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Overall Migration and Kinetics of Release of Antioxidant Compounds from Citrus Extract-Based Active Packaging

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ABSTRACT: Overall migration (OM) tests were conducted on an antioxidant active packaging prepared by coating plasma pretreated and untreated polyethylene terepthalate (PET) trays with a citrus extract. The release of antioxidant compounds into food simulants was measured to permit their subtraction from OM values in line with active packaging legislation. The results demonstrated the compliance of the packaging with the limit for OM for plastic material in contact with food. The validity of the procedure for OM in aqueous food simulants was questioned, with the loss of volatile compounds during evaporation of the simulant resulting in an underestimation of total compounds released. The study showed a total release of 75% of the citrus extract coating into water and 25% into oil, which decreased to 45 and 12.5%, respectively, following plasma pretreatment of the trays.

KEYWORDS: *active packaging, migration, polyethylene terephthalate tray, carboxylic acids, flavanones*

INTRODUCTION

Lipid oxidation is one of the main degradation processes in fresh and processed meats and leads to the formation of off-flavors, with adverse effects on organoleptic properties.[1] This process can be controlled by the addition of antioxidant compounds that scavenge the free radicals associated with initiation and propagation of lipid oxidation.[2] In recent years, active packaging has been developed in which antioxidant compounds are introduced into the packaging to protect fatty foods from oxidation.[3,4] With regard to the choice of antioxidants, there has been particular interest in extracts from fruits and herbs because of their strong antioxidant efficacy and consumer preference for compounds of natural origin over synthetic substances.[5−7] Polyphenols are the most common constituents of these natural extracts with their antioxidant activity being attributed to a concomitant effect of free radical scavenging and iron chelating.[8−10]

Active packaging materials prepared with different plant extracts such as oregano, salvia, and rosemary have been shown to be effective in preventing spoilage processes in different food products.[11−15] In our laboratory, an antioxidant active packaging surface capable of reducing lipid oxidation in cooked turkey meat has been developed.[16,17] The packaging consists of polyethylene terephthalate (PET) trays coated with a commercial citrus extract, composed of a mixture of carboxylic acids and flavanones, previously used as a biocide agent.[18] However, the introduction of a new packaging, such as this, to the food market requires adherence to legislation on the use of active and intelligent packaging and legislation on materials in contact with foods, which apply limits to the migration of substances from the packaging polymer into the food.

The overall migration (OM) test aims to quantitate the amount of packaging polymer (monomer, oligomers, and plastic additives) that is released from the bulk plastic to the food in contact with the packaging, to ensure compliance of the plastic material with the legislation requirements for OM.[19] Standardized OM tests are conducted with food simulants that represent the characteristics of a real food at specific conditions of time and temperature and represent the real use of the product.[20] However, the possible release of compounds into food, inherent to the mechanism of action of active packaging, is somehow at odds with the principle of the inertness of the packaging. Until now, the European Union (EU) legislation on active packaging[21] has adhered to the procedures for overall migration with the obvious specification that the intentionally added compounds, which can be released into food (citrus extract in this case), should be excluded from the migration result. Nevertheless, these compounds have to be authorized; for example, they can be food additives,[22] or otherwise covered in a positive list within the food contact legislation. Although active packaging that reduces food spoilage using active substances coated on the packaging surface has promising potential applications for the food industry, its use in the market is limited, and only a few studies on its compliance with the legislation on materials in contact with food can be found in the literature.[23,24] This study is a first evaluation of the applicability of migration testing, in accordance with the principles established for active packaging for food contact,[21] to a citrus extract-coated packaging material.

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MATERIALS AND METHODS

Safety. Boron trifluoride and potassium hydroxide are corrosive and cause burns.

Reagents. Pentane (98%), heptane (≥96%), methanol (HPLC grade), cyclohexane (99.9%), boron trifluoride (14% in methanol), potassium hydroxide (87.8%), glyceryl triheptadecanoate (99%), naringin (≥95%), neohesperidin (≥90%), rhoifolin (apigenin 7-*O*-neohesperidoside, ≥97%), hesperidin (≥97%), and neohesperidin dihydrochalcone (≥95%) were purchased from Sigma-Aldrich (Steinheim, Germany). Citrus extract (trade name Citrox), containing a mixture of flavonoids and carboxylic acids, was obtained from Citrox Biosciences (Kimbolton, Cambridgeshire, UK). The composition of the citrus extract was determined in our laboratory: naringin, 1.5 g; neohesperidin, 1.4 g; citric acid, 25.8 g; and salicylic acid, 36.3 g in 100 g.[25] Ultrapure water was obtained from a Direct Q Millipore water purifier (Vimodrone, Italy). Sunflower oil was purchased from SALOV S.p.A. (Lucca, Italy).

Samples. Holfeld Plastics (Arklow, Co. Wicklow, Ireland) supplied recycled PET trays (100 × 150 × 25 mm). The antioxidant packaging was prepared by nebulizing a methanolic solution of the citrus extract (CIT) on the interior surface of the tray, using a Burgener Mira Mist high-pressure nebulizer (Burgener Research Inc., Ontario, Canada) following the procedure described in a previous study.[16] This packaging is hereafter referred to as PET-CIT. The effect of an air atmospheric pressure plasma jet activation of the PET using a PlasmaTreat jet system (Plasmatreat GmbH, Steinhagen, Germany) was also investigated.[26] This polymer activation of the surface used pretreatment conditions similar to those described previously.[17] The citrus extract-coated PET trays activated in this way are denoted PET-PL-CIT. Optical profilometry and focused ion beam/scanning electron microscope analysis, performed in a previous study,[17] showed that the components of the citrus extract formed a coating visible as a continuous film on the surface of the PET.

Any residual methanol on PET, PET sprayed with methanol, PET-PL, PET-PL sprayed with methanol, PET-CIT, or PET-PL-CIT trays was quantified using headspace analysis with an Agilent 6890 gas chromatograph and MSD 5973 mass spectrometer. A J&W Scientific DB-Wax column (30 m × 0.25 mm, 0.5 μm film thickness) was used with helium at 1.3 mL min^{-1} as carrier gas. Trays were cut into duplicate 0.5 g pieces and placed in 22 mL vials for headspace analysis with an incubation time of 20 min at 100 °C. Methanol was quantified in single ion monitoring mode using target ion m/z 32. The instrument was calibrated with methanol at six concentrations, giving a regression coefficient (R) >0.99 in the range of 0.15−1.58 μg of methanol in the headspace and an LOQ of 0.1 ng g^{-1}. The amounts of methanol present on the surface of the trays in contact with food simulants (sunflower oil and water, see below) were 0.41 ± 0.01, 0.84 ± 0.16, 1.08 ± 0.16, 0.98 ± 0.12, 29.68 ± 4.81, and 30.02 ± 3.64 μg for PET, PET sprayed with methanol, PET-PL, PET-PL sprayed with methanol, PET-CIT, and PET-PL-CIT, respectively. Methanol is included in the positive list of substances permitted for food contact materials, and the amounts detected on the surface are well below the applicable specific migration limit (the generic value of 60 mg/kg food).[19]

For the experimental procedures, the PET-CIT and PET-PL-CIT trays were compared to untreated trays (PET) and plasma-treated PET trays with no citrus extract application (PET-PL), respectively.

Overall Migration from Packaging in Sunflower Oil. The test was carried out according to the official procedure for OM from packaging into oil.[27] Three samples of each tray type (PET, PET-PL, PET-CIT, PET-PL-CIT) were weighed (initial weight), filled with sunflower oil (240 mL) to 5 mm from the rim, covered with a glass plate, and stored in a convection incubator at 5 °C for 10 days. One tray for each treatment was stored without food simulant to evaluate any weight loss due to the evaporation of volatile compounds. Sunflower oil was chosen as food simulant because of its higher stability at low temperature compared to olive oil. It also complies with the requirements for simulants for testing plastic materials and articles in contact with foodstuffs under the new Regulation EU No. 10/2011.[19] Following the 10 day exposure to the tray surface, the sunflower oil was transferred into a glass beaker and the remaining oil allowed to drain from the trays. Any adhering oil was removed by gently pressing the tray between filter papers. The trays were weighed (weight after exposure) and cut into pieces, and the adhering oil was removed by Soxhlet extraction for 7 h, using pentane spiked with glyceryl triheptadecanoate as extraction solvent. The Soxhlet extraction cycle was repeated to ensure the complete removal of the adhering oil. The extracted oil was redissolved in heptane and methylated with potassium hydroxide (KOH) and boron trifluoride (BF_3) at boiling temperature under reflux. The adhering oil was quantified by analyzing the methyl esters by gas chromatography with a flame ionization detector, using methyl heptadecanoic acid as internal standard. The OM expressed in milligrams was calculated as follows: initial weight of trays − (weight of the trays after exposure to food simulant − weight of adhering oil). The results for OM for the trays coated with the citrus extract (PET-CIT and PET-PL-CIT) were then corrected by subtracting the total amount of the coated compounds released into the food simulant (oil), quantified by the extraction and analytical procedures described in the kinetic study below. The mean OM value of three samples of each kind of tray (expressed in mg dm^{-2}) was considered as the final result for the migration test.

Overall Migration from Packaging in Water. The test was carried out according to the official procedure for OM from packaging in aqueous simulants[28] on the same number and type of trays as described for OM in oil. Water was chosen as food simulant because of the high compatibility of the active components with water. The trays were filled to 5 mm from the rim with deionized water (240 mL), covered with a glass plate, and stored in a convection incubator at 5 °C for 10 days. Following exposure, 200 mL of water was removed from the trays and transferred into a 250 mL glass beaker. Aliquots of 50 mL were poured consecutively into preweighed glass dishes (200 mL), and the water was slowly evaporated on a heating plate. When the simulant was almost completely evaporated, the dishes were placed in an oven at 110 °C for 30 min to complete the process, and the glass dishes were than weighed again. The OM expressed in milligrams was calculated by applying the following formula: (final weight of glass dish − initial weight of glass dish)/200 mL × total volume of the food simulant (240 mL). The mean value of the three samples of each kind of tray (expressed in mg dm^{-2}) was considered as the final result for the migration test.

Kinetic Study on the Release of Specific Coating Constituents during the Overall Migration Test in Sunflower Oil and Water. Using the trays containing the simulants as described in the sections above, the kinetics of release of citrus extract compounds was studied. This involved removing 0.5 g aliquots of the food simulants from each tray at intervals over 10 days and analyzing the content of citrus extract compounds therein. The reduction in the simulant volume due to the sequential sampling over the duration of the kinetic experiments amounted to <10% of the total volume, and its effect on the migration values could thus be considered negligible (considering the uncertainty of the measurement itself). For sunflower oil, six aliquots of 0.5 g of oil were collected, after gentle agitation with a pipet tip, from each tray after 1 and 6 h and 1, 2, 3, 7, and 10 days. After 10 days, an additional six 0.5 g aliquots of oil were collected from the residual simulant and mixed with a glass rod, having been transferred from each tray into a beaker (as described above for OM in sunflower oil). For each set of six aliquots, three aliquots were analyzed for carboxylic acids and three for flavanone content using the methods described below. The sum of the values calculated for all compounds in the residual simulant at day 10, together with the content calculated in the aliquots of simulant collected over the course of the kinetic study, gave the total release of citrus extract from the coating of each tray.

For the kinetics of migration into water, six 0.5 mL aliquots of food simulant were collected, following gentle agitation with a pipet tip, from each tray after 10 and 30 min; 1, 2, and 5 h; and 1, 2, 3, 7, and 10 days. An additional six aliquots of water were collected from the residual simulant transferred into a glass beaker (as described above for OM in water) and thoroughly mixed. For each set of six aliquots, three

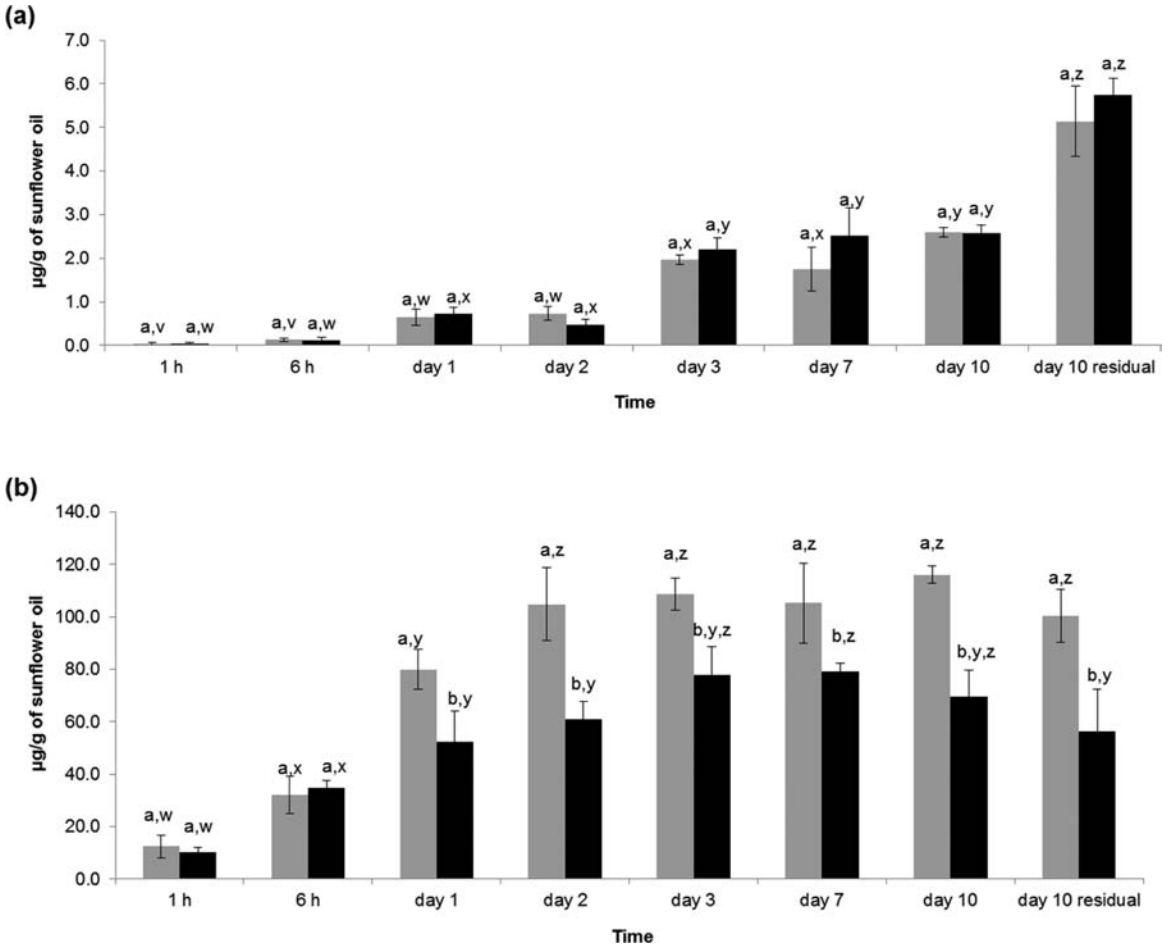

Figure 1. Kinetics of release of (a) citric acid and (b) salicylic acid into sunflower oil from PET-CIT trays (gray bars) and PET-PL-CIT trays (black bars). Within each storage time, bars with different letters (a, b) are significantly different due to the treatment. Within each treatment, bars with different letters (w–z) are significantly different due to the storage time. Bars at 1 and 6 h and days 1, 2, 3, 7, and 10 represent values obtained following analysis of three 0.5 g aliquots taken at these time points. The day 10 residual bars represent values obtained following triplicate analysis of the residual oil at day 10.

aliquots were analyzed for carboxylic acids and three for flavanone content using the methods described below. The sum of the values calculated for all compounds in the residual simulant at day 10, together with the content calculated in the aliquots of simulant collected over the course of the kinetic study, gave the total release of citrus extract from the coating of each tray.

Analysis of Citric Acid and Salicylic Acid by HPLC. For the quantitation of citric and salicylic acid in oil, 0.5 mL of water was added to 0.5 g of simulant which was mixed by vortex and centrifuged at 2500 rpm for 10 min. An aliquot of the separated aqueous phase was directly injected onto the HPLC column. A recovery study performed on oil samples spiked with known amounts of each compound gave values of 98.4 ± 3.7% for citric acid and 62.7 ± 3.8% for salicylic acid. The final results were corrected using the recovery values. For the quantitation of the two carboxylic acids in the aqueous simulant, the aliquots collected during the kinetic were directly injected onto the HPLC column. The analysis of carboxylic acids was performed on an Agilent HPLC 1200 (Palo Alto, CA, USA) equipped with an Agilent Eclipse XDB C18 (250 mm × 0.4 mm, 0.5 μm particle size) column. The mobile phase was 0.1% phosphoric acid and methanol in a gradient from 99.5:0.5 to 10:90 in 20 min at 25 °C. The detector was a diode array monitoring at wavelengths of 208 and 260 nm for the quantitation of citric acid and salicylic acid, respectively. The instrument was calibrated with six-point calibration curves for the two carboxylic acids prepared in ultrapure water. Aliquots of the two simulants from noncoated trays (PET and PET-PL) were analyzed as blanks. The mean values for the analysis of three samples represented the concentration of the compounds at each kinetic sampling time. The correlation coefficients (R) calculated for the calibration curve for the carboxylic acids were >0.99 in concentration ranges of 0.5–50 μg/mL for citric acid and 25–200 μg/mL for salicylic acid, and LOQs of 1.8 and 0.7 μg g^{-1} were calculated for citric and salicylic acid, respectively.

Analysis of Flavanone Content by Ultrahigh-Performance Liquid Chromatography–Mass Spectrometry (UPLC-MS). Flavanones are minor constituents of the citrus extract,[25] and an UPLC-MS method was developed to obtain the high analytical sensitivity necessary for their detection. For their quantitation in oil, an aliquot of 0.5 mL of methanol, spiked with neohesperidin dihydrochalcone as internal standard, was added to 0.5 g of simulant. The samples were mixed by vortex and centrifuged at 2500 rpm for 10 min. The methanol phase, which was separated from the oil phase, was analyzed by UPLC-MS. A recovery study performed on oil samples spiked with known amounts of each compound gave values of 94.6 ± 4.9% for naringin and 96.2 ± 3.2% for neohesperidin. The final results were corrected using the recovery values. For the quantitation of flavanones in the aqueous simulant, aliquots of simulant collected were mixed with 50% methanol spiked with the internal standard, to enhance the instrument response, and injected directly onto the UPLC-MS. Aliquots of the two simulants from noncoated trays were analyzed as blanks. The mean values of analysis of the three aliquots collected for the flavanone analysis represented the concentration of the compounds at the corresponding kinetic time. The analysis of flavanones was performed with a Waters Acquity UPLC (Milford, MA, USA), coupled to a triple-

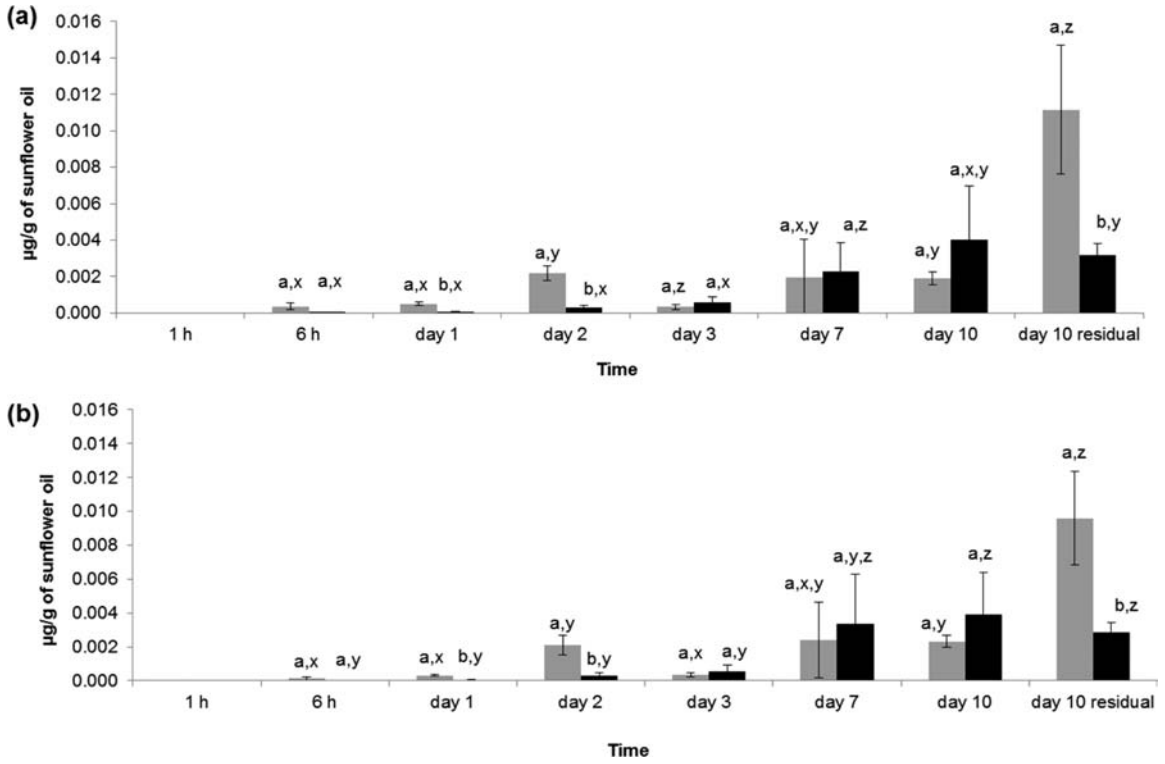

Figure 2. Kinetics of release of (a) naringin and (b) neohesperidin into sunflower oil from PET-CIT trays (gray bars) and PET-PL-CIT trays (black bars). Within each storage time, bars with different letters (a, b) are significantly different due to the treatment. Within each treatment, bars with different letters (x–z) are significantly different due to the storage time. Bars at 1 and 6 h and days 1, 2, 3, 7, and 10 represent values obtained following analysis of three 0.5 g aliquots taken at these time points. The day 10 residual bars represent values obtained following triplicate analysis of the residual oil at day 10.

quadrupole mass-spectrometer Xevo TQ Waters-Micromass (Manchester, UK). An Acquity BEH C18 (50 mm × 2.1 mm, 1.7 μm particle size) column (Waters Corp., Milford, MA, USA) was used with 0.1% formic acid and 0.1% methanolic formic acid as mobile phases at a gradient from 80:20 to 10:90 in 10 min, at 25 °C. The analysis was performed in negative electrospray ionization mode (ESI^-) and multiple-reaction monitoring mode (MRM) at 500 °C desolvation temperature and 1000 L/h desolvation gas flow. Transitions from m/z 579 to 151, at 54 V (cone voltage) and 36 V (collision energy), were monitored for naringin, from m/z 609 to 164 at 56 V and 58 for neohesperidin, from m/z 577 to 269 at 54 and 36 V for rhoifolin, from m/z 609 to 164 at 36 and 52 V for hesperidin, and from m/z 611 to 166 at 56 and 56 V for neohesperidin dihydrochalcone. The instrument was calibrated with methanolic solutions of pure standards of flavanones at six concentrations, in the same range as the samples. The calibration curve of flavanones gave a regression coefficient (R) >0.99 in a concentration range of 0.50–50 ng/mL, and the LOQ was calculated as 0.1 ng g^{-1} for each flavanone.

Statistical Analysis. Each experiment was conducted in triplicate, and results were expressed as the mean value of the three replicates. Statistical analysis of the data was performed using SPSS. Analysis of variance (ANOVA) was performed on the data, and Duncan's test was used to identify significant differences between samples.

RESULTS AND DISCUSSION

Kinetics of Release in Sunflower Oil. The kinetics of release from PET-CIT trays showed an increase in citric acid release over the first three days of contact with sunflower oil (Figure 1a). Significantly higher values were found at day 1 compared to 6 h ($p < 0.05$) and at day 3 compared to day 2 ($p < 0.01$). There was no significant difference in citric acid release between days 3 and 7. At day 10, the PET-CIT release was significantly higher ($p < 0.05$) compared to day 7, whereas the PET-PL-CIT was unchanged. No differences were found between the citric acid released from PET-CIT and PET-PL-CIT trays at any of the sampling times. The concentration of citric acid in the residual sunflower oil at day 10 (day 10 residual) was significantly higher than that found in the 0.5 g aliquot taken at day 10. The low solubility of citric acid in sunflower oil (log octanol/water partition coefficient = −1.7)[29] probably led to a very slow diffusion into the food simulant, with a higher concentration in the boundary layer in contact with the tray so that the 0.5 g aliquots taken periodically, despite the gentle agitation prior to sampling, may not have been representative of citric acid dispersed throughout the simulant. The kinetic results may therefore be an underestimation of true migration, whereas the real release could be represented by the value calculated at day 10 residual. The amounts of citric acid released by day 10 were 5.14 ± 0.81 and 5.75 ± 0.37 μg g^{-1} oil for the PET-CIT and PET-PL-CIT trays, respectively.

The kinetics of release of salicylic acid was faster than that of citric acid; this may be attributed to differences in the molecular structure of the two compounds, which suggests a higher solubility in oil for the salicylic acid (log octanol/water partition coefficient = 2.2).[30] The PET-CIT trays showed a complete release after day 2, with 1 h significantly lower than 6 h ($p < 0.05$), 6 h lower than day 1 ($p < 0.01$), and day 1 lower than day 2 ($p < 0.05$) (Figure 1b). The PET-PL-CIT tray release was complete at day 3, with significantly lower values at 1 h compared to 6 h ($p < 0.01$), 6 h compared to day 1 ($p < 0.05$), and day 2 compared to day 3 ($p < 0.05$). The release from PET-CIT trays was significantly higher than PET-PL-CIT trays ($p < 0.01$), even at day 1. The amounts released after 10 days

Table 1. Migration from Trays into Sunflower Oil over 10 Days of Storage at 5 °C[a]

	PET-CIT	PET-PL-CIT	PET	PET-PL
A: raw data of weight loss (mg)	31.08 ± 1.78	20.69 ± 2.64	8.53 ± 0.96	3.99 ± 1.28
B: citrus extract release (mg)	22.79 ± 1.88	13.56 ± 3.32		
A − B: migration (mg)	8.28 ± 0.46	7.13 ± 0.84	8.53 ± 0.96	3.99 ± 1.28
overall migration value[b] (mg/dm^2)	3.69 ± 0.20	3.18 ± 0.37	3.81 ± 0.43	1.78 ± 0.57

[a]A, raw data of weight loss from the trays following removal of simulant (sunflower oil); B, sum of citric acid, salicylic acid, naringin, neohesperidin, hesperidin, and rhoifolin released. [b](A − B)/(tray area, 2.24 dm^2).

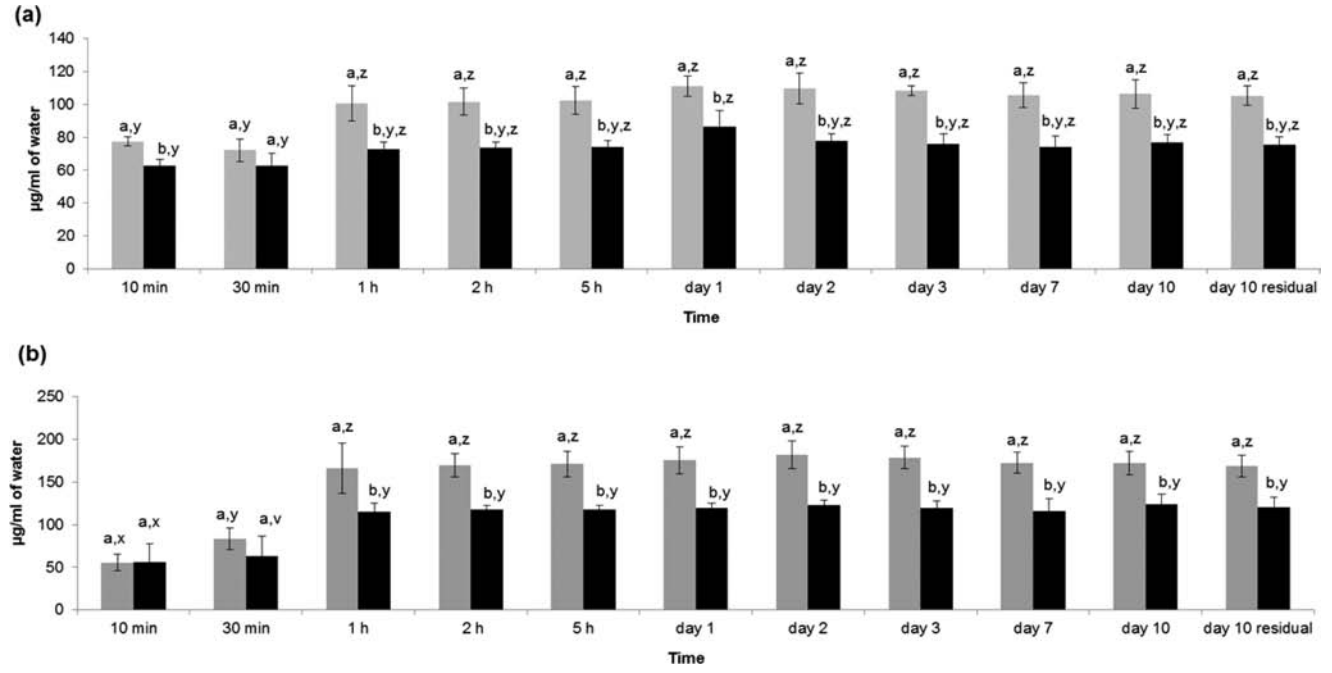

Figure 3. Kinetics of release of (a) citric acid and (b) salicylic acid in water from PET-CIT trays (gray bars) and PET-PL-CIT trays (black bars). Within each storage time, bars with different letters (a, b) are significantly different due to the treatment. Within each treatment, bars with different letters (x–z) are significantly different due to the storage time. Bars at 10 and 30 min; 1, 2, and 5 h; and days 1, 2, 3, 7, and 10 represent values obtained following analysis of 0.5 g aliquots taken at these time points. The day 10 residual bars represent values obtained following analysis of the residual oil at day 10.

were 100.41 ± 10.10 μg g^{-1} oil for the PET-CIT trays and 56.19 ± 16.18 μg g^{-1} oil for PET-PL-CIT trays, respectively. The lower content of the flavanones, compared to the carboxylic acids, in the citrus extract and their lower solubility in oil, due to the high polarity of their sugar moiety, were probably the cause of a low and uneven release for naringin (Figure 2a) and neohesperidin (Figure 2b). The kinetics of release of the two flavanones showed very similar trends, with the maximum release reached only at day 7. The concentration of flavanones released from PET-CIT trays into the residual oil at day 10 was significantly higher ($p < 0.01$) than the value obtained from the 0.5 g aliquot taken at day 10, probably due to an uneven distribution of the flavanones in the simulant during sampling, over the course of the kinetic study. Significant differences ($p < 0.01$) in flavanone release between PET-CIT and PET-PL-CIT trays were found at days 1, 2, and 10 in the residual oil. Traces of rhoifolin and hesperidin were found in the sunflower oil only after 7 days of exposure to food simulant.

The efficacy of the packaging in reducing lipid oxidation in cooked meat samples has already been demonstrated, with a higher antioxidant activity being associated with the PET-PL-CIT trays compared to the PET-CIT trays.[16] However, it is unclear whether the citrus coating exerts its antioxidant properties at the packaging surface/meat interface or whether release of antioxidant compounds from the citrus-coated packaging surface into the meat is necessary for the antioxidant effect. The results of the kinetic study shown here demonstrate release of the antioxidant compounds into a food simulant. The average total amounts of citric acid released into oil after 10 days were 1.89 ± 0.16 mg for the PET-CIT trays and 1.93 ± 0.07 mg for the PET-PL-CIT trays. The total amounts of salicylic acid released were 20.90 ± 2.02 mg for the PET-CIT trays and 11.63 ± 3.23 mg for the PET-PL-CIT trays. These values represented 23 and 12% of the total amount of the citrus extract coated on the PET-CIT and PET-PL-CIT trays, calculated as 98.01 ± 16.04 and 109.89 ± 0.96 mg, respectively, on the basis of the measurement of the amount expressed in milligrams per square centimeter for each coating performed on small PET disks.[17] The PET-PL-CIT tray, which is characterized by a higher antioxidant activity,[17] showed a lower release of the compounds, thus suggesting that a contribution to the antioxidant effect by the molecules immobilized onto the polymer surface cannot be excluded. Further investigations are currently underway to better clarify the relative importance of the two potential mechanisms of the antioxidant effect.

Overall Migration in Sunflower Oil. The differences between the weight of the trays before and after exposure to the

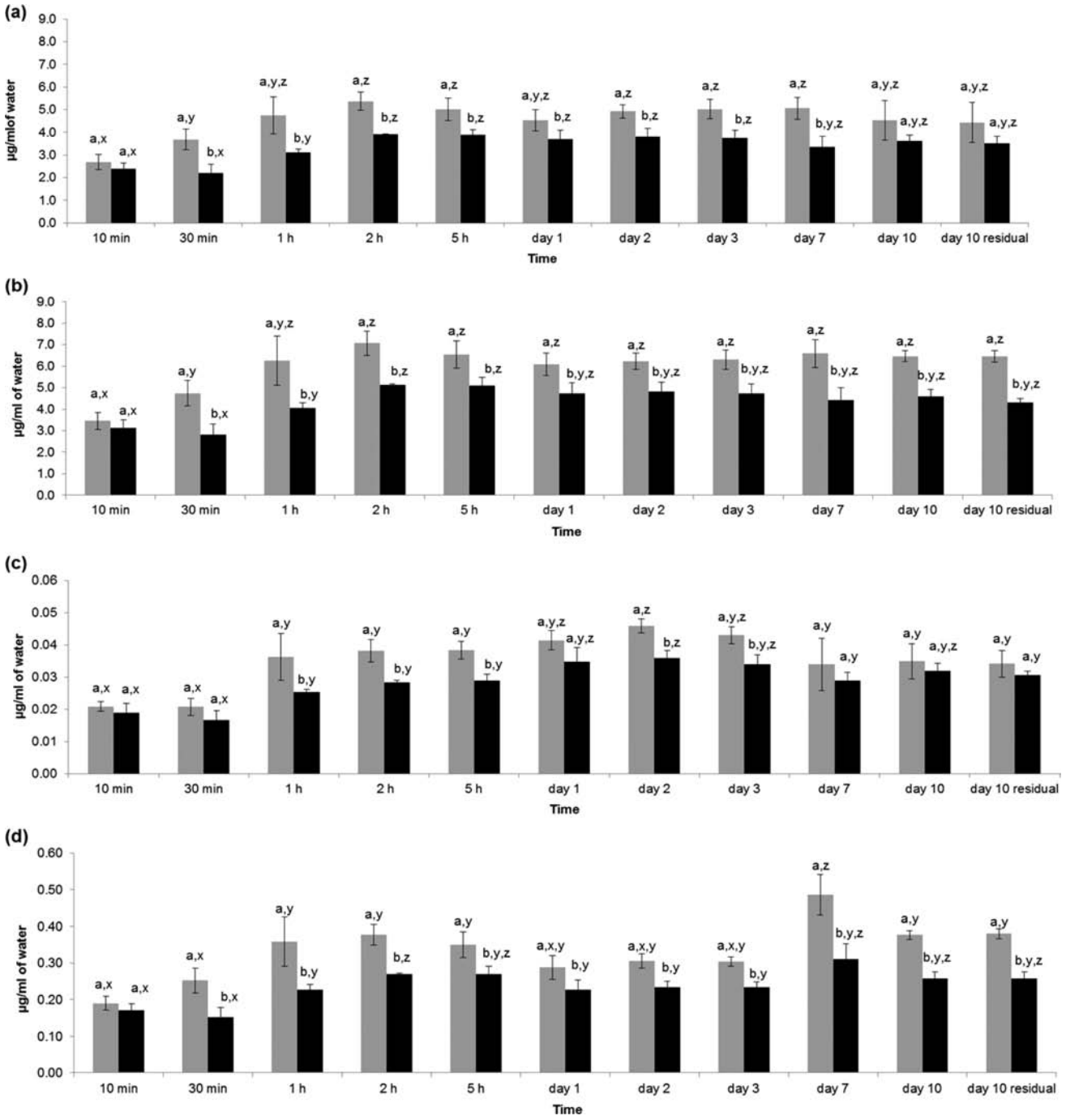

Figure 4. Kinetics of release of (a) naringin, (b) neohesperidin, (c) rhoifolin, and (d) hesperidin in water from PET-CIT trays (gray bars) and PET-PL-CIT trays (black bars). Within each storage time, bars with different letters (a, b) are significantly different due to the treatment. Within each treatment, bars with different letters (x–z) are significantly different due to the storage time. Bars at 10 and 30 min; 1, 2, and 5 h; and days 1, 2, 3, 7, and 10 represent values obtained following analysis of 0.5 g aliquots taken at these time points. The day 10 residual bars represent values obtained following analysis of the residual oil at day 10.

food simulant were reported as weight loss in milligrams (Table 1). For the PET-CIT and PET-PL-CIT trays, the weight loss was corrected for the citrus components released (total amounts of citric acid, salicylic acid, naringin, neohesperidin, hesperidin, and rhoifolin released from the coating) to give OM expressed in milligrams. The OM, expressed in milligrams per square decimeter, was calculated as the ratio of the amount released (in mg) to the surface area of the trays (2.24 dm^2) in contact with the food simulant. The results of the OM for the uncoated trays (PET and PET-PL) showed that the plasma pretreatment reduced the migration of the components from the passive part of the packaging. This suggests that modification of the structure of the PET polymer after plasma pretreatment probably serves as a barrier to migration of passive components during the exposure to the food simulant. Another explanation for the lower OM values found in PET-PL trays compared to PET trays could be the removal of small residual components from the external layer of the trays induced by the plasma pretreatment, which reduced the migration from the polymer. These differences were not

present in the coated trays, PET-CIT and PET-PL-CIT, where it seems that the passive components migrated to similar degrees, 3.69 and 3.18 mg dm^{-2}, respectively. Interactions between the PET surface and the citrus extract coating, in the PET-PL-CIT trays, may have led to the loss of the barrier effect introduced by the plasma pretreatment. It is worth noting that the presence of unknown minor components of the citrus extract, which were not considered in the calculation of citrus extract release, could have led to a slight overestimation of the OM results for the coated trays. Nevertheless, the results of OM were below the limit of the legislation for plastic material in contact with food of 10 mg dm^{-2},[19] for both the uncoated and coated trays.

These results of the OM in sunflower oil showed the suitability of the migration test used for the components of the passive part of the packaging. However, Regulation EC 450/2009 stipulates that the amount of released active substances must be subtracted from the value obtained by the classical migration test. Therefore, this in practice means that the quantitation of the intentionally released compounds must also be performed to properly evaluate compliance with OM limits, making the experimental work more laborious. The release of multiple compounds, as in the present study, further increases the complexity of the procedure. In addition, the methods for the quantitation of the intentionally released substances require interlaboratory harmonization to ensure standardization and reliability of the analyses associated with the OM measurement.

Kinetics of Release in Water. Preliminary results showed a fast release of citrus extract compounds in water due to their high polarity; for this reason, the kinetics of migration into water was specifically studied during the first hours of exposure. The maximum release of citric acid was obtained after 1 h of exposure to the simulant for the PET-CIT trays (Figure 3a). Samples taken at 30 min were significantly lower than those at 1 h ($p < 0.05$). In contrast, the release of citric acid from PET-PL-CIT trays was immediate, with similar values between all of the time points except 10 and 30 min, which were significantly lower than at day 1 ($p < 0.01$). The release from PET-CIT trays was always significantly higher than PET-PL-CIT ($p < 0.05$), except at 30 min, and the total amounts of citric acid released during the 10 days were 25.33 ± 1.29 and 18.25 ± 0.99 mg for PET-CIT and PET-PL-CIT trays, respectively.

With regard to the kinetics of salicylic acid release, the release from PET-CIT trays was complete after 1 h, with significantly lower values at 10 min compared to 30 min ($p < 0.05$) and at 30 min compared to 1 h ($p < 0.01$) (Figure 3b). For the PET-PL-CIT trays a significant difference was found only between 30 min and 1 h ($p < 0.01$). The total amounts of salicylic acid released during the 10 days were 40.35 ± 2.76 mg for PET-CIT trays and 28.79 ± 2.43 mg for PET-PL-CIT trays.

Migration trends similar to those observed for the carboxylic acids were found for the kinetics of flavanone release (Figure 4). The release was complete at 1 h for all of the compounds, with significantly lower release from PET-PL-CIT trays compared to PET-CIT trays. The total amounts of release of flavanones during the 10 days were 1.08 ± 0.19 mg in PET-CIT trays and 0.85 ± 0.06 mg in PET-PL-CIT trays for naringin and 1.55 ± 0.05 mg in PET-CIT trays and 1.05 ± 0.04 mg in PET-PL-CIT trays for neohesperidin. The amounts of rhoifolin and hesperidin were approximately 0.01 and 0.1 fold lower than those of naringin and neohesperidin, respectively.

The kinetic study demonstrated a faster release of the citrus extract components into water compared to sunflower oil, with the maximum concentrations already obtained after 1 or 2 h. Furthermore, the final release into water was higher than into oil, with total releases of citrus extract of 68.40 ± 4.6 mg for PET-CIT trays and 49.00 ± 3.6 mg for PET-PL-CIT trays, which represented, respectively, 67 and 54% of the total amount of the coating. A higher adhesion to the surface of the coating obtained after plasma pretreatment, already shown above by the lower release of the citrus extract components into sunflower oil, was confirmed. The higher total release into water was primarily due to a much higher release of flavanones and especially of citric acid, whereas the difference in release of the salicylic acid component between the two simulants was less pronounced. These results suggest a different affinity of the compounds for the different food simulants, depending on their chemical properties. In particular, the polarity of citric acid, due to its three carboxylic groups, and of flavanones, due to their sugar moiety, would confer a higher hydrophilicity compared to salicylic acid. In this perspective, the water content of a meat packaged in the trays should facilitate the release of flavanones and citric acid, the activity of which in the prevention of lipid peroxidation is well documented.[8,31−34] Similarly, salicylic acid was also released in a higher amount into water than into sunflower oil, and its ability to increase antioxidant enzyme activity in fruits[35] and to reduce oxidative stress in biological samples[36] has also been shown.

Overall Migration in Water. The total weight loss from the trays was calculated as the difference in weight between the glass dishes before and after evaporation of the simulant (water) (Table 2). The values of OM for PET and PET-PL were <1 mg and, therefore, lower than the legislative limit used for plastics of 10 mg dm^{-2}.[19] The values of citrus extract release reported in the table were calculated by multiplying the sum of the amount in micrograms per milliliter at day 10 of all the components of the citrus extract, by the final volume of the simulant. The calculation also included the amount of citrus extract contained in the 0.5 mL aliquots collected during the 10 days of the kinetic study. The difference between weight loss of the trays and citrus extract released gave the OM of the coated trays (PET-CIT and PET-PL-CIT). The negative results obtained for the OM suggest an underestimation of the total amount of the antioxidant compounds released into the water following the evaporation step. A further experiment conducted on an aqueous solution of the two main compounds of the citrus extract, citric and salicylic acid, at the same concentrations as at day 10 demonstrated that evaporation of the citrus extract components occurred during the drying of the glass dishes at 110 °C. In particular, the residual amount of citric acid in the glass dishes after the evaporation step was <10% of its

Table 2. Migration from Trays into Water over 10 Days of Storage at 5 °C[a]

	PET-CIT	PET-PL-CIT	PET	PET-PL
A: raw data of weight loss (mg)	34.53 ± 3.3	22.16 ± 3.5	<1	<1
B: citrus extract release (mg)	68.40 ± 4.6	49.00 ± 3.6		
A − B: migration (mg)	−33.86 ± 2.49	−26.84 ± 1.16	<1	<1

[a]A, raw data of weight loss from the trays following removal and drying of simulant (water); B, sum of citric acid, salicylic acid, naringin, neohesperidin, hesperidin, and rhoifolin released from trays.

initial amount. Therefore, although the availability of uncoated trays permitted us to assess the compliance of the packaging material to the OM limit of 10 mg dm^{-2} and it is reasonable to assume that the antioxidant coating should not affect the inertness of the material, the current procedure for the calculation of the OM in aqueous simulants is not always applicable, and alternative techniques have to be developed.

In conclusion, the procedures for testing migration from packaging material in contact with food were successfully applied to PET trays coated with citrus extract using sunflower oil as food simulant. The uncoated PET trays were in compliance with the limit of the OM for plastic material in contact with food,[19] with a reduction of the migration induced by plasma pretreatment. The analytical procedures developed for the quantitation of the citrus extract were fit for purpose and permitted correction of the OM values for the amount of released compounds. The kinetics of release of the antioxidant compounds showed a faster and higher release into water than into oil and a significant effect of the plasma pretreatment in enhancing the adhesion of the compounds to the polymer surface. Although the release of the citrus extract quantified in both food simulants was high, it does not imply a concern for the safety of the packaging. Currently, for the EU market, both citric acid and salicylic acid are authorized for plastics in contact with foods equally as monomer or additive according to Regulation (EU) 10/2011.

Citric acid is already allowed as a food additive under Regulation (EC) 1333/2008 for fresh minced meat in quantities "quantum Satis". Salicylic acid is not included in the list of additives in Regulation (EU) 1129/2011. Therefore, for it to be used in the composition of an active coating for food contact where it is released in the food as preservative (under Regulation (EC) 450/2009), it needs to be subjected to an evaluation by the European Food Safety Authority to obtain authorization as a food additive in an amendment of Regulation (EC) 1333/2008.

A fast release of the antioxidant coating does not reduce the efficacy of the packaging because the citrus extract constituents can perform their antioxidant activity also after their release. Similarly, the slower release induced by the plasma pretreatment could ensure a prolonged efficacy of the antioxidant active packaging. Moreover, the procedures for OM testing are developed to represent worst-case conditions, and it is reasonable to expect a lower release of these components in real foods. It is worth noting that extensive studies on the stability of the active coating demonstrated that the coating substances were not lost during 6 months of storage, and the antioxidant efficacy remained unaltered during that period.[17]

On the basis of the results, the active packaging under investigation complies with the regulation on OM from plastic material in contact with food, and it can be considered as safe for future commercial use.

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Notes

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