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Development of a rapid micro-Raman spectroscopy approach for detection of NIAS in LDPE pellets and extruded films for food packaging applications

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Abstract

Identification and quantification of Non Intentionally Added Substances (NIAS) at low con- centrations and eventually their distribution on Low Density Polyethylene (LDPE) films is an important issue. As Raman spectroscopy techniques are innovative and sensitive analyt- ical methods and do not require pretreatment and manipulation of the sample, these were used for the identification of NIAS in LDPE pellet and films, which are commonly used for food packaging applications. The aggregation state of NIAS in LDPE were observed by Raman imaging with micrometric resolution. Four main NIAS compounds were identified in granule and film surfaces by confocal Raman spectroscopy: calcite (CaCO3), calcium sulphate (CaSO4), polystyrene (PS) and titanium dioxide (TiO2) in anatase form. By a semi-quantitative evaluation of the inorganic NIAS, 9.93 mg/kg of CaCO3, 1.62 mg/kg of CaSO4 and 0.17 mg/kg of TiO2 were estimated. The experimental findings achieved by Ra- man analysis were confirmed by the results obtained by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Moreover, the toxicity of the identified compounds was examined according to the Cramer rules.

Keywords	NIAS; Confocal Raman spectroscopy; Micro-Raman imaging; Food packaging;		
Manuscript category	PLASTICS (chemical analysis including chemical analysis of Rubbers, polymers used in biomedical applications, scattering, spectroscopy, NMR)		
Corresponding Author	chiara portesi		
Order of Authors	chiara portesi, Davide Visentin, Francesca Durbiano, Maria Cesarina Abete, Marco Rizzi, Valter Maurino, A. M. Rossi		
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Data for: Development of a rapid micro-Raman spectroscopy approach for detection of NIAS in LDPE pellets and extruded films for food packaging applications

Identification and quantification of Non Intentionally Added Substances (NIAS) at low concentrations and eventually their distribution on Low Density Polyethylene (LDPE) films is an important issue. As Raman spectroscopy techniques are innovative and sensitive analytical methods and do not require pretreatment and manipulation of the sample, these were used for the identification of NIAS in LDPE pellet and films, which are commonly used for food packaging applications. The aggregation state of NIAS in LDPE were observed by Raman imaging with micrometric resolution. Four main NIAS compounds were identified in granule and film surfaces by confocal Raman spectroscopy: calcite (CaCO3), calcium sulphate (CaSO4), polystyrene (PS) and titanium dioxide (TiO2) in anatase form. By a semi-quantitative evaluation of the inorganic NIAS, 9.93 mg/kg of CaCO3, 1.62 mg/kg of CaSO4 and 0.17 mg/kg of TiO2 were estimated. The experimental findings achieved by Raman analysis were confirmed by the results obtained by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Moreover, the toxicity of the identified compounds was examined according to the Cramer rules.

April 29st, 2019

Dear Editor,

Please consider our manuscript for publication as an Article in "Polymer Testing" journal.

Manuscript Title: "Development of a rapid micro-Raman spectroscopy approach for detection of NIAS in LDPE pellets and extruded films for food packaging applications"

Category: Article

Authors: Chiara Portesi*, Davide Visentin, Francesca Dubiano, Maria Cesarina Abete, Marco Rizzi, Valter Maurino, and Andrea Mario Rossi

*c.portesi@inrim.it, Strada delle cacce 91 10135 Torino (Italy) +390113919332

In this work Raman spectroscopy has been applied for the first time for detecting and mapping with micrometric resolution the distribution of Non-Intentionally Added Substances (NIAS) present at the surface of Low Density Poly Ethylene (LDPE) pellets and extruded films intended for food packaging applications. Calcite (CaCO₃), calcium sulphate (CaSO₄), polystyrene (PS) and TiO₂ anatase were detected. For validating this method, the results of Raman spectroscopy analysis were compared to those obtained by ICP-MS and thus a semi-quantitative evaluation of the inorganic contaminants was possible, leading to the values of 0.167 mg/kg for TiO₂ and of 9.931 mg/kg CaCO₃ and 1.622 mg/kg of CaSO₄ respectively.

This work presents an original application of the Raman spectroscopy, suitable, due to its rapid and non-destructive character, for in-line measurements in food packaging industry.

The manuscript is submitted on an exclusive basis and its contents have not been published elsewhere.

Thank you for your consideration.

Sincerely,

Chiara Portesi

August 9th, 2019

Dear Editor, Dear Reviewers,

Thank you for considering for publication the manuscript "Development of a rapid micro-Raman spectroscopy approach for detection of NIAS in LDPE pellets and extruded films for food packaging applications".

The manuscript has been revised according to the comments of the reviewers.

Please find below the list of changes and comments

- The pellet provided by the buyer was guarantee as pure material, without any added substances. Appling Raman spectroscopy and ICP-MS we found presence of other elements, for this reason the term "NIAS" is used in the manuscript since pellets and films were provided to the buyer without any additive added in the samples in any stage of the production, except commercial antioxidants (as stated in rows 138-139). Therefore, all other material found can be considered as nonintentionally added.
- About polystyrene, due to the fact that it has been detected both on the surface of the pellets and in the bulk, its presence come from the production process of the granules, such as, for instance, from previous batch of other material (as stated in rows 281 282
- About TiO2, even if the authors agree with the comment of the reviewer, several reports have confirmed that crystalline phases of TiO2-NPs could play an important role in toxicity. However, there is still controversy over the degree or even the presence of effects due to the different surface properties and reactivity of the two allotropic phases of TiO2-NPs, namely, anatase and rutile. Any case, the discussion in details of the toxicity of TiO2 (or other NIAS) is beyond the purposes of this work. The aim of the manuscript is to propose Raman spectroscopy as simple, rapid, non-destructive and low cost for the detection on non-intentionally added substances in food packaging materials.
- The authors agree with the reviewers: the simple presence of a determined substance in FCM doesn't mean a risk. However, the proposed approach for the calculation of the transfer to food has the limit that the migration of the NIAS (as for any substance in contact with food) strongly depends on the type of food (foods with hydrophilic properties, such as aqueous, acidic and alcoholic foods, foods with lipophilic properties, such as dairy and non-dairy fatty foods, dry food, etc). Moreover, the migration tests are always performed in different conditions (temperature, time of storage, time of contact with the FCM). All this tests are beyond the purposes of this work.
- "Sensitivity" has been changed all along the manuscript with the more correct term "limit of detection", according with the reviewer comment.
- The references have been written in the adequate way.
- In rows 167-169, the choice of the calcination temperature of 500°C has been justified. In rows 169-170, the cleaning procedure of the crucible is described, performed in order to avoid any contamination of the samples. In rows 180.182, the choice of the glass crucible is justified.
- In row 228, the size of the sack is specified.
- Thank to the reviewer for the comments, in this work we decided to use the method of uncertainties type B. The 20% of uncertainty is a considerable value and it is the uncertainty of the technique (ICP-MS) for the given metals, in trace concentration.
- The determination of Al, Zn, Mg, Fe and Cu is done by ICP-MS but not confirmed by Raman spectroscopy – this is due to the fact that in the pellets they are present in a non-Raman active molecular (metallic) form. The measurements have been carried out in an analytical lab equipped for routine ICP-MS measurements avoiding any possible contamination during preparation and analysis process. The value of the blank is subtracted in the measurements.

- According to the reviewer comments, the captions have been condensed and table 1 removed.

Thank you for your consideration.

Sincerely,

Chiara Portesi

- Rapid and non-destructive identification of Non-Intentionally Added Substances by Raman spectroscopy;
- Determination of the aggregation state of the NIAS by Raman Imaging;
- Determination of the distribution of the NIAS in the LDPE matrix by Raman imaging;
- Semi-quantitative analysis of the NIAS;
- Comparison of Raman data and ICP-MS data

Development of a rapid micro-Raman spectroscopy approach for detection of NIAS in LDPE pellets and extruded films for food packaging applications

4

5 Chiara Portesi^{a,*}, Davide Visentin^b, Francesca Durbiano^a, Maria Cesarina Abete^c, Marco 6 Rizzi^c, Valter Maurino^d, Andrea Mario Rossi^a

- ^aQuantum Metrology and Nanotechnology, INRIM, Strada delle cacce 91, Torino, 10135, Italy.
 Tel.: +390113919332
- 9 ^b Plastotecnica SpA, Via dell'Artigianato, 1/3, Bagnoli di Sopra (PD), 35023, Italy
- ^c Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, via Bologna 148, Torino, 10154, Italy
- 11 *dDepartment of Chemistry, University of Turin, Via P. Giuria 5, Torino, 10125, Italy* 12
- **13** *corresponding author.
- 14 Email addresses: c.portesi@inrim.it (Chiara Portesi), visentin.d@gmail.com (Davide Visentin),
- 15 f.durbiano@inrim.it (Francesca Durbiano), mariacesarina.abete@izsto.it (Maria Cesarina Abete),
- 16 Marco.Rizzi@izsto.it (Marco Rizzi), valter.maurino@unito.it (Valter Maurino), a.rossi@inrim.it
- 17 (Andrea Mario Rossi)
- 18
- 19
- 20

21 ABSTRACT

22 Identification and quantification of Non Intentionally Added Substances (NIAS) at low concentrations and eventually their distribution on Low Density Polyethylene (LDPE) films is an important issue. As 23 Raman spectroscopy techniques are innovative and sensitive analytical methods and do not require 24 25 pretreatment and manipulation of the sample, these were used for the identification of NIAS in LDPE pellet and films, which are commonly used for food packaging applications. The aggregation state of 26 NIAS in LDPE were observed by Raman imaging with micrometric resolution. Four main NIAS 27 compounds were identified in granule and film surfaces by confocal Raman spectroscopy: calcite 28 29 (CaCO₃), calcium sulphate (CaSO₄), polystyrene (PS) and titanium dioxide (TiO₂) in anatase form. By 30 a semi-quantitative evaluation of the inorganic NIAS, 9.93 mg/kg of CaCO₃, 1.62 mg/kg of CaSO₄ and 0.17 mg/kg of TiO₂ were estimated. The experimental findings achieved by Raman analysis were 31 confirmed by the results obtained by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). 32 33 Moreover, the toxicity of the identified compounds was examined according to the Cramer rules.

34

35 Keywords: NIAS; Confocal Raman spectroscopy; Micro-Raman imaging; Food packaging.

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38 1. INTRODUCTION

In the last decades, concerns over food safety issues, such as possible contaminations due to migration 39 40 of dangerous substances in food matrices from food packaging materials, have increased among 41 consumers. Non Intentionally Added Substances (NIAS) in Food Contact Materials (FCMs) represent a major issue for the food packaging and FCM industry. In general, NIAS are chemical compounds that 42 can be present in food packaging matrices and whose presence is unexpected and often undesired [1]. 43 44 NIAS originate from break-down products of food contact materials, impurities of starting materials, unwanted side-products and various contaminants from recycling [2]. It is generally accepted that only 45 46 compounds below 1000 Da are considered NIAS. Substances with a higher molecular weight are 47 regarded as inert towards migration due to their larger sizes [3].

48 The safety of FCM must be evaluated on the basis of the amount of chemicals that can migrate from the 49 materials into food. The materials must be manufactured in compliance with EU regulations, including good manufacturing practices, so that any potential transfer into food does not raise safety concerns. 50 The main risks concern the presence of unexpected and undesired chemicals which could be threaten 51 52 human health; other possible risks regard the change of the food composition with adverse effects on the taste and odor. The Commission Regulation (EU) No. 10/2011 on plastic materials and articles 53 54 intended to come into contact with food states that potential health risks in the final food packaging products or articles should be assessed by the manufacturer in accordance with the internationally 55 56 recognized principles of risk assessment. In that regard, the European Food Safety Authority (EFSA) 57 published a draft scientific opinion on recent developments in the risk assessment of chemicals in food related to the safety assessment of substances used in FCM, for public consultation [4] in which 58 59 assessment of NIAS is briefly discussed. At the same time, the International Life Sciences Institute 60 (ILSI) published a guidance on best practices for NIAS evaluation in FCM [5,6]. Central to NIAS risk assessment and the first step towards demonstrating NIAS compliance is to determine which substances 61 are present in the FCM. While NIAS derived from side reactions and degradation products can broadly 62 63 be predicted and used as a starting point for developing an analytical strategy, the NIAS due to 64 contamination can occur at any point in the life cycle of the FCM and their identification is considered 65 a significant challenge. Consequently, their individuation and quantification have to be carried out on the finished materials and articles. 66

An effective approach to the NIAS testing and risk assessment solution is based on the following steps:
information gathering, analytical screening, identification of hazardous substances and final risk
assessment. Currently, screening programs to identify and semi-quantify the presence of NIAS are
executed with different sample preparation techniques (e.g. extraction with solvents under controlled

71	conditions) and analytical techniques (e.g. gas chromatography - quadrupole time-of-flight mass
72	spectrometry (GC/Q-TOF), gas chromatography - mass spectrometry (GC-MS), liquid chromatography
73	- mass spectroscopy (LC-MS) and inductively coupled plasma - mass spectrometry (ICP-MS)). By
74	using sensitive advanced analytical techniques most NIAS are regularly detected but not their
75	distribution. In the food packaging industry, the analytical techniques that are usually employed for
76	detecting NIAS rely on mass spectrometry, mainly GC-MS to quantify volatile [7-10] and semi-
77	volatile substance and triple quadrupole - Liquid Chromatography Mass Spectrometry (QqQ-LCMS) to
78	quantify non-volatile and polar compounds [11–13]. These are targeted analytical methods which need
79	internal standards composed of the same or similar chemical structures of the NIAS under
80	investigation. Moreover, for an accurate determination of the amount of NIAS present in the food
81	package, internal standards with also similar concentrations should be used.
82	Although GC-MS and LC-MS are powerful techniques, they have some limitations. In general, these
83	techniques are more suited for the evaluation of targeted chemical compounds or at least belonging to
84	the reference database for this reason unexpected chemical compounds could be difficult to identified.
85	Moreover, these two techniques have limitations with regard to the range of analytes that can be
86	handled. In addition, relatively to the ionization techniques compatible with to the LC-MS, MS
87	fragment determination is difficult when there is no <i>a priori</i> knowledge on the structure and origin
88	[1,3]. Lastly, all these determinations based on mass spectrometry are expensive and time-consuming,
89	thus their use in a lab as a possible in-line detection is infrequent. In addition, these techniques do not
90	provide any information about the distribution and the aggregation state of the contaminants in the
91	sample, which is an important parameter the packaging industry production process and for the risk
92	assessment purposes [4].

93 To overcome these issues, alternative techniques can be taken into account. Vibrational spectroscopies 94 have already proven to be powerful techniques for the identification of chemical compounds, thanks to the unique chemicals spectra they provide. Moreover, these are able to perform spectra in few seconds, 95 and no pre-treatment of the sample is required. In particular, confocal Raman spectroscopy has the 96 97 additional advantages of not undergoing water interference and having a high spatial resolution, thus 98 allowing a chemical mapping of Raman active compounds on x-y plane with spatial resolution below 1 99 μm. In principle, confocal Raman spectroscopy enables an in-line characterization of granules and 100 films for what concern Raman active NIAS, since a single spectrum can be recorded in few seconds 101 and can provide information about their presence, their distribution and their aggregation state in the 102 FCM.

Thanks to these characteristics, Raman spectroscopy techniques have already been widely used in food science for many years ([14–20] and they are promising candidate techniques for analysis in the food packaging industry [21] to quickly detect the possible contamination of raw material and final products by NIAS and their distribution in the FCM. Furthermore, by implementing these analytical methods, i.e. using signal enhancement techniques such as Surface Enhanced Raman Spectroscopy (SERS) and Tip-Enhanced Raman Spectroscopy (TERS), the limit of detection is further increased of orders of magnitude and the resolution reaches the nanometer scale.

Nowadays, at industrial level the routine procedure for a preliminary screening of possible inorganic
impurities in granules and films intended for food packaging uses is the gravimetric ashes analysis [22].
However, this analysis has the main limitation of being a destructive technique, with a poor limit of
detection (limited to 0.01 mg for a commercial analytical balance) and no specificity. The operating
procedure consists in heating the organic matrix at high temperature (500 °C) so that this burns and, if

the resulting inorganic residues are significant by weight, these latter are subjected to further analysisbased on mass spectrometry.

117 In this work a versatile, simple and reproducible procedure to detect, discriminate and semi-quantify 118 NIAS in granules and extruded films of Low Density Polyethylene (LDPE) for food packaging 119 applications was proposed. The analytical procedure was set up on the raw material as a model system 120 to standardize the methodology for both qualitative and quantitative analyses. Moreover, micro-Raman 121 mapping was exploited to measure the distribution and the identification of contaminants on industrial 122 LPDE granule and films. The foreseen advantages rely on the rapidity and the low limit of detection of 123 the technique, on the possibility to use untreated raw materials, to not use internal standards and to map 124 the distribution of the contaminants and their aggregation state.

For a tentative semi-quantitative analysis of the inorganic NIAS, the ashes of the pellets were measured
by Raman spectroscopy. For a comparison, NIAS were determined also with established analytical
methods such as by gravimetric analysis and by ICP-MS. The toxicity of the identified compounds was
estimated on the basis of the Cramer classes.

129

130 **2.** Materials and methods

LDPE was chosen in this study as a representative of the common FCMs for food packaging
applications. In particular, the surface of 50 LDPE granules, their cross sections, and 10 portions of a
LDPE film were investigated for the possible presence of NIAS.

134 The LDPE samples were provided by Plastotecnica SpA. The film (width 100 cm and thickness 40 μ m)

135 was obtained from the processing of the granules using a production blown line (die diameter 280 mm,

die gap 1.2 mm, mono-extrusion, line's speed 41 m/min). The density of LDPE was in the range of

137 $0.921 \text{ g/cm}^3 - 0.923 \text{ g/cm}^3$ and the melt flow index was in the range of 1.8 g/10 min - 2.0 g/10 min

138 (measured at 2.16 kg). The pellets were colorless and no additives were added in the samples in any139 stage of the production, except commercial antioxidants.

140 Nitric acid (HNO₃ 68%) was purchased from Sigma-Aldrich (Milan, Italy) and was used for the

141 preparation of the external standard solutions (1% in water) by dilution of a certified reference material

142 and for the preparation of the solution samples to be injected in the ICP-MS. The solutions were

143 prepared with Milli-Q quality water ($18 \text{ M}\Omega \text{cm}$).

144

145 **2.1** Qualitative analysis of LDPE granules and films

146 The LDPE granules and the extruded film were analyzed by Raman spectroscopy techniques for 147 detection of possible NIAS both on the surface and in the bulk material. The samples were 148 characterized without any preliminary treatment. In order to perform bulk material analyses, the granules were cut by a disposable scalpel and the internal surfaces were investigated by Raman 149 spectroscopy. Confocal Raman spectra were obtained by a Thermo ScientificTM DXRTM Raman 150 151 instrument. The microscope was equipped with a motorized stage with 1 µm of resolution in the X-Y 152 plane and a charge-coupled device (CCD) detector. A preliminary optical investigation by using a $10\times$ magnification objective of the surface of granules and film was performed for detecting possible 153 154 inhomogeneities in the LDPE matrix. The spectra were then acquired in different points of the samples 155 surface, by using a laser excitation source of 532 nm, 3 mW laser power and a 100× microscope objective, in the range from 50 cm⁻¹ to 3500 cm⁻¹ with a grating resolution of 5 cm⁻¹. In the case of 156 detection of agglomerations which include contaminants, these latter were further characterized by 157 158 micro-Raman imaging, which was carried out by a Thermo ScientificTM DXR^{xi} Imaging microscope, 159 considering the same experimental parameters set for confocal spectra. This microscope is equipped with an Electron Multiplying CCD camera that allows to collect 600 spectra per second. In this way, 160

optical and chemical mapping of the contaminants was obtained, thus providing information of theagglomeration state and dimensions of the NIAS with real-time data visualization.

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2.2 Ashes analysis of the LDPE granules

For the ashes analysis, a 1.5 g of sample taken from LDPE granules were weighted on a glass crucible
and brought to a temperature of 500 °C. Then, the possible inorganic residues detected by gravimetry.
The calcination temperature of 500 °C has been chosen since all the organic components are thus
burned and removed, without any possible contamination from the crucible (the glass melting
temperature is 1723°C). As preliminary cleaning procedure, the crucible was soaked in aqua regia
(HCl:HNO₃ 3:1 v/v), rinsed thoroughly in water and dried with nitrogen. Then, it was degassed,
keeping it in the furnace at 105 °C for 2 h, and then it was weighted. These steps were repeated as

172 many time as needed for the crucible to be stable in weight.

173 An analytical balance SI 114 from Denver Instruments (maximum weight 110 g and resolution 0.1 mg)

174 was used to estimate the weight of the LDPE granules after thermal treatment. The thermal treatment

175 was carried out with a muffle Carbolite furnace RHF1400 and it consisted of a heating ramp from 20°C

to 500°C in 30 min and then in keeping the temperature at 500°C for 30 min. By this thermal treatment,

177 all the organic compounds of the samples burned and only inorganic residues, oxides and metals,

178 remained for the analysis.

After the thermal treatment, the inorganic residues possibly present in the crucible were detected by optical inspection and analyzed by Raman spectroscopy and Raman imaging. The transparent glass crucible allowed the back-illumination of the sample, thus helping in the detection even of the smallest residual particles. 183 Then, ICP-MS technique was used for confirming by elemental analysis the presence of contaminants 184 detected by Raman characterization. A Thermo Scientific ICAP-Qs ICP-MS equipped with a 185 quadrupolar mass analyzer and a quadrupolar collision/reaction cell with flat electrodes (flatpole) was 186 used for the detection of Ca and Ti. This instrument was calibrated with the external standard solutions 187 composed of HNO₃ (1% in water) prepared by dilution of a certified reference material at 1 g/l, by using ⁴⁵Sc (100 ppb) as internal standard. Interference from polyatomic ions were removed by 188 operating the collision cell He mode (the presence of He gas in the collision cell) with discrimination of 189 190 the ions kinetic energy (He – KED). The following ions were used for quantitation: ⁴⁴Ca, ⁴⁶Ti and ⁴⁷Ti. 191 Other operating parameter were: RF power 1450 W, Argon plasma gas flow 15 l/min, auxiliary gas 192 flow 1.0 l/min, nebulizer gas flow 0.90 l/min, sample flow rate 1.0 ml/min, nebulizer type concentric, 193 spray chamber impact bead, interface cones (sampler and skimmer) Ni collision cell, He flow 5.0 194 ml/min, extraction lenses potentials and KED bias optimized though the auto-tuning procedure by 195 using the tuning solution suggested by manufacturer.

196

2.3 Evaluation of the toxicity of the identified NIAS

Cramer classes were applied in order to estimate the toxicity of the identified compounds. Toxtree
v3.1.0 (Ideaconsult Ltd., Sofia, Bulgaria), based on a decision tree approach, was the used software.
The substances are classified into three classes taking into account their chemical structures: class I
corresponds to a low toxicity, class II corresponds to an intermediate toxicity and class III corresponds
to a high toxicity ([23]).

- 203
- **3.** Results and discussion

205 After a preliminary investigation with an optical microscope of the whole surface of granules and film, 206 Raman spectra of the homogenous parts of LDPE samples were carried out (Fig. 1). The inset of Fig. 1 207 summarizes the assignment of the Raman bands to LDPE. The assignments are supported by Raman 208 spectra of several kinds of polyethylene obtained in previous studies [24]. The characteristic bands of 209 LDPE, clearly visible in Fig. 1, are: the symmetric CH₂ stretching mode at 2847 cm⁻¹, the asymmetric CH₂ stretching mode at 2882 cm⁻¹, the unresolved feature near 1439 cm⁻¹ due to anisotropic parts (CH₂ 210 211 Fermi resonance), CH₂ twisting features near 1295 cm⁻¹ and the amorphous C-C stretching at 1128 cm⁻¹ 212 and 1062 cm⁻¹.

213 Thanks to the micro Raman spectroscopy was possible to observe inhomogeneous parts in LDPE 214 granules and films, composted of agglomerations with dimension of the order of μ m². In fact, in the 215 optical image of Fig. 2 the agglomerations are visible in white, while the black background represents 216 the homogeneous parts of LDPE. The relatively large dimensions of these agglomerations are probably 217 due to the fact that contaminants tend to migrate within the polymer and to form aggregates. By 218 confocal Raman spectra four main compounds were identified as contaminants, as reported in Fig. 3 219 (a), (b), (c) and (d): calcite (CaCO₃), calcium sulphate (CaSO₄), polystyrene (PS) and titanium dioxide 220 (TiO_2) in anatase phase. Fig. 2 also shows the micro-Raman maps with CaCO₃, CaSO₄ and TiO₂ 221 superimposed on the optical images. The combination of these images demonstrates the consistency of 222 the chemical and the morphological information. The same study was repeated for the bulk of the 223 granules. It was not possible to analyze inclusions of PS by micro-Raman imaging because in the time 224 needed to take the maps the laser degrades the organic materials, even if the laser power is reduced to 225 the minimum (1mW). The CaCO₃ was found on the surfaces both of the granules and film. Traces of 226 CaSO₄ were detected only on the surface of the granules. PS was found both on the surface and in the 227 bulk of the granules. TiO₂ anatase was found both on the surface of granules and film.

In order to establish if the possible source of contamination was the 25 kg sack containing the LDPE pellets, a homogeneity study of the contaminants in granules accommodated in different location of a sack was performed. Ten granules were allocated at different distance with respect to inner surface of the sack and were analyzed in order to establish a possible contamination by the sack. Since no differences were found in this sense, as an alternative it was assumed that the contamination originate from the production process.

After the thermal treatment, micro-Raman imaging were used for the characterization of residues. An

area of about 1 cm \times 2 cm of the crucible containing the residues was characterized (See Fig. 4(a)). In

this area 22 aggregates were observed. Fig. 4(b) reports that 9 aggregates were composed of CaCO₃

237 (yellow squares), 2 of CaSO₄ (green squares), 8 of TiO₂ anatase (blue squares) and 3 of TiO₂ rutile

238 (black squares). The presence of TiO_2 rutile was due to the polymorph changes caused by the thermal

treatment, where a portion of TiO_2 anatase changed in rutilo phase. Fig. 4 (c) and (d) show the residues

of CaCO₃ and of TiO₂ in anatase obtained by optical images and superimposed to the corresponding

241 micro-Raman images. No traces of PS were detectable after the thermal treatment.

Since these compounds, already present before the thermal treatment, were neither expected nor desired,they can actually be considered as NIAS.

By gravimetric analysis, i.e. by weighting the crucible before and after the thermal treatment, no

residues were determined because the residues amount is below the resolution of the used analytical

246 balance (0.1 mg).

247 The analysis of residues of the LDPE granules by ICP-MS after the thermal treatment shows the

248 presence of Ca and Ti, with a measured concentration of 0.10 mg/kg and 3.585 mg/kg respectively,

249 with an instrumental uncertainty of about 20 % for both them. In this sense, the Raman and ICP-MS

analysis are consistent, since the results of Raman analysis show the presence of CaCO₃, CaSO₄ and

251 TiO₂ in the LDPE matrices, and the ICP-MS data confirm the presence of Ca and Ti in the ashes 252 residues. Despite the concentration of these compounds is very low, at trace levels, the following 253 consideration can be done: i) assuming that all the Ti present in the LDPE samples is in form of titania polymorph (TiO₂), a semi-quantitative estimation of the concentration of TiO₂ in the granules and film 254 255 can be carried out. Indeed, considering that the ratio between the atomic weights of O (15.999 u) and Ti 256 (47.867 u) in TiO₂ is 0.334 and that for each Ti atom two O atoms are present, from the results obtained 257 by ICP- MS that for Ti was of 0.1 mg/kg, a concentration of 0.17 mg/kg for TiO₂ was calculated. ii) The large amount of Ca found in granules and film by elemental analysis is confirmed also by Raman 258 259 analysis by the presence of two compounds containing Ca (40.078 u): CaCO₃ and CaSO₄. The 260 deductions on the respective concentration of the latter are more difficult than in case of TiO₂. On the 261 other hand, by micro Raman imaging of the ashes on the crucible, a large abundance of CaCO₃ was found compared to that of CaSO₄ (9 and 2, respectively, out of 22 aggregates in the investigated area). 262 263 Based on this consideration, a preliminary evaluation of the concentration of CaCO₃ and CaSO₄ was done, with a similar consideration as above in the case of TiO₂. If all the Ca present in the granules is in 264 265 form of either CaCO₃ (molecular weight 100 mg/mol) or CaSO₄ (molecular weight 136 mg/mol) in the 266 proportion of 9:2 then the concentration of $CaCO_3$ and $CaSO_4$ can be evaluated of 9.93 mg/kg and 1.62 267 mg/kg, respectively.

According to the Cramer classes, the inorganic NIAS, CaCO₃, CaSO₄ and TiO₂, detected by Raman

spectroscopy belong to the class III because they are not the normal constituents of a human body.

270 Moreover, NIAS contains substances other than C, H, O, N, divalent S, which don't curs only as a Na,

271 K, Ca, Mg, N salt, phosphate, sulphamate, sulphonate, sulphate and hydrochloride.

272 Concerning the traces detected by ICP-MS, any Raman active molecule was also detected by

273 spectroscopic analysis, therefore any further consideration can be done in this respect, except to the fact

- the some NIAS are present in the samples, in the form of chemical compounds containing both
- transition metals and an alkaline earth metals (Mg). The largest abundance is measured for Al (1.19

276 mg/kg), Zn (0.67 mg/kg), Mg (0.56 mg/kg), Fe (0.45 mg/kg) and Cu (0.41 mg/kg).

277 In conclusion, Raman spectroscopy was demonstrated to be a sensitive technique for detecting NIAS in

the LDPE granules and films intended for food packaging applications. The distribution of the NIAS

between the surface and the cross-section of LPDE granules seem to indicate that CaCO₃ and CaSO₄

280 originate from the environmental contamination, since their presence was detected only at the surface

281 of the granules, while the PS and TiO_2 contamination came from the production process of the

granules, such as, for instance, from previous batch of other material, as they were found in bulk

283 material as well.

By micro Raman imaging it is possible to understand the distribution of inclusions in the sample, andtheir agglomeration state. On this base it could be considered as a suitable technique for a semi-

286 quantitative analysis of inorganic impurities in the surface of granules and films.

The limit of detection of Raman spectroscopy is below that of the gravimetric method, and it is able to
discriminate the different types of contaminant. The results obtained by Raman spectroscopy were
confirmed by the ICP-MS analyses.

This approach can be implemented for performing non-destructive and in-situ analysis of the surface of granules and films without any pre-treatment of the sample, in a little time-consuming and in very much effective way, thus addressing the stringent needs in food packaging industry related to food safety and risk assessment issues.

294

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299 Data availability

The raw data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study. The processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

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304 REFERENCES

- 305 [1] C. Nerin, P. Alfaro, M. Aznar, C. Domeño, The challenge of identifying non-intentionally added
 306 substances from food packaging materials: A review, Anal. Chim. Acta. 775 (2013) 14–24.
 307 doi:10.1016/j.aca.2013.02.028.
- 308 [2] M. Aznar, A. Rodriguez-Lafuente, P. Alfaro, C. Nerin, UPLC-Q-TOF-MS analysis of non-

309 volatile migrants from new active packaging materials, Anal. Bioanal. Chem. 404 (2012) 1945–

310 1957. doi:10.1007/s00216-012-6247-5.

- 311 [3] C.C. Wagner, Non-intentionally added substances (NIAS), Food Packag. Forum. (2014).
- 312 http://www.foodpackagingforum.org/food-packaging-health/non-intentionally-added-

313 substances-nias.

314 [4] EFSA, Recent developments in the risk assessment of chemicals in food and their potential

- 315 impact on the safety assessment of substances used in food contact materials, EFSA J. 14 (2016)
 316 4357. doi:10.2903/j.efsa.2016.4357.
- 317 [5] S. Koster, M.-H. Bani-Estivals, M. Bonuomo, E. Bradley, M.-C. Chagnon, L. Garcia M., F.
- 318 Godts, T. Gude, H. Rüdiger, P. Paseiro-Losada, G. Pieper, M. Rennen, T. Simat, L. Spack,

- Guidance On Best Practices On The Risk Assessment Of Non-Intentionally Added Substances
 (Nias) In Food Contact Materials And Articles, (2016).
- 321 http://www.contactalimentaire.com/fileadmin/ImageFichier_Archive/contact_alimentaire/Fichier
- 322 s_Documents/guide/Guidance_on_Best_Practices_NIAS__2015.pdf.
- W. Leeman, L. Krul, Non-intentionally added substances in food contact materials: How to
 ensure consumer safety, Curr. Opin. Food Sci. 6 (2015) 33–37. doi:10.1016/j.cofs.2015.11.003.
- 325 [7] S. Koster, M. Rennen, W. Leeman, G. Houben, B. Muilwijk, F. van Acker, L. Krul, A novel
- 326 safety assessment strategy for non-intentionally added substances (NIAS) in carton food contact
- 327 materials, Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess. 31 (2014)
- **328** 422–43. doi:10.1080/19440049.2013.866718.
- 329 [8] C. Nerín, E. Canellas, M. Aznar, P. Silcock, Analytical methods for the screening of potential
 330 volatile migrants from acrylic-base adhesives used in food-contact materials, Food Addit.
- 331 Contam. Part A Chem. Anal. Control. Expo. Risk Assess. 26 (2009) 1592–1601.
- doi:10.1080/02652030903161572.
- 333 [9] F. Hernández, T. Portolés, E. Pitarch, F.J. López, Gas chromatography coupled to high-
- resolution time-of-flight mass spectrometry to analyze trace-level organic compounds in the
- environment, food safety and toxicology, TrAC Trends Anal. Chem. 30 (2011) 388–400.
- doi:10.1016/j.trac.2010.11.007.
- V. García Ibarra, A. Rodríguez Bernaldo de Quirós, P. Paseiro Losada, R. Sendón, Identification
 of intentionally and non-intentionally added substances in plastic packaging materials and their
 migration into food products, Anal. Bioanal. Chem. 410 (2018) 3789–3803. doi:10.1007/s00216018-1058-y.
- 341 [11] M.J. Martínez-Bueno, S. Cimmino, C. Silvestre, J.L. Tadeo, A.I. Garcia-Valcárcel, A.R.

342		Fernández-Alba, M.D. Hernando, Characterization of non-intentionally added substances
343		(NIAS) and zinc oxide nanoparticle release from evaluation of new antimicrobial food contact
344		materials by both LC-QTOF-MS, GC-QTOF-MS and ICP-MS, Anal. Methods. 8 (2016) 7209-
345		7216. doi:10.1039/c6ay01972b.
346	[12]	M.J. Martínez-Bueno, M.D. Hernando, S. Uclés, L. Rajski, S. Cimmino, A.R. Fernández-Alba,
347		Identification of non-intentionally added substances in food packaging nano films by gas and
348		liquid chromatography coupled to orbitrap mass spectrometry, Talanta. 172 (2017) 68-77.
349		doi:10.1016/j.talanta.2017.05.023.
350	[13]	H. Gallart-Ayala, O. Núñez, P. Lucci, Recent advances in LC-MS analysis of food-packaging
351		contaminants, TrAC - Trends Anal. Chem. 42 (2013) 99-124. doi:10.1016/j.trac.2012.09.017.
352	[14]	E.C.Y. Li-Chan, The applications of Raman spectroscopy in food science, Trends Food Sci.
353		Technol. 7 (1996) 361–370. doi:10.1016/S0924-2244(96)10037-6.
354	[15]	L.G. Thygesen, M.M. Løkke, E. Micklander, S.B. Engelsen, Vibrational microspectroscopy of
355		food. Raman vs. FT-IR, Trends Food Sci. Technol. 14 (2003) 50-57. doi:10.1016/S0924-
356		2244(02)00243-1.
357	[16]	A.M. Herrero, Raman spectroscopy a promising technique for quality assessment of meat and
358		fish: A review, Food Chem. 107 (2008) 1642–1651. doi:10.1016/j.foodchem.2007.10.014.
359	[17]	N.P. Mahalik, A.N. Nambiar, Trends in food packaging and manufacturing systems and
360		technology, Trends Food Sci. Technol. 21 (2010) 117-128. doi:10.1016/j.tifs.2009.12.006.
361	[18]	Y.S. Li, J.S. Church, Raman spectroscopy in the analysis of food and pharmaceutical
362		nanomaterials, J. Food Drug Anal. 22 (2014) 29-48. doi:10.1016/j.jfda.2014.01.003.
363	[19]	A.M. Giovannozzi, F. Rolle, M. Sega, M.C. Abete, D. Marchis, A.M. Rossi, Rapid and sensitive
364		detection of melamine in milk with gold nanoparticles by Surface Enhanced Raman Scattering,

¹⁶

365	Food Chem.	159 (2014) 250–256. doi:10.1016/	j.foodchem.2014.03.013.
			/	,

- 366 [20] S. Lohumi, S. Lee, H. Lee, B.K. Cho, A review of vibrational spectroscopic techniques for the
- detection of food authenticity and adulteration, Trends Food Sci. Technol. 46 (2015) 85–98.
- 368 doi:10.1016/j.tifs.2015.08.003.
- 369 [21] H. Sato, K. Shimoyama, Masahiko Taeko, T. Amari, S. Šašic, T. Ninomiya, H.W. Siesler, Y.
- 370 Ozaki, RamanSpectraofHigh-Density,Low-Density,andLinearLow-
- 371 DensityPolyethylenePelletsandPredictionofTheirPhysicalPropertiesbyMultivariateDataAnalysis,
- 372 J. Appl. Polym. Sci. 86 (2002) 443–448.
- 373 https://onlinelibrary.wiley.com/doi/epdf/10.1002/app.10999.
- 374 [22] ASTM International, D5630 13: Standard Test Method for Ash Content in Plastics, ASTM
 375 Stand. Guid. (2013). doi:10.1520/D5630-13.1.
- 376 [23] G.M. Cramer, R.A. Ford, R.L. Hall, Estimation of toxic hazard-A decision tree approach, Food
 377 Cosmet. Toxicol. 16 (1976) 255–276. doi:10.1016/S0015-6264(76)80522-6.
- 378 [24] H. Sato, M. Shimoyama, T. Kamiya, T. Amari, S. Šašić, T. Ninomiya, H.W. Siesler, Y. Ozaki,
- 379 Near infrared spectra of pellets and thin films of high-density, low-density and linear low-
- density polyethylenes and prediction of their physical properties by multivariate data analysis, J.
- 381 Near Infrared Spectrosc. 11 (2003) 309–321. doi:10.1255/jnirs.376.
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388 FIGURE CAPTIONS

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Fig. 1 Raman spectra of the LDPE matrix and the assignments of Raman bands in the inset.
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- 392 Fig. 2. Micro-Raman imaging of inhomogeneous parts of LDPE samples (white parts) inclosing the
- **393** agglomerations with $CaCO_3$ (a), $CaSO_4$ (b) and TiO_2 in anatase phase (c).
- 394
- Fig. 3. Raman spectra of CaCO₃ (a), CaSO₄ (b), TiO₂ in anatase phase (c) and PS (d).

396

397 Fig. 4. Distribution of inorganic residues on the crucible (a) and their abundance (b). Optical images and micro-

398 Raman maps of $CaCo_3$ (c) and TiO_2 in anatase phase (d) residues in the crucible after thermal treatment.

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400





406 Fig. 2 (printed in colors)











