





Article

Low-Density Polyethylene Migration from Food Packaging on Cured Meat Products Detected by Micro-Raman Spectroscopy

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Abstract: Food packaging has been demonstrated as a crucial issue for the migration of microplastics (MPs) into foodstuffs, concerning human health risk factors. Polymeric materials called plastics are continuously utilized in food packaging. Polyethylene (PE) is commonly used as a food packaging material, because it offers easy handling during transportation and optimal storage conditions for food preservation. In this work, three types of cured meat products of different fat compositions and meat processing methods—bacon, mortadella, and salami—were studied using spectroscopic methods (Raman and FT-IR/ATR) to determine the migration of low-density polyethylene (LDPE) from plastic packaging to the surface of the meat samples. The experimental duration of this study was set to be 28 days owing to the selected meat samples' degradation, which started to become visible to the human eye after 10 days of storage in vacuum LDPE packaging, under refrigerated conditions at 4 °C. Spectroscopic measurements were performed at 0, 3, 9, 12, 15, and 28 days of storage to obtain comparative results. We demonstrated that the Raman spectral peaks of LDPE firstly appeared as a result of polymeric migration on day 9 in Bacon, on day 15 in Salami, and finally on day 28 in Mortadella. On day 28, all meat samples were tainted, with a layer of bacterial outgrowth developed, as proven by bright-field microscopic observation. Food packaging migration to the surface of cured meat samples was validated using Raman vibrational spectroscopy. To ensure minimal consumption of MPs in cured meat products stored in plastic packaging, while at the same time maintaining good food quality, they should be kept in refrigerated conditions and consumed within a short period of time. In this work, the migration of MPs from food packaging to the surface of cured meat samples was observed using micro-Raman spectroscopy.

Keywords: food packaging; low-density polyethylene (LDPE); microplastic migration; micro-Raman spectroscopy; cured meat



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1. Introduction

Plastic packaging is eco-toxic and unhealthy for humans [1–4]. However, the use of plastic for food packaging has many advantages, such as being lightweight and thus allowing easy transportation, design freedom, durability, and cost-effectiveness [5]. The most common plastics used by the food packaging industry are as follows: polyethylene terephthalate (PET), high-density polyethylene (HDPE), low-density polyethylene (LDPE), polycarbonate (PC), polyvinylidene chloride (PVDC), polypropylene (PP), and polystyrene (PS) [6–11]. From polymer sciences, we know that different polymers have different properties. PE and especially LDPE is produced in thinner layers compared with some other polymers, but it remains stable in our experimental temperature range of 4–40 °C.

Moreover, LDPE is very flexible and tough. For this reason, it is used in many applications of food packaging, in grocery stores for fruit and vegetable bags, in beverages, as well as in cured meat products under vacuum packaging [12], among others [13,14]. It can be observed from the literature that LDPE is a very flexible material by its nature, has no addition of plasticizers, and has a melting point at a temperature of 85 °C [15].

However, we already know that plasticizers and MPs [16] have been identified in food, migrating [17] from food plastic packaging to the food surface [18,19]. Migration of plasticizers [20] from plastic packaging to roasted chicken meat was observed in 2018. Moreover, various studies have been conducted concerning the migration of MPs and nanoparticles from plastic packaging to food simulants [21–24]; since 1989 [25], olive oil and corn oil have been used as food simulants, instead of real samples, for fatty foods [26]. It is known that MPs are more prone to migrate into oily foodstuffs owing to hydrophobic interactions [27]. This is the reason that, in this work, we chose to study LDPE migration to real meat samples to prove that oily meats are more prone to experience faster migration of MPs. The choice of the cured meats for LDPE migration from plastic packaging was based on the popularity of their consumption; bacon, mortadella, and salami were selected. Vibrational spectroscopic methods (ATR/FT-IR and Raman) are commonly used for food studies [28], as well as MP analysis [29–31] and meat discrimination [32]. For this reason, as in a previous cheese study [33], Raman spectroscopy was used to determine the migration of MPs to three types of cured meat (bacon, mortadella, and salami).

According to our results, it is evident that LDPE surely migrates from food packaging to all of the cured meat samples while being kept at refrigeration temperatures (~4 °C). For each meat sample, migration was observed in different time periods, detectable by the Raman spectroscopy technique. In these experiments, real food samples were used for the migration of MPs to the cured meat surface, so the results simulate high-rate, real-life situations.

2. Materials and Methods

2.1. Samples and Experiment Preparation

The experimental design was based on the fat composition and the meat processing methods of our samples. The experimental duration was set to 28 days to observe the cured meats' behavior and to check if bacterial growth could affect MPs' detection on the oily surface of the meat. This approach resulted in six time points for each cured meat, named as follows: day 0, where the MPs' migration should be zero; day 3, day 9, day 12, and day 15, where the migration should be detectable for bacon, mortadella, and salami; and finally day 28, for the observation of cured meat degradation using Raman microscopy.

Generally, bacon has up to 50% fat [34], mortadella has up to 30% fat [35], and salami has up to 50% fat [36]. In our experiment, the materials used were as follows: (i) smoked and fatty bacon with a 20% fat content, (ii) boiled mortadella with a 26% fat content, and (iii) air-dried fatty salami with a 29.5% fat content. Owing to the meat processing of mortadella, the water content is preserved, and the lipid distribution into mortadella's meat volume is more uniform than that of bacon and salami. On the contrary, during air drying, water is not preserved, and the lipid distribution is sparse, resulting in denser areas. Smoked bacon has a greater water content than salami, but lower water content than mortadella. Eighteen (18) resealable air-tight LDPE pouches, commercially available in Greek stores as packaging pouches, were prepared and pre-marked with the assigned measurement day and meat type. Those polymeric pouches have previously been characterized for their chemistry (FT-IR/ATR and Raman) and structure (XRD) [33]. For this experiment, three different kinds of cured meats, bacon, mortadella, and salami, were prepared. Initially, to avoid any contamination with MPs from the environment, the work area was under a hood, sterilized with pure ethanol; nitrile gloves and a cotton lab coat were used each time during the entire experimental process, and hair was caught in a bun. Consequently, all of the surfaces of the cured meat samples in contact with the original packaging were removed. The cleaned meat was cut into small square pieces with a sterilized metallic knife and then placed into

the pre-marked LDPE pouches, as shown in Figure 1. Only fatty areas of the meat samples were used for this experiment. Air was manually removed from the plastic package with a vacuum pump. To accomplish this, a needle was placed at the end of the vacuum pump tube to remove all of the air from the plastic pouches, ensuring direct contact with the sample's surface. To avoid any possibility of polymer migration on day 0, the first sample from each cured meat type was measured right after being cut, without coming into contact with the LDPE pouch. For reference purposes, the meat surface spectral information was acquired on day 0, where the meat surface was intact and free of any LDPE contamination. The remaining samples were stored under refrigerated conditions at 4 °C, and they were measured on the corresponding measurement day that was previously marked on the LDPE pouch.

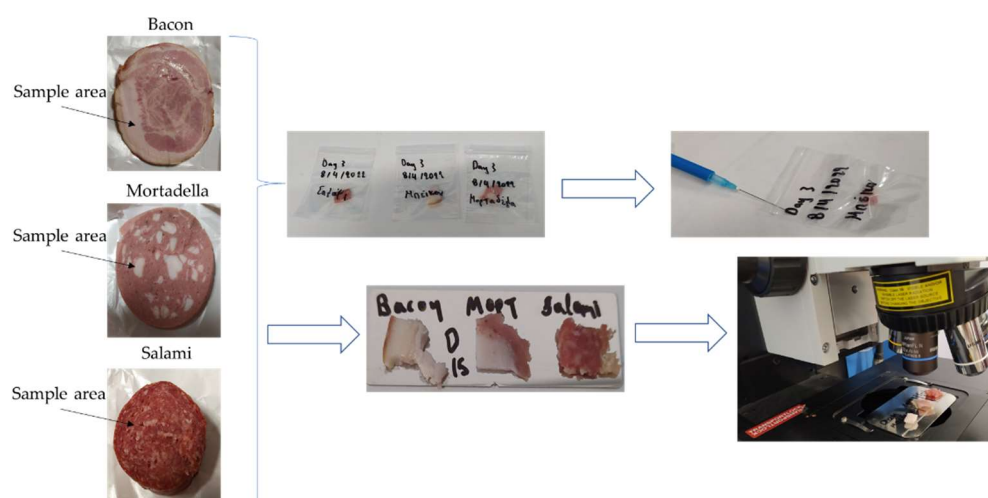


Figure 1. Sample preparation process.

On the measurement day, each sample was carefully taken out of the plastic packaging and placed on a sterilized stainless metallic microscopic slide for Raman measurements. Based on the previously mentioned fatty characteristics of the samples, it was expected that migration of MPs from the plastic packaging to the fattier surface areas of the meats would take place earlier [27], so the measurement points selected focused on those sample areas. Only one side of the sample area was measured in multiple different places to increase the possibility of MPs' detection, as the other side was touching the metallic plate, and MPs from the metal–sample interface could be left on the metallic slide.

2.2. Data Acquisition

Measurements were performed with a state-of-the-art instrument located in our premises; specifically, we used a modified Raman microscope (LabRAM HR; HORIBA FRANCE SAS, Longjumeau, France).

LabRAM HR Raman Microscope Instrument Description and Acquisition Settings

Raman measurements were performed with a modified LabRAM HR Raman Spectrometer (HORIBA Scientific, Kyoto, Japan). Raman excitation was achieved with a 532 nm central wavelength solid-state laser module with a maximum laser output power of 90 mW. The microscope was coupled with a 50× microscopic objective lens with 0.5 numerical aperture and 10.6 mm working distance (LMPlanFLN 50X/0.5, Olympus), delivering the excitation light and collecting the Raman signals. A neutral density filter of 5% transmittance was used, which resulted in 1 mW of power on the sample (3.2% from a maximum power of 35 mW). The laser spot size, referring to the microscope resolution, was approximately 1.7 μm laterally and 2 μm axially. A 600 grooves/mm grating resulted in a Raman spectral resolution of around 2 cm⁻¹. The Raman spectral range was set to be from 40 to 3050 cm⁻¹,

resulting in two optical windows per point. The acquisition time for each measurement was 15 s, with three accumulations in each point.

The Raman signal detector was the Sincerity CCD Deep Cooled Camera (HORIBA Scientific, Lille, France), operating at $-50\text{ }^{\circ}\text{C}$. Before each experiment, spectral calibration of the Raman instrument was performed with an Si reference target, presenting a single peak at 520.7 cm^{-1} . All measurements were performed under a constant environmental temperature at $22.5\text{ }^{\circ}\text{C}$ and a humidity range between 32 and 48%. At the time of measurement, samples were placed onto a stainless-steel microscope slide using metallic forceps, maintaining the orientation they had in the LDPE pouches. From each sample, nine measurements at different points were acquired to check for Raman signal consistency. All measurements were acquired from the fattiest areas of each sample. Good signals were received only with absolute focused laser light and in flat areas.

2.3. Data Processing and Analysis

2.3.1. Raman Spectral References

In Figure 2, the Raman spectrum of LDPE is presented. The major LDPE Raman peaks are indicated with dashed green lines. Raman peak numbering is according to the ascending wavenumber order.

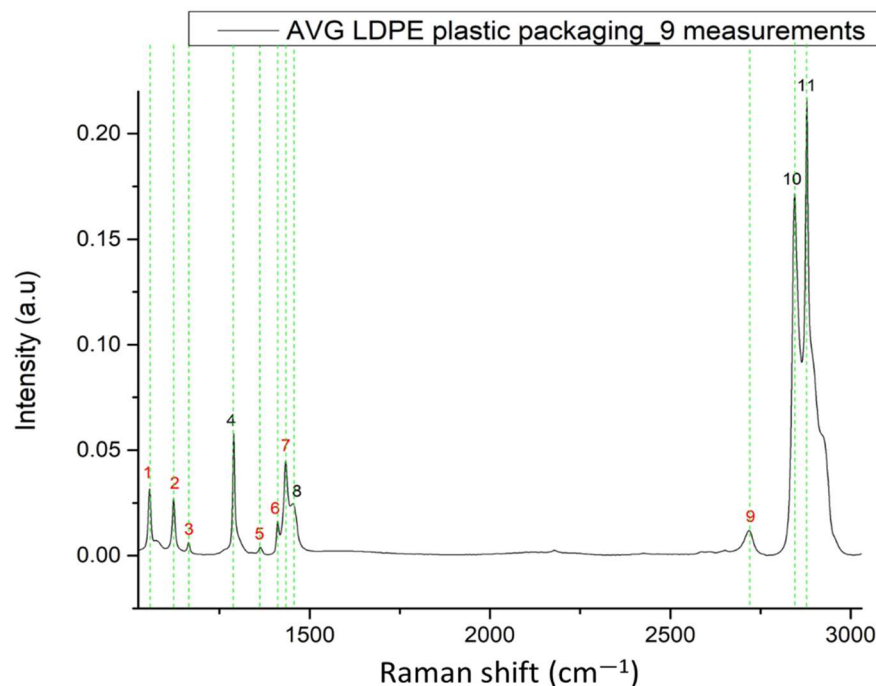


Figure 2. Raman spectrum of LDPE. Peaks in bold do not exist in meat samples.

According to KnowItAll Informatics System by Bio-Rad Laboratories database, the identified Raman peaks of LDPE are presented in Table 1. For the identification of the LDPE Raman assignments also found in meat studies, a literature study was performed. These assignments together with the associated references are presented in Table 1. Bold peaks did not exist in meat samples.

Table 1. List of the major Raman peak assignments commonly found in meat and LDPE.

| No | LDPE Major Raman Peaks (cm ⁻¹) | Raman Peak Assignments Found in Meat Studies (cm ⁻¹) |
|----|--|--|
| 1 | 1056 | 1056 → it does not exist in meat samples 1064 → $\nu(\text{C-C})$ of lipids [37] |
| 2 | 1123 | 1123 → it does not exist in meat samples 1129 → Proteins, lipids, $\nu(\text{C-N})$, $\nu(\text{C-C})$ [37] |
| 3 | 1164 | 1164 → it does not exist in meat samples 1171 → Proteins, $\nu(\text{C-N})$, $\nu(\text{C-C})$ [37] |
| 4 | 1290 | 1297 → Amide III, protein α -helix [37] 1225–1350 → Amide III vibrational modes [38] |
| 5 | 1363 | 1363 → it does not exist in meat samples |
| 6 | 1411 | 1411 → it does not exist in meat samples |
| 7 | 1435 | 1435 → it does not exist in meat samples 1443 → CH ₂ twisting modes of lipids [38], scissoring mode of methylene $\delta(\text{CH}_2)$ in fats [37] |
| 8 | 1453 | 1451 → CH ₂ and CH ₃ bending vibration in lipids and protein [39] 1449 → Proteins, lipids, $\delta(\text{CH}_2, \text{CH}_3)$ [37] |
| 9 | 2718 | 2718 → it does not exist in meat samples |
| 10 | 2844 | 2850 → CH ₂ symmetric stretching motion of lipids [38] 2950~2800 → C-H (CH ₂ and CH ₃) stretching vibrations of lipids [40] |
| 11 | 2879 | 2950~2800 → C-H (CH ₂ and CH ₃) stretching vibrations of lipids [40] |

2.3.2. Spectra Processing and Analysis

LabSpec v6 Raman software, made by Horiba (HORIBA FRANCE SAS, Longjumeau, France), was used for all Raman spectra, visualization, procedure, and analysis. All manuscript figures were generated through OriginLab 2021 pro.

Raman Spectra Processing and Analysis

The processing methodology is very important for comparing signaling results. The following processing methodology was used for each Raman spectrum: (a) smoothing under a Gaussian filter with a kernel of five points (denoise at 5) was used, where cosmic rays were removed; (b) background was removed using a baseline correction at the ninth order polynomial function; (c) a shift to zero was applied; and, finally, (d) a unit vector. Moreover, for each meat sample on every measurement day, an average spectrum from nine measurement points was determined. Finally, all of the original average day 0 Raman spectra were then removed by the average day x spectrum (day x to day 0) to observe only the Raman spectral changes throughout the experiment.

3. Results and Discussion

3.1. Raman Spectroscopic Analysis

3.1.1. Consistency/Repeatability Tests

Initially, consistency/repeatability tests of the Raman measurements on the same sample were performed. For this reason, nine measurements at different locations were acquired from each meat sample. The averages of these measurements are presented in Figure 3. As shown, measurement repeatability was found to be consistent. Some of the inconsistencies were mainly due to the spatial inhomogeneity of the sample, most probably due to the protein/fatty ratio of the tissue. In general, Raman measurements followed the same pattern between the same sample, presenting a strong level of repeatability. Day

0 was chosen for the consistency/repeatability measurements, where there was no MP migration or bacterial growth.

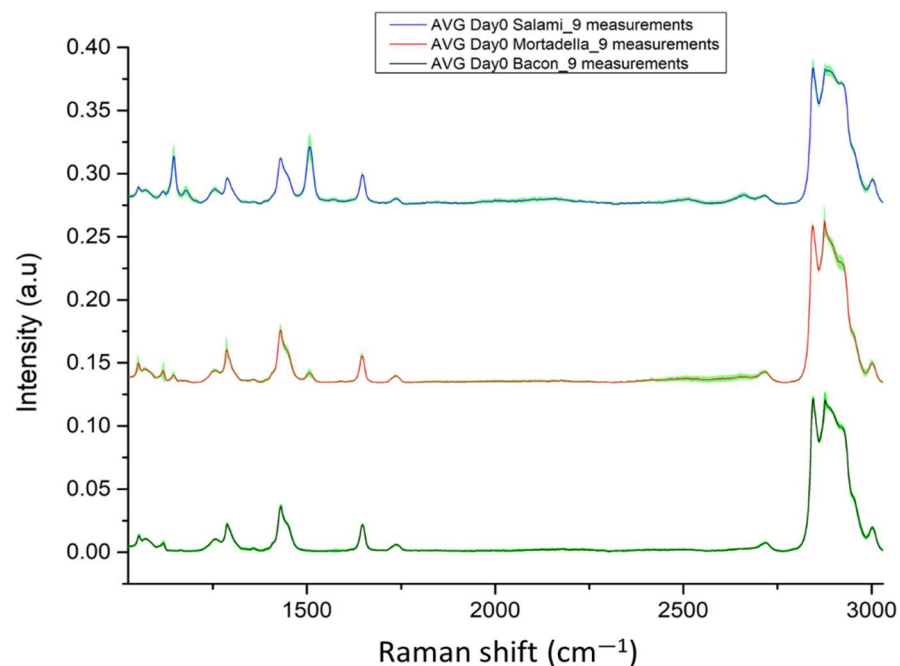


Figure 3. Raman consistency/repeatability average measurements of meat samples, from the nine different measurement points on day 0. Spectra are presented in stack-line format, with an offset between them, in order to present the full spectral details in each sample. For this reason, the intensity axis is in arbitrary units. Standard deviation is depicted as green shadows.

Furthermore, on day 9, bacon and mortadella samples presented some microstructural differentiations in fatty measurement areas, observed by Raman microscope. These differentiations are possibly related to the presence of bacterial growth such as *Salmonella* [41], lactic acid bacteria, *Carnobacterium maltaromaticum*, *Staphylococcus* [42], and other bacteria. In bacon, the most common bacteria during prolonged storage are *Serratia*, *Carnobacterium*, and *Leuconostoc* [42]. In vacuum-packed and refrigerated meat products, meat spoilage arises owing to the interaction among lactic acid bacteria (the dominant flora) and *Enterobacteriaceae*, *Pseudomonads*, *Brochothrix thermosphacta*, and other bacterial species [42]. In salami, some common bacteria in abundance are *Lactobacilli* and *Staphylococci* [43].

Salami samples did not seem to have such bacterial growth at the early stages of the experiment. On day 28, all samples had some spherical and fibrous microstructures. These structures were not present before day 9; therefore, we assume that they indicate the start of bacterial growth. Three selected pictures are presented in Figure 4 below.

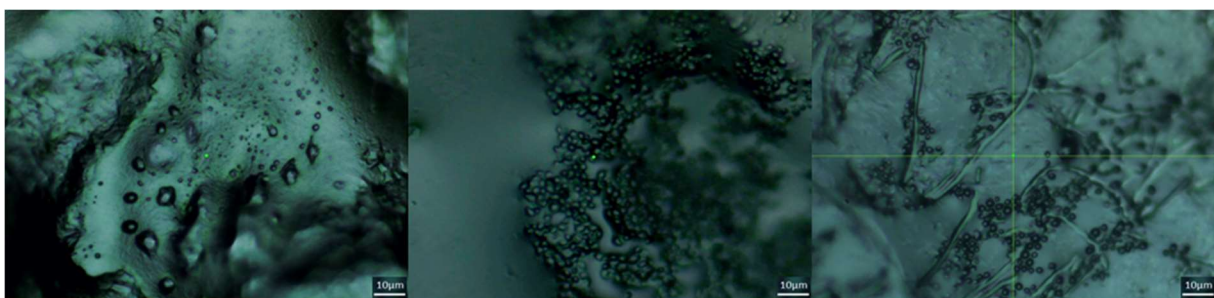


Figure 4. Bright-field picture of the meat samples from the Raman microscope: left picture, mortadella on day 12; middle picture, salami on day 28; and right picture, bacon on day 28. The point of the Raman measurement is depicted as a green dot in the center of the picture.

3.1.2. LDPE Detection

Initially, all meat samples were measured on day 0 and compared with the characteristic Raman signal of LDPE to understand if there were any overlapping peaks. The Raman measurements are presented in Figure 5. As can be observed, all 11 Raman peaks of LDPE are not present in the sample's spectrum, except from number 4 at 1290 cm^{-1} , number 10 at 2844 cm^{-1} , and number 11 at 2879 cm^{-1} , where their wavenumbers are close to sample peaks (1288, 2845, and 2877 cm^{-1} , respectively). Although these Raman peaks are not different from the ones of LDPE, MPs' migration can be detected through changes in those peak intensities.

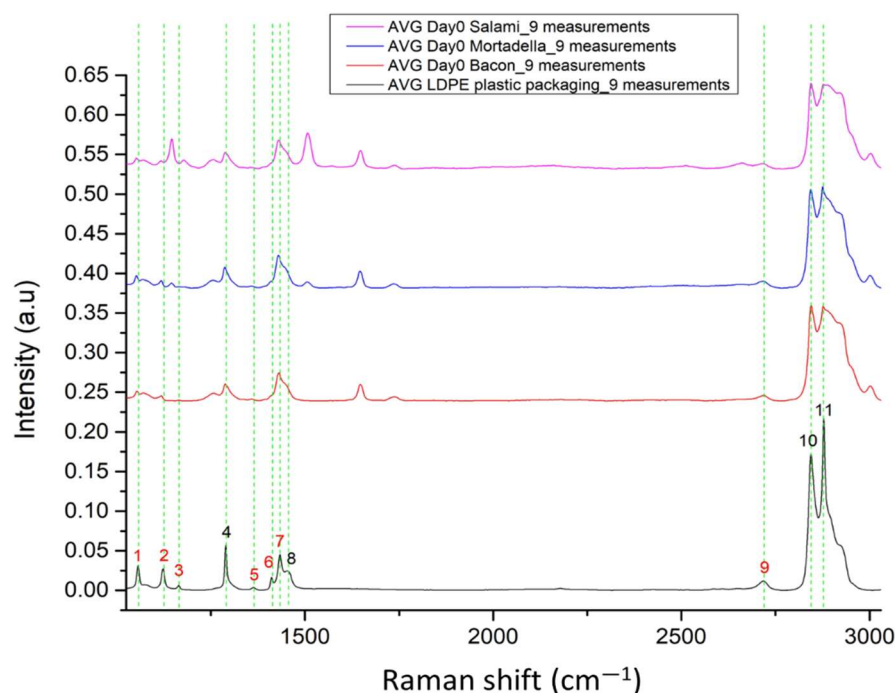


Figure 5. Average of reference Raman spectra from meat samples at day 0 and LDPE. Spectra are presented in stack-line format, with an offset between them, in order to present the full spectral details in each sample. For this reason, the intensity axis is in arbitrary units.

Important Note: All of the meat samples were also measured by ATR/FT-IR spectroscopy, but, most probably because of the large spatial spot size, MPs were not detected. Nevertheless, all ATR/FT-IR instrument description and settings are described in S2.1 and Figure S1. The results from the sample measurements can be found in the Supplementary Document (S3.2 and Figure S2) of this work.

As we observe in Figure 6 below, almost all Raman LDPE peaks start to appear at on day 9 in bacon and, on day 12, these peaks continue to appear. This means that we have detectable migration of MPs from plastic packaging to the bacon surface on day 9. On day 15, the LDPE peaks are reversed, probably because of the presence of bacterial growth of some developing species [42], causing bacon spoilage [44]. Finally, on day 28, we observe again the appearance of LDPE peaks, because MPs have accumulated on so many days. Concurrently, these peaks are not less prominent on day 28, owing to the parallel extended bacterial growth (see Figure 4).

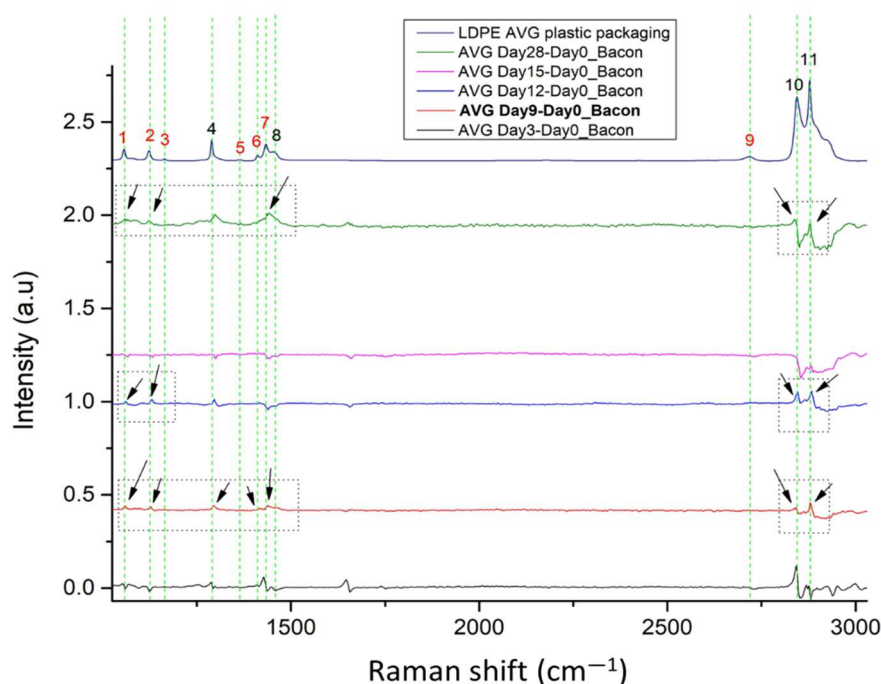


Figure 6. Raman spectra of bacon on different days, in comparison with LDPE spectrum. The Raman spectrum is presented in stack-line format, with an offset between them, in order to present the full spectral details in each sample. For this reason, the intensity axis is in arbitrary units.

Different behavior was found in mortadella meat. As we observe in Figure 7, the first detection of the migration of MPs was on day 28. Before then, we did not observe any LDPE peak on days 3, 9, 12 or 15. This fact can be explained as follows: mortadella has a more uniform fat content distribution with less dense areas than bacon, where the migration of LDPE was first detected on day 9. Moreover, mortadella was boiled, not smoked or dried, which has probably caused a significant amount of lipids to be redistributed during the boiling process. For these reasons, mortadella can be preserved for longer periods in the fridge, the development of bacterial growth is more difficult, and the migration of MPs is delayed because of the uniform distribution of lipids. It is noteworthy that mortadella is a cooked meat product with a shelf life of up to 60 days when stored at 4 °C [27,45].

Salami meat was the most stable, concerning the development of bacterial growth, compared with mortadella and bacon. Migration of MPs was detected sooner compared with mortadella, but later compared with bacon samples. On day 15, LDPE peaks increased and were observed for the first time, as can be seen in Figure 8 below. On day 28, these peaks were less distinct, probably because of the bacterial growth that covers the LDPE signal (see Figure 8 below).

Based on the spectral differences from day 0, we believe that an interplay between the LDPE characteristic peaks and the behavior of the Raman signals from the meat samples could not possibly exist throughout the days. Additionally, the measured LDPE spectral features have no direct correlation with the spectral features from Raman spectra present in the later experimental days, where the bacterial growth starts to develop and the spoilage of the meat begins. These observations, in combination with the fact that the most important LDPE peaks are present from day 9 in bacon, day 15 in salami, and day 28 in mortadella, prove that the Raman spectra identified are most probably from the polymer itself. However, this result is due to Raman instrument sensitivity, which managed to detect those peaks only after day 9. Migration of MPs from plastic packaging to the surface of cured meat samples could have possibly started earlier in the timeline even though the concentration of the polymeric migration may have not been sufficient to be detected with Raman spectroscopy.

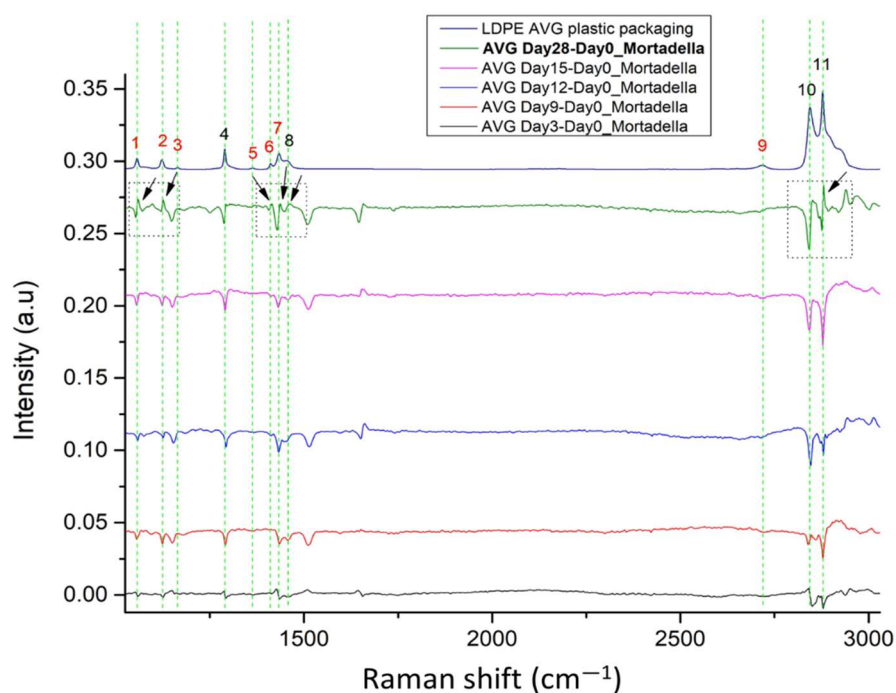


Figure 7. Raman spectra of mortadella on different days, in comparison with the LDPE spectrum. The Raman spectrum is presented in stack-line format, with an offset between them, in order to present the full spectral details in each sample. For this reason, the intensity axis is in arbitrary units.

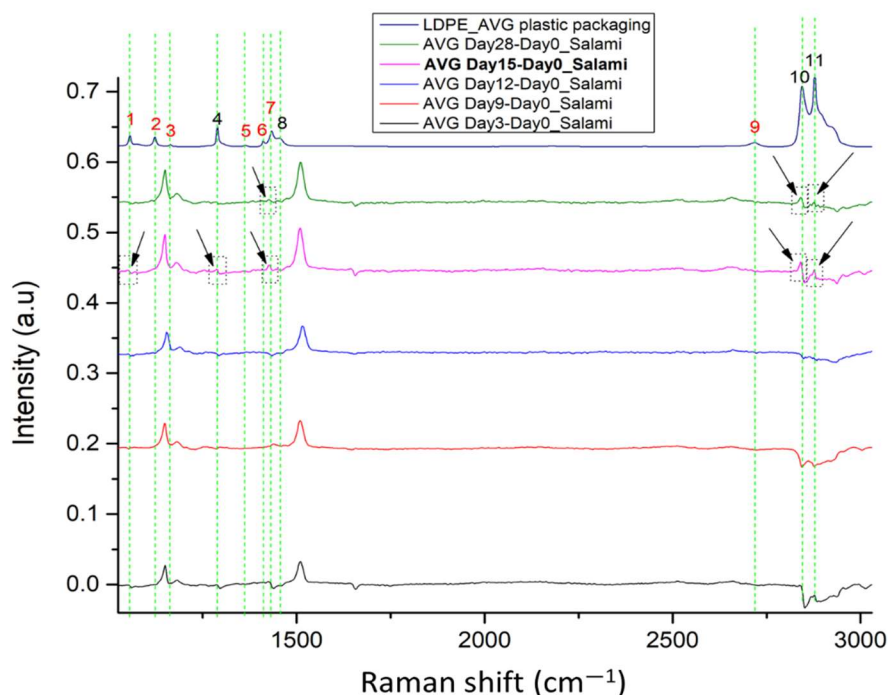


Figure 8. Raman spectra of salami on different days, in comparison with the LDPE spectrum. The Raman spectrum is presented in stack-line format, with an offset between them, in order to present the full spectral details in each sample. For this reason, the intensity axis is in arbitrary units.

It must be noted that the aim of our work is to show that microplastics indeed migrate from food packaging to processed food (fatty food) while being kept under refrigerated conditions. Any correlation with human health is out of the scope of this work and should be studied in the future.

4. Conclusions

In this work, we showed that LDPE migration from LDPE food packaging indeed occurs in three different cured meat samples being kept under refrigeration temperatures (4 °C). We managed to observe this polymeric migration over time, based on Raman spectroscopy instrumentation. MPs were first identified on day 9 in bacon, later on day 15 in salami, and finally on day 28 in Mortadella. LDPE migration was validated with Raman spectroscopy, but not through ATR/FT-IR spectroscopy. With Raman spectroscopy, a simple, fast, and efficient vibrational spectral measurement methodology was developed, which enabled us to detect the migration of MPs to foodstuffs through Raman signal analysis. Our findings indicate that MPs migrate from LDPE packaging to processed meat while being kept under refrigerated storage conditions.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/microplastics1030031/s1>, Figure S1: Sample preparation process for ATR measurement; Figure S2: ATR measurements of meat samples compared with LDPE peaks.

Author Contributions: Conceptualization and methodology, G.K. and V.M.P.; bibliographic investigation, K.K., G.K., E.A. and V.M.P.; experimental characterization and analysis, K.K., G.K. and V.M.P.; manuscript reviewing, G.K., E.A. and V.M.P.; funding acquisition, G.K. and V.M.P. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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