Zinc migration into liver pâté and pâté with ham packaged in black colored polypropylene containers

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Abstract. The European food contact legislation requires verification of compliance for migration of substances from plastics food contact materials with both overall and specific migration limits. To do so in this study, migration tests were carried out using food simulant and food under well specified time and temperature conditions. A total of 28 samples of liver pâté or pâté with ham packaged in black polypropylene containers were examined for the presence of Zn according to Serbian regulation after 14 months’ storage from their production dates, and data were compared to results obtained by testing empty, unused containers according to EU legislation. Elevated Zn concentrations of 161.27-327.54 mg/kg (above the maximum prescribed limit of 100 mg/kg) were registered in pâté from zones in contact with the polypropylene packaging, while pâté in zones not in contact with packaging had lower Zn levels (9.98-15.08 mg/kg).

1. Introduction
Migration is a global term to describe a net mass transfer of chemical substances from a packaging material into the food [1]. Migration of substances from food contact materials to food must not occur in amounts that endanger human health. Migration tests can be divided into two distinct phases. The first is the migration exposure itself, i.e. the contact of the plastic material to the food simulant. The second is the quantification of the migrants by chemical analysis for specific migration and by gravimetric analysis for overall migration [2].

In many cases, migration is governed by a mass transfer process called diffusion that can be described by Fick’s law:

$$ \frac{\partial c}{\partial t} = D_p \frac{\partial^2 c}{\partial x^2} $$

where C is the concentration of migrant in the food contact material or article (P) at Time t and at distance x from the origin of the x-axis, and $D_p$ is the diffusion coefficient in the food contact material or article. In practice, a monolayer, homogenous, plastic food contact material or article (P) can be regarded as a film or sheet of finite and constant thickness ($D_p$) being in contact with food of finite volume (VF) and contact area (A). It is assumed that at the time of bringing the migrant into contact...
with food \((t = 0)\), the migrant is distributed homogeneously in the plastic \([3]\). Therefore, the key parameter necessary for migration modeling is the diffusion coefficient of the migrant in the plastic. One problem is that for some metals, knowledge on actual release of metal ions is limited, and hence, no inorganic compounds, metals, metal oxides etc. are eligible to have their migration into the packaged food product modeled. \([4]\) To aid industry and national food authorities, the Council of Europe has suggested specific release limits (SRLs), and for Zn, this is \(= 5\)mg/kg \([5]\). Zn is a nutrient that plays many vital roles in mammal bodies \([6]\), but that in higher concentrations can be toxic. The main role of food simulants is that they can be used for estimating migration/release, but food simulants will not always provide correct estimation of the release of metal ions from a food contact material to food.

The main goal of this case study (Zn migration into meat pâté products) was to demonstrate in practice the above-mentioned claim that the results of the previous testing of the packaging should be taken with some reservation if the intention is to keep the product for a prolonged time.

2. Materials and methods

2.1. Samples

Empty, unused polystyrene packaging containers, 90 ml (Figure 1) were filled to the top with and exposed to olive oil for 10 days at 60°C. The analysis of metals was performed according to SS EN ISO 17294-1,2 (modified) and EPA method 200.8 (modified) using ICP-SFMS technique. These data were supplied by a laboratory based in Sweden.

![Figure 1. Examined container for pâté made from cast black colored polypropylene](image1.png)

![Figure 2. Discoloration of liver pâté surface that had been in contact with the polypropylene container](image2.png)

A total of 28 samples of liver pâté or pâté with ham were examined for the presence of Zn (Table 2) at 14 months after their production dates and storage at up to 25°C, when visible discoloration of the liver pâté surfaces was visible via experimental, periodic visual controls. For analysis, samples of the two different pâtés were taken in an identical manner from two different zones (inner layer of pâté not in contact with the packaging and pâté layer with visible discoloration in contact with the packaging container wall). Pâté samples were homogenized and then prepared for instrumental analysis.

The Zn content of these test portions of the two kinds of pâté were obtained in the manner described above for the packaging. Briefly, test portions were mineralized by adding 5 mL of 65% HNO\(_3\) and 1.5 mL of 30% H\(_2\)O\(_2\) (Merck, Darmstadt, Germany). Microwave assisted digestion was performed in START D (Milestone, Italy). The following temperature program was used (default food program): 5 min – ramp up from room temperature to 180 °C; 10 min hold at 180 °C; 15 min cooling. After cooling, digested samples were quantitatively transferred into 100 mL polypropylene volumetric flasks and diluted to volume with ultrapure water. The analysis was performed by inductively-coupled plasma mass spectrometry (ICP-MS) using the instrument iCap Q (Thermo Scientific, Bremen,
Germany), equipped with a collision cell and operating in kinetic energy discrimination (KED) mode. The isotope measured was zinc ($^{66}$Zn). Torch position, ion optics, and detector settings were adjusted daily using a tuning solution (Thermo Scientific Tune B), in order to optimize measurements and to minimize possible interferences. For the quantitative analysis of the samples, a five-point calibration curve (including zero) was constructed in the concentration range of 0.1–2.0 mg/L for $^{66}$Zn. An additional line of the peristaltic pump was used for online introduction of a multi-element internal standard ($^{45}$Sc – 10 ng/mL; $^{71}$Ga – 2 ng/mL). Concentrations of measured isotope were corrected for response factors of both higher and lower mass internal standards using the interpolation method. The quality of the analytical process with respect to the accuracy and precision was assessed by analysis of the standard reference material SRM 1577c (NIST, Gaithersburg, MD, USA). Reference material was prepared in a random manner during microwave digestion of each sample batch and run at the beginning, in the middle and at the end of each sample list. This method is accredited by Accreditation Body of Serbia (ATS) according to ISO/IEC 17025:2006, Accreditation number: ATS 01-049.

2.2. Statistics
Statistical analysis was performed by the MINITAB software package, version 16.0. Concentrations were expressed as mean values, standard deviations, median and range of minimum to maximum. Before choosing the appropriate statistical analysis, four different individual distribution identification methods were conducted as the first step to identify the native distribution. Probability plots were used for visualization of graphical goodness of fit test. The one way ANOVA and post hoc Tukey’s honestly significant difference test (Tukey’s HSD) were used to test the significance of differences between means for the groups of samples analyzed. The differences were considered statistically significant when the p-value was less than 0.05. Interval plots were used to illustrate both a measure of central tendency and variability of the data.

3. Results and discussion
The contents of specific metals that migrated into the simulant, olive oil, from the new, previously unused polypropylene containers in which product can be packaged are presented in Table 1. Initially, there were no significant concentrations of the measured metals registered in the simulant used (olive oil).

Table 1. Heavy metal concentrations in olive oil simulant filled in previously unused polypropylene containers and stored for 10 days at 60°C

| Simulant | Metal content (mg/kg simulant) |
|----------|--------------------------------|
|          | Zn | Cd | Cr | Hg | Pb |
| Olive oil| <0.4 | <0.002 | <0.04 | <0.004 | <0.008 |

Zinc concentrations (mg/kg) measured in different zones of canned liver pâté or pâté with ham (packaged in black polypropylene containers, hot sealed with lids made from aluminum foil, and originally top covered with [apparent] polypropylene black lids, net weight 90g) are shown in Table 2.
Table 2. Zinc concentrations in different zones of two pâté products packaged in PP containers

|                          | Declared expiry date (months) | Duration of storage after production (months) | N  | Zn* (mg/kg) | Standard deviation | Minimum Zn level (mg/kg) | Maximum Zn level (mg/kg) | Median Zn level (mg/kg) | Range of Zn levels (mg/kg) |
|--------------------------|-------------------------------|-----------------------------------------------|----|-------------|---------------------|--------------------------|--------------------------|--------------------------|---------------------------|
| Liver pâté mass (inner layer) | 36                            | 14                                            | 8  | 16.2**      | 6.4                 | 9.9                      | 28.7                     | 13.9                     | 18.7                      |
| Ham pâté mass (inner layer)   | 36                            | 14                                            | 6  | 29.5**      | 3.5                 | 23.7                     | 34.6                     | 29.7                     | 10.9                      |
| Liver pâté mass in contact with (container wall) | 36                            | 14                                            | 8  | 201.6**     | 53.6                | 161.3                    | 327.5                    | 185.3                    | 166.3                     |
| Ham pâté mass in contact with (container wall) | 36                            | 14                                            | 6  | 55.2**      | 8.6                 | 41.6                     | 70.4                     | 54.4                     | 28.9                      |

* number of examined samples; ** maximum permissible concentration of Zn in the content is 100 mg/kg [7]< ** means that do not share a letter are significantly different (p<0.05)

Visual observation of the content was performed after removing the sealed aluminum lid from the top of the containers and vertical deliberation of the pâté onto a white plate. Pâté with ham, with production dates close to those of liver pâté and packaged in the same polypropylene containers, had no visible discoloration, but this was not the case for liver pâté. Inspection of liver pâté revealed spotted greyish-black discolorations in the form of a continual stripe, approximately 5-6 mm wide, on surfaces that were in contact with the polypropylene packaging material, immediately below the internal rim of the container. Some liver pâté samples also showed visible discolorations on the surface that had been in contact with the internal refracted bottom rim. Zn concentrations higher than maximum prescribed value (100 mg/kg as defined by Serbian regulation) were measured in liver pâté portions obtained from the zones of visible discoloration (Table 2).

The examined liver pâté samples had significantly higher (p<0.05) Zn concentrations (range from 161.27 to 327.54 mg/kg) in the product mass taken from contact zones with visible discoloration, than in the product mass taken from zones of product without discoloration (9.98-15.08 mg/kg). Pâté with ham had significantly (p<0.05) lower Zn levels both in contact (41.6-70.4 mg/kg) and in non-contact zones (23.7-34.6 mg/kg) than did liver pâté. There were no statistically significant differences (p>0.05) of measured Zn concentrations in pâté with ham derived from contact and non-contact zones.

Taking into account the identical expression of visible discoloration (geometric locus) and the much higher Zn concentrations (10 to 20 times) in contact zones than in non-contact zones, in the case of liver pâté, it can be strongly concluded that migration of Zn occurred from the curved container’s wall material into the product during the 14 months’ storage period. Such migration was also measured, but was not visibly detectable in pâté with ham, in which Zn concentrations were lower (maximum measured Zn concentration in the contact zone was 70.4 mg/kg) (Table 2). Lower Zn concentrations in product mass taken from contact zones could be explained due to the different formulation of this product in comparison to liver pâté [8]. Pâté with ham could contain a higher content of animal protein, and the stability of such a meat emulsion is better.
The very nature of the reaction is based on the fact that, for example, zinc stearate is an extremely polar molecule, which, when embedded in otherwise nonpolar polypropylene, leads to electrical incompatibility between the additive and the plastic material, in this case, polypropylene [9]. This assertion, first of all, takes into account that during the production of polypropylene containers, zinc-containing compounds (e.g., zinc stearate or zinc oxide) are used as additives, which have multiple roles in the process of making the packaging, such as: facilitating the removal of molds, lubrication, regulation of acidity of molten polymeric mass, improvement of pigment dispersion and dyeing, and effect on polymer rheology [10]. This Zn added to the packaging material can specifically migrate to the inner surface of the plastic material, which is, in turn, in contact with the food product.

The multiple-fold increased Zn content in the zone of product discoloration could also be associated with container geometry-related variables (thicknesses, contact area topography, volumes) as well as time and temperature of product storage (discoloration was visible where product had contact with packaging curvature) [11]. Numerous factors influence the speed of this reaction, especially pH and the nature of the food. If we suppose the pâté mass is highly polar in nature (since it contains a degree of fat), the conditions for the migration of polypropylene additives are created.

It should also be borne in mind that the pâté itself contains vegetable oil, added to improve pâté lubricity, and so the food itself becomes an exceptionally good migration medium (for example, olive oil serves as a migration simulator according to European Directive 85/572/EEC, so-called simulant D) for components used in plastic container production [12]. It is recognized that the use of metallic stearates is associated with problems with the discoloration of foods in contact with polymeric material [13]. The chemical examination of empty, unused containers (Figure 1) did not determine any malfunction of the finished product according to prescribed food simulants, accredited methods of packaging material testing or legal MRL limits (Table 1).

A general conclusion from this study is that results from migration/release testing with food should prevail over results from tests with approved simulants. Therefore, if analyses are available with the food itself and also in simulants, the results from analysis with food will be considered as documentation for packaging material compliance. Consequently, it was deemed necessary to establish guidelines to regulate these situations. The guidelines take into account the fact that some inorganic compounds, metals, metal oxides, metal salts, etc., are not eligible for modeling [14].

Results from this case study also indicate the elevated Zn concentrations in liver pâté might endanger human health if the total content of the metal in food exceeds the health-based guideline values or brings about an unacceptable change in the composition of the food or a deterioration of its organoleptic characteristics.

As a final recommendation, it is possible to review the defined shelf life and storage conditions taking into account the nature of packaging and the specific product formulation.

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