Chemical imaging of contaminated recycled paper and board: towards identification of the causality of food contamination

Lucas BIANT¹, Murielle HAYERT¹, Hawraa AYOUB¹, Phuong-Mai NGUYEN², Olivier VITRAC¹

¹ Université Paris-Saclay, INRAE, AgroParisTech, UMR SayFood, 91120, Palaiseau, France
² LNE, Laboratoire National de Métrologie et d’Essais, 78190, Trappes, France

Abstract

Contamination of recycled paper and board food packaging by mineral oil hydrocarbons caused several crises in the past years. Although the migration from paper and board to foodstuffs has been proved many times, the contamination mechanisms and causalities remain undetermined and face unresolved issues. This study takes part in the collaborative project FoodSafeBioPack, which aims at evaluating the migration of potentially carcinogenic polyaromatic molecules from recycled paper and board packaging to food. New chemical imaging methods are used to follow this contamination at the microscopic scale (fibers and fibrous network). The first images acquired of polyaromatic molecules distribution on fibers were used to develop a methodology that will help to follow their migration. The influence of using different solvents and molecules on the initial chemical potential is discussed. The description of the paper structure and initial conditions of transfer will support the development of a microscopic mass transfer model.

1. Introduction

Cellulosic fibrous materials (i.e., paper and board) offer a unique biobased, recyclable, and biodegradable solution to reduce the environmental impact of plastic food packaging. However, recycled paper and board (P&B) are not subjected to the same safety rules as recycled plastics: no decontamination step and no obligation of closed-loop recycling. P&B used for food contact are printed, coated, and glued in mass. The 2009 German market survey (Vollmer et al., 2010) revealed that recycled P&B were highly contaminated by mineral oil hydrocarbons (MOH). Recycled P&B are, therefore, thought to be a primary exposure source of MOH (Foodwatch, 2015 & 2021). The aromatic fraction or MOA (16-35 w%) should be considered potentially carcinogenic (EFSA, 2012). Due to the lack of MOH refining and the extensive use of MOA-based ink solvents in copy papers, contaminants and residual solvents accumulate in the recycling loop. As of January 1, 2025, using mineral oils for printing intended for the public is prohibited in France (article 112 of the French “anti-waste” law 2020-105). MOH mass transfer can occur without contact and across plastic layers. They have been characterized and modeled only macroscopically (Nguyen et al., 2017). The distribution of contaminants at the scale of fibers (e.g., inside or outside fibers) and of the porous network (accumulation in closed or open pores) are unknown as well as the consequences of the release kinetics with temperature and relative humidity. The presented research is part of the collaborative project FoodSafeBioPack “Evaluating and managing the migration of contaminants from cellulosic materials” (ANR-20-CE21-0009, 2020-2024), funded by the French National Research Agency and addresses specifically unresolved issues: i) How to detect and quantify traces of MOA below the detection limits of golden-standard LC-GC-FID techniques (Weber et al., 2018)(Koster et al., 2020)? ii) How to design cellulosic structures, which are barriers to MOA while recyclable and compatible with food contact? How are almost non-volatile MOA compounds transferred between and across fibers?

This article explores the potentialities of chemical imaging to reconstruct the contamination pathways in model and commercial paper and board. Chemical imaging combines vibrational spectroscopy techniques (mid-infrared, Raman, fluorescence) and microscopy to detect the signature of chemicals along the cross-section of materials in a dry state without solvent extraction and dilution. Confocal Raman and fluorescence laser scanning spectroscopy, respectively UV and visible lasers, offers 3D
reconstructions at micrometric resolution. They can be coupled with polarization spectroscopy to separate crystallized/precipitated MOA in solvent menisci from absorbed ones at the surface of fibers in an amorphous state alone or in mixtures. This work uses a neat blotting paper impregnated with model surrogates for validation and commercial recycled kraft paper to demonstrate the feasibility of laser-scanning chemical imaging. The conditions of impregnation in good and bad solvents are especially discussed.

2. Background

2.1. Transfer mechanisms between paperboard and food

Because of the absence of regulation for decontamination during the recycling process of paper and board, recycled cellulosic material used for producing P&B food packaging is a reservoir of contaminants. The contamination during recycling comes mainly from printed magazines, newspapers, or even printed packaging. The most common contaminants are mineral oils (aromatic or MOAH and saturated or MOSH), printing inks constituents (photo-initiators), plasticizers (phthalates), solvents, or per-fluorinated compounds (PFOA) (Nguyen et al., 2017). Isomers of DIPN (Di-iso-Propyl Naphthalene) are also markers of recycled P&B, even after several recycling loops. Contaminants in P&B food packaging can migrate to food and cause health issues for consumers. The transfer mechanisms from packaging to food and inside the packaging are not well defined. Food contamination comes from packaging in direct contact with the food, but other macroscopic transfer mechanisms are involved (Figure 1b). Besides primary packaging, migration can occur from secondary packaging and other transport boxes, increasing total contamination (Barp et al., 2015). Moreover, it has been proved that substances can migrate without direct contact with food, i.e., through the gas phase (Eicher et al., 2015) and a plastic barrier film (Biedermann et al., 2013).

Figure 1 – Description of multiscale mechanisms involved in risk assessment of food contamination by recycled P&B
One of the biggest challenges regarding food contamination by packaging is the determination of causalities, i.e., identifying the precise source of contamination and the involved mechanisms. Transfer mechanisms combine both microscopic and macroscopic phenomena (Figure 1 a-b). Paper's complex fibrous structure (described by the example of a blotting paper real 3D structure – Figure 1d) involves several microscopic mechanisms. Two types of mechanisms are involved regarding the volatility of contaminants, the initial state of contamination (solute fugacity at the surface of fibers – Figure 1c) and the distance between fibers (Figure 1a). Volatile compounds are thought to transfer over large distances before being reabsorbed on the next fiber.

2.2. Chemical imaging for evaluating food packaging

Vibrational spectroscopic techniques based on light-matter interactions, such as infrared or Raman scattering and fluorescence emission, help us determine the chemical composition of samples. Chemical imaging uses these techniques to acquire spectral information, simultaneously as spatial and time information, creating real-time images of a sample's chemical structure and component distribution. Although they are complementary, not all techniques are generally applied to each sample, and they are used regarding the searched molecules. The characteristics, strengths, and weaknesses of each of the three main chemical imaging techniques are presented in Table 1. References of studies using one or several of these techniques in food packaging, fibrous and/or cellulosic materials are also given. The presented methods pave the way for the use of chemical imaging in the observation of diffusion behavior in P&B packaging.

Table 1 – Main characteristics, strengths, and weaknesses of the techniques used in chemical imaging

<table>
<thead>
<tr>
<th>Chemical imaging technique</th>
<th>Principle</th>
<th>Pros</th>
<th>Cons</th>
<th>Example studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-Infrared (MIR)</td>
<td>Vibration of molecules by absorption, diffusion or emission of light. Spectral range 2500 - 25 000 nm.</td>
<td>Low cost, applicable to many molecules. Rapid and non-destructive analysis. Easy spectral interpretation.</td>
<td>Poor sensitivity. Strong absorption: need sample preparation care. Very sensitive to the presence of water.</td>
<td>Lagaron et al., 2004</td>
</tr>
<tr>
<td>Near-Infrared (NIR)</td>
<td>Vibration of molecules by absorption, diffusion or emission of light. Spectral range 780 - 2500 nm.</td>
<td>Low cost, applicable to many molecules. Rapid and non-destructive analysis. Lower absorption: applicable to many samples (thick or thin).</td>
<td>Overlapping of characteristic bands of absorption.</td>
<td>Lagaron et al., 2004, Mauricio-Iglesias et al., 2009</td>
</tr>
<tr>
<td>Raman</td>
<td>Diffusion of light: inelastic scattering.</td>
<td>High sensitivity. Little or no sample preparation. Insensitive to the presence of water in the sample.</td>
<td>High cost. Samples can be damaged by laser source during measurement. Weak Raman effect, not detectable for all molecules. Fluorescence can overwhelm Raman effect.</td>
<td>Lagaron et al., 2004, Mauricio-Iglesias et al., 2009, Portesi et al., 2019, Yang et al., 2020</td>
</tr>
</tbody>
</table>

3. Materials and methods

3.1. Studied fibrous materials and contaminants

The solutes used were selected regarding their ability to be detected in Raman spectroscopy or fluorescence microscopy and their chemical structure. Polyaromatic molecules with a melting point between ambient temperature and 215°C were used as models for MOAH. Table 2 summarizes the identities of these model contaminants. All solutions were prepared by solubilizing each solute either in dichloromethane (stabilized with ethanol) or in ethanol 96%.

Paper samples were provided by the French Pulp and Paper Research & Technical Center (CTP). A recycled kraft paper (produced with the kraft process, i.e., with the addition of NaOH/Na2S mixture to
the pulp to remove excess lignin and keep the cellulose) and a virgin blotting paper (100% cellulose fibers, no additives, 90 g/m²) were used as reference materials.


<table>
<thead>
<tr>
<th>Name</th>
<th>CAS number</th>
<th>M (g.mol⁻¹)a</th>
<th>Pᵥ sat (Pa) (25°C)b</th>
<th>MP (°C)a</th>
<th>BP (°C)b</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nile Red</td>
<td>7385-67-3</td>
<td>318</td>
<td>/</td>
<td>203-215</td>
<td>485</td>
<td></td>
</tr>
<tr>
<td>para-Terphenyl</td>
<td>92-94-4</td>
<td>230</td>
<td>4.6 10⁻⁵</td>
<td>212-213</td>
<td>389</td>
<td></td>
</tr>
<tr>
<td>Benzophenone</td>
<td>119-61-9</td>
<td>182</td>
<td>2.6 10⁻¹</td>
<td>47-51</td>
<td>305</td>
<td></td>
</tr>
</tbody>
</table>

3.2. Imaging techniques and samples preparation

Contaminated paper samples were prepared using two different techniques. The impregnation in contaminant solutions is inspired by the donor paper preparation done by Biedermann-Brem and Grob (2014). For CLSM, Nile Red solutions (0.20 g/L in EtOH or DCM) were poured into 100 ml closed glass bottles. Paper samples (1 cm x 1 cm, approximately) were soaked into those solutions for 24 hours. Impregnated papers were used for imaging after 2 minutes of solvent evaporation under the airflow of a fume hood. For Raman analyses, drops of p-terphenyl, t-stilbene, and benzophenone solutions (0.05 g/L in DCM) were deposited at the surface of a paper sample just before imaging. Raman spectra and images were acquired with two Raman imagers (models LabRam Soleil, Horiba, France and DXR3, ThermoFisher Scientific, USA). Both devices were equipped with a 785 nm laser source to reduce the autofluorescence of fibers’ cellulosic residues. Epifluorescence was collected in confocal mode with a 488 nm excitation source (model TCS SP5, Leica, Germany). The 3D surface of fibers was reconstructed from the natural fluorescence at 500-550 nm.

4. Results

The different studies on fiber structures, molecules diffusion behavior, or plastic and paper food packaging using chemical imaging techniques encourage their use for assessing the contamination of recycled P&B by MOH and their mass transfer to food. First, images were obtained using the vibrational structure of molecules that can be differentiated from the signal of paper fibers.

4.1. Initial contaminant distribution observed by confocal fluorescence

Typical effects of contaminant redistribution can be observed in CLSM with Nile Red, a fluorescent polyaromatic molecule (Figure 2, a-b). The redistribution is affected by two independent factors: the intrinsic properties of the substance and the method of incorporation. The nature of the solvent used to prepare the contaminant solution has an impact on the ability of molecules to penetrate the paper. A solvent entering in contact with fibers undergoes meniscuses effects: a wetting solvent can enter narrow angles while a non-wetting solvent minimizes its contact with fibers and only recover the fibers’ surface in open angles (Figure 2, bottom). The solute was incorporated using a wetting and a non-wetting solvent, respectively ethanol and dichloromethane. A solvent capable of wetting cellulosic fibers recovers the entire fibers and penetrates inside their lumen. On the contrary, a non-wetting solvent leaves the contaminant molecules at the fibers’ surface and forms meniscuses between fibers. The initial distribution of contaminants after solvent evaporation is the first key parameter to understanding transfer mechanisms. Images obtained with Nile Red allow the identification of solute molecules redistribution in a blotting paper sample, but the conclusions cannot be generalized before confirmation with other model contaminants.
Figure 2 – Impregnation profile of Nile red on blotting paper fibers immersed in: a) solution of Nile red in dichloromethane, b) solution of Nile red in ethanol.

4.2. Use of Raman imaging for distribution and migration pathways observation

The solute distribution assessment was supported by observing separated signals of cellulose and model contaminants in Raman spectroscopy. The reference spectra of cellulose and pure molecules (in solutions) were obtained first (Figure 3, b). Raman imaging allowed the acquisition of fiber contamination images by associating each pixel with the spectrum of cellulose, pure molecule, or a mix of both. Using a laser source at 785 nm ensures the detection of the actual Raman signal of cellulose and avoids the detection of cellulose fluorescence.

Figure 3 (a-c) – Raman chemical imaging of p-terphenyl distribution in a blotting paper sample.

Images obtained after contamination of a drop of p-terphenyl solution are presented in Figure 3 (a, c). Typical redistribution concluded in CLSM is not observed for this molecule. Still, areas of higher solute concentration appear close to the paper surface, where the drop was deposited (in orange in Figure 3, c). We can assume that in this area, molecules with high melting points (p-terphenyl, MP = 212-213°C) crystallize after solvent evaporation. The heterogeneous distribution of contaminant molecules in the fibrous network results from the technique of contamination (drop deposition), the rapid solvent
evaporation, and the high melting point of p-terphenyl. The distribution of p-terphenyl through the paper’s thickness is shown in Figure 3, c. The signal of amorphous molecules (in yellow) towards the inner part of the paper suggests the presence of a diffusion profile. Their ability to migrate depends on their melting point and vapor pressure.

The same experiment was performed with a benzophenone solution, a polyaromatic molecule with a lower melting point (47-51 °C). Its distribution on paper fibers, displayed in Figure 4, differs from p-terphenyl because no high concentration areas are detected. Because of its low melting point, the crystallization of benzophenone molecules at the paper surface may not happen during solvent evaporation. Nevertheless, the heterogeneous distribution in the paper is confirmed by the presence of virgin fibers (in blue, only Raman signal of cellulose) even after contamination.

![Chemical structure of benzophenone](image)

**Figure 4** – Raman chemical imaging of benzophenone distribution in a blotting paper sample.

### 4.3. Detection of aromatic contaminants in recycled paper sample

Such distribution was evaluated in a commercial sample, *i.e.*, a recycled kraft paper contaminated by aromatic molecules. Figure 5 displays the paper cross-section’s distribution of (poly)aromatic compounds. The spatial distribution of high and low concentration areas (respectively in red and blue) is determined quickly from the signal intensity of obtained spectra. As for contamination by drop deposition, the distribution of aromatic molecules in the paper is heterogeneous. The ability of Raman imaging to observe contaminated samples originating from recycled or virgin paper is highly satisfactory for further experiments with other molecules.

![Raman spectra of aromatic compounds](image)

**Figure 5** – Distribution of aromatic compounds along the cross-section of a recycled kraft paper sample.
5. Conclusions and perspectives

The contamination of paper and board during the recycling loop, the storage, the stacking, or directly by solutes of printing inks is a significant issue for replacing single-use plastic food packaging with P&B materials. Following many studies reporting critical levels of food contamination by substances originating from P&B packaging and against the lack of specific regulation for P&B materials, the French DGCCRF set an obligation of risk assessment for the contamination of dry and fatty food by organic materials made from vegetal fibers (fiche MCDA n°4 - V2,01/01/2019). Risk assessment is supported by measuring contamination levels by analytical techniques, the gold standard being extraction in a solvent followed by chromatographic measurements. These evaluation methods are limited by limits of quantification and can only give information at the macroscopic scale.

The direct imaging of contaminants in P&B samples opens new possibilities for analyzing contaminants’ mass transfer in materials and food. The analysis of contamination at the scale of fibers allows the determination of transfer causalities, i.e., the origin and mechanisms of migration. The distribution of model contaminants (polyaromatic molecules) in a blotting paper was studied with Raman imaging and confocal laser scanning microscopy. The heterogeneous distribution of solutes in the fibrous network of paper was highlighted in fluorescence and confirmed by observations in Raman. This technique can determine high concentration areas, possibly created by the crystallization of molecules during solvent evaporation. Such effect was observed for a molecule with a high melting point (p-terphenyl) but not for a molecule with a melting point closer to ambient temperature (benzophenone).

The first images obtained show the capability of chemical imaging for helping the determination of transfer mechanisms in paper and board. Further work is needed to observe the migration of molecule that will explain the causalities of food contamination by recycled paper and board. The obtention of molecular distribution profiles and initial states of molecules at the surface of fibers will support the development of a microscopic model. The establishment of this model will be done in parallel, using information of the fibrous network structure acquired by chemical imaging of paper and board. Its aim is to describe transfer mechanisms at the scale of fibers and fibrous network.

Acknowledgments

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References


