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Packaging Related Food Safety Aspects – Analytical Contributions to the Characterization of Food and Food Contact Materials

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“Believe you can and you're halfway there”

Theodore Roosevelt

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Abstract

Food packaging materials (FCM) based on cellulosic fibres are one of the most commonly used food packaging materials. With the current trend to reduce plastic materials for food packaging, paper and board as well as new bio-based materials will get even more important.
The advantages of paper and board FCMs are obvious – they are renewable, biodegradable and inexpensive and assure the strength and stability needed for a packaging. The disadvantage is the porous cellulose structure, resulting into a lack of most barrier functionalities needed, like resistance against grease, water vapour or oxygen. Furthermore, the use of recycled paper or board is of special importance, regarding its renewable character. However, during the recycling process fibres of different qualities are mixed together and a huge pool of possible contaminants is created. These contaminants are not removed during the recycling process, but enriched in the final product instead. From there they can be transferred into the packed food – even from secondary or tertiary packaging – and the safety of the product can no longer be guaranteed. For example, mineral oil hydrocarbons (MOH) are one of the most discussed contaminants present in food nowadays and food packaging made of recycled paper is considered to be one of the main sources. Special treatments or additional barrier layers are applied to improve the barrier properties of paper and board and to fulfil the complex requests. The application of functional barriers within the packaging is one of the most often used solution attempts. Various materials were used as barrier, including aluminium or different polyolefins, also in complex multi-layer materials. This makes recycling of the resulting packaging materials difficult and further desire for more sustainable materials arose.
Therefore, the aim of this work was to combine the above-mentioned aspects: On the one hand, recycled paper and board should be used as FCM and functional barriers applied. On the other hand, bio-based or biodegradable raw materials should be applied as barrier, while still ensuring the safety of the packed good. The first step was to comprehensively characterise different industrially produced paper samples for the present contaminants, able to be transferred into a packed good. Second, biopolymers to be used as functional barriers were identified and applied on paper and board FCMs. The resulting barrier properties were characterised using a newly developed two-sided migration test. Third, different food types were analysed for their contamination with mineral oil hydrocarbons (MOH).
Since the needed analyses are a complex task, a wide range of analytical techniques – mainly gas chromatography-based ones – were used and the gathered information combined, to provide a comprehensive overview of packaging related food safety aspects.

Vorwort

Zellulosebasierende Materialien sind im Moment bereits eine der am häufigsten verwendeten Lebensmittelkontaktmaterialien (Food Contact Materials, FCM). Mit dem aktuellen Trend zur Reduzierung von Kunststoffen werden sie zusammen mit anderen biobasierten Materialien aber noch wichtiger. Dabei liegen die Vorteile von FCMs aus Papier und Karton auf der Hand: Sie zählen zu den erneuerbaren Rohstoffen, sind biologisch abbaubar, kostengünstig und gewährleisten die erforderliche Festigkeit und Stabilität der Verpackung. Ihr Nachteil ist allerdings die poröse Struktur des Zellulosenetzwerkes. Diese führt dazu, dass viele der benötigten Barrierefunktionen, wie die Beständigkeit gegen Fett, Wasserdampf oder Sauerstoff, fehlen. Darüber hinaus ist die Verwendung von Recyclingfasern von besonderer Bedeutung. Einerseits, gerade wegen des umweltfreundlichen Charakters, andererseits wegen der resultierenden Gefahren: Während des Recyclingprozesses werden Fasern unterschiedlicher Qualität miteinander vermischt und es entsteht ein riesiger Pool möglicher Kontaminationen. Diese werden während des Recyclingprozesses nicht entfernt und stattdessen im Endprodukt angereichert. Von dort können sie - auch aus Sekundär- oder Tertiärverpackungen - in das verpackte Gut übergehen und die Sicherheit desselben kann nicht mehr gewährleistet werden. So sind z.B. Mineralölkohlenwasserstoffe (MOH) eine der am häufigsten diskutierten Kontaminanten in Lebensmitteln, mit Verpackungen aus Recyclingfasern als eine der Hauptquellen. Spezielle Behandlungen oder zusätzlich eingebrachte Schichten werden daher verwendet, um die Barriereeigenschaften zu verbessern und die komplexen Anforderungen zu erfüllen. Als Barrieren wurden bereits vielerlei Materialien verwendet, darunter z.B. Aluminium oder verschiedene Polyolefine, auch als Kombinationen in komplexen Mehrschichtmaterialien. Diese erschweren allerdings das Recycling der resultierenden Verpackung und der Wunsch nach umweltfreundlicheren Materialien wurde laut.

Ziel dieser Arbeit war es daher, die oben genannten Aspekte zu kombinieren: Einerseits die Verwendung von Recyclingpapier und -karton als FCM, in Kombination mit funktionellen Barrieren. Andererseits, sollten aber biobasierte und/oder biologisch abbaubare Rohstoffe verwendet werden, wobei die Sicherheit des verpackten Gutes im Mittelpunkt stand. Deshalb wurden zunächst industriell hergestellte Papierproben auf ihre vorliegenden Verunreinigungen untersucht. Zweitens wurden verwendbare Biopolymere identifiziert und anschließend auf verschiedene Papiere als Barriere aufgebracht. Die resultierenden Barriereeigenschaften wurden mit einem neu entwickelten, zweiseitigen Migrationstest charakterisiert. Drittens wurden verschiedene Lebensmittelarten auf Kontamination mit MOH untersucht.

Da die erforderlichen Analysen sehr komplex sind, war eine Vielzahl von verschiedenen Analysetechniken - hauptsächlich auf Grundlage von Gaschromatographie – nötig. Die gesammelten Informationen wurden vereint, um einen umfassenden Überblick über die Aspekte der Lebensmittelsicherheit in Bezug auf Verpackungen zu erhalten.

Content

List of Frequently Used Abbreviations

FCM = food contact material
HS-SPME = head space-solid phase micro extraction
SPE = solid phase extraction
GC= gas chromatography
MS = mass spectrometry
qMS = quadrupole mass spectrometer
TOF = Time-of-Flight mass spectrometer
FID = flame ionization detector
HPLC = high pressure liquid chromatography
GC×GC = comprehensive two-dimensional GC
MOH = mineral oil hydrocarbons
MOSH = mineral oil saturated hydrocarbons
MOAH = mineral oil aromatic hydrocarbons
POSH = polyolefin oligomeric saturated hydrocarbons
PAH = polyaromatic hydrocarbons
g= grams
ng = nanograms
pg = picograms
mg = milligrams
kg = kilograms
m = meter
mL = millilitres
µL = microliters
L = litres
ms = millisecond
µs = microsecond
sec = second
min = minute
h = hours
d = days
Da = Dalton
°C = degree Celsius
kV = kilovolts
(v/v) = volume per volume
(w/w) = weight per weight
ADI = acceptable daily intake
b.w. = body weight
MOE = margin of exposure

NOAEL = no-observed-adverse-effect level
RP = reference point
PE = polyethylene
LLDPE = linear, low density polyethylene
PP = polypropylene
PTFE = polytetrafluoroethylene
MTBE = methyl tert-butyl ether
m-CPBA = meta-Chloroperbenzoic acid
HCl = Hydrochloric acid
Al_2O_3 = aluminium oxide
MPPO = Tenax® TA a poly(2,6-diphenyl-p-phenylene oxide)
EtOH = Ethanol
C_n = hydrocarbon with a chain length of *n* C-atoms
5B = 5-Pentylbenzene
1-, 2-MN = 1-, 2-Methylnaphthalene
TBB = tri-tert-Butylbenzene
Per = perylene
CyCy = cyclohexylcyclohexane
Cho = cholestane
DIPN = Diisopropylnaphthalene
DEHP = bis(2-ethylhexyl) phthalate
DnBP = di-*n*-butyl phthalate
DiBP = di-*iso*-butyl phthalate
IS = internal standard
CHT = chitosan
MFC = microfibrillated cellulose
EFSA = European Food Safety Authority
BfR = German Federal Institute of Risk Assessment

1. Introduction

1.1. Motivation and Aim

Food contact materials (FCMs) and articles are materials and articles that are considered to come into contact with food. They should not change the food in any unacceptable way, neither in terms of taste or quality nor in terms of contaminations (REGULATION (EC) No 1935/2004; US FDA 2018)).

FCMs made from cellulosic fibres are versatile, from paper and board to single use tableware, coffee filters and tissues, to name just a few examples. Food packaging materials based on cellulosic fibres are one of the most commonly used food packaging materials (Muncke 2012). They are renewable, biodegradable and cheap and assure the strength and stability needed for a packaging. With the current trend to reduce plastic materials for food packaging, paper and board as well as new bio-based materials will get even more important (Team Media Relatins REWE International AG, 2018) (Nestle Deutschland, 2019). However, they have to keep their functionality under permanently changing conditions, like varying temperature or moisture, and over long storage periods, thereby protecting the food till the end of its shelf-life. To meet these expectations, the food packaging needs various barrier functions, like resistance against grease, water vapour, water, oxygen, nitrogen or volatile organic compounds. Due to its porous cellulose structure, paper lacks most of them and needs special treatment or additional barrier layers to improve the barrier properties (Andersson, 2008).

Of special importance is the use of recycled paper or board as FCM. The recycling rate of paper and board is above 70% in Austria at the moment. For the production of e.g. folding boards recycled fibres are added in 90% of cases ([ERPC] European Recovered Paper Council, 2015). These folding boards are often used in secondary or tertiary food packaging material and pose one problem: during the recycling process fibres of different purities and sources (e.g. newspapers, packaging materials, tissues) are mixed together and a huge pool of possible contaminants is created. These contaminants, as e.g. mineral oil residues or plasticizers, are not removed during the recycling process and enriched in the final product instead. From there they can be transferred into the packed food – even from secondary or tertiary packaging- and the safety of the product can no longer be guaranteed (Mariani, et al., 1999) ([BfR] German Federal Institute for Risk Assessments, 2016) (Geueke, et al., 2018).

This work focused mainly on recycled board, on contaminants present, transfer processes, and the way to reduce contaminations. The only fast and practical solution for reducing contaminations seems to be the application of functional barriers in the packaging (Ewender, et al., 2013). Recently applied barriers include aluminium or different synthetic materials like various polyolefins. Since polyolefins lack on satisfying barrier properties, they are often integrated into multilayer materials with complex structures encountered in recycled paper or board (Richter, et al., 2014).

Based on the increasing criticism on plastic materials in Europe and the sustainability reputation of cellulose-based materials, bio-based alternatives are needed. Aim of this project was

therefore on the one hand to find bio-based or biodegradable materials that can be used as functional barriers. Different sorts of paper were coated with varying barrier materials at the Institute Paper, Pulp and Fibre Technology and the physical parameters evaluated (PhD-Thesis of Kopacic, S.; (Kopacic, 2019)). From the analytical side, comprehensive testing of present contaminants and their possible migration into food was evaluated. Since testing methods are rare, mostly time consuming and complicated, different analytical attempts for testing functional barriers were discussed, tested and applied on the coated papers.
On the other hand, the migration of mineral oil hydrocarbons (MOH) from recycled paper and the presence in food was evaluated. MOHs are one of the most discussed contaminants present in food nowadays and food packaging made of recycled paper is considered to be one of the main sources. In general, MOH are divided into a saturated and an aromatic fraction (Biedermann, et al., 2009). Mineral oil saturated hydrocarbons (MOSH) consist of branched and unbranched open chain hydrocarbons, called paraffines, and cyclic hydrocarbons with at least one saturated ring, called naphthenes. They can accumulate in the human body and cause granuloma formation, inflammatory diseases and organ weight increase. The mineral oil aromatic hydrocarbons (MOAH) consist of aromatic substance classes with mainly 1-7 aromatic (or partly aromatic) ring systems with an alkylated side chain. Usually the MOAH fraction can be found in a ratio of 15-30% of the whole mineral oil fraction (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012). Routine analysis is done using online coupling of high-pressure liquid chromatography and gas chromatography with flame ionization detection (HPLC-GC-FID), but this method lacks on detailed information (Biedermann, et al., 2012a). Therefore, multidimensional techniques are used, like comprehensive 2D-GC×GC with mass spectrometry detection (MS) to further characterize these fractions. Analysis of not only paper and board and migrates was done, but also of various food matrices, to get an overview of current levels of contamination. All generated information was combined to the resulting packaging related food safety aspects.

The results of this work were presented at various occasions in oral or written form; a list is given in the appendix. Following peer-reviewed publications are accessible:

- Walzl, A., Kopacic, S., Bauer, W., & Leitner, E. (2019). Characterization of natural polymers as functional barriers for cellulose-based packaging materials. Food Additives & Contaminants: Part A, 36(6), 976–988.
- Kopacic, S., Walzl, A., Hirn, U., Zankel, A., Kniely, R., Leitner, E., & Bauer, W. (2018.). Application of Industrially Produced Chitosan in the Surface Treatment of Fibre-Based Material: Effect of Drying Method and Number of Coating Layers on Mechanical and Barrier Properties. Polymers, 10(11), 1232.
- Kopacic, S., Walzl, A., Zankel, A., Leitner, E., & Bauer, W. (2018.). Alginate and Chitosan as a Functional Barrier for Paper-Based Packaging Materials. Coatings, 8(7), 235.

1.2. Migration

The transfer of volatile organic compounds from packaging into food is called migration. These migration phenomena are complex processes and depend on the nature of the migrant, the material from which it is released, the packed good or used simulant and the conditions of storage. The process is fast and depends also on the surrounding atmosphere of the packaging (Pocas, et al., 2011) (Triantafyllou, et al., 2007) (Mariani, et al., 1999). It can occur through two ways: by direct (wetting) contact or indirect contact (through the gas phase) (Mariani, et al., 1999) (Jickells, et al., 2005).

Transfer through direct contact is called migration. The migration process starts with the evaporation of the contaminants from the packaging, followed by their migration to the food and re-condensation on the food. The transfer of contaminants through direct contact can occur for substances with chain lengths of up to C_{35} or even C_{40}, depending on the storage time and temperature (Mariani, et al., 1999) (Lorenzi, et al., 2013).

The transfer through indirect contact is called permeation. The process of permeation requires the contaminants from e.g. a secondary packaging to be vaporized into the gas phase ("indirect contact") permeate through the primary packaging (and a potentially applied functional barrier) and re-condense on the food. Since evaporation at room temperature is only relevant for substances with a chain length of up to C_{24}, permeation is limited to these substances (Diehl, et al., 2015).

Since paper and board are very heterogeneous and porous materials, the transfer of substances through it is determined by adsorption and desorption on the fibres, transfer across fibres and diffusion into the air pores (Pocas, et al., 2011). Therefore, grammage and thickness play an important role on the migration rate: low grammage and thickness showed faster migration, since contaminants are present at the surface more readily (Nerin, et al., 2007) (Diehl, et al., 2015). Furthermore, the chemical characteristics of the paper play an important role: cellulose fibres are hydrophilic and non-ionic, while the lignin fractions present in paper includes aromatic phenols with carboxyl- and hydroxyl- groups, being mainly anionic. This results into a negatively charged paper surface, influencing the migrant making its way through the paper (Pocas, et al., 2011). Another important factor is the amount of recycled fibres present; on the one hand they are themselves sources for organic compounds able to migrate; on the other, they again change the papers chemical properties, depending on which and how much contaminants are in them. Studies trying to identify the influence of the amount of recycled fibres showed versatile results, suggesting that the influence is not very clear (Triantafyllou, et al., 2002) (Triantafyllou, et al., 2005) (Triantafyllou, et al., 2007),

The migrant itself needs to be volatile, which means it needs a high enough vapour pressure at the temperature of use, lower than its boiling point (Johns, et al., 1996). Studies suggest that there is a cut-off threshold for volatility, meaning that substances with a volatility lower the threshold are not transferred, while substances with a higher one may be transferred (Jickells, et al., 2005). Also, the polarity and the molecular weight and structure of the compound have influence on the amount of migration (Triantafyllou, et al., 2005).

To predict migration, the partition coefficient of substances between paper and air is probably the most important parameter (Triantafyllou, et al., 2005). The coefficient depends on the boiling point and structure of the migrant and increases with the temperature. However, it was shown that there is no linear relationship between boiling point of a substance and its partitioning behaviour (Jickells, et al., 2005). It seems that volatile substances partition more easily in the air, resulting in a low partition coefficient, but the influence of the paper and its properties, as mentioned above, is significant. For example, electron rich substances, like naphthalene, are not retained by the negatively charged paper, while e.g. methyl stearate or the very polar acetophenone with much higher boiling points partitioned strongly with the paper because of the high affinity to the fibres surface. The adsorption behaviour of substances in cellulosic fibres shows a Langmuir shape, depending on saturation of the fibres. Adsorption into paper decreases with higher temperature, resulting into higher migration levels (Triantafyllou, et al., 2005). Diehl, et al. (2015) also investigated the influence of the partition coefficient between the paper and the foodstuff.
Mathematical calculations, modelling and descriptions were done by several authors as e.g. (Piringer, et al., 1998) (Pocas, et al., 2011) (Franz, 1993) (Diehl, et al., 2015), but resulted into no clear statement and should not be discussed any further in this work.
Summarizing, migration and permeation are complex processes; their influencing parameters are manifold and diverse. General rules could not be found by the authors studied this topic before. Theses phenomena cannot be described easily and need careful considerations and testing case by case.

1.3. Challenge Recycled Board

The challenge of the analysis and risk assessment of recycled paper and board is well summarized in (Biedermann, et al., 2013a) and (Biedermann, et al., 2013b):
The problem is that today's analytical techniques are mainly focused on the determination of known substances. Either, the substances of interest are important constituents of a product or they are potentially harmful. With the right extraction procedures, highly selective clean-up steps, derivatisation and selective chromatographic techniques and detections, it is possible to determine known substances in low concentrations (down to the nanogram (ng) and picogram (pg) level and even lower). However, when talking about migration from recycled paper or board, the analytical challenge is the comprehensive analysis of largely unknown mixtures. Due to the fact that fibres of different quality match during the recycling process and impurities are not quantitatively removed, but enriched in the final product, the use of such materials as food contact material is critical. The enriched contaminants can migrate or permeate into the packed good. These migrates represent a large pool of possible analytes, e.g. from high to low polarity and molecular weight. To ensure the safety of a packaging, these complex mixtures need to be characterized comprehensively in an untargeted approach to find potentially harmful substances. Though, this attempt is unrealistic due to the limitation of our analytical techniques. Therefore, the EU Regulation 10/2011 sets a limit of potential toxicological relevance as 10 µg kg^{-1} food

for unknown or not evaluated substances (Commission Regulation (EU), 2011). Substances present below this limit are considered to be of no toxicological concern. This limit was also the result of a project of the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) started in 2009 ([BMELV], German Federal Ministry of Food, Agriculture and Consumer, 02.03.2010-31.05.2012). From this limit, a detection limit in paper and board is derived by the fact, that about 70% of the present contaminants below C_{24} migrate into food (Lorenzin, et al., 2010) (Biedermann, et al., 2013b) and that the mass ratios of paperboard to food in commonly encountered packs range from 1:4 to 1:25, with an average of around 1:10. Assuming a mass ratio of 1:7 (Fiselier, et al., 2010) and a 70% transfer resulted in a detection limit of 100 µg kg^{-1} in paper and board (Biedermann, et al., 2013a). Comprehensive analysis of all substances above this limit is not possible within a quick and easy procedure. As Biedermann et al. (2013a) states, a direct injection of a paperboard extract on a GC gives an estimate of how complex the task is. Many bulk substances in concentrations of 1-10 mg kg^{-1} are present (e.g. mineral oil, triglycerides or carboxylic acids), forming humps of unresolved substances, overloading the chromatogram and co-eluting with peaks of interest in concentrations of toxicological relevance. They tried to remove the bulk substances by several attempts, as described in (Biedermann, et al., 2013a) and explained the results of the final method in (Biedermann, et al., 2013b). In short, they used liquid extraction of the board with hexane/ethanol 1:1. The extract was concentrated resulting in an ethanol solution, which was turbid mainly because of the extracted triglycerides and waxes. This extract was filtered through a basic aluminium oxide (Al_2O_3) column and the analytes eluted with 10% water/methanol. The carboxylic acids, such as the resin acids (diterpenic structures like abietic and pimaric acid) remained on the column. For re-concentration, pyridine was added as a keeper and suitable solvent for silylation and HPLC injection. The HPLC was used to separate the extract into six fractions, applying a gradient with hexane, dichloromethane and MTBE. The fractions were analysed separately and showed that the first fraction mainly consisted of MOSH, the second of MOAH. The third fraction eluted ethers, the fourth esters, in the 5th phthalates, photoinitiators, diesters, phenols, carbonyls and branched alcohols were found, while linear alcohols, triesters and acrylates eluted in the 6th fraction. Silylation of the extract led to earlier elution of many compounds, as well as identification of new ones.

They summarized that it was possible to detect the most of the present substances in this recycled paperboard, but not all of them. It was impossible to identify all present substances in a concentration above 100 µg kg^{-1} in the paperboard, although using comprehensive 2D-GC×GC-MS to analyse the six fractions. The comparison of four different recycled paperboards showed that the composition of recycled board varies widely. Doing such comprehensive characterizations for every paperboard intended to come into contact with food would result into an immense unrealistic workload. Furthermore, the development of a comprehensive technique to characterize recycled paperboard quick and easily failed, since they found more than 250 substances, which can migrate into food in relevant concentrations. They concluded that safety assessment of the finished board is not possible by evaluating every single substance.

Therefore, they suggest the application of a functional barrier, which should reduce the migration (Biedermann, et al., 2013a) (Biedermann, et al., 2013b).

1.4. Functional Barriers

1.4.1. Need for Barriers

To prevent the migration and contamination of food by the packaging, several ways were thought of and discussed by e.g. Ewender et al. (2013). They discussed following five solutions for the problem (Ewender, et al., 2013):

1. Substitution of recycled fibres by fresh fibres: the idea of this solution is, if no recycled fibres are used anymore for food packaging, no migration can take place. However, there are several disadvantages. First, it does not help to exchange only the primary packaging with fresh fibres, because migration from secondary or tertiary packaging will still occur. So, second, every packaging needs to be from fresh fibre and the amount of needed fibre material would be immense. Third, it is known today, that migration from recycled fibre packaging is one major source of contamination, but not the only one. Contaminations by other sources throughout the whole production chain of the food would still be possible and present.
2. Elimination of mineral oil components from printing inks for newspapers: since newspapers and their printing inks are not foreseen to be used as food contact materials, there are no regulations for them. Nevertheless, they end up as recycled fibres in FCMs, having a negative influence in terms of contaminations. An exchange of the printing inks would be possible and mineral oil free printing inks e.g. low migration inks for FCM applications are already available. However, the requirements on printing inks for newspapers are high (e.g. printing speed, viscosity…) and exchange difficult and expensive.
3. Decontamination during paper and cardboard recycling: a cleaning step, which results in clean, contamination free fibres, similar to fresh fibres, would be a great solution solving the whole problem of migration from FCM. However, including such a step into the existing processes is difficult, a quick and easy cleaning step so far not available and considering the technical possibilities also unrealistic.
4. Sorting of the input streams of paper and board recycling: there is the possibility of sorting newspapers with mineral-oil-based printing inks from the fibre material for FCMs prior to the recycling process. This seems to be a complicated, time intensive step needing change of the existing processes. However, according to a talk by Traussnig H. given at an ILSI Workshop on mineral oil risk assessment in 2019, this is state of the art nowadays, resulting into a decrease of fibre contamination (Traussnig, 2019).
5. Application of functional barriers: Bbarriers implemented into the food packaging as coating or internal bag can reduce the migration significantly, without changing the recycling process. However, after opening the package or internal bag by the consumer,

migration can again occur. Also, finding suitable barrier materials can be difficult. This problem is solved by the use of complex multilayer materials. These materials have again disadvantages, as e.g. the recyclability of the resulting packaging material. A detailed discussion on used materials will be made in chapter 1.4.2.

Careful consideration of the above-mentioned opportunities led to the decision that the only fast and practical solution seems to be the application of the functional barriers. As described in the EU Regulation 10/2011 *"a 'functional barrier' means a barrier consisting of one or more layers of any type of material, which ensures that the final material or article complies with Article 3 of Regulation (EC) No 1935/2004 and with the provisions of the Regulation 10/2011'"*. This means, the barrier is placed between the food and the food contact material and should prevent migration from the packaging into the food. Besides, the use of non-authorized substances is allowed behind a barrier, as long as their migration remains below 10 µg kg^{-1}. Substances that are mutagenic, carcinogenic or toxic to reproduction should not be used without authorization and are not covered by the concept (Commission Regulation (EU), 2011). On the other hand, the barrier should also preserve the quality and the taste of the packed good, without doing any changes to it (Diehl, et al., 2015) (Ewender, et al., 2013). A scheme of the functional barrier concept can be seen in Figure 1:

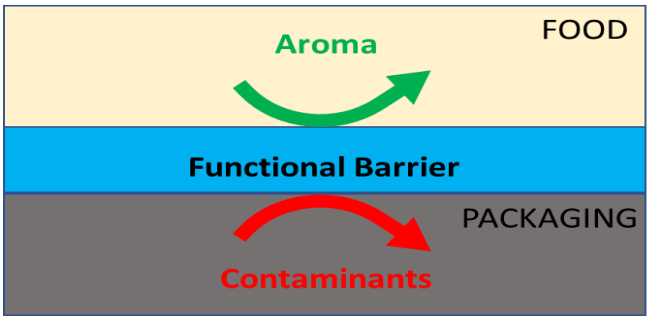

Figure 1: Scheme of functional barrier concept.

Materials used as functional barriers are e.g. aluminium, polyethylene (PE), polypropylene (PP) or combinations in multilayer materials. Metal foils are considered to be tight barriers, stopping migration completely, but are not environmental and food friendly. Synthetic polymers are favoured, but are no tight barriers and testing methods for their performance needed (Richter, et al., 2014) (Biedermann-Brem, et al., 2016). An overview of the conventional materials used and the possible new bio-based alternatives is given in chapter 1.4.2.

As just mentioned, to ensure that a material is an adequate functional barrier, test methods are needed. Simulation of barrier efficiency could be done, but is not satisfying in terms of real application (e.g. influence of pinholes, scratches, etc.). Therefore, one method was particularly developed to fit this purpose and first described by Fiselier and Grob and toady known as "Taped Barrier Test" (Fiselier, et al., 2012). One important definition they made in their publication is the breakthrough concept. It states that during long-term storage of food there may be a time period, where the functional barrier stops the migration completely and no

contaminants reach the food. This period is named lag time or breakthrough time. After this period, the migration into the food starts, but a good barrier slows migration down, so that migration limits are not reached within the shelf life of the product. The Taped Barrier Test and other possible testing concepts are described in the chapter 1.6.

1.4.2. Barrier Materials

1.4.2.1. Conventional Materials

As already mentioned, conventional barrier materials include aluminium and polyolefins. While aluminium is a tight barrier, the various plastic materials have highly diverse barrier functions. Most plastics were used as barriers against gases or humidity only; some others were used against permeation of organic substances to prevent e.g. the loss of aroma of the packed food. To render the porous material paper into a suitable food packaging material, several layers of various polymers may be needed. This was investigated and discussed by (Richter, et al., 2014). The design of internal bags used for recycled paperboard packaging of dry foods and their barrier properties against migration were described. The samples investigated were collected in Dresden and Zurich in June 2013. They identified the layers of the bags using microtome cuts and Fourier Transform Infrared Spectroscopy. The barrier efficiency of the bags was tested with the taped barrier test. The composition of the tested internal bags varied from a PP monolayer to complex multilayer materials with up to seven different materials. The materials found were paper, aluminium, PE, PP, polyethylene terephthalate (PET), glycol modified PET, polyamide (PA), polyethylene vinyl alcohol (EVOH), PE vinyl acetate (EVA), cellophane, polymethyl methacrylate and polyvinylidene chloride. Some of the layers were co-extruded, some had adhesion-promoting layers, others were applied as lacquers. PE multilayers often had pigments for their appearance filled between them, also ionomers were applied. Aluminium was used as a foil, laminated by an adhesive or as a vapour coating on PP, PA or PET. To protect it from scratches, it mainly was placed in the middle of multilayer barriers. The thickness of the barriers varied depending on their constructions and can be seen in the examples given below. The barrier properties of the materials were investigated using the Taped Barrier Test, which will be explained in detail later. The materials were divided into five classes by Richter, et al. (2013), depending on their barrier efficiency:

Class 1 had no relevant barrier efficiency with strong breakthrough of above 80% of the surrogate substances after one week at room temperature. Materials with Class 1 barrier properties were PE, paper and paper laminated with PE or EVA. For PE films it was also shown in (Biedermann, et al., 2013a) and (Biedermann, et al., 2013b) that they had hardly any real barrier efficiency, but reduced migration by absorption, similar to a sponge soaking up water.

Class 2 materials were oriented PP (OPP), used as monolayer or in combination with PE. Internal bags made of OPP kept migration of mineral oils low for about six months at room temperature, but migration increased significantly afterwards. Still, migration was about ten times lower than in comparable packaging systems with PE barriers.

Class 3 materials kept tight at room temperature, but had substantially more breakthrough than class 4 materials at elevated temperatures. Examples for class three materials are as given in (Richter, et al., 2014), Table 1: Paper co-extruded with 26 µm PE and 15 µm PVDC-PMMA or 2 µm PVDC co-extruded with 28 µm PP and 2 µm PVDC or 13 µm PET laminated by polyurethane to a 30 µm PE layer. As can be seen from these examples, the construction of the barriers is already getting complex.

Even more complex were the class 4 materials, which already had high barrier efficiency. They were tested at elevated temperature and kept tight for the duration of the experiment, equivalent to 150 weeks storage at room temperature. As given also in Table 1 in (Richter, et al., 2014), class 4 materials are e.g. paper printed with a 2 µm acrylate varnish, glued by an acrylate adhesive to 17 µm PP with vapour-deposited aluminium and a 3 µm layer of PE on the food contact side or a 17 µm PA layer co-extruded with 50 µm PE.

Class 5 were all materials including an aluminium foil layer (not including vapour-deposits). They were considered as tight without testing.

The examples given in (Richter, et al., 2014) show that it is a highly complex task to develop barrier materials, having all the needed properties and high barrier efficiency. Often, every single layer in a multilayer material is needed for its own characteristics to create does high-tech barriers. Consequently, most of the well performing barriers were highly complex ones, made up of several layers. In comparison, the poor barriers were mainly single layers of just one material.

The big disadvantage of these functional barriers is the additional packaging material needed. Often, they are incorporated in the paper and board material, recycling of the resulting FCM is, if possible, complicated and expensive. Consequently, cellulose-based FCMs lose their sustainable and environmentally friendly character. Furthermore, the bad reputation of plastic materials rising lately in Europe, results into the need for green alternatives (Nestle Deutschland, 2019) (Team Media Relatins REWE International AG, 2018).

1.4.2.2. Bio-Based Materials

To maintain the good reputation of paper and board food packaging materials, alternatives for the above-mentioned conventional barrier materials are needed. Biopolymers can be a good alternative. They have the potential to replace petroleum-based coatings, thereby strengthening the agricultural economy and reducing the importance of petroleum and its products and derivatives (Khwaldia, et al., 2010).

In general, biopolymers are defined as materials synthesised in the cells of living organism, meaning that they are polymers of natural origin. Therefore, they are divided into the three possible classes of their origin: polynucleotides (RNA and DNA), amino acid-based polymers (peptides and proteins) and polysaccharide-based polymers (carbohydrates) as summarised in (Khwaldia, et al., 2010) and (Hassan, et al., 2019). For biopolymers used as a raw material no general definition is available. They are mostly considered as biopolymer, if they are either bio-based and/or biodegradable. This means that also mineral oil-based polymers are considered to be biopolymers, if they are biodegradable (Hassan, et al., 2019).

Natural polymers used for coating of paper are mainly divided into protein-based, sugar-based and lipid-based ones (Khwaldia, et al., 2010).

1.4.2.2.1. Protein-Based Polymers

Proteins had been used for film formation or in coatings by several authors as e.g. (Gupte, et al., 2015), (Gennadios, 2002), (McHugh, et al., 1994) or (Sun, 2005). They have been coated on various different food matrices, but also on paper. They show excellent oxygen barrier properties and good mechanical properties. Their disadvantage is the water sensitivity due to the hydrophilic nature, and the resulting poor barrier properties against water vapour. Examples for protein-based polymers are as given in (Khwaldia, et al., 2010) and (Gennadios, 2002):

- Whey protein is a by-product of cheese industry. It represents about 20% of the milk proteins and remains in the milk serum after cheese production. It is made up of a protein mixture, the main ones being α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulin and protease-peptones (Dybin, et al., 1991). Whey protein is already known as excellent barrier against oxygen, aroma and oil due to their film formation properties. They form water-insoluble films out of aqueous solutions, due to the formation of intermolecular disulphide bonds after heat denaturation of the proteins (McHugh, et al., 1994).
- A further milk protein is casein. It is a phosphoprotein, with only little amount of cysteine. Consequently, it cannot form disulphide bonds and formed films are water soluble (Dumont, 1998). Still, due to its random coil nature, its film formation properties are excellent (McHugh, et al., 1994). Casein is commercially available as acid casein or converted to soluble casein through neutralization with salts. It was shown that coating of paper with casein improved paper strength and reduced water vapour transmission (Khwaldia, 2010).
- Soy protein is a globulin protein of soybeans. It is mainly composed of glycinin and conglycinin (Sun, 2005). It is widely used in the United States in paper and paper coatings, since it improves mechanical strength, grease barrier properties and extends the shelf life of food products (Park, et al., 2000).
- Further polymers are e.g. corn zein or wheat gluten.

1.4.2.2.2. Polysaccharide-Based Polymers

Polysaccharide-based polymers show excellent gas, aroma and lipid barrier properties. They show strong interactions and therefore have good film-formation properties. The big disadvantage again is their hydrophilicity and the resulting water solubility. Examples for polymers are as described in (Khwaldia, et al., 2010) and (Cazon, et al., 2017)

- **Chitosan (CHT)** is an edible and biodegradable, positively charged polysaccharide, which is derived from deacetylation of chitin (see Figure 2). Chitin is the 2nd most abundant naturally occurring biopolymer after cellulose (No, et al., 1995). CHT has excellent film-formation properties, due to the high crystallinity and strong hydrogen bonding's between the molecular chains. Those result into robust and flexible films,

with good barrier properties against grease and oxygen (Kittur, et al., 1998) (Gällstedt, 2001). It has already been used as a paper-making additive, due to its positive effects on the printability. Furthermore, it was possible to develop water insoluble sheets of CHT and pulp fibres. CHT coating of paper showed good moisture barrier properties but still too high hydrophilicity for food packaging applications (Thomson, 1985), (Gällstedt, et al., 2006) .

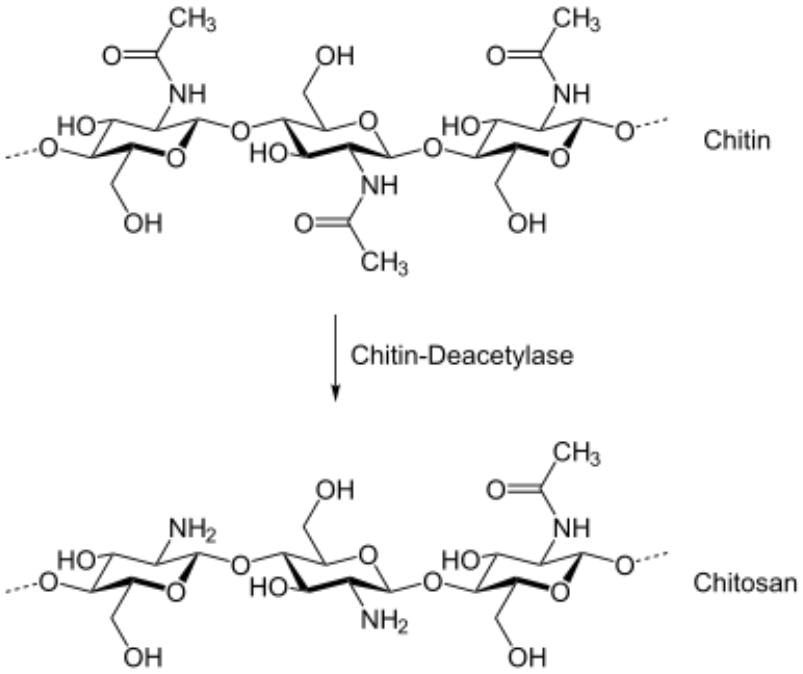

Figure 2: Structure of chitin, made of *N*-acetylglucosamine monomers. Deacetylation results into chitosan. Source of image (NEUROtiker, 2008).

- **Alginates** are salts of the alginic acid (see Figure 3), which is extracted from brown algae of the Phaeophycean class (King, 1983). They have excellent film-forming properties and are used in sizing and coating of paper for surface treatment, because they show resistance against solvents, oil and grease (Khwaldia, et al., 2010). However, it was reported that alginate coating decreased tensile strength of paperboard and increased hydrophilicity (Rhim, et al., 2006).

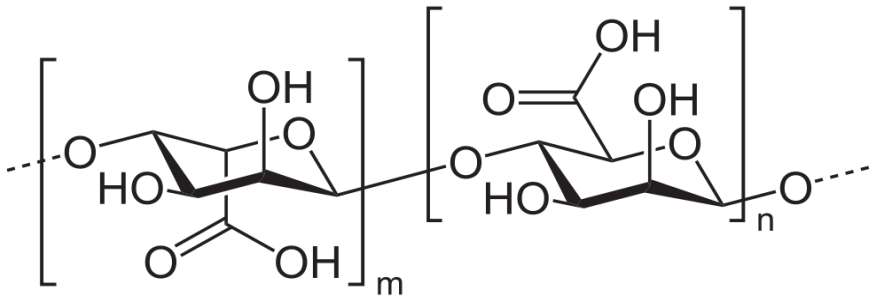

Figure 3: Structure of alginic acid, built from α-L-guluronate und β-D-mannuronate monomers. Source of image (Neurotiker, 2008).

- **Starch** is inexpensive, widely available and routinely used in paper production for surface treatment, without changing the papers properties (Matsui, et al., 2004). The structure, made of α-D-glucose monomers, can be seen in Figure 4. However, films have poor physical properties. Therefore, modification of starch, such as acetylation to starch acetate, is performed to improve the properties. Those modifications can have influence on the physical, oil, grease and water resistance and the optical properties of the resulting product (de Graaf, et al., 1995) (Frignant, et al., 1998) (Larotonda, et al., 2005) .

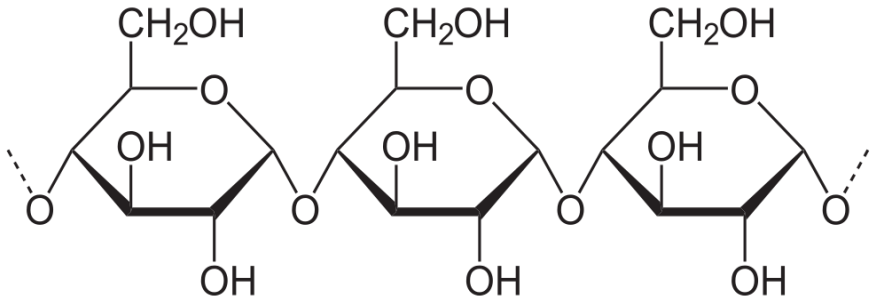

Figure 4: Structure of starch, which is built from α-D-glucose monomers. Source of image (NEUROtiker, 2008).

- **Cellulose** and its derivatives: cellulose is the most abundant natural polymer available, since it is present in any plant-based material. Its structure and properties can be adopted by different treatments. For example, microfibrillated cellulose (MFC) consists of cellulosic fibrils of plant cell walls. They are produced by disintegration of wood pulp, resulting into cellulose that is degraded and opened-up into its elementary fibrils and micro fibrils. The end product still has the properties of cellulose fibres, but is made of fibrillar structures with a width of lower than 20 nm and a length of up to several µm, respectively. They are partly crystalline and have therefore the ability to from a dense network by strong inter and intra-fibrillar bonds (Turbak, et al., 1983) (Herrick, et al., 1983). The resulting films are transparent and should have interesting barrier properties: because of the strong hydrogen bonding, they have high strength properties, the polar films should have good resistance against oils and grease and due to the crystallinity, the permeability should be low, although diffusion of gases is possible through the voids of the network. The disadvantages of MFC include the water sorption and high sensitivity against humidity, due to the polysaccharide properties of cellulose. (Aulin, et al., 2010) (Fukuzumi, et al., 2009) (Syverud, et al., 2009)

1.4.2.2.3. Lipid-Based Coatings

Lipids and waxes have the big advantage to be hydrophobic and therefore reduce moisture permeability. Paraffin wax coating of paper and board is routinely done for food packaging applications to improve water resistance. Also, lipid coatings have good moisture barrier properties, but are brittle, lack homogeneity and lead to pinholes and cracks. Hence, they are often incorporated into multilayer materials, rather than being used alone (Khwaldia, et al., 2010) (Rhim, et al., 2005)

1.4.2.2.4. Reference Materials

Several suppliers already offer bio-based barrier materials. In this study, several of them were used to compare their behaviour with the one of conventional polymers and the tested biopolymers. Unfortunately, we are not allowed to give any names of suppliers or products used. Consequently, they are just revered to as “reference material” and a consecutive number as will be described in detail in chapter 2.3.1.2.

1.4.2.2.5. Summary

The materials described above can be used alone or also in mixes or multilayers. As with conventional barrier materials, these materials need a lot of know-how to achieve the best barrier properties. The coating procedure, coat weight and additives are thereby key points. For example, it may be needed to apply several layers of one barrier material to achieve a full coverage of all fibres, addition of plasticizers, pigments, antioxidants or other agents may be needed to improve their performance.
Common to all mentioned materials is that most of them had only been tested as air, oxygen or grease barriers. They were hardly ever applied as barrier against migration of volatile organic substances. Although, some were tested as barrier against aroma active substances, they had never been tested as barrier against migration of MOSH and MOAH.

1.5. Testing Migration

1.5.1. Regulations and Guidelines

So far, there are no regulations being concentrated only on paper and board food contact materials. However, there are several general documents focusing on food safety and FCMs share of it. The two most important general documents are probably the Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food and the Commission Regulation (EC) No 2023/2006 on good manufacturing practice (GMP) for materials and articles for food contact. In consequence of the lack on regulations on paper and board, the Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food is commonly used as a benchmark for testing methods and limits. In addition, widely accepted is the German Federal Institute of Risk Assessments (BfR) recommendation XXXVI. on paper and board food contact materials in regard to substances that can be safely used during production of a material ([BfR] German Federal Institute for Risk Assessments, 2016). Furthermore, paper and board industry tried to align their quality standards and testing methods, publishing the food contact guideline for the compliance of paper and board materials and articles ([CEPI] Confederation of European Paper Industries, 2019) and the DIN SPEC 5010- Testing of paper and board ([DIN] Deutsches Institut für Normung eV, 2018-05).
The different documents are discussed in detail in the following chapters:

1.5.1.1. Commission Regulation (EC) No 1935/2004

The Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food regulates the general principle that any material or article intended to come into direct or indirect contact with food must be sufficiently inert to not transfer any substances to food in quantities large enough to endanger human health, to bring unacceptable changes in the composition of the food or to change its organoleptic properties in a negative way (Commision Regulation (EC) No 1935/2004). Furthermore, measures to prevent such unacceptable changes are described. Among traditional FCMs, it also includes coatings for food that are not intended to be consumed together with food, as well as active or intelligent FCMs, which both will not be discussed further in this work. Besides the FCMs itself, the regulation also covers substances that may be used in their manufacture. These substances have to undergo a safety assessment followed by a risk management decision prior to their authorization and inclusion in the community list of authorized substances. Further topics covered are e.g. the traceability at all stages of manufacture, processing and distribution, the use of recycled materials, labelling and safeguard measures.

Annex I of the regulation lists a number of materials and articles intended to come into contact with food. Among those materials are e.g. ceramics, cork, rubbers, glass, plastics, paper and board, but also adhesives, printing inks or coatings. Special measures may be taken for these materials, as e.g. including a list with purity standards, conditions of use, specific and overall migration limits for the transfer into food, provisions to protect human health and rules to ensure and check compliance, collecting samples and methods of analysis. Furthermore, the Regulation (EC) No 2023/2006 refers to these special measures for FCMs.

1.5.1.2. Commission Regulation (EC) No 2023/2006

The Commission Regulation (EC) No 2023/2006 lays done the rules on good manufacturing practice (GMP) for the groups of materials and articles intended to come into contact with food that are listed in Annex I of Regulation (EC) No 1935/2004. GMP is defined by the Regulation (EC) No 2023/2006 as those aspects of quality assurance, which ensure that FCMs are consistently produced and controlled in conformity with the rules and quality standards applicable and appropriate to them and their intended use. It applies to all sectors and stages of manufacture, processing and distribution of FCMs. To fulfil this, a quality assurance and control system should be used to ensure the conformity to GMP. It includes all documented arrangements made and takes account of e.g. adequacy of personnel and equipment, but also of the used starting materials. The main aim is to monitor the system and to identify measures to correct any failures and problems that may inhibit fulfilling the GMP requirements. Appropriate documentation has to be done and shall be made available, if requested (Comission Regulation (EC) No 2023/2006).

Detailed rules on GMP are set for printings inks applied on the non-food contact site of FCMs in the annex of the regulation. They should in particular ensure that substances are not transferred into food by migration through the substrate or by set-off in a stack or reel.

1.5.1.3. Commission Regulation (EU) No 10/2011

Commission Regulation (EU) No 10/2011 of the 14 January 2011 on plastic materials and articles intended to come into contact with food, sets basic rules for the use of these materials as FCM and the testing of compliance of them. Resulting from the lack of similar regulations for paper and board FCMs, the principals, methods and limits set are used as a benchmark for cellulose-based materials as well. Therefore, it is one of the most important documents regarding this topic.

The regulation refers first of all to the Regulation (EC) No 1935/2004 and Regulation (EC) No 2023/2006 and the principal rule that materials directly or indirectly in contact with food must be sufficiently inert to not transfer substances to food in quantities that can endanger human health, bring unacceptable changes to the composition of the food or deteriorate it in its organoleptic properties. The traceability of used substances and materials has to be ensured. The use of recycled materials and articles should be favoured, but strict requirements have to be established to ensure food safety and consumer protection. Priority should be given to the harmonisation of rules on recycled materials and articles as their use is increasing and national laws and provisions are lacking or are divergent.

According to the good manufacturing practice, the Regulation 10/2011 states that it should be feasible to manufacture FCMs in a way that they are not releasing more than 10 mg of substances per 1 dm^2 of surface area. This limit is set as a generic overall migration limit (OML). For a cubic packaging of 6 dm^2 surface area, containing 1 kg of food, it results to a migration of 60 mg kg^{-1} food. For small packages, where the surface to volume ratio is higher, the resulting migration into food is higher. To receive the same protection as for large packages, the overall migration limit should be linked to the limit in food and not to the surface area of the packaging. The OML is only applicable, if the risk assessment of individual substances present in the materials is not indicating lower limits. For the risk assessment of individual substances, it has to be considered that only substances with a molecular weight smaller than 1000 Da can be absorbed in the body. Therefore, only the migration of substances with a molecular weight <1000 Da are a potential health risk. The risk assessment of a substance should be performed by the European Food Safety Authority (EFSA). Two main things should be covered by it: first, the substance itself, relevant impurities and foreseeable reaction and degradation products in the intended use. Second, the possible migration of the substance from a FCM into food and the resulting toxicity under the worst foreseeable conditions of use. Based on the risk assessment, the authorization should, if necessary, set out specifications for the substance and restrictions of use, quantitative restrictions or migration limits to ensure the safety of the final material or article. Also, there should be a differentiation between substances that have a function in the final polymer and additives only needed during the manufacturing process (polymer production aids, PPA). PPAs are substances added intentionally during manufacturing (IAS), but not intended to be present in the final product. Furthermore, they may contain impurities of different origins, which are non-intentionally added with them (NIAS). If NIAS of an additive are relevant for the risk assessment, they have to be considered and included in the risk assessment of the additives and in its specifications. NIAS are also formed as reaction

and degradation products during manufacturing and use, so also these substances should be included in the assessment. However, it is not possible to list and consider all impurities, reaction and degradation products. Only the substances included in the resulting Union list of authorized and assessed substances in manufacturing of plastics, given in Annex I of the regulation (in short "Union list"), may be intentionally used. They must be of a technical quality and purity suitable for the intended and foreseeable use. The composition must be known to the manufacturer of the substance and made available to the authorities on request. Consequently, plastic materials and articles shall not transfer their constituents to foods in quantities exceeding the specific migration limits (SML) set out in the Unions List. Those SMLs are expressed in mg of substance per kg of food, applying the real surface to volume ratio in actual or foreseen use. For samples not yet in contact with food, the surface to volume ratio of 6 dm^2 per kg of food should be used. If substances are present in the list, but no specific migration limit is provided, the generic specific migration limit of 60 mg kg^{-1} is applied. The overall transfer of substances should also not exceed this migration limit. If substances are not included in the Unions List, their migration shall not be detectable with a limit of detection of 10 µg kg^{-1}. That limit shall always be expressed as concentration in food or food simulant. It is also applied on groups of compounds, if they are structurally and toxicologically related (e.g. isomers or compounds with the same functional group), but then expressed as the total specific migration limit (SML(T)). Finally, it is not allowed to use substances being classified as 'mutagenic', 'carcinogenic' or 'toxic to reproduction' and therefore, they should not migrate into food or simulant at all (Commission Regulation (EU), 2011).

In recent years plastic food contact materials are developed that not only consist of one plastic but combine several plastic layers to obtain the needed functionality and protection of the food. In such a multi-layer material layers may be separated from the food by a functional barrier. This barrier is a layer within food contact materials or articles preventing the migration of substances from behind that barrier into the food. Behind a functional barrier, non-authorised substances may be used, provided their migration remains below the limit of 10 µg kg^{-1} in food. The use of substances being classified as mutagenic, carcinogenic or toxic to reproduction is again not allowed without previous authorisation. Consequently, they are not covered by the functional barrier concept (Commission Regulation (EU), 2011).

With regard to the established overall and specific migration limits, basic principles of the testing procedures are given. The material or article shall be treated as given in its instructions or by provisions given in the declaration of compliance. Migration is determined on the material or article or on a specimen taken from or representing it. For each test performed a new test specimen is used. Only those parts of the sample which are intended to come into contact with food in actual use shall be placed in contact with the food or food simulant in a manner representing the worst foreseeable conditions of use, regarding contact time and contact temperature. In order to achieve comparable results, testing should be performed under standardized test conditions including testing time, temperature and test medium. Standard test conditions for temperature and time are given in Annex V of Regulation 10/2011 and can be seen and are discussed in Table 2 and Table 3 later in the chapter. In regards to the test medium

the use of food simulants is recommended, since food is a complex matrix and the analysis of migrating substances in food may pose analytical difficulties. Food simulants should represent the chemical composition and the physical properties of the food, intended to come into contact with the FCM. Research data is available for certain food types as representatives, comparing migration into food with migration into food simulants (see also chapter 1.5.2 of this work). On the basis of these results, food simulants had been assigned. The food simulants that shall be used for different types of foods are described in Annex III of the regulation and are listed in Table 1 below. For fat rich food the results obtained with food simulants may significantly overestimate migration and should be corrected by a reduction factor.

Table 1: Food simulants as given in (Commission Regulation (EU), 2011).

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

* Any vegetable oil with a fatty acid distribution as defined in Annex III/Table 1 of the regulation.

Food simulants A, B and C are assigned for foods having a hydrophilic character and are able to extract hydrophilic substances. Food simulant B shall be used for food stuff having a pH below 4.5. Food simulant C shall be used for alcoholic foods with an alcohol content of up to 20% and for those, which contain organic ingredients rendering the food more lipophilic. Food simulants D1 and D2 are assigned for foods that have a lipophilic character and are able to extract lipophilic substances. In addition, food simulant D1 shall be used for alcoholic foods with an alcohol content of above 20% and for oil in water emulsions. In contrast, food simulant D2 shall be used for foods with free fats at the surface. Food simulant E is assigned for testing specific migration into dry foods. For further information it is referred to the detailed description of the food simulants assigned to the different food categories as given in Table 2 of Annex II of Regulation 10/2011.

Annex V of Regulation 10/2011 gives the general rules of storage time and temperature conditions used for testing migration. Shown in Table 2 below are the contact times in worst foreseeable use and the resulting testing time. For contact times above 30 days at room temperature and below the specimen shall be tested in an accelerated test at elevated temperature for a maximum of 10 days at 60°C. Testing time and temperature conditions shall be calculated based on the Arrhenius equation given in Equation 1.

Equation 1:
$$t_2 = t_1 * e^{-\left(\frac{E_a}{R}\right)*\left(\frac{1}{T_1}-\frac{1}{T_2}\right)}$$

E_a is defined as the worst-case activation energy of 80 kJ mol^{-1} and R is a factor of 8.31 Joule Kelvin mol^{-1}, resulting into Equation 2:

Equation 2: $$t_2 = t_1 * e^{-(9627)*(\frac{1}{T_1}-\frac{1}{T_2})}$$

t_1 is the contact time, t_2 is the testing time, T_1 is the contact temperature in Kelvin (K). For storage at room temperature, this is set at 298 K (25 °C). For refrigerated and frozen conditions, it is set at 278 K (5 °C). T_2 is the testing temperature in Kelvin.

Table 2: Contact Time in worst foreseeable use as given in (Commission Regulation (EU), 2011).

Contact Time in Worst Foreseeable Use	Testing Time
t ≤ 5 min	5 min
5 min < t ≤ 0,5 hours	0,5 hour
0,5 hours < t ≤ 1 hour	1 hour
1 hour < t ≤ 2 hours	2 hours
2 hours < t ≤ 6 hours	6 hours
6 hours < t ≤ 24 hours	24 hours
1 day < t ≤ 3 days	3 days
3 days < t ≤ 30 days	10 days
Above 30 days	See Arrhenius equation below

Regarding the testing temperature, Table 3 shows the contact temperature in worst foreseeable use and the resulting test temperature given in the regulation.

Table 3: Contact temperatures as given in (Commission Regulation (EU), 2011).

Contact Temperature in Worst Foreseeable Use	Test Temperature
T ≤ 5°C	5°C
5°C < T ≤ 20°C	20°C
20°C < T ≤ 40°C	40°C
40°C < T ≤ 70°C	70°C
70°C < T ≤ 100°C	100 °C or reflux temperature
100°C < T ≤ 121°C	121°C (*)
121°C < T ≤ 130°C	130°C (*)
130°C < T ≤ 150°C	150°C (*)
150°C < T < 175°C	175°C (*)
T > 175°C	Adjust the temperature to the real temperature at the interface with the food (*)

(*) This temperature shall be used only for food simulants D2 and E. For applications heated under pressure migration testing under pressure at the relevant temperature may be performed. For food simulants A, B, C or D1 the test may be replaced by a test at 100 °C or at reflux temperature for duration of four times the time selected according to the conditions in Table 1.

Table 4 gives examples for standardized test conditions resulting from Table 2 and 3 and the Arrhenius equation:

Table 4: Resulting standardized test conditions as given in (Commission Regulation (EU), 2011).

Test Conditions	Storage Conditions in Worst Foreseeable Use
10 d at 20°C	all storage times at frozen condition
10 d at 40°C	all storage times at refrigerated and frozen conditions, including heating up to 70°C for up to 2 hours, or heating up to 100°C for up to 15 minutes.
10 d at 50°C	all storage time at refrigerated and frozen conditions including heating up to 70°C for up to 2 hours, or heating up to 100°C for up to 15 minutes and storage times of up to 6 months at room temperature.
10 d at 60°C	long term storage above 6 months at room temperature and below including heating up to 70°C for up to 2 hours, or heating up to 100°C for up to 15 minutes.

If tests carried out under the contact conditions specified in Tables 2, 3 and 4 cause any changes in the test specimen, which do not occur under worst foreseeable conditions of use, the migration tests shall be carried out under the worst foreseeable conditions of use in which these changes do not take place. For polyolefins the maximum testing temperature is depending on the phase transition temperature. For long term storage at room temperature testing time can be reduced to 10 days at 40°C, if there is scientific evidence that migration of the respective substance in the polymer has reached equilibration under this test condition. In addition, if a material or article is intended for different applications with different combinations of contact time and temperatures, testing should be done at the most severe ones. If the material or article is intended to come into repeated contact with food, the migration test(s) shall be carried out three times on a single sample using a new portion of food for each test. Its compliance is checked on the basis of the level of the migration found in the third test. However, if there is conclusive proof that the level of the migration does not increase in the second and third tests and if the migration limits are not exceeded on the first test, no further test is necessary.
At the end of a migration test, the specific migration is analysed in the food or food simulant using an analytical method in accordance with the requirements of Article 11 of Regulation (EC) No 882/2004.

An extra chapter is related to screening approaches. To screen if a material or article complies with the migration limits, any approach can be applied which is considered more severe than the verification methods described earlier. To screen for specific migration of non-volatile substances, determination of overall migration using test conditions at least as severe as the ones for specific migration can be applied. To screen for specific migration, the migration potential can be calculated based on the residual content of the substance in the material or article assuming complete migration or by applying generally recognised diffusion models. To

screen for overall migration, the migration potential can be calculated based on the residual content of migrate-able substances determined in a complete extraction of the material or article.

In conclusion, the tests performed, should as far as possible mimic migration, which may occur from the article into the food. It is reproduced by the food simulant, the testing time and temperature used.

1.5.1.4. BfR Recommendation XXXVI. Paper and Board for Food Contact

This document is valid for single or multi-layer materials made from paper or board, which are intended to come into contact with food. It gives recommendations on raw materials, production aids and refining agents that may be used in the production of FCMs. If available, also restrictions and limits are given for these substances. Therefore, it is an important document for producers of paper and board FCMs as well as for quality assurance and risk assessment ([BfR] German Federal Institute for Risk Assessments, 2016).
As an example, the migration of certain phthalates from recycled fibre raw materials is mentioned. The three phthalates bis(2-ethylhexyl) phthalate (DEHP), di-*n*-butyl phthalate (DnBP) and di-*iso*-butyl phthalate (DiBP) should not be transferred into food or food simulant in an amount larger than 1.5 mg kg^{-1} and 0.3 mg kg^{-1} for the latter two, respectively. The sum of the three phthalates (SML(T)) must not exceed 0.3 mg kg^{-1} either. In comparison, the isomers of diisopropylnaphthalene (DIPN) should not be present in a finished FCM at all ("as low as technically feasible") ([BfR] German Federal Institute for Risk Assessments, 2016).

1.5.1.5. Food Contact Guidelines for the Compliance of Paper & Board Materials and Articles

Since the European Union did not introduce a specific Union measure for paper and board food contact materials, in 2010 the aegis of the Confederation of European Paper Industries (CEPI) and the International Confederation of Paper and Board Converters in Europe (CITPA) published the Food Contact Guideline for the Compliance of Paper & Board Materials and Articles for Food Contact ([CEPI] Confederation of European Paper Industries, 2019). After the first publication, the document was once revised in 2012 and a second time in the beginning of 2019. The guideline is aimed for all those interested in ensuring the safety of paper and board intended to come into contact with food. It was well accepted by organisations and authorities at the European level, but also on national level, but still is just a guideline with voluntarily use rather than having a legal force.
The guideline covers materials and articles consisting mainly of paper and board, including untreated paper and board, coated papers, tissue products, multi-material-multi-layer materials (MMML), paper and board for filtering and baking applications, release papers, bags, boxes, wrappings and secondary and tertiary transport packaging's. Chapter 1 gives general aspects of the guideline including the scope, GMP, risk management, use of IAS during manufacturing and the presence of NIAS, traceability and labelling. These topics are further discussed in the related chapters of the guideline. Chapter 2 gives a graphical representation of the scope of the

guideline. General aspects for testing of compliance are described in chapter 3, which should be discussed in more detail here:

The chapter is divided into general considerations, recommendations for paper and board materials, including IAS and NIAS, for testing MMMLs and converted paper and board materials as well as testing of tissues. In general, testing should be done using internationally recognised and validated methods like EN, ISO or equivalent, if available. Some of the widely used and generally accepted methods are listed in Table 1 of the guideline, e.g. methods for compliance testing of certain IAS and NIAS. For some IAS there are quantitative limits like a maximum usage rate, residual content in paper, maximum concentration of known impurities or SMLs. They are included in various national and European regulations. Information of included IAS (and NIAS present in IAS) should be communicated from suppliers to producers of paper and board materials. NIAS can originate from several sources throughout the production chain (impurities, reaction or degradation products…). Some NIAS are known to be present only in recycled fibre; therefor testing of fresh fibres may not be needed, although as risk assessment still recommended. A table of general purity requirements and current state-of-the-art known NIAS is given. Not included are NIAS present in IAS, since they should be communicated by the supplier (e.g. dialkylketones in AKD sizing).

Testing of migration should be done with modified polyphenylene oxide (MPPO), which is the only known simulant that can be applied to paper and board to simulate a one-sided migration test with food contact. High temperature applications should be simulated according to EN 14338. It is not necessary to test for all foreseen conditions of use, only the worst-case conditions should be considered. Since paper and board is not intentionally used to have contact with liquids, testing with liquid food simulants is not relevant or appropriate. Testing with them is considered to be a total extraction, rather than a one-sided migration test. Exceptions from this are filter papers like coffee filters or tea bags. However, total extraction results can be used to reflect the total composition and purity of the FCM and may therefore be applied on certain samples of interest. If the given migration limits are exceeded by the total extraction, more realistic test can be done or correction factors applied. Polar solvents like acetone or ethanol are efficient for extractions of cellulose-based materials. They swell fibres and dissolve/release substances, which are bound to the surface and have initially a low migration potential. For contact with fatty food iso-octane is the most relevant solvent, water extraction should be done for moist or aqueous food.

MMML refers to all paper and board materials coated with two or more layers of different materials, which are intentionally bound together (“bag-in-box” is not included) and of which one layer is paper or board. The coating usually consists of plastic films and/or aluminium foil. In general, paper and board coated with MMMLs have to comply with the Regulation No 1935/2004 and the GMP Regulation. They do not need to comply with the overall migration and specific migration limits according to Article 14 of the Regulation 10/2011. However, risk assessment may still be needed and migration testing can still be performed using the conditions given in the Regulation 10/2011. Converted paper and board materials are all glued, printed or varnished materials, such as corrugated board, bags, wrappings, cups and so on. Extraction

testing is not feasible, since they may include MMML, printing ink, glues etc. Therefore, either testing of only the MMML when in contact with food or only migration testing with MPPO should be done.

For the use of recycled fibres for food contact materials and articles several points need to be assessed: the quality, source and proportion of the recycled fibres must be appropriate, unwanted substances and materials must be removed and the final materials must be suitable for the intended use and should not transfer constituents during the use. Further requirements are given in the legal regulations (Comission Regulation (EC) No 2023/2006).

The stated and discussed issues of the guideline, especially the ones for compliance testing, had been further stressed by the establishment of the DIN SPEC 5010.

1.5.1.6. DIN SPEC 5010

In May 2018, the DIN SPEC 5010 on "Testing of paper and board- Determination of the transfer of mineral oil hydrocarbons from food contact materials manufactured with portions of recycled pulp" was published ([DIN] Deutsches Institut für Normung eV, 2018-05). It is stated that at the moment, there are no standards covering this topic. A DIN SPEC is therefore used, as a fast way to develop a standardized method, in small specialist working groups. They are widely accepted in respect to the DIN "brand", published on Beuth Verlag and can be used as a basis for a full standard at any time ([DIN] Deutsches Institut für Normung e. V.).

Aim of the DIN SPEC 5010 is to determine the migration of mineral oil hydrocarbons with a standardized method and to evaluate the functional barrier properties of coated papers. The basic principle of the proposed method is, to bring the side of intended food contact in contact with a food simulant using defined and standardised conditions in a thermostatically controlled oven. Afterwards, the simulant is extracted with n-hexane and the migrated MOH content determined.

In more detail, 1 dm^2 of the material to be tested should be cut and placed in a petri dish with an outside diameter of 114 mm. As an alternative, migration cells can be used instead of petri dishes. The sample should be covered with 4 g of the food simulant poly(2,6-diphenyl-p-phenylene oxide) (in short MPPO; food simulant E of Regulation 10/2011; available e.g. under the trade name Tenax®). For blank values empty petri dishes or migration cells with the same amount of MPPO should be used. The test conditions should be 10 days at 40°C to simulate storage at room temperature for up to 12 months or 30 days at 40°C to simulate storage at room temperature for up to 24 months. The samples are placed in the preheated oven for the testing time necessary. At the end of the storage time the vessels are removed from the oven and cooled to room temperature, without opening them. Extraction can be done by adding an extraction standard or alternatively with internal standard for quantitation only. The MPPO is transferred into a 50 mL Erlenmeyer flask and the extraction standard and 20 mL of n-hexane are added. The extraction standard consists of *n*-dodecane, *n*-pentacosane and hexylbenzene in concentration of 300 µg mL^{-1} in hexane. The flask is agitated by hand for 1 minute and allowed to stand for at least 5 minutes afterwards. The extract is then transferred through a filter funnel into a 50 mL volumetric flask and the procedure repeated twice with 20 mL n-hexane. At the

third extraction step, the flask should be shaken at the end of the 5 minutes, the extract and the MPPO poured in the filter funnel and rinsed with n-hexane. The quantitation standard is added to the flask and the flask filled to the mark.
Without the use of an extraction standard, the procedure stays the same, but the quantitation standard is added prior to extraction, 10 mL of n-hexane are used and extraction is done four times.
Measurement of MOSH and MOAH should be done according to the procedures published by the German Federal Institute for Risk Assessment and the Zurich Cantonal Laboratory ([BfR] German Federal Institute for Risk Assessment, 2012) (Biedermann, et al., 2012a). For data evaluation, an n-alkane standard from e.g. C_{10}-C_{40} separated by gas chromatography on a dimethyl polysiloxane phase should be used. On the basis of the retention of the standards, the integration intervals should be set, e.g. from C_{10}-C_{16}, C_{16}-C_{20}, C_{20}-C_{24}, C_{24}-C_{35} or from C_{16}-C_{25} and C_{16}-C_{35}. Integration of the n-alkanes can be done from the beginning of the peak at the rising edge, in the maximum of the signal or after complete elution at baseline height. The method used should be clearly documented.

This DIN SPEC was published only in May 2018, at a time were the migration methods described in this work had already been developed and validated (see chapter 2.2. Method Development and Validation). It has to be mentioned that both methods are in good accordance to each other and therefore no changes to the methods developed in this work were made.

1.5.2. Use of Food Simulants

As already discussed before, the EU Regulation 10/2011 introduced several food simulants to be used for the testing of FCMs (see chapter 1.5.1.3 and (Commission Regulation (EU), 2011)). The advantages are the standardised matrices used for simulation of migration, the easier handling and analysis and the generation of more reliable and comparable data. However, the question arose, if these simulants are suitable for the intended use. Two of the most discussed ones were the simulant D2 – vegetable oil and E – poly(2,6-diphenylene oxide).
Food simulant E should be used as a simulant for dry, non-fatty food. It is a fine yellowish powder, with a highly porous structure. The porous polymer is based on poly (2,6-diphenylene oxide), also referred to as modified polyphenylene oxide (MPPO) or Tenax® TA. Uptake of possible migrants was seen to be slightly higher, when compared to food, due to the high surface area. However, this was considered to be acceptable by the Regulation 10/2011, which referred to the available literature. For example, Zurfluh, et al. 2013 showed that simulation of migration using MPPO at 40°C or 60°C for 10 days overestimated the migration into real food by a factor of up to 27, which was not acceptable for him. It is suggested that an estimation of migration is much easier by doing a total extraction of the packaging and using the fact that 70-80% of the total amount of <nC_{24}-MOSH migrates during long-term storage. They further studied the influence of higher temperature on the acceleration of migration. It was found that for a complex mixture of substances, having a broad range of molecular mass and volatility, the increase of

temperature results to a broadened band of migrating compounds. Thus, resulting in an overestimation, depending on the mass and volatility distribution of the substances. However, the publication also compares the data of total extraction, migration with storage at 40°C and 60°C for 10 days. While storage of 40°C simulates roughly one and a half month at room temperature, storage at 60°C simulates about 10 months. Comparison therefore seems to be critical and should be done only with samples stored long-term at room temperature for a comparable time period (Zurfluh, et al., 2013).
For food simulant D2 the disadvantages and the following discussion are clear: any vegetable oil with a certain fatty acid distribution, as it is given in the Regulation 10/2011, can be used as simulant. It is questionable, if the big advantages of using food simulants still exist, if a highly varying matrix like vegetable oil is used. Furthermore, the purity of the oil needs to be checked carefully and the analysis of the fatty matrix poses an analytical challenge. Therefore, the food simulant D2 should be replaced by alternatives as e.g. 95% ethanol or isooctane as already had been discussed by several authors earlier (De Kruijf, et al., 1988) (Baner, et al., 1992) (Papaspyrides, et al., 1998) (Feigenbaum, et al., 2000).

In general, studies showed that the use of food simulants may overestimate the real migration. On the other side, the many advantages of food simulant outweigh this slight disadvantage: since food is a complex, highly varying matrix the use of standardised food simulants allows an easier performance of the migration test. The extraction and analysis of food simulants can also be standardised and more easily done as performing analysis of the highly varying food matrix. The most important point is probably the generation of comparable results.

1.5.3. Different Limits for Migration

Several different limits had been discussed and set in the past, based on the one hand, on the risk assessment and evaluation of single substances and on the other hand, on the non-targeted approach or non-evaluated substances, respectively.
Most relevant in the European Union are probably the limits given in the Regulation 10/2011 on plastic materials and articled intended to come into contact with food. The regulation sets a generic overall migration limit for evaluated substances of 60 mg kg^{-1} food. It actually is defined as the maximum permitted amount of non-volatile substances released from a FCM into food or food simulants and is determined gravimetrically. Furthermore, the specific migration limits (SML) for individual substances and the total specific migration limits (SML(T)) for substance groups are given. For unknown or not assessed substances, a limit of 0.01 mg kg^{-1} food is defined (Commission Regulation (EU), 2011). This limit, widely used in the European Union, has no toxicological background. It was set pragmatically as a limit of detection for target analysis, at times where it was a challenge to reach it, and has nowadays become a convention and a real challenge for untargeted analysis (Biedermann-Brem, et al., 2016).

The US Food and Drug Administration (FDA) introduced the Threshold of Regulation (ToR) for non-evaluated substances. It was statistically derived from a large database on the toxicity of chemicals and defines that components with a concentration <0.5 µg kg^{-1} in food are likely to be safe, provided they are non-carcinogens. Compounds migrating into food at lower concentrations can be used virtually without restrictions (Rulis, 1989) (Begley, 1997).
The International Life Science Institute (ILSI) defined a Threshold of Toxicological Concern (TTC) and proposed for known substances without structural alert for potential genotoxicity the limit of 1.5 µg per person and day (Kroes et al. 2000). It results into the ToR for a consumption of 300 g of a contaminated food per day. For unknown substances (worst case scenario), the TTC is set as 0.15 µg per day for a person of 60 kg body weight (corresponding to 0.0025 µg kg^{-1} body weight), which results in a consumption of 30 g day^{-1} for the contaminated food to reach the ToR (Grob, 2002).
In comparison, the widely used limit of 0.01 mg kg^{-1}, having no toxicological evaluation as background, would result in the TTC if only 15 g of food containing a given substance are consumed daily.
The limits for mineral oil migration will be discussed in the respective chapter 1.7.3.

Summarising, the limits set are mostly in the range of mg kg^{-1} food contact material or µg kg^{-1} food for individual substances. For overall migration or total specific migration of groups they may be higher. As already mentioned in this work, Biedermann, et al. (2013a and 2013b) tried to assess, whether recycled paper and board is suitable for food contact, if such limits are applied. They concluded that if an untargeted approach is used, it is not possible to evaluate all substances present in relevant concentrations in a recycled fibre material. Therefore, functional barriers should be applied to prevent migration.

1.6. Testing of Functional Barriers

As already discussed in chapter 1.4, methods for testing functional barriers are rare. Most of the methods available had been developed and adapted to the purpose in the last few years.

1.6.1. Taped Barrier Test

The taped barrier test is a newly developed test for functional barriers, which had been first described by Fiselier, et al. (2012). It was further developed by Biederman-Brem in the following years (Biedermann-Brem, et al., 2014) (Biedermann-Brem, et al., 2016) (Biedermann-Brem, et al., 2017a) (Biedermann-Brem, et al., 2017b).
Fiselier described in 2012 that the taped barrier test is a test for functional barriers, which does not need migration cells or MPPO (Fiselier, et al., 2012). The test set up includes a donor, the barrier to be tested and an acceptor, all wrapped together in a test pack. The first tests used a sheet of copy paper as donor, which was soaked in a n-hexane solution containing 2 g mineral

oil and 0.1 g of the dye Sudan Red II in 200 g solvent. The present concentration of MOH was determined by extraction, after drying the donor paper. The dye is added to check on the one hand, the homogenic distribution on the spiked paper and on the other hand, to detect gaps present in the pack; pin holes, cracks or scratches in the barrier would be detected as red spots on the barrier or acceptor, if present. A sheet of clean paper was used as a spacer to rule out wetting contact between the donor and the barrier. Donor, spacer and barrier were wrapped into aluminium foil and fixed gas tight with a tape, representing the donor-package. To check for interferences during the analysis (e.g. polyolefin oligomeric saturated hydrocarbons; POSH) all barrier films were extracted and analysed prior to preparation of the test packs. A low-density PE film was chosen as an acceptor, since it is easily extractable and because of its low content of POSH interfering with the analysis. It was loosely fixed onto the donor-package, being in direct contact with the barrier, and everything wrapped into aluminium foil. Several packages were stored at room temperature or in an oven at 40°C or 60°C to accelerate the test. During the test period, the mineral oil spiked on the donor, should permeate through the barrier and be adsorbed in the attached acceptor film. From this only loosely fixed film, periodically, strips of about 2 cm were cut (~250 mg) and extracted using n-hexane at room temperature for two hours, to control the extent of permeation. Analyses of the extracts were done with HPLC-GC-FID and the results were determined as breakthrough levels. The breakthrough is described as the time, in which no mineral oil reaches/migrates into the food or simulant (=lag time or breakthrough time). However, an efficient barrier also slows migration down, which means that even if there is breakthrough, because the barrier is not tight anymore, the migration could stay low till the end of shelf life. To fit this matter of fact, they defined breakthrough limits as 1% and 10% of the equilibrium concentration. The equilibrium concentration is considered to be the worst-case situation with 100% breakthrough of mineral oil. It was not determined for every test, since they believed it stays similar for all packs of the same size and film. For the estimation of and comparison with real migration into food, they took some assumptions: First, they assumed that barrier and receptor have similar thickness and affinity to mineral oil. At equilibrium the residual mineral oil content in the donor is negligible. When reaching the 10% breakthrough, they found about 5% of the total mineral oil content in the acceptor. This 5% are hydrocarbons of sufficient volatility to migrate through the gas phase ($<C_{24}$). If there is a packaging with a surface area of 10 dm^2, a grammage of 300 g m^{-2} and a MOSH contamination of 350 mg kg^{-1}, then there would be a migrate-able amount of MOSH of 10.5 mg. If there are 250 g food packed into this packaging and the transfer of MOSH is about 70% (Lorenzin, et al., 2010), it reaches 29 mg kg^{-1} in the food. Consequently, a reduction to 5% migration would result into 1.5 mg kg^{-1}, which is two and a half times above the 0.01 mg kg^{-1} b.w. ADI of JECFA for intermediate molecular mass hydrocarbons (Joint FAO/WHO Expert Committee on Food Additives (JECFA) , 2002). Another assumption starts at the observation that the limit of 0.6 mg kg^{-1} food (ADI for a 60 kg b.w. person) is exceeded sometimes by the 100-fold (Vollmer, et al., 2011). A barrier would need to reduce the migration by the same factor, which would be represented by a 2% breakthrough in their test. As a result of these assumptions they used the respective breakthrough limits of 1% and 10%.

In addition, the general approach of analysis was described and examples given: the analysis started with the 60°C pack and breakthrough was determined and controlled later with the 40°C pack. The pack stored at room temperature was used for verification, if there was suspicion that testing of 60°C could have done changes to the barrier. Acceleration factors were calculated using the Arrhenius equation. As example, a PP film tested at 60°C is given. It reached equilibration after 120 h with a concentration of 7150 mg kg^{-1} in the receptor foil. The 1% and 10% breakthrough amounted to 72 and 715 mg kg^{-1}, which was reached after 1.6 and 4.2 h testing time. As further example, they describe the results of a PE film as barrier, stored at 40°C. After 30 min of test time, the MOSH migration into the receptor was 50 mg kg^{-1} and little below the 1% breakthrough limit (62 mg kg^{-1}), after 1 h and 2 h the migration was 210 and 880 mg kg^{-1} (10% breakthrough limit of 620 mg kg^{-1}). In a routine attempt, they prepared three packs and stored them at room temperature, 40°C and 60°C. Acceleration factors were calculated using the Arrhenius equation, but not always showed satisfying results by comparing results of the three test temperatures. They concluded that elevated temperatures sometimes destroyed the barrier efficiency present at room temperature. If a barrier has a bad performance at 60°C, its performance needs to be checked at lower temperatures. However, if a barrier meets the requirements at 60°C, it is valid also at room temperature.
The advantages of this method were described as the simple experimental set-up, with regard to the easy availability of the materials and the possibility of preparing and storing many packs at once. The disadvantage was the co-elution of POSH from the PE film in the MOSH region. The level of MO in the donor paper was quite high compared to levels discussed nowadays (see chapter on MOSH & MOAH), but was chosen so high, to overcome the problem of POSH migrating from the barrier film and extracted from the PE acceptor. Integration was therefore done of the unresolved hump, without the POSH signal on top. The limit of quantification (LOQ) for MOSH and MOAH were determined as 20 and 5 mg kg^{-1}, respectively, resulting in a concentration of 1% breakthrough of 50-70 mg kg^{-1}. Lower concentrations could be used, but than a clean acceptor film would have been needed. Analysis could also be done with only e.g. GC (instead of HPLC-GC-FID), since MOSH and MOAH showed similar migration behaviour and no further information can be generated by using the additional LC separation.

Biedermann-Brem introduced some changes to the test two years later (Biedermann-Brem, et al., 2014): Since mineral oil is not the only possible contaminant migrating from paper and board, a wide range of substances should be simulated by surrogate substances, as described by Ewender, et al. (Ewender, et al., 2013). They selected a set of surrogate substances by their polarity and molecular mass (volatility). Among them were n-alkanes, benzophenones, phthalates and an alkyl citrate. For simplification of the test, carboxylic acids or amines were not used. From their studies they concluded, to choose n-heptadecane (C_{17}), 4-methyl benzophenone (MBP), di-*n*-propyl phthalate (DPP) and triethyl citrate (TEC) as surrogate substances. They were of adequate molecular mass and volatility to migrate, but not too volatile to result in losses during handling, giving a robust test. Also, they are not present in interfering amounts in the tested samples. By choosing only four surrogate substances in concentrations of

250 mg kg^{-1} the load for the barrier gets much lower and simulates the conditions of a real paperboard much better than in the previous described test. They still used the dye Sudan Red II to visually check the homogeneity of the components after soaking the donor paper. They showed that lighter regions on the donor had fewer surrogate concentrations than the darker boarders of the board in one of their next publications (Biedermann-Brem, et al., 2017b).

They also tested the need for a spacer between the donor and the barrier in order to rule out wetting contact. They concluded that a spacer is not needed since there was no difference in the results. Furthermore, they exchanged the PE-acceptor and used a siliconised paper instead, which would uptake all substances without discrimination. The solvent used was changed from n-hexane to methyl tert-butyl ether (MTBE), for the same reason. In terms of result evaluation, they kept the reduction of migration by a factor of 100, compared to the concentration present at the end of the shelf life of a product without a barrier. Results were expressed as percentage of breakthrough (limit of 1% breakthrough). However, it was now justified with the conventional EU detection limit of 0.01 mg kg^{-1}, because the ADI of 0.01 mg kg^{-1} b.w. was withdrawn by JECFA in 2012 (Joint FAO/WHO Expert Committee on Food Additives (JECFA) , 2012). The needed barrier efficiency is discussed in detail in their next publication (Biedermann-Brem, et al., 2016).

The test was characterized using only polymer films with low barrier efficiency to obtain results in reasonable time. Analysis was done using GC-FID with 50 µL injection volume. Probably a little controversial is the use of silicon paper as an acceptor. They used a paper coated with 25% of dimethyl polysiloxane, to provide stability and higher polarity and because it was easy to handle. In comparison with the special prepared paper, regular baking paper only had about 3-5% silicone and migration showed lower concentration levels, but higher deviation. Comparison with polenta showed that the amount of substances migrating to polenta was 2- to 3- times lower than the results with the siliconised paper. On the other hand, it was stated in the conclusion that it should make no difference which acceptor is used, since the test measured the amounts of surrogates becoming available to food. One problem of the silicon paper was, however, that high boiling polysiloxanes had been extracted with the surrogates. They could not be removed by using heating for 1h at 160°C as cleaning method. A special injection method with concurrent solvent condensation (CSR), was used to prevent interferences with the GC-FID analysis.

Rather than assuming the same equilibrium concentration for every pack as done before by (Fiselier, et al., 2012), they now determined the concentration of the surrogates in the siliconised paper at 100% breakthrough. Since it is often not possible to wait, until the equilibrium is reached in a test pack, especially for good barriers, they made an alternative set up: all parts of a test pack (donor, spacer, barrier, and receptor of same area) were loosely placed in a flask without contact to each other. The flask was stored at room temperature, 40°C or 60°C for up to two weeks and the time needed to reach equilibrium was checked. The concentration measured at equilibrium in the receptor was used as a reference point for the migration and for the determination of 1% and 10% breakthrough.

Their results obtained from testing different barrier materials were varying. The tested polymers showed different selectivity for polar or non-polar substance. Furthermore, they sometimes saw high amounts of migration in the first days, which levelled off and increased again afterwards. They concluded that the early migration has its origin from pinholes or gaps in the barrier. In this region there was strong and fast migration through the hole, which continues, until the donor is locally depleted. Only after some time, the real migration started, leading to the homogeneous increase of migration. This theory was strengthened by the presence of red spots from Sudan Red II on the receptor paper (Biedermann-Brem, et al., 2014).

In another publication in 2016, they discussed the needed barrier efficiency for a functional barrier used for/in recycled paper and board applications (Biedermann-Brem, et al., 2016). They stated that a functional barrier needs to reduce the migration for all substances to a level not being of toxicological concern. For evaluated substances this may be an ADI or SML. For non-evaluated and unknown substances, the level can be the limit of the EU Regulation 10/2011, being 0.01 mg kg^{-1}, but the TTC would be lower for non-evaluated substances (0.0025 µg kg^{-1} b.w. per person per day). In terms of barrier efficiency, they discussed for non-evaluated substances that some substances present in paper board may reach a concentration of 10 mg kg^{-1}. If the assumption is taken that the packaging to food ratio is in average 1:10 and the substance migrates to 100%, resulting into 1 mg kg^{-1} food, a reduction of migration of the 100-fold would be necessary to reach the level of 0.01 mg kg^{-1} in food, equivalent to 1% of migration from the FCM into food. Considering evaluated substances that may be present also in higher concentrations, they concluded that the 1% migration limit would be applicable too, since in no study known to them the proposed limits for an evaluated substance were reached.
Applied to the taped barrier test, this new derived limit would mean that as soon as one surrogate reached the 1% breakthrough within the proposed testing time, the test is failed. However, since the actual composition of the paper or board used is mostly unknown, an accurate description of the needed barrier efficiency is not possible. Therefore, they suggested a benchmark, which will be discussed in detail in chapter 1.6.4.

Biedermann-Brem discussed the role of the paper for preparing the donor in another work (Biedermann-Brem, et al., 2017b). In a real packaging, not only the permeation through the barrier, but also the adsorption and release of the contaminants from the packaging needs to be considered, influencing the (vapour)-pressure on the barrier. Therefore, they prepared three test packs: the first one with an OPP barrier and a copy paper donor with the surrogate substances as described in (Biedermann-Brem, et al., 2014), the second, with the OPP barrier, a copy paper donor and an additional recycled board (350 g m^{-2}) and the third one with copy paper donor and recycled board, but without a barrier. They found that migration into the receptor was lowest in the second attempt, while highest in the third. The reason for the low migration in the second pack was on the one hand the higher amount of fibre material present, but more important adsorption into the recycled board decreased the vapour pressure on the barrier. They analysed the test components by GC×GC measurements before the test and afterwards. From the

recycled board, the non-polar n-alkanes of up to C_{25} and other MOSH substances migrated nearly completely, while the more polar substances remained in it. The OPP barrier included most of the MOSH substances after the test, while only a little amount migrated into the acceptor. They concluded that the results show the partitioning of the contaminants in the different parts of a packaging. However, in a real food packaging situation, the ratio between food and packaging would be much higher and stronger absorption into the food would take place. They admitted that the result derived with the siliconised paper, are only comparable with food or food simulants, when looking at low migration of about 1%.

In another test using two packs consisting either of a copy paper donor, OPP and the receptor or a copy paper donor, recycled board, OPP and the receptor, they traced the migration of C_{17} and MBP and the influence of the inserted board. Without the board C_{17} migrated to nearly 100% into the OPP film in a few days and very slowly further into the acceptor afterwards. In comparison, C_{17} was distributed nearly equally in board and barrier in the second pack, leading to much lower migration. For MBP the results were even more significant. Only about 60% migrated into the OPP film after a few days, further migration into the receptor was quicker and larger, resulting into a partitioning of about 45% each in barrier and receptor, 10% remained in the donor. With the inserted recycled board, the MBP was adsorbed to 90% in the paperboard rapidly and remained there over the tested period of time nearly completely. They concluded that the copy paper as donor should be replaced by a recycled board and that the barrier efficiency for polar substances needs to be low, because they are anyway absorbed in the paperboard nearly completely. Furthermore, the attempt of setting the migration limit to 1% of the donor's concentration is better than a 100-fold reduction, because migration of polar substances does not need to be reduced by a factor of 100 (as discussed in detail in (Biedermann-Brem, et al., 2017a)). They then tested, if standardisation of the recycled board is necessary and found that there was little variation between four tested recycled boards.

In their next studies, they examined the adsorption and extraction behaviour in the donor board after preparation and storage for up to 5 months. Shortly after the preparation all surrogates could be extracted completely using MTBE and the conditions room temperature for 1 h. After storage for two months or five months C_{17} could still be extracted without problems, there were certain losses for DPP, but MBP and TEC were not extractable from the paper matrix at all. They had been adsorbed into the paper completely. Different extraction methods were tested, also to find the best method for the test stripes, and extraction with MTBE with addition of 5% MeOH as swelling agent overnight at 40°C were chosen. Furthermore, to represent real conditions in a better way, the donor should be condition after preparation for 2 weeks at 40°C, so that absorption of the surrogate substances into the paper could take place. The influence of humidity in the donor board and the lab air was also investigated. Present humidity could swell the barrier and block adsorption sites in the donor paper, thus resulting in higher vapour pressure again. In first tests, there was only little influence seen, but they saw need for further tests.

Finally, TEC was removed from the surrogate substances, since it never was released from the donor after conditioning. For DPP and MBP it was decided to keep them, although C_{17} was the surrogate determining the barrier efficiency for all tested materials, when the conditioned donor

was used. The concentration in the donor was reduced to 40 mg kg^{-1}. To achieve higher sensitivity in analysis high volume injection with FID or as an alternative MS detection should be used.

Biedermann-Brem published a further update of the method in the same year (Biedermann-Brem, et al., 2017a). They decided to calculate the results of the test as percentage migrated from donor, instead of using the 100% breakthrough attempt (= concentration in acceptor at equilibrium). The criterion for a good barrier were therefore change from "reduction of a factor 100" to "migration of surrogate substances <1%". The biggest part of the publication mainly focuses on a detailed description of the method, the experimental set up and the analysis:
The donor was prepared from an unprinted recycled paperboard of 300 g m^{-2} cut to A5 format and soaked for 30 sec in a beaker containing 2 L of MTBE solution with 40 mg L^{-1} C_{17}, DPP and MBP as well as 250 mg L^{-1} Sudan Red II. Soaking for shorter times gives lower concentrations of the surrogates and also inhomogeneous distribution of them. After withdrawing and drying in a fume hood for 2 min (insufficient drying can influence the test by changes in donor and barrier and falsify the results), several donors were wrapped in aluminium foil and conditioned in an oven for 2 weeks at 40°C. Prior to preparing the test packs, a strip of 2 cm width was cut from the donor, to determine the maximum amount of possible migration.
The sampling times and storage temperature should be chosen in a way, to meet the 1% migration limit. Reference data on used temperatures and time for different polymers could be found in the literature by their earlier studies (e.g. (Richter, et al., 2014)). The test should be continued until the 1% limit was clearly exceeded or the barrier was tight for the time of interest.
Sampling was done by cutting stripes of 2 x 9 cm from the receptor. To avoid an influence from sideways, a 1cm strip was cut and discarded prior to every analysis stripe. Extraction of the stripes was done by cutting them into pieces, addition of 2 ml MTBE/MEOH 95:5 and internal standard (5µL of 300 mg L^{-1} cyclohexylcyclohexane in hexane) and extraction overnight at 40°C. The donor stripe was extracted with 10 mL solvent and 20 µL internal standard in the same way. Analyses of the extracts were done with GC-FID and 50 µL injection volume and with GC-MS and 1 µL injection volume. The result was given as percentage of the amount migrated into the receptor to the original amount in the donor for the same surface area. The concentrations found in donor and receptor were therefore corrected by the ratio of mass per surface area. Calculation of response factors was not needed, since areas of the same detection methods are compared. The LOD was for both methods about 0.05 mg kg^{-1} siliconised paper, LOQ at 0.2 mg kg^{-1} siliconised paper, which equals 0.2% migration.
They also did further tests for the identification of an appropriate acceptor material. The receptor should adsorb everything migrating from the barrier completely, without further influence on the test. Papers with 13% and 29% of dimethyl polysiloxane, a paper unilaterally laminated with silicone and the food simulant MPPO were compared in tests using migration cells. They found that migration into the tested materials is similar in the first days with low migration of the surrogates. For long term storage and increasing migration of the surrogates, MPPO showed the highest adsorption, probably because of the highest surface area and the

limited adsorption sides on the siliconised paper. Considering the limited active groups on the siliconised papers, this theory was confirmed by the results. They decided, for testing only low concentrations of up to 1%, the siliconised paper seems to be appropriate, regardless to the underestimation of migration at higher concentrations. They strengthened their decision by the argument of the safety margin built in the approach. Another detail mentioned in the paper is that the used siliconised paper was now cleaned in a vacuum oven at 0.1 bar at 130°C for 8 hours. This is probably to overcome the problem of extracting interfering siloxanes.
They also discussed the repeatability of the method, which was tested on 5 equal test packs and found standard deviations of up to 18%. They explained additional uncertainty may be gained by the extrapolation of the data to the 1% value, depending on the number of stripes cut and on how close they were at the limit.

Richter et al. used the test to evaluate the barrier efficiency of internal bags and barriers from the German and Switzerland market (Richter, et al., 2014). For identification and characterisation of the barrier materials and multilayer materials they used microtome cuts, Fourier transform infrared spectroscopy and transmission microscopy combined with a digital camera. Multilayer materials and/or adhesives were separated by dissolving.
Test packs of the taped barrier test were kept at room temperature, if the breakthrough was more than 50% after one week, the test was stopped, otherwise continued at room temperature or at elevated temperature. The barrier efficiency was divided into classes. Class 1 barriers showed no significant barrier properties with breakthrough of 80% for the surrogate substances after one week at room temperature. Such barrier include paper, paper laminated with PE or pure PE films. Class 2 barriers had a 1% breakthrough time at room temperature of 10-40 days, at 40°C breakthrough was complete after one week. Such barrier includes oriented polypropylene (OPP) or combinations of OPP and PE. Class 3 barriers were tight during one week at room temperature and one week at 40°C, but most of them showed significant breakthrough afterwards and a lot faster than class 4 barriers. Class 4 barriers were pretty good barriers. Test at 40°C for 22 weeks showed less than 1% breakthrough, tests at 60°C for 5 weeks showed, that they still were tight. Conversion of these data to room temperature, results in tightness of at least 100-150 weeks. Class 4 barriers mainly were multilayer materials with more than 2 layers. Class 5 barriers were aluminium foils, which were considered as tight without testing.

1.6.2. Purge & Trap Attempt

Ewender, et al. (2013) defined another attempt to test functional barriers: They stated, since permeation depends on the molecular weight and the vapour pressure, the design of a permeation test should be done with resolved, single substances and permeation should be seen as a kinetic process. The kinetic part is necessary to determine individual permeation rates that are needed for a correct correlation of molecular weight, vapour pressure and permeation. Such correlations might be useful to extrapolate the experimental determined permeation rates to other non-tested substances of interest. Therefore, their study used model compounds,

analytically determined as single compounds. However, it is necessary to test the permeation of every single substance through films over time to generate the needed kinetic points of the permeation curve.

To reduce the workload and the time needed, automated detection by GC was used to detect the kinetic points of the permeation curves with high precision. As model substances they used n-alkanes from C_{12}-C_{24}, representing the MOSH. For MOAH they used one to three ring aromatic compounds and for substances typically included in printing inks, DIPN was used. The experiments were performed in migration cells made of aluminium. The cell consisted of an upper and a lower part, with two sealant rings in the middle, where the sample was placed. In the bottom of the cell a board soaked with the model substances was placed in direct contact with the film to be tested. For soaking of the board, a 1 g L^{-1} solution with the model substances in diethyl ether was prepared and the board soaked in 4 mL of this solution. After evaporation of the solvent at room temperature for a few minutes, the concentration of the substances on the board was approximately 750 mg kg^{-1}, in sum 11.3 g kg^{-1} for the 15 substances and about 30-fold higher than the concentrations usually found for MOs in recycled board. The permeation test was performed at 40°C in an oven. The upper part of the cell was constantly washed with nitrogen, thereby transferring all permeated substances out of the cell and onto traps, prior to GC-FID analysis. Linear permeation curves are received, giving the permeation per time. From the slope the permeation rates in mg d^{-1} dm^{-2} were determined. The test should be considered as a worst-case scenario, since the concentrations spiked on the cardboard are far higher than the ones recycled board and an equilibrium state between food, barrier and board is never reached.

The method was demonstrated on a set of polymer films used as inner liner of cardboard such as OPP, high-density PE as well as PET, PA or metalized OPP films. The experimental determined permeation rates were correlated with the vapour pressures of the individual substances. The results showed that there is a huge difference in the permeation rates depending on molecular weight. High volatile compounds permeated completely after 1 day at 40°C. Permeation of n-dodecane occurred so fast, that a steady state is not reached and permeation rates cannot be determined. Also, aromatic compounds permeated to similar extend or slightly lower. The barrier efficiency could only be given as a comparison between different films. Determining the release from the spiked board without the permeation through a barrier as a blank-value was not possible, due to too fast permeation. However, it was possible to establish a good correlation between vapour pressure and permeation rate for all compounds. This gives the possibility to predict the permeation of a given substance through a film. For the tested barriers, they concluded that PE and OPP are worse barriers, while PET and PA showed slow permeation and therefor good barrier properties (Ewender, et al., 2013).

1.6.3. Hexane Vapour Transmission Rate (HVTR)

Diehl et al. (2001) presented a semi-quantitative rapid test to determine the barrier efficiency of a material. They designed a test cell, in which a sponge was placed in the bottom. On the

sponge, a mineral-oil-simulant was spiked. The sample was placed in the lid of the cell and the cell closed gas tight using sealants. The permeation of the simulant through the barrier is tested at room temperature and determined gravimetrically after 1, 2, 3 and 4 hours and the permeation rate calculated as g m^{-2} d^{-1}. The used simulant should have similar properties than *n*-alkanes in the range of C_{16}-C_{24}, but with higher diffusion rate at room temperature. They decided that *n*-hexane would be appropriate, but also mixtures of *n*-pentane to *n*-tetracosane were tested.
Using this method, different materials were investigated; paper, low-density PE, HDPE and PP showed no barrier properties with a permeation rate of about 1000 g m^{-2} d^{-1}. Good barrier properties showed PET and aluminium films with a permeation rate of about 10 g m^{-2} d^{-1} (Diehl, et al., 2011).
The disadvantages of the test are the swelling of several polymers induced by hexane and that calibration of the permeation, also in terms of mineral oil, may be difficult (Biedermann-Brem, et al., 2014).

1.6.4. Benchmark by Biedermann-Brem et al. (2016)

Since most of the compounds migrated from paper and board are still unknown and the state of knowledge is constantly changing, a benchmark was proposed by (Biedermann-Brem, et al., 2016). The advantages of benchmarks in comparison to regulations are that they are much more flexible and anyone can bring arguments of why to change, improve or even drop them. If a benchmark is widely accepted by producers, customers and authorities, its influence can be as similar as a regulation's one.
(Biedermann-Brem, et al., 2016) proposed a benchmark for testing the barrier efficiency of internal bags for paper and board food packaging materials. The method starts with the characterisation of the food contact material itself. They used the methods described by (Biedermann, et al., 2013a), being summarised earlier in this work. The characterisation gives the number of substances present in the FCM and their concentrations. Subsequently, an assumption on which and how many substances may potentially migrate in relevant concentration and the needed barrier efficiency can be taken. The second step is the determination of the maximum tolerable migration. For example, the substances present in highest concentration were about 10 mg kg^{-1} in a recycled paper. For complete migration of these substances a contamination of 1 mg kg^{-1} food is assumed, if a typical packaging to food ratio of 1:10 is applied. This would be 100 times above the limit of 0.01 mg kg^{-1} for non-evaluated substances. Therefore, an applied barrier needs to reduce the migration by a factor of 100 or to 1%, respectively. Taking the assumption of a board, heavily contaminated by 1000 mg kg^{-1} MOSH, a reduction of migration to 1% would lead to a migration level of 1 mg kg^{-1}, using the proposed packaging/food ratio. This level of migration is considered to be safe at the moment ([BMEL] Federal Ministry of Food and Agriculture , 2014) ([BMEL] Federal Ministry of Food and Agriculture, 2017). Third, it has to be checked experimentally, if the barrier efficiency of a material complies with the needed one. They recommend the taped barrier test, which was already extensively described in this work. Fourth, the extract received

from the siliconised paper after the taped barrier test should be analysed by GC×GC to get further information about migrated substances.
With the consideration of these steps, the packaging and the internal bag should be intensively characterized. The benchmark was adopted as guideline from the Schweizerisches Verpackungsinstitut (SVI) ([SVI] Schweizerisches Verpackungsinstitut, 2016).

1.6.5. Conclusion on Test Methods for Functional Barriers

Summarizing the information generated on the available test methods shows that an ideal test for the evaluation of the barrier efficiency of coated paper samples is not available so far. All of the presented tests, except the hexane vapour transmission test, have the disadvantage to focus on polymer films only. The disadvantage of the vapour transmission test is that no estimations or comparisons in terms of concentrations migrating for other substances can be done. Besides, each test has its advantages and disadvantages:
The taped barrier test is a newly developed test that may need some more evaluation and testing. Nevertheless, it sounds promising and with some adoptions it may also be possible to apply it to coated paper samples. It was decided to do a closer experimental evaluation also as part of this work.
The Benchmark proposed by (Biedermann-Brem, et al., 2016) may be a good and comprehensive attempt. However, it is questionable, if it is possible to perform all mentioned and described analysis on the samples, since it would be very time consuming. It is also questionable, if the paperboard to which a barrier should be applied is available all the time. Last, the benchmark would have the disadvantages mentioned and found for the taped barrier test too (see later in this work in chapter 3.1.2).
The purge and trap attempt has some promising points, but also disadvantages. The used method of washing the upper part of the cell with nitrogen is needed to determine kinetic permeation rates. However, it does not reflect a real packaging situation, since equilibration can never be reached. In contrast, testing the permeation through samples in migration cells by spiking substances in the bottom of the cell is a good call. Surrogates can be used as marker substances, similar to the taped barrier test. Instead of a siliconised paper or a trap, simply a food simulant could be used to measure the amount of migration and permeation from the sample and express it as amount per kg of food simulant. Based on these principals the two-sided migration experiment was developed as described in chapter 2.2.2 of this work.

1.7. MOSH & MOAH

1.7.1. Introduction

Mineral oil residues in food gained a lot of concern in the last years since e.g. levels of up to 6000 mg kg^{-1} were found in edible oil or up to 1300 mg kg^{-1} in chocolate as reported e.g. by

(Moret, et al., 1997) (Biedermann, et al., 2009) and summarised by (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012) or (Grob, 2018). Due to the often-cited statement that mineral oil hydrocarbons may include potential mutagenic and carcinogenic substances these levels are unacceptable and on the one hand, huge efforts were made to identify the sources of mineral oil and on the other, to reduce the levels to acceptable values. The problems we face are the ubiquitous sources of contaminations (as discussed in chapter 1.7.2) and the analytical challenge for correct analysis of the residues as will be discussed in chapter 1.7.4 (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012).

In general, mineral oil hydrocarbons (MOH) are complex mixtures of hydrocarbons, which are defined as substances of unknown and variable composition (UVCB substances) in the REACH Regulation (Commission Regulation (EC) No 1907/2006). According to the definition by the JRC Guide, they originate from crude mineral oils or are produced from coal, natural gas or biomass through Fischer-Tropsch synthesis. They do not include hydrocarbons naturally present in food, like odd numbered *n*-alkanes with a carbon number of C_{21} to C_{35}, natural olefins like squalene, sterene or carotenoids, nor the fractions considered to be POSH, present in e.g. plastic packaging ([JRC] , 2019). The MOHs are divided into two main fractions: mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) (Biedermann, et al., 2009). Usually, the MOAH fraction can be found in a ratio of 15-30% of the whole mineral oil fraction. MOSH consist of branched and unbranched open chain hydrocarbons, called paraffines and cyclic hydrocarbons with at least one saturated ring, called naphthenes (Vollmer, et al., 2011) (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012) (Moret, et al., 2014). Paraffines build of linear *n*-alkanes with at least 20 carbon atoms are called waxes ([JRC] , 2019).

Regarding the health effects, MOSH can accumulate in the human body and may cause micro granuloma formation, inflammatory diseases and organ weight increase (Grob, 2018). The MOAH consists of substances with at least one aromatic ring; typically, it includes substance classes with 1-7 aromatic or partly aromatic rings with highly alkylated side chains. There is no acute toxicity and no accumulation after chronic intake, but they are considered to be mutagenic, because of the present 3-7 ring-MOAH. They are not mutagenic as such, but undergo metabolic activation and can lead to cancer (genotoxic carcinogenicity) (Roy, et al., 1988) (Carillo, et al., 2019). These substances are often referred to as 3-7 ring polyaromatic hydrocarbons (PAHs), but MOAH should be strongly differentiated from the PAHs. PAHs are substances mainly formed by incomplete combustion and decomposition of organic matter at high temperatures by both, natural and anthropogenic sources. They are composed of two or more fused aromatic rings and occur in complex mixtures. In contrast to MOAH, they do not include heteroatoms, are only slightly alkylated and can be analysed as single peaks (Grob, et al., 1991) (EFSA Panel on Contaminants in the Food Chain (Contam), 2008). Still, the 3-7 ring MOAH substances have similar structure and toxicological properties and should therefore be removed in production of mineral oil products. In the EFSA opinion 2012, all MOH are considered to be mutagenic unless they have been treated to remove MOAH (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012). Subsequently, MOHs are distillate as a first step for fractionation into

individual products. The raw products are hydrogenated in another step to remove the aromatic MOAH. Quantitative removal is hardly possible and some MOAH substances always remain in the final product. According to theory, these substances should only have one or two isolated aromatic rings left and are highly alkylated ones, not allowing metabolic activation. Latest studies show that MOAH present in food is mainly made up of 1-2 aromatic ring substances and therefore there may not be any threat from them at all (Grob, 2018) (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2009) (Koch, et al., 2019). Still, there are no mixture toxicity data for MOAH, no marker molecules can be used and so far, there are no response-dose data and no toxicology reference point (van den Ven, et al., 2017). Further discussion on the health effects of MOSH and MOAH are made in chapter 1.7.3.1.

Also in terms of analysis, mineral oil hydrocarbons are a huge challenge. Since MOH are complex mixtures of hydrocarbons, it is not possible to separate them into single components during analysis. EFSA stated in their scientific opinion that the possible number of hydrocarbon compounds in mineral oil products easily exceeds 100 000 for those with less than 20 carbon atoms. The number further increases exponentially with the number of C-atoms. To illustrate the complexity the EFSA shows Figure 5 in their scientific opinion (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012). It gives the number of isomers resulting from a given number of C-atoms. Considering the EFSA figure and using e.g. a C-atom number of 10 would result into about 50 possible mono-aromatic isomers, 500 cycloalkane isomers and already about 5000 alkane isomers. Thinking about the fact that MOH present in food and FCMs includes the C-atom range from C_{10} to about C_{50}, the analytical task of resolving the resulting millions of peaks is not possible with today's analytical techniques. Humps of unresolved substances are received, even when using multidimensional chromatography techniques, as 2D-comprehensive GC×GC. An online LC-GC-FID method for the determination of MOH in food was first published by Grob, K. et al. in 1991.

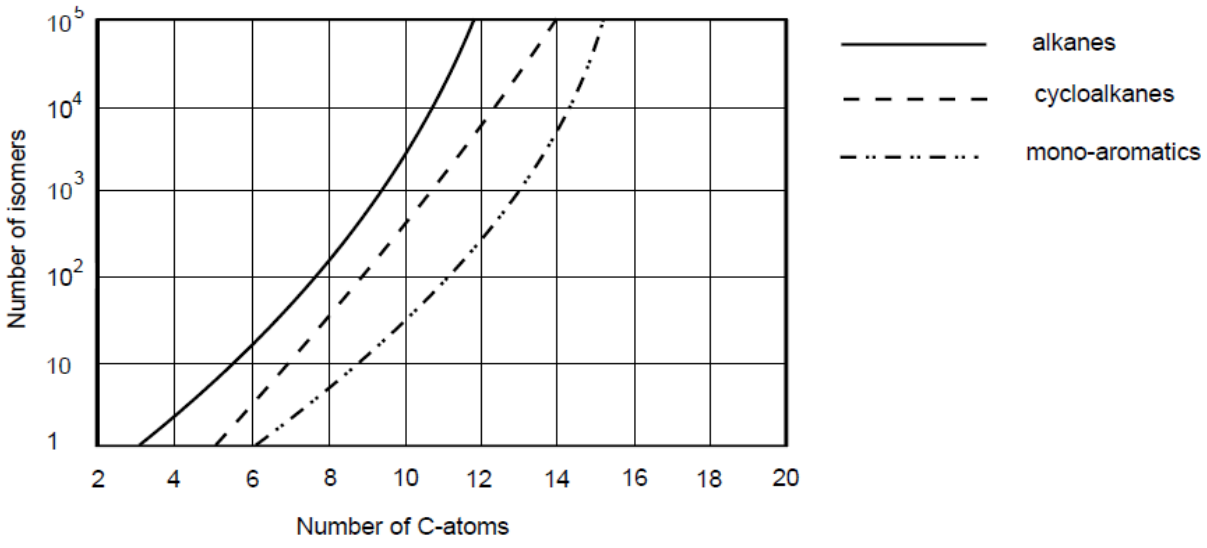

Figure 5: Illustration of MOHs complexity; taken from (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012 p. 17).

The recently applied state of the art analysis method uses normal phase high pressure liquid chromatography (HPLC) to pre-separate the two fractions, online coupling and high-volume injection to a dual-column gas chromatograph (GC) for further separation and flame ionization detection (FID) for quantification (Grob, et al., 1991) (Biedermann, et al., 2012a). Aim of the method is to increase sensitivity by forming humps of unresolved mixtures. A fast GC program is therefore used, generating no further information than the total amount of MOSH and MOAH as will be discussed in the chapter about HPLC-GC-FID, according to (Biedermann, et al., 2012a). A further problem of the analysis is the complex food matrix. High fat content and highly varying matrix effects lead to very complex analysis tasks. Auxiliary methods are often necessary to remove interfering naturally occurring substances and to enrich the fractions to lower LOQs. For example, epoxidation with *meta*-Chloroperbenzoic acid (m-CPBA) or flash chromatography with silica gel and/or aluminium oxide is used to remove naturally occurring olefins, *n*-alkanes and fat in e.g. edible oils or chocolate. Besides the already mentioned challenges, there is another one, being probably the most severe: the lack of standardize sample preparation methods. The only method existing so far is the EN16995 on the analysis of edible oils and fats, but it has shortcomings in terms of LOQs (Austrian Standards Institute, 2017). A detailed discussion on the analytical challenge and the available methods is given in chapter 1.7.4.

In summary, mineral oil residues in food are one of the biggest and most discussed challenges nowadays. There are hardly any approved methods, the existing ones being complex and time consuming. The information generated is often small, due to the formation of unresolved humps. To get detailed information, more complex methods and additional dimensions have to be applied (e.g. two-dimensional comprehensive GC×GC-MS after HPLC pre-separation). Sources are ubiquitous and negative health effects still discussed. The following chapters should give a quick overview of the state-of the art and the on-going discussions.

1.7.2. Sources of MOH in Food and Food Contact Materials

As already mentioned in the introduction, MOH are ubiquitous in our daily life. They are used e.g. for printing newspapers, as additives in many cosmetic products and food stuff, as fuel for our vehicles, as lubricants for machinery and as raw material for daily life products. Since this work is related to food and food contact materials, sources for them are discussed shortly:

1.7.2.1. Sources in Food

MOH contamination can enter food from many sources in the whole production chain – from the raw material throughout the production to the finished product. Some examples of sources should be mentioned here:

- environmental contribution to the raw product from the air (e.g. exhaust gases from vehicles, smoke, debris from tires and road tar)

- environmental contribution to the raw product from the aquatic ecosystem (e.g. accidents and oil spills, dust washed into water, debris)
- contribution from harvesting and processing machinery (e.g. fuels, lubricating oils)
- processing additives (MOH as food additive e.g. release agents in bakery, de-dusting agents during transport and storage of grain and rice, or MOH as contamination in additives)
- transport- and storage- packaging of raw, intermediate and final products (e.g. recycled board, jute and sisal bags, waxed packaging materials).

Depending on the source and the way of entering, the present contaminations are versatile – in concentration and in present fractions (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012).

The German Federation of Food Law and Food Science (BLL) published in December 2017 a 34-page document called "*Toolbox for preventing the transfer of undesired mineral oil hydrocarbons into food"*. The document summarised the available information on occurrence, routes and sources of contaminations, and analysis and risk assessment in tabular form, called the "toolbox". The toolbox gives the potential routes of entry as either migration from packaging, contamination during production or as intentional use as additive or processing aid. It then lists possible sources, the route of entry, found substances, tools to prevent contaminations, notes and examples and references ([BLL] German Federation of Food Law and Food Science, 2017). An example is given in Figure 6.

NO.	SOURCE	ROUTE OF	SUBSTANCES	TOOL	NOTES/EXAMPLES	REFERENCE
19	Metal foil/metal sheets (lacquered/ laminated)	Primary packaging	MOSH analogues (MORE)	Avoid surface lubricants on MOH or MORE basis, if possible Do not allow inevitable residues of rolling oils or rolling emulsions Both sides in the case of rolled or stacked products Use MOSH/MOAH-free coatings and lacquers	Rolling oils or rolling emulsions are used for the production of metal foils, in general paraffin oils which may introduce MOSH analogues. In the case of lacquered or laminated applications, the foils/sheets must be annealed and the rolling oils used evaporated. Consider lacquer and laminating components because they may contain MOSH analogues (MORE). Consider printing ink specifications for printing and avoid contact with the inside of cans.	[Expert] [36] [44]
20	Composite materials Laminates	Primary packaging	MOSH/MOAH	Use suitable materials with respective layer thickness as barrier materials; for aluminium foil, a thickness of 6 µm is considered to be suitable depending on the other composite materials Review packaging tightness under consideration of the closing technology	Specify aluminium tightness (pin holes/defects) for aluminium foils and composite materials. For beverage cartons, a common layer thickness is 6.25 µm. Other than metal or aluminium foils, metal coatings, are in general not a barrier for MOSH/MOAH.	[24] [Expert] [44]
21	Laminated composite foils	Primary packaging	MOSH/MOAH MOSH analogues (POSH)	Use suitable carrier materials (plastics) with appropriate layer thickness	E.g. composite bag as inside bags. The requirements of the Plastics Regulation No. 10/2011 in combination with the declarations of compliance apply.	[Expert] [24]

Figure 6: Example of BLL Toolbox; given are possible sources of contamination via packaging as route of entry, substances found, tool to prevent, notes and examples and references ([BLL] German Federation of Food Law and Food Science, 2017).

1.7.2.2. Sources in FCMs

Migration of MOH from food contact materials was considered to be one of the major sources of food contamination (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012). As already discussed in this work, contamination can occur through direct contact between the food and the FCM or through indirect contact (permeation through the gas phase).

Recycled paperboard usually contains *n*-alkanes in the range of C_{13}-C_{45}. The main reason is that during the recycling process fibres of different quality are mixed together; contaminations are spread and -instead of removed- enriched in the final product. The main sources of MO contamination are the offset printing inks of newspapers. For the migration into food only the volatile part is of interest, although migration increases also for low volatile substances during long storage times. It was determined empirically that migration takes place up to C_{24}, but may reach C_{28}. About 70% of the hydrocarbons eluted below C_{24} are transferred from the packaging to the food, when packed several months in it. The transferred substances are mainly re-condensing on the foods surface and should be easily extractable without extensive sample preparation (Biedermann, et al., 2010) (Vollmer, et al., 2011).

Besides the use of recycled fibre, the use of mineral-oil-based printing inks was one of the major sources of food contamination through FCM. The most commonly used inks for printing paper and board were offset printing inks, containing 20-30% mineral oil. Contaminations of 100 mg kg^{-1} were sometimes found (Droz, et al., 1997) (Biedermann, et al., 2010) (Vollmer, et al., 2011). Today, food grade mineral oils ("low-migration inks") replaced the conventional offset printing inks for FCM, omitting at least one of the many possible sources.

Food stuff packed in jute and sisal fibres was often heavily contaminated. Those fibre materials were batched with about 7% mineral oil to improve spinning properties and elasticity. The MOH was transferred to the packed food such as cocoa beans, coffee and rice, during storage and transport (Grob, et al., 1991). Today, sacks for the European market are batched with plant oils or had been replaced by alternatives to reduce contamination of the products (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012).

Also waxed packaging, such as paper for meat or bakery products or cheese and candy coatings, release waxes into the product. The transfer mainly depends on the surface ratio of food and packaging, on the fat content of the food and the temperature. For cheese it was shown that the wax penetrates through 2-5 mm of the surface with average concentrations of up to 27 mg kg^{-1} cheese (Grob, et al., 1991) (Castle, et al., 1993). Still, the use of certain MO fractions as food additives, including waxes, is allowed by the European Union as will be discussed in more detail in the next chapter 1.7.3.

For plastic materials the use of mineral oils as additive is approved according to Regulation (EU) No 10/2011. Besides, plastic materials are known to release the MOSH analogues POSH, consisting of branched alkanes and forming patterns of unresolved peaks in the LC-GC-FID chromatograms (Commission Regulation (EU), 2011) (Fiselier, et al., 2012).

1.7.3. Guidelines, Regulations and Opinions

As already mentioned in the introduction, the problem of MOH is a very complex one.
There are hardly any approved methods (except the one for fat and oils (Austrian Standards Institute, 2017)), still unclear toxicological evaluations and a big lack of data concerning the amounts present in food. There had been several attempts to derive a common sense so far, but none did solve the problem.

1.7.3.1. EFSA Scientific Opinion of 2012 and Technical Report of 2019

The scientific opinion on mineral oils from EFSA was published in 2012 and gave a quick history, definition of MOs and summarized literature and studies available on analysis, occurrence, exposure and hazard and risk assessment (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012). In 2019, an update and rapid risk assessment was made and published as technical report, due to the contamination of infant formulas with MOAH (EFSA, et al., 2019).
The historical background given in the scientific opinion starts in 2008. The rapid alert system for food and feed (RASFF) was notified that sunflower oil originating in Ukraine was contaminated with high levels of mineral oil. Similar cases on contamination of oils and other products (e.g. cakes, cocoa beans, presence of diesel in red wine …) had been reported to the RASFF system before. The Commission Regulation (EU) No 37/2010 classifies mineral hydrocarbons (aliphatic, branched aliphatic and cyclic ones) of low to high viscosity, including microcrystalline waxes with a carbon range of approximately C_{10}-C_{60}, with exclusion of aromatic and unsaturated compounds, as with no maximum residue limit (MRL) for all food producing species (Commission Regulation (EU) No 37/2010, 2009). The Directive 95/2/EC on food additives other than colours and sweeteners permitted the use of microcrystalline wax for surface treatment for certain food types under number E905 with no specified maximum level, but in accordance with GMP. The used fractions must have an average molecular weight above 500 Da, a viscosity above 11 centistokes at 100°C and must not contain more than 5% of molecules with less than 25 C-atoms (European Parliament and Council Directive No 95/2/EC, 1995). In addition, the Commission Regulation (EC) 10/2011 and the Council Directive 91/414/EH give provisions to use certain fractions of mineral oils (Commission Regulation (EU), 2011) (Council of the European Communities, 1991). The regulation 10/2011 on plastic materials and articles for food contact gives a positive list of additives. It includes FCM substance No 95 (white mineral oils, paraffinic, derived from petroleum-based hydrocarbon feedstock, average molecular weight above 480 Da), FCM substance No 94 (waxes, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, average molecular weight above 500 Da) and FCM substance No 93 (waxes, paraffinic, refined, derived from petroleum-based or synthetic hydrocarbon feedstock, average molecular weight above 350 Da). EFSA classifies those fractions of paraffines and naphthenes accepted to be used in food had been classified according to their viscosity as medium and low viscosity (C_{10}-C_{25}; viscosity at 100°C is 3-8,5 centistokes; molecular weights of 300-500), high viscosity (C_{30}; viscosity at 100°C not less than 11 centistokes; molecular weights above 500) and microcrystalline waxes

(C20-C60; viscosity at 100°C is 10-30 centistokes; molecular weights of 300-750+). In 2002, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) set ADIs for the high viscosity fraction (0-20 mg kg-1 b.w.) and temporary ones for the medium and low viscosity fraction (Class I 0-10 mg kg-1 b.w. and Class II and III 0-0.01 mg kg-1 b.w.) (Joint FAO/WHO Expert Committee on Food Additives (JECFA) , 2002). The temporary group ADIs for classes II and III were withdrawn in 2012, because of a lack of data (Joint FAO/WHO Expert Committee on Food Additives (JECFA) , 2012). ADIs for the high viscosity white mineral oils were set by the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) as 12 mg kg^{-1} b.w. per day in 2009 (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2009). The German Federal Institute for Risk Assessment (BfR) evaluated hydrocarbon solvents used for the formulation of additives in paper and board production. Those fractions were liquid paraffinic oils with a chain length of $<C_{17}$, not containing MOAH. Transfer from food packaging into food of those hydrocarbons should not exceed 12 mg kg^{-1} (7. Sitzung der BfR-Kommission für Bedarfsgegenstände (in German), 2011).

The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) delivered its scientific opinion on MOH in food in 2012. They considered hydrocarbons containing 10 to about 50 C-atoms. They concluded that MOH occur in food as a result of either contamination or from intended use. Contaminations were divided into environmental-based ones and FCM-based ones. The main source was identified to be contamination from FCM, especially from those FCMs made from recycled paper and board and the printing inks applied to them. Furthermore, intended use of MOH during manufacture of FCMs or the food itself plays an important role. Besides, the environmental sources of contamination are manifold (see in more detail chapter 1.7.2 above).

Of the commercially available MO products considered, little was known about the composition, since most information was given as physico-chemical properties (e.g. viscosity). However, there were also huge differences on the composition of products with the same physico-chemical properties. The positive is that food grade MOH products were treated to minimize the typically MOAH content of 15-35% to as low as possible.

As the most efficient analysis method HPLC-GC-FID is described. Depending on the matrix and the enrichment steps used, detection limits as low as 0.1 mg kg^{-1} can be reached. For further classification GC×GC methods can be used, but are not practical for routine analysis. Due to the high complexity of the MOH mixtures the definition of a certified standard, being generally applicable, is not possible. Thus, data on MOH in food was only available for a limited number of food types and only from a few countries. Furthermore, most data only referred to total MOSH without MOAH data or additional differentiation into groups or sub-classes. The typical carbon range found (or evaluated) was between C_{12} and C_{40}. The food was divided into different groups. The highest levels were found for “bread and rolls” and “gains for human consumption”. It was assumed that they resulted from the use of food grade white oils, being MOSH, with virtually no MOAH, as release agents or spraying agents and calculated to be 532 mg kg^{-1} and 977 mg kg^{-1}, respectively.

The estimated daily intake was calculated as 0.03-0.3 mg kg^{-1} bodyweight (b.w.) per day, based on the mean occurrence levels of different food groups (considered to represent the background concentration, normally to be expected in each group) and the consumption data of the European dietary survey.

The available toxicity data was also summarised. It was concluded that MOSH and MOAH have low acute oral toxicity. However, the MOSH fraction accumulated in rats, leading to the formation of microgranulomas in the liver, being associated with inflammatory reactions, and the mesenteric lymph nodes (MLN), the latter being considered of having low toxicological concern. Microgranuloma formation has also been observed in humans exposed to MOSH in liver, spleen, lymph nodes and other organs, but with no association of inflammatory reactions or other negative effects. Furthermore, long chain MOSH can act as tumour promoters at high doses. The absorption for n- and cycloalkanes varies from 90% for the fraction of C_{14}-C_{18} to 25% for the fraction of C_{26}-C_{29} and decreases with increasing carbon number, until it is negligible for C-numbers above C_{35}. The fractions between C_{16} and C_{35} accumulate in different tissues and produced, as, already mentioned, liver microgranulomas in Fischer 344 rats, which was considered to be the critical effect. Existing toxicological studies from which the current ADIs are derived had poorly characterized MO products in terms of chemical composition. Therefore, the existing ADIs (especially the group ADI by JECFA for medium- and low-viscosity mineral oil class II and III) were revised in 2012. The existing ADIs for microcrystalline waxes, high viscosity mineral oils and medium- and low- viscosity class I mineral oils should not be revised. However, none of the existing ADIs was adequate for the risk characterization of MOSH in food (from C_{12} to C_{40}, centred at C_{18} to C_{34}). Therefore, a margin of exposure (MOE) approach was used and for the background exposure as a reference point (RP) the no-observed-adverse-effect level (NOAEL) of 19 mg kg^{-1} b.w. per day for the most potent MOSH (low and intermediate melting point waxes) was chosen. For the high exposure scenarios in bread and rolls and grains a NOEAL of 45 mg kg^{-1} b.w. per day was chosen as RP. MOAH data indicates that the fraction is well absorbed, rapidly distributed to all organs and metabolized. There is no accumulation in tissue. The 3-7 ring fractions of the MOAH are known to be mutagenic (including the non-alkylated PAHs), since the substances with no or low alkylation can be activated by P450 enzymes into reactive genotoxic carcinogens, forming DNA adducts. Some of the highly alkylated MOAH fractions can act as tumour promoters, although not being carcinogens themselves. Simple MOAH, like naphthalene, are carcinogenic by a non-genotoxic mode of action, involving cytotoxicity and proliferative regeneration. Therefore, all MOH are considered to be mutagenic, as long as there was no treatment to remove MOAH. Although MOAH may be mutagenic and carcinogenic, there are no dose-response data on the carcinogenicity and a MOE could not be derived.

In summary, due to the high complexity of the MOHs, the lacking composition information and the presence of humps, it was recommended to evaluate the health-based guidance on whole-mixture studies. The scientific opinion states that none of the current methods of analysis has been formally validated. Certified reference standards and materials for MOH are needed to allow method development and validation. Furthermore, EFSA set a call for data on the

occurrence of MOH in food. In monitoring, MOSH and MOAH should be separately determined in sub-classes, based on molecular mass ranges and structures. For MOSH, those classes should be up to n-C_{16} and from C_{16} to C_{35} with a distinction of *n*-alkanes, branched alkanes and cyclic alkanes. POSH should be distinguished from MOSH. For MOAH improvement of the analytical techniques is needed to further separate it into subclasses. Also, measures to reduce contamination should be taken; as recycled paper and board used as packaging material was considered to be a main source for contamination, functional barriers should be applied to prevent it.

Following the EFSA call for data, the European commission published a recommendation on the monitoring of MOH in food and FCMs (Commission Recommendation (EU) 2017/84, of 16 January 2017). It is stated that the generation of reliable analytical results has to be ensured. Therefore, the member states should care for the availability of the analytical equipment and the experience needed. To gain uniformity, the European Union Reference Laboratory for Food Contact Materials should establish further guidance to the competent authorities and parties interested on sampling and analysis. This guidance was only published on the 23th February 2019 and will be discussed in chapter 1.7.3.1 of this work ([JRC] , 2019). However, the data should be collected during the years 2017 and 2018 in various kinds of food and latest provided on the 28th February 2019. Therefore, the attempt is still lagging with too less data provided so far, for a new comprehensive attempt. The deadline for providing data was postponed several times afterwards.

In 2019, when foodwatch published a project-report on the test results for MOH in canned baby milk products. The values found for MOSH/POSH were in the range of 0.5 – 8.4 mg kg^{-1}, those for MOAH in the range of 0.5 – 3 mg kg^{-1} (foodwatch international, 2019). Following this report, EFSA published a rapid risk assessment (EFSA, et al., 2019). The Commission services asked the competent authorities to re-sample contaminated batches and draw conclusions on the presence of MOAH and the possible source. No MOAH was found by the authorities. Member states were asked to provide available data, including chromatograms of LC-GC-FID. No state reported data, but some were available from Specialised Nutrition Europe (SNE), the Austrian Agency for Health and Food Safety (AGES) and the Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit; BVL) from earlier reports (e.g. via the RASFF). However, those ones were without the needed chromatograms. Also, foodwatch did not disclose the reports of the analysed samples, due to contractual agreements with the analysing laboratories. Therefore, they concluded that the origin of the MOAH in positively analysed samples is not clear (e.g. crude oil from fuels would include 3-7 ring MOAH, refined oils would not contain them). No additional information on the detected MOAH in food was available (chromatograms or information on presence of 3-7 ring MOAH). Therefore, and in accordance with the previous EFSA opinion, MOAH in food should be considered as possible concern for human health. However, even if data on the excluded presence of 3-7 ring MOAH would have been available, characterization of possible

non-neoplastic hazards would not be feasible. They recommended that analytical methods to identify 3-7 ring MOAH should be routinely applied when MOAH is detected. Chromatograms should be made available upon request for better evaluation of the risk (EFSA, et al., 2019).

Recently, an analytical method to separate the mono- and diaromatic fraction (MDAF) from the tri- and poly aromatics (TPAF) was published by Koch et al. (2019). They used a manual SPE approach with a bed made of silica gel loaded with 0.3% silver nitrate to pre-separate MOSH and MOAH. The MOAH fraction was further separated into the MDAF and TPAF by donor-acceptor complex chromatography with a Nucleosil Chiral-2 LC column. GC×GC-TOF-MS was used for the identification of the substances present in the individual fractions. The method was applied onto two petrolatum and two paraffinum liquidum samples. They concluded that the present fractions were mainly alkylated mono- and diaromatic compounds. The TPAF fraction was quantified using LC-GC-FID and made up <1% of the whole MOAH fraction. GC×GC-TOF was used for the qualitative approach. About two thousand peaks were identified, giving a mean concentration of about 50 µg kg^{-1} for individual substances. The substances present had mainly a high degree of alkylation. Therefore, the resulting risk was considered to be low. The next steps would be an automated online LC-LC-GC-FID approach for improved sensitivity and an easier analysis procedure, also in food stuff (Koch, et al., 2019).

1.7.3.1. Draft of MO Regulation

The Federal Ministry of Food and Agriculture in Germany (BMEL) tried to enforce a legal regulation for MOSH and MOAH. In general, the (Commision Regulation (EC) No 1935/2004), the (Comission Regulation (EC) No 2023/2006) and the national Foodstuffs, Consumer Goods and Feed law are valid. Furthermore, they tried to draft a "mineral oil regulation" as part of the consumer good ordinance ("Bedarfsgegenständeverordnung"). The new ordinance should set regulations and limits for the migration of mineral oil from food contact materials into food. There had been four drafts of the regulation since then; the most cited ones are probably the 3th draft of the 24th July 2014 and the 4th draft of the 7th March 2017.
In the 3th draft it is stated that food on the German market may be contaminated by mineral oil. One of the biggest sources is the use of recycled paper and board as food contact material. To protect consumers, the transfer of mineral oils from recycled FCMs should be minimized and limited. Therefore, on the one hand, limits for MOSH and MOAH present in the FCMs should be set. The maximum amount of MOSH being present in a FCM should be 24 mg kg^{-1}, the amount of MOAH 6 mg kg^{-1}. If a material or article exceeds the limits, it is prohibited to be use as a FCM. On the other hand, if it can be proven that under worst foreseeable conditions of use the transfer of MOSH and MOAH from the FCM into food remains below 2 mg kg^{-1} and 0.5 mg kg^{-1} food, it can still be used. The application of the functional barrier concept is possible and allowed to be able to comply with these limits. The limits are derived from the assumptions that the usual food to FCM ratio is 12:1, transfer into food is 100%, the background contamination of food is 20 mg MOH per kg and that only 10% of this contamination should be related to the FCM. For dry, non-fatty food stored at room temperature or lower, the

evaluation should be done in the range of C_{16}-C_{25}. For all other applications it should be done from C_{16}-C_{35} ([BMEL] Federal Ministry of Food and Agriculture , 2014).
In the 4th draft the limit for MOSH is completely withdrawn and only one for MOAH retained. To keep the level of MOAH migrating into food as low as possible, the use of functional barriers is mandatory. The transfer of MOAH from a FCM into food, determining the barrier efficiency needed, should not be detectable with a limit of detection of 0.5 mg kg^{-1}. The C-range to be analysed it C_{16}-C_{35}. No barrier is need, if the transfer is not possible at all (e.g. no MOAH in the FCM used) or if special requirements are taken to prevent the transfer of MOAH (e.g. by the food producer or if the food producer declares that no barrier is needed).
So far, none of the drafts came into force. The website of the BMEL states that the 4th draft was sent to the federal states and authorities for their statements and opinions in March 2017 ([BMEL] Bundesministerium für Ernährung und Landwirtschaft, 2019). Nevertheless, the limits of 2 mg kg^{-1} MOSH and 0.5 mg kg^{-1} MOAH proposed in the two drafts, are an often-used benchmark for the maximum migration from FCMs, but also as a general limit for food.

1.7.3.1. JRC Guideline

The latest try to derive a common sense for MOHs was published by the Joint Research Centre (JRC) in 2019. It provides guidance for the sampling and analysis of MOH and FCM in the frame of the Commission Recommendation (EU) 2017/84, as already discussed above. The document includes guidance on sampling, the minimum performance requirements of the analytical methods used for the generation of reliable data on the occurrence of MOH, guidance on reporting the results as total amount and in fractions and references to literature describing current analytical approaches. The guidance does not aim to give any standard operating procedures, which are planned to be given in hands-on training courses in future.
The most important chapters of the guideline are the ones on sampling, analysis and reporting of results. Those will be discussed in more detail here:

Sampling
In general, the guideline refers to Regulation (EC) No 333/2007 on methods of sampling and analysis of food. However, only some of the sections are relevant for mineral oil, since the regulation is mainly related to control of lead, cadmium, mercury, inorganic tin and arsenic, 3-MCPD and PAHs in food. Special focus is given to sampling products from different suppliers as this is a main focus of the recommendation (EU) 2017/84. It advises e.g. to collect unused packaging materials from the same batch as used to package the sampled food, if it is available. It may provide useful information for the identification of contaminating sources.
Regarding the sampling procedure, precautions must be taken to avoid contamination of the sample. Recommendations given are e.g. avoidance of the use of cosmetics like hand creams, use of collection tools free from mineral oil or use of inert storage containers, such as glass or polyethylene terephthalate (PET). The materials and articles used should be carefully checked for contamination prior to use and/or rinsed with *n*-hexane and dried at the highest temperature possible (400°C recommended for glass materials). Paperboard boxes are not suitable as transport or storage packaging, even not for secondary ones. Containers should be closed with

a polytetrafluoroethylene (PTFE)-layered lid or a glass stopper. Wrapping in aluminium foil can be done for different kind of samples and also for covering containers. However, also the foil needs to be checked for contamination and rinsing or heating out prior to use may be needed. No tapes, paper or plastic labels should be used to seal or label a packaging, since they can also contaminate the sample. The use of a permanent marker is recommended.
Furthermore, the section gives information on the required recording of information during sampling, such as description of product, packaging, treatment, date of expiry and sampling.

Analysis
This section firstly defines the analytes – MOSH and MOAH, which were already given in the introduction on MOSH and MOAH in this work. The analysis is based on the determination of the amounts present after pre-separation of the two fractions, integration of the hump from the peak start of n-C_{10} to the peak end of n-C_{50} and subtraction of all present interferences. The MOSH fraction may include POSH, since clear separation and/or subtraction is not always possible. In such cases the presence should be clearly reported. As internal standard cyclohexylcyclohexane (CyCy) is used in the MOSH fraction and 1- or 2- methylnaphthalene should be used as internal standard in the MOAH. The analytical determination results into a value expressed in mg MOSH or MOAH per kg sample.
Furthermore, general sample preparation possibilities, the background of the analysis with LC-GC-FID with its advantages and disadvantages, the selection of internal and verification standards and the calculation of results are explained shortly, with reference to the relevant literature. Most of the cited literature is also discussed in this work in the corresponding chapters and should therefore not be discussed in detail here (see chapter 1.7.4 - sample preparation techniques; chapter 2.1.5.5 - LC-GC-FID).
One of the key points of analysis and data evaluation is the integration of the chromatograms. The humps present are divided into sub-fractions, which varied widely in past. With this guide, standardization of the reported fractions was aimed: the total amount of MOSH and MOAH gives the integration from beginning of the *n*-C_{10} peak till the end of the *n*-C_{50} peak, after subtraction of interferences. Each sub-fraction starts at the retention time of the peak end of the first *n*-alkane of the range and ends at the retention time of the peak end of the last *n*-alkane within the fraction. Only the first fraction starts at the retention time of the peak start of *n*-C_{10}. The fractions as given in the guideline are:

Table 5: Integration intervals as given in the JRC Guideline ([JRC] , 2019).

MOSH	**MOAH**
total MOSH ≥ *n*-C_{10} to ≤ *n*-C_{50}	total MOAH ≥ *n*-C_{10} to ≤ *n*-C_{50}
MOSH ≥ *n*-C_{10} to ≤ *n*-C_{16}	MOAH ≥ *n*-C_{10} to ≤ *n*-C_{16}
MOSH > *n*-C_{16} to ≤ *n*-C_{20}	MOAH > *n*-C_{16} to ≤ *n*-C_{25}
MOSH > *n*-C_{20} to ≤ *n*-C_{25}	MOAH > *n*-C_{25} to ≤ *n*-C_{35}
MOSH > *n*-C_{25} to ≤ *n*-C_{35}	MOAH > *n*-C_{35} to ≤ *n*-C_{50}
MOSH > *n*-C_{35} to ≤ *n*-C_{40}	
MOSH > *n*-C_{40} to ≤ *n*-C_{50}	

At the end of the analysis section performance requirements of the analytical methods are given. It is stated that the final procedure should undergo thorough validation to prove its fitness for purpose using e.g. the Eurachem Guide (2014). As general performance requirements the maximum allowable limit of quantification for each integration fraction (LOQ_{max}), the target LOQ for each integration fraction (LOQ_t), the acceptable ranges for recovery (R_{rec}) and the intermediate precision for different types of samples are defined.

Since the LOQ that can be reached with the LC-GC-FID method is mainly depending on the sample's matrix (more precisely the fat content of the sample), a differentiation into categories was made: dry food with low-fat content (< 4% fat), food with higher fat content (> 4% fat), fats and oils and an additional category for paper and board materials. Resulting from the complex sample preparation steps, which may be necessary, there is the differentiation between the LOQ_t and the LOQ_{max}. The LOQ_t gives thereby the lowest, analytically achievable LOQ for the majority of matrices of its category, the LOQ_{max} the limit that should at least be succeeded. The recovery is in general corrected by the added internal standards, being used for quantification. However, when inclusion of MOH happens in certain food types, quantitative extraction is hardly possible and also the internal standard correction is not effective. Those food types are e.g. food with high crystallinity (e.g. food with high sugar content, powdered drinks or milk powders), pasta or paperboard (swelling with ethanol needed) and plastic packaging's. Checking of recovery can be performed by rinsing the extracted sample and subsequent extractions under harsher conditions.

The intermediate precision is the precision within a laboratory ("in-house") and should be calculated using e.g. the Eurachem Guide (2014).

Table 6: method performance requirements as given in the JRC Guide (2019).

Categories	**Associated Foods**	**LOQ_{max} [mg/kg]**	**LOQ_t [mg/kg]**	**R_{rec} [%]**	**Intermediate Precision [%]**
Dry, low-fat content (< 4% fat/oil)	bread and rolls; breakfast cereals; grains for human consumption; pasta, products derived from cereals	0.5	0.1	80-110	15
Higher fat/oil content (> 4% fat/oil)	fine bakery ware; confectionery (incl. chocolate) and cocoa; fish meat, fish products (canned fish); oilseeds; pulses; sausages; tree nuts	1	0.2	70-120	20
Fat/oils	animal fat (e.g. butter); vegetable oils	2	0.5	70-120	20
Paper and Board	Reporting only up to C_{35} (extraction optimised up to C_{35})	10	5	80-110	10

Reporting of results
The last section gives guidance on the reporting of results. They shall be reported in mg kg^{-1} with two significant figures and rounded. A short description of the analytical steps applied should be given in the report, too. For reporting to the EFSA database further guidance and an example is given.

In conclusion, the JRC guideline is a rather general document. It describes the main principals of the analysis with its advantages and disadvantages. However, no detailed information on the sample preparation methods that should be used for certain food types are given. Criticism quickly arose, since only giving the methods requirements with literature references, but without any further details, does not help to align (sample preparation)-methods for the individual categories and to generate more reliable results. Interpretation of how to choose and/or which sample preparation is needed, is still possible and problems not solved by the guideline. A detailed discussion on the sample preparation methods is therefore made in the next chapter.

1.7.4. Sample Preparation Techniques and Analysis

1.7.4.1. General Scheme

The Joint Research Centre (JRC) of the European Commission's science and knowledge service published a guideline on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials (JRC, 2019). They defined a general scheme of sample preparation, which is given in Figure 7.
There is no further information or detailed descriptions on the methods given, except the performance requirements for the methods and literature references, as was discussed in the previous chapter. Therefore, most laboratories still use their own developed methods, based on the little literature and the only standardised method available. Those are e.g. the BfR Compendium, giving an overview on sample preparation methods, as will be discussed in chapter 1.7.4.2, the EN 16995, which is the only available certified method, but also associated with many disadvantages and criticism, as will be discussed in chapter 1.7.4.3 and a new attempt made by German Society for Lipid Science (Deutsche Gesellschaft für Fettwissenschaft; DGF) and a group of expert laboratories, trying to align their analysis and developing the method discussed in chapter 1.7.4.4.

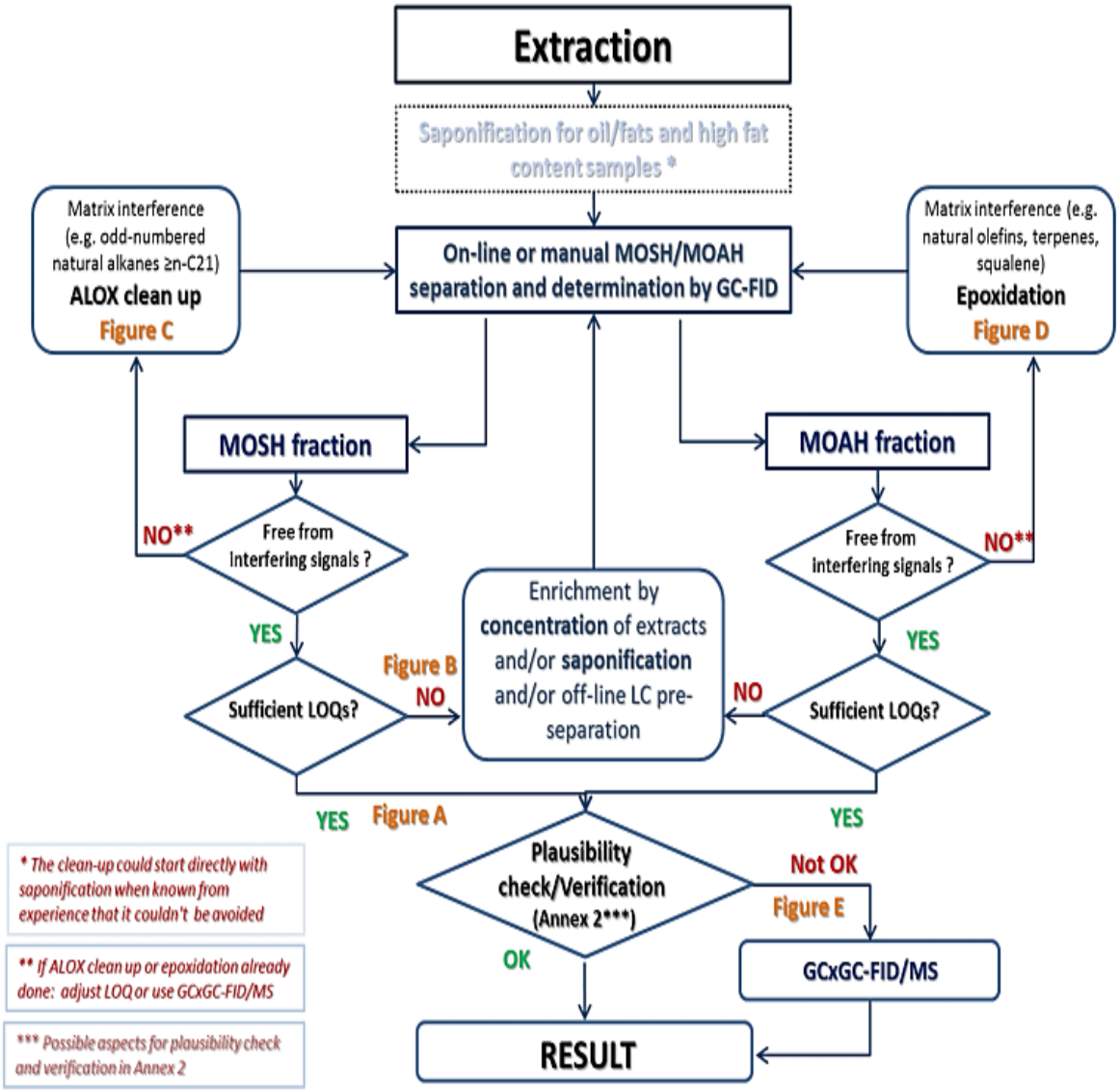

Figure 7: Decision tree suggested from JRC for sample preparation and auxiliary methods ([JRC] , 2019).

1.7.4.2. BfR Compendium

The BfR Compendium is a document published by the German Federal Institute of Risk Assessment (BfR) and the Kantonales Labor Zürich (KLZH). Its aim is the development and harmonisation of analysis methods for the determination of MOH in food and food contact materials. The document comprises 103 pages and is published only in German language. It mainly describes the HPLC-GC-FID online coupling, the measurements principal and interpretation and integration of the resulting chromatograms, thereby giving a lot of instructive examples. Furthermore, sample preparation steps are described, focusing on the extraction of MOH from different food matrices and with reference to further clean-up steps, which may be needed ([BfR] German Federal Institute for Risk Assessment, 2012).

The food is divided into different categories, depending on their properties, similar to the JRC guide. It is important that the fat content of the extract remains below 400 mg mL^{-1} to prevent damage of the HPLC-GC-FID system (20 mg fat capacity of a 5 µm × 250 mm × 2.1 mm silica gel column; see (Biedermann, et al., 2012a)). Described sample preparation methods for the respective matrices are:

- Dry food like pasta, rice or cereals are finely milled, 20 g of sample weight in and extracted with 20 mL of hexane overnight. Afterwards, the supernatant can be injected directly or after enrichment using solvent evaporation.
- Of edible oils and fats 200 mg are weight into an autosampler vial and dissolved in 300 µL of hexane. The extract is analysed as such.
- Any kind of food containing water is immersed in ethanol first, to supress the water by ethanol. The resulting extract is decanted and the sample extracted additionally with hexane overnight. Both extracts are combined afterwards and water is added to separate the aqueous (water + ethanol) from the organic (hexane) phase. The aqueous phase is discarded and the organic one analysed using the HPLC-GC-FID.
- Milk and milk products need to be digested using hydrochloric acid. 5 mL of acid are added to 10 g of milk and the sample digested at 80°C for up to 60 min. The resulting solution is extracted with 10 mL of pentane or hexane twice, the solvent evaporated to dryness and the remaining residue of fat weight. Afterwards, 200 mg of the fat residue are used for the analysis, as described for edible oils and fats.
- Milk powder or infant formulas can be analysed for the surface contamination or their total MOH content. The samples can be extracted with hexane overnight to determine the surface content (e.g. contamination by migration from packaging). For the total content the samples need to be digested with hydrochloric acid, as described for milk and milk products. As compromise, the samples can be extracted with hexane at 60°C overnight to reach about a fourth of the total content.
- Confectioneries and sweets can either be analysed for their surface contamination or for their total MOH content, as well. For the surface contamination, they are extracted as such with hexane for 1h and the supernatant analysed afterwards. For the total content the confectioneries are dissolved in warm water, the resulting solution extracted with hexane and the hexane phase analysed afterwards.
- Chocolate is analysed in a way similar to the confectioneries: 10 g of chocolate are deposited in pieces in 20 mL hexane. The fat dissolves, sugar and cocoa powder precipitate. The supernatant is analysed.
- Food contact materials and articles made from paper or board are cut into pieces, 1 g weight into a glass vial and extracted with 10 mL of an ethanol/hexane 1:1 (v/v) solution for 2h at ambient temperature. To 5 mL of the extract 10 mL of water are added afterwards to remove the ethanol and the resulting hexane phase analysed.
- Plastic materials are cut and 200-300 mg extracted with 10 mL of hexane at ambient temperature overnight.

- Of mineral oil products a 10 g L^{-1} stock solution in hexane is prepared. 10 µL of the stock solution are diluted with 10 mL hexane and the dilution analysed on the HPLC-GC-FID.

Additional clean-up and enrichment steps may be needed to reach the proposed LOD of 0.1 mg kg^{-1}. To remove naturally occurring, long chain *n*-alkanes activated Al_2O_3 is used. For 1 g of edible oil or fat a column with 20 g Al_2O_3 and 6 g of silica gel should be appropriate. The capacity of Al_2O_3 to retain alkanes is small; about 10 g are needed to retain 1 mg of alkanes. The MOAH fraction may need epoxidation and an additional enrichment using a silica gel column with 12 g of silica gel for 1 g of sample.

In summary, the compendium ([BfR] German Federal Institute for Risk Assessment, 2012) gave a good overview on the sample preparation and extraction steps that may be needed for the individual food categories. However, as can be seen by the on-going discussions, a general overview is not sufficient to homogenise sample preparation methods and giving reliable data. Therefore, standardised methods should be developed, as was done in 2017 for vegetable oils and foodstuff on basis of vegetable oils ([CEN] European Committee For Standardization , 2017).

1.7.4.3. EN 16995 - Vegetable Oils and Foodstuff on Basis of Vegetable Oils

The only standardised method available so far is the EN-16995 on vegetable oils and foodstuffs on basis of vegetable oils ([CEN] European Committee For Standardization , 2017). The methods principal should only be explained shorty, without giving details here, since a discussion will be made later, in the experimental and results section of this work:
The method starts with weighing 300 mg of a liquid or solid fat, which soluble in *n*-hexane, into an auto sampler vial, with addition of internal standard and 600µL of *n*-hexane. An additional distribution with ethanol needs to be done for fatty food matrices containing water to repress the water and make the mineral oils extractable with hexane. For solids non-soluble in *n*-hexane, a dichloromethane/hexane solution should be used and the dissolved sample cleaned on a silica gel column. The analysis of the resulting extracts should be done using HPLC-GC-FID. If the analysis shows strong interferences in the MOSH chromatogram by natural, odd numbered *n*-alkanes in the range of C_{23}-C_{33}, an additional clean-up with aluminium oxide needs to be done (300 mg sample transferred to a 10 g Al_2O_3 and 3 g silica gel column, eluted with 25 mL hexane). If the analysis of this sample on the LC-GC-FID shows strong interferences in the MOAH chromatogram by natural olefins, such as squalene or terpenes, an additional epoxidation step is necessary (300 mg of sample epoxidized with 1 mL of a 100 mg mL^{-1} *m*-CPBA in dichloromethane).
Criticism arose shortly after publication of the method, since the method has been proven to be suitable only for MOSH and MOAH concentrations above 10 mg kg^{-1}. This was five times higher than the MOSH limit of 2 mg kg^{-1} discussed in the mineral oil regulation draft and even 20 time higher than the MOAH limit ([BMEL] Federal Ministry of Food and Agriculture , 2014)

([BMEL] Federal Ministry of Food and Agriculture, 2017). Compared to the JRC guideline published in 2019, where the target LOD to be reached is 0.5 mg kg^{-1} in fats and oils, the EN method is also 20 times too less sensitive ([JRC] , 2019). Therefore, the method is hardly used in the published form.

1.7.4.4. Draft of DGF Method

The German Society for Lipid Science (Deutsche Gesellschaft für Fettwissenschaft; DGF) made several attempts towards a standardised sample preparation and analysis method for edible fats and oils. A group of in total eleven individual laboratories (private and official control laboratories from Germany and Austria) participated in the so called "Think Tank – MOSH/MOAH". The project started with an inter-laboratory survey to test the state of the art in 2017. In further meetings and surveys the individual laboratories methods were aligned, with the aim to develop a comprehensive validated analysis procedure with a LOQ in the needed range of 0.5 mg kg^{-1}. As a first step, the participating laboratories concluded that a saponification step is crucial to get a reasonable low LOQ. Second, the sample amount needed to achieve the required sensitivity was settled as 3 g fat or oil. Proceeding from these general arrangements, the EN-16995 and the substantial know-how of the participating labs, the method was developed step by step, tested, validated and further adjusted through several trials. The final method should be published as a standardised DGF-Method, as soon as it is available (April/May 2020). The use of the drafted method for other food matrices seemed promising and it was tested and applied on various food types within this work (although not validated so far). A more detailed description and discussion is given in the corresponding chapters 2.5 and 3.4.

1.8. Analysis Methods – Theoretical Background

As was already seen and discussed in the first chapters of this work, the possible contaminants in food and food contact materials are versatile (see e.g. (Castle, et al., 1993) (Biedermann, et al., 2013b) ([BfR] German Federal Institute for Risk Assessments, 2016). Therefore, different analytical approaches are needed to comprehensively characterize the substances present in food contact materials, the migration into food and the food itself to generate the needed information of packaging related food safety aspects. The principals of the instrumentations used, including certain advantages and disadvantages, will be discussed in this chapter.

1.8.1. Gas Chromatography with Flame Ionisation Detection (GC-FID)

In this work, as a first attempt the overall migration of the volatile substances from a food contact material into a food simulant was determined using GC-FID. The FID is a universal detector and responds with similar sensitivity to a wide range of organic chemicals (Grob and Barry 2004). This means that quantification of the present substances can be done by simply integrating across the detected peaks, without the need for the calculation of a response factor.

The disadvantage of the FID is the fact that it is difficult or impossible to identify unknowns using an untargeted approach. One possibility is the determination of retention indices (Kovats, 1958), but the approach is impractical for e.g. MOH, which form humps of unresolved substances.

1.8.2. Gas Chromatography with Mass Spectrometry (GC-MS)

Since the FID, which is used for the determination of the overall migration, does not give detailed information, especially regarding the identification of unknowns, qualitative information is generated using a MS detector. The principal of a MS detector is the splitting of a substance into ion fragments, separation of the fragments regarding their mass to charge (m/z) ratio and detection of the fragments. Various methods are available, differentiated by e.g. the generation of ions or the principal of detection (Gross, 2011).
In this work, electron ionisation (EI) was used for ion generation. The molecules in gas phase are ionised by electrons with high kinetic energy. Mostly 70 electron Volts (eV) are used, having the big advantage that the generated ions are highly reproducible and generation and comparison with databases is possible (Gross, 2011).
Besides the ionisation, the used mass filter is a determining factor of the MS analysis. Mass filters used in this work were the linear quadrupole (qMS) and a time-of-flight (TOF) mass filter. The differences, advantages and disadvantages will be described in detail later in this work (chapter 1.8.4.2 and 1.8.4.3).
The use of a MS detector allows the identification of unknown substances in an untargeted approach (especially when done with EI). This methodology allowed to obtain qualitative information about the constituents of the sample, but also to quantify substances with a SML by internal standard approach.

1.8.3. Online-Coupled High Pressure Liquid Chromatography - Gas Chromatography with Flame Ionisation Detection (HPLC-GC-FID)

Analysis of MOH is done using an online-coupling of HPLC-GC-FID in routine attempts. Biedermann and Grob developed this method and published a comprehensive review on it (Biedermann, et al., 2012a) (Biedermann, et al., 2012b).
In gas chromatographic analysis, MOSH and MOAH form irregular humps of unresolved substances. Therefore, the method of choice is on-line coupled HPLC-GC-FID, which combines and uses the advantages and principals of the single techniques. HPLC provides the needed separation efficiency to separate MOSH and MOAH, allowing on-line control of the MOSH and MOAH cuts via UV-detection. Using HPLC saves resources in terms of solvent, material and time consumption, when compared to manual LC sample preparation methods (like SPE). On-line coupling to GC results in a fully automatized closed system. A high-volume injection of the entire LC fractions is possible. This gains sensitivity, thereby ruling out

contamination or losses of samples during pre-separation. Furthermore, GC allows the identification of interferences in the two fractions, such as POSH in MOSH or natural olefins in MOAH. FID is the detector of choice, because it is an universal detector, providing virtually the same response per mass for components of similar structure, as was already discussed in the GC-FID chapter (1.8.1).

As already mentioned, high volume injection of the entire LC fractions (450µL) into the GC is done to gain the sensitivity needed. The solvent is evaporated there, using two effects: the solvent trapping and the retention gap effect (Grob, 1983). Solvent trapping is the trapping of volatiles in a condensed film. By injecting liquids into a capillary column plugs or lenses are formed, which are pushed through the column by the carrier gas leaving behind an irregular film on the column wall. This film has a high retention power and traps the sample components. Evaporation of the solvent film happens, when the carrier gas is saturated continually with solvent vapours. High boiling compounds remain stacked on the column wall, while volatile substances are evaporated and re-condensed in the remaining film. This effect continues until all of the solvent is evaporated. The volatiles are released at the moment, in which the last solvent is vaporized, forming a sharp band (Grob, 1982). However, the higher boiling compounds are retained on the column forming long initial bands, which would result into peak broadening. These bands can be shortened by using the second effect – the retention gap effect. As the name implicates, a pre-column is needed as retention gap. The pre-column is an inert uncoated tube with no or only low retention power. High carrier gas pressure (about 150 kPa) is used to transfer the high boiling substances through the retention gap at an oven temperature below the chromatography temperature (in theory at least 100°C below). The components are re-concentrated at the beginning of the analysis column, because of its high retention power, until the start of the oven program and the raising of the temperature (Grob, et al., 1985). Wetting of the retention gap has to be possible with the used solvent to form a film. To enable the high gas flow rates needed, a column with 0.53 mm inner diameter is chosen (Biedermann, et al., 2012a). The injected solvent needs to be evaporated to prevent flushing of the analysis column. Evaporation is done through so-called solvent vapour exits (SVE) installed between the pre-column and the analysis column. Transfer of the fractions from LC to GC is done by a Y-interface. A fused silica capillary acts as transfer line and feds the eluent from LC into a Y piece. To the other two legs of the Y-piece, the carrier gas and the pre-column are joined. The Y-piece is placed outside the GC oven; this means the pre-column is passed through an unused detector base block to the roof of the oven. The SVEs are opened during injection to allow the evaporation of the solvent. They are closed, when the oven program starts. After the transfer, the transfer line is back-flushed with carrier gas through restriction capillaries placed at the transfer valves (Biedermann, et al., 2009).

1.8.3.1. HPLC-Pre-Separation

The HPLC separates MOSH and MOAH and isolates interferences of the sample matrix. On the normal phase silica gel column, high molecular mass paraffins elute first, followed by low molecular mass paraffin’s due to the size exclusion effect. The napthenes are partially separated

from the paraffin's, with polycyclic naphthenes eluting last in MOSH. The MOAH starts with highly alkylated single aromatic compounds, where the aromatic ring is sterically shielded and retention therefore still low. The lower alkylation gets and the higher the number of aromatic rings the later MOAH substances elute. Closely after the MOAH fraction, wax esters elute, which consist of long chain fatty acids esterified with long chain fatty alcohols. The elution is controlled by a set of internal standards. In the MOSH fraction the linear paraffin *n*-tridecane (C_{13}) elutes first, followed by n-undecane (C_{11}) and the naphthenic substance cyclohexylcyclohexane (CyCy). Cholestane (Cho), a polycyclic compound ends the MOSH, while tri-tert-butylbenzene (TBB), a single aromatic compound which is protected by three tert-butyl groups, starts the MOAH. TBB is followed by *n*-pentyl benzene (5B) and the pair of 1-, and 2-methylnahphthalene. The end of MOAH is marked by perylene, a compound consisting of 5 aromatic rings without any shielding. Cho needs to be present in MOSH completely and absent in the MOAH, whereas vice versa for TBB. Full presence of perylene in the MOAH fraction can be controlled with UV-detection. As packing material silica gel with small pores is used to achieve high retention power. To keep the retention power the hexane used must be free of polar impurities. To elute the MOAH a gradient with a more polar solvent is used, with dichloromethane as solvent of choice. The capacity to retain lipids is about 20 mg for a 25 cm × 2 mm i.d. HPLC column with gradient elution with up to 30% dichloromethane. The column needs to be back flushed with dichloromethane afterwards, to remove the lipids and to protect the GC system from be flooded by triglycerides (Biedermann, et al., 2012a).
In a conventional online coupling of LC-GC-FID, the flow rate used is 0.5 mL min^{-1}. The gradient is optimised to elute the MOSH and the MOAH fraction separated within 90 sec each. This results into a fraction volume of 450 µL, being transferred to the GC. As an alternative, it is possible to fractionate the extract in an offline attempt and use it either further for analysis on GC-FID or e.g. multidimensional analysis of the pre-separated fractions (Biedermann, et al., 2012a).

1.8.3.2. GC-System

The separation column has a dimethyl polysiloxane stationary phase to achieve adequate separation of the standards. The requirements to the column are a flat baseline (low column bleeding), also at the used high temperatures of about 400°C, and the separation of MOSH and MOAH from interferences. The film thickness is a compromise between the column bleed (thin film, lower bleed), the baseline drift (horizontal baseline needed over the range of integration), column capacity and assurance of a sufficient retention gap effect (thicker films gave higher capacity and a more robust film). Delayed elution of high mass compounds should not be a problem, because of the late elution of high mass compounds after C_{35} (Biedermann, et al., 2012a).
To gain sensitivity, which means the maximum height of humps, a rapid temperature program is used (20-25°C min^{-1}). To keep the column bleed low, to avoid peak broadening and to achieve the retention gap effect, the carrier gas flow must be high to bring the analytes as fast as possible through the columns. For a program of 20-25°C min^{-1} and a 10 m × 0.25 mm id analysis

column an inlet pressure of 60 kPa has to be achieved. Hydrogen is preferable over other carrier gases, because of the good separation efficiency at high flow rates, according to Van Deemter equation (Biedermann, et al., 2012a) .

1.8.3.3. Measurement Uncertainty, Limit of Quantification and Auxiliary Methods

The measurement uncertainty consists of three parts: extraction, HPLC-GC analysis and integration/interpretation of chromatogram. The analysis part is considered to be small and not relevant for the final results. The influence during extraction depends on the completeness of the extraction and possible contaminations. To gain sensitivity and lower the limit of quantitation especially in terms of high fat content, auxiliary methods are needed. The used methods and their influence on the results already had been further described in the chapter on sample preparation (1.7.4). Besides, interpretation and integration are the predominant parts of uncertainty and often result in uncertainties of about 20% (Biedermann, et al., 2012a).

1.8.4. Comprehensive 2D-GC×GC

1.8.4.1. General Aspects

Two-dimensional comprehensive GC×GC-MS, in short comprehensive GC-MS or GC×GC-MS, is a method used, when a complex mixture of analytes has not been separated efficiently in one-dimensional GC-MS (Shimadzu Corporation, 2019). The comprehensive GC-MS uses two columns with different selectivities to separate the analytes efficiently. The name comprehensive refers to a method being able to transfer and separate the entire sample eluting from a primary column on a secondary column, rather than separating just a specific fraction as in “heart-cut” multidimensional GC. The components separated in the first dimension must remain separated in the second one (Liu, et al., 1991) (Cortes, 1992).

The comprehensive GC×GC is build-up of an injection port, a primary column, a resampling device (modulator), the secondary column and the detector. The two columns can be placed in the same or in different ovens resulting in the same or in different temperature programs (Tranchida, et al., 2009). The first column is a conventional, usually nonpolar GC-column and separates the analytes by their boiling point or rather volatility. The second column has opposite polarity to the first, is much shorter and uses the polarity of the analytes to separate them. These actually results into three dimensions: the first column gives the separation through boiling point, the second column the separation through polarity and the detector gives the mass spectrum or intensity as the third dimension (Mondello, 2011 p. 96).

The two columns are connected in series by a modulator. The modulator is the most important unit of the comprehensive GC-MS since it controls the flow from the first column into the second. The analytes leaving the first column are trapped in the modulator for a defined time, before they are re-focused and injected on the second column. The time needed to complete these steps is called modulation time or frequency. The modulation frequency is between two and ten seconds long. In this time, the analytes coming from the first column are trapped,

focused and re-injected on the second column. This means that separation on the second column has to be fulfilled between two modulations, otherwise there will be a phenomenon called wrap-around leading to peak broadening. Therefore, the dimensions of the second column are usually short; they are between 0.5 m and 1.5 m length (Mondello, 2011).

Modulators are divided into two main groups – valve based (flow modulators) and thermal modulators. The thermal modulators are divided into cryogenic modulators and heat-based modulators. Many different modulators had been developed in the past, each one having its own characteristics, advantages and disadvantages (Shimdazu Corporation, 2014). However, they should not be discussed any further at this point.

The detector of a GC×GC instrument has several requirements needed, the most important one probably being the fast acquisition rate needed to be able to detect the peaks separated from each other. Considering that the peak width after 2D-separation is about 40-300 ms and that at least 10 data points per peak should be detected for quantification, an acquisition rate of about 250 Hz is needed (250 spectra s^{-1}). The acquisition rate mainly depends on the detectors shape and the used gas flows, the response mechanism, the process of responding and the processing of the signal. Besides, the detector needs a small internal volume to prevent peak broadening, but also the mass spectrometric resolution and accuracy are important (the two latter ones mainly for qualitative tasks). The first detector used and still one of the most popular ones is the FID. It is the "working horse", a fast (up to 500 Hz), universal detector, with a small internal volume and a large linearity range. However, it lacks on the structural information (besides the already present one from the 2D-separation). Thus, for qualitative tasks mass spectrometers are the detectors of choice. A TOF-MS is known by its fast acquisition rate of about 500 spectra per second in full scan (newest ones can do even more). The use of deconvolution software to resolve overlapping peaks allows even higher acquisition rates. The disadvantages of a TOF are the higher costs compared to a quadrupole and the resulting huge amounts of data. Therefore, and because of its easier availability, quadrupole mass filters (qMS) are often preferred, especially in routine analysis. The speed of a quadrupole depends on the scan speed, given in amu s^{-1}, the interscan delay (time between the end of one scan and the start of the next), given in ms, and the scanned mass range, given in amu. New quadrupoles can have scan speeds of up to 20 000 amu s^{-1}. If masses are scanned e.g. from 50-450 amu, this would result into 50 full scan spectra per second. As already mentioned, for qualitative tasks the resolution and accuracy are important, where TOF is considered to give the better performance (Mondello, 2012) (Shimdazu Corporation, 2014). The qMS and TOF-MS used in this work are discussed in more detail below.

The result of a two-dimensional comprehensive GC×GC-MS analysis are a series of two-dimensional chromatograms derived from modulation of the one-dimensional separation (Step 1; Figure 8). These series are transformed to a second-dimensional chromatogram with the axis giving the retention time on the first and the second column (Step 2; Figure 8). The second-dimensional chromatogram is transformed into colour, contour or 3D plots that visualize the peak intensity by different colours or shades (Step 3; Figure 8). To visualize peaks with different intensities it is often important to change the contrast settings, when working with visualization

through colour or shading. Specially designed software programs are used to create the two-dimensional image (Dallüge, et al., 2003).

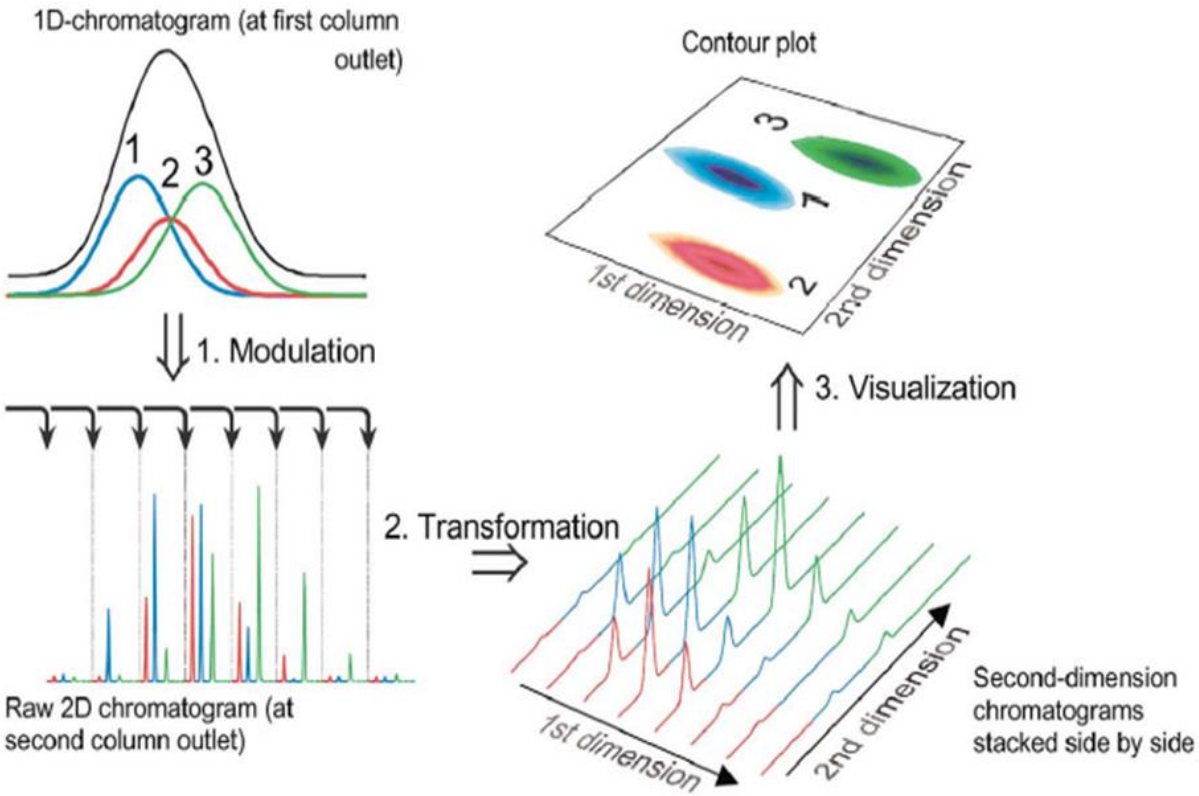

Figure 8: Creating a two-dimensional comprehensive GC×GC-MS image. Source of image (Dallüge, et al., 2003)

The advantages of comprehensive GC×GC are considered to be excellent selectivity, high sensitivity, enhanced separation power, speed and structured chromatograms (Mondello, 2011 p. 8).

1.8.4.2. Comprehensive 2D-GC×GC-qMS

The first comprehensive 2D-GC×GC instrument used in this work was one with a quadrupole mass analyser (qMS). The quadrupole consists of four rods positioned parallel in a radial array. Using the ramping of direct-current and radio-frequency voltages the ions are sorted by their mass to charge ratio while passing the rods (always just one m/z at a time). Therefore, achieving really fast acquisition rates is limited by the physical laws (Mondello, 2011 p. 176).

The modulator used was a cryogenic two-stage loop modulator. It traps the analytes in a modulation loop, by using a continuous cold-jet, creating two cold spots in the loop having a temperature below that of the GC oven (Figure 9). The first cold spot traps the analytes from the first dimension, a short hot-jet injects the analytes into the loop and another cold-jet refocuses them. This modulation cycle prevents interferences by analytes eluting from the first column during reinjection and peak broadening in the second dimension. The cold-jet was produced by heat exchange using liquid nitrogen. The disadvantages of loop modulators are the

handling; the loop has to be in exactly the right shape, size and place for the modulation to be efficient (Mondello, 2011) (Shimdazu Corporation, 2014) (ZOEX Corporation, 2013).

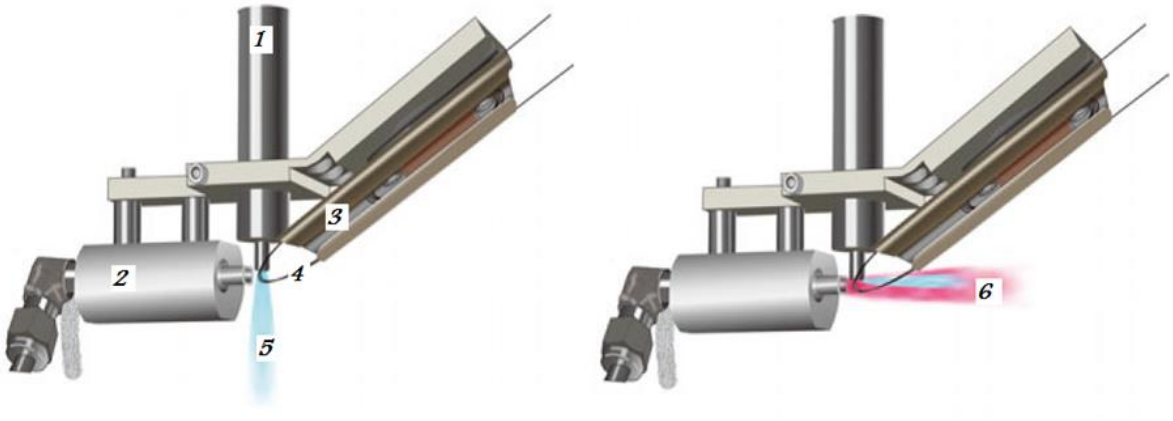

Figure 9: two-stage loop modulator showing (1) cold-jet assembly, (2) hot jet assembly, (3) column holder, (4) modulation loop, (5) cold-jet, (6) hot-jet; source of image (Shimdazu Corporation, 2014).

1.8.4.3. Comprehensive 2D-GC×GC-TOF-MS

A time-of-flight mass analyser (TOF) accelerates the ions into a field-free flight tube using an electric field. In this tube the ions are separated by their mass to charge ratio by the time needed to pass the tube, using the physical principal that their velocity is directly coupled to their mass (Gross, 2011).

The advantages of the TOF are the very fast data acquisition (travel time in µs range), unlimited mass range, the excellent mass resolution and its accuracy. A complete spectrum is produced from every ion pulse reaching the detector, producing very sensitive full scan spectra. Generation of the ions using EI gives high speed and mass accuracy, still allowing data base searches of the spectra. This has the big advantage that the whole mass information is recorded throughout the analysis run, enabling a qualitative and a quantitative analysis within one run, resulting in a nearly unlimited number of peaks without the need of an additional targeted analysis. According to (Koch, et al., 2019), GC×GC-TOF is the most powerful tool for compound identification. The nontargeted deconvolution (NTD®) ensures the separation of co-eluting peaks and easier differentiation between matrix and analytes (Leco Corporation, 2019).

The modulator used in this work for the GC×GC-TOF measurements was a two-stage quad-jet thermal modulator. The difference to the loop modulator is that modulation is done on-column, rather than in a loop. Handling is therefore easier, since the loop has to be created and placed exactly in right position. Furthermore, it is possible to control the jets separated from each other, allowing e.g. short hot-jet pulses for volatile substances in the begin of a run and longer hot-jet pulses for high boiling substances at the end of a run (LECO Corporation, 2019).

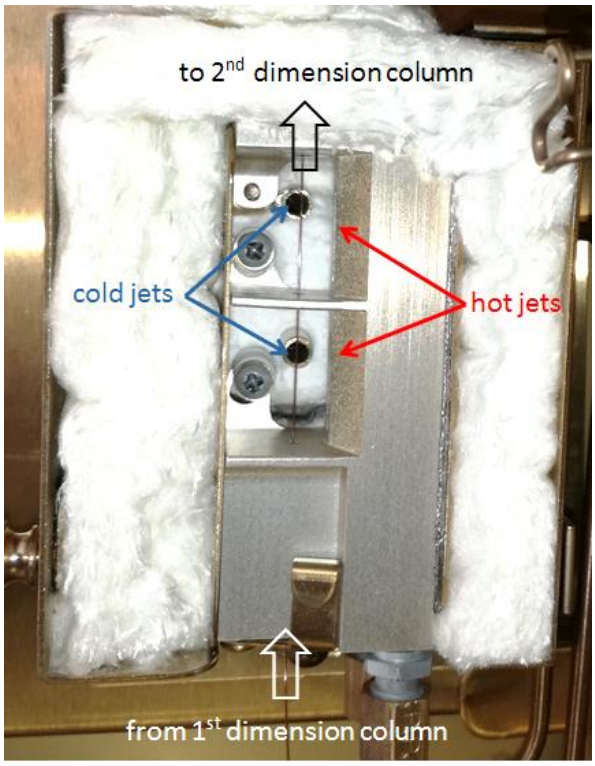

Figure 10: Two stage quad-jet modulator of Pegasus® BT 4D-GC×GC-TOF mass spectrometer.

2. Experimental Part

2.1. General Experimental Aspects

2.1.1. Materials

Extractions were performed in 10-60 mL glass vials with screw cap equipped with silicone/PTFE septa (N22; 3.2 mm; Macherey-Nagel GmbH&Co. KG, Düren, Germany). Measurements were done in 1.5 mL glass vials with screw cap equipped with silicone/PTFE septa (N9; with hole; 1 mm; Macherey-Nagel GmbH&Co. KG, Düren, Germany) and micro inserts (15 mm tip; for wide opening; 0.2 mL/ 6×31 mm; clear; Macherey-Nagel GmbH&Co. KG, Düren, Germany). Filter papers were of 150 mm diameter, MN 615 ¼ (Macherey-Nagel GmbH&Co. KG, Düren, Germany) and were washed with *n*-hexane and stored at 100°C over night in an oven to reduce their MOSH/MOAH contamination, which was derived from their recycled board packaging. Syringe filters were PTFE-45/13 CHROMAFIL^{R} Xtra (Macherey-Nagel GmbH&Co. KG, Düren, Germany). Pasteur Pipettes were from Fisherbrand, made of glass, unplugged and of 150 mm length from Fisher Scientific GmbH, Schwerte, Germany. Micropipettes of different volumes were used (1 µL-200 µL) for e.g. standards dilution. They were supplied from BLAUBRAND®, intraMARK, DE-M, CE, BRAND GMBH + CO KG, Wertheim, Germany. The lab oven used for drying and baking out glass ware was a Heraeus T 6060 (Heraeus Instruments, Thermo Scientific, Vienna, Austria). The climate oven used for migration and permeation experiments was an APT.line® KBF-ICH climatic chamber for constant conditions with program control and with ICH compliant illumination in the doors from Binder GmbH, Tuttlingen, Germany. The automatic solvent evaporator used was a TurboVap® II (Biotage, Uppsala, Sweden) operated at 40°C water bath temperature and a nitrogen stream of 1-10 bar, depending on the solvent to be evaporated. Evaporation tubes with a total volume of 50 mL and a 0.5 mL end-point from the same supplier were used. The silicone coated paper used for the taped barrier test was from BGB Analytik AG (Boeckten, Switzerland) and referred to as "Div-Paper-PDMS-PK10; A4 Cellulose Paper Sheet, PDMS coated; for use in taped barrier test". The microwave used was a microwave extraction reactor "Monowave EXTRA" from Anton Paar GmbH (Graz, Austria). It was equipped with a "MAS24" autosampler for 24 vials of 30 mL each. The vials were made of glass and equipped with PEEK caps with silicone/PTFE septa.

2.1.2. Chemicals and Reagents

Tenax® a poly 2,6-diphenyl-p-phenylene oxide (Tenax TA (refined), 60–80 mesh; SUPELCO, Bellefonte, USA) was used as a simulant for dry, non-fatty food. It was applied to the samples in an amount of 4 g dm^{-2}. Silica Gel 60, extra pure for column chromatography with particle size from 60 µm to 200 µm (70 mesh to 230 mesh) was purchased from Merck KGaA (Darmstadt, Germany). It was activated at 400°C for 16h and stored in a glass bottle to prevent

contaminations. Aluminium oxide 90, alkaline, for column chromatography 0.063 mm to 0.2 mm was purchased from the same supplier. It was activated at 500°C for 16h and also stored in a glass bottles to prevent contaminations. 3-Chloroperbenzoic acid (≤77%) was purchased from SIGMA-ALDRICH Co. (St.Louis, USA) and used without further clean-up. Potassium hydroxide pellets (GPR RECTAPUR, 85.7%), anhydrous sodium sulphate (AnalaR NORMAPUR; 99.9%) and hydrochloric acid 37% (AnalaR NORMAPUR; Reag. Ph. Eur.) were purchased at VWR Internationale (Radnor, Pennsylvania, USA). Sodium thiosulfate anhydrous (EMPLURA®; ≥97.0%) and sodium carbonate anhydrous (pro analysi; 99.9%) were purchased at Merck KGaA (Darmstadt, Germany).

2.1.3. Solvents

All solvents used were purchased in glass bottles: acetone (ROTISOLV® ≥99.9 %, UV/IR-Grade; Carl Roth GmbH + Co. KG, Karlsruhe, Germany), ethanol (abs. 100%, HPLC grade; CHEM-LAB NV, Zedelgem, Belgium), dichloromethane (ROTISOLV® ≥99.9 %, GC Ultra Grade; Carl Roth GmbH + Co. KG, Karlsruhe, Germany), *n*-hexane (Picograde® for Residue Analysis; LGC Promochem GmbH; Wesel, Germany), methanol (HiPerSolv CHROMANORM for HPLC; LC-MS Grade; VWR Internationale; Radnor, Pennsylvania, USA) and tert-butyl methyl ether (MTBE, for HPLC, ≥99.8%, SIGMA-ALDRICH Co., St.Louis, USA).

2.1.4. Standards

2.1.4.1. MOSH/MOAH Internal Standard Mix

The standard was purchased by Restek Corporation (Bellefonte, US) and contained the following substances in 1 mL ampules in toluene: *n*-undecane (300 µg mL^{-1}), *n*-tridecane (150 µg mL^{-1}), cyclohexylcyclohexane (300 µg mL^{-1}), cholestane (5-alpha-cholestane; 600 µg mL^{-1}), 1-methylnaphthalene (300 µg mL^{-1}), 2-methylnaphthalene (300 µg mL^{-1}), *n*-pentylbenzene (300 µg mL^{-1}), perylene (600 µg mL^{-1}) and 1,3,5-tri-*tert*-butylbenzene (300 µg mL^{-1}). Ten µL were usually added to the samples to reach a final concentration of 1.5–6 µg mL^{-1}.

2.1.4.2. Migration Internal Standard Mix

The internal standard for migration experiments consisted of *n*-dodecane-d_{26} ($C_{12}D_{26}$; EURISOTOP SAS, Saint-Aubin, France), *n*-nonadecane-d_{40} ($C_{19}D_{40}$; 98%; Cambridge Isotope Laboratories, Inc.; Tewksbury, Massachusetts), benzophenone-d_{10} ($C_{13}D_{10}O$; 99atom%; SIGMA-ALDRICH Co., St.Louis, USA), bis(2-ethylhexyl)phthalate-3,4,5,6-d_4 and di-*n*-butylphthalate-3,4,5,6-d_4 (both "analytical standards", purchased from SIGMA-ALDRICH Co., St. Louis, USA), each substances in a concentration of 200 mg L^{-1} in acetone. 25 µL of this stock solution were added before the extraction of the food simulant, resulting in 93.8 µg kg^{-1} food, if the tested area is 0.32 dm^{-2}.

2.1.4.3. Permeation Standard Mix

The permeation standard for two-sided migration experiments consisted of *n*-tetradecane-d_{30} ($C_{14}D_{30}$, 98%; Cambridge Isotope Laboratories, Inc.; Tewksbury, Massachusetts) *n*-hexadecane-d_{16} ($C_{16}D_{34}$, 98%; Cambridge Isotope Laboratories, Inc.; Tewksbury, Massachusetts) *n*-eicosane-d_{42} ($C_{20}D_{42}$, Cambridge Isotope Laboratories, Inc.; Tewksbury, Massachusetts) *n*-tetracosane-d_{50} ($C_{24}D_{50}$, 98%; Cambridge Isotope Laboratories) *n*-octacosane-d_{58} ($C_{28}D_{58}$, CDN Isotopes), l- menthol (99%, SIGMA-ALDRICH Co., St.Louis, USA), eugenol (99%, SIGMA-ALDRICH Co., St.Louis, USA), vanillin (97%, SIGMA-ALDRICH Co., St.Louis, USA) and acetovanillone (≥ 98%; SAFC), each 100 mg L^{-1} in acetone. 100 µL of the standard were spiked in the bottom of the migration cell, resulting for complete permeation into the food simulant in 187.5 µg kg^{-1}, if the tested area is 0.32 dm^{-2}

2.1.4.4. n-Alkanes

For calculation of retention indices and determination of cutting fractions in HPLC-GC-FID a "C_7-C_{40} Saturated Alkanes Standard" from Supelco (Bellefonte, USA) was used. It contained each *n*-alkane between C_7 and C_{40} in a concentration of 1000 mg L^{-1} in hexane. It was further diluted using *n*-hexane to a working solution of 10 mg L^{-1} for GC-FID measurements and to 1 mg L^{-1} for HPLC-GC-FID or GC-MS measurements. Since the integration range was extended up to C_{50} by the JRC Guideline ([JRC] , 2019), an additional standard was purchased, covering the range from C_{12} to C_{60}. The standard purchased at Supelco (Bellefonte, USA) was the ASTM® D5442 Quantitative Linearity Standard containing *n*-C_{12}, *n*-C_{14}, *n*-C_{16}, *n*-C_{18}, *n*-C_{20}, *n*-C_{22}, *n*-C_{24}, *n*-C_{26}, C_{28}, *n*-C_{30}, C_{32}, *n*-C_{36}, *n*-C_{40}, *n*-C_{44}, *n*-C_{50} and *n*-C_{60}, each in a concentration of 100 mg L^{-1} in cyclohexane. It was further diluted using *n*-hexane to a working solution of 1 mg L^{-1} and used only for HPLC-GC-FID measurements.

2.1.5. Instrumentation

2.1.5.1. Head Space-Solid Phase Micro Extraction (HS-SPME) - GC-MS

Sample enrichment was done using a 50 µm/30 µm divinylbenzene/Carboxen® on polydimethylsiloxane (PDMS) on a 2 cm StableFlex^{TM} fibre at 70°C for 30 min. The GC was an Agilent 7890 A series gas chromatograph equipped with a PAL CTC Analytics autosampler and a mass spectrometer, 5975 C VLMSD with Triple Axis Detection. The oven was equipped with a Zebron 5MSplus column (30 m × 0.25 mm × 0.25 µm) and the temperature programmed from -10°C (1 min) to 310°C (1 min) with 8°C min^{-1}. Carrier gas was helium, total flow 20.4 mL min^{-1} and a constant velocity of 31.5 cm sec^{-1}, septum purge flow 3 mL min^{-1}. Injection temperature was 270°C, gas saver and split was opened after 1 min with a flow of 15 mL min^{-1}. Detection was done using a quadrupole mass filter scanned from m/z 20 to m/z 300, detector voltage was relative to tune. Ions were generated using 70 eV. Data evaluation was done using "MSD ChemStation Data Analysis Application" version G1701EA- Revision E.02.01.

2.1.5.2. GC-FID

Separation was done using a Hewlett Packard 6890 Series GC system equipped with an Optima delta-6 capillary column (7.5 m × 100 µm × 0.10 µm, Macherey-Nagel, Germany). The oven was programmed to 60°C (hold 1 min) and raised at 15°C min^{-1} to 300°C (3 min). Carrier gas was hydrogen with constant flow and a linear velocity of 48 cm sec^{-1}. Aliquots of one microliter were injected with a split of 1:20; injection port temperature was set to 280°C. The detector temperature was set to 320°C, air flow was 450 mL min^{-1}, hydrogen flow 40 mL min^{-1} and make up gas was nitrogen with a flow of 25 mL min^{-1}. Data evaluation was done using "GC ChemStation" version B.04.03[16].

2.1.5.3. GC-MS in Scan-Mode

To determine the specific migration of individual substances a GC with MS detection was used. Gas chromatographic separation was done using a Shimadzu GC2010 system equipped with an Rxi-5Sil MS capillary column (30 m × 250 µm; 0.25 µm). Helium was used as carrier gas in the linear velocity flow control mode with a constant flow of 40 cm sec^{-1}. A high-pressure splitless injection with 250 kPa for 0.8 min and column flow of 4.74 mL min^{-1} was used, gas saver was on in a ratio of 1:5 after 2 min. Aliquots of one microliter were injected using an injection temperature of 270°C, sampling time 1 min. Initial oven temperature was 70°C (1min) and was raised at 6°C min^{-1} to 340°C (5 min). Detection was done with GCMS-QP2010 PLUS mass selective detector. Ions were generated with electron ionisation (70 eV), detector voltage was relative to tune, mass spectrometer scanned from m/z 35 to 500 with a scan rate of 1666 amu sec^{-1} and an event time of 0.3 sec. The software used was "GC-MS Solution" version 4.42.

2.1.5.4. GC-MS in Single-Ion-Monitoring (SIM)-Mode

To determine the permeation of the surrogate substances in the taped barrier test, the same GC-MS as described in chapter 2.1.5.3 was used. Helium was used as carrier gas in the linear velocity flow control mode with a constant flow of 40 cm sec^{-1}. A high-pressure splitless injection with 250 kPa for 0.8 min and column flow of 4.74 mL min^{-1} was used, gas saver was on in a ratio of 1:5 after 2 min. Aliquots of one microliter were injected using an injection temperature was 270°C and a sampling time 1 min. Initial oven temperature was 70°C (1min) and was raised at 6°C min^{-1} to 340°C (5 min). Detection was done with GCMS-QP2010 PLUS mass selective detector. Ions were generated with electron ionisation (70 eV), detector voltage was relative to tune, mass spectrometer operated in SIM and scan mode in parallel. Scan was done from m/z 35 to 500 with a scan rate of 1666 amu sec^{-1} and an event time of 0.3 sec throughout the run (7.00 min to 50.0 min runtime). Acquisition in SIM mode was done as given in Table 7. The software used was "GC-MS Solution" version 4.42.

The ions used for detecting the deuterated internal standards were m/z 50, 66 and 82 for deuterated n-alkanes, m/z 82, 110 and 192 for deuterated benzophenone, m/z 153, 171 and 283 for the deuterated phthalates. The surrogate substances were detected using m/z 57, 71 and 85

for C_{17}, m/z 77, 105 and 182 for benzophenone, m/z 149, 167 and 279 for bis(2-ethylhexyl) phthalate and m/z 115, 143 and 276 for Sudan Red II.

Table 7: GC-MS measurement parameters of SIM mode

Start Time (min)	End Time (min)	Event Time (sec)	m/z
7.00	10.0	0.3	50, 66, 82
17.0	23.0	0.3	50, 57, 66, 71, 77, 82, 85, 105, 110, 182, 192
30.0	36.0	0:3	115, 143, 149, 153, 167, 171, 279, 283, 276

2.1.5.5. HPLC-GC-FID

The HPLC was a Shimadzu LC 20AD equipped with an Allure Silica 5 µm column (250 × 2.1 mm). Gradient elution was used, started with 100% *n*-hexane (flow 0.3 mL min^{-1}) and raised to 35% dichloromethane within 2 min (hold for 4.20 min). The column was backflushed at 6.30 min with 100% dichloromethane (flow 0.5 mL min^{-1}; hold for 9 min) and reconditioned to 100% *n*-hexane (flow 0.5 mL min^{-1}; hold for 10min). Flow was decreased afterwards to 0.3 mL min^{-1} until next injection. The UV-detector was equipped with a D_2-lamp set a 230 nm and 40°C cell temperature. The program details can be seen in Figure 11.

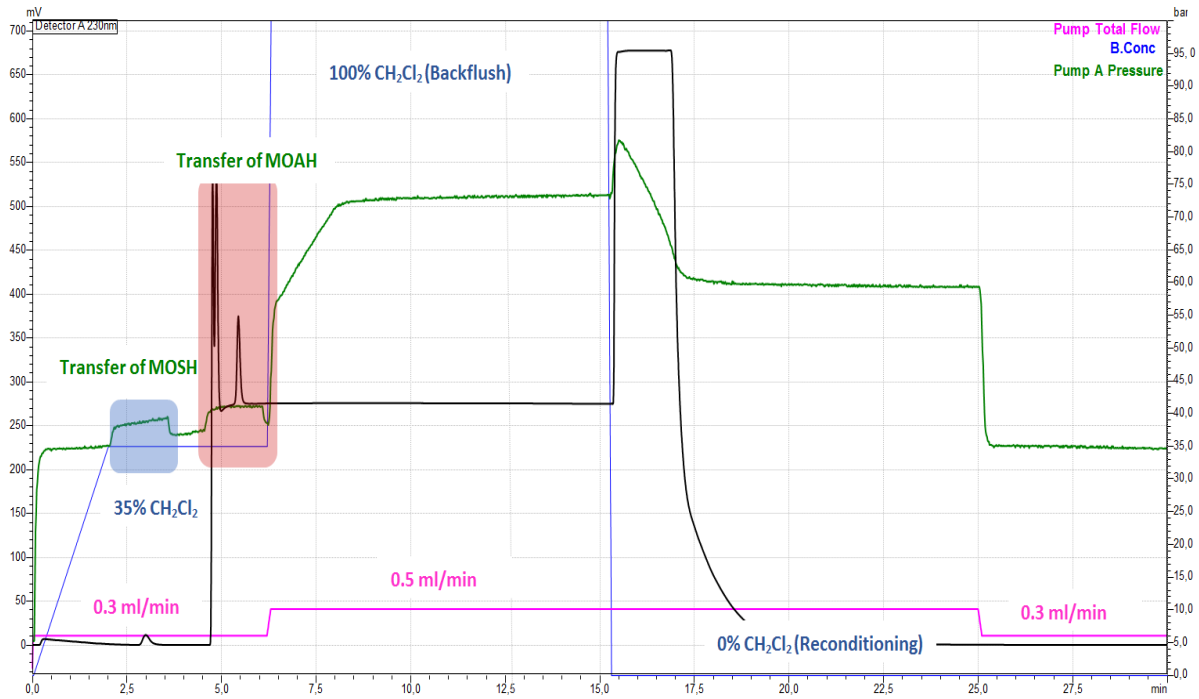

Figure 11: Example of LC chromatogram: pressure line of pump A in green, showing the two transfer windows of MOSH and MOAH, the backflush and reconditioning; UV-detectors trace in black, with the CH_2Cl_2 breakthrough and the typical pattern of the MOAH substances. The flow is indicated and labelled in pink and the pump B concentration in blue.

The GC was a Shimadzu GC 2010 dual-column and dual-FID system, equipped with two guard columns (Restek MXT® Siltek (10 m × 0.53 mm id)) and two analysis columns (Restek MTX®-1 (15 m × 0.25 mm id × 0.1 µm df)). Carrier gas was hydrogen with 150 kPa constant analysis pressure and an evaporation pressure of 87 kPA for MOSH and 85 kPA for MOAH. The oven was programmed to 60°C (hold 6 min), raised at 20°C min^{-1} to 100°C (0 min) and followed by 35°C min^{-1} to 370 °C (9.29 min). The LC-GC interface was controlled by "Chronect-LC-GC" by Axel-Semrau. Data evaluation was done using "LabSolutions" of Shimdazu Corporation for LC and GC data in version 5.92.

2.1.5.6. Comprehensive 2D-GC×GC-MS

Due to the formation of unresolved humps, it is often necessary to use more comprehensive techniques to analyse the samples. The comprehensive GC×GC-MS was composed of the following units: an OPTIC-4 Multimode GC Inlet and an AOC- 5000 Plus autosampler, a Shimadzu gas chromatograph for mass spectrometer GC-2010 Plus. The columns were placed in the same oven. The modulator used was a cryogenic modulator (Zoex ZX1 two-stage loop thermal modulator; Zoex Corporation, Houston, Texas, USA). Detection was done using the mass spectrometer GCMS-QP2010 Ultra. Ions were generated with electron ionization (70 eV). The ion source temperature was set at 200°C, the interface temperature at 310°C. The mass spectrometer was scanned with a scan speed of 20.000 amu sec^{-1}, resulting into 33 full scan spectra recorded from m/z 50 to 500. The software used for data evaluation was Version 2.7 of "GC Image" from the Zoex Corporation.

Since the column combinations, the oven program and the modulation used changed several times throughout this work, they are summarised individually below.

- **Conditions A:** The GC was equipped with a HT1 column (30 m × 0.25 mm × 0.25 µm) and a BPX5 column (2.5 m × 0.15 mm × 0.15 µm) placed in the same oven. The columns were connected directly by using a push-fit connector and sealing of the connection with a polyimide resin. Injection was done in splitless mode, with a sampling time of 1 min. The split was opened afterwards, with a split ratio of 30. Injector port temperature was 270°C and a purge flow was 1.0 mL min^{-1}. Helium was used as a carrier gas in the linear velocity flow control mode with a constant flow of 40 cm sec^{-1}. The initial oven temperature of 40°C (1 min) was increased to 80°C (0 min) with a ramp of 10°C min^{-1} and then on to 280°C (5 min) with a ramp of 3°C min^{-1}. Modulation frequency was 5 sec with a hot- jet pulse of 350 ms. The temperature of the hot-jet was programmed at 320°C (32 min), followed by 280°C till the end of the run. The detector voltage was relative to tune. Data were acquired at 8-72 minutes runtime.

  The HS-SPME measurements were done using a 50 µm/30 µm divinylbenzene/Carboxen® on polydimethylsiloxane (PDMS) on a 2 cm StableFlex™ fibre. Enrichment was done at 80°C for 30 min or at 150°C for 30 min.
- **Conditions B:** The column combination of "conditions A" were still used. However, the oven program was changed. The initial oven temperature was 40°C (1 min), was

increased to 120°C (0 min) with a ramp of 15°C min^{-1} and then on to 310°C (2 min) with a ramp of 4°C min^{-1}. Aliquots of one microliter were injected in splitless mode, with a sampling time of 1.5 min. The split was opened afterwards, with a split ratio of 5. Helium was used as carrier gas in the linear velocity flow control mode, with a constant flow of 35 cm sec^{-1}. Modulation frequency was 6 sec with a hot-jet pulse of 350 ms. The temperature of the hot-jet was programmed at 320°C (40 min), followed by 345°C (5 min) and 280°C till the end of the run. The detector voltage was 1.1 kV absolute and data were acquired at 11-53 minutes runtime.

- **Conditions C:** The column combination was changed to a Rxi®-17Sil MS (15 m × 0.25 mm × 0.25 µm) in the first dimension and to a Rxi®-1MS (2 m × 0.15 mm × 0.15 µm) in the second dimension. The initial oven temperature was 60°C (1 min) and was raised to 300°C (1 min) with a ramp of 5°C min^{-1}. Aliquots of one microliter were injected in splitless mode, with a sampling time of 1.5 min. The split was opened afterwards, with a split ratio of 5. Helium was used as a carrier gas in the linear velocity flow control mode, with a constant flow of 35 cm sec^{-1}. Modulation frequency was 10 sec with a hot-jet pulse of 350 ms. The temperature of the hot-jet was programmed at 330°C (35 min), followed by 280°C till the end of the run. The detector voltage was 0.8 kV absolute and data were acquired at 4-48 minutes runtime.

2.1.5.7. Comprehensive 2D-GC×GC-TOF-MS

TOF measurements were done on a Pegasus® BT 4D-GC×GC-Time-of-Flight Mass Spectrometer. The GC×GC was an Agilent 7890 equipped in the first dimension with a Rxi®-17Sil MS (12 m × 0.25 mm × 0.25 µm) and in the second dimension with a Rxi®-1MS (1.91 m × 0.15 mm × 0.15 µm) placed in an extra oven. Of the second dimension 0.45 m were still in 1st oven, 0.10 m in the modulator, 0.31 m were used as transfer line. Therefore, only 1.05 m were really in the 2nd oven. The carrier gas was helium with a constant flow of 1.4 mL min^{-1}. Gas saver was on after 2 min with 20 mL min^{-1}. Septum purge flow was 3 mL min^{-1}, inlet purge was 60 sec with a flow of 50 mL min^{-1}. Total flow was 15.4 mL min^{-1}. Injection was done splitless or with a split of 1:10. The inlet temperature was set to 280ºC. The oven was programmed at 40ºC (1.01 min) and raised with 5ºC min^{-1} to 360ºC (5 min). The secondary oven offset was +5ºC, the modulator offset +15ºC. From the start of the run to 998 sec the modulation frequency was 5 sec, with a 0.9 sec hot-jet and 1.6 sec cold-jet. From 998 sec to the end of run the modulation frequency was also 5 sec, but with 1.6 sec hot-jet and 0.9 sec cold-jet. The transfer line was set at 330ºC, the acquisition delay was 200 sec and the extraction frequency 30 kHz. Acquisition rate was 100 spectra sec^{-1} or 200 spectra sec^{-1} in the mass range of 40-600 amu, resulting into a scan speed of 56 000 amu sec^{-1} and 11 2000 amu sec^{-1}, respectively. The ion source was set to 250°C with an emission current of 1 mA. Ions were generated via electron ionization with an electron energy of 70 eV. The software used for data evaluation was “ChromaTOF” Version 5.40.12.0 for Pegasus® BT from Leco Corporation.

2.2. Method Development and Validation

Method development and validation was described partly in (Walzl, et al., 2019).

2.2.1. Total Extraction

To determine the total amount of migrate-able substances in the raw paper, a total extraction of the paper was performed similar to Biedermann and Grob (2012a).
In brief, the paper was cut into small pieces, 1 g was weighed into a glass vial with a screw cap and 10 µL of a MOSH/MOAH internal standard mix were added, resulting in a concentration of 1.5–6 µg mL^{-1}. Ten mL of hexane/ethanol 1:1 (v:v) were added and the paper was extracted with occasionally shaking at room temperature for 2 h. Afterwards, the extract was washed two times with water and subsequently centrifuged. The hexane phase was collected in a 50 mL Turbovap® evaporation vial and the solvent was evaporated to 0.5 mL using an automatic solvent evaporator. The extract was then transferred into a 1.5 mL glass vial with screw cap, 0.5 mL of fresh hexane used to wash the evaporation vial and the solvent combined with the initial extract to achieve an end volume of 1 mL. All extracts were stored in a refrigerator until analysis, only a small amount of about 100 µL was filled into a 1.5 mL glass vial with a screw cap and a micro inlet for measurements. Those were performed using an online-coupled HPLC-GC-FID as described in the section 2.1.5 Instrumentation.
Correctness of the analysis was proven by an inter-laboratory comparison, but will not be discussed in detail here.

2.2.2. Migration Experiments

2.2.2.1. General Procedure

As discussed in the introduction, different publications and regulations described migration experiments for testing FCMs. Specified therein was that MPPO as food simulant for dry, non-fatty food is the only appropriate food simulant in testing of paper and board FCMs (e.g. ([DIN] Deutsches Institut für Normung eV, 2018-05), ([CEPI] Confederation of European Paper Industries, 2019)). Since this work focuses on paper and board food contact materials, this work followed the recommendations for the use of MPPO as food simulant for dry, non-fatty food.
The used MPPO had to be cleaned prior the use, which was done by placing a stir bar and about 50 g of the material in a 250 mL glass flask with glass stopper and adding a mixture of acetone/hexane 1:1 (v/v). The added volume varied, depending on the amount needed to disperse and extract the MPPO properly. As a rule of thumb, the MPPO should at least be covered by a supernatant of about 2 cm solvent. The flask is then placed on a magnetic stirrer and stirred, with exchange of the solvent from time to time, depending on the purity of the MPPO used. If it is used for the first time, the solvent needs to be replaced about every 15 min at first, since it will turn into a dark brown solution. Later on, it should be replaced at least every time it gets yellow/brownish. The cleaning procedure should continue, till the supernatant is

colourless. After this first cleaning, it was sufficient to stir the MPPO with solvent two times for about 4h and a third time overnight, since there should not be any residues of the samples anyway. In both cases, the MPPO is transferred into a crystallizing dish of about 30 cm diameter afterwards, as much solvent as possible is decanted and the stir bar removed. Drying can be done at room temperature, but can also be accelerated by placing the dish in an oven at about 60°C. The MPPO needs to be shaken regularly to prevent boiling delays and lumping of it. The drying and cleaning process is finished, when there is no smell of solvent left.

In this work, the migration experiments were performed in migration cells (MigraCell® MC 60 contact are ~0.5 dm^2; FABES Forschungs-GmbH, Munich, Germany). To cut the samples into appropriate circles, scissors and as template, the metal plate of the cell were used. The cell was assembled according to the manufacturer's instructions, as will be discussed in detail in the chapter's 2.2.2.1.1-One-Sided-Test: Migration and 2.2.2.1.2-Two-Sided Test: Permeation.
Acceleration of the migration experiments was done by calculating acceleration factors and storage conditions using the Arrhenius equation, as described in the introduction and in the Commission Regulation (EU) 10/2011. Calculated and tested conditions will be discussed in the chapter 2.2.2.2.2 Test of Storage Conditions.
Validation of the developed procedure was done in the end, as described in the chapter's 2.2.2.2.3 Validation of Migration and 2.2.2.2.4 Validation of Permeation.

2.2.2.1.1. One-Sided-Test: Migration

As a general procedure, the migration cell was assembled for a one-sided migration experiment as per following description: in the lid of the bottom part of the cell a colourless silicone O-ring was placed, the bottom part covered with the metal plate, the sample to be tested placed on the plate and the cell closed with the upper lid, fitted with a red FEP-coated O-ring. The cell was fixed with a clamping ring. The sample was covered with MPPO through the screw plug on the top of the cell. The cell was placed in an oven for a defined period at elevated temperature, as described in chapter 2.2.2.2.2 Test of Storage Conditions. Afterwards, the MPPO was drained into a glass vial with screw cap and 25 µL of migration internal standard mix were added. The MPPO was extracted three times with 10 mL of solvent (see 2.2.2.2.1 Test of Solvent for MPPO Extraction) and three minutes of vortexing. The extracts were combined through a folded filter in a 50 mL evaporation vial and the solvent evaporated to 0.5 mL in an automatic solvent evaporator. The extract was then transferred into 1.5 mL autosampler glass vial with screw cap, 0.5 mL of fresh hexane used to wash the evaporation vial and the solvent combined with the initial extract to achieve an end volume of 1 mL. All extracts were stored in a refrigerator, for measurements only a small amount was filled into a 1.5 mL glass vial with screw cap and a micro inlet. The extracts were measured on GC-FID, GC-MS, HPLC-GC-FID and GC×GC-MS as described in the chapter 2.1.5 Instrumentation.

2.2.2.1.2. Two-Sided Test: Permeation

When performing a two-sided test, three changes of the experimental set up were made: first, in the bottom of the cell a piece of cellulose was placed, on which the modelling substances for the permeation were spiked. Second, the metal plate in the middle of the cell was removed and therefore, third, the colourless silicone ring had to be replaced by a FEP-coated red ring to prevent migration of siloxanes into the MPPO. Test conditions and extraction procedure stayed the same as described above.

As modelling substances, deuterated *n*-alkanes of different chain lengths were chosen (*n*-C_{14}, *n*-C_{16}, *n*-C_{20}, *n*-C_{24} and *n*-C_{28}), because they simulate best a possible migration of mineral oil hydrocarbons through the sample. Deuterated substances were used to prevent interference of permeation and migration test, because these *n*-alkanes were also initially present in most of the tested paper samples. To simulate aroma permeability, a set of four aroma compounds was chosen (menthol, eugenol, vanillin and acetovanillone).

100 µL of the permeation standard mix in acetone, containing each of the mentioned substances in a concentration of 100 mg L^{-1}, were spiked into the bottom of the cell, resulting in 10 µg absolute per sample or 10 ng μL^{-1} in the final extract.

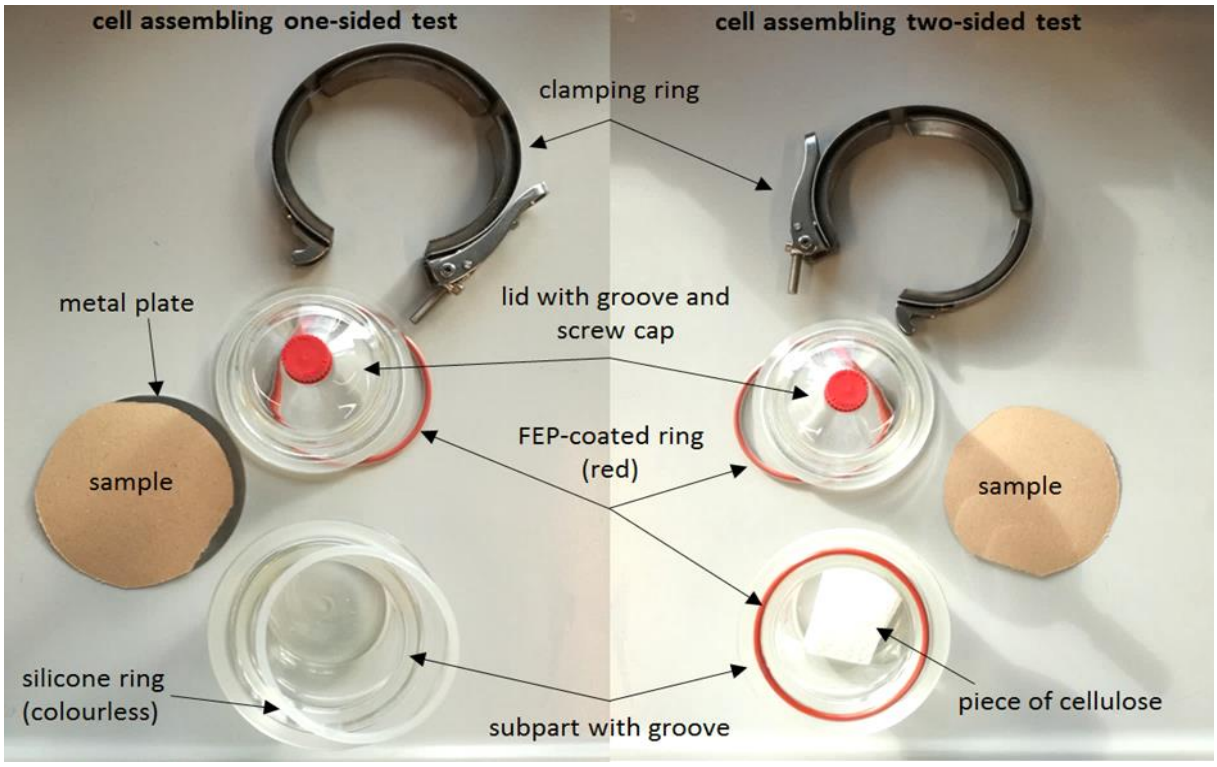

Figure 12: Differences in cell assembling: the one-sided test (migration) uses a colourless silicone ring and a metal plate to place the sample; the two-sided test uses no metal plate, a FEP-coated ring and a piece of cellulose in the subpart of the cell.

2.2.2.2. Method Development

2.2.2.2.1. Test of Solvent for MPPO Extraction

The experimental set-up was done as described above for a one-sided migration. As sample, a recycled paper was used. The solvents tested for the extraction of the MPPO were acetone, hexane and a mixture of acetone/hexane 1:1 (v/v).

2.2.2.2.2. Test of Storage Conditions

As described in the EU Regulation 10/2011 the Arrhenius equation can be used to calculate conditions for accelerated tests. The standard conditions described are either 40°C for 10 days or 60°C for 10 days. However, for a quick screening of migration and barrier properties of many different samples and materials, it is not practical to wait such long times. Therefore, as an alternative, quicker conditions were calculated and 80°C for 2 days tested. Furthermore, in industry widely used conditions such as 40°C or 60°C for just one day and the influence of humidity (20% relative humidity and 50% relative humidity) were tested, too. Since not only migration, but also the permeation was investigated (see also later in chapter 2.2.2.2.4), two-sided tests were performed in triplicates as described above, using a recycled paper as sample and *n*-hexane as solvent for extraction of the food simulant. The used and tested conditions are given in Table 8.

Table 8: Storage conditions for migration experiment tested during method development.

Temperature	Time	Simulated Storage Time at RT	Humidity
80°C	2d	307d	20%
80°C	2d	307d	50%
80°C	1d	153d	20%
80°C	1d	153d	50%
60°C	1d	30d	20%
60°C	1d	30d	50%
40°C	1d	5d	20%
40°C	1d	5d	50%
60°C	10d	299d	20%
60°C	10d	299d	50%
40°C	10d	47d	20%
40°C	10d	47d	50%

2.2.2.2.3. Validation of Migration

Validation of the proposed method was done by spiking a transformer oil (Trafoöl Technol 2000), DIPN and a set of phthalates (di-ethylhexylphthalate, di-*n*-butylphthalate and di-*iso*-butylphthalate) directly on a clean fresh fibre paper. Prior to use this paper was analysed and

showed no results in the range to be validated (< LOD) for total amount of MOSH and MOAH, overall migration, specific migration and migration of MOSH and MOAH. The MOSH and MOAH content of the transformer oil was analysed prior to use, too. A 10 g L^{-1}stock solution of the oil in hexane was prepared. It was further diluted by adding 20 µL of the described stock solution and 10 µL of MOSH/MOAH internal standard mix to 980 µL of hexane, to achieve a final concentration of 200 mg L^{-1}. The sample was analysed in a single measurement only with HPLC-GC-FID as described in section 2.1.5 Instrumentation. The MOSH content of the oil was determined as 619 mg kg^{-1} in the range of C_{16}-C_{25} and 637 mg kg^{-1} in the range C_{16}-$_{35}$. The MOAH content is 105 and 108 mg kg^{-1} in the ranges of C_{16}-C_{25} and C_{16}-$_{35}$, respectively. For validation, volumes of 10, 25, 50, 125 and 250 µL of the 10 g L^{-1} stock solution in *n*-hexane were spiked directly on the fresh fibre paper of 0.32 dm^2 surface area. Taking the assumption that 1 kg of food is packed in a cubic packaging of 6 dm^2 the spiked concentrations result e.g. for the lowest one into 2 mg per 6 dm^2 paper or 2 mg kg^{-1} food. Considering the levels of MOSH and MOAH allowed to migrate in the draft of the mineral oil regulation (2 mg kg^{-1} MOSH and 0.5 mg kg^{-1} MOAH; (BMEL, 2014; BMEL, 2017)), this was considered to be an adequate lowest point. The other levels would result into a concentration of 5, 10, 25 and 50 mg of mineral oil per kg of food simulant, respectively. Taking the same assumptions for DIPN and the set of phthalates, the spiked concentrations would result into a possible migration into food of 10, 50, 200, 500 and 1000 µg kg^{-1}.

After spiking the substances on the paper, the solvent was allowed to dry and the paper used immediately for the migration experiments. The cell was assembled as described above in chapter 2.2.2.1.1 and migration experiments performed at 80°C for 2 days. Experiments were performed in triplicates and extraction of the MPPO was done with *n*-hexane. Overall migration was determined using GC-FID, specific migration using GC-MS and migration of mineral oil using HPLC-GC-FID, as described in chapter 2.1.5 Instrumentation. Permeation was not tested during validation of migration experiments.

2.2.2.2.4. Validation of Permeation

For the validation of permeation, as a first attempt, the permeation standard mix was spiked into the bottom of the cell either on a piece of cellulose or without the cellulose. Aim was to test the adsorption of the model substances in the donor, as it was seen by Biedermann-Brem, et al. (2017b) in their studies. Concentrations used were 1, 2, 5, 10 and 15 µg absolute per sample, resulting into a final concentration of 1-15 ng μL^{-1} in the extract. These levels were chosen to fit the concentrations of the *n*-alkanes migrated from the recycled paper sample in the range of C_{16}-C_{35} (determined with GC-MS using the internal standard *d*-C_{19}). The permeation experiment was performed at 80°C for two days and extraction of the MPPO done with hexane and analysis as described above in chapter 2.2.2.1. As proof of concept, the cellulose used as donor was extracted once after a two-sided migration experiment, to analyse it for remaining permeation standard. The cellulose was cut into smaller pieces, placed in a 10 mL glass vial with screw cap and extracted with acetone at 40°C over night. The supernatant was analysed with GC-MS.

Second, the influence of humidity on permeation (and also migration) was investigated, using the same test series described in chapter 2.2.2.2.2 – Test of Storage Conditions. The permeation experiments were performed at either 20% or 50% relative humidity in an oven at varying temperature and defined storage times (see Table 9). The intermediate level of 10 µg permeation mix per sample was chosen for this test series and spiked either directly into the cell or on a piece of cellulose. Extraction of the MPPO and analysis was again done as described above in chapter 2.2.2.1. General Procedure. The used nomenclature will be explained with the results in section 3.1.1.2.

Table 9: Sampling plan for influence of humidity, storage time and donor on permeation and migration.

Temperature	Time	Simulated Storage Time at RT	Humidity	With Donor	Without Donor
80°C	2d	307d	20%	80_2_20_m	80_2_20_o
80°C	2d	307d	50%	80_2_50_m	80_2_50_o
60°C	10d	299d	20%	60_10_20_m	60_10_20_o
60°C	10d	299d	50%	60_10_50_m	60_10_50_o
80°C	1d	153d	20%	80_1_20_m	80_1_20_o
80°C	1d	153d	50%	80_1_50_m	80_1_50_o
40°C	10d	47d	20%	40_10_20_m	40_10_20_o
40°C	10d	47d	50%	40_10_50_m	40_10_50_o
60°C	1d	30d	20%	60_1_20_m	60_1_20_o
60°C	1d	30d	50%	60_1_50_m	60_1_50_o
40°C	1d	5d	20%	40_1_20_m	40_1_20_o
40°C	1d	5d	50%	40_1_50_m	40_1_50_o

Third, since in the coating trials some coated papers showed a higher permeation than the raw paper samples, this was further investigated. It was hypothesized that the papers being wet from the coating procedures are allowed to shrink freely while drying. This shrinking can result into a deformation of the fibres network and more open spaces between them. The process would not have any influence on the overall migration, since the present substances do not change in their concentration, but it would allow a higher permeation through the samples. To test this hypothesis the free shrinking was simulated by wetting a piece of recycled raw paper with 0.5 mL of water and allowing it to dry to constant weight again at room temperature. The dried sample was then used to perform a two-sided migration experiment, as described in chapter 2.2.2.1.2 at 80°C for two days.

2.2.3. Taped Barrier Test

The taped barrier test was extensively described in theory in the introduction. To assess the applicability of the test, several attempts were made using conventional polymers, but also biopolymers.

2.2.3.1. General Procedure

The taped barrier test was performed according to the last published revisions of the method by Biedermann-Brem, et al. (2017a and 2017b):
The donor solution contained the surrogates DEHP (40 mg L^{-1}), benzophenone (Bzp; 40 mg L^{-1}), *n*-heptadecane (C_{17}; 40 mg L^{-1}) and Sudan Red II (1-(2,4-dimethylphenylazo)-2-naphthol; 250 mg L^{-1}) in MTBE.
The donor paper was prepared from a recycled board sample of 300 g m^{-2} grammage. It was cut into pieces of about 15 × 21 cm. The pieces were soaked in the donor solution for 30 sec, without contact to the wall of the container, removed afterwards, dripped-off and dried in a fume hood for 2 min. Several pieces of the donor were then wrapped together into aluminium foil and conditioned in an oven at 40°C for at least two weeks. Prior to preparing the test packs, about 1 cm of the donor is cut from each side. An extra stripe of about 1 cm is cut for the determination of the initial surrogate concentration; it was cut into small pieces, weight into a 10 mL glass vial, 25 µL of the migration internal standard solution added and extracted with 4 mL MTBE/EtOH (95:5, v:v) at 40°C for 8h. The amount of solvent used was 2-fold of the amount described (Biedermann-Brem, et al., 2017a), but using only 2 mL did not cover the paper properly.
The silicon paper used as acceptor was purchased from BGB Analytik AG (Div-Paper-PDMS-PK10; A4 Cellulose Paper Sheet, PDMS coated) and cleaned either in an vacuum oven at 130°C and 0.1 bar vacuum for 8h, as described in (Biedermann-Brem, et al., 2017a) or at 160°C for 1h as described in (Biedermann-Brem, et al., 2014). Afterwards, it was wrapped and stored in aluminium foil until used. The purity of the paper was checked by extracting 250 mg with MTBE/EtOH (95:5, v:v) at 40°C for 8h and analysing it with GC-MS in scan-mode as described in 2.1.5 Instrumentation. .
The test packs were prepared as described, without any changes (Biedermann-Brem, et al., 2017a). Also cutting of the stripes was done equally. The test conditions (temperature) and time between cutting of the stripes were chosen depending on the tested barrier. Extraction of the stripes was done as described for the donor – the stripe was cut into small pieces, weight into a 10 mL glass vial, 25 µL of internal standard solution added and extracted with 4 mL MTBE/EtOH (95:5, v:v) at 40°C for 8h. Afterwards, about 50 µL of the extract were filled into a 1.5 mL autosampler vial with micro insert and analysed using GC-MS in SIM-Mode, as described in 2.1.5 Instrumentation.

2.2.3.2. Comparison of Siliconized Paper and MPPO

To compare the acceptor behaviour of MPPO and the siliconized paper, proposed as acceptor for the taped barrier test by Biedermann-Brem et al. (2014), a two-sided migration experiment

was performed as described above. As paper sample (donor) a recycled paper was used, but instead of covering it with MPPO, a piece of siliconized paper, cleaned in a vacuum oven at 0.1 bar prior to use, was cut into the same size as the sample and the silicone-treated side brought into direct contact with it. The migration experiment was performed in triplicates at 80°C for two days. Afterwards, the siliconized paper was removed, the whole sheet cut into pieces (~600 mg) and weight into a 30 mL glass vial with screw cap and silicone/PTFE septum. 25 µL of the migration internal standard mixture were added and the paper was extracted with 10 mL of MTBE/EtOH (95:5, v:v) for 8h at 40°C. The extract was evaporated to 0.5 mL, the evaporation vial washed with 0.5 mL of MTBE and combined with the extract. Analysis was done as described in the section 2.1.5 Instrumentation on the GC-FID for the overall migration and on GC-MS in scan-mode to get more detailed information on the migrated substances.

2.2.3.3. Taped Barrier Test Applied on Chitosan Free-Standing Film

The taped barrier test was first applied on a chitosan free-standing film, produced at the Institute of Paper, Pulp and Fibre Technology, TU Graz. No further specifications were known for these films, except the material.

The test packs were prepared in triplicates as described in (Biedermann-Brem, et al., 2017a) and above in the section 2.2.3.1. The first tests packs with chitosan barrier were stored at 80°C and stripes were cut every 4h or 12h overnight, respectively. For the second test, packs were stored at 40°C with stripes cut every week. A proof of concept was made by puncturing one of the 40°C test packs with a needle three times one week prior to cutting the last stripe.

2.2.3.4. Determination of Breakthrough Concentration for Chitosan Film

Since no information is known on the soaking behaviour of the chitosan film and the distribution of the surrogate substances in the test pack, a 100% breakthrough test was performed as described in (Biedermann-Brem, et al., 2014).

In short, stripes of 2×10 cm were cut from the donor, the chitosan film and the acceptor and placed in a 60 mL glass vial with screw cap and silicon/PTFE septum with as less contact to each other as possible (see Figure 13). The Arrhenius equation was used to calculate different acceleration factors and storages conditions, resulting into two series of tests: One series was stored at 40°C for 1 day, 3 days, 7 days, 10 days and 35 days (five vials prepared in total), the second series was stored at 60°C for 1 day, 3 days, 7 days, 10 days, 17 days and 24 days (6 vials prepared in total). The vials were stored for the designated time and the donor, the barrier and the acceptor cut into pieces and extracted with 4 mL of MTBE/EtOH (95:5, v:v) at 40°C for 8h, afterwards. The extracts were analysed at the GC-MS in SIM mode as described in 2.1.5 Instrumentation and the results of the batches compared to each other and to the results of the taped barrier test.

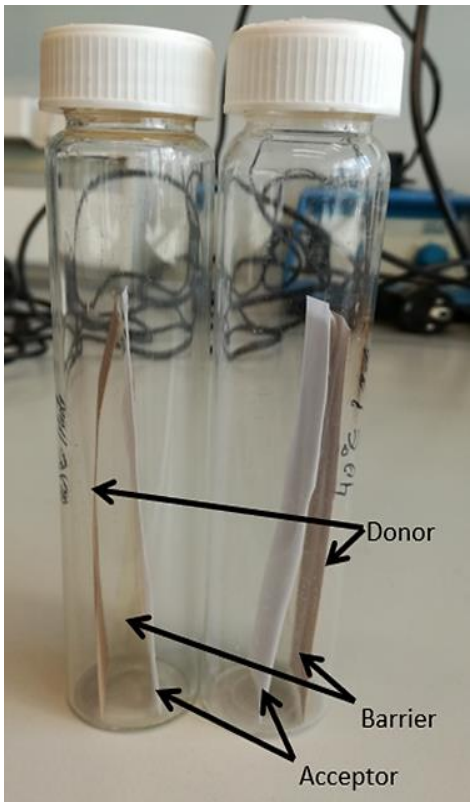

Figure 13: Set-up of breakthrough test.

2.2.3.1. Taped Barrier Test Applied on Conventional Polymer Films

For easier method validation and comparison of the chitosan behaviour, three conventional polymer films were used; 40 µm linear, low density polyethylene film (LLDPE), a 70 µm LLDPE film and a 50 µm polypropylene film (PP) were received from a local producer. Test packs were prepared in triplicates as described in section 2.2.3.1 and stored either at 40°C or at room temperature, as described in the publications of Biedermann-Brem, et al. (2014, 2017a, 2017b).

2.2.3.2. Comparison of Taped Barrier Test and Two-Sided Migration Experiment

As a last step for the evaluation of the taped barrier test its test procedure, handling and the results were compared to the two-sided migration experiment, which was developed in this work. Therefore, the three conventional barrier films and the chitosan biopolymer film were tested in two-sided migration experiments using the standard conditions of 40°C for 10 days and MPPO as food simulant, as described in section 2.2.2.1.2. Following experiments were performed to generate the needed information on the films and the two tests:

To determine the migration of substances initially present in the films, a migration experiment was done without an additional donor first (barrier film – food simulant). Afterwards, the recycled raw paper was used as a donor to simulate a real packaging situation, similar to the taped barrier test (recycled paper – barrier film – food). The MPPO was extracted with *n*-hexane in both attempts and analysis done on GC-FID to determine the overall migration, on GC-MS

to determine the permeation and on HPLC-GC-FID to determine the migrated mineral oils, as described in chapter 2.1.5 Instrumentation.

2.3. Characterisation of Papers and Biopolymers

2.3.1. Characterisation of Raw Papers and Biopolymer Raw Materials

2.3.1.1. Raw Papers

In order to find industrially produced raw papers that could be used in the coating trials, the industry partners were asked to hand in paper or board samples being relevant for food contact applications for them. A summary of the received samples is given in Table 10. At this point it has to be mentioned that most of the samples received from the industry partners had been transported and later stored in a recycled fibre folding box for about a year, prior to the start of this work. Usually, all samples were packed carefully in aluminium foil to prevent contamination by migration from outside, but also losses of volatile substances to the surroundings.

Table 10: Raw paper samples and coated reference paper samples received from industry partners.

No.	Description of Sample	Grammage [g*m^{-2}]	Thickness [µm]
1	white, non-printed, fresh fibre raw paper, A3	72.1	96.1
R1	PE coated, white, non-printed fresh fibre paper, A3	82.1	93.6
2	white, non-printed fresh fibre raw paper, A3	39.4	51.8
R2_1	PE composite, white, non-printed fresh fibre paper, A4	63.6	72.6
R2_2	“tight” - white, non-printed fresh fibre paper, A3	39.5	47.5
R2_3	waxed paper – 40 g m^{-2} fresh fibre + 4-8 g m^{-2} wax coating, white, non-printed, A3	42.8	50.0
3	brown, non-printed liner, recycled and fresh fibre mix, raw paper, A3	137	183
4	brown, non-printed liner from recycled fibre, raw paper, A3	130	197

The papers were pre-characterised in different ways: HS-SPME with GC-MS detection was used to determine the sum of volatile substances, total extraction and HPLC-GC-FID measurements were done to determine the total amount of MOSH and MOAH and the two-sided migration experiment with various detection methods was used to determine the overall migration, permeation and migration of MOSH and MOAH. It is important to mention that the two-sided migration experiment had not been developed completely, when the characterisation of the papers was done as a first step in the project. In contrast to the describe method in chapter

2.2.2.1.2, the permeation standard was spiked directly into the bottom part of the cell and analysis and data evaluation was done only on GC-FID. Furthermore, the HPLC-GC-FID was not available in a routine attempt; therefore, only for some raw papers migration data for MOSH and MOAH are available. The generated data will be discussed in detail in the results section.

2.3.1.2. Biopolymers

An overview of the biopolymers available and tested in this work was given in the introduction in chapter 1.4.2.2. Table 11 shows in which ways (summarised as trials) they were tested in this work.

Table 11: Summary of testing trials and the materials used in them.

Coating Principal	**Pouring- Method**		**Hand Squeegee**	**Summet Coater**		
Trial // Material	*HS-SPME-GC-MS*	*free-standing films*	*6 g m^{-2} coating*	*3 g m^{-2} drying + layering*	*layering*	*sizing and calendering*
Chitosan	x	x	x	x		x
Alginate	x	x	x		x	x
MFC	x	x	x			
Casein	x	x	x			
EVOH[1]	x	x	x			
Ref. I[2]		x	x			
Soy			x			
CNC[3]			x			
Ref. II[4]			x			
Gluten			x			
Xylan			x			
Ref. III[5]			x			
Starch			x			

[1]EVOH = ethylene vinyl alcohol copolymer; [2]micro emulsion; [3]cellulose nano-crystals; [4]ready to use solution for barrier coating of recycled and fresh fibre boards for food contact, based on aqueous acrylate dispersion; [5]acrylate dispersion with butadiene copolymers used for paper and board coating

A basic characterisation of the polymers was done to get an overview of the present volatile substances in the different materials, using HS-SPME as sample preparation method. The samples were cut into small pieces of 5×5 mm and 250 mg were weight into head space glass vials. 10 µL of a 10 mg L^{-1} *d*-dodecane solution in methanol as internal standard and a magnetic stir bar were added and the vials closed with crimp caps. The samples were measured using HS-SPME and GC-MS detection as described under 2.1.5.1. Only the first received free

standing films were characterised with this method, since later on, the films and coated papers were directly tested in the migration experiment, developed in this work (seen Table 11). The following chapter 2.3.2. summarised the coating trials and analyses done.

2.3.2. Trials

The following chapters give an overview of the most important trials performed during this work. All film formation and paper coating work was done at the Institute of Paper, Pulp and Fibre Technology of the TU Graz. For further information on the coating procedures or the physical properties of the resulting samples (e.g. film formation properties, grammage, thickness, density, roughness, air permeability, water-vapour transmission rate, water absorptiveness, contact angle, grease resistance, …) may be referred to the institute and their published work e.g. (Kopacic, et al., 2018a) (Kopacic, et al., 2018b) (Kopacic, 2019).
After pre-testing of the raw papers, as described above in chapter 2.3.1.1, it was decided to use following two papers in our coating studies as examples for industrially produced food contact (raw) materials: the secondary fibre raw paper 4, which did not had any pre-treatment, and the paper 1, which was completely made from primary fibre and already mass-sized with liquid alkenyl succinic anhydride (ASA) and starch surface-sized using a calender machine. They were chosen as substrate to simulate both, a worst-case paper without any further treating and a clean surface sized primary fibre paper.
The results of the different trials will be discussed in the results section starting with chapter 3.2.2.

2.3.2.1. Free-Standing Films

First, it was tried to produce a free-standing film of the biopolymer raw materials to characterise their general physical behaviour, the present migrate-able volatile substances and their barrier efficiencies. Therefore, their overall migration and the barrier efficiency against permeation was tested using the developed two-sided migration experiment.

2.3.2.2. 6 g m^{-2} Coat Weight with Hand Squeegee

Second, the materials were coated on the two chosen raw papers using a hand squeegee and a coat weight of 6 g m^{-2}. After coating of the papers, two-sided migration experiments were performed to evaluate the barrier efficiency of the materials.

2.3.2.3. Chitosan with Drying and Layering

Third, after getting a general overview of the materials using a high coat weight of 6 g m^{-2}, the material chitosan was chosen to be tested further in more specific tests. The coat weight was reduced to 3 g m^{-2}, which is a more industrial-near approach, and the results were compared with the once gained before. Furthermore, these were the first tests were coating was done using a summet coater. The coater offered the possibility to use different drying methods like infrared, hot air or a combination of infrared and hot air. The influences of the reduced coat weight and

the different drying methods were evaluated. Two-sided migration experiments were performed to evaluate two aspects: First, the influence of the used coating parameters on the barrier efficiency of the material and second, the best coating parameters for further trials.

Table 12: Samples of trial 3 – raw papers coated with chitosan (5% solid content) using a summet coater; investigation of influence of drying method, layering and coat weight.

No.	Paper	Drying Type	Temperature [°C]	Drying Time [s]	Layers	Coat weight [g m^{-2}]
1A	Primary Fibre	IR	-	4	1	3
2D	Primary Fibre	IR	-	4	2	6
3B	Primary Fibre	HL	100	120	1	3
4E	Primary Fibre	HL	100	120	2	6
5C	Primary Fibre	HL+ IR	100	120+4	1	3
6F	Primary Fibre	HL+IR	100	120+4	2	6
7G	Secondary Fibre	IR	-	4	1	3
8J	Secondary Fibre	IR	-	4	2	6
9H	Secondary Fibre	HL	100	120	1	3
10K	Secondary Fibre	HL	100	120	2	6
11I	Secondary Fibre	HL+ IR	100	120+4	1	3
12L	Secondary Fibre	HL+IR	100	120+4	2	6

2.3.2.4. Alginates and Influence of Layering

In the 4th trial, the influence of building layers was further investigated. The recycled raw paper was coated using alginate, which showed the best barrier behaviour against MOH migration before. In a first attempt, coating was done in either one or two layers of the same coat weight using the coating parameters determined in the 3rd trial.

Table 13: Samples of trial 4.1 – secondary fibre paper coated with different coat weight and layers of alginate.

Sample	Coat weight total	Number of Layers	Coat Weight per Layer
6SL	6 g m^{-2}	1	6 g m^{-2}
6DL	6 g m^{-2}	2	3 g m^{-2}
4.5SL	4.5 g m^{-2}	1	4.5 g m^{-2}
4.5DL	4.5 g m^{-2}	2	2.25 g m^{-2}
2SL	2 g m^{-2}	1	2 g m^{-2}
2DL	2 g m^{-2}	2	1 g m^{-2}

In a second attempt, the influence of the coating temperature and the used solid content, both influencing the viscosity of the coating solution, were investigated using a 3 g m^{-2} single layer coating on the secondary fibre paper. The nomenclature refers to coating at 40°C with a solid content of 4%, coating at room temperature with a solid content of 7.7% and coating at 40°C with a solid content of 7.7%

Table 14: Samples of trial 4.2 – secondary fibre paper coated with same coat weight and one layer of alginate, but at varying conditions influencing the viscosity of the coating solution.

Sample	Solid Content	Coating Temperature	Coat Weight	Layers
40°C-4%	4%	40°C	3 g m^{-2}	1
RT-7.7%	7.7%	RT	3 g m^{-2}	1
40°C-7.7%	7.7%	40°C	3 g m^{-2}	1

2.3.2.5. Influence of Sizing and Calendering

Since the used secondary fibre raw paper was not treated any further prior to coating so far, the influence on the barrier properties of such pre-treatment was investigated in this 5th trial. Both raw papers were calendered in the lab with 3000 decanewton at 65°C once and coated afterwards. Due to the compression of the papers, they did not take up the foreseen 6 g m^{-2} coating anymore. Therefore, the papers were coated with 3 and 5 g m^{-2} instead. The influence of sizing with alkenyl succinic anhydrides (ASA) was investigated for the recycled raw paper only, because the primary fibre raw paper was already surface sized. The sizing with ASA was performed by one of the industry partners.

Table 15: Samples of 5th trial – influence of sizing and calendering on secondary and primary fibre raw paper coated with alginate or chitosan.

Sample	Paper	Material	Coat Weight
RRPs	sized secondary fibre raw paper	-	-
RRPc	calendered secondary fibre raw paper	-	-
RRPs + 6Alg	sized secondary fibre raw paper	alginate	6 g m^{-2}
RRPc + 5Cht	calendered secondary fibre raw paper	chitosan	5 g m^{-2}
RRPc + 3Cht	calendered secondary fibre raw paper	chitosan	3 g m^{-2}
RRPc + 5Alg	calendered secondary fibre raw paper	alginate	5 g m^{-2}
RRPc + 3Alg	calendered secondary fibre raw paper	alginate	3 g m^{-2}
FFPc	calendered primary fibre raw paper	-	-
FFPc + 5Alg	calendered primary fibre raw paper	alginate	5 g m^{-2}
FFPc + 3Alg	calendered primary fibre raw paper	alginate	3 g m^{-2}
FFPc + 5Cht	calendered primary fibre raw paper	chitosan	5 g m^{-2}
FFPc + 3Cht	calendered primary fibre raw paper	chitosan	3 g m^{-2}

2.4. Characterisation of Migrated Fractions

2.4.1. General Aspects

Two different comprehensive 2D-GC×GC-MS systems were tested in this work: The first system was a Shimdazu QP2010 Ultra quadrupole-MS with Version 7.2 of “GC-Image” from the Zoex Corporation for data evaluation. The second one was a Leco Pegasus® BT TOF-MS

with “ChromaTOF” Version 5.40.12.0 for Pegasus® BT also from Leco Corporation. The most important differences between the two instruments are probably the modulators and the detectors used. For detailed descriptions of the two instruments see chapter 2.1.5. Instrumentation. It has to be mentioned that the GC×GC-qMS was available permanently at the institute. The measurements using the GC×GC-TOF were performed during a visit at the LECO European Application and Technology Center in Berlin, Germany. Therefore, the measurements done and the amount of data available for the TOF are limited.

2.4.2. GC×GC-qMS

2.4.2.1. HS-SPME

To get a first impression, the fresh fibre raw paper 1 and the recycled fibre raw paper 4 were measured on the instrument using HS-SPME extraction. The papers were cut into small pieces, 100 mg weighed into a glass vial and extraction, enrichment and measurement of the samples done with “Conditions A”, as described in chapter 2.1.5.6. For testing different extraction conditions, the HS-SPME was performed at either 80°C for 30 min or at 150°C for 30 min and compared using the recycled raw paper sample.

2.4.2.2. Migrated Total Fraction

The second step was to measure a migrated fraction of substances, which was transferred from the paper samples into the food simulant MPPO during a two-sided migration experiment. The migration experiments were performed as described in chapter 2.2.2, using the recycled raw paper and the same paper coated with 6 g m^{-2} alginate as sample (taken from hand squeegee trial). The resulting extracts were measured on the instrument using the “Conditions B” as described in chapter 2.1.5.6.

2.4.2.3. Migrated Fractions Before and After Pre-Separation

The last step done was to have a closer look into the two pre-separated fractions of MOSH and MOAH. Again, extracts of two-sided migration experiments were used for these tests. The extracts resulted from the migration of the uncoated recycled raw paper sample and the same paper coated with two times 3g m^{-2} alginate (total coat weight 6 g m^{-2}; sample 6DL of trial 4.1). The extracts were used as such (referred to as “total MOH”) or pre-fractionated into MOSH and MOAH using the Fract & Collect tool of the HPLC-GC-FID systems. Measurements were done using the “Conditions C” as described in chapter 2.1.5.6.

2.4.3. GC×GC-TOF

The main aim of visiting the LECO European Application and Technology Center in Berlin, Germany and doing measurements on the TOF instrument was, to compare the two different GC×GC instruments.

To check the sensitivity of the TOF system and to compare it with the qMS instrument, the MOSH/MOAH internal standard mix with an initial concentration of 150-600 ng μL^{-1} (equals 150-600 mg L^{-1}) was used and measured on both instruments. It was diluted with *n*-hexane to obtain the concentration levels of 15–60 pg μL^{-1} (referred to as 10 pg μL^{-1} level) and 1.5-6 pg μL^{-1} (referred to as 1 pg μL^{-1} level). The injection volume used was 1µL, resulting to injected absolute amounts of the individual substances for the 10 pg μL^{-1} level of C_{13} 15 pg; C_{11}, CyCy, 5B, 1-, and 2-MN and TBB 30 pg, Cho 60 pg absolute and for the 1 pg μL^{-1} level in C_{13} 1.5 pg; C_{11}, CyCy, 5B, 1-, and 2-MN and TBB 3 pg, Cho 6 pg absolute.
The samples measured on the TOF were the same as in chapter 2.4.2.3, to allow comparison of the data: the used extracts resulted from a two-sided migration experiment of the uncoated recycled raw paper sample and the same paper coated with two times 3g m^{-2} alginate (total coat weight 6 g m^{-2}; sample 6DL of trial 4.1). The extracts were used as such (referred to as "total MOH") or pre-fractionated into MOSH and MOAH using the Fract & Collect tool of the HPLC-GC-FID systems. Measurements were done as described in chapter 2.1.5.7.

2.5. Analysis of MOSH & MOAH in Food

2.5.1. General Aspects

As already discussed in the introduction, the analysis of MOSH and MOAH in food is a big challenge. The complex matrix food is highly variable; therefore, different sample preparation techniques for different food types are needed.
At the beginning, the available documents and methods were the BfR-compendium, published only in German language and the EN16995 ([BfR] German Federal Institute for Risk Assessment, 2012) (Austrian Standards Institute, 2017). On basis of these two documents the method development and analysis of MOSH and MOAH was started. Later on, the "ThinkTank" collaboration led to the development of a saponification method, which was a better approach and routinely used for sample preparation from that point on. Furthermore, the JRC guideline was published in 2019, as already discussed in the introduction ([JRC] , 2019). The recommendations given on sampling, handling of samples, data evaluation and reporting were also considered in this work.

The first analyses were done for edible fats and oils, since there was already the EN16995 published. As a general approach, 300 mg of sample were weight into a vial and 600 µL of hexane added. The dissolved sample was transferred to a glass column packed and compressed with 10 g Al_2O_3 and 3 g silica gel, and the MOSH fraction eluted with 25 mL hexane. For removal of fat in the MOAH fraction 12g of silica were used, because of the higher polarity of the eluent, which includes up to 30% dichloromethane. Al_2O_3 was not used for MOAH fractions, since they are retained on it ([CEN] European Committee For Standardization , 2017). However, since the stated limits of detection were >10 mg kg^{-1}, soon the amount of sample used was increased to 1 g instead of the stated 300 mg. According to the published literature, the retention

capacities of the described 10 g Al_2O_3 + 6 g silica gel columns should still be appropriate. If still higher sensitivity was needed, larger columns than the proposed ones were used (Fisilier, et al., 2009) (Biedermann, et al., 2012a). Furthermore, it was tried to apply the method also on other matrices, as e.g. chocolate.

An alternative sample preparation procedure was developed within the "ThinkTank" collaboration: For the removal of fat, saponification was applied. As a general approach, 3g of crushed (if needed) and homogenised sample were weight into a 60 mL glass vial with screw cap. 30 mL of a mixture of hexane and ethanol (1:1, v/v) were added, the vial placed in a heating block at 60°C for 30 min and occasionally shaken, to allow the sample to get evenly distributed. Afterwards, 3 mL of an aqueous potassium hydroxide solution (50%; w/w) were added and the sample allowed to saponify at 60°C over night. On the next day the samples were cooled to room temperature, another 5 mL of a mixture of hexane/ethanol (1:1, v/v) added and the vial shaken. After phase separation, the upper organic hexane-phase was used for direct analysis or further sample preparation steps for the MOSH and/or MOAH fraction and the lower aqueous ethanol/water mix disposed.

In clean-up and enrichment of the MOSH fraction the naturally occurring odd numbered *n*-alkanes needed to be removed. Column chromatography with 7g Al_2O_3, 3 g silica gel and 1 g of Na_2SO_4 was used. A glass column was packed with the materials in this order and compressed. Afterwards, the extract was transferred to the column and the MOSH fraction eluted with 25 mL of hexane. At the end, the solvent was evaporated to reach an end volume of 1 mL and used for analysis of MOSH on the HPLC-GC-FID. MOAH could not be analysed from this extract, since it is retained on the Al_2O_3 column. Therefore, there was another sample preparation path for the MOAH fraction.

In clean-up and enrichment of the MOAH fraction naturally occurring olefins, such as carotenoids and squalene needed to be removed, the method of choice being epoxidation. As preparatory step, a SPE clean-up using 3 g silica gel and 1 g Na_2SO_4 was done. A 6 mL glass column was packed with the materials and compressed. The sample was transferred to the column and eluted with 15 mL of hexane. The solvent was then evaporated to a volume of 1 mL and used further for the epoxidation. Various epoxidation methods had been tried within the method development in the "Think Tank" project and are described in the next paragraphs:

First of all, automated epoxidation was performed according to (Nestola, et al., 2017), using 1 mL of a 200 g L^{-1} m-CPBA solution in ethanol. Epoxidation was done at 40°C for 20 minutes and 2 mL of an aqueous 100 g L^{-1} sodium thiosulfate (Na_2SO_3) solution were added to reduce the excess of m-CPBA. 500 µL of ethanol were added to enhance phase separation in the next step and the vial centrifuged. The upper hexane phase was then transferred into a 1.5 mL autosampler vial with screw cap and a spade point of Na_2SO_4, the fraction allowed to dry for 5 min and used for analysis of the MOAH fraction, afterwards.

Second, epoxidation was done with 350 µL of a 30 g L^{-1} m-CPBA solution in dichloromethane for 15 min at 40°C. The excess of m-CPBA was reduce by adding 4 mL of a solution consisting of 5 g sodium thiosulfate and 5 g sodium carbonate in 100 ml of water (50 g L^{-1} solution of salts in water). The solvent was evaporated to an end volume of 1 mL afterwards and used for

analysis of the MOAH fraction on the HPLC-GC-FID. This method was considered to be a "soft" one.
Third, epoxidation was done as described for the second try, but 1 mL of the 30 g L^{-1} m-CPBA solution in dichloromethane were added. This method was considered to be "hard".

If the analysis of the samples using HPLC-GC-FID did not give a clear result, although using the different clean-up and enrichment steps, comprehensive 2D-GC×GC-MS was used to verify the results. The analysis was done either directly from the resulting total extract (MOSH and MOAH fraction combined), from the resulting extract after clean-up and enrichment steps (e.g. after MOSH clean-up with Al_2O_3) or after pre-separation of MOSH and MOAH on the HPLC and collection of the fractions afterwards (called "Fract & Collect").

In conclusion, for analysing MOSH, the sample was saponified, an alox clean-up made, solvent evaporated to 1 mL and the extract measured on HPLC-GC-FID. For MOAH, the sample was also saponified, an SPE clean-up with silica gel done, solvent evaporated to 1 mL, epoxidized using m-CPBA and measured on HPLC-GC-FID, too. For this procedure limits of detection (LOD) and quantification (LOQ) were determined using seven blank samples for MOSH and MOAH, each. The blanks consisted only of the solvents and reagents used and the added internal standard. All clean-up and enrichment steps were applied to them. They were measured on HPLC-GC-FID afterwards and their blank values determined by comparing them to a directly injected internal standard. LOD was determined by the mean value of the individual fractions of the blank (y_B) plus three times the standard deviation (s_B) of them. LOQ was determined by the mean value of the individual fractions plus ten times the standard deviation, as can be seen in Equation 3 and 4:

Equation 3: $$LOD = y_B + 3s_B$$

Equation 4: $$LOQ = y_B + 10s_B$$

LOD and LOQ were determined according to the JRC Guideline for the MOSH fractions C_{16}-C_{20}, C_{20}-C_{25}, C_{25}-C_{35}, C_{35}-C_{40} and C_{40}-C_{50}. For the MOAH, the fractions were C_{16}-C_{25}, C_{25}-C_{35} and C_{35}-C_{50} ([JRC] , 2019).

In the following chapters, the sample preparation and analysis procedure will be discussed specifically for different sample types and food matrices, divided into dry food, fats, oils and oil-based products, meat & milk, chocolate, spices and lubricating oils. The results, as well as the advantages and disadvantages of the applied methods are discussed in the results and discussion section in chapter 3.4.

2.5.2. Dry Food

Dry food, as rice or noodles, are probably the easiest food matrix for the analysis of MOSH and MOAH. They only have a low amount of fat (<4%) and therefore extensive sample preparation steps for fat removal as e.g. saponification or epoxidation to remove olefins from the MOAH are not needed. It may however be needed to remove naturally present odd numbered *n*-alkanes from the MOSH fraction using Al_2O_3.

As a general attempt, the food was homogenized, milled (e.g. noodles) or weight in directly (e.g. rice and grains) in an amount of 10 g. The food was covered with hexane, 10µL of MOSH/MOAH internal standard solution added and extracted over night at 40°C. Of the supernatant about 100 µL were taken and analysed on the HPLC-GC-FID, afterwards. If the resulting chromatogram showed present odd numbered *n*-alkanes, the whole extract was used for the clean-up of the MOSH fraction on an Al_2O_3 column. If no interferences could be seen, the extract was decanted and the solvent evaporated to e.g. 1 mL to increase the sensitivity for the analysis of MOSH and MOAH.

For evaluation of the methods performance, a rice sample was spiked with 5 mg kg^{-1} of transformer oil (Trafoöl Technol 2000) and analysed with the proposed method. To clearly demonstrate the difference between applied and not applied clean-up and enrichment, the sample was analysed once directly and once after Al_2O_3 clean-up and enrichment.

2.5.3. Fats, Oils and Oil-Based Products

Within this food category are e.g. edible fats and oils, but also oil and fat-based products, like butter or pesto. The fat content varies from 30-40% for the pesto sample to nearly 100% for the edible oils and fats. Method development was done with edible oils for this category and applied on the other fat rich matrices afterwards. Consequently, sample preparation steps are the same, although the fat content, consistency and ingredients may vary a lot.

As a screening approach, 300 mg of the samples were solved in 600 µL hexane, 10 µL of internal standard added and analysed on HPLC-GC-FID. The resulting chromatograms were used to identify the present natural interferences and therefore gave important information on the sample preparation, clean-up and enrichment steps needed. Again, if the chromatogram showed present odd numbered *n*-alkanes in the MOSH fraction, cleaned-up using an Al_2O_3 column was done. If the chromatograms showed an overload of naturally present olefins in the MOAH fraction, epoxidation with m-CPBA was applied to them. The general sample preparation procedure, as described in the introduction of this chapter (chapter 2.5.1) was applied:

3g of the sample were homogenised and weight into a 60 mL glass vial with screw cap. 10 µL of the internal standard and 30 mL of a mixture of hexane and ethanol (1:1, v/v) were added and the sample homogenised at 60°C for 30 min. Solid samples melted during this time, resulting into a homogeneous solution. Afterwards, saponification with KOH was performed

and the resulting extract used for the further clean-up with Al_2O_3 for the MOSH and the SPE clean-up and epoxidation with m-CPBA in ethanol for the MOAH fraction.

2.5.4. Meat and Milk

Meat and meat-products, as well as milk and milk-products were put together into one category, since these food types need an additional sample preparation step: Digestion of the matrix using hydrochloric acid (HCl) is needed to allow a complete extraction of the mineral oils, which may be present. The digestion was done according to the BfR compendium ([BfR] German Federal Institute for Risk Assessment, 2012):
As a general attempt, 3g of sample were milled, homogenised and weight into a 60 mL glass vial with silicone/PFTE septum. 5 mL of concentrated HCl were added and the vial placed in a heating block at 80°C for about 3h. The digestion was complete, when the flakes formed after addition of the acid dissolved again and a mostly clear solution remained. Afterwards, the solution was extracted with 10 mL of hexane two times, the two hexane fractions combined and 10µL of internal standard added. The resulting extract was further used for saponification. 20 mL of ethanol and 3 mL of KOH (50%, w/w, in water) were added and saponification done at 60°C over night. Afterwards, 5 mL of hexane and 5 mL of an ethanol/water 1:1 (v/v) mixture were added, the vial shaken and after the phase-separation the hexane was used for the further MOSH or MOAH clean-up (see 2.5.1.General Aspects).

2.5.5. Chocolate

Chocolate seems to be a comparable matrix to fats, cheese or milk. However, sample preparation did not work as well as it did for them. Several different types of chocolates, as well as different ways of saponification were tried, including microwave assisted methods similar to (Moret, et al., 2016):
Frist, a chocolate sample was milled, 5 g weight into a microwave glass vial with silicone/PTFE septum, 5 mL of hexane, 10 mL of saturated methanolic KOH, 10µL of internal standard and a PTFE coated stir bar were added. The vial was placed in the autosampler rack and the microwave was programmed to perform the simultaneous extraction and saponification at 120°C for 20 min automated. The vial was heated with maximum power of 850 W, till the temperature was reached and held for 20 min. Stirring was done at full speed of 1200 rpm, the maximum pressure in the vial was 30 bar. Cooling to room temperature was done with a stream of compressed air (5 bar). The resulting dark-black, slimy residue was transferred into a new glass vial, the microwave glass vial washed with 10 mL of water and 10 mL hexane and both fractions combined with the chocolate residue. The vial was shaken, after phase-separation the upper hexane phase transferred into a fresh glass vial and dried with Na_2SO_4. Afterwards, it was tried to evaporate the solvent to achieve an end volume of 1 mL but this was not possible due to precipitation of large amounts of fat.

Second, 5 g of milled chocolate were weight into a microwave glass vial and saponification done as described in the first try, but only the 10 mL of saturated methanolic KOH and 10 µL of internal standard were added. After cooling of the vial three separated layers were observed: in the bottom there was a dark-brown liquid layer, a colourless solid layer in the middle and a clear solution on top of the solid layer. It was tried to transfer the extract into a new vial by washing it with water and hexane, but this resulted in a complete solidified residue.
Third, the amount of chocolate was reduced to 2.5 g. This resulted into a light-brown residue with mousse-like consistency. Adding 20 mL of water and hexane again led to a solidified residue.
So, in the fourth attempt the amount of chocolate was decreased further to 1 g. This time a homogeneous and liquid extract in chocolate-brown colour was received. It was possible to transfer it to a new vial, to extract it with 20 mL of hexane and to evaporate the hexane to an end volume of 1 mL. The extract was measured on the HPLC-GC-FID and showed that further clean-up and enrichment of the MOSH and MOAH fraction was needed.
Fifth, the method developed for oils and fats was applied on chocolate, too. 3g of milled chocolate were weight into a glass vial and homogenised in 30 mL hexane/ethanol (1:1; v/v) at 60°C for 30 min. The chocolate melted during this time, resulting into a brown, drinking-chocolate-like solution with a small amount of non-soluble residue. For the MOSH fraction saponification and Al_2O_3 clean-up was done and the extract evaporate to 1 mL end volume. For the MOAH fraction SPE clean-up and epoxidation using m-CPBA in ethanol were done. No problems occurred during sample preparation and the extracts were measured on the HPLC-GC-FID.

2.5.6. Spices

Spices are a matrix not really comparable to the others. They are dry food, without fat (except the present essential oils), but include loads of natural olefins interfering the MOSH and MOAH fraction. Therefore, to successfully remove all interferences, a comprehensive clean-up is necessary.
3 g of sample were weight into a glass vial and 30 mL of the hexane/ethanol 1:1 (v/v) mixture and internal standard added. The samples were saponified with KOH to remove the essential oils present. For the MOSH fraction an Al_2O_3 clean-up needs to be done, for the MOAH fraction a SPE clean-up with silica gel, followed by epoxidation. The epoxidation needs to be done carefully to quantitatively remove the present interferences. This means that depending on the kind of sample, longer reaction times or even further addition of m-CPBA may be needed, as will be discussed in chapter 3.4.1 General Aspects.

2.5.7. Lubricating Oils

For lubricating oils, it needs to be differentiated, if the oil present is mineral-oil-based or plant-based.

If the oil was mineral-oil-based, sample preparation was easy. A 10 g L^{-1} stock solution of the oil or fat in n-hexane was prepared and further diluted 1:20 in *n*-hexane (e.g. 50 µL of stock solution diluted in 950 µL of hexane). 10 µL of the dilution were directly injected into the HPLC-GC-FID for a screening approach, 50 µL were used to gain more sensitivity, if needed.

If the oil was plant-based, sample preparation had to start from the premise that the same interferences are present as in edible oils and fats. To achieve the needed lubricating oil properties, many of the samples were mixtures of different natural fats or oils. As described for the mineral oil-based samples, a screening was done to decide, which clean-up steps were needed. A comprehensive clean-up as described for edible oils and fats in chapter 3.4.3 was applied to most samples.

Performing the screening as described in chapter 3.4.3 (300 mg sample in 600 µL hexane) would also be possible and would give a similar result.

3. Results and Discussion

3.1. Results Method Development and Validation

3.1.1. Test of Solvent for MPPO Extraction

For extraction of MPPO after the migration experiments, the solvents acetone, *n*-hexane and a mixture of acetone and *n*-hexane (1:1; v/v) were tested.
Using the rather polar solvent acetone to extract the mainly non-polar substances of interest (MOSH and MOAH) was not appropriate. It resulted into cloudy extracts or even precipitation after solvent evaporation to 1 mL. It was seen in the GC-MS analysis that acetone was not able to keep the long chain n-alkanes in solution. Therefore, the precipitation occurred. In contrast, there was no significant difference in the extraction of the polar substances, such as e.g. DIPN or DEHP (see Figure 14; DIPN and DEHP marked with circles).

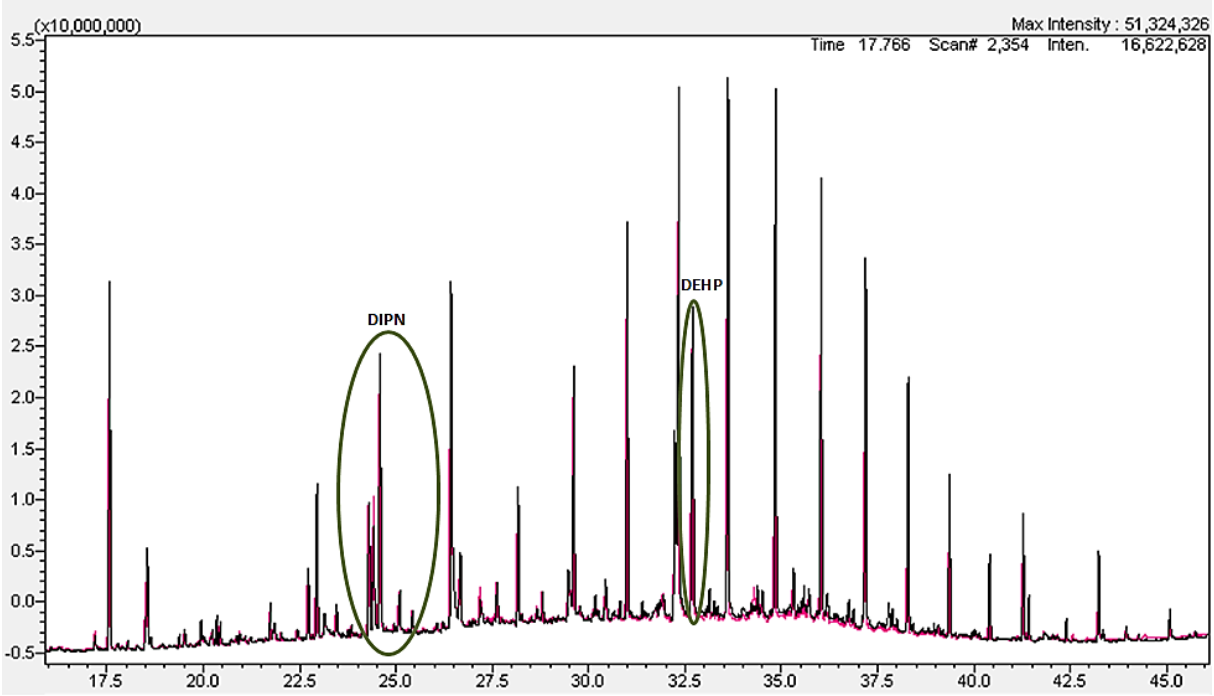

Figure 14: Comparison of *n*-hexane (black) and acetone (red) for the extraction of MPPO.

When using the mixture of *n*-hexane and acetone an interesting phenomenon was seen: after solvent evaporation to 0.5 mL and addition of the 0.5 mL solvent mixture used for washing the evaporation vial, a phase separation occurred. GC-MS measurements of both fractions were performed. It seemed that the lower phase was made up from acetone, not including a significant number of analytes. The upper phase was made up from hexane, including all analytes of interest. Obviously, the analytes were enriched in the hexane, leading to such a non-polarity that the acetone separated from the mixture.
Only the extraction with n-hexane gave reliable results and was therefore chosen as solvent of choice for extraction of the MPPO.

3.1.2. Test of Storage Conditions

As already discussed, the standard testing conditions for migration experiments are either 10 days at 40°C or 10 days at 60°C. Since such long testing times are inconvenient for the screening of the barrier properties of many samples, alternatives were tested. The Arrhenius equation was used to calculated storage conditions being equal to the standard ones. Furthermore, conditions widely used for screening approaches in industry were tested. All used conditions were summarised in Table 8 in the experimental section and the results in Figure 15.

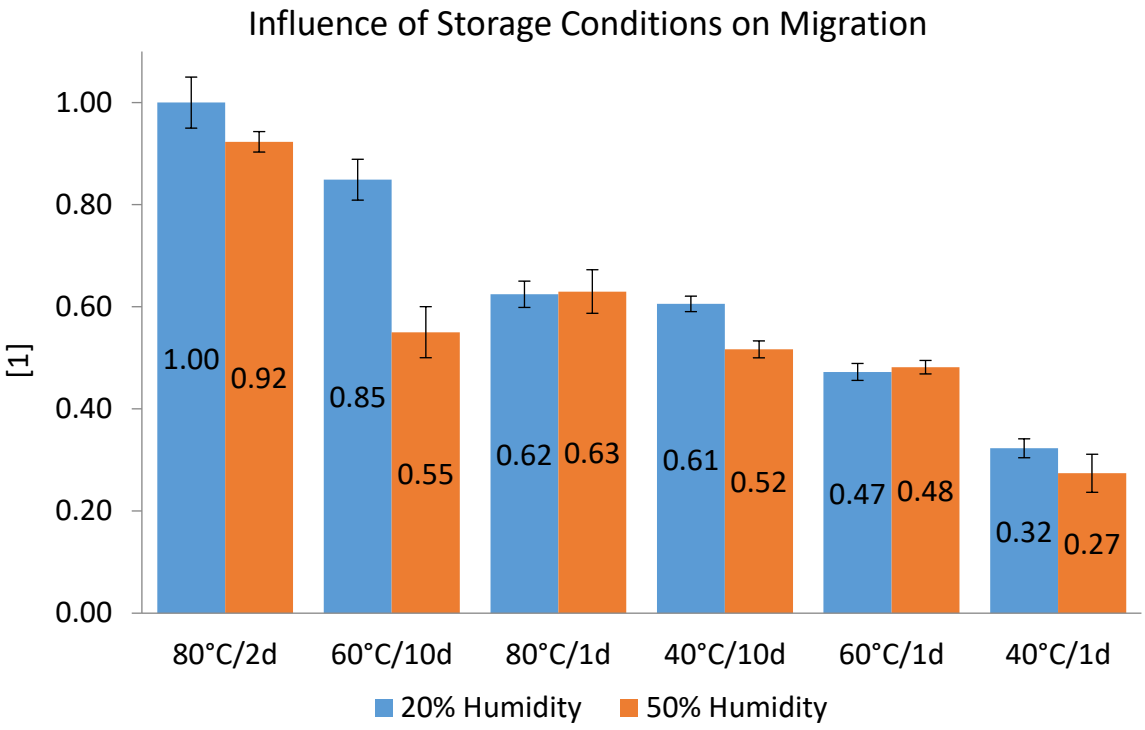

Figure 15: Results for test of storage conditions for overall migration. Experiments performed at the given conditions in triplicates at 20% relative humidity and 50%, respectively. Values are normalized to highest result. n=3.

As can be seen in Figure 15, the highest migration was seen for the 80°C/2 d with 20% relative humidity test. All other results were normalized to that. The same temperature and time conditions but with 50% relative humidity, as well as the 60°C for 10 days with 20% humidity, gave comparable, but little lower results. Those conditions would simulate storage of 299 and 307 days (~10 months) at room temperature, respectively, and are considered to be long time storage. According to the calculated conditions, storage at 80°C for one day would simulate storage at room temperature for 153 days (~ 5 months). Migration at these conditions did not show half the value of the 10 months simulation as one might think; it is only decreased by about 30-40%, compared to the long-term storage conditions. Surprisingly, the conditions 40°C for 10 days, simulating 47 days at room temperature, and 60°C for 1 day, simulating 30 days at room temperature, showed also similar migration. Lowest migration was seen for the 40°C/1 day test, simulating only 5 days storage at room temperature.

From the results it can be concluded that the conditions 80°C for 2 days with 20% relative humidity are comparable to the 60°C for 10 days conditions. A slight overestimation of results occurs by using them, but it was considered that this is more appropriate than using 80°C and 1 day and receiving an underestimation of migration.
The influence of the changed humidity on the overall migration of volatile substances into the MPPO seems to be small. It looks like that the 50% humidity led to slight decrease of the migration in some cases. However, the results for 10 days at 60°C with 50% humidity was significantly lower than the value for 20% humidity, which was neither seen for any other condition, nor could it be explained. It probably resulted from any non-detected issue during sample preparation or analysis.

For the migration of MOSH and MOAH the various conditions were evaluated as well and the results are summarised in Figure 16.

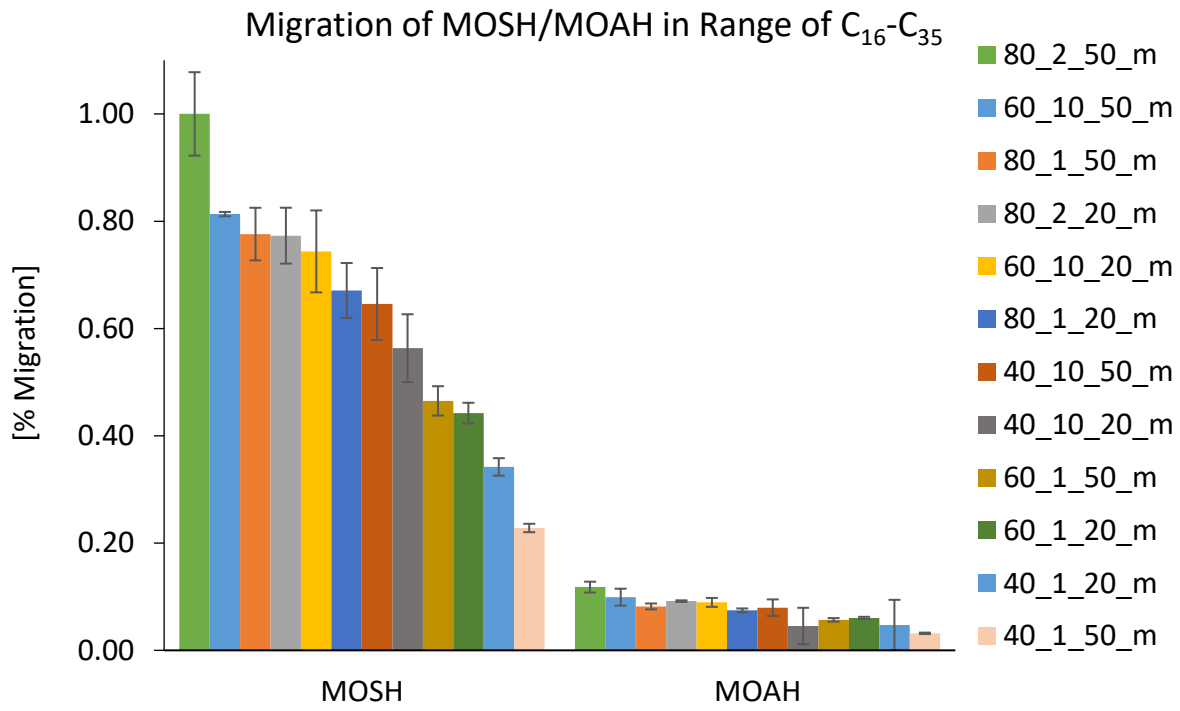

Figure 16: Results for test of storage conditions for migration of MOSH and MOAH in range of C_{16}-C_{35}. Experiments performed at the given conditions in triplicates at 20% relative humidity and 50%, respectively. Values are normalized to highest result.

In general, the data showed the same results as for overall migration. The conditions 80°C/ 2 days with 50% relative humidity are the most severe for the paper sample, resulting into the highest migration. The conditions 60°C for 10 days with 20 and 50% humidity, 80°C/ 2 days with 20% humidity and 80°C/ 1 day with 50% humidity gave comparable results. Migration of MOSH and MOAH than started to decrease, depending on the simulated storage

time. Least migration again was seen for the conditions 40°C/ 1 day, simulating only 5 days storage at room temperature.
For the migration of MOSH and MOAH it seemed that increasing the relative humidity from 20% to 50% increased migration at least slightly, except for the 40°C/ 1 day test.

In summary, since the standard conditions are 60°C for 10 days are inconvenient for an easy and quick screening approach of many samples, an alternative was needed. Therefore, the aim of this study was to find storage conditions, which would simulate long time storage at room temperature as fast as possible. It was decided to use 80°C for 2 days at 20% relative humidity to generate results in short time, while still being comparable with the standard test conditions. It was accepted and considered in results evaluation that these conditions would probably result in a slight overestimation of migration.

3.1.1. Validation

Validation of migration and permeation experiments was partly described in (Walzl, et al., 2019)

3.1.1.1. Validation of Migration

Validation of the migration experiment was done by spiking a mineral oil on a fresh fibre paper sample, performing the migration experiment at 80°C for 2 days, extracting the MPPO with *n*-hexane and measuring the resulting extracts on GC-FID for the overall migration, on HPLC-GC-FID for the migration of MOSH and MOAH and on GC-MS for specific migration of the phthalates and DIPN. The experiments were performed in triplicates. As an example, the resulting GC-MS chromatograms for the levels 50 mg kg^{-1}, 25 mg kg^{-1}, 5 mg kg^{-1} and the blank value are given in Figure 17.

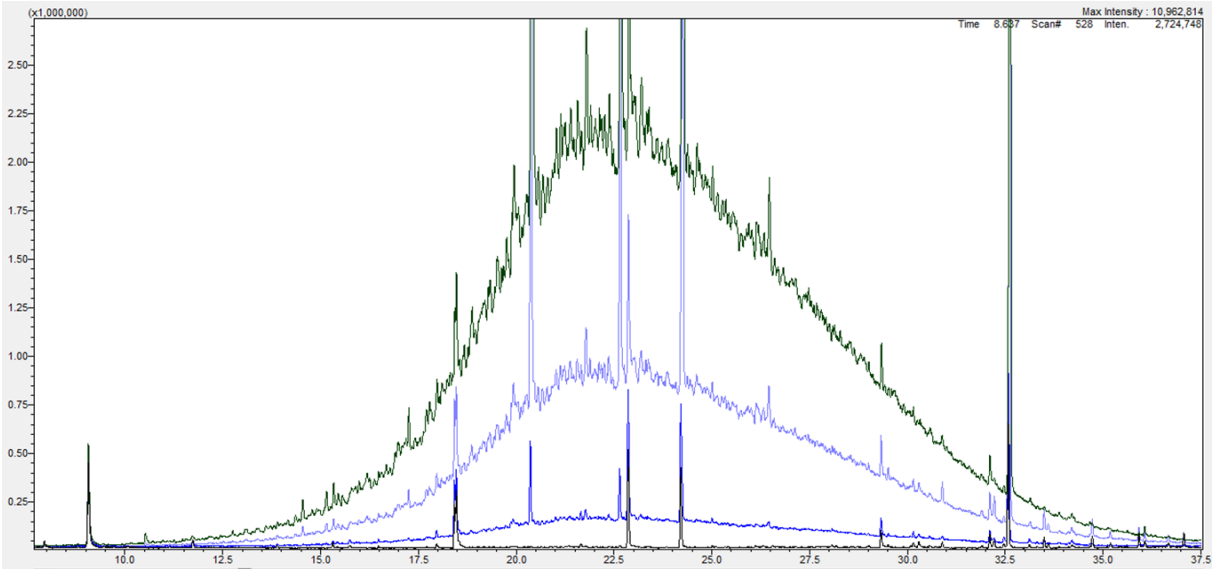

Figure 17: GC-MS chromatogram of validation experiments: in green, spiked level of 50 mg kg^{-1}, light blue 25 mg kg^{-1}, dark blue 5 mg kg^{-1} and black the blank value (non-spiked paper sample); spiked phthalates and internal standards sitting on top of the hump.

Evaluation of the received data was done with "ValiData", an Excel-macro for statistical evaluation of analyses data sets (Version 3.02.48; by W. Wegscheider, et.al). Validation of permeation was done separately (see chapter 3.1.1.2).

Overall migration was validated at the levels 0, 2, 5, 10, 25 and 50 mg kg^{-1} food. Limit of detection and quantification were determined via the calibration method as 1.04 and 3.45 mg kg^{-1}. The variance test and the linearity test showed no significant differences at a level of 99%. Relative standard deviation of the method was 18.7%. The overall migration limit for a food contact material is 60 mg kg^{-1} and far above our limit of detection.

Migration of MOSH was evaluated between C_{16} and C_{35} at the levels 0, 1.6, 4, 8, 20 and 40 mg kg^{-1}. An example for the resulting HPLC-GC-FID chromatograms is given in Figure 18. Limit of detection and quantification were determined via calibration method as 0.81 and 2.88 mg kg^{-1}. The variance test and the linearity test showed no significant differences at a level of 99%. Relative standard deviation of the method was 5.73%. The maximum amount of MOSH considered to be acceptable at the moment is 2 mg kg^{-1} ([BMEL] Federal Ministry of Food and Agriculture , 2014), which is easy detectable with the obtained limit of detection.

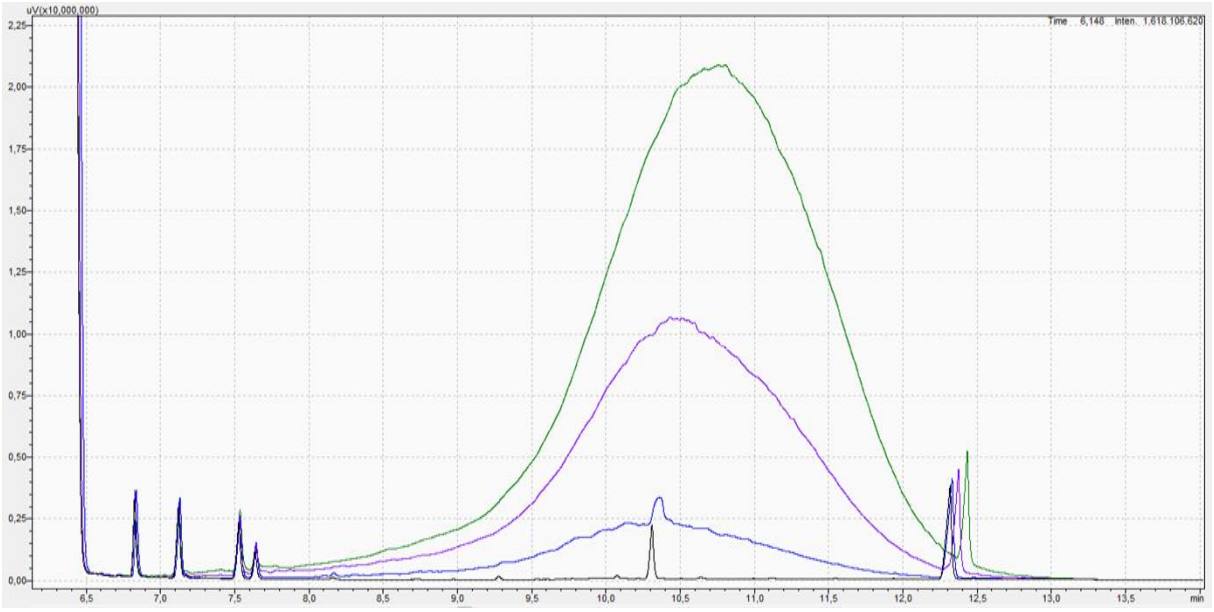

Figure 18: HPLC-GC-FID chromatogram of MOSH fraction resulting from validation experiments: in green, the highest spiked level of 40 mg kg^{-1}, in purple 20 mg kg^{-1}, dark blue 4 mg kg^{-1} and black the blank value (non-spiked paper sample).

Migration of MOAH was evaluated between C_{16} and C_{35} at the levels 0, 1, 2, 5, 10 mg kg^{-1}. An example for the resulting HPLC-GC-FID chromatograms is given in Figure 19. Limit of detection and quantification were determined via calibration method as 0.30 and 1.05 mg kg^{-1}. The variance test and the linearity test showed no significant differences at a level of 99%. Relative standard deviation of the method was 6.78%. The maximum amount of MOAH

considered to be acceptable at the moment is 0.5 mg kg^{-1} ([BMEL] Federal Ministry of Food and Agriculture, 2017), which is easy detectable with the obtained limit of detection.

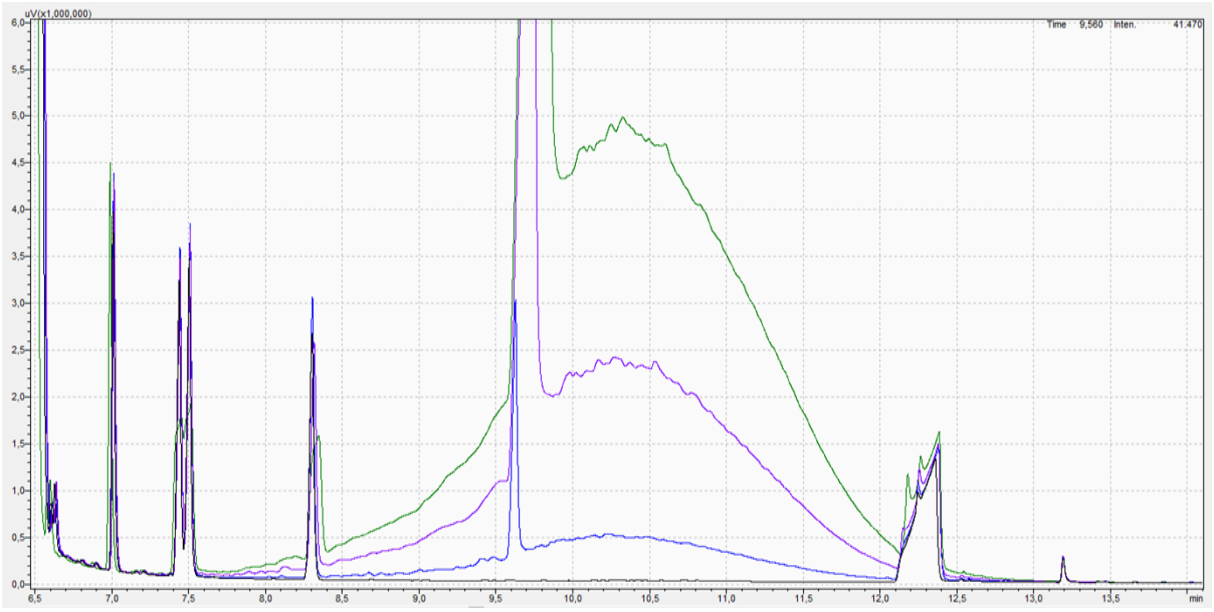

Figure 19: HPLC-GC-FID chromatogram of MOAH fraction resulting from validation experiments: in green, the highest spiked level of 10 mg kg^{-1}, in purple 5 mg kg^{-1}, dark blue 1 mg kg^{-1} and black the blank value (non-spiked paper sample).

As proof of concept, the three phthalates DnBP, DiBP, DEHP and the DIPN were validated for their specific migration at the levels 0, 10, 50, 200, 500 and 1000 µg kg^{-1}. Results are summarised in Table 16. The specific migration limits for DEHP, DiBP and DnBP are 300 µg kg^{-1} foods, whereby also the sum of DiBP and DnBP should not be higher than this level. These SMLs are again far higher than our detection limit.

Table 16: Summary of validation results for phthalates and DIPN (n=3).

	LOD [µg kg^{-1}]	**LOQ [µg kg^{-1}]**	**Linearity at 99%**	**Variance at 99%**	**Rel. Stddev. of method [%]**
DnBP	2.59	9.42	ok	ok	3.20
DiBP	3.68	13.3	ok	ok	4.54
DEHP	3.10	11.3	ok	ok	3.83
DIPN	3.39	12.3	ok	ok	4.20

In summary, the achieved limits of detection allow to determine the relevant parameters with statistical certainty. The linear range of the method was assessed and the variances and the standard deviation of the method showed good results as well. The validation experiments showed that the migration method is appropriate for its intended use.

3.1.1.2. Validation of Permeation and Two-Sided Migration Test

The use of deuterated *n*-alkanes made it possible to perform two-sided tests in the migration cells. This means the determination of migration and permeation was possible in one experimental setup without any interferences. This saved a lot of time and resources and provided a quick and easy screening method for examining the behaviour of the natural polymers used as functional barriers. Furthermore, it was decided to use cellulose as a donor in the bottom of the cell, similar to the taped barrier test (see chapter 3.1.2), since it better simulates a real packaging situation (e.g. the contaminants aren't just present in the air, but released from a secondary packaging instead).

As a first step in the validation procedure, the experiments done for the raw paper characterisation (see chapter 3.2.1.1) were evaluated. Permeation was analysed simultaneously with overall migration on the GC-FID for these experiments. However, the FID lacked on the possibility of clearly identifying individual substances in the hump of unresolved substances. This made the evaluation of permeation difficult. It was decided to use GC-MS for the analysis of the permeation instead, since it is more sensitive and a clear identification of the substances is possible by the mass spectrum. If any results given in this work were still determined on GC-FID, it is indicated with them.

Figure 20 gives an example of a GC-MS chromatogram resulting from a two-sided migration experiment: The overall migration of the recycled raw paper sample is given as black trace. It includes the internal standards added for quantitation and the substances of the permeation standard, which permeated through the paper sample into the MPPO during the experiment. For comparison, the internal standards are given as red trace (direct injection of 1 µL standard in a concentration of 5 µg mL^{-1}; in order of elution: $C_{12}D_{26}$, benzophenone-d_{10}, $C_{19}D_{40}$, DEHP-d_4 and DnBP-d_4) and permeation standards as green traces (direct injection of 1 µL standard in a concentration of 10 µg mL^{-1}; in order of elution: menthol, eugenol, $C_{14}D_{30}$, vanillin, acetovanillone, $C_{16}D_{34}$, $C_{20}D_{42}$, $C_{24}D_{50}$, $C_{28}D_{58}$).

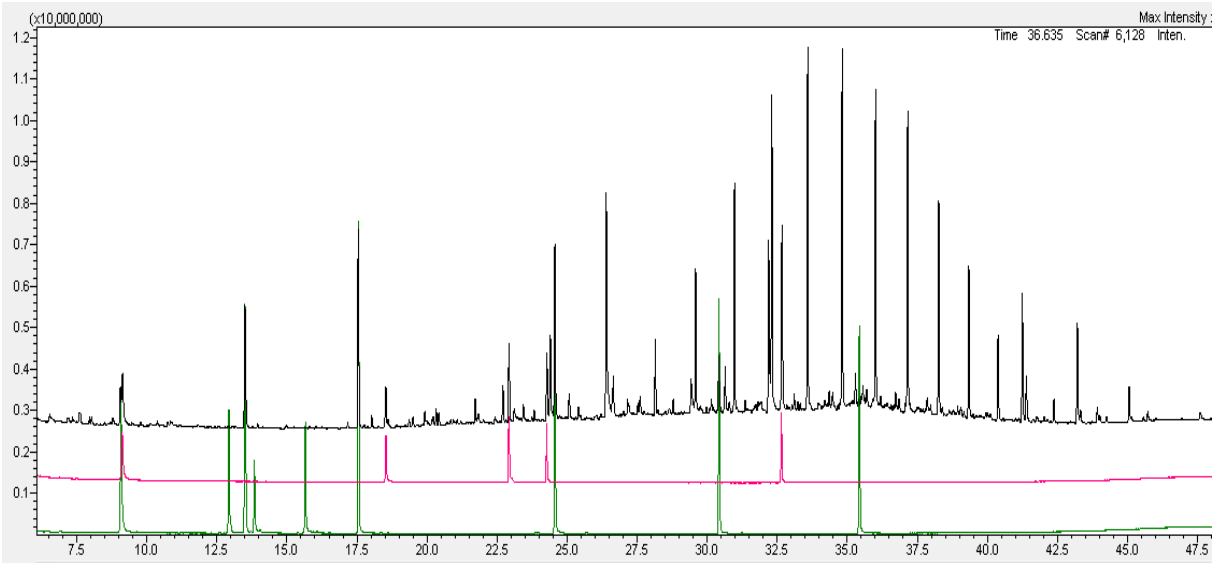

Figure 20: Chromatogram of recycled raw paper showing the overall migration (in black), internal standard (red) and the spiked permeation substances (green).

As already discussed in the introduction, the gas-phase migration and permeation of substances into dry food are limited by their volatility. It has been shown that it is relevant for substances up to a chain length of C_{24} and no longer detectable for those with a chain length of C_{28}. Consequently, higher-boiling substances do not migrate into food, but remain in the packaging material (Lorenzin, et al., 2010) ([BfR] German Federal Institute for Risk Assessment, 2012). In comparison, compounds with a chain length of up to C_{35} can be transferred through wetting contact (Mariani, et al., 1999) (Lorenzi, et al., 2013). The results found in this study were according to theory. The transfer from the recycled raw paper into the food simulant (direct contact) was found for compounds of chain lengths up to C_{35}, while the highest levels for permeation – which takes place through the gas phase – were found for the d-C_{14} and d-C_{20} compounds, the permeation of d-C_{24} was low and that of d-C_{28} was no longer detectable (see Figure 20 and Figure 21). The behaviour of the added aroma active substances yielded an interesting observation: although all four compounds have similar volatility and boiling points in the same range as C_{12}-C_{16} (see Table 17), only menthol permeated through the papers.

Table 17: Given are the boiling points and polarity of the aroma active substances and the n-alkanes.

Substance	Boiling Point [°C]
Menthol	212
C_{12}	216
C_{14}	254
Eugenol	254
Acetovanillone	265
Vanillin	285

The polar groups of the aroma active compounds apparently interact strongly with the polar groups of the paper. Therefore, it was decided to do further tests and comprehensively validate this behaviour.

The evaluation started with the facts seen and described for the taped barrier test: It was described that the polar surrogate substances di-propyl phthalate, 4-methyl benzophenone and triethyl citrate hardly ever permeated into the acceptor in significant amounts. Tests showed that they were adsorbed into the recycled paperboard used as donor. This effect was significantly more distinct, when the donor was conditioned at 40°C for two weeks (Biedermann-Brem, et al., 2017b). In this work, the theory arose that also without conditioning the polar aroma active substances are adsorbed on the donor and not released during the migration experiment. Therefore, two tests series were made to confirm this theory: one, with a piece cellulose as donor in the bottom of the cell and one, without cellulose. The results of the permeation experiment using cellulose as a donor are shown in Figure 21. It can clearly be seen that the polar aroma active substances eugenol, vanillin and acetovanillone and the high boiling *n*-alkane *d*-C_{28} are nearly completely lost. For the substances menthol and d-C_{24} only about one third could be found for the highest concentration, which means that two thirds are

lost. In general, for none of the substances 100% (15 ng) of the spiked amount could be found at the end of the experiment.

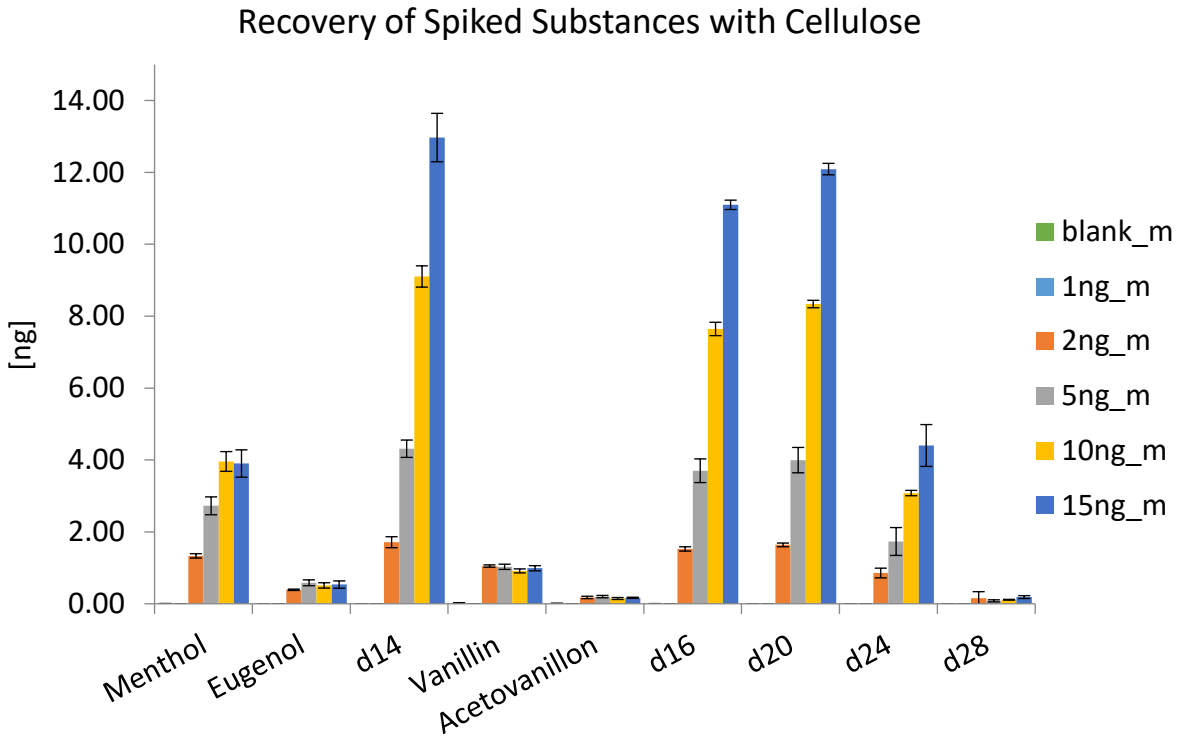

Figure 21: Permeation experiment using cellulose as a donor. n=3, given are the found absolute amounts of the substances after spiking 1, 2, 5, 10 and 15 ng absolute on the donor (labelled as "_m").

One might now think that the loss could originate from several critical steps throughout the experiment; a not properly assessed migration cell, adsorption in the paper or losses through the gas phase during the storage period, losses during the extraction step, during solvent evaporation or analysis. However, since the added internal standards would compensate for the latest three points, the reason must be found during the storage experiment. To proof the theory that similar to the taped barrier test, polar substances are strongly retained in the donor and not released anymore (Biedermann-Brem, et al., 2017a), the same test series was performed again. This time the donor in the bottom of the cell was removed and the results were summarised in Figure 22.

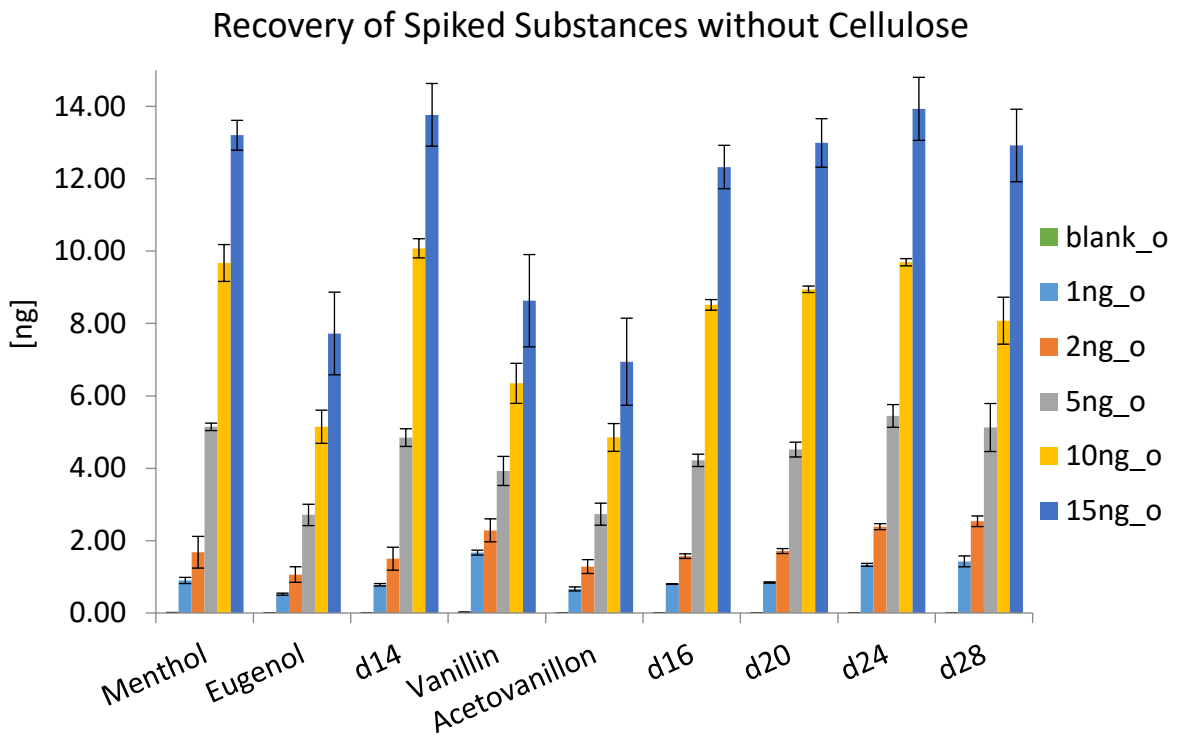

Figure 22: Permeation experiment without using cellulose as a donor. n=3, given are the found absolute amounts of the substances after spiking 1, 2, 5, 10 and 15 ng absolute without the donor (labelled as "_o").

In contrary to the experiment with cellulose, in the second series no substance was lost completely. There were still losses for the polar aroma active substances eugenol, vanillin and acetovanillone, but these were not as drastic as before. There were also still losses for all substances, but they were much smaller and might be explained by the adsorption in the tested paper sample.

To get further proof of the theory that the aroma active substances are adsorbed in the cellulose, it was tried to extract them after performing a two-sided migration experiment. The results showed that it was possible to extract the missing amounts of the non-polar d-C_{24} and d-C_{28} giving a summed recovery of 92% and 115%. However, the polar aroma active substances could not be extracted from the cellulose anymore using this simple attempt. In comparison, it was described just shortly later by (Biedermann-Brem, et al., 2017b) that they had to use the combination of MTBE/MeOH (95:5, v/v) and extraction at 40°C over night to get satisfying extraction efficiencies for the donor paperboards they used.

Following these test series, the influence of humidity in combination with varying storage temperatures and periods was assessed. The industry partners of this project had several concerns on the influence of humidity on the barrier properties. Humidity was only shortly mentioned in the publications of Biedermann-Brem, were it was concluded that further tests had to be done (Biedermann-Brem, et al., 2017b). Barnkob & Petersen described in their work that significantly higher migration of benzophenone from paperboard into the food simulant

MPPO was found, if the relative humidity was increased at constant temperature (Barnkob, et al., 2013).

The data pool and information gained in the performed test series of this work were immense, involving several complex relations needing to be discussed. First of all, the behaviour of the polar aroma active substances is completely different from the non-polar alkanes, as can be seen in the results above. Therefore, the changing humidity influenced the two substance groups in another way. Secondly, the substances have different volatilities from low (e.g. d-C_{14}, d-C_{16}) to high (e.g. d-C_{28}) as was also already discussed above. Consequently, the variation of storage conditions had a big influence depending on the one hand on the simulated storage time and on the other hand on the used temperature or volatility of the substances, respectively. The correlations are discussed in detail with the results shown in Figure 23 to Figure 26.

At this point the used nomenclature should be explained: the first number refers to the temperature used, the second to the days of storage, the third to the used humidity and the letter at the end either to experiment performed with cellulose as donor (marked as “m”) or to performed without donor (marked as “o”). The individual conditions are separated by an underline. For example, an experiment performed with cellulose as donor at 80°C for 2 days at 50% relative humidity would result in 80_2_50_m. An experiment performed without a donor at 60°C for 10 days at 20% relative humidity in 60_10_20_o.

The first results shown represent the already discussed relation of decreasing volatility with increasing chain length of the *n*-alkanes (Figure 23).

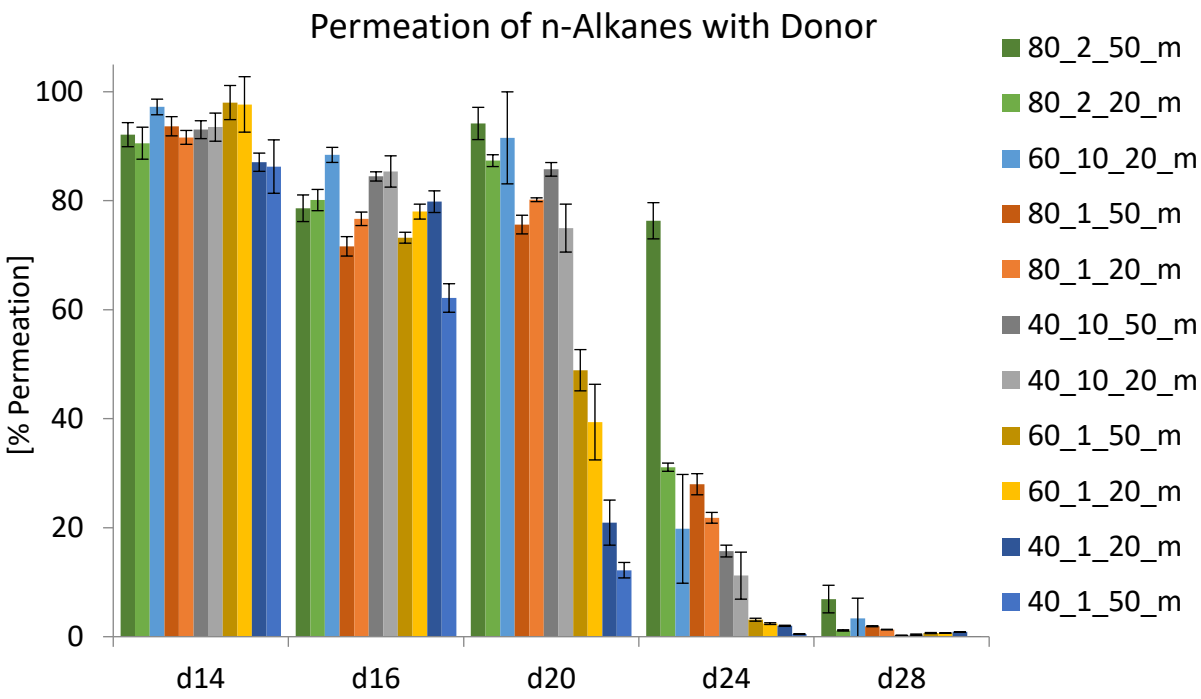

Figure 23: Permeation results for *n*-alkanes with cellulose used as donor for varying conditions. n=3, given as percentage of permeation through the tested sample into the food simulant MPPO for the individual substances. Sorted in descending order of simulated storage period.

The d-C_{14} is rather volatile permeated to about 90% for all used conditions. The d-C_{16}, which is a little bit lesser volatile, did not permeate at such high amounts anymore and results evened out at about 80% permeation. No clear trend on the influence of humidity and storage temperature could be drawn. For d-C_{20} the temperature/time relation started to hit in more clearly. It only permeated to about 20% for the weakest conditions of 40°C for 1 day (simulating 5 days as room temperature). Also, for the 60°C/ 1 day experiment (simulating 30 days at room temperature) the permeation decreased to about 40%. For the less volatile d-C_{24} the influence of temperature could be seen very severely, while d-C_{28} was not released from the donor anymore in amounts higher than 10%. This trend could only be explained by the decreasing volatility by increasing chain length. Similar relations were seen for the influence of humidity; while for d-C_{14} and d-C_{16} no clear differences were seen, there seemed to be a little difference for d-C_{20} but a much clearer one for d-C_{24} and d-C_{28}. The permeation of the experiments performed at 50% humidity were for certain conditions 10% to 50% higher than for those performed at 20% humidity. In conclusion, for certain temperature, time and humidity conditions was still be possible to "force" the long chain alkanes into the gas phase, but high standard deviations between the triplicates, as e.g. for the C_{28}, indicate that this behaviour is neither stable nor repeatable.

In comparison, the results of the experiments without the use of cellulose as donor were more consistent. They are given in in Figure 24 below.

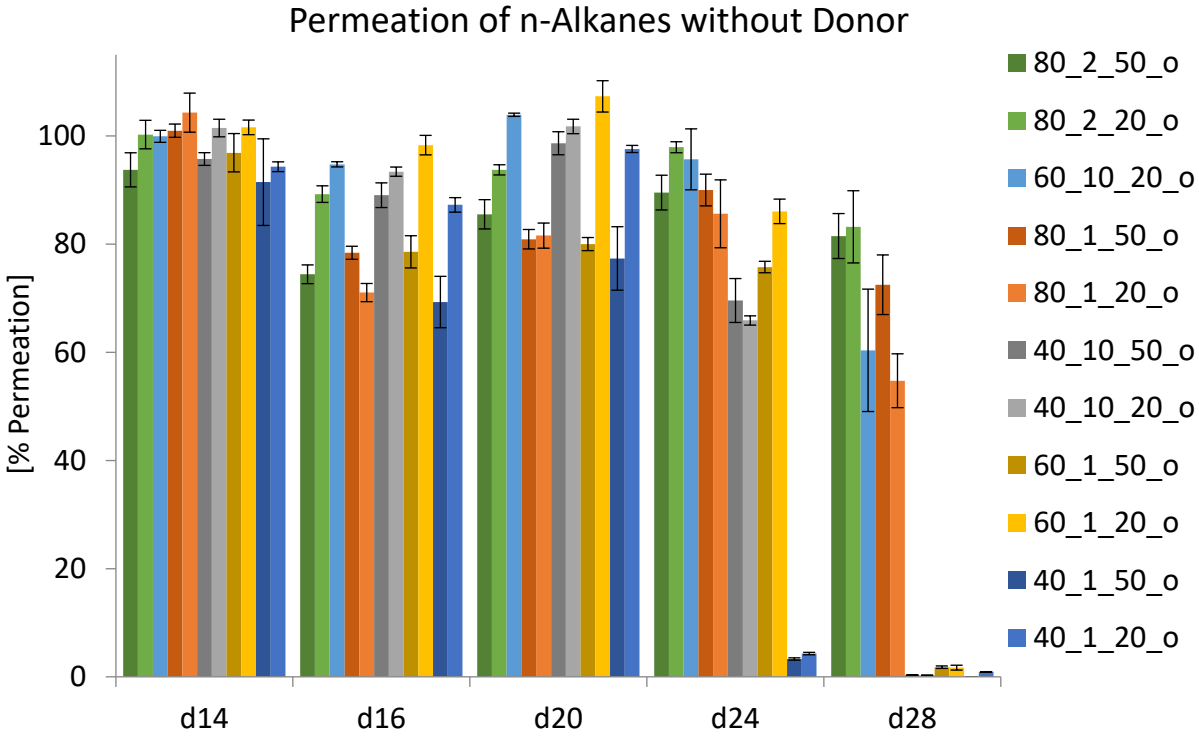

Figure 24: Permeation results for *n*-alkanes without cellulose used as donor for varying conditions. n=3, given as percentage of permeation through the tested sample into the food simulant MPPO for the individual substances. Sorted in descending order of simulated storage period.

As can be seen in Figure 24 without the donor, the permeation did not break in for the less volatile substances, except for the experiments performed at 40°C for 1 day (C_{24}) and 40°C for 1 and 10 days and 60°C for 1 day (C_{28}), respectively. This behaviour also needs to be considered, when performing experiments at elevated temperature (>40°C), since those less volatile substances will migrate/permeate in the experiment, but not during storage at room temperature. No clear trend between could be drawn from the experiments at 20% relative humidity and 50% relative humidity in Figure 24. For some results, the 50% humidity data showed higher results, for other the 20% humidity ones did. Comparison of the general behaviour of the substances when performing the experiment with and without cellulose as donor, let to the conclusion that without cellulose the *n*-alkanes permeated much easier and more consistent. This can probably be explained by the fact, that they did not need to be released from a donor anymore. A change in humidity had higher influence when cellulose was used as donor, indicating that humidity may influence the adsorption behaviour on the donor. However, influence of humidity was low, the indicated trends could not be clearly proven and only hypothesis were drawn from this data set.

In contrary to the *n*-alkanes, the results for the aroma active substances showed a completely different behaviour: The influence of humidity got much more distinct, as can be seen in Figure 25 and conclusions were drawn easier.

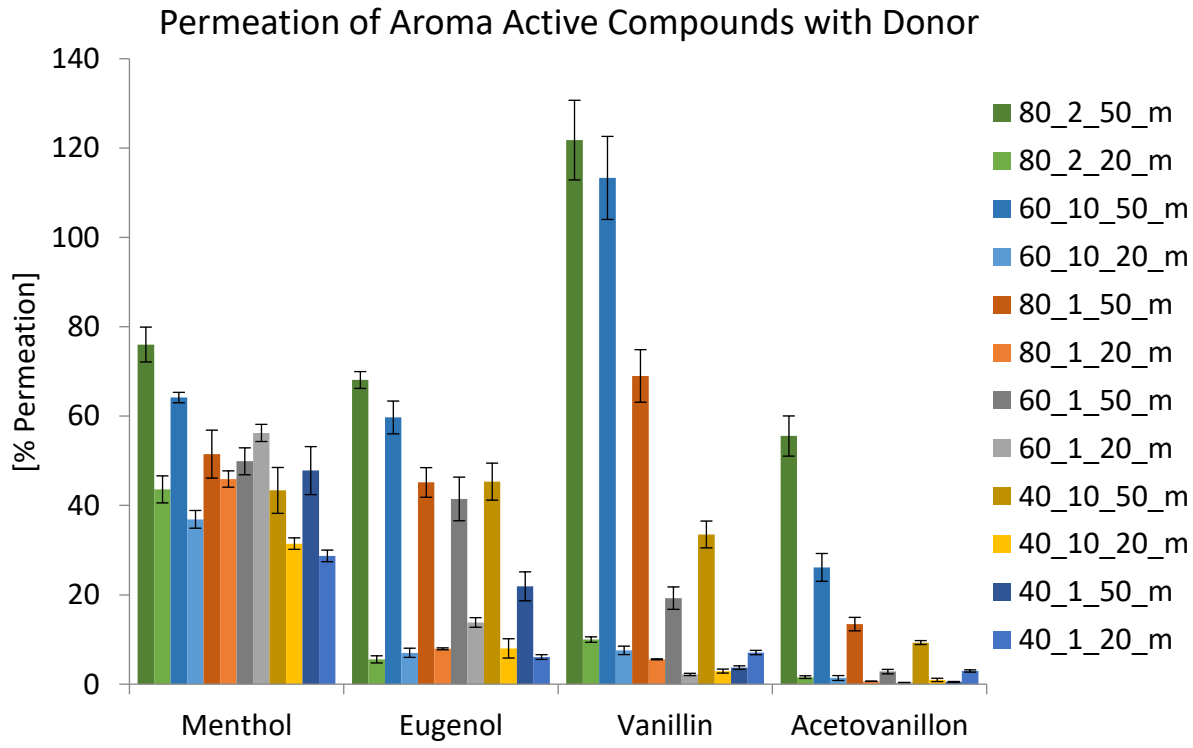

Figure 25: Permeation results for aroma active compounds with cellulose used as donor for varying conditions. n=3, given as percentage of permeation through the tested sample into the food simulant MPPO for the individual substances. Sorted in descending order of simulated storage period.

While most of the substances did hardly permeate for the experiment performed at 20% relative humidity (<10% permeation; except menthol), there was a clearly higher permeation for the experiment performed at 50% humidity. This strengthens the hypothesis that adsorption on the sides of the donor or paper sample is inhibited at high humidity. It is assumed that the highly polar water molecules present are blocking the adsorption sides. However, permeation still remained relatively low in the experiments, when compared to the high amounts of *n*-alkanes found. The highest amounts permeated through the samples and into the MPPO for vanillin, followed by menthol (mean of about 50% permeation) and eugenol. Acetovanillone permeated to only small amounts (mean of ~10-15%). The values of about 120% for vanillin may be explained by the fact that it is initially present in recycled board as degradation product of lignin. Figure 26 shows that without the use of cellulose as donor, permeation for the aroma active substances got significantly higher, when compared to the results with cellulose as donor in Figure 25. This is comparable to the first test series done (see Figure 21 and Figure 22).

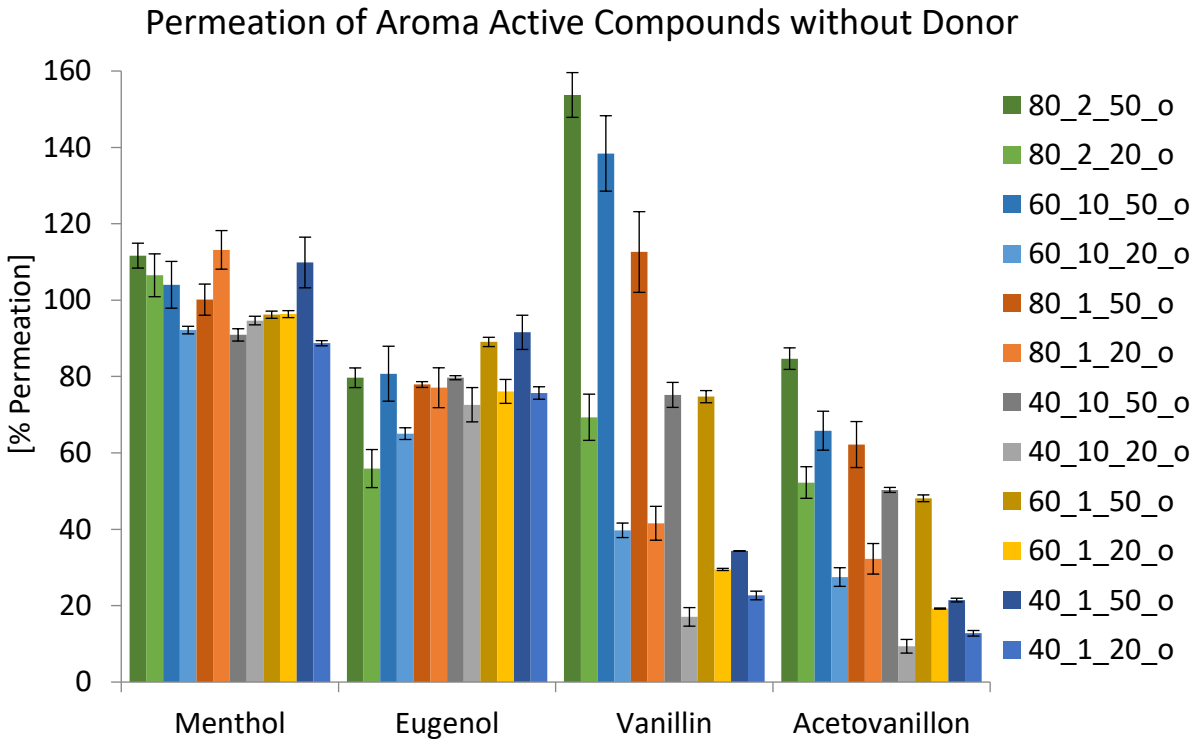

Figure 26: Permeation results for n-Alkanes without Cellulose used as Donor for varying conditions. n=3, given as percentage of permeation through the tested sample into the food simulant MPPO for the individual substances. Sorted in descending order of simulated storage period.

Menthol now permeated to about 100%, eugenol to about 70% in average. The influence of the humidity or the changing storage conditions on them was nearly neglectable. More severe was the influence of humidity on vanillin and acetovanillone. The results for vanillin showed partly huge amounts of permeation of up to nearly 160%, strengthening the theory that at these severe conditions of e.g. 80°C/ 2 days at 50% relative humidity the vanillin present as degradation product of lignin in the paper sample was also released. Similar were also the results for the

60°C/ 10 days and 80°C/ 1 day at 50% relative humidity. Acetovanillone did not show such a huge dependence on the humidity, but still permeated to a much higher amount, when the experiment was performed at 50% relative humidity. The influence of humidity decreased again for the least severe storage conditions used – 40°C for 1 day, simulating only 5 days at room temperature.
Concluding the results for the aroma active substances, the behaviour of them was much more influenced by the humidity and the presence of cellulose as donor, than the non-polar alkanes. Without cellulose the substances permeated to a higher amount and in contrast to the alkanes, the change in humidity had a big influence. This influence was very pronounced for the experiment with cellulose, were at low humidity low permeation happened, while at higher humidity the substances also permeated at higher amounts. Again, it was concluded, that a change in humidity mainly influences the adsorption behaviour on the donor.

The last point evaluated for the permeation experiment was the fact that for some coated paper samples the permeation was higher than for the non-coated samples. The test on the wetted and dried paper sample showed a clear result. Permeation increased to about 10-20% for the deuterated *n*-alkanes, as can be seen illustrative for deuterated alkanes in Figure 27. These results confirm the theory that free shrinking of wetted paper loosens the fibre network, leading to larger voids between the fibres and therefore increased permeation through it.

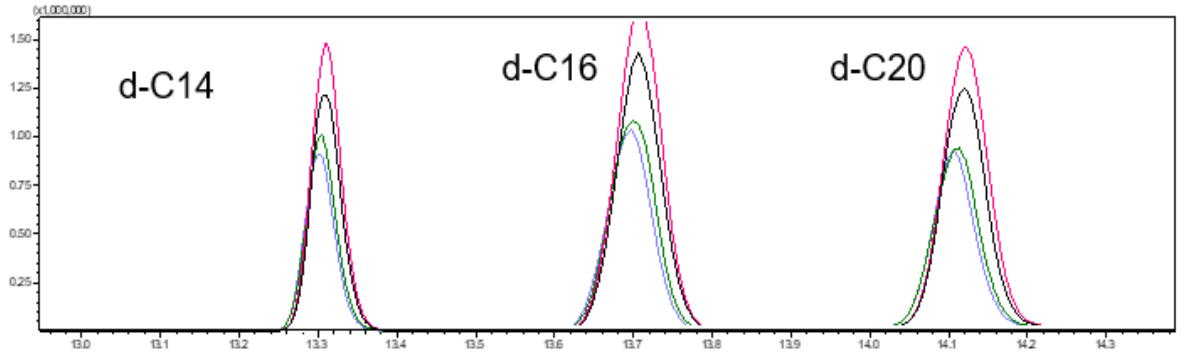

Figure 27: Permeation increased after wetting and drying of a paper sample (upper two lines in chromatogram) in comparison to a non-treated sample (lower two lines in chromatogram).

In summary, this chapter shows that permeation is a complex phenomenon being influenced by the test temperature and time, the humidity, and the nature of the substances in terms of polarity and volatility. No general rules can be established, since the behaviour is varying from substance to substance and from test condition to test condition. Further work needs to be done to understand the complex relations between paper, substances and test conditions.

3.1.2. Taped Barrier Test

3.1.2.1. Comparison of Siliconized Paper and MPPO

The taped barrier test was described as a quick and easy test for the barrier properties of films, not needing special migration cells or the difficult to handle MPPO. Instead, test packs were made, a PE film used as acceptor and the packs wrapped simply in aluminium foil (Fiselier, et al., 2012). The advantages are clear and promising, but a few years later an adaption of the method was published. The PE film was replaced by a special prepared silicone-coated paper, which should act as a better acceptor for the newly introduced surrogate substances of different polarities (Biedermann-Brem, et al., 2014). The siliconized paper was extensively tested and adjusted several times afterwards and its advantages and suitability pointed out in the following publications (Biedermann-Brem, et al., 2017a) (Biedermann-Brem, et al., 2017b).

The siliconized paper used in this work was purchased from BGB Analytik AG. It was sent to us after requesting the paper used for the taped barrier test in the publications of Biedermann-Brem, et al. It was labelled as "A4 Cellulose Paper Sheet, PDMS coated, for use in taped barrier test", without any further specification. The price was quite high, in our study comparable with the one of MPPO. However, MPPO has the big advantage that it can be cleaned and re-used again and again. The second disadvantage was the cleaning of the siliconized paper; while MPPO can be simply stirred with solvent at room temperature, dried and re-used afterwards, the siliconized paper was much more challenging. If used without further clean-up, lots of high boiling siloxanes were present, overloading the chromatogram (see Figure 28). Several ways to overcome this problem were described in the respective publications: first, cleaning at 160°C for 1h was used. However, the paper was not cleaned efficiently. Therefore, a concurrent solvent recondensation (CSR) splitless injection was described and used, because of the present high boiling siloxanes (Biedermann-Brem, et al., 2014). Later, cleaning the paper during 8h at 0.1 bar and 130°C in a vacuum oven was used and gave good results (Biedermann-Brem, et al., 2017a).

In this work similar results were achieved: heating at 160°C for 1h had hardly any influence on the siloxanes present in the chromatograms after extracting the paper. Some of the more volatile impurities present before cleaning were removed, but most of them stayed. Cleaning the paper during 8 h at 0.1 bar and 130°C in a vacuum oven showed much better results. Most of the impurities were removed giving a clean acceptor (Figure 28). However, using a vacuum oven for cleaning paper is a pretty excessive method, having several disadvantages. First, the availability of a vacuum oven is limited. Second, it is costly and complex (e.g. liquid nitrogen needed, handling of oven) and third, it is time consuming, when only doing for a few sheets of paper needed. The third described method, using CSR injection, was not available for this work. To overcome the cleaning problem, it was decided to use the paper as it is and analyse the extracts in SIM-mode, so that present impurities would not interact with the analysis of the surrogates in any way. The disadvantages of this decision are that the analysis system still needs to deal with the present impurities (contamination of liner, column, etc.) and that any further information on the barrier (e.g. POSH migration from the barrier) is lost.

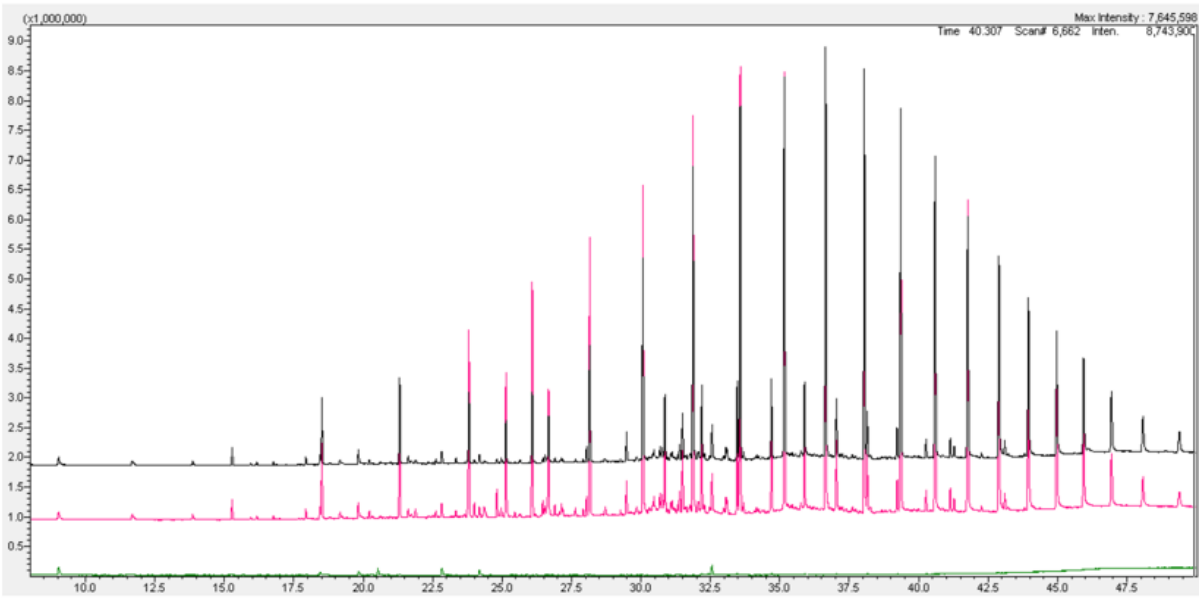

Figure 28: GC-MS chromatogram of extracted siliconized paper without cleaning (red), cleaning for 1h at 160°C (black) and cleaning in a vacuum oven at 0.1 bar, 130°C for 8h (green). Present peaks are mainly high boiling siloxanes.

The second matter to be discussed is the adsorption efficiency of the siliconized paper. Since it seems to be a good alternative to MPPO, the question arose, if it could also replace the MPPO in migration experiments. However, as already described by Biederman-Brem, the siliconized paper has only limited active sites on its surface. Therefore, it is not comparable with the food simulant MPPO or real food, since the surface area and the uptake into the latest is much higher. Nevertheless, they concluded, if only a small amount of e.g. polenta was used in their tests, which would then have a similar surface area and adsorption properties as the silicon paper, the behaviour would also be similar. They proposed that for low migration in the range of about 1% the siliconized paper acts similar to food and food simulants and changed the evaluation criterions of their test accordingly. In this work it was tried to compare the behaviour of the silicon paper with MPPO, too. A two-sided migration experiment was performed, using a recycled paper as donor and the siliconized paper cleaned in a vacuum oven as acceptor. The results were than compared with a two-sided migration experiment performed with MPPO as acceptor. The GC-MS chromatogram resulting from the migration with siliconized paper as acceptor showed the typical fingerprint of the tested recycled paper sample. However, the amounts migrated into the siliconized paper were far below the ones migrated into the MPPO as can be seen in Figure 29. The deuterated *n*-alkanes spiked in the bottom of the cell to monitor the permeation through the sample were not adsorbed at all in the siliconized paper. A large peak is present at 20 min, which was identified as diallyl phthalate, originating probably from the paper itself. The small hump between 30 and 32.5 min originates from abietic acid and degradation products. However, the hump of mineral oil and other substances typical for recycled board (e.g. benzophenones, other phthalates) are not captured. A determination of an overall migration value from the GC-FID measurements was not possible. The residuals of the

siloxanes and other substances initially present in the siliconized paper, had a much higher concentration than the detected migration into it and strongly adulterated the result.

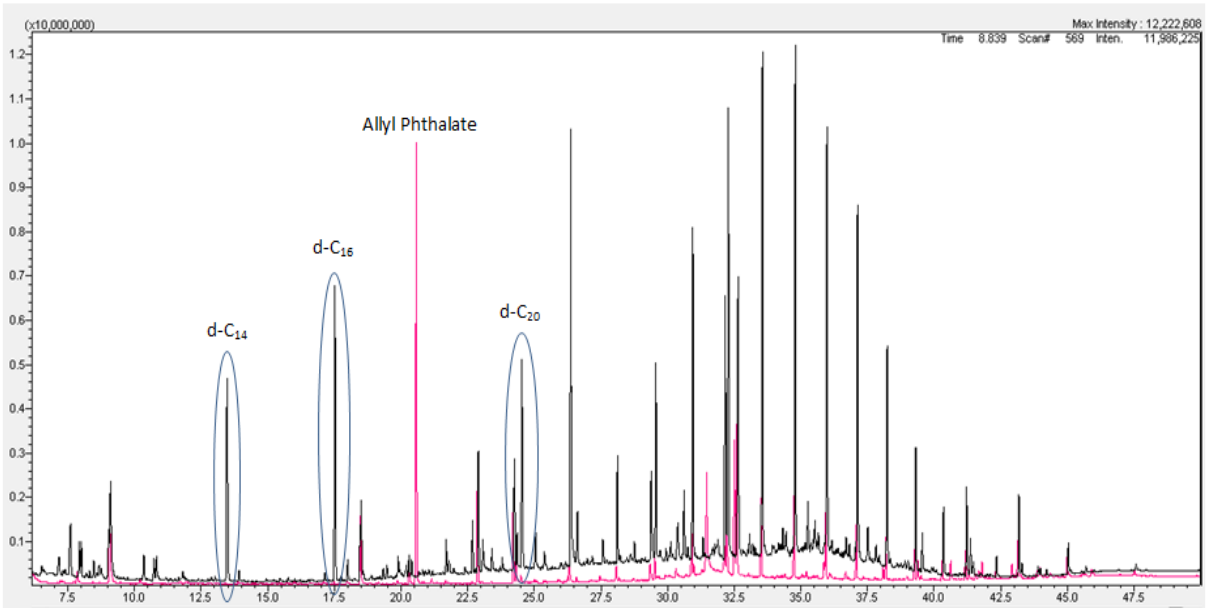

Figure 29: GC-MS chromatogram of comparison of migration into MPPO (black) and siliconized paper (red). Deuterated n-alkanes from permeation only present in MPPO, as marked with circles. Large peak in siliconized paper at 20 min is diallyl phthalate.

Since the amounts of migrated substances into the siliconized paper are not comparable with the MPPO at all, it is not possible to give any migration values in terms of e.g. mg kg^{-1}. Furthermore, replacing the MPPO with the siliconized paper as acceptor in a routine attempt would be more expensive, would strongly underestimate the real migration and is therefore not appropriate. For the use in the taped barrier test, it may be so, since the tests criterions were changed over time and better suit the behaviour of the siliconized paper.

3.1.2.1. Taped Barrier Test Applied on Chitosan Free-Standing Film

Since it was known from previous tests that chitosan is a good barrier against migration (compare with chapter 3.2.2), the first tests were done at severe conditions: Test packs (n=3) with a chitosan film as barrier were placed in an oven at 80°C and stripes cut every 4 h or 12 h overnight within two days. This temperature had already been used before in migration experiments and did not show any changes to the material. However, when applied on the pure film in the taped barrier test, the film got sticky after about 24 h and brittle after about 36 h. Interestingly, the barrier efficiency did not collapse in terms of a complete breakthrough. In detail, the barrier was tight for benzophenone till the end of the test (migration stayed <1%). C_{17} migrated to about 0.8% in the first 16 h and reached the 1% limit, afterwards. The stripe cut after 20h already showed a migration of 1.5%, the stripe at 24 h showed 2.5% and the highest value was seen after 40 h with 6.5% migration. The stripe at 48 h showed a lower concentration of about 3.8% again. The phthalate showed already high migration in the first stripe (8.3%) and migration increased consequently till the end of the test to 18%. The deviation between the

triplicates was high with e.g. up to 50% deviation between the three packs for C_{17}. This was related and explained by the high temperature and the brittleness of the barrier film.
The second test was therefore carried out at 40°C within ten weeks of test time and in triplicates again. Stripes were cut at day 5, 9, 14, 21, 28, 34 and after 72 days. The results are given in Table 18. It can be seen that DEHP is present from the first stripe to the last one in similar amounts in every test pack. It was concluded that DEHP probably is a contamination, whose origin could be the siliconised paper, the barrier film, the aluminium foil or the tape used to seal the packs. Furthermore, the deviation between the three packs and also within the same pack is again large. For example, the first replicate shows no migration of C_{17} within 9 days. On day 14 the 1% limit is reached, but the levels decrease again on day 21 and stayed below 1% till day 34. After 72 days the limit is clearly exceeded with 4.9 % migration. Similar behaviour is also seen for benzophenone, which is detected after 14 days, but not on the following tested days except the 72nd. The second replicate was tight till the 28th day and migration of C_{17} increased up to 11% afterwards. The third pack showed the worst behaviour: already the first stripe after 5 days showed a migration of 1.6% of C_{17}. However, it decreased afterwards and stayed below 1% till day 34. The third pack was also the one, where the barrier was punctured with a needle, after cutting the stripe at day 34. The proof of concept went well, the last stripe of this pack showed the highest migration for Bzp (2.7%) and C_{17} (28%).

Table 18: Results of taped barrier test for chitosan film at 40°C over 72 days (n=3); given in % migrated from donor; n.i. means not detected (LOQ of 0.2%). Values underlined reached the 1% migration limit, DEHP excluded.

Values in % of Donor		5d	9d	14d	21d	28d	34d	72d
Replicate 1	*Bzp*	n.i.	n.i.	0.20	n.i.	n.i.	n.i.	0.59
	C17	n.i.	n.i.	<u>1.0</u>	0.34	0.74	0.68	4.9
	DEHP	5.7	5.5	4.0	4.5	5.6	4.4	3.1
Replicate 2	*Bzp*	n.i.	n.i.	n.i.	n.i.	0.23	0.23	1.1
	C17	n.i.	n.i.	n.i.	0.35	<u>1.2</u>	0.96	11
	DEHP	5.2	5.0	4.1	4.2	4.6	4.7	3.4
Replicate 3	*Bzp*	0.45	n.i.	n.i.	n.i.	n.i.	n.i.	*2.7**
	C17	<u>1.6</u>	0.46	0.30	0.33	0.64	1.1	*28**
	DEHP	5.7	5.3	4.0	4.7	4.8	4.6	*3.4**

*Barrier punctured for proof of concept

In summary, no real conclusion could be drawn from these results, because they are to inconsistent. The theory of pinholes, leading to hot spots of migrations on one stripe and decreasing at the next one would fit the results (Biedermann-Brem, et al., 2014). All three packs would have been tight till day 28 or 34 and afterwards, showing a migration of C_{17} far above

1% at day 72. However, neither on the barrier, nor on the receptor, red spots of Sudan Red II had been observed. It was also never detected by the GC-MS analysis (and therefore not given in Table 18). Using the Arrhenius equation, it was calculated that a storage for 28 days at 40°C would equal a storage at room temperature for about 130 days (about 4 months), storage for 34 days at 40°C 150 days at room temperature (5 months), respectively. When comparing test pack replicate 1 and 2 with the punctured 3rd one, it could be seen that even after 72nd days, simulating about 11 months of storage at room temperature, no complete breakthrough could be seen. It seems that chitosan lowers migration down, although not being tight over the tested time period. It therefore could be considered at least as a modest barrier. Furthermore, it was decided to evaluate this behaviour more closely, since so far, no information on the barrier properties of chitosan against migration of e.g. MOSH/MOAH is available (see chapter next chapter, 3.1.2.1). Following these results, also the question arose, if the self-made barrier films were too inhomogeneous to do a reliable evaluation of the taped barrier test. Therefore, it was decided to do further tests of the method with conventionally available polymer films (see chapter 3.1.2.2).

3.1.2.1. Determination of Breakthrough Concentration for Chitosan Films

Since no information was known on the barrier behaviour of the chitosan film a breakthrough test was performed. Aim was to investigate, if the film works as a real barrier or just reducing the permeation by soaking up the substances, similar to the OPP film used for validation of the test by (Biedermann-Brem, et al., 2017b). Therefore, the constituents of the test (donor, barrier and acceptor) were placed together in a glass vial, with the possibility of free permeation in each constituent via the gas phase. Two batches of vials were tested, one batch was stored at 40°C for 1 day, 3 days, 7 days, 10 days and 35 days (five vials in total), the second batch was stored at 60°C for 1 day, 3 days, 7 days, 10 days, 17 days and 24 days (6 vials in total) as was described in the experimental section. After storage, the distribution of the surrogates was determined.

The *n*-heptadecane was found from the first vial on, which was stored at 40°C for 1 day, to about 90% in the acceptor with about 10% remaining in the donor. This distribution did not change during the test period. Even the last vial in the more severe test at 60°C stored for 24 days, showed the same result.

In comparison, the dye Sudan Red II hardly ever permeated through the air into the acceptor or the barrier and was found to 100% in the donor for the 40°C test series. For the storage at 60°C, also only about 1.5% permeated into the acceptor after storage for 24 days, with 98.5% still remaining in the donor.

Benzophenone showed an interesting behaviour in the test. The first vial extracted from the 40°C batch after 1 day of storage showed a benzophenone distribution of about 80% presence in the donor and 20% in the acceptor. After 10 days at 40°C, the concentrations started to align; only 65% were left in the donor and 35% were found in the acceptor. Using storage conditions at 60°C, benzophenone is already distributed to 60% in the donor and 40% in the acceptor. The

distribution further aligned and ended with 52% being present in the acceptor and 48% left in the donor at the end of the test after 24 days at 60°C.
The phthalate was the only surrogate found in the barrier with a concentration of about 4%. However, since the 4% level stayed nearly the same in all vials and similar concentrations were found also in the test packs, it is likely that it is a contamination present in the film intentionally. The permeation and therefore distribution of the phthalate between donor and acceptor was slow; it remained to nearly 100% in the donor throughout the test at 40°C and only started to permeate between 3 and 7 days at 60°C. In the last vials after 24 days at 60°C the permeation was still on-going with a distribution of 83% in the donor and 14% being present in the acceptor (constant level of 3% in the barrier film).
In conclusion, it was seen that none of the surrogates permeated into the barrier. Therefore, chitosan can be considered as a real barrier, rather than being a sponge, just soaking up substances that will start to leak sooner or later as e.g. described for the OPP film in (Biedermann-Brem, et al., 2017b). The results are given graphically in the attachment (Figure 80).

3.1.2.2. Taped Barrier Test Applied on Conventional Polymer Films

As already described in the experimental section, three conventional barrier films (LLDPE of 40 µm and 70 µm thickness and a 50 µm PP film) were tested in triplicates for their behaviour in the taped barrier test and for the evaluation of the test. Similar to the conditions applied by Biederman-Brem, et. al. for the OPP film, packs were stored at 40°C and at room temperature. In the first series, stripes were cut on the 1st and 2nd day, afterwards on the 7th, 15th, 21st and 35th day. Already the stripes on day 1 showed extensive breakthrough for all three films and for all surrogates. This could also be seen visually, since the Sudan Red II coloured the receptors evenly red. The red colour intensified over the test period, as can be seen in Figure 30.

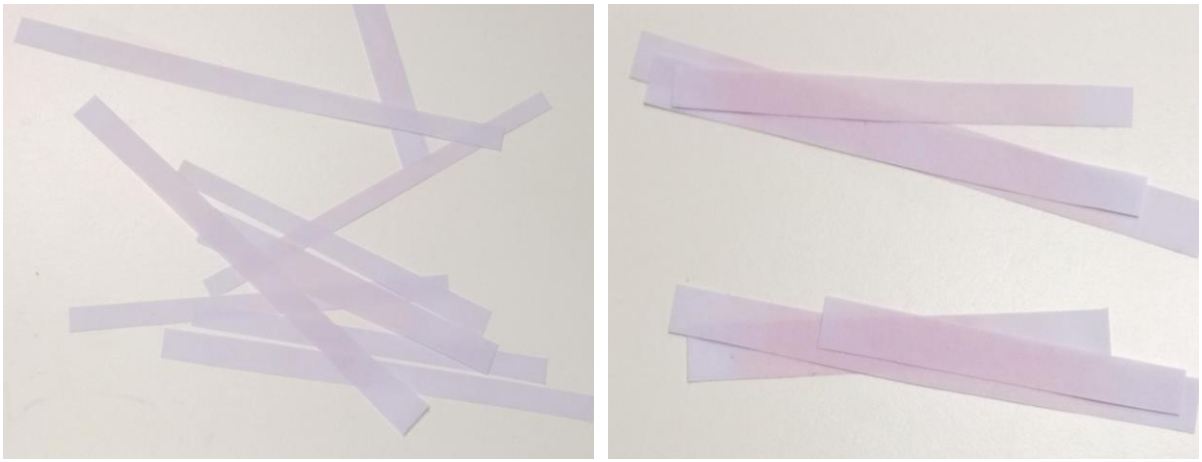

Figure 30: Stripes cut from the test packs after 1 day at 40°C (left) and stripes cut from the test packs after 7 days at 40°C (right).

The results of the GC-MS analysis are given in Table 19. Although levels are high this time and the tested films are conventionally produced ones, standard deviation between triplicates

was up to 50% for certain values. Particularly Sudan Red II showed high deviations; therefore, the values are not given in the table below. It was detected in levels of about 3-20% in the samples. Migration of C_{17} was for some stripes above 100%. This can be easily explained by the fact that it is present intentionally in the donor and the barrier. The values for Bzp and C_{17} reached a maximum between the 2nd and the 7th day and decreased again afterwards. The levels for DEHP decreased constantly till the end of the test.

Table 19: Results of taped barrier test of three conventional barrier films at 40°C. Results given in % migration from donor (n=3).

Values in % of Donor		1d	2d	7d	15d	21d	35d
LLDPE 40µm	*Bzp*	6.5 ± 3.1	27 ± 2	20 ± 2	20 ± 2	20 ± 3	14 ± 1
	C_{17}	29 ± 10	99 ± 14	109 ± 13	102 ± 9	100 ± 9	81 ± 1
	DEHP	4.7 ± 1.3	27 ± 9	59 ± 9	72 ± 9	77 ± 9	75 ± 12
PP 50µm	*Bzp*	8.1 ± 1.2	33 ± 4	25 ± 3	25 ± 3	24 ± 2	18 ± 3
	C_{17}	53 ± 6	100 ± 23	130 ± 4	119 ± 2	112 ± 1	90 ± 7
	DEHP	4.2 ± 0.5	20 ± 2	41 ± 1	58 ± 6	65 ± 8	70 ± 6
LLDPE 70µm	*Bzp*	5.4 ± 3.4	28 ± 2	20 ± 2	21 ± 3	20 ± 2	15 ± 4
	C_{17}	23 ± 11	70 ± 13	75 ±11	75 ± 10	71 ± 4	60 ± 11
	DEHP	4.4 ± 0.8	22 ± 3	52 ± 7	65 ± 5	68 ± 6	65 ± 13

Based on the quick breakthrough seen in the 40°C test, the second test was performed at room temperature. The stripes were cut daily on the 1st, 2nd, 3rd, 4th and 7th day. Still, breakthrough was seen immediately with the first stripes, with breakthrough levels also being far above 1% (25-46% for C_{17}; 0.9-3% for Bzp). Interestingly, the levels of C_{17} did not further increase till the end of the test. They stayed on the same level for 2-3 days and decreased slightly afterwards. On the contrary, the levels of Bzp increased constantly with final a concentration of 2-5%. The results are in good accordance with those published earlier by Fiselier and Grob (2011).

Comparing the behaviour of the polymers with the biopolymer chitosan showed that the biopolymer has excellent barrier properties compared to the conventional barriers. Unfortunately, repeatability did not get better with the conventional barrier films, indicating that the origin lies in the test itself, rather than in the barrier films. Therefore, the four films (the biopolymer and the three conventional polymers) were used in two-sided migration test to get on the one hand, more information about their behaviour and on the other hand, to compare the taped barrier test with the two-sided migration experiment.

3.1.2.3. Comparison of Taped Barrier Test and Two-Sided Migration Experiment

As mentioned above, the four barrier films were tested in a two-sided migration experiment to allow comparison of the two tests.

The big disadvantage of the siliconized paper and therefore also of the taped barrier test is the limited acceptor capacity. Compared to MPPO, which is a standardised food simulant in the EU (Commission Regulation (EU), 2011), it does not give information on e.g. what migrates from the barrier itself and which and how much of other substances migrate from the secondary fibre donor. In contrary, the two-sided migration experiment allows to generate a lot more information by using following procedure:
The films were tested without an additional donor first, to see the migration of the initially present substances (mainly polyolefin oligomeric saturated hydrocarbons, POSH). Second, a secondary fibre donor was added to obtain a real packaging situation; a recycled paper or board is used as food packaging for dry, non-fatty food and a barrier film is placed as internal bag between the packaging and the food to prevent migration. The barrier properties of the films were evaluated in terms of what and how much migrated into the food simulant MPPO from

1. the packaging (=donor) into the food simulant without barrier
2. the barrier into the food simulant without donor
3. the packaging into the food simulant with an applied barrier.

Since the polymer films used are known to undergo changes when tested at higher temperature, the migrations were performed using the standard conditions of 40°C for 10 days (Commission Regulation (EU), 2011). Therefore, when evaluating the results, it has to be considered that the simulated storage time is only about 2 months, compared to the 10 months simulation when using higher temperatures (60°C/ 10 d or 80°C/ 2 d). The results for the overall migration for the three tested polymers are given in Figure 31.

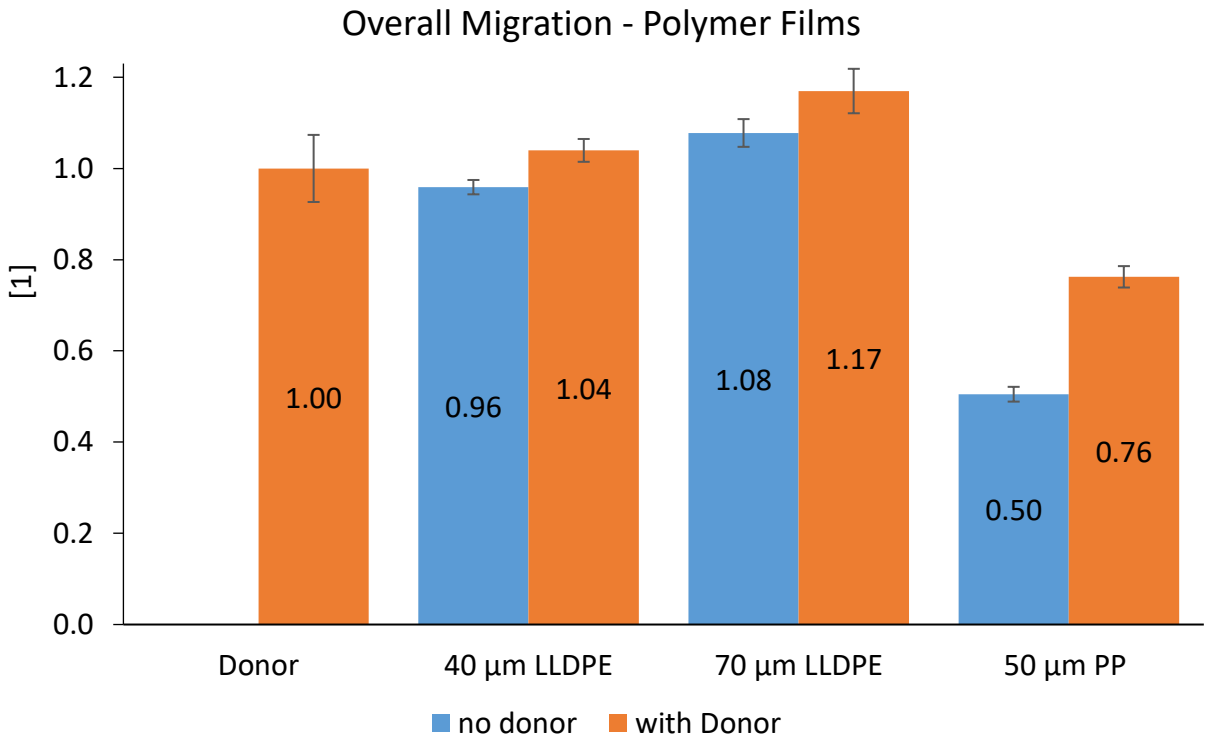

Figure 31: Results overall migration for films only (representing POSH) and films with donor (POSH + MOSH/MOAH); values normalized to the values of the donor. n=3.

As can be seen in Figure 31, the differences between donor, barrier and donor plus barrier are minor. This seems to be a little odd, because one might think there should be a difference. By looking at Figure 32, showing an example of the resulting chromatograms from the 70 µm LLDPE films, the reason becomes clear: The film itself already showed an overall migration in similar concentration to the migration from the donor. It mainly originates from the present even numbered *n*-alkanes, characteristically for the material PE. The recycled raw paper however, showed a completely different composition – the migration had its origin mainly from a hump of unresolved substances of MOSH/MOAH. The combination of both only showed slight increase of the migration value- the migration from the film stayed the same, but migration from the recycled paper was nearly completely reduced, meaning that the film has good barrier efficiency against migration of MOSH and MOAH in these tests.

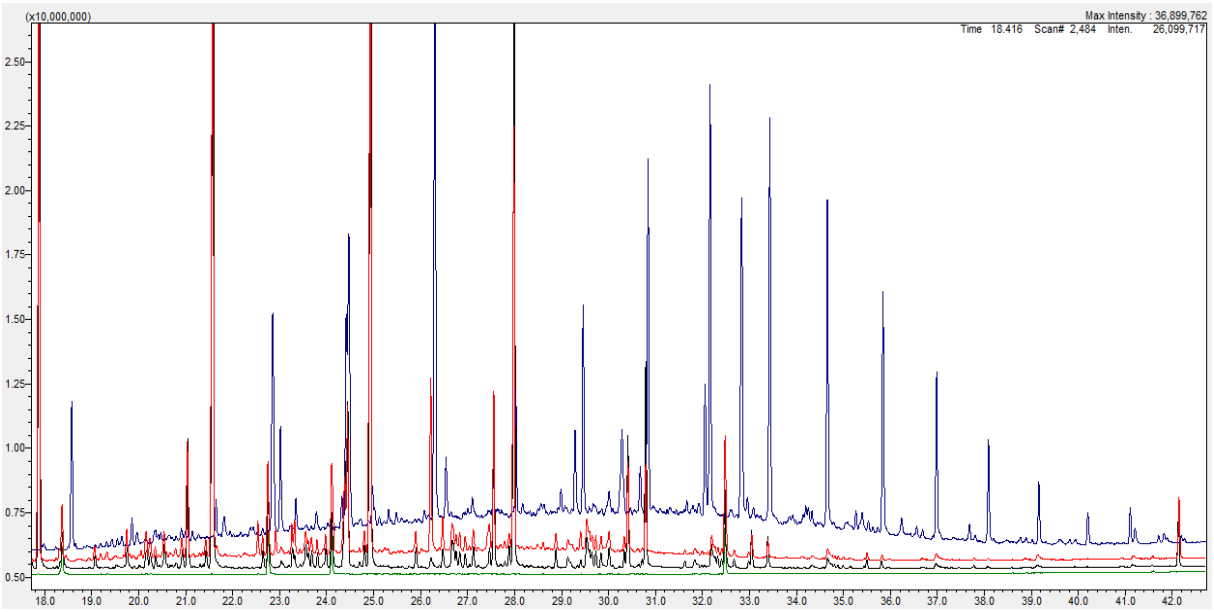

Figure 32: Comparison of migration in two-sided migration experiment: recycled raw paper without barrier (blue), recycled raw paper with 70µm LLDPE barrier (red), barrier only (black) and blank value (green).

This fact becomes even clearer, when looking at the MOSH and MOAH measurements. The film itself shows a clear pattern of POSH in the chromatogram. By combination with the recycled paper the value increased, but in a different region of the chromatogram and with different pattern. This is shown more clearly in the MOSH and MOAH chromatograms in Figure 33. The light blue trace gives the typical pattern of POSH migrating from a PE sample (compare with (Biedermann, et al., 2012a) (Biedermann, et al., 2012b)). No migrating substances can be seen in the MOAH fraction (light green trace in Figure 33). When performing the migration with the recycled board as donor the pattern changes: the POSH pattern of PE is now overlaid by MOSH migrating from the recycled board. The MOAH trace also shows a hump, originating from the recycled board.

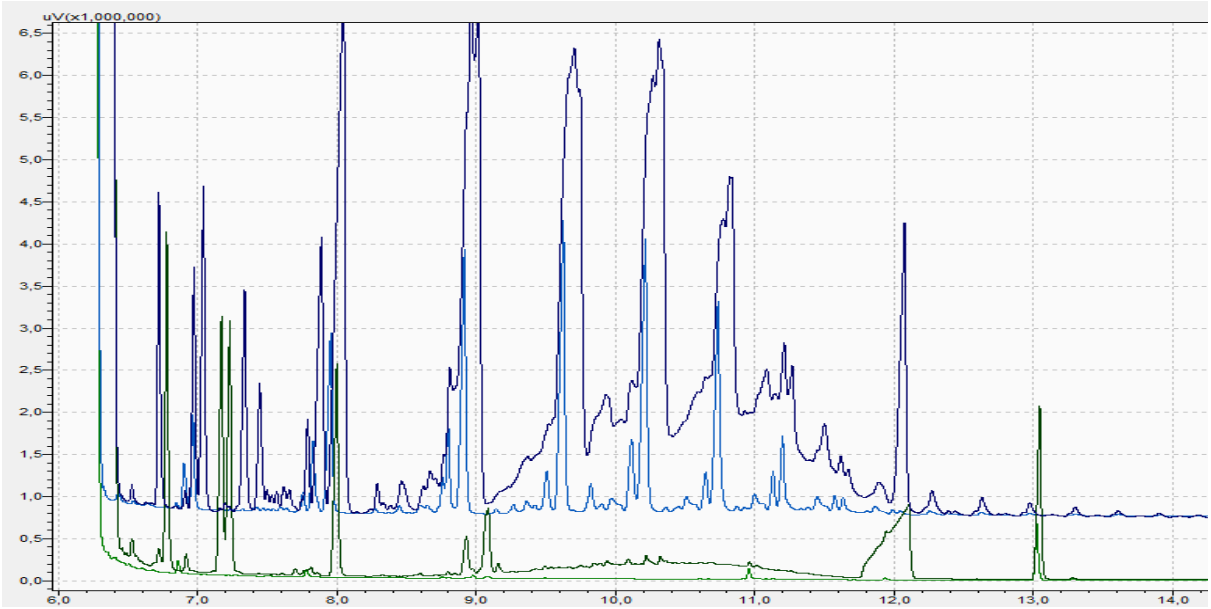

Figure 33: LC-GC-FID chromatogram comparing MOSH (dark blue) and MOAH (dark green) from recycled paper and POSH from PE present in MOSH fraction (light blue), but no MOAH (light green).

When subtracting the "blank"-value of the film from the value of donor plus film, one can calculate the amount of migration through the barrier and use it to assess the barrier properties against MOSH and MOAH. This was done for the three conventional barrier films and the results presented in Figure 34.

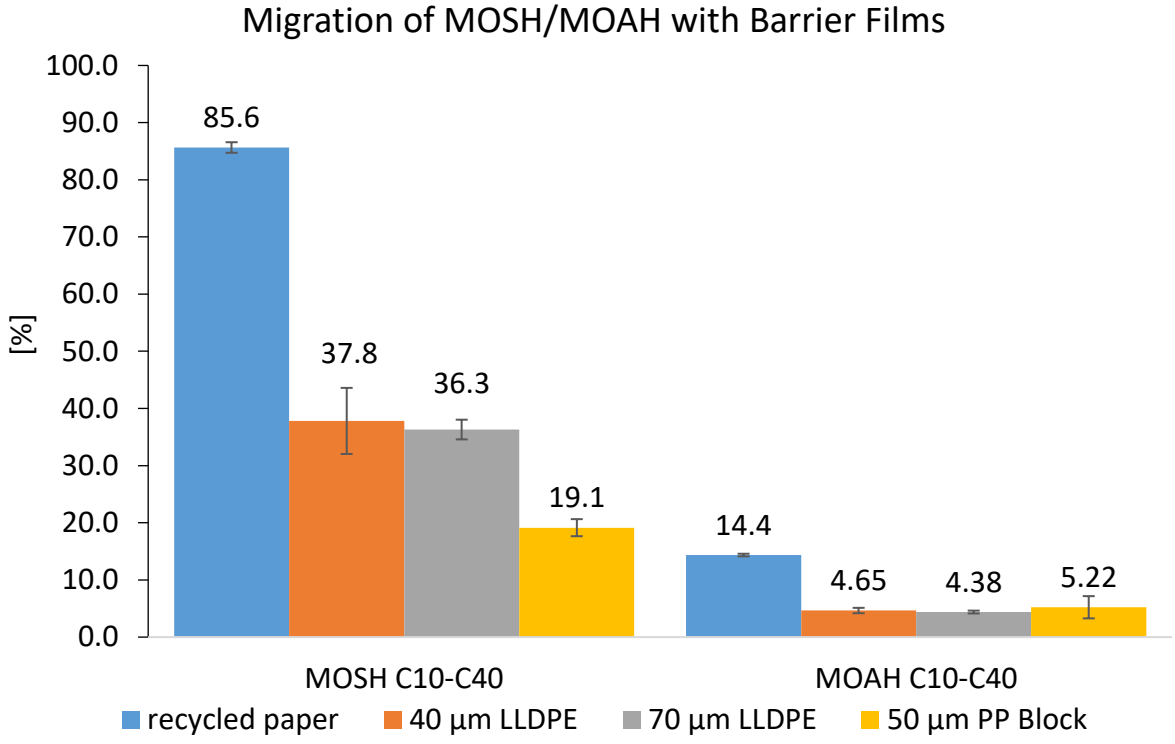

Figure 34: Barrier properties of polymer films against migration of MOSH and MOAH given in %. n=3, values normalised to recycled paper.

The chitosan film was not mentioned in the considerations above. The reason behind is that the chitosan film did not show any detectable migration itself and was also tight as barrier applied on the donor paper.
In conclusion, the polymer films showed modest to good barrier efficiency against the migration of MOSH and MOAH, but had high intrinsic migration. Therefore, it is questionable, if the situation is improved by using such polymer films as barrier. In contrast, the chitosan film showed excellent properties with no detectable migration at all. A conclusion on the taped barrier test and the detailed comparison with the two-sided migration experiment is given in the next chapter.

3.1.2.4. Conclusion on Taped Barrier Test

In general, the taped barrier test was so far described as a good alternative to the standard test methods used to determine migration and barrier efficiency (e.g. migration experiments in migration cells). There is no need for special and therefore often pricey migration cells and no need for difficult to handle and also often expensive food simulants like MPPO (Tenax®). The only materials needed are aluminium foil, tape, the donor, which can be easily prepared in the lab, and the siliconized paper. With these materials an unlimited amount of test packs can be prepared and stored as stacks, space saving at room temperature or in an oven. Furthermore, the test seems to be really quick. First results given in the publications were already received after a few hours of testing time (Fiselier, et al., 2012) (Biedermann-Brem, et al., 2014) (Biedermann-Brem, et al., 2016) (Biedermann-Brem, et al., 2017a) (Biedermann-Brem, et al., 2017b).
In practice, things get a little bit more complicated. The first problem faced in this work was the availability of the siliconized paper. Biedermann-Brem, et al. states it as purchased from a supplier. Comparison of the used paper in the publications showed inconsistency in grammage and coating and it is not clear, if it really was purchased as conventional product or specially prepared. Upon request we received 10 sheets of siliconized paper from the stated supplier. However, the only specifications received were “A4 Cellulose Paper Sheet, PDMS coated, for use in taped barrier test” without any further information on grammage or coat weight. Further discussion on the siliconized paper and comparison with MPPO was done in chapter 3.1.2.1 and it was concluded that MPPO was the better acceptor for this work and besides a standardised food simulant.
Furthermore, during preparation of the test packs, it soon went clear that the task is far more complex and time consuming than assembling a migration cell. The donor, barrier and acceptor need to be cut precisely and carefully, without doing any harm or damage to any part of the pack, especially not to the barrier, since scratches or tiny holes would lead to false results. Taping aluminium foil, donor and barrier together can also be a challenging task, particularly when dealing with a very thin and electrostatically charged barrier film. Of course, experience and training make things easier, but preparing dozens of packs is still a time-consuming task, if e.g. about 10-15 min were needed for one pack.

Another disadvantage was seen, when testing a good barrier material. The conditions and sampling times described by Biedermann-Brem et. al. were mostly for a biaxial oriented polypropylene film (BOPP), which is a weak barrier and was therefore used to get results in a short period of time. When doing the test for good barrier materials, days and weeks are needed to reach the breakthrough limit of 1%, except the barrier is considered to be tight before. The fact that there are no standardised test conditions and that they should be chosen to fit the barrier materials, let room to influence the test – either in positive or in negative way. Therefore, standardised test conditions should be set and used as described e.g. in EU Regulation 10/2011 to make results comparable and reliable.

Comparable and reliable results are also good keywords, when talking about repeatability. The first publications only mentioned repeatability once, when testing two barrier films in five packs. Standard deviation within the five packs was up to 18% and additional uncertainty is gained by the extrapolation of the data to the 1% value. They also mentioned that some barriers showed immediate breakthrough, which levelled off and increased again during the duration of the test. This phenomenon was explained by pinholes. The theory was strengthened by the presence of red spots from Sudan Red II on the acceptor (Biedermann-Brem, et al., 2014). Similar behaviour was seen in our tests. In three replicates of a barrier film, the film showed in one pack immediate breakthrough, modest breakthrough in a second pack and a third was tight till the end of the test. Compared with the presented results of Biedermann-Brem in 2014, there were no red spots from pinholes on our barrier or receptor (except the test at 40°C, where the acceptor was evenly covered). Furthermore, beside in the first introduction and description of the test in 2012 and the observations in 2014, the use of Sudan Red II for detection of barrier damages had not been further mentioned (Fiselier, et al., 2012) (Biedermann-Brem, et al., 2014). Probably the changes in the test since then (revisions in 2017; conditioning of donor and absorption of polar substances, far lesser concentrations of surrogates) led to the vanishing of the effect. This was not further discussed in the newer publications, although removing TEC as surrogate and also thinking of just keeping the non-polar C_{17}. In 2017, an interlaboratory comparison was performed to further evaluate the test (Funk, et al., 2017). Two polymer films, being a biaxially oriented polypropylene (BOPP) bilaterally coated with an acrylate to be tested at 40°C and a 8 µm BOPP film to be tested at 25°C, were sent to the participating laboratories. All labs used the same siliconized paper, but prepared the donor themselves. Stripes were cut after 3, 4 and 5 weeks for the acrylate coated and after 2, 3 and 4 weeks for the BOPP film. Twelve labs reported results. For the acrylate coated film, the migration of the surrogates MBP and DPP remained low and were considered to be irrelevant. Migration of C_{17} showed a deviation of 16-24%. The results of the 8 µm BOPP film gave higher deviations. Those are explained by inconstantly temperatures used by the participating labs and it is referred to the change of the BOPPs barrier properties in the range of 22-28°C (Biedermann, et al., 2017b). Therefore, no detailed results are given in the publication (Funk, et al., 2017). Considering the mentioned facts and the results in this work, it was questionable, rather the test is robust, repeatable and reproducible.

The last part needing discussion is the used migration limit for results evaluation. It was changed several times during the revisions of the method. First, the barrier should reduce the migration by a factor of 100 compared to the equilibrium concentration, to stay beneath the ADI of 0.06 mg kg^{-1}b.w., resulting in 1% and 10% breakthrough limits. After withdrawal of the ADI, the results were still given as 1% breakthrough levels from the equilibrium concentration, but now justified with the conventional EU detection limit of 0.01 mg kg^{-1}. In the next revision it was seen that polar substances are adsorbed in the recycled board used as donor, which lead to the conclusion that a 100-fold reduction is not necessary. Furthermore, it was seen that the siliconized paper does not simulate food very well at levels above 1% migration. Thus, for the new limit is was concluded that the surrogates should not be detected in an amount higher than 1% of the donor's concentration. Whether this is reliable should not be further discussed her.
For this work it was decided to take some parts and generated information to improve the newly developed two-sided migration test, but to do no further experiments with the taped barrier test.

3.2. Results Characterisation of Papers and Biopolymers

3.2.1. Characterisation of Raw Papers and Biopolymer Raw Materials

3.2.1.1. Raw Papers

The received papers were a mix of conventionally available products, aimed to be food contact materials. The samples represented clean fresh fibre papers, papers made from fibre mixtures, as well as "worst-case" ones, made from 100% recycled fibre. Since the paper samples had been stored in a recycled folding box at the beginning of the project, all of the samples showed a slight mineral oil contamination, which was probably not there initially.
The first characterisation was done using HS-SPME and GC-MS detection to get an overview of the present substances and contaminations. The advantages of this method are that it is possible to generate quickly and easily information on the present contaminations. The only sample preparation steps are cutting the samples and weighing them into the vials. The disadvantages are that the extraction through the head-space does not catch high boiling substances, since they are too less volatile. Furthermore, quantification and correlation to real migration is difficult. This can also be seen in the presented results: From the resulting chromatogram, one can estimate the quality of the paper, in terms of present contamination e.g. the presence of an unresolved hump of mineral oil and its size as can be seen in Figure 35 to Figure 38.

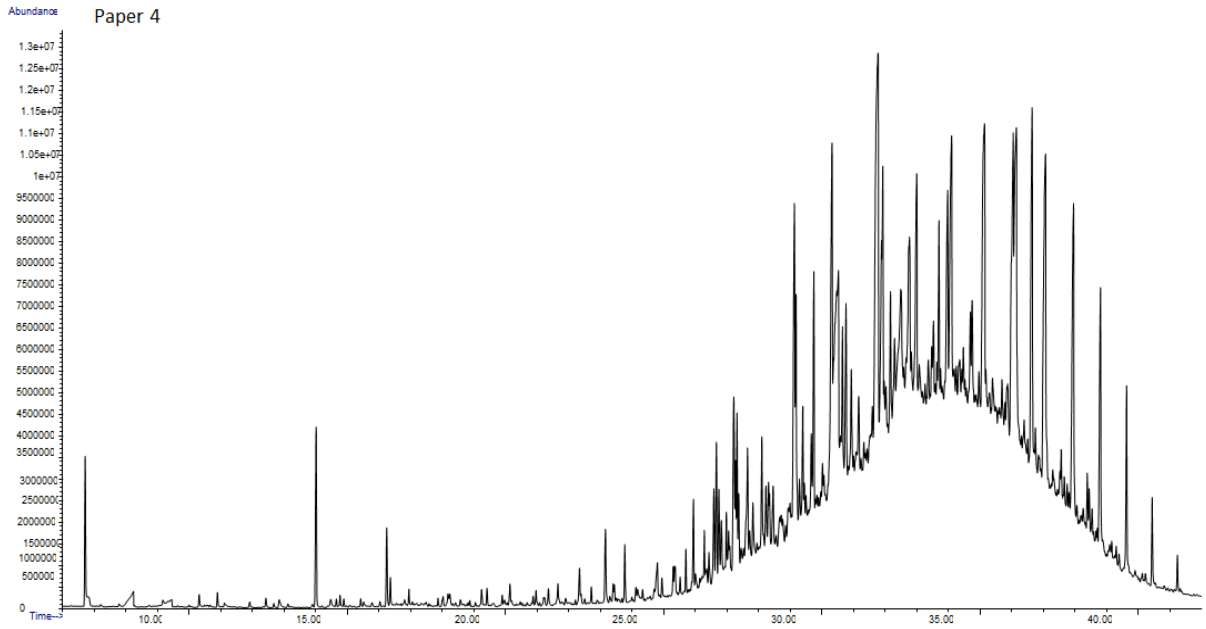

Figure 35: HS-SPME-GC-MS chromatogram of paper 4: clearly visible MOSH/MOAH hump from recycled fibre.

Figure 35 shows the resulting chromatogram of paper 4, a liner made from recycled fibre and aimed to be used for corrugated board folding boxes. Clearly visible is the already mentioned unresolved hump of mineral oil hydrocarbons (MOH). Comparing the area of the added internal standards with the area of the hump, already allows an estimation of the concentration of the present contaminants.

In comparison, Figure 36 shows the chromatograms of the fresh fibre paper sample 1 and its PE coated reference. The overall amount of present substances is much lower. The hump of MOH that can be seen may, as already mentioned, originate from the storage in a recycled board box. The comparison between the paper and the reference shows a similar pattern in the present contaminations. There is a slight difference in the substances sitting on the hump. The reference has a more distinct pattern that is probably POSH originating from the PE coating (see differentiation POSH/MOSH in chapter 3.1.2.3).

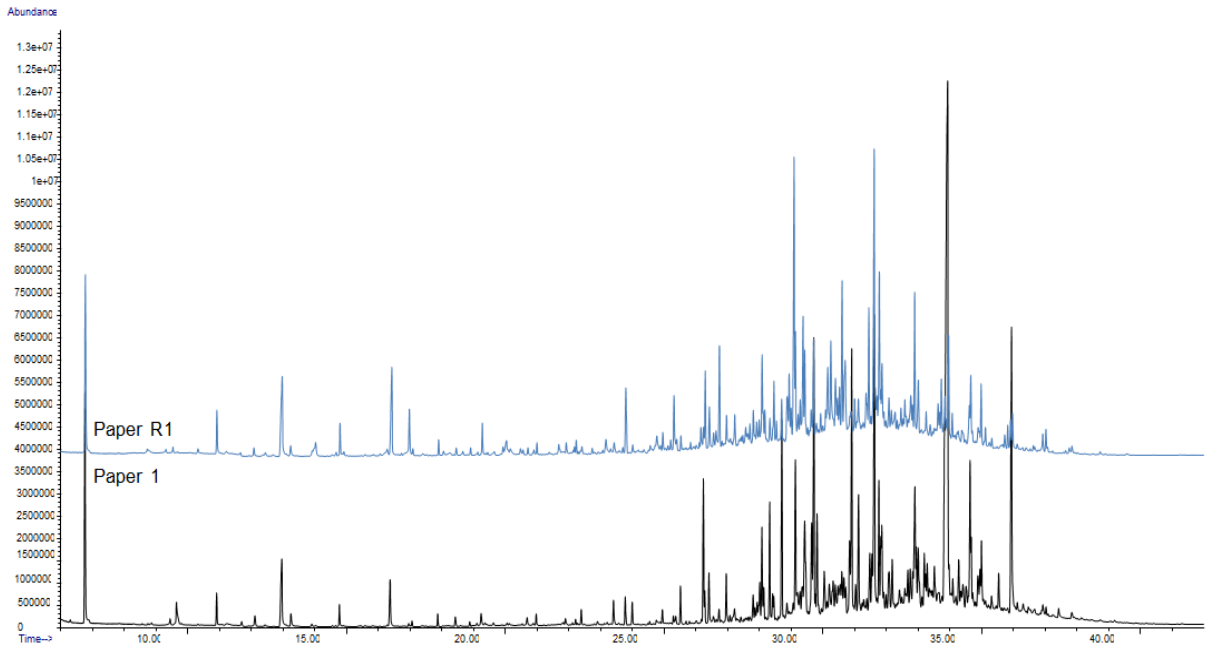

Figure 36: HS-SPME-GC-MS chromatogram of fresh fibre paper 1 and R1: slight hump visible.

Figure 37 shows the chromatogram of paper sample 3, made from a mix of recycled and fresh fibre material. In comparison with paper 4, made completely from recycled fibre (see Figure 35), the hump is smaller, but with more peaks in higher concentrations sitting on top of it.

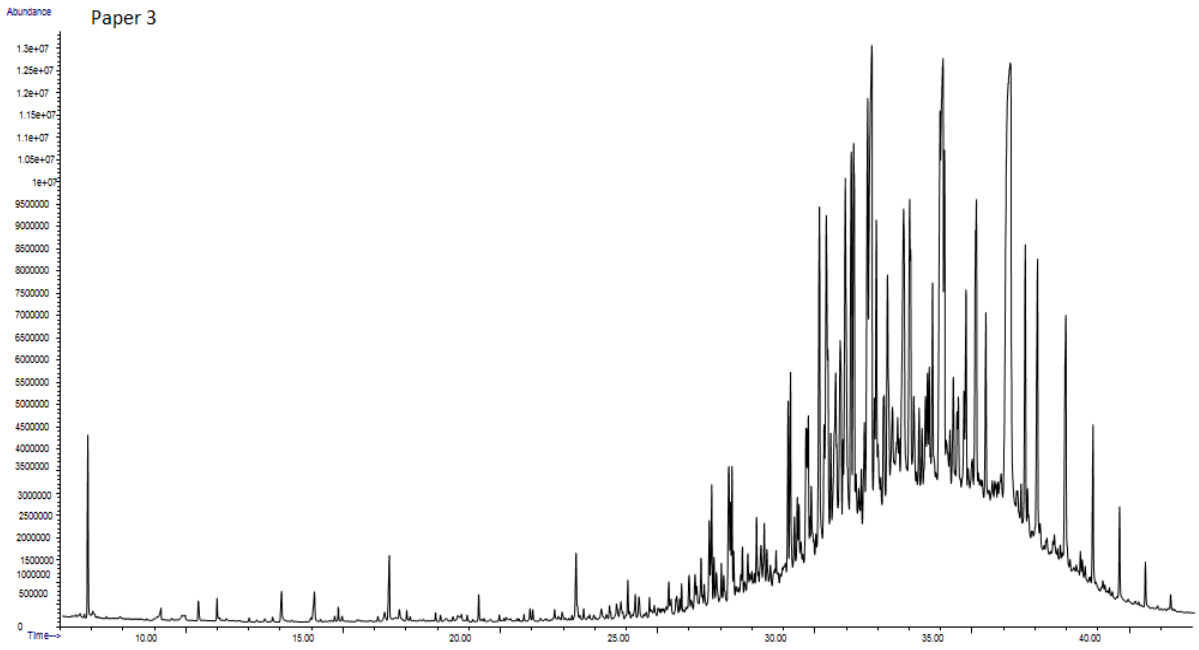

Figure 37: HS-SPME-GC-MS chromatogram of paper 3: clearly visible MOSH/MOAH hump from recycled fibre.

The chromatograms of paper sample 2 given in Figure 38 show partly different pattern; the raw paper shows a hump, but with an unexpected form. It is irregular, not showing the Gaussian

distribution typical for a MOSH/MOAH hump. The origin is unclear, since the paper is a non-printed fresh fibre paper. The reference R2_1 shows a similar pattern, while R2_2 and R2_3 were more similar to a clean fresh fibre paper.

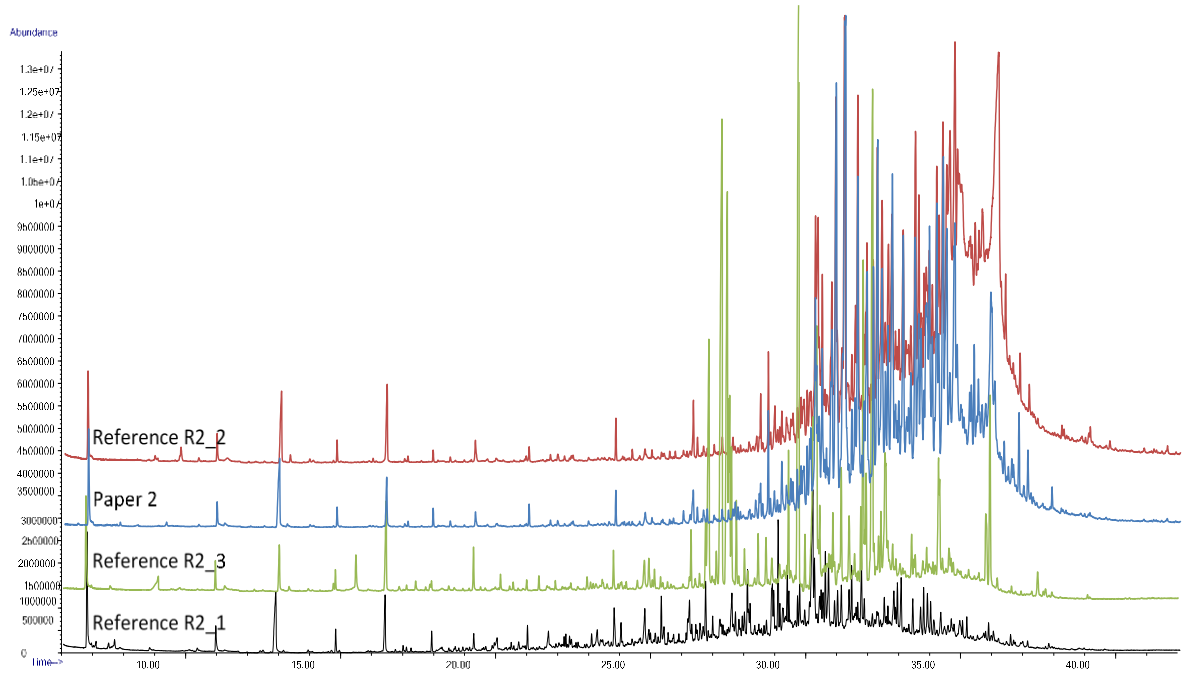

Figure 38: HS-SPME-GC-MS chromatogram of paper 2 and its references: irregular humps of unclear origin for R2_2 that is not present in R2_1 and R2_3.

Table 20 shows the sum of all volatile substances detected. No further information than this general overview can be generated from the results. It would be possible to e.g. identify the most abundant peaks, but those are mostly harmless bulk-substances, such as aldehydes, acids or fatty acids as was already described by (Biedermann, et al., 2013a) and in the introduction of this work.

Table 20: Sum of volatile compounds detected using HS-SPME-GC-MS in the papers and in their references.

Paper	**1**	**R1**	**2**	**R2_1**	**R2_2**	**R2_3**	**3**	**4**
sum of all volatile substances	530	602	2100	740	2700	1680	4200	6200

A further attempt to estimate the contamination potential of a food contact material is the total extraction with hexane/ethanol 1:1 (v/v) to determine the total amount of mineral oil hydrocarbons present. Since the specification of absolute values is prohibited by the industrial partners the values are normalized to the highest MOSH concentration (paper 4). The results are given in Figure 39. The MOAH values, given as insert in Figure 39, should be about 20% of the total MOH content. The papers made with a certain amount of recycled fibre can be clearly identified by their MOAH value. These are, as already mentioned, paper 4 and paper 3. The others have no significant MOAH content, although e.g. paper R2_1 and R1 have a

considerably high MOSH content. This however results from the co-extraction of POSH from their PE coating. For the waxed reference paper R2_3 it was not possible to determine the MOSH/MOAH values, since the liquid extraction with n-hexane mainly extracted the high boiling waxes from the coating.

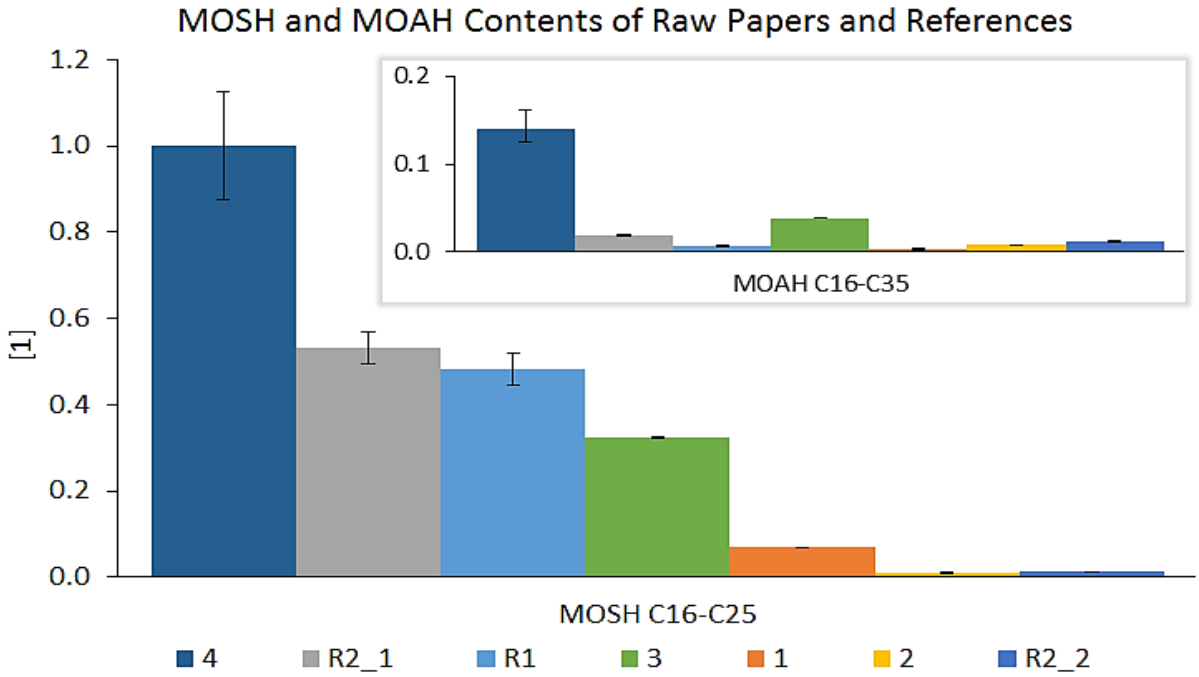

Figure 39: Total amount MOSH and MOAH in the raw papers and reference papers, determined by total extraction. n=3, normalised to the highest value of MOSH in paper 4.

Although, the total amount of MOSH and MOAH present is an indication for the possible contamination of food, it does not give a definite result on the possible migration. It can be estimated that about 70% of the total amount migrates into food during long term storage at room temperature (Lorenzin, et al., 2010) (Vollmer, et al., 2011). Therefore, if a paper has a low content of MOSH and MOAH and a transfer of 70% into food would not pose any risk, no further tests have to be performed. However, especially for papers with high contamination, migration experiments should be used to determine an absolute value of migration. This was also done in this work. The overall migration of volatile substances into the food simulant MPPO showed a similar pattern to the ones described for the total amount of MOSH/MOAH: The fresh fibre paper samples 1 and 2 showed the lowest migration, while the papers 3 and 4 made partly or completely from recycled fibres, showed the highest migration. The reference papers R1 and R2_1, both coated with PE, showed a higher migration of volatile substances, since the PE coating released POSH substances. The “tight” reference paper R2_2 showed a migration similar to the raw paper, while the wax coated one showed a slight increase in migration. The results are summarized in Figure 40 and were again normalized to sample 4.

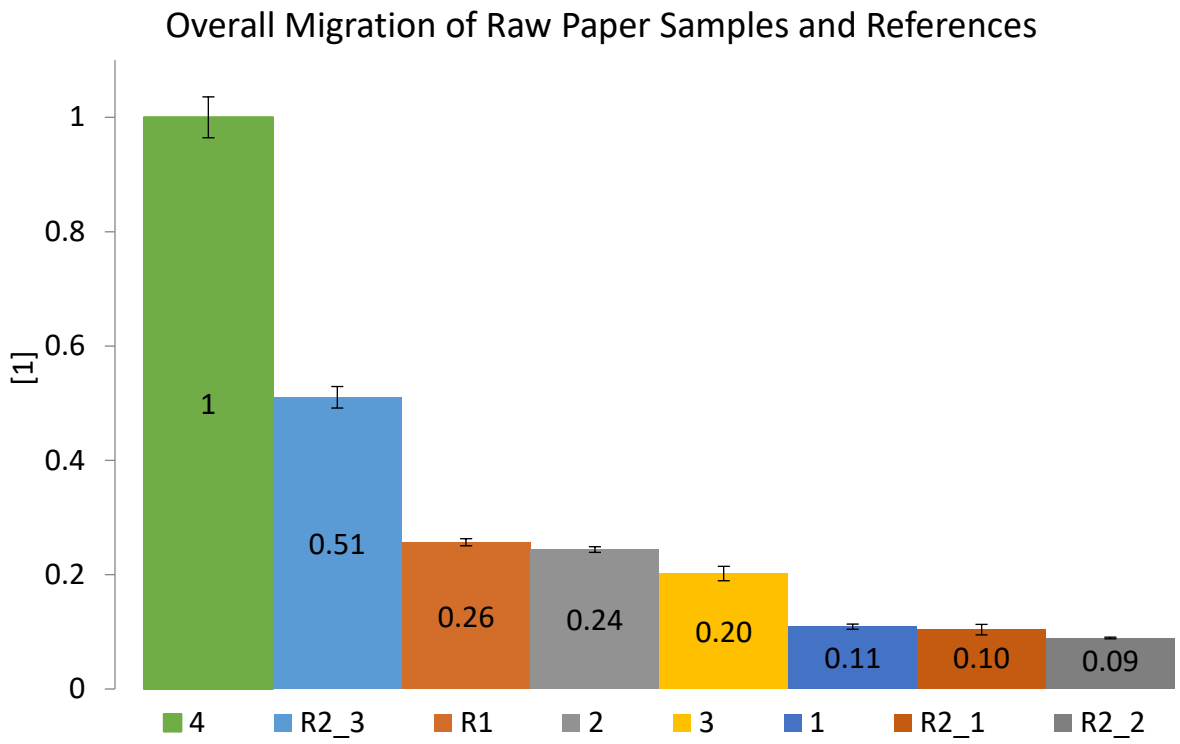

Figure 40: Overall migration of paper samples into MPPO. Values normalized to Sample 4 (n=3).

The permeation results showed nearly equal transfer of the spiked deuterated n-alkanes and the aroma compound menthol for each sample. All found levels were between 50-70% for the raw papers 1-4. The reference papers with coating did not show any reduction of migrations, except the paper R1, which reduced permeation of the deuterated n-alkanes slightly by about 10-20%, but not the one of menthol. It was not possible to determine the permeation of the other aroma compounds (eugenol, vanillin, acetovanillone) reproducible with GC-FID, since the concentrations were low and a clear identification not possible. As described in the experimental section, the two-sided migration experiments of these samples were performed as one of the first, when the method had not been fully characterized and validated. Subsequently to the results of the raw paper characterization, it was decided to analyses future permeation experiments on GC-MS. Comparison of the results of sample 1 and 4, done with the validated method afterwards, showed similar results. It was therefore decided to not redo the entire paper samples, since the newly generated information would be limited and the decision on which papers would be used for the coating trials had already been made.
The situation was similar for the analysis of MOSH and MOAH migration: The online-coupled HPLC-GC-FID had not been available for the routine analysis of migration extracts at the time of the characterization of the raw papers. Therefore, also only data for the papers 2 and 4 are available.

As already mentioned, it was decided to use two of the received and characterized raw papers for the coating trials: the fresh fibre paper sample 1 and the secondary fibre raw paper 4. Paper 4 was used without any further treatment, paper 2 was mass-sized using active liquid alkenyl

succinic anhydride (ASA) and surface-sized using starch and a calender machine. They were chosen as substrate to simulate both, a worst-case paper without any further treating and a clean, already surface sized primary fibre paper.

3.2.1.2. Biopolymers

The characterization of the barrier materials with HS-SPME gave following concentrations of volatile organic substances in mg kg^{-1} material:

Table 21: Concentration of all volatile compounds in the barrier materials received as free-standing film.

Material	**mg kg^{-1}**
Alginate	5.37
Casein	13.5
Chitosan	3.70
EVOH	7.60
MFC	1.91

The most abundant substances were identified. For MFC and alginate the substances present were mainly aldehydes. Chitosan and EVOH showed a similar pattern, but with a large hump of the acetic acid use for pH adjustment during film formation. Casein showed the highest value of volatile substances, because it had – besides aldehydes – a large hump of glycerine in the chromatogram. The chromatograms of the materials are given in the attachment (Figure 81 - Figure 85).

The characterisation using HS-SPME was only done for the first available biopolymers, since the generated information was low and not helpful in terms of determining barrier efficiency. Therefore, for all further characterisation of these materials and the new ones, migration experiments were performed directly.

3.2.2. Trials

3.2.2.1. Free-Standing Films

Two-sided migration experiments were performed for all received free-standing films. The overall migration showed values below the LOD for all materials, except casein. Some single substances migrated from the casein film, mainly being high boiling triglycerides. However, the casein film got brittle during the test at 80°C and developed an odour of sour milk. Also, the EVOH film used as reference got brittle and it was concluded that these two films will need a substrate for better evaluation.

The permeation results showed that all tested materials were able to reduce the permeation of the spiked substances to <10%. This was promising and it was decided to use all materials in the following trial, in which the biopolymers were coated on the two chosen raw papers using a hand squeegee.

3.2.2.2. 6 g m^{-2} Coat Weight with Hand Squeegee

Using the hand squeegee, the raw papers chosen in the preliminary experiments were coated with the different available barrier materials. The main aim was to generate information on the barrier materials concerning the coating behaviour, behaviour of the materials on the two raw papers and the resulting barrier efficiency. Also new materials, which had not been available for the free-standing film trial, were used in this trail directly, without any pre-tests.

The results of the two-sided migration experiments are given in the following figures (Figure 41- overall migration recycled paper; Figure 42- permeation recycled paper; Figure 43- permeation fresh fibre paper).

To render the recycled paper suitable for food contact, the migration should be reduced to at least 25%. Reference III did not show any barrier efficiency against migration. The materials xylan, CNC, reference II, gluten, starch, MFC and soy protein showed only minimal barrier efficiency. EVOH showed bad repeatability, but could be a good barrier. The materials casein, reference I and chitosan showed good barrier properties and alginate performed excellent, reducing the migration to the level of a clean fresh fibre paper.

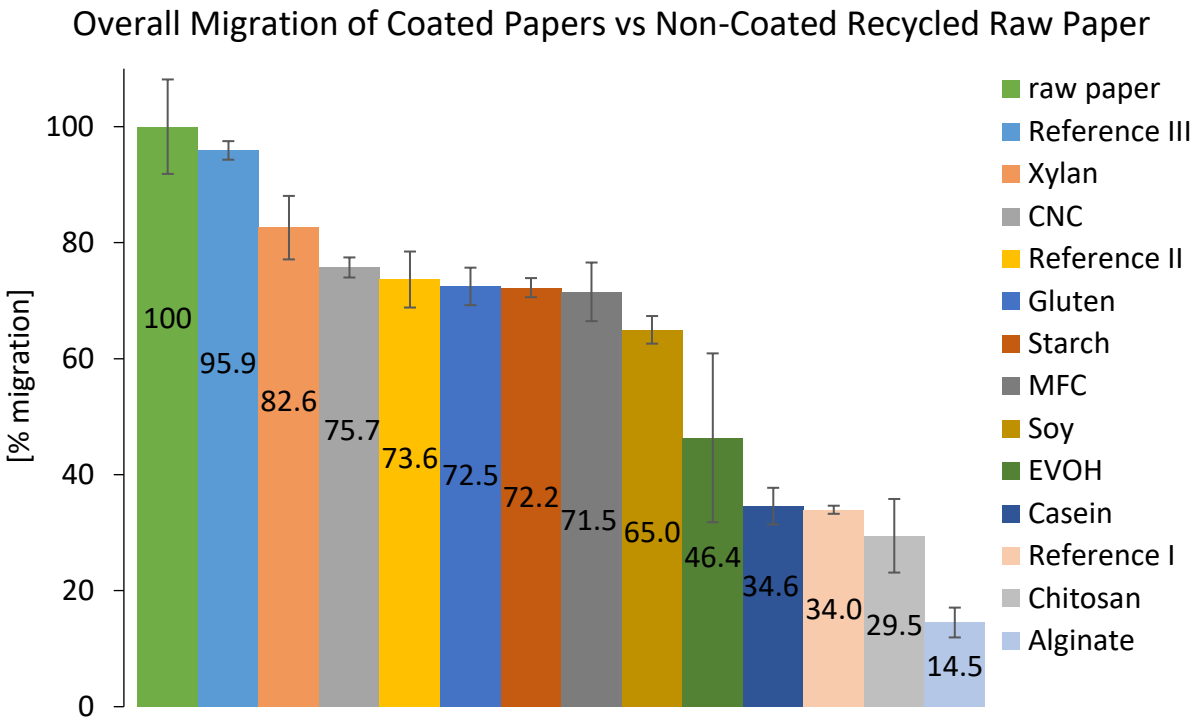

Figure 41: Overall migration of recycled raw papers compared to coated recycled raw papers. n=3, values given as percentage of the non-coated recycled raw paper.

The permeation experiment gave results similar to the overall migration. Chitosan and alginate had the best performance on the recycled board, while several others, like soy protein, MFC or reference I showed no barrier properties at all. The results for the other materials are modest, as can be seen in Figure 42. As discussed in the chapter on validation of permeation (chapter 3.1.1.2), when a piece of cellulose is used in the bottom of the cell to simulate secondary

packaging, the aroma active substances, except menthol, do not permeate, but get adsorbed by the donor. In addition, the long chain C_{28} does not permeate anymore, because of lack of volatility. This fact can already be seen for C_{24} in the results. Furthermore, it has to be mentioned that the results given in Figure 42 had been analysed on GC-FID, rather than GC-MS, but this should not have any influence on the general behaviour of the materials seen.

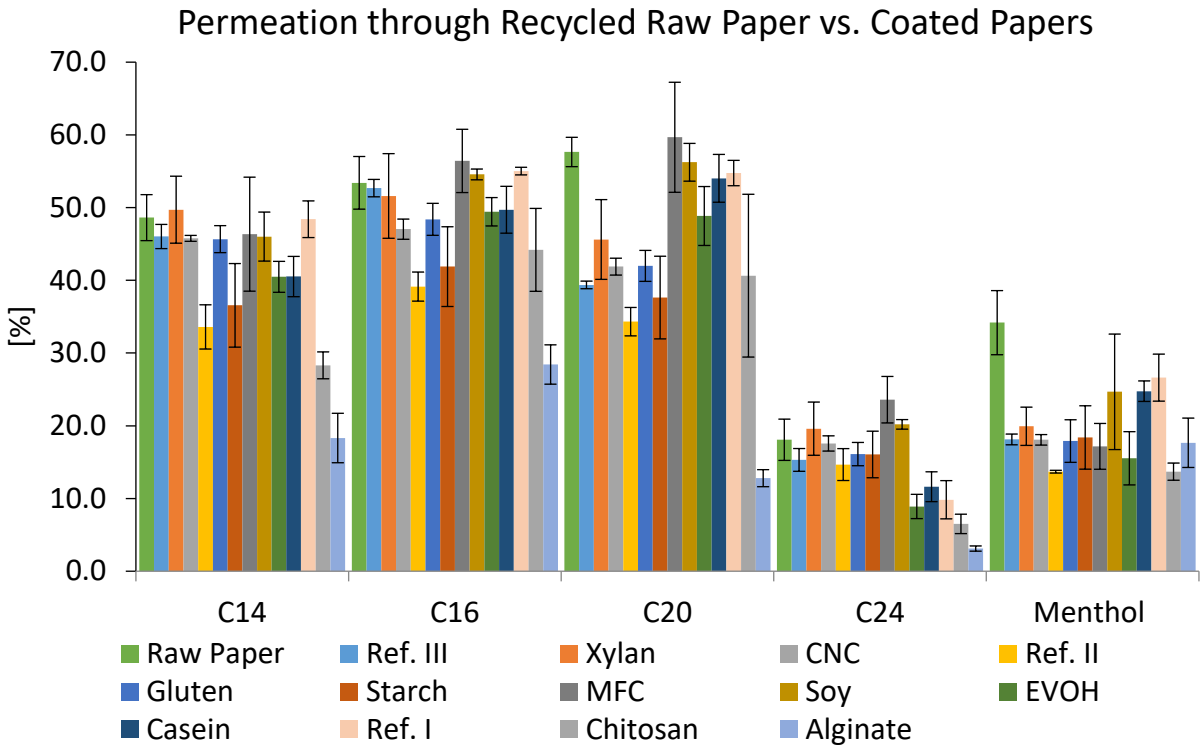

Figure 42: Permeation through recycled raw paper and comparison with coated papers. Values determined with GC-FID. n=3, values given as % recovery of the spiked amount. Vanillin, eugenol, acetovanillone and C_{28} were <LOD.

For the fresh fibre raw paper, whose overall migration is low to begin with and barriers against migration are not needed, all materials except MFC and xylan, reduced the migration to below the LOD. The residual migration for MFC still was 54.2%, the one of xylan 87.4%. Therefore, the more powerful tool in terms of barrier efficiency evaluation is the permeation through the sample. The results for the experiment are given in Figure 43. The best performing material was the reference material EVOH, which reduced the permeation of all substances below the LOD and is therefore not given in Figure 43. It was followed by casein and chitosan, which both also showed good barrier properties, but chitosan showed bad repeatability this time. So did alginate, but much worse: on the one hand it reduced the permeation to a mean of about 20%, on the other, it showed high standard deviations of up to 120% for certain substances. It is therefore also not given in Figure 43. MFC, soy protein and the reference I showed again no to only little barrier efficiency.

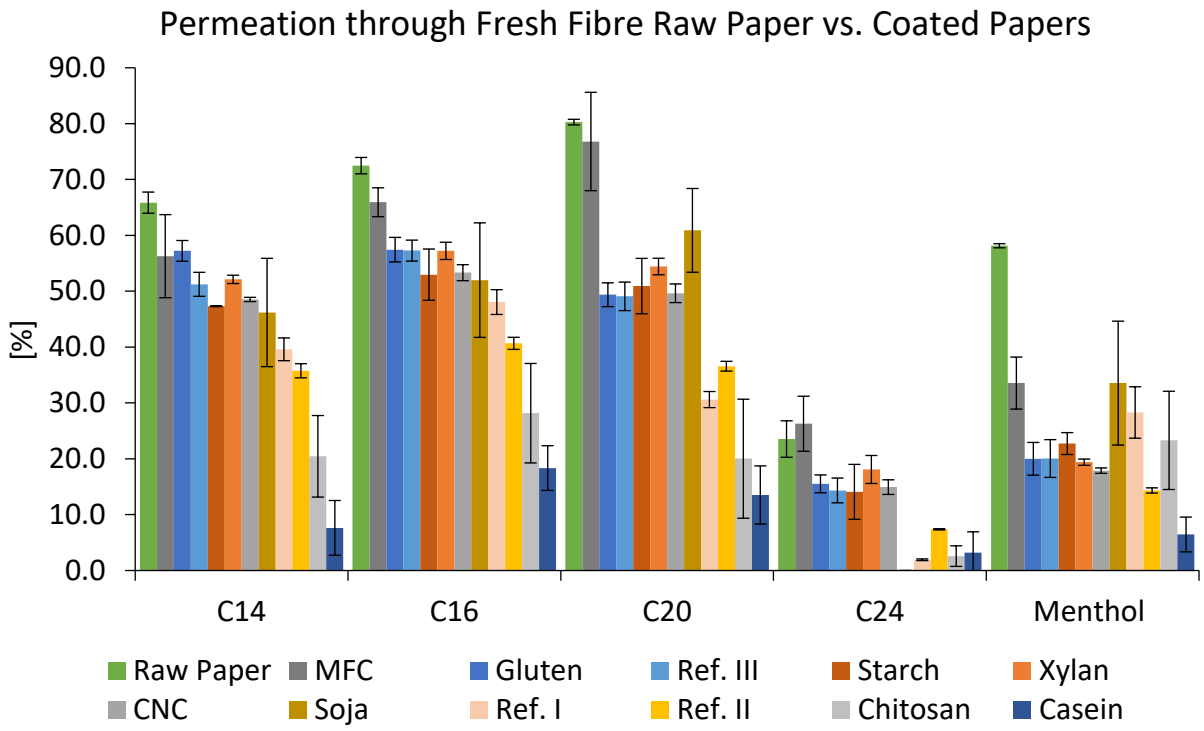

Figure 43: Permeation through fresh fibre raw paper and comparison with coated papers. Values determined with GC-FID; n=3; values given as % recovery of the spiked amount; Vanillin, Eugenol, Acetovanillone and C_{28} were <LOD.

Comparing the permeation results for the two raw papers, it can be seen that the permeation through the fresh fibre paper is generally higher. This can be explained by the lower grammage and thickness of the raw paper and therefore, the faster and easier permeation through it. On the other side, the barrier properties resulting after coating were better for several materials and permeation even decreased to not detectable by e.g. EVOH. This can also be easily explained by the papers properties: the recycled raw paper was not treated any further (considered to be a "real" raw paper) before coating. The fresh fibre raw paper was mass-sized and surface-sized, therefore caoting conditions were better, which also resulted in better barrier behaviour. The high standard deviations between the triplicates of some materials may result from the manually performed coating procedure.

Migration of MOSH and MOAH could not be analysed for those samples, because it was not available at this time.

After generating all the information on coating behaviour and barrier efficiency needed, the most promising materials were used for the further tests. Well performing on both raw papers were the biopolymers alginate and chitosan and the reference material EVOH. Casein was excluded from further tests, because of the intensive sour milk like odour.

First aim of the following tests was to reduce the rather high coat weight of 6 g m^{-2}, which would be far too high to be used in an industrial coating process. Since the usage of the hand squeegee reaches its limits at this point the next tests were performed using a summet coater.

3.2.2.1. Chitosan with Drying and Layering

Aim of this study was on the one hand to reduce the coat weight and compare the barrier properties of 6 g m^{-2} with 3 g m^{-2}. On the other hand, this was the first trial performed on a summet coater and the new opportunities derived from it were tested. Among them are besides the reduced coat weight, different possible drying techniques. In this study the influence of drying by hot air (HL), infrared radiation (IR) or a combination of both was tested.

Again, both raw papers were used in the coating trails to receive as much information as possible, but only one barrier material was chosen as a start. Since chitosan performed well on both papers in the hand squeegee trails, it was chosen for this one. Of the coated papers two-sided migration experiments were performed to determine the overall migration, permeation and migration of MOSH and MOAH. The results are summarised for both papers in Figure 44 to Figure 46. For the fresh fibre raw paper, the overall migration was reduced to <LOD for all coated samples. Therefore, no further information could be generated from these results and the permeation results are the significant ones.

Figure 44 summarises the results for the overall migration of the recycled raw papers.

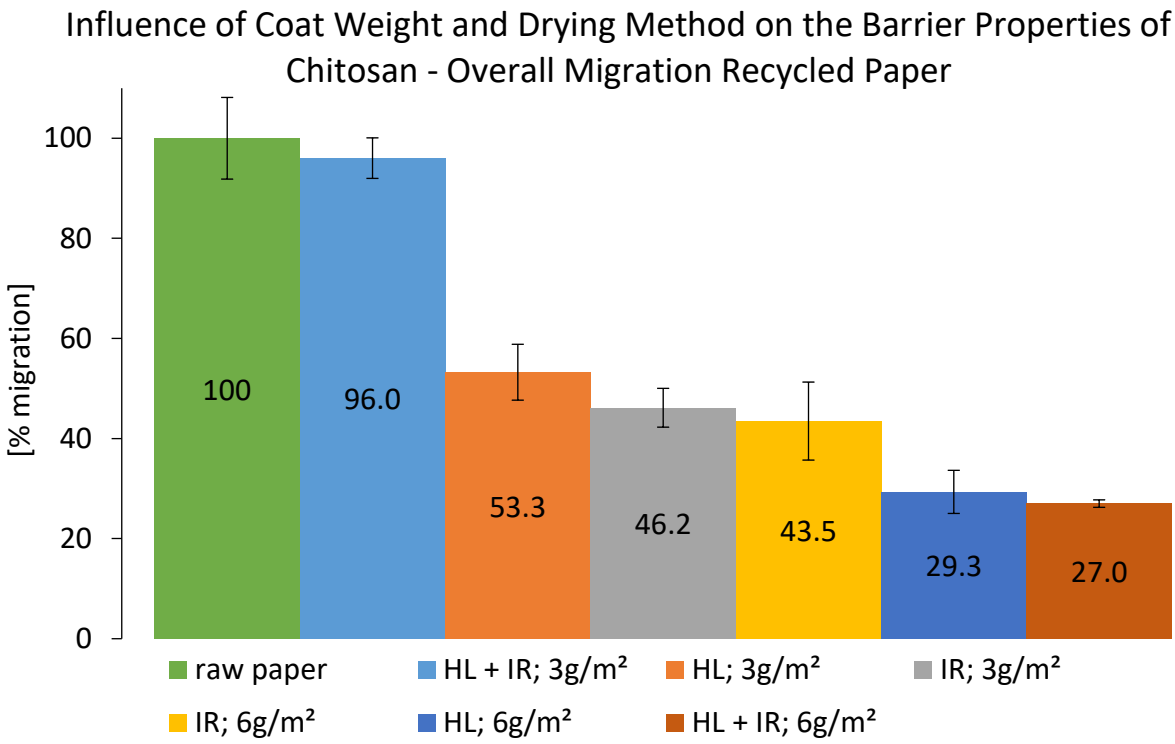

Figure 44: Influence of coat weight and drying - overall migration of recycled raw paper compared to chitosan coated recycled raw paper; n=3, values given as percentage of the uncoated recycled raw paper.

The influence of the used coating parameters was clearly visible. Using a coat weight of 6 g m^{-2} gave the better results than using only 3 g m^{-2}. The 6 g m^{-2} samples dried with hot air and the one dried with hot air + infrared radiation reduced migration to a level comparable to the first trial. Interestingly, the sample with IR drying only did not show barrier properties as good as the other two. Migration was increased by about 15%, resulting in values comparable with the

3 g m^{-2} coating (43.5% – 53.3% migration). However, again one sample showed significantly worse results; the combination of hot air + infrared radiation drying showed hardly any reduction of migration.

The permeation data showed comparable results, which are summarised in Figure 45: The sample coated with 3 g m^{-2} chitosan and the combinational drying showed again the worst results, even worse than the non-coated paper. The negative influence of the drying method on the 6 g m^{-2} + IR drying was even more distinct in the permeation – it showed as bad results as the 3 g m^{-2} sample with IR+HL drying (although the deviation within three replicates was high). Interestingly, there was only little difference between the 3 g m^{-2} sample with IR drying and the 6 g m^{-2} samples with HL and IR+HL drying.

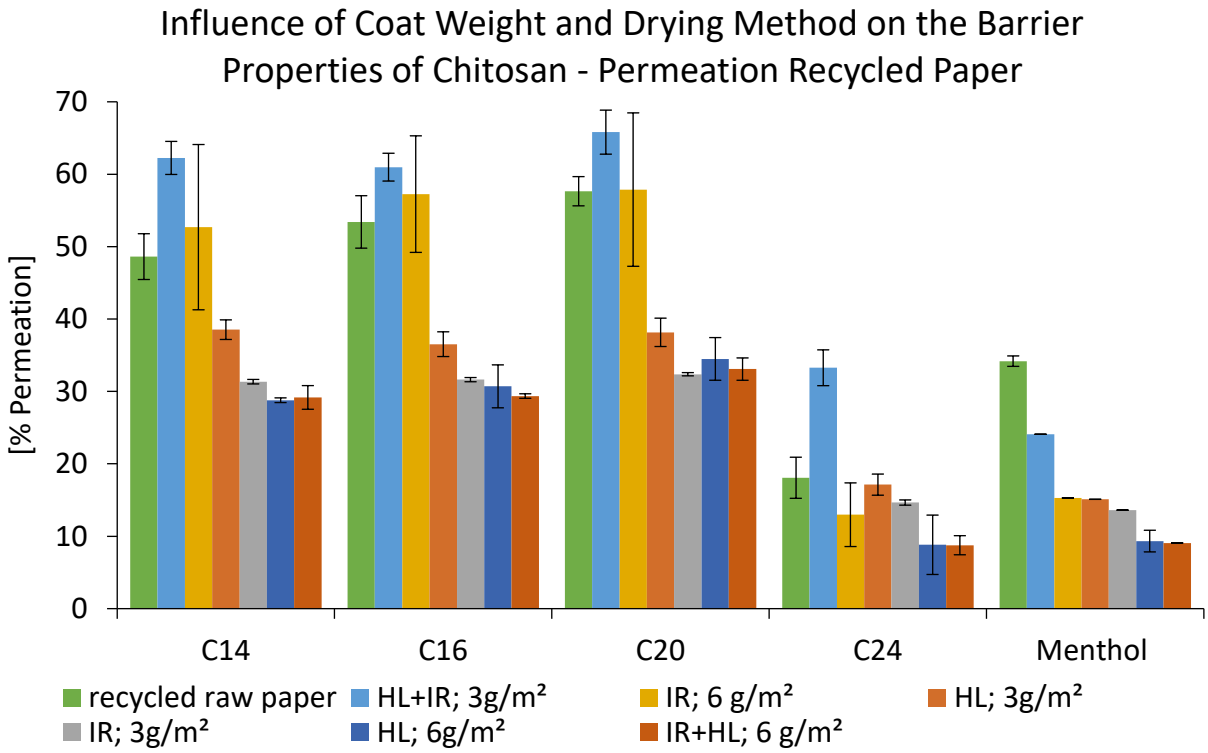

Figure 45: Influence of coat weight and drying on permeation through non-coated and chitosan coated recycled raw paper. Determined on GC-FID with n=3; Values given as % recovery of the spiked amount; vanillin, eugenol, acetovanillone and C_{28} were <LOD.

For the fresh fibre paper sample, the 6 g m^{-2} always gave better results than coating with only 3 g m^{-2} (see Figure 46). However, repeatability was even worse for some data points of those samples, than it had been for the recycled paper samples. Whether the reason is the coating procedure or the migration-, permeation experiment could not be evaluated, since only a limited number of samples was available (maximum of 3 replicates) and coating trials where not repeated. Still the results indicate that the drying procedure can have a big influence on the barrier and the barriers performance. Using a coat weight of 3 g m^{-2} the combinational drying completely destroys the barrier properties of chitosan, resulting in even poorer performance

than the raw paper. Repeatability was especially bad for the hot air + infrared drying of the 6 g m^2 chitosan sample. Interpreting this fact lead to the conclusion that the behaviour within the triplicates seems to vary a lot; while one sample still showed good barrier properties, the next one seemed to be weakened. A second reason that may be responsible for the loss of the barrier properties was identified later in the work. The phenomenon of higher permeation through the coated paper samples in comparison with the raw paper lead to the conclusion that free shrinking of the wet paper after coating may lead to the higher permeation. Whether the results had been influenced by the drying or the free shrinking or probably by a combination of both, could not be told from the resulting data.

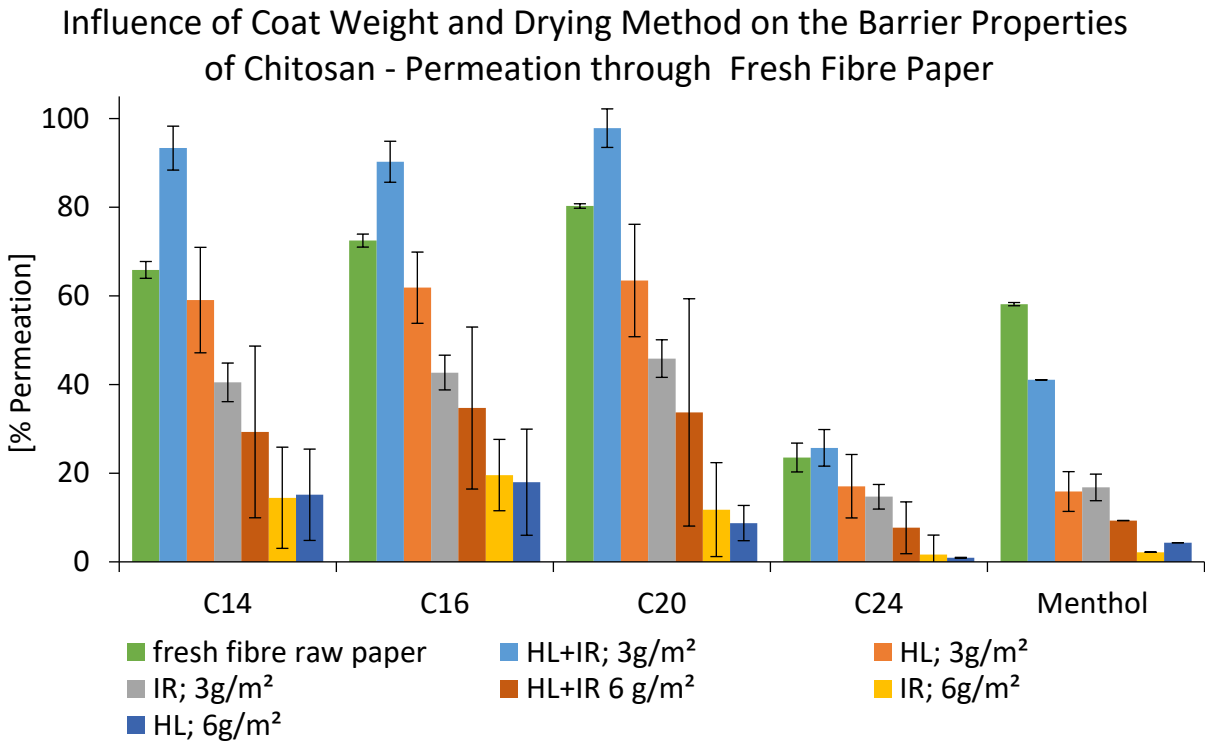

Figure 46: Influence of coat weight and drying on permeation through non-coated and chitosan coated fresh fibre paper. Determined on GC-FID; n=3, values given as % recovery of the spiked amount. Vanillin, eugenol, acetovanillone and C_{28} were <LOD.

In conclusion, the results show that the influence of the drying method is not neglectable. However, no clear pattern could be found, whether one method is better than the other. In discussion with the industry partners, they stated that the combination of hot air and infrared drying is the state of art drying method during paper and board production. Therefore, it was decided to use this combination in the following trials considering the screening approach of the project. For future applications of a biopolymer coating on paper or board it clearly has to be stressed, that the parameters must be chosen carefully and specifically for every single coating attempt.

3.2.2.1. Alginates and Influence of Layering

In the 4th trial the coating parameters set in the 3rd were used, meaning that all samples were dried with the combination of HL + IR. Furthermore, it was investigated how far the coat weight could be reduced to still get acceptable barrier efficiency and which influence the implementation of layers has on them (trial 4.1). Since the trial before was done with chitosan, alginate was used as a barrier material for this one, to generate more information on its behaviour too. Again, two-sided migration experiments were performed for each sample and the overall migration, the permeation and the migration of MOSH & MOAH determined and compared with the raw papers, afterwards. In a second attempt (trial 4.2), it was tried to find optimal viscosity conditions for the coating process.

The results of trial 4.1 revealed important and promising outcomes, summarised in Figure 47: when using only a single layer (SL) of coating, the decrease of migration was small. When applying double layers (DL), covering of the paper fibres was better and the barrier efficiency strongly improved (compare with (Kopacic, 2019)). As example, the 4.5 g m^{-2} single layer sample (4.5SL) showed barrier properties as good as the 6 g m^{-2} single layer sample (6SL) and both samples showed the same efficiency as the 2 g m^{-2} double layer sample (2DL, coated two times with 1 g m^{-2} coat weight). It was concluded that just applying more coating per square meter without optimisation of the coating paramteres does not help to further improve the barrier efficiency. In contrary, the double layer samples showed a steady increase in the barrier efficiency with increasing coat weight. The two times 3 g m^{-2} (6DL) sample gave the best result.

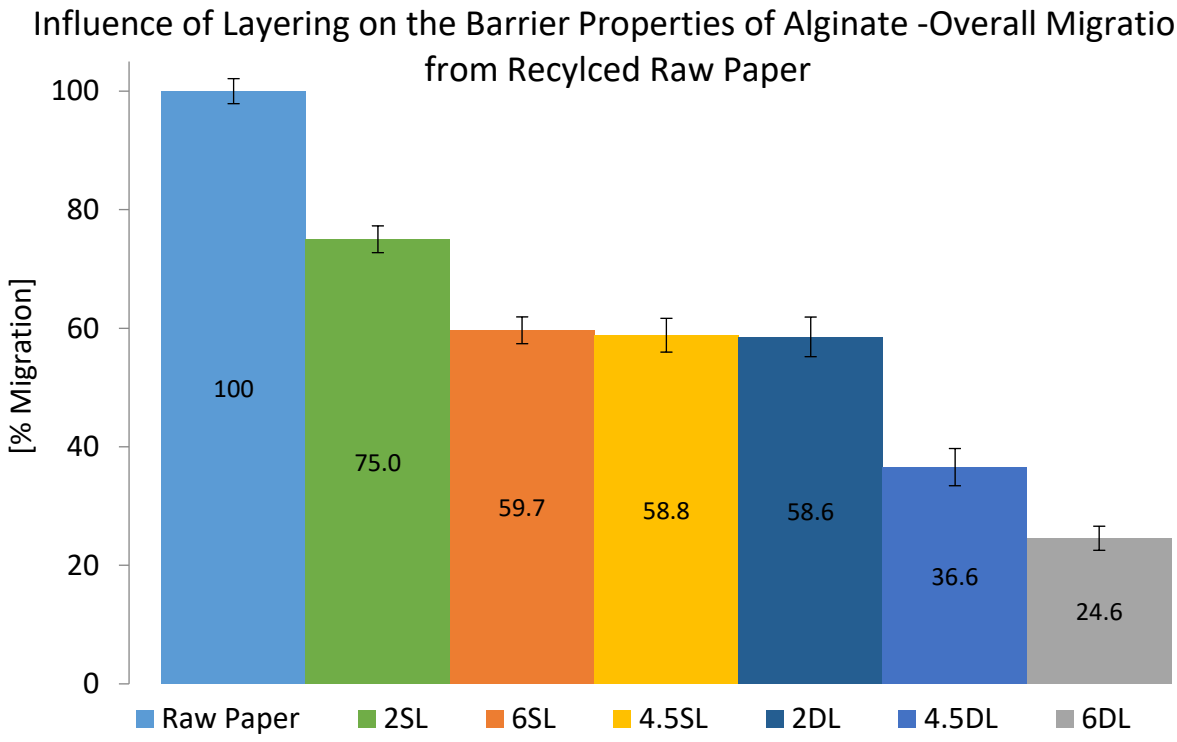

Figure 47: Overall migration results for Trial 4.1- layering experiment with alginate. n=3, values given as percentage of the uncoated recycled raw paper.

The data from the permeation experiment did not show exactly the same behaviour; the permeation is nearly the same for the 2SL, 6SL and 2DL samples (as can be seen pretty well for d-C_{16} and d-C_{20}), but the 4.5SL sample performs slightly better for all substances. Furthermore, the difference for the double layer coated samples with higher coat weight (4.5DL and 6 DL) is not as remarkable as it had been for the overall migration. In contrary, it nearly stays the same for menthol and d-C_{16} not having big influence on the permeation. The data is summarised in Figure 48.

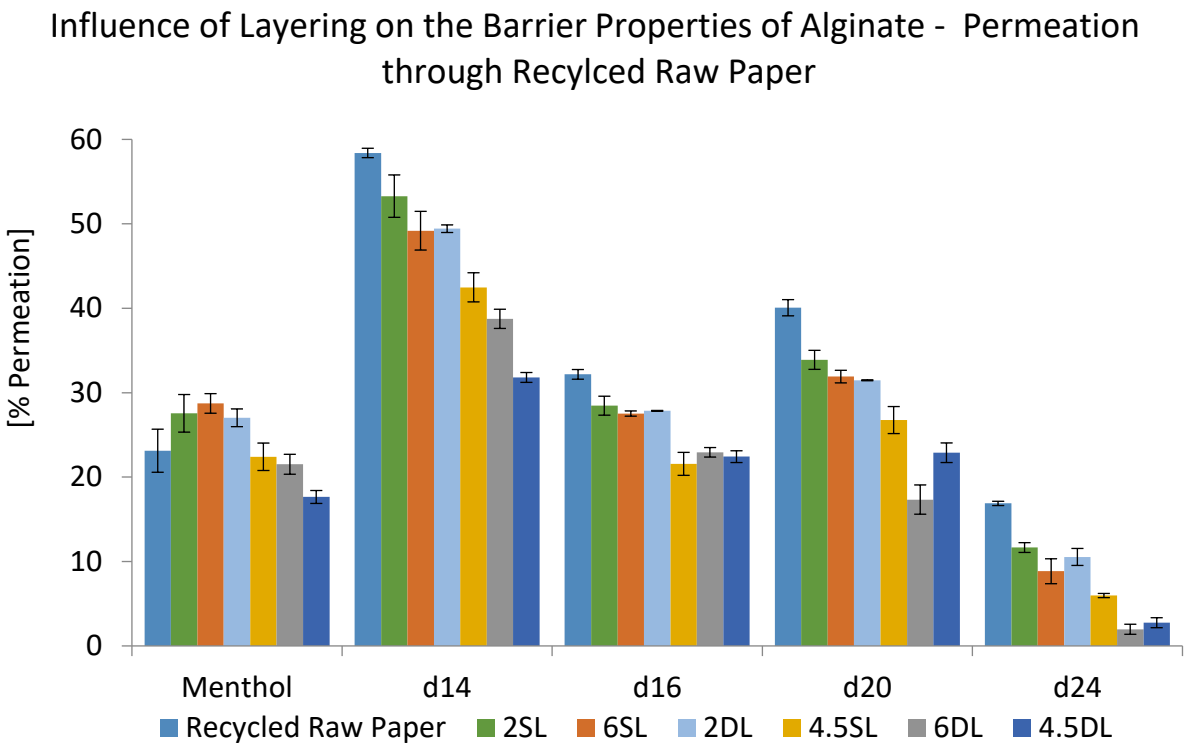

Figure 48: Permeation results for layering experiment with alginate. Determined on GC-MS; n=3, values given as % recovery of the spiked amount. Vanillin, eugenol, acetovanillone and C_{28} were <LOD.

The results for the migration of MOSH and MOAH, given in Figure 49, were comparable with the overall migration. The 2 g m^{-2} single layer sample (2SL) again showed the poorest result, the 4.5 g m^{-2} single layer sample (4.5SL) showed barrier properties nearly as good as the 6 g m^{-2} single layer sample (6SL) and the 2 g m^{-2} double layer sample (2DL). The increase of the barrier efficiency for the double layer samples with higher coat weight was even more distinct, making them to a good solution in terms of barrier coating for food contact materials.

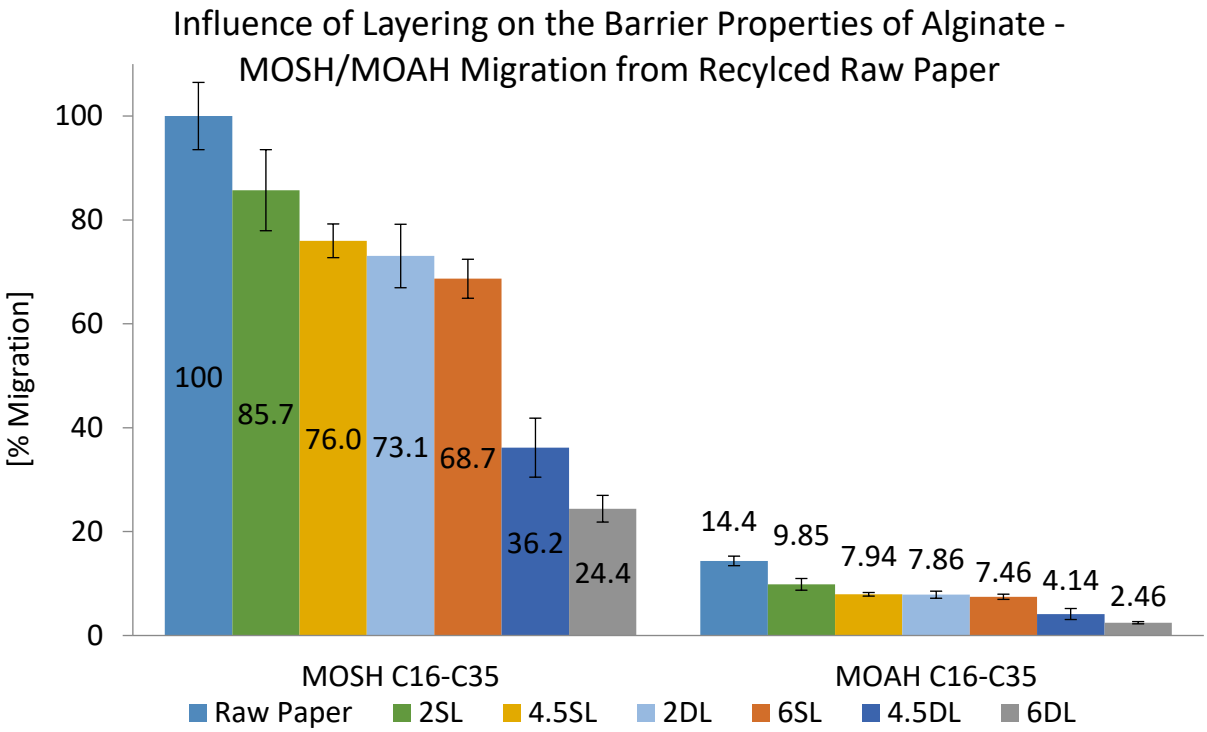

Figure 49: Results of migration of MOSH and MOAH for layering experiment with alginate. n=3, values given as percentage of the uncoated recycled raw paper.

Next, the results described above were compared with the results of the 3 g m^{-2} single layer samples, which had been coated at different temperatures and with varying solid content in trail 4.2. It was concluded, that they fit together quite well, as can be seen in Figure 50.

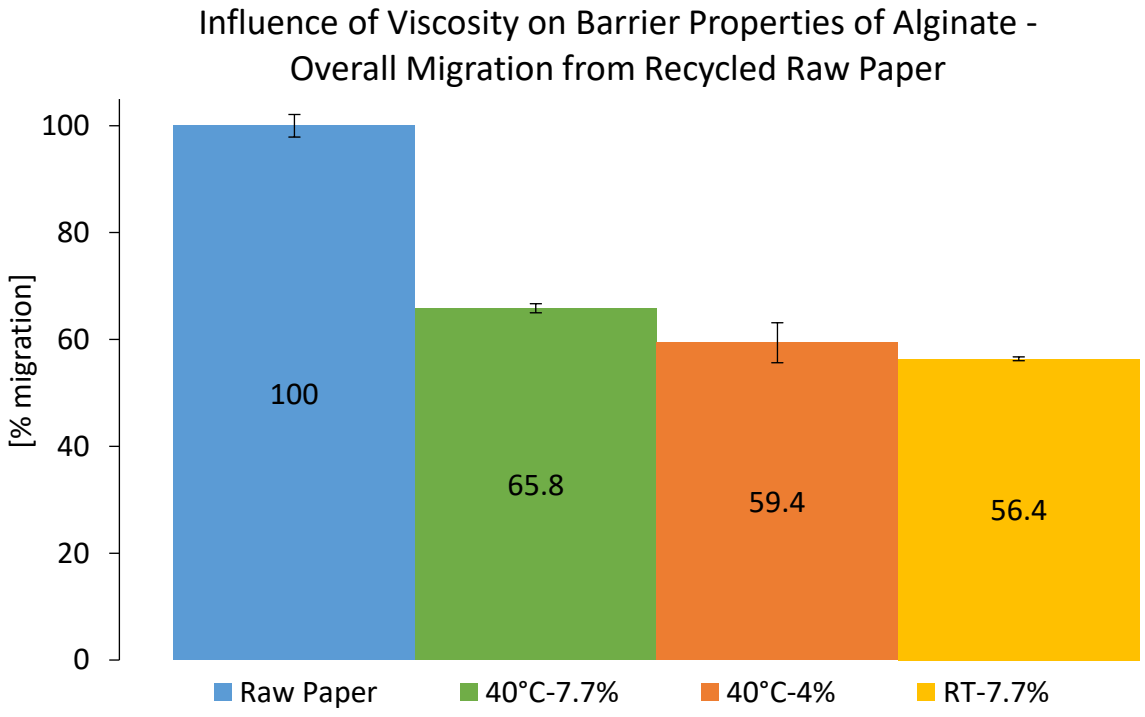

Figure 50: Overall migration results of trial 4.2. - Influence of coating conditions. n=3, values given as percentage of the uncoated recycled raw paper.

Coating with 3 g m^{-2} in a single layer shows a barrier efficiency that is comparable to the 4.5 and 6 g m^{-2} single layer sample, also strengthening the theory that at one point the barrier efficiency does not increase with the coat weight. The influence of the varying coating conditions is present, but with about ± 10% reduction not immense. It seems, that the sample coated with a higher viscosity solution (RT-7.7%) results in a slightly better barrier performance.

The permeation results and the results of MOSH/MOAH migration for trial 4.2 are in alignment with the overall migration results and therefore not shown separately.
In conclusion, this trial showed, that just increasing the coat weight does not help to improve the barrier efficiency. It is much more valuable to use low coat weight and apply several layers. This is comparable to already used functional barriers; most of them being complex multi-layer materials. Again, it can be concluded, that the optimisation of the coating conditions can be an important detail for the key to success in real food contact applications.

3.2.2.2. Influence of Sizing and Calendering

To test the influence of different paper treatment possibilites on the barrier properties, in the 5th trial the recycled raw paper (RRP) was surface sized using alkenyl succinic anhydrides (RRPs) and calandared (RRPc). The fresh fibre raw paper was already surface sized and was additionally calandered prior to the coating procedure (FFPc). Coating materials were again alginate and chitosan, which were applied in an amount of 3 and 5 g m^{-2} for the calandered samples and 3 and 6 g m^{-2} for the surface sized papers, respectively. For the results of this trial it has to be mentioned that the influence of layering on the barrier properties are immense. Therefore, by using only one layer of coating, the barrier properties decrease and are not comparable with the good results achieved in the 4th trial.
First, the inlfucene of sizing and calendering onto the raw papers was investigated. The results for the RRP are given in Figure 51. It can be seen, that sizing did not have any influence on the migration. The calandered raw paper showed a slightly higher overall migration as the untreated raw paper, probably due to contamination of the paper during the calandering procedure. For the fresh fibre paper sample, which was already sized with starch, only the influence of calendering was tested. The overall migration showed no differences and the results are therefore not given.

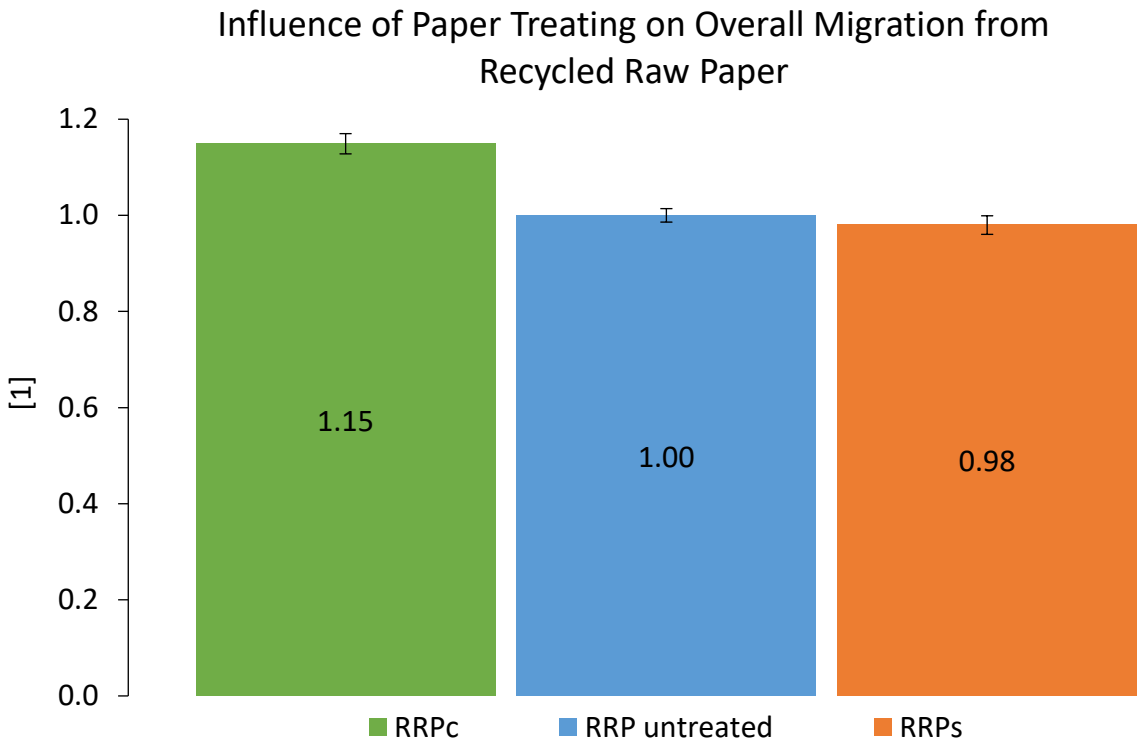

Figure 51: Influence of paper treating on the overall migration of the recycled raw paper. n=3, results normalised to the untreated paper.

Second, the influcene of sizing and calendering on the barrier properties of alginate and chitosan was investigated. The results of the overall migration for the recycled raw paper are summarised in Figure 52, the results of the permeation experiment in Figure 53 and the ones for the MOSH and MOAH migration in Figure 54. The change of barrier efficiency was as expected for those: it drastically decreases to a maximum reduction of 30%, due to the application of only one coating layer. No difference between alginate and chitosan could be seen when using 5 g m^{-2} coat weight (RRPc + 5Cht and RRPc + 5Alg) and interestingly the use of 3 g m^{-2} chitosan (RRPc + 3Cht) showed barrier properties as good as using 5 g m^{-2}. This again indicates that just applying more coating per square meter without the optimisation of the coating paramteres does not help to further improve the barrier efficiency. Furthermore, sizing or calandering of the recycled raw paper with ASA did not help to further improve the coating's barrier efficiency.

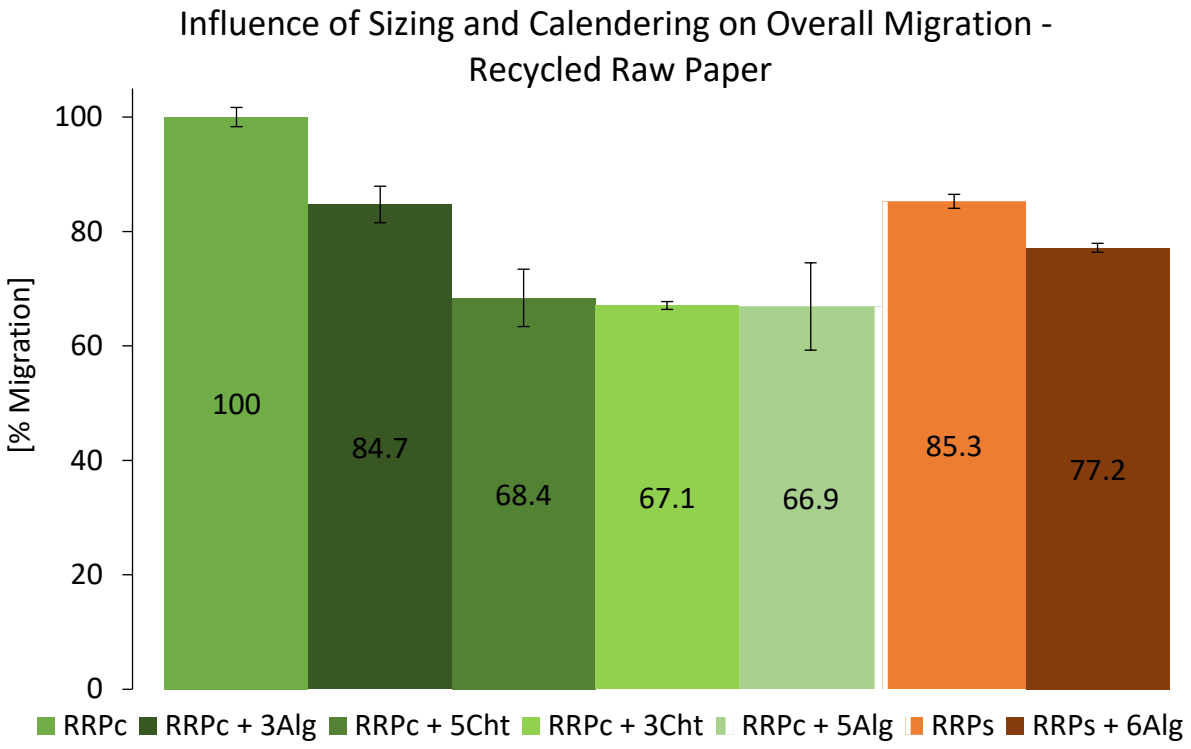

Figure 52: Influence of sizing and calendering on overall migration of the recycled raw paper. n=3, results normalised to the calendered raw paper (RRPc).

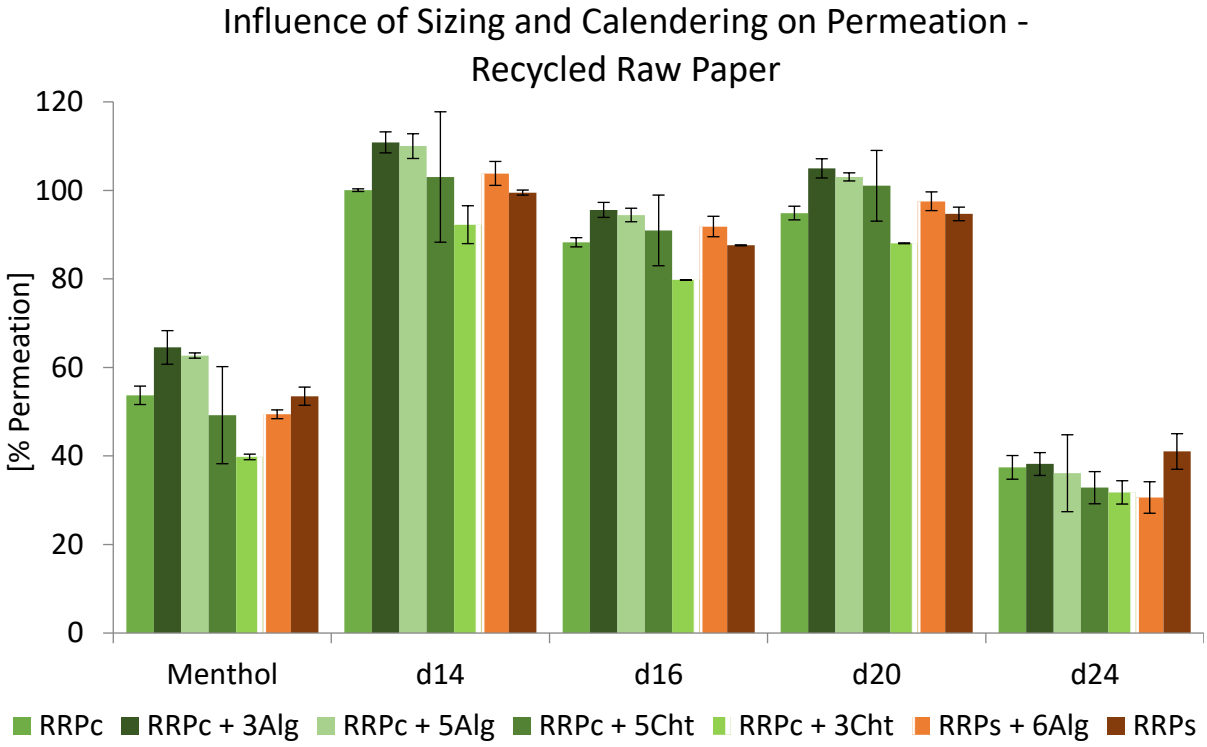

Figure 53: Influence of sizing and calendering on permeation through recycled raw paper determined with GC-MS; n=3; values given % recovery of the spiked amount; canillin, eugenol, acetovanillone and C_{28} <LOD.

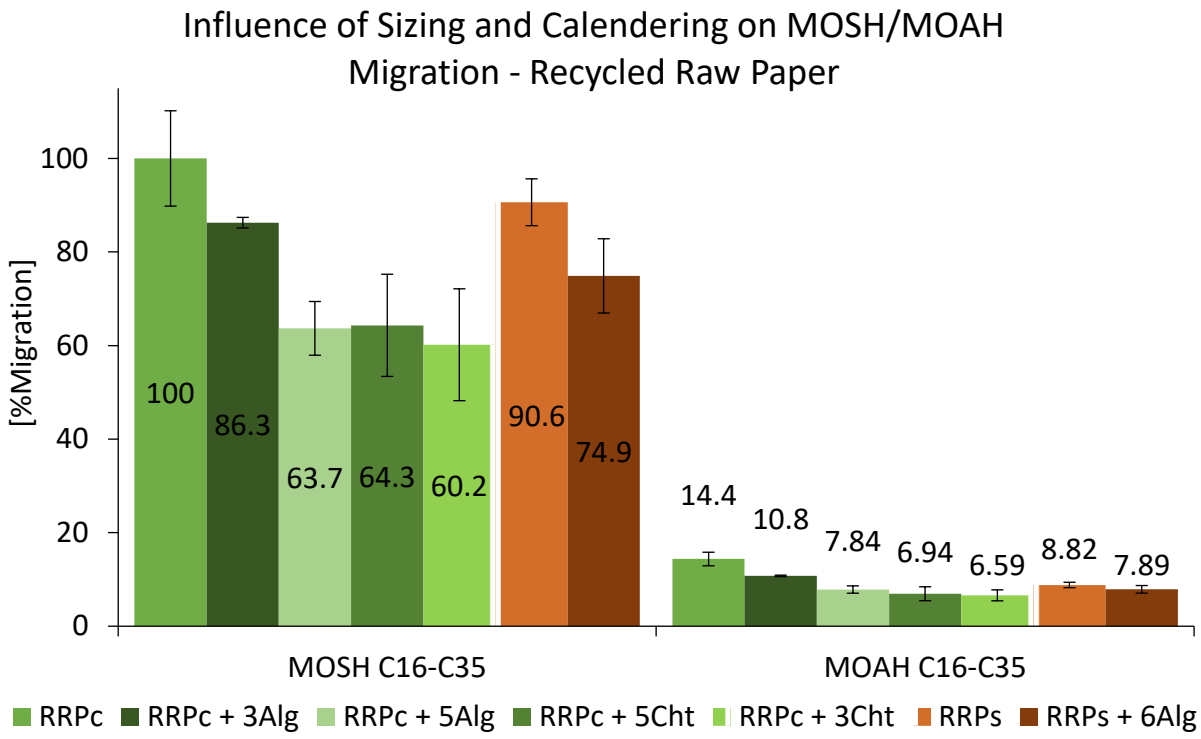

Figure 54: Influence of sizing and calendering on the migration of MOSH/MOAH from the recycled raw paper; n=3; results normalised to the calendered raw paper (RRPc).

Compared to the recycled fibre paper, the influence of calendering on the permeation through the FFP and on the coating was bigger. The results are summarised in Figure 55.

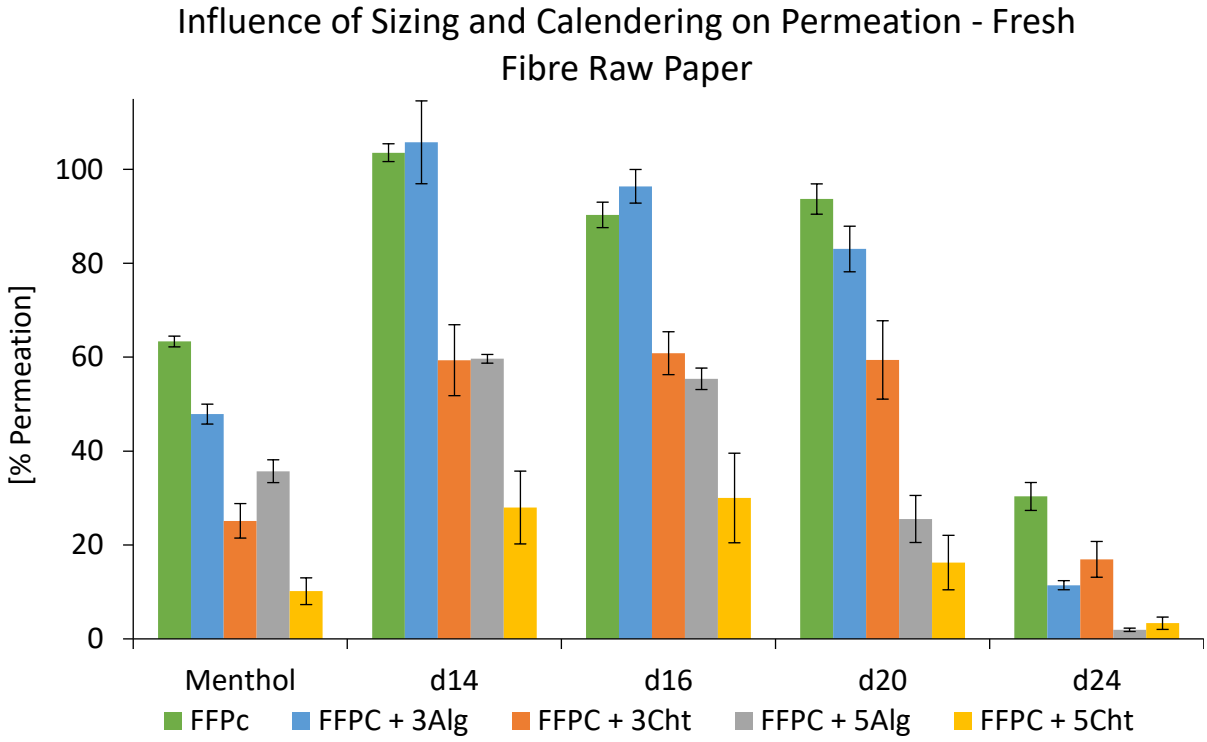

Figure 55: Influence of sizing and calendering on permeation through fresh fibre raw paper; n=3; values given as % recovery of the spiked amount; vanillin, eugenol, acetovanillone and C_{28} < LOD.

The resulting barrier efficiencies for the 5 g m^{-2} chitosan samples (FFPC + 5 Cht) were good, despite using only one layer of coating. The 3 g m^{-2} chitosan sample gave results similar to the 5 g m^{-2} alginate sample. The 3 g m^{-2} sampled again showed the lowest barrier effect.

In summary, sizing and calendering influences the paper surface and can therefore have an additional influence on the coating procedure and the resulting barrier efficiency. The paper gets denser and the surface smoother, which results in a more even distribution of the coating and a better coverage of the fibres. In general, the barrier efficiencies seen in this trial would be too low for the application in food packaging materials. One layer of coating is simply not enough to cover all fibres evenly, which can be seen by the far better results achieved before, when using two coating layers. Furthermore, it could be shown, that chitosan gave better results than alginate for the fresh fibre paper sample and both gave comparable results for the recycled fibre sample.

3.3. Results Characterisation of Migrated Fractions

3.3.1. General Aspects

After performing a (two-sided) migration experiment with a sample and analysing and characterising it using GC-FID, GC-MS and HPLC-GC-FID, there is already a lot of information on the migrated substances generated. However, for recycled paper and board with huge amounts of MOSH and MOAH present in the extract, it is not possible to separate all substances of interest, due to the presence of unresolved complex mixtures and the formation of humps. Applying an additional chromatographic dimension, by using comprehensive 2D-GC×GC may help to overcome this problem. However, it soon went clear that even comprehensive GC×GC is not able to separate those humps completely. Nevertheless, it is possible to generate further information by e.g. identification of substance classes. Classification can be done more easily, if the total migrated fraction is pre-separated into the MOSH and MOAH fraction using the normal-phased HPLC.
Furthermore, two instruments of different suppliers were tested for their chromatographic performance, their detector, as well as for the data evaluation software available. It has to be mentioned that the GC×GC-qMS is permanently available at the institute for measurements, while the GC×GC-TOF measurements were performed during a visit at the LECO European Application and Technology Centre in Berlin, Germany. Therefore, most data available is from GC×GC-qMS.
Since the instruments used were both based on mass spectrometry, a quantification of individual substance classes was not possible. As already discussed in the introduction, a universal detector, such as the FID, would be needed for this attempt.

3.3.2. GC×GC-qMS

3.3.2.1. HS-SPME

To get a first idea of the instrument's capabilities, the paper samples were measured using HS-SPME as sample preparation method. Since the present substances were known to reach chain lengths of C_{35}-C_{45} two extraction conditions were tested: extraction at 80°C for 30 min transferred substances till a chain length of about C_{20}, for the extraction at 150°C substances with a chain length of up to C_{30} were seen. The impressive images resulting from the recycled raw paper sample 4 at the tested conditions are given in Figure 56.

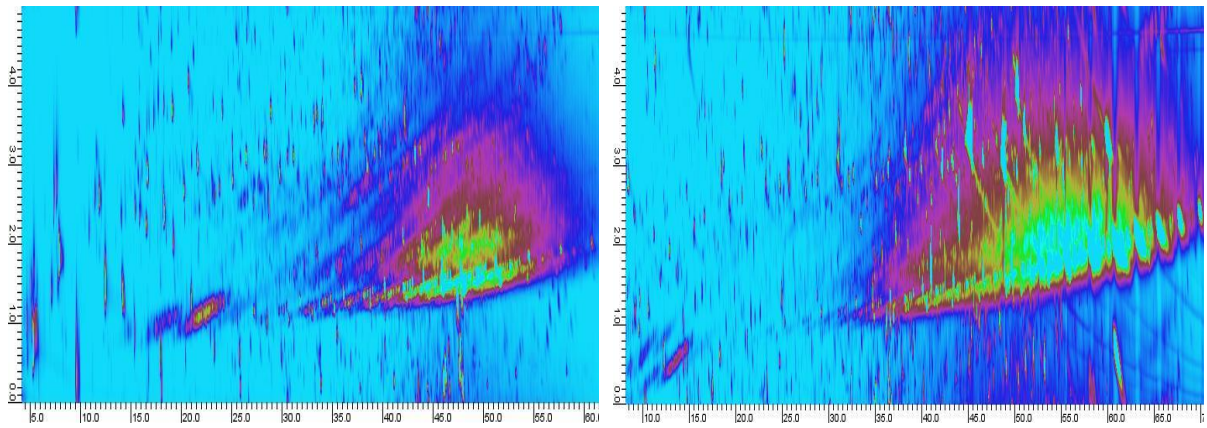

Figure 56: Recycled fiber raw paper sample 4; HS-SPME extraction done at 80°C for 30 min (left) and at 150°C for 30 min (right)

Since it is clearly visible from the resulting chromatograms, that the higher temperature released more substances from the recycled raw paper, those conditions were chosen to analyze the fresh fiber raw paper sample 1, too. For comparison of the chromatogram of the fresh fiber sample with the immense hump of substances present in the recycled raw paper, it is given in Figure 57 on the next page.

The conclusion of this first measurements was that even though a 2D-separation was applied onto the recycled raw paper sample, it was not possible to separate the huge hump of unresolved substances. The chromatogram was heavily overloaded, although HS-SPME was only used as a quick and easy attempt to generate first information. It was decided to perform the next measurements on extracts of migration experiments, since this would better represent the real situation.

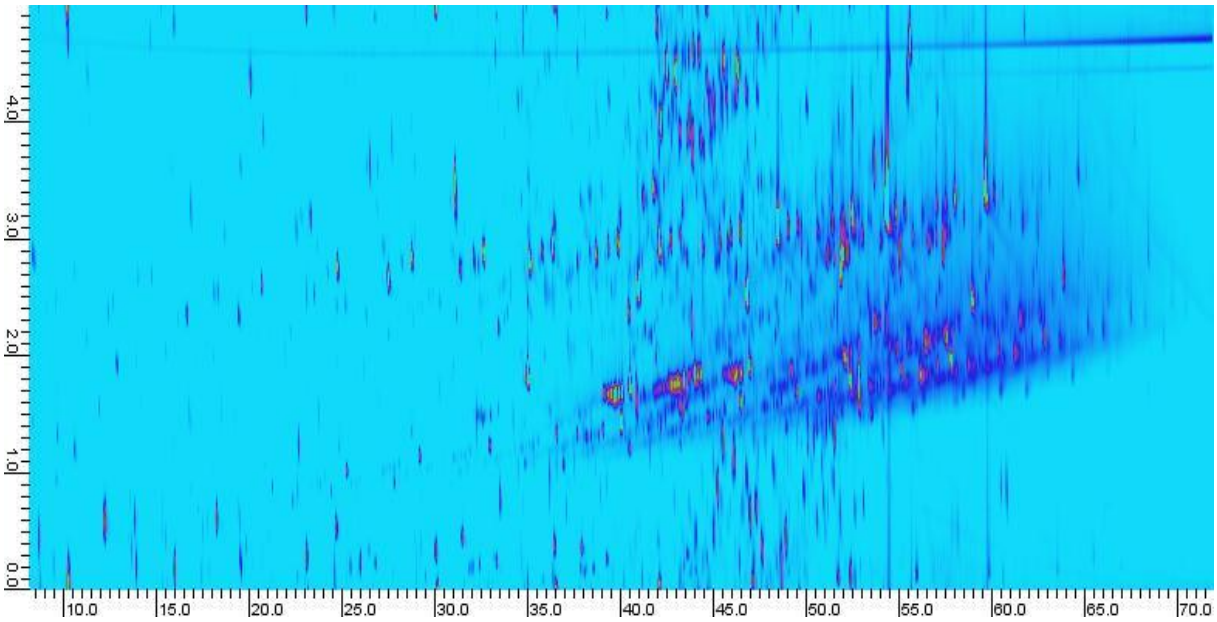

Figure 57: Fresh fiber paper sample 1; extraction done at 150°C for 30 min.

3.3.2.2. Migrated Total Fraction

The second step was to analyses a total migrated fraction of substances transferred from the paper samples into the food simulant. Examples of the overall migration of the recycled raw paper and the same raw paper coated with 6 g m^{-2} alginate are given in Figure 58 and Figure 59. The scaling of the images is for the left figures the same as for Figure 56 and Figure 57 to allow a comparison of the hump's intensity. One can already see that by far lesser substances are present in the migrated fraction. The right figures of Figure 58 and Figure 59 show the same chromatogram, but with enhanced scaling.

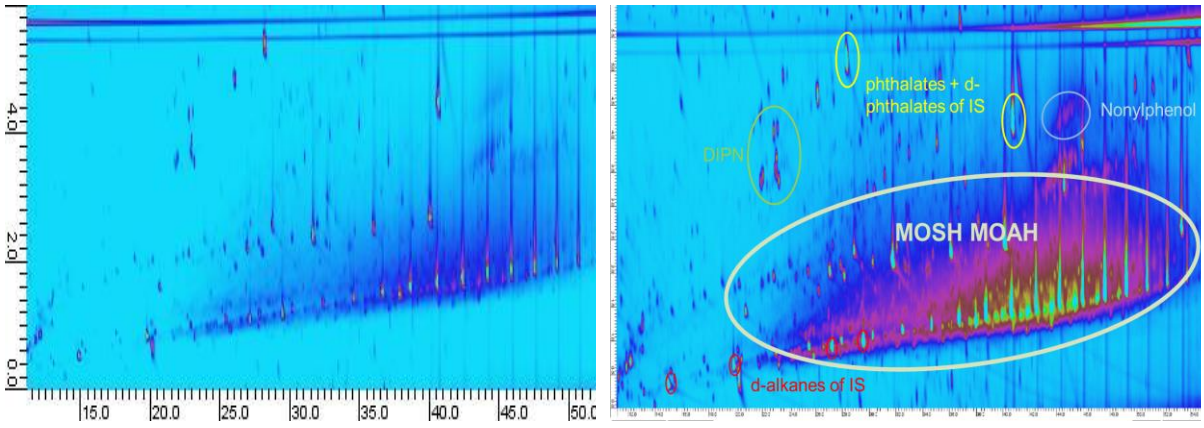

Figure 58: Total migrated fraction of recycled raw paper with same scale (left) as figure 56-57 and intensified scale (right).

By comparing the non-coated sample with the alginate coated one the successful reduction of the overall migration by 90% is visible. The non-coated sample in Figure 58 still shows a large hump of MOSH and MOAH, marked with a colorless circle. Marked in green are the clearly

visible DIPNs, also other phthalates are present, but mainly overlaid by the added deuterated internal standards. In comparison, the coated sample in Figure 59 shows only little residues of the substances mentioned before. The cloud of MOSH and MOAH is nearly gone.

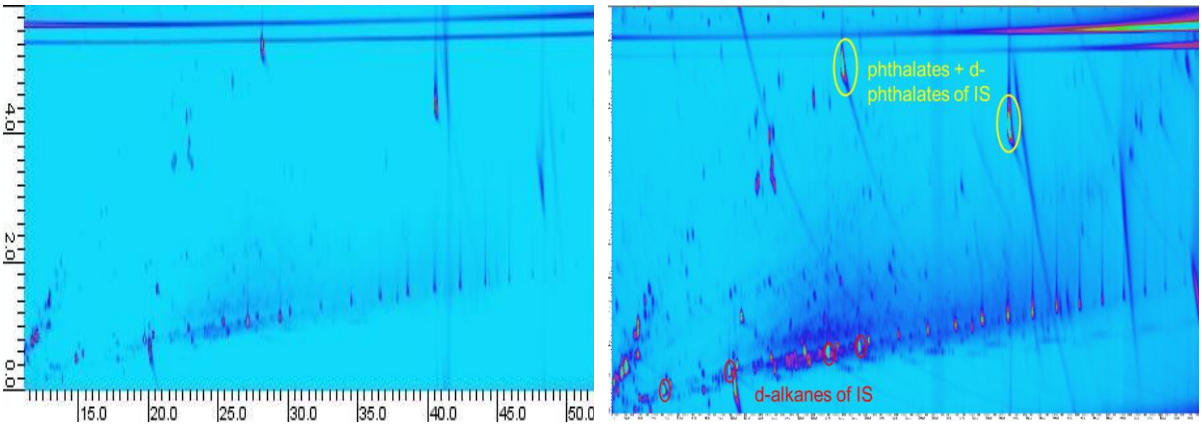

Figure 59: Total migrated fraction of recycled raw paper coated with 6 g m^{-2} alginate with same scale (left) as figure 56-57 and intensified scale (right).

Although these chromatograms show pretty good results, a more detailed characterization of the substances still present in the migrated fraction is needed. Therefore, pre-fractionation of the total migrated fraction into MOSH and MOAH was done in the next step.

3.3.2.3. Migrated Fractions Before and After Pre-Separation

To allow a better separation of individual groups, the column combination was changed for these measurements. The usual combination for GC×GC is a non-polar first dimension column, separating by volatility, and a polar second dimension column, separating by polarity (Mondello, 2011). The combination used for these measurements was a reversed one: a polar column in the first dimension and a non-polar in the second one. It allows a better separation of the individual substance groups, as can be seen for the total migration extract in Figure 60 and the MOAH fraction in Figure 61.

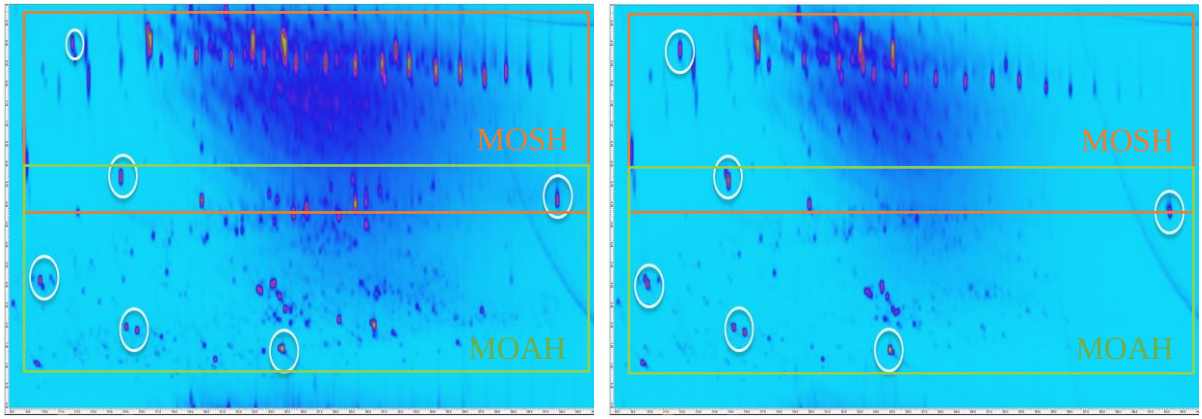

Figure 60: Total migrated fraction of recycled raw paper non-coated (left) and alginate coated (right). Internal standards indicating separation of MOSH (in orange) and MOAH (in green) marked with circles.

The pre-fractionation into MOSH and MOAH facilitates the evaluation of individual substance classes without disturbance by the other fraction. This is especially helpful for the MOAH fraction, since MOSH substances would interfere with the analysis, which is indicated by the overlapping area in Figure 60. The resulting chromatograms for the pre-fractionated MOAH fraction are given in Figure 61.

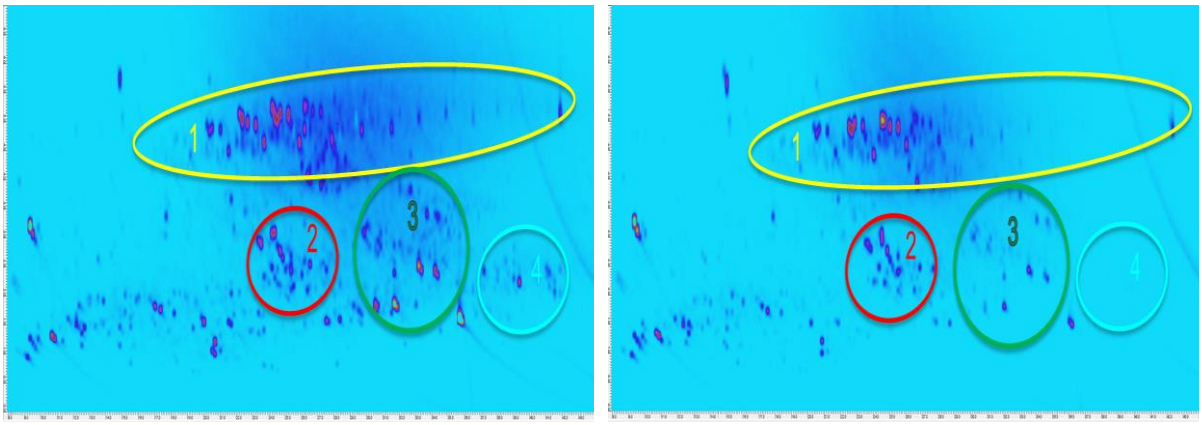

Figure 61: migrated MOAH fraction of recycled raw paper non-coated (left) and alginate coated (right). Alkylbenzene marked in yellow, DIPN und 2-ring aromatics in red, 3-ring aromatics in green and 4-ring aromatics in blue.

The used column combination allows a clear differentiation of the aromatic substances by their ring number. The one-aromatic ring substances (yellow circle in Figure 61) are rather volatile and therefor clearly separated from the other MOAH classes in the second dimension. The two-ring and higher-ring aromatics are separated by their polarity in the first dimension.
Aim of these experiments was, to get information on the substances present in the residual migration of a recycled board after applying a functional barrier on it. Since the three- and higher-ring aromatics are considered to be the most dangerous part of the MOAH fraction, their presence in food should be as low as possible (EFSA, et al., 2019). The GC×GC data shows that the MOAH substances still present are mainly one- and two-ring aromatics. There are still some three-ring aromatics left, but the migration of the four-ring aromatics was completely stopped by applying alginate as functional barrier.

3.3.3. GC×GC-TOF

As already discussed in the introduction, the big advantage of the TOF is, that a complete spectrum is produced from every ion pulse reaching the detector, resulting in very sensitive full scan spectra. Therefore, it is an important tool to not only identify substance classes, but also individual substances within one class. It was also used in this work as tool to identify present substances and substance classes in the migrated fractions.

As a first step, the sensitivity of the TOF detector was compared to the qMS, described in the chapter before. To do so, the signal to noise (S/N) ratio of the measured internal standards were determined and compared to each other at two levels, as given in Table 22.

Table 22: Signal to noise ratios of MOSH/MOAH Internal Standard Mix for TOF and qMS.

	10 pg*µL^{-1}		**1 pg*µL^{-1}**	
	TOF	*qMS*	*TOF*	*qMS*
C_{11}	840	338	84	115
CyCy	2061	811	60	215
C_{13}	507	624	71	133
Cho	2209	417	159	56
5B	1072	476	109	100
1MN	5058	437	128	88
2MN	5572	277	157	63
TBB	15819	859	382	114
Per	1860	64	52	46

For the 10 pg µL^{-1} level, the TOF gives clearly the better S/N ratios, especially for the high-mass compounds cholestane (~5-fold better), tri-tert-butylbenzene (~18-fold) and perylene (~29-fold better). For the 1 pg µL^{-1} level, the qMS gave similar, to slightly the better results for the low-mass substances as e.g. C_{13} (~2-fold better than TOF). However, the TOF again performs better for the high-mass substances Cho and TBB (both ~3 fold better).
In the next step, the chromatograms of the overall migration extracts of the coated and the non-coated paper were compared to each other, as can be seen in Figure 62. The analysis of the non-coated paper (left chromatogram in Figure 62) revealed nearly 20 000 present peaks with a S/N ratio of ≥2, when applying the automated signal detection. After subtracting non-relevant peaks, such as siloxanes from e.g. column bleed and all peaks with a S/N <10, about 10 000 detected peaks remained to be evaluated. In comparison, the analysis of the alginate coated paper sample (right chromatogram in Figure 62) revealed a similar total number of peaks. However, after subtracting the non-relevant peaks and all peaks with a S/N <10, only about 7000 detected peaks remained to be evaluated.

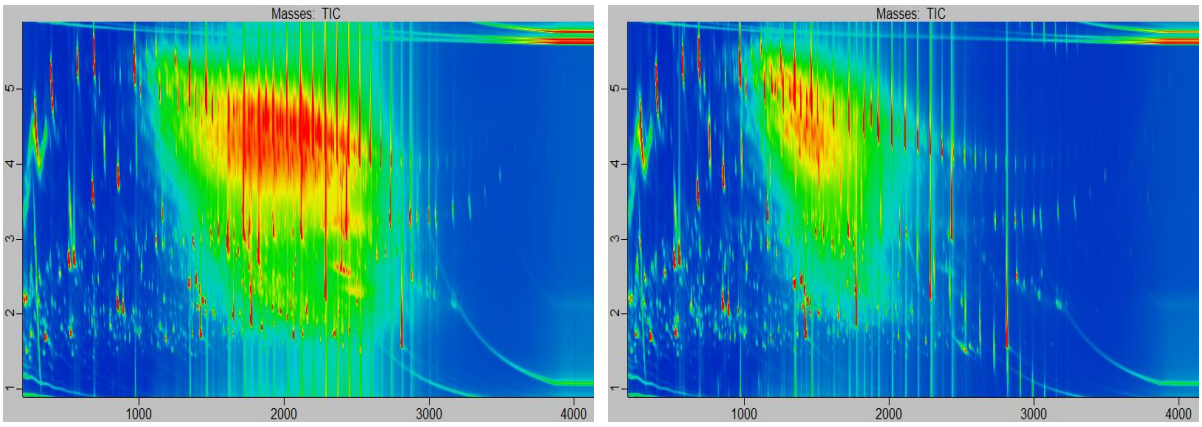

Figure 62: GC×GC-TOF chromatogram of overall migration of non-coated recycled raw paper sample (left) and the same paper coated with 6 g m^{-2} alginate (right).

Since the MOAH fraction has the higher toxicological relevance, the following characterisation and classifications mainly focused on it (EFSA, et al., 2019). The present substances were divided according to the PhD-thesis of Jurek A. (2017) into following substance groups:

- Phenylalkanes: they were divided depending on the α-C atom of the alkyl-chain into primary phenylalkanes with a base peak of m/z 91, secondary phenylalkanes with a base peak of m/z 105 and tertiary phenylalkanes with a base peak of m/z 119. The individual alkanes were identified by using their molecular ions, which were present for most of the detected substances (see appendix Table 24 and Table 25).
- Naphthalenes: divided based on their alkylation into methyl and/or isopropyl (m/z 184 and 196) and into trimethyl naphthalenes (m/z 170 and 155; Table 26 in the appendix).
- Biphenyls: divided into tetramethyl- and diethyl biphenyls with the m/z 195, 210 and 165, the trimethyl biphenyls with the m/z 196, 181 and 165 and the dimethyl biphenyls with the m/z 182 and 167 (see appendix Table 27).
- Phenanthrenes and anthracenes: divided based on their alkylation into methyl-, dimethyl, trimethyl and tetramethyl phenanthrenes and anthracenes. The m/z used were the base mass of m/z 178 + m/z 14 for every methyl group added (see Table 28 in the appendix).
- Non-MOAH substances: such as abietic acids and derivatives, steranes, hopanes and DIPN were subtracted from the MOAH fraction and are not given separately.

As example the identification and classification procedure for the phenylalkanes are given in Figure 63. Primary phenylalkanes were identified by their base peak of m/z 91, α-methyl phenylalkanes by the base peak of m/z 105, all higher α-alkyl phenylalkanes by the base peak of m/z 91 and the 2nd most abundant peak of m/z $105+(14)_{n-1}$, where n= number of C-atoms in the α-side chain. For example, α-ethyl phenylalkanes is identified by the base peak of m/z 91 and the 2nd most abundant peak of m/z 119 (105+14), α-butyl phenylalkanes by the base peak of m/z 91 and the 2nd most abundant peak of m/z 147 (105+3*14) (Jurek, 2017).

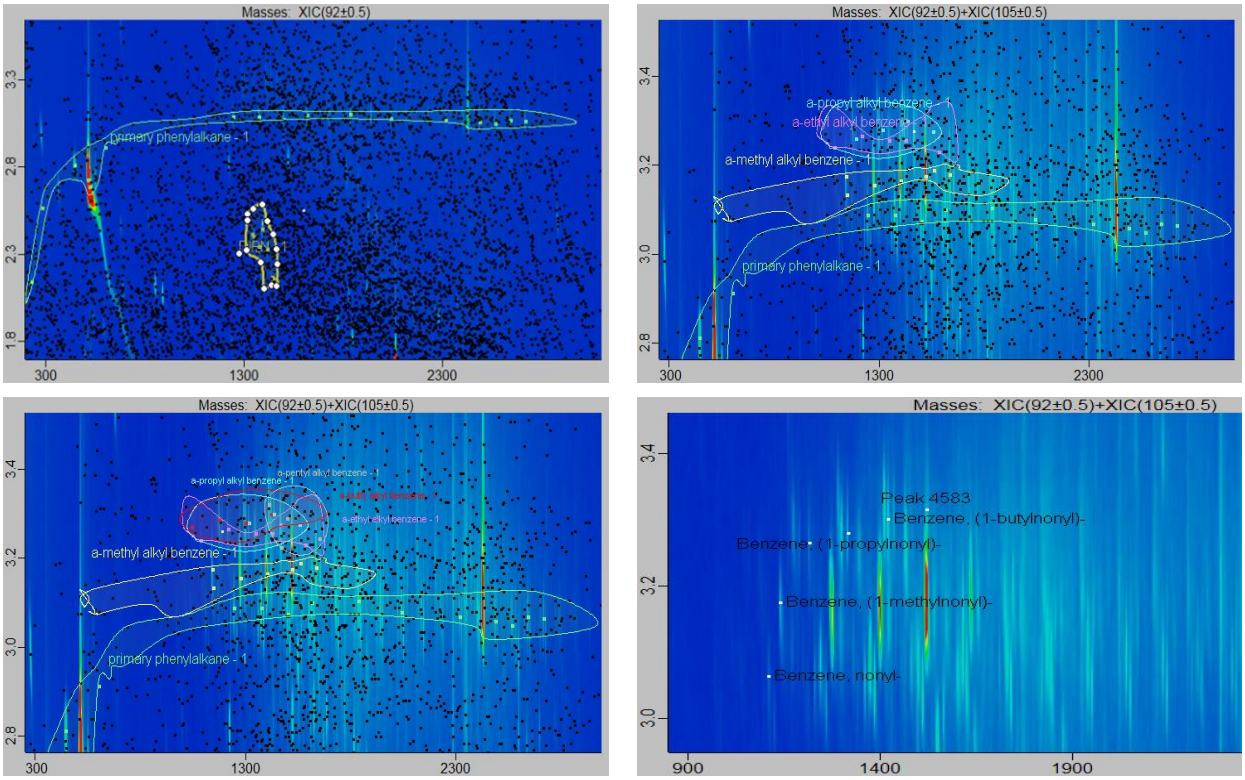

Figure 63: Example for phenylalkane-classification: starting with primary phenylalkanes (upper left) and identifying α-methyl-, α-ethyl-, α-propyl-, α-butyl- and α-pentyl- alkanes (upper right and lower left); example for phenyl, nonyls- (lower right). Peak 4583 in the lower right chromatogram is Benzene, (1-pentylnonyl)-.

In comparison with the results of Jurek, A. (2017) the alkyl chain length of the phenyl alkanes showed similar values of 10 to 20 C-atoms for recycled fibre materials. The tables of the identified substances given in the appendix represent only a quick overview of substances migrated from the recycled raw paper into the food simulant MPPO, since detailed identification of all substances was not the prior aim. It has to be stressed, that these lists are by far not complete. The semi-quantitative evaluation using the area percentage showed that these identified peaks are still <10% of the MOAH fraction. The comparison of the non-coated and the coated paper sample revealed that there were only slightly changes in terms of present substances for the 1-, and 2-ring MOAH substances. However, the higher ring substances (as well as the long-chain MOSH) were reduced to a substantial part, as was also visible in the chromatograms of Figure 62.

In conclusion, the GC×GC-TOF is a powerful tool for the identification of unknown substances in the migrated mineral oil fractions, since full-scan spectra are available at each point of the chromatogram. The used software allowed to set filters and mark regions of interest, allowing to identify substances of interest quickly and easily. Nevertheless, still only small parts of the total MOAH fraction are known and the toxicological evaluation therefore difficult. Further work will be done in the FFG project “MOSH/MOAH Reduktion von Mineralöl in Lebensmitteln”, which will be continued till 2022. HPLC-GC×GC-FID/MS analysis will be

used to further characterize individual substance groups and to provide those pre-separated groups to toxicological evaluation.

3.4. Analysis of MOSH & MOAH in Food

The presented data is part of the work done in the first year of the FFG project "MOSH/MOAH Reduktion von Mineralöl in Lebensmitteln". The project and the work will be continued till 2022. Therefore, presented methods and data are preliminary state of the art.
At this point I want to stress again that parts of the method development and optimisation, mainly saponification and epoxidation, were done as part of the collaboration "ThinkTank-MOSH/MOAH" and the information generated further applied to other food types.

3.4.1. General Aspects

In the beginning, the only available documents and methods were the BfR-compendium, only published in German and the EN16995 ([BfR] German Federal Institute for Risk Assessment, 2012) (Austrian Standards Institute, 2017). Based on these two documents the method development and analysis of MOSH and MOAH in this work were started. However, as already discussed in the introduction, it soon became clear that the presented methods reach their limits for MOSH and MOAH concentrations below 10 mg kg^{-1}. The used Al_2O_3 and silica gel columns do not have high enough capacities to retain large amounts of fat. If for example, the targeted detection limit of 0.5 mg kg^{-1} for fats and oils of the JRC guideline should be reached, one would need about 3 g of sample to be sensitive enough. The clean-up and enrichment of the MOSH fraction would need a column with at least 18 g of silica gel to retain the fat and about 30 g of Al_2O_3 for retaining the naturally present n-alkanes (capacity of 3 mg alkanes), calculated according to (Biedermann, et al., 2012a). For the MOAH fraction, one would need even more material, since dichloromethane is used for elution of the MOAH, which decreases the retention of fat. For 3 g of sample 36 g of silica gel would be needed. Consequently, the resulting columns would get enormous, so would the needed amount of resources, which are the bed materials, solvents and especially time needed to do one column. Further disadvantages are the higher risk of contaminations, since larger amounts of resources used result in higher blank values too, and the high influence of the operator. The results for the tests performed in this work were also improvable: for several matrices/samples (especially for chocolate and palm oil) a fat breakthrough was seen, resulting e.g. in oily or even solid residues after solvent evaporation, which could not be used for further analysis. If the sample preparation showed no complications, the chromatograms often showed residues of fat, sometimes so high that they completely devastated the HPLC-GC-FID. It seemed that the self-prepared and used columns did not have high enough capacities to retain the fat. Furthermore, high blank values or even bad contaminations of the blank and the sample were seen, which again made the evaluation of results impossible. Repeatability was also bad; analysis of the same sample on three different

days showed three completely different results in most cases. Therefore, it was decided to look for alternatives for these sample preparation methods and saponification was identified as the most suitable one.

During saponification the ester bonds of the triglycerides are broken by using a base, resulting in glycerol and the fatty acid salts (soap), which are water soluble. This is used when doing a saponification for MOSH and MOAH analysis to remove fat from the matrix. The saponification is performed in an aqueous medium. Hexane is used afterwards to extract the non-polar mineral oils, while the saponification products remain in the aqueous medium. In theory, this is a simple process, but in practice it can be hard to perform. Depending on the food's origin and sources, the present triglycerides can be varying. Consequently, not every food matrix behaves the same during saponification. Method optimisation for every single food type may therefore be needed. For example, the developed method worked well for many edible oils, fats, and fat/oil-based foods, but not for chocolate, as will be discussed in the chapter 3.4.5 on chocolate below. However, also within one food type it is not guaranteed that each sample will work with the same method. Method development and optimisation can therefore get really challenging and time consuming. For example, while the method was applied successfully for many different edible oils like olive oil, sunflower oil, rapeseed oil or fats like butter or coconut butter, it did not work at all for palm oil, resulting for many tried conditions in a solid, orange mess (see Figure 64). However, if the optimal conditions are found, the saponification has many advantages: it is quicker (many samples can be done parallel), easier, solvent and material consumption is much lower and repeatability is much better compared to column clean-up and enrichment.

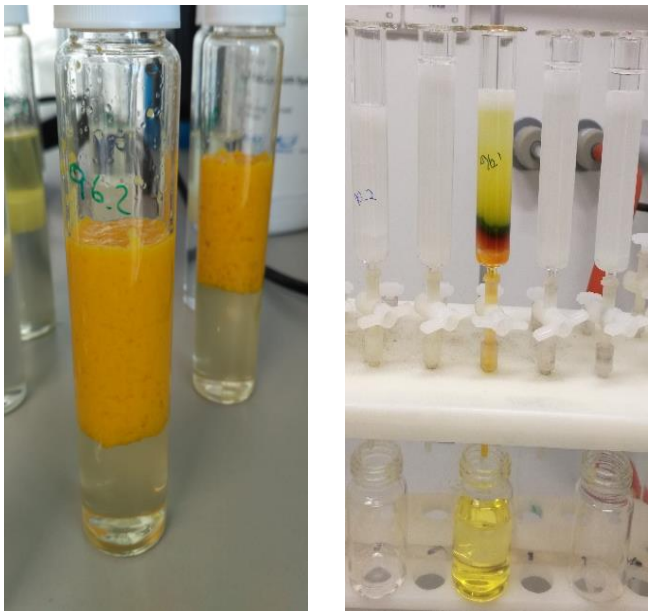

Figure 64: Challenge palm oil: saponification and SPE clean-up.

Since saponification only removes triglycerides and fats, performing manual column chromatography can still be needed for the further clean-up and enrichment of the samples. In

the MOSH fraction natural odd-numbered n-alkanes may be present, needing an Al_2O_3 clean-up to remove them. In the MOAH fraction, natural olefins may be present, needing an SPE clean-up and epoxidation using m-CPBA. Clean-up of the MOSH fraction, using columns with 7 g Al_2O_3, 3 g silica gel and 1 g of Na_2SO_4 showed good performance. In the MOAH fraction the SPE clean-up using 6 g of silica gel and 1 g of Na_2SO_4 prior to epoxidation showed more selective and therefore better epoxidation results.

The test of the three different epoxidation methods showed the following results, explained on an olive oil sample in Figure 65: The "soft" method, using 350 µL of m-CPBA in dichloromethane showed incomplete epoxidation (middle). The added internal standards were still heavily disturbed and the chromatogram still shows a large peak of present olefins. Therefore, this method is not suitable and was rejected. The "hard" method showed complete epoxidation and good results (right), so did the automated approach, using epoxidation in ethanol (left).

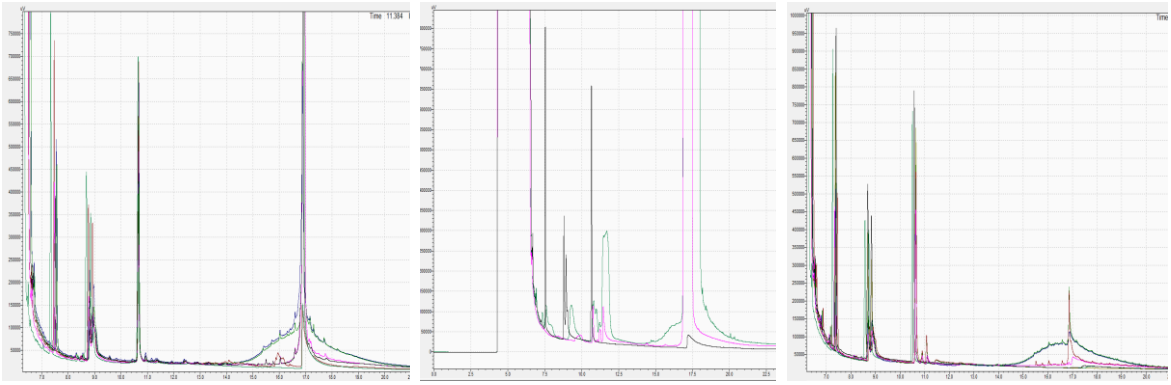

Figure 65: Comparison of epoxidation methods using olive oil: automated ethanolic epoxidation (left), "soft" epoxidation in dichloromethane (middle) and "hard" epoxidation in dichloromethane (right).

The automated approach showed fewer losses of the added internal standards after epoxidation, if compared to the hard method in dichloromethane. This can also be seen in the chromatograms: perylene is still quantitatively present in the left one, while it is nearly completely vanished in the right. In conclusion, it was decided to use the automated approach, since it gives good and repeatable results and because it is the most comfortable, time and resources conserving one. Although, comprehensive testing of the epoxidation procedure was done, it again was not perfect for all types of food and matrices. Sometimes epoxidation was not completed with the proposed procedure. In these cases, the change of colour was often a good indicator (Figure 66): fully epoxidized samples were nearly colourless, if there still was colour a harder epoxidation (e.g. performing a 2nd epoxidation on the same extract, longer reaction times, higher temperatures, etc.) was needed and applied from case to case.

Figure 66: Coloured samples before epoxidation (left) vs. colourless samples after quantitative epoxidation (right).

The determination of LOD and LOQ from the blank values (n=7) are given in Table 23. It was seen that still some improvement is needed to reach the LOQ of 0.5 mg kg^{-1} for fats and oils required in the JRC guideline. For food types with lower fat content it needs to be even lower ([JRC] , 2019). The significantly higher LOD and LOQ for the MOAH in the range of C_{16}-C_{25} can be explained by residues of the epoxidation reagent m-CPBA interfering with the analysis.

Table 23: LOD and LOQ for MOSH and MOAH in the respective range, when applying clean-up for fatty matrices. Determined from the deviation of the blank with n=7. Values given in mg kg^{-1} food.

MOSH	LOD	LOQ	MOAH	LOD	LOQ
C_{16}-C_{20}	0.56	1.1	C_{16}-C_{25}	1.9	3.7
C_{20}-C_{25}	0.73	1.6	C_{25}-C_{35}	0.78	1.8
C_{25}-C_{35}	0.90	1.8	C_{35}-C_{50}	0.37	0.91
C_{35}-C_{40}	0.36	0.76			
C_{40}-C_{50}	0.39	1.0			

It can be concluded, although the discussed methods had been developed, tested and optimised carefully, there is no solution for all different types of samples. They worked well for some food types, but did not for others. In addition, further sample preparation steps as e.g. digestion with hydrochloric acid may be needed and LOD and LOQ need further improvement.
All used sample preparation steps, advantages and disadvantages, problems occurring during analysis and results, will be discussed matrix specific in the next chapters.

3.4.2. Dry Food

As an example for the discussed sample preparation, the chromatograms of a spaghetti sample are shown in Figure 67. The direct analysis of the supernatant shows that odd number n-alkanes are present in the MOSH fraction. An Al_2O_3 clean-up is need. A MOSH contamination cannot be seen without an enrichment step (black line). After the clean-up and enrichment step, the

chromatogram clearly shows a MOSH hump with a concentration of 0.4 mg kg^{-1}, which would have been overlaid by the odd numbered n-alkanes otherwise.

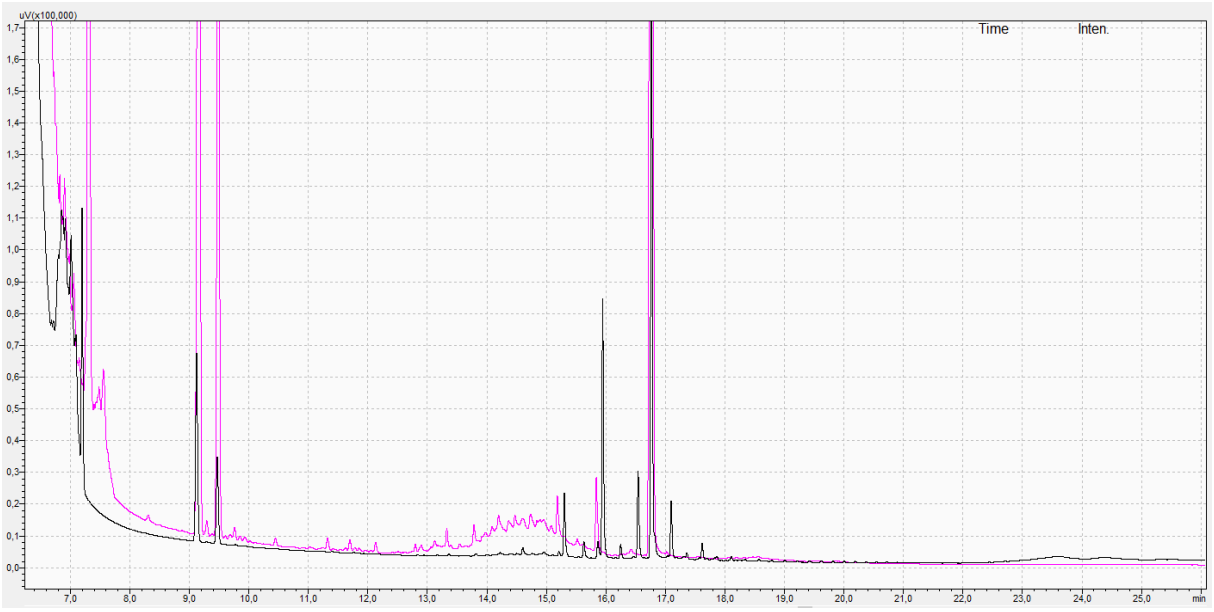

Figure 67: Chromatograms MOSH of spaghetti without Al_2O_3-clean up (black) und with (pink).

The performance of the method was evaluated by spiking a rice sample with 5 mg kg^{-1} of a mineral oil. Figure 68 shows the resulting two chromatograms of the MOSH fraction with and without Al_2O_3 clean-up. The extracts were brought to an end volume of 1 mL in both attempts to make the results and chromatograms better comparable. Before the clean-up with Al_2O_3 mainly odd numbered n-alkanes in the range of C_{25}-C_{35} are present and overloading the chromatogram (black line). Integration of the present humps would give a MOSH value of 10.8 mg kg^{-1} and a MOAH value of 0.64 mg kg^{-1}. The MOSH and MOAH ratio would already indicate a false positive value, since the MOAH fraction is only about 6.5% of the whole mineral oil fraction. After the clean-up the n-alkanes are completely removed and data evaluation is possible without any interferences. The result for the MOSH concentration is now 4.31 mg kg^{-1}, the MOAH fraction would be 15%. Adding up the MOSH and the MOAH concentration would give a total MOH contamination of 4.95 mg kg^{-1} and a recovery of 99% of the spiked amount.

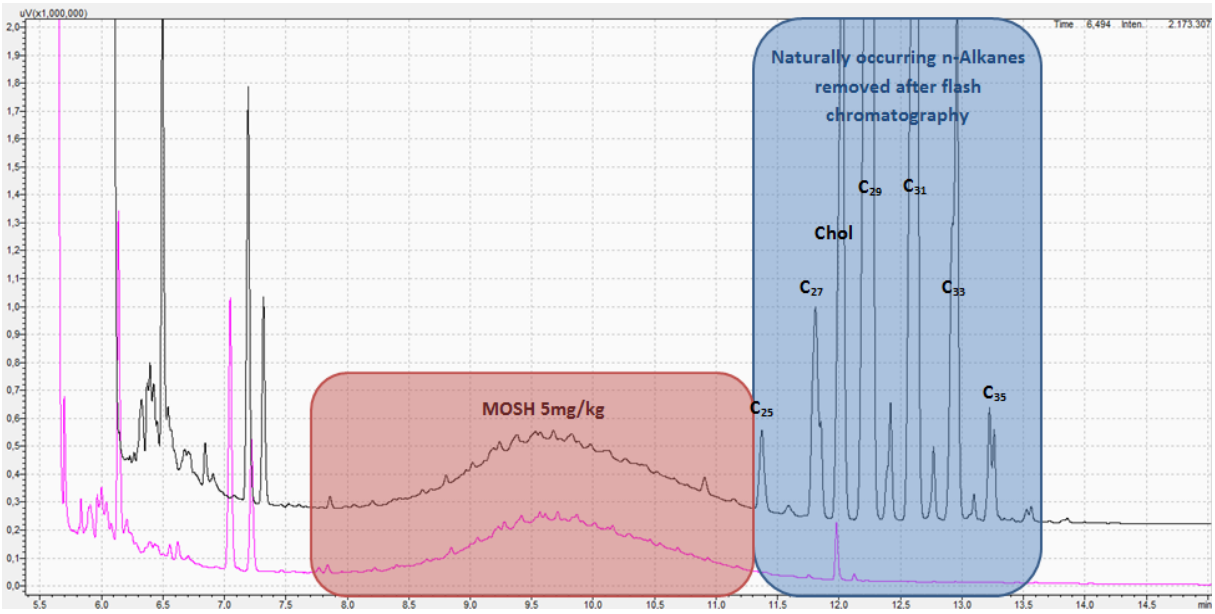

Figure 68: Chromatograms of a rice sample spiked with mineral oil before and after Al_2O_3 clean-up.

3.4.3. Fats, Oils and Oil-Based Products

As already discussed, this category may vary a lot depending on the fat content, composition and ingredients of the food sample. However, the applied method was tested and optimised carefully, especially for edible fats and oils in the joint working group "ThinkTank-MOSH/MOAH". As an example, the analysis of a sunflower oil is shown:
Screening of the oil sample showed in the MOSH fraction of the oil naturally occurring, mainly odd numbered n-alkanes. They were overloading the chromatogram, thereby forming a little hump. A contamination of the oil with mineral oil can neither be confirmed nor excluded (see Figure 69, black trace). Saponification and Al_2O_3 clean-up were performed, resulting in the removal of the n-alkanes and an enrichment of the sample. The resulting chromatogram (pink trace in Figure 69) now shows a clear hump of MOSH. Data evaluation is easy and integration of the hump gives a MOSH concentration of 3.2 mg kg^{-1}.

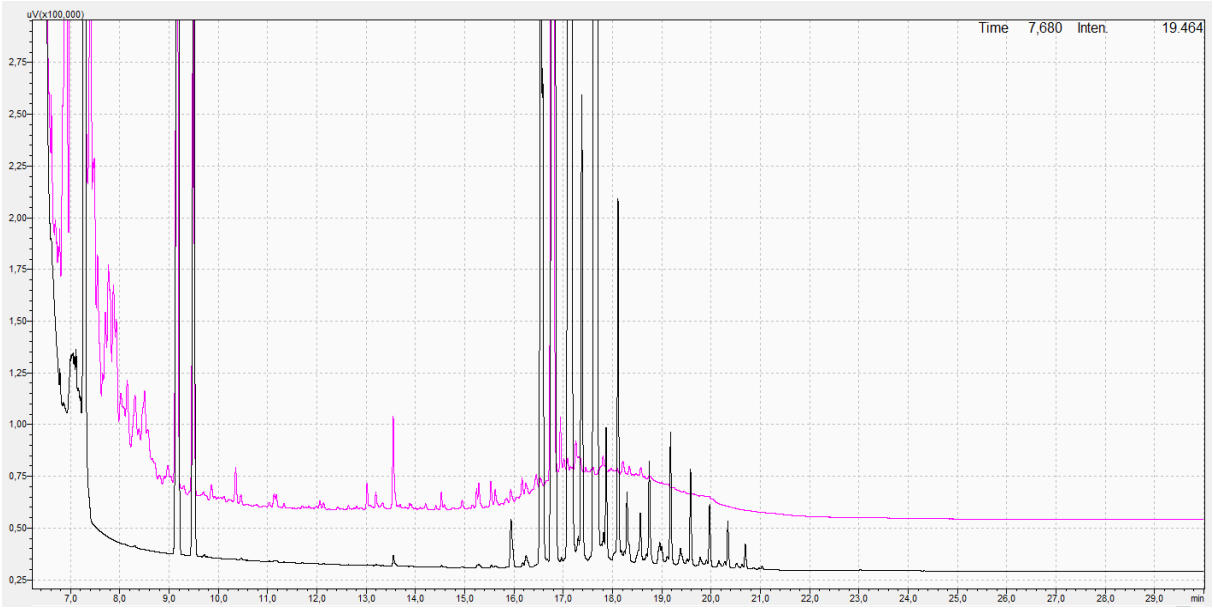

Figure 69: Chromatograms MOSH fraction of sunflower oil before (black) and after (pink) Al_2O_3 clean-up.

More difficult is the analysis of the MOAH fraction. The screening of the oil showed present olefins, again overloading the chromatogram and forming irregular humps (black trace in Figure 70). SPE clean-up and epoxidation removed some of the present interferences, but not all of them (pink trace in Figure 70). By comparison with the blank's chromatogram (blue trace in Figure 70) and subtraction of the still on the hump remaining peaks, a MOAH contamination of 0.9 mg kg^{-1} was determined. The MOAH fraction makes up about 19% of the whole detected mineral oil contamination, which indicates a correct analysis.

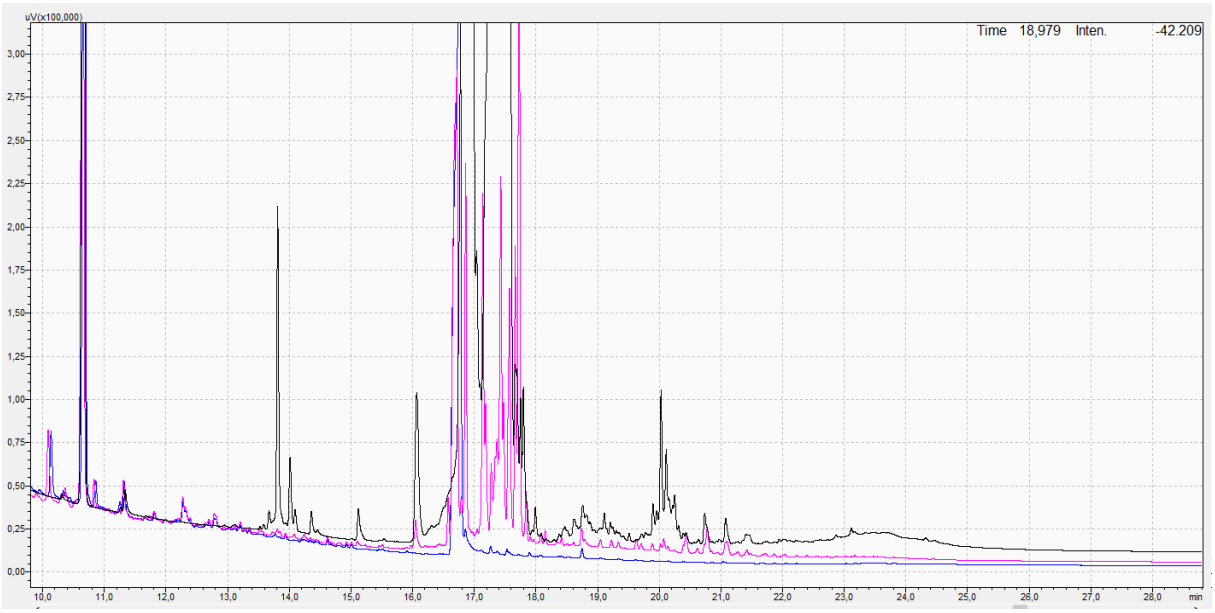

Figure 70: Chromatograms MOAH-fraction of sunflower oil before (black) and after (pink) SPE clean-up and epoxidation, compared to the blank value (blue).

Since the analysis of edible oils worked well with the developed method, it was also applied to other fat rich matrices. No problems occurred during the sample preparation procedure, when applied to e.g. pesto. The resulting chromatograms were appropriate, interferences had been removed mostly and data evaluation could be done. The hump present showed the same pattern for all four tested samples, indicating a MOSH contamination from the same source, but to a different extent. The results for the four samples gave a MOSH contamination between <0.5 and 30 mg kg^{-1}, which seemed odd, since the sample were received from the same producer and there would not be an explanation for such varying contamination. After having a closer and more careful look at the chromatograms, a non-typical hump form was identified. They did not show the typical Gaussian distribution, but as can be seen in Figure 71, the humps of all four samples showed two spikes at about 16.5 min and 18.5 minutes, marked with blue circles in the chromatograms.

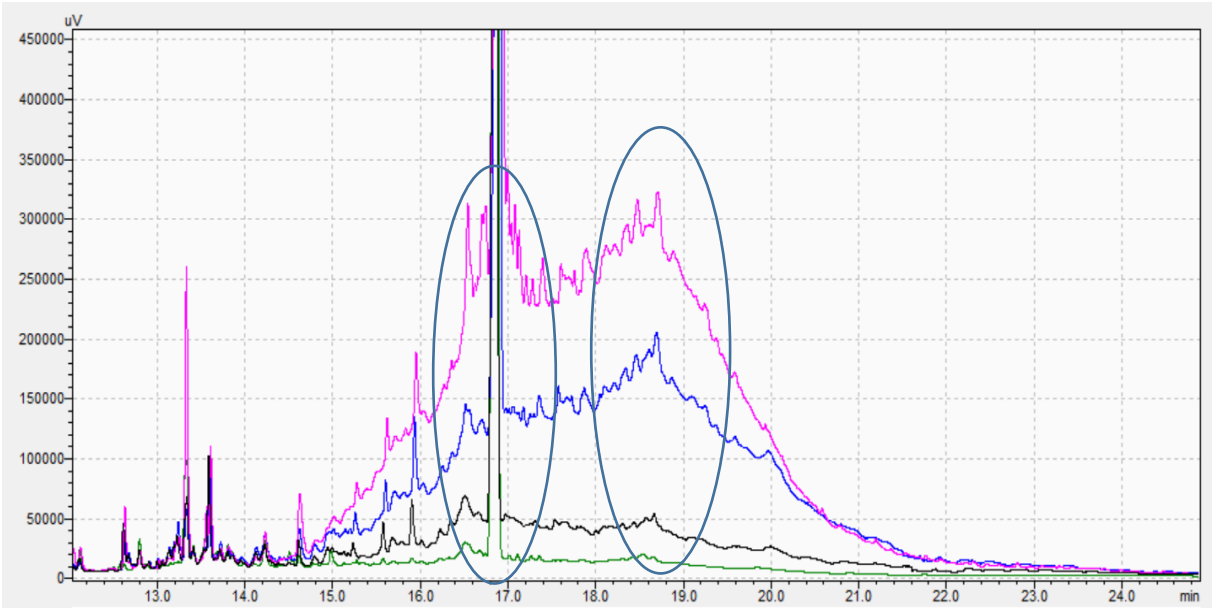

Figure 71: MOSH chromatograms of four pesto samples, showing non-typical spikes, as marked with circles.

To verify these findings and the determined contamination, 2D-GC×GC-MS measurements were performed on the MOSH extract of the pesto sample with the highest contamination. The resulting GC×GC chromatograms showed a clear result (Figure 72): in the range of C_{20} to about C_{35} there is a MOSH contamination present (marked with a yellow circle in Figure 72). However, starting at about C_{30} there is another hump present, made up by highly polar substances of natural origin (marked with a green circle in Figure 72). Those highly polar substances of natural origin are clearly separated in the 2D-chromatogram from the non-polar MOSH. Both substance classes form humps in different regions of the chromatograms 2nd dimension, where separation is done based on polarity. The humps are overlapping in the 1st dimension, since separation is only done based on volatility. Their overlapping can only be identified by the formation of the non-typical spikes.

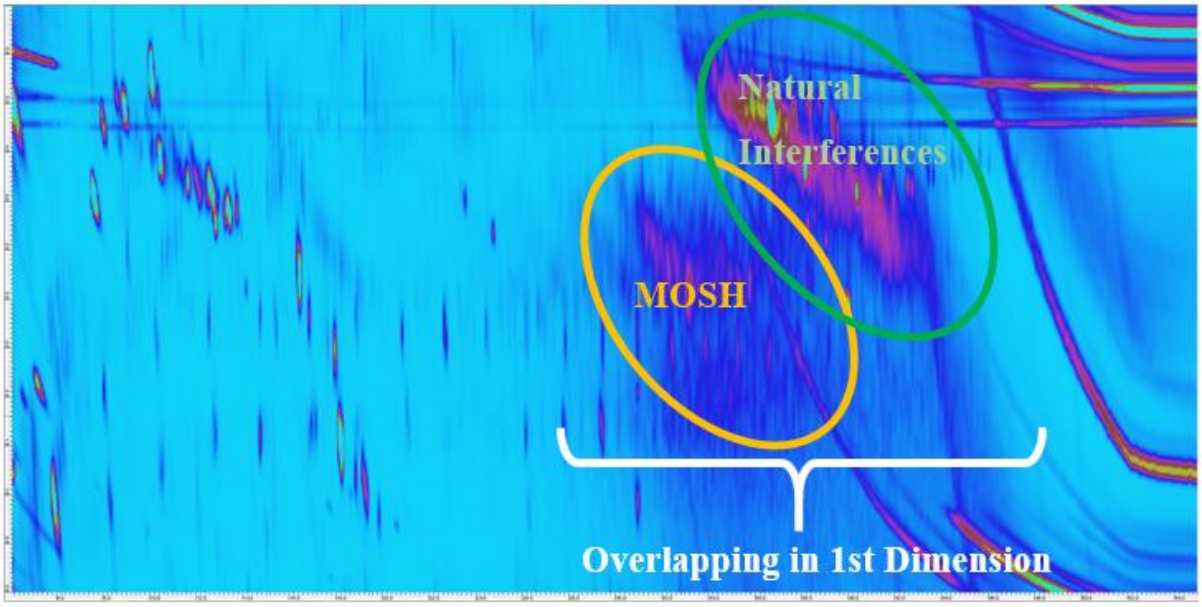

Figure 72: 2D-GC×GC chromatogram of pesto sample, showing natural interferences marked in green and MOSH, marked in yellow. Both would overlap in the 1st dimension.

Therefore, the values for MOSH determined with the HPLC-GC-FID would have been false positive to a disastrous extent. Consequently, how small or large the contamination really is cannot be determined from the HPLC-GC-FID chromatogram, but also not from the GC×GC analysis because of the non-universal MS detector. An analysis with GC×GC-FID would be needed to determine the results of these samples.

3.4.4. Meat and Milk

Meat and milk, as well as meat- and milk-products seem to be complex matrices. The crucial step is the digestion using HCl, for which it is important to continue the digestion until all of the flakes formed after addition of the HCl are gone. The rest of the procedure (saponification and further clean-up) is done as described for the general procedure, afterwards.
The digestion is needed to release all of the mineral oil that may be present within the matrix. This was tested and shown for a cheese sample: Sample preparation was done once with an HCl digestion and once without. All other sample preparation steps, being saponification and MOSH clean-up using Al_2O_3, were done in the same way. The resulting chromatograms are shown in Figure 73. Without the HCl digestion (black trace) no MOSH contamination can be seen. Using the HCl digestion of the matrix released the present MOSH contamination of 1.9 mg kg^{-1}.

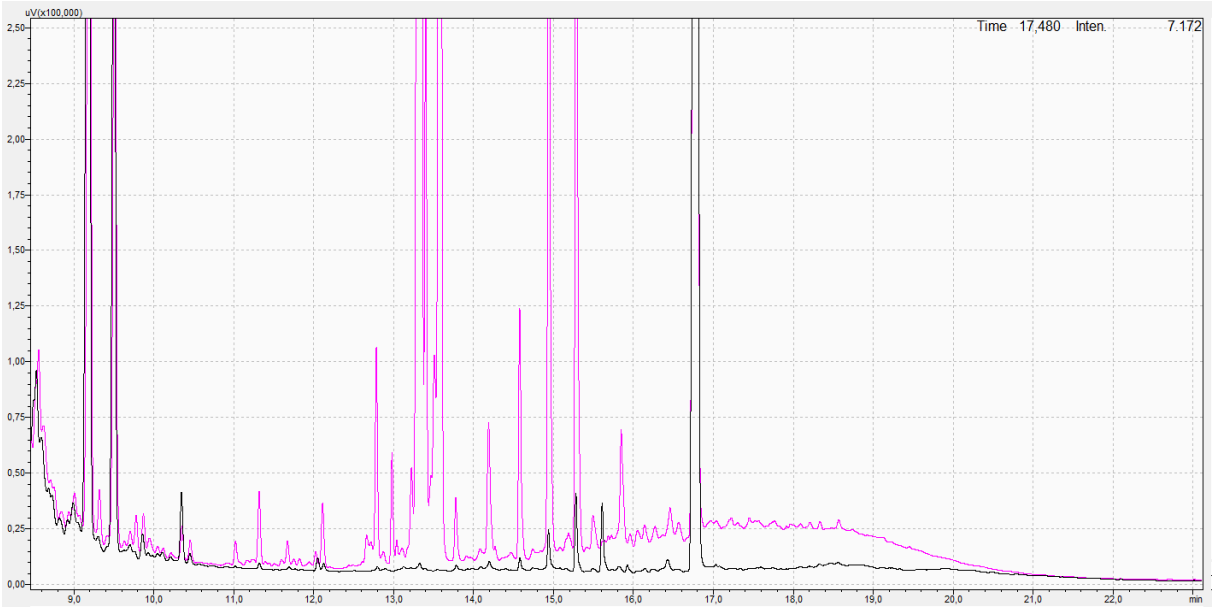

Figure 73: Comparison of sample preparation of cheese; with HCl digestion (pink) and without (black).

3.4.5. Chocolate

The work with chocolate showed early that it is probably the most difficult matrix for MOSH and MOAH analysis. Different types of chocolate were used for the method development (e.g. milk chocolate, white chocolate or dark chocolate; cocoa percentage of 70-100%), hoping that method development could be done with one and applied to the others afterwards. However, none of the described attempts led to a successful analysis. Different colours and consistencies resulted from the same chocolate sample, when the attempts describe in chapter 2.5.5 were performed, as can be seen as example in Figure 74.

Figure 74: Appearances of the same chocolate sample during method development.

Problems like solid residues, no phase separation, insufficient saponification, total loss or contamination of samples were seen.
The fourth method showed that using only 1g of sample is not sufficient and that further clean-up and enrichment of the MOSH and MOAH fraction were needed. The most promising method was the last one, when the method for fats and oils was applied to the chocolate. However, the HPLC-GC-FID chromatogram showed again an unusual pattern for the measured extracts, as can be seen in the example given in Figure 75. GC-MS analysis was done to identify the still present interferences. The humps mainly consisted of fatty acid esters, which indicated that saponification was not sufficient enough.

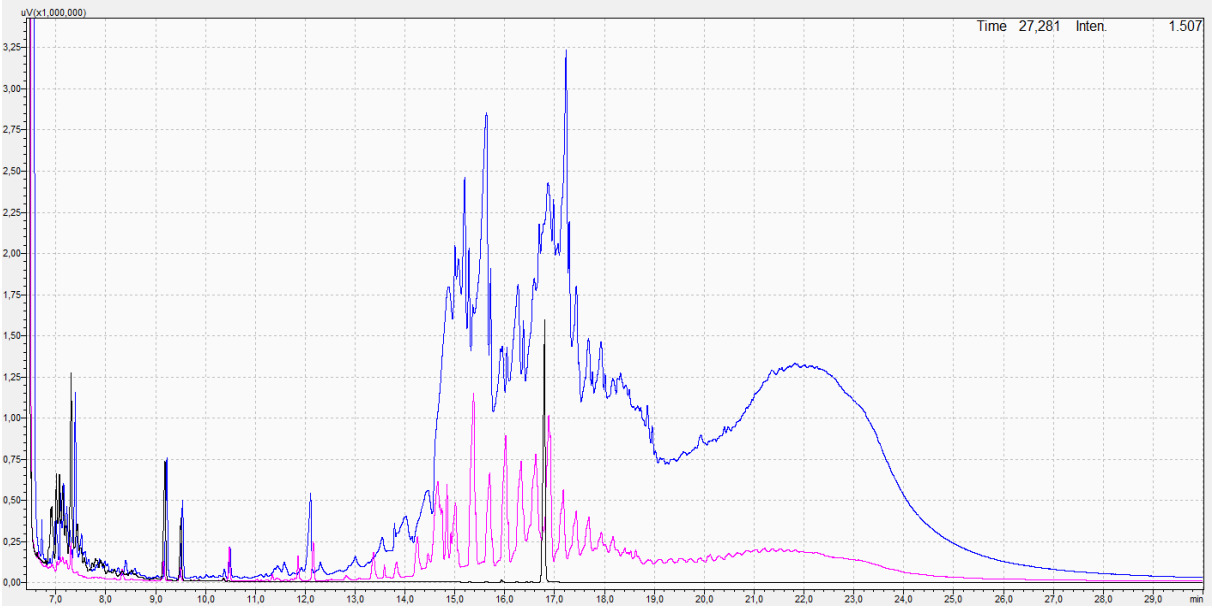

Figure 75: Examples of residues of fatty acid esters in HPLC-GC-FID analysis.

In conclusion, so far it was not possible to develop a promising strategy for the MOSH and MOAH analysis of chocolate and additional effort needs to be undertaken.

3.4.6. Spices

Spices do not seem to be a complex matrix, but the load of natural substances interfering with the analysis may be immense. The MOSH fraction needs Al_2O_3 clean-up, since naturally present odd numbered n-alkanes may interfere with it. This can be seen on the example of granulated onions in Figure 76. While the MOAH fraction is without any interference, the MOSH needs clean-up.

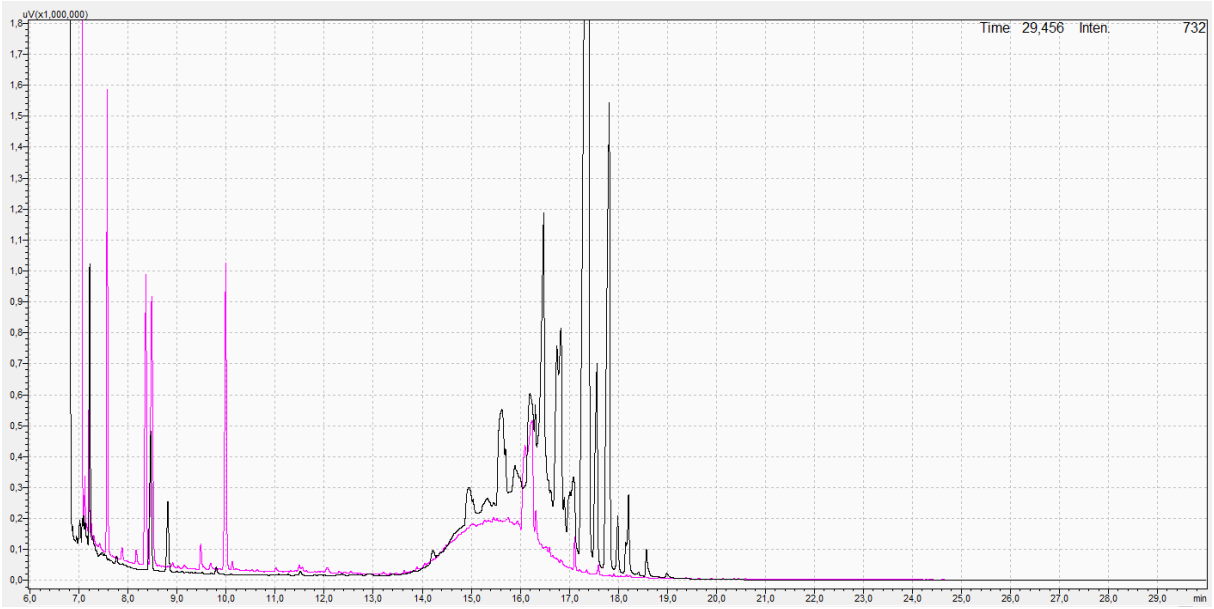

Figure 76: MOSH (black) and MOAH (pink) chromatograms of granulated onion. MOSH needs clean-up, MOAH is without interference.

For other types of spices, as e.g. cumin or turmeric the MOAH fraction contains naturally present olefins like carotenoids. They are not only colouring the extracts, but also heavily overloading the chromatograms. Therefore, it is important to perform the epoxidation carefully to remove them, without having too much loss of MOAH. The progress of the epoxidation can be nicely controlled by the colour of the extract, as was already discussed before. However, also after applying the epoxidation, there are often non-epoxidizeable residues left interfering with the analysis. In such cases, an additional analysis of the extracts with GC×GC-MS was done to verify the results. As example, Figure 77 shows the chromatograms of a turmeric powder after epoxidation. The MOSH shows a mineral oil hump without interference and no further clean-up is needed. In the MOAH, even after the epoxidation heavy interferences are present, overloading the chromatogram. An evaluation of a present MOAH contamination is not possible. Therefore, the GC×GC-MS was used and it was concluded that the sample is only minimally contaminated with MOAH.

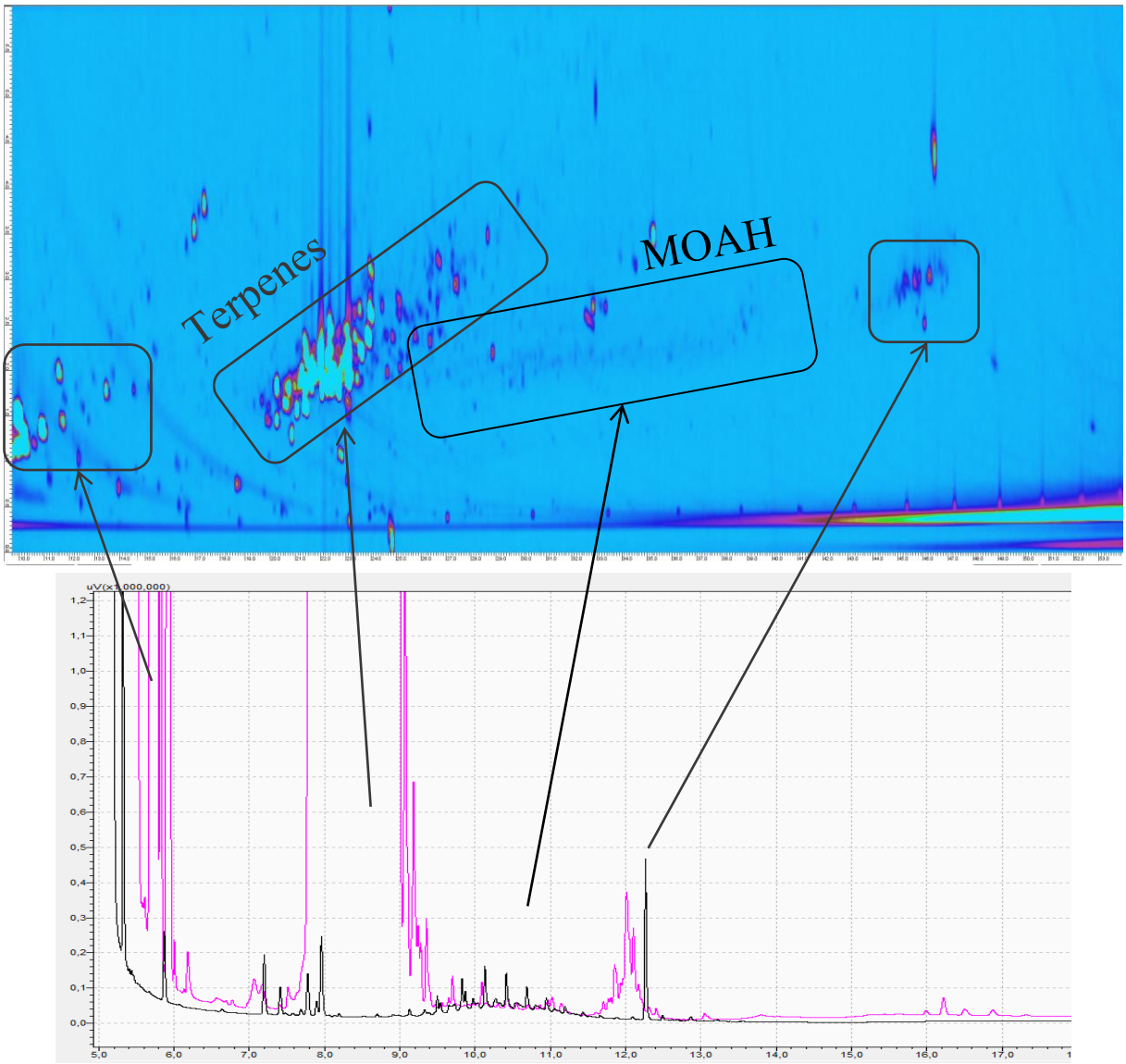

Figure 77: Chromatograms of turmeric sample. Lower chromatograms show the MOSH trace in black and the MOAH trace with heavy interferences in pink. The GC×GC chromatogram above shows that interferences are terpenes and that a slight MOAH contamination is overlaid by them in the LC-GC.

In conclusion, spices are another widely varying matrix. Therefore, again a careful case-by-case evaluation is needed for the analysis of each sample.

3.4.7. Lubricating Oils

As already mentioned in the experimental part, for lubricating oils a differentiation between mineral-oil-based and plant-based products is important for the sample preparation.
The mineral-oil-based products simply need to be diluted, giving a nice MOSH and MOAH hump in the HPLC-GC-FID analysis that can be easily integrated for the result. An example can be seen in Figure 78.

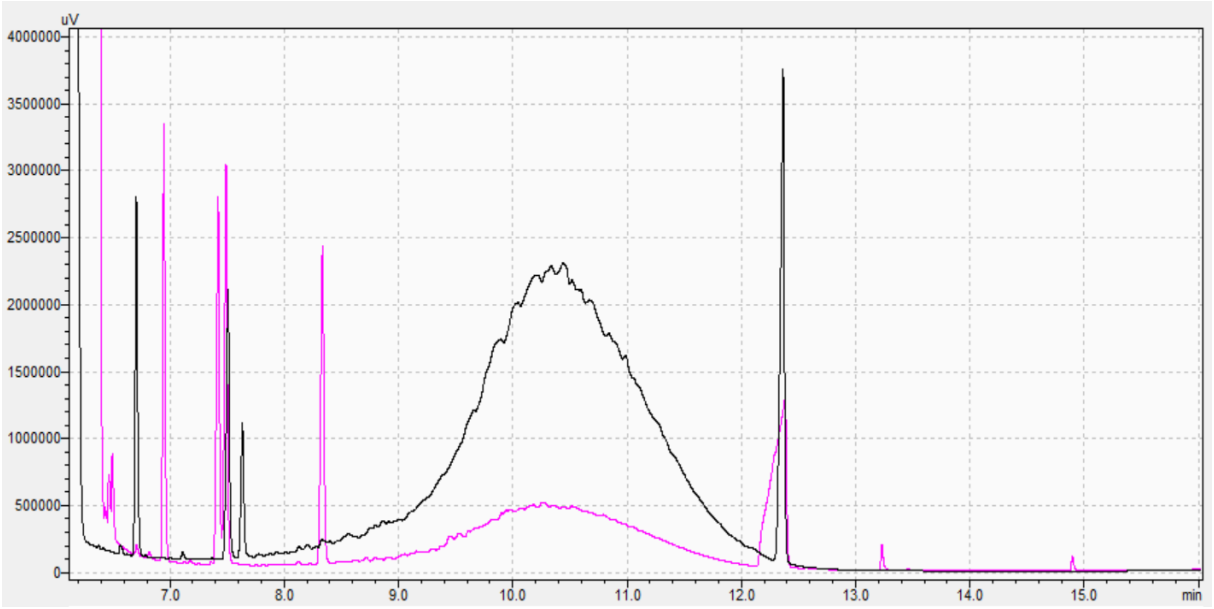

Figure 78: Mineral-oil-based lubricating oil sample: MOSH (black) and MOAH (pink).

In comparison, plant-based products are much more complex. Figure 79 shows an example for the screening result of a plant based lubricating oil. Huge irregular humps are present in both fractions, unusual for MOSH and MOAH. Unfortunately, clean-up of the sample and identification of the sources of the humps is work in progress and no further results can be presented so far.

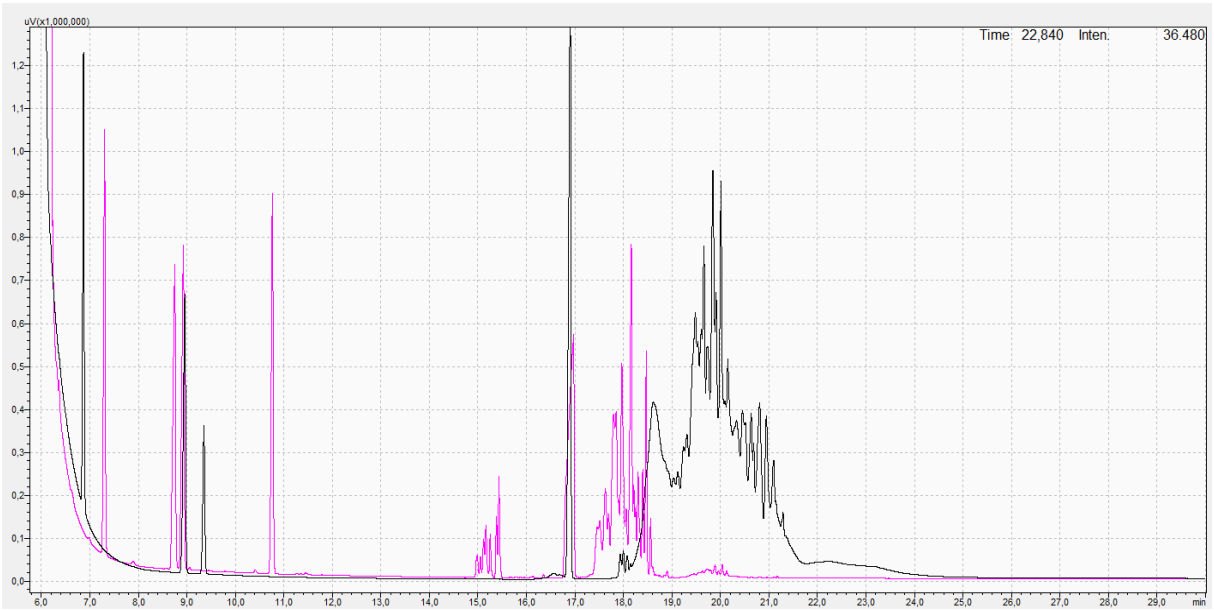

Figure 79: Plant-based lubricating oil sample: MOSH (black) and MOAH (pink).

4. Conclusion & Outlook

The aim of this work was to generate an overview of packaging related food safety aspects. Considered were mainly paper and board food contact materials and the application of bio-based or biodegradable functional barriers on them. To ensure the safety of a packed good, comprehensive tests are necessary.

The first step was to characterise different industrially produced paper samples. HS-SPME-GC-MS was used to generate a quick overview of the present contaminants but has several disadvantages. Total extraction gave a more comprehensive overview, but migration tests are needed to get a realistic evaluation of the possible transfer of the contaminants into a packed good.

Second, a screening of the barrier properties of different biopolymers was done, by coating them on two different papers. Promising ones, such as alginate and chitosan were used for further coating trials to evaluate their behaviour and properties more carefully. Both biopolymers showed good barrier properties in general. However, it needs to be stressed that there is no simple solution. Each material used has its own properties and functionalities. A careful evaluation of the many parameters influencing the coating procedure has to be done, prior to performing coating trials. For example, the used raw paper played an important role in terms of pre-treatment and surface structure. Furthermore, it was shown that barrier properties do not increase linearly with the coat weight and applying several layers of low coat weight is the much better approach.

Third, a newly developed two-sided migration test was used to test both- permeation through the sample and migration from it- in one attempt. The new method was characterised and validated carefully and compared to the tape barrier test, proposed some years earlier.

Last, different food types were analysed for their contamination with mineral oil hydrocarbons (MOH). Sample preparation and analyses are a complex task. The presented results are preliminary; the applied methods are not fully tested and validated so far and more work needs to be done, especially for the MOAH fraction. Most important of the MOSH/MOAH discussion is probably an alignment of analysis methods to generate reliable results for risk assessment. Therefore, one of the most important steps is the collaboration at national and international level with experts in the field. Only if a common definition and sense of the problem is found, it is possible to work together to find appropriate solutions.

In conclusion, a lot of information on contaminants present in paper and board and on the barrier properties of biopolymers was generated. Real packaging applications will need further careful testing and evaluation, but promising results were obtained during this work. Mineral oil hydrocarbons are still a widely discussed topic with no common view at the moment. Work needs to be continued in the following years to develop the analytical certainty needed, to perform hazard and risk assessment and to find a final conclusion in terms of legal regulations.

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6. Appendix

To chapter 3.1.2.1: Determination of Breakthrough Concentration for Chitosan Film

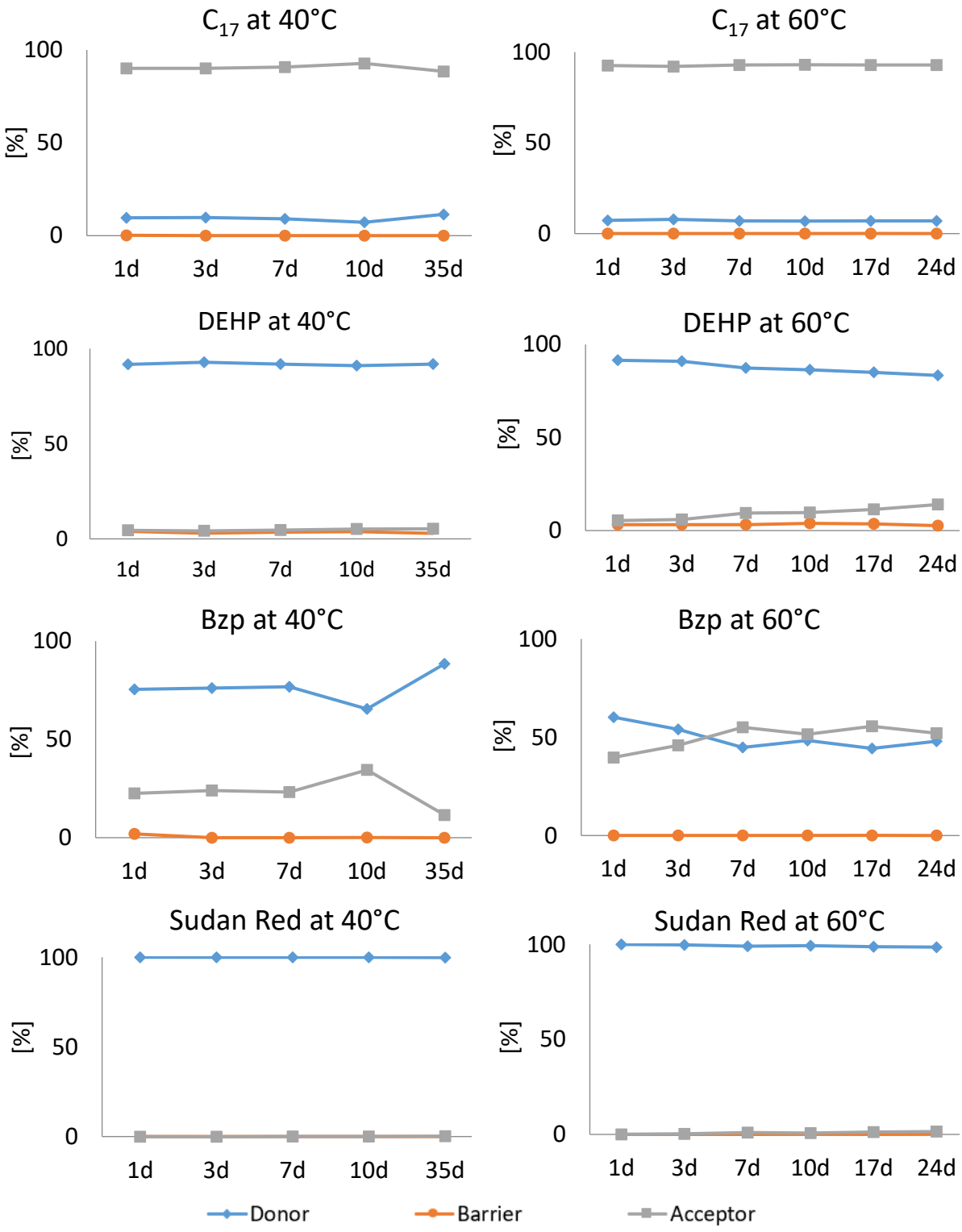


Figure 80: Summary of Breakthrough Test results for series stored at 40°C and 60°C.

To chapter 3.2.1.2: GC-MS Chromatograms of Free-Standing Films

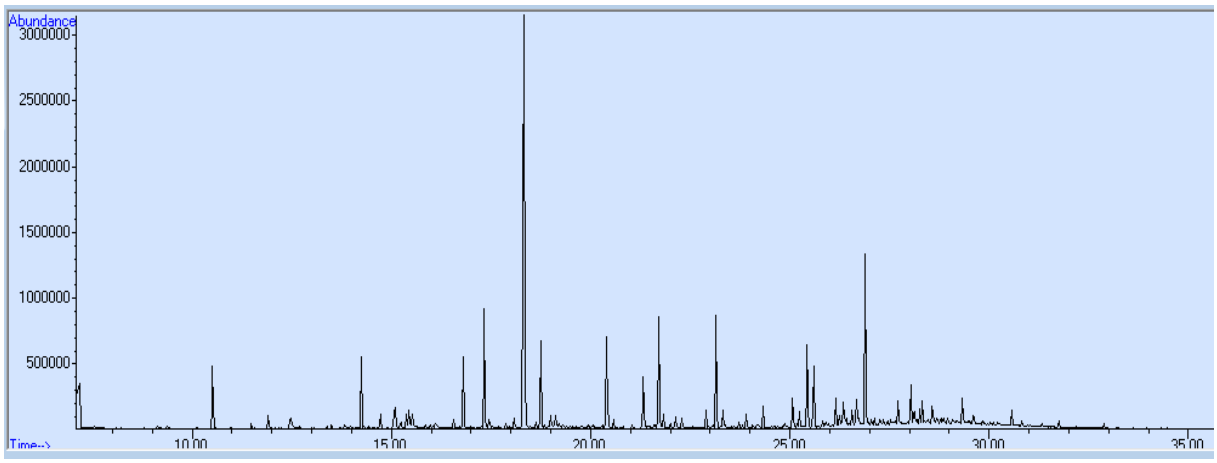


Figure 81: HS-SPME-GC-MS chromatogram of free-standing MFC film.

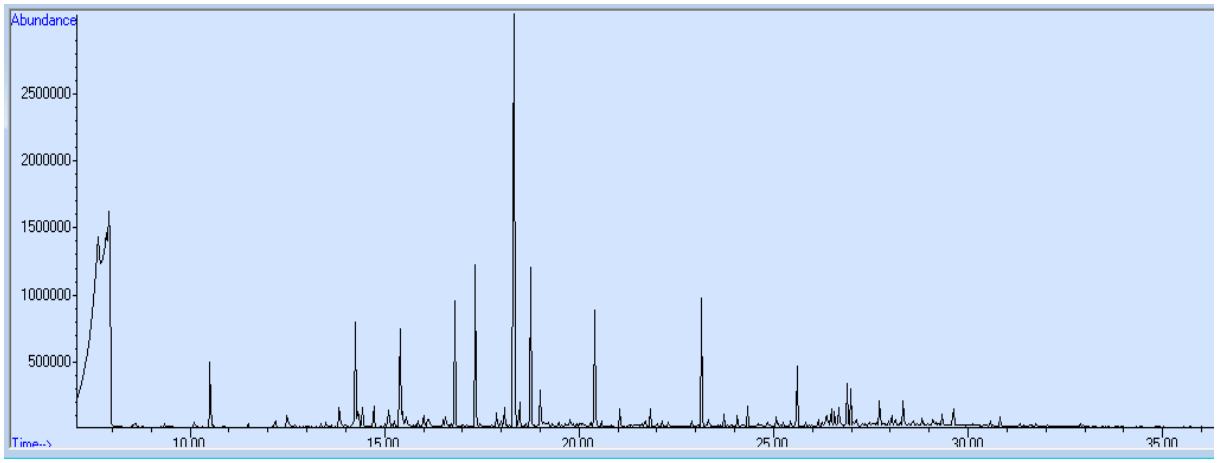


Figure 82: HS-SPME-GC-MS chromatogram of free-standing chitosan film.

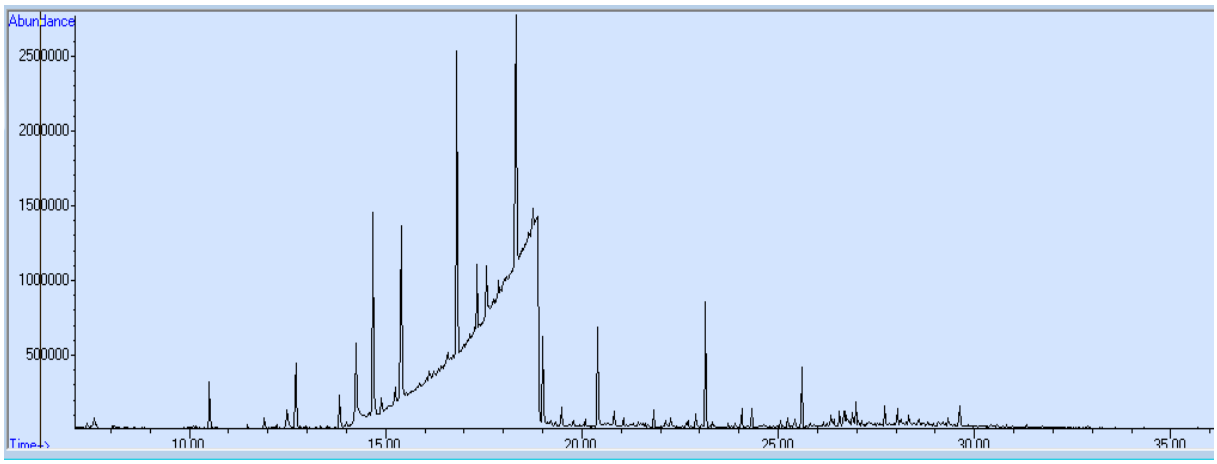


Figure 83: HS-SPME-GC-MS chromatogram of free-standing casein film.

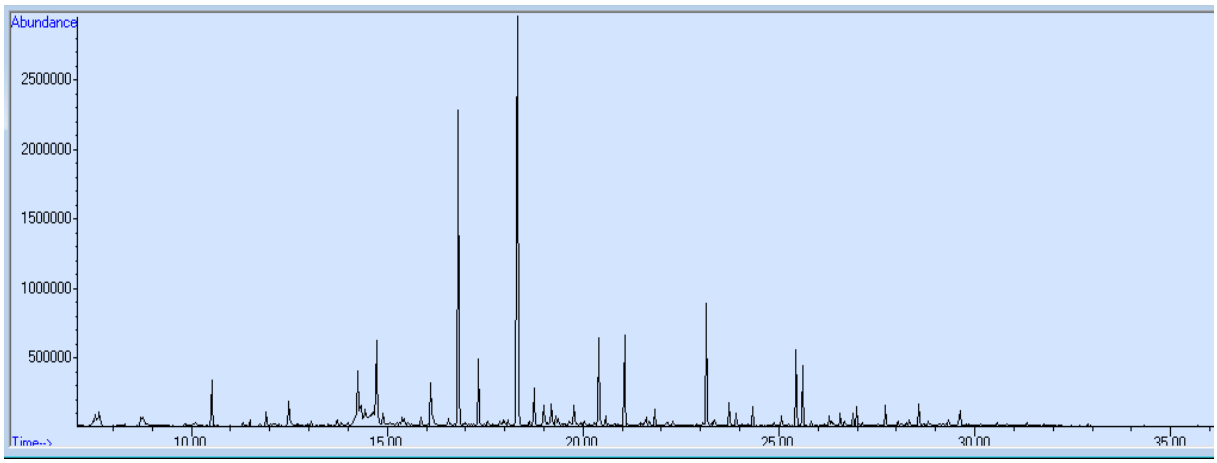


Figure 84: HS-SPME-GC-MS chromatogram of free-standing alginate film.

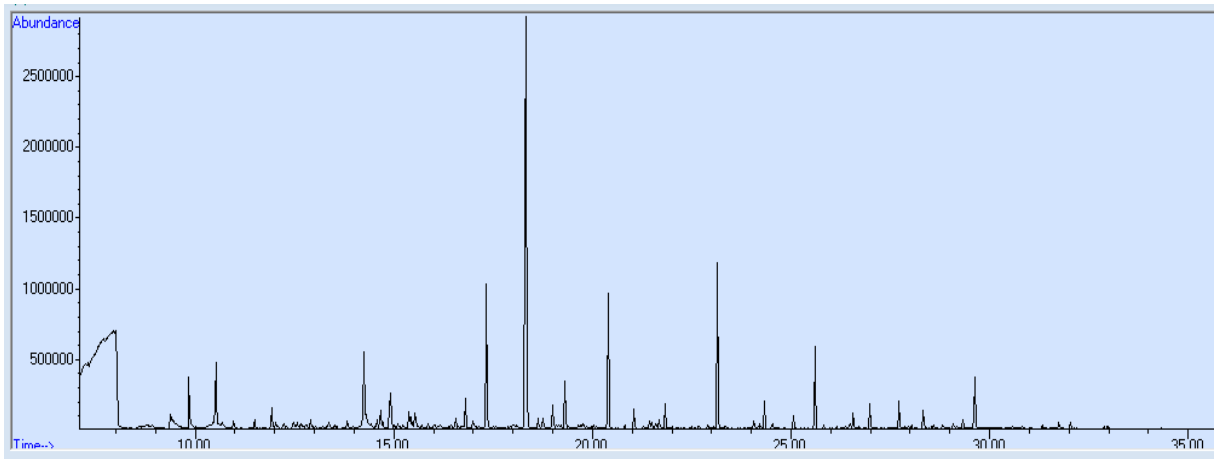


Figure 85: HS-SPME-GC-MS chromatogram of free-standing EVOH film.

To chapter 3.3.3. GC×GC-TOF

Table 24: Identified primary phenylalkanes in total migration extract of recycled raw paper.

Name	R.T. (s)	Area	Base Masses	Molecular Ion
Benzene, propyl-	230.002 s, 2.145 s	9641221	91/92	120
Benzene, pentyl- (IS)	515.025 s, 2.733 s	1275632333	91/92	148
Benzene, hexyl-	600.032 s, 2.914 s	416406	91/92	162
Benzene, nonyl-	1110.07 s, 3.067 s	518662	91/92	204
Benzene, decyl-	1245.08 s, 3.093 s	1898820	91/92	218
Benzene, undecyl-	1375.09 s, 3.089 s	2294453	91/92	232
Benzene, dodecyl-	1500.1 s, 3.082 s	1651694	91/92	246
Benzene, tridecyl-	1615.11 s, 3.104 s	1521462	91/92	260
Benzene, tetradecyl-	1730.12 s, 3.081 s	1355075	91/92	274
Benzene, pentadecyl-	1835.13 s, 3.104 s	2003549	91/92	288
Benzene, hexadecyl-	1940.14 s, 3.087 s	1121835	91/92	302
Benzene, heptadecyl-	2040.15 s, 3.081 s	1200139	91/92	316
Benzene, octadecyl-	2135.15 s, 3.083 s	1071854	91/92	330
Benzene, nonadecyl-	2315.17 s, 3.078 s	972041	91/92	344

Benzene, uncosanyl-	2400.18 s, 3.071 s	1104159	91/92	372
Benzene, docosanyl-	2485.18 s, 3.051 s	799931	91/92	386
Benzene, tricosanyl-	2565.19 s, 3.050 s	890010	91/92	400
Benzene, tetracosanyl-	2640.2 s, 3.064 s	912658	91/92	414
Benzene, pentacosanyl-	2715.2 s, 3.062 s	850853	91/92	428
Benzene, hexacosanyl-	2790.21 s, 3.055 s	470227	91/92	442
Benzene, heptacosanyl-	2860.21 s, 3.061 s	327899	91/92	456

Table 25: Identified secondary phenylalkanes in total migration extract of recycled raw paper.

Name	R.T. (s)	Area	Base Mass	Molecular Ion
Benzene, (1-methylnonyl)-	1140.08 s, 3.177 s	2798440	105.1	218
Benzene, (1-methyldecyl)-	1275.09 s, 3.151 s	9652829	105.1	232
Benzene, (1-methylundecyl)-	1400.1 s, 3.166 s	11854945	105.1	246
Benzene, (1-methyldodecyl)-	1520.11 s, 3.177 s	23235146	105.1	260
Benzene, (1-methyltridecyl)-	1635.11 s, 3.179 s	6488458	105.1	274
Benzene, (1-methyltetradecyl)-	1745.12 s, 3.180 s	1312856	105.1	288
Benzene, (1-methylpentadecyl)-	1765.13 s, 3.156 s	3288192	105.1	302
Benzene, (1-ethyloctyl)-	1085.07 s, 3.244 s	989414	91	218
Benzene, (1-ethylnonyl)-	1215.08 s, 3.269 s	4478923	91	232
Benzene, (1-ethyldecyl)-	1345.09 s, 3.255 s	5481136	91.1	246
Benzene, (1-ethylundecyl)-	1465.1 s, 3.270 s	6457378	91.1	260
Benzene, (1-ethyldodecyl)-	1540.11 s, 3.249 s	1962372	91	274
Benzene, (1-propylheptyl)-	1060.07 s, 3.261 s	971163	91	218
Benzene, (1-propyloctyl)-	1190.08 s, 3.258 s	5819095	91	232
Benzene, (1-propylnonyl)-	1315.09 s, 3.285 s	7104049	91	246
Benzene, (1-propyldecyl)-	1435.1 s, 3.301 s	6456016	91.1	260
Benzene, (1-propylundecyl)-	1550.11 s, 3.298 s	585350	91.1	274
Benzene, (1-butylhexyl)-	1045.07 s, 3.283 s	1750512	91	218
Benzene, (1-butylheptyl)-	1175.08 s, 3.286 s	9841496	91	232
Benzene, (1-butyloctyl)-	1300.09 s, 3.284 s	17876856	91	246
Benzene, (1-butylnonyl)-	1420.1 s, 3.305 s	5790167	91	260
? Benzene, (1-butyldecyl)-	1535.11 s, 3.302 s	962209	91.1	274
?Benzene, (1-butylundecyl)-	1640.12 s, 3.304 s	393932	91.1	288
?Benzene, (1-pentyloctyl)-	1410.1 s, 3.322 s	10596493	91	missing
?Benzene, (1-pentylnonyl)-	1530.11 s, 3.286 s	1066996	91.1	missing

Table 26: Identified napthalenes in total migration extract of recycled raw paper.

Name	R.T. (s)	Area	Base Mass
1-Naphthalenol	755.044 s, 2.090 s	31340735	115.1
Naphthalene	690.039 s, 2.035 s	21225343	128.1

Naphthalene, 1-propyl-	1120.07 s, 2.234 s	184056	141.1
Naphthalene, 1-propyl	1105.07 s, 2.076 s	245986	141.1
Naphthalene, 1-hexyl-	1260.08 s, 2.281 s	333399	141.1
Naphthalene, 1-hexyl-	1515.11 s, 2.307 s	424593	141.1
Naphthalene, 2-ethyl-	995.064 s, 2.176 s	689157	141.1
Naphthalene, 2-methyl-	890.055 s, 2.067 s	414519062	141.1
Naphthalene, 1-methyl-	900.056 s, 1.933 s	25317343	142.1
Naphthalene, 2-methyl-	960.061 s, 1.751 s	76357448	142.1
Naphthalene, 1-methyl-	890.055 s, 2.072 s	885860093	142.1
Naphthalene, 1-methyl-	855.052 s, 2.122 s	918594525	142.1
Peak 2847	1330.09 s, 2.532 s	539656	142.2
Peak 4102	1670.12 s, 2.309 s	45043	155.1
Naphthalene, 2-(1-methylethyl)-	1095.07 s, 2.157 s	89790	155.1
Naphthalene, 2-(1-methylethyl)-	1285.09 s, 2.075 s	100510	155.1
Naphthalene, 2-methyl-1-propyl-	1250.08 s, 2.235 s	83506	155.1
Naphthalene, 2-(1-methylethyl)-	1320.09 s, 2.064 s	254766	155.1
Naphthalene, 2-(1-methylethyl)-	1100.07 s, 2.137 s	240094	155.1
Peak 3046	1380.09 s, 2.293 s	463450	155.1
Peak 3677	1545.11 s, 2.382 s	682667	155.1
Peak 3589	1520.11 s, 2.350 s	573514	155.1
Peak 4002	1640.12 s, 2.376 s	910784	155.1
Naphthalene, 2-methyl-1-propyl-	1295.09 s, 2.241 s	461543	155.1
Naphthalene, 2-(1-methylethyl)-	1145.08 s, 2.233 s	739153	155.1
Naphthalene, 2-(1-methylethyl)-	1070.07 s, 2.260 s	698249	155.1
Peak 4221	1705.12 s, 2.355 s	1051003	155.1
Naphthalene, 1,6,7-trimethyl-	1215.08 s, 2.187 s	2278521	155.1
Naphthalene, 2,3,6-trimethyl-	1245.08 s, 2.149 s	2446378	155.1
Naphthalene, 1,2-dimethyl-	1020.07 s, 2.137 s	79321	156.1
Naphthalene, 1,6-dimethyl-	1010.06 s, 2.181 s	784982	156.1
Naphthalene, 1,8-dimethyl-	1045.07 s, 2.134 s	1777399	156.1
Naphthalene, methyl-isopropyl	1390.1 s, 2.133 s	74826	169.1
Naphthalene, methyl-isopropyl	1235.08 s, 2.238 s	69259	169.1
Naphthalene, 1-methyl-7-(1-methylethyl)-	1205.08 s, 2.356 s	83103	169.1
Peak 2447	1210.08 s, 2.305 s	136911	169.1
Naphthalene, methyl-isopropyl	1315.09 s, 2.186 s	229965	169.1
Peak 3607	1525.11 s, 2.327 s	436750	169.1
Peak 3676	1545.11 s, 2.349 s	519829	169.1
Napthalene, methylethyl -	1715.12 s, 2.357 s	720563	169.1
Naphthalene, 1-methyl-7-(1-methylethyl)-	1245.08 s, 2.264 s	439184	169.1
Peak 4081	1665.12 s, 2.375 s	1038947	169.1
Naphthalene, 1-methyl-7-(1-methylethyl)-	1325.09 s, 2.214 s	422116	169.1
Peak 3126	1400.1 s, 2.306 s	362920	169.1
Naphthalene, 1-methyl-7-(1-methylethyl)-	1330.09 s, 2.240 s	870414	169.1
Peak 3170	1410.1 s, 2.213 s	773935	169.1
1-Methyl-7-isopropylnaphthalene	1310.09 s, 2.241 s	1389477	169.1
Peak 3171	1410.1 s, 2.325 s	977358	169.1

Naphthalene, propyl-methylethyl	1385.09 s, 2.398 s	1097258	169.1
Peak 2959	1355.09 s, 2.461 s	1891293	169.1
Naphthalene, 2,3,6-trimethyl-	1115.07 s, 2.042 s	26516	170.1
Naphthalene, 1,4,6-trimethyl-	1105.07 s, 2.095 s	272164	170.1
Peak 2707	1290.09 s, 2.080 s	584883	170.1
Naphthalene, 1,6,7-trimethyl-	1185.08 s, 2.206 s	2931023	170.1

Table 27: Identified biphenyls in total migration extract of recycled raw paper.

Name	R.T. (s)	Area	Base Mass
2,2'-Dimethylbiphenyl	1155.08 s, 2.142 s	57499	182.1
3,3'-Dimethylbiphenyl	1280.09 s, 2.104 s	361390	182.1
4,4'-Dimethylbiphenyl	1285.09 s, 2.099 s	1025250	182.1
4,4'-Dimethylbiphenyl	1305.09 s, 2.082 s	1220913	182.1
4,4'-Dimethylbiphenyl	1315.09 s, 2.081 s	162138	182.1
Peak 3543	1365.09 s, 2.042 s	571861	182.1
Peak 3557	1475.1 s, 1.963 s	1318891	182.1
1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	1375.09 s, 2.115 s	13030674	195.1
1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	1390.1 s, 2.045 s	1010845	195.1
1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	1405.1 s, 2.090 s	36585057	195.1
1,1'-Biphenyl, 3,4-diethyl-	1420.1 s, 2.056 s	14909714	195.1
1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	1455.1 s, 2.029 s	25592965	195.1
1,1'-Biphenyl, 3,4-diethyl-	1515.11 s, 2.284 s	351816	195.1
Peak 3588	1520.11 s, 2.261 s	321195	195.1
1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	1525.11 s, 2.035 s	4891179	195.1
Peak 3675	1545.11 s, 2.187 s	229536	195.1
Peak 3693	1550.11 s, 2.154 s	336600	195.1
1,1'-Biphenyl, 3,3',4,4'-tetramethyl-	1580.11 s, 2.120 s	484081	195.1
Peak 3865	1600.11 s, 2.108 s	729425	195.1
Peak 3878	1605.11 s, 2.078 s	319393	195.1
Peak 3897	1610.11 s, 2.127 s	795203	195.1
Peak 3899	1615.11 s, 2.127 s	575294	195.1
Peak 3396	1475.1 s, 2.098 s	615157	196.1
Peak 3453	1485.1 s, 2.082 s	413214	196.1
Peak 3468	1490.1 s, 2.086 s	874562	196.1
Benzene, 1-methyl-3-[(4-methylphenyl)methyl]-	1495.1 s, 2.070 s	617101	196.1
Peak 3504	1500.1 s, 2.058 s	257457	196.1
Peak 3524	1505.1 s, 2.102 s	366302	196.1
Peak 3811	1585.11 s, 2.001 s	290627	196.1
Peak 3829	1590.11 s, 1.985 s	93835	196.1
Peak 3863	1600.11 s, 2.006 s	532931	196.1
Peak 4044	1655.12 s, 2.038 s	143746	196.1

Table 28: Identified phenanthrenes and anthracenes in total migration extract of recycled raw paper.

Name	R.T. (s)	Area	Base Mass
Anthracene	1615.11 s, 1.808 s	178752597	178.1
Anthracene	1625.11 s, 1.819 s	8028555	178.1
Phenanthrene, 1-methyl-	1735.12 s, 1.874 s	48611349	192.1
Phenanthrene, 4-methyl-	1740.12 s, 1.858 s	2636107	192.1
Phenanthrene, 1-methyl-	1745.12 s, 1.860 s	56356411	192.1
Anthracene, 2-methyl-	1775.13 s, 1.825 s	82555618	192.1
Phenanthrene, 2,3,5-trimethyl-	1790.13 s, 1.868 s	1331589	220.1
Peak 16929	1815.13 s, 1.898 s	867903	220
Peak 17053	1825.13 s, 1.884 s	2173485	220.1
Peak 17230	1840.13 s, 1.939 s	751273	234.1
Phenanthrene, 3,6-dimethyl-	1860.13 s, 1.914 s	32096364	206.1
Phenanthrene, 3,6-dimethyl-	1870.13 s, 1.895 s	21146537	206.1
Phenanthrene, 2,3-dimethyl-	1890.14 s, 1.886 s	134386155	206.1
Phenanthrene, 1,7-dimethyl-	1910.14 s, 1.870 s	45675038	206.1
Phenanthrene, 2,3-dimethyl-	1925.14 s, 1.856 s	12476131	206.1
Phenanthrene, 3,6-dimethyl-	1940.14 s, 1.854 s	7900213	206.1
Phenanthrene, 3,6-dimethyl-	1945.14 s, 1.846 s	2003975	206.1
3,4-Dimethylphenanthrene	1965.14 s, 1.834 s	2908144	206.1
Phenanthrene, 2,3,5-trimethyl-	2000.14 s, 1.951 s	111146730	220.1
Phenanthrene, 2,3,5-trimethyl-	2030.15 s, 1.918 s	34540907	220.1
Peak 19537	2040.15 s, 1.921 s	17803389	220.1
Peak 19694	2055.15 s, 1.904 s	3915302	220.1
Peak 19797	2065.15 s, 1.900 s	5050597	220.1
Phenanthrene, 2,3,5-trimethyl-	2070.15 s, 1.885 s	9990591	220.1
Peak 20006	2085.15 s, 1.973 s	5788340	234.1
Peak 20104	2095.15 s, 1.869 s	2094449	220.1
Phenanthrene, 3,4,5,6-tetramethyl-	2095.15 s, 1.988 s	7256693	234.1
Peak 20161	2100.15 s, 1.975 s	4249985	234.1
Phenanthrene, 2,3,5-trimethyl-	2110.15 s, 1.863 s	3871349	220.1
Peak 20313	2115.15 s, 1.852 s	10605900	220.1
Phenanthrene, 2,4,5,7-tetramethyl-	2120.15 s, 1.975 s	8836444	234.1
1,3,6,8-Tetramethylanthracene	2125.15 s, 1.968 s	7077555	234.1
Peak 20425	2125.15 s, 2.028 s	75398024	178
Peak 20559	2140.16 s, 1.964 s	8151529	234.1
1,3,6,8-Tetramethylanthracene	2150.16 s, 1.973 s	13441401	234.1
Peak 20929	2175.16 s, 1.939 s	6477176	234.1

7. List of Figures

8. List of Tables

9. List of Publications and Activities

Peer-Reviewed Publications

- *Characterization of natural polymers as functional barriers for cellulose-based packaging materials (attached at the end of this work)*
  Walzl, Andrea; Kopacic, Samir; Bauer, Wolfgang; Leitner, Erich.
  In: Food Additives & Contaminants/ Part A, Vol. 36, No. 6, 04.2019, p. 976-988.

- *Application of Industrially Produced Chitosan in the Surface Treatment of Fibre-Based Material: Effect of Drying Method and Number of Coating Layers on Mechanical and Barrier Properties.*
  Kopacic, Samir; Walzl, Andrea; Hirn, Ulrich; Zankel, Armin; Kniely, Rudolf; Leitner, Erich; Bauer, Wolfgang.
  In: Polymers, Vol. 10, No. 11, 1232, 07.11.2018.

- *Alginate and chitosan as a functional barrier for paper-based packaging materials.*
  Kopacic, Samir; Walzl, Andrea; Zankel, Armin; Leitner, Erich; Bauer, Wolfgang.
  In: Coatings, Vol. 8, No. 7, 235, 03.07.2018.

Non-Peer-Reviewed Publications

- *Charakterisierung von Biopolymeren: Funktionelle Barrieren für Lebensmittelkontaktmaterialien.*
  Walzl, Andrea; Kopacic, Samir; Bauer, Wolfgang; Leitner, Erich.
  In: Chrom+Food Forum, Vol. 2019, No. 3, 2019, p. 30-31.

- *Mineral oil residues in food: Determination of MOSH and MOAH using LC-GC-online technique and comprehensive chromatography.*
  Leitner, Erich; Walzl, Andrea.
  In: SHIMADZU News, Vol. 2018, No. 2, 2018, p. 12-15.

- *MOSH and MOAH: Mineral Oil Residues in Food.*
  Walzl, Andrea; Leitner, Erich.
  In: Chrom+Food Forum, Vol. 2018, No. 4, 2018, p. X-XII.

Talks and Posters

- *Analyse von Mineralölrückstände in der komplexen Matrix "Lebensmittel".*
  Kolb, Larissa Patricia; Walzl, Andrea; Leitner, Erich. 2019. Poster session presented at Anakon 2019, Münster, Germany.

- *Charakterisierung von Biopolymeren als Funktionelle Barrieren für Lebensmittelkontaktmaterialien.*
  Walzl, Andrea; Leitner, Erich. 2019. Poster session presented at Anakon 2019, Münster, Germany.

- *Evaluation of Biopolymers used as Functional Barriers against Mineral Oil Hydrocarbons Migration from Recycled Paper and Board.*
  Walzl, Andrea; Leitner, Erich. 2019. Poster session presented at Analytica Vietnam 2019, Ho Chi Minh City, Viet Nam.

- *Mineral Oil Residues in Food-Sample Preparation for Critical Matrices.*
  Kolb, Larissa Patricia; Walzl, Andrea; Leitner, Erich. 2019. Poster session presented at Analytica Vietnam 2019, Ho Chi Minh City, Viet Nam.

- *Characterisation of Functional Barriers for Food Contact Materials.*
  Walzl, Andrea; Leitner, Erich.
  2017. Poster session presented at XIX. Euroanalysis 2017, Stockholm, Sweden.

- *Biopolymers used as Functional Barriers – An Analytical Point of View*
  2019, Andrea Walzl (Speaker), Samir Kopacic (Contributor), Wolfgang Bauer (Contributor), Erich Leitner (Contributor), presented as Talk at Paper & Biorefinery Conference 2019, Graz, Austria.

- *Characterization and Application of Biopolymers as Functional Barriers for Cellulose Based Materials*
  2019, Andrea Walzl (Speaker), Samir Kopacic (Contributor), Wolfgang Bauer (Contributor), Erich Leitner (Contributor), presented as Talk at 43rd International Symposium on Capillary Chromatography & the 16th GCxGC Symposium, Fort Worth, United States

- *MOSH/MOAH Analytics 2.0 – LCGC-Coupling and Comprehensive GCMS for the Analysis of Mineral Oil in Food and Food Packaging*
  Sep 2018, Andrea Walzl (Speaker), Erich Leitner (Contributor), presented as Talk at Food Analysis Applications Seminar, Basel, Switzerland

- *MOSH/MOAH Analytik 2.0 - LCGC-Kopplung und Comprehensive GC-MS für die Analytik von Mineralölen in Lebensmitteln und deren Verpackungen*
  Jun 2018, Andrea Walzl (Speaker), Erich Leitner (Contributor), presented as Talk at Anwenderseminar Lebensmittelanalytik, Vienna, Austria

- *Characterisation of Functional Barrier Properties*
  May 2018, Andrea Walzl (Speaker), Samir Kopacic (Contributor), Wolfgang Bauer (Contributor), Erich Leitner (Contributor), presented as Talk at 42nd International Symposium on Capillary Chromatography and 15th GCxGC Symposium, Riva del Garda, Italy.

- *Charakterisierung von Funktionellen Barrieren*
  Apr 2018, Andrea Walzl (Speaker), Samir Kopacic (Contributor), Wolfgang Bauer (Contributor), Erich Leitner (Contributor), presented as Talk at Österreichische Lebensmittelchemietage 2018, Seggau, Austria

- *Mineral Oil Hydrocarbons in Food - An Analytical Challenge*
  May 2017, Andrea Walzl (Speaker), Erich Leitner (Contributor), presented as Talk at 13. ASAC JunganalytikerInnen Forum, Vienna, Austria

Other Activities

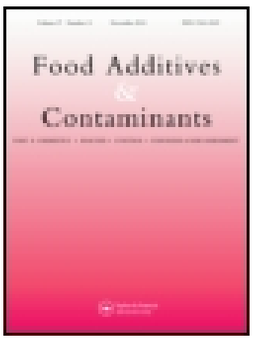
- *Mineral Oil Risk Assessment: Knowledge Gaps And Roadmap - Workshop*
  Feb 2019, as Participant, Brussels, Belgium

- *29. Doktorandenseminar Hohenroda*
  Jan 2019, as Participant, Hohenroda, Germany

- *15th Weurman Flavour Research Symposium*
  Sep 2017, as Participant, Graz, Austria

Prizes

LESLIE S. ETTRE AWARD 2018
"The Leslie Ettre Award was established in 2008 in honor of the late Leslie S. Ettre who worked at PerkinElmer for 32 years and made major contributions to gas chromatography (GC), including writing and editing over 40 books and almost 400 articles and papers. The Leslie Ettre award is presented to a scientist 35 years old or younger who presented the most interesting original research in capillary gas chromatography with an emphasis on environmental and food safety."
Andrea Walzl (Recipient), at 42nd International Symposium on Capillary Chromatography and 15th GCxGC Symposium



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Characterization of natural polymers as functional barriers for cellulose-based packaging materials

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ABSTRACT

Cellulose-based packaging materials are currently the most commonly used food packaging materials due to their light weight, stability and affordable price. However, the use of recycled paper and board adds to the risk that undesirable substances migrate into the packed goods, since contaminants are not completely removed during the recycling process and can accumulate in the final product. The only available fast and practical solution that can be used to reduce the migration of these substances is the application of functional barriers in the packaging. The applied barriers are currently mostly synthetic, which either serve only a moderate barrier function and/or have the disadvantage that it is often more complex and expensive to recycle the resulting packaging material. The aim of this project is to evaluate different bio-based or biodegradable polymers with regards to their barrier properties. Due to the fact that the transport phenomena are mainly driven by (gas phase) migration, methods based on gas chromatography (GC), including GC coupled with mass spectrometry (GC-MS) and flame ionization detection (GC-FID), GC-FID coupled online with high pressure liquid chromatography (HPLC-GC-FID), and comprehensive GCxGC-MS were used to qualify and quantify the migrated substances. This use of a wide range of different methods and instruments yielded excellent results, allowing us to comprehensively characterize the biopolymers and their barrier function.

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Functional barrier; overall migration; specific migration; mineral oil hydrocarbons; barrier materials; HPLC-GC-FID; GC-MS; GC-FID

Introduction

Food contact materials (FCMs) and articles are materials and articles that are considered to come into contact with food. They should not change the food in any unacceptable way, either in terms of taste or quality or in terms of contaminations (REGULATION (EC) No 1935/2004; US FDA 2018)). Paper and board are currently the most commonly used food packaging materials (Muncke 2012). Due to their low price and environmentally friendly characteristics, recycled paper and board is often preferred, especially for secondary and tertiary packaging (Andersson 2008). However, it is challenging to use these materials and meet the above-mentioned requirements. During the recycling process fibers with different purities and sources (e.g. newspapers, packaging materials, tissues) are mixed together and a huge pool of possible contaminants is created. These contaminants can be transferred from the packaging into the packed food – even from secondary or tertiary packaging- and the safety of the product can no longer be guaranteed (EC 2011; ERPC 2015; Geueke et al. 2018). These migration phenomena are complex processes and can occur through two ways: by direct (wetting) contact or by migration or permeation (Boccacci Mariani et al. 1999). The process starts with the evaporation of the contaminants from the packaging, followed by their migration or permeation to the food and re-condensation on the food. The process of permeation requires the contaminants from e.g. a secondary packaging to be vaporized into the gas phase, permeate through the primary packaging (and a potentially applied functional barrier) and re-condense on the food. Since evaporation at room temperature is only relevant for substances with a chain length of up to C_{24}, migration and permeation is limited to these

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substances (Diehl and Welle 2015). However, the transfer of contaminants can also take place through direct contact (wetting contact), and substances with chain lengths of up to C_{35} or even C_{40} can be transferred in this way, depending on the storage time and temperature (Boccacci Mariani et al. 1999; Lorenzini et al. 2013).

The conditions that are widely used to perform migration testing originate from a regulation for plastic materials and articles that are considered to come into contact with food (EC 2011). The tests and limits described for migration in this regulation are also used for paper and board food contact materials (e.g. DIN 2018). The regulation states that, food simulants should be used for testing the migration, since food is a complex, highly varying matrix. The simulants should mimic the food, which is considered to be packed in the sample as good as possible. For dry, non-fatty food like rice, noodles or spices TenaxTA® (poly(2,6-diphenyl-*p*-phenylene oxide)) is the given simulant. It should be applied in an amount of 4 g dm^{-2} to the surface area to be tested. The test conditions should be chosen to simulate the worst foreseeable use situation. To simulate long-term storage, testing at elevated temperature is allowed to accelerate the test. As an example, the effects of a long-term storage for up to or more than six months at room temperature are simulated by using the test conditions of 60°C for 10 days.

Since the possible contaminants are versatile, different analytical techniques are needed to comprehensively characterize the substances that have migrated into the food simulant during a migration test (Castle et al. 1997; Biedermann and Grob 2013). In this study, the overall migration of the volatile fraction out of the sample into the food or food simulant was determined using GC-FID as a first step. The FID is a universal detector and responds with similar sensitivity to a wide range of organic chemicals (Grob and Barry 2004). This means that quantification of the overall amount of substances that have migrated can be performed by simply integrating across all detected peaks, without the need for the calculation of a response factor. However, it is difficult or impossible to identify unknowns using this untargeted approach.

The maximum allowed amount of migrated substances is defined by the overall migration limit (OML). It is set as 10 mg of substances that are allowed to be released from $1dm^2$ of packaging material. It is assumed that a person with a 60-kg bodyweight consumes 1 kg of food daily, which is packed in a cubic packaging that has 6 dm^2 of surface area, resulting into a calculated maximum allowed migration of 60 mg kg^{-1} food (EC 2011).

In the addition to the OML, a specific migration limit (SML) is defined for individual substances. The SML is the maximum concentration of a specific substance that is allowed to migrate from the packaging material into the food. It is derived from toxicological data for the specific substances. Examples for substances with an SML are diethylhexylphthalate (DEHP; max. 1.5 mg kg^{-1} migration into food or simulant) or di-*n*-butylphthalate and diisobutylphthalate (DnBP and DiBP; both max. 0.3 mg kg^{-1} migration into food or simulant) (BFR 2016).

Since the FID, which is used for the determination of the overall migration, does not give detailed information, especially regarding the identification of unknowns, qualitative information is generated using a MS detector. Therefore, the analysis of specific substances with low SMLs was performed using GC with MS detection in this study. This methodology allowed us to obtain qualitative information about the constituents of the sample, but also to quantify substances with an SML (Biedermann and Grob 2013).

The contaminants that gained the most public concern in the last years are the mineral oil hydrocarbons (MOH). They are divided into a saturated and an aromatic fraction. The MOSH fraction (mineral oil saturated hydrocarbons) contains branched and unbranched open chain hydrocarbons (paraffins) and saturated cyclic hydrocarbons (naphthenes). The MOAH (mineral oil aromatic hydrocarbons) consists of highly alkylated aromatic ring compounds, which make up 15–30% of the whole mineral oil fraction. Routine analysis of MOH is performed using online coupled HPLC-GC-FID, but no further information than the overall concentration of MOSH and MOAH can be generated using this method (CONTAM 2012; Biedermann and Grob 2012a, 2012b). Furthermore, no regulated limits for the migration of MOSH and MOAH exist so far, but several studies have dealt with their toxicity and influence on human health (e.g. CONTAM Trimmer 2012; Barp et al. 2014, 2017; Trimmer et al. 2004). The MOSH is known to be enriched in different

human tissues and is thought to be responsible for the formulation of granulomas and organ weight increase. The MOAH consist of the often stated "potentially mutagenic and carcinogenic substances", but the main concern is their genotoxicity. It is currently discussed, whether the MOAH fraction should be differentiated by ring number. While one and two ring systems are considered to have no or only low genotoxic effects, the higher ring-number systems are of more concern. However, most of the MOAH contaminations found in food are believed to consist of mainly 1–2 aromatic ring-systems, but we lack suitable analytical techniques to characterize these easily in more detail (CONTAM 2012; Grob 2018).

To protect the food from all these possible contaminants and to protect the food's quality, texture and flavor, functional barriers are used (BFR 2009; EC 2011; Bordenave et al. 2014). Functional barriers are materials that are placed between the food and its packaging to prevent, on the one hand, the unwanted migration from the packaging into the food and, on the other hand, to prevent, for example the losses of aroma. Materials that are used at the moment are mostly synthetic ones, which are often used in combinations of multi-layered materials, such as polyethylene (PE), polypropylene (PP) or aluminium. A disadvantage of these commonly used coating materials is the fact that they make the recycling of the resulting packaging material more complex and expensive (Ewender et al. 2013; Richter et al. 2014; Diehl and Welle 2015).

Authors of several publications have recently discussed the barrier properties needed to render a recycling paper suitable for food contact. As Biedermann-Brem *et. al.* note, an effective barrier must reduce the migration of all substances to a level at which they are no longer of toxicological concern. For known and assessed substances this may be the SML, a tolerable daily intake (TDI), or a level of acceptable risk (Biedermann-Brem et al. 2016; p.728). For non-evaluated substances, the limit of the (EC 2011) is often used, whereby non-evaluated substances should be undetectable with a limit of detection of 0.01 mg kg^{-1}. The German Federal Ministry of Food Agriculture and Consumer Protection (BMELV) also used this limit in their project on the migration of substances from recycled paper and board and defined it as a threshold of toxicological relevance in food (Harling et al. 2012). However, this limit has no toxicological background but was derived when it was still a challenge to reach this LOD in a target analysis. For the "taped barrier test" developed by Biedermann-Brem et al., a reduction of the migration of spiked surrogate substances to below 1% of the content in the donor board was defined, meaning that the migration of all non-evaluated or unknown substances also remain below 0.01 mg kg^{-1} food (Biedermann-Brem et al. 2016).

The aim of this project was to characterize and apply natural polymers as functional barriers for cellulose-based food packaging materials. Different materials like microfibrillated cellulose (MFC), casein, soy protein, alginate and chitosan were tested in the form of free-standing films first. Afterwards an industrially produced recycled paper was coated with the best performing materials. The recycled raw paper is considered to be the most challenging scenario with regard to food packaging, with comparatively high loads of mineral oil hydrocarbons and other contaminants. The raw paper was first characterized by total extraction, from all samples (coated and uncoated) migration tests were performed to test for the migration of the above mentioned contaminants. Regarding the barrier efficiency needed, we tried to reduce the migration of contaminants to meet the current regulatory requirements, giving a special focus on the planned and often stated levels for MOSH and MOAH (BMEL 2014, 2017). To reach the proposed limits of 2 and 0.5 mg kg^{-1} in food, a reduction of the migration of MOH from the recycled paperboard of at least 70% for MOSH and 45% for MOAH had to be achieved.

To reduce the sample preparation and analysis time of the migration experiment and gain more information, a two-sided test in migration cells was performed, to test migration and permeation in one attempt. To differentiate the amount of overall migration of the tested sample from the simulated permeation, deuterated *n*-alkanes were used for the permeation test. Furthermore, the Arrhenius equation with an activation energy of 80 kJ mol^{-1} (as given in EC 2011) was used to calculate conditions that were equal to the standard test conditions of 10 days at 40°C or 60°C. After extensive pre-tests, conditions of 80°C for 2 days were chosen. The analyses were performed using the different instruments described to

comprehensively characterize the extracts obtained after migration experiments. GC-FID was used to determine the overall migration, GC-MS for specific migration and LC-GC-FID for the migration of MOSH/MOAH, respectively.

The application of the developed method is shown for three materials alginate, chitosan and microfibrillated cellulose (MFC).

Materials and methods

Description of paper samples

The uncoated raw paper used is an industrially produced packaging paper (liner), made of 100 % secondary (recycled) cellulosic fibers. The paper was not treated with sizing or coating chemicals. The thickness of the paper was 200 ± 3 µm, the measured bulk density of the sheet was 660 ± 10 kg m^{-3} and the grammage was 130 ± 1 g m^{-2}. This raw paper was coated at standard ambient conditions using a draw-down coater (RK Printcoat Instruments LtD (UK)). The coating was performed one-sided and in our case, the top side of the paper was used, whereas the bottom side remained uncoated. The target coating weight of 6 g m^{-2} with a deviation of less than 5 % was achieved for all bio-based barrier materials used in this work.

The aqueous coating solutions were prepared without any additives using MFC, chitosan and alginate. The solid content of the MFC coating solution was 3 % (w/w), and four thin layers were applied in total to the paper in order to reach desired coating weight of 6 g m^{-2}. The two other materials, alginate and chitosan, were dissolved in water at 70°C and the solid content of the solution was adjusted to 4% (w/w). Two layers of alginate and chitosan were applied in order to accomplish total coating weight of 6 g m^{-2}. After the coating trial, all samples were conditioned at 23°C and 50% relative humidity in a climate room and prepared for further characterizations.

General test procedures

Determination of MOH in raw paper

To determine the total amount of MOSH and MOAH in the raw paper, a total extraction of the paper was performed similar to Biedermann and Grob (2012a).

In brief, the paper was cut into small pieces, 1 g was weighed into a glass vial with a screw cap, and 10 µL of a MOSH/MOAH Internal Standard Mix was added to reach a concentration of 1.5–6 µg mL^{-1}. The standard that was purchased by Restek Corporation (Bellefonte, US) contained the following substances in 1 mL ampules in toluene: *n*-undecane (300 µg mL^{-1}), *n*-tridecane (150 µg mL^{-1}), bicyclohexyl (300 µg mL^{-1}), cholestane (5-alpha-cholestane; 600 µg mL^{-1}), 1-methylnaphthalene (300 µg mL^{-1}), 2-methylnaphthalene (300 µg mL^{-1}), *n*-pentylbenzene (300 µg mL^{-1}), perylene (600 µg mL^{-1}) and 1,3,5-tri-*tert*-butylbenzene (300 µg mL^{-1}). Ten mL of hexane/ethanol 1:1 were added and the paper was extracted at room temperature for 2 h with occasionally shaking. Afterwards the extract was washed two times with water and subsequently centrifuged. The hexane phase was collected in a 50 mL turbovap evaporation vial, and the solvent was evaporated to 0.5 mL using an automatic solvent evaporator. The extracts were then transferred into 1.5 mL glass vials with screw caps, the evaporation vial was rinsed with 0.5 mL of hexane and this also added to the 1.5 mL vial. The extracts were stored in a refrigerator until analysis, only a small amount was filled into a 1.5 mL glass vial with a screw cap and a micro inlet for measurements that were performed using an online coupled HPLC-GC-FID as described in the section "Instrumentation".

Migration experiments

Migration and permeation experiments were performed as described in Kopacic et al. (2018) and in compliance with the (EC 2011).

In brief, the experiments were performed in triplicates using migration cells (MigraCell®; FABES Forschungs-GmbH, Munich, Germany). Tenax® a poly(2,6-diphenyl-p-phenylene oxide (Tenax TA (refined), 60–80 mesh; SUPELCO, Bellefonte, USA) was used to simulate dry food, such as rice, cereals, cocoa, coffee and spices and was applied in an amount of 4 g dm^{-2}. The cell was assembled according to the manufacturer's instructions and placed in an oven at 80°C for 2 days. After this period, the Tenax® was placed in a glass vial with a screw cap, and 25 µL of an internal standard mix was added. The internal standard for migration experiments consisted of dodecane-d_{26} ($C_{12}D_{26}$; EURISO-TOP SAS, Saint-Aubin, France),

nonadecane-d_{40} ($C_{19}D_{40}$; 98%; Cambridge Isotope Laboratories, Inc.; Tewksbury, Massachusetts), benzophenone-d_{10} ($C_{13}D_{10}O$; 99atom%; SIGMA-ALDRICH Co., St.Louis, USA), bis(2-ethylhexyl) phthalate-3,4,5,6-d_4 and di-n-butylphthalate-3,4,5,6-d_4 (both "analytical standards", purchased from SIGMA-ALDRICH Co., St. Louis, USA), each 200 mg L^{-1} in acetone (ROTISOLV® ≥99.9 %, UV/IR-Grade; Carl Roth GmbH + Co. KG, Karlsruhe, Germany). The Tenax® was extracted three times with 10 mL of *n*-hexane (Picograde® for Residue Analysis; LGC Promochem GmbH; Wesel, Germany) and vortexed for three minutes. The extracts were filtered through a folded filter into a 50 mL evaporation vial and the solvent was evaporated to 0.5 mL using an automatic solvent evaporator (TurboVap ® II; Biotage, Uppsala, Sweden). The extracts were then transferred into 1.5 mL glass vials with screw caps, the evaporation vials were rinsed with 0.5 mL of hexane and this solvent added to the 1.5 mL vials. The extracts were stored in a refrigerator until the analysis was performed; only a small amount was filled into a 1.5 mL glass vial with a micro insert and screw cap for measurements. The extracts were analyzed with GC-FID to determine the overall migration, GC-MS to determine the specific migration and HPLC-GC-FID to determine the migration of MOSH and MOAH as described in the section "Instrumentation".

Permeation experiments

The migration cell used allows a one-sided migration experiment (as described above) and a two-sided application for testing migration and permeation to be done at once, as already described in Kopacic et al. (2018). Three changes in the experimental set up were made compared to migration experiments (see also Figure 1): first, a piece of cellulose is placed at the subpart of the cell, simulating a secondary or tertiary packaging, and where the modelling substances for the permeation were spiked. Second, the metal plate in the middle of the cell was removed and therefore, third, the colourless silicone ring, which was placed in the subpart of the cell, had to be replaced by a FEP-coated red ring to prevent siloxanes from contaminating the Tenax®. As modelling substances, deuterated *n*-alkanes of different chain lengths were chosen because they simulated the possible migration of mineral oil hydrocarbons through the sample most effectively ($C_{14}D_{30}$, $C_{20}D_{42}$ and $C_{24}D_{50}$, 98%-atom% D, purchased from Cambridge Isotope Laboratories, Inc.; Tewksbury, Massachusetts; $C_{16}D_{34}$, 99%-atom% D, purchased from abcr GmbH, Germany; $C_{28}D_{58}$ 98%-atom%D, purchased from C/D/N/Isotopes, Inc. Quebec, Canada). Deuterated substances were used to prevent interference of permeation and migration tests, because these n-alkanes are also intentionally included in the tested recycled paper sample. To simulate aroma permeability, a set of four aroma

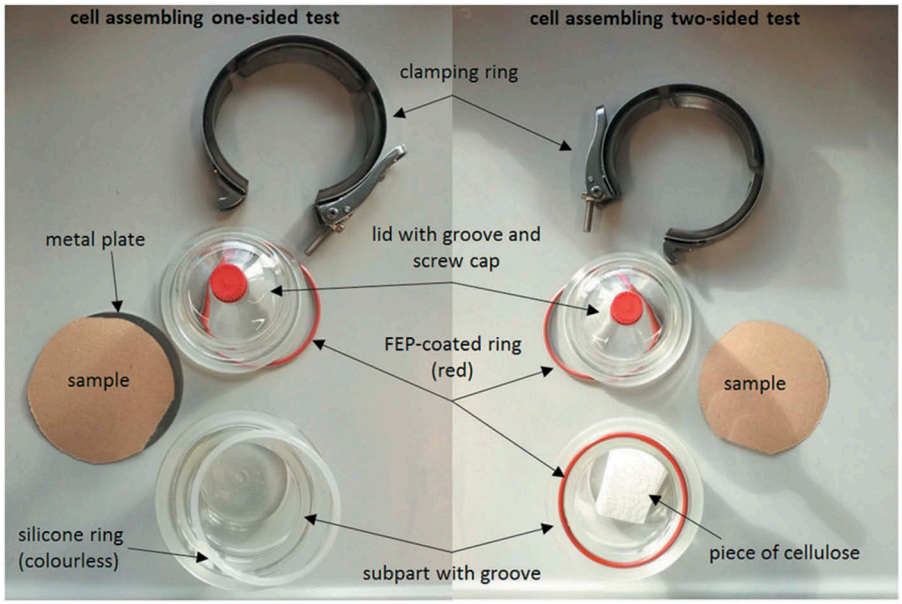

Figure 1. comparison of cell assembling of one-sided and two-sided migration test.

compounds was selected (dl-menthol, ≥95%; eugenol, ReagentPlus®, 99%; vanillin, ≥97% and acetovanillone, ≥98%; purchased from SIGMA-ALDRICH Co., St. Louis, USA). 100 µL of a stock solution containing each of the mentioned substances in a concentration of 100 mg L^{-1} in acetone were spiked onto the piece of cellulose at the bottom of the cell. The test conditions, extraction and analysis remained the same those described in "Migration Experiments".

Instrumentation

GC-FID

The extracts were measured using a gas chromatograph with a flame ionization detector (GC-FID) to determine the overall migration. Separation of substances was done using a Hewlett Packard 6890 Series GC System equipped with an Optima delta-6 capillary column (7.5 m × 100 µm × 0.10 µm, Macherey-Nagel, Germany). The oven was programmed to 60°C (hold 1 min), raised at 15°C min^{-1} to 300°C (3 min). The carrier gas was hydrogen with a linear velocity of 48 cm sec^{-1}. Aliquots of one microliter were injected with a split of 1:20; the injection port temperature was set to 280°C. The detector temperature was set to 320°C, the air flow was 450 mL min^{-1}, hydrogen flow 40 mL min^{-1} and make-up gas was nitrogen with a flow of 25 mL min^{-1}. Data evaluation was performed using "GC ChemStation" version B.04.03[16].

GC-MS

To determine the specific migration amounts of individual substances, a GC with MS detection was used. Gas chromatographic separation was done using a Shimadzu GC2010 system equipped with an RXI 5Sil MS capillary column (30 m × 250 µm × 0.25 µm). Helium was used as a carrier gas with a constant flow of 40 cm sec^{-1}. A high-pressure splitless injection with 250 kPa for 0.8 min and column flow of 4.74 mL min^{-1} was used; the gas saver at a ratio 1:5 was turned on after 2 min. The analysis pressure was 73.2 kPa; column flow was 1.17 mL min^{-1}, septum-purge flow was 3 mL min^{-1}. Injection temperature was 270°C and sampling time was 1 min. The initial oven temperature was 70°C (1 min) and was raised at 6°C min^{-1} to 340°C (5 min). Detection was done with a GCMS-QP2010 PLUS mass selective detector. Ions were generated with electron ionization (70 eV). The detector voltage was relative to tune (0.82 kV), the mass spectrometer scanned from *m/z* 35 to 500 with a scan rate of 1666 amu sec^{-1} and an event time of 0.3 sec. The software used was "GC-MS Solution" Version 4.42.

HPLC-GC-FID

For the analysis of MOSH and MOAH, an online-coupled HPLC-GC-FID instrument was used as described in Biedermann and Grob (2012a). The HPLC used was a Shimadzu LC-20AD equipped with an Allure Silica 5 µm column (250 × 2.1 mm). A gradient elution program was used, started with 100% *n*-hexane (flow 0.3 mL min^{-1}), increased to 35% CH_2Cl_2 within 2 min (hold for 4.20 min), then the column was back flushed at 6.30 min with 100% CH_2Cl_2 (flow 0.5 mL min^{-1}; hold for 9 min) and reconditioned to 100% *n*-hexane (flow 0.5 mL min^{-1}; hold for 10 min). The flow was decreased afterwards to 0.3 mL min^{-1} until the next injection. The UV-Detector was equipped with a D_2-lamp set at 230 nm and 40°C cell temperature. The GC was a Shimadzu GC 2010 dual FID equipped with two Restek MXT Siltek guard Columns (10 m × 0.53 mm id) and two Restek MTX ®-1 analytical columns (15 m × 0.25 mm id × 0.1 µm df). The carrier gas was hydrogen with 150 kPa analysis pressure and an evaporation pressure of 87 kPa for MOSH and 85 kPa for MOAH. The oven was programmed to 60°C (hold 6 min), raised at 20°C min^{-1} to 100°C (0 min) and followed by 35°C min^{-1} to 400°C (8.43 min). The LC-GC interface was controlled by Chronect-LC-GC by Axel-Semrau, and data evaluation was done using LabSolutions vs. 5.92. For the calculation of retention indices and determination of cutting fractions in HPLC-GC-FID, a "C_7-C_{40} Saturated Alkanes Standard" from SUPELCO (Bellefonte, USA) was used. The concentration of the alkanes was 1000 µg mL^{-1} in hexane and was diluted to 1 µg mL^{-1} with hexane prior to the analysis. According to a proposed method published by the German Federal Institute for Risk Assessment (BfR), quantification was done by integrating the hump for different molecular-weight regions. They proposed the ranges of C_{16}-C_{25} and C_{25}-C_{35} for dry non-fatty food that comes in contact with a food contact material at room temperature (BFR 2012).

2D-comprehensive GCxGC-MS

Due to the formation of unresolved humps, it is often necessary to use more comprehensive techniques to analyse the samples. The comprehensive GCxGC-MS was composed of the following units: an OPTIC-4 Multimode GC Inlet and a AOC-5000 Plus autosampler, a Shimadzu gas chromatograph for mass spectrometer GC-2010 Plus equipped with a HT1 column (30 m × 0.25 mm; 0.25 µm) and a BPX5 column (2.5 m × 0.15 mm; 0.15 µm) placed in the same oven. The columns were connected directly by using a push-fit connector and sealing of the connection with a polyimide resin. The injection was done in splitless mode, the split was opened after 1.5 min with a split ratio of 10, and the injector port temperature was 270°C. Helium was used as a carrier gas in the linear velocity flow control mode. The pressure was set at 150 kPa and the purge flow was 1.0 mL min^{-1}. The initial oven temperature of 40°C (1 min), was increased to 120°C (0 min) with a ramp of 15°C min^{-1} and then on to 310°C (2 min) with a ramp of 4°C min^{-1}. The modulator used was a cryogenic modulator (Zoex ZX1 2 stage loop thermal modulator; Zoex Corporation, Houston, Texas, USA). Modulation frequency was 6 sec with a hot- jet pulse of 350 ms. The temperature of the hot jet was programmed at 280°C (40 min), followed by 320°C for 5 minutes and finally set to 360°C till the end of the run. Detection was done using the mass spectrometer GCMS-QP2010 Ultra. Ions were generated with electron ionization (70 eV). The ion source temperature was set at 200°C, the interface temperature at 310°C; the detector voltage was 0.85 kV. The mass spectrometer was scanned with a scan speed of 20.000 amu sec^{-1}, resulting into 33 full-scan spectra recorded from *m/z* 50 to 500. Data were acquired during 11 and 53 min runtime. The software used for data evaluation was Version 2.7 of "GC Image" from the Zoex Corporation.

Validation of migration experiments

Validation of the proposed method was accomplished by spiking a transformer oil (Technol 2000, 3632-RÜ; TECHNOL Mineralöl-Veredelungs-Ges. m.b.H., Vienna, Austria), di-*iso*-propylnaphthalene (DIPN) and a set of phthalates (DEHP, DnBP, DiBP) directly onto a clean fresh fiber paper. The total amount of MOSH and MOAH, overall migration, specific migration and migration of MOSH and MOAH of this fresh fibre paper were analyzed prior to use. No contaminants were detected in the concentration range used for validation. The MOSH and MOAH content of the transformer oil was also analyzed prior to use. A stock solution of 10 g L^{-1} in hexane was prepared. To 970 µL of hexane, 20 µL of the described stock solution and 10 µL of MOSH/MOAH Internal Standard Mix were added to achieve a final volume of 1 mL and a concentration of 200 mg L^{-1}. The sample was analyzed in a single measurement only with HPLC-GC-FID as described in "Instrumentation". The MOSH content of the oil was determined as 619 mg kg^{-1} in the range C_{16}-C_{25} and 637 mg kg^{-1} in the range $C_{16\text{-}35}$. The MOAH content was 105 and 108 mg kg^{-1} in the ranges of C_{16}-C_{25} and $C_{16\text{-}35}$.

For validation, volumes of 10, 25, 50, 125 and 250 µL of the 10 g L^{-1} stock solution in *n*-hexane were spiked directly onto the fresh fiber paper with a 0.3 dm^2 surface area. Taking the assumption that 1 kg of food is packed in a cubic packaging of 6 dm^2, the lowest spiked concentrations resulted in 2 mg per 6 dm^2 paper or 2 mg kg^{-1} food. Considering the levels of MOSH and MOAH that are considered to be safe at the moment (2 mg kg^{-1} MOSH and 0.5 mg kg^{-1} MOAH; (BMEL 2014, 2017)), this seemed to be an adequate lowest point. The other spiked concentrations gave 5, 10, 25 and 50 mg of mineral oils per kg of food simulant, respectively. Taking the same assumptions for DIPN and the phthalates, the spiked concentrations resulted in a potential migration of 10, 50, 200, 500 and 1000 µg kg^{-1} food.

After spiking the substances onto the paper, the solvent was allowed to dry, and the paper was used immediately for the migration experiments. The cell was assembled, and the migration experiments were performed as described above. Permeation was not tested during the validation experiments; experiments were performed in triplicates. The overall migration was determined using GC-FID, the specific migration using GC-MS and the migration of mineral oil using HPLC-GC-FID. For data evaluation the software "ValiData" (excel-macro; Version 3.02.48; ′W. Wegscheider, et.al) was used.

Application on coated and uncoated papers

The raw paper was characterized by total extraction with hexane/ethanol to determine the total MOSH/MOAH content and by a two-sided test in migration cells to determine the overall migration, the specific migration of DEHP, DnBP, DiBP, DIPN, the migration of MOSH and MOAH, as well as the permeation of selected marked compounds through the paper into the food simulant. The original amounts of the raw paper were compared afterwards with the results of the samples coated with alginate, chitosan and MFC.

Results and discussion

Validation

Overall migration was validated at the levels 0, 2, 5 and 10 mg kg^{-1}food (n = 3; performed in three separate migration experiments). The limit of detection and quantification were determined with the calibration method as 1.04 and 3.45 mg kg^{-1}. The variance test and the linearity test showed no significant differences at a level of 99%. The relative standard deviation of the method was 18.7%. The overall migration limit for a food contact material is 60 mg kg^{-1}, which is far above our limit of detection and range of interest.

The migration of MOSH between C_{16} and C_{35} was evaluated at the levels 0, 1.6, 4, 8, 20 and 40 mg kg^{-1} (n = 3; performed in three separate migration experiments). The limit of detection and quantification were determined with the calibration method as 0.81 and 2.88 mg kg^{-1}. The variance test and the linearity test showed no significant differences at a level of 99%. The relative standard deviation of the method was 5.73%. The maximum amount of MOSH considered currently to be acceptable is 2 mg kg^{-1}, which is easy detectable with the obtained limit of detection.

The migration of MOAH between C_{16} and C_{35} was evaluated at the levels 0, 0.4, 1, 2, 5 and 10 mg kg^{-1} (n = 3; performed in three separate migration experiments). The limits of detection and quantification were determined with the calibration method as 0.30 and 1.05 mg kg^{-1}. The variance test and the linearity test showed no significant differences at a level of 99%. The relative standard deviation of the method was 6.78%. The maximum amount of MOSH considered currently to be acceptable is 0.5 mg kg^{-1}, which is easy detectable with the obtained limit of detection.

Table 1. Summary of validation results for phthalates and DIPN (n = 3).

	LOD [µg kg^{-1}]	LOQ [µg kg^{-1}]	Linearity at 99%	Variance at 99%	Rel. Std. Dev. of Method [%]
DnBP	2.59	9.42	Ok	Ok	3.20
DiBP	3.68	13.3	Ok	Ok	4.54
DEHP	3.10	11.3	Ok	Ok	3.83
DIPN	3.39	12.3	Ok	Ok	4.20

DnBP, DiBP, DEHP and DIPN were validated at the levels 0, 10, 50 and 200 µg kg^{-1} (n = 3; performed in three separate migration experiments). Results are summarized in Table 1. The specific migration limit for DEHP, DiBP and DnBP is 300 µg kg^{-1} food, whereby the sum of DiBP and DnBP should also not be higher than this level. These SMLs are again far above our detection limit and limit of interest.

General procedure

The use of deuterated n-alkanes allows us to perform two-sided tests in the migration cells. This means the determination of migration and permeation was possible in one experimental setup without any interferences. This saved a lot of time and resources and provided us a quick and easy screening method for examining the behavior of the natural polymers used as functional barriers.

Figure 2 gives an example of a GC-MS chromatogram of an overall migration extract of the recycled raw paper sample (black trace), which includes internal standards and the permeation standard. For comparison, the internal standards are given as red traces (direct injection of 1µL standard in a concentration of 5 µg ml^{-1}; in order of elution: $C_{12}D_{26}$, benzophenone-d_{10}, $C_{19}D_{40}$, bis (2-ethylhexyl)phthalate-3,4,5,6-d_4 and di-n-butylphthalate-3,4,5,6-d_4) and permeation standards as green traces (direct injection of 1 µL standard in a concentration of 10 µg ml^{-1}; in order of elution: menthol, eugenol, $C_{14}D_{30}$, vanillin, acetovanillone, $C_{16}D_{34}$, $C_{20}D_{42}$, $C_{24}D_{50}$, $C_{28}D_{58}$).

Application to coated paper samples

Overall migration

Since the substances released from food contact materials should not cause unacceptable changes in

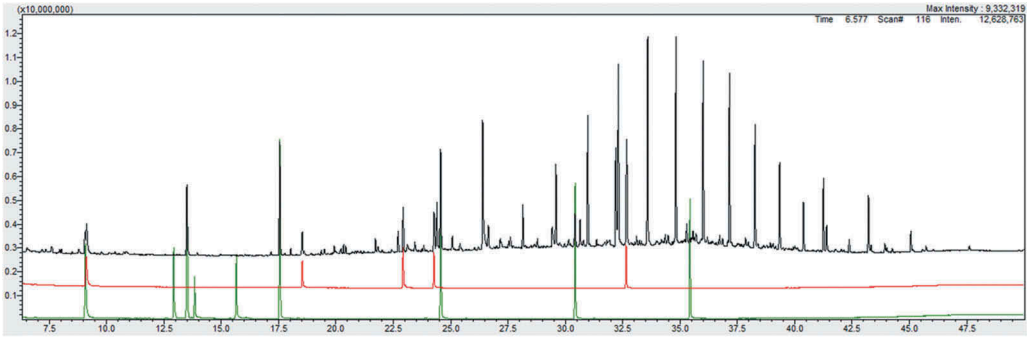

Figure 2. GC-MS chromatogram of raw paper sample (black trace), with shown internal standard (red trace) and permeation standard (green trace).

the composition of the food, the overall migration should be kept as low as possible (REGULATION (EC) No 1935/2004). A paper produced from secondary fiber is considered to be the most challenging material, especially in terms of its mineral oil hydrocarbons amount and barriers are needed to reduce migration. Taking the approach of using alginate and chitosan coatings successfully reduced the migration from the packaging material into the food simulant in our study: The two materials showed good barrier efficiencies. The overall migration of substances from the recycled packaging materials (given as 100%) could be reduced to 16.3 ± 1.0% by the alginate coating and to 29.5 ± 1.6% by the chitosan coating. MFC reduced the migration only to 71.5 ± 0.5% and did not show the barrier efficiency desired.

Permeation

The gas-phase migration and permeation of substances into dry food are limited by their volatility. It has been shown that it is relevant for substances up to a chain length of C_{24} and no longer detectable for those with a chain length of C_{28}. So higher-boiling substances remain in the packaging material and do not migrate (Lorenzini et al. 2010; BFR 2012). In comparison, compounds with a chain length of up to C_{35} can be transferred through wetting contact (Boccacci Mariani et al. 1999; Lorenzini et al. 2013). According to theory, the transfer from the recycled raw paper into the food simulant (direct contact) was found for compounds of chain lengths up to C_{35}, while the highest levels for permeation – which takes place through the gas phase – were found for the d-C_{14} and d-C_{20} compounds. In comparison, the permeation of d-C_{24} was low and that of d-C_{28} was no longer detectable. The behavior of the added aroma active substances yielded an interesting observation: Although all four compounds have a volatility and a boiling point in the same range as C_{12}-C_{16} (Table 2), only menthol permeated through the papers (coated and uncoated). The polar groups of the aroma compounds apparently interact strongly with the polar groups of the paper. In further tests using head-space solid-phase micro extraction (HS-SPME), the permeation standards were tried to be extracted directly after spiking onto the cellulose. We confirmed that the polar substances could not be desorbed from the paper anymore (data not shown).

Of the three tested biopolymers, the alginate-coated samples again showed the best barrier properties (Figure 3). The permeation rate of d-C_{14} to d-C_{20} were between 8.54 and 14.49 µg dm^{-2} day^{-1} for the uncoated recycled fiber, for d-C_{24} 3.39 µg dm^{-2} day^{-1}and 5.39 µg dm^{-2} day^{-1} for menthol. For the alginate coating the values were reduced to 1.81–3.80 µg dm^{-2} day^{-1} (reduction of 63–83%) for d-C_{14} to d-C_{20}, to 0.11 µg dm^{-2} day^{-1}for d-C_{24} and 0.58 µg dm^{-2} day^{-1} for menthol (reduction by 97% and 90%). For the chitosan coating, the permeation of d-C_{14} to d-C_{20} was only reduced by 44–62%; for

Table 2. Boiling points of aroma substances and *n*-alkanes in the same elution range.

Substance	Boiling Point [°C]
Menthol	212
C_{12}	216.2
C_{14}	253.5
Eugenol	254
Acetovanillone	265
Vanillin	285
C_{16}	286.8

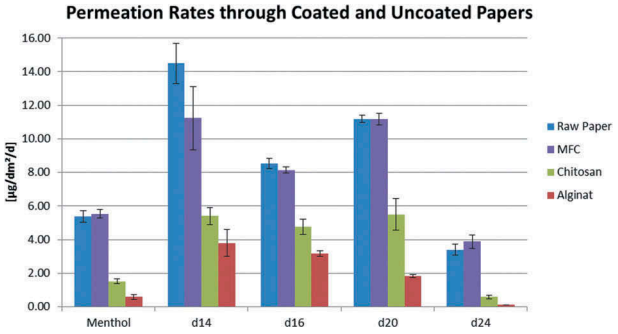

Figure 3. Comparison of permeation rates of deuterated *n*-alkanes of different chain lengths and menthol through coated and uncoated paper (µg dm^{-2} day^{-1}; n = 3).

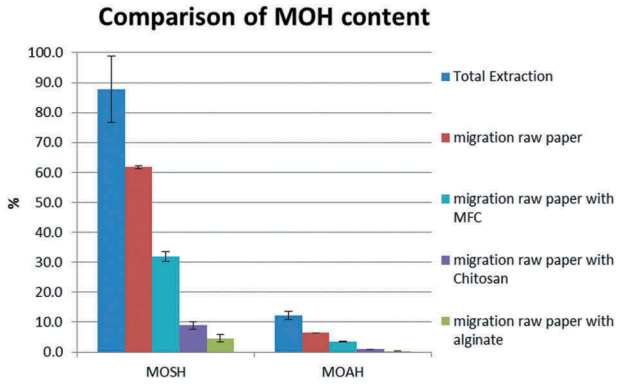

Figure 4. Comparison of migration of MOSH and MOAH in the range of C_{16}-C_{35} through coated and uncoated paper (%; n = 3).

d-C_{24} by 83% and for menthol by 72%. The MFC coating showed no significant reduction at all.

Specific migration

DnBP, DiBP, DEHP and DIPN were quantified using GC-MS and the deuterated internal standards added. The best results were again achieved with the alginate coating with a reduction of the migration of up to 98%. Chitosan showed also good barrier efficiency, reducing migration by 53 to 94%. The worst barrier efficiency was again observed by MFC with reductions of 8–42%. Details are given in Table 3.

Migration of MOSH and MOAH

The migration of MOSH and MOAH from the packaging are probably the most important values recently. The values considered to be safe in food are currently 2 mg kg^{-1} MOSH and 0.5 mg kg^{-1} MOAH, but no regulated limits have yet been defined (BMEL 2014, 2017). According to literature, about 70–80% of the total MOH content of the paper migrates into the food or food simulant (Lorenzini et al. 2010; Vollmer et al. 2011; Fiselier and Grob 2012; Biedermann-Brem et al. 2017). This was also observed in our investigation. Of the total amount of MOSH and MOAH in the recycled raw paper determined by total extraction, 68.2 ± 0.6% migrated into the food simulant without a barrier. From this 68%, 61.7 ± 0.6% were made up of MOSH and 6.4 ± 0.1% of MOAH. With the alginate barrier, the migration was reduced to 5.0 ± 1.4% (4.6 ± 1.3 % MOSH, 0.4 ± 0.1% MOAH) and with the chitosan coating, to 9.8 ± 1.3 % (8.9 ± 1.3 % MOSH, 0.9 ± 1.3 % MOAH). The MFC coating again showed a low barrier efficiency; about 35.4 ± 1.8% MOH still migrated into the food simulant (made up of 31.9 ± 1.7% MOSH and 3.5 ± 0.1% MOAH). The results are summarized in Figure 4. As stated in the introduction, a reduction of the migration of at least 70% for MOSH and 45% for MOAH needs to be achieved to render the raw paper suitable for food contact. Therefore, the alginate and chitosan barriers showed excellent results, with reductions of above 90%, while the barrier efficiency of MFC was not sufficient.

Table 3. Migration of DiBP, DnBP, DEHP and DIPN out of raw paper given as 100% compared to migration out of coated papers (*n* = 3).

	Raw paper [%]	Alginate [%]	Chitosan [%]	MFC [%]
DiBP	100 ± 1	7.93 ± 0.10	18.3 ± 1.4	50.7 ± 8.0
DnBP	100 ± 3	6.20 ± 1.15	15.5 ± 3.4	53.7 ± 10.1
DEHP	100 ± 5	1.69 ± 0.11	5.54 ± 0.81	92.4 ± 3.9
DIPN	100 ± 3	35.3 ± 3.3	47.3 ± 2.4	58.6 ± 6.2

Further characterisation using GCxGC-MS

GCxGC-MS is an important tool that can be used to further characterize the samples in more detail and, in particular, to identify unknowns and false positive values.

The method was used to compare the extracts of the migration experiments of coated and uncoated samples, without further sample preparation. Figure 5 shows the migration from the recycled paper sample, Figure 6 shows the overall migration from the same paper coated with alginate. The comparison of the chromatograms shows, how the migration of the MOSH/MOAH, present in the recycled paper board, can be almost completely reduced by the use of the alginate coating. As discussed earlier, DIPN was reduced to about 35%, but is still present in the

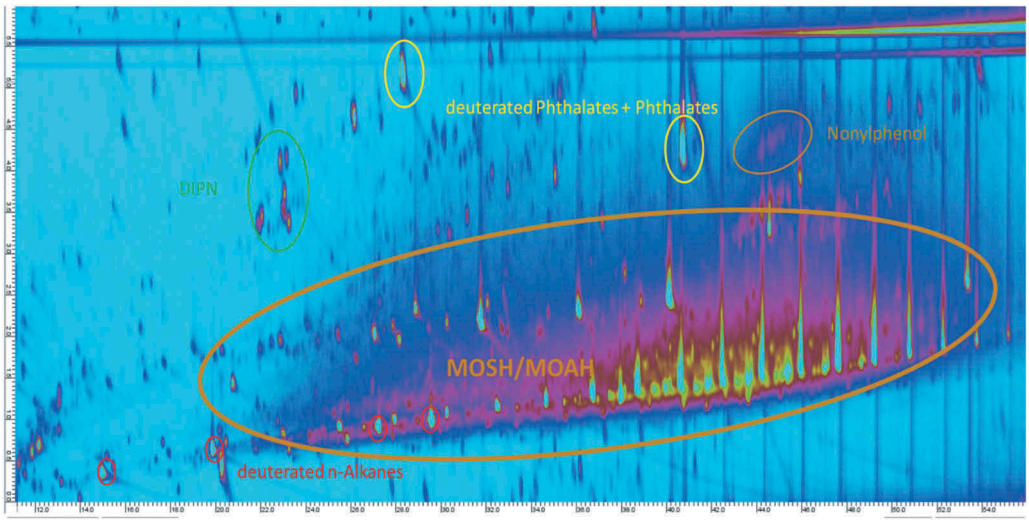

Figure 5. GCxGC-Chromatogram of substances migrating into food simulant from recycled raw paper.

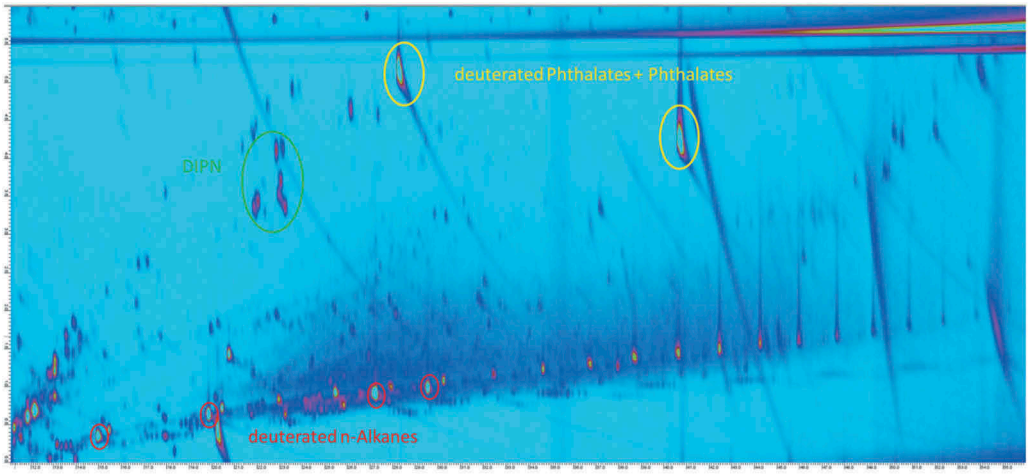

Figure 6. GCxGC-Chromatogram of substances migrating into food simulant from recycled raw paper coated with alginate.

coated sample, while the migration of the phthalates could be nearly completely stopped.

Conclusion

The implementation of parallel migration and permeation experiments in migration cells using deuterated alkanes is new to our knowledge. The extracts received from one experiment can be used to determine the overall migration using GC-FID, while the specific migration and permeation can be determined using GC-MS, and the migration of mineral oil hydrocarbons using online-coupled HPLC-GC-FID. Even further characterization and identification of unknowns can be accomplished using comprehensive GCxGC-MS. The method used showed well linearity and repeatability, with limits of detections for the validated substances and parameters that were lower than those specified in the current regulations.

The developed method was used to characterize a recycled raw paper that was either uncoated or coated with one of the three biopolymers alginate, chitosan, or microfibrillated cellulose. The polymers varied in their behavior as barriers. Alginate had the best performance due to the fact that it is the most hydrophilic material, followed by chitosan, which is a moderate, but still acceptable barrier. MFC showed a poor barrier behavior. The alginate coating reduced the overall migration in comparison to those measured in the uncoated recycled paper by 84%. The permeation of deuterated *n*-alkanes and menthol could be reduced by 63–97%, the specific migration

of selected phthalates by up to 98% and migration of MOHs by 95%. The chitosan coating reduced the overall migration still by 70%, the permeation by 44–83%, the specific migration by 53–94% and the migration of MOHs by 90%. In comparison, MFC reduced the overall migration by only roughly 30%, the specific migration by 8–42% and the migration of MOHs by 65%. MFC showed no reduction in the permeation at all. Our results show that alginate had the best barrier properties, but chitosan also performed well. It was possible to hold the migration of the evaluated substances and substance groups below the currently accepted regulatory limits that were summarized in the introduction.

In summary, the presented method gives the possibility of comprehensively characterizing the barrier properties of functional barriers, but this method can also be used for other food contact materials. In this study, it was applied on coated and uncoated recycled paper, but it can also be used to characterize board, barrier films or plastic materials. The method fulfils the requirements of the current regulations.

Author Contributions

Andrea Walzl performed the migration and permeation experiments including all measurements, the validation of method, application onto the samples and data evaluation. Samir Kopacic performed the coating of the paper. All authors mentioned have analyzed and summarized the data and helped writing the paper.

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Disclosure statement

No potential conflict of interest is reported by the authors

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