EW: “Mass spectrometry in support of the environment, food, and health interaction and disease”

Characterization of non-intentionally added substances in food packaging nanofilms by analytical approaches based on HRMS
ERANET SUSFOOD: Sustainable food production

Title: Improved resource efficiency throughout the post-harvest chain of fresh-cut fruits and vegetables

The Fruit and Vegetable Sector is an important segment of the European Agroindustry, with a weight of about 18% of the value of EU agricultural production.

Fresh cut products:
This sector is a major user of water; about 70% of the water consumption is for cleaning and decontamination.

Proper disinfection technology is required to ensure microbial safety avoiding at the same time the formation of disinfection by-products such as chlorinated chemicals.

Growth of microorganisms is favoured by cutting/slicing, which remove the natural barrier.

The quality of fresh-cut products also depends critically on packaging technology, which has to preserve good appearance and flavour, as well as meet safety requirement.
Exporters of food products.
Water stressed areas, Mediterranean basin.
Resource-efficient food processing:
• Water savings
• Reduce use of chemicals
• Valorization of food residues

Food quality & safety:
• Extension of shelf-life
• Avoid harmful disinfection by-products
• Good appearance and flavour
These goals are to be achieved by the combination of the following nanotechnology-based solutions for fresh-cut products:

**Washing & desinfection:**

Introduction of new membranes with biocidal functionality to allow operating in closed loop and the reduction or elimination of chemical disinfectants.

Development of a hybrid technology in which the use of ozone combined with ultrafiltration using ceramic membranes with (eventually) active surface.

**Shelf-life improvement:**

Development of packaging materials with antimicrobial components, to increase shelf life and, to reduce wastes.
Institute of chemistry and technology of polymers

CEREAL approach

To design the new packaging materials mainly monolayers by directing the structure and properties of the polymer matrix during crystallisation taking into consideration the material characteristics, the nanoparticles shape (rod shape and plate-like), the conditions of the manufacturing packaging process (extrusion and blowing).

To develop materials having improved physical, mechanical, barrier and antibacterial activity properties that contribute to extend shelf-life and quality of the food

Development of industrial scale processes for the synthesis and the post-treatments of metal oxide nano-powders, and fabrication of nanocomposites by melt processing.
Packaging materials

1. Development
2. Optimization of the processing conditions
3. Assessment of properties of the materials:
   - filler/matrix interactions, thermal stability and rheological behaviour of the nanocomposites, optical properties, UV-absorption and degradation upon illumination, thermal and mechanical properties and processability
4. Evaluation of new materials
   - Migration of substances
   - Antimicrobial activity, shelf life prolongation and impact on fresh-cut produce quality. Relevant test strains: Listeria monocytogenes, E. coli, Pseudomonas fluorescens, Bacillus subtilis (spores) and Aspergillus niger (spores).
Polypropylene –based nanocomposites

(PP)-based nanocomposites functionalized with zinc oxide nanoparticles (ZnO NPs) and polylimonene (PL)

<table>
<thead>
<tr>
<th>Composition</th>
<th>PPR3221 (wt%)</th>
<th>PL (wt%)</th>
<th>ZnONPs (wt%)</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPR</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>PPR</td>
</tr>
<tr>
<td>PPR/PL 95/5</td>
<td>95</td>
<td>5</td>
<td>0</td>
<td>PPR/PL 95/5</td>
</tr>
<tr>
<td>PPR/ZnO 97/3</td>
<td>97</td>
<td>0</td>
<td>3</td>
<td>PPR/ZnO 97/3</td>
</tr>
<tr>
<td>PPR/PL/ZnO 92/5/3</td>
<td>92</td>
<td>5</td>
<td>3</td>
<td>PPR/PL/ZnO 92/5/3</td>
</tr>
</tbody>
</table>

Biopolymer metal oxide nanocomposites

Nanocomposites: Poly(lactic acid) (PLA), PL, ZnO NPs, and ZnONPs coated with stearic acid

<table>
<thead>
<tr>
<th>Code</th>
<th>PLA (wt%)</th>
<th>PL (wt%)</th>
<th>ZnONPs (wt%)</th>
<th>mZnONPs (wt%)</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>PLA</td>
</tr>
<tr>
<td>PLA/ZnO3%</td>
<td>97</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>PLA/ZnO3%</td>
</tr>
<tr>
<td>PLA/ZnO5%</td>
<td>95</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>PLA/ZnO5%</td>
</tr>
<tr>
<td>PLA/mZnO3%</td>
<td>93</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>PLA/mZnO3%</td>
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<tr>
<td>PLA/mZnO5%</td>
<td>95</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>PLA/mZnO5%</td>
</tr>
<tr>
<td>PLA/PL/ZnO</td>
<td>85</td>
<td>10</td>
<td>5</td>
<td>-</td>
<td>PLA/PL/ZnO</td>
</tr>
</tbody>
</table>
Non-intentionally added substances (NIAS) are compounds present in food contact materials (FCM), not added for a technical purpose during manufacture.

Relevant issue for the food packaging industry

**Impurities** - bulk materials
**Reaction intermediates** formed during manufacture
**Degradation** of products
**Contaminants**

NIAS-Non-intentionally added substances
## Test conditions for overall migration

<table>
<thead>
<tr>
<th>Test</th>
<th>Expected contact with food</th>
<th>Migration test conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM1</td>
<td>Contact with frozen and cooled food</td>
<td>10 days @ 20°C</td>
</tr>
<tr>
<td>OM2</td>
<td>Long storage at room temperature + short heating</td>
<td>10 days @ 40°C</td>
</tr>
<tr>
<td>OM3</td>
<td>Short heating</td>
<td>2 hours @ 70°C</td>
</tr>
<tr>
<td>OM4</td>
<td>High temperature use</td>
<td>1 hour @ 100°C</td>
</tr>
</tbody>
</table>
| OM5  | High temperature use (up to 121°C) | 2 hours @ 100°C  
Or 1 hour refluxing @ 121°C |
| OM6  | Use of simulant A, B or C at a temperature of more than 40°C | 4 hours @ 100°C  
or 4 hours refluxing |
| OM7  | Use of fatty food at a high temperature | 2 hours @ 175°C |

<table>
<thead>
<tr>
<th>Simulant</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol 10% (v/v)</td>
<td>Simulant A</td>
</tr>
<tr>
<td>Acetic acid 3% (w/v)</td>
<td>Simulant B</td>
</tr>
<tr>
<td>Ethanol 20% (v/v)</td>
<td>Simulant C</td>
</tr>
<tr>
<td>Ethanol 50% (v/v)</td>
<td>Simulant D1</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>Simulant D2</td>
</tr>
<tr>
<td>Modified polyphenylene oxides, particle size 60-80 mesh, pore size 200 nm</td>
<td>Simulant E for dry foods</td>
</tr>
</tbody>
</table>

2007/42/EC
Regenerated cellulose film

(2023/2006) Good Manufacturing Practice

84/500/EEC
Ceramics, as amended

1895/2005/EC
BADGE/BFDGE/NOGE

(2009) Active and intelligent materials

93/11/EEC
Nitrosamines and nitrosatable substances

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BADGE/BFDGE/NOGE

(2009) Active and intelligent materials

93/11/EEC
Nitrosamines and nitrosatable substances

(2011) restriction of use of Bisphenol A in plastic infant feeding bottles

(EC) No 10/2011
Plastics Implementation Measure, as amended/corrected

(EC) No 282/2008
Recycled plastics

(2011) polyamide and melamine plastic kitchenware

EU legislation
LC-QTOF-MS

Qualitative analysis

Screening analysis

full-scan

Fragmentation
LC-QTOF-MS

Qualitative analysis

Screening analysis

Full-scan

Fragmentation
Simulation of fragmentation

I) 2,4,6-Triamino-1,3,5-triazine

II) Azepan-2-one

III) (2E)-3-phenylprop-2-enal

rH: Charge-Site Rearrangement; i: inductive cleavages; π: π-Bond Dissociation

Tentative assignation of fragment ions
## LC-QTOF-MS

### Polypropylene –films

<table>
<thead>
<tr>
<th>RT</th>
<th>Molecular ion</th>
<th>Accurate mass [H⁺] (m/z)</th>
<th>Error (ppm), scope (%)</th>
<th>Product ion (loss)</th>
<th>Accurate mass [H⁺] (m/z)</th>
<th>Error (ppm)</th>
<th>NIAS (tentative identification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>C₃H₆N₆</td>
<td>127.0727</td>
<td>−0.82 ppm, &gt;86%</td>
<td>C₃H₃N₅ (−NH₃)</td>
<td>110.0467</td>
<td>−2.7</td>
<td>2,4,6-Triamino-1,3,5-triazine</td>
</tr>
<tr>
<td>1.5</td>
<td>C₆H₁₁NO</td>
<td>114.0913</td>
<td>−0.70 ppm, &gt;98%</td>
<td>C₆H₉N (−H₂O)</td>
<td>96.0807</td>
<td>−8.9</td>
<td>Azepan-2-one</td>
</tr>
<tr>
<td>8.9</td>
<td>C₉H₈O</td>
<td>133.0648</td>
<td>1.50 ppm, &gt;76%</td>
<td>C₈H₈ (−CO)</td>
<td>105.0698</td>
<td>5.0</td>
<td>(2E)-3-Phenylprop-2-enal</td>
</tr>
<tr>
<td>Simulant A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>C₃H₆N₆</td>
<td>127.0727</td>
<td>2.5 ppm, &gt;65%</td>
<td>C₃H₃N₅ (−NH₃)</td>
<td>110.0467</td>
<td>9.0</td>
<td>2,4,6-Triamino-1,3,5-triazine</td>
</tr>
<tr>
<td>1.5</td>
<td>C₆H₁₁NO</td>
<td>114.0913</td>
<td>−1.35 ppm, &gt;85%</td>
<td>C₆H₉N (−H₂O)</td>
<td>96.0807</td>
<td>−9.7</td>
<td>Azepan-2-one</td>
</tr>
<tr>
<td>8.9</td>
<td>C₉H₈O</td>
<td>133.0648</td>
<td>0.3 ppm, &gt;76%</td>
<td>C₈H₈ (−CO)</td>
<td>105.0698</td>
<td>7.9</td>
<td>(2E)-3-Phenylprop-2-enal</td>
</tr>
<tr>
<td>Simulant B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Data processing LC-QOrbitrap-MS

*Filters: intensity signals: $1 \times 10^{5}$

mass error: 5 ppm

Screening analysis

principal component analysis

(A) PLA/ZnO
(B) PLA/PL/ZnO

(A) control
(B) control
structural elucidation
N,N-Diethyldodecanamide

<table>
<thead>
<tr>
<th>#</th>
<th>Checked</th>
<th>ΔMass [Da]</th>
<th>ΔMass [ppm]</th>
<th>CSD</th>
<th>Formula</th>
<th>Molecular Weight</th>
<th>Name</th>
<th>Structure</th>
<th># References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00011</td>
<td>0.44</td>
<td>17736</td>
<td>C16 H33 N  C</td>
<td>255.29621</td>
<td>N,N-Diethyldodecanamide</td>
<td></td>
<td></td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>0.00011</td>
<td>0.44</td>
<td>62629</td>
<td>C16 H33 N  C</td>
<td>255.29621</td>
<td>Palmitamide</td>
<td></td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>0.00011</td>
<td>0.44</td>
<td>66404</td>
<td>C16 H33 N  C</td>
<td>255.29621</td>
<td>N-Laurylmorpholine</td>
<td></td>
<td></td>
<td>37</td>
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<tr>
<td>4</td>
<td>0.00011</td>
<td>0.44</td>
<td>68863</td>
<td>C16 H33 N  C</td>
<td>255.29621</td>
<td>N,N-Dimethyltetradecanamide</td>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>0.00011</td>
<td>0.44</td>
<td>84518</td>
<td>C16 H33 N  C</td>
<td>255.29621</td>
<td>N,N-Dibutyloctanamide</td>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>0.00011</td>
<td>0.44</td>
<td>85623</td>
<td>C16 H33 N  C</td>
<td>255.29621</td>
<td>N-Butyldodecanamide</td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>0.00011</td>
<td>0.44</td>
<td>81853</td>
<td>C16 H33 N  C</td>
<td>255.29621</td>
<td>N,N-Dipropyldecanamide</td>
<td></td>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>
Tentative fragmentation pathway

**N,N-Diethyldodecanamide**

\[ \text{m/z } 256.26 \rightarrow \text{H}_2\text{O} \rightarrow \text{m/z } 256.26 \]

\[ \text{m/z } 116.11 \rightarrow \text{rH}_\text{B} \rightarrow \text{m/z } 88.08 \]

\[ \text{m/z } 256.2635 \rightarrow \text{rH}_\text{B} \rightarrow \text{m/z } 102.0913 \]

\[ \text{C}_{16}\text{H}_{34}\text{NO} \rightarrow \text{C}_{16}\text{H}_{34}\text{NO} \rightarrow \text{C}_{16}\text{H}_{34}\text{NO} \]

\[ \text{C}_{4}\text{H}_{10}\text{NO} \rightarrow \text{C}_{5}\text{H}_{12}\text{NO} \rightarrow \text{C}_{6}\text{H}_{14}\text{NO} \]
## LC-QOrbitrap-MS

### Biopolymer metal oxide nanocomposites

<table>
<thead>
<tr>
<th>Rt</th>
<th>accurate mass value [M+H]+</th>
<th>formula proposed</th>
<th>mass deviation* (ppm)</th>
<th>accurate mass value (m/z)</th>
<th>formula proposed</th>
<th>mass deviation* (ppm/mDa)</th>
<th>Candidate compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.7</td>
<td>256.2635 C_{16}H_{34}NO</td>
<td>-1.2</td>
<td></td>
<td>116.1070</td>
<td>C_{6}H_{14}NO</td>
<td>-2.6 / 0.3</td>
<td>N,N-Diethylldodecanamide</td>
</tr>
<tr>
<td>11.9</td>
<td>331.2843 C_{19}H_{39}O_{4}</td>
<td>-1.0</td>
<td></td>
<td>313.2737 99.0441</td>
<td>C_{19}H_{37}O_{3}</td>
<td>-2.3 / 0.7</td>
<td>1-Palmitoylglycerol</td>
</tr>
<tr>
<td>12.2</td>
<td>359.3156 C_{21}H_{43}O_{4}</td>
<td>-0.7</td>
<td></td>
<td>341.3050 285.2788</td>
<td>C_{21}H_{41}O_{3}</td>
<td>-0.6 / 0.2</td>
<td>Glycerol stearate</td>
</tr>
<tr>
<td>12.5</td>
<td>310.3104 C_{20}H_{40}NO</td>
<td>-0.9</td>
<td></td>
<td>268.2999 210.1852</td>
<td>C_{18}H_{38}N</td>
<td>0.2 / 0.1</td>
<td>N-[(9Z)-9-Octadecen-1-yl]acetamide</td>
</tr>
</tbody>
</table>

Characterization of NIAS; use of standards. Simulant B: 2.7 – 7.6 ng.g\(^{-1}\)
GC-HRMS

Deconvolution
- Min. intensity signals: 2e6
- Mass Error (MS): 5 ppm
- Signal to noise: 10
- Ion overlap window: 98
- RT Alignment: 10 sec

Mass spectral library
(250,000 chemicals)
- # Top matches: 4
- SI Threshold: 800
- Score Threshold: 95

Characterization

Screening analysis
GC-QTOF-MS

Characterization NIAS in PP films

candidate structure of the parent compound

Phthalates
Phthalic acid, butyl undecyl ester

Diethyl Phthalate

Benzyl butyl phthalate

Bis(2-ethylhexyl) phthalate

Fragmentation pattern of phthalates
GC-QOrbitrap-MS

α-Tocopherol acetate

Full MS [50-550 Da]

NIST
## GC-QOrbitrap-MS

<table>
<thead>
<tr>
<th>Rt</th>
<th>Precursor Ion</th>
<th>Fragment Ions</th>
<th>Mass Deviation* (ppm/mDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.8</td>
<td>C\textsubscript{15}H\textsubscript{24}O\textsubscript{6}</td>
<td>C\textsubscript{6}H\textsubscript{9}O\textsubscript{2}</td>
<td>1.4 / 0.2</td>
</tr>
<tr>
<td></td>
<td>300.1567</td>
<td>C\textsubscript{3}H\textsubscript{3}O</td>
<td>1.6 / 0.1</td>
</tr>
<tr>
<td></td>
<td>tripropylene glycol diacrylate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.0</td>
<td>C\textsubscript{21}H\textsubscript{42}</td>
<td>C\textsubscript{3}H\textsubscript{9}</td>
<td>0.5 / 0.03</td>
</tr>
<tr>
<td></td>
<td>294.3281</td>
<td>C\textsubscript{6}H\textsubscript{11}</td>
<td>0.3 / 0.02</td>
</tr>
<tr>
<td></td>
<td>10-Heneicosene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29.6</td>
<td>C\textsubscript{31}H\textsubscript{52}O\textsubscript{3}</td>
<td>C\textsubscript{29}H\textsubscript{50}O\textsubscript{2}</td>
<td>0.9 / 0.4</td>
</tr>
<tr>
<td></td>
<td>472.3911</td>
<td>C\textsubscript{10}H\textsubscript{13}O\textsubscript{2}</td>
<td>0.8 / 0.1</td>
</tr>
<tr>
<td></td>
<td>alpha-Tocopherol acetate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
α-Tocopherol acetate

Use of standards: Identification, quantification

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>M/Z</th>
<th>Mass</th>
<th>Control_A_1 MS Area</th>
<th>Control_A_2 MS Area</th>
<th>Control_B_1 MS Area</th>
<th>Control_B_2 MS Area</th>
<th>PLA_A_1 MS Area</th>
<th>PLA_A_2 MS Area</th>
<th>PLA_B_1 MS Area</th>
<th>PLA_B_2 MS Area</th>
<th>PLA_PLA_A_1 MS Area</th>
<th>PLA_PLA_A_2 MS Area</th>
<th>PLA_PLA_B_1 MS Area</th>
<th>PLA_PLA_B_2 MS Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.45</td>
<td>71.0855</td>
<td>71.0855</td>
<td>13,052.046</td>
<td>15,748.047</td>
<td>4,228.435</td>
<td>4,618.454</td>
<td>7,094.313</td>
<td>7,470.742</td>
<td>6,676.613</td>
<td>6,561.106</td>
<td>8,343.610</td>
<td>8,147.282</td>
<td>3,092.596</td>
<td>2,946.050</td>
</tr>
<tr>
<td>26.65</td>
<td>71.0855</td>
<td>71.0855</td>
<td>11,112.346</td>
<td>11,662.670</td>
<td>5,808.105</td>
<td>6,091.809</td>
<td>7,403.231</td>
<td>7,576.025</td>
<td>7,007.032</td>
<td>7,080.239</td>
<td>8,530.663</td>
<td>8,134.808</td>
<td>3,461.096</td>
<td>3,296.232</td>
</tr>
<tr>
<td>27.82</td>
<td>71.0855</td>
<td>71.0855</td>
<td>5,037.915</td>
<td>9,642.655</td>
<td>6,901.563</td>
<td>2,892.324</td>
<td>5,146.173</td>
<td>4,076.177</td>
<td>4,431.101</td>
<td>3,934.276</td>
<td>4,678.423</td>
<td>4,872.961</td>
<td>2,130.416</td>
<td>1,792.576</td>
</tr>
<tr>
<td>39.21</td>
<td>71.0855</td>
<td>71.0855</td>
<td>6,603.453</td>
<td>2,209.004</td>
<td>2,255.612</td>
<td>1,191.226</td>
<td>2,540.297</td>
<td>2,651.372</td>
<td>2,320.035</td>
<td>2,259.485</td>
<td>2,312.559</td>
<td>5,176.497</td>
<td>1,890.298</td>
<td>2,215.720</td>
</tr>
</tbody>
</table>

Sample List:
- Control A
- PLA
- PLA/PL

Peak Identifications:
- α-Tocopheryl acetate
- (-)-α-Tocopherol acetate
- α-Tocopherol-β-D-mannoside

535 ng g⁻¹
Reference scenarios:
- Ecoinvent database
- European reference Life Cycle Database ELCD (European Commission’s Joint Research Centre)

Data provided by the CEREAL partners

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**Intro to LCA**

- ISO 14040, 14044
- Functional Unit
- Cut-off criteria
- Foreground vs Background data

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**LCA Philosophy**

Environmental improvements are quantified by comparing the damages caused by a complete system using the newly developed technologies against reference systems representative from current-use technologies.
Montecarlo analysis
PLA/ZnO vs Ref.

Midpoints show better performance in most cases for proposed package

Single score ReCiPe (H/H) shows that the use of PLA+ZnONP reduces the environmental impact of lettuce consumption by 10% while the package made from PP+ZnONP entails reductions of a 9%.
The production stage is by far the most environmentally damaging. The contribution of packaging to the whole environmental impact, including disposal stage, is not relevant. The production stage is by far the most environmental damaging.
HRMS approach is useful for the optimization process and development of films

Migration of NIAS from PP based films in both simulators A and B, and from PLA films in simulant A and B.

NIAS detected are not included in the candidate EU list of chemicals to be evaluated for risks

Based on ZnONPs release test (ICP-MS), the nanopolymers composed of PP/PL/ZnONPs, 92/5/3, and PLA/PL/ZnONPs provided the most suitable contact material with improved functionality

ZnONPs release test (ICP-MS): solubilization in the form of $\text{Zn}^{2+}$ is below SML (25 mg.kg$^{-1}$)

PL additive appears to hinder $\text{Zn}^{2+}$ release
EW: “Mass spectrometry in support of the environment, food, and health interaction and disease”

Thanks