# Testing nano-silver food packaging to evaluate silver migration and food spoilage bacteria on chicken meat Supplementary Data

## Silver Nanoparticle size distribution by Transmission Electron Microscopy:

6 µL of water dispersion of silver nanoparticles (dispersion nanoparticles, 20 nm particle size, 0.02 mg/mL in aqueous buffer, contains sodium citrate as stabilizer) (Sigma-Aldrich® St Louis, MO, USA) was deposited on carbon coated 400 mesh copper grids. After one minute, the suspension was removed by filter paper and the grid was left to dry overnight. The sample was imaged with Fei Tecnai 12 G2 transmission electron microscope equipped with tungsten filament and operated at 120 kV. Images were recorded on a side-mounted Olympus Morada CCD at instrumental magnifications ranging from 135000 to 360000×. A total of 769 individual particles were manually selected from 23 representative micrographs and the distribution of their area-equivalent circular diameters (ECD) was calculated. Final particle size distribution was obtained by log-normal fit to ECD. AgNPs particle size distribution (obtained from these 769 particles) showed the presence of a single population, with median (± MAD) diameter of 17 nm (± 2 nm) and with first and third quartiles at 15 nm and 20 nm respectively (Figure S-1).

## Atomic Absorption Spectrometer (AAS)

Silver quantification was performed on an Electrothermal (ETAAS) using an M6 mkII Atomic Absorption Spectrometer (Thermo Electron, Cambridge, UK) with D2 and Zeeman background correction, equipped with a GF95 Graphite Furnace atomizer (GFAAS). Analytical conditions are reported in Supplementary tables S-1 and S-2. Calibration solutions were prepared within the range of 0.3-2.4 µg kg-1 (corresponding to 15- 120 µg kg-1 in sample) by dilution of a Silver Nitrate (AgNO3) stock solution 1000 mg L-1 (Ultra ScientificNorth Kingstown, RI, USA).

## Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

Silver quantification was performed on a ICP-MS (NexION 300D, Perkin Elmer) used in standard mode, as Ag is not affected by severe polyatomic interferences. Samples were introduced in the ICP-MS by a quartz cyclonic spray chamber (Perkin Elmer), a concentric quartz nebulizer (Type A0.5, Meinhard) and a quartz torch and injector (Perkin Elmer); instrumental parameters were daily optimized by following an autotune procedure. The final operating conditions are reported in table S-3.

The analyses were carried out with internal standard correction to compensate for instrumental drift: the internal standard solution (10 µg kg-1 of rhodium, obtained by dilution of a 1000 mg kg-1 stock solution provided by Romil) was added on-line to the samples before being introduced into the nebulizer. Calibration solutions were prepared within the range of 0.1 - 5 µg kg-1 (corresponding to 2.5- 125 µg kg-1 in sample) for ICP-MS, by dilution of a silver stock solution 1000 mg L-1 (Ultra Scientific North Kingstown, RI, USA).

## AAS and ICP-MS methods validation

Analytical methods were fully validated in agreement with the guidelines laid down by Commission Decision 2002/657/EC, following the conventional validation approach required for quantitative confirmation. The following parameters were evaluated: specificity, linearity, recovery, repeatability, within-laboratory reproducibility, decision limit (CCα), detection capability (CCβ) and ruggedness.

Specificity was assessed by analysis 20 different blank samples, verifying the absence of interfering signals.

Linearity was studied by means of calibration curves within the range of 0.3-2.4 µg kg-1 (corresponding to 15 - 120 µg kg-1 in a sample) for AAS and within the range of 0.1 - 5 µg kg-1 (corresponding to 2.5- 125 µg kg-1 in a sample) for ICP-MS. Calibration curves were plotted using the absorbance versus the analyte concentration including zero level in the curve construction. Linear regression analysis was carried out and the linear calibration model was verified by correlation coefficients (Pearson’s R) better than 0.992 and by Mandel’s test.

Recovery, repeatability, within-laboratory reproducibilitywere estimated by analyzing 18replicates at 4 concentration levels (20, 50, 75 and 100 µg kg-1) on three different days (6 replicates per level per day).

Decision limit(CCα), and detection capability (CCβ) were estimated by the calibration procedure according to ISO 11843-2 (2000), considering the approach proposed by Verdon (Verdon et al. 2007) and Lega et al. (2013). Estimated CCα and CCβ were 8.1 and 19.7 µg kg-1  for AAS and 5.1 and 8.7 µg kg-1  for ICP-MS. In all cases CCα and CCβ were verified by spiking a minimum of six blank matrices at the concentration corresponding to the calculated decision limits.

Detailed information about parameters evaluated within the validation session are reported in tables S-5 and S-6.

# Supplementary Figures and Tables

## Supplementary Figures

Figure S-1. Silver, dispersion – nanoparticles size distribution.

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## Supplementary Tables

Table S-1 Digestion Parameters

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **POWER** | | **RAMP (min)** | **C° CONTROL** | **time(min)** |
| **MAX** | **%** |
| 1600 | 100 | 20 | 180 | 15 |

Table S-2 Instrumental parameters for GFAAS determination

|  |  |
| --- | --- |
| **Parameter** | **Ag** |
| Wavelength (nm) | 328.1 |
| Slit (nm) | 0.5 |
| Measurement time (sec) | 3.0 |
| Background correction | D2 |
| Atomisation (t °C) | 1500 |

Table S-3 GFAAS temperature program

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Step**  **n°** | **Temp**  **(°C)** | **Time**  **(sec)** | **Ramp**  **(°C sec-1)** | **Air flow**  **(L min-1)** |
| 1 | 110 | 20 | 5 | 0.1 |
| 2 | 160 | 10 | 5 | 0.1 |
| 3 | 800 | 30 | 30 | 0.2 |
| 4 | 1500 | 3 | 0 | Off |
| 5 | 2700 | 3 | 0 | 0.3 |

Table S-4 Operating conditions for Ag determination by ICP-MS.

|  |  |
| --- | --- |
| **Argon auxiliary gas flow rate** | 1.2 l·min−1 |
| **Argon plasma gas flow rate** | 12 l·min−1 |
| **Argon nebulizer flow rate** | 1 l·min−1 |
| **RF Power** | 1500 W |
| **Spray chamber** | Quartz cyclonic |
| **Nebulizer** | Concentric |
| **Sampler and Skimmer** | Ni |
| **Dwell time** | 50 ms |
| **Sweeps/reading** | 30 |
| **Readings/replicate** | 1 |
| **Replicates** | 3 |
| **Isotope (m/z)** | 107Ag |

Table S-5. Method Recovery, Precision, Repeatability, within Laboratory reproducibility, for AAS

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Matrix** | **Nominal concentration**  **µg Kg-1** | **Recovery%** | **Repeatability CV %** | | | **Within Laboratory Reproducibility (CV %)** |
| **1st day** | **2nd day** | **3rd day** |
| **Meat** | 20 | 89,1 | 9,46 | 7,60 | 3,05 | 6,95 |
| 50 | 95,2 | 3,05 | 1,84 | 0,67 | 4,70 |
| 75 | 97,4 | 3,86 | 1,76 | 1,74 | 6,34 |
| 100 | 97,5 | 2,00 | 1,92 | 2,08 | 7,77 |

Supplementary Table 6. Method Recovery, Precision, Repeatability, within Laboratory reproducibility for ICPMS

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Matrix** | **Nominal concentration**  **µg Kg-1** | **Recovery%** | **Repeatability CV %** | | | **Within Laboratory Reproducibility (CV %)** |
| **1st day** | **2nd day** | **3rd day** |
| **Meat** | 20 | 107,8 | 6,90 | 4,92 | 1,20 | 11,5 |
| 50 | 91,8 | 3,13 | 0,90 | 1,04 | 2,6 |
| 75 | 90,1 | 2,70 | 1,35 | 1,78 | 2,3 |
| 100 | 88,5 | 1,07 | 1,50 | 0,57 | 1,7 |

References

Verdon E, Couedor P, Sanders P. 2007. Multi-residue monitoring for the simultaneous determination of five nitrofurans (furazolidone, furaltadone, nitrofurazone, nitrofurantoine, nifursol) in poultry muscle tissue through the detection of their five major metabolites (AOZ, AMOZ, SEM, AHD, DNSAH) by liquid chromatography coupled to electrospray tandem mass spectrometry—In-house validation in line with Commission Decision 657/2002/EC. Anal Chim Acta. 586(1–2):336-347

Lega F, Contiero L, Biancotto G, Angeletti R. 2013. Determination of thyreostats in muscle and thyroid tissues by QuEChERS extraction and ultra-performance liquid chromatography tandem mass spectrometry. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 30(6):949-957