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MIGRATION OF ORGANIC SUBSTANCES THROUGH BIO-COATED PAPER AND BOARD FOR FOOD PACKAGING

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Dove è amore e sapienza, ivi non è timore né ignoranza.
Dove è pazienza e umiltà, ivi non è ira né turbamento.

(San Francesco, FF 177)

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ABSTRACT

Migration of organic substances through Bio-Coated Paper and Board for Food Packaging

This PhD thesis is aimed to generate data on migration of typical contaminants from Paper and Board (P&B) packaging into food and on the effectiveness of bio-based polymers coated onto paper as barrier materials. In the first part of the project, an analytical survey of P&B materials intended for food use was carried out with the aim to identify chemicals with a potential to migrate into foods. A screening was applied by means of Solid Phase Micro Extraction (SPME) and solvent extraction (SE) with subsequent analysis by Gas Chromatography-Mass Spectrometry (GC-MS) to determine volatile and non-volatile molecules. A large number of analytes were detected and a chemometric approach was used to explore the data. PCA (Principal Component Analysis) was used to identify and select some compounds as markers for sample classification. The chosen analytical method coupled with chemometrics proved to be an effective way in processing these data. A literature survey for safety data or legislative restrictions of the identified substances was performed. The semi-quantification of the compounds in the packaging allowed a worst case estimation of food contamination by means of the infinite total migration model; occasionally, migration estimations overcame the specific migration limits. Additionally, a study to investigate the occurrence of diisobutyl phthalate (DiBP) in polyvinyl chloride (PVC) cling films for food contact applications was carried out. It demonstrated the contamination pathway from the secondary paper packaging (contaminated materials, such as folding cardboards and inner cores made of recycled fibres) used for distribution and storage of these primary plastic packaging that will be in contact with food.

In the second part of the project, coatings of different biopolymers onto paper substrates were developed and characterized. Focus was directed to water-based, renewable biopolymers, such as: modified starches (cationic starch and cationic waxy starch), plant and animal proteins (gluten and gelatine), poured onto paper with an automatic applicator. Optical contact angle measurements and microstructural observations of the bio-coated paper allowed the characterization of the samples. At the same time, partition and diffusion studies of selected substances of toxicological concern were carried out between paper/coated paper and air or food simulants, additionally, a comparison with a polyethylene laminated paper was performed. The aim was to evaluate the physicochemical behaviour and the barrier properties of bio-coatings against migration of typical contaminants from recycled paper packaging. From the partitioning studies, considerable differences in the adsorption behaviour of the selected contaminants between bio-coated or uncoated paper and air were highlighted. Lowest values of partition coefficients were achieved when paper was bio-coated, making evident that biopolymers reduced the affinity of the paper substrate for the tested migrants. These findings were discussed considering the characteristics of the tested biopolymers. Diffusion studies into the solid food simulant poly 2,6-diphenyl-p-phenylene oxide, also known as Tenax®, confirmed that all the tested biopolymers slowed down migration. The Weibull kinetic model was fit to the experimental data to compare migration from paper and bio-coated paper.

Finally, research activity was focused on the migration of contaminants like MOSH (mineral oil saturated hydrocarbons) and on the evaluation of starch based bio-coatings as barrier materials. Migration test series were performed up to 10 days at 60 °C using spiked model substances (n-alkanes C_{10}-C_{40}) and Tenax® as food simulant. HPLC-GC-FID system was used to analyse extracts and its relative performances were compared with an automatic permeation system. Existing predictive models for migration were preliminary applied for comparison with measured data. Finally, migration test series with real contaminated packaging materials were developed.

Migrazione di sostanze organiche attraverso rivestimenti bio-polimerici da imballaggi in carta e cartone per uso alimentare

Obiettivi perseguiti durante il presente dottorato di ricerca sono stati: l'ottenimento di dati sulla migrazione dei contaminanti tipicamente presenti in imballaggi di carta e cartone negli alimenti e la valutazione dell'efficacia di bio-polimeri deposti su carta come materiali barriera. Nella prima parte del progetto, è stata eseguita un'indagine analitica di materiali cartacei per uso alimentare allo scopo di identificare le sostanze chimiche più comuni con un potenziale di trasferimento verso gli alimenti. Tali materiali sono stati selezionati in considerazione di una vasta gamma di utilizzi (dal confezionamento primario e secondario agli articoli per fast food). È stato condotto uno screening analitico mediante tecniche di micro estrazione in fase solida (SPME) ed estrazione con solvente (SE) entrambe seguite da successiva analisi mediante gascromatografia-spettrometria di massa (GC-MS) al fine di determinare le principali molecole volatili e semi-volatili in essi presenti. Un gran numero di analiti sono stati rilevati, conseguentemente, per analizzare tali dati, è stato utilizzato un approccio di tipo chemiometrico. Attraverso l'analisi delle componenti principali (PCA) sono stati identificati e selezionati alcuni composti come marker per la classificazione dei campioni. Il metodo analitico utilizzato, combinato con l'approccio chemiometrico, si è dimostrato essere efficace per la trattazione di tali dati. In seguito è stata eseguita una ricerca documentale dedicata a proprietà tossicologiche o restrizioni legislative delle sostanze individuate. La semi-quantificazione dei composti negli imballaggi, ha consentito, mediante l'applicazione del modello di migrazione totale a tempo infinito, una stima della contaminazione di alimenti in condizioni limite; occasionalmente, tali stime portano al superamento dei limiti di migrazione previsti dalla legge. In aggiunta, è stato condotto uno studio sulla presenza di di-isobutile ftalato (DiBP) in film di polivinil cloruruo (PVC) destinato al contatto alimentare. Si è dimostrato come l'origine della contaminazione di tale imballaggio plastico primario per alimenti, fosse costituita dall'imballaggio secondario in cartone (astucci e mandrini in fibra riciclata) utilizzato per la distribuzione e lo stoccaggio di tali tipologie di materiali.

Nella seconda parte della ricerca, sono stati sviluppati e caratterizzati rivestimenti a base di biopolimeri applicati su substrati di carta. In particolare, sono stati considerati rivestimenti a base acquosa, costituititi da biopolimeri rinnovabili, tra i quali: amidi modificati (waxy e ad alto contenuto di amilosio), proteine animali (glutine e gelatina). La caratterizzazione dei campioni di carta bio-rivestita è stata condotta mediante misure di angolo di contatto e osservazioni microstrutturali. Allo stesso tempo, sono stati realizzati studi di ripartizione e diffusione di molecole selezionate tra carta o carta bio-rivestita e aria o simulanti alimentari, inoltre; quale confronto, sono stati considerati diversi rivestimenti plastici. Notevoli differenze sono state evidenziate nell'adsorbimento dei contaminanti tra carta bio-rivestita o non rivestita e aria. I minori coefficienti di ripartizione sono stati raggiunti nella carta bio-rivestita, rendendo evidente come i biopolimeri testati siano stati capaci di ridurre l'affinità del substrato carta nei confronti di tali contaminanti. Tali risultati sono stati discussi in relazione delle caratteristiche proprie di ogni biopolimero. Studi di diffusione nel simulante alimentare solido poli 2,6-difenil-p-fenilene ossido, noto anche come Tenax®, hanno confermato che tutti i biopolimeri testati rallentano la migrazione. I dati sperimentali sono stati interpretati mediante modello di Weibull.

L'ultima parte dell'attività di ricerca è stata dedicata alla migrazione di composti similari agli oli minerali ed alla valutazione dell'efficacia di rivestimenti a base amido su carta come materiali barriera. La cinetica di migrazione di composti modello (n-alcani: C_{10}-C_{40}) in Tenax® è stata studiata sia mediante HPLC-GC-FID che un sistema di misurazione della permeazione in continuo. L'applicazione di modelli attualmente disponibili ha permesso il confronto con i dati misurati sperimentalmente. Infine, sono stati condotti test di migrazione utilizzando materiali realmente contaminati da oli minerali.

0. PREFACE

This PhD project focused on: *i)* the understanding of migration phenomenon of chemicals from recycled Paper and Board (P&B) packaging into food; *ii)* the evaluation of bio-coatings as barrier materials, pursuing a "safe and sustainable" approach.
Nowadays, P&B represent a large sector of the food and drink packaging market; they originate from natural sources and are thus regarded as environmentally friendly and perceived by consumers as safe. However, printing inks and recycled paper are likely to contain harmful substances that could give raise to migration and endanger human health.
An immediate solution to improve the safety of this kind of food packaging can be seen in the use of a functional barrier which is a layer placed between food and contaminated material which reduces the migration from any layer beyond.
The increasing worldwide interest in bio-macromolecules as polymer materials led to the idea that their applications as coatings on P&B can be an interesting way to optimize the current packaging solutions. The application of a biopolymer coating has the potential to replace synthetic coatings of oil origin, achieving the overall performance of the packaging structure in terms of protective barrier to chemicals contamination; moreover, the existing coating formulation can be exploited and, finally, a "safer and greener" packaging structure can be obtained.
To achieve these goals, target migrating substances have been chosen by means of an analytical screening of P&B packaging, afterwards the development and testing of bio-based coating materials has been undertaken with the overall goal of improving the performance of the final packaging, in particular the barrier against migration of harmful substances from recycled P&B. At the same time, bio-coatings were characterized with suitable techniques, in order to find out a linkage between their performances and physicochemical properties; among which microscopic morphology deserve to be mentioned.
Summarizing, the development of the project has been done following three main steps:

1. Phase 1: Screening for chemical contaminants in P&B
An analytical survey of P&B materials intended for food use was carried out by GC-MS techniques with the aim to identify and quantify volatiles and non-volatiles chemicals with a potential to migrate into foods. Risk evaluation was performed and PCA was used to select some compounds as markers for further migration studies.

2. Phase 2: Bio-based coatings development and migration testing
Different biopolymers were tested on the basis of their attitude to form coatings onto paper and to behave as barrier materials against migration of selected chemicals. In addition to some analyses made to characterize the final bio-coated paper; a relevant part of the work was devoted to test the coatings barrier properties against migration, by means of partition and diffusion experiments with selected spiking substances and food simulants at different time/temperature conditions.

3. Phase 3: Barrier properties of starch-coated paper
Physical and chemical characteristics of starch based coatings were specifically considered and linked to their obtained best performances in terms of barrier to migration. Research was focused on the optimization of the formulation to gain functional barrier properties against mineral oil compounds (still on-going research).

1. STATE OF THE ART

P&B is the oldest and most versatile packaging materials available on the market today. It has a long and successful history of use in the food industry in a wide range of applications. These include applications where intimate contact with food is involved, such as tea bags, baking papers, and filters, and direct contact packaging such as butter wrapping, sugar bags, and cartons for dry and frozen foods. In addition it has a very wide range of uses in transport and distribution packaging.

In 2012, the Confederation of European Paper Industries (CEPI) member countries produced 94 million tonnes of paper and board, 38 million tonnes of pulp, and used 43 million tonnes of recovered paper (CEPI, 2013).
During the same year, the CEPI member countries produced:

- 24.9 million tonnes of case materials,
- 8.3 million tonnes of carton boards,
- 3.8 million tonnes of other paper for packaging,
- 3.9 million tonnes of wrappings.

The use of recovered paper and board by sector is:

- 23.5 million tonnes of recovered paper used in “case materials sector”,
- 3.6 million tonnes of recovered paper used in “carton boards sector”,
- 4 million tonnes of recovered paper used in “other paper for packaging/wrapping sector”.

P&B production involves two steps. First, the fibres need to be produced. This is done in a pulp mill where pulp is produced using chemical or/and mechanical processes. Pulp production can be integrated with paper production, or the pulp can be produced in a separate pulp mill. The paper itself is then produced on a paper machine from a mixture of fibres (which can be primary or recycled fibres), chemicals and additives.
Figure 1.1 presents the different stages of the manufacture of P&B:

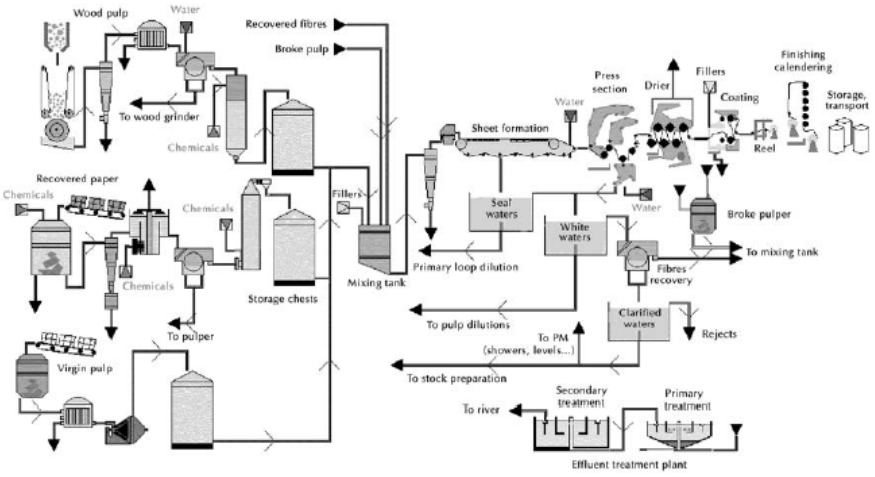

Figure 1.1 *Overall paper process (ILSI Europe, 2004)*

Recovered paper is an important raw material in terms of volume and utilisation for the paper industry in many countries. The recycling of paper is an example of sustainable use of resources. Although recycling is both economically and ecologically sound, recovered paper cannot be used in all paper grades. Broadly speaking, the final production process for recycled paper is the same as the process used for paper made from primary fibres. The main difference is that recovered paper fibres have already been used, so that non-fibre material, originating from previous uses, will have to be removed. 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. There are different grades of recovered P&B to satisfy the needs of different producers. More than 50 grades of recovered P&B are defined in the European List of Standard Grades of Recovered Paper and Board (EN 643).
The additional technical demands (mechanical strength, optical properties…) placed on the P&B are normally obtained through the use of chemical additives which are combined with the fibrous raw materials. The amount required to achieve the technical effect is very small, i.e., for most additives significantly less than 1% by weight of the paper. The main challenge facing the papermaker is retaining the chemical additives in the paper so that they can perform their intended technical function.

P&B 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 substance transfer between food and the base paper material. The subject of functional barriers in itself would be the core object of the present research project, and will be here treated in more detail.

Migration is a major factor in regulating the safety and the quality of packaged food. Migration of chemicals from packaging materials can have an impact both on food safety and on food quality. This is recognised in the European Framework Regulation 1935/2004, Article 3, which states that: "*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:*

- *Endanger human health, or*
- *Bring about an unacceptable change in the composition of the food, or*
- *Bring about deterioration in the organoleptic characteristics of the food.*"

This is the core European Union (EU) legislative requirement for all the materials and articles intended for food contact. However so far no specific measure at the level of the EU has been introduced for P&B, although national and other provisions exist, among which "Resolution AP (2002)/1" of the Council of Europe (CoE, 2002), "Recommendation XXXVI" (plus parts I, 2 and 3) of the German Federal Institute for Risk Assessment (BfR, 2012) and "Industry Guideline for the Compliance of Paper & Board Materials and Articles for Food Contact" published by CEPI and International Confederation of Paper and Board Converters in Europe (CITPA) in 2012, deserve to be mentioned.

For some years, research work has been undertaken to assess the migration phenomena from P&B materials to foodstuffs.
Migration of chemicals is a diffusion process that is subject to both kinetic and thermodynamic control. The migration of contaminants from paper packaging into food is a complex and difficult process to study because fibre-based materials are heterogeneous, and because the process takes place by direct contact and gas-phase diffusion via air pores in the matrix (Poças et al., 2011).

Some of the earliest studies on recycled P&B showed the presence of phthalates (Aurela et al., 1999) and naphthalenes (Boccacci et al., 1999). Phthalates, benzophenone, and diisopropyl naphthalenes (DIPNs) are considered the most profound contaminants in a wide range of paper samples tested (Summerfield & Cooper, 2001). Bisphenol A has also been found in recycled papers (Cederberg, 2002; Vinggaard et al., 2000). Recently it was found that mineral oils originating from the recycled fibre in P&B are able to migrate into food (packaged in recycled packaging) via the vapour phase (Biedermann & Grob, 2010). This raised major concern, as these mineral hydrocarbons are often not food grade approved, and toxicological assessments of this complex mixture of compounds are still uncertain at present. The presence of potentially toxic compounds in P&B, therefore, needs to be monitored for their amounts in the paper, but also in terms of their migration into foodstuff.

The focus of current efforts to harmonise EU legislation regarding food contact materials, is firmly on the development of a positive list of permitted ingredients. Such a list will not, however, encompass the multitude of chemicals that could be present in packaging materials and articles as intrinsic or contaminant substances such as feedstock impurities and reaction intermediates. Other packaging's, such as multi-layer materials, are likely to contain substances from the adhesives and printing inks used. Recycled paper could in principle contain contaminant residues from their earlier use. In all of these instances, if it could be demonstrated that a layer of material functions as an effective barrier to migration, then substances beyond (outside) that barrier would not have the potential to contaminate the packaged food. In this context, a functional barrier has been defined as "*any integral layer which under normal or foreseeable conditions of use reduces all possible material transfers (permeation and migration) from any layer beyond the barrier, into food, to a toxicologically and organoleptically insignificant and to a technologically unavoidable level*" (Feigenbaum et al., 2005).

When attempting to devise a general method of test for functional barriers, the main problem becomes the number of potential contaminants and the fact that their identity and propensity to migrate are not always known. To test for the general case, it is advantageous to employ a small set of model contaminants that can be selected to cover a range of physical and chemical characteristics that could influence migration behaviour, such as polarity, volatility and molecular size.

It is self-evident that the majority of barrier layers will not prevent all migration for all time. Rather, they will first delay migration during the "lag-phase" which is the time required for substances to migrate through the thickness of the layer and then appear at the food contact surface. Thereafter, migration into the food will occur but will be attenuated by the presence of the barrier layer. Migration is a process that occurs according to Fick's Law of diffusion. Migration is driven by a concentration gradient and limited by the diffusion coefficient of the substance in the medium through which it migrates. The performance of a barrier material will thus be determined by its intrinsic resistance to migration coupled with its thickness.
In the absence of pinholes, glass and aluminium are considered absolute functional barriers, whereas internal paper bags offer no protection (Gärtner et al., 2009). Polymers like polyethylene terephthalate (PET) and polyamides (PA) have low diffusion rates (Feigenbaum et al., 2005) and act as virtual absolute barriers for average shelf lives (Fiselier & Grob 2011). Polyethylene (PE) is a poor barrier, whereas polypropylene (PP) is fairly effective at ambient temperature, but rapidly loses efficiency above it (Johns et al., 2000; Choi et al., 2002; Song et al., 2003; Pastorelli et al., 2008). A new technical approach creates a functional barrier by coating the paperboard with a plastic film. It must be taken into account that high-process temperatures can contaminate the barrier from the very beginning (Franz et al., 1997) for plastic multi-layered systems.

In contrast, any literature is available on improving barrier properties of P&B towards contaminant substances via coating with biomaterials. The association of biopolymers to paper provides interesting functionalities while maintaining environment-friendly characteristic of the material.

According to their origin, biopolymers can be grouped into three main categories (Petersen et al., 1999). Figure 1.2 schematically summarizes the classification of biopolymers.

- Polymers directly extracted from natural materials such as polysaccharides (e.g. cellulose, starch, chitin), lignins, proteins and lipids.
- Polymers produced by classical chemical synthesis from renewable bio-derived monomers (e.g. polylactide or PLA, which is polymerized from lactic acid obtained from dextrose).
- Polymers produced by microorganism or genetically transformed by bacteria. This category includes polyhydroxyalkanoates or PHAs. Commercially, these principally consist of polyhydroxybutyrate (PHB) and copolymers of hydroxybutyrate and hydroxyvalerate (PHBV).

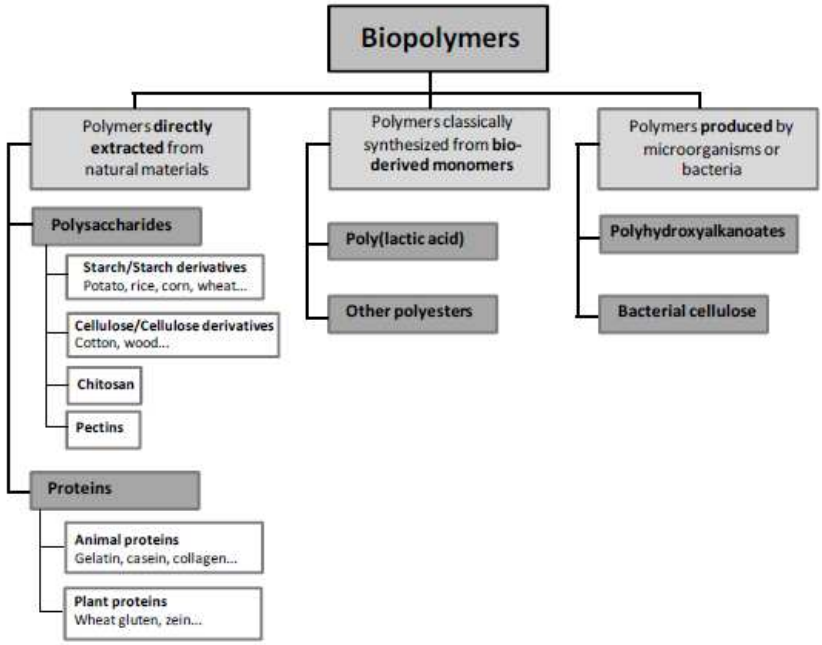

Figure 1.2 *Classification of biodegradable polymers (Petersen et al. 1999)*

Several studies have already focused on the potential of bio-based materials, but only for controlling permeation of water vapour and oxygen from food paper packaging (Andersson, 2008).

In conclusion, the main objectives of this PhD project were to test different types of renewable biopolymers as paper coating materials, to evaluate their barrier performances against migration and finally to discuss their possible optimization to achieve functional barrier properties. Focus was directed to water-based, renewable coatings made of starch, plant and animal proteins, considering model chemicals and emerging contaminant substances from recycled P&B, among them mineral oils.

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2. AIM OF THE STUDY

GENERAL AIM

The general aim of this PhD project was to evaluate the effectiveness of bio-based coatings for preventing migration of chemicals from P&B packaging to food. It arises from the greater interest in the safety assurance and at the same time in the sustainability aspects of the food packaging market and of the legislation. In other words, the aim was to test feasible "green" alternatives to the synthetic polymer solutions for controlling migration of contaminant substances that are of concern for human health. To provide such evaluations, selected biopolymers were studied under different point of views:

- Attitude to form coatings onto P&B materials
- Physical and chemical characterization
- Partition coefficients of selected chemicals
- Barrier properties

Different techniques were exploited to gain the broadest information about the developed coatings, especially:

- Micro-Visco-AmyloGraphic (MVAG) tests
- Microstructural observations
- Adsorption isotherms
- Diffusion kinetics

They were used on one hand to obtain measurements of coating characteristics, on the other hand to assess migration performances, and finally to obtain the necessary information for their optimization.

SPECIFIC AIMS

The main specific aim of this PhD project was to evaluate bio-based coatings for preventing migration of target compounds, identified as the most representative for food P&B packaging contaminants, on the basis of their potential of migration into food in a worst case scenario.

3. Part 1

CONTAMINANTS IN PAPER AND BOARD FOR FOOD PACKAGING

This first part of the PhD project has a twofold objective: to propose a fast screening procedure for Paper and Board (P&B) packaging, allowing samples classification and to estimate food contamination by prevailing dangerous migrants.
To the scopes a screening analysis of volatile and non-volatile compounds present in a set of twenty types of P&B samples of different characteristics was performed coupling solvent extraction and solid phase micro extraction (SPME) gas chromatography-mass spectrometry (GC-MS) techniques. A chemometric approach was then used to analyse the obtained analytical data. In particular Principal Component Analysis (PCA) was chosen as it allows the simultaneous consideration of all the investigated analytes and the individuation of those contaminants that could be used as "markers" for a given type of packaging. A literature search for safety data or legislative restrictions of the identified substances was performed. Moreover the obtained semi-quantification of the compounds in the packaging allowed a worst case estimation of food contamination applying the infinite total migration model; occasionally, migration estimations overcame the specific migration limits. The chosen analytical method coupled with a chemometric approach proved to be an effective way to process these data.
Finally further analyses were undertaken with the aim of investigating the occurrence of diisobutyl phthalate (DiBP) in PVC cling films and its source of contamination along a converting process. Although raw plastic materials (for direct food contact applications) used by producers are free from phthalates and analytical evidences confirm their absence after the extrusion process, it can be found in final rolls packaged into cardboard packaging (secondary packaging) during storage. Different cardboard cores and folding cartons made of recycled fibres were analysed and some of them resulted highly contaminated. To investigate the possible DiBP transfer mechanism from contaminated paper and adsorption by plastic materials through the gas phase, kinetic experiments were performed in a model system. Additionally, to evaluate the partitioning behaviour, adsorption isotherms of DiBP into paper and plastic materials were obtained at 40 °C.

3.1 ANALYTICAL SCREENING

This experimental section was carried out through an analytical survey of twenty P&B materials intended for food direct and indirect contact. Samples were analysed to identify chemicals with a potential to migrate to foods. Representative materials covering a range of uses such as primary and secondary packaging or article for take away foods were obtained from distributors. A screening approach was applied by means of two different analytical techniques which included determination of volatiles by SPME and non-volatiles by solvent extraction, with subsequent analysis by GC-MS. A large number of both volatile and non-volatile substances were identified with aliphatic hydrocarbons (from C_{12} to C_{40}); aromatic cyclic, in which several phthalates, such as common plasticizers, as well as styrene and DiPNs, were included; aldehydes (from C_6 to C_{30}), which are very common in paper samples; ketones, such as butylacetone, acetophenone; alcohols, such as 2-heptanol-5-ethyl and 2-ethyl-1-hexanol; and, finally, some acids, as well as other compounds such as triacetin, 2-pentylfuran or phosphoric acid tributyl ester.

Multivariate analysis was applied on data obtained from the analytical screening of P&B food packaging materials.

Principal Component Analysis (PCA) allows to emphasize the presence of residual solvents, probably coming from printing inks, as well as hydrocarbons and aromatic compounds, mainly toluene and plasticizers linked to the recycled pulp content such as DiBP or DiPNs, in the corrugated and pigmented packaging; whereas in the plastic coated samples triacetin and hexanal were identify as the prevailing compound.

Chemical's quantification, that was obtained in the material, and it was referred to the relative response factor of the internal standards used, allowed a preliminary worst case estimation of food contamination.

3.1.1 Materials and methods

3.1.1.1 Materials

3.1.1.1.a Paper and Board packaging samples

In Table 3.1.1 the characteristics of the P&B samples studied are listed. All of them were obtained from distributors and were chosen according to their application (in direct or indirect food contact), their different surface treatments and their pulp characteristics in order to represent a wide spectrum of paper samples.

code	Type	Food Contact	Surface treatment	Pulp
1a	Paper	D	U	Unbleached kraft
2a	Paperboard SBS	D	G	Bleached chemical
3a	Paperboard SBS	D	G	Bleached chemical
4a	Paperboard SBS	D	G	Bleached chemical
5a	Paper	D	G	Unbleached kraft
6a	Paper	D	U	Chemical/mechanical
7a	Corrugated medium	D	CB	Recycled
8a	Paperboard WLC	D	G	Recycled
9a	Laminated Paper	DS	PC	Chemical/mechanical
10b	Laminated Board	DS	PC	Chemical/mechanical
11b	Laminated Board	DS	PC	Chemical/mechanical
12b	Laminated Board	DS	PC	Chemical/mechanical
13b	Laminated Board	DS	PC	Chemical/mechanical
15c	Paperboard FBB	I	G	Chemical/mechanical
16c	Corrugated medium	I	U	Chemical/mechanical
17c	Paperboard WLC	I	G	Recycled
18c	Corrugated medium	I	CB	Recycled
19c	Paperboard WLC	I	G	Recycled
20c	Paperboard WLC	I	G	Recycled

Table 3.1.1 *Characteristics of the P&B packaging samples*

Since a great variety of grades are commercially available and the terms used to describe them vary from market to market, in our type classification the grade categories are based on fibre grades and production technology. Moreover, type classification of P&B (according to Robertson & Gordon 2005; paperboard is a paper with a grammage above 224 g m^{-2}) was made according to DIN Standard 19303-2009 "Paperboard – Terms and grades"; in which are defined: solid bleached board (SBS), white lined chipboard (WLC) and folding boxboard (FBB). As regard classification of the surface treatments: uncoated samples (U), corrugated board (CB), pigmented coated (G) and plastic

laminated (PC) were considered. As regard the type of food contact, direct contact (D), direct and short time (DS) and indirect (I) (secondary packaging) were considered.
A particular sample (14C), was not included in the previous table; it was a paperboard packaging for plastic freezing bags, so it was not directly related to food but it was included in the study to find comparison with other samples. It was a white lined chipboard (WLC), pigmented coated (G), made of recycled pulp.
Additional characteristic like food capacity, grammage and thickness were measured for each packaging. Respectively TAPPI Test Methods T410 and T411 were used in order to determine grammage and thickness.

Table 3.1.2 reports the obtained measurement that allowed a better description of the samples.

ID code	Grammage (g m^{-2})	Thickness (µm)	Surface (dm^2)	Food capacity (ml)
1a	80	110	4.9	320
2a	385	500	9	500
3a	385	500	11.25	500
4a	385	530	10.52	500
5a	200	250	5.55	1000
6a	150	210	6.3	270
7a	270	330	11.15	300
8a	400	570	5.8	1000
9a	40	50	9.2	500
10b	300	380	3.8	150
11b	280	350	2.15	300
12b	320	410	2.85	390
13b	300	380	3.6	216
14c	280	350	4.9	100
15c	320	410	5.7	320
16c	150	210	6.1	400
17c	310	390	4.6	200
18c	310	390	33.8	750
19c	350	450	16.9	375
20c	300	380	4.6	500

Table 3.1.2 *Main measurements made on P&B packaging samples*

All the materials were handled with gloves free from phthalates and stored in LDPE bags before analysis.

3.1.1.1.b Solvents and reagents

All the solvents and reagents used in this study were purchased from Sigma-Aldrich, Italy.

3.1.1.2 Methods

3.1.1.2.a Solvent Extraction (SE)

Duplicate portions of samples were cut from each packaging. Each portion (1 g) was transferred to a screw-cap glass vial (20 ml capacity) and was extracted with 10 ml ethanol/hexane 1:1 during 2 h at ambient temperature. This method, already reported in the literature (Grob et al., 2010), aims at low extraction efficiency for high molecular hydrocarbons which could disturb the GC system.
Then, as internal standard 25 µl of a metyl heptadecanoate solution in ethanol/hexane 1:1 (2.5 µg μl^{-1}) were added. Vials were sealed with septa and screw caps and kept at room temperature overnight. Samples were sonicated for 30 min and then a portion of supernatant was transferred to a glass autosampler vial ready for the CG-MS analysis.

3.1.1.2.b Solid Phase Micro Extraction (SPME)

Duplicate portions of samples were cut from each packaging. Each portion (3 cm^2) was placed in a 20 ml sealed vial with screw top (manual HS-SPME) and an internal standard addition of 1 µl of methanol solution with a defined concentration of chlorobenzene (4 µg μl^{-1}) was performed. The samples were incubated at 130°C for 30 min, as it represents the standard condition for solvent residual analysis for plastic materials (UNI EN 13628-2).
Afterwards a 50/30µm divinylbenzene-carboxen-polydimethilsiloxane (DVB/CAR/PDMS) fibre was immersed in the headspace of the vials for 15 min at room temperature.
The influence of the type of coating on the analytes extraction by HS-SPME was previously studied by others authors (Ezquerro et al., 2003) and DVB/CAR/PDMS has been selected for its better behaviour for the volatile organic compounds (VOCs) with higher molecular masses. The VOCs were thermally desorbed in the injector port of the chromatograph and the fibre was maintained for 20 min during the GC-MS analysis.

3.1.1.2.c GC-MS Conditions

A Perkin Elmer Autosystem XL gas chromatograph equipped with a DB-5MS (30 m, 0.25 mm i.d., film thickness 0.25 µm) column, a Merlin Microseal™ Septum Kit installed on the Capillary Inlet system and a Turbomass mass spectrometer was used for the analytical determination. Helium was used as the carrier gas (flow rate 1 ml min^{-1}).
A blank solution was analysed prior to any sample measurement to avoid possible memory effects. The mass spectrometer was used in the total ion current (TIC) mode, recording electron ionization (EI) mass spectra at 70 eV and 250 °C ion source and transfer line temperature.
GC-MS parameters for the liquid mode were: injected volume 2 µl (automatic sampler HT300A), split ratio:19, Injector temperature 270 °C, Oven program temperature 40 °C (2 min) - 15 °C/min to 290 °C (11min). GC-MS parameters for the SPME mode were: Injection mode Splitless, Injector temperature 250 °C, Oven program temperature 40 °C (4 min) - 5 °C/min to 90 °C - 12,5 °C/min to 270 (5min) - 5 °C/min to 290 °C (10min).

3.1.1.2.d Principal Component Analysis (PCA)

In order to examine the results from a multidimensional point of view, the obtained data were analysed by Principal Component Analysis (PCA) (Wold et al., 1987). PCA is a well-known method for the extraction of the relevant information in multivariate data sets. It is based on the

construction of a small number of orthogonal variables called principal components (PCs) which are linear combinations of the original variables. The first PC accounts for the maximum information and is oriented towards the maximum variation within the data; the second PC is orthogonal to the first and oriented in the second direction of maximum variation and so on. The Score Plot and the Loading Plot are the graphical results of the model. The first one shows the sample projections in the PCs space, allowing the detection of groups or tendencies between samples. The second plot gives information concerning the correlation between the investigated variables and the joint interpretation of the two plots could allow discovering variables that mainly characterize a given group of samples.
Autoscaling was performed prior to the calculation in order to give to all the variables the same possibility to influence the PCs directions. PCA modeling was performed using The Unscrambler X (CAMO, Norway).

3.1.1.2.e Potential of migration into food

Semi quantitative evaluation of each analyte concentration in each packaging was obtained, in the case of non-volatile substances, considering the response factor of the internal standard metyl heptadecanoate, as reported in equation 3.1.2.1.

$$\frac{C_a}{C_{IS}} = \frac{A_a}{A_{IS}} \rightarrow C_a = \frac{C_{IS}}{A_{IS}} * A_a \qquad (3.1.2.1)$$

Where:

C_a = analyte concentration (mg l^{-1})
C_{IS} = internal standard concentration (mg l^{-1})
A_a = analyte area
A_{IS} = internal standard area

Semi-quantitative evaluation was already used in literature (Gruner et al. 2008) and it could be considered a simple and fast method to obtain a screening of the unknown compounds present in packaging samples.
Moreover, an estimation of the worst case food contamination (assuming that all amounts of the substance initially present in the direct contact packaging material is able to migrate) was performed. This type of estimation was already used in literature and defined as "potential of migration" (Lorenzini et al., 2013).

In particular, the "infinite total migration model" (Piergiovanni, 2010) was applied (Equation 3.1.2.2) to derive $C_{F,\infty}$, the concentration of the chemicals into the food at infinite time, considering $C_{p,0}$ and α, being the initial concentration in the packaging and the ratio between the food and the packaging real mass.

$$C_{F,\infty} = \frac{C_{p,0}}{\alpha} \qquad (3.1.2.2)$$

It must be highlighted that this is a screening method leading to a considerable overestimate of exposure; since it is based on the worst case assumption that all amounts of the substance initially present in the direct contact packaging material is able to migrate into food.

3.1.1.2.f Literature survey

Obtained results were compared with legislative or guidance limits set in different national and European documents, particularly to:

- EU Plastic Regulation on plastic materials and articles intended to come into contact with food (Regulation EU 10/2011 - Annex I);
- Guidelines on paper and board materials and articles, made from recycled fibres, intended to come in contact with foodstuffs (CoE Resolution 2002 - Annex 3);
- Recommendation XXXVI of the German Federal Institute for Risk Assessment (BfR): Paper and board for food contact (BfR 2012);
- Industry Guideline "CEPI guide for good manufacturing practice for paper and board for food contact" (CEPI 2012);
- Lists of permitted substances for the manufacture of packaging inks, subject to the requirements set out therein (Annex 6 of the Ordinance of the FDA on articles and materials - Swiss ordinance).

3.1.2 Results and discussion

3.1.2.1 Performance overview of the analytical methods

The application of the two analytical techniques, solvent extraction and SPME coupled to GC and MS detection, provided two different chromatograms, which allowed defining two different profiles of characteristic substances for each sample of packaging.
The identification of the detected compounds was achieved through the mass spectrum and through comparison with the National Institute of Standard and Technology (NIST) library of spectra. Only when match comparison with recorded spectra in the NIST library was higher than 40%, compounds were considered.
A large number of both volatile and non-volatile substances were identified. All the compounds could be grouped into seven groups according to their chemical families: aliphatic hydrocarbons, aromatic hydrocarbons, aromatic cyclic, aldehydes, ketones and ethers, alcohols and other (Figure 3.1.1A, B).

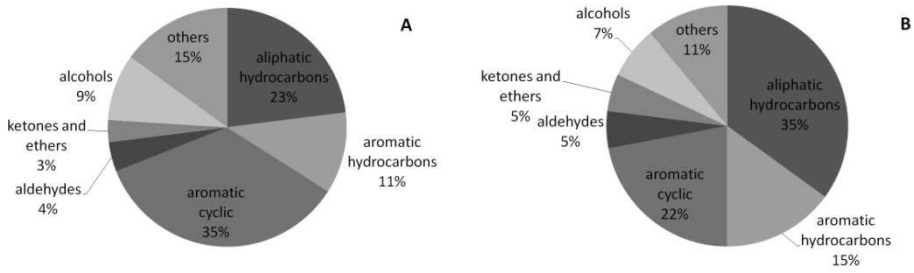

Figure 3.1.1 *Solvent extraction (A) and SPME (B) profiles of identified substances*

The analysis of paper packaging using the solvent extraction techniques was proficient in detecting different chemical compounds (mainly aromatic cyclic), while the SPME analysis allowed to identify a large number of aliphatic hydrocarbons with low molecular weight, however it was inadequate for non-volatile compounds. Moreover, in the majority of samples analysed, in the case of SPME, the substances were below the limit of detection and therefore these results were used only for qualitative evaluations.
Both the analyses were carried out using internal standards. For solvent extraction technique, metyl heptadecanoate was used, in the case of SPME chlorobenzene was selected. As a result the semi-quantitative evaluation of each compound was achieved by considering the response factor of one of them equal to one, considering the similarity of the chemical family within each group of substances. This kind of semi-quantitative evaluation was already used in literature (Gruner et al., 2008) and it could be considered a simple and fast method to obtain a screening of the unknown compounds present in packaging samples.

3.1.2.2 Semi quantitative evaluation of non-volatile substances

The calibration graph was constructed for the internal standard metyl heptadecanoate after analysis of a series of five standards of known concentrations. The calibration curve exhibited a linear response over the concentration range of interest and a determination coefficient equal to 0.998. The limit of detection (LOD) was established according to the ISO/IEC 17025 Guidelines15 and corresponds to 0.04 mg kg^{-1}.

Obtained results varied considerably for each sample analysed; compounds identified in some packaging were few even if abundant (Figure 3.1.2A) whereas in others they were numerous and in some cases led to a “forest of peaks” (Figure 3.1.2B) in which identification of single compounds was difficult to be reached.
Lack of homogeneity in the sampled paper and board used for food packaging was the main out finding of this screening that leaded to a multivariate evaluation of results in order to find analytes that could be used as "markers" for a given type of packaging, considering their main characteristics.

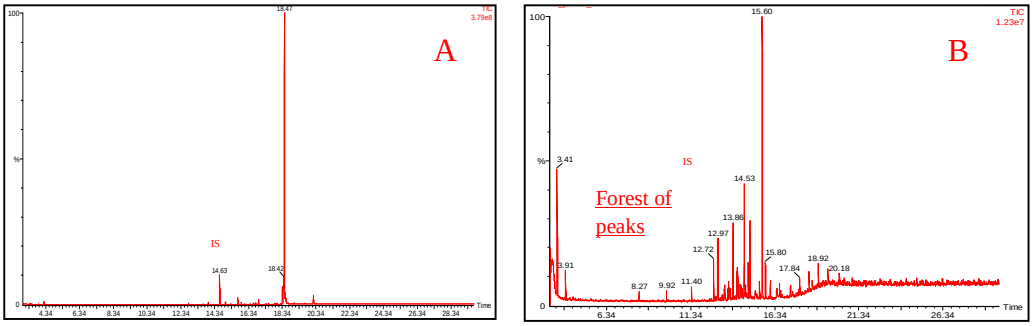

Figure 3.1.2 *SE GC-MS chromatograms of two representative samples*

In the following tables, results obtained by solvent extraction of samples, for each type of packaging are shown: direct contact (Table 3.1.3), indirect contact (Table 3.1.4) and article for take away food (Table 3.1.5); the mainly identified compounds are listed, together with the highest concentration ($C_{p,0}$) detected in the “worst case” packaging sample, that is listed as well.

Identification	**“worst case” Sample**	$C_{p,0}$ **(mg kg^{-1})**
Toluene	6a	13.98
Ethanol, 2-(2-butoxyethoxy)	3a	242.90
2,6-Diisopropylnaphthalene	8a	30.57
Di-isobutil phthalate (DIBP)	4a	73.90
2-ethyl hexanol (2EH)	2a	6.66
Dibutil phthalate (DBP)	7a	23.34

Table 3.1.3 *Main identified compounds in direct contact packaging*

Identification	**“worst case” Sample**	$C_{p,0}$ **(mg kg^{-1})**
Toluene	18c	20.12
Triacetin	17c	75.76
2,6-Diisopropylnaphthalene	17c	56.78
Di-isobutil phthalate (DIBP)	18c	616.62
2-Butenedioic acid (E)-, bis(2-ethylhexyl) ester	18c	102.93
2-ethyl hexanol (2EH)	17c	7.34
Furan, 2-pentyl	17c	0.67
Diethylene glycol dibenzoate	18c	668.83

Table 3.1.4 *Main identified compounds in indirect contact packaging*

Identification	"worst case" Sample	$C_{p,0}$ (mg kg^{-1})
Toluene	13b	26.96
Di-isobutil phthalate	10b	75.65
2-ethyl hexanol (2EH)	13b	6.07
Furan, 2-pentyl	13b	3.58

Table 3.1.5 *Main identified compounds in article for take away food*

Data shown in the previous tables refer only to the main abundant compounds identified; the concentrations found in the packaging ranged between 0.7 and 669 mg kg^{-1}, as previously stated, this confirms the lack of homogeneity in the chemical's content in different paper packaging.

3.1.2.3 Principal Component Analysis (PCA)

Data obtained from solvent extraction and GC-MS analysis were studied with Principal Component Analysis. All the samples were investigated but the packaging without surface treatment were excluded. Chemicals detected in more than five samples were used as variables: the thirteen considered compounds were listed. Resulting score plot and loading plot (PC1 vs PC2) are shown in Figure 3.1.3 Samples were labelled dependently to their surface treatment.

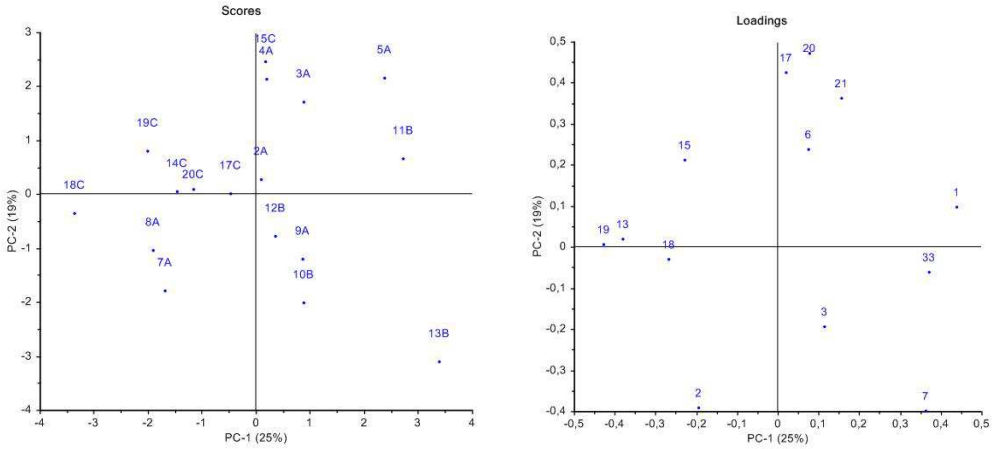

Figure 3.1.3*. Scores and loadings plots.Where:1= ethane, 1,1-diethoxy; 2= toluene; 3= Hexanal; 6= ethanol, 2-(2-butoxyethoxy)-; 7= triacetin; 13= 2-ethylhexanol; 15= 2,6-diisopropylnaphthalene; 17= n-C11; 18= diisobutyl phthalate; 19= dibutyl phthalate; 20= n-C16;21= n-hexadecanoic acid; 33= 2-ethylhexyl methacrylate.*

In this graph, that explains 44 % of the total variance, it is immediately evident that the samples are quite well grouped in the PCs space according to their surface treatment. In particular almost all pigmented coated samples are located along the positive part of PC2: two sub-groups of pigmented coated samples could be instead detected along PC1. Corrugated samples appear on the left bottom part of the graph and the plastic laminated packaging are distinguished in the underside right hand pane. The model is thus able to find the major differences between the investigated materials with respect to the variables chosen for their characterization. In other words, the chosen variables proved to be adequate to differentiate the samples under study.

From the loading plot, in which the investigated variables assume certain positions according to the relationship between their selves and their influence on the system, several useful information can be extracted.
A direct correlation between dibutyl phthalate (DBP) and diisobutyl phthalate (DiBP) together with 2-ethyl hexanol (2-EH) emerges: they are located at negative values of PC1 close to diisopropyl naphthalene (DIPN). These molecules could be found in high level both into the corrugated samples and into pigmented coated packaging samples sub-group (19C, 14C, 20C, 17C, 8A) as well. Particularly linked to the former type of packaging, toluene is displayed in the negative part of both PC1 and PC2 as commented afterwards.
Phthalates are widely used as plasticizers and additives in many daily used products and due to their widespread use, relatively large amounts of these compounds are released in the environment (Steiner et al. 1998, Kambia et al. 2001, Li et al. 2004). In particular, due to their similarities, DBP and DiBP can be used in combination as plasticizer in adhesives, printing inks and coloured laminated films; frequently they are detected as migrants into food packaged in paper that contains recycled fibres (Jickells et al. 2005, Gartner et al. 2009, Aurela et al. 1999, Bononi and Tateo 2009).
Moreover, 2-ethyl hexanol is mainly used in the manufacture of ester plasticizers and in the manufacture of coating materials, adhesives, printing inks, and impregnating agents (Elvers et a1. 1989).
The above mentioned literature therefore confirmed the correlations found in the loadings plot. As for DIPN, in literature studies it was being proposed as marker of recycled pulp such as DBP, because it is used as solvent in carbonless copy paper (Asensio and Nerin 2009), in our study it was detected in most of the pigmented coated packaging samples.
It is worth emphasizing that the abundance of these molecules in corrugated samples suggests that this type of packaging could contain recycled fibres or other impurities. It could be assessed that they are not of food grade quality, even though one of them (7A) is intended for food direct contact use.
Concerning this issue, it is well known how it is frequent in papermaking process of corrugated structure to use virgin paper only for the top layers, whereas the fluting board is made mainly of recycled pulp. Interestingly, the pigmented coated sample with the higher grammage (8A) is located close to the corrugated samples; it is a board packaging intended for direct contact with salt and it can be hypothesized that its poor purity could be linked both to the grammage and to the cheapness of the product being packed.
Oppositely, at positive values of PC1, ethane,1-1-diethoxy (used as solvent, for fragrance of cosmetic manufacture and for synthesize of dyestuff) and 2-ethylhexyl methacrylate (mainly employed as a raw material for resins, paints, coating materials, adhesives, fibre-treating agents, lubricant additives) are located. Together with triacetin and hexanal (negative value of PC2) they reach high values in plastic laminated samples, contributing to differentiate these samples from all the others. Plastic laminated samples showed in fact positive PC1 values and negative PC2 ones. Sample 13B, belonging to plastic laminated group, is completely separated from the other packaging being predominantly characterized by the highest content in triacetin.
Concerning PC2, triacetin (already commented) and toluene show high negative values while hexanal showed an "intermediate" negative PC2 value. Hexanal is considered the dominant oxidation product from resins and wood flavour (Ziegleder et al. 2001), and it was also linked to oxidation of inks (Hamalainen et al. 2005). In our study hexanal was linked with plastic laminated samples, therefore it could be related both to the plastic coating and inks, probably underwent to oxidation. Toluene, used as a solvent in paints, paint thinners, adhesives, inks, resins and cleaning agents, and also to manufacture polymers (nylon, plastic soda bottles, polyurethanes) and for pharmaceuticals, presents high values in corrugated packaging being potentially a marker for this kind of packaging but it was found in plastic coated samples as well. This confirms that only the

simultaneous consideration of several chemicals with a multivariate approach allowed distinguishing the investigated packaging materials.
At positive values of PC2 in the loading plot a group of chemicals could be considered as positively correlated. By comparison with scores plot they could be linked to pigmented coated samples. This type of packaging was the most sampled in our study because is the widely used type for food packaging; it could be considered divided into two sub-types: for direct and indirect contact (where often a plastic bag contains the food and the function of the paper packaging is secondary). Looking at the score and loadings plots we obtained in our study, this sub-type can be clearly identified.
One group of samples (19C, 20C, 17C, 8A) containing high concentration of phthalates, 2EH and DIPN could be classified as pigmented coated packaging for indirect food contact usage; the sample 14C could be grouped together as well, even if it was a paper packaging for freezing bags therefore it was not intended for food applications. Another group of packaging (3A, 4A, 15C) resulted characterized by different compounds. Among the chemicals identified: n-Hexadecanoic acid, hydrocarbons and ethanol, 2-(2-butoxyethoxy). Samples 3A and 4A are distinguishable by the previous group of packaging for their food direct contact application, as for 15C it was a secondary packaging. However in this case the food was contained into an inner paper bag, therefore it could be assumed that there was not a functional barrier material (like a plastic polymer) able to avoid migration phenomena from food to packaging.
In fact, n-Hexadecanoic acid, commonly named as palmitic acid, is widely used as a lubricant and as an additive in industrial preparations; it constitutes between 20 and 30 percent of most animal fats and is also an important constituent of most vegetable fats. It is used in the manufacture of metallic stearates, pharmaceuticals, soaps, cosmetics, and food packaging. Its presence in the analysed sample could be due to a contamination from the food itself, moreover it was not considered as a harmful substance, and therefore it was not considered as marker for the classification of packaging. Contrary, ethanol, 2-(2-butoxyethoxy) was noticed as an important marker for these samples, it is a well-known printing solvent and previous studies linked its concentration to the quantity and type of waste material in the paperboard (Ziegleder 1998).
As regard hydrocarbons, they had low molecular weight and could not be distinguished for their origin (from food itself or mineral oils).

3.1.2.4 Qualitative evaluation of volatile substances

In the case of SPME, most of the detected compounds were lower than the limit of quantification; therefore these data were used only for qualitative evaluations in order to find other information about characterization of samples on the basis of their chemicals content. As for solvent extraction technique, compounds identified were numerous and led to a “forest of peaks” in the obtained GC chromatogram, as it is shown for example in Figure 3.1.4.

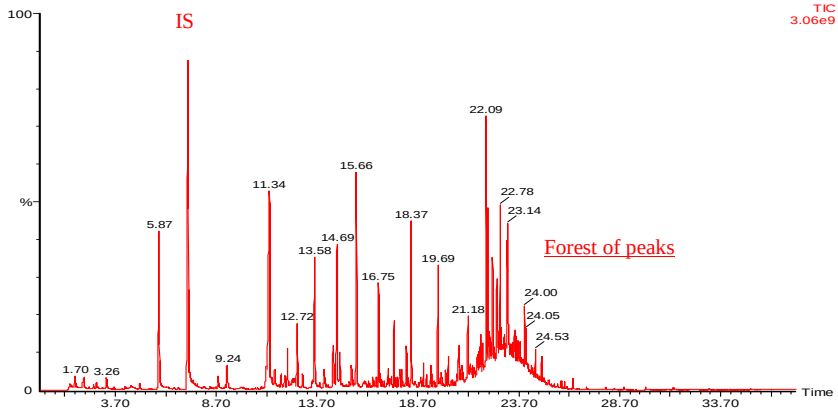

Figure 3.1.4 *SPME GCMS chromatogram of a representative sample*

Generally speaking, any class of substance could be identified as representative for a specific typology of packaging, but some substances are more often present in some packaging. Sometime these compounds are the same identified from solvent extraction data, while additionally other substances were detected.

Among alcohols, 2-ethyl hexanol (2-EH) was the most frequent; regarding the surface treatment classification it was found in pigmented coated, plastic laminated and corrugated packaging.

Among aldehydes, hexanal, heptanal, decanal and bezaldehyde were detected in most of the analysed packaging. In detail, decanal was the most frequent in all the samples while only pigmented coated, plastic laminated and corrugated samples were characterized by a higher content (more than 60 %) of hexanal, heptanal and benzaldehyde strongly different from uncoated samples (30 %) which were more characterised by pentadecanal.

With SPME technique it was possible to identify acetophenone among ketones as a characteristic compounds (regarding the surface treatment) for pigmented coated, plastic laminated and corrugated, while it was not found for uncoated samples.

Considering the family of aliphatic hydrocarbons, a lot of compounds was detected and particularly tetradecane, hexadecane, heptadecane and nonadecane were present in more than 60 % in all of the samples.

As regards to the other compounds, such as DIPN, it was found in a lot of samples but in the case of pigmented coated and corrugated it was detected with an higher frequency, confirming as already with solvent extraction analyses.

For other substances, like in the case of DBP and DiBP, the SPME technique does not allowed any classification of samples, since it was found ubiquity in all the samples, this result is in apparent contrast with data obtained through solvent extraction and PCA analysis, however it is well known how such not volatile compounds cannot be efficiently detected with the first analytical device.

3.1.2.5 Estimation of the migration into food

Considering that in Europe, the safety and quality of paper and board for food contact are not under control of a specific directive, at present, manufacturers of materials and food packers, who are responsible for the safety of their product, should establishing compliance with Regulation 1935/2004 (EC) and following the requirements of the Regulation 2023/2006 (EC). In order to do that a risk assessment approach have to be applied. It consists of three phases: risk identification, risk characterization and risk evaluation; therefore in the case of food packaging, the first phase of the assessment must be migration, packaging usage and food consumption data collection.

In our study we performed some risk analysis steps for all the main chemicals detected with the analytical screening. In the following tables, as regard results obtained by solvent extraction of samples, for each type of packaging: direct contact (Table 3.1.5), indirect contact (Table 3.1.6) and direct short time (Table 3.1.7); the identified compounds and the estimated concentration of the chemicals into the food at infinite time are listed. Only data about compounds recognized as hazardous or potentially hazardous and determinations in "worst case" samples are here reported. References to limitations set in the selected legislative or guidance documents are provided as Specific Migration Limit (SML) and discussed, considering the foreseen food contact application.

Identification	$C_{F,\infty}$ **(mg kg^{-1})**	**Limitation**
Toluene	0.52	SML = 1.2 mg kg^{-1} (CoE 2002) SML = 0.01 mg kg^{-1} (FDHA ordinance)
Ethanol, 2-(2-butoxyethoxy)	17.35	SML = 3 mg kg^{-1} (FDHA ordinance) SML = 3 mg kg^{-1} (CoE 2002)
2,6-Diisopropylnaphthalene (DIPN)	0.71	As low as technically possible (XXXVI BfR)
Di-isobutil phthalate (DIBP)	5.98	SML = 1 mg kg^{-1} (XXXVI BfR)
Dibutil phthalate (DBP)	2.33	SML = 1 mg kg^{-1} (XXXVI BfR)
2-ethyl hexanol (2EH)	0.61	SML = 0.01 mg kg^{-1} (FDHA ordinance) SML = 30 mg kg^{-1} (CoE 2002) SML = 60 mg kg^{-1} (EU Reg. 10/2011)
Acetophenone	0.02	SML = 0.01 mg kg^{-1} (FDHA ordinance)

Table 3.1.5 *Estimation of the potential of migration for direct contact packaging*

Identification	$C_{F,\infty}$ **(mg kg^{-1})**	**Limitation**
Toluene	2.87	SML = 1.2 mg kg^{-1} (CoE 2002) SML = 0.01 mg kg^{-1} (FDHA ordinance)
Triacetin	5.36	SML = 0.01 mg kg^{-1} (FDHA ordinance)
2,6-Diisopropylnaphthalene (DIPN)	4.06	As low as technically possible (XXXVI BfR)
Di-isobutil phthalate (DIBP)	88.09	SML = 1 mg kg^{-1} (XXXVI BfR)
2-Butenedioic acid (E)-, bis(2-ethylhexyl) ester	14.70	SML = 0.01 mg kg^{-1} (FDHA ordinance)
Diethylene glycol dibenzoate	95.55	SML = 0.01 mg kg^{-1} (FDHA ordinance)
Furan, 2-pentyl-	0.05	SML = 0.01 mg kg^{-1} (FDHA ordinance)
2-ethyl hexanol (2EH)	1.05	SML = 0.01 mg kg^{-1} (FDHA ordinance)
Acetophenone	0.05	SML = 0.01 mg kg^{-1} (FDHA ordinance) SML = "to be fixed" (Appendix C CoE 2002)

Table 3.1.6 *Estimation of the potential of migration for indirect contact packaging*

Identification	$C_{F,\infty}$ (mg kg^{-1})	Limitation
Toluene	1.35	SML = 1.2 mg kg^{-1} (CoE 2002)
Di-isobutil phthalate (DIBP)	5.82	SML = 1 mg kg^{-1} (XXXVI BfR)
Furan, 2-pentyl-	0.08	SML = 0.01 mg kg^{-1} (FDHA ordinance)
2-ethyl hexanol (2EH)	0.30	SML = 0.01 mg kg^{-1} (FDHA ordinance)
Acetophenone	0.18	SML = 0.01 mg kg^{-1} (FDHA ordinance) SML = "to be fixed" (Appendix C CoE 2002)

Table 3.1.7 *Estimation of the potential of migration for article for direct contact and short time packaging*

Toluene (CAS n. 108-88-3) is a common solvent; it is used to produce benzene and the synthesis of organic chemicals (ATSDR 2000). Toluene is, however, much less toxic than benzene, and has, as a consequence, largely replaced it as an aromatic solvent in chemical preparation. For example, benzene is a known carcinogen, whereas toluene has very little carcinogenic potential (Dees et al. 1996). Comparing $C_{F,\infty}$, the estimated concentration of the chemical into the food at infinite time, with the SML proposed by CoE it was below this limit, but in case of the FDHA ordinance this limit is exceed. It should be noted here how guidance or legislative restrictions for chemicals migration from packaging materials into food still could be different at present in the European Union. Toluene was also found in secondary packaging, in sample 18C, exceeding the SML when considering the estimated $C_{F,\infty}$, in this case however it must be noted that the effectiveness of the internal plastic bag (polyethylene) acting as a functional barrier slowing the transfer should be checked.

Ethanol, 2-2-butoxyethoxy (CAS n. 112-334-5) may be harmful if inhaled or swallowed, and cause respiratory tract and skin irritation. However it is not identified as probable, possible or confirmed human carcinogen by IARC. The SML foreseen for it is equal to 3 mg kg^{-1} (FDHA ordinance and CoE), this is exceeding in case of sample 3A, a pasta paper box, intended for direct contact with food. It should be emphasised that this chemical was mainly detected in primary packaging where an intimate with food is foreseen; therefore a complete safety assessment should be awaited for further conclusion.

2,6-Diisopropylnaphthalene (CAS n. 2027-17-0) is widely used as a substitute for PCB, it was reported also as a plant growth regulator used to prevent sprouting of stored potatoes (EPA 2003). Toxicological studies are being conducted which may lead to restrictions in P&B materials, however until now any SML is established and it was remarked only as "low as technically possible" in the XXXVI Recommendation of BfR as regard the packaging content. It was found both in the primary and secondary packaging collected in our study and it could lead to a migration up to 0.71 and 4.06 mg kg^{-1} of food respectively.

Diisobutyl phthalate (CAS n. 84-69-5), dibutyl phthalate (CAS n. 84-74-2) and 2-ethyl hexanol (CAS 104-76-7) were considered marker for corrugated and pigmented coated packaging, moreover they were detected in most of the packaging, regardless the food contact application. It could be stated that their presence is linked to the surface treatment of the material but not to the foreseen usage. It is worth emphasizing that the industry should take care of the presence of proven harmful compounds, such as DiBP and DBP; while in the case of 2EH no harm resulted from aggregate exposure (EPA 2006). This is particularly important to be considered when an intimate contact is implied or there is not an adequate functional barrier able to protect from migration into food. In fact, our estimation lead to overcoming of the SML in all the cases analysed.

Acetophenone (CAS 98-86-2) was not highlighted by liquid extraction but only using SPME technique. It is used for fragrance in soaps and perfumes, as a flavouring agent in foods (Burdock

2005), and as a solvent for plastics and resins. It was detected in P&B food packaging and linked to the recycled fibres content (Asensio and Nerin 2009; Ziegleder 2001). Acute (short-term) exposure to acetophenone vapour may produce skin irritation and transient corneal injury in humans. No information is available on the chronic (long-term), reproductive, developmental, or carcinogenic effects of acetophenone in humans. EPA has classified acetophenone as a Group D, not classifiable as to human carcinogenicity (EPA 2000). However the SML proposed by the FDHA ordinance correspond to 0.01 mg kg^{-1} and even if not by far, it was exceed in many cases of our study.

Triacetin (CAS n. 102-76-1) was another compound already mentioned with PCA analysis, it is also known as glycerol triacetate (GTA) is an antifungal agent used in the perfumery and pharmaceutical industries, although it is also used as a plasticizer in cellulose. US Food and Drug Administration (FDA) has approved it as Generally Regarded as Safe (GRAS), however in Europe, the FDHA ordinance settled a SML of 0.01 mg kg^{-1} and it was exceed in case of sample 17C, a primary packaging for chocolates.

2-Butenedioic acid (E)-, bis(2-ethylhexyl) ester (CAS n. 141-02-6) is used in paint lacquers and varnishes, in cosmetics and personal care products; it was reported as an irritant to eyes, respiratory system and skin. It was detected in sample 18C and the estimation of migration lead to the overcoming of the SML.

Diethylene glycol dibenzoate (CAS 120-55-8) is a high solvating plasticizer used for PVC, vinyl flooring and in elastomers. FDA has approved it only as component for adhesives and component of coatings (FDA 2012), in Europe considering the FDHA Ordinance, it has a SML equal to 0.01 mg kg^{-1}, that is far exceed in sample 18C.

Furan, 2-pentyl- (CAS 3777-69-3) is a flavour and fragrance agents, to the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated. However a SML of 0.01 mg kg^{-1} was decided for it by the FDHA ordinance.

3.1.3 Conclusions

The analytical screening performed on the packaging samples using simple solvent extraction coupled with GC-MS technique revealed an high content in different type of chemicals. A great lack of homogeneity among packaging samples was found. The concentration of some analytes were significantly high, therefore a deepen evaluation of the risk of migration into food was requested and at the same time the assessment of their toxicological effect.

Multivariate analysis was applied on data obtained from the analytical screening of P&B food packaging materials. Using PCA, some molecules were found characteristic of certain packaging. Some molecules, that were recognized of toxicological concern through the literature survey, could be used as markers for recycled pulp content, plastic coating or printing inks. A linkage between the materials type and their production technique was proposed. As instance, aromatic compounds, mainly toluene and DiBP or DiPNs were linked to the recycled pulp content and to the corrugated and pigmented packaging; whereas in the plastic coated samples triacetin and hexanal were identify as the prevailing compound.

From the obtained results for SPME, it could be stated that it is an interesting technique to perform screening of paper packaging samples, and for sure it reveals important information in addiction to solvent extraction data, but it needs to be optimized in order to perform a finer statistical evaluation as a multivariate analysis.

3.1.4 References

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3.2 CONTAMINATION FROM SECONDARY PAPER PACKAGING

An analytical screening was undertaken with the aim of investigating the occurrence of diisobutyl phthalate (DiBP) in polyvinyl chloride (PVC) cling films for food contact applications and its source of contamination along a converting process. Although raw plastic materials used by producers are free from phthalates and analytical evidences confirm their absence after the extrusion process, DiBP can be found in final rolls packaged into cardboard packaging during storage.
A solvent extraction GC-MS analysis was applied on several intermediate products and at different stages taken from the converting process, with the aim of identifying the source of contamination. Different cardboard cores and folding cartons made of recycled fibres were analysed and some of them resulted highly contaminated by DiBP. The storage of final cling films with these materials increased DiBP transfer into PVC.
To investigate the possible DiBP transfer mechanism from contaminated paper and adsorption by plastic materials through the gas phase, kinetic experiments were performed in a model system. Results obtained at 20, 30 and 40 °C showed a considerable uptake of DiBP into PVC; Weibull model parameters estimated from the experimental data suggested an initial rate of the process dependent on temperature. Additionally, to evaluate the partitioning behaviour, adsorption isotherms of DiBP into paper, PVC and low low density polyethylene (LLDPE) cling film were obtained at 40 °C.

3.2.1 Materials and methods

3.2.1.1 Materials

The converting process of two typologies of cling films for household and professional applications was considered and an analytical screening was carried out on selected materials collected from different steps of the converting process, as listed in Table 3.2.1.

The base materials used for the entire production of cling films are large rolls of PVC (also named jumbo rolls) that could hold several kilometres of wrapped plastic protected by a LDPE film on the external side, during transport and storage. For the sampling, jumbo rolls from two different suppliers were considered. Several meters of film were sampled from the external, medium and inner depth of the large rolls (codes A, B, C respectively from supplier 1 and D, E, F respectively from supplier 2).

The second step of the converting process is the preliminary dimensioning of the jumbo rolls to obtain intermediate rolls which are wrapped around a cellulosic core. Two intermediate rolls from the two different jumbo rolls were sampled at the inner layer (codes G and H), close to the cellulosic cores, which were also analysed (codes G_1 and H_1, respectively).

Following the converting process, the plastic film of these intermediate rolls are unrolled, cut to the proper length (about 18 m, 36 m, 50 m for household and 300 m for professional application) and width (about 0.33 m), and re-rolled onto small cellulosic cores. The rolls of cling films are finally placed inside printed folding cartons provided with a serrated edge at the opening to enable the consumer to tear off the desired length. The final products were then stacked into different board transport boxes for the shipping to retailers.

Different kinds of cellulosic material (all made with recycled fibres) are provided to the converter. In particular, folding cartons for household PVC rolls consist in a multilayer material with a printed top layer and a reverse layer made by Kraft or grey/white (g/w) paper, depending on the supplier (Table 3.2.1, codes I_1 and I_2). The cores of the household and professional cling films differ in the reverse layer made by Kraft or grey/white (g/w) paper (codes L_1-L_3). Whereas, the folding cartons for professional PVC rolls (Table 3.2.1, code I_3) are corrugated board characterized by a wall/flute/wall structure.
As final products, two typologies of cling films (household and professional), each wrapped onto cellulosic cores and placed inside their folding cartons were sampled at the last step of the converting process (Table 3.2.1, code M-N). The same products, both for household and professional application, were also sampled after a storage of about two months (Table 3.2.1, code O-P).
Considering that rolling, unrolling and re-rolling operations tend to give a slight negative charge of static electricity to the wrapped cling film, which can cause trim fragments and fibres to stick to films after slitting, dusts were also sampled from machineries (code Q). Also the adhesive used to glue the folding cartons was taken as a sample (code R).
For the sampling, the first few meters of the PVC rolls were rejected. All the materials was handled with gloves free from phthalates, each sample was put into different LDPE bags, and analyzed (code S).
Three samples of each material listed in Table 3.2.1 were picked up and all analytical measurements were carried out in triplicate.

Level	Code	Sample description	DiBP (mg kg^{-1}) +/- st dev
Base materials	A	PVC Jumbo roll from supplier 1 (external part)	Nd
	B	PVC Jumbo roll from supplier 1 (medium part)	Nd
	C	PVC Jumbo roll from supplier 1 (inner part)	Nd
	D	PVC Jumbo roll from supplier 2 (external part)	Nd
	E	PVC Jumbo roll from supplier 2 (medium part)	Nd
	F	PVC Jumbo roll from supplier 2 (inner part)	Nd
Intermediate converting materials	G	PVC intermediate roll from supplier 1	Nd
	G_1	Cellulosic core of intermediate PVC roll G	Nd
	H	PVC intermediate roll from supplier 2	Nd
	H_1	Cellulosic core of intermediate PVC roll H	Nd
Cellulosic materials for final products	I_1	Folding carton for household use (kraft)	Nd
	I_2	Folding carton for household use (g/w)	Nd
	I_3	Folding carton for professional use (multilayer)	2956 +/- 133
	L_1	Cellulosic core for household roll (kraft)	287 +/- 115
	L_2	Cellulosic core for household roll (g/w)	Nd
	L_3	Cellulosic core for professional roll (g/w)	Nd
Final product	M	PVC roll for household use	Nd
	N	PVC roll for professional use	Nd
Final product after storage	O	PVC roll for household use	78 +/- 16
	P	PVC roll for professional use	132 +/- 52
Various	Q	Dust	Nd
	R	Adhesive/Glue	Nd
	S	LDPE bags for samples recovery	Nd

Table 3.2.1 *DiBP average recovery in PVC and cellulosic materials (mg kg^{-1}) along the converting and distribution steps. Note: Nd = no detected (below LOD)*

Diisobutyl phthalate (DiBP, CAS number 84-69-5, purity ≥ 98%), all reagents and the internal standard, methyl heptadecanoate (CAS number 1731-92-6, purity ≥ 99%), were purchased from Sigma-Aldrich, Italy.

The main chemical physical features of DiBP are the follows: molecular weight 278.5 Da; boiling point 327 °C; vapour pressure (at 20 °C) $4.73*10^{-3}$ Pa; the logarithm of octanol-water partition coefficient (log K_{ow}) 4.27.

3.2.1.2 Methods

3.2.1.2.a DiBP screening in PVC cling film and cellulosic materials

Packaging samples were cut into small pieces (about 1 × 1 mm) and approximately 50 mg of paper or 100 mg of PVC films were weighted for each extraction. A solution of internal standard (methyl heptadecanoate) in heptane was added to the cut pieces, so that the amount of internal standard corresponded to 50 mg l^{-1} in the extract. Extraction was performed in a 20 ml vial with tetrahydrofuran (2.5 ml) and subsequently methanol (7.5 ml) was added providing gentle agitation for 10 min at room temperature and finally a sonication for 10 min to improve contact between solvent and sample. The vials were sealed and left for ca. 24 h in vertical position at room temperature. The recovery of the method was determined in the range 89.5–97.0%. DiBP was determined in the extract by GC-MS (Autosystem XL equipped with Turbomass, Perkin Elmer, Italy) using SIM detection. The characteristic ions m/z 149, 57, 223 for DiBP were chosen for quantitative studies. The column was Agilent DB-5MS (30 m, 0.25 mm i.d., film thickness 0.25 µm). An injection was made in the splitless mode and helium was used as carrier gas (flow rate 1 ml min^{-1}). The column temperature was held at 50 °C for 3 min, than raised at 15 °C/min to 270 °C (held for 15 min). A Merlin Microseal™ Septum Kit was installed onto the Capillary Inlet system of the GC; injections (2 µl) were performed by means of a HT300A Autosampler (HTA, Italy). Each sample injection was preceded by a blank to avoid possible memory interferences. The confirmation of DiBP identity was achieved by comparison of the observed mass spectra with those recorded in standard spectrometry libraries and by identical retention time of standard injected under the same conditions. Concentrations were determined from the calibration graph, constructed after analysis of a series of five DiBP standards (in tetrahydrofuran and methanol, 1:3) of known concentrations, in the range of 0.1-20 µg ml^{-1}, using the internal standard method. The calibration curve exhibited a linear response over the concentration range of interest and a determination coefficient equal to 0.998. The calibration levels were analyzed randomly in every sequence; the response factor of the phthalate together with the area of the internal standard peak were monitored using SIM detection. In addition, one reagent blank was analyzed in every sequence. The limit of detection (LOD) and the limit of quantification (LOQ) were established according to the ISO/IEC 17025 Guidelines, using Equations (3.2.1) and (3.2.2):

$$LOD = 3.3 \cdot \sigma B/m \quad (3.2.1)$$

$$LOQ = 10 \cdot \sigma B/m \quad (3.2.2)$$

Where σB is the standard deviation of the blank sample and m is the slope of the regression line.

The limit of detection and the limit of quantification correspond respectively to 2 and 4 mg kg^{-1} in the polymeric samples and to 4 and 8 mg kg^{-1} in the cellulosic samples, taking into account the real ratio between the weight of the sample and the volume of solvent. The relative standard deviation (R.S.D.) of the method was 0.1-2 %.

3.2.1.2.b Kinetic of DiBP transfer from contaminated paper to PVC through gas phase in a model system

A paper disk was inserted into a 0.5 l glass jar and spiked with 0.5 ml of a DiBP solution at concentrations such that the paper would contain this substance at 4000 mg kg^{-1}, assuming total incorporation. The paper disk used was obtained from household folding cartons free from phthalates, as verified with preliminary analysis; it had a diameter of 5 cm and weighed approximately 0.63 g. PVC multilayer samples were obtained from base materials, they were free from phthalates as verified via preliminary analysis, and had parallelepipeds geometry, with a surface of 16 cm^2 and weight of 0.46 g. Three PVC multilayer samples were placed inside each glass jar with no direct contact between them and the contaminated paper using a steel rack. This steel rack was placed at the bottom of the jar, it had a prism triangular shape, with a 4.5 cm side, empty in the middle to allow the three samples to stand up vertically and to face the paper disk at the same distance, about 0.5 cm, without direct contact.

The glass jars were then closed with caps (internally covered with aluminium foil to avoid interferences with the plastisol inside) and stored at different temperatures (20, 30 and 40 °C). After fixed intervals, the glass jars were opened and the PVC films taken out for extraction and DiBP quantification as previously described.

3.2.1.2.c Adsorption isotherm of DiBP at 40 °C in paper, PVC and LLDPE

Strips of paper, PVC and LLDPE cling films, with approximate dimensions 1 × 2.5 cm and an average weight of approximately 130, 140 and 100 mg respectively, were placed into 20 ml septum glass vials. The three materials used were obtained respectively from household folding cartons (code I_1), jumbo rolls (code A), and LLDPE rolls cling film obtained from the same manufacturer. They were free from phthalates, as verified via preliminary analysis. The vials were sealed with Teflon-coated septa and 1 µl of ethanol solution containing DiBP at appropriate concentrations (ranging from 10 to 150 g kg^{-1}) was injected with a five microliters Hamilton micro syringe. The solution was carefully introduced on the walls of the vial, according to the glass injection technique (Franz, 2002), in order to prevent liquid droplets from coming into direct contact with the paper strip. After the injection, the glass vials were placed inside the oven under the following optimum kinetic condition: 40 °C for 5 days. The effectiveness of this condition has been described elsewhere (Nerin & Asensio 2004). After equilibration under the desired test condition, the packaging material samples were removed and analyzed for adsorbed DiBP by the solvent extraction technique previously described.

3.2.1.2.d Statistical analysis and data modelling

The software Table Curve 2D (Jandel Scientific, version 4) was used to estimate the coefficients of the Weibull model (β, τ, C_∞) by non linear estimation.

3.2.2 Results and discussion

3.2.2.1 DiBP screening in PVC cling film and cellulosic materials

All materials collected from the converting process and described above (Table 3.2.1) were investigated. Analyses carried out at different depths of the jumbo rolls and collected from different suppliers confirmed the absence of DiBP after the extrusion process, transport and storage in the manufacturer's warehouse (Table 3.2.1; codes A-F). Furthermore, after the unwinding, dimensioning and wrapping around the cores in the converting machines (Table 3.2.1; codes G and H) as well as the respective cellulosic cores (Table 3.2.1; codes G_1 and H_1), the intermediate PVC rolls were free from DiBP.

Considering the final products (i.e. the PVC film wrapped around its cellulosic core and put inside its folding carton) picked up from the manufacturer on the same day of production, no DiBP was detected (Table 3.2.1, samples M and N). On the contrary, analyses on products stored for some weeks, revealed an important DiBP contamination (Table 3.2.1; codes O and P): the mean DiBP value measured in three different samples for household use was 78 mg kg^{-1} while those for professional use showed a content of about 130 mg kg^{-1}. Data from literature on DIBP contamination in plastic products such as cling film are quite limited but determinations of this non-authorized phthalate in package bags for biscuits were found to be in the order of 2.4 and 7.8 mg kg^{-1} (Shen, 2005).
The Bundesinstitut fur Risikobewertung (BfR – German Safety Authority) has recommended a specific restriction of migration into foods of 1 mg kg^{-1} food and suggested that German industry should agree on a common strategy to reduce and phase out the use of glues, printing inks and other products containing DiBP in order to reduce its levels in recycled paper packaging materials for food contact applications (BfR, 2007).
However, when contamination occurs from sources other than food contact materials, this has to be taken into account when testing for compliance of the food contact materials, in particular for phthalates (Reg EU 10/2011). For this reason, in our investigation in-depth studies were performed on cellulosic articles and packaging collected from manufacturers, such as cores and folding cartons for cling films that, at the time of analysis, had never been in contact with PVC (Table 3.2.1, codes I_1-I_3 for folding cartons and L_1-L_3 for cores). In fact, the highest recoveries of DiBP in literature, up to 450 mg kg^{-1}, were registered in P&B in direct contact with food, especially those made of recycled fibres (Aurela et al., 1999). In contrast, no information on the presence of DiBP in folding cartons and cores used as packaging for plastic materials (such as cling film), is at present available.

As shown in Table 3.2.1, they often presented a significant contamination of DiBP. In particular, the core for household PVC rolls, made of Kraft paper, revealed the presence of the phthalate at a level of 287 mg kg^{-1} (Table 3.2.1; code L_1), but the other type, the grey/white core, revealed its absence (Table 3.2.1; codes L_2 and L_3).

With regard to the folding cartons for the PVC roll, the household one did not present a detectable DiBP content (Table 3.2.1; codes I_1 and I_2), whereas the professional one showed the highest contamination (Table 3.2.1; code I_3), reaching about 3000 mg kg^{-1}. The possible reason for this difference is due to the structure of the materials and to the quality of the recycled cellulosic fibres. In fact, the household folding carton is a multilayer material with a top layer and a reverse layer, with a total average thickness of 0.5 mm, whereas the professional one consists of a wall/flute/wall structure, with an average thickness of 1.75 mm. This fact led to further investigations on the

structure of professional folding carton in order to thoroughly evaluate the source of DiBP contamination.

Stratigraphy of the professional folding cartons was also carried out considering both external and internal walls of the structure (coded as I_{3i}, and I_{3e} in Table 3.2.2) and the flute between them (I_{3m}). In this case, the results were expressed as mg of DiBP per kg of each single layer. As shown in Table 3.2.2 (code I_{3m}), the DiBP content in the flute reached values up to 6000 mg kg^{-1}. This high concentration is probably due to the low quality of adhesives used in the manufacture of this material but also to the efficiency of the recycling process in producing a cleaner material.

Description	**Code**	**DiBP**
Internal cover	I_{3i}	1080 +/- 180
Corrugated medium	I_{3m}	6315 +/- 55
External cover	I_{3e}	1994 +/- 511

Table 3.2.2 *DiBP content in different sections of folding carton for professional roll (mg kg^{-1})*

Since PVC cling films packaged in their final folding cartons and obtained after a storage of about two months showed a high DiBP contamination, an in-depth analysis was also carried out on these products and on the respective cellulosic materials in contact with them till analysis. In particular, the external, medium and inner layers of the PVC rolls and their respective cellulosic cores and folding cartons were analyzed. The results in Table 3.2.3 confirmed that the cellulosic materials were the source of contamination of the cling films: if the core had a high content of DiBP (as in the case of the household product), the inner layers in direct contact with it were more highly contaminated; differently, if the folding carton was the cause of contamination (as for the professional product), the external layers of PVC presented a high level of DiBP as a consequence of the transfer.
The absence of DiBP in all the other materials analyzed, including cellulosic dust formed during converting operations (code Q, Table 3.2.1), industrial glue for folding carton closure (code R, Table 3.2.1), bags and gloves used for sampling (sample S, Table 3.2.1), proved that the results were not affected by contaminating interferences.

Product size	Portion	Code	DIBP
Household	External PVC	O_1	46 +/- 13
	Medium PVC	O_2	78 +/- 16
	Inner PVC	O_3	81 +/- 31
	Folding carton (g/w)	O_b	Nd
	Core (kraft)	O_c	767 +/- 54
Professional	External PVC	P_1	206 +/- 29
	Medium PVC	P_2	132 +/- 52
	Inner PVC	P_3	29 +/- 14
	Folding carton	P_b	2228 +/- 217
	Core (g/w)	P_c	Nd

Table 3.2.3 *DiBP content in different portion of household and professional cling film picked up after a storage of two months (mg kg^{-1}). Note: medium PVC was sampled at 0.5 cm and 1 cm from core for household and professional size respectively.*

3.2.2.2 Kinetic of DiBP transfer from contaminated paper to PVC through gas phase in a model system

Since PVC base materials and intermediate products as well as the final products did not contain DiBP at the end of the manufacturing process, it can be considered a post-contaminant derived from cellulosic articles, whose source and transfer needs to be evaluated. So far, two main contamination pathways have been surmised: migration via direct contact and via gas phase transfer. In this study a possible transfer mechanism through gas phase from cellulosic to plastic materials was investigated.

It has been demonstrated that DiBP is a component of sufficient volatility to evaporate from the packaging material and re-condense into the food or simulant (Gartner et al., 2009; Aurela et al., 1999; Castle et al., 1989). Partition coefficients between paper and air and adsorption isotherms were provided in literature for a series of volatile compounds used as model substances to represent different families of contaminants commonly present in paper (Nerin & Asensio 2004; Franz, 2002; Nerin et al., 2007). Different studies have been carried out on the migration of contaminants from P&B to food even where foodstuff is packaged in a plastic wrap or when plastic lamination on the paper acts as a functional barrier (Triantafyllou et al., 2005; Nerin et al., 2007; Triantafyllou et al. 2002; Triantafyllou et al., 2007; Franz et al., 1996; Franz et al., 1997; Piergiovanni et al., 1999; Anderson et al., 2003).

However not much information is available in literature about the factors determining the equilibrium sorption of chemical substances between different packaging materials.

The procedure proposed in our study involves the use of a three phase model system (paper/gas phase/PVC), the contamination of paper with a solution of contaminant (DiBP) in order to allow measurement of the phthalate uptake by the plastic material.

The results of the experiments obtained at three temperatures (20, 30 and 40 °C) were fitted with the Weibull kinetic model (Pocas et al., 2011a; Pocas et al., 2011b), as expressed in Equation 3.2.3.

$$\frac{C_t - C_\infty}{C_0 - C_\infty} = \exp\left[-\left(\frac{t}{\tau}\right)^{\beta}\right] \qquad (3.2.3)$$

where C_t is the concentration of the DiBP into PVC sample changing with time t; C_∞ is the concentration at equilibrium and C_0 is the initial concentration of DiBP into PVC sample and equal to zero in this specific test. The parameter β describes the shape of the curve and relates to the initial rate of the phenomenon, quantifying the pattern of the curvature. The parameter τ is associated to the process rate and related to the diffusion coefficient and the material thickness.

The Weibull model can be used to describe the migration from packaging with the advantage of significant simplicity of calculation compared to Fick's 2nd law. The model has been used to describe different processes in food processing, quality and safety. Recently, it has been applied in packaging system with mass transfer processes more complex than those that simple diffusional phenomena can describe; such as the migration of components from paper-based materials (Pocas et al., 2011a; Pocas et al., 2011b).

Figure 3.2.1 shows the experimental data and the Weibull model fitting curves at the three different temperatures. In Table 3.2.4, the Weibull model parameters estimated from the experimental data are listed ($p<0.01$). Usually, in the experiments where the migration from contaminated plastics in contact with food are considered, the β values are lower than 1 ($0.5< \beta <1$) because the mass transfer is primarily controlled by the diffusion. In our system, β values obtained by the Weibull model are always higher than 1, suggesting that the overall rate is controlled by external resistance. This behaviour has been observed in migration from paper-based materials into foods (Pocas et al., 2011b). The presence of the headspace between the contaminated paper sample and the PVC creates an external resistance that controls the rate of transfer. In fact, two parallel mass transfer processes are taking place: one from the contaminated paper sample to the surrounding air and the other from the surrounding air into PVC.

T (°C)	β *	τ (hour)*	C_∞ (mg kg^{-1})*	R^2
20	2.6 *(0.4)*	136 *(8)*	147 *(5)*	0.994
30	2.3 *(0.1)*	144 *(2)*	218 *(6)*	0.999
40	1.8 *(0.2)*	136 *(9)*	194 *(7)*	0.977

Table 3.2.4 *Weibull model parameters and determination coefficients (*Standard error between the brackets)*

As reported by different Authors (Cunha et al., 2001; Blasco e al., 2006) the β parameter was found to be independent of temperature in mass transfer processes that involve drying or dehydration, and so in phenomena very different from migration. In this study it was observed that the parameter β, related to the initial rate of the phenomenon, was dependent on temperature, decreasing progressively from 40 to 20 °C, supporting the fact that the limiting step is the evaporation of the contaminant from the paper, when the headspace obstacles the direct contact between the source of contamination and the plastic.

The rate of the process (described by the τ parameter) was not affected by the temperature in the range considered. Also other Authors (Pocas et al., 2011a; Pocas et al., 2011b) highlighted that τ depends on the temperature only for lighter phthalates (with boiling points lower than 300 °C), probably because the vapour tension of the heavier substances like DiBP does not change in a significant way between 20 and 40 °C.

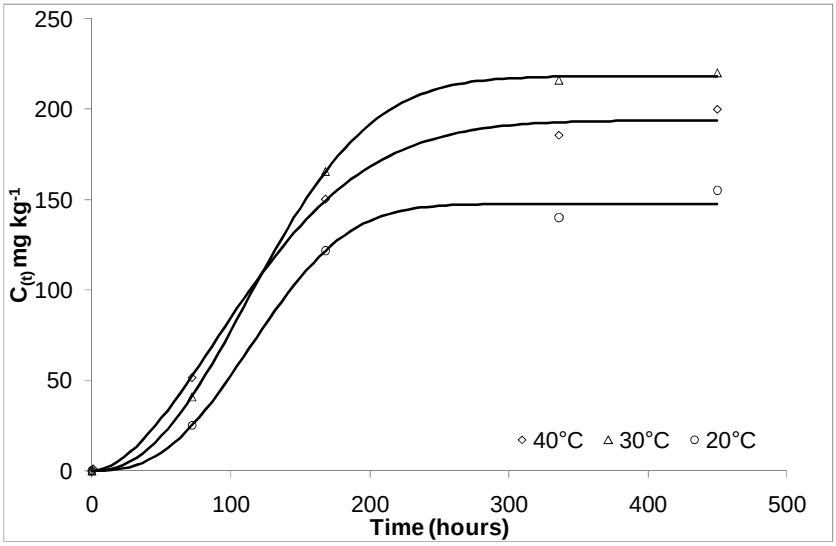

Figure 3.2.1 *Kinetic of DiBP transfer into PVC via gas phase at different temperatures*

3.2.2.3 Adsorption isotherm of DiBP at 40 °C in paper, PVC and LLDPE

The adsorption isotherms of DiBP in paper, PVC and LLDPE cling film were determined at 40 °C. In this study, also LLDPE film was considered to evaluate the partition behaviour and the affinity of the phthalate in this material, which represent a common alternative to PVC in cling film production. Figure 3.2.2 shows the curves plotted as concentration of DiBP in each material (mg kg^{-1}), determined with the solvent extraction method previously described, versus concentration in air (mg l^{-1}), calculated as the difference from the total initial concentration of DiBP generated in the vial minus the quantity that resulted from the extract analysis, as suggested by Triantafyllou et al. (Triantafyllou et al., 2007).

The adsorption data at equilibrium for the range of concentrations considered were fitted to the experimental data in accordance to the Langmuir sorption model (Equation 3.2.4), and the corresponding curves are shown in Figure 3.2.2.

$$q_e = \frac{A_{\max} \cdot k \cdot C_e}{1 + k \cdot C_e} \tag{3.2.4}$$

Where q_e is the DiBP concentration on adsorbent at equilibrium time (mg kg^{-1}); C_e is the DiBP concentration in air at equilibrium (mg kg^{-1}); A_{max} is the maximal amount of DiBP adsorbed per kg and k is an adsorption constant (kg mg^{-1}).

The Langmuir adsorption isotherm assumes that fixed individual sites exist on the surface of the adsorbent. Each of this sites is capable of adsorbing one molecule, resulting in a layer one molecule thick over the entire adsorbent surface. Once the sites are filled no further sorption will occur.

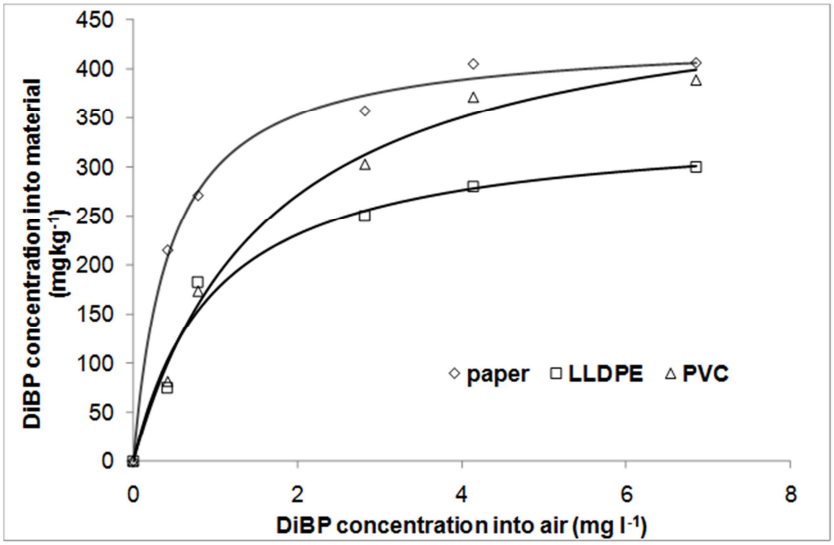

Figure 3.2.2 *Adsorption isotherm for DIBP in paper, PVC and LLDPE cling films*

The agreement between the experimental points and the model curves is satisfactory for all the materials considered, as can be seen from the determination coefficient R^2 reported in Table 3.2.5, together with the resulting values of the fitting parameters A_{max} (maximal amount of adsorbate or DiBP per kg) and k (adsorption constant).

Material	A_{max} (mg kg^{-1})*	k (l mg^{-1})*	R^2
Paper	433 *(10)*	2.2 *(0.2)*	0.99
PVC	494 *(30)*	0.6 *(0.1)*	0.98
LLDPE	344 *(25)*	1.0 *(0.3)*	0.98

Table 3.2.5 *Langmuir parameters and fitting parameters for the DiBP adsorption isotherms on paper, PVC and LLDPE cling films (*Standard error between the brackets)*

We can observe that PVC presented the highest amount of DiBP adsorbed at saturation (about 495 mg kg^{-1}), followed by paper that showed a comparable value (about 433 mg kg^{-1}). This similarity could explain the possible partitioning of the DiBP in the plastic material when it is in close contact with a contaminated medium like P&B. This situation is particularly of concern during the transport and distribution of cling film rolls, for food contact applications, inside cellulosic packaging. DiBP adsorption on LLDPE cling film also occurred, but the maximal adsorption was lower, as evidenced by the sorption isotherm in Figure 3.2.2, where the upper limit of saturation corresponded to about 340 mg kg^{-1}. The different molecular structure of this kind of polymer, which belongs to the polyolefin material group, is probably involved in the lower affinity for a contaminant like DiBP, even if further investigations are necessary.

3.2.3 Conclusions

Several interesting assumptions can be emphasized from this study:

- The screening along the manufacturing process proved to be an important instrument for the detection of possible contamination pathways of a food contact material.
- Cardboard packaging (folding cartons and cores), typically used as packaging for cling film rolls, showed high DiBP contents. This is especially true for the flute inside the professional folding carton structure and its external printed cover, which contained the greater amount of the phthalate.
- For this reason, food contact materials, such as PVC cling film, when put in contact with cardboard packaging made of recycled fibres could be contaminated by non intentionally added substances or contaminants like DiBP, even if base materials are produced in accordance with regulatory requirements.
- Kinetic transfer studies showed considerable migration potency of DiBP from contaminated paper into PVC trough gas phase at different temperatures. In addition, no considerable differences between the adsorption behaviour of this phthalate in cellulosic material and PVC could be recognized. Lower adsorption in LLDPE matrix was noticed.

In conclusion, a post-contamination of DiBP into PVC cling film, after the extrusion process, is possible being the cardboard packaging used for final products the major source of pollution. The approach presented in this study can be used by the manufacturers of primary packaging to assess the safety of own products and to select the suppliers guaranteeing the suitability of the entire packaging system for the food that will be packed. This is a case study that demonstrates the occurrence of contamination pathways for non-intentionally added substances into plastic materials used in direct contact with foods, generated by the P&B packaging, for which no specific legislative requirements or limitations are nowadays in force, even if they are indirectly related to food.

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4. Part 2

BIO-COATED PAPER AS BARRIER TO MIGRATION

In this second part of the PhD project, the aim was to evaluate the physicochemical behaviour and the barrier properties of bio-coatings against migration of typical contaminants from recycled paper packaging. Focus was directed to water-based, renewable biopolymers, such as: modified starches (cationic starch and cationic waxy starch), plant and animal proteins (gluten and gelatine), poured onto paper with an automatic applicator; additionally, a comparison with a polyethylene laminated paper was performed.

The Micro-Visco-AmyloGraphic (MVAG) test was used to highlight gelatinisation and retrogradation properties of starch gels; optical contact angle measurements and microstructural observations of the bio-coated paper allowed the characterization of obtained samples.

Partition and diffusion experiments demonstrated the validity of starch based coatings both for a polar and a non-polar compound, often present in recycled paper packaging, such as benzophenone (BP) and diisobutyl phthalate (DiBP).

Diffusion studies into the solid food simulant poly 2,6-diphenyl-p-phenylene oxide, also known as Tenax®, confirmed that all the tested biopolymers slowed down migration. The Weibull kinetic model was fit to the experimental data to compare migration from paper and bio-coated paper.

In particular, cationic starch with high amylose content seems to be the most promising choice. Gelatinisation and retrogradation properties and polarity properties of biopolymers should be considered key factors in the improvement of barrier properties of paper packaging for food contact application.

4.1 BIO-COATED PAPER DEVELOPMENT AND CHARACTERIZATION

Considering the whole classification of biopolymers (Figure 1.2), in this PhD project firstly focus was directed to polymers directly extracted from natural materials, in order to perform a screening of their ability to form coating onto paper and to behave as barrier materials against chemical migration. From the polysaccharides group starches were tested; belonging to the class of protein, wheat gluten as vegetal and gelatine as animal proteins were selected. They were prepared as coating solution following base formulation already published in literature, they were poured onto paper and paperboard sheet using an automatic applicator (lab scale). A preliminary characterization of the starch gels was obtained through the Micro-Visco-AmyloGraphic test; moreover obtained samples of bio-coated paper were studied using micrometer and microscopic analyses in order to measure thickness; optical contact angle technique was adopted to better define their surface properties.

4.1.1 Materials and methods

4.1.1.1 Materials

4.1.1.1.a Biopolymers

The following polymers were considered:

- wheat gluten
- two different starches
- gelatine

Wheat gluten powder (CAS n 8002-80-0), was purchased from Sigma Aldrich, Italy, with a minimum protein content of 80% and moisture content between 5.5 and 8.0%.

Two different starches were considered:

- cationic waxy starch
- cationic starch with high amylose content.

Both starches (HI-CAT 260® the former, HI-CAT 5283A® the latter) were supplied by Roquette - Italy (Figure 4.1.1). Both starches were from maize but different in amylose content: cationic waxy starch contained about 1% of amylose, whereas cationic starch contained about 75% of amylose.

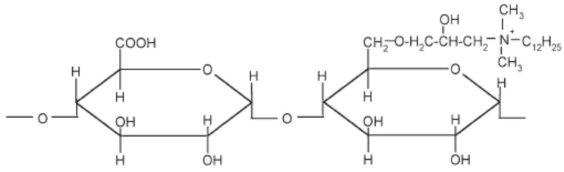

Figure 4.1.1 *Representative part of a cationic starch molecule*

As an animal protein (Figure 4.1.2), pig skin gelatine powder type A, 133 Bloom (Weishardt International, Grauliet Cedex, France) was used.

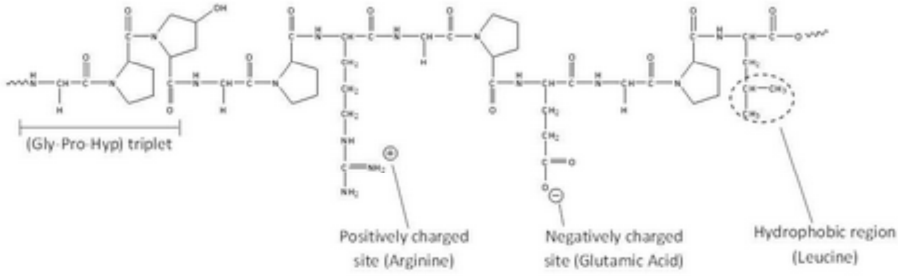

Figure 4.1.2 *Representative part of a gelatine molecule*

4.1.1.1.b Paper and board substrates

The paper substrate used for coating was of food grade quality, unprinted and provided in A4 sheet form by an Italian industrial supplier (Burgo, Italy). The paper was 100% virgin kraft grade, one side calendered and it had a grammage of 100 g m^{-2}. The paper was coated with each biopolymer onto the non calendered face and the obtained samples were used for partition and diffusion experiments. Additionally a 100 g m^{-2} virgin kraft grade paper laminated with 20 g m^{-2} polyethylene (PE) was used to make comparison with the performances of the bio-coated paper samples.

The paperboard substrate used for coating was a fully coated white lined chipboard with kraft back, made by 95% of recycled fibres and had a grammage of 430 g m^{-2}; it was provided by an Industrial producer company. In this work, it was coated onto the kraft back side with cationic starch and cationic waxy starch and it was used only for migration experiments after long time storage, without spiking procedure. In fact, previous analysis, performed using a methodology described by Guazzotti et al. (2012) allowed the determination of an initial concentration of diisobutyl phthalate (DiBP) equal to 57.4 mg kg^{-1}. The presence of DiBP in the paperboard substrate could be explained considering the recycled fibre content declared by the producer. DiBP is considered in literature (Bononi and Tateo 2007; Asensio and Nerin 2009), as many other phthalates, a typical contaminant in paper and board for food packaging; frequently it could be linked to the presence of inks and recycled fibre content.

4.1.1.2 Methods

4.1.1.2.a Coating dispersions preparation

Starch coatings were prepared by dispersion of 10% w/w cationic waxy starch (HI-CAT 260®) or cationic starch (HI-CAT 5283A®) in distilled water and heating to 95 °C for 30 min.

The gelatine coating was obtained mixing 5 g of Type A, 133 Bloom, pharmaceutical and food grade pigskin gelatine powder in 25 ml of milli-Q water, then heating to 55 °C for 20 min at 200 rpm to obtain a complete solubilisation and denaturation of the protein (Farris et al. 2011).

Wheat gluten coating solution was prepared at room temperature, according to a literature procedure (Mascheroni et al. 2010). Firstly, 20 g of wheat gluten powder was poured into an hermetic box and dispersed, under shacking, in 50 ml of deionised water containing sodium sulfite as a reducing agent of disulfide bonds. Secondly, after 30 min of settling, the solution was adjusted to 100 ml by slowly adding deionised water with acetic acid to reach a pH value of 4, under magnetic stirring.

The soy protein isolate (SPI) coating solution was prepared by dissolving 5 g of SPI in a final volume of 30 ml of water. SPI-solution was homogenized at 200 rpm for 30 min at 40 °C, with an ultraturrax (DI-25 Basic, IKA Yellow Line, Germany).

Chitosan coating solution was prepared by dissolving shellfish chitosan 2% (w/v) in 1% (v/v) hydrochloric acid solution with agitation using a magnetic stirrer at 45 °C (Vartiainen et al., 2004).

4.1.1.2.b Characterization of starch gels

The Micro-Visco-AmyloGraphic (MVAG) (Brabender OHG, Duisburg, Germany) test was used for evaluating the changes in viscosity of starch during heating and cooling. An aliquot of 10 g of starch was dispersed in 100 ml of distilled water, scaling both sample and water weight on a 14% (w/w) initial moisture basis. The temperature profile was similar to that used in the preparation of coatings: heating from 30 to 95 °C, holding at 95 °C for 5 min, cooling from 95 to 50 °C, holding at 50 °C for 5 min and cooling to 30 °C. The heating and cooling rate was 6.0 °C min^{-1}, the speed 250 rpm, and a measurement range of 300 cmg (value referred to the cartridge used, it stands for the sensitivity in terms of torque and correspond to 0.029 Nm). Viscosity was expressed in Brabender units (BU). The following indices were considered: pasting temperature (temperature at which an initial increase in viscosity occurs), peak viscosity (maximum viscosity achieved during the heating cycle), peak temperature, breakdown (decrease in viscosity during the first holding period, corresponding to the peak viscosity minus the viscosity after the holding period at 95 °C); setback at 50 °C (increase in viscosity during cooling, corresponding to the difference between the viscosity at 50 °C and the viscosity reached after the first holding period) and setback at 30 °C (increase in viscosity during cooling, corresponding to the difference between the final viscosity and the viscosity reached after the first holding period). Measurements were performed in duplicate.

4.1.1.2.c Coated paper and board preparation

The bio-based coating solutions so obtained were cooled at room temperature and poured onto the paper with an automatic applicator (Ref. 1137, Sheen Instruments, Kingston/UK) equipped with a steel horizontal rod. Coating deposition was performed according to ASTM D823-07-Practice C, 25 at a constant speed of 150 mm min^{-1}.
Coated paper sheets were dried using a constant and perpendicular flux of mild air (25 ± 0.3°C for 2 min) at a distance of 40 cm from the applicator. Before the migration experiments, all bio-coated samples were stored in a desiccator at 25°C for one week.

4.1.1.2.d Thickness determination

Average thickness of paper and board substrates and of the coated samples was determined at 25 °C and 0% RH with a micrometer (Dialmatic DDI030M, Bowers Metrology, Bradford/UK) from ten measurements taken randomly over the materials surface. Apparent thickness of the coated layers was calculated by subtracting the total thickness of uncoated paper from the total thickness of coated paper.

4.1.1.2.e Scanning electron microscopy analysis

Cross section and surface of coated and uncoated paper and board were examined using a scanning electron microscopy (SEM) LEO-1430 in order to acquire information on the homogeneity, thickness and overall physical organization of the material network. Surface test specimens (10 mm × 10 mm) were directly mounted with carbon tape on stubs. For cross sectional views, samples were fractured in a frozen state in liquid nitrogen and mounted on a specimen split mount holder. Before insertion into the microscope, samples were degassed under vacuum and gold coated (thickness of 10 nm). All micrograph were obtained using an accelerating voltage of 15 kV.

4.1.1.2.f Contact angle analysis

Contact angle analysis was performed using an optical contact angle apparatus (OCA 15 Plus – Data Physics Instruments GmbH, Filderstadt, Germany) equipped with a video measuring system with a high-resolution CCD camera and a high performance digitizing adapter. SCA 20 software (Data Physics instruments GmbH, Filderstadt, Germany) was used for data acquisition. Rectangular (5×2 cm^2) coated and uncoated paper samples were fixed and kept flat during the analysis by means of a special sample holder with parallel clamping jaws. The contact angle of water in air at 23°C and 50%RH was measured by the sessile drop method by gently placing a droplet of 3 ± 0.5 µl of liquid according to the so-called pick-up procedure (Farris et al. 2011). A minimum of 10 droplets were examined for each sample and the resulting mean θ values were measured.

4.1.1.2.g Statistical analysis

Statgraphics Plus 4.0 software (STSC, Rockville, USA) was used for the one-way ANOVA to check for differences between samples.

4.1.2 Results and discussion

4.1.2.1 Micro-Visco-AmyloGraphic properties of starch gels

Among the tested biomaterials, starch is yet widely used for packaging applications, in the sizing and coating of paper and board, as a thickener or adhesive.
The complex structure of the starch in its native form due to the fraction of the amylose and the amylopectin greatly influences the film forming properties. In fact, the linear amylose fractions in starch are able to form continuous, strong and flexible films through hydrogen bonding, while the branched amylopectin structure prevents the molecules from coming close enough to form hydrogen bonds, giving harder and more brittle films (Andersson, 2008). For this reason, the pasting properties of starch have to be investigated for papermaking application. In this study, the MVAG test was used to highlight gelatinisation and retrogradation properties of cationic waxy and non-waxy starches. The test simulated the conditions adopted during coating preparation, thus providing useful information on starch behavior during processing. The representative viscoamilographic profiles of starch samples are shown in Figure 4.1.3 and the results are presented in Table 4.1.1.
When starch is heated in excess of water, the granules absorb water and swell losing their molecular order and resulting in an increase in viscosity slope. The temperature at which the slope magnifies indicates the beginning of irreversible starch swelling and the onset of gelatinization (the pasting temperature). As well known, the conversion of starch into a thermoplastic material by extrusion or by gel casting into films leads to a loss of the natural organization of the starch polymers. During the heating step, cationic starch showed great gelatinisation properties: in particular, it exhibited lower pasting temperature and higher peak viscosity, when compared to cationic waxy starch sample. During the holding period at 95 °C, the product slurries were subjected to high temperatures and mechanical shear stress which further disrupted starch granules, resulting in amylose leaching out. At a macroscopic level, this period is commonly associated with a breakdown in viscosity, that was higher in cationic starch than in cationic waxy starch, indicating low stability during heating and mechanical stresses. The introduction of cationic groups into the starch results in a weakening of the structure due to repulsion between neighboring groups inhibiting interchain associations (Liu et al. 1999). The obtained results confirmed that the effect of cationization was particularly strong in the case of high amylose starches (Liu et al. 1999). During cooling, the viscosity increased as a result of the formation of a gel structure and re-association between starch molecules, especially amylose. Cationic starch showed a higher setback value, indicating a higher retrogradation rate than cationic waxy starch, likely due to the amylose release from the swollen granules that can re-associate to produce a strongly linked viscous paste, confirming previous studies (Liu et al. 1999).

	Pasting Temperature (°C)	Peak Viscosity (BU)	Peak temperature (°C)	Breakdown (BU)	Setback at 50°C (BU)	Setback at 30°C (BU)
Cationic waxy starch	58.8	960.5	66.4	439.0	278.5	400.5
Cationic starch	47.9	1469.5	58.3	982	359	494.5

Table 4.1.1 *Pasting properties of starch samples*

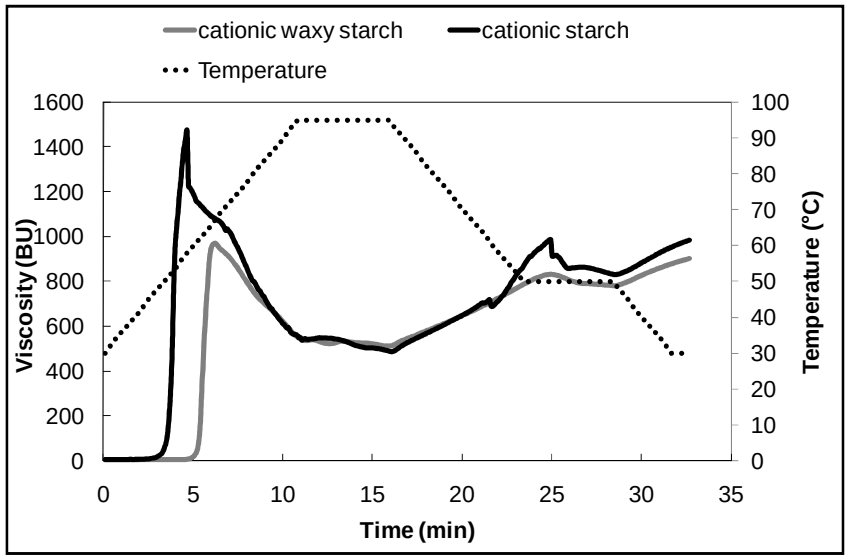

Figure 4.1.3 *Viscoamilographic profiles of starch samples*

4.1.2.1 Coating thickness

Measurements of apparent coating thickness made by micrometer are reported in Table 4.1.2. Deposition of the coating solutions onto cellulosic substrate led to a thickness increase considering all the studied biopolymers. This is due to the formation of a coated layer for which thickness is influenced by the nature of coating polymer, the solid content of coating solution and the applied wet coating thickness. The increase in the apparent coating thickness of starches was more important in the paperboard than in the paper substrate, with a decrease in the second case. It could probably be related to a higher penetration into the non calendered side of the paper rather than into the kraft back of the paperboard substrates, as already highlighted in literature (Guillaume 2010).

Materials	Total Thickness (µm)	Apparent Coating Thickness (µm)
Uncoated paper	110 ± 7	
PE laminated paper	128 ± 7	18 ± 6.6 (α = 0.05; ν = 18)
Waxy starch coated paper	121 ± 9	11 ± 6.6 (α = 0.05; ν = 17)
Starch coated paper	122 ± 8	12 ± 6.6 (α = 0.05; ν = 17)
Gelatin coated paper	125 ± 7	15 ± 6.6 (α = 0.05; ν = 18)
Gluten coated paper	126 ± 6	16 ± 6.6 (α = 0.05; ν = 18)
Uncoated paperboard	430 ± 5	
Waxy starch coated paperboard	450 ± 6	20 ± 6.6 (α = 0.05; ν = 18)
Starch coated paperboard	458 ± 5	28 ± 6.6 (α = 0.05; ν = 18)

Notes: Variability of the difference was calculated between replicates and the Welch-Satterthwaite formula was used to compute degrees of freedom (ν).

Table 4.1.2 *Total and apparent thickness of coated and uncoated paper and board samples*

4.1.2.2 Scanning electron microscopy observations

Measurements of apparent coating thickness made by micrometer were confirmed with scanning electron micrographs (SEM) of the cross sections of coated and uncoated paper (Figure 4.1.4).
A simple bi-layer structure was observed for the analysed bio-coatings poured onto paper, a continuous layer of coating material was deposited onto paper which is a porous with rough surface material. This suggested that the tested coatings were able to cover and fill the porous structure of paper. As observed on scanning electron micrographs of coated and uncoated paper surface (Figure 4.1.5a, 4.1.5b, 4.1.5c), coating with bio-polymers led to a rather smooth surface compared to a rougher and mottled appearance due to the entanglement of cellulosic fibres (Figure 4.1.5d). These observations were in agreement with those obtained from other Authors (Guillame et al. 2010). Fractures zones (Figure 4.1.5b) were clearly identified in the case of gelatin coating and pointed out a lack in integrity of the layer, consequently to permeable zones altering the barrier performances. Whereas, no fracture zones were evidenced in the case of starch and gluten coatings, suggesting that the integrity of the coating was maintained.

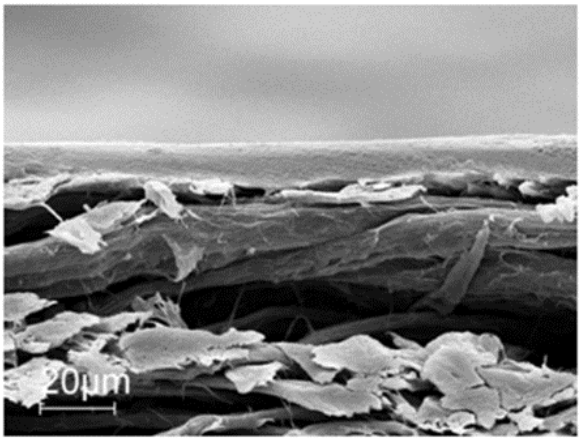

Figure 4.1.4 *Cross section view of coated paper by scanning electron microscopy*

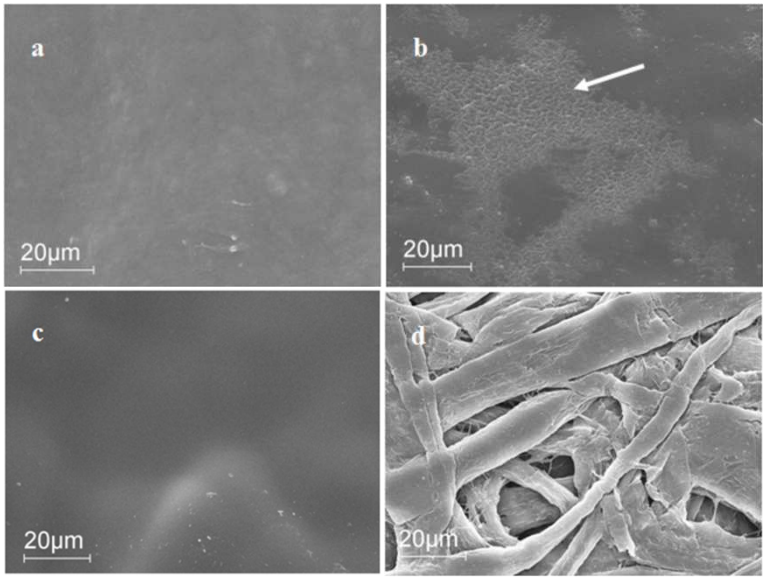

Figure 4.1.5 *Surface views of coated and uncoated paper by scanning electron microscopy: (a) starch coating; (b) gelatin coating; (c) gluten coating and (d) uncoated paper*

4.1.2.3 Contact angle measurements

Surface properties of bio-coated paper were evaluated by contact angle measurements. Results are presented in Table 4.1.3.
Initial contact angle between water and bio-coated paper varied from 71.2 to 73.8°. According to the widely accepted relationship between the contact angle and hydrophobicity (Vogler 1998), the initial contact angles suggested that all the bio-coated surfaces exhibited hydrophobicity, even if not particularly important. Moreover, ANOVA test ($p>0.05$) between replicates did not reveal significant differences between the different bio-polymers tested.
This finding highlighted a comparable hydrophilic/hydrophobic nature of the two proteins and polysaccharides studied.

	Gelatin coated paper	**Gluten coated paper**	**Waxy starch coated paper**	**Starch coated paper**
Contact angle with water (θ, °)	71.2 ± 4.0	72.0 ± 2.4	73.8 ± 4.2	72.7 ± 7.8

Table 4.1.3 *Contact angles of bio-coated paper with water*

4.1.3 Conclusions

In conclusion of this section of the research, it could be stated that the ability of different biopolymers to form coating onto paper was tested and obtained samples were characterized for further explaining their performances. Starches, wheat gluten and gelatine were selected. They were prepared as coating solution following base formulation already published in literature, they were poured onto paper and paperboard. A preliminary characterization of the starch gels, obtained through the Micro-Visco-AmyloGraphic test, allowed to discriminate their gelatinization and retrogradation properties; confirming that the effect of cationization was particularly strong in the case of high amylose starches, in fact cationic starch showed a higher setback value, indicating a higher retrogradation rate than cationic waxy starch, likely due to the amylose content.

Moreover obtained samples of bio-coated paper were studied using micrometer and microscopic analyses in order to measure thickness. The increase in the apparent coating thickness was more important in the paperboard than in the paper substrate, with a decrease in the second case. It could probably be related to a higher penetration into the non calendered side of the paper rather than into the kraft back of the paperboard substrates.

As regard SEM evaluations, a simple bi-layer structure was observed for all the analysed bio-coatings poured onto paper, a continuous layer of coating material was deposited onto paper which is a porous with rough surface material. This suggested that the tested coatings were able to cover and fill the porous structure of paper.

Finally, optical contact angle technique was adopted to better define their surface properties; they highlighted a comparable hydrophilic/hydrophobic nature of the two proteins and polysaccharides studied.

4.1.4 References

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4.2 PARTITION AND DIFFUSION STUDIES

In this section of the phD project, special aims were directed to investigate the physico-chemical behaviour of selected contaminants on chosen paper and other packaging materials, taking into account the effect of a bio-coating layer on the affinity of migrants. The partition behaviour of all possible organic compounds between paper and individual foodstuffs is difficult and too time consuming for being fully investigated. Therefore it may be more efficient to determine these partition coefficients indirectly through experimental determination using targeted substances. In this work, the behaviour of selected organic pollutants (benzophenone and diisobuytl phthalate) at 40 °C was studied. Whereas the first represents the polar migrant, the second one is a non polar, hydrophobic compound.

Partitioning studies were developed following a literature technique for determine partition coefficient of model substances between the tested material and air. Bio-coated paper, neat paper, polyethylene laminated paper and plastic polymers were compared. Afterwards, diffusion experiments into the food simulant Tenax® were performed and discussed. Moreover, a kinetic model was applied to experimental data in order to derive fundamental parameters regarding the kinetic of migration and the lag phase introduced by bio-coatings materials.

4.2.1 Materials and methods

4.2.1.1 Materials

Four biopolymers were considered: gelatine, gluten and two types of starch. See paragraph 4.1.1.1.a for details.

The substrate for coating deposition was paper or board: see paragraph 4.1.1.1.b for details.

4.2.1.2 Methods

4.2.1.2.a Partition experiments between bio-coated paper and air

From uncoated and coated paper samples, strips with approximate dimensions 1 cm × 7 cm were cut, weighted, and placed in 20 ml septum glass vials. The vials were sealed with Teflon-coated septa and one microliter of diethyl ether solution containing the mixture of each contaminants: di-isobutyl phthalate DiBP, (Sigma-Aldrich, Italy, CAS n 84-69-5, purity ≥ 98%, MW 278 Da, b.p. 327 °C, log K_{ow} 4.27) and benzophenone BP, (Sigma-Aldrich, Italy, CAS n 119-61-9, MW 182 Da, b.p. 305 °C, log K_{ow} 3.18) at seven appropriate concentrations (5, 10, 20, 40, 80, 160, 200 µg µl^{-1}) was injected with a five microliters Hamilton micro syringe, in order to obtain different initial concentrations in the gas phase (ranging from 0.25 to 10 µg ml^{-1}). The solution was carefully introduced on the walls of the vial, following a on glass injection technique, as showed in Figure 4.2.1 (Franz 2002), in order to avoid liquid droplets to come in direct contact with the paper strips. Triplicate determinations were carried out.

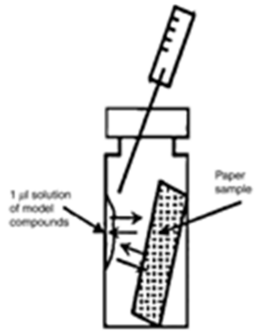

Figure 4.2.1 *On glass injection technique*

After the injection, the glass vials were placed in an oven at 40 °C for 5 days corresponding to the established condition to reach equilibration, previously used also by other Authors (Nerin and Asensio 2004) in similar experiments. To define the equilibration time, two series of vials containing an uncoated paper sample were prepared, each spiked with the lowest and the highest concentration levels of each contaminant solution. The vials were then thermostatted for different times at 40 °C. After different times (fixed in advance), the sample was removed and analysed for adsorbed surrogates as described in the following paragraph entitled "solvent extraction and GC-MS analysis". Plotting the obtained peak area against the thermostatting time, the time needed for equilibration was individuated as the time until a constant peak area was obtained (Kolb and Ettre

1997). After 3-5 days at 40 °C, a plateau was reached for the tested concentration levels and 5 days were taken as the maximum time. Partition coefficients, $K_{paper/air}$ were then calculated by equation (4.2.1).

$$K_{paper/air} = \frac{C_{paper}}{C_{air}} = \frac{C_{paper} \cdot V_{air}}{V_{paper} \cdot (C_{paper,t=0} - C_{paper})} \quad (4.2.1)$$

Where C_{paper} is the concentration of adsorbed contaminant at equilibrium, C_{air} is the concentration in headspace at equilibrium, $C_{paper,t=0}$ is a fictive value (assuming the added amount of surrogates being absorbed by the sample via spiking), V_{paper} is the volume of paper sample and V_{air} is the difference between the volume of the empty vial and the V_{paper}. This kind of procedure was already presented in literature (Triantafyllou et al. 2005; Franz 2002).

4.2.1.2.b Kinetic diffusion experiments into Tenax® using a cell

The diffusion experiment was carried out using a glass migration cell developed by FABES Forschungs-GmbH and Gaßner (Germany). It was formed by a glass beaker with an inner diameter of 6.5 cm and a height of 9 cm closed with a glass lid, hold together by a metal clamp and silicon sealant. Spiked filter paper was used as the source of contamination (donor). It was spiked by immersion for 2 h in a solution in ethanol containing DiBP and BP at a concentration of about 300 mg l^{-1} each, then it was removed and allowed to dry for 15 min. Similar type of spiking has already been presented in literature (Poças et al. 2011; Zülch and Piringer 2010). Three specimens of the spiked filter paper were analysed, after contamination and drying to verify the initial concentration of the target compounds. Each piece of material to be tested (uncoated and coated paper samples) was cut into round pieces with 9 cm of diameter and placed in a glass cell between the spiked disk of filter paper and 1 g of poly 2,6-diphenyl-p-phenylene oxide (Tenax®, Sigma-Aldrich, Italy, 60-80 mesh), as food simulant, that was distributed upwards the test material. Tenax® was cleaned up before use by solvent extraction using acetone for complete purification, according to the European standard EN 14338/2003. As regard the use of Tenax® for migration studies from paper and board, it is the most commonly referred simulant to mimic the contact with dry foods and it is already foreseen in the EU legislation for testing migration from plastics. The spiked filter paper and samples to be tested were placed such that the former faced the empty glass cell on the bottom, while the latter, covered with Tenax®, faced the upper glass cell. As a result, like in real packaging cases, the transfer of surrogates from the contaminated paper takes place both towards Tenax® through the sample and towards air to the other empty side of the cell. The test cells were stored at 40 °C for kinetic migration experiments. Triplicate determinations were carried out. A blank test cell with paper samples without any spike treatment of surrogate was also prepared as a control. At the end of the storage time the glass cell was opened and Tenax® analysed for the migrants concentration as afterwards discussed.

4.2.1.2.c Migration from non spiked paperboard

Due to the initial concentrations of DiBP in the board samples (grammage equal to 430 g m^{-2}), determined as previously explained in section “Bio-based coated paper”, these materials were not spiked with contaminated filter paper. Uncoated and starch-coated board samples were tested for migration of DiBP, initially present in the material, into Tenax® after long time storage (3 months) at 40 °C using the migration cells previously described.

4.2.1.2.d Solvent extraction and GC-MS analysis

Migrants were determined after solvent extraction from the paper strips or from the receptor Tenax®. The paper was cut into small pieces and extracted with 5 ml of ethanol, while Tenax® was extracted with 10 ml of ethanol during overnight under agitation at room temperature. After sonication for 15 min, the extracts were analysed by GC-MS. Absolute ethanol was found to give complete extraction of adsorbed contaminants under these conditions as continued extraction and repeated analyses revealed no increase in their concentrations. The recovery of the method was determined in the range of 80.5-90.5%.
Analyses were performed using a Perkin Elmer Gas Chromatograph mod. Autosystem XL, coupled to a Turbomass Mass Spectrometer. The column was Agilent DB-5MS (30 m, 0.25 mm i.d., film thickness 0.25 µm). Injection was made in the splitless mode (20 ml min^{-1} after 0.5 min) and helium was used as carrier gas (flow rate 1 ml min^{-1}). The column temperature was held at 40 °C for 2 min, than raised at 15 °C min^{-1} to 290 °C (held for 11 min). Merlin Microseal™ Septum Kit was installed on the Capillary Inlet system of the GC, injections (2 µl) were performed by means of an HT300A Autosampler. The mass spectrometer was operated in the full-scan mode, recording electron ionization (EI) mass spectra in the range of 45-450 (*m/z*) at 70 eV electron energy and 250 °C ion source and transfer line temperature. Peak areas were monitored using selected-ion monitoring (SIM) mode, target ions monitored were 57, 149, 223 (*m/z*) for DiBP and 77, 105, 182 (*m/z*) for BP, maintaining a dwell time of 100 ms for each ion. The confirmation of the substances was achieved by identical retention time and by comparison of the observed mass spectra with those of standards injected under the same conditions. Each measure obtained for samples was preceded by a blank measure to avoid possible memory interferences. Concentrations of the two substances were determined from each respective calibration graphs, constructed after analysis of a series of five standards of known concentrations. The calibration curves exhibited a linear response over the concentration range of interest and a determination coefficient equal to 0.998. The limits of detection (LOD) were established according to the ISO/IEC 17025 Guidelines15 and correspond for BP and DiBP, respectively, to 0.04 and 0.12 mg kg^{-1}.

4.2.1.2.e Statistical analysis

The kinetic migration curves and the coefficients β, τ, C_∞ were obtained by fitting the Weibull distribution function (4.2.2) to the experimental data using the software Table Curve 2D (Jandel Scientific, version 4).

$$\frac{C_{(t)}}{C_\infty} = 1 - exp\left[-\left(\frac{t}{\tau}\right)^{\beta}\right] \qquad (4.2.2)$$

Where $C_{(t)}$ is the concentration of the migrant in food simulant changing with time *t*, C_∞ is the concentration at equilibrium. The model has two parameters: τ, the system time constant, associated to the process rate, being the time required to accomplish a one log cycle (63.8%) of the process (expressed in days in our study); β is the shape parameter and relates to the initial rate of the process, quantifying the pattern of curvature observed (Cunha et al. 2001; Poças et al. 2011)). Statgraphics Plus 4.0 software (STSC, Rockville, USA) was used for the one-way ANOVA to check for differences between samples.

4.2.2 Results and discussion

4.2.2.1 Partition coefficients ($K_{paper/air}$) estimation

Partition Coefficients of chemical compounds in polymers have been broadly studied in the literature in order to provide the tools necessary to predict migration from the plastic packaging materials to the food using the appropriate mathematical models (Canellas et al. 2010). Migration from paper and board is a different phenomenon from the one occurring in plastics, due to the adsorption/desorption process of the migrant on the fibre surface; up to now only few authors reported partition coefficients for these kind of packaging materials, therefore, the retention behaviour of substances in the paper matrix is especially interesting to measure and model. Moreover, the comparison of uncoated paper with bio-coated paper allows the affinity of the materials for different substances to be recognized, thus to assess the potential barrier properties of coating layers.

Evaluation of the partition coefficient of a substance between a packaging substrate and air ($K_{paper/air}$) is considered a simple and efficient approach to measure the behaviour of organic pollutants (Franz 2002; Nerin and Asensio 2004; Triantafyllou et al. 2005, 2007). It can be intended as the ratio between the concentration in the paper sample and the concentration in the air at equilibrium. Partition coefficient can be determined at a specific temperature from the respective adsorption isotherm and it is defined as its slope (Nerin and Asensio 2004). Previous works reported the dependency of this parameter on the structure of the packaging samples, the chemical nature, especially the polarity, of both the compound and the matrices, and on the time and temperature conditions (Franz 2002; Triantafyllou et al. 2005,2007; Nerin and Asensio 2004); additionally a correlation with the Hildebrand solubility was found (Canellas et al. 2010).

In our study, adsorption isotherms at 40 °C were plotted as concentration of contaminant in paper or coated paper, C_{paper} (y-axis), expressed as mg kg^{-1} versus concentration in air after the test, C_{air} (x-axis), expressed as mg l^{-1} (Figure 4.2.2 and Figure 4.2.3). The C_{air} was calculated as the difference from the total quantity of contaminant added via spiking to each sample, $C_{paper,t=0}$ (fictive value assuming the added amount of surrogates being absorbed by the sample) minus the quantity resulted from the analysis of the paper extract at equilibrium, C_{paper} (Triantafyllou et al. 2005).

In particular, in order to calculate partition coefficients values as described in equation (1), only the initial part of the isotherm was considered (Figure 4.2.2 and Figure 4.2.3), when the relationship $C_{paper} = f(C_{air})$ was linear, therefore partition coefficient corresponded to the slope of the adsorption isotherm (Nerin and Asensio 2004).

From the obtained results, in the case of BP, the polar compound, considerable differences in partitioning behaviour between the coated and non-coated paper and air can be highlighted. Partitioning coefficients decreased significantly when paper was bio-coated, making evident that biopolymer dispersions acted as net repulsive layers (Table 4.2.1). The high partitioning into the non-coated paper can be justified by the affinity of this substance for the cellulose fibres that have an overall negative charge due to carboxyl groups from the carbohydrates and the hydroxyl groups of the lignins. Moreover, the adsorption of this polar substance decreased significantly in PE laminated paper. This finding is in agreement with the known behaviour of polar organic compound, with no good solubility in unpolar plastic like PE (Franz 2002). Interestingly, for BP strongly reduction of adsorption onto all bio-coated paper samples was observed, it was significantly lower than the one observed for PE paper.

For all the tested samples when the concentration of BP and DiBP in air increased, it also increased in paper, reaching a plateau that corresponds to saturation (Figure 4.2.2 and Figure 4.2.3). An empirical approach was adopted in order to describe the shape of each isotherms, by fitting an

equation to the experimental data with the software Table Curve 2D. It is worth emphasizing that, although in literature (Triantafyllou et al. 2005; Guazzotti et al. 2012) adsorption isotherms of similar compounds onto paper are demonstrated being of Langmuir type, poor fitting using this sorption model was obtained with our samples (data non reported), mainly due to the presence of a sigmoidal shape, highlighted at a broad range of concentration levels. Moreover, the adsorption isotherms obtained for paper samples seem to be of V type according with Brunauer theory (Brunauer et al. 1940): this is the typical behaviour of meso-porous sorbent with capillary condensation phenomena.

Considering BP, lower values of the maximum saturation were achieved when paper was coated compared to uncoated paper for which great adsorption was observed.

In literature, it was demonstrated that BP molecules interact with paper by maximizing stacking interactions between aromatic rings of BP and the apolar CH groups of cellulose (Mazeau and Vergelati 2002). The oxygen atom of the carbonyl group of BP interacts with a surface hydroxyl group of a glucose unit through a hydrogen bond; despite the obvious electrostatic character of this interaction due to the creation of a hydrogen bond, the dominant component of the interaction is the van der Waals term. The interaction is hydrophobic; however the hydroxyl groups of the cellulose chains are involved in interstrand hydrogen bonds to maintain cohesion within the crystal structure (Mazeau and Vergelati 2002).

In the case of the non-polar compound, DiBP, adsorption into the packaging material decreased only when starch or waxy starch dispersions are coated onto paper; while PE, gluten and gelatin coatings did not seem to be effective.

The presence of bio-polymers onto the paper surface creates interactions that can be either attractive or repulsive, depending on the coverage amount of molecules and how they are attached to the surface.

Protein coatings considered in this study, enhanced hydrophobic characteristics at the solid/air interface and reduced interactions between BP molecules and the surface hydroxyl group of cellulose, but they were not so effective against DiBP adsorption. In the case of gelatin, it exposes hydrophobic amino acid side chains (leucine, valine, phenylalanine, isoleucine, and methionine) (Holly et al. 1975); in the case of gluten, it exposes hydrogen bonds and covalent disulfide bonds.

Differently from the protein coatings, cationic starch onto paper lowered adsorption of both the contaminants. BP and DiBP affinity reduction could be justified by the decrease of OH groups at the surface of cellulose due to the interactions with cationic groups of starch; moreover the physical structure of starch gel could be hypothesised as another important characteristic. In fact, cristallinity properties were enhanced in starch coatings, especially with high amylose content, as a result of microcrystals and helix formation between the polysaccharide chains by hydrogen bonding during the retrogradation phenomenon (Westling et al 1998). Additionally cationic starch showed better performances than cationic waxy starch, this fact is in agreement with the higher setback value obtained for cationic starch rather than cationic waxy starch by visco-amylographic measurements (Table 4.1.3).

	Uncoated Paper	**PE laminated Paper**	**Gelatin coated paper**	**Gluten coated paper**	**Waxy starch coated paper**	**Starch coated paper**
BP	$406^{a} \pm 56$	$223^{b} \pm 106$	$80^{c} \pm 34$	$74^{c} \pm 23$	$35^{c} \pm 2$	$78^{c} \pm 37$
DiBP	$75^{a} \pm 40$	$157^{a} \pm 93$	$50^{a} \pm 25$	$80^{a} \pm 31$	$59^{a} \pm 44$	$24^{b} \pm 18$

Notes: Different superscripts within a group (i.e. within each parameter) denote a statistically significant difference ($p < 0.05$).

Table 4.2.1 *Values of partition coefficients of target contaminants between different matrices and air*

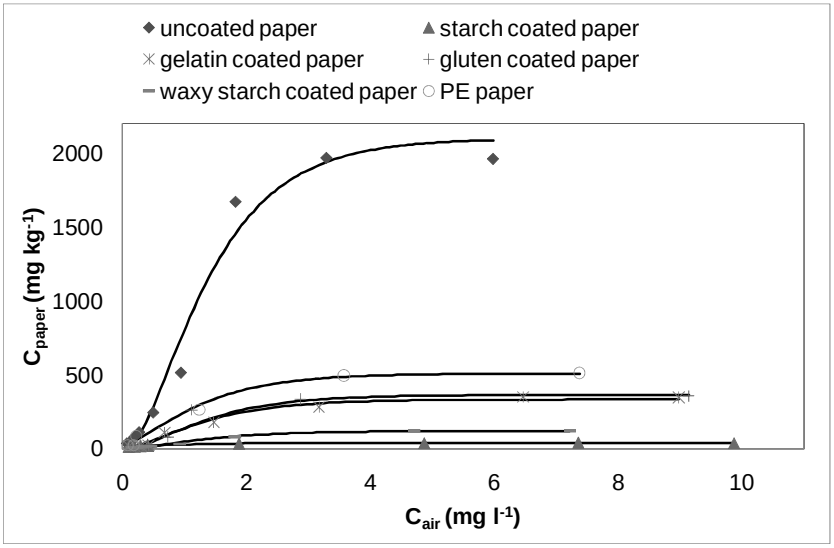

Figure 4.2.2 *Adsorption isotherms at 40°C for benzophenone in paper, PE paper, waxy starch, starch, gluten and gelatin coated paper*

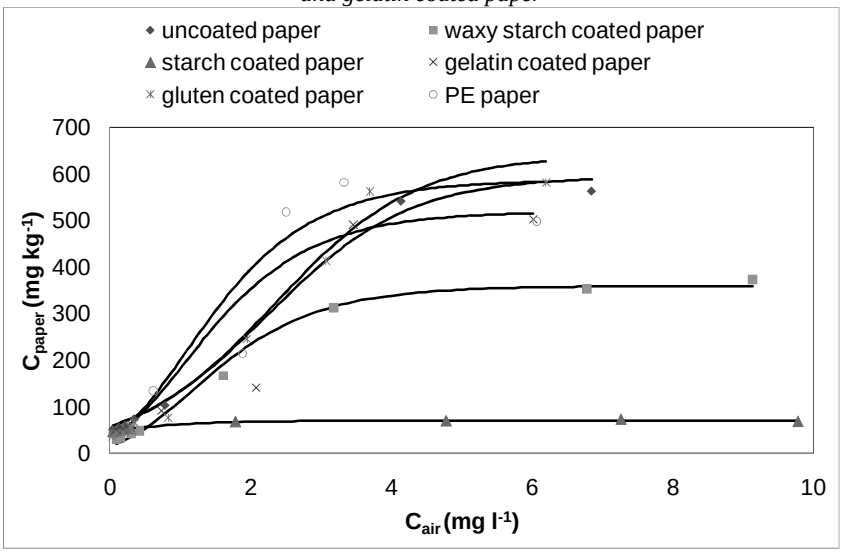

Figure 4.2.3 *Adsorption isotherms at 40°C for diisobutyl phthalate in paper, PE paper, waxy starch, starch, gluten and gelatin coated paper*

4.2.2.2 Weibull modelling of migration kinetics

The concentration of the selected migrants into the solid simulant Tenax® was determined for different times of contact during eleven days at 40 °C. The migration results are presented as the percentage relative migration over time, defined as the % ratio of concentration of migrants in the Tenax® to the initial concentration in the spiked filter paper. The Weibull kinetic model was fit to the experimental data.

The migration curves and the Weibull model fit obtained for DiBP and BP are presented in Figure 4.2.4 and Figure 4.2.5 respectively. The values of the model parameters and determination coefficients for the same migrants are presented in Table 4.2.2.

The Weibull model was found to be very effective in describing the shape of the migration curves for coated and uncoated paper. The goodness of fit was evaluated by the determination coefficient R^2.
Both for DiBP and BP higher values of migration into Tenax® at equilibrium (C_∞) were obtained when paper was uncoated if compared to the bio-coated paper. These results confirmed that bio-coatings onto paper acted as net repulsive layers towards the migrants considered in this study. In particular, for DiBP, migration at equilibrium through PE laminated paper did not differ significantly from uncoated paper, while the ones occurred from bio-coatings were significantly lower. In the case of BP, migration through PE laminated paper differed significantly from uncoated paper and from bio-coated paper. Starch coating onto paper lead to a 20% decrement of relative migration of DiBP into Tenax® compared to uncoated paper, at the tested temperature. The same coating allowed the reduction of BP migration up to 17% of relative migration.
The starch with higher content in amylose exhibited slightly better functional properties than those of amylopectin (waxy starch), especially regarding barrier to migration of DiBP. In literature, it was reported that the linear amylose and the branched amylopectin polymers exhibit different behavior with regard to gelation and development of cristallinity (Westling et al. 1998). The amylose gel was suggested to contain rigid, crystalline, double-helical junction zones connected by more mobile amorphous, single-chain segments; the gelation of amylopectin, on the other hand, is a slow process taking several weeks; thus, in the presented study, it could be hypothesized able to form totally amorphous structures, since only one week of storage was observed before experiments. For synthetic polymers, it is well known that a linear polymer crystallizes more easily than a branched polymer based on the same monomer. In the case of biopolymers further characterization analyses should be awaited in order to acquire the necessary knowledge. As preliminary evaluation, the viscoamilographic test proposed in the present work, allowed to show an higher retrogradation rate for high amylose than waxy starch (Table 4.1.3, Figure 4.1.6); it could be linked to the amylose release from the swollen granules that can re-associate to produce a strongly linked viscous paste.

The obtained values of τ are higher for bio-coated samples, indicating that the rate of mass transfer was lower than that one that occurred from uncoated or PE laminated paper. Values found for β, index determining the pattern of curvature, ranged from 1.1 to 1.7 for uncoated and PE paper whereas for bio-coated paper ranged from 2.2 to 4.9. It has been noted that the Weibull model is very effective at describing processes with atypical curve patterns by simply having β values higher than 1 (lag phase). In phenomenological terms, a diffusional process may exhibit this pattern when the equilibrium conditions are not established instantaneously at the interface. This type of shape was observed sometimes in migration from paper-based materials into solid foods (Poças et al. 2011). Interestingly, β values increased when paper was bio-coated, corresponding to the presence of an evident lag phase due to barrier properties of the tested bio-coatings and quantifying the importance of the interface resistance.

		Uncoated Paper	PE laminated Paper	Gelatin coated paper	Gluten coated paper	Waxy starch coated paper	Starch coated paper
DiBP	C_∞ **(%)**	$26.8^{a} \pm 0.5$	$27.0^{a} \pm 0.8$	$10.4^{b} \pm 0.4$	$12.8^{b} \pm 1.4$	$7.8^{b} \pm 0.2$	$5.4^{b} \pm 0.1$
	τ **(days)**	$2.7^{c} \pm 0.1$	$3.7^{cd} \pm 0.2$	$6.7^{de} \pm 0.2$	$6.6^{d} \pm 0.8$	$5.9^{d} \pm 0.2$	$8.8^{e} \pm 0.1$
	β	$1.4^{f} \pm 0.1$	$1.7^{f} \pm 0.2$	$4.6^{g} \pm 0.8$	$2.2^{f} \pm 0.5$	$2.8^{fg} \pm 0.3$	$4.9^{g} \pm 0.1$
	R^2	0.990	0.990	0.981	0.970	0.994	0.998
BP	C_∞ **(%)**	$8.2^{c} \pm 2.8$	$4.0^{b} \pm 0.6$	$1.7^{a} \pm 0.1$	$1.0^{a} \pm 0.1$	$1.4^{a} \pm 0.2$	$1.5^{a} \pm 0.2$
	τ **(days)**	$3.5^{b} \pm 2.0$	$4.8^{b} \pm 1.2$	$7.1^{a} \pm 0.3$	$6.0^{a} \pm 0.7$	$6.7^{a} \pm 1.0$	$8.0^{a} \pm 1.0$
	β	$1.1^{c} \pm 0.1$	$1.4^{b} \pm 0.3$	$3.2^{a} \pm 0.4$	$2.3^{a} \pm 1.0$	$2.2^{a} \pm 0.5$	$2.4^{a} \pm 0.4$
	R^2	0.992	0.959	0.989	0.919	0.960	0.988

Notes: Different superscripts within a group (i.e. within each parameter) denote a statistically significant difference ($p < 0.05$).

Table 4.2.2 *Weibull model parameters and determination coefficients ($\mu \pm$standard error)*

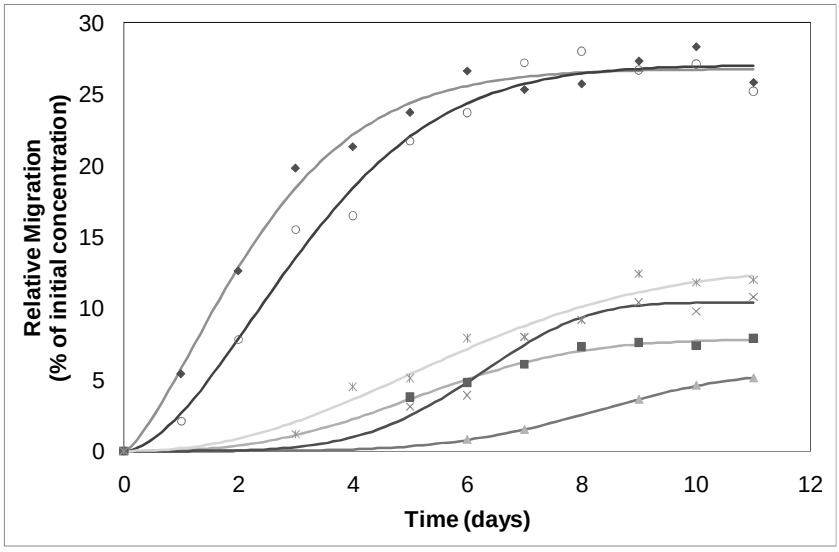

Figure 4.2.4 *Kinetics of migration at 40°C for diisobutyl phthalate in paper (♦), PE paper(○), waxy starch (■), starch (▲), gluten (*) and gelatin (×) coated paper and Weibull model fit*

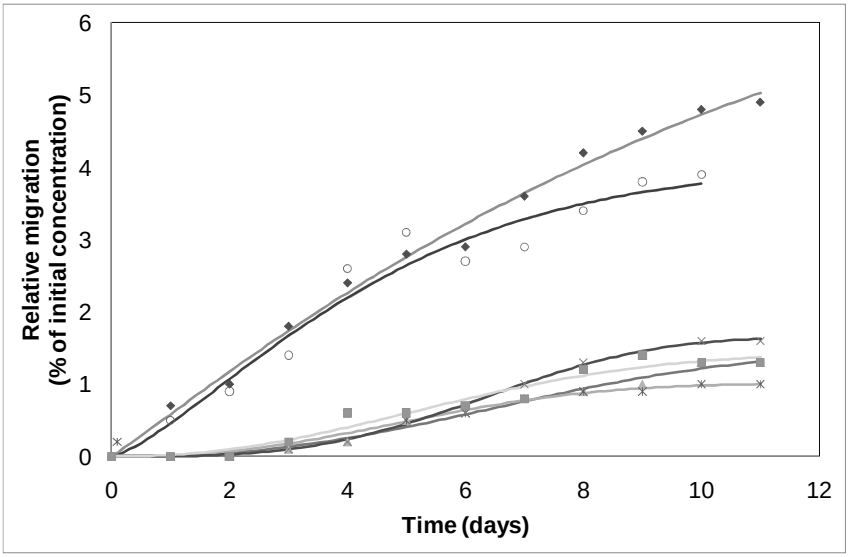

Figure 4.2.5 *Kinetics of migration at 40°C for benzophenone in paper (♦), PE paper(○), waxy starch (■), starch (▲), gluten (⁎) and gelatin (×) coated paper and Weibull model fit*

4.2.2.3 Migration from non spiked paperboard

Migration of DiBP from non spiked uncoated paperboard was compared to that obtain for the starch-coated one. Results after three months of storage at 40 °C demonstrated that cationic starch-based coatings slowed down migration of this compound that is among the contaminants, one of the most often present in paper and board packaging. For DiBP, migration into Tenax® was reduced to 20.8 and 24.6 mg kg^{-1} when starch and waxy starch coatings respectively were coated onto paperboard, while for the uncoated paperboard DiBP migration reached 47.8 mg kg^{-1}. Considering the initial content of the migrant into the material, it can be stated that in the case of paperboard, starch-based coatings slowed down relative migration of DiBP of about 50%.

4.2.3 Conclusions

In conclusion, results on the efficiency of the selected bio-based coatings against migration of typical contaminants from recycled paper were obtained for the first time in our study.
The modification of paper surface by means coating techniques is a well-known solution adopted by the paper making companies. The application of bio-coatings could be proposed for paper and paper-board used to package dry foods, like semolina, pasta, sugar, flour, milk powder, rice, salt, etc. All the bio-polymers used in this study have been previously studied for food contact applications because of their food-grade nature and their ability to increase gas barrier properties. Specifically, in our study, some different performances have been evaluated to acquire a basic knowledge about the potentiality of green alternatives towards functional barrier properties.
Partition and diffusion results demonstrated the validity of starch based coatings both for a polar and a non polar compound, often present in recycled paper packaging, such as BP and DiBP. In particular, cationic starch with high amylose content seems to be the most promising choice. Gelatinisation and retrogradation properties and polarity properties of biopolymers should be considered key factors in the improvement of barrier properties of paper packaging for food contact application. Again, the application of starch instead of protein-based coatings could avoid potential allergenic issues (like using wheat gluten coating) and could respect religious habit (if pigskin gelatine is used). In the presented paper the good fitting obtained with the Weibull model was demonstrated for DiBP and BP molecules; this information should considered promising as regard the possibility to derive the model parameter relationships, even if further data, obtained through systematic experiments for validation and expansion to other substances/conditions should be additionally performed. For final conclusions, optimization of base-formulation of starch coating, together with functional barrier testing in different conditions (different substances, simulants or foods, type of contact, type of paper, temperature and moisture) should be awaited to confirm the fulfilment of functional barrier requirements, as set for plastic materials.

4.2.4 References

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5. Part 3

MINERAL OIL MIGRATION THROUGH STARCH-COATED PAPER

In this second part of the PhD project, the aim was to improve research activity especially focused on the migration of contaminants like MOSH (mineral oil saturated hydrocarbons) and MOAH (mineral oil aromatic hydrocarbons) from paper and board for food packaging and on the evaluation of functional barrier materials, in particular bio-coatings.
Migration test series were performed under varying conditions (kinetics up to 10 days, 60 °C) using spiked model substances (n-alkanes C_{10}-C_{40}) and Tenax® as food simulant. HPLC-GC-FID system was used to analyse extracts and its relative performances were compared with an automatic permeation system. Migration testing of barrier materials, in particular bio-coatings made of starch and applied onto paper, was evaluated and discussed considering the information gained through characterization of the same materials via micro-structural observations.
Existing predictive models for migration were preliminary applied for comparison versus measured data. Finally, migration test series with real MOSH-contaminated packaging materials were developed.

This 3rd part of the PhD research project was partially developed within a ***Lifelong Learning Programme*** *"Erasmus" cooperation with the "****Fraunhofer Institute for Process Engineering and Packaging – IVV****" (Giggenhauser Str. 35 - 85354 Freising, Germany,) under the supervision of* ***Dr. Roland Franz*** *(Head of Department Product Safety and Analysis).*

5.1 Materials and methods

5.1.1 Materials

4.1.1.1.a Paper and board packaging samples

For screening purpose, five samples were analysed; they were obtained respectively from three Italian Papermaking Suppliers: "Reno de Medici", "Ghelfi Ondulati" and "Burgo Mosaico". In Table 5.1.1 their main characteristics are reported.

code	Description	Gram mage ($g\ m^{-2}$)	Thickness (mm)	Name	Company
MO-0078	Fully coated white lined chipboard with kraft back suitable for dry food	320	0.405	paperboard recycled type 962	Reno de Medici
MO-0079	Fully coated white lined chipboard with manilla back suitable for dry food	320	0.425	paperboard recycled type 963	Reno de Medici
MO-0080	Paperboard with kraft back suitable for dry food	135	0.300	paperboard 100% virgin	Ghelfi Ondulati
MO-0081	One side machined glazed white paper suitable for dry food	50	0.060	paper G50	Burgo Mosaico
MO-0082	One side machined glazed kraft paper suitable for dry food	50	0.060	paper raw G50	Burgo Mosaico

Table 5.1.1 *Main characteristics of paper and board packaging samples*

A representative sample of each paper or board in triplicate was cut to pieces of no more than 2 cm edge length, followed by homogenisation in an adequate inert glass vessel.
Two grams (± 0.1 g) of the homogenised paper or board sample were weighted into a 70 ml screw cap glass test tube (with PTFE septum). Twenty µl of the MOSH/MOAH standard solution 150-600 µg ml^{-1} each in toluene (Restek, USA) and 10 ml ethanol/hexane (1:1; v/v) were added. The tube was vigorously shaken, then allowed to stand at room temperature for 2 hours. Prior to sampling the extract, the tube was agitated over again. In order to remove the ethanol, approximately 4 ml of the extract were taken and shaken with 10 ml water. Finally, an aliquot of the supernatant hexane phase was taken and placed in a vial for auto sampler in order to perform the HPLC-GC-FID analysis.

5.1.1.b Solvents and reagents

All the solvents and reagents used in this study were purchased from Sigma-Aldrich, Germany and Restek, USA.

5.1.1.c Starches and other ingredients

Starch coatings were obtained from maize cationic waxy starch (HI-CAT 21370®, Roquette, Italy) or maize cationic starch (HI-CAT 260®, Roquette, Italy) or cationic starch mixture with high amylose content based on cereal and tuber starch (HI-CAT 5283A®, Roquette, Italy). Additionally, an aqueous solution 70% of D-sorbitol (Neosorb 70/70®, Roquette, Italy) was used to obtain plasticized coatings.

5.1.2 Methods

5.1.2.a HPLC-GC-FID analysis

Extracts were analysed by online normal HPLC-GC-FID as described by Biedermann et al. (2009), Biedermann and Grob (2012a and b).

Briefly, 50 µl extract were injected into a 250 x 2 mm i.d. HPLC silica gel column (Luna 5u Silica 2 100A, Phenomenex) with a flow rate of 300 µl min^{-1} using a gradient starting with 100% hexane and reaching 30% dichloromethane. HPLC-GC transfer occurred by the retention gap technique and partially concurrent eluent evaporation. A 7 m x 0.53 mm i.d. uncoated, deactivated precolumn was followed by a steel T-piece union connecting to the solvent vapour exit and a 15 m x 0.25 mm i.d. x 0.25µm separation column (Rxi-5Sil MS, Restek). From HPLC, MOSH were eluted between 2.0 and 3.5 min, MOAH between 4.0 and 5.5 min. GC oven temperature was programmed from 65 °C (6 min) to 350 °C at the rate of 20 °C min^{-1}.

Single analyses were performed using an Axel Semrau on-line coupled LC-GC system (Figure 5.1.1). The LC system (HP) was equipped with a UV lamp (Gynkotek), and the GC was by Dani. Data were processed using Chronos 3.5 software by Axel Semrau.

Figure 5.1.1 *Coupled LC-GC system used at Fraunhofer IVV*

The MOSH/MOAH standard solution 150-600 µg ml^{-1} each in toluene (Restek, USA) was used for quantification and normalisation of GC-related variations, Bicyclohexyl (92-51-3) or Cholestane (481-21-0) (added with the Restek standard) were used as non-interfering internal standards. The

internal standards have two functions: measurement of the target material and verification of method performance.

Firstly, blanks and diluted solutions obtained in hexane (Fluka) were analysed with coupled HPLC-GC-FID, in order to create a calibration curve to be saved into the software.
Compositions of the Standard Solution Restek (MOSH/MOAH, 2 ml in Toluene) and of the obtained dilutions are reported in Table 5.1.2.

MOSH/ MOAH	**Compound name**	**Standard Solution (µg ml^{-1})**	**dilution 1:5000 (ng ml^{-1})**	**dilution 1:2500 (ng ml^{-1})**	**dilution 1:1000 (ng ml^{-1})**	**dilution 1:500 (ng ml^{-1})**
MOSH	n-undecane (C11)	300	120	150	200	600
MOAH	n-pentylbenzene	300	120	150	200	600
MOSH	n-tridecane (C13)	150	60	75	100	300
MOAH	1-methylnaphathalene	300	120	150	200	600
MOSH	bicyclohexyl	300	120	150	200	600
MOAH	2-methylnaphthalene	300.2	120.08	150.08	200.08	600.4
MOAH	1,3,5 - tri-tert-butylbenzene	300	120	150	200	600
MOSH	cholestane	600	240	300	400	1200
MOAH	perylene	600	240	300	400	1200

Table 5.1.2 *Compositions of the Standard Solution RESTEK and of the obtained dilutions*

5.1.2.b Cleaning of Tenax® with Accelerated Solvent Extractor

Accelerated solvent extraction (ASE) was the technique used for regenerating Tenax® before usage. It combines elevated temperature and pressures with liquid solvents to achieve fast and efficient removal of analytes from the matrices.
For an efficient extraction, the solvent must be able to solubilise the target analytes while leaving the sample matrix intact. The polarity of the extraction solvent should closely match that of the target compounds. Mixing solvents of differing polarities can be used to extract a broad range of compound classes.

For extracting Tenax®, ASE 300 DIONEX was used (Figure 5.1.2) as solvent Ethanol and a mixture of Hexane/Ethyl Acetate 56:44 were used.

Figure 5.1.2 *ASE system*

The method parameters used for cleaning Tenax®, are reported in Table 5.1.3.

Oven temperature	110 °C Ethanol – 100 °C Hexane/Ethyl Acetate
Flush	120 sec
Purge Time	100 sec
Cycles	3
Pressure	100 bar

Table 5.1.3 *ASE parameters*

After this cleaning Tenax® was collected in an Erlenmeyer flask and allowed to dry in a ventilated oven at 160 °C for 6 hrs.

5.1.2.c Development of starch coated paperboard

Starch coatings were prepared as 10% w/w dispersion in distilled water. After heating to 95 °C for 15 min, the bio-based coating solutions so obtained were cooled at room temperature and poured onto each type of selected paper (MOSH contaminated and MOSH free, respectively sample MO-0079 and MO-0080) with an automatic applicator (Ref. 1137, Sheen Instruments, Kingston/UK) equipped with a steel horizontal rod. Coating deposition was performed according to ASTM D823-07-Practice C, 25 at a constant speed of 150 mm min^{-1}, using a suitable steel rod to obtain a wet coating thickness equal to 50 µm.

Coated paper sheets were dried using a constant and perpendicular flux of mild air (25 ± 0.3 °C for 2 min) at distance of 40 cm from the applicator. Before the migration experiments, all bio-coated samples were stored in a desiccator at 25 °C for one week.

Additionally, following the same procedure, the same starch coatings lab made were prepared with addition of sorbitol as plasticizer. In this case an aqueous solution 70% of D-sorbitol (Neosorb 70/70®, Roquette, Italy) was used to reach a final concentration equal to 10% w/w.

Table 5.1.4 summarizes the descriptions and the codes used for the identification of the samples..

Code	Description
A	maize cationic waxy starch
B	maize cationic starch
X	cationic starch mixture with high amylose content
AS	maize cationic waxy starch + sorbitol
BS	maize cationic starch + sorbitol
XS	cationic starch mixture with high amylose content + sorbitol

Table 5.1.4 *Descriptions and codes for the different starches used as coatings*

5.1.2.d Scanning electron microscopy observations

Cross section and surface of coated and uncoated paper and board were examined using a scanning electron microscopy (SEM) ISIABT-55 in order to acquire information on the homogeneity, thickness and overall physical organization of the material network.

Surface test specimens (5 mm × 5 mm c.a.) were directly mounted with tape on iron stubs. For cross sectional views, samples were cut with a ceramic blade or with a microtome.

Before insertion into the microscope, samples were degassed under vacuum (20 minutes) and gold coated (3 minutes).

All micrograph were obtained using an accelerating voltage of 3 kV.

Additionally, elemental analyses were obtained through an Energy Dispersive Spectrometer (EDS) an accelerating voltage of 23 kV in order to acquire information of the elements pattern of particular zones and particles.

5.1.2.e Migration kinetics of n-alkanes through spiked neat and coated paperboard

A migration test scheme was performed, considering the possibility of studying the migration kinetics at 20, 40, 60 °C of n-alkanes from a spiked donor (blue paper, industrial towel 60 g m^{-2}, provided from Würth, Germany) throughout a virgin white kraft paperboard 135 g m^{-2} (sample code MO-0080 in Table 5.1.1) into the receptor Tenax®, placed in direct contact with the paperboard; the experiments were carried out using glass migration cells (MigraCell – Fabes & Gaßner, Germany) in thermostatic ovens. The substances were chosen as representatives for MOSH.

After each time-temperature conditions point each cell was opened, both blue paper and Tenax® were extracted following the BfR methodology for quantification of alkane's residue or migration,

respectively in the donor and receptor. Quality control parameters, such as blanks, recovery and LDR were evaluated.

More in detail, time dependant investigations (kinetic measurements) were carried out in triplicate at:

- 60 °C after 4, 5, 15, 24, 48 hrs.
- 40 °C after 3, 5, 15, 24, 48, 72, 96, 120 hrs and 10 days.
- 20 °C after 5, 10, 15, 24, 60, 90 days.

Glass migration cells were prepared fixing a 0.40 dm^2 round piece of paperboard between a spiked 0.40 dm^2 round piece of blue blotting paper and covered with 2 g of the food simulant Tenax®, previously cleaned with ASE, on its food contact side.

The blue paper was spiked with 100 µl of a mixture of all even n-alkanes from C_{10} to C_{40}, 50 mg l^{-1} in n-heptane each using a micro syringe and allowed to dry under a fume-hood for 3 min.

After each time-temperature exposure´s condition, blue paper and Tenax® were collected and weighted into a 70 ml screw cap glass test tube (with PTFE septum). Twenty µl of the MOSH/MOAH standard solution 150-600 µg ml^{-1} each in toluene (Restek, USA) was added with a micro syringe (2x10µl) and 10 ml ethanol/hexane (1:1; v/v) were added. The tube was vigorously shaken, then allowed to stand at room temperature for 2 hours in the case of paper extract or 24 hours in the case of Tenax® extract. Prior to sampling, the tube was agitated over again. In order to remove the ethanol, approximately 4 ml of the extract were taken and shaken with 10 ml water. Finally, an aliquot of the supernatant hexane phase was taken and placed in a vial for auto sampler in order to perform the HPLC-GC-FID analysis.

Blank evaluations of Tenax® and blue paper were performed in triplicate.

Determination of the total initial concentration of alkanes spiked in the blue paper (donor) using the described procedure was evaluated by performing 5 extractions of spiked of 0.40 dm^2 round piece of blue blotting paper.

Additionally, following the same procedure, migration experiments were developed for each type of starch-coated paperboards (see Table 5.1.4), in order to investigate their potential barrier properties against MOSH.

In these cases, time dependant investigations were carried out in triplicate at 40 °C after 3, 10 and 12 days.

5.1.2.f Permeation analyses with automated method

Fifteen model substances were chosen according to their physical and chemical properties; n-alkanes from dodecane up to tetracosane were used as representatives for MOSH.
One to three aromatic ring compounds were used to represent the MOAH compounds. To involve also substances typically used in printing inks, also DIPN and 2,2,4-trimethyl-1,3-pentanediol-di-iso-butyrate (TXIB) were included into the list of substances.

For contaminating the cardboard used for the permeation testing, a 1000 ppm solution of each of the substances in diethylether was prepared. A disc of the cardboard with 15.4 cm in diameter was soaked with 4 ml of this solution. After evaporation of the diethylether just by waiting a few minutes at room temperature, a concentration of approximately 750 mg kg^{-1} of each substance resulted in the cardboard.
Coated paperboard samples were placed in special permeation cells made of aluminium and 15.7 cm in diameter. On both sides, a sealant ring was embedded into the aluminium cell. The test materials were clamped between both sealant rings. In the lower space of the cell, the spiked cardboard was placed. The tested coatings had direct contact with the cardboard. Samples A, B and X (see Table 5.1.4) were investigated. Duplicate analyses were observed.

The upper side of the cells was rinsed with pure nitrogen. The constant nitrogen flow moved the permeated substances out of the cell. The nitrogen stream was analysed for the substances by using a 16-position valve that passes the gas stream of each cell one after the other to a pre-trap with a connected enrichment unit and gas chromatograph with flame ionization detection (Ewender et al. 2012).

Permeation was measured at a constant temperature in a climate chamber of 40 °C.
As regard gas chromatographic conditions; column was EQ 5; length: 30 m; internal diameter: 0.25 mm; film thickness: 0.5 mm; carrier gas is 120 kPa helium. Temperature programme: 90 °C (2 min); rate 10 °C min^{-1} to 180 °C; rate 20 °C min^{-1} to 320 °C hold for 9 min (total 27 min). Pre-trap: substances collected at room temperature on 20 mm length by 5 mm diameter of 10% OV 101 on Supelcoport, desorbed at 320 °C. Main trap: substances focused at - 45 °C on 20 mm length by 1.4 mm diameter of 10% OV 101 on Supelcoport, desorbed at 340 °C.

5.1.2.g Migration testing from real contaminated packaging

After considering the migration behaviour of n-alkanes as MOSH likes compounds, using the spiking procedure in order to simulate a donor phase, further studies were conducted focusing on real contaminated packaging materials. They were recycled paperboard samples for contact with dry foodstuffs (sample code MO-0079 in Table 5.1.1) in which an average content of MOSH equal to 486 µg g^{-1} was detected via HPLC-GC-FID analysis.

Single sided migration investigations were performed by means of the same type of glass migration cells as previously described with Tenax® as food simulant. They were provided with a metal lid at the bottom and 2 g of Tenax® on the upper side (in direct contact with the neat or the coated sample); migration tests were performed in triplicate at 40 °C for 3 days for neat paper and lab scale coated paper samples.

After the experiments, both paperboard and Tenax® were extracted and analysed for quantification of MOSH hump residual or migration respectively, using the HPLC-GC-FID system.

5.2 Results and discussion

5.2.1 MOSH content in paper and board packaging samples

From the blank analyses of a series of hexane (Fluka) tests, no interferences were observed, both for MOSH (Figure 5.2.1) and MOAH (Figure 5.2.2) analyses.

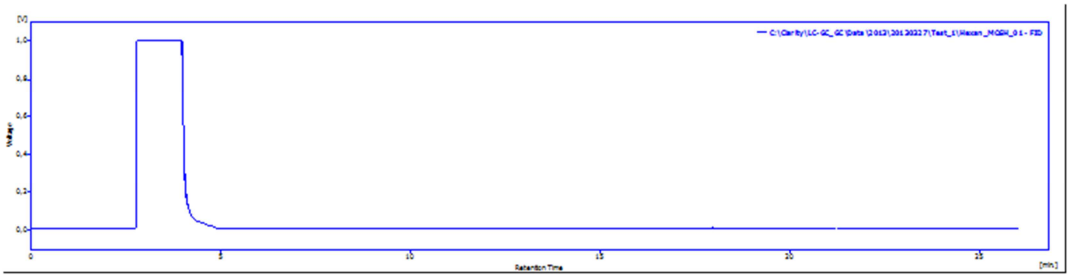


Figure 5.2.1 *Blank analysis for MOSH*

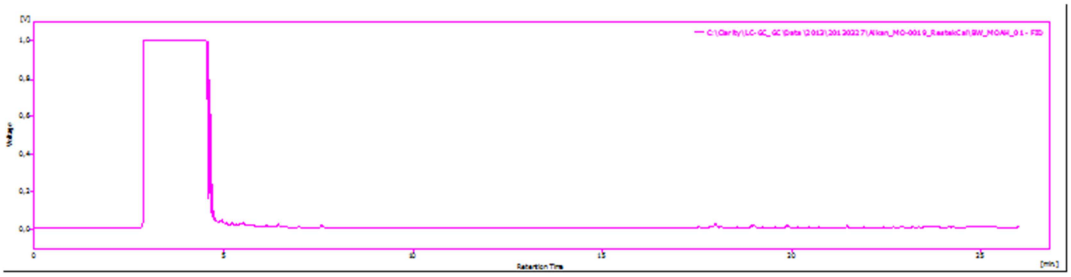


Figure 5.2.2 *Blank analysis for MOAH*

As example, GC chromatograms obtained for the standard solution Restek (150-600 µg ml^{-1}) are presented with integration of MOSH (Figure 5.2.3) and MOAH (Figure 5.2.4) standard peaks.

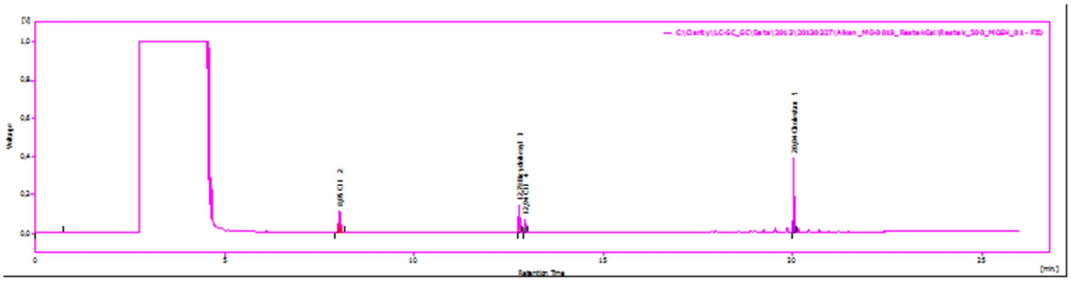


Figure 5.2.3 *Integration of MOSH standard compounds chromatogram*

The following standards are relevant for the MOSH analysis (from BfR Methodology):

- Bicyclohexyl (Cycy) is the main internal standard. It does not occur in relevant amounts in mineral oils. In the presented GC chromatogram it showed a retention time equal to 8.05 min.

- n-Tridecane (n-C13) ensures the separation from Cycy: coeluted n-C13 from the sample would increase the peak area for Cycy and, hence, result in too low concentrations calculated for the target material.
- The even more volatile n-undecane (n-C11) serves as a guard: if volatiles were lost during sample evaporation or injection, the losses would be higher for n-C11 than for Cycy. Hence, the peak area of n-C11 should not be significantly reduced compared to Cycy. However, the area of Cycy may also be increased due to co-elution with a sample component.
- For samples without an overcrowded region around n-C28, also cholestane (Cho) can be used for verification of the Cycy area, but Cho primarily serves the verification of the separation between the MOSH and MOAH.

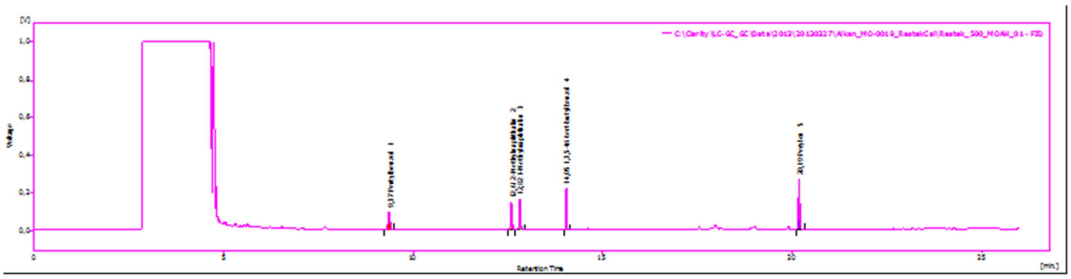


Figure 5.2.4 *Integration of MOSH standard compounds chromatogram*

As regard the MOAH fractions, the following standards are relevant for the MOAH analysis:

- 1- and 2-methylnaphthalene (1-MN and 2-MN) are the main internal standards added at the same concentration. They are easily recognized as a closely eluted pair. They should have identical areas. Otherwise, the larger peak is co-eluted with a sample component, i.e. the smaller peak should be used for calculation.
- Pentylbenzene (5B) is utilized as a guard for loss of volatile substances (analogous to n-C11).
- 1,3,5-Tri-tert-butylbenzene (TBB) assures the MOSH/MOAH separation (start of the MOAH-fraction).
- Perylene marks the ending of the MOAH fraction and assures the retention strength of the liquid chromatography.

As regard the handling of the obtained chromatograms, a substantial part of the integration, such as the positioning of the baseline and the integration of peaks to be subtracted, had to be carried out by the usage of Chronos 3.5 software by Axel Semrau. The baseline from a blank chromatogram was transferred into the chromatogram of a sample. Ideally, a horizontal line can be placed to the lowest point in the chromatogram, either before or after the elution of the MOSH/MOAH fraction. Subsequently, integration of the total areas by mass ranges had to be performed and subtraction of shoulder peaks from components naturally occurring in foodstuffs and components not belonging to the MOAH-fraction (e.g. diisopropylnaphthalene; DIPN) and subtraction of internal standards from the total area.

Finally, calculation of the concentrations was achieved using the formula (5.2.1) and the internal standard known concentrations, as the FID response of the MOSH and of the MOAH is approximately equal and the FID response is linear (verified by means of calibration).

$$Conc.\,Paper_{MOSH\ or\ MOAH}(ppm) = \frac{(Area_{MOSHorMOAH})\cdot 3ppm}{Area_{Internal-Standard}} \qquad \textbf{(5.2.1)}$$

Figures 5.2.5, 5.2.6, 5.2.7, 5.2.8, 5.2.9 report the chromatographic results for integration of MOSH humps, respectively for samples MO-0078, MO-0079, MO-0080, MO-0081and MO-0082.

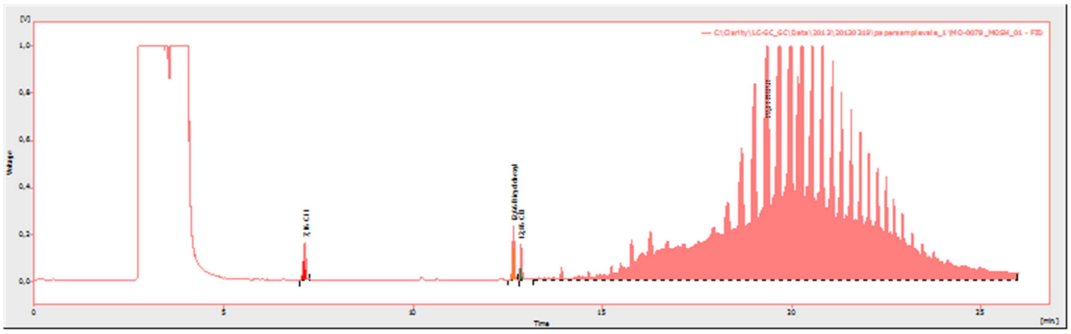


Figure 5.2.5 *Integration of MOSH hump for sample MO-00778*

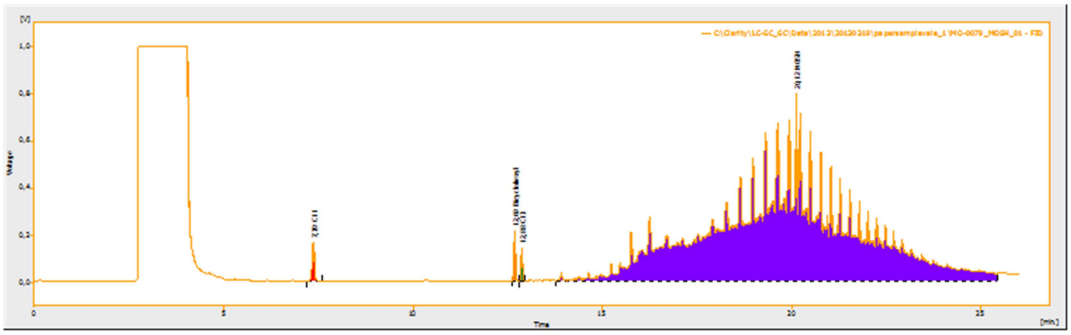


Figure 5.2.6 *Integration of MOSH hump for sample MO-00779*

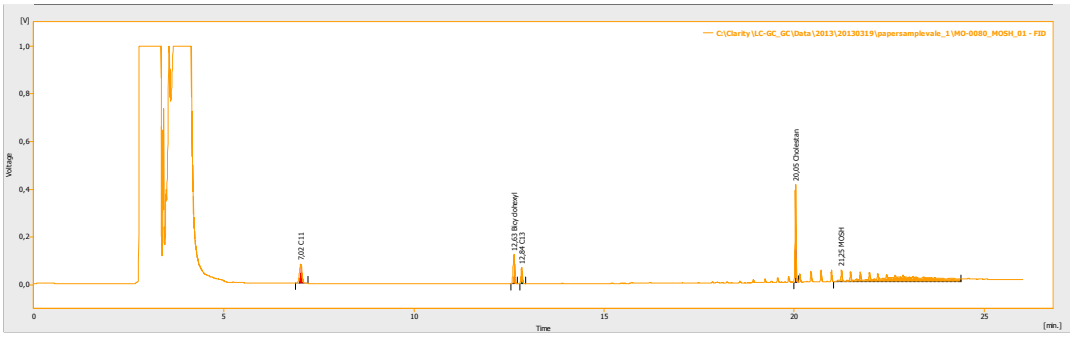


Figure 5.2.7 *Integration of MOSH hump for sample MO-00780*

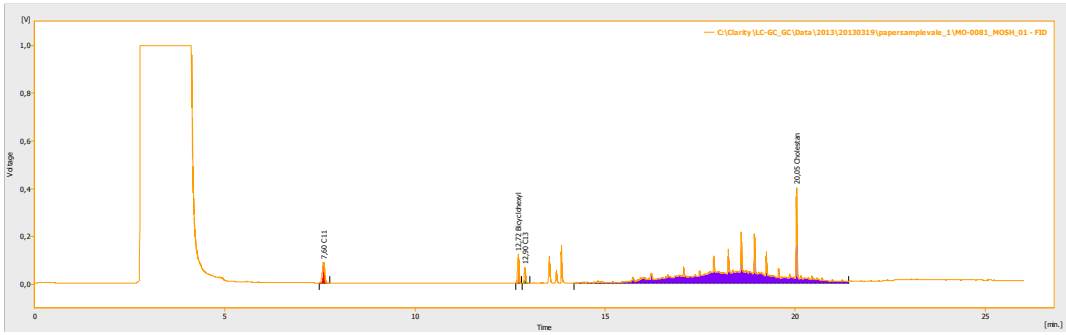


Figure5.2.8 *Integration of MOSH hump for sample MO-00781*

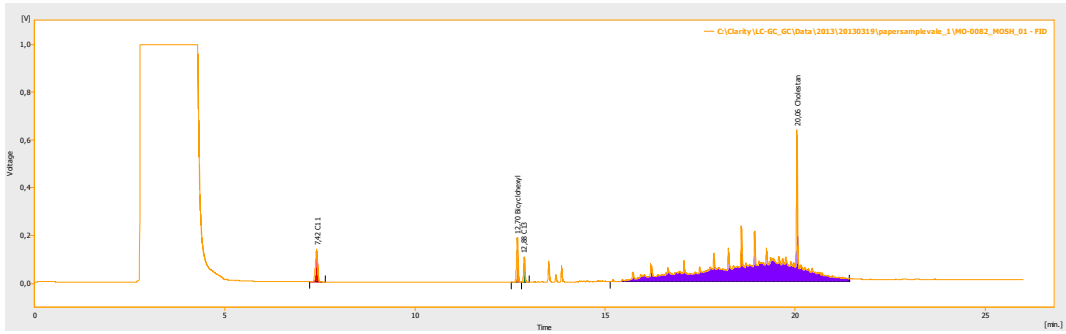


Figure 5.2.9 *Integration of MOSH hump for sample MO-00782*

Table 5.2.1 reports the concentration found for MOSH calculated from the integration of each whole hump of largely unresolved peaks and successive subtraction of internal standards and shoulder peaks from component not belonging to the MOSH fraction. Figure 5.2.10 shows integration of subtracted peaks, as an example for sample MO-0078).

code	MOSH (µg g^{-1})
MO-0078	484.37 ± 13.21
MO-0079	486.53 ± 10.21
MO-0080	< LOQ
MO-0081	84.95 ± 7.51
MO-0082	91.57 ± 9.271

Notes: Limit of quantification (LOQ) was estimated considering 10 time the S/N ratio.

Table 5.2.1 *Concentration of MOSH in the analysed samples*

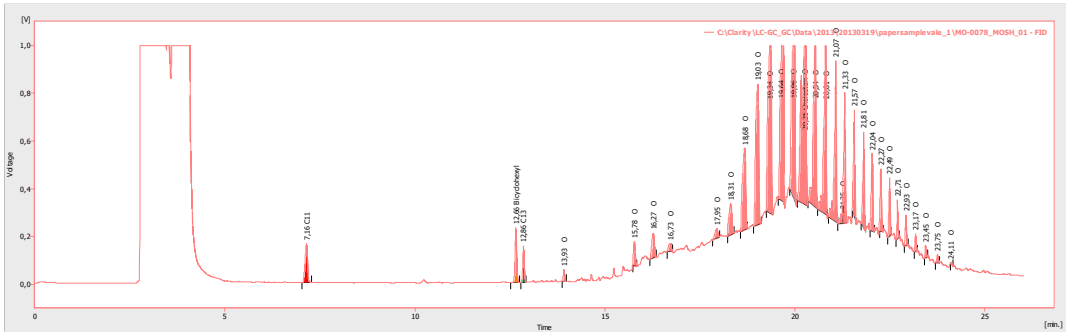


Figure 5.2.10 *Integration of subtracted peaks for sample MO-0078*

From the obtained results it can be stated that MOSH were identified in all the paper and board samples. Paperboard from recycled fibres, such as MO-0078 and MO-0079 resulted highly contaminated, reaching values above 480 µg g^{-1} in both cases, moreover paper from virgin fibres, MO-0081 and MO-0082, revealed a lower content of MOSH, close to 90 µg g^{-1} in both cases. It could be supposed that these materials went through a possible post contamination, occurred during storage in carton boxes for long time.

Among these paperboards, sample MO-0079 was selected to perform real migration studies of MOSH.

On the contrary, sample MO-0080, a paperboard made of virgin fibres, showed an integrable hump above C24 and it could be considered not related to MOSH but to naturally occurring hydrocarbons, such as odd numbered n-alkanes starting from C21.

GC-MS analyses would be performed in the future in order to clearly identify these substances.

This kind of paperboard sample MO-0080 was selected for kinetics studies using spiking substances.

5.2.2 Scanning electron microscopy observations of starch-coated paper

In order to characterize the overall physical appearance of the obtained coated paperboard samples, Scanning Electron Microscopy (SEM) observations of surfaces and cross sections were performed.

Deposition of the bio-polymer solutions onto cellulosic substrate led to different surface appearance, in dependence of the type of coating, as observed on scanning electron micrographs. Generally, coating with bio-polymers led to a rather smooth surface compared to a rougher and mottled appearance due to the entanglement of cellulosic fibres. These observations were in agreement with literature data (Guillame et al. 2010). Any fracture zones were evidenced in the case of lab scale coated paperboard, suggesting that the integrity of the coating was maintained (Figures from 5.2.11 to 5.2.17).

Differences could be pointed out considering the type of starch and additional ingredients used in the formulations.

In the case of coated paperboard B (Figures 5.2.11, 5.2.12, 5.2.13), a mottled appearance of the surface, due to the entanglement of cellulosic fibres, could be observed, even if bio-polymers deposition led to a rather smooth surface compared to a rougher surface of raw paperboard. This observation was clearly identified in detailed images of samples (Figures 5.2.11).

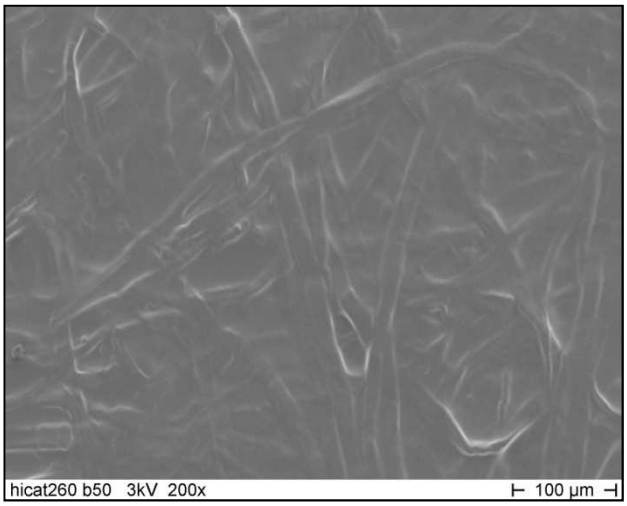


Figure 5.2.11 *SEM observation for surface of coated Paperboard B (detail)*

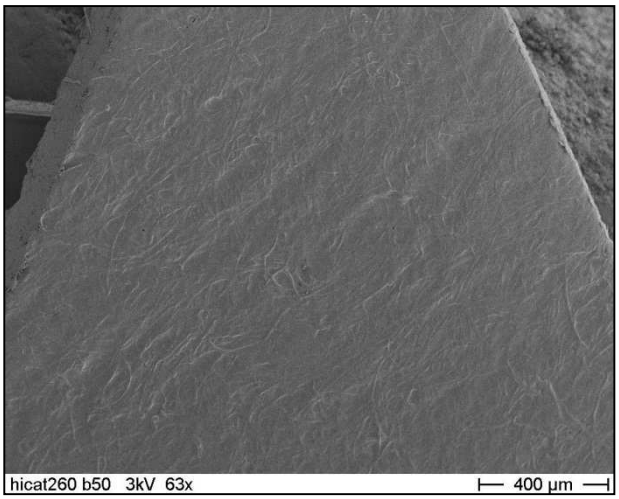


Figure 5.2.12 *SEM observation for surface of coated Paperboard B (overview)*

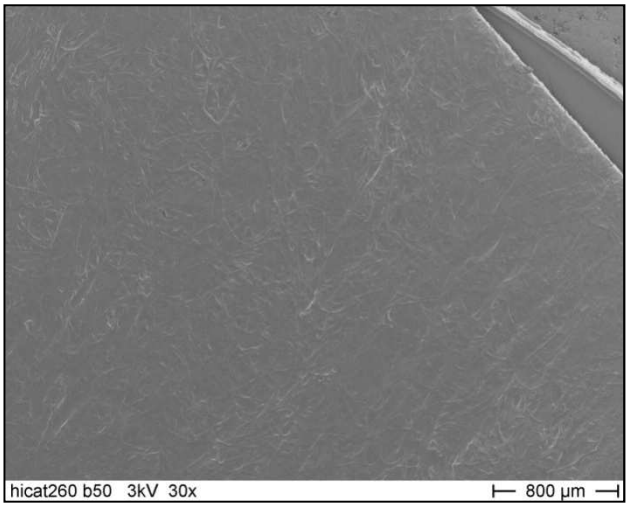
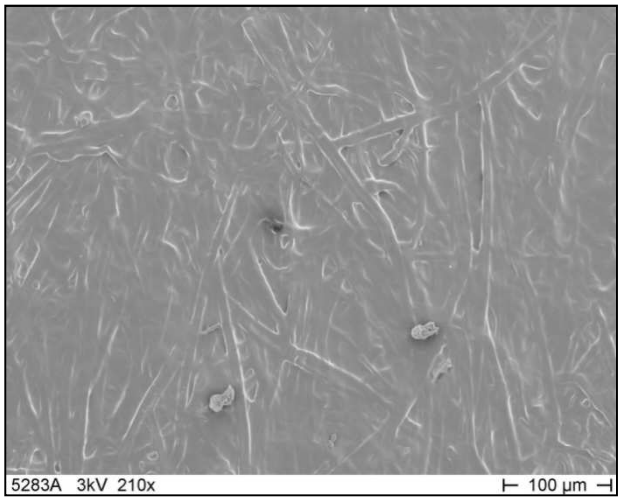


Figure 5.2.13 *SEM observation for surface of coated Paperboard B (overview bis)*

In the case of coated paperboard X a comparable surface appearance with the previous one was observed (Figures 5.2.14, 5.2.15). Some particles at the surface were noticed (Figures 5.2.14), probably linked to the presence of insoluble granules of starch or to the presence of dust deposited after the coating deposition; moreover few black spots were seen. They were supposed to be evidence of valleys in the coating surface.



Figures 5.2.14 *SEM observation for surface of coated Paperboard X (detail)*

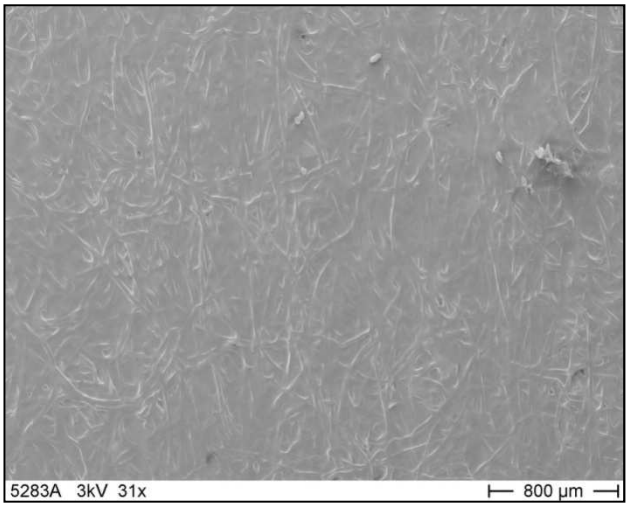


Figure 5.2.15 SEM observation for surface of coated Paperboard X (overview)

In the case of coated paperboard A (Figures 5.2.16, 5.2.17), a rather smooth surface compared to the other samples was observed; suggesting that a continuous layer of coating material was deposited onto paperboard. Some particles at the surface were observed, maybe due to insoluble granules of starch. This observation was clearly identified in detailed observation (Figure 5.2.17).

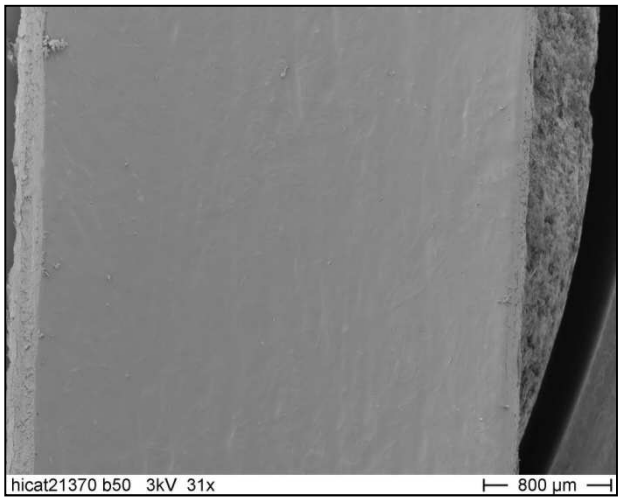


Figure 5.2.16 *SEM observation for surface of coated Paperboard A (overview)*

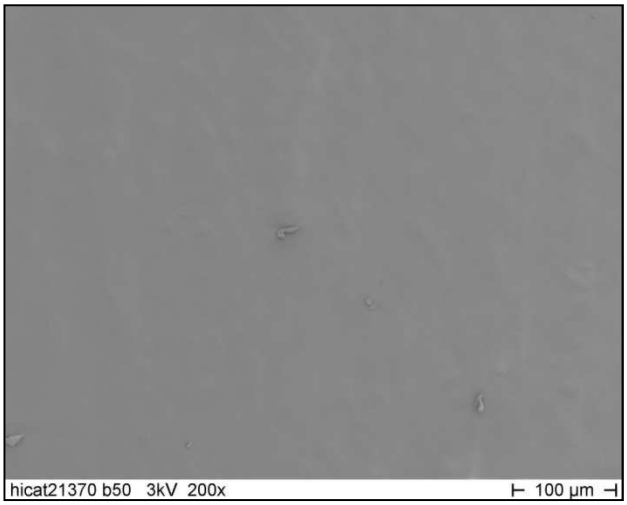


Figure 5.2.17 *SEM observation for surface of coated Paperboard A (detail)*

The observed differences in the ability of the bio-polymeric coating to cover and hide the entanglement of the cellulosic fibres of the raw paper substrate below it could be linked to its penetration and interaction with the paper matrix, therefore to the real homogeneous thickness obtained for each type of starch.

Furthermore, preliminary observations of cross section of coated paperboard were carried out, with the aim of investigate coating thickness and the bi-layer structure obtained (Figure 5.2.18).

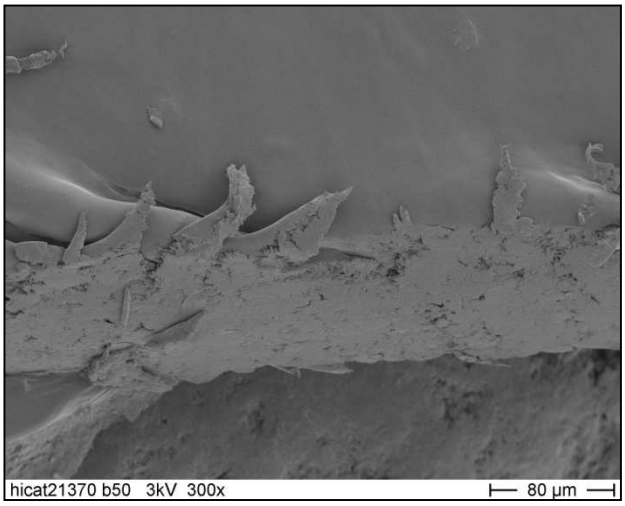


Figure 5.2.18 *SEM observation for cross section of coated Paperboard A*

Due to imprecision of blade cuts (ceramic knife), detailed information could not be obtained, even if a simple by-layer structure could be hypothesized.

Further observations of samples cut with microtome (steel blade) were made, however also in this case the inaccuracy of the obtained cross sections did not allowed a perfect differentiation and measurement of coating thickness.

Preliminary measurements made for the entire sample section (Figure 5.2.19) and particular zones with coating (Figure 5.2.20) showed an apparent thickness around 5-10 µm for the bio-polymer layer. These findings are in agreement with theoretical calculation, considering the dry weight of coatings (10 w/w) and the used steel rod (to obtain a wet coating thickness equal to 50 µm).

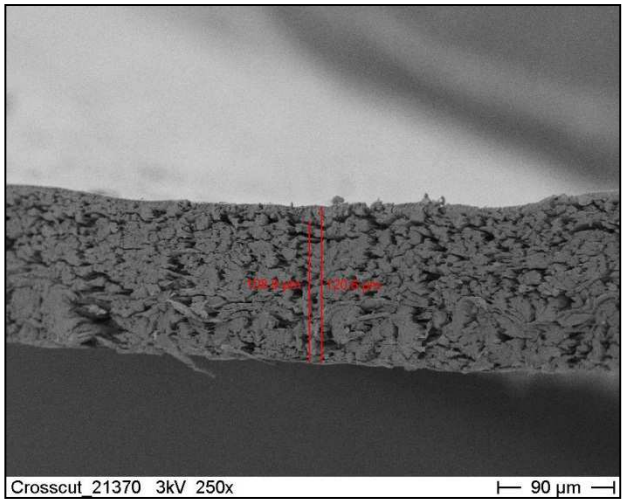


Figure 5.2.19 *SEM observation for cross section of coated Paperboard A with measurements (overview)*

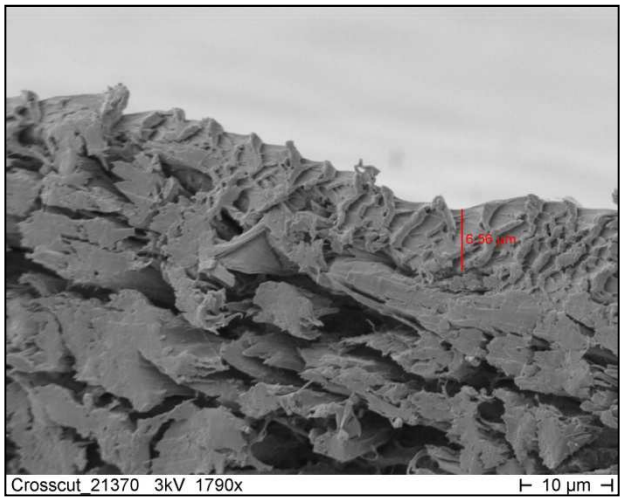


Figure 5.2.20 *SEM observation for cross section of coated Paperboard A with measurements (detail of coating)*

Measurements of coating thickness using a suitable micrometre would be required.

At the same time, starch coated paper with addition of sorbitol as plasticiser was analysed by SEM in order to acquire information on the overall appearance of the obtained structure and to compare with the same type of starch coated paper without sorbitol addiction.

Plasticised coatings (Figures 5.2.21, 5.2.22, 5.2.23) showed smooth surfaces compared with formulations without plasticizers without pores and cracks. These results are in agreement with literature data (Garcia et al. 1999; Mali et al. 2002).
Plasticizers, hydrophilic compounds such as polyols, are frequently added to bio-coatings to enhance flexibility. Among them, sorbitol was found a more effective plasticizer than other (ex. glycerol) (Garcia et al. 1999).
Moreover, data from the literature proved that the A-type crystalline structure of native corn starch was transformed to B-type in the plasticised films due to the effect of a facilitated movement of starch chains (Pushpadass et al. 2009).

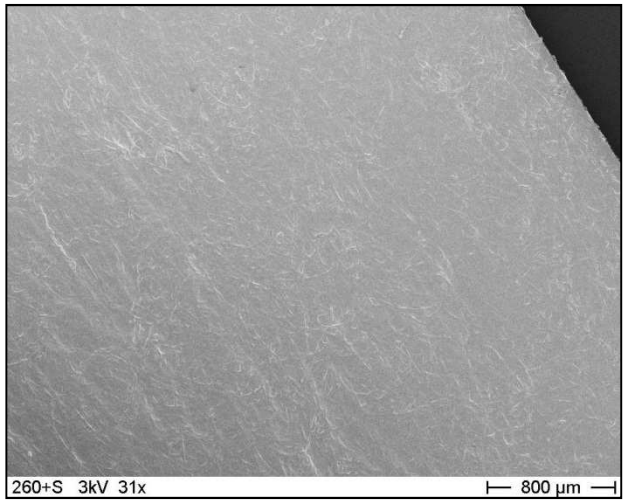


Figure 5.2.21 *SEM observation for surface of coated Paperboard BS (overview)*

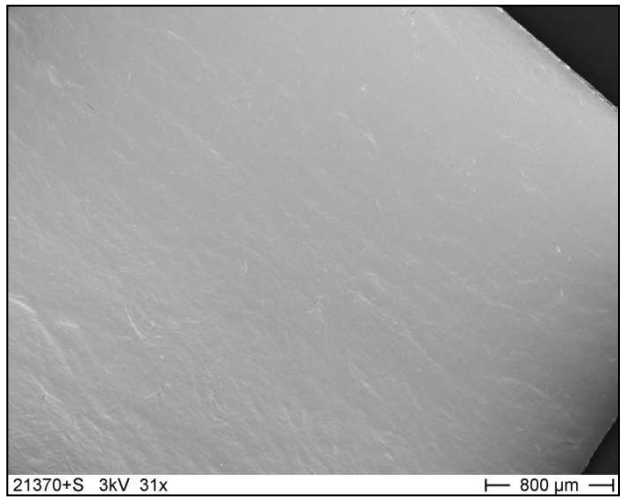


Figure 5.2.22 *SEM observation for surface of coated Paperboard AS (overview)*

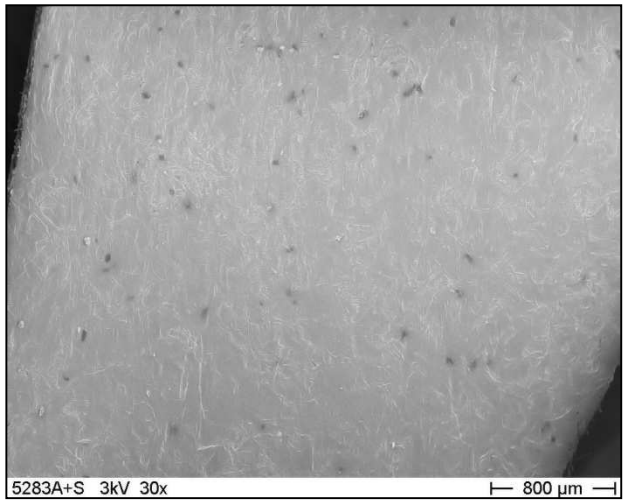


Figure 5.2.23 *SEM observation for surface of coated Paperboard XS (overview)*

Concerning coated paperboard XS, the high amylose content starch with addition of sorbitol, particles at the surface were observed, they were homogeneously distributed (Figure 5.2.23) and from detail view they were measured as 20 µm in diameter (Figure 5.2.24).

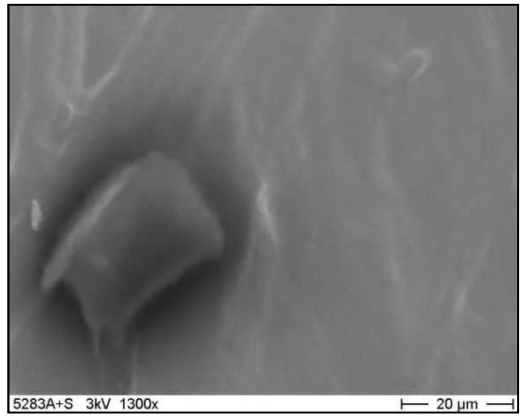


Figure 5.2.24 *SEM observation for particle on top of coated Paperboard XS (detail)*

EDS spectra (Figure 5.2.25) recorded for these particles using an accelerating voltage of 23 kv, showed a significant content in Calcium, Chlorine and Potassium, elements likely deriving from salts maybe arising from a contamination by mineral water. Since these particles were not observed for the same starch coating without the addiction of sorbitol, the sorbitol solution could be hypothesized as the source of these substances.

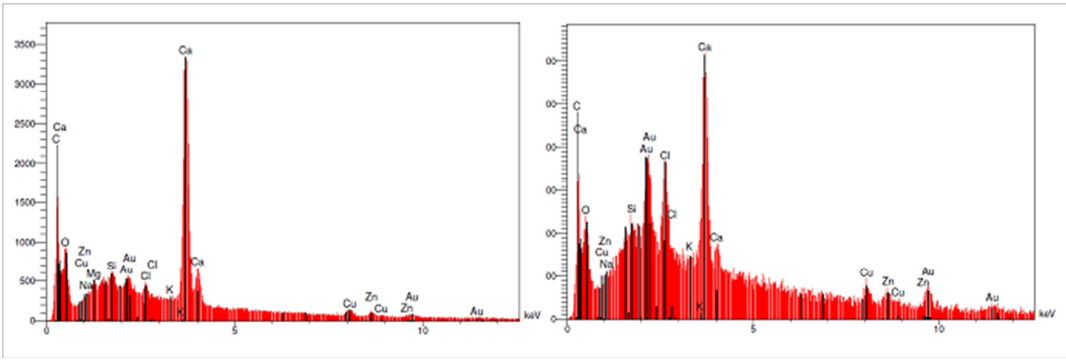


Figure 5.2.25 *EDS spectra for particle on top of coated Paperboard XS (left) and covered (right)*

5.2.3 Migration kinetics of n-alkanes through spiked neat paperboard

For quantification and normalisation of GC-related variations, Bicyclohexyl (92-51-3) or Cholestane (481-21-0) (added with the Restek standard) were used as non-interfering internal standards.

Firstly, determination of the total initial concentration of alkanes spiked in blue paper was evaluated.

As can be seen in Table 5.2.2, the average concentration found for the five samples analysed is comparable only for alkanes from C18 to C40, while for C10 and C12 any quantification was possible (< LOQ) and in the case of C14 and C16 an average concentration significantly lower compared with other alkanes was detected.

Statgraphics Plus 4.0 software (STSC, Rockville, USA) was used for the one-way ANOVA to check for differences among samples. Table 5.2.3 reports the results for ANOVA test for concentration by sample.

The ANOVA table decomposes the variance of concentration into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 180.777, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean concentrations from one level of sample to another at the 95.0% confidence level.

Compound Name	(µg g^{-1}) BP 1	(µg g^{-1}) BP 2	(µg g^{-1}) BP 3	(µg g^{-1}) BP 4	(µg g^{-1}) BP 5	Mean ± St dev.
C14	1.56	2.40	2.18	1.97	2.24	2.07 ± 0.291
C16	6.95	8.70	8.21	8.03	8.64	8.11 ± 0.631
C18	11.50	11.80	11.69	12.23	12.42	11.93 ± 0.341
C20	11.93	11.88	12.13	12.72	12.90	12.31 ± 0.421
C22	11.83	11.72	11.89	12.61	12.89	12.19 ± 0.470
C24	12.09	11.90	12.16	12.89	13.86	12.58 ± 0.719
C26	12.21	11.97	12.27	13.32	13.35	12.63 ± 0.588
C28	11.93	11.69	12.00	12.42	12.61	12.13 ± 0.334
C30	12.19	11.85	12.19	12.50	12.59	12.27 ± 0.262
C32	11.76	11.36	11.74	11.99	11.99	11.77 ± 0.231
C34	11.69	11.35	11.70	12.04	12.20	11.80 ± 0.300
C36	11.37	11.20	11.46	11.73	11.84	11.52 ± 0.235
C38	11.57	11.17	11.40	11.66	11.72	11.50 ± 0.201
C40	11.44	11.07	11.21	11.58	12.18	11.50 ± 0.387

Table 5.2.2 *Initial concentration of alkanes found in spiked blue paper samples*

Source	Sum of squares	DF	Mean square	F-Ratio	p-value
Between groups	510.552	13	39.2732	180.78	0.0000
Within groups	12.1658	56	0.217247		
Total (corr.)	522.718	69			

Table 5.2.3 *ANOVA table for concentration by spiked blue paper samples*

Main reasons of these findings could be linked to the loss of highly volatile substances during spiking and drying from heptane from the blue paper sample, as already explained by Ewender et al. (2012). Therefore a nominal concentration is not reached for C10 to C12 compounds. These results suggest avoiding further evaluation of these substances, because they could be affected by a non-homogeneity concentration in the donor phase.

Secondly, kinetics extracts of Tenax® collected from migration cells stored at 60 °C 40 °C and 20 °C in triplicate were analysed and evaluated.

In Table 5.2.4 results for the average quantifications for n-alkanes from C18 to C36 are reported for experiments at 60 °C.

Time (hrs)	4	5	15	24	48
Compound Name	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})
C18	0.424	0.793	2.380	2.624	2.297
C20	0.381	0.713	2.312	2.635	2.360
C22	0.228	0.402	2.133	2.476	2.306
C24	0.115	0.159	1.571	2.063	2.248
C26	0.042	0.049	0.808	1.243	1.750
C28	0.042	0.049	0.231	0.492	0.825
C30	< LOQ	< LOQ	< LOQ	< LOQ	0.041
C32	< LOQ	< LOQ	< LOQ	< LOQ	0.086
C34	< LOQ	< LOQ	< LOQ	< LOQ	0.038
C36	< LOQ	< LOQ	< LOQ	< LOQ	0.017

Notes: Limit of quantification (LOQ) was estimated considering 10 time the S/N ratio.

Table 5.2.4 *Concentrations found in Tenax® for the kinetics at 60 °C*

In Figure 5.2.26 results for measured data and curves obtained fitting the Weibull kinetic model are reported as migration versus time.

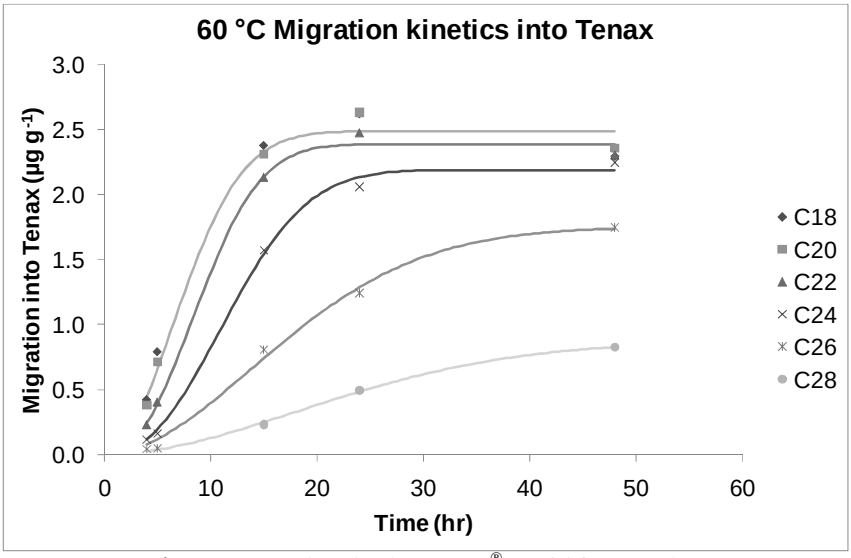


Figure 5.2.26 *Migration into Tenax® at 60 °C versus time*

The kinetic migration curves were obtained by fitting the Weibull distribution (see function 4.2.2) to the experimental data using the software Table Curve 2D (Jandel Scientific, version 4).

The values of the model parameters and determination coefficients for the same migrants are presented in Table 5.2.5.

	C_∞	τ	β	R^2
C18	2.43	6.67	3.24	0.986
C20	2.49	9.05	2.00	0.990
C22	2.39	10.56	2.31	0.997
C24	2.19	13.79	2.35	0.998
C26	1.75	20.64	1.88	0.994
C28	0.87	26.79	1.89	0.998

Table 5.2.5 *Weibull model parameters for n-alkanes at 60 °C*

The Weibull model was found to be very effective in describing the shape of the migration curves for uncoated paper. The goodness of fit was evaluated by the determination coefficient R^2.

The obtained values of τ are higher for heavier n-alkanes (C26-C28), indicating that the rate of mass transfer was lower than that one that occurred for lighter compounds.

In Table 5.2.6 results for the average quantifications for n-alkanes from C18 to C28 are reported for experiments at 40 °C.

Time (hrs)	**3**	**5**	**15**	**24**	**48**	**72**	**96**	**120**
Compound Name	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})
C18	2.01	2.07	2.09	2.13	2.14	2.17	2.23	2.27
C20	1.02	1.42	1.79	1.91	2.09	2.13	2.16	2.18
C22	0.33	0.76	1.23	1.35	1.82	2.04	2.05	2.06
C24	< LOQ	0.26	0.43	0.66	1.05	1.45	1.59	1.64
C26	< LOQ	0.05	0.07	0.17	0.31	0.62	0.71	0.88
C28	< LOQ	< LOQ	< LOQ	0.05	0.07	0.15	0.21	0.19

Notes: Limit of quantification (LOQ) was estimated considering 10 time the S/N ratio.

Table 5.2.6 *Concentrations found in Tenax® for the kinetics at 40 °C*

In Figure 5.2.27 results for measured data and curves obtained fitting the Weibull kinetic model are reported as migration versus time.

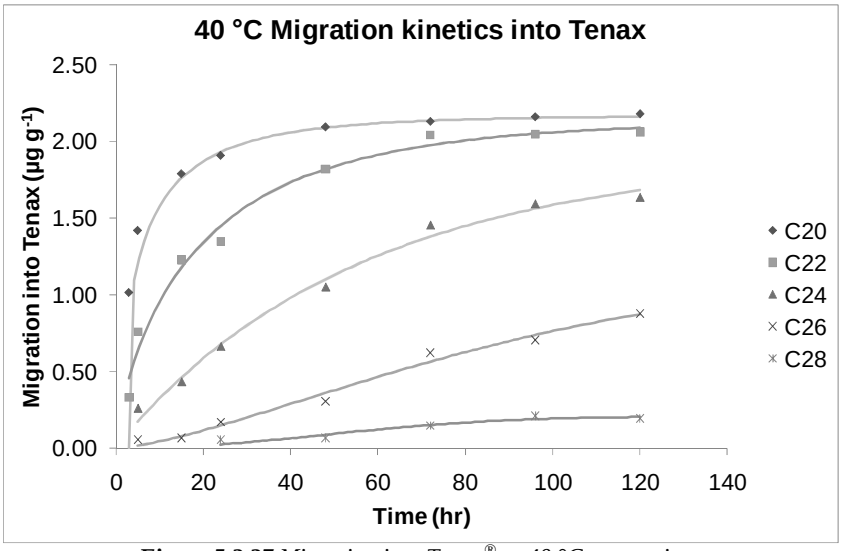


Figure 5.2.27 *Migration into Tenax® at 40 °C versus time*

The values of the model parameters and determination coefficients for the same migrants are presented in Table 5.2.7.

	C_∞	τ	β	R^2
C20	2.17	5.58	0.56	0.986
C22	2.14	20.29	0.75	0.983
C24	1.93	56.82	0.97	0.990
C26	1.18	97.55	1.43	0.989
C28	0.21	63.61	2.11	0.922

Table 5.2.7 *Weibull model parameters for n-alkanes at 40 °C*

The Weibull model was found to be very effective in describing the shape of the migration curves for uncoated paper, also in the case of experiments performed at 40 °C. The goodness of fit was evaluated by the determination coefficient R^2.

Values found for β, index determining the pattern of curvature, ranged from 0.56 to 0.97 for n-alkanes from C20 to C24 whereas for C26 and C28 ranged from 1.43 to 2.11. It has been noted that the Weibull model is very effective at describing processes with atypical curve patterns by simply having β values higher than 1. In phenomenological terms, a diffusional process may exhibit this pattern when the equilibrium conditions are not established instantaneously at the interface. This type of shape it was observed sometimes in migration from paper-based materials into solid foods (Poças et al. 2011). Interestingly, β values increased for higher n-alkanes, corresponding to the presence of an evident lag phase.

Considering experiments at 20 °C, only preliminary evaluations were made. In this case, only n-alkanes C24 and C26 were evaluated since a possible lost of lighter molecules (from C18 to C22) was hypothesised during long time storage using glass cells.

In Table 5.2.8 results for the average quantifications for n-alkanes C24 and C26 are reported for experiments at 20 °C.

Time (days)	**5**	**10**	**15**	**20**	**60**	**90**
Compound Name	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})
C24	0.31	0.55	0.64	1.05	1.13	2.27
C26	0.04	0.07	0.11	0.34	0.39	0.71

Table 5.2.8 *Concentrations found in Tenax® for the kinetics at 20 °C*

Moreover, extraction of the donor system after all experiments, the spiked blue paper, was performed and allowed to define the decrement of each n-alkane during time.

Table 5.2.9 and 5.2.10 report the remaining concentration (µg g^{-1}) and %, found for each alkane in blue paper respectively for the experiment at 40 and 60 °C at 5 and 2 days.

Migration and remaining percentage values were calculated considering the average of initial contaminant concentration found for each n-alkane in spiked blue paper.

Compound Name	**(µg g^{-1}) in blue paper**	**Reamaining %**
C18	< LOQ	-
C20	< LOQ	-
C22	0.89	10.9
C24	3.26	27.3
C26	7.59	61.6
C28	10.57	86.7
C30	11.95	94.9
C32	11.94	94.5
C34	11.93	98.3
C36	11.69	95.2
C38	11.58	98.4
C40	11.44	96.9

Notes: Limit of quantification (LOQ) was estimated considering 10 time the S/N ratio.

Table 5.2.9 *Concentration of n-alkanes found in blue paper after 5 days at 40 °C*

Compound Name	(μg g^{-1}) in blue paper	Reamaining %
C18	< LOQ	-
C20	< LOQ	-
C22	< LOQ	-
C24	< LOQ	-
C26	2.36	18.6
C28	6.06	49.9
C30	9.43	76.9
C32	10.95	93.0
C34	11.48	97.3
C36	11.23	97.4
C38	10.99	95.5
C40	10.72	93.2

Notes: Limit of quantification (LOQ) was estimated considering 10 time the S/N ratio.
Table 5.2.10 *Concentration of n-alkanes found in blue paper after 2 days at 60 °C*

It is clear how the temperature and molecular weight of the chemicals influence the migration behaviour. In fact, as regard 60 °C only heavier substances, such as from C30 to C40 could be detected with a percentage above 50% in blue paper (donor) while alkanes from C18 to C28 could be detected with a percentage above 5% into Tenax® (receptor). These findings are in agreement with literature data (Lorenzini et al. 2013).

Figure 5.2.28 reports the migration % and the remaining % respectively into Tenax® and blue paper at 60 °C after 2 days. It could be clearly state that a mass balance missing occurred for the n-alkanes from C18 to C30, it was approximately equal to 80%. Possible explanations of these results could be a consistent trapping of molecules into the test material, the neat paperboard sandwiched between the donor (blue paper) and the receptor (Tenax®) phases; in fact it was not analysed after the test but it was supposed able to trap such compounds.

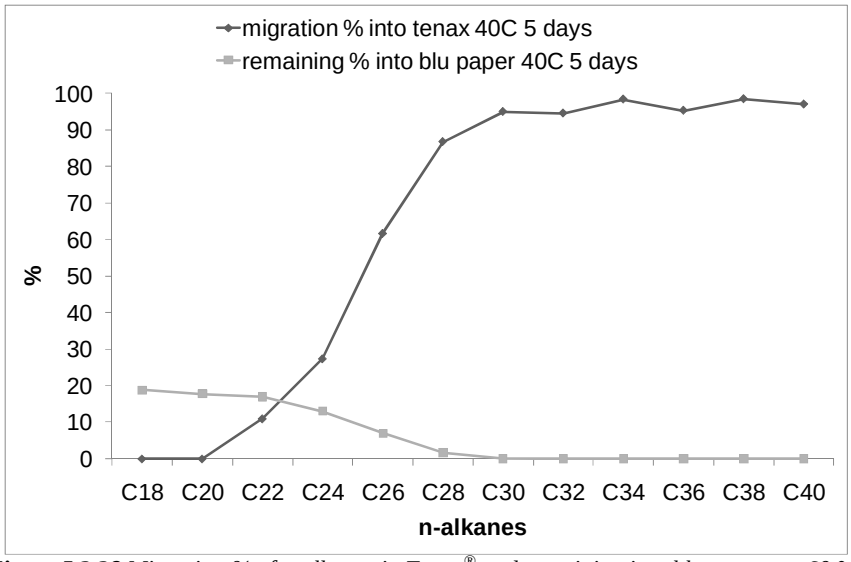


Figure 5.2.28 *Migration % of n-alkanes in Tenax® and remaining into blue paper at 60 °C*

Also in the case of 40 °C (Table 5.2.9), a net separation of n-alkanes into blue paper or Tenax®, considering their molecular weight, could be observed. Particularly, it is worth emphasising how alkanes from C30 to C40 remained quite totally in the spiked donor, alkanes from C24 to C28 could be detected with a percentage above 20%; contrary only alkanes from C18 to C26 were found able to migrate into Tenax® almost for 5% of the original spiking. These findings are in agreement with literature data (Lorenzini et al. 2013).

Correlation with molecular weight and vapour pressure for n-alkanes migration seems to be an important factor; it needs to be fully investigated.

To conclude, it was proved as the upper migration limit depends on temperature; it influences the carbon number up to which mineral oil hydrocarbons are volatile enough to be significantly transferred to food: over C28 at 60 °C and around C26 at 40 °C and at room temperature (preliminary analyses).

Additional considerations could be made regarding the correspondence of the obtained results with the "formula for accelerated test" settled in the EU Reg. 10/2011 (5.2.1) and to the relative accelerating factors:

$$t_2 = t_1 * \text{Exp}\left((-E_a/R) * (1/T_1 - 1/T_2)\right) \qquad (5.2.1)$$

where:

E_a is the worst case activation energy 80kJ mol^{-1}
R is a factor 8,31 J/Kelvin/mol
Exp -9627 * $(1/T_1 - 1/T_2)$
t_1 is the contact time
t_2 is the testing time
T_1 is the contact temperature in Kelvin
T_2 is the testing temperature in Kelvin.

In fact, taking into account the reaching of equilibrium into the migration of alkanes, as instance n-C24, at 40 °C it was reached approximately after 6 days while at 60 °C after 1 day, according to the EU Reg. the accelerating factor in this case would be exactly 6.3.

5.2.4 Migration of n-alkanes through spiked coated paperboard

Firstly, lab made coated paperboard were analysed themselves in order to identify a possible contamination of mineral oil compounds. Figures from 5.2.29 to 5.2.31 report the obtained chromatograms.

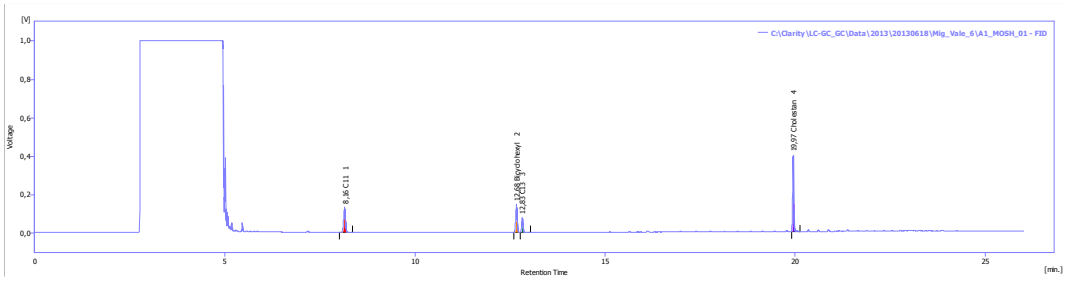


Figure 5.2.29*Chromatogram obtained for coated paperboard A, with integration of restek standard*

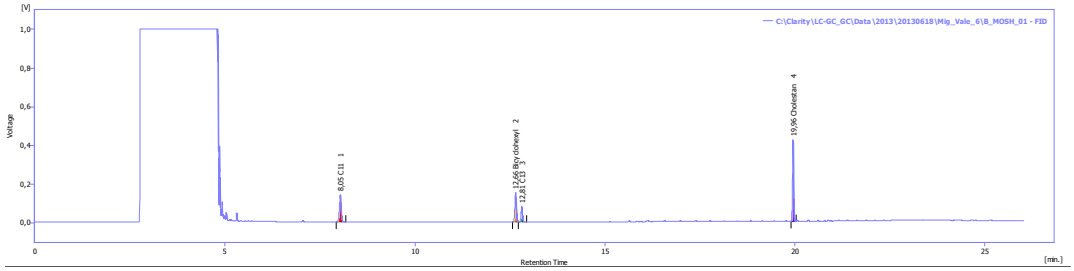


Figure 5.2.30 *Chromatogram obtained for coated paperboard B, with integration of restek standard*

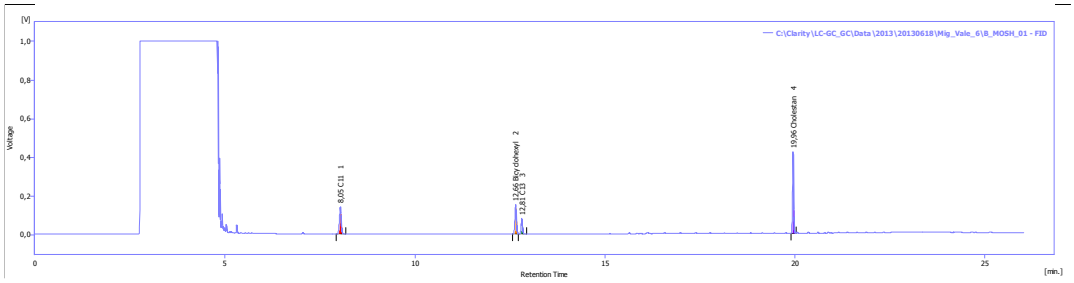


Figure 5.2.31 *Chromatogram obtained for coated paperboard X, with integration of restek standard*

From the obtained results, it was concluded that bio-coated paperboard lab scale made (type A, B, X) could be considered for further investigations of the barrier properties against mineral oil components, since any contamination from mineral oils was quantified (< LOQ).
Migration kinetics experiments were developed for testing the different types of bio-coated paperboard using glass cells, following the same procedure used for uncoated paperboards, in order to investigate their potential barrier properties against n-alkanes.

Hereafter, results obtained for n-alkanes concentration found in the extracted Tenax® after 10 days at 40 °C are presented and discussed.
In Table 5.2.11, average results and relatives standard deviations for neat and the different type of coated paperboards are reported.
In Figure 5.2.32, the same results are represented as histograms.

Compound Name	Neat	Coated A	Coated B	Coated X
C18	2.63 ± 0.27	0.16 ± 0.09	0.450 ± 0.19	0.117 ± 0.09
C20	2.38 ± 0.26	0.072 ± 0.025	0.252 ± 0.078	0.061± 0.03
C22	2.35 ± 0.30	0.049 ± 0.002	0.116 ± 0.031	0.032 ± 0.01
C24	2.24 ± 0.36	0.048 ± 0.036	0.073 ± 0.031	0.020 ± 0.005
C26	1.20 ± 0.26	0.055 ± 0.046	0.060 ± 0.041	0.029 ± 0.014

Table 5.2.11 *Mean concentration and standard deviation of alkanes found in Tenax® (µg g^{-1}); comparison between neat and coated paperboard*

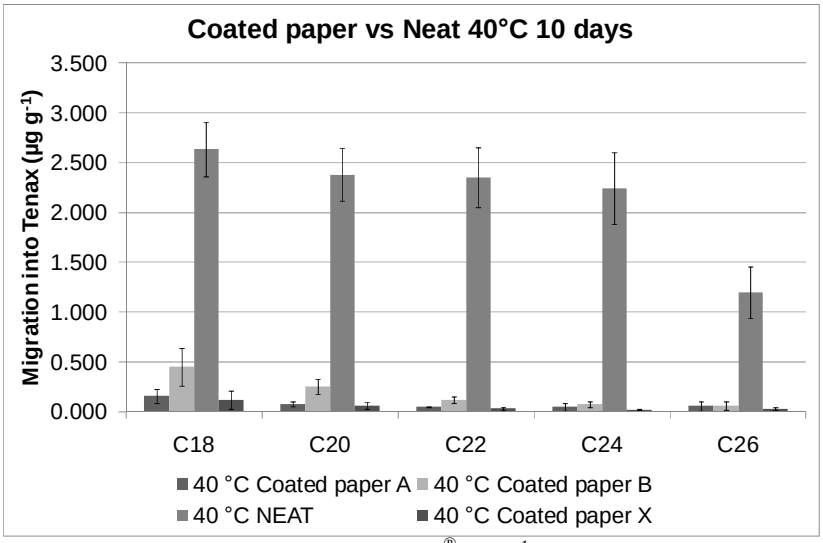


Figure 5.2.32 *Concentration of alkanes found in Tenax® (µg g^{-1}); comparison between neat and coated paperboard*

From the obtained results, it could be noted that important differences in the migration behaviour of n-alkanes through neat and bio-coated paperboard occurred, in particular as regards alkanes from C18 to C26. Focus was particularly related to this class of n-alkanes as previously done for neat paperboard. In fact to an hand, adopting the described methodology of spiking with a mixture solution under a fume hood lighter alkanes were easily lost or did not reach a constant concentration in the donor phase, thus affecting comparison of results; to the other hand heavier alkanes, such as above C26, difficulty could migrate through paperboard at 40 °C, even after 10 days.

Additional evaluations of the residual n-alkanes content into the spiked blue paper, after the migration test at 40 °C 10 days, were developed; they are in agreement with previous results, for

uncoated paper only n-alkanes from C30 to C40 seemed to remain in the donor phase, while when paper is bio-coated as regard alkanes from C18 to C26, they seemed to remain in the donor phase, up to 50% of their initial spiking concentration.

As for neat paperboard, also for the tested bio-coated paper an higher migration into Tenax® was observed, in particular for C18 and C20. One-way ANOVA evaluations were performed for all the compounds using Statgraphics Plus 4.0 software (STSC, Rockville, USA) to check for differences between samples (the neat and the different coated paperboards) . Table 5.2.12 reports the results for ANOVA test for concentration by sample. The ANOVA table decomposes the variance of concentration into two components: a between-group component and a within-group component. The F-ratio is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, for all the compounds considered (in the range from C18 to C26) there is a statistically significant difference between the mean concentrations from one level of sample to another at the 95.0% confidence level. Therefore migration through bio-coated paperboard is statistically lower than through neat paperboard at the tested conditions.

Source	Sum of squares	DF	Mean square	F-Ratio	p-value
C18					
Between groups	10.1447	3	3.3816	111.19	0.0000
Within groups	0.2433	8	0.0304		
Total (corr.)	10.3880	11			
C20					
Between groups	11.9179	3	3.9726	208.94	0.0000
Within groups	0.1521	8	0.0190		
Total (corr.)	12.0700	11			
C22					
Between groups	11.8831	3	3.9610	170.00	0.0000
Within groups	0.1864	8	0.0233		
Total (corr.)	12.0695	11			
C24					
Between groups	10.8707	3	3.6236	109.83	0.0000
Within groups	0.2640	8	0.0330		
Total (corr.)	11.1346	11			
C26					
Between groups	3.00141	3	1.0005	56.52	0.0000
Within groups	0.1416	8	0.0177		
Total (corr.)	3.1430	11			

Table 5.2.12 *ANOVA table for concentration by samples*

Only slight differences could be empathized between the different coatings. A multiple comparison procedure, a Fisher Least Significant Difference (LSD) test, was applied to determine which means were significantly different from which others at the 95.0% confidence level. The obtained results showed any differences among the tested biopolymers. Therefore, the different starches used in the formulations: waxy starch (A), normal starch (B) and starch with high amylose content (X) seemed to be comparable as regard barrier properties against these molecules at the tested conditions. However for final conclusions, a more detailed characterization of the surface and cross section morphology of each material should be awaited; in particular information about the thickness of each bio-layer and its integrity should be defined since they could considered key factors for the comparison of different formulations.

Main reasons for the interest in studying which kind of starch should be used, for achieving functional barrier applications, layered in the importance of the amylose/amylopectin proportions present. Previous studies showed improvements in material properties when amylose and amylopectin films were allowed to crystallize, especially as barrier to oxygen and carbon dioxide, these films have the potential to replace conventional petrol-based films (Pushpadass et al. 2009).
Films made from high amylose starch showed lower water vapour and gas permeability than regular starch film (Garcia et al. 2000).

As regard bio-coated paper with sorbitol addiction, as plasticizer, a comparison between the migration behaviour of n-alkanes (range from C18 to C26) into Tenax® after 10 days at 40 °C was performed.

Figures 5.2.33, 5.2.34, 5.2.35 report the obtained comparison for formulations with and without sorbitol addiction.

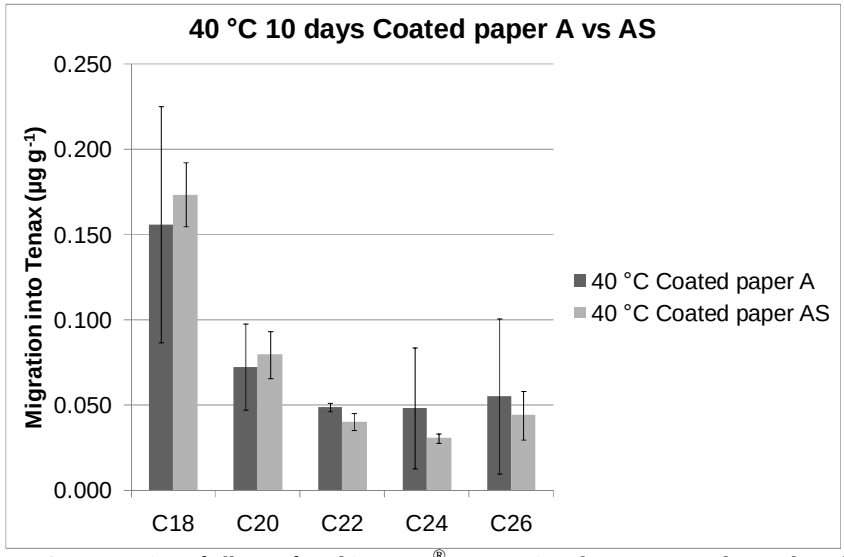


Figure 5.2.33 *Concentration of alkanes found in Tenax®; comparison between coated paperboard A and AS*

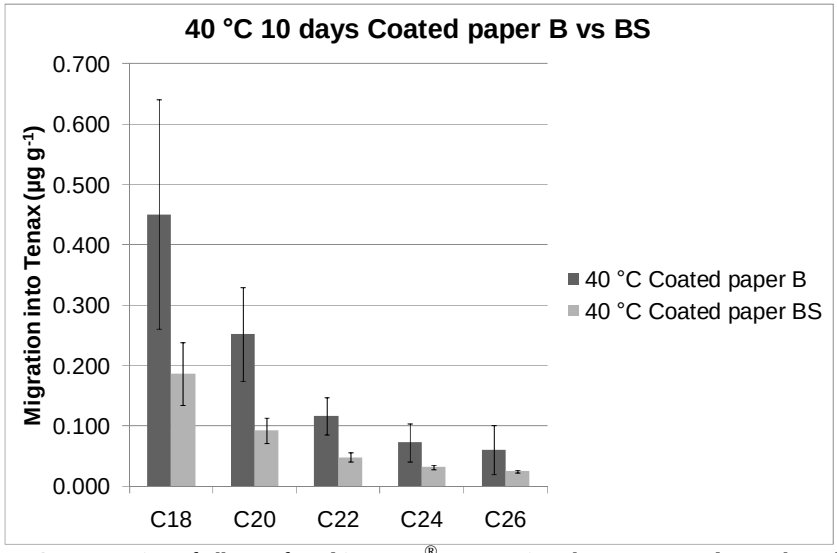


Figure 5.2.34*Concentration of alkanes found in Tenax®; comparison between coated paperboard B and BS*

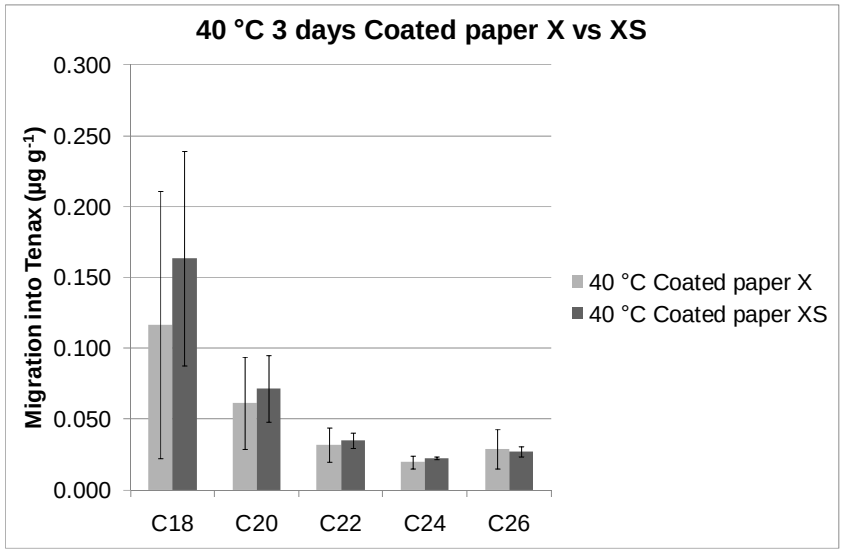


Figure 5.2.35 *Concentration of alkanes found in Tenax®; comparison between coated paperboard X and XS*

Pure starch films are brittle due to the strong intermolecular and intramolecular hydrogen bonding and need to be plasticized to make films (Domingo & Morris 1999). Addition of plasticizers overcomes the brittleness and improves the flexibility and extensibility characteristic by reducing the intermolecular hydrogen bonding between polymer chains and by increasing the mobility of the polymer (Forssell et al. 2002). The amount of plasticizer may affect the crystallinity of the polymer and the mechanical properties of the film. More importantly, molecular weight and chemistry of the plasticizer influence the physical and functional properties of starch films (Roz et al. 2006). sorbitol

was reported to be able to decrease intermolecular attraction and interfering with the amylose packing (Donhowe and Fennema 1993). Sorbitol was chosen among polyols used as plasticizers since it was reported in literature as able to interact better than others with polymeric chains of polysaccharides (Garcia et al. 2000).

In this preliminary study, it was highlighted a significant difference with the base formulation (without sorbitol) only in the case of coated paper type B (normal starch). Possible explanations of these findings layer in the occurrence of cocrystallization between amylose and amylopectin when a plasticizer is added, found by few authors (Leloup et al. 1991).

Further studies (i.e. cristallinity and mechanical properties evaluations) considering different characterization techniques and at the same time migration testing of the different formulations preliminary tested seemed to be fundamental to obtain the necessary knowledge to assess the effect of amylose and plasticizer content on the functional barrier performances of starch based bio-coatings.

5.2.5 Permeation analyses with automated method

In order to choose the best solution among different barrier materials already available on the market, food packaging companies need quantitative data to assess their effects on reduction of mineral oils migration. Data for barrier properties of polymer films against mineral oil components are rare in the scientific literature. At the moment

At the moment, only two publications are dealing with barrier improvement factors for mineral oil substances (Fiselier & Grob 2011; Ewender et al. 2012). Two other studies have been published on permeation of other cardboard contaminants through polymer liners (Anderson & Castle 2003; Song et al. 2003). In addition, some studies for the barrier testing of aroma compounds are published (Franz 1993). At first glance, the barrier behaviour of polymer film for aroma components may be regarded similar to mineral oil substances. However, because of the inherently particular high lipophilicity of mineral oil components, these substances might behave different compared with aroma compounds.

Methods for the determination of the permeation of mineral oil components through polymer films or cardboard coatings are also rare in the scientific literature. Only Fisilier and Grob (Fiselier & Grob 2011) described a method for the determination of the barrier properties of polymer films against mineral oil. The method is working with mineral oil spiked cardboard, which is placed below the polymer film. On the other side of the barrier material, a polyethylene receptor film is placed. After certain storage times, samples from the polyethylene receptor film were extracted and analysed for their content of mineral oil compounds. From the results, the barrier properties of the investigated polymer films can be evaluated.

As mentioned earlier, mineral oil is a complex mixture that cannot be resolved by chromatographic separation steps into single compounds. As a consequence, the permeation curves can only be measured as a sum parameter for the whole unresolved mineral oil peak. Therefore, an evaluation of the barrier properties is difficult because low molecular weight compounds with higher vapour pressures will permeate much faster in comparison with the high molecular weight compounds with lower vapour pressures. This situation has several consequences for the design of a permeation test for mineral oil components: (a) only substances that can be resolved as single substances should be used and (b) the permeation should be determined as a kinetic. The kinetic is necessary because otherwise an evaluation of the different permeation rates is not possible. The individual permeation

rates are necessary for a correlation, i.e. with the molecular weight or the vapour pressure of the permeants. Such correlations might be useful for the extrapolation of the experimental determined permeation rates to other not tested substances of interest.
In this part of the study, a new method, recently published in literature for the barrier testing of polymer film (Ewender et al. 2012), was adopted to evaluate bio-coated paper. It consisted of (a) an automated detection of the permeated compounds by GC and (b) model compounds that can be analytically determined as single compounds. By following this automated approach with model substances, much more kinetic points can be determined per sample over time (i.e. eight points per day), and the permeation curve can be determined more precisely for each film and each model substances.

Table 5.2.12 reports the obtained results, as permeation rates for the applied compounds.

For compound C24 the permeation rates are below the analytical detection limit (0.01 µg d^{-1} dm^{-2}).

(µg/d dm^{-2})	C12	Naphthalin	1-Methylnaphthalin	C14	1-Ethylnaphthalin	C16	TXIB	Benzophenone	2,7-Diisopropylnaphthalin	C18	4-Methylbenzophenone	Phenanthren	C20	C22	C24
Coated paper B	1024	46	659	892	444	273	127	15	61	57	13	29	7	1	0
Coated paper B	702	41	672	911	491	305	161	48	95	62	24	45	6	1	0
Coated paper X	947	38	673	913	494	335	168	45	101	75	27	51	7	1	0
Coated paper X	678	28	490	721	407	233	106	17	54	49	13	25	7	1	0
Coated A	981	63	869	1647	513	377	210	49	109	82	30	56	8	1	0
Coated A	1054	33	720	964	474	287	138	18	61	60	16	29	9	1	0
HDPE 20µm	1050	2340	1740	1270	1560	204	167	116	76	22	36	30	2	0.2	0

Table 5.2.12 *Experimental determined permeation rates for the model compounds (µg d^{-1} dm^{-2})*

Representative examples of the measured permeation curves for the n-alkanes C14, C20 and C22 are given in Figure 5.2.36, 5.2.37 and 5.2.38 for the different tested coated papers. From the slope of these curves at linear permeation increase over time, the permeation rates in µg d^{-1} dm^{-2} can be determined.

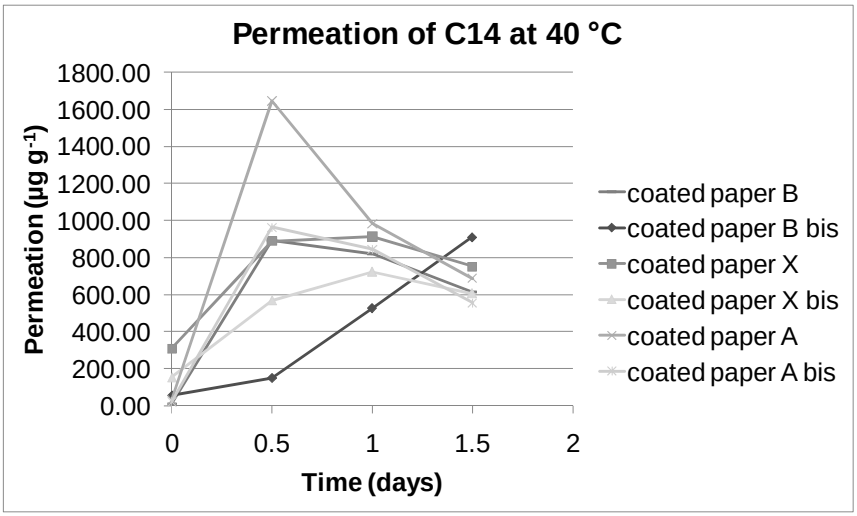


Figure 5.2.36 *Permeation curves at 40 °C of C14 over time for the tested bio-coated papers*

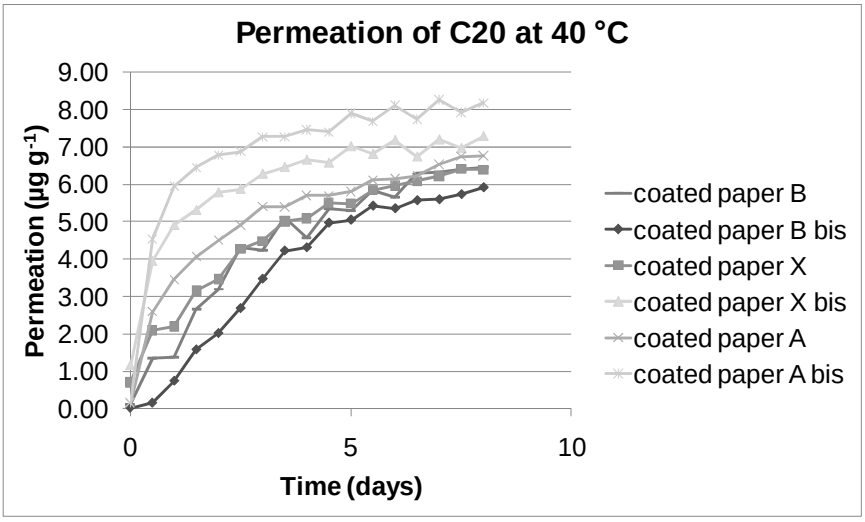


Figure 5.2.37 *Permeation curves at 40 °C of C20 over time for the tested bio-coated papers*

Every 12 hours the permeated amount of compounds was determined. It should be noted here that the permeation tests applied in this study do not allow the loss of substances to the environment. Therefore, the applied method is simulating the worst case for the permeation.

In addition, because of the constant nitrogen stream on the other side of the permeation cell, the equilibrium state between compounds on the cardboard and compounds in the foodstuffs can never be reached. This can be also considered as a worst case.

The sum of the concentrations of the 15 model substances in the cardboard was about 11250 µg g^{-1}. The applied concentration is therefore a factor of about 30 higher than the average values for the sum of mineral oil components found in the literature. Such high concentrations for each individual substance can be considered as a worst-case scenario. Additionally, mixture effect between aromatic and non-aromatic compounds could be hypothesised.

The permeation rates show some differences according to the different molecular properties of the permeants. High volatile compounds, such as C14, showed within about 1 day storage at 40 °C a total mass transfer.
As regard C22, an initial lag phase has been detected (3 days).

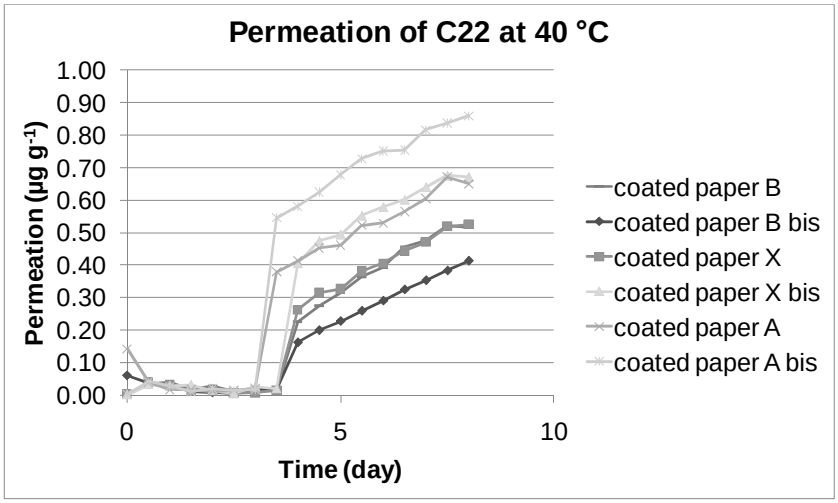


Figure 5.2.38 *Permeation curves at 40 °C of C22 over time for the tested bio-coated papers*

For compounds with higher molecular weight than C22, such as C24, this technique does not allowed to make considerations, since their higher vapour pressures do not allowed gas phase transfer permeation.

The range of final permeation for the considered compounds is comparable to polyolefin, such as HDPE, therefore the tested coatings could not be able to act as good barriers, from the comparison with PET and PA.

However the thickness effect must be taken into consideration when comparing different materials.

To conclude, results obtained through the permeation system could be combined with results obtained via HPLC-GC-FID system since the first highlighted the weakness of the tested coated paper against lighter molecular weight compounds, while the second one showed their good barrier properties versus higher molecular weight n-alkanes, as well as versus real MOSH contaminated paperboard.

For final conclusions further experiments should be carried out.

5.2.6 Migration into Tenax® from real contaminated packaging

As regard residual content of MOSH in the paperboard (donor phase), a significant higher amount of residual was detected in the coated paperboard compared to neat paperboard, in which the corresponding n-alkanes fraction C16 – C26 was significantly lower, thus hypothesised able to migrate into Tenax®. Figures 5.2.39 and 5.2.40 show the resulting chromatograms for an overlay view of the extracted neat paper (with integration of MOSH hump) and coated paper (background).

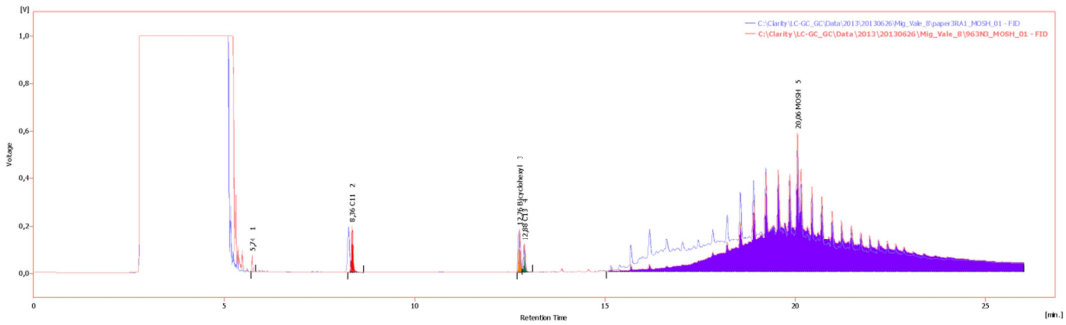


Figure 5.2.39 *Chromatograms overlay view of neat and coated recycled paper after the test*

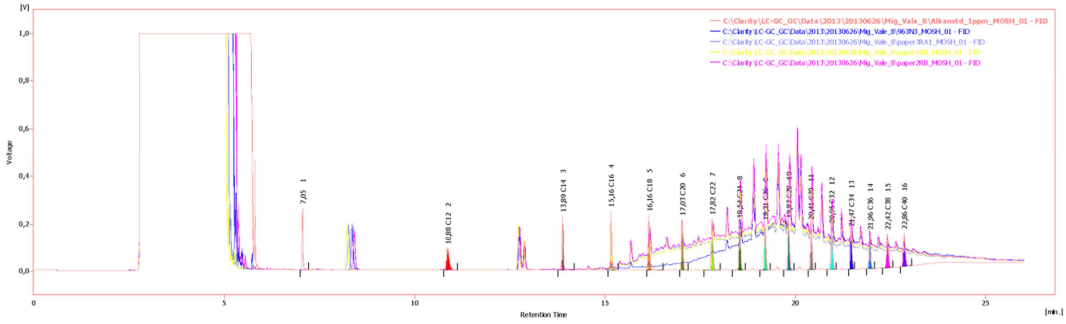


Figure 5.2.40 *Chromatograms overlay view of neat and coated recycled papers after the test with background of n-alkanes (C10 – C40, 1 mg l^{-1})*

Considering the obtained results for extraction of Tenax® in direct contact with neat paperboard after 3 days at 40 °C, an average amount equal to 69,70 µg g^{-1} of MOSH was detected. It was comparable to the n-alkanes fraction C16 – C26. Therefore a correspondence with the missing fraction of MOSH hump revealed in the donor phase was proved. Figure 5.2.41 shows an overlay view of Tenax ® and the respective paperboard into direct contact (background) after the migration test.

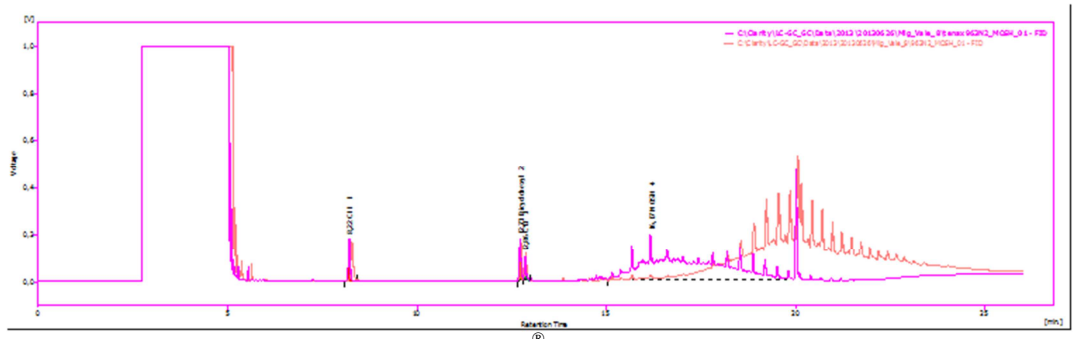


Figure 5.2.*41Chromatograms overlay view of Tenax® and neat recycled paper (background) after the test*

As regard the obtained results for extraction of Tenax® in direct contact with coated paperboard after 3 days at 40 °C, any peak related to MOSH was detected. It must be highlighted that these

analyses were performed in triplicate. Figure 5.2.42 shows the obtained chromatogram with evidence of absence of MOSH related humps.

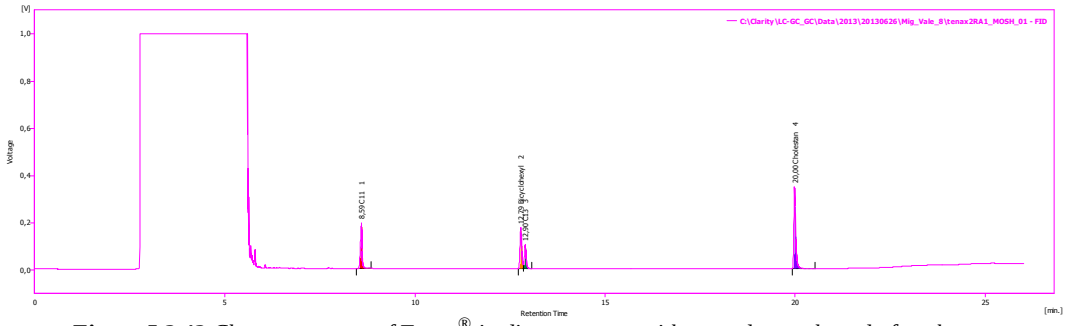


Figure 5.2.42 *Chromatograms of Tenax® in direct contact with coated paperboard after the test*

5.3 Conclusions

In this work, the evaluation of different starch-based coatings onto paper was considered to investigate their ability in reducing the transfer of MOSH like contaminants into foods.
Films can be made from starch or its components, amylose and amylopectin, by various techniques. These two components can be separated from each other and new blends with various proportions can thus be made.
Amylose is known to have a fast gelation; the gelation of amylopectin, on the other hand, is a slow process taking several weeks. In addition to the gelation of amylose, crystallinity is also formed. The A and B type crystalline structures are made up of specific arrangements of double helices. Amylose reaches a relatively high final crystallinity when dried from a water solution to a film. In contrast to amylose, amylopectin forms totally amorphous films under the same conditions but can crystallize, for instance when a plasticiser such as sorbitol is added.
The overall objective of this research project was to gain knowledge about the migration kinetics of n-alkanes trough neat paper at different temperatures and about the starch type to be used in the development of new bio-coated paper materials to prevent migration of these substances.
Previous studies showed improvements in material properties when amylose and amylopectin films were allowed to crystallize. The influence of amylose/amylopectin proportions on the properties is of importance when choosing raw materials among different starches and considering newly developed starches with various amylose contents.
The specific aim of this work was therefore to study how the proportions of amylose and amylopectin and the presence of sorbitol affect the morphology and the barrier properties of starch-coated paper against migration of n-alkanes.
Obtained results for the migration kinetics of n-alkanes from spiked paper into direct contact with Tenax® showed the effect of temperature and molecular weight of the considered migrant, correspondence of the obtained results with the "formula for accelerated test" settled in the EU Reg. 10/2011 and to the relative accelerating factors was demonstrated at 40 and 60 °C and a first modelling of the kinetics was performed using the Weibull distribution function.
Future perspectives for this part of the research would be the improvement of the modelling aspects using Migratest EXP software (FABES, GmbH), the additional evaluation of room temperature experiments (20 °C), as well as the consideration of different conditions (gas phase transfer, relative humidity, different contaminants, real food matrices…).

As regard the evaluation of starch-coated paper materials, the obtained results at 40 °C after 10 days, using glass migration cells with Tenax® in direct contact and HPLC-GC-FID analyses demonstrated the effectiveness of lab scale coatings onto paper against n-alkanes (range C18-C26) migration compared to uncoated paperboard. Any statistical significant difference was highlighted among the tested starch formulations (waxy, normal and high amylose content). Migration tests of real MOSH hump from coated recycled paperboard in the same conditions, confirmed the absence of detectable signals related to migration of MOSH. Addiction of sorbitol to base formulation did not reveal significant differences in the barrier properties, except for normal starch, in which a slight improvement was observed.
Scanning Electron Microscopy analyses allowed a clear differentiation of the coated materials as regard their morphology, particularly in the capability of waxy starch and of sorbitol added formulations to appear rather smooth surface compared with high amylose content and normal starch. Preliminary measurements of coating thickness lead to an average evaluation equal to 5 µm.

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6. Future Perspectives

The information obtained with the presented PhD study should considered promising as regard the possibility to deepen understanding the migration processes of migrants from paper and board and to derive model parameter relationships to explore migration modelling as a tool for compliance assessment of this kind of packaging.

However, further data, obtained through systematic experiments for validation and expansion to other substances/conditions should be additionally performed. The evaluation of migration through the different type of papers, testing different conditions (different substances, simulants or foods, type of contact, type of paper, temperature and moisture) would be considered as further perspectives.

Moreover, chemical and physical properties of bio-coatings can be further exploited in order to complete the necessary knowledge to select criteria for materials suitable in reducing migration of contaminants. As instance, mechanical properties could additionally be studied in order to obtain fundamental data for future applications as pilot scale development.

Finally, the optimization of starch coating formulations, together with improvement of the coating techniques (focused in the achievement of thicker layers, for example by layer by layer coating) and the migration testing in different conditions should be awaited to confirm the fulfilment of functional barrier requirements for food packaging.

Acknowledgements

I would like to thank my tutor Sara Limbo and my co-tutor Luciano Piergiovanni. Thanks for all the valuable opportunities you have offered me.

I am grateful to all the people of the "Packlab Team" for making it a friendly place to study and work.

Ich möchte Roland Franz und die Gruppe der Fraunhofer IVV aus Freising danken. Eine Erfahrung und eine Begegnung, die immer in meinem Herzen bleiben wird.

Special thanks to all my dear friends, who are always able to transform shadows in sunbeams.

Grazie di cuore alla mia famiglia, ai miei meravigliosi genitori Dina e Massimo che mi sostengono da sempre e a cui va tutto il mio affetto!

Grazie

Valeria

Appendix 1

Conference's abstract

CONTAMINAZIONE DA DIBP IN FILM ESTENSIBILE FOOD GRADE: INDAGINE ANALITICA LUNGO IL FLOW-SHEET DEL PROCESSO PRODUTTIVO

Valeria Guazzotti[1], Luciano Piergiovanni[1], Gianluigi Vestrucci[2], Sara Limbo[1]

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Nel presente lavoro di ricerca è stata condotta una mappatura analitica dei diversi manufatti, lungo il processo produttivo di films estensibili *food grade*, all'interno di una azienda *converter*. L'indagine ha rilevato la presenza di DIBP nei prodotti finiti nonostante le materie prime polimeriche ne fossero esenti. Elevati livelli di DIBP si sono riscontrati in mandrini ed astucci di natura cellulosica, pertanto, questi sono stati identificati come possibile fonte di contaminazione. In un sistema modello, appositamente ideato, si è realizzata la verifica dell'ipotizzato fenomeno di trasferimento di DIBP da matrice cellulosica a plastica, attraverso il mezzo aereo in funzione del tempo e della temperatura. Infine è stata costruita l'isoterma di adsorbimento di DIBP in diversi matrici (PVC, PE e carta) in funzione della tensione di vapore.

DIBP CONTAMINATION IN CLING FILM FOOD GRADE: ANALYTICAL EVALUATION OF A TYPICAL CONVERTING PROCESS

In the present research study an analytical screening has been performed on several intermediate products and at different stages of the converting process of PVC cling films. Despite DIBP recovery in final products, analytical evidences confirmed its absence in raw materials. Internal cores and boxes made of recycled cellulose used as transport and distribution packaging where identified highly contaminated of DIBP. To evaluate possible DIBP transfer mechanism from contaminated paper and adsorption by polymeric packaging materials, migration experiments have been performed in a model system. Results from kinetic studies show considerable migration potency of DIBP into PVC over the period of 3 weeks. Finally, adsorption isotherms in different materials (PVC, PE and paper) were obtained.

IDENTIFICAZIONE E VALUTAZIONE SEMIQUANTITATIVA DI COMPOSTI ORGANICI VOLATILI E NON VOLATILI IN IMBALLAGGI CELLULOSICI DESTINATI AL FOOD PACKAGING.
STUDIO PRELIMINARE DELLE PROPRIETA' DIFFUSIONALI

Erika Tasca[1], Valeria Guazzotti[1], Luciano Piergiovanni[1], Sara Limbo[1]

[1] Distam, Università degli Studi di Milano,Via Celoria, 2, 20133 Milano

In un'ottica di Risk Assessment, l'identificazione semiquantitativa di composti volatili e non volatili, è stata ottenuta attraverso l'applicazione di uno screening analitico condotto su 20 campioni tra imballaggi primari, secondari e articoli destinati ad un contatto breve. La valutazione della potenziale contaminazione dell'alimento è stata ottenuta attraverso l'applicazione di un modello previsionale limite. Per una più realistica stima del fenomeno è stata indagata la capacità di adsorbimento di una matrice carta e una matrice carta politenata a confronto con il polimero PET.

IDENTIFICATION AND SEMIQUANTITATIVE EVALUATION OF VOLATILE AND NON VOLATILE ORGANIC COMPOUNDS IN CELLULOSIC FOOD PACKAGING MATERIALS. PRELIMINARY STUDY OF DIFFUSION PROPERTIES

The semiquantitative identification of organic and non organic compounds has been obtained through an analytical screening on 20 samples (both primary and secondary packaging and articles for take away products). The potential contamination of the food has been calculated with a worst case model. Finally adsorption behavior of paper, paper/PE and PET has been studied.

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Evaluation of Transference Capacity of Contaminants from Cellulosic Primary or Secondary Packaging and Innovative Approach to Improve Food Contact Material Safety

This PhD thesis research project is aimed to generate an advanced scientific understanding in migration from cellulosic food packaging and in the use of a functional barrier for the appropriate protection design against chemical contaminants. Barrier's properties will be achieved by means of different technological solutions, taking into account both functionality and sustainability.

Valutazione del potenziale di trasferimento di contaminanti da imballaggi cellulosici primari o secondari e soluzioni innovative per garantire la sicurezza dei materiali a contatto con gli alimenti

Questo progetto di tesi di dottorato mira all'accrescimento della conoscenza scientifica del fenomeno di migrazione dagli imballaggi cellulosici di contaminanti chimici agli alimenti e lo sviluppo di una barriera funzionale che garantisca una adeguata protezione durante la shelf life. Diverse soluzioni tecnologiche saranno ricercate e valutate in termini di adeguatezza funzionale e sostenibilità ambientale.

1. State-of-the-Art

A variety of chemicals may enter our food supply, by means of intentional or unintentional addition, at different stages of the food chain. These chemicals include food additives, pesticide residues, environmental contaminants, mycotoxins, flavouring substances and micronutrients. Packaging systems and other Food Contact Materials (FCMs) are also a source of chemicals in food products and beverages. Nowadays, monitoring of these chemical's migration has become an integral part of food safety insurance.

The European framework Regulation 1935/2004 sets up general requirements for all FCMs. Considerable scientific progress has been made in understanding and modeling the diffusion and migration of adventitious substances with hazardous potential from plastics into foodstuffs in direct or indirect contact with the food, thus European legal requirements in respect to plastic are clearly and uniformly defined (Commission Regulation 10/2011). Whereas with paper and board (P&B) the situation is practically the reverse and nowadays the safety and quality of paper for food use in Europe are not under the control of EC Directives.

P&B are essentially composed of pulp from different vegetable sources and are used as primary, secondary and tertiary (transport) packaging, most often employed in contact with dry foods but also with fatty foodstuffs like fast food items. Chemical hazards from potential migrants include additives intentionally added in this type of material (fillers, starch and derivates, wet strength sizing agents and grease-proofing agents) and unintentionally added substances due to incorporation of recycled pulp. According to the literature (Droz et al., 1997; Binderup et al., 2002; de Fatima Pocas et al., 2007), recycled fibre is considered a major source of migrants, this route of contamination is officially recognised in the Resolution RESAP

PARTITION BEHAVIOUR OF MODEL CONTAMINANTS BETWEEN COATED PAPER AND AIR: PRELIMINARY EVALUATION OF BIO-BASED LAYERS AS POTENTIAL BARRIER FOR PAPER FOOD PACKAGING

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INTRODUCTION

Paper and Board (P&B) represent a large sector of the food and drink packaging market. They originate from natural sources and are thus regarded as environmentally friendly and perceived by consumers as safe. In addition, they are recyclable and, to attain a more sustainable economy, P&B partly or fully produced from recycled fibers are already being used in direct or indirect contact with certain foodstuffs in many countries in Europe. However, according to the literature (1), chemical hazards from potential migrants must be considered, such as additives intentionally added during manufacture and non intentionally added substances due to printing inks and incorporation of recycled pulp. P&B have to comply with basic legislative criteria concerning safety; this means that they should not give raise to migration of components which can endanger human health. Considerable scientific progress in preventing the transference of contaminants from cellulosic packaging into direct or indirect contact foodstuffs is one of the strongest arguments coming also from the industrial sector. Several options at different stages of the process could be studied to improve the safety of cellulosic food packaging. An attractive immediate solution can be the use of a functional barrier (FB) which is a layer placed between food and contaminated material which reduces the migration from any layer beyond to 10 ppb (2). Layers based on synthetic polymers may potentially represent a FB: they are low-cost, readily available and widely used for controlling permeation of water vapour and oxygen. In recent years, sustainability has become the subject of greater attention and both legislation and market aspects (customer orientation) look for the replacement of petrol-based raw materials with environmentally friendly, recyclable or biodegradable materials.

Until now, any literature is available on improving barrier properties of P&B towards contaminant substances via coating with biomaterials. In this work, focus was directed to the evaluation of partitioning behaviour of selected contaminants between bio-coated paper and air. Water based, renewable coating biopolymers, such as starch, plant and animal proteins were tested, with the aim to consider the possibility of using these materials to improve the safety of cellulosic materials for food contact applications.

MATERIALS AND METHODS

Reagents and Solutions: Benzophenone (BP, CAS n 119-61-9) and diisobutyl phthalate (DiBP, CAS n 84-69-5) were chosen as the selected contaminants, they were of analytical grade. Standard solutions of these reagents in absolute ethanol at

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Evaluation of Transference Capacity of Contaminants from Cellulosic Packaging and Innovative Approach to Improve Food Contact Material Safety

The aim of this PhD thesis is to study migration from cellulosic packaging to foods, developing bio-based coatings onto paper with barrier properties against chemical contaminants. Firstly, a gas chromatographic screening on paper and paperboard packaging intended for food use was performed to identify typical contaminants. Secondly, coatings of different biopolymers onto paper substrates were developed and characterized. At the same time, partition and diffusion studies of selected substances were carried out between paper/coated paper and air or food simulants.

Valutazione del potenziale di trasferimento di contaminanti da imballaggi cellulosici e soluzioni innovative per garantire la sicurezza dei materiali a contatto con gli alimenti

Obiettivo della presente tesi di dottorato è lo studio del fenomeno di migrazione dagli imballaggi cellulosici agli alimenti e lo sviluppo di un coating bio-based in grado di offrire una adeguata barriera verso contaminanti chimici. È stato condotto uno screening analitico su imballaggi di carta e cartone destinati al contatto con alimenti. In seguito sono stati sviluppati e caratterizzati differenti coatings biopolimerici su substrati cellulosici. Sono stati inoltre allestiti esperimenti di ripartizione e diffusione di sostanze target tra carta o carta con coating, in aria o simulanti alimentari.

1. Introduction

In accordance with the PhD thesis project previously described (Guazzotti, 2011), this poster reports some results of the first three activities concerning:

(A1) Semi-quantitative evaluation of volatile and non volatile organic chemicals in paper packaging intended for food use by SPME and solvent extraction GC/MS technique;
(A2) Development and characterization of bio-based renewable coatings onto paper substrates;
(A3) Partition and diffusion experiments of selected substances between paper and air or food simulants. Comparison of paper, bio-coated paper and plastic materials.

2. Materials and Methods

2.1 Sampling of packaging - SPME and Solvent Extraction GC/MS analysis

Twenty representative samples, such as primary and secondary packaging or article for take away foods were obtained from distributors. All of them have different food capacity, surface treatment, grammage and thickness.

SPME was performed with a 50/30 µm DVB/CAR/PDMS fibre immersed in the headspace of manual-HS vials, heated at 130°C for 30 min. Solvent extraction was achieved with ethanol/hexane solution, by sonication for 30 min. Analysis were carried out using a Perkin Elmer Autosystem XL GC equipped with a Turbomass MS. The column was DB-5MS (30 m, 0.25 mm i.d., film thickness

Bio-based coatings as potential barrier to target chemical compounds from recycled paper and board

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Paper and Board (P&B) represent a large sector of the food and drink packaging market. To attain a more sustainable economy, P&B partly or fully produced from recycled fibers are already being used in contact with certain foodstuffs in many countries in Europe. However, several studies (1) make evident that there are many contaminants that can potentially migrate from recycled paper into food posing health concern for the consumers. In this work, focus was directed to the evaluation of partition and diffusion behaviour of target migrants into air and the food simulant poly(2,6-diphenyl-p-phenylene oxide) through bio-coated paper to consider the possibility of using biopolymers to improve barrier properties of these packaging materials for food contact applications.
Two contaminants were selected: diisobutyl phthalate (DiBP) as a non polar compound and benzophenone (BP) as a polar one. Paper and board used as substrates for bio-coating were unprinted and of food-grade quality. Gelatin and wheat gluten hydro gel coating dispersions were obtained as described in published methods (2) (3); cationic starch and cationic waxy starch were dispersed in distilled water and kept at 95°C for 30 minutes. All the Hydrogel coating dispersions were poured onto paper with an automatic applicator (Ref. 1137, Sheen Instruments, Kingston/UK) equipped with a steel horizontal rod. Before the experiments, all bio-coated paper were stored in a dessicator at 25°C for one week. Adsorption isotherms were obtained applying an “on glass injection technique” (4) and the partition coefficients between coated paper and air were calculated. Briefly, strips of coated or no coated paper were placed into sealable glass vials, followed by the addiction of 1 µl of spiking solutions at different concentrations and, after equilibration at 40°C, solvent extraction and GC/MS analysis allowed adsorption of the contaminants into each material. For diffusion studies, test samples of coated paper were placed in glass cells sandwiched between a filter paper, previously spiked with the migrants, and 1 g of poly(2,6-diphenyl-p-phenylene oxide) as food simulant. Several time steps were observed in kinetic migration determinations. At each time cells have

Migration of organic substances through Bio-coated Paper and Board for Food Packaging

The aims of this PhD thesis were to study the potential of migration from paper and board packaging to foods, to develop and test bio-based coatings onto paper with barrier properties against chemical contaminants, among them phthalates, photoinitiators, diisopropyl naphthalenes and mineral oils.

Migrazione di sostanze organiche attraverso rivestimenti bio-polimerici da imballaggi in carta e cartone per uso alimentare

La presente tesi di dottorato ha riguardato lo studio del fenomeno di migrazione dagli imballaggi in carta e cartone agli alimenti, lo sviluppo e la valutazione di differenti coatings bio-based in grado di offrire un'adeguata barriera verso contaminanti chimici, tra i quali ftalati, fotoiniziatori, diisopropilnaftaleni e oli minerali.

Key words: Food packaging; migration; risk assessment; paper and board; bio-polymers.

1. Introduction

In accordance with the PhD thesis project previously described (Guazzotti, 2011), this oral communication reports the main results of the following activities directed to:

(A1) Screening for organic chemicals in paper and board packaging intended for food use by SPME and solvent extraction GC/MS technique, chemometric approach and risk assessment;
(A2) Development and characterization of bio-based renewable coatings onto paper and board substrates;
(A3) Migration studies of selected substances between paper and food simulants, comparison of paper, bio-coated paper and plastic materials;
(A4) Evaluation of plastic and bio-based barriers coated onto paper against mineral oils migration.

2. Migration of chemicals from paper-based packaging materials to food

Paper and Board (P&B) represent a large sector of the food and drink packaging market. They originate from natural sources and are thus regarded as environmentally friendly and perceived by consumers as safe. In addition, they are recyclable and, to attain a more sustainable economy, P&B partly or fully produced from recycled fibres are already being used in direct or indirect contact with certain foodstuffs in many countries in Europe. In recent years, several studies have evaluated the suitability of P&B for food use focus on using recycled fibre, which is currently between 40 and 90% depending on the material's use (Simoneau *et al.* 2003). Recycled fibre is considered a major source of migrants (Poças and Hogg 2007; Guazzotti *et al.* 2012a), among which the

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via Velleia 2 - MONZA

SCREENING CHIMICO-ANALITICO DI IMBALLAGGI IN CARTA E CARTONE PER USO ALIMENTARE: APPROCCIO CHEMIOMETRICO E VALUTAZIONE DEL RISCHIO DI MIGRAZIONE

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Il presente lavoro di ricerca riporta i risultati di un'indagine analitica condotta su una ventina di tipologie d'imballaggio in carta e cartone per uso alimentare, allo scopo di identificare i composti chimici più comuni e caratteristici. Tali materiali sono stati selezionati in considerazione di una vasta gamma di utilizzi (dal confezionamento primario e secondario agli articoli per fast food). È stato dapprima condotto uno screening analitico mediante tecniche di Micro Estrazione in Fase Solida (SPME) ed estrazione con solvente (SE) entrambe seguite da successiva analisi in gascromatografia/spettrometria di massa (GC/MS) al fine di determinare le principali molecole volatili e non volatili presenti. È stato possibile rilevare la presenza di solventi residui, probabilmente proveniente da inchiostri di stampa, così come idrocarburi e composti aromatici, principalmente toluene e plastificanti, legati al contenuto di carta riciclata, come diisobutil ftalato (DIBP) o diisopropil naftaleni (DiPNs) in differenti tipologie d'imballaggio. L'analisi dei dati è stata condotta mediante approccio statistico multivariato. Attraverso l´analisi delle componenti principali (PCA) sono stati individuati e selezionati alcuni composti chimici come marker per la classificazione dei campioni di imballaggio in carta e cartone in funzione della presenza di materiale da riciclo, del trattamento di superficie e delle loro caratteristiche di stampa. Il metodo analitico utilizzato, combinato con l´approccio chemiometrico, si è dimostrato essere un modo efficace per la trattazione di tali dati. In seguito, seguendo la procedura di Risk Assessment, è stata eseguita una ricerca documentale dedicata a proprietà tossicologiche o restrizioni legislative vigenti per tutte le sostanze individuate (identificazione dei pericoli). Inoltre, la semi-quantificazione dei composti negli imballaggi, ha consentito, mediante l´applicazione del modello di migrazione totale a tempo, una stima della contaminazione di alimenti in condizioni limite; occasionalmente, tali stime portano al superamento dei limiti di migrazione previsti dalla legge.

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BIO-COATINGS PER IMBALLAGGIO ALIMENTARE: STUDIO DELLA RIPARTIZIONE E MIGRAZIONE DI CONTAMINANTI TIPICI DA CARTA RICICLATA

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Il presente lavoro di ricerca riporta i principali risultati riguardanti lo sviluppo e la caratterizzazione di coatings a base di biopolimeri applicati su carta e cartone a uso alimentare. Tali materiali sono stati testati ai fini delle proprietà barriera nei confronti della migrazione di contaminanti chimici. In particolare, sono stati considerati rivestimenti a base acquosa, costituititi da biopolimeri rinnovabili, tra i quali: amidi modificati, proteine animali e vegetali. La caratterizzazione dei campioni di carta bio-rivestita è stata condotta mediante misure di angolo di contatto e osservazioni microstrutturali. Allo stesso tempo, sono stati realizzati studi di ripartizione e diffusione di molecole selezionate tra carta o carta bio-rivestita e aria o simulanti alimentari, inoltre, quale confronto, sono stati considerati diversi rivestimenti plastici. L'obiettivo di tali esperimenti è stato valutare il comportamento fisico-chimico e le proprietà barriera dei bio-rivestimenti verso la migrazione dei contaminanti più tipici degli imballaggi in carta riciclata. Notevoli differenze sono state evidenziate nell´adsorbimento dei contaminanti tra carta bio-rivestita o non e aria. Sia per i composti polari che per quelli non polari (benzofenone e diisobutilftalato, rispettivamente), i minori coefficienti di ripartizione sono stati raggiunti nella carta bio-rivestita, rendendo evidente come i biopolimeri testati siano stati repellenti nei confronti di tali contaminanti. Tali risultati sono stati discussi in relazione delle caratteristiche proprie di ogni biopolimero. Studi di diffusione nel simulante alimentare solido poli 2,6-difenil-p-fenilene ossido, noto anche come Tenax®, hanno confermato che tutti i biopolimeri testati rendono possibile un rallentamento della migrazione. I dati sperimentali sono stati interpretati mediante l'applicazione del modello cinetico di Weibull. I valori trovati per β, indice corrispondente alla presenza di una fase di latenza, variano tra 1,1-1,7 per la carta tal quale o rivestita da polietilene mentre per la carta bio-rivestita variano tra 2,2-4,9, dimostrando un'evidente proprietà barriera da parte dei biopolimeri testati.

VALUTAZIONE DI RIVESTIMENTI A BASE AMIDO SU CARTONE CONTRO LA MIGRAZIONE DI OLI MINERALI

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Il presente lavoro di ricerca riporta i risultati riguardanti la valutazione della proprietà barriera di differenti rivestimenti a base amido contro la migrazione di oli minerali provenienti da carta e cartone per imballaggio alimentare. Lo studio è stato focalizzato su differenti formulazioni, in considerazione del contenuto in plasticizzante (sorbitolo), tecnica di deposizione (applicatore a barra su scala di laboratorio vs size press su impianto pilota) e alla tipologia di amido (contenuto in amilosio/amilopectina, caratteristiche visco-amilografiche e grado di modificazione). I materiali sviluppati sono stati utilizzati per prove di migrazione condotte in celle di vetro (Migracell – Fabes) a diverse combinazioni di tempo/temperatura. L'utilizzo di tali celle ha permesso la valutazione delle proprietà barriera alla migrazione di tali bio-rivestimenti posti tra una carta appositamente contaminata da idrocarburi saturi (donatore) e il simulante alimentare Tenax (recettore) in contatto diretto o indiretto. Inoltre è stato realizzato un test di confronto con una situazione reale di stoccaggio, nella quale è stata utilizzata una carta riciclata contenente oli minerali come donatore. I risultati emersi sono stati evidenziati e discussi insieme alle caratteristiche chimico-fisiche proprie dei bio-polimeri testati, valutate mediante differenti tecniche di caratterizzazione. In conclusione, attraverso il presente studio, è stato possibile acquisire un necessario patrimonio di conoscenze riguardo alle potenziali proprietà di barriera alla migrazione di materiali allo stesso tempo biodegradabili.

Appendix 2

Publications

Food Packages n°40, pg.26-28

ULTIME NOVITÀ DALLA RICERCA

Rassegna CISETA 2011

GSICA GSICA

GSICA ha patrocinato la sessione di packaging del congresso Italiano di Scienza e Tecnologia degli Alimenti, giunto alla sua decima edizione

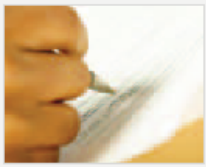
Nell'ambito di una delle più importanti manifestazioni fieristiche sugli alimenti, Tuttofood, si è svolta, il 9-10 maggio 2011, la decima edizione del congresso Italiano di Scienza e Tecnologia degli Alimenti, CISETA. Nel corso dei due giorni scientifici sono stati affrontati, con elevato rigore metodologico e professionale, i temi di maggiore interesse per l'intero comparto alimentare. In particolare, nella sessione intitolata: "Sicurezza ed efficacia delle soluzioni di confezionamento alimentare", sotto il coordinamento di **Luciano Piergiovanni**, presidente GSICA e direttore del diSTAM di Milano, sono stati presentati i migliori risultati della ricerca scientifica sul food packaging e sono stati tracciati gli indirizzi su cui fondare il trasferimento tecnologico nei prossimi anni. Sette i contributi scientifici presentati durante la sessione nella forma di comunicazioni orali e otto i poster. Numerose le università italiane rappresentate, ma non è mancata la partecipazione di laboratori privati e istituti indipendenti di ricerca.

Quanto ai temi trattati, l'orientamento alla sostenibilità ambientale è stato rimarcato in molti interventi, dall'applicazione del Life Cycle Assessment (LCA) al settore degli imballaggi, presentato da **Miguel Angel Silva** di Neotron spa, alla valutazione del possibile utilizzo di PET di riciclo all'interno di strutture multistrato coestruse (PET/R-PET/PET), condotta da **Alberto Taffurelli** per CSI spa. Proprio l'approccio di analisi delle capacità barriera di materiali multistrato nei quali la parte interna è di riciclo risulta oggi l'obiettivo di molti studi sui materiali food grade plastici e non solo. Si ricerca la sicurezza e l'assicurazione dell'idoneità di questi nuovi materiali, ma con un occhio di particolare riguardo alla loro

IN PRIMO PIANO

Le dichiarazioni di conformità

a cura di Valeria Guazzotti e Erika Tasca ricercatrici al DISTAM di Milano



La conformità alle norme vigenti deve essere dimostrata da una documentazione appropriata: la Dichiarazione di Conformità (DC). La DC deve essere disponibile su richiesta delle autorità competenti. È sempre obbligatoria. In Europa per: plastiche, ceramiche, imballaggi intelligenti e attivi. A livello nazionale per: plastiche multistrato, carte e cartoni, gomme, vetro, acciaio inox, alluminio metalli e leghe rivestiti e non. La dichiarazione di conformità deve, inoltre, essere collegata alla relativa documentazione tecnica a supporto (DS). Quest'ultima non deve essere obbligatoriamente tenuta in azienda bensì può essere disponibile anche presso un fornitore o un laboratorio. Spesso la DC risulta generica e i controlli vertono proprio sulle DS. In pratica la DC è il veicolo legale, la DS è la prova di quanto dichiarato. La DS è un documento privato dell'azienda, redatta da un consulente o da un laboratorio di analisi a seconda delle esigenze del cliente e può contenere accordi commerciali. Non è dunque accessibile ad altri fornitori o clienti ma solo alle autorità di controllo. Anche il sistema europeo sta evolvendo e presto l'onere della dimostrazione di conformità non graverà più sulle autorità pubbliche ma sulle imprese. Le dichiarazioni di conformità dovranno quindi essere sempre più complete e le evidenze documentali in house dovranno essere sempre più accurate. Come aiutare le aziende di piccole e medie dimensioni a preparare documenti corretti? Sono disponibili le Linee Guida per l'applicazione del Regolamento 2023/2006/CE alla filiera dei materiali e oggetti destinati al contatto con gli alimenti, il documento frutto della prima fase del progetto CAST, ora tradotte in lingua inglese per tutta Europa. I prossimi passi? In autunno verrà pubblicato un nuovo documento guida che consentirà agli operatori del settore di disporre di uno strumento pratico e completo per redigere le DS. Anche per questo secondo progetto si prevede già, oltre a quella italiana, la versione in inglese.

Modifiche al Reach

Pubblicati due aggiornamenti al Reach (a fine notizia i riferimenti alle gazzette) acronimo di Registration, Evaluation, Authorisation of Chemicals.

La norma armonizza tutte le disposizioni ad oggi esistenti sulle sostanze chimiche. Tra le novità l'inserimento di otto sostanze di estrema criticità classificate come cancerogene e tossiche per la riproduzione, aggiunte alla lista delle sostanze soggette ad autorizzazione, prima di essere utilizzate. Ricordiamo che grazie al Reach è stato istituito un sistema più efficiente e specifico per ottimizzare la gestione delle conoscenze (eco)tossicologiche di oltre 30'000 chemicals. Il produttore di una particolare sostanza diventa il responsabile delle informazioni fornite e le autorità sono chiamate alla valutazione dei dati forniti dall'industria. Per sostanze di particolare rischio sono poi previsti dei programmi di test ad-hoc.

Per adeguarsi al Reach, prima di tutto, è necessario elaborare l'inventario delle materie prime (sostanze e preparati). Nel caso di preparati è opportuno identificare la composizione dichiarata dal fornitore. Per ogni sostanza tal quale o presente nei preparati, va identificato il numero CAS e, se possibile, il numero EINECS o ELINCS. Bisogna poi stabilire le quantità acquistate annualmente e stilare una lista di tutti i fornitori, con verifica della sede di produzione e cioè controllare se il fornitore è stabilito al di fuori dell'UE. Segue la verifica della disponibilità delle schede con i dati di sicurezza e la conformità di queste alla legislazione esistente. Infine si devono raccogliere le informazioni sugli utilizzi e le condizioni d'uso delle materie prime all'interno della propria impresa e verificare l'eventuale disponibilità di informazioni relative al rilascio nell'ambiente, all'esposizione nel luogo di lavoro. La maggior parte delle aziende di packaging sono da considerare utilizzatori di sostanze chimiche. Qualche esempio? I produttori di vernici, inchiostri, adesivi, le industrie grafiche, le industrie chimiche di formulazione; gli utilizzatori di lubrificanti e oli, i produttori di articoli quali i componenti elettronici.

Anche l'industria dei polimeri plastici rappresenta una delle categorie direttamente coinvolte nell'ottemperanza del Reach. Recentemente è decollato il progetto Polymer Reach con l'obiettivo di sviluppare nuovi contenuti, una piattaforma di e-learning dedicata specificatamente all'industria europea delle materie plastiche, per supportare le aziende, soprattutto le PMI, nella comprensione del regolamento Reach e nel miglioramento delle loro competenze in materia (www.polymer-reach.eu).

Regolamento (UE) n. 109/2012 della Commissione, del 9 febbraio 2012, recante modifica del regolamento (CE) n. 1907/2006 del Parlamento europeo e del Consiglio concernente la registrazione, la valutazione, l'autorizzazione e la restrizione delle sostanze chimiche (REACH) per quanto riguarda l'allegato XVII (sostanze CMR). Gazzetta Ufficiale Europea L37 del 10/02/2012.

Regolamento (UE) n. 125/2012 della Commissione, del 14 febbraio 2012, recante modifica dell'allegato XIV del regolamento (CE) n. 1907/2006 del Parlamento europeo e del Consiglio concernente la registrazione, la valutazione, l'autorizzazione e la restrizione delle sostanze chimiche («REACH»). Gazzetta Ufficiale Europea L41 del 15/02/2012. ■

Food Packages n°46, pg.12-17

Packaging sicuro

Trasparenza, collaborazione, competenza e condivisione. Sono questi gli ingredienti per alimenti sicuri in confezioni sicure

VALERIA GUAZZOTTI
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AIDEPI (Associazione delle Industrie del Dolce e della Pasta Italiani) ha organizzato, in occasione di Ipack-Ima 2012, il workshop "*La scelta del packaging nella sicurezza alimentare*" con l'obiettivo di approfondire la conoscenza della documentazione di supporto a dimostrazione della conformità degli imballaggi a contatto con gli alimenti. Importanti esponenti istituzionali, del mondo della produzione del packaging e della distribuzione moderna, hanno partecipato all'incontro per affrontare i principali temi riguardanti i materiali non regolamentati in modo specifico, tra i quali carta e cartone e le sostanze non listate. Comune denominatore nelle relazioni proposte, la valutazione del rischio attraverso il dialogo di filiera.

"La sicurezza alimentare è correlata alla presenza di pericoli che possono essere introdotti in qualsiasi punto della filiera alimentare. Le organizzazioni che vi operano devono avere la capacità di controllarli soddisfacendo tutti i requisiti necessari e attuando le opportune misure di controllo. Questo obiettivo si può raggiungere combinando tre elementi che devono funzionare in sinergia: un sistema di gestione integrato, un laboratorio interno dedicato alla sicurezza e alla verifica della conformità e, infine, un dipartimento di ricerca e sviluppo che tenga conto della sicurezza alimentare sin dalla progettazione di ogni nuova soluzione o sistema", ha spiegato **Andrea Cassinari**, presidente AIBO-FCE (Associazione italiana business operator – food contact expert) e Quality & GMP Manager presso Cellografica Gerosa spa. "In particolare la costituzione di un laboratorio interno di food

Keywords
Linea guida
Normativa
Sicurezza alimentare

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Contamination of Polyvinyl Chloride Cling Films from Cardboard Packaging

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An analytical screening was undertaken with the aim of investigating the occurrence of di-isobutylphthalate (DIBP) in polyvinyl chloride (PVC) cling films for food contact applications and its source of contamination throughout a converting process. Although raw plastic materials used by producers are free from phthalates and analytical evidences confirm their absence after the extrusion process, DIBP can be found in final rolls packaged into cardboard packaging during storage.

A solvent extraction Gas Chromatography/Mass Spectrometry (GC/MS) analysis was applied on several intermediate products and at different stages taken from the converting process, with the aim of identifying the source of contamination. Different cardboard cores and folding cardboards made of recycled fibres were analyzed, and some of them resulted highly contaminated by DIBP. The storage of final cling films with these materials increased DIBP transfer into PVC.

To investigate the possible DIBP transfer mechanism from contaminated paper and adsorption by plastic materials through the gas phase, kinetic experiments were performed in a model system. Results obtained at 20 °C, 30 °C and 40 °C showed a considerable uptake of DIBP into PVC; Weibull model parameters estimated from the experimental data suggested an initial rate of the process dependent on temperature. In addition, to evaluate the partitioning behaviour, adsorption isotherms of DIBP into paper, PVC and low low density polyethylene (LLDPE) cling film were obtained at 40 °C.

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KEY WORDS: cling film; cardboard packaging; contamination of packaging films; di-isobutylphthalate

INTRODUCTION

The European Union legislation for testing the transfer of constituents from plastics intended to come into contact with food has recently been revised.[1] Authorized substances are specifically listed in Annex I of the Regulation 10/2011/CE; however, compounds not included in the list may still be resent in the finished product in the form of impurities, decomposition products or reaction intermediates from the authorized starting substances. A risk assessment for these compounds is necessary to ensure compliance with the Framework Regulation 1935/2004/CE, which states in Article 3 that all materials and articles intended to come into contact with foods should be safe for human health. This assessment is the responsibility of the producer of the material or article.

An analytical surveillance of contaminants from plastic food contact materials (polyolefin drinking bottles, water boilers, polyamide cooking utensils and plastic multilayer materials) performed by Norwegian Food Safety Authority[2] demonstrated that most migrants were substances not intentionally added to the plastic or originating from nonplastic components, such as printing inks, adhesives, not-listed additives, solvents and coatings.

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Parole chiave: imballaggio in carta e cartone, materiali a contatto con alimenti, migrazione, risk assessment

Keywords: paper and board packaging, food contact materials, migration, risk assessment

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Food Additives and Contaminants

Bio-based coatings as potential barrier to chemical contaminants from recycled paper and board packaging

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Abstract:	In this study partition and diffusion experiments with paper and board samples coated with different biopolymers were carried out. The aim was to evaluate the physicochemical behaviour and the barrier properties of bio-coatings against migration of typical contaminants from recycled paper packaging. Focus was directed to water-based, renewable biopolymers, such as: modified starches (cationic starch and cationic waxy starch), plant and animal proteins (gluten and gelatine), poured onto paper with an automatic applicator; additionally, a comparison with a polyethylene laminated paper was performed. Optical contact angle measurements and microstructural observations of the bio-coated paper allowed the characterization of samples. From the partitioning studies, considerable differences in the adsorption behaviour of the selected contaminants between bio-coated or uncoated paper and air were highlighted. For both the polar and the non polar compounds considered (Benzophenone and Diisobutyl phthalate, respectively) the lowest values of partition coefficients were achieved when paper was bio-coated, making evident that biopolymers acted as net repulsive layers. These findings were discussed