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Electrospray Ionization-Mass Spectrometry Analysis Reveals Migration of Cyclic Lactide Oligomers from Polylactide Packaging in Contact with Ethanolic Food Simulant

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Electrospray ionization-mass spectrometry analysis revealed rapid migration of cyclic oligomers from polylactide (PLA) packaging when stored in contact with 96% ethanol. The mass losses in contact with water, 3% acetic acid, 10% ethanol and isooctane were 3 to 5 times smaller and no migration of cyclic oligomers was observed. The presence of cyclic oligomers in the original PLA films and their solubility in ethanol, thus, explains the rapid mass loss for PLA in contact with ethanolic food simulant. On prolonged ageing no further mass loss was observed in 96% ethanol, whereas mass loss in aqueous food simulants increased because of hydrolysis of PLA matrix or the cyclic oligomers to water-soluble linear products. The mass losses were generally somewhat smaller for the stereocomplex material compared with the poly-L-lactide materials. Similar trend was observed for solvent uptakes, which is easily explained by the higher degree of crystallinity and stronger secondary interactions in the stereocomplex material. The use of ethanol as a fatty food simulant for PLA materials could, thus, lead to overestimation of the overall migration values.

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KEY WORDS: polylactide; food packaging; migration; food simulant; ESI-MS

INTRODUCTION

Although all food contact materials must maintain the quality of the food and protect it from harmful external influences, they must also be safe during storage and not release migrants to the food inside the package. European community regulations state that 'food contact materials should not transfer to foodstuffs any of their constituents in quantities that could endanger human health or cause deterioration in the organoleptic characteristics of the foodstuff'.[1] The migration from the packaging materials can be stimulated or promoted by food-polymer interactions, chemical affinity of migrant to a contacting phase and external conditions.[2] Because of the increased waste problems and reduction of petroleum-resources, new investigations are focused on biodegradable packaging materials from renewable sources. In this respect, one of the most attractive materials is polylactic acid or polylactide (PLA), which in many aspects is comparable with PET.[3,4]

Polylactide is approved by the Food and Drug Administration for use as food contact material. In packaging applications, PLA is suitable for e.g. cups, bottles, films and containers. Applications include rigid thermoforms such as trays and lids, bottles for water, milk or oil, clamshells for food

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packaging, shrink wraps for packaging, candy and flower wraps, disposable salad cups and cold drink cups.[5,6] PLA coatings can also be used to increase the water barrier properties of paperboard.[7] More thermally and hydrolytically stable, PLA materials can be obtained through stereocomplexation after blending poly(D-lactide) and poly(L-lactide).[8]

Degradable packaging is more sensitive to external conditions and heat, moisture and other factors could cause degradation resulting in formation of degradation products.[9] Chemical hydrolysis of PLA leads to lactic acid and its oligomers,[10,11] whereas thermal degradation during e.g. processing or polymerization could lead to formation of cyclic degradation products.[12] Cyclic oligomers can also be added as plasticizers. Lactic acid is included in the list of authorized monomers in the European Directive 2002/72/EEC with no restrictions.[13] However, cyclic lactide and linear or cyclic oligomers are not included in this list. Although the hydrolysis, biodegradation and thermal degradation of PLA have been rather extensively studied, there are only a few studies where migration from PLA to different food simulants was evaluated. Lactic acid, lactide and lactoyl lactic acid were identified as migrants in short-term trials between 0.5 and 24 h at 26–43°C.[14] The overall migration was highly dependent on the original level of lactide in the samples. The remaining monomer rests as well as the chemical changes induced during polymerization and processing are, thus, crucial parameters for controlling the properties of the PLA materials.[15] Migration of lactic acid, lactide and in some cases oligomers from different PLA materials to water, 4% acetic acid and 20% ethanol at 20–95 C was also shown.[16] The migrants were quantified by overall migration and by alkaline decomposition of all migrants to lactic acid. PLA was found to be relatively stable at 40 C, however at higher temperatures the migrant levels increased rapidly.

The purpose of our study was to further investigate the interactions and stability of PLA in contact with different food simulants by following mass loss, changes in molecular mass and more detailed identification of the migrants by electrospray ionization-mass spectrometry (ESI-MS). Especially, we aimed to explain the unexpectedly high overall migration values reported previously for PLA in contact with ethanol.[17]

EXPERIMENTAL

Food simulants

Five different food simulants were used. Water (LC grade, Fisher Scientific) represented aqueous food having pH higher than 4.5, 3% acetic acid (Acros Organics) represented aqueous food having pH lower than 4.5 and 10% ethanol (v/v) (Merck) was used instead of alcoholic food. Furthermore, 96% ethanol (VWR) and isooctane (LC grade, Merck) represented oily food.

Samples

Two different poly-L-lactide (PLLA) films were prepared by dissolving 8 g of PLA granules from Hycail (HM1011), denoted PLLA-H and NatureWorks (5200 D), denoted PLLA-N into 40 ml chloroform (HPLC grade-Fisher Scientific). The solutions were then solution casted on 18.5 cm diameter glass petri plates. A poly-L-lactide/poly-D-lactide stereocomplex film was prepared in the same way from granules obtained from Hycail and denoted PLA-S. The chloroform was first evaporated at room temperature, and then the remaining traces were evaporated by drying several days in vacuum oven at room temperature. After drying, the average thicknesses of the PLLA-H, PLLA-N and PLA-S were 0.25 mm, 0.23 mm and 0.43 mm, respectively. Even though same amount of each PLA was solution casted on the glass plate of same size, the stereocomplexation during drying caused shrinking of the PLA-S film resulting in thicker films. Glass transition temperatures and degree of crystallinity for the prepared films were determined by differential scanning calorimetry, which were as follows $T_g = 33°C$ and $X_c = 10\%$ for PLLA-H, $T_g = 29°C$ and $X_c = 13\%$ for PLLA-N and $T_g = 44°C$ and $X_c = 45\%$ for PLA-S. According to sample specifications density of PLLA-N and PLLA-H was 1.24 g/cm^3, whereas PLA-S is expected to have slightly higher density typically around 1.27 g/cm^3.[18]

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Storage stability

Samples were cut into small pieces weighting approximately 11 mg. These pieces were put into clear glass vials. A 10 ml of food simulant was added into each vial. The vials were sealed with butyl/PTFE aluminium caps and stored at 25°C for 7 or 50 days. After the ageing samples were withdrawn from the simulants and dried in the vacuum oven (~0 Pa at 25°C) until constant weight. Mass losses for each sample were calculated according to:

$$\text{Mass loss } \% = \frac{W_{\text{initial}} - W_{\text{dry}}}{W_{\text{initial}}} \times 100\% \quad (1)$$

where W_{initial} is the first weight of the samples before immersion into the simulant and W_{dry} is the dry weight after storage and drying under vacuum. The solvent uptake for each sample was calculated according to Equation 2

$$\text{Solvent uptake } \% = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \times 100\% \quad (2)$$

where W_{wet} is the wet weight of the sample immediately after the storage. Overall migration for each sample was calculated according to Equation 3

$$\text{Overall migration} = \frac{W_{\text{initial}} - W_{\text{dry}}}{2 \times \left(\frac{W_{\text{initial}}}{\delta \times L}\right)} = \text{mg/cm}^2 \quad (3)$$

where δ is the density of the sample and L is the thickness of sample.

Size exclusion chromatography

A Verotech PL-GPC 50 Plus system (Agilent Technologies, Sweden) equipped with a PL-RI Detector and two PolarGel-M Organic (300 × 7.5 mm) columns from Varian was applied to follow the possible changes in molecular weight and polydispersity index. The samples were injected with a PL-AS RT Autosampler for PL-GPC 50 Plus and THF was used as mobile phase (1 ml/min, 35°C). The calibration was created using polystyrene standards with a narrow molecular weight distribution.

Electrospray ionization-mass spectrometry

Finnigan LCQ ion trap mass spectrometer (Finnigan, USA) was used to identify the migrants in food simulants. The food simulants were evaporated to dryness, and the residues were diluted with water/methanol (2 : 1, v/v) (LC grade, Fisher Scientific). The solutions were filtered through PTFE filters (4 mm × 0.45 μm) and continuously infused by the instrument syringe pump at a rate of 5 μl/min. Capillary temperature and voltage were set to 175°C and 5.00 V, respectively.

RESULTS AND DISCUSSION

The effect of aqueous, alcoholic, acidic and fatty food simulants on the storage stability of PLA films was evaluated by determining mass loss, solvent uptake and changes in molecular weight. To understand the interactions between different food simulants and PLA, the low molecular weight compounds that migrated from the materials to food simulants were identified by ESI-MS.

The effect of food simulants on the mass loss

Mass losses for the three PLA materials after 7 days of storage in different food simulants at room temperature are shown in Figure 1. The mass losses increased in the order PLA-S < PLLA-N < PLLA-H. The mass losses for PLLA-N and PLLA-H in water, 10% ethanol, 3% acetic acid and isooctane were around 5%, whereas the mass loss for PLA-S stereocomplex in the same solvents were around 3–4%. However, the

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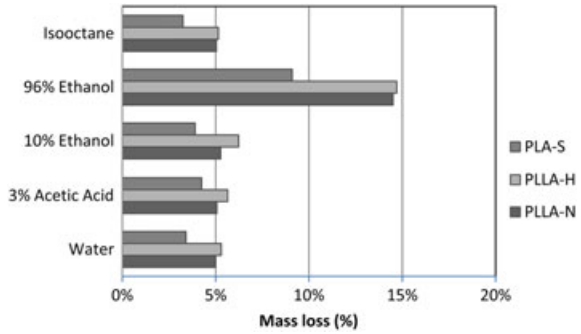

Figure 1. Mass loss after 7 days of storage in contact with aqueous, alcoholic, acidic and fatty food simulants.

mass losses during storage in 96% ethanol were approximately three to five times larger compared with the mass losses in the other simulants. To see the effect of prolonged contact, ageing was continued for up to 50 days (see Figure 2). The mass losses increased for the samples aged in the aqueous simulants indicating some hydrolysis of the polyester chains leading to formation of water-soluble products. However, interestingly on prolonged ageing, no additional mass losses were observed in 96% ethanol. The overall migration values calculated as mg/cm^2 sample were generally between 0.05 and 0.11 mg/cm^2 after 7 days and between 0.07 and 0.19 mg/cm^2 after 50 days depending on the food simulant. However, after ageing in 96% ethanol, somewhat higher values between 0.19 and 0.24 mg/cm^2 were measured. These values can be considered acceptable as lactic acid is used as additive in food industry such as meat, poultry and fish products, beverages, confectionery, dairy products, savoury flavours productions. The general overall migration limit from food contact material to the food stuff according to the European Commission directive 2002/72/EC is 10 mg/dm^2, or 0.1 mg/cm^2.[13]

Solvent uptakes

After 7 days, there were generally no large differences in the absorption of the different food simulants by polylactide (Figure 3). The exception was the uptake of 96% ethanol, which was in correlation with the mass loss results higher compared with the other food simulants. Considering the different PLA materials solvent uptake was generally somewhat smaller in the case of PLA-S, intermediate for PLLA-H and largest for PLLA-N. However, PLLA-H absorbed more 96% ethanol than the other materials.

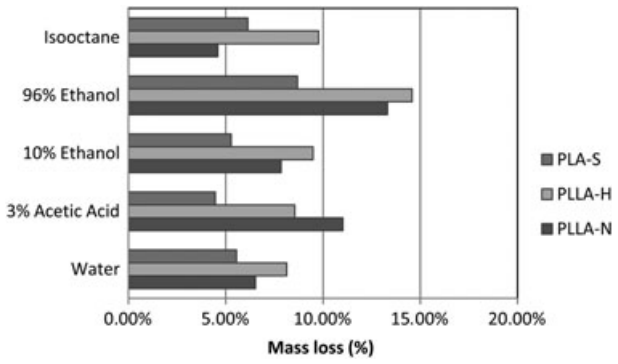

Figure 2. Mass losses after 50 days of storage in contact with aqueous, alcoholic, acidic and fatty food simulants.

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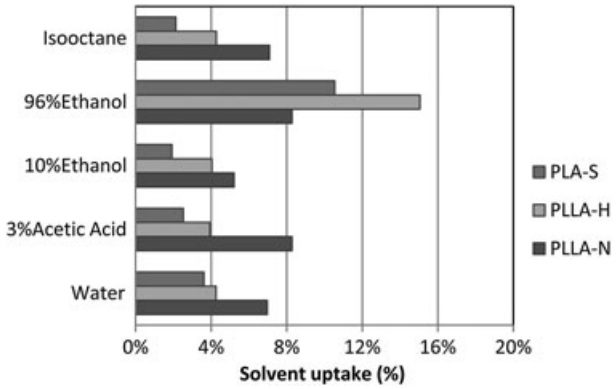

Figure 3. Food simulant uptakes by the polylactide materials after 7 days.

Electrospray ionization-mass spectrometry analysis of migrants

To further explore the reason for the high mass loss observed in contact with ethanol, the low molecular weight compounds that had migrated from the polymer films into the different food simulants were identified by ESI-MS. No significant peaks were detected after 1 week of storage when the food simulant was water, 10% ethanol, 3% acetic acid or isooctane. However, after 1 week storage of the PLAs (PLLA-N, PLLA-H and PLA-S) in 96% ethanol homologous series of cyclic lactic acid oligomers were detected. The most dominant oligomers contained between 6 and 17 lactic acid units. The ESI-MS mass spectra showing the migrants from PLA-S is shown as an example in Figure 4. The cyclic lactic acid oligomers appear at $m/z = 23 + 72 \times n$. In addition, some potassium adducts of cyclic oligomers appearing at $m/z = 39 + 72 \times n$ were detected. In an earlier study, we have shown that these cyclic oligomers are already present in the unaged PLLA-H, PLLA-N and PLA-S films.[19] However, they are not soluble in the water-based simulants or in isooctane, which explains the originally lower mass losses in these simulants. On prolonged storage in aqueous simulants, hydrolysis of PLA and the cyclic oligomers can take place leading to increasing mass losses as a function of storage time. In previous studies where the same PLAs were subjected to long-term ageing in water at 37°C and 60°C linear lactic acid and its oligomers up to 13–14 repeating units were identified as migrants into water.[20]

Molecular weight changes

Figure 5 represents weight average molecular weights for PLLA-H and PLLA-N after 1 week storage in contact with different food simulants. It was not possible to run size exclusion chromatography

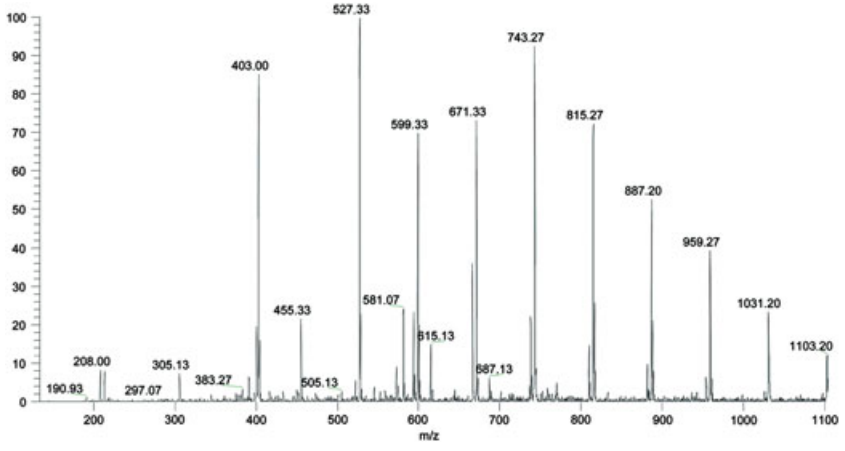

Figure 4. Positive ESI-MS spectra showing migrants from PLA-S after 1 week storage in contact with 96% ethanol.

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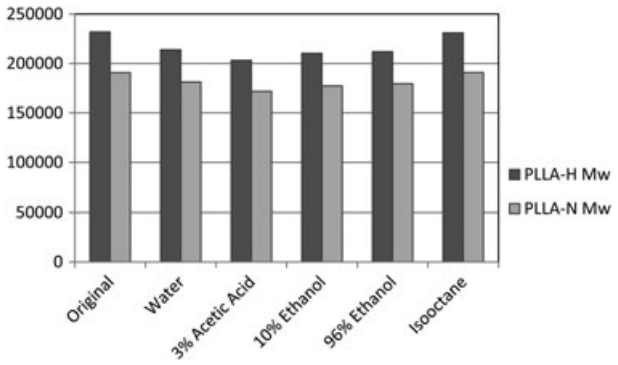

Figure 5. Weight average molecular weight of the samples after 1 week storage in contact with food simulants.

analysis of PLA-S because of the insolubility of the stereocomplex in chloroform. Same trend can be observed both for PLLA-H and PLLA-N. Both materials show 7–10% molecular weight decrease in contact with water, 10% ethanol and 96% ethanol. The molecular weight decrease was a few percent higher in contact with the acidic food simulant, 3% acetic acid, which could catalyse the hydrolysis of ester groups. No changes could be observed during ageing of PLLA-H in isooctane.

CONCLUSION

Storage of PLA at room temperature in contact with 96% ethanol led to a rapid mass loss of approximately 15% already after 7 days at room temperature. Interestingly prolonged ageing in 96% ethanol did not significantly increase the mass loss. Storage in contact with water, 3% acetic acid, 10% ethanol or isooctane resulted only in 3–5% mass loss after the same period. Longer ageing time of 50 days in the aqueous food simulants increased the mass loss to 8–12%. As expected the mass losses were somewhat lower for PLA stereocomplex compared with PLLA-H and PLLA-N. The fast original mass loss in 96% ethanol was explained by ESI-MS analysis of the migrants into the food simulants. Analysis of the 96% ethanol food simulant revealed a homologous series of cyclic lactide oligomers, whereas the low molecular weight products in the other food simulants were under detection limits. The rapid initial mass loss in 96% ethanol is thus explained mainly by migration of cyclic oligomers present in the original samples. These cyclic oligomers are not soluble in the aqueous food simulants leading to lower initial mass loss. On prolonged ageing in the 96% ethanol, there was no significant hydrolysis of the PLA materials, and no further mass loss was observed. However, during ageing in aqueous food simulants, water-soluble hydroxyacids were formed by hydrolysis, and mass loss increased with time. Because cyclic oligomers are commonly present in PLA materials, the use of ethanol as a fatty food simulant for PLA materials might lead to considerable overestimation of the overall migration values.

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