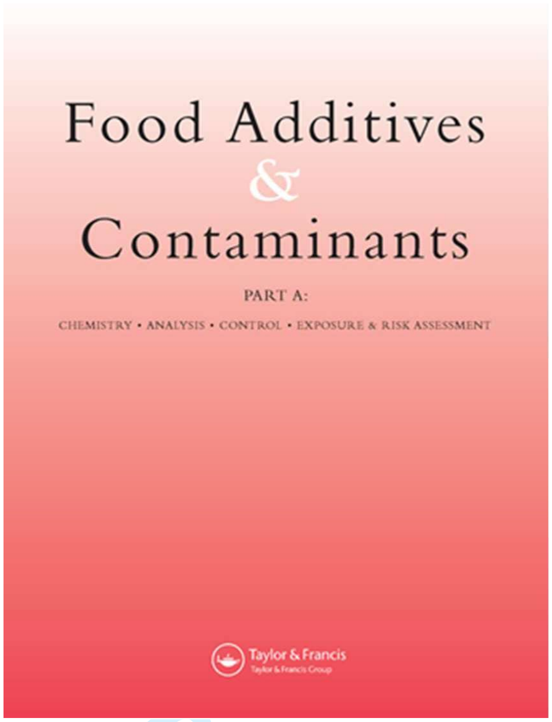

Study on the release of nanocarbon particles of different size and shape from nanocomposite poly(lactic) acid film into food simulants

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Complete List of Authors:	Velichkova, Hristiana; Institute of Mechanics, Bulgarian Academy of Sciences Kotsilkov, Stanislav; Institute of Mechanics, Bulgarian Academy of Sciences Ivanov, Evgeni; Institute of Mechanics, Bulgarian Academy of Sciences Kotsilkova, Rumiana; Institute of Mechanics, Bulgarian Academy of Sciences Gyoshev, Stanislav; Institute of Information and Communication Technologies, Bulgarian Academy of Sciences Stoimenov, Nikolay ; Institute of Information and Communication Technologies, Bulgarian Academy of Sciences Vitanov, Nikolay ; Institute of Mechanics, Bulgarian Academy of Sciences
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Abstract:	Poly(lactic) acid (PLA) film with 2 wt % mixed nanocarbons of graphene and carbon nanotubes in ratio 1:1 with traces of impurities of fullerenes and carbon black was produced by layer to layer deposition and hot pressing. The release of nanocarbons from the film was studied as varying time-temperature conditions and simulant solvents. Migrants in solvents were examined with laser diffraction analysis and transmission electron microscopy. The film integrity and the presence of migrants on the film surfaces were visualized by scanning electron microscopy. The partial dissolution of PLA film in the solvents was confirmed by swelling test and differential scanning calorimetry. Nanocarbon migrants were not detected after migration test at 40oC for 10 days. However, high temperature migration test at 90oC for 4 hours and above provoked a release of few layer graphene nanoplatelets in the ethanol, acetic acid and oil-based food simulants. In contrast, only traces of short carbon nanotubes, fullerenes and carbon black were observed to release in the most aggressive acetic acid solvent. Obviously, the enhanced molecular mobility at temperatures above the glass transition and the partial dissolution of PLA ingredients by the simulant solvents facilitate the diffusion processes. Moreover, shape, size, entanglement, and concentration of nanoparticles play significant role in the release process. Asymmetric graphene platelets (100-1000 nm) easily migrate dispersed in the dissolved PLA organic substances. While fibrous MWCNTs formed entangled network on the film surfaces as the PLA polymer matrix dissolve, which prevent their release into food simulants. Thus, small amounts of carbon nanotubes may release fixed with organic substances in large agglomerates (> 1 μm), due to polymer dissolution. The fullerenes and carbon black nanoparticles (10-20 nm) were of insufficient concentration in polymer therefore their migration was low or undetectable. The total amount of detected migrants is below overall migration limit.

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Hristiana Velichkova[1], Stanislav Kotsilkov[1], Evgeni Ivanov[1], Rumiana Kotsilkova[1]*,
Stanislav Gyoshev[2], Nikolay Stoimenov[2], Nikolay K. Vitanov[1]

[1]*Institute of Mechanics, Bulgarian Academy of Sciences, Acad. G. Bonchev Street, Bl.4, Sofia, Bulgaria*
[2]*Institute of Information and Communication Technologies, Bulgarian Academy of Sciences,*
Acad. G. Bonchev Street, Bl.2, Sofia, Bulgaria

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*Corresponding author: Rumiana Kotsilkova
Institute of Mechanics, Bulgarian Academy of Sciences,
Acad. G. Bonchev street, Block 4, 1113 Sofia, Bulgaria
E-mail: kotsilkova@imbm.bas.bg

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Abstract:

Poly(lactic) acid (PLA) film with 2 wt % mixed nanocarbons of graphene and carbon nanotubes in ratio 1:1 with traces of impurities of fullerenes and carbon black was produced by layer to layer deposition and hot pressing. The release of nanocarbons from the film was studied as varying time-temperature conditions and simulant solvents. Migrants in solvents were examined with laser diffraction analysis and transmission electron microscopy. The film integrity and the presence of migrants on the film surfaces were visualized by scanning electron microscopy. The partial dissolution of PLA film in the solvents was confirmed by swelling test and differential scanning calorimetry. Nanocarbon migrants were not detected after migration test at 40°C for 10 days. However, high temperature migration test at 90°C for 4 hours and above provoked a release of few layer graphene nanoplatelets in the ethanol, acetic acid and oil-based food simulants. In contrast, only traces of short carbon nanotubes, fullerenes and carbon black were observed to release in the most aggressive acetic acid solvent. Obviously, the enhanced molecular mobility at temperatures above the glass transition and the partial dissolution of PLA ingredients by the simulant solvents facilitate the diffusion processes. Moreover, shape, size, entanglement, and concentration of nanoparticles play significant role in the release process. Asymmetric graphene platelets (100-1000 nm) easily migrate dispersed in the dissolved PLA organic substances. While fibrous MWCNTs formed entangled network on the film surfaces as the PLA polymer matrix dissolve, which prevent their release into food simulants. Thus, small amounts of carbon nanotubes may release fixed with organic substances in large agglomerates (> 1 μm), due to polymer dissolution. The fullerenes and carbon black nanoparticles (10-20 nm) were of insufficient concentration in polymer therefore their migration was low or undetectable. The total amount of detected migrants is below overall migration limit.

*Key words***:** migration, PLA, graphene, nanocarbons, food simulants, swelling, dissolution, laser diffraction, TEM, SEM, DSC.

Introduction

Applications of nanomaterials in active and intelligent food packaging are rapidly becoming a commercial reality and already make up the largest share of the current and short-term predicted nanofood market (Chaudhry et al. 2008). Incorporation of graphene and carbon nanotubes (ranging from 1-2 wt.%) in polymers is a promising approach for food packaging applications leading to several benefits, such as improved mechanical and antimicrobial properties, and also able to trace and monitor the condition of food during transport and storage (Ivanov & Kotsilkova 2015). Graphene and its derivatives are identified as a powerful candidate for gas-barrier materials because perfect graphene do not allow diffusion of small gases through its plane (Du & Cheng 2012, Cui et al. 2016). Recently, poly(lactic) acid (PLA) has received attention as a sustainable, biocompatible, biodegradable material with good mechanical and optical properties (Jamshidian et al. 2010). However, the large-scale use of PLA as packaging material is hindered by its poor gas barrier properties compared to commodity polymers that may be improved by nanocomposites technology (Wu et al. 2014). Incorporation of graphene and carbon nanotubes in PLA is expected to enhance not only gas-barrier properties but also increase mechanical strength and improve thermal properties when properly dispersed in a polymer matrix (Huang et al. 2014). The use of 2D graphene nanoplatelets as a surface coating instead of bulk additives overcomes common issues related to dispersion of nanofiller in a polymer matrix, and gives a clear advantage in preserving the mechanical properties of the bulk polymer (Pierleoni et al. 2016). Such graphene based coatings placed on the surface of several industrially relevant commodity polymers significantly improve the gas barrier properties of polymeric films for large-scale applications. However, by our knowledge it is insufficiently presented in the reviewed literature whether graphene and carbon nanotubes can migrate into food from polymeric films and what is the potential hazard after such migration

A contributing factor to the rapid commercial development in polymer nanocomposite food packaging materials is the expectation that, due to the fixed or embedded nature of nanoparticles in polymer, they will not pose any significant risk to the consumer (Chaudhry et al. 2008). Recent research on nanoparticle migration from packaging film into food staff show contradicting results. From both the experimental findings and theoretical modeling, Bott et al. (2014) concluded that carbon black does not migrate into food once it is incorporated into low density polyethylene and polystyrene based films, when test conditions of 10 days at 60°C were applied. Schmidt et al. (2009, 2011) found that asymmetric nanoclay platelets at 50-800 nm in radius embedded in poly(lactic) acid nanocomposites indeed migrate from the nanocomposite in 95% ethanol after 10 days a 40^{o}C and it was attributed to the weak filler to polymer interfacial interactions. Lin et al. (2014) found that the migration of Ti from nano-TiO_2-polyethylene packaging films into food simulants might occur via dissolution from the surface and cut edges of the film under different temperature and migration time conditions. Detailed investigations on the effect of high pressure thermal treatments (e.g. pasteurization and sterilization) on food/packaging interactions focusing on migration from the PLA/gluten/montmorillonite nanocomposite materials into food stuff found that the overall migration and protein migration were high, while the migration of montmorillonite was low or not detectable (Mauricio-Iglesias et al. 2010, 2010a). The reported results lead to the conclusion that a partial migration of nanoparticles from packaging films into food or food simulant cannot be excluded. Therefore, the confirmation of the estimated migration value by experimental testing is obligatory in order to demonstrate the non-compliance of a nanomaterial.

To answer the needs for greater information on release and potential hazard associated with nanoparticles for food packaging applications, the objective of this study is to assess whether the embedded nanofiller of different size and shape can transfer in nanoform from polymeric film into food-simulating solutions under different migration conditions. Poly(lactic) acid (PLA) based polymer nanocomposite incorporating mixed nanocarbons (mostly graphene and carbon nanotubes, and some impurities of fullerenes and carbon black) was chosen in order to study how the distinct geometric shapes, aspect ratios and concentration of nanofiller affect the nanoparticle release from the film into the food simulant. The swelling and dissolution of the polymer film into acidic, ethanol and oil-based food simulants under various time-temperature migration conditions were studied by scanning electron microscopy and differential scanning calorimetry, and assumed to be the driving force for the diffusion process. The released migrants from the polymeric films into the food simulants were detected by laser nanoparticles sizer and transmission electron microscopy. Safety concerns of released nanocarbon particles into food and drinks for consumer at long term exposure were discussed.

Materials and Methods

Materials

Commercial poly(lactic) acid polymer doped with nanocarbon filler (by Graphene 3D Lab) and neat PLA were used as row materials. Films were prepared by layer to layer deposition using 3D printing

FDM technique, followed by hot pressing to obtain ~30 μm thickness. The total amount of the nanocarbon filler in the PLA nanocomposite film was ~2 wt%, consisting mainly of graphene and multiwall carbon nanotubes in ratio 1:1 with some impurities of fullerenes and carbon black. **Figure 1(a)** presents example TEM micrograph of the PLA film doped with nanocarbons, showing the presence of few layers graphene nanoplatelets (GR) of size above 200 nm; multiwall carbon nanotubes (MWCNTs) of length around 1 μm and diameter ~ 30 nm; as well as traces of fullerenes (~ 10 nm) and carbon black (~20 nm) as impurities. **Fig. 1(b)** shows the SEM micrograph of the cross section of the PLA-nanocomposite film of thickness ~ 30 μm, visualizing the homogeneous dispersion of nanocarbon fillers in the matrix polymer. The test samples were further indicated as the nanocomposite PLA/GR/NC film and the neat PLA film used as a control.

---------------------- Figure 1(a,b) place here -----------------------------

Migration tests

The migration test involves 2 steps. First, the film was immersed into the food simulant(s) allowing the substances from the packaging material to migrate into the simulant(s) at definite time-temperature conditions. The second was to detect the nanocarbon migrants transferred into a food simulant in terms of specific migration, according to the European Standard EN 13130-1:2004 (EN 2004). In this study we were interested to detect nanocarbons as specific migrants that could migrate from the composite film into the simulant solutions. Film samples were thin round plates with diameter of about 0.3 dm and thickness of 30 μm. The total film surface to simulant volume ratio was ~ 0.14 dm^2 of film contact area totally immersed in 30 ml food simulant. Four samples were tested for each of the simulants. Pre-treatment of test specimens for dust removing was performed by washing in distilled water and drying (JRC 2009). The migrant transferred from the films into food simulants was detected by laser diffraction analysis and TEM.

Four standard food simulants were used in this study: ethanol/water 10% (v/v) as a simulant for aqueous foods (simulant A); acetic acid/water 3% (v/v) for acidic foods (simulant B); and 50% (v/v) ethanol/water (simulant D1) for alcoholic drinks, as well as olive oil (simulant D2) for fatty foods, as prescribed in the EU regulation 10/2011 (EU 2011).

Migration tests were performed in a temperature controlled chamber, under static and dynamic conditions, as follows: (1) 10 days at 40^oC (standard static test); (2) 4 hours at 90^oC (high temperature static test), and (3) 4 hours at 90°C and subsequent storage for 10 days at 40°C, including ultrasonic treatment 5 min daily (combined high temperature-long time dynamic test). The standard static test was set accordingly with the prescription in EU regulation 10/2011 (EU 2011). While the high temperature static and ultra-high temperature dynamic migration conditions were chosen based on literature sources (Xu et al. 2010; Mutsuga et al. 2008) aiming to mimic the use of nanocomposite films in extreme conditions, such as high temperature processing, incl. microwave, and subsequent long-term storage and transportation. In our study we investigate if different time-temperature migration tests may cause mass transfer of nanocarbon particles due to physical or other changes in the film sample. **Table 1** summarizes the migration conditions and the food simulants applied in this study.

----------------------- Table 1 place here ----------------------

Swelling test

For swelling experiment, dry PLA based film samples were weighted and fully immersed in food simulant. The film was stored in the simulant liquids at the three time-temperature migration conditions, listed in **Table 1.**The weight of the swollen sample was measured after blotting excessive solvent gently with filter paper. Sample weights before and after exposure to food simulants were used to conclude if any diffusion took place. The films were weighted in high precision analytical balance to ±0.1 mg. The overall mass transfer due to swelling/dissolution was presented in mg/per dm^2 of the film surface immersed in the simulant. The degree of swelling was calculated by the relative change (S %) of mass of the film. All these tests were performed in triplicate and the overall swelling/migration was calculated as the average.

Laser diffraction analysis

The detection of the migrants in the food simulants was performed by a laser nanoparticles sizer Analysette 22 Nano Tec plus (FRITSCH), using wet dispersion unit. After migration test, amount of 30 ml simulant solution was added into the wet dispersion unit of laser nanoparticles sizer and gently stirred during the laser analysis. In order to obtain repeatable and reliable results all measurements were repeated at least 3 times with different combination of optical parameters. Submicron and micron size migrants within the test range from 0.1 to 100 μm were analyzed and the results are presented as a histogram representing the number of detected particles in number percentage (n%) versus particle size (µm). The laser diffraction analysis was limited to the ethanol and acetic acid food simulants A, B and D1 as the device cannot be used for characterization of the oil based food simulant D2.

Electron Microscopy

Transmission Electron Microscope (TEM) at accelerating voltage 200 kV was used for analysis of the colloids of migrated nanoparticles into the food simulants. For this study a preliminary preparation technique was applied. A micro-quantity of colloid was dropped on standard copper TEM grid covered by membrane from amorphous carbon; after that the grid was dried in dust-free atmosphere at ambient conditions, and then visualized at different magnifications. The phase composition of the dried colloids was determined by selected area electron diffraction (SAED) mode of the microscope. Because of the specificity of the test, only simulants A, B and D1 was dried and subjected to TEM analysis.

Scanning Electron Microscope (SEM) Philips 515 at accelerating voltage 25 kV and 5 kV was performed to study the film surface morphology. Before the examination in the microscope, the samples were covered with metal coating for better conductivity of the surface and to avoid the discharge effects. The neat PLA and the PLA/GR/NC films before and after migration tests in the four food simulants (A, B, D1 and D2) were subjected to the SEM surface analysis.

Differential Scanning Calorimetry (DSC)

The calorimetric analysis was performed by Differential Scanning Calorimeter DSC Q20 TA Instruments in nitrogen atmosphere, with double cycle of heating from 30 to 200°C at 10°C/min separated by a single cooling cycle at 10°C/min. Sample of about 5 mg in weight was put in aluminum pan for the DSC analysis. The glass transition temperature (T_g), the crystallization temperature (T_c), the total crystallinity (χ%) and the melting temperature (T_m) were determined from the 1st and 2nd run DSC curves and their first derivative. The neat PLA and the PLA/GR/NC films before and after migration tests in the four food simulants were subjected to the DSC test.

Results and Discussion

Swelling of PLA and PLA/GR/NC films in food simulants

The sorption and desorption processes in the film during migration tests was characterized by control on the swelling of the films in the three food simulants: 10% ethanol (A), 3% acetic acid (B) and 50%

ethanol (D1), as varying time-temperature conditions. **Table 2** summarizes both the mean values of equilibrium percentage swelling *S*% and the total concentration of migrant (mg/dm^2) from 0.14 dm^2 film surface after the standard static and the high temperature static migration regimes. The reproducibility of the measurements is given by the standard deviation of the results of the movement in and out of the film of an unspecified mixture of substances and simulant.

----------------- Table 2 place here ---------------

As seen from **Table 2**, the swelling of the neat PLA and the nanocomposite PLA/GR/NC films by the food simulants was insufficient after 10 days storage at 40^{o}C (standard static test). The ethanol based solutions (food simulants A and D1) do not permeate within the films, but only the 3% acetic acid (simulant B) in a minor extent diffuse in the composite films, in terms of sorption (*S*%=0.84%), i.e. movement of simulating liquid in the film. In contrast, a decrease of mass of the films was observed after 4 hours storage at 90^{o}C (high temperature static test) in the three food simulants A, B, and D1. A slight desorption (with mass transfer of 1.8 to 4.2 mg/dm^2 out of the film) was observed for the neat PLA film, while the nanocomposite PLA/GR/NC film show higher amounts of mass transfer (2.4-4.9 mg/dm^2) depending on the food simulants (Velichkova et al. 2016).

We consider the mass transfer from the films mainly due to partial dissolution of some organic substances from the PLA polymer by the aggressive food simulants and their subsequent diffusion out of the film. The higher mass transfer from the nanocomposite PLA/GR/NC film compared to the neat PLA film might be associated with the release of nanocarbon particles, which diffuse from the film dispersed in the PLA organic substances.

Our results for dissolution of the neat PLA are similar to those found out by Mutsuga et al. (2008), who reported for 49.63 μg/cm^2 of lactic acid products to migrate from polylactide food-contact materials into 4% acetic acid and 20% ethanol at temperatures of 95◦C. The results also confirmed Jamshidian et al. (2010) reporting that different thermodynamic properties such as polarity and solubility of the solvents play an important role on swelling and dissolution processes. In our case, 3% acidic acid (simulant B) seems to be more aggressive for the PLA polymer compared to 10% and 50% ethanol simulants (A and D1).

The swelling and dissolution results give no detailed information for the movement of specific film ingredients like organic substances or nanoparticles out of the polymer film. However, if consider a homogeneous dispersion of the embedded ~ 1 wt% graphene in the PLA/GR/NC film and taking into account that mostly graphene nanoplatelets release from the film, we calculate the approximate amounts of graphene migrants to vary within the range 0.024-0.049 mg/dm^2, depending on the food simulants (**Table 2**).

Characterization of migrants in the food simulant solutions

Reviewed studies on migration reported that the small amount of any material that migrate from the neat PLA film into food will be lactic acid based organic substances that will be subsequently hydrolyzed in aqueous systems to lactic acid (Jamshidian et al. 2010). The rate of migrate of lactic acid products from PLA polymer into food simulants is augmented by high temperatures and long-time tests; thus sorption of certain organic solvents could cause dissolution of one or more components of the polymer matrix (Mutsuga et al. 2008). The ethanol and polar solvents are found aggressive to the PLA films due to the polymer hydrolysis leading to release of PLA degradation products. Zygoura et al. (2011) reported that the polymer degradation is followed by increased additive diffusion rates, thereby significantly higher additive migration levels into the surrounding medium. In some studies, the migration level is controlled below the overall migration limits by incorporation of nanofillers, such as cellulose nanocrystals in PLA film (Fortunati et al. 2012).

Experimental and theoretical studies reported that nanoparticles larger than 3 - 4 nm in diameter, when fully incorporated in plastic film, cannot migrate (following Fick‘ian law of diffusion) from commodity plastics films (Franz 2015). Duncan & Pillai (2015) considered two nanoparticle release paradigms: (1) the release of nanoparticles via passive diffusion, desorption, and dissolution into external liquid media; and (2) the release of nanoparticles assisted by matrix degradation. However, it is still not explored in depth if swelling of the polymer at the film surface and partial dissolution of some organic ingredients may cause physical release of nanoparticles of different size, shape and entanglement, such as graphene, carbon nanotubes, fullerenes and carbon black from plastic films towards foods/or food simulants.

Migrants detected in food simulants after high temperature static migration

Our study demonstrates that nanoparticle migrants from the nanocomposite PLA/GR/NC film were not detectable in the three food simulants (A, B and D1) after the standard static migration test of 10 days at 40^{o}C. However, during the high temperature static migration test conditions (at 90^{o}C for 4 hours), nanocarbon migrants indeed were detected by TEM and laser diffraction analysis to migrate at different degree in the acidic and alcohol based food simulants. The size distribution histograms and the TEM micrographs of the migrants are compared in **Figures 2(a-f)**.

---------------------------- **Figure 2 (a-f) place here** --

The diagrams in **Figs. 2(a-c)** show laser diffraction size distribution histograms representing number percentage (n%) of migrants versus their size. The diagrams compare migrants from both the nanocomposite PLA/GR/NC film (light bars) and the control neat PLA film (dark bars) within the size range 0.1-100 µm, detected in the three food simulants A, B and D1. The histograms for the neat PLA film have bi-modal size distribution with a small peak in the size range 1-10 µm and a sharp main peak within 10 – 100 µm. The migrants from the neat PLA film are associated with lactic acid based substances, commonly dissolved from the PLA polymer into the used food simulant solutions (Jamshidian et al. 2010; Mutsuga et al. 2008). In contrast, migrants from the composite PLA/GR/NC film show multi-modal size distribution in the measured size range. Particularly, migrants in nanoform appear in the size range 0.1 – 1 µm, that are of ~ 0.4 n% in simulants B and D, as well as about 0.2 n% in simulant A. Moreover, the micron size migrants from the PLA/GR/NC film within the range 1 –20 µm are twice larger amount than those detected from the neat PLA. The nanoscale migrants might be associated with release of nanocarbon particles from the nanocomposite film, as such were not found from the neat PLA film.

When correlate the laser diffraction analysis results in **Figs. 2 (a-c**) with the TEM micrographs in **Figs. 2(d-f)**, we consider that the dedicated small size migrants in nanoform in the three food simulants are primary nanoparticles of few layers graphene and their small aggregates of size ~200 nm to 1 µm. Obviously, asymmetric graphene platelets indeed migrate from the PLA film into ethanol and acetic acid food simulants. Exceptionally, in the most aggressive 3% acetic acid (simulant B) not only graphene but also carbon nanotubes fixed in agglomerates with organic substances **(Fig. 2e)** appear as migrants from the PLA/GR/NC film. Concentration of the nanocarbon type as a filler in the polymer is also of importance. The fullerenes and carbon black are of insufficient amounts in the polymer therefore they are detected although rarely in the most aggressive acetic acid food simulant.

Figs. 3(a-d) characterize the variety of nanoscale structures identified as migrants in the 3% acetic acid (simulant B) after high temperature static migration test. Intercept in the micrographs presents the selected area electron diffraction (SEAD) patterns identifying different crystalline allotropes of carbon. In **Fig 3(a)**, the TEM micrograph visualize few layers graphene platelets, and the electron diffraction pattern confirms the Graphite 2H, hexagonal phase with d = 2.139 [Å] and 1.235 [Å], identifying graphene. In **Fig 3(b)**, short carbon nanotubes of ~ 20 nm outer diameter, ~ 3 nm inner diameter and length above 100 nm are visible to release in agglomerates fixed with organic substances. The SEAD pattern identifies these crystalline carbon allotropies as multiwall carbon nanotubes (Lucas et al. 1998). In **Fig 3(c)**, fairly monodisperse clusters ~ 10 nm compacted in loose agglomerate of size ~100-200 nm are detected and the SEAD pattern shows that clusters are polycrystalline in nature and phase determined was C60 and C70 (Lucas et al. 1998; Deguchi et al. 2001). Finally, **Fig. 3(d)** visualizes the amorphous carbon black migrants of average size around 30-50 nm.

------------ **Figure 3 (a-d) place here** --------------------

Migrants detected after high temperature-long time dynamic migration

Migrants in the three simulating solutions after the high temperature-long time dynamic migration test (for 4h at 90°C and subsequent storage for 10 days at 40°C, incl. 5 min/daily ultrasonic treatment) are analyzed by laser diffraction analysis and TEM. Example results are shown in **Figs. 4(a-f)**, comparing laser diffraction histograms and TEM micrographs of migrants detected in the three food simulants.

------------ **Figure 4 (a-f) place here** ------------------

The size distribution of migrants from both the nanocomposite PLA/GR/NC film (light bars) and the neat PLA film (dark bars) is compared for the three food simulants A, B and D1 in **Figs. 4(a-c).** Similar to the results from the high temperature static migration test, here the migrants from the neat PLA film show bi-modal size distribution with two peaks within the size range (1-100 µm). In contrast, the nanoscale migrants obtained from the PLA/GR/NC film of size 0.1-1 µm appear in larger amounts (0.5-0.7 n%) and the migrants of micron size 1-20 µm in **Fig. 4(a-c)** show more complex multimodal size distribution at high temperature-long time dynamic migration test compared to those of the static test in **Fig. 2(a-c)**. The main peak of size distribution of migrants for the nanocomposite PLA/GR/NC film is shifted towards higher size compared to those of the neat PLA.

The corresponding TEM micrographs in **Fig. 4(d-f)** show large amounts of single graphene platelets of wide size range (from nano to micron scale) released into the three food simulants A, B and D1, due obviously to the facilitating effect of dynamic treatment on dissolution processes. Graphene migrants in 3% acetic acid and 50% ethanol release in larger amount compared to 10% ethanol. The high temperature-long time dynamic test in the acetic acid simulant (B) also extracts not only graphene, but also carbon nanotubes and other nanocarbon particles that are mostly fixed with organic substances in large agglomerates around 10 μm (**Fig 4e**).

Characterization of nanocomposite film integrity after migration tests

Thermal analysis

Calorimetric analysis was performed in order to characterize the structural changes of the polymer films produced by the high temperature migration tests. The **Fig. 5(a,b)** show example DSC thermograms (heat flow vs. temperature), 1st run (a) and 2nd run (b), of the nanocomposite PLA/GR/NC film after the high temperature-long time dynamic migration test in the four food simulants: 10% ethanol (simulant A), 3% acetic acid (simulant B), 50% ethanol (simulant D1), and olive oil (simulant D2), compared with the control PLA/GR/NC film (before migration test). **Table 3** summarizes the thermal characteristics, such as: glass transition temperature (Tg), melting temperature (Tm), crystallization temperature (Tc) and total crystallinity (χ%), determined from the 1st and the 2nd run thermograms.

------------ **Figure 5 (a,b) place here** ------------------

----------- **Table 3 place here** ------------

In **Fig. 5a**, the DSC 1st run thermogram of the control nanocomposite PLA/GR/NC film show well defined peaks for the glass transition temperature (T_g = 61^{o}C) and the crystallization temperature (T_c = 103^{o}C) before migration test. Double melting peak (T_m) was observed for the control film, where the large peak represents the melting temperature of the neat PLA polymer, while, the small peak might be associated with the melting of the PLA molecules attracted to the surfaces of nanocarbon fillers. In contrast, the thermal characteristics of the migrated PLA/GR/NC films are changed after the high temperature migration test in the four food simulating solutions. The T_g and T_c peaks disappear; the small melting peak (T_m) is shifted slightly towards lower temperatures (in simulants A and B) or disappeared (in simulants D1 and D2), due probably to the swelling of the film by the simulant solutions.

The 2nd run DSC thermograms in **Fig. 5b** show a presence of crystallization peak temperature (T_c) for all migrated films, but it is shifted (with 5-6^{o}C) towards higher temperatures, compared to the control film. The small melting peak (T_m) is slightly shifted with 2-3^{o}C to higher temperatures. The total PLA crystallinity (χ%) of the migrated films decreases about 1.3 to 1.75 fold in comparison with the control film. The effect of 3% acetic acid on the degree of crystallinity is slightly higher compared to other food simulants, due probably to different degree of dissolution of the PLA polymer by the simulant solutions during the migration test.

SEM analysis of the migrated film surfaces

SEM analysis was performed in order to prove the film surface before and after high temperature-long time dynamic migration test in the four food simulants. The **Figures 6(a-f)** show example SEM micrographs for the migrated PLA/GR/NC film, compared to the control film before migration. The control PLA/GR/NC film **in Fig. 6(a)** has smooth polymer surface without nanocarbon particles on it. While, in **Figs. 6(b,c)** micron size holes (≥ 10 µm) and graphene nanoplates are visible on the surfaces of migrated films in 10% ethanol and 3% acetic acid, indicating for diffusion of the dissolved substances from volume towards the film surfaces. Large amounts of graphene plates released on the film surfaces are observed after migration in 50% ethanol and olive oil, (**Fig. 6d-f**). Although rarely, a local degradation of the PLA film surface is visible (**Fig. 6d**), where the fibrous MWCNTs formed entangled network as the PLA polymer matrix dissolve, which prevent their release into food simulant.

Based on both DSC and SEM results we consider that the partial dissolution of the PLA polymer by the aggressive simulant solutions at high temperature migration conditions at 90^{o}C (both static and dynamic migration tests) resulted in migration of the dissolved organic substances, which diffuse out of the film and locally destroy the integrity of the film surface. Obviously, the enhanced dynamics of molecules above the glass transition facilitate the diffusion processes. Following the release mechanisms of nanoparticles proposed by Duncan & Pillai (2015), we assume that such physical changes of the PLA polymer related to polymer dissolution provoke a diffusion of the dissolved organic substances doped with nanocarbon particles, mainly graphene, out of the film towards the food simulant.

------------ Figure 6 (a-f) place here ------------------

4. Safety concerns on graphene

Graphene detected as the main nanoscale migrant from the PLA/GR/NC film fall under the EU adopted definition 2011/696/EU (EU 2011a) of a nanomaterial having one or more external dimensions in the size range 1 nm - 100 nm. According to (EC) No 450/2009 (EC 2009), substances in nanoform shall only be used in active and intelligent plastic food contact materials when they are explicitly authorized and included in the European Plastics Regulation specifications (Ebnesajjad 2013). Currently, only carbon black, TiN, and SiO_2 are approved (with some restrictions) as nanomaterials for safety use in contact with food. There is no compliance standards set by regulatory agencies limiting the specific migration limits of graphene for migration from food packaging materials into food.

If consider the PLA matrix polymer, several authors observed that the migrated organic ingredients from the neat PLA films do not exceed the overall migration limit of substances from food packaging materials (OML=10 mg/dm^2), established by current EU legal standards (Mutsuga et al. 2008; Mattiolia et al. 2013). According to Conn et al. (1995), the migration of the PLA degradation products is not arising safety concerns as these products are lactic acid monomers, dimers and oligomers that will be subsequently hydrolyzed in aqueous systems to lactic acid, which is natural product and food ingredient. However, exempted from upper rules are the nanoparticles that may release from the nanocomposite PLA based film.

In our study it was determined that the total amounts of migrant from the nanocomposite PLA/GR/NC film into the food simulants A, B and D1 at the high temperature migration test (90^oC for 4 h) are of 2.8; 4.9 and 2.4 mg/dm^2, respectively, while the corresponding amounts of the migrated nanocarbon particles, if calculated as 1 wt% of the total migrants, are approximately of 0.028, 0.049, and 0.024 mg/dm^2, respectively. Our results show that the nanofiller migrant in the ethanol based food simulants (A and D1) is mainly nanosized graphene platelets, while the higher amount of nanofiller migrant in the acidic based food simulant (B) is probably due to the release of graphene and other nanocarbons. Such small amounts of graphene migrant would probably be in compliance with Article 30(1)(b) of Regulations (EC) No. 1935/2004 (EC 2004). Nevertheless, the hazard from the long term exposure of consumer to graphene via thermally treated packaged food should not be ignored.

Graphene as a novel composite additive have two primary categories of negative human and environmental impact – the toxicity (Smolander & Chaudhry 2010) and the life cycle (Arvidsson et al. 2013), which are recently subjected to intensive study. Few peer-reviewed publications related to toxic effects from exposure to graphene show that the shape, high surface area, surface chemistry and purity may lead to unknown toxicological effects and uncertainties on consumer safety (Singh 2016; Ahmed & Rodrigues 2013; Arvidsson et al. 2013). Researchers agree that graphene toxicity might be lower compared to the toxicity of carbon nanotubes (Guo & Mai 2014). However, dose is one of the most important factors and some researchers believe that low doses of graphene may be safe (Ruiz et al. 2011). Concerning hazard from migration of graphene from packaged film into food or drink, little information is currently available concerning the uptake of nanoparticles following oral exposure by ingestion directly from food and drink. Authors reported that some nanoparticles (e.g. fullerenes), diffuse through gastrointestinal tract mucus to reach the cells of the intestinal lining and the blood (Jani et al. 1990; Food Safety Authority of Ireland 2008), but information on translocation route of graphene is still not available.

The second category of negative human and environmental impact arises from processes along the life cycle. Life cycle analysis (LCA) provides information in relation to exposure, as well as analysis of nanoparticles release and monitoring throughout the whole product life cycle (Sweet & Strohm 2006). Life cycle behavior of graphene remains at the very early stages of development. Arvidsson et al. (2014) demonstrate the possibility to conduct a life cycle assessment study based mainly on information from patents and scientific articles on graphene production for use in composite bulk materials. The results show that the ultrasonication production route has lower energy and water use, but higher human and ecotoxicity impacts, compared to the chemical reduction route. For the time being, the available LCA studies and environmental assessments support the further development of bio-based polymers, however researchers agree that the effect of nanofiller on the environment has to be considered case by case (Patel et al. 2005; Hottle et al. 2013). More studies are needed on the effects of graphene, as additive in PLA composites for food packaging application, as well as on the distribution of PLA/graphene packaging materials in waste stream in order to analyze their life cycle environmental impacts and to prognosis hazard of graphene for humans and environment.

5. Conclusions

The study presents important findings indicating that few layers graphene platelets of about 200 – 1000 nm in length indeed release from the PLA/GR/NC film at high temperature migration conditions of 90^{o}C for 4 hours and above. About 2.4 - 4.9 mg/dm^2 organic substances doped with approximately 0.024 - 0.049 mg/dm^2 nanocarbons, mainly graphene, release in the food simulants 10% ethanol, 3%

acetic acid and 50% ethanol, respectively. The partial dissolution of PLA polymer by the simulant solutions, as well as the enhanced dynamics of molecules above the glass transition temperature was considered as the driving force for the diffusion processes. As a result, asymmetric graphene platelets (100-1000 nm) easily migrate in the three food simulants together with the dissolved PLA organic substances. While fibrous MWCNTs formed entangled network on the film surfaces as the PLA polymer matrix dissolve, which prevent their release as single nanoparticles into the food simulants. The fullerenes and carbon black nanoparticles (10-20 nm) were of insufficient concentration in the nanocomposite therefore their migration was low or undetectable.

The amount of migrant was found in compliance with the overall migration limit of 10 mg/dm^2 applied for food packaging materials. However, release of graphene in nanoform from the PLA-based nanocomposite films into food has to be taken into account at high temperature processing and subsequent long-time storage and transportation of packaged food in order to prognoses the risk from graphene in the food chain in long term.

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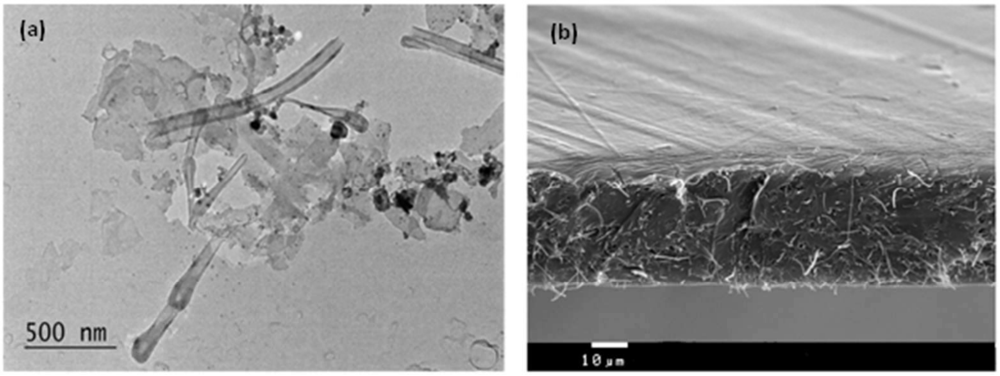

Fig.1 (a,b). TEM micrograph of the PLA/GR/NC nanocomposite film doped with graphene and carbon nanotubes with traces of impurities like fullerenes and carbon black (a); SEM micrograph of the cross section of the film (b).
Materials
133x49mm (120 x 120 DPI)

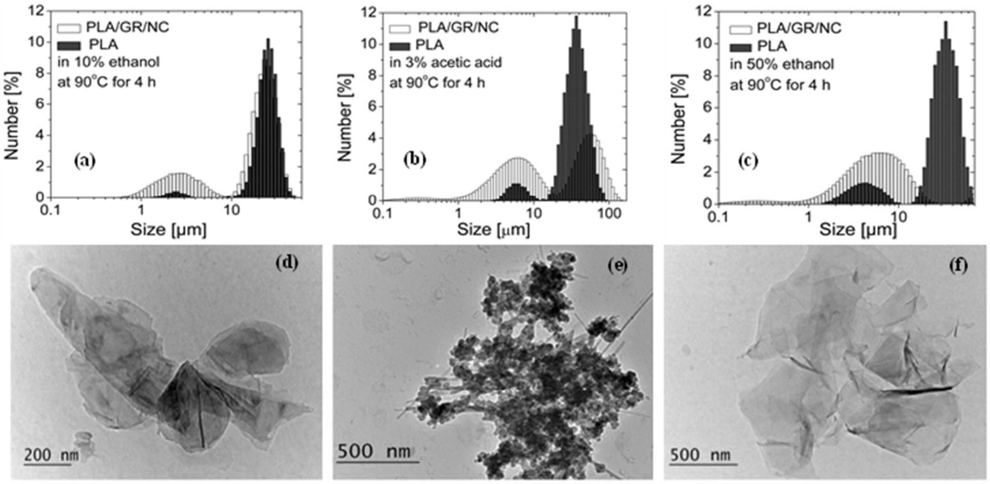

Fig. 2 (a-f). Size distribution histograms and TEM micrographs of migrants after high temperature static migration test at 90oC for 4 hours into three simulating solutions: (a,d) 10% ethanol, (b,e) 3% acetic acid, and (c,f) 50% ethanol. Histograms of the neat PLA film (dark bars) and nanocomposite PLA/GR/NC film (light bars) are compared. Corresponding TEM micrographs of dried simulants present the migrants from the PLA/GR/NC film
Migrants detected in food simu
155x75mm (120 x 120 DPI)

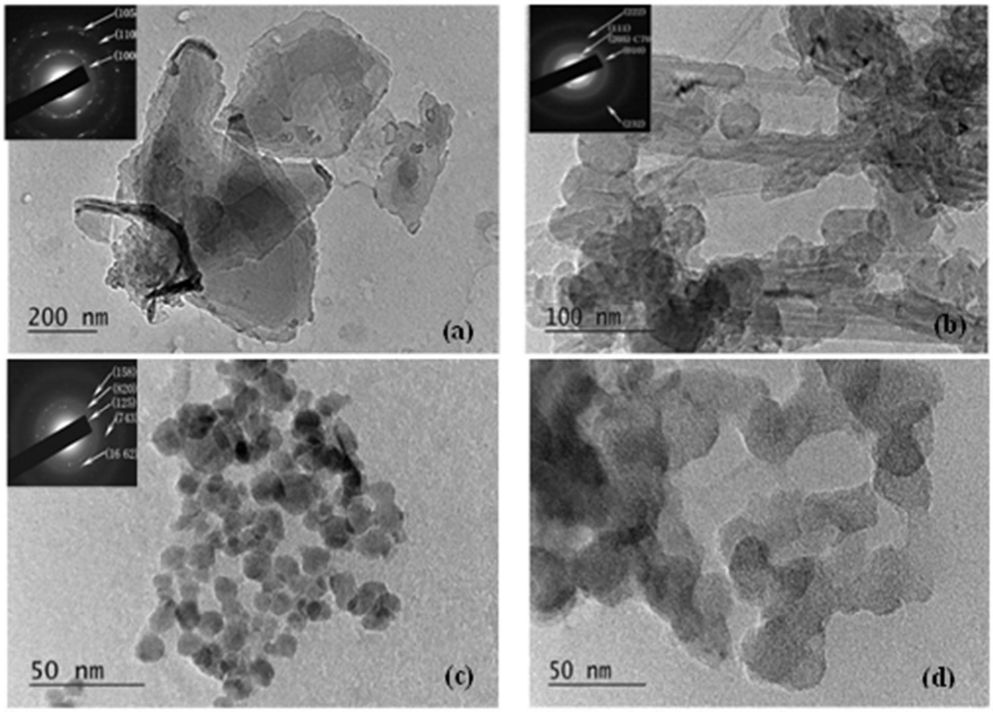

Fig. 3 (a-d). TEM micrographs of different migrants from nanocomposite PLA/GR/NC film identified in the food simulant B (3% acetic acid) after the high temperature static test at 90oC for 4h: (a) graphene; (b) carbon nanotubes, (c) fullerene-like C60/C70 crystals, and (d) carbon black. The intercepts present the electron diffraction patterns of different crystalline allotropes of carbon
Migrants detected in food simu
107x77mm (120 x 120 DPI)

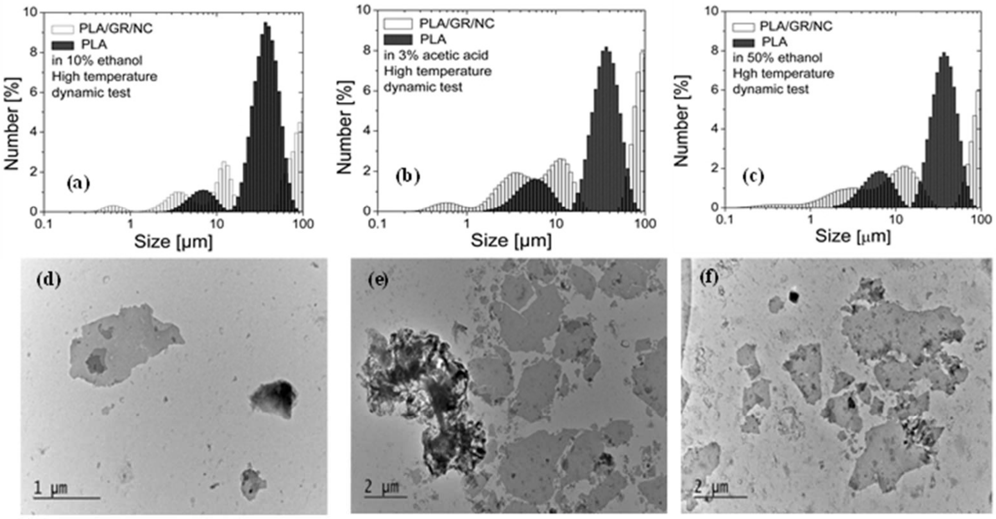

Fig. 4(a-f). Size distribution histograms and corresponding TEM micrographs after high temperature-long time dynamic test: First row: comparison of the particle size distribution histograms of migrants from the neat PLA film (open bars) and the nanocomposite PLA/GR/NC film (full bars); Second row: TEM micrographs of dried simulants of the PLA/GR/NC film in the three food simulants: (a,d) 10% ethanol (A), (b,e) 3% acetic acid (B), and (c,f) 50% ethanol (D1).
Migrants detected after high t
153x79mm (120 x 120 DPI)

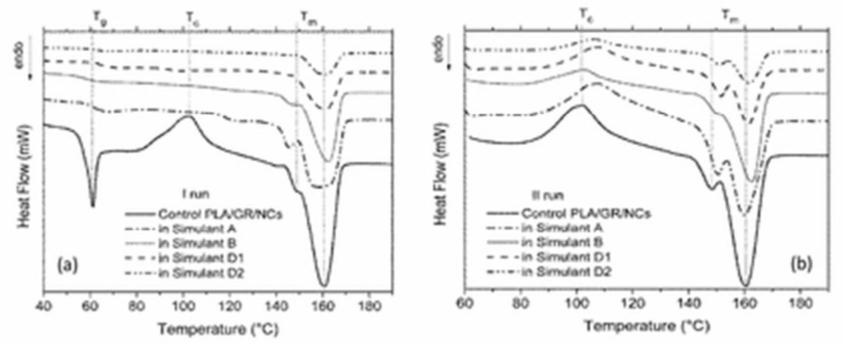

Fig. 5(a,b). DSC thermograms from: (a) 1st run (a) and (b) 2nd run of the nanocomposite PLA/GR/NC film comparing control sample (first full line) and migrated films into four food simulants: Simulant A (10% ethanol), Simulant B (3% acetic acid), Simulant D1 (50% ethanol), and Simulant D2 (olive oil), after the high temperature-long time dynamic migration test.
Thermal analysis
35x14mm (300 x 300 DPI)

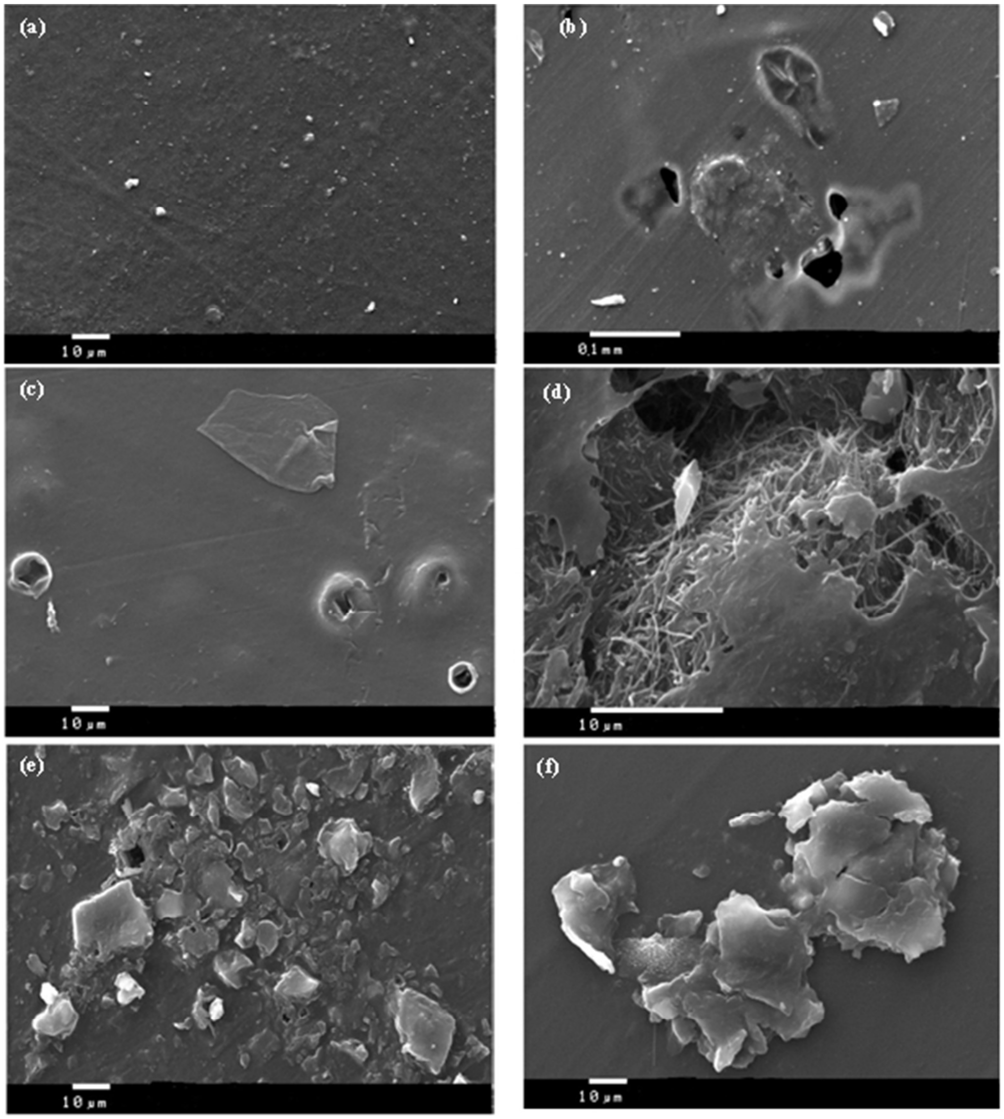

Fig 6 (a-f). SEM micrographs of nanocomposite PLA/GR/NC film surfaces after high temperature–long time dynamic migration test into food simulants: (a) the control film; (b) in10% ethanol; (c) in 3% acetic acid; (d,e) in 50% ethanol at high and low magnifications; and (f) in olive oil. Scale bars (wait line) and size is seen in the bottom of the pictures.
SEM analysis of the migrated f
129x143mm (120 x 120 DPI)

Table 1. Migration testing conditions and food simulants applied in this study

Test	Migration testing conditions in this study	Food simulants
No.1	10 days at 40^{o}C (standard static test)	10% v/v ethanol (simulant A) 3% v/v acetic acid (simulant B) 50% v/v ethanol (simulant D1) Olive oil (simulant D2)
No.2	4 hours at 90^{o}C (high temperature static test)	
No.3	4 hours at 90^{o}C, and subsequent storage for 10 days at 40^{o}C, incl. dynamic treatment 5 min daily (combined high temperature-long time dynamic test)	

Table 2. Mean values of percentage equilibrium swelling and mass transfer from the neat PLA and the nano-composite PLA/GR/NC films into food simulants A, B and D1. Migrants from 0.14 dm^2 contact film surface were estimated.

Test No.	**Migration testing conditions**	**Food simulants**	**Neat PLA film**			**Nancomposite PLA/GR/NC film**			
			Swelling *S* (%)	**Total concentr. of migrant** *mg/dm^2*	**Standard deviation**	**Swelling** *S* (%)	**Total concentr. of migrant** *mg/dm^2*	**Standard deviation**	**Approx. graphene migrant** *mg/dm^2*
1	after 10d at 40 °C (standard static test)	10%ethanol Simulant A	0	0	0	0	-	0	-
		3%acetic acid Simulant B	0	0	0	0.84	1.3	±0.010	-
		50% ethanol Simulant D1	0	0	0	0	-	0	-
2	after 4h at 90°C; high temperature static test	10% ethanol Simulant A	- 1.65	- 2.1	±0.024	- 1.95	-2.8	±0.038	0.028
		3% acetic acid Simulant B	- 2.37	- 4.2	±0.010	- 2.99	- 4.9	±0.025	0.049
		50% ethanol Simulant D1	- 1.36	- 1.8	±0.012	- 1.80	- 2.4	±0.019	0.024

Table 3. Thermal characteristics of PLA/GR/NC film from the 1st and 2nd run: glass transition (T_g), melting (T_m) and crystallization (T_c) temperatures; total crystallinity (χ%) after high temperature static migration tests in the food simulants: 10% ethanol (A), 3% acetic acid (B), 50% ethanol (D1) and olive oil (D2), compared to the control film

Thermal characteristics of the PLA/GR/NC film before and after migration tests					
Characteristics	Control film	Simulant B 3% acetic acid	Simulant A 10% ethanol	Simulant D1 50% ethanol	Simulant D2 Olive oil
T_g I run (°C)	61	-	-	-	-
T_c I run (°C)	103	-	-	-	-
T_c II run (°C)	102	102	107	108	107
χ I run (%)	9.99	-	-	-	-
χ II run (%)	7.49	4.28	5.73	4.55	4.73
T_m I run (°C)	148 161	146 163	145 163	- 162	- 162
T_m II run (°C)	148 161	150 163	150 161	151 162	151 162