Introduction

Natural polymers exhibit certain barrier and mechanical properties inherent to their chemical composition, porosity and thickness, which can be improved with some additives or fillers. Poly(lactic acid) (PLA) represents the most investigated bio-polyester for a wide range of applications, due to its biodegradability, renewability, good mechanical properties, processability and low cost [1]. However, high brittleness, slow crystallization rate and low barrier properties of PLA compared to those of fossil-based polymers strongly limit its applications in food packaging. Over the past years, various nanomaterials have been investigated for reinforcing PLA, including layered silicates, carbon nanotubes, hydroxyapatite, layered titanate, aluminium hydroxide, etc. Unlike macro-scaled particles (e.g. talc, glass fibre, carbon particles), nanoparticles can improve material properties at much lower added quantities (2-8 % wt.). Among the nanomaterials investigated, layered silicate clays have been studied in the greatest detail by researchers from both academia and industry due to their ability to dramatically improve material properties including mechanical and flexural properties, elevated heat distortion temperature, enhanced barrier properties and controlled biodegradation [2]. Nanoscaled layered silicates (nanoclay) are known to improve a series of polymer properties already at small concentrations. In particular, permeation properties can be substantially improved [3].

However, in the case of food contact materials (FCM), it is fundamental to evaluate their suitability towards food contact. Regarding nanoparticles incorporation, the potential risk arising from nanoscience and nanotechnology in food and FCMs has to be clarified, and approval for the application of new FCMs depends on migration assessment [4]. In fact, the increased mobility of nanoparticles, driven by their reduced size, raises concerns regarding their potentially enhanced release from packaging into food and then increased human exposure. Furthermore, these concerns are worsened by insufficient data on mode of toxicity, persistence and possible bioaccumulation of nanoparticles [5], with the European Union and Switzerland being the only world regions to incorporate nano-specific provisions in food legislation [6]. No official analytical methods exist for assessment of nanoparticles migration and it is necessary to use multiple techniques, as no single technique is capable of providing all of the necessary information, such as size, shape and chemical composition. In general, besides analytical techniques to evaluate the total content of specific chemical component (such as atomic absorption spectroscopy), specific techniques to identify the released nanoparticles are to be applied, such as inductively coupled plasma-optical emission spectroscopy [7], scanning electron-microscope (SEM) or transmission electron microscopy (TEM) [8].

Another important and hot-topic field in food packaging is the development of innovative active packaging, based on the incorporation of certain components

into packaging systems that release or absorb substances from or into the packed food or the surrounding environment so as to prolong shelf-life and sustain the quality, safety and sensory characteristics of the food. Most important active packaging concepts applied to foods include antimicrobial and antioxidant packaging [9], [10].

As consumers are more and more concerned about the presence of synthetic chemical compounds, one of the major emerging technologies for the development of active packaging is the application of natural compounds [11], [12].

The European project SusFoFlex investigated the development of innovative and sustainable PLA-based packaging solutions for food applications, including the addition of nanoclay additives and natural antioxidant compounds recovered from agro-food wastes. According to current legislation, as commented above, while for nanoparticles migration should be avoided, natural antioxidants/antimicrobials should migrate to transfer their function on the food or the environment surrounding the food [13]. In both the cases, migration has to be assessed, therefore in this paper the results of preliminary evaluation of nanoclay and antioxidants release from some of the tested additivated PLA solutions are reported.

Materials and methods

PLA formulation with nanoclay

A commercial PLA (Hycail HM 1011) was used for the production of PLA specimens. Different formulation tests were carried out (not reported data) to select the type of nanoclay and to screen the best suitable liquid media (plasticizers and surfactants) to improve the dispersability and homogeneity of nanoclay in the polymer. Based on the evaluation of mechanical and barrier properties and optical examination (TEM analysis) of dispersion of the nanoadditive, nanoclay Cloisite® 30B was finally selected together with a formulation without any dispersant. For PLA samples production, nanoclay was added at a 1 % wt. dosage. Following a pre-mixing in a ball mill (3 h, 300 rpm, spin range 30 min, pause 5 min), PLA compounding was performed by extrusion in a co-rotating twin screw extruder (Brabender TSE 20/40) with a temperature profile between 175-180 ℃ and a rotational speed of 100 rpm.

Both PLA filled with nanoclay (PLA_Clay, 5 m*5 cm) and control unfilled PLA (PLA_UF, 2 m*5 cm) samples were prepared.

PLA formulation with natural antioxidants

For the production of active PLA enriched with natural antioxidants recovered from agro-food wastes, commercial PLA (Hycail HM 1011) and extracts from waste orange peels were used. The orange peels extract (OPE) was obtained from waste milled (< 2 mm) dried orange peels through extraction with aqueous 80 % ethanol [14] at 50 °C, 1 h under stirring, using a 1:10 g:mL solid to solvent ratio. A crude extract was separated by centrifugation, concentrated 4-fold under vacuum and then purified through an absorption process on an Amberlite resin (IRA-410 from Rohm and Haas, Philadelphia) to remove sugars (fructose, glucose and sucrose), organic acids (citric acid) and minerals (Ca, K, Na, Mg) [15]. The overall optimised process (not detailed here) included the following steps: resin regeneration with 1 % NaOH, ethanol 96 % and water; loading with an aqueous dilution of the crude OPE; rinsing with water; desorption of adsorbed phenolic compounds with ethanol 96 %; concentration under vacuum of the obtained purified extract. The purified extract was finally encapsulated with β-cyclodextrin (Kleptose, βCD, kindly supplied by Roquette Services Techniques et Laboratories, France) by spray-drying. βCD were selected as carrier materials since previous trials (not shown data) revealed they provided a higher thermal resistance to the OPE than citrus pectin and maltodextrins. Spray-drying was carried out in a Mini Spray drier (B-290, Buchi, Switzerland) at the following operation conditions: 4 mL/min feeding rate; 667 L/h drying air flow rate; aspirator rate set at 100%; 120 °C inlet air temperature. βCD were added as a water solution 2 % w/v at a solid ratio of 0.75 solid βCD / solid OPE. The obtained spray-dried powder was analysed for the total phenols content according to the Folin's assay [14] giving a value of 370 mg /g as hesperidin equivalents (HE, being hesperidin the main phenolic compound present in the OPE).

PLA_OPE specimens (10 cm*10 cm) were prepared mixing the PLA with 1 % wt. of the spray-dried extract (OPE) in a Brabender internal mixer (50 cm^3 capacity) at 175 °C and then by hot pressing at 175 °C, 100 bar.

Migration assessment

To evaluate the influence of clay filling on migration, both PLA_Clay and PLA_UF samples were cut in 1 dm^2 samples, weighted to obtain the specific weight (g/dm^2) and submitted to overall migration assessment using 100 mL of distilled water for each piece according to the total immersion method [16]. Testing conditions were 10 days at 40 °C which are the standardised testing conditions set by EU legislation [17] for overall migration to simulate "any long term storage at room temperature or below, including heating up to 70 °C for up to 2 hours, or heating up to 100 °C for up
to 15 minutes". Blank samples were run simultaneously in triplicate containing only the simulant to check for contamination and make the blank correction in the evaluation of migration.

After the contact time, the overall migration was assessed by the evaporation method: the contact solution was transferred to a pre-weighed Erlenmeyer flask (100 mL) and allowed to completely evaporate in an oven at 105 °C. The flask was removed from the oven, placed in a desiccator, allowed to cool to ambient temperature, and then weighed. The overall migration was expressed as mg of residue per dm^2 of the surface of the sample and calculated as the difference between the mass of the residue from the film after evaporation of the simulant and the mass of residue from the blank simulant, divided by the surface area of the test specimen which was in contact with the simulant during the exposure. Even though both the sides of the specimen were in contact with the simulant, only the value of 1 dm^2 was used in the calculations, as indicated in [16].

Specific migration of nanoparticles was based on evaluation of specific migration of silica, aluminium, sodium and calcium which are the main components of nanoclay. At the end of the overall migration test an aliquot of the samples of distilled water was concentrated 5 times under vacuum and analysed by atomic absorption spectroscopy. Another aliquot was filtered with vacuum filtration system on a polycarbonate membrane filter (Sartorius) of 25 mm diameter and 0.4 µm porosity. The filter was dried at 40 °C and observed by SEM (scanning electron microscopy) at environment pressure (Fei Quanta FEG 250 ESEM) carrying out spectrum microanalysis (Microanalysis EDAX TEAM system) on some of the observed particles. This analysis provides the percent composition, in terms of elements, of selected sample areas.

To evaluate migration from PLA_OPE, the same procedure reported for overall migration from PLA_Clay was adopted. In this case, overall migration was assessed also in 3 % acetic acid and 20 % ethanol. Specific migration of phenolic compounds was assessed analysing the simulant for the total phenols content by Folin's assay [14].

All the migration tests were carried out in triplicate.

Results and discussion

Migration from PLA filled with nanoclay

The PLA_Clay showed a specific weight of 5.31 ± 0.85 g/dm^2, while PLA_UF an average value of 4.20 ± 0.29 g/dm^2. Distilled water was selected as the testing simulant since preliminary trials revealed an higher overall migration in this than in 3 % acetic acid and 20 % ethanol. PLA_Clay samples were already visually different from PLA_UF, being more opaque and with opacity enhanced by distilled water contact (Figure 1). The visual change after simulant contact suggests dissolution or degradation of the matrix polymer may have occurred and,

indeed, the chemical stability of the matrix polymers in food simulant solvents is an important characteristic that has to be taken into account when analyzing the migration. Such phenomena, in fact, may assist the release of nanoparticles from the nanocomposite film into the surrounding media [18], [19].

The overall migration was always < 0.1 mg/dm^2 for PLA_UF, while for PLA_Clay it was < 0.1 mg/dm^2 except for one sample for which a migration of 0.2 mg/dm^2 was evaluated. The overall migration was then definitely far below the allowed limit (10 mg/dm^2) set by the EU regulation (10/2011).
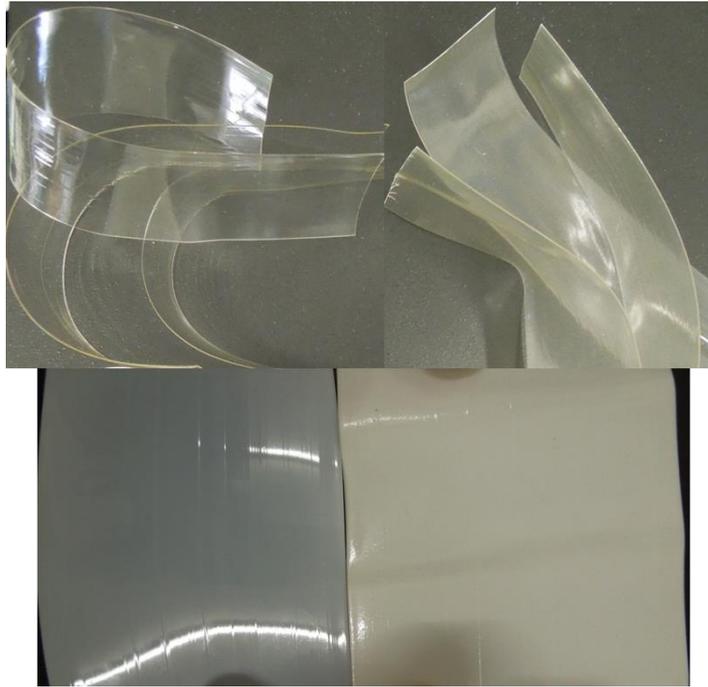

Fig. 1 - Samples of unfilled PLA films (on the left) and of PLA films with nanoclay (on the right) before (on the top) and after the migration test with full immersion in distilled water for 10 days at 40 °C.

In order to investigate the nanoclay migration, it was decided to analyse the presence of nanoparticles and of typical clay elements in the simulant. Cloisite® 30B is a natural montmorrilonite with a quaternary ammonium salt as expansor molecule. A typical clay composition is 20.5 % Si, 9.8 % Al, 0.9 % Na and 0.7 % Ca, plus N, C, O and H from the expansor. Based on this composition and on the specific weight of the samples used in the migration tests, the maximum concentration to be expected in the simulant for each component, supposing a total migration, was calculated (Table 1) and compared with the measured values.

The measured values in the simulant from PLA_Clay were always definitely lower than the calculated maximum migration but also higher than for PLA_UF, suggesting release of nanoclay components. However, the relative percentages of the migrated elements did not reflect the average composition of nanoclay with Ca > Na > Si > Al. This can mean that some elements of the clay are released more easily than others.

Table 1 - Si, Ca, Al and Na specific migration from unfilled PLA (PLA_UF) and nanoclay filled PLA (PLA_Clay) in distilled water after 10 days contact at 40 °C (atomic absorption analysis). Max: maximum theoretical migration.

	max mg/L	mg/L
		Si
PLA_UF		0.008 ± 0.001
PLA_Clay	106.09 ± 0.017	0.047 ± 0.005
		Ca
PLA_UF		0.036 ± 0.006
PLA_Clay	3.62 ± 0.001	0.242 ± 0.132
		Al
PLA_UF		< 0.001
PLA_Clay	50.72 ± 0008	0.003 ± 0.005
		Na
PLA_UF		0.072 ± 0.010
PLA_Clay	4.14 ± 0.001	0.108 ± 0.033

Typical dry particle sizes (by volume) of Cloisite® 30B is reported by the manufacturer as 10 % less than 2 µm, 50 % less than 6 µm and 90 % less than 13 µm, therefore it was used a filter with porosity < 2 µm (0.4 µm) for filtration of the distilled water after PLA contact. SEM observation of the sediments showed the presence of some particles in the water left in contact with PLA_Clay (particle size from 1 to 40 µm with some particles also < 1 µm) (Figure 2) and only a very few particles on the filters used to filtrate water from PLA_UF contact.
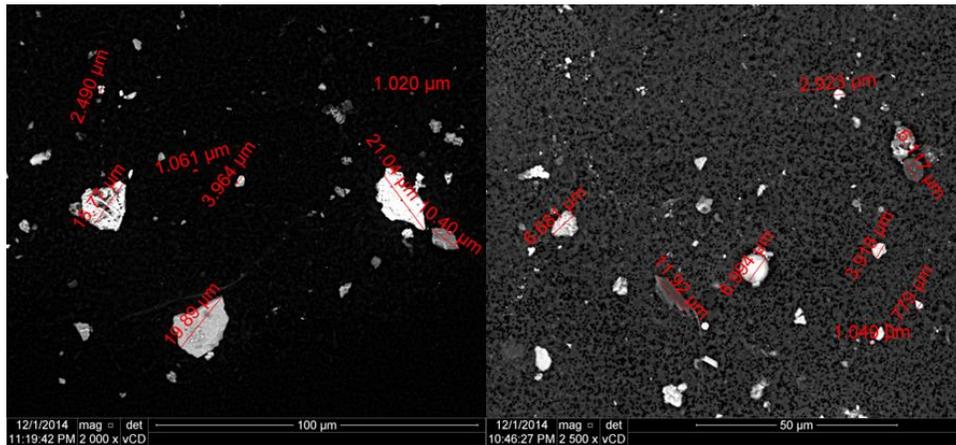
Fig. 2 – Examples of SEM observation at different magnitude (mag) of some filter area spots (sediment obtained from filtration of distilled water after contact with PLA_Clay).

SEM microanalysis was then applied to investigate the element composition of some of the observed particles and see if they could be clay particles. Many particles released from PLA_Clay films showed the presence of Si and Al, suggesting they could have originated from clay release (Figure 3). However, presence of these elements was also found in particles from unfilled PLA. Since only some spots of the filter areas could be observed and analysed, a more extensive application of microanalysis will be required to achieve a more quantitative result.

Ca presence, in percentage higher than Si and Al, was observed only in a few larger particles (Figure 4), suggesting it is released from PLA not in nanoparticle form.
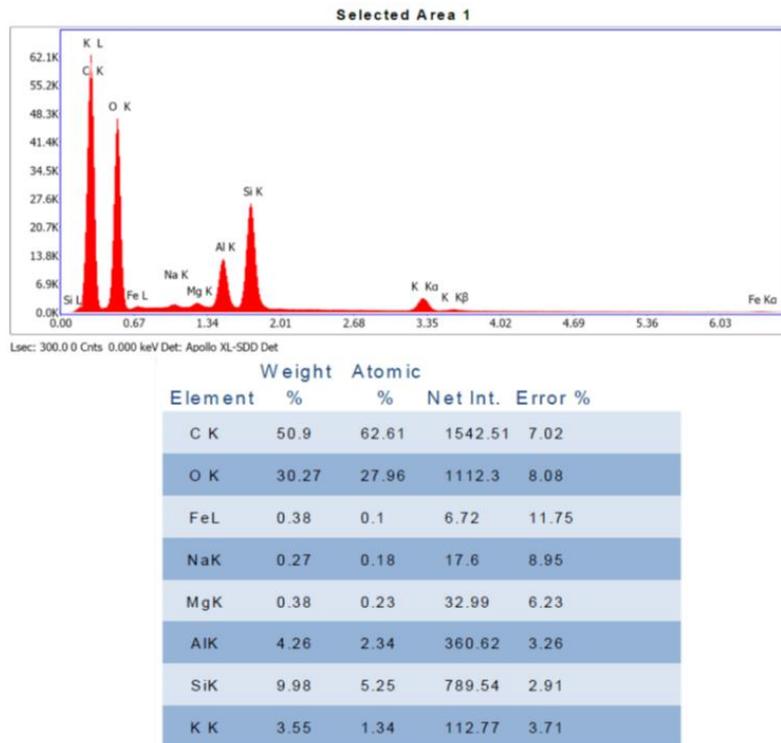
Element	Weight %	Atomic %	Net Int.	Error %
C K	50.9	62.61	1542.51	7.02
O K	30.27	27.96	1112.3	8.08
FeL	0.38	0.1	6.72	11.75
NaK	0.27	0.18	17.6	8.95
MgK	0.38	0.23	32.99	6.23
AlK	4.26	2.34	360.62	3.26
SiK	9.98	5.25	789.54	2.91
K K	3.55	1.34	112.77	3.71

Fig. 3 – Example of composition (evaluated by microanalysis) of a particle released from PLA_Clay with the presence of Si and Al.

Migration from PLA enriched with orange peels extract

The specific weight of the PLA_OPE specimens used for overall migration into distilled water, 3 % acetic acid and 20 % ethanol was 6.30 ± 3.54 g/dm^2, 5.55 ± 1.56 g/dm^2 and 4.95 ± 1.56 g/dm^2, respectively. Since the SusFoFlex project under which the PLA materials were manufactured set fresh cut-fruit as target food for PLA application, 3 % acetic acid and 20 % ethanol were also assayed as food simulants for migration of OPE components.

Overall migration from PLA_OPE was always below the set limit by EU regulation (10 mg/dm^2) and ranged from 1.35 ± 0.21 mg/dm^2 for 20 % ethanol, to 1.35 ± 1.48 for distilled water, to 2.70 ± 0.25 for 3 % acetic acid. Furthermore, it must be said that in case of active packaging the EU regulation [13] specifies that the release of active components must not be included in the overall migration.

Original PLA_OPE samples were orange coloured due to the addition of the OPE and it was observed that the colour slightly decreased after migration test, in particular for the 20 % ethanol simulant (Figure 5), suggesting a higher release of OPE substances in this simulant, in spite of the measured lower overall migration compared to 3 % acetic acid.
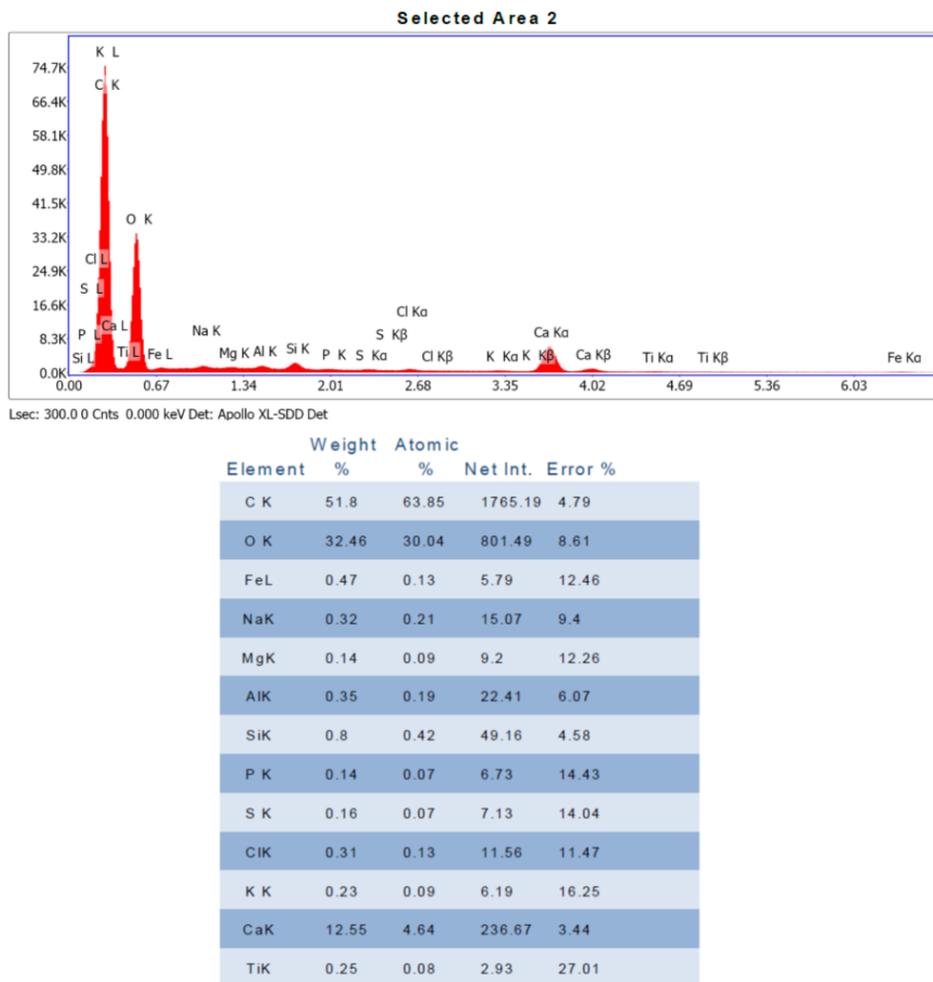
Selected Area 2

Lsec: 300.0 0 Cnts 0.000 keV Det: Apollo XL-SDD Det

Element	Weight %	Atomic %	Net Int.	Error %
C K	51.8	63.85	1765.19	4.79
O K	32.46	30.04	801.49	8.61
FeL	0.47	0.13	5.79	12.46
NaK	0.32	0.21	15.07	9.4
MgK	0.14	0.09	9.2	12.26
AlK	0.35	0.19	22.41	6.07
SiK	0.8	0.42	49.16	4.58
P K	0.14	0.07	6.73	14.43
S K	0.16	0.07	7.13	14.04
ClK	0.31	0.13	11.56	11.47
K K	0.23	0.09	6.19	16.25
CaK	12.55	4.64	236.67	3.44
TiK	0.25	0.08	2.93	27.01

Fig. 4 – Example of composition (evaluated by microanalysis) of a particle released from PLA_Clay with a high Ca content.

As in the case of migration from PLA_Clay, based on the total phenols content of the original OPE, the additivation level (1 % wt. on PLA) and the specific weight of the PLA_OPE, the maximum total phenolic release (assuming a total migration) was calculated and compared with the measured one. It resulted that only about 0.1 %, 1 % and 2.5 % of the OPE phenols migrated into 3 % acetic acid, water and 20 % ethanol, respectively confirming the higher release in 20 % ethanol hypothesized from Figure 5. This result was actually expected since OPE had been obtained from orange peels using aqueous ethanol as extraction solvent. Higher percent release of natural antioxidants from active food packaging are reported in the literature depending on the adopted simulant [20], [21].
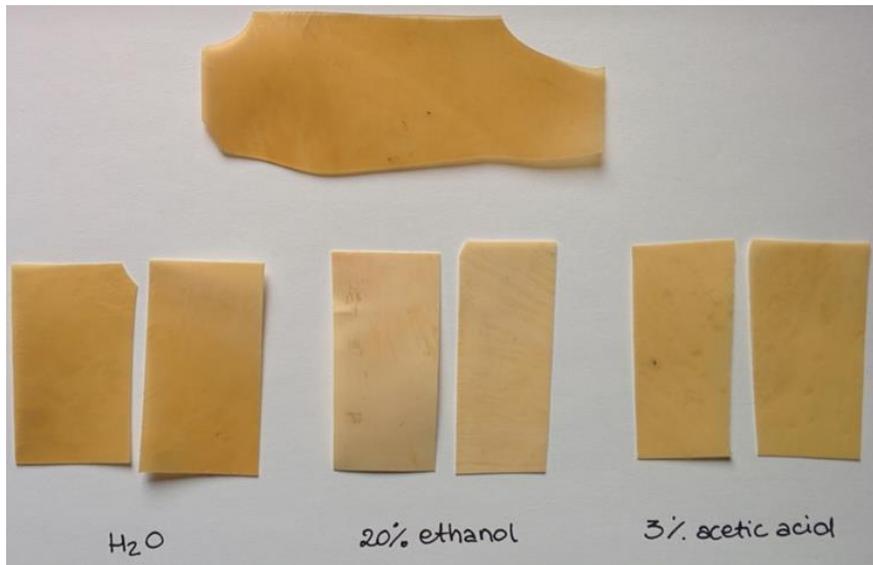
Fig. 5 – PLA_OPE samples before (on the top) and after contact with different food simulants.

Conclusions

The overall migration from PLA filled with 1 % wt. nanoclay was far below the limit of 10 mg/dm^2 set by EU regulation. The results from specific migration assay suggest that some nanoclay elements are released more easily than other or that the nanoclay incorporation makes the polymer less stable and that Ca is released but not in nanoparticle size. Contact with distilled water at 40 °C for 10 days brought to a swelling of the PLA, which was visually enhanced by nanoclay filling.

Also overall migration from PLA enriched with orange extract showed very low values (well below the limit of 10 mg/dm^2) in all the three investigated simulants, with a slightly higher value for 3 % acetic acid, than for distilled water and 20 % ethanol. Similarly, the release of orange extract components (phenolic compounds) was extremely low compared to the maximum theoretical release (from 0.1 % to 2.5 % of the total phenols). In this case, 20 % ethanol determined the highest release of total phenols compatibly with the fact that the OPE was obtained through an ethanol extraction. This simulant is indicated for alcoholic foods with alcohol content below 20 % but also for slightly lipophilic food products. Selection of specific food applications is then required to enhance the antioxidant release and then, exploit PLA_OPE as an effective active packaging.

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