discussion will take place in the experimental part of this thesis.

1.3.2 TOXICOLOGICAL EVALUATION OF MINERAL OIL

Hydrocarbons are the most abundant xenobiotics in our body (ca. 1 g accumulated in our fat tissue)⁹⁵, probably due to dietary intake combined with use of low quality cosmetics⁹⁶ such as body lotions and hand creams, containing paraffin, petrolatum and mineral oil as main ingredients.

Despite being a well acquainted food contaminant, a full toxicological evaluation of mineral oil is not available as yet, due to the multitude and variety of molecules present in mineral oil and thus the complexity of the evaluation. To obtain complete toxicological data, various mutagenicity studies *in vitro* have to be carried out, together with studies on oral toxicity, absorption, distribution, metabolism, excretion, bioaccumulation, effect on

⁹² Biedermann *et al.*, 2011a.

⁹³ Droz and Grob, 1997.

⁹⁴ Biedermann-Brem and Grob, 2011.

⁹⁵ Concini *et al.*, 2008.

⁹⁶ Concini *et al.*, 2011.

reproduction and developmental toxicity, and studies on long term toxicity/carcinogenicity. Just some of these data are available for mineral oil or for some of its hydrocarbons.

The European Food Safety Authority (EFSA) has recently issued a toxicological evaluation for 34 hydrocarbons⁹⁷, which are just a little piece in the mineral oil hydrocarbons full picture. Therefore this is just a very little official step forward from the previous official EU position, of 15 years before: in 1995 an Opinion of the Scientific Committee for Food (SCF) on mineral and synthetic hydrocarbons was issued⁹⁸. Examining the studies available at that time, both on animals and humans, the SCF stated that: “it is clear that some mineral and synthetic oils and waxes not only accumulate with repeated dosing, but also give rise to effects which are not confined solely to localized foreign body reactions and provide clear evidence of toxicity in animals. In those oils and waxes which did show effects, the effects seen were similar in nature but differed in severity, i.e. some only gave rise to significant effects at a 2% level in the diet whereas others produced effects at 0.02%, with very occasional findings at 0.002%. The following effects were observed: increased organ weights, especially liver and lymph nodes; altered serum enzyme levels; increased monocyte and neutrophil counts; reduced red blood cells, hemoglobin and haematocrit; and the accumulation of hydrocarbon material in tissues. The main histopathological findings were granulomatosis in the liver and focal collections of vacuolated macrophages (histiocytosis) in the lymph nodes. In animals dosed with certain of the waxes, an inflammatory lesion at the base of the mitral valve in the heart was observed. It was characterized by increased cellularity of the valve with destruction of the fibrous core. In some animals given these waxes, birefringent hydrocarbon material was detected in the mitral valve region, but the inflammatory lesion was not always accompanied by a significant level of hydrocarbon material in the valve; similarly, the presence of birefringent material was not always accompanied by an inflammatory lesion. None of the oils tested produced this lesion. In those studies which included a withdrawal phase, most of the toxic effects were still evident at the end of the withdrawal period but there was limited evidence that the severity of some of these effects had decreased during this phase. In all studies, female rats appeared to be more susceptible than male rats. Samples of liver tissue from a small number of rats were analyzed and the accumulated

⁹⁷ EFSA Scientific Opinion - Flavouring Group Evaluation 25, Rev. 1: Aliphatic and aromatic hydrocarbons from chemical group 31. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). EFSA Journal 2010; 8(5): 1334.

⁹⁸ Opinion of the EU Scientific Committee for Food (SCF) “to advise on the safety-in-use of mineral and synthetic hydrocarbons oils and waxes for use as food additives, in food processing and for use in food packaging materials”, 1995 (http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_37.pdf).

mineral hydrocarbons were found to be comparable but not identical to the original oil/wax administered. Lower and higher molecular weight hydrocarbons were under-represented in the liver extracts compared with the original test sample and the branched chain content of the residues was relatively higher than in the original test sample. The data indicate that toxicity is correlated with accumulation. In animal studies, of those mineral and synthetic hydrocarbons which did accumulate, the degree of accumulation was generally highest in those showing most toxicity and lowest in those materials producing little or no toxicity. In all groups tissue levels declined following withdrawal of dosing. Two of the human population studies also showed a clear correlation between the extent of the lesions and the amount of mineral hydrocarbons which could be extracted from the tissues. We have concluded that it is largely the amounts of lower molecular weight, shorter chain-length substances, which are absorbed and only slowly cleared from the body, that most probably determine the occurrence or absence of toxicity. Accordingly, we consider that, for practical purposes for the time being, mineral and synthetic hydrocarbons could be defined by physical specifications which are sufficiently tightly drawn so as to ensure that only a small proportion of any product conforming to these specifications will have carbon chain-lengths in the absorbable range”.

In fact, the hydrocarbons toxicity is directly related to their physical properties, specifically viscosity, surface tension, volatility, and chemical activity of the side chains. Substances with a lower viscosity and/or surface tension, besides being those of higher toxicological concern⁹⁹, can easily migrate through the gaseous phase of a packaging to the food content, thus leading to a higher gastrointestinal exposure through food ingestion. Organ systems that can be affected by hydrocarbons include pulmonary, neurologic, cardiac, gastrointestinal, hepatic, renal, dermatologic, and hematologic. As part for professional and intentional exposure, usual quantities to which the population can be exposed are small, thus the most likely toxicity profiles are the chronic ones.

A 2001 study reviewed the effect of feeding mineral oil hydrocarbons (without aromatic fraction) to rats, concluding that the low molecular weight ones produced dose-dependent lesions as inflammation and necrosis in the mesenteric lymph nodes and in liver¹⁰⁰: a panel of pathologists reviewed published and unpublished data on MOSH (white mineral oils) and waxes administered to different strains of rats. The panel agreed that certain of the

⁹⁹ Opinion of the EU Scientific Committee for Food (SCF) “to advise on the safety-in-use of mineral and synthetic hydrocarbons oils and waxes for use as food additives, in food processing and for use in food packaging materials”, 1995 (http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_37.pdf).

¹⁰⁰ Carlton *et al.*, 2001.

mineral hydrocarbons produced lesions described as granulomas and microgranulomas, in the mesenteric lymph nodes and liver of rats, varying in severity with dose and type of mineral hydrocarbons. The hepatic lesions had inflammatory cell infiltration, necrosis, and fibrosis in the case of low molecular weight paraffin waxes. The microgranulomas were similar in subchronic and chronic studies. Some slight reversibility existed for these lesions, but complete resolution was unlikely as regression of the lesions would be too slow. The panel also agreed that a minimal severity infiltrate of mononuclear inflammatory cells occurred in the base of the mitral valve but the focal infiltrate was minimal in severity. Quite significant differences in metabolism and relative toxicity are seen depending on rat strains, and it is therefore difficult to extrapolate toxicity data to apply to humans. The panel also reviewed some available studies on chronic and subchronic toxicity on human tissues (liver, hepatic lymph nodes and spleen), which were considered of little significance and not similar to those seen in rats.

In another study on toxicity after oral exposure¹⁰¹, several white (with no aromatic fraction) mineral oils, some of which were food grade, and some waxes were fed to rats at 2% level in the diet, for up to 90 days. The hydrocarbons were present in most tissues (including intestine, heart and kidney), and the histopathological findings on target organs (liver and lymph nodes) were the same as found in previous studies. MOSH are not present in urine and are mostly excreted unaltered with faeces. Besides being the more toxic, low to medium molecular weight hydrocarbons are also those with the highest tendency to accumulate into tissues, probably because the higher molecular weight ones are poorly absorbed by gastrointestinal tract and skin. Preferential accumulation was in the alkane range approximately from C20 to C35¹⁰². Therefore, size and structure of individual components play a role both in determining their propensity to accumulate in different tissues and in the severity of any damage that they cause once they have accumulated. These data also suggest that mineral oil should not be used for food applications, or at least food grade mineral oil should not contain material which can accumulate, between C20 and C35.

Another study considered the toxicity of mineral oil when directly injected in tissues¹⁰³, using P2X₇ receptor activation in macrophages and other immune cells as a marker of pro-inflammatory response. It was demonstrated that mineral oil treatment reduces P2X₇

¹⁰¹ Scotter *et al.*, 2003.

¹⁰² *Ibid.*

¹⁰³ Marques da Silva *et al.*, 2008.

receptor expression, down-modulating ATP-induced apoptosis, membrane permeabilization and nitric oxide production. These effects might be associated with the unpleasant side effects already described during long-term administration of mineral oil for cosmetic purposes or as a laxative.

A different study evaluated the toxicity of mineral oil not by direct administration to animals but through *in vitro* assessment. Extract of different kind of paper based materials (fresh fibres, recycled fibres of different quality, recycled fibres but de-inked) were analyzed to establish the level of contaminant and then used to perform four different *in vitro* toxicity tests, with different endpoints¹⁰⁴: a cytotoxicity on human fibroblasts, Ames test on *Salmonella* to screen mutagenic and carcinogenic potential, a test on yeast cells to assess oestrogenic activity, and CALUX assay for compounds with dioxin-like activity. The extract from fresh fibres showed both a much lower level of contamination (determined by GC-MS) and a lower cytotoxicity. The extract from the lower quality paper material (containing the highest amount of recycled fibres) had the highest cytotoxic effect, and also showed some activity at the dioxin-like effect test. None of the extracts showed mutagenic activity. No conclusion on the oestrogenic potential could be made because the extract where toxic to the test organism (yeast cells). A more extended study on *in vitro* toxicity of paper based material extracts was carried out by a joint European project called BIOSAFEPAPER^{105,106}, with researchers from UK, Finland, France, Sweden and Italy joining forces, with the aim of developing quick and reliable tests to be used mainly by paper producers and end-users in order to assess the quality and safety of paper products. The emphasis is on cost-effective tests with toxicologically relevant end-points and sample preparation reflecting actual end uses. The tests involved have already been validated in other areas of safety evaluation. Thus the innovative aspect is to optimize them for paper & board and to develop a test battery applicable to actual food packaging. Nineteen food contact papers and boards and one non food contact board were extracted using either hot or cold water, 95% ethanol or Tenax[®], according to the end use of the sample. Tenax[®] is modified polyphenylene oxide (MPPO), a porous substance often used to test migration of volatile compounds from paper and board. Analyses were performed in GC/MS. The main substances extracted with water were timber natural products such as fatty acids, resin acids, natural wood sterols and alkanols. Substances extracted with ethanol were

¹⁰⁴ Binderup *et al.*, 2002.

¹⁰⁵ EU Biosafepaper project. Application of bioassays for safety assessment of paper and board for food contact (<http://www.uku.fi/biosafepaper/>).

¹⁰⁶ Bradley *et al.*, 2008.

diisopropylnaphthalenes, alkanes and phthalic acid esters. The non food contact board showed the greatest number and highest concentrations of contaminants. The extracts were then subjected to a battery of *in vitro* toxicity tests measuring acute and sublethal cytotoxicity, and genotoxic effects. None of the water or Tenax[®] extracts was positive in cytotoxicity or genotoxicity assays. The ethanol extract of the non-food contact board gave a positive response in the genotoxicity assays, and all four ethanol extracts gave different levels of positive responses in the cytotoxicity assays. These responses could not be linked to any specific compound, but there was a correlation between the total amount of contaminants and the toxicity level.

Also other contaminants potentially migrating from paper-based food packaging have been toxicologically evaluated, e.g. photoinitiators as benzophenones, and bisphenol A. The genotoxicity of 28 paper products, either from fresh or recycled fibres, has been assessed¹⁰⁷. GC/MS analysis confirmed that such contaminants are at least 10 times more abundant in recycled fibres than in fresh fibres. The genotoxicity of paper and paperboard extracts and compounds found in them were investigated by Rec-assay (using *Bacillus subtilis*): of the 28 products, 13 possessed DNA-damaging activity, 75% of which were made from recycled material. However, the levels of the chemicals in the recycled products could not explain such high genotoxic effects.

According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) Acceptable Daily Intake (ADI) values reported in Table 2, for the mineral oil hydrocarbons relevant for this work there is a limit of 0.01 mg kg⁻¹ per body weight. Considering an average body weight of 60 kg, and 1 kg of potentially contaminated food consumed daily, the specific migration limit (SML) of mineral oils in food will be 0.6 mg kg⁻¹.

It has to be underlined that this JECFA evaluation is based on white mineral oil, which contains no MOAH: for technical grade mineral oil (with up to 30% MOAH), SML should be even lower. The aromatic fraction of mineral oil is more concerning because of its higher toxicity^{108,109}, but alkylated aromatic hydrocarbons are insufficiently investigated to date. Data on occurrence, metabolism and toxicological effects are limited to few congeners, only. Alkylation of aromatic ring systems may influence metabolism and biological activity of the compounds and may result in different toxicological properties compared to polycyclic aromatic hydrocarbons, generally considered more toxic (some of

¹⁰⁷ Ozaky *et al.*, 2004.

¹⁰⁸ Khan *et al.*, 1986.

¹⁰⁹ Brown-Woodman *et al.*, 1994.

them have been proven carcinogenic in human according to IARC - International Agency for Research on Cancer) compared to MOAH. There is insufficient data on genotoxic and carcinogenic effect of mineral oil containing an aromatic fraction. Alkylation of aromatic rings can sometimes decrease toxicity but in other cases increase it.

Table 2. Toxicological evaluation by JECFA¹¹⁰. Mineral oils with the red circle, and with the lower Acceptable Daily Intake (ADI), are those used as solvent for printing inks, thus potentially migrating to food. Table prepared by Pfaff and Wölfle (Bundesinstitut für Risikobewertung - BfR). Akademie Fresenius, 17-18 October 2011.

WHO/JECFA, 2002



Name	Viscosity at 100 °C (mm ² /s)	Viscosity at 40 °C (mm ² /s)	Average mol. weight (g/mol)	Carbon number at 5% distillation point	ADI (mg/kg b.w.)
High viscosity mineral oil (P100)	> 11	> 99.8	> 500	> 28	20
Medium and low viscosity mineral oil, Class I	8.5 - 11		480 - 500	> 25	
P70	9.0	70	480	27	10
P70H	8.6	7	480	27	
Medium and low viscosity mineral oil, Class II	7.0 – 8.5		400 - 480	> 22	0.01
N70(H)	7.7	70	420	23	0.01
Medium and low viscosity mineral oil, Class III	3.0 – 7.0		300 - 340	17	0.01

There is still a long way to go before a complete toxicological evaluation on mineral oil is achieved: any new toxicity data has to be evaluated, it has to be established if certain classes (or subclasses) are more relevant due to their toxicity or to differences in the way they are metabolised by the human body, identified the different sources of the background presence of mineral oil in food other than adulteration or misuse, contain a dietary exposure assessment for the general population and specific groups of the population (in particular infants and children) by taking into account the background presence of mineral oil (e.g. pre-packaging) in food, and advise on new classes to be included if monitoring would be set up for the presence of mineral oil in food. Furthermore, when food contact materials substances are assessed for their health risk, they are not routinely tested for their

¹¹⁰ JECFA. 59th Report of the Joint FAO/WHO Expert Committee on Food Additives (2002), WHO TRS n. 913.

endocrine disrupting potential¹¹¹, but this effect should be taken into account, at least for foods aimed at sensitive population groups as infants, children and pregnant woman.

1.3.3 LEGISLATION, GUIDELINES AND STANDARDS

At present no European harmonized legislation has been issued for paper-based packaging¹¹², but in October 2010 EFSA has issued a call for data on mineral oil hydrocarbons¹¹³, which usually precludes the starting of a legislative process on the matter. Even in the absence of specific European legal limits on mineral oil contaminants, the implementation of Article 3 of Reg. (EC) No 1935/2004 requires every packaging to be safe for consumers: “Materials and articles..., shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could: a) endanger human health; or b) bring about an unacceptable change in the composition of the food; or c) the labeling, advertising and presentation of a material or article shall not mislead the consumers.” It is therefore clear that no excuse can be used by manufacturers of contaminated paper-based materials. Italy is one of the few European Countries to have a specific legislation on paper for food contact, which was long considered among the most complete at European level¹¹⁴: DM 21/3/73¹¹⁵, despite being dated, it is still valid for the parts where no European legislation is available. This law fixes specific quality requirements for paper-based materials for food contact, e.g. limits in the presence of Pb and polychlorinated biphenyls (PCB). However, no specific mention is dedicated to mineral oil hydrocarbons. Recently the Swiss Confederation has issued an Ordinance¹¹⁶ containing a positive list of substances for the manufacture of printing inks for food packaging: Swiss producers and also producers exporting to Switzerland have to fulfill this Ordinance requirements when printing their packaging. Germany has no specific law but the Bundesinstitut für Risikobewertung (BfR, the German Food Safety Authority) Recommendation n. XXXVI on “Paper and Board for Food Contact”, specific for paper based packaging, lists in specific detail which ingredients and additives are allowed in

¹¹¹ Muncke, 2011.

¹¹² Lorenzini R. Contaminazione da olio minerale: tossicità, riferimenti normativi, metodi analitici. *Macchine Alimentari (Tecniche Nuove)*, February 2011.

¹¹³ <http://www.efsa.europa.eu/en/dataclosed/call/datex100806.htm>.

¹¹⁴ Escabasse and Ottenio, 2002.

¹¹⁵ Decreto Ministeriale del 21/03/1973: “Disciplina igienica degli imballaggi, recipienti, utensili, destinati a venire in contatto con le sostanze alimentari o con sostanze d’uso personale”.

¹¹⁶ Swiss Ordinance on Materials and Articles of the FDHA SR 817.023.21 for Printing Inks of 23 November 2005 and following updates and revisions.

papermaking processes, depending on paper product final use (e.g. temperature of usage as for papers resistant to oven or microwave cooking). This Recommendation also includes a final Annex with quality specifications, in form of limitation of specific contaminants, for recycled paper materials.

Other official documents, as the Good Manufacturing Practice Guide of the European Carton Makers Association (ECMA) and the Industry Guideline for the compliance of paper & board materials and articles for food contact published by the Confederation of European Paper Industries (CEPI) and the International Confederation of Paper and Board Converters in Europe (CITPA), can help paper based food packaging producers to comply with safety and quality of their products. Also the European Printing Ink Association (EuPIA) issues guidelines, dedicate to the quality of printing inks for food contact materials, which are again used as reference for many packaging producers and their clients.

Some international standards to evaluate the transmission of off-odours and off-flavours from paper-based products to food are available for industry and laboratory testing. The Robinson Test is among the more widespread and applied, using milk chocolate (very sensitive to the presence of volatile compounds) as test food. The packaging to be tested is placed in a sealed container with freshly ground milk chocolate, at controlled temperature and relative humidity conditions. After 48 hours the chocolate is tasted by a trained panel against a blank chocolate sample. The standard EN 1230 (2010) “Paper and board intended to come in contact with foodstuff – Sensory analysis” has two parts: Part I: Odour. Part II: Off flavour. It gives an evaluation of quality and safety of paper and board based on the volatile compounds they release, thus migrating through the gas phase, not by direct contact. Therefore, this test is ideal for dry food. More general standards are available, for all kind of packaging including paperboard, as ISO 13302 (2003) “Sensory Analysis – Method for assessing the modifications of foodstuffs flavour due to packaging” and DIN 10955 (2004) “Sensory Analysis – Testing of packaging materials and packages for food products”.

Regarding more in general the safety assessment of paper and board intended for food contact, there are no validated and official harmonized testing methods, but the European project BIOSAFEPAPER, discussed in previous paragraph, has being carried out to investigate possible approaches. It has developed, validates and intercalibrates a battery of short-term biological tests for the safety assessment of paper and board intended for food contact. The developed methods are tuned to help the European paper industry to reduce

the contents of unwanted substances in packaging materials based on renewable resources; and the final aim of this project is to create a basis for scientifically sound recommendations for a harmonized risk evaluation and product testing and to increase the confidence of consumers in the will and ability of European industries to continue to provide safe food contact materials. Hopefully, the project will also represent a pre-normative research effort which will be used to launch regulatory harmonization at EU level on the safety of food contact paper and board, representing a renown standard for safety evaluation.

1.3.4 GAS PHASE MIGRATION STUDIES

Chemical migration is a diffusion process that is subject to both kinetic and thermodynamic control, influenced by:

- Temperature: migration increases with increased temperature of contact.
- Time: migration is higher for contact of long duration.
- Surface and thickness of food packaging material.
- Chemical and physical characteristics of migrating compound: migration usually decreases with substances of higher molecular weight, because they are less mobile. In the case of migration through the gas phase, volatility will be a determinant characteristic.
- Chemical and physical nature of food (lipid content, surface to weight ratio, etc.)

Two types of migration are conventionally considered: migration by direct contact between packaging and food, and migration of volatile compounds through the gas phase inside the packaging. Often both of them occur at the same time, but usually one migration mechanism is more relevant than the other: e.g. in the case of a yogurt contained in a PS jar the direct contact migration will prevail, whereas for breakfast cereals packed in paperboard the migration through the gas phase will be far more important. Both migration mechanisms (by direct contact and through the gas phase) have been investigated by Boccacci Mariani and coworkers¹¹⁷ for DIPN: the main factors influencing migration were time, food characteristics and initial board contamination.

The conditions of use of paper and board for food packaging range from short contact time (usually less than 1 hour for pizza delivered in cartons) to prolonged shelf life (2-3 years for some dry foods), and covers nearly all temperature ranges, from the -18°C of frozen

¹¹⁷ Boccacci Mariani *et al.*, 1999.

foods to refrigerated temperature, to room temperature (probably the most represented), to microwave and oven applications for some special paper trays. The characterization of paper or paperboard inertness should vary according to the nature of food, the temperature and the duration of contact, and the specific conditions of use. In many Countries, direct contact with paper materials is used only for dry foods, more for the poor wet strength typical of paper than to avoid potential migration. For humid food usually plastic coated paperboard is used, but as discussed in the experimental part of this work, many plastic polymers do not offer sufficient protection against migration from the paper layer. Presently, in contrast to what is the case for plastic materials, few analyses of paper and board materials intended to come into contact with foodstuffs take into account the product's foreseeable use conditions (short or long contact time with foodstuffs)¹¹⁸.

As seen in previous paragraph, legislation and standards regarding migration of contaminants from packaging to food are focused on migration by direct contact between the packaging material and the food or beverage. Migration through the gas phase is considered by some standard but mostly for the off-odour issues that packaging can release to (usually dry) food, not from a toxicological point of view. The exposure of population to substances migrating from packaging to dry foods through the gas phase is underestimated¹¹⁹, probably due to the fact that dry food is considered of low extractive power towards packaging contaminants, totally ignoring its adsorption attitude toward volatile compounds, especially if the food has high surface and long shelf life, it is porous and rich in lipids. For the transfer of contaminants from paperboard into dry food, migration by direct contact is negligible compared to migration through the gas phase. The latter is restricted to components of sufficient volatility to evaporate from the packaging material and recondense in the food^{120,121,122}. The process depends on the vapour pressure (determining migration rate) and the partitioning between the packaging material and the food, but also on situational factors: from a box standing alone on a shelf, evaporated hydrocarbons are largely removed into ambient air, whereas this is not possible for boxes packed into larger units and stacked on pallets. In the latter case, vapours are primarily transferred into the packed food unless there is an internal bag of a material stopping this migration. For a worst case assumption, escape of vapors should be assumed to be

¹¹⁸ ILSI Europe Report Series. Packaging materials. 6: Paper and board for food packaging applications. 2004.

¹¹⁹ Muncke, 2011.

¹²⁰ Shepherd, 1982.

¹²¹ Droz and Grob, 1997.

¹²² Jickells *et al.*, 2005

negligible, i.e. the vapor pressure is likely to approach saturation in the air within the packs and between the packs. Migration is driven towards equilibrium with the food. Triantafyllou and coworkers investigated this mechanism by determining the partitioning between paperboard and air and the uptake into model foods as a function of temperature. In one of their works¹²³, they studied the migration kinetics of phthalates, DIPN and other paper contaminants, from paperboard to Tenax[®], then developed a rapid test method using GC/MS identification and GC/FID quantification. Time and temperature (70°C for 20 to 360 min, and 100°C for 10 to 120 min were tested) were the most important variables for mass transfer. In another of their works¹²⁴, the partition behaviour between paperboard and air of several contaminants was studied, to estimate their attitude to migrate toward food through the gas phase. The more volatile substances partition mainly in the gas phase (air), where their concentration is mostly influenced by temperature (again, 70 and 100°C were the tested temperatures). Values of partition coefficients ($K_{\text{paper/air}}$) ranged from 47 to 1207 at different T. The adsorption isotherms of the studied contaminants onto paper samples are of Langmuir type. In one of their more recent works¹²⁵, they investigated the mobility of selected contaminants typical of recycled fibres materials towards dry food of different fat content; food contamination was quantified by GC/FID. The proportion of contaminants migrating to food was highly depended on the nature of paper samples, the nature of food (fat content in particular) and the chemical nature (volatility in particular) of contaminant. Therefore, the partitioning coefficient depends on the materials properties. However, since the mass of the food exceeds that of the packaging material by a factor typically ranging from 5 to 25¹²⁶, most of the hydrocarbons may end up in the food fairly independently of this partitioning coefficient.

As seen, migration from paper and board is often tested using modified polyphenylene oxide (MPPO - Tenax[®]) placed on the material during 10 d at 40°C¹²⁷. Tenax[®] may be considered as an adsorbent adequately simulating food¹²⁸, especially for volatile contaminants¹²⁹, but it is doubtful whether a standard laboratory simulation test, e.g. testing the paper based material 10 d at 40°C, really reflects migration of mineral oil over up to several years at room temperature (usually storage condition for many products packaged

¹²³ Triantafyllou *et al.*, 2002.

¹²⁴ Triantafyllou *et al.*, 2005.

¹²⁵ Triantafyllou *et al.*, 2007.

¹²⁶ Fiselier *et al.*, 2010.

¹²⁷ Aurela *et al.*, 1999.

¹²⁸ Lin *et al.*, 2011.

¹²⁹ Nerin *et al.*, 2007.

in paperboard). This method could be suitable for single contaminants, but not for a complex mixtures of thousands of hydrocarbons as mineral oil is. Since the more volatile components are transferred faster, the hydrocarbons found in young products range to lower carbon numbers than in aged packs. Similar transfer was observed for jute bags made with mineral batching oil: comparing a new jute bag to an old one, the concentration of the hydrocarbons was diminished up to *n*-C24 and the contaminants found in hazelnuts, chocolate, coffee and rice ranged to *n*-C21, *n*-C31, *n*-C24 and *n*-C21, respectively¹³⁰. Summerfield and Cooper¹³¹ investigated the migration of hydrocarbons and phthalates from paper products to Tenax[®] simulant, using a mixture of dichloromethane and ethanol for the Soxhlet extraction of paperboard and GC/MS for the instrumental analysis. They tested an accelerated temperature condition (80°C) and found it representative, but they only tested single molecules and not a mineral oil mixture.

In many scientific studies, considerable effort has been devoted to identify and quantify contaminants in paper and board, particularly if containing recycled fibers; however, much less effort has been dedicated to the development of predictive migration models for such materials in contact with food. The non homogeneity of fiber based materials makes modeling difficult. A few works had as objective correlating migrant content in the paper and the final values for direct contact migration¹³² and comparison of mass fraction of migrant under different pack formats and storage conditions¹³³. Aurela and Ketoja¹³⁴ followed a different approach and compared experimental results from transfer of certain volatiles through paper with computer simulations in which the fibre network is simulated by a virtual network of paper fibres, finding that for most contaminants the gas diffusion rate is very sensitive to sheet porosity. Sendòn Garcia and coworkers¹³⁵ applied a migration models already under development for plastic (FOODMIGROSURE European project). Poças and coworkers¹³⁶ identified the most important factors affecting the migration rate of phthalates and a variety of organic molecules (octane, naphthalene, xylene, methyl caproate, di-*iso*-butyl ketone, acetophenone, octanal, benzyl alcohol and 2-ethyl-1-hexanol) from paperboard into Tenax[®] and contributed to the development of a mathematical model to describe such migration, with an experimental design based on the

¹³⁰ Grob *et al.*, 1991b.

¹³¹ Summerfield and Cooper, 2001.

¹³² Boccacci Mariani *et al.*, 1999.

¹³³ Anderson and Calste, 2003.

¹³⁴ Aurela and Ketoja, 2002.

¹³⁵ Sendòn Garcia *et al.*, 2006.

¹³⁶ Poças *et al.*, 2011.

Taguchi method and the Weibull kinetics model instead of Fick's 2nd law of diffusion. They tested various temperatures but not over 40°C, to remain into the real packaging conditions of use. They concluded that migration from paper is much faster than from plastic, and contaminants molecular weight strongly influences their gas phase migration attitude. In many cases, mass transfer from paper into Tenax[®] cannot be described by diffusion models.

To conclude, paperboard packed dry foods particularly prone to migration of contaminants through the gas phase are those with:

- high surface/weight ratio;
- high lipid content;
- placed in small boxes (small food weight/packaging weight ratio);
- with long shelf life;
- stored at room temperature or above (summer months);
- not protected by a functional barrier between paperboard and food (direct contact).

1.3.4.1 ROLE OF FUNCTIONAL BARRIERS

Many food and beverages require barrier applications. "Barrier" is a nonspecific word that indicates a material's attitude to prevent substances (e.g. gases or contaminants) from permeating through the material. Barrier efficacy is usually defined against oxygen and moisture (water vapour), being those the most common agents causing quality loss in the product (i.e. oxidation in fat-rich products, loss of crispiness in baked products, mold formation, etc.). According to the American Society for Testing and Materials (ASTM), the barrier toward oxygen (standard D3985) and moisture (standard F1249) is low if over 100 cm³/m²/24 h pass the material, medium if such amount is >6 and <100, high if >1 and <5, very high if <1. As it can be seen in Table 3, often a material which is an excellent barrier against oxygen is not at all a good barrier against moisture (with some exceptions). This makes it necessary to produce multilayer materials to obtain both characteristics, e.g. the very common film formed by simple a polyolefin (good moisture barrier) on both sides, with an EVOH (excellent oxygen barrier) core.

Another class of substances against which barrier properties are vital is volatile compounds: in the case of desired compounds (e.g. aromas and flavours), the barrier has to prevent their loss from the product, whereas in the case of undesired compounds (e.g. volatile contaminants as seen in previous paragraph), the barrier has to protect the food against them.

Table 3. Comparison of barrier properties of selected films (Source: Constantia flexible).

Film	Film thickness (μm) necessary for O ₂ barrier (1 cm ³ /m ² /24 h/bar)	Film thickness (μm) necessary for water vapour barrier (1 g/m ² /24 h)
EVOH	4	200
PVDC-Lacquer	20	8
OPA	450	600
PET	1800	550
OPP	36000	25
PP	75000	70
HDPE	75000	40
LDPE	200000	100

In Figure 9 some multimaterial combinations are shown, with their oxygen and moisture barrier properties.

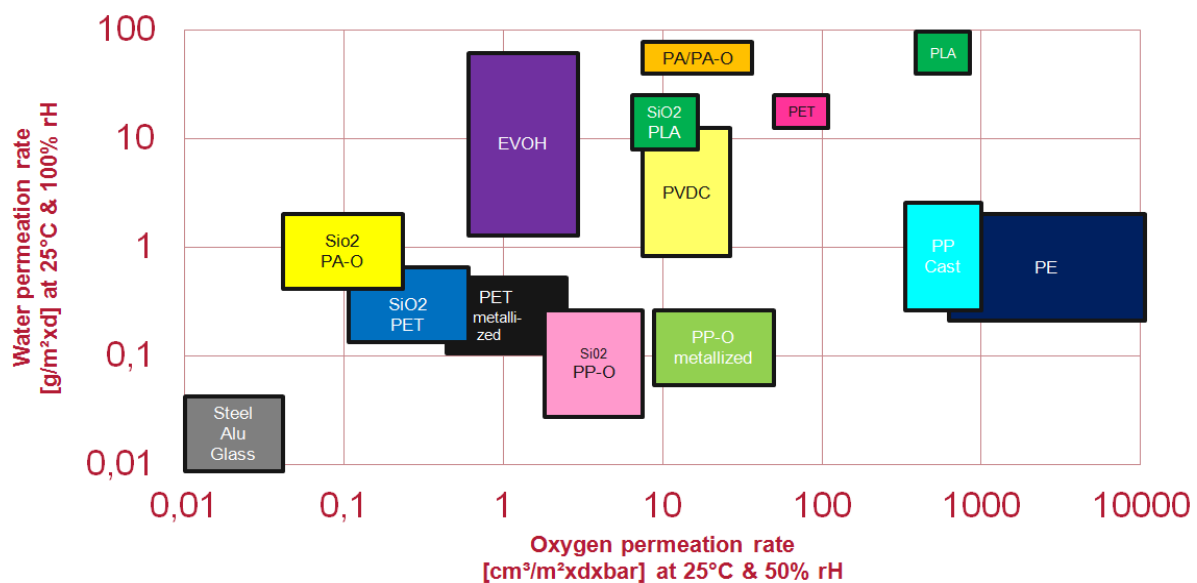


Figure 9. Moisture and oxygen barrier offered by some common materials and some multimaterial combinations (Source: Constantia flexible).

Among packaging materials, only glass and intact aluminum (over 10 μm of thickness) are considered absolute barriers. Paper-based materials offer virtually no barrier properties to volatile contaminants¹³⁷. In the case of plastic polymers, as seen in Table 3, barrier properties depend on thickness and chemical nature of plastic and contaminant. As a rule of thumb, a certain polymer will make a good barrier for a gas or substance with very

¹³⁷ Gärtner *et al.*, 2009.

different chemical nature: e.g. polyolefins (PE and PP) are poor barrier against mineral oil, and organic compounds in general (even at low temperatures¹³⁸), because they have very high chemical affinity with it, but on the other hand they are good moisture barriers. In general, a good oxygen barrier will also be a good barrier for carbon dioxide and volatile organic vapors. Mineral oil will be much more efficiently stopped by polymers bearing polar groups as PA, PET, EVOH, etc. Other variables must be considered when assessing plastic barrier properties: permeability of plastic films can increase dramatically at high temperature and relative humidity, therefore the transportation and storage temperature and relative humidity of food packaging must be carefully taken into account. Temperature inside a truck on a hot summer day can increase permeability by three or four times¹³⁹.

Studies on functional barriers protection properties against contaminants from paperboard are not a new entry: the subject was already investigated over 15 years ago¹⁴⁰. Various scientific groups have published studies^{141,142,143} on the barrier efficacy of plastic layers against contaminants diffusion, sometimes extrapolating mathematical functions able to represent the interaction between contaminants and barriers depending on different variables such as temperature. If the plastic layer is not a good barrier, the migration will only be slowed down: in fact these weak barriers introduces a lag time, with low migration until the migrant passed through the plastic layer^{144,145}. Diffusion and migration through functional barriers depends on many factors such as diffusion coefficient of migrating contaminants, time and temperature of processing, storage conditions of the empty material, and conditions of filling and of storage of the food. The assessment of the efficiency of functional barriers should rely heavily on prediction of migration¹⁴⁶.

A good functional barrier has to be free of defects (e.g. pinholes), have an high degree of crystallinity and a glass transition temperature much higher than the storage temperature of food, and have a very different polarity to whose of the contaminants. Along with functional barrier in form of plastic bags to be inserted inside paperboard boxes, also special coatings for the inner paperboard surface are under development. Both paperboard and plastic or coating manufacturers are working hard to put on the market a successful

¹³⁸ Choi *et al.*, 2002.

¹³⁹ Soroka, 2009.

¹⁴⁰ Johns *et al.*, 1996.

¹⁴¹ Piringner *et al.*, 1998.

¹⁴² Galotto *et al.*, 2011.

¹⁴³ Welle and Franz, 2012.

¹⁴⁴ Franz *et al.*, 1997.

¹⁴⁵ Piergiovanni *et al.*, 1999.

¹⁴⁶ Feigenbaum *et al.*, 2005.

product in case migration limits for mineral oil are enforced by the European Union. Substances suitable to be used as coatings for paperboard are polyacrilates, polyamides, aliphatic-aromatic copolyesters based on terephthalic acid and adipic acid, possibly blended with polylactic acid, etc. If protection (lag time) offered by PE against mineral oil is just a few d, these coatings promise to extend lag time to several years. Some of them are to be used as extrusion coatings (or coextrusion coatings if applied to plastic), other as dispersion coating. The way the protective coating is applied to paperboard is not negligible, because if extreme conditions are used (e.g. high coextrusion temperatures), the functional barrier can be contaminated during this step due to the strong contaminants diffusion acceleration given by temperature. As a consequence, contaminants might already have penetrated the protective coating and from there easily reach the food once the finished box is filled¹⁴⁷. Sometimes the plastic barrier itself can become a source of contaminants, especially if not properly produced and/or stressed (e.g. with high temperatures) after packaging. Such contaminants can be e.g. volatile plastic additives as plasticizers and/or plastic monomers and oligomers. In the case of polyolefins, such oligomers are in fact very similar to MOSH, and are defined Polyolefin Oligomeric Saturated Hydrocarbons (POSH)¹⁴⁸. On a GC/FID chromatogram, MOSH and POSH are seen together due to their very similar chemical nature, but MOSH is usually represented by a hump of fairly volatile hydrocarbons, whereas POSH have typically spaced peaks (slightly different if the plastic is LDPE, HDPE or PP) representing the oligomers. In the next paragraph the analytical techniques for mineral oil hydrocarbons are discussed.

Other possibly effective functional barriers might be represented by natural substances of polar nature (e.g. polysaccharides), suitable to be applied as thin coatings on paperboard, as modified cellulose, chitosan, pullulan, pectin and gelatin. They have the plus of being water soluble and thus compostable, and recyclable along with paper (on the contrary of plastic coated paperboard), making the choice of internally coated recycled paperboard even more eco-friendly.

1.3.5 ANALYTICAL CHALLENGE

Food packaging safety analyses are usually more complicated and challenging compared to food safety analyses, due to many reasons. Food packaging evaluation from a safety point of view is a fairly young science. Furthermore, while food composition is declared, by law,

¹⁴⁷ Franz *et al.*, 1997.

¹⁴⁸ Biedermann-Brem *et al.*, 2012.

in the ingredient list, packaging composition is very often a secret recipe jealously guarded by packaging companies: this is the reason why, when analyzing a packaging sample extract with chromatographic techniques, often we find a “forest of peaks”, mostly unexpected and unknown. Analyzing heterogeneous materials such as paper and board is even more challenging, especially if containing recycled fibres, with changing characteristics and contamination levels from batch to batch. For this reason, in USA the Recycled Paperboard Technical Association (RPTA) has implemented a sampling and analysis protocol that paper mills can use to determine whether a recycled paper batch is suitable for food contact¹⁴⁹: among others contaminants, Polycyclic Aromatic Hydrocarbons (PAH) are tested, but not mineral oil. In Europe, there are numerous CEN (European Committee for Standardization) analytical methods to assess various aspects of paper based products intended to come into contact with foodstuff (see Table 4), but at present none of them is dedicate to mineral oil contaminants. However, the Committee is preparing a GC/MS method for the determination of polycyclic aromatic hydrocarbons in paper.

Table 4. CEN published standards regarding paper based products intended to come into contact with foodstuff¹⁵⁰.

Standard reference	Title
CEN/TR 15645-1:2008	Paper and board intended to come into contact with foodstuffs - Calibration of the odour test - Part 1: Odour
CEN/TR 15645-2:2008	Paper and board intended to come into contact with foodstuffs - Calibration of the off flavour test - Part 2: Fatty food
CEN/TR 15645-2:2008/AC:2008	Paper and board intended to come into contact with foodstuffs - Calibration of the off-flavour test - Part 2: Fatty food
CEN/TR 15645-3:2008	Paper and board intended to come into contact with foodstuffs - Calibration of the off-flavour test - Part 3: Dry food
CEN/TR 15645-3:2008/AC:2008	Paper and board intended to come into contact with foodstuffs - Calibration of the off-flavour test - Part 3: Dry food
EN 1104:2005	Paper and board intended to come into contact with foodstuffs - Determination of the transfer of antimicrobial constituents
EN 1230-1:2009	Paper and board intended to come into contact with foodstuffs — Sensory analysis — Part 1: Odour
EN 1230-2:2009	Paper and board intended to come into contact with foodstuffs — Sensory analysis — Part 2: Off-flavour (taint)
EN 12497:2005	Paper and board - Paper and board intended to come into contact with foodstuffs - Determination of mercury in an aqueous extract

¹⁴⁹ Hagenbarth, 2005.

¹⁵⁰ <http://www.cen.eu/CEN/Sectors/TechnicalCommitteesWorkshops/CENTechnicalCommittees/Pages/Standards.aspx?param=6153&title=CEN/TC+172>.

EN 12498:2005	Paper and board - Paper and board intended to come into contact with foodstuffs - Determination of cadmium and lead in an aqueous extract
EN 13676:2001	Polymer coated paper and board intended for food contact - Detection of pinholes
EN 14338:2003	Paper and board intended to come into contact with foodstuffs - Conditions for determination of migration from paper and board using modified polyphenylene oxide (MPPO) as a simulant
EN 14719:2005	Pulp, paper and board - Determination of the Diisopropylnaphthalene (DIPN) content by solvent extraction
EN 1541:2001	Paper and board intended to come into contact with foodstuffs - Determination of formaldehyde in an aqueous extract
EN 15519:2007	Paper and board intended to come into contact with foodstuffs - Preparation of an organic solvent extract
EN 15845:2010	Paper and board - Determination of the cytotoxicity of aqueous extracts
EN 643:2001/AC:2002	Paper and board - European list of standard grades of recovered paper and board
EN 645:1993	Paper and board intended to come into contact with foodstuffs - Preparation of a cold water extract
EN 646:2006	Paper and board intended to come into contact with foodstuffs - Determination of colour fastness of dyed paper and board
EN 647:1993	Paper and board intended to come into contact with foodstuffs - Preparation of a hot water extract
EN 648:2006	Paper and board intended to come into contact with foodstuffs - Determination of the fastness of fluorescent whitened paper and board
EN 920:2000	Paper and board intended to come into contact with foodstuffs - Determination of dry matter content in an aqueous extract
EN ISO 15318:1999	Pulp, paper and board - Determination of 7 specified polychlorinated biphenyls (PCB) (ISO 15318:1999)
EN ISO 15320:2011	Pulp, paper and board - Determination of pentachlorophenol in an aqueous extract (ISO 15320:2011)

For many paper based materials contaminants as phthalates¹⁵¹, benzophenones photoinitiators¹⁵², di-isopropyl naphthalenes¹⁵³ and others it is possible to apply a powerful and relatively straight forward analytical techniques such as GC or LC coupled to Mass Spectrometry detector (MS). However, such detector is not ideal for mineral oil contaminants, for various reasons, despite being applied by several laboratories (e.g. paper mills internal laboratories to assess pulp quality). The MS detector looks for characteristic fragments of a certain analyte; it is a powerful detector able to scan for hundreds of analytes at the same time, but mineral oil is composed by thousands of different analytes, most of which are isomers, forming a “hump” of unresolved peaks in the chromatogram (see Figure 10). MS is a very sensitive detector, but its response depends on analytes

¹⁵¹ Gärtner *et al.*, 2009.

¹⁵² Castle *et al.*, 1997.

¹⁵³ Sturaro *et al.*, 2006.

chemical structure: response increases with mass owing to higher yield of larger fragments. Therefore, correction factors should be applied for every hydrocarbons or class of hydrocarbons: this is of course not feasible due to the multitude of molecules in mineral oil. Furthermore, mineral oil composition may vary significantly: the hump could be mainly composed by very volatile hydrocarbons (e.g. from a “new” paperboard), or on the contrary be centered on heavier MW substances (e.g. from an “old” paperboard), or sometimes it even presents a “double hump”. This variability is a problem for MS, because as said the response factor of this detector varies slightly depending on the hydrocarbon MW and chemical nature (e.g. aliphatic or aromatic). Finally, with MS also other substances present in the sample extract can be detected and quantified together with mineral oil contaminants. This is because the commonly researched fragments are quite small and therefore not very specific: MW 43, 57 and 71 for *n*-alkanes and *iso*-alkanes, MW 69 for cycloalkanes, MW 91 for aromatics¹⁵⁴. Therefore, the GC/MS methods for mineral oil contaminants can only be considered semi quantitative.

A completely different analytical approach consist in analyzing the mineral oil contaminants with GC/FID (Flame Ionization Detector), prior to an online LC cleanup of samples extracts. Dr. Konrad Grob, of the Kantonaes Labor of Zurich (Food Control Authority for the Canton of Zurich), has been developing this approach for at least two decades.

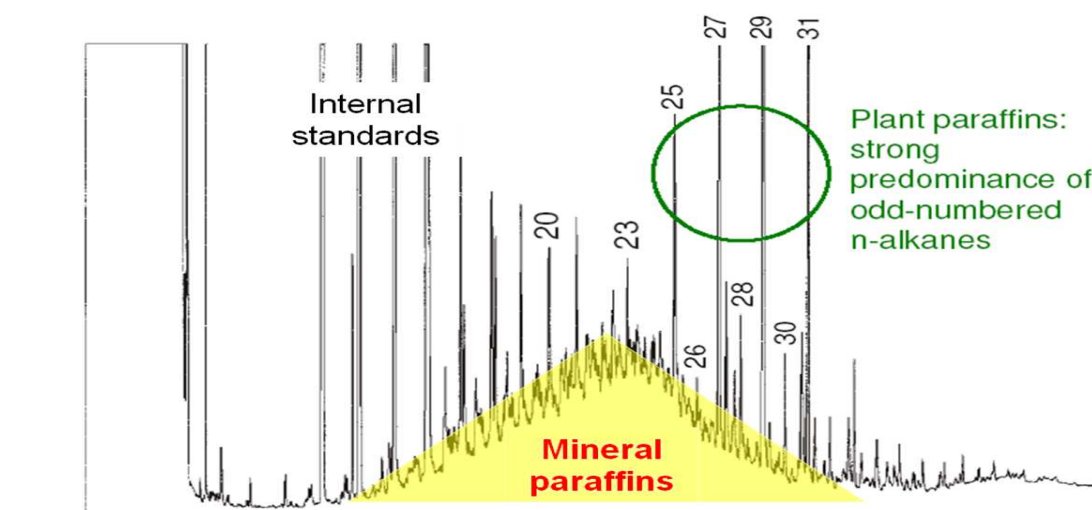


Figure 10. LC-GC/FID chromatogram of MOSH hydrocarbons (mineral paraffins), showing the typical hump of unresolved peaks due to the presence of many isomers. Chromatogram from Kantonaes Labor, Zurich.

¹⁵⁴ Silverstein *et al.*, 2006.

The peculiarity of this chromatographic method is the uncommon combination of LC and GC¹⁵⁵. The LC allows for an online clean up and pre-separation of analytes, with improved repeatability compared to off-line clean up (e.g. using SPE columns), lower risk of sample contamination and of human errors in general. Furthermore, the LC clean up step allows to inject also “dirty” extracts (e.g. from highly contaminated packaging or from complex food matrixes), including samples with as much as 20% of lipid content. The packaging or food hexane extract is injected through an autosampler into the LC normal phase column: the silica stationary phase retains all chemical substances with a certain grade of polarity, whereas hydrocarbons, which are nonpolar, are eluted by the hexane mobile phase dosed via a syringe pump. The strongly nonpolar MOSH are eluted first, then a few minutes later MOAH and DIPN come out of the chromatographic system. All other compounds present in the sample, not of interest for GC analysis and quantification, are retained into the LC column, which is backflushed with dichloromethane, that ensures column cleanliness for the following sample injection. Now the two fractions of interest (MOSH and MOAH + DIPN) are transferred into the GC system: during transfer the eluant is fed into the GC precolumn through a Y-pressfit connector. Carrier gas and solvent are mixed without entering a dead volume.

GC capillary columns and precolumns are best prepared and internally coated in the laboratory, to avoid any contamination from mineral oil (e.g. from their paperboard casing when purchased). GC capillary column length does not need to be over 10 m (7 m is ideal), and its stationary phase coating has to be thin in order to limit column “bleeding” at high temperatures, even if this causes diminished retention power.

Previously two different LC injection had to be performed for MOSH and MOAH, but at present there are chromatographic systems able to exploit the same LC injection for both GC analyses. Both fractions are accompanied by several internal standards, among which an UV detectable one to make sure all relevant analytes are transferred to GC. Anyway, the quantity to be transferred is still a quite big volume for a capillary GC system: if no vapor exit is present, it is difficult to inject volumes over 50 μL . In the LC-GC/FID system, 250 μL are transferred from LC to GC, therefore a special large volume injection (LVI) system has to be applied, to avoid a sample concentration step prior to GC analysis.

There are different techniques to perform a large volume injection (LVI) in GC, among which¹⁵⁶:

¹⁵⁵ Tranchida *et al.*, 2011.

¹⁵⁶ Grob, 1991.

- Concurrent solvent evaporation technique: even very large volumes can be injected because the solvent is completely evaporated prior to entering the GC capillary column; the downturn of this technique is that any highly volatile analytes are lost, together with the solvent. In the case of hydrocarbons, they are lost up to ca. C16-C18, representing an important fraction of mineral oil contamination. This technique is however applicable to higher MW analytes such as sterols, ESBO, etc.
- Partially concurrent solvent evaporation (or “retention gap”) technique: it is similar to the previous one, but a little amount of solvent is retained. In practice, this result can be achieved placing, ahead of the separating column, a precolumn (without stationary phase) long enough to contain the full volume injected. A suitable precolumn will be 0.53 mm of diameter and 5 to 10 m long (for a carrier gas flow of 50 mL min⁻¹), uncoated and deactivated (“wettable” but low in retention power). In this way, the solvent at first completely floods the precolumn, then starts to evaporate, and is let into the separating columns just a few seconds prior to complete evaporation, allowing retention of even the more volatile analytes. This technique is ideal for mineral oil contaminants analysis, and allows the injection of up to 250 µL.

Coming to the final part of the analysis, the use of FID can seem obsolete, but in fact this detector is ideal for mineral oil quantification. It is a robust detector, with a high range of linearity for these contaminants. This is important also because some samples, especially if from packaging, can bear a very high contamination (thousands of mg kg⁻¹), which would be detrimental for a sensitive MS detector. Furthermore, FID has the same response factor for all hydrocarbons, so no calculations and adjustments are necessary during quantification.

Finally, the use of internal standards is ideal for this chromatographic method because it allows a precise quantification even in case of errors during the volumes handling in sample extractions, or in case of concentration of solvent in vial. This is not uncommon due to the high volatility of hexane, the final solvent for injection into the chromatographic system.

It is possible to perform the analysis simply using a GC/FID and substituting the online LC pre-separation and cleanup steps with a Solid Phase Extraction (SPE) cartridge^{157, 158}. This method is simplified from the instrumental point of view, but repeatability can suffer and there is a risk of sample contamination during handling. Furthermore, reconcentration of

¹⁵⁷ Fiorini *et al.*, 2010.

¹⁵⁸ Moret *et al.*, 2011.

sample is more difficult (unless it is done manually prior to injection), with higher limits of detection and quantification.

2. AIM OF THE THESIS

Food contact materials can contain various substances able to migrate into their food content, thus posing a risk for public health. European legislation is particularly focused on plastic materials, whereas other materials as paperboard and corrugated board are much less regulated. Paper based materials, especially if from recycling, can contain a variety of contaminants a part of which with sufficient volatility to easily pass from the board to the food: mineral oil is one of them. Particularly sensitive foods are those with a high surface to weight ratio, rich in lipid, with a long shelf life and in direct contact with the paper material (no plastic or aluminum protective barrier).

Mineral oil is one of the many products derived from petroleum. It is formed by a complex mixture of thousands of hydrocarbons: this is why the appearance of a mineral oil GC chromatogram is not given by some clear peaks but it is instead a hump of unresolved isomers. It is widely used as solvent for printing inks applied on paper based products such as newspapers, books and packaging (included food packaging). This is the reason why in paperboard packaging obtained from recycled materials, amounts up to thousands of mg kg^{-1} of mineral oil hydrocarbons can be found: they derive both from the recycled material (mostly made up of heavily printed newspapers) and from the paperboard packaging printing itself. The most volatile of these hydrocarbons can easily migrate to paperboard food content through the gas phase.

Mineral oil is mostly composed by saturated hydrocarbons (MOSH), either linear, branched or cyclic, but up to 30% of total hydrocarbons can be represented by aromatic compounds (MOAH), usually with 1, 2 or 3 heavily alkylated aromatic rings. A definitive

toxicological evaluation of mineral oil hydrocarbons, as well as legal specific migration limits, are not available as yet, but several food contamination data show that the problem cannot be ignored. The European Food Safety Agency (EFSA) has been working on these issues for some years and time for an official position is approaching.

Along with this lack of legislative references, food contamination with mineral oil also poses other problems, starting with the extractive and quantification methods: being this contaminant formed by a myriad of different molecules, an effective analytical method must be ideally able to extract them all together and instrumentally quantify them with a wide range of linearity and robustness.

The aim of the first part of this work was to design an multiextraction method able to extract at the same time analytes with quite different chemical and physical behaviour, both from paper based packaging and from food. A fine balance between extracting “enough” but not “too much” is needed: all the analytes of interest have to be quantitatively recovered, but high boiling compounds (either hydrocarbons, plastic oligomers, or other substances) has to be avoided as much as possible. These compounds are of scarce interest because less toxic (scarcely absorbed by gastrointestinal tract) and much less volatile thus unlikely to be transferred to food. Furthermore, they are able to seriously damage GC capillary column and pre-column, leading to frequent and time consuming maintenance of the chromatographic system. The extractive and analytical methods for mineral oil hydrocarbons and other contaminants, one optimized, have been applied to the analysis of over 100 products from the Swiss and Italian market.

The second part of this work was dedicated to a comprehensive migration study under controlled conditions of temperatures and storage, where two representative food products packaged in paperboard have been followed from production to end shelf life. Scientific studies on contaminants migration through the gas phases are not many, and some more knowledge is needed to understand these migration processes, their kinetics and the influencing parameters such as time and temperature. Deeper understanding of mineral oil (and other volatile contaminants) migration process will give valuable information to food control authorities and to packaging and food producers in order to increase the safety level of food packaged in paper-based materials.

3. MATERIALS AND METHODS

The experimental part of this work has been carried out with the cooperation of three structures: Agroenvironmental Sciences and Technologies Department (University of Bologna, Italy), Coop Italia (Casalecchio di Reno, Bologna, Italy) and Kantonales Labor ZH (Food Control Authority for the Canton of Zurich, Switzerland).

3.1 PACKAGING AND FOOD EXTRACTIONS

Food samples packed in paperboard boxes were collected from the Italian and the Swiss retail markets in spring and summer 2009. Only products without any kind of aluminium internal bag (aluminium foil or metalized plastic) were analysed. Both packaging, and their food content in case of severe contamination of packaging, have been analysed. For some items also plastic parts and glues have been analyzed.

Solvents and internal standards. HPLC-grade methanol, ethanol and dichloromethane were purchased from J.T. Baker (Deventer, Holland), respectively. Technical grade methyl *tert*-butyl ether (Merck, Darmstadt, Germany) was distilled before use. Hexane from Brenntag (Schweizerhall AG, Basel, Switzerland) consisting of some 60% *n*-hexane and 40% *iso*-alkanes, was purified filtering it through silica gel activated at 400°C column (400g silica for 10L solvent) to remove polar compounds traces and then distilled to increase the purity of *n*-hexane. Silica gel was from Merk (Darmstadt, Germany). MOSH and MOAH internal standards solutions were prepared as described by Biedermann and

coworkers¹⁵⁹: 1,1,2-trichloroethane, *n*-dodecane (C12), *n*-tetradecane (C14), *n*-hexadecane (C16), hexyl-benzene (6B), nonyl-benzene (9B), biphenyl (BP), 1,3,5-tri-*tert*-butylbenzene (TBB), perylene (Per) and 5- α -cholestane (Cho) were purchased from Fluka/Sigma-Aldrich (Buchs, Switzerland). The stock solutions of hydrocarbons internal standards containing 100 mg of the components in 10 mL of 1,1,2-trichloroethane were: mixture 1 = 6B, 9B, BP and TBB; mixture 2 = C-12, C-14 and C-16; mixture 3 = Per and Cho. The hydrocarbons internal standards solution contained 100 μ L mixture 1, 300 μ L mixture 2, and 500 μ L mixture 3 in 10 mL 1,1,2-trichloroethane.

Packaging extraction. Extraction of paper-based packaging was performed manually chopping paper or paperboard into small pieces, and weighing 1 g into a 20 mL amber vial with PTFE-lined screw cap. To prevent contamination, samples were handled without gloves; hand creams were avoided. Working up virgin fibre paperboard free of mineral oil inks verified the absence of sample contamination during manipulations. After adding 20 mL of internal standards solution and 10 mL of different solvents or solvent mixtures, the vial was shaken on a vortex (Haidolph, Germany) and allowed to stand during various periods of time. Before injection into the chromatographic system, paperboard pieces and ethanol (or methanol), if present, were removed from the extract by adding approximately 10 mL of water into the amber vial and vortexing: water addition causes those polar solvents to separate from hexane where they were previously miscible. Finally, pure hexane extract was obtained by centrifuging (ALC 4239R by Thermo ScientificTM, USA): hexane lays at the top while water and other polar solvents, along with packaging pieces, lay at the bottom. For plastic extraction, the same method was used except for sample weight: only 0.2 g of sample was weighed instead of 1 g, due to the often higher mineral oil contamination of plastic compared to paperboard, along with the presence of polyolefin oligomeric saturated hydrocarbons (POSH), as typical plastic oligomers.

Food extraction. When analyzing food, it must be considered that many plants contain little amounts of natural hydrocarbons, which of course have to be deducted from mineral oil contamination quantification. However, natural paraffins nearly exclusively consist of odd-numbered *n*-alkanes of fairly high molecular weight (e.g. *n*-C21, *n*-C23, etc.), and thus are easily distinguished from paraffins originating from mineral oil. Food extraction approach is different depending on food moisture content:

- **Extraction of dry foods.** The full packaging food content, or a representative amount, was thoroughly ground (Osterizer by Sunbeam, USA). 10 g of ground food

¹⁵⁹ Biedermann *et al.*, 2009.

was placed into a 50 mL glass flask with PTFE-lined screw cap. Internal standards solution (20 µl) and hexane (20 mL) were added to food sample and the flask was thoroughly shaken on a vortex (Haidolph, Germany), then allowed to stand at room temperature for 3 h. Before chromatographic analysis, hexane extract was centrifuged if necessary.

- **Extraction of moist or liquid foods.** The full packaging food content, or a representative amount, was thoroughly minced, if necessary. 5 g of food was placed into a 100 mL glass flask with a glass tight top. 25 mL of ethanol were added, along with 20 µL of internal standards solution. The flask was capped, vigorously shaken for about 10 s, then placed on a shaker (Unimax 2010 by Heidolph, Germany) for 30 min. 20 mL hexane were then added to the flask, which was again vigorously shaken for about 10 s and placed on the shaker for other 30 min. Finally, as for the packaging extraction, water was added (ca. 40 mL) and flask was vigorously shaken once again, forcing ethanol to join the aqueous phase and thus separate from hexane. The flask was left at room temperature for 3 h. Prior to chromatographic analysis, the liquid phase was centrifuged if necessary (e.g. if not perfect phase separation reached, due to presence of natural emulsifiers in the food).

To prevent contamination, samples were handled without gloves and hand creams were avoided.

3.2 CONTROLLED MIGRATION PLAN CONDITIONS

Foods and their packaging characteristics. Breakfast cereals (müesli) and dry egg pasta (taglioline) were chosen as food models for their high surface to weight ratio in order to represent worst case scenarios. Lipid content was 16% in müesli and 4% in egg pasta. Müesli packaging consisted in a printed paperboard box made of recycled fibers, measuring 14.5 x 4.5 x 21 cm and weighing 38 g, containing an unprinted polyethylene plastic bag (2 g weight) with 375 g muesli inside (16% fat content). Egg pasta packaging consisted in a unprinted paperboard tray with sides but no top, made of recycled fibers, measuring 21 x 13.5 x 5.5 cm and weighing 24 g; 250 g egg pasta (4% fat content) was put in direct contact with the tray, and a polypropylene plastic printed wrap (6 g weight) was around the tray. Both food models were obtained immediately after food production and

packaging to have a “time zero” condition. All boxes were from the same production batch.

Temperatures and storage conditions. Dispatched müsli and egg pasta packs were immediately stored at chosen conditions. Some packs were wrapped into aluminum foil and stored in 4, 20, 30, 40, and 60°C conditioned cells. Such temperatures were chosen to represent refrigerated condition, usual room temperature, storage during warm months in not-conditioned facilities and simulation of accelerated migration, respectively. The remainder of packs were disposed in four different storage conditions at unconditioned room temperature: some of them were stored with all sides exposed to air except for bottom (from now on called “free packs”), to simulate normal domestic storage condition; some other packs were piled together with only sides and top exposed to air (“shelved packs”), to simulate supermarket on-shelf storage; lastly, some other packs were left inside the shipping cartons made of corrugated board (“boxed packs”), to simulate warehouse facilities storage: packs at the centre of cartons were analyzed separately from those at the corner of cartons.

Both müsli and egg pasta were obtained in sufficient quantity to undergo the different temperature and storage conditions up to the end of their shelf life. At every test time, a whole pack was withdrawn from the experimental condition and tested. After analysis, the remainders of sample were discharged.

Analyses scheduling. Test times for müsli were: 1, 5, 8, 15, 28, and 57 d for 60°C condition; 1, 5, 12, 28, 57 and 113 d for 40°C condition; 7, 14, 28, 64, 113, 233 and 397 d (end of shelf life) for 30, 20 and 4°C conditions, and for “free”, “shelved” and “boxed” packs conditions. Test times for egg pasta were: 2, 5, 8, 15, 26 and 64 d for 60°C condition; 2, 6, 12, 26 and 64 d for 40°C condition; 7, 14, 26, 64, 240 and 404 d (end of shelf life) for 30, 20 and 4°C conditions, and for “free”, “shelved” and “boxed” packs conditions. Food was analyzed at every test time for both food models; packaging (paperboard and plastic) only at some selected test times. About 185 samples have been analyzed in total.

Extraction of packaging and food. Paperboard and plastic were extracted according to methods explained in paragraph 3.1, using a mixture of ethanol:hexane 1:1 by volume as solvent (solvent mixture optimized by Lorenzini and coworkers¹⁶⁰ to extract low to medium molecular weight hydrocarbons (roughly up to 40 carbon atoms), with limited extraction of poorly volatile high molecular weight substances, potentially damaging GC

¹⁶⁰ Lorenzini *et al.*, 2010.

capillary column), and 2 h at RT as extracting conditions. Müesli and taglioline, being both dry foods, were extracted according to the “extraction for dry foods” method explained in paragraph 3.1. Food content of either müesli and taglioline was withdrawn from the various controlled condition at the planned test times and ground. The remainder ground food was left for future reference into a glass jar, capped with an aluminum-lined screw lid to avoid any contamination from contaminants in lid gasket.

3.3 INSTRUMENTAL ANALYSIS

Hexane packaging and food extracts were analysed for MOSH, MOAH and DIPN, as described by Biedermann and coworkers¹⁶¹. A fully automated instrument from Thermo Fisher[®] (Milano, Italy) was used, assembled with on-line normal phase high performance liquid chromatography system coupled with a capillary gas chromatography separation with flame ionisation detector (NPLC-CGC-FID), shown in Figure 11.



Figure 11. An example of LC-GC/FID system, consisting of a GC/FID instrument (left) equipped with an on line LC cleanup system (right).

The LC component worked at room temperature at a flow rate of $300 \mu\text{L min}^{-1}$. 20 (for MOSH) or 50 (for MOAH/DIPN) μL of sample extracts were injected in a 25 cm x 2 mm internal diameter (i.d.) silica gel NPLC column (Lichrospher Si 60, 5 μm). The eluant

¹⁶¹ Biedermann *et al.*, 2009.

gradient started with 100% hexane and reached 30% dichloromethane, both dosed via dedicated syringe pumps. A specific fraction from the LC column, containing respectively purified MOSH (first chromatographic run) and purified MOAH + DIPN (second chromatographic run), was monitored by UV detection on the basis of Per and Cho reference standards retention times. The breakthrough fraction (200 μL) was transferred via a glass Y-piece to the GG precolumn, by the retention gap technique and partially concurred eluant evaporation, using a 10 m x 0.53 mm i.d. uncoated deactivated precolumn, followed by a steel T-piece connection to the solvent vapour exit and a 10 m x 0.25 mm i.d. separation column, coated in the laboratory with dimethyl polysiloxane PS-225 (Fluka, Buchs – Switzerland), in order to avoid any contamination from commercial columns paperboard packaging. Transfer and solvent evaporation occurred at an oven starting temperature of 65°C (6 min, starting from injection into LC), then the oven temperature was increased at the rate of 20°C min^{-1} up to 350°C. In the mean time, the LC column was backflushed with dichloromethane. MOSH fraction was detected from 2.0 to 3.5 min and MOAH fraction from 4.0 to 5.5 min from beginning of LC-GC transfer. DIPN are included in MOAH chromatogram. The chromatographic areas representing MOSH and MOAH were integrated as whole “humps”, applying relevant deductions for internal standards and food naturally occurring hydrocarbons (typically odd carbon numbered *n*-alkanes as C21 and C23). Quantification was performed referring to the mean value of internal standards area: C12, C14 and C16 for MOSH, and 6B, 9B BP and TBB for MOAH and DIPN. A pure and white mineral paraffin oil, centered on *n*-C23, used in the past as a release agent by a candy manufacturer, was used as an external standard for recovery tests (average 90%). The quantification and detection limits were 1 and 0.2 mg kg^{-1} , respectively. In absolute terms, the detection limit is 50 ng. For MOSH contamination in müesli, integration was also detailed (“slashing the hump”) for every single carbon atom fraction, representing the *n*-alkane of that carbon number plus all the branched isomers. This detail was needed in order to monitor the migrating fractions proportions depending on time, temperature and storage conditions. Single analyses have been performed, except for some selected samples of the “shopping trolley survey” and samples of the controlled migration plan (müesli and egg pasta) stored at 20°C, for which analyses have been run in double: results from repeated analyses had a standard deviation below 10%. This uncertainty is not ideal, but it is reasonable if considering the complexity of the analysis, of the analytes (mixtures of hundreds of compounds) and of the extracted matrixes (packaging and food).

4. RESULTS AND DISCUSSION

4.1 EXTRACTION METHODS OPTIMIZATION

The aim of this first part of PhD work was to optimize extraction methods, both for paper-based packaging and for food.

An ideal extraction method should be simple, rapid and multiextractive, thus being able to extract at the same time all analytes of interest. In this case, analytes are represented by highly nonpolar saturated hydrocarbons (MOSH) and slightly more polar aromatic hydrocarbons (MOAH) and di-isopropyl naphthalenes (DIPN). DIPN are usually present in paper-based materials, and in food if migration occurred, as a group of isomers. Both MOSH and MOAH can be represented by hundred, or even thousands of different compounds: all of them have to be extracted and quantified.

4.1.1 PACKAGING EXTRACTION

An additional achievement, required specifically to the packaging extraction method, was to avoid the extraction of high molecular weight molecules, as high molecular weight mineral oil fractions, plastic oligomers, waxes, etc. Those substances pose virtually no risk for the consumer, because their poor volatility render their migration unlikely, and anyway they would not be easily absorbed by human gastrointestinal tract. Furthermore, those substances, if present in the extract, are quickly “clogging” and damaging the GC capillary column due to their poor volatility, with loss of chromatographic quality and thus need to replace the column.

1) Optimization of extraction length and temperature

In a first extraction experiment, both extraction length and temperature were varied in order to extract the analytes of interest, which have relatively small molecular weight, avoiding as much as possible the extraction of high boiling undesired packaging components. Supposing that the discrimination between analytes of different molecular weight could be obtained applying adequate extraction length and temperature, we compared the results for a 100% hexane extraction at different extracting conditions. In order of increasing extraction power, the time and temperature combinations were:

- 15 min at room temperature;
- 30 min at room temperature;
- 1 h at room temperature;
- 1 h at 60°C;
- 3 h at 60°C;
- 8 h at 60°C;
- 24 h at 60°C.

Figure 12 shows overlapping LC-GC/FID chromatograms of the MOAH fraction obtained extracting with 100% hexane a printed recycled paper box (breakfast cereals). A progressive increasing yield of extraction can be noticed, especially on the second part of the chromatograms, confirming that prolonged time and high temperature (24 h 60°C) are undesirable extraction conditions because they improve the extraction of undesired high boiling substances. On the other hand, extraction times shorter than 1 h are unable to sufficiently extract MOAH, therefore extraction time should be at least 1 h at RT. For MOSH (of the same paperboard sample), no significant quantitative extracting yield could be obtained prolonging the time over 1 h RT, confirming that these conditions are effective enough to quantitatively extract MOSH, avoiding at the same time extraction of high boiling hydrocarbons and polymers. Furthermore, heat (60°C) application is not advisable because it seems to provoke a certain loss of the more volatile MOSH. The extraction of DIPN (extracted and LC eluted together with MOAH) needs a special consideration: these ink additives have a particular physical nature, being often added to thermal and pressure sensitive paper in an encapsulated form, therefore their release from paperboard matrix is quite slow. Some experiments (data not shown) have confirmed that their extraction is slower compared of that of MOAH of similar volatility. For these reason, the final extraction conditions are precautionary extended to 2 h at RT. To check whether these extraction conditions guarantee a good recovery of analytes, the same paperboard extracted

for 2 h at RT was re-extracted overnight (over 10 h extraction), showing no significant analytes residues.

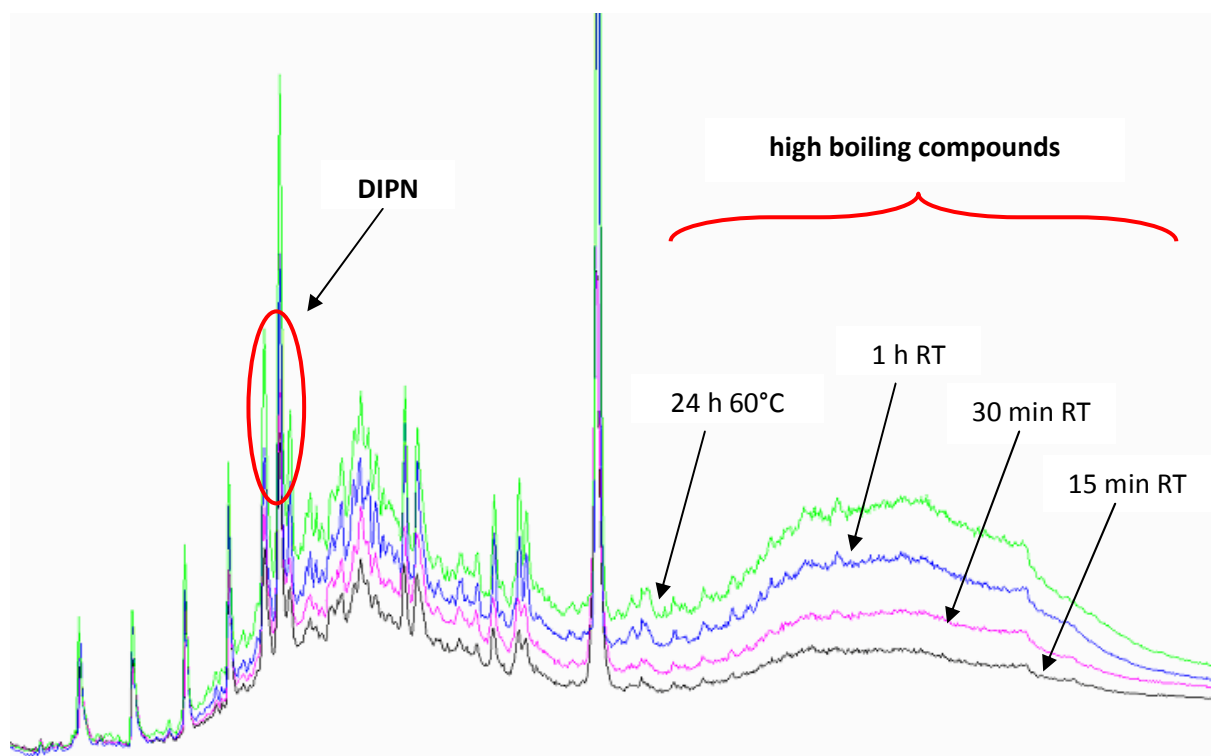


Figure 12. MOAH LC-GC/FID chromatograms of extraction with 100% hexane for 15 min RT (black line), 30 min RT (pink line), 1 hour RT (blue line) and 24 h 60°C (green line).

2) Choice of extraction solvent

Several organic solvents were tested for extracting power towards analytes of interest:

- methyl *tert*-butyl ether (MTBE);
- hexane (C6);
- methanol (MeOH);
- ethanol (EtOH).

All solvents were tested at RT, a part for C6, tested at -18°C . In fact, extracting power of C6 was already known to be very good from the previous experiments, so the extraction at freezer temperature was tried to investigate whether the low temperature could hinder the release of high molecular weight undesired compounds from paperboard matrix.

Before chromatographic analyses, all extracts were converted to C6 extracts adding to each of them an equal amount of hexane and separating it from the more polar solvent with water addition (provoking phase separation), and finally passing the C6 converted extract on silica powder to eliminate all traces of polar solvents, detrimental for the LC system.

An initial attempt was made to perform the extraction with MTBE, being it a very good solvent for hydrocarbons. As a result, the GC capillary column was very quickly damaged and chromatographic quality consequently decreased, probably due to the high solvent ability of MTBE towards high boiling hydrocarbons and polymers, which damaged the column.

We then compared, for both MOSH and MOAH, the results obtained using C6, MeOH and EtOH. For the last two solvents the extraction was performed at RT, whereas for the hexane it was performed at freezer temperature (-18°C) in an attempt to slow down extraction of high molecular weight compounds. For MOAH fraction, LC-GC/FID chromatograms (Figure 13) show that cool temperature hexane extraction is not able to discriminate between low and high boiling compounds: part of the first ones is lost and still most of the second one is extracted, as shown by the pronounced hill in the second part of the chromatogram. Methanol discriminates very well the high boiling components, not extracting them at all, but also loses some of the low boiling ones. Ethanol offers a very good extraction pattern, giving the highest yield for the compound of interest (low boiling) and avoiding the extraction of most of the high boiling compounds. This experiment demonstrate that ethanol is the ideal solvent for the MOAH fraction.

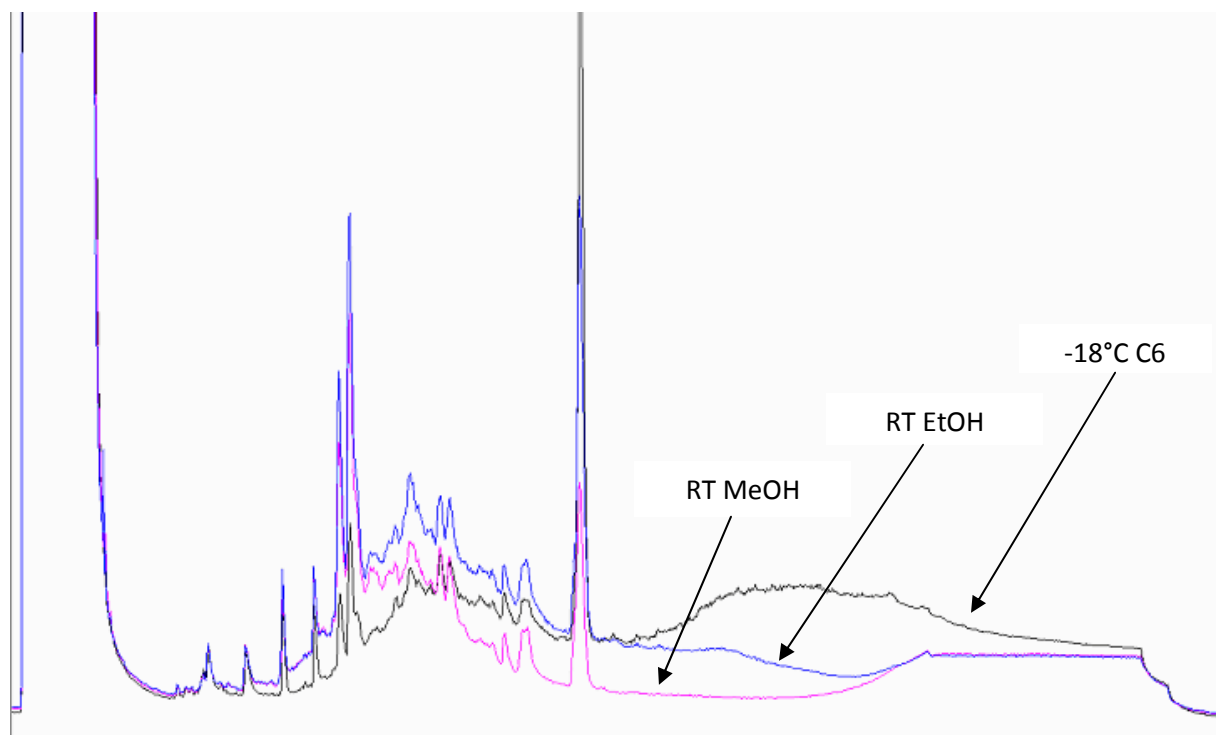


Figure 13. MOAH LC-GC/FID chromatograms of extraction performed with -18°C hexane (black line), room temperature methanol (pink line) and room temperature ethanol (blue line).

The three solvent (C6, MeOH and EtOH) extracts were analyzed also for the MOSH fraction, showing again some very interesting results (Figure 14). Cool temperature (-18°C) C6 extraction gives an optimal and complete extraction of the MOSH, whereas EtOH only manages to extract the very low molecular weight ones and MeOH is unsatisfactory as a solvent for these analytes, probably due to his high polarity. This experiment demonstrate that C6 is the ideal solvent for the MOSH fraction.

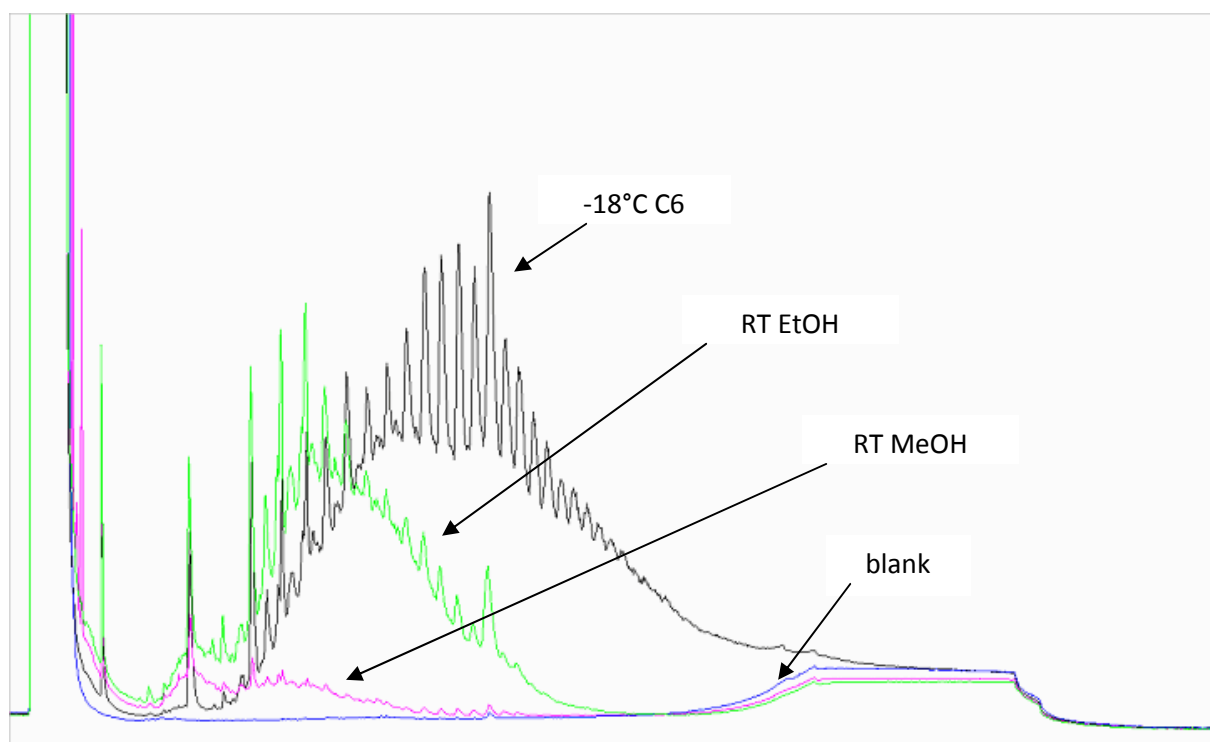


Figure 14. LC-GC/FID chromatograms of MOSH extraction performed with -18°C hexane (black line), RT methanol (pink line) and RT ethanol (green line); the blue line shows a blank injection.

The combination of these results shows that is not easy to find a solvent ideal for both MOSH and MOAH extraction, unless heavy compromises in one of the two extraction yields are accepted. On the other hand, adopting a unified extraction method for both analytes fractions would be more practical, cost saving and time saving compared to have two different and dedicated methods. Being EtOH ideal for MOAH and C6 ideal for MOSH, we tested mixtures of the two solvents, in different proportions, to find a mix that could have the advantages of both nonpolar C6 (optimal extraction of analytes) and polar EtOH (hindering extraction of undesired compounds). The tested solvent mixtures of C6 and EtOH were:

- 10% C6 - 90% EtOH by volume;
- 20% C6 - 80% EtOH by volume;
- 50% C6 - 50% EtOH by volume;
- 80% C6 - 20% EtOH by volume.

Also in this case, before chromatographic analyses, the extracts were brought back to 100% C6 with two water additions, the first one to provoke phase separation and the second one to wash C6, in order to completely remove EtOH traces.

For MOSH, Figure 15 shows extraction results for EtOH 100% (from previous experiment) and C6 10%, 20%, 50% and 80%. The 50:50 mixture seems the best compromise to have good extraction of the analytes of interest, without extracting the high boiling undesired compound, as clearly done by the 80% C6 mixture.

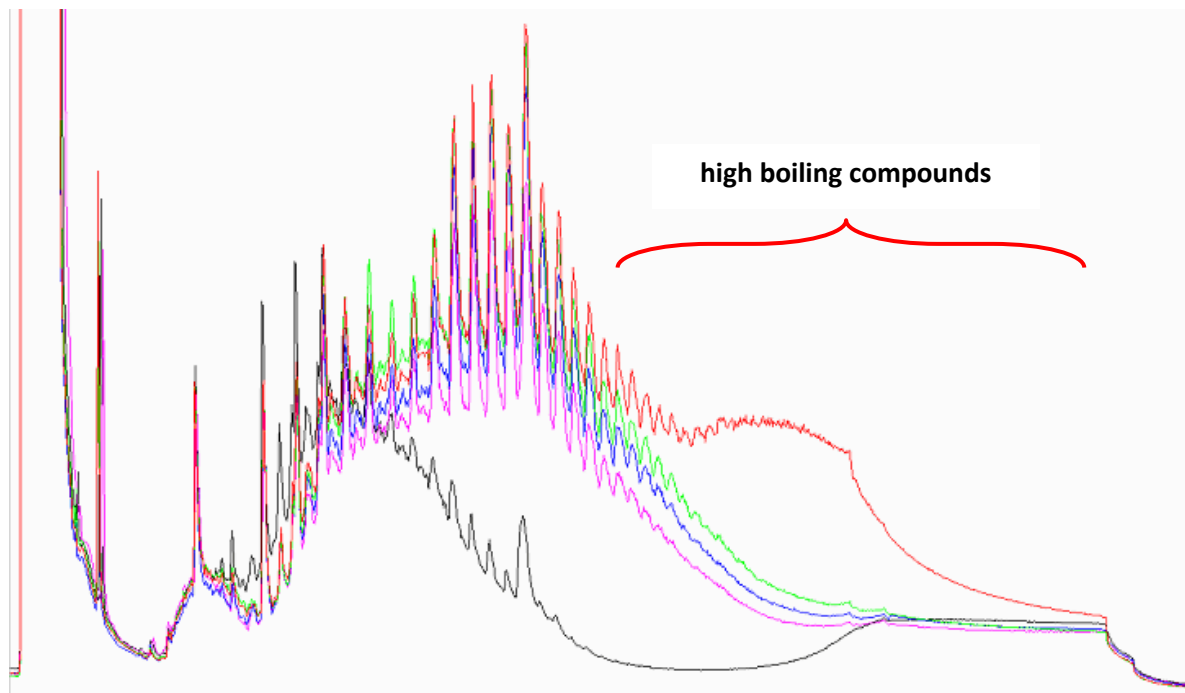


Figure 15. LC-GC/FID chromatograms of MOSH extracted with ethanol 100% (black line), hexane 10% (pink line), hexane 20% (blue line), hexane 50% (green line) and hexane 80% (red line).

For MOAH, the supremacy of ethanol 100% as the best extraction solvent keeps strong, because it is able to avoid, at the same time, undesired compounds (Figure 16). The 50:50 mixture gives a good yield of the analytes of interest, but also extracts part of the high boiling substances. Hopefully the discrimination against them will increase at higher molecular weights (not visible with this chromatography), as we can expect ideally continuing the red (still ascending) and the green (already descending) lines.

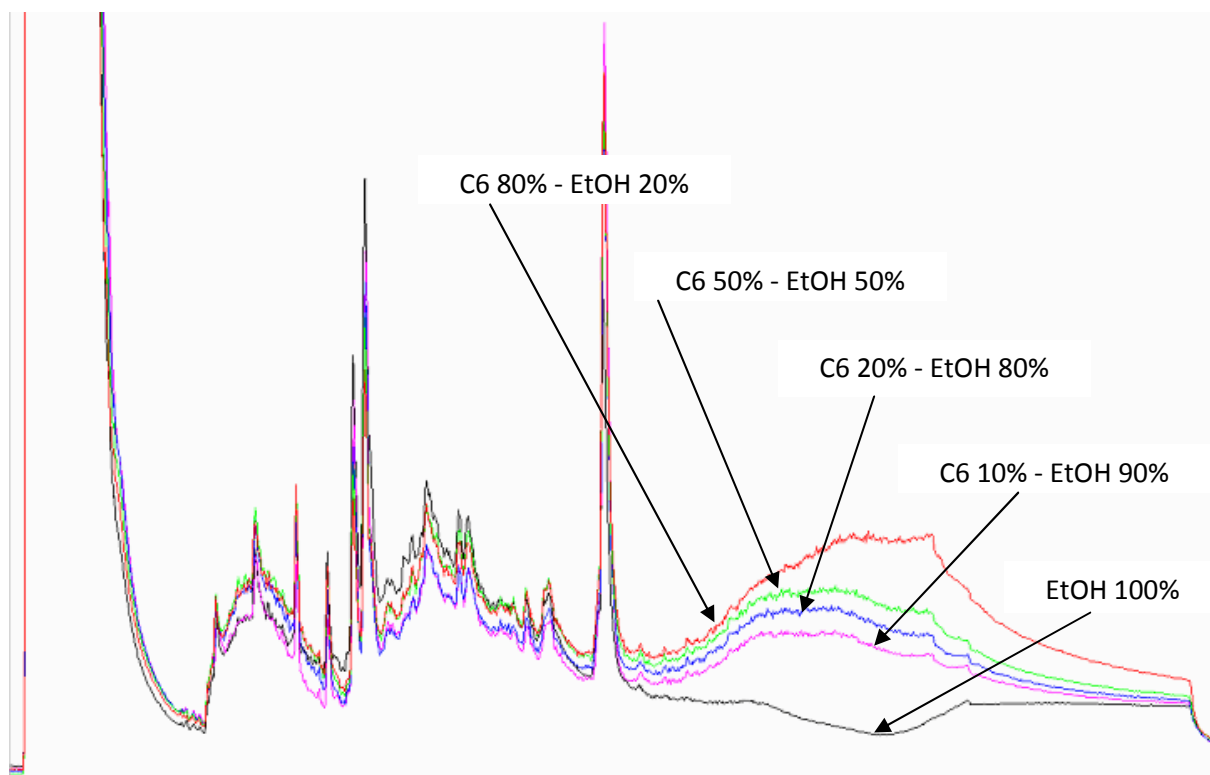


Figure 16. LC-GC/FID chromatograms of MOAH extracted at RT with ethanol 100% (black line), hexane 10% (pink line), hexane 20% (blue line), hexane 50% (green line) and hexane 80% (red line).

3) Final adjustments

The C6:EtOH 50:50 (1:1) extraction mixture was identified as the best compromise in order to extract at the same time MOSH and MOAH. It was then necessary to verify again the extraction length, previously verified with C6 (step 1). Therefore, the mixture was tested at RT for 1 h, 2 h, 5 h and 24 h. Focalizing on the different extraction times, differences are not great (see Figure 17). Therefore, the previously identified extraction length of 2 h at RT was confirmed as ideal.

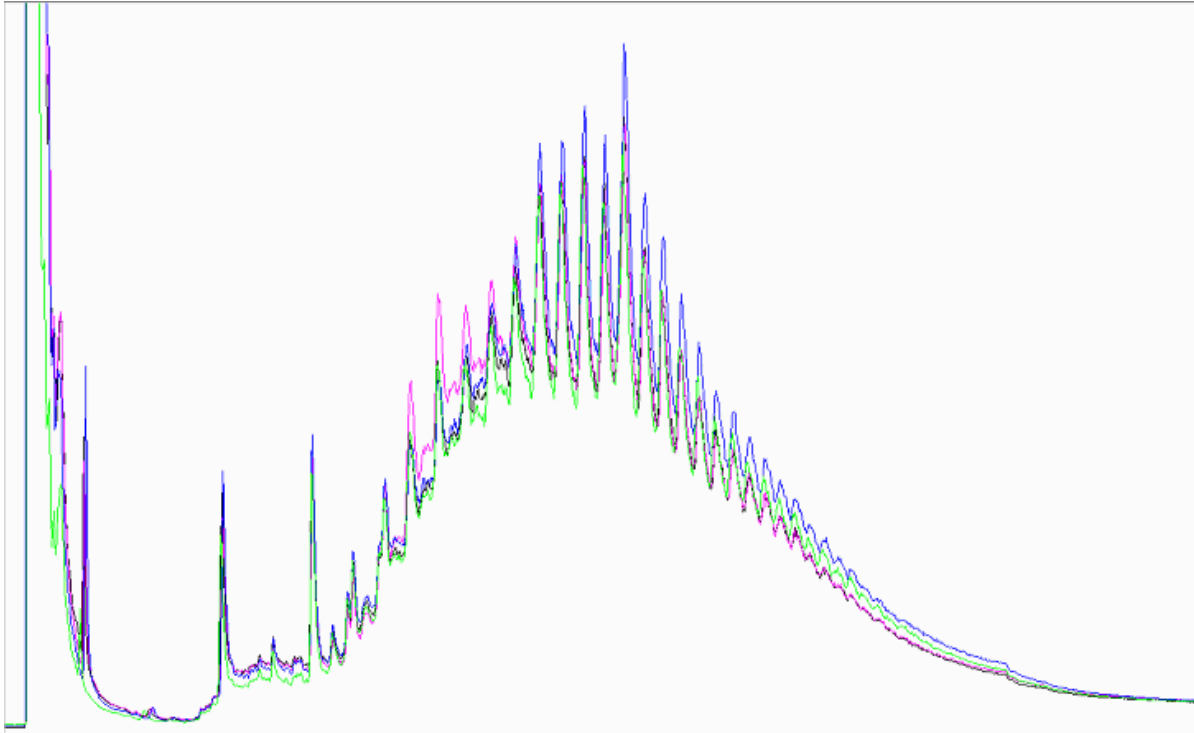


Figure 17. MOSH LC-GC/FID chromatograms of: in black 1 h extraction, in pink 2 h, in blue 5 h and in green 24 h, all at RT and with an C6:EtOH 1:1 extraction mixture. No significant differences can be noticed at varying of extraction length.

Furthermore we wanted to determine whether variability in the part of packaging chosen for analysis (e.g. printed or not printed, shiny or opaque) could significantly affect the final result. For MOSH, Figure 18 shows the 1 and 2 h RT extraction of a shiny multicoloured paperboard part, the 2 h RT extraction of a white shiny part of the same paperboard and the 2 h RT extraction of a white opaque part. Confronting the extraction length of 1 or 2 h, it is clear that the second option gives a better extraction yield, thus confirming the choice of 2 h RT as the ideal extraction conditions. For MOAH there are no significant differences but the white opaque paperboard again shows a smaller amount of analytes in the first part of the chromatogram (see Figure 19).

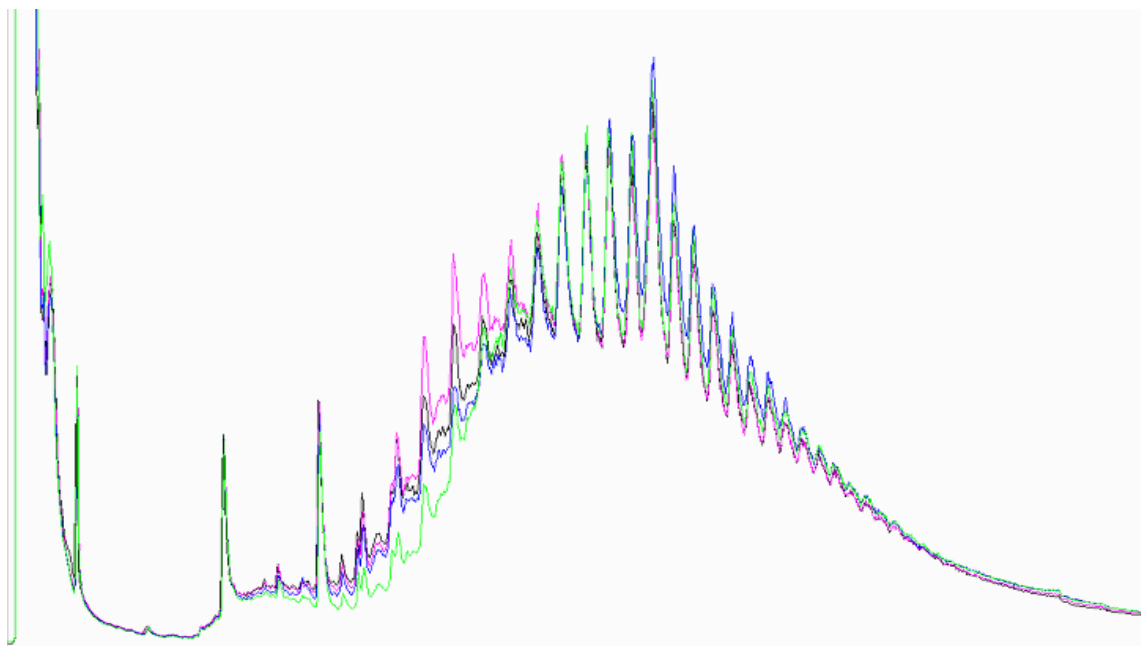


Figure 18. MOSH LC-GC/FID chromatograms of: in black the 1 h RT extraction of a shiny multicoloured paperboard part of the packaging, in pink the 2 h RT one (more effective length of time), in blue the 2 h RT extraction of a white shiny part of the same paperboard and in green the 2 h RT extraction of a white opaque part: in green line, some of the analytes in the first part of the chromatogram are missing, so we can deduct they probably belong to the final shiny lacquering of packaging.

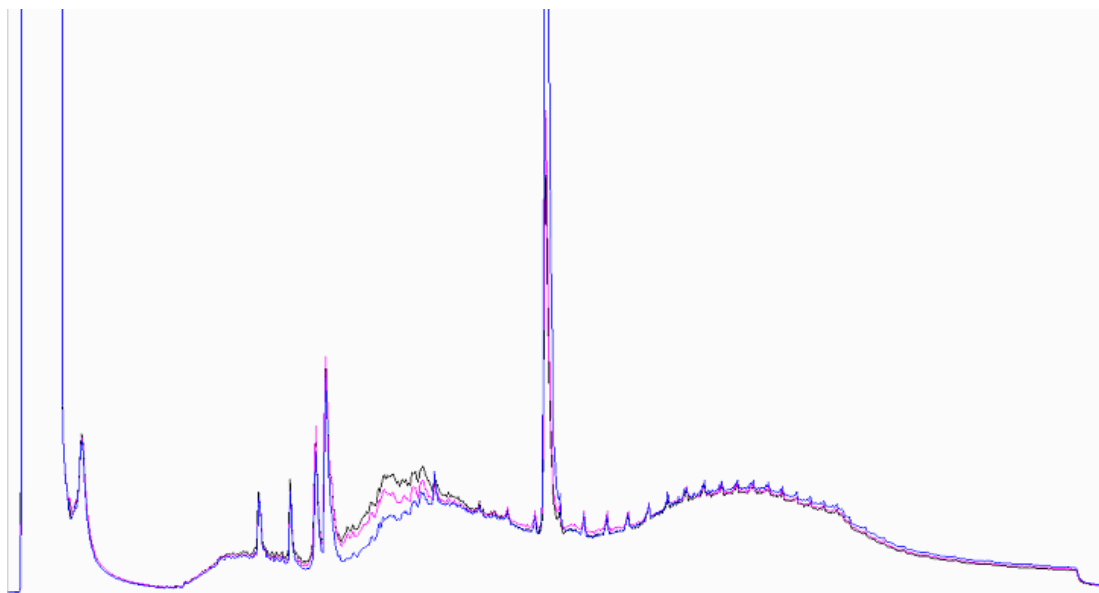


Figure 19. MOAH LC-GC/FID chromatograms of extraction with C6:EtOH 1:1 for 2 h at RT. The black line corresponds to the multicoloured board, the pink to the white shiny part and the blue to the white opaque part and: there are no significant differences but the opaque again shows a lesser in the first part of the chromatogram.

Overall, it can be concluded that length of extraction is not significantly affecting the final yield and kind of analytes extracted, whereas extracting mixture composition is the most important factor to take into account.

As a final example, Figures 20 and 21 show the MOSH and MOAH fraction chromatograms, respectively, from different paperboards, all extracted with the final optimized method.

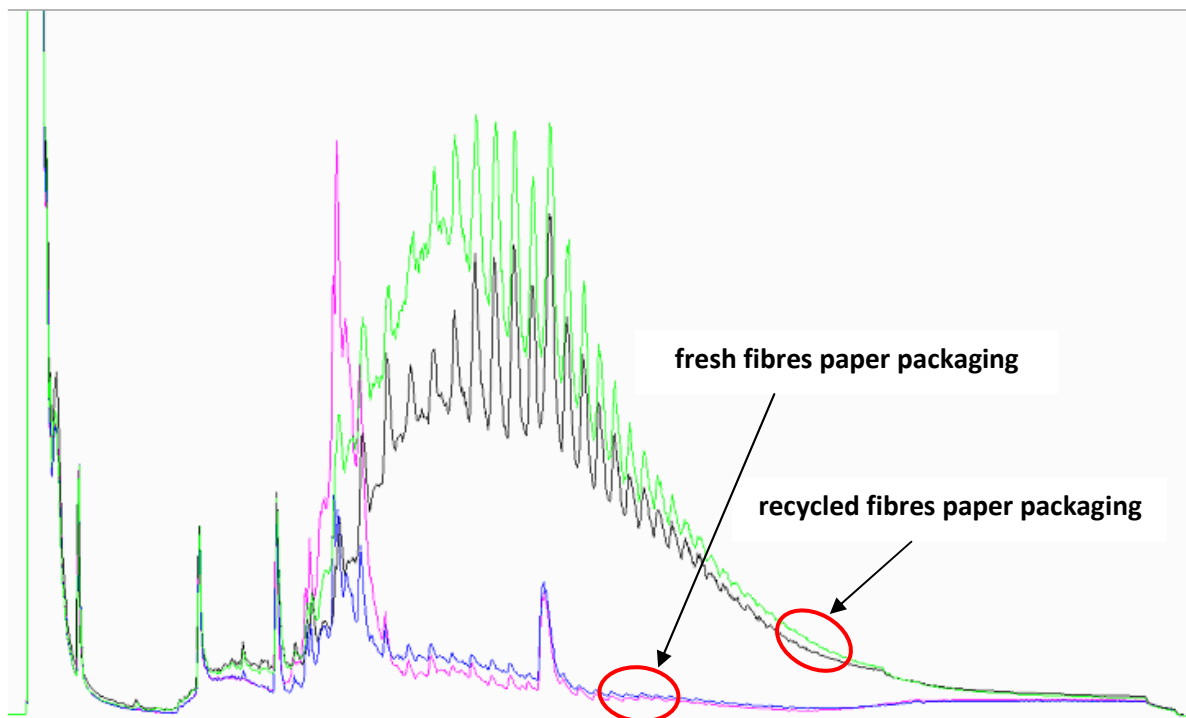


Figure 20. LC-GC/FID chromatograms of MOSH: in black breakfast cereals with red fruits, in pink Luxemburgerli packaging, in blue Cappuccino ice cream cake, in green artisanal cake packaging. It can be distinguished clearly the two packaging made of recycled paper (higher contamination) from those made of virgin paper (lower contamination).

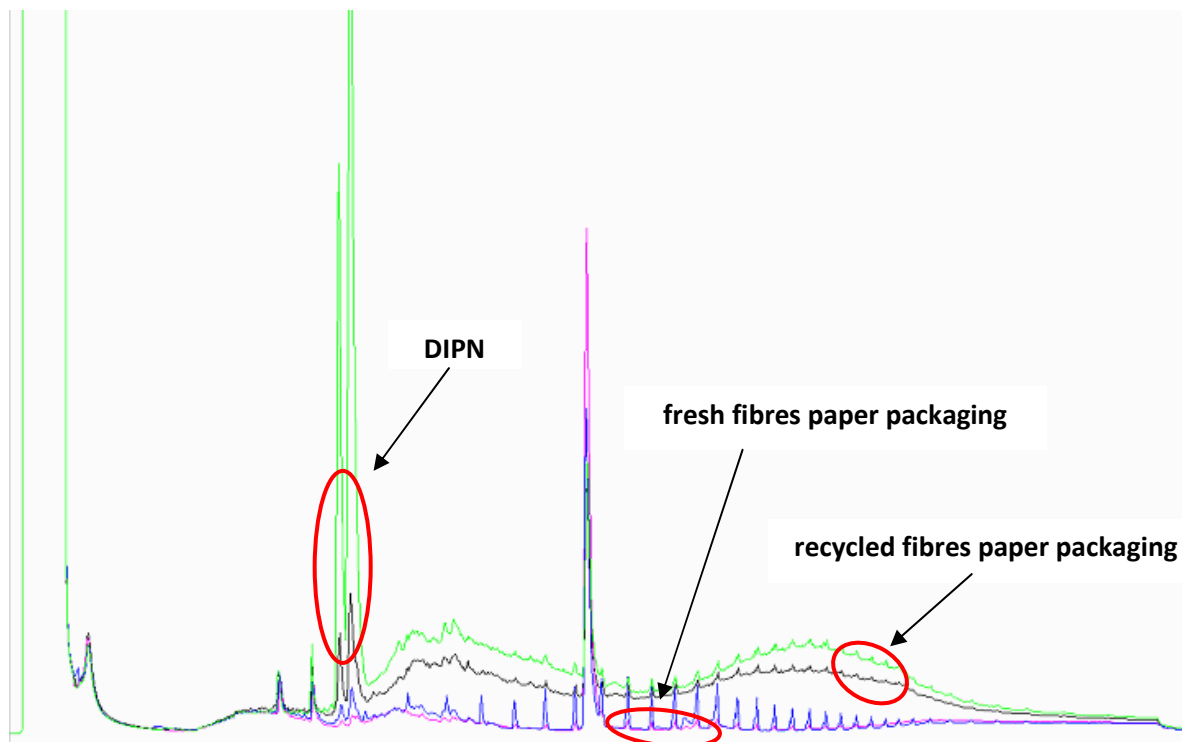


Figure 22. MOAH LC-GC/FID chromatograms: in pink the Luxemburgerli box (virgin paper, violet ink, opaque), in blue the Cappuccino ice cream cake box (virgin paper, multicoloured and shiny), in green the box for artisanal cakes (recycled paper, pink and blue inks) and in black the breakfast cereals with red fruits box again (recycled paper with multicolour inks and shiny finish). For the first two it is noticeable the low amount of MOAH compared to the last two, very probably due to the recycled paper nature of them; for the Cappuccino box the DIPN (two big peaks plus sometimes minor peaks, depending on isomers proportion), typical recycling markers, are evident.

The final method for paperboard packaging analysis is as follow: $1 \text{ g} \pm 0.01$ of paper/paperboard/corrugated board, finely cut into pieces, is weighed directly into a 20 mL screw top amber flask. A representative area of the pack (e.g. multicoloured portion of a coloured packaging) should be chosen. No gloves or hand cream were used during the sample manipulation and the flask caps had a PTFE inner surface to avoid external contamination. A mixture of hexane:ethanol (10 mL) 1:1 by volume is added into the flask to the board pieces, along with 20 μL of internal standards solution for mineral oil hydrocarbons. The flask is closed tightly and vortexed for 5 s, then left for 2 h at RT. After that time, ca. 10 mL of water is added into the flask, ensuring thorough mixing by manually shaking or vortexing. The water ensures the removal of ethanol from hexane owing to the polar character of the protonated solvents. After separation of the two phases (hexane and hydroalcoholic solution) obtained either by allowing 10 min time or by

centrifugation, the upper hexane phase is removed and then washed again with water in a separate and clean glass flask, to ensure complete removal of ethanol that would be detrimental to the chromatographic analyses. Finally, ca. 1 mL of the clean hexane extract is transferred into autosampler vials for the chromatographic analysis, ensuring the vial cup is well tight to avoid any solvent loss through evaporation. For the same reason, vials are better kept into a refrigerator if not immediately analyzed.

For plastic extraction, the same method was used except for sample weight: only 0.2 g of sample was weighed instead of 1 g, due to the often higher mineral oil contamination of plastic compared to paperboard, along with the presence of polyolefin oligomeric saturated hydrocarbons (POSH), as typical plastic oligomers.

This extraction method has also been successfully applied to other paperboard contaminants, such as phthalates and other plasticizers, photoinitiators, rosin components, etc. The same extract can be analyzed by LC-GC/FID for MOSH, MOAH and DIPN and in GC/MS for all those other contaminants (data not shown in this thesis), making optimal use of one single extraction.

4.1.2 FOOD EXTRACTION

Food extraction approach is very different depending on food moisture content. Dry food can easily be extracted with pure hexane, which has very high chemical affinity for MOSH and MOAH. In the case of moist or liquid foods (frozen foods, eggs, teas, but also foods considered quite “dry” as dry plums and apricots), the presence of water in the food matrix hinders the hexane capacity to extract the mineral oil contaminants, so a more complex extraction procedure must be applied.

1) Extraction of dry foods

The volatile contaminants migrating from paper-based packaging to its food content are adsorbed by food surface, without a deep penetration into food matrix. Therefore a simple hexane extraction is ideal, having this solvent high affinity for hydrocarbons. In the case of packaging, the extraction of high molecular weight compounds had to be avoided in order to protect GC capillary column and precolumn, but in the case of food, those compounds are not present because they are not sufficiently volatile to migrate to food. Therefore, the addition of ethanol to extracting mixture in order to avoid them is not necessary: pure hexane is the best choice.

The chosen amount of food to be extracted is tenfold the amount of packaging (10 g instead of 1 g): this is because food contamination is non homogeneous inside the packaging, being highest near the packaging and lowest at the product core, due to the diffusion character of migration through the gas phase. For this reason, 10 g is considered a more representative amount for food extraction. To further minimize the problem, all food packaging content has to be ground and mixed to homogenize contamination content, then the 10 g aliquot is taken. In some selected cases, just specific parts of the food have been tested: e.g. for some pastry just the bottom part has been analyzed, in order to verify the use of mineral oil as antisticking agent (see Table 5). The 10 g of food is then placed into a 50 mL glass flask with PTFE-lined screw cap. Internal standards solution (20 µl) and hexane (20 mL) are added and the flask is thoroughly shaken on a vortex, then allowed to stand at room temperature for 3 h. Before chromatographic analysis, hexane extract is centrifuged if necessary.

2) Extraction of solid dry foods for detection of pre-packaging contamination

Some foods can have a multiple mineral oil contamination, originating from different sources (see paragraph 1.3.1): i.e. pasta and bakery products can have a contamination from packaging plus a primary contamination from cereal kernels dust binding; chocolate and other colonial goods can have a contamination from packaging plus a primary contamination from jute bags, etc.

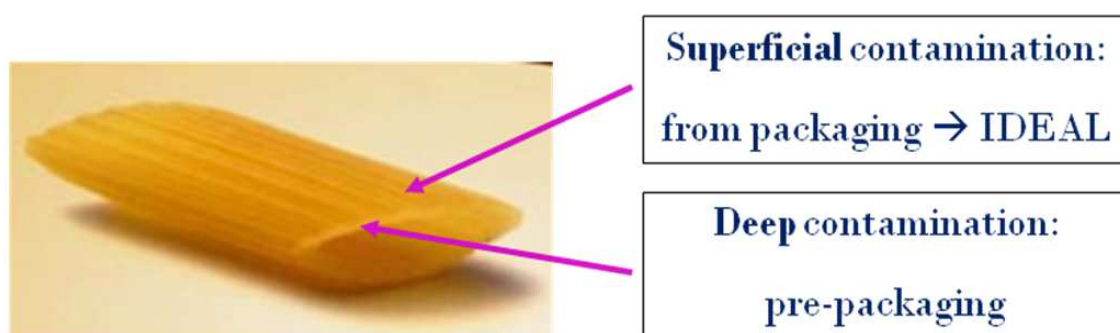


Figure 23. In many foods, among which pasta, mineral oil contamination can be both superficial (migration from paper-based packaging) and deep (e.g. dust binding on wheat kernels, with flour contamination and all derived products).

While the contamination from packaging is superficial, and therefore it is easily extracted by hexane, any primary contamination will be present deep inside the food matrix (see Figure 23), which will need to be thoroughly swollen and disaggregated in order to extract those contaminants. In order to further investigate this subject, we prepared handmade pasta (“strozzapreti”), spiking the dough with a known amount of mineral oil, and following a preparation procedure as much similar as possible to artisanal dry pasta production: 100 g of flour have been mixed with 50 g of water and additionated 4.5 mg of white mineral oil of known composition and chromatographic behaviour, dissolved in a little amount of hexane:ethanol 1:1 in order to facilitate mineral oil incorporation into dough aqueous matrix. The dough has then been shaped into “strozzapreti” (long thins pasta shapes) and dried overnight at 60° to reproduce artisanal dry pasta manufacturing. The loss of pasta weight due to desiccation has been considered. Also some blank pasta, obtained from unspiked dough, have been prepared, to take into account any possible flour contamination. Handmade pasta, once dry, has been ground and treated with different approaches: part was suspended in hexane and part in water, both for 3 h at room temperature. After that time, Scanning Electron Microscope (SEM) pictures were taken (see Figure 24). Only in this latter case the food matrix appear to be completely smooth, to indicate that food matrix was properly swollen ad dispersed by water action. Therefore, a first dispersing step with water and/or ethanol is necessary, prior to hexane extraction, in order to detect any pre-packaging mineral oil contamination in dry food matrixes. If water is used for such first step, then an “interface solvent” (e.g. ethanol) is necessary in order to successfully perform the contaminants extraction, due to the non mixability of water and hexane. Different combinations, amounts and sequences of these three solvents have been applied in order to find the more effective and simple extracting procedure, e.g.:

- water, ethanol and hexane directly added to food, for different length of time;
- just water added to food for different length of time, followed by ethanol, then hexane;
- as previous but adding ethanol and hexane together;
- water and ethanol added to food for different length of time, followed by hexane.

The best extraction sequence, recovery-wise, is the application of water alone for 18 h, despite the SEM appearance of food matrix is already smooth and even after 3 h. The following addiction of EtOH and C6, either in sequence or already mixed, is not a critical step from the length of time point of view, probably because the passage of mineral oil from water-disaggregated food matrix to organic solvents is fast and easy.

To evaluate deep contamination → preparation of spiked “handmade strozzapreti”:



Figure 24. SEM images of handmade dry pasta after grounding (left), suspension in nonpolar solvent (middle) and in polar solvent (right). Hexane is not able to provoke food matrix swelling and opening. On the contrary, water allows to obtain a smooth food suspension.

In conclusion, the critical step in order to extract a pre-packaging mineral oil contamination from a solid dry food is the food matrix disaggregation, which has to be performed with a long water contact. The following passage of hydrocarbons from water dispersed food to ethanol (interface solvent) and hexane (final extraction solvent) is easy and fast, performed with thorough agitation of mixture followed by phase separation.

3) Extraction of moist or liquid foods

The presence of water in the food matrix hinders the hexane capacity to extract the mineral oil contaminants, because they cannot be reached by hexane even with a thorough vortexing of finely minced food with such solvent. Therefore, an “interface” solvent, mixable with both water and hexane (as seen in previous paragraph), is necessary: ethanol has the ideal polarity to perform this function. We already knew from previous experiments (extraction of pre-packaging contaminants from solid dry food) that the critical step is the water disaggregation of food matrix. In the present case, such “step” is unnecessary because the food is already moist, or liquid. Therefore, it is sufficient to perform the hexane extraction, preceded by the use, again, of ethanol as interface solvent.

The final extraction method for moist/liquid foods and for pre-packaging contamination in dry foods, is as follow: the full packaging food content, or a representative amount, is thoroughly minced/ground (if necessary) and mixed. 5 g of food is placed into a 100 mL glass flask with a glass stopper. 25 mL of ethanol is added, along with 20 μ L of mineral oil internal standards solution. The flask is capped, vigorously shaken for about 10 s, then

placed on an horizontal shaker for 30 min. 20 mL hexane are then added to the flask, which is again vigorously shaken for about 10 s and placed on the shaker for another 30 min. Finally, as for the packaging extraction, water is added (ca. 40 mL) and flaks is vigorously shaken once again, forcing ethanol to join the aqueous phase and thus separate from hexane. The flask is left at room temperature for 3 h and proper phase separation occurs. Prior to chromatographic analysis, the liquid phase is centrifuged if necessary.

4.2 “SHOPPING TROLLEY” SURVEY

As discussed in paragraph 1.3.1, the presence of mineral oil as a food contaminant is not rare, and can originate from many different sources. Paper-based packaging is one of them. Applying the optimized extraction methods for both packaging and food, detailed in previous paragraphs, we analyzed a variety of samples collected from Italian and Swiss supermarkets, to represent what it can be called a “shopping trolley survey”. In fact, the chosen products are very common and are often present in every average consumer food shopping, therefore it was interesting to investigate to which level of mineral oil contamination, from packaging migration, we are exposed as consumers. Among the food categories investigated are:

- rice, pasta and flour;
- sweet and savoury bakery products;
- breakfast cereals;
- dry fruits and tree nuts;
- eggs;
- frozen food (breaded and not);
- “colonial” foods (chocolate products, cocoa, tea).

This survey did not take into account important variables as the product age: many product packaged in paper or paperboard have a long or very long shelf life, and the food content contamination increases considerably over time, depending on many factors as temperature and food characteristics. Nevertheless, the aim of this part of the work was simply to give a picture of the average food contamination. All the mentioned variables will be taken into account in the last part of this work, the “migration study”, where the migration kinetics is observed under specific controlled conditions.

The contaminants measured by LC-GC/FID analyses in the “shopping trolley survey” were MOSH, MOAH and DIPN, however not necessarily all three contaminants were determined for each sample. Being a survey, the major aim was to collect information on a wide number of products, and this approach was preferred instead of having complete data on a much lower number of products:

- if the contamination found in packaging was very low, it was not so compelling to perform the analysis also on food content;
- if MOSH found in food was very low, it was not a priority to measure MOAH (usually no more than 30% of MOSH).

Applying the same extraction methods, also GC/MS analyses have been performed, looking for other typical paper-based materials contaminants. In particular the following have been researched and quantified:

- Plasticizers: acetylated tributylcitrate (ATBC), trimethyl pentadiol diisobutyrate (TXIB), dibutylphthalate (DBP), diisobutylphthalate (DIBP), diethylhexylphthalate (DEHP), diisoethylhexylmaleate (DEHM), oleic acid methyl ester, isopropylallurate, isobutylallurate, 2-ethylhexilpalimtate, 2-ethylhexilstearate.
- Photoinitiators: benzophenone, 4-methylbenzophenone.

Quantification for these analytes are not reported in this work.

In Table 5 are reported the mineral oil (MOSH and MOAH) and DIPN (“recycling markers”) packaging and food contamination data for 68 products, chosen on the Italian market in 2009. For MOSH and MOAH, only the contamination that chromatographically corresponds to up to 24 C atoms is reported, as these are relevant for migration into food via the gas phase¹⁶². For packaging, the board has been analysed being the main source of food contamination, but sometimes also other parts have been analysed, e.g. plastic parts and glues. Plastic, if present, often bears a very high contamination, often higher than the paperboard: this might induce to think that plastic is the real source of contamination. Despite being sometimes a partial source of contamination (e.g. releasing hydrocarbons as plastic oligomers - POSH), plastic is much more often a reservoir of contamination absorbed from paperboard, what we call “sponge effect”. This phenomenon is only seen for certain plastic polymers, which have high chemical affinity towards hydrocarbons: in particular polyolefins as PE and PP. Also glues used to shape up paperboard boxes, and to

¹⁶² Biedermann *et al.*, 2011a.

seal them after they are filled, can contribute to food contamination. There are 2 main groups of glues:

- the “hot melt” glues, easily visible tearing apart the paperboard boxes at the top and bottom, often contain waxes and other hydrocarbons;
- the “liquid” glues, of difficult identification because they sink into the paperboard once applied (usually to sew the side part of boxes), usually do not contain hydrocarbons, but at a GC scan their extracts show a multitude of peaks.

However, the contribution of glues to overall food contamination is probably not of primary importance.

Table 5. Mineral oil contamination of paper-based packagings and their food content (“shopping trolley survey”) for the Italian market samples. Highest packaging contamination is found in paperboards which are printed and produced from recycled fibres, and in polyolefinic plastics. Highest food contamination is found in foods packaged in recycled paperboard printed with mineral oil based inks, especially if food is placed either in direct contact with the board, or inside a plastic bag unable to perform as a protective barrier.

PRODUCT	SAMPLE	mg kg ⁻¹		
		MOSH < C24	MOAH < C24	DIPN
originario rice, PB	R P board	413	173	n.d.
	food	1	< LOQ	n.d.
	glue "side"	< LOD	n.d.	n.d.
	glue "top"	112	n.d.	n.d.
arborio rice, PB	R P board	297	n.d.	n.d.
	food	1	n.d.	n.d.
vialone nano rice, PB	R P board	628	219	n.d.
	food	< LOQ	n.d.	n.d.
carabinieri rice, PB	R P board	296	117	n.d.
ribe rice, PB	R P board	218	73	n.d.
thaibonnet rice, PB	R P board	177	n.d.	n.d.
	food	< LOQ	n.d.	n.d.
basmati rice, PB	R P board	593	n.d.	n.d.
	food	1	n.d.	n.d.
long wild rice, PB	R P board	201	57	n.d.
	glue "side"	< LOD	n.d.	n.d.
	glue "top"	128	n.d.	n.d.
ribe organic rice, PB	R P board	532	204	n.d.
parboiled Roma rice	R U board, intern. coated	40	1	n.d.

	R P board, intern. coated	44	1	n.d.
lasagne (dry egg pasta foils), DC	F P board	52	n.d.	n.d.
	glue	602	n.d.	n.d.
tagliatelle paglia-fieno (dry egg pasta), DC	R U board	243	81	present
	food	37	n.d.	n.d.
	glue "sides"	159	n.d.	n.d.
spaghetti chitarra (dry egg pasta), DC	R P board	420	30	present
	glue "top"	257	n.d.	n.d.
	glue "side"	< LOD	n.d.	n.d.
	glue "side top"	189	n.d.	n.d.
	glue "window"	< LOD	n.d.	n.d.
	food	36	n.d.	n.d.
taglioline (dry egg pasta), DC	R U board,	517	41	present
	glue "sides"	248	n.d.	n.d.
	food	37	n.d.	n.d.
wheat flour "0", DC	paper	48	12	n.d.
	glue "top"	211	n.d.	n.d.
organic wheat flour "00", DC	paper	38	n.d.	n.d.
chocolate covered hazelnuts, PB	F P board	31	n.d.	n.d.
chocolate covered nougat, PB	F P board	60	n.d.	n.d.
	single plastic wrap	1028	n.d.	n.d.
	food	< LOD	n.d.	n.d.
organic milk chocolate, Al foil	external paper	71	n.d.	n.d.
	food	1	n.d.	n.d.
organic dark chocolate 70% cocoa, Al foil	food	5	n.d.	n.d.
dark chocolate Dominican Republic, Al foil	food	2	n.d.	n.d.
dark chocolate Ecuador, Al foil	food	3	n.d.	n.d.
cantucci mandorle pinoli (hazelnuts biscuits), PB	R P board	223	55	n.d.
	food	< LOD	< LOD	n.d.
	glue "side"	< LOD	n.d.	n.d.
	glue "bottom"	65	n.d.	n.d.
wholemeal rusks, DC	F U board	87	n.d.	n.d.
	internal paper	158	n.d.	n.d.
	external plastic	1025	n.d.	n.d.
	food	1	< LOD	n.d.

plum cakes, PB	R U board	165	50	present
	paper cooking mould	11	n.d.	n.d.
	food	< LOQ	< LOD	n.d.
gluten free cocoa plum cake, PB	R U board	334	66	present
	external plastic	1950	n.d.	n.d.
	paper cooking mould	178	29	present
	food	< LOQ	< LOD	n.d.
pain au chocolat, PB	R U board	n.d.	n.d.	n.d.
	food	6	n.d.	n.d.
apricot little jam tarts, PB	R U board	284	26	present
	external plastic	1248	n.d.	present
	glue	257	n.d.	n.d.
	food (just bottom & sides)	1	< LOD	n.d.
organic little sponges, PB	R U board	271	n.d.	present
	external plastic	1767	n.d.	present
	paper cooking mould	98	< LOD	n.d.
	food	< LOQ	< LOD	n.d.
	food (bottom)	4	n.d.	n.d.
organic baby biscuits, PB	R P board	480	n.d.	n.d.
	food (bottom)	< LOQ	< LOD	n.d.
chocolate cake, PB	R P board	384	51	present
	paper cooking mould	25	n.d.	n.d.
jam tart, PB	R P board	353	29	present
	glue "side"	< LOD	n.d.	n.d.
	glue "bottom"	95	n.d.	n.d.
unsalted crackers, PB	R P board	210	n.d.	n.d.
gluten free crackers, PB	R P board	350	n.d.	n.d.
wholemeal rice and wheat flakes, PB	R P board	434	n.d.	n.d.
rice and wheat flakes with red fruits, PB	R P board	580	25	n.d.
rice and wheat flakes with chocolate bits, PB	R P board	552	n.d.	n.d.
breakfast cereals with milk, PB	R P board	460	93	n.d.
crispy rice with chocolate, PB	R P board	436	n.d.	n.d.
	food	31	n.d.	n.d.

organic corn shells with chocolate, PB	R P board	332	109	n.d.
organic wholemeal cereals sticks, PB	R P board	592	n.d.	n.d.
organic müsli with fruit, PB	R P board	367	95	12
	glue "side"	< LOD	n.d.	n.d.
	glue "top"	341	n.d.	n.d.
	food	13	< LOQ	< LOQ
organic crispy müsli, PB	R P board	283	79	n.d.
	food	23	n.d.	n.d.
Turin bread sticks, PB	R P board	411	156	8
	internal plastic	1694	296	34
	food	2	1	< LOD
Turin bread sticks, PB (after 180 d)	R P board	249	56	6
	internal plastic	1395	126	22
	food	13	1	< LOQ
fresh medium eggs, DC	R P outside board	413	122	39
	R U inside board	923	293	14
	food	9	n.d.	n.d.
pre-packaging eggs, producer 1	food	2	< LOQ	< LOQ
pre-packaging eggs, producer 2	food	3	< LOD	< LOD
pre-packaging eggs, producer 3	food	4	< LOQ	< LOD
organic brazil nuts, PB	R P board	526	158	62
	internal plastic	137	n.d.	n.d.
	food	1	< LOQ	n.d.
organic brazil nuts, PB (after 180 d)	R P board	309	64	42
	internal plastic	138	6	4
	food	4	< LOQ	< LOD
rolled puff pastry (refrigerated)	F P board	9	1	n.d.
	external plastic	128	28	n.d.
	food	< LOQ	< LOQ	< LOD
tiramisù cake (frozen)	F P board	37	20	4
	plastic tray	90	28	< LOD
	food (whole)	1	< LOQ	< LOD
	food top ½ cm	9	n.d.	n.d.
	food bottom ½ cm	9	n.d.	n.d.






mixed fried fish (frozen), DC	F P board	62	9	n.d.
	food (whole)	1	< LOQ	< LOQ
	food breading	14	n.d.	n.d.
	food shrimps	11	n.d.	n.d.
	food squids	17	n.d.	n.d.
	food cod	23	n.d.	n.d.
fish fingers (frozen), DC	F P board	33	9	< LOQ
cordon bleu (frozen), DC	F P board	42	10	< LOQ
breaded little mozzarellas (frozen), DC	R P board	377	90	16
	food (whole)	< LOQ	< LOD	< LOD
	food breading	9	n.d.	n.d.
breaded little mozzarellas (frozen), DC (after 180 d)	R P board	346	68	17
	food breading	5	< LOQ	< LOD
rice arancini (frozen), DC	R P board	661	158	5
	food (whole)	< LOQ	< LOD	< LOD
	food breading	12	n.d.	n.d.
rice arancini (frozen), DC (after 180 d)	R P board	556	109	8
	food breading	21	2	< LOQ
black tea	R P board	390	136	5
	external plastic	3221	367	10
	paper bag	184	27	< LOQ
	tag	42	8	< LOQ
	filter paper	415	45	3
	dry tea leaves	44	12	1
	dry tea leaves (after 180 d)	58	7	1
	tea drink	1	< LOQ	< LOQ
green tea	R P board	284	n.d.	n.d.
	filter paper	349	n.d.	n.d.
	dry leaves	44	n.d.	n.d.
unsweetened cocoa powder, PB	R P board	295	94	35
	internal plastic	104	19	< LOQ
	food	1	< LOQ	< LOQ
unsweetened cocoa powder, PB (after 180 d)	R P board	150	30	19
	internal plastic	66	2	1
	food	20	7	6
croissants (end shelf life), PB	R P board	103	n.d.	n.d.
	internal plastic	232	n.d.	n.d.
	food	13	n.d.	n.d.
baby pasta, PB	R P board	243	n.d.	n.d.
	internal plastic	1585	n.d.	n.d.
	food	4	n.d.	n.d.

baby pasta, PB (after 180 d)	R P board	26	3	9
	internal plastic	1859	142	94
	food	3	< LOQ	< LOD
dry plums California	F P board	22	5	3
	internal plastic	239	8	6
	food	1	< LOD	< LOD
dry plums XL California	food	1	< LOD	< LOD
dry plums Italy	food	1	< LOD	< LOD
dry raisins producer 1	food	2	< LOD	< LOD
dry raisins producer 2	food	1	< LOD	< LOD
dry apricots	food	1	< LOQ	< LOQ
dry apricots bio	food	6	< LOQ	< LOQ

Abbreviations: PB = plastic protective barrier between paperboard and food; DC = direct contact paperboard/food; R = recycled paper fibres; F = fresh paper fibres; P = printed board; U = unprinted board; LOQ = limit of quantification; LOD = limit of detection; n.d. = not determined.

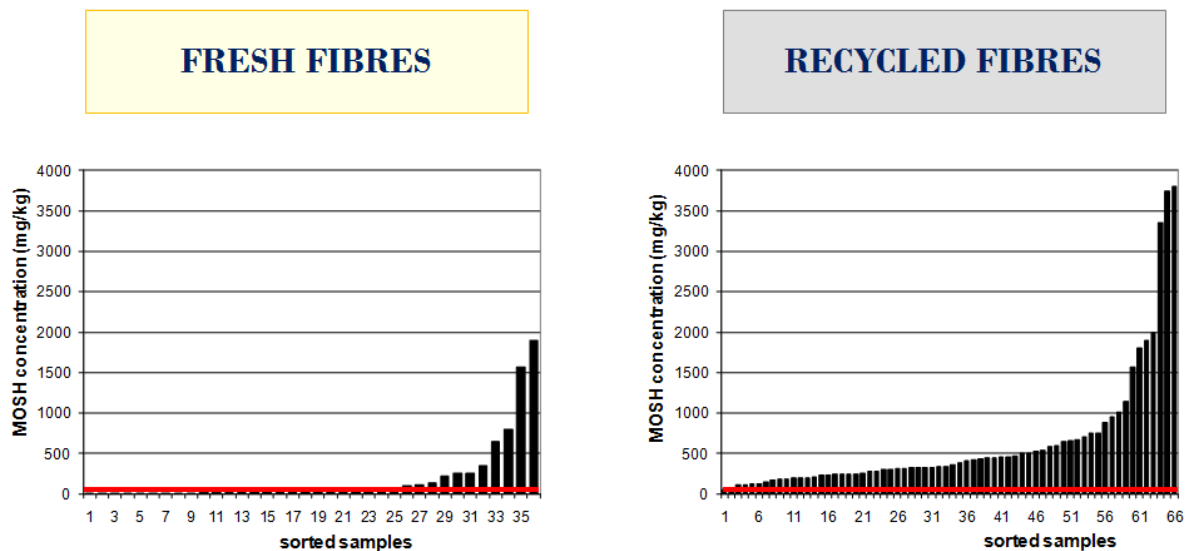
In Table 6 a selection of the most interesting data are shown, with comments.

Table 6. Some of the more representative results of the “shopping trolley survey”, grouped according to food kind, with comments on the nature of contamination. Values represent mg kg⁻¹ of MOSH. Green writing stands for adequate food protection, whereas red writing signals a food packaging safety issue.

	from 200 to 600 in board	barrier (thick PA) is working
	no more than 1 in rice	
	from 30 to 200 in board (virgin)	virgin fibres = cleaner
	up to 1000 in plastic wrap	“sponge effect”
	max 5 in chocolate	jute bags?
	up to 500 in board and up to 2000 in plastic	“sponge effect”
	up to 6 in sweet snacks with chocolate	jute bags?
	from 300 to 600 in board	high contamination: barrier is not enough
	up to 30 in “kids cereals”	
	up to in board	high contamination with direct contact!
	up to 40 in egg pasta	

	up to 900 in board	through egg shell or feed contamination
	9 in eggs (some already present pre-pack)	
	62 in board (virgin fibres)	fish more contam. that breading
	14 in breading, up to 23 in fish (cod)	
	37 in board (virgin fibres)	good quality board & low T not protecting enough
	90 in plastic tray	
	9 in cake	
	284 board, 349 filter bag	mineral oil poorly extracted by hot H ₂ O
	44 in tea, dry leaves	
	1 in tea, drink	
	295 in paperboard	much higher if paper bag instead of plastic
	1 in cocoa powder	

Figure 25 shows the sorted contamination results for all analyzed paper-based packagings, divided into fresh fibres based and recycled fibres based.



safety limit: 4 ppm in board → < 0.6 ppm in food

Figure 25¹⁶³. Sorted results for MOSH contamination in paper-based food packaging made of fresh fibres (left), reaching up to ca. 2000 mg kg⁻¹, and recycled fibres (right), reaching up to nearly double the contamination compared to fresh fibres paper based materials.

¹⁶³ modified from Lorenzini *et al.*, 2010.

Recycled fibres show overall much higher contamination, due to the fact that mineral oil contamination from printing inks (sole contamination source in the case of fresh fibres paperboard) is summing up with mineral oil present in recycled paper material. The red line in both graphics roughly represents the paperboard contamination estimated safety threshold that should not be exceeded, in case the JECFA ADI limit (see paragraph 1.3.2) is enforced at legal level.

Finally, in Figures 26 and 27 some MOSH and MOAH chromatograms are reported, to introduce the subject of migration kinetics, core of the last part of present PhD work (see next paragraph). In Figure 26 the migration of the most volatile fraction of mineral oil from a contaminated paperboard towards its food content is illustrated, and in Figure 27 the loss of MOSH and MOAH over time from a contaminated paperboard is shown.

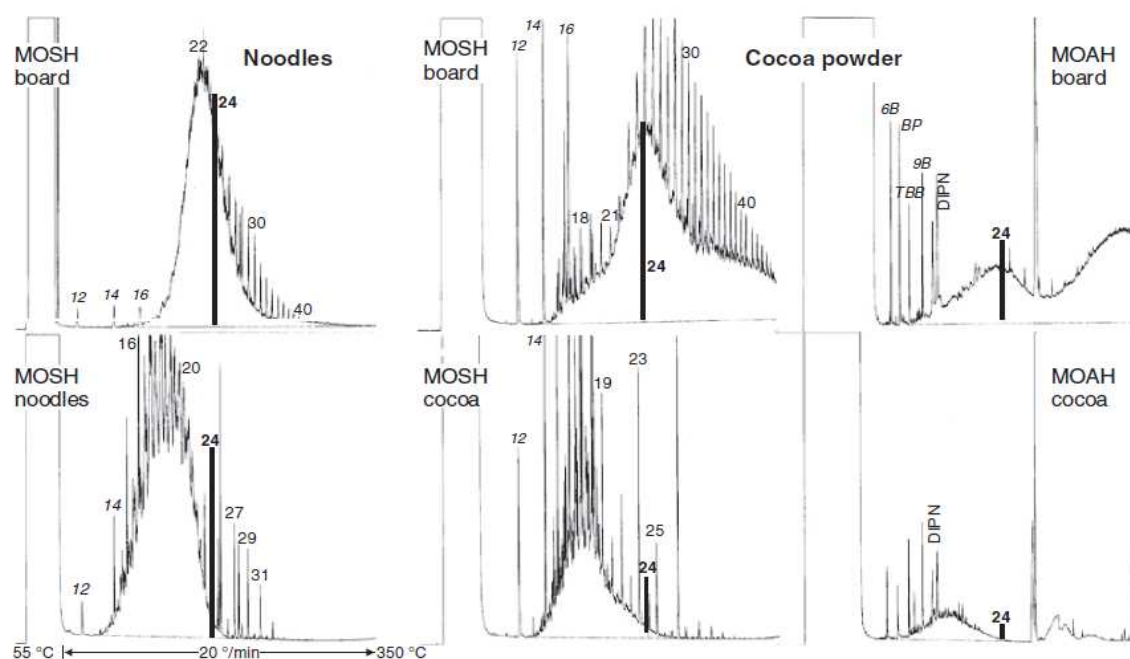


Figure 26¹⁶⁴. LC-GC/FID chromatograms of MOSH in noodles paperboard packaging (top left) and its food content (bottom left), and of MOSH and MOAH in cocoa powder paperboard packaging (top middle for MOSH, top right for MOAH) and its food content (bottom middle for MOSH, bottom right for MOAH). Paperboard chromatograms are not in scale with food chromatograms, whose contamination is much lower compared to packaging. It can be observed that only the most volatile part of the hump, positioned at the beginning of the paperboard chromatograms, is able to migrate to food: over C24 no significant migration occurs.

¹⁶⁴ from Lorenzini *et al.*, 2010.

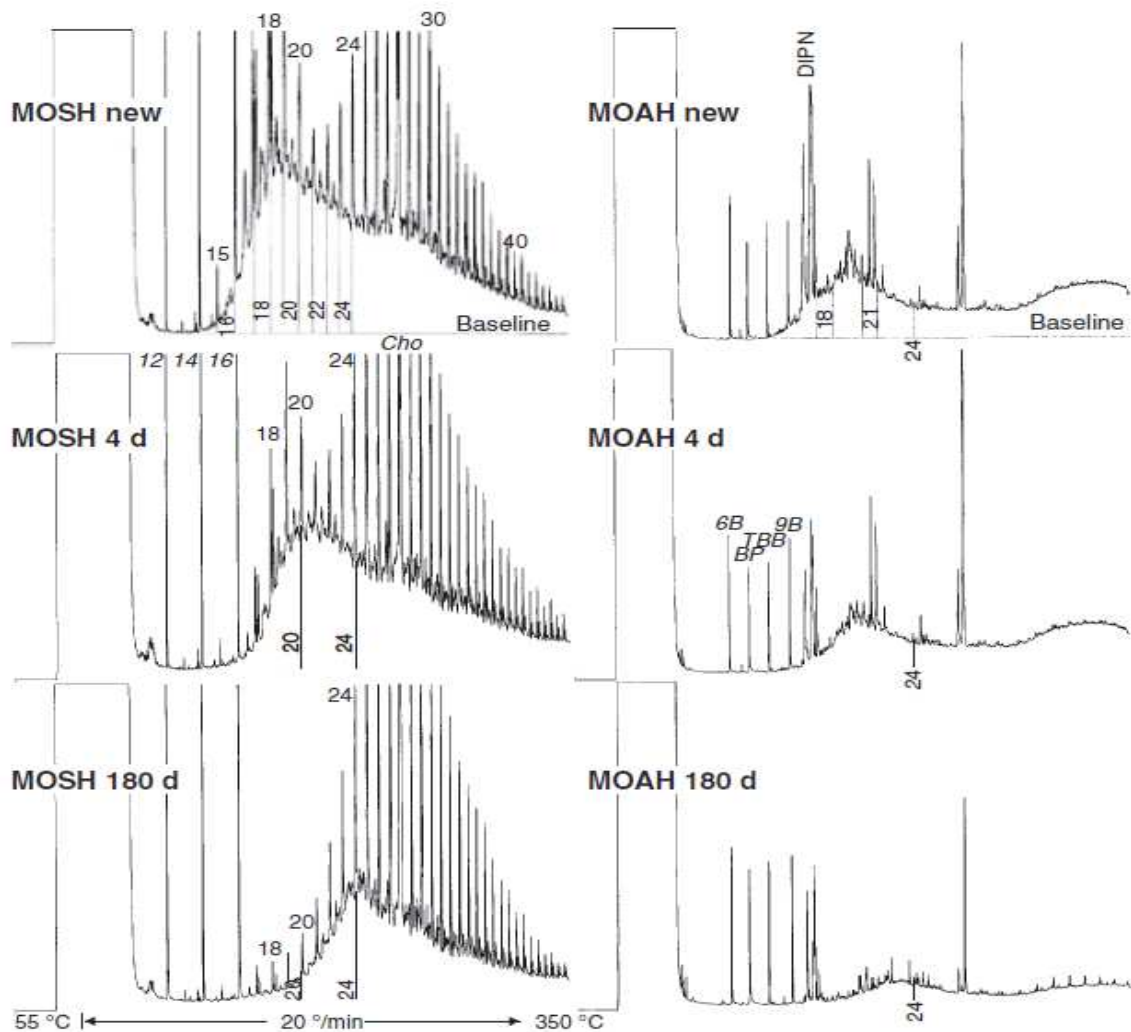


Figure 27¹⁶⁵. LC-GC/FID chromatograms of MOSH (left) and MOAH (right) contamination of an average paperboard made of recycled paper, left at the air for a certain amount of time. At time zero (top chromatograms) the presence of volatile contaminants, up to C24, is abundant. After only 4 d (middle chromatograms) such “hump” of volatile hydrocarbons has already diminished, and after 6 months (180 d, bottom chromatograms) has nearly disappeared.

4.3 MIGRATION STUDY

The “shopping trolley” survey gave important and interesting results about a range of paperboard packaged food products, giving an idea of consumers exposure to mineral oil contamination. However, the “age” of the boxes purchased at supermarket was not known, so data were missing on the migration stage of each product. In order to fill this knowledge gap, a detailed migration study was designed to follow up two food models during their

¹⁶⁵ from Lorenzini *et al.*, 2010.

entire shelf life, monitoring the influence of time, temperature, storage conditions and presence of a plastic barrier on the migration kinetics.

In previous studies it was concluded that the fraction corresponding to *n*-C24 is the upper end of the MOSH and MOAH with a significant potential to evaporate and migrate into food, so, again, only hydrocarbons up to this molecular weight are considered in this discussion.

4.3.1 MIGRATION KINETICS AT DIFFERENT TEMPERATURES

Müesli. At time zero, the contamination of printed paperboard made of recycled fibres was 16.8 mg of MOSH (corresponding to 442 mg kg⁻¹), 4.2 mg of MOAH (110 mg kg⁻¹), and 0.7 mg DIPN (19 mg kg⁻¹). Considering paperboard and food weight, the maximum theoretical contamination transferable to food is of 44.8 mg kg⁻¹ MOSH, 11.2 mg kg⁻¹ MOAH and 1.9 mg kg⁻¹ DIPN. All contaminants concentration decrease in paperboard as a function of time and temperature, being faster at higher temperatures, slower and incomplete at lower temperatures (Figure 28). Their migration kinetic from paperboard is linear for a long time (ca. 200 d), corresponding to over half of total transferrable contamination. Only when their residual content in paperboard is fairly small, migration pace decreases. Figure 28 also shows that DIPN contamination decrease in paperboard is considerably slower compared to MOAH and especially MOSH. This is probably because DIPN are added as ingredient of carbonless copy paper and thermal paper in an encapsulated form: their migration from paperboard is thus hindered, despite a high volatility according to GC retention time. This observation is confirmed by the longer extraction time needed for DIPN compared to MOAH of similar volatility (Lorenzini et al. 2010).

Half of the transferrable MOSH have already migrated to food in less than 5 d at 60°C and in ca. 2 and 8 months at 40 and 30°C, respectively (Figure 29). Migration is complete only for the highest temperature (60°C), after about 2 months. After 113 d (ca. 4 months), 63% of MOSH migration from paperboard to food was reached at 40°C, 48% at 30°C, 29% at 20°C and only 12% at 4°C. Migration kinetics of MOAH and DIPN were similar to those of MOAH, with an initial fast migration speed followed by a slower speed, a plateau or, in some cases, a decrease in contamination.

Egg pasta. At time zero, the contamination of unprinted paperboard made of recycled fibres was 6.7 mg MOSH (279 mg kg⁻¹), 1.9 mg MOAH (80 mg kg⁻¹) and an impressive 7.8 mg DIPN (327 mg kg⁻¹). Considering paperboard and food weight, these data lead to

the maximum theoretical contamination transferable to food of 26.8 mg kg⁻¹ MOSH, 7.6 mg kg⁻¹ MOAH and 31.2 mg kg⁻¹ DIPN. Figure 30 shows paperboard contamination decrease, which is not as fast as for müsli: this is probably due to the fact that egg pasta paperboard is wrapped by a plastic bag that hinders the volatilization toward atmospheric air, whereas in the müsli plastic bag is inside the paperboard. Also for the egg pasta can be noticed that DIPN contamination decrease in paperboard is considerably slower compared to that of MOSH. Despite a slower contamination decrease in paperboard compared to müsli, food contamination increase is extremely fast in egg pasta (see Figure 31). In this model, food is in direct contact with paperboard: migration is so fast that pasta at time zero (2 d travel from production plant immediately after production, to laboratory) already had a background contamination of 2.1 mg kg⁻¹, compared to the lower than LOD contamination level of blank egg pasta (no paperboard contact). As shown in Figure 31, half of the transferrable MOSH up to 24 carbon atoms is already migrated to food in less than 2 d at 60°C, ca. a week at 40°C and ca. a month at 30°C: this migration pace is extremely faster compared to müsli model. The internal plastic bag present in müsli slowed down the beginning of migration compared to egg pasta, which lacking this protective layer. Migration kinetics in egg pasta decreases at early stages for all temperatures (Figure 31). Migration in this food model is already complete after ca. 1 month at 60°C and ca. 3 months at 40°C. At lower temperatures migration resulted much slower and incomplete: 8 months were necessary to transfer to food half of MOSH at 20°C, and this value was never reached at 4°C, where even after more than 1 year only about 1/3 of transferable MOSH migrated to food.

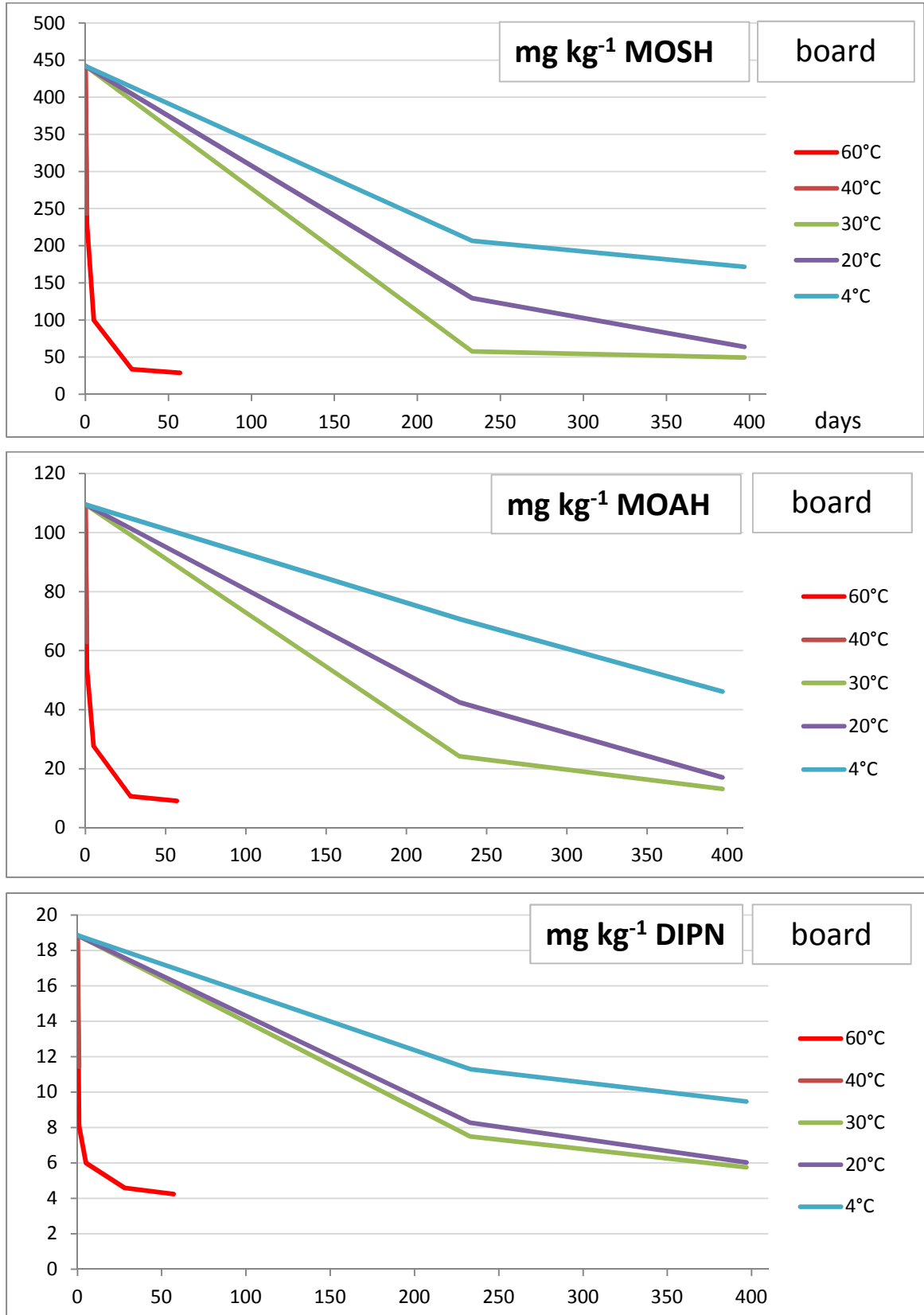


Figure 28. Contamination decrease in muesli paperboard as a function of time and temperature, which has a determining effect on the kinetics. DIPN decrease is slower due to the physical nature of these additives.

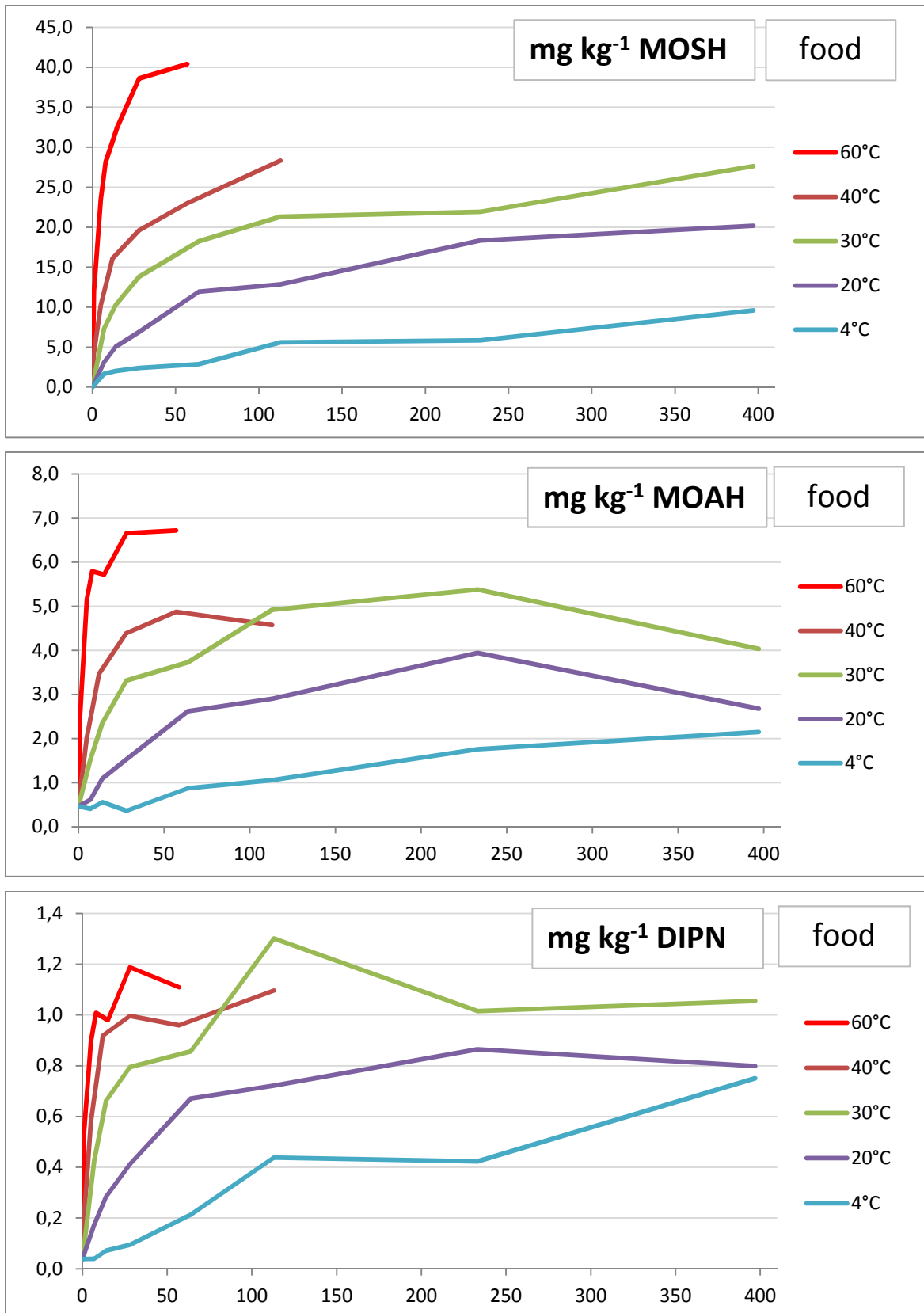


Figure 29. Contamination increase in muesli (food) as a function of time and temperature, which again has a determining effect on the kinetics. The migration is fast despite the protective plastic barrier present between paperboard and food.

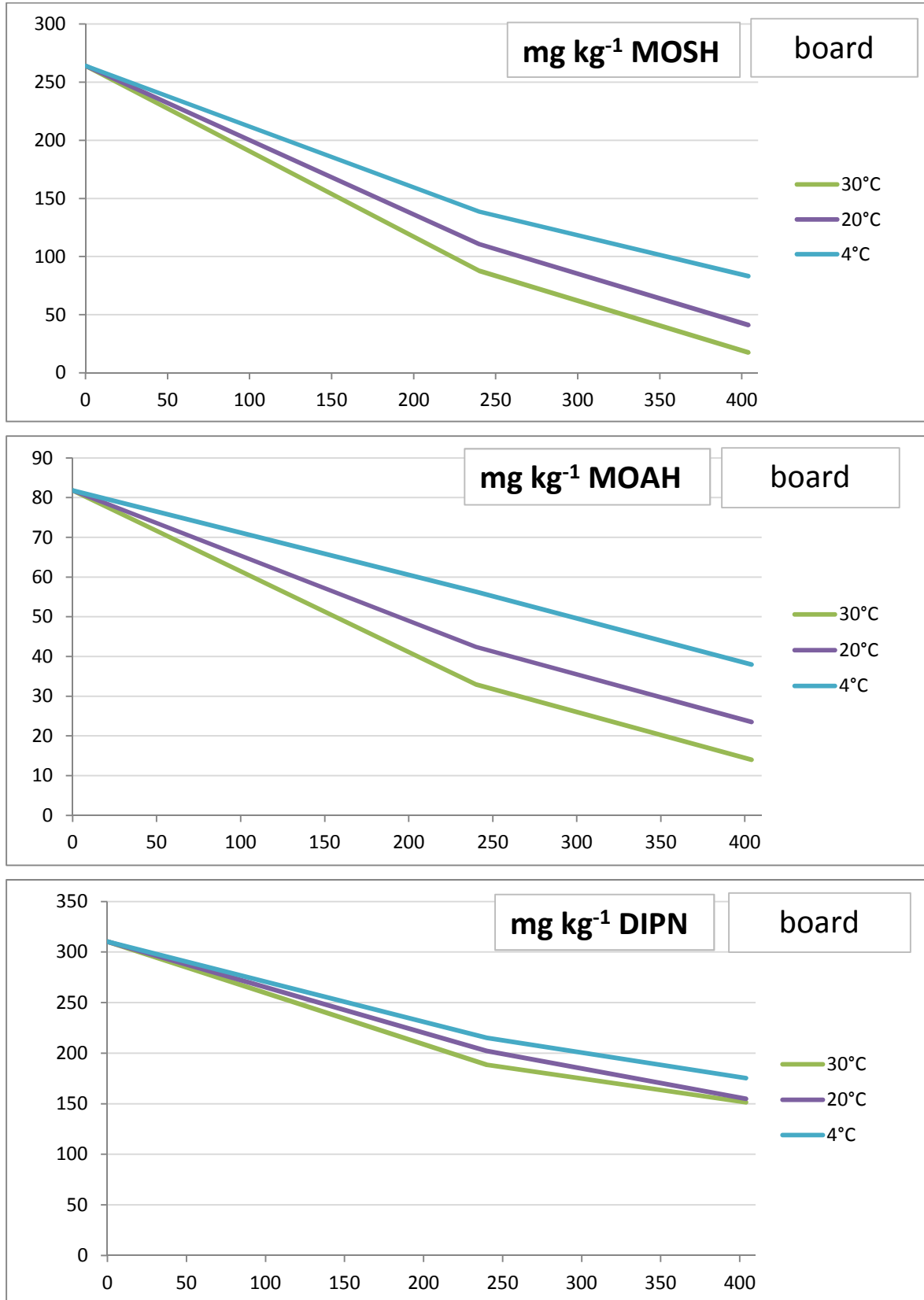


Figure 30. Contamination decrease in egg pasta paperboard as a function of time and temperature, which has a determining effect on the kinetics. DIPN decrease, as in müsli, is slower due to the physical nature of these additives.

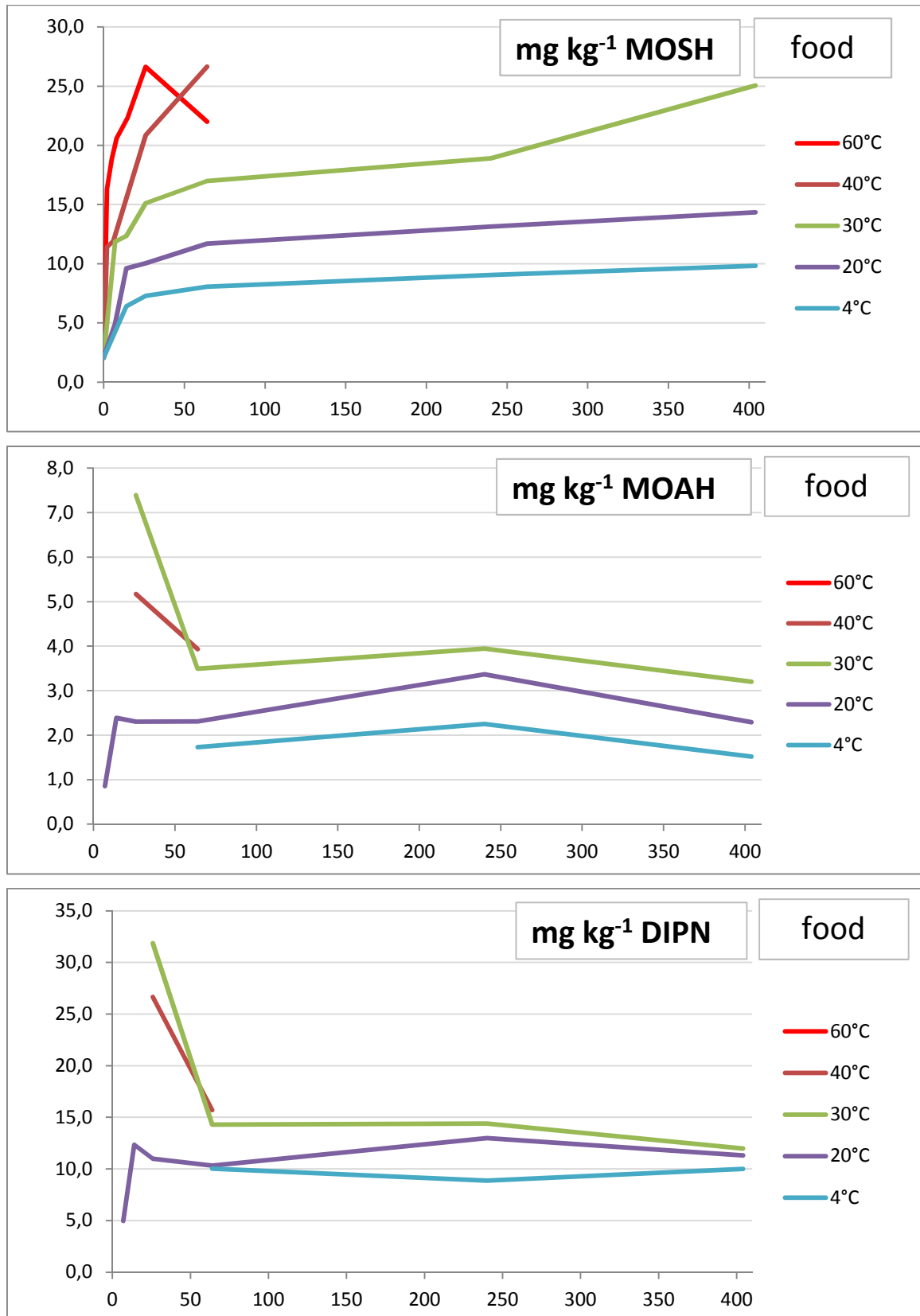
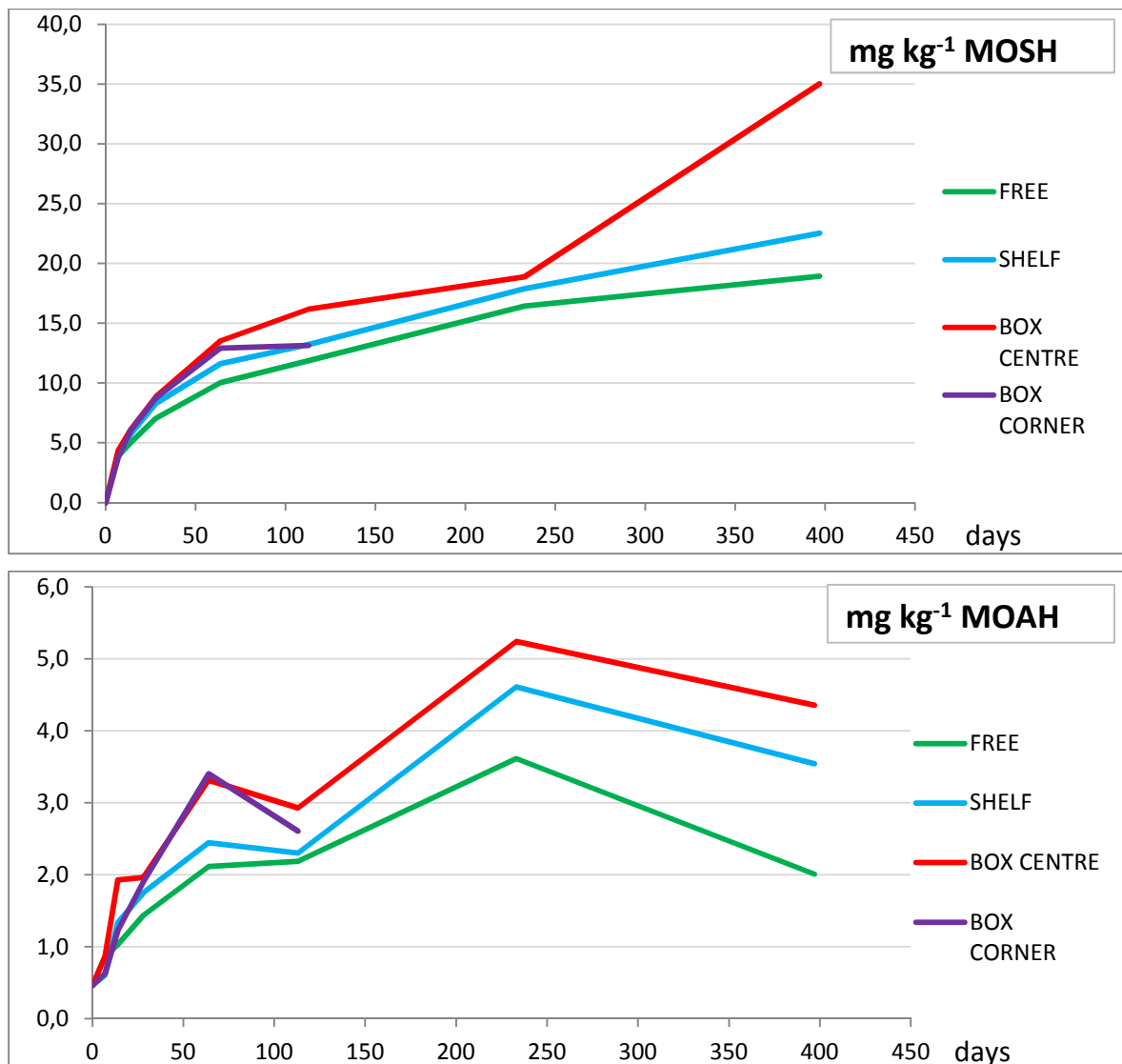


Figure 31. Contamination increase in egg pasta (food) as a function of time and temperature, which again has a determining effect on the kinetics. The migration is even faster than in müsli, and the initial “lag time” is absent because egg pasta is in direct contact with paperboard.

4.3.2 MIGRATION KINETICS AT DIFFERENT STORAGE CONDITIONS

Figure 32 shows increase of all considered contaminants (MOSH, MOAH and DIPN) in food (müesli) over time, at the tested storage conditions, all at RT: “free” packs (as in domestic storage), “shelved” packs (piled up as in supermarket storage) and “boxed” packs (inside corrugated board boxes, as in warehouse storage). Food contamination is maximum for packs situated at the centre of corrugated board boxes, and minimum for “free” packs, whereas “shelved” packs have an intermediate contamination level. MOSH and DIPN migration toward food of packs stored at the center of boxes (Figure 32) does not seem to reach a plateau: it continues to increase even after many months of storage, showing a very high long-term contamination. In egg pasta (data not shown), a sharper migration onset is noticed compared to müesli, with no “lag time”, as already observed in Figure 31.



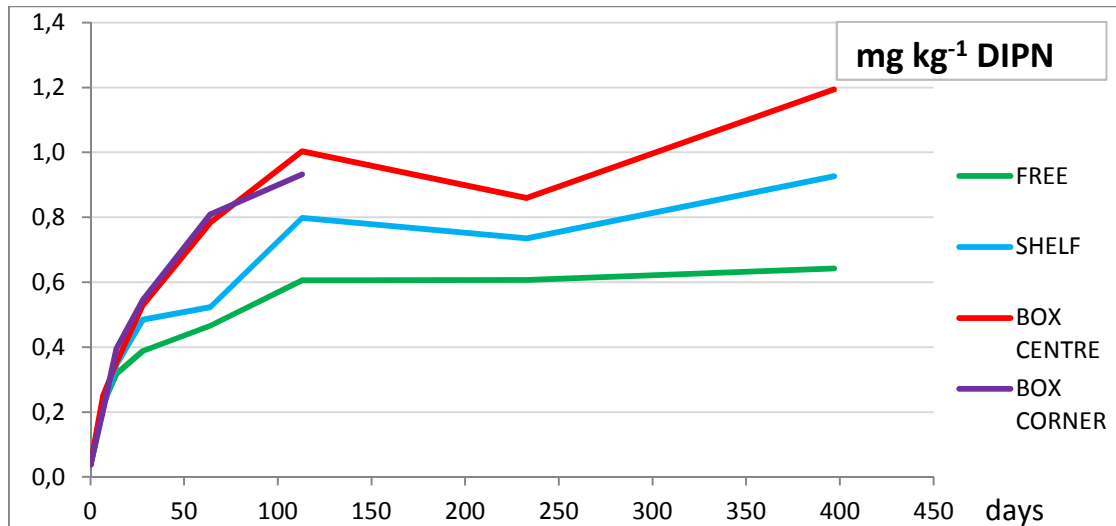


Figure 32. Müsli (food) contamination increase depending on the storage conditions. For all the three contaminants considered, the contamination is maximum for storage inside the corrugated board box, especially after a prolonged period of time.

4.3.3 ROLE OF PLASTIC PROTECTIVE BARRIER

In Figure 33, LC-GC/FID chromatograms of müsli MOSH contamination in paperboard, internal plastic bag and food after different times at 60°C are shown. At day 1 contamination of paperboard is massive compared to plastic and food, but at day 5 the most volatile MOSH fraction has been already relevantly transferred to food content. This process is even more evident at day 28 and day 57. The migration kinetics “lag time” observed in Figure 28 for müsli can be explained by the trapping power of the internal plastic bag towards contaminants: the most volatile fraction of them is accumulated into the plastic layer then later on released towards food content.

In Figure 34, end shelf life (ca. 1 year) müsli and egg pasta chromatograms at different temperatures are shown. It can be noticed that MOSH concentration in plastic bag (inside paperboard in müsli, outside it in egg pasta) is higher at 4°C compared to 20° and 30°C. Plastic layer thus acts as a “sponge”, especially at low temperatures, trapping part of contaminants. For müsli, the most volatile fraction of mineral oil is initially accumulated in plastic and then released towards the food; this is probably the main factor creating a “lag time” in food contamination kinetics for foods protected by a polyolefinic plastic bag. The same phenomenon can be observed also for MOAH and DIPN.

The typical POSH pattern (regularly spaced peaks representing oligomers groups) can be observed, particularly in egg pasta chromatograms.

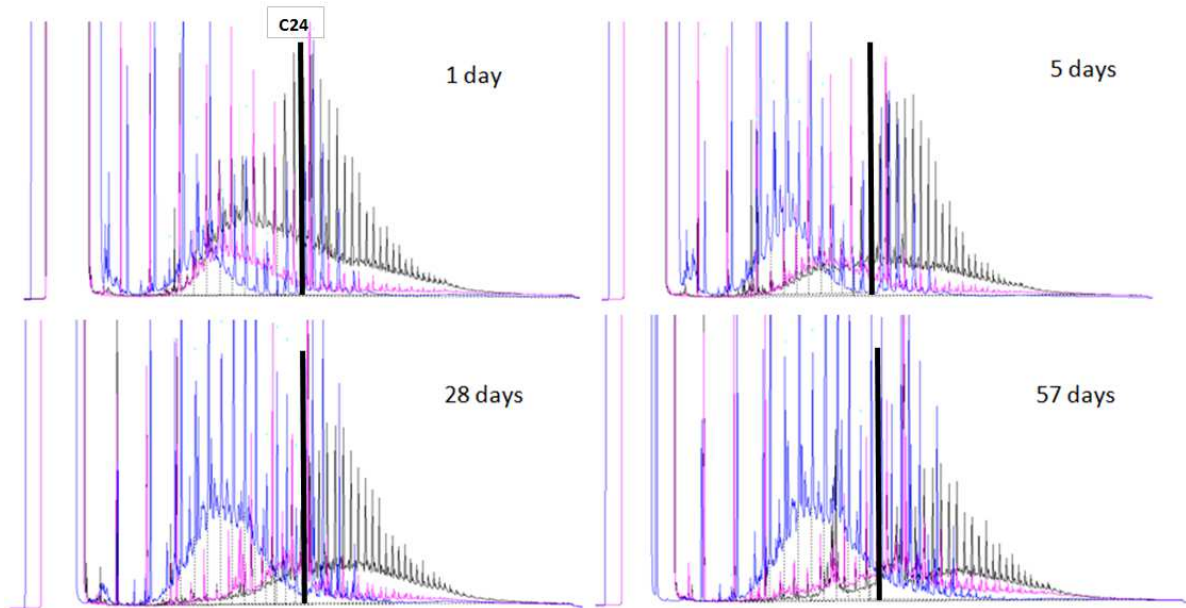


Figure 33. LC-GC/FID chromatograms of müsli paperboard MOSH contamination at 60°C.

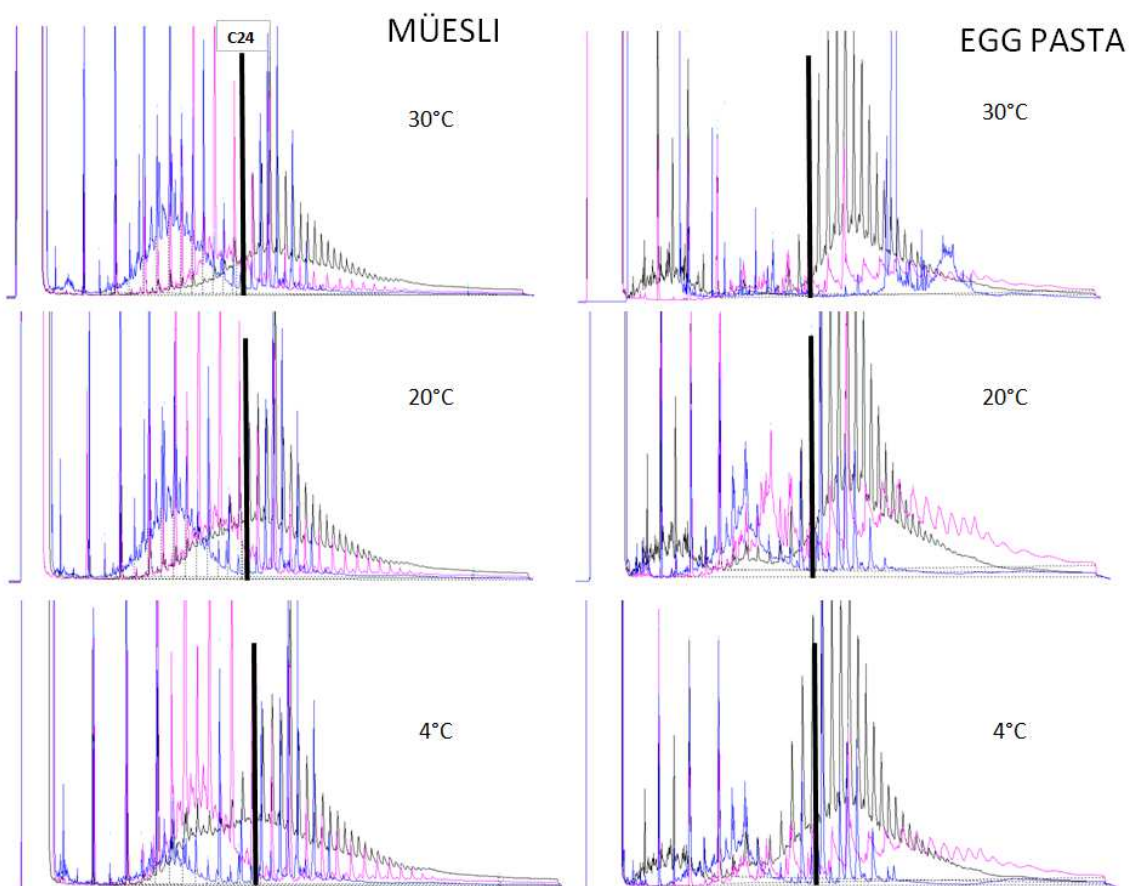


Figure 34. LC-GC/FID chromatograms at end shelf life (ca. 1 year) for müsli and egg pasta.

4.3.4 MIGRATION PATTERN OF DIFFERENT MOSH FRACTIONS

Muesli MOSH chromatograms (food) have been integrated, as shown in Figures 33 and 34, quantifying every single C atom fraction, in order to monitor the relative abundance of every fraction at different times and temperature. Figures 36 and 37 show, with two different graphical representations, such relative abundances at different temperatures. With an absolute abundance graph (as shown in figure 35) it is more difficult to compare migrating fractions. Low MW hydrocarbons of the transferable fraction (up to 24 carbon atoms) migrate from the very first days and are then rapidly depleted, whereas higher molecular weight hydrocarbons of the transferable fraction only migrate significantly at higher time and temperature conditions. This different behaviour is evident especially at high temperature storage (60 and 40°C), but is much less noticeable at 20°C (RT, and normal storage temperature for most paperboard packaged foods). Using high temperature as a mean to obtain fast food packaging safety assessment results through accelerated migration, is therefore not viable for paperboard packaging: such approach would lead to an overestimation of migration because higher MW hydrocarbons, poorly volatile at RT, would migrate to food at higher temperatures, altering the results. Nevertheless, a simple and fast method for a paper based packaging safety assessment consists in testing the paper material and establishing its mineral oil content. On the result, the worst case scenario estimate of 70% volatile MOSH and MOAH (<C24) able to migrate from that packaging to food content, if no effective functional barriers are present, could be applied¹⁶⁶.

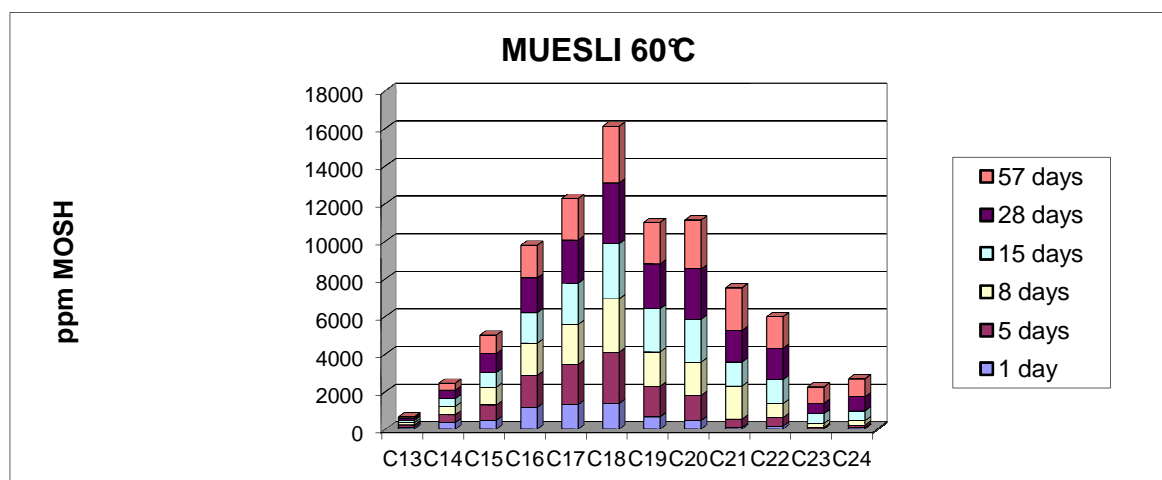
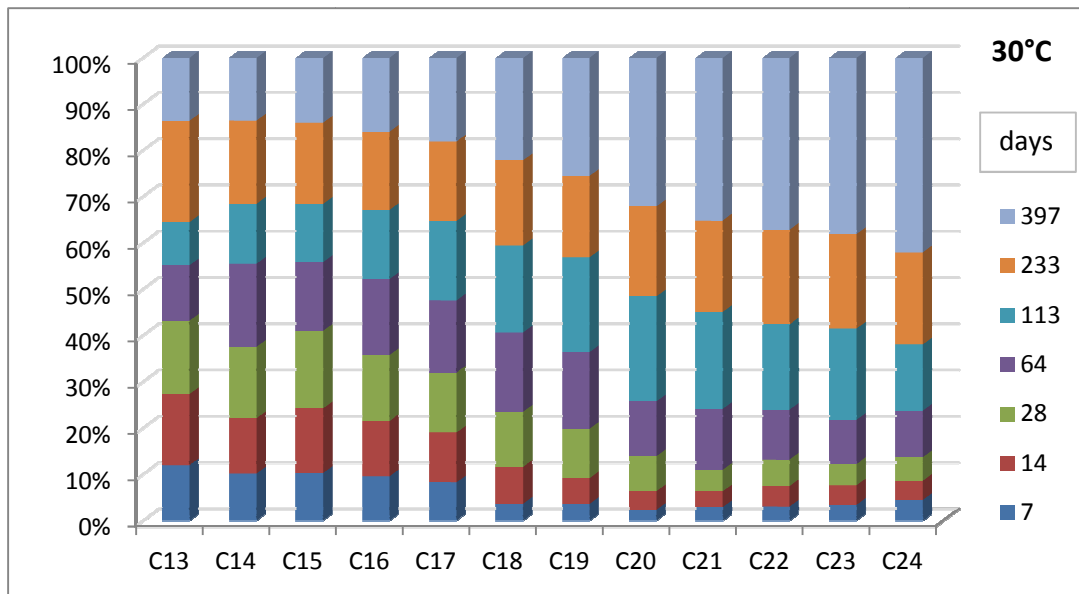
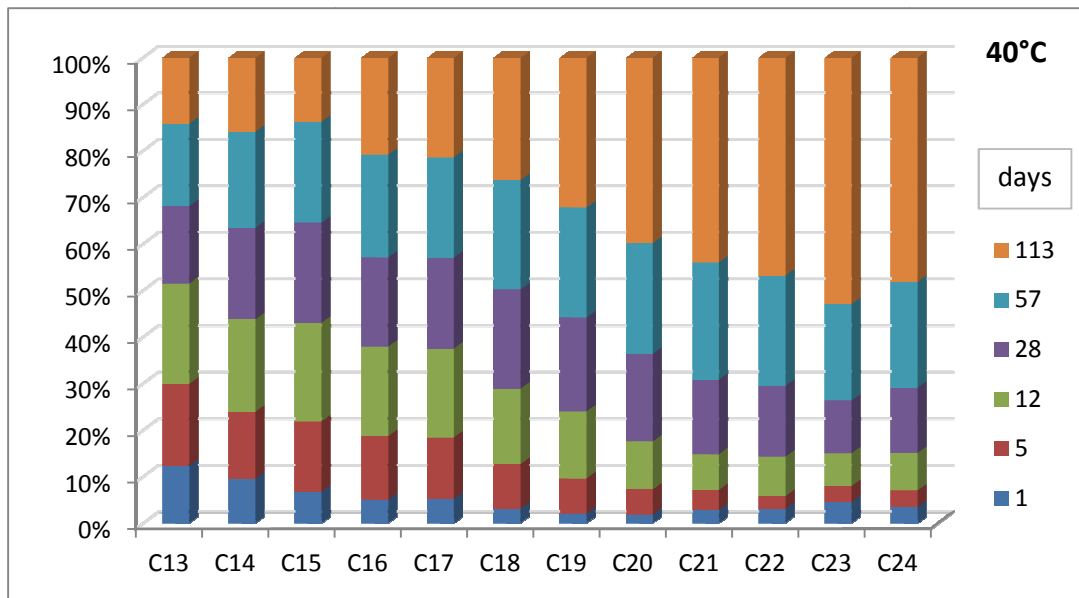
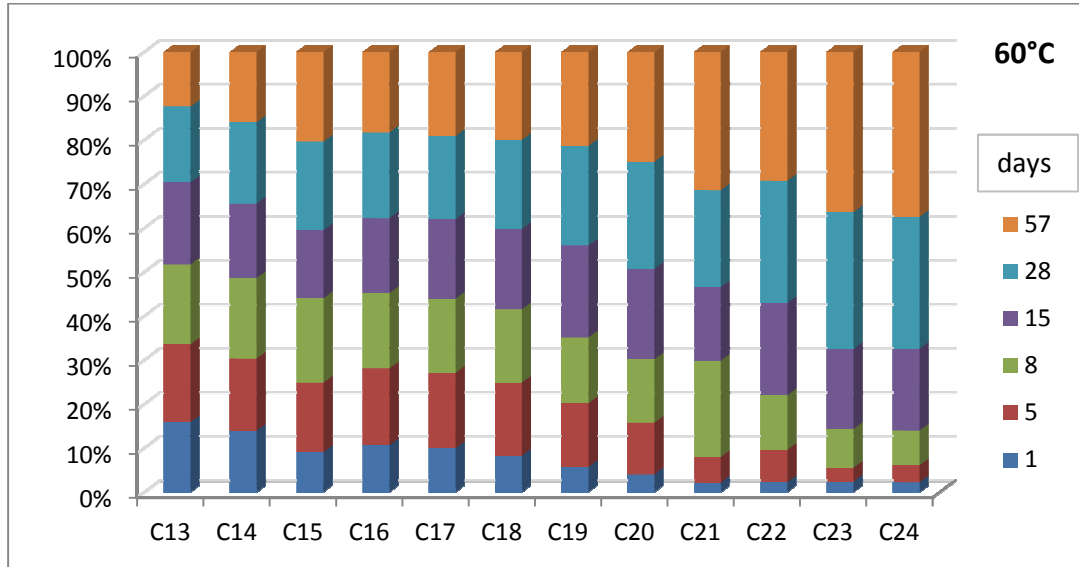


Figure 35. Example of absolute abundance representation of MOSH fractions.

¹⁶⁶ Lorenzini *et al.*, 2010.



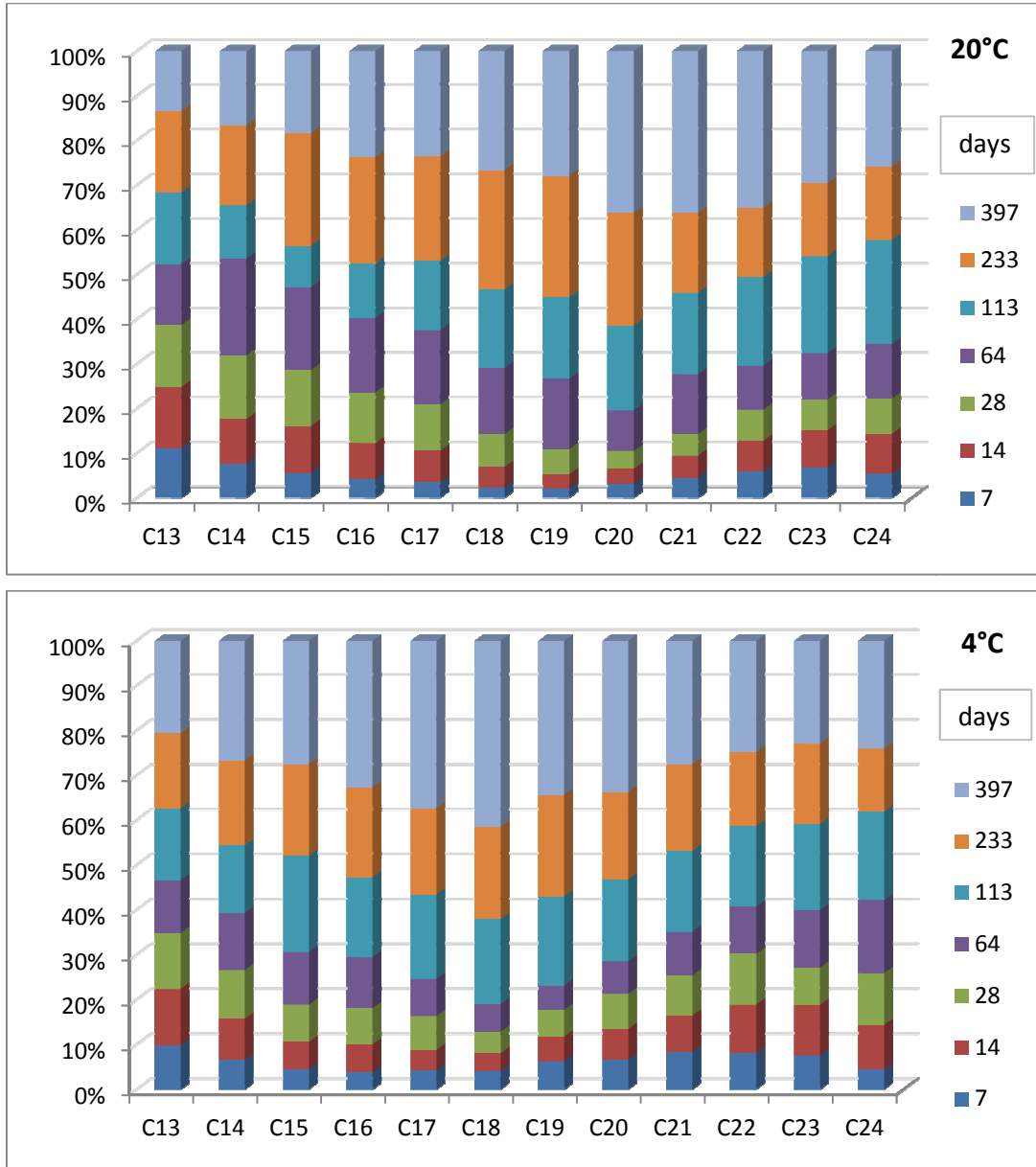
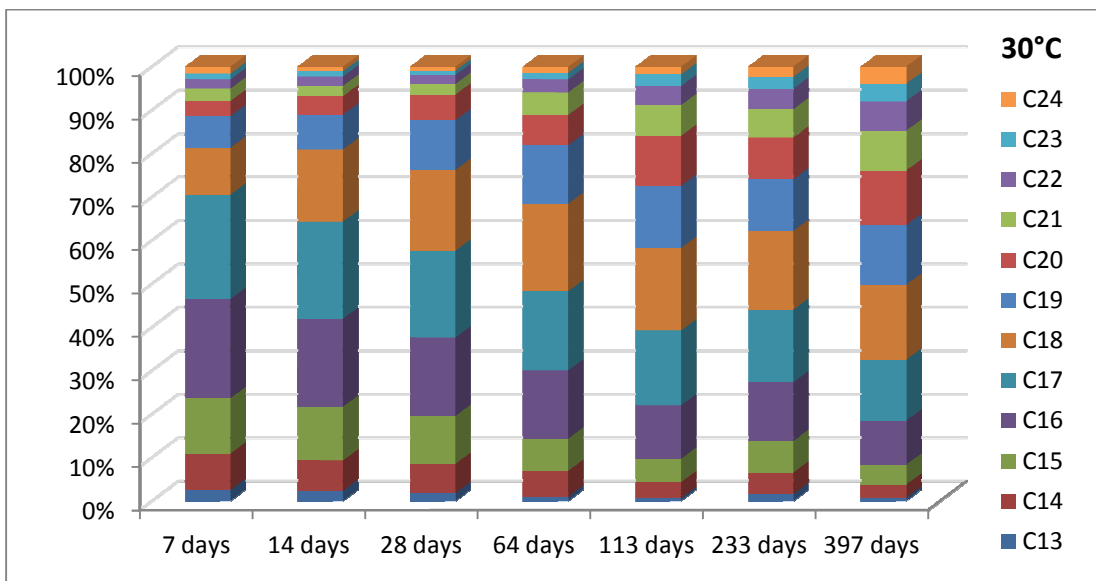
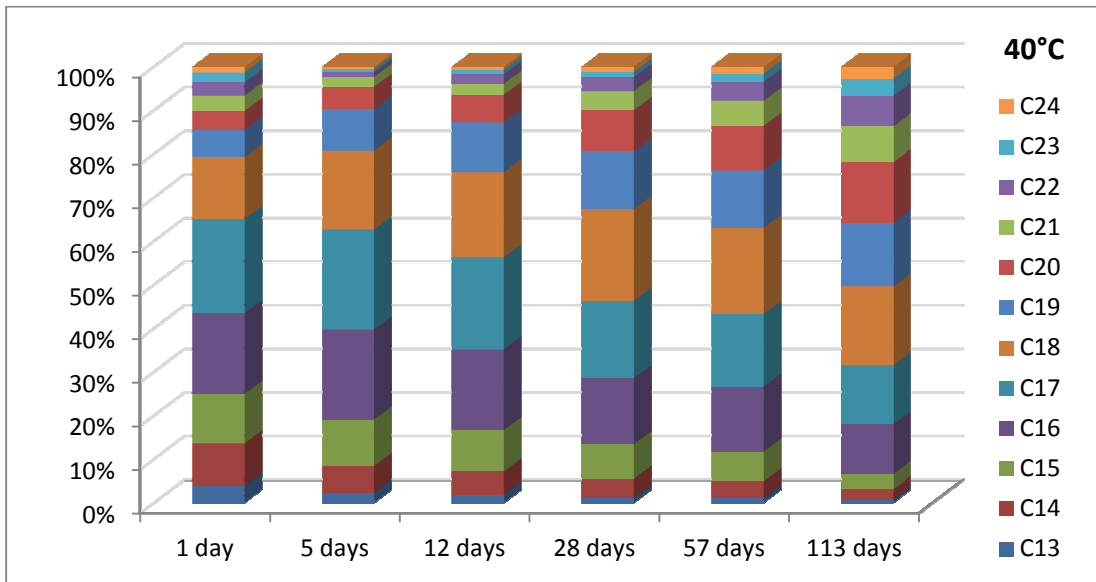
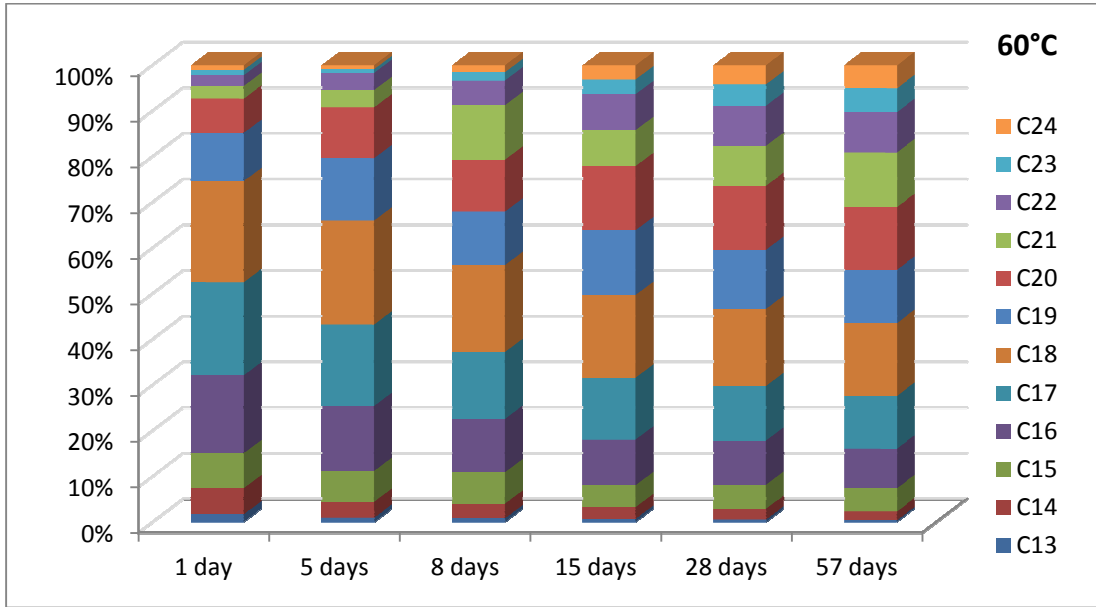


Figure 36. Muesli MOSH fractions migrating over time at different temperatures. At 60°C (1st graph) it is evident that very volatile hydrocarbons, roughly from C13 to C17, massively migrate during the first days. On the contrary, less volatile hydrocarbons, roughly from C20 to C24, migrate significantly after some weeks. This effect is evident only for accelerated migration temperatures (60 and 40°C), and to a lesser extent for 30°C, but cannot be observed for 20 and 4°C temperatures.



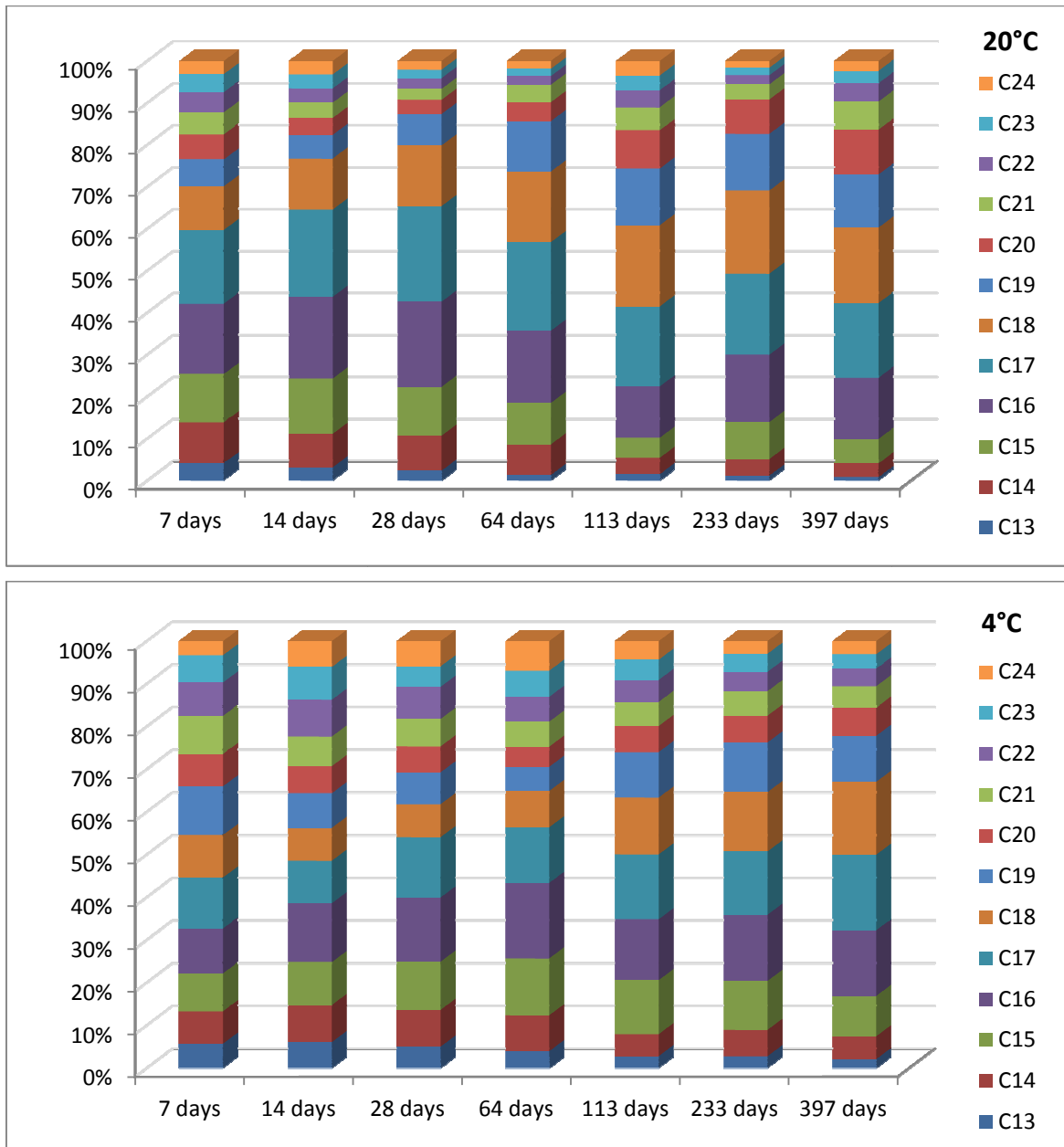


Figure 37. Muesli MOSH fractions migrating over time at different temperatures, as shown in Figure 36, but with a different representation.

5. CONCLUSIONS

Despite being commonly perceived and “natural” and safe, paper based packaging can contain a variety of contaminants, especially if made up with recycled materials. Some of these contaminants have sufficient volatility to migrate from the packaging to food content (even if dry) through the gas phase, with the evaporation-recondensation mechanism. Food particularly prone to contamination from packaging are those with high superficial area and in direct contact with the board (no protecting barrier between paperboard and food).

Mineral oil is a product of petroleum, widely used for many industrial applications. Its presence as food contaminant is not uncommon, and migration from packaging is just one of the possible sources. The main cause of mineral oil presence in paperboard packaging is offset printing inks, often used to print paperboard boxes. Furthermore, mineral oil is used also to print books, magazines and newspaper, therefore recycled paper has high mineral oil contamination levels, among other contaminants as di-isopropyl naphthalenes (DIPN), phthalates and other plasticizers, printing ink photoinitiators, etc. Mineral oil is mainly constituted by saturated hydrocarbons (MOSH), but it can contain up to 30% aromatic hydrocarbons (MOAH), more toxic. A conclusive toxicological evaluation of MOSH and MOAH is still on the way (due to the complexity of hydrocarbon mixtures they are formed by), as is the European legislation on paper based packaging materials. Based on the temporary Acceptable Daily Intake (ADI) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the presence of mineral oil should not exceed 0.6 mg kg^{-1} of food. This evaluation was based on white mineral oils, refined to eliminate

MOAH, whereas the mineral oils in recycled board and most printing inks are of technical grade, thus also contain MOAH.

In the first part of this work, extractive and analytical methods to determine simultaneously MOSH, MOAH and DIPN have been optimized, both for packaging (paper based and plastic) and food. These methods have proven simple and effective, thus ideal for the everyday routine and for a high throughput results laboratory, allowing the consecutive injection of tens of samples without deterioration of chromatographic quality. Furthermore, the same extract can be analyzed by liquid chromatography coupled to gas chromatography with flame ionization detector (LC-GC/FID) for MOSH, MOAH and DIPN and by gas chromatography with mass spectrometry detector (GC/MS) for many other contaminants, making optimal use of one simple extraction.

The optimized extraction methods were then applied to the analysis (packaging and food content) of over 100 products from Italian and Swiss market packed in paper based materials. These products were chosen to represent the average “shopping trolley” of customers, with pasta, rice, flour, breakfast cereals, sweet and savoury snacks, eggs, chocolate, tea, frozen food, etc. On the basis of mineral oil contamination found during this survey, the limit in food derived from the JECFA ADI is commonly exceeded tens or even hundreds of times, therefore this “shopping trolley” survey gave valuable data to have a picture of the mineral oil food contamination level to which consumers are exposed.

The age of products at the moment of purchase was unknown, therefore it was impossible to know at which point the migration from packaging to food was. For producers as well as enforcement authorities it is important to predict long term migration from paperboard into foods (many products have shelf lives of 1-3 years). With the second part of this work we tried investigated more deeply into this subject, designing a systematic migration plan to take into account variables such as time, temperature, storage conditions and packaging structure, in order to monitor the migration kinetics. Two representative food models have been chosen, both with high surface to weight ratio: egg pasta in direct contact with paperboard, and breakfast cereals protected by a polyolefinic bag. Results of this migration study show that migration of mineral oil from packaging paperboard to food content is a fast process, mostly influenced by temperature. In absence of a protecting barrier between paperboard and food, e.g. a plastic bag, half of MOSH up to 24 C atoms is transferred to food in about a month at 30°C (not uncommon in summertime in many warehouse storage facilities), and even in weeks higher temperatures as 40 and 60°C (accelerated migration). At those temperatures, higher molecular weight mineral oil fractions migrate to food,

which would migrate much less and slowly at room temperature. This phenomenon causes a misrepresentation of the real migration pattern, making it unadvisable to apply the accelerated migration temperatures to obtain quick laboratory tests results, as routinely done for other materials (e.g. plastics).

The role of a plastic barrier between paperboard and food is controversial, because its protective role depends on its composition and thickness. The most common bags are made of polyolefinic plastics such as PE and PP, with high chemical affinity towards mineral oil. Therefore they act as a sponge, absorbing mineral oil and thus releasing it towards food after diffusion equilibrium is reached, causing a slower contamination onset in food (a sort of “lag time”) compared to foods in direct contact with paperboard.

Some advice for food packaging producers and final users can thus be drawn. When paper based materials are chosen for food packaging, a risk assessment has to be performed, taking into consideration food characteristics, package characteristics and size, presence of an internal protective barrier, storage temperature, length of shelf life, etc. Being temperature the main variable conditioning migration speed and magnitude, different considerations are needed e.g. for frozen foods (often paperboard packed) compared to room temperature stored foods: the latter pose higher migration risk. For foods more sensitive to migration of volatile contaminants (i.e. foods with high surface to weight ratio, in small packets where the food amount is little compared to packaging, foods highly porous and rich in lipids, with unrefrigerated and prolonged storage, etc.), the packaging should consist of fresh fibres printed with mineral oil free inks. Alternatively, an efficient protective barrier should be placed between paperboard and food (i.e. aluminum, PET, PA, etc.). If a polyolefinic plastic barrier is used, the protection is only temporary (depending on plastic thickness), therefore shelf life should be reduced according to migration kinetic studies. As a general consideration for paperboard food packaging, a more widespread risk assessment approach is advisable.

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