Final Report on the NanoPack project (DSF Grant File No. 08-037946)

Introduction

The NanoPack project was financially supported by the Danish Council for Strategic Research in the period 2007-2011 and was focused on the development of new bioplastic-based, thermoformed meat packaging trays. Partners in the project were Risø DTU, two departments from the University of Copenhagen (KU), Faculty of Life Sciences (Food Science (IFV) and Basic Sciences and Environment (IGM)), two departments from DTU Food (Chemistry, and Toxicology and Risk Assessment), Færch Plast A/S and the Danish Meat Research Instutute (DMRI, now part of the Teknologisk Institut). The overall goal was to develop polylactide (PLA) nanocomposite films which could be used in meat packaging and which would meet consumer requirements for sustainability, functionality and safety. The NanoPack objectives covered:

- Development of PLA/montmorillonite (MMT) clay nanocomposites
- Development of PLA/layered double hydroxide (LDH) clay nanocomposites
- Transfer of knowledge from laboratory to pilot-scale for film and food tray production
- Development of new analytical methods
- Knowledge dissemination

Approach

From a materials perspective, the approach to the project involved the use of commercial polylactides from NatureWorks LLC (Ingeo™ extrusion/thermoforming grades) in combination with organomodified MMT (OMMT) clays from Southern Clay Products (SCP) under the Cloisite™ trade name. After initial laboratory and pilot-scale work, Cloisite™ 30B from SCP was selected as the preferred OMMT. In parallel, a PhD student project at KU explored the synthesis and characterization of layered double hydroxide clays (LDHs) which were modified using long-chain fatty acids. As with the OMMTs, the objective was to hydrophobize the clay and to insert the organomodifier in the clay galleries, both of which should enhance dispersability in a PLA matrix. In 2009-2010, two series of pilot-scale melt processing trials using PLA in combination with Cloisite™ 30B or a laurate (C12)-modified LDH were carried out at Kunststof Kemi in Nykøbing Mors. The resulting films were fully characterized and, as an outcome, a thermoformed meat packaging shelf life trial was designed and carried out in the final year of the project. In addition, although PLA/Cloisite™ 30B films were selected for this trial, the oxygen barrier improvement in these films was not at the project target level (x10 versus PLA reference) and therefore research on an alternative surface coating approach to achieve the barrier effect was included in the project.

Overview of methods and key research findings

1. *The properties of PLA films produced at Kunststof Kemi*

Following an initial period in which PLA/nanoclay films were prepared in the laboratory and also using extruders located at the Danish Polymer Center, DTU , Lyngby and at the Thermofisher company in the UK, preferred facilities for melt extrusion experiments at Kunststof Kemi were utilized. Clays and PLA were melt compounded either by direct mixing or by preparation of laboratory masterbatches and films with 5 wt% clay content were then extruded. The oxygen permeability properties of the PLA films are shown in Figure 1, which indicates the significant permeability reduction obtained in films containing 5 wt% Cloisite™ 30B. The line marked PLA/Cloisite-CS-LA in Figure 1 refers to film prepared using chitosan-modified MMT clay, synthesised in the laboratory at Risø DTU as an alternative to Cloisite™ 30B. On the basis of these and other results, the three film types indicated in Figure 1 were included in the meat packaging shelf life trial (see later).

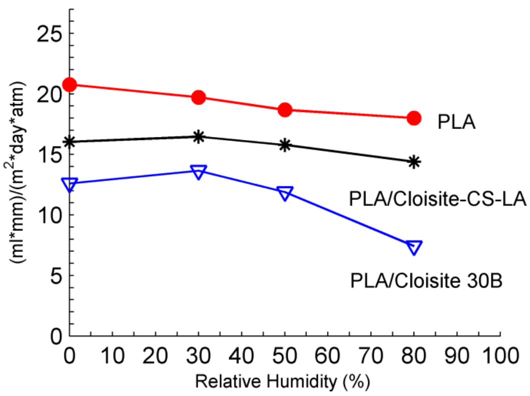

Figure 1: Oxygen permeability of PLA films produced at K-Kemi as a function of relative humidity

Granulates and films from the processing work at Kunststof Kemi were analysed using gel permeation chromatography to determine PLA molecular weights. PLA is sensitive to degradation during processing even when rigorously dried; however, the differences in weight-average molecular weights (Mw) values for films with and without Cloisite™ 30B or Cloisite™CS-LA were not statistically significant. In contrast, when a laurate (C_{12})-modified layered double hydroxide (LDH), synthesised at KU Faculty of Life Sciences, was melt processed with PLA, significant reductions in polymer molecular weight were detected even though the clay and the polymer had been previously dried in the prescribed manner. This finding might be due to the presence of tightly bound water in the LDH structure but is more likely attributed to metal centers in the LDH acting as catalysts for PLA deploymerisation. As a result of this effect, research on laurate-modified LDH as nanofiller in PLA was not pursued in the direction originally planned.

2. *Nanoparticle migration and characterisation*

A multi-instrument technique based on combining asymmetric flow field-flow fractionation with light scattering and inductively-coupled plasma mass spectrometry (ICP-MS) was set up at DTU Food, validated using gold nanoparticle standards and applied to the characterisation of Closite™ 30B and its migration from PLA films. The results in Table 1 point to the following: i) total migration values were less than the allowable 10 mg/ dm^2 standard, ii) clay quantities detected were as expected for the spike reference samples, iii) significant migration was detected from all PLA/Cloisite™ film samples although clay could not be found chemically using ICP-MS, suggesting that migrating PLA oligomers were responsible, iv) clay was chemically detected in migrates from PLA-LDH films, which could be a consequence of PLA degradation in these cases.

Sample	Clay load	Total migration	Clay detection (ICP-MS)
PLA	None	1.7±0.6 mg/dm^2	No
PLA/Cloisite™ 30B	5%	6.7±0.5 mg/dm^2	No
Cloisite™ 30B spike	1.9 mg	2.1±0.5 mg	Yes
PLA/Cloisite™ 15A	5%	11.5±1.9 mg/dm^2	No
Cloisite™ 15A spike	2.8 mg	3.2±1.0 mg	Yes
PLA/Cloisite™ 20A	5%	5.4±0.3 mg/dm^2	No
Cloisite™ 20A spike	3.3 mg	3.0±1.3 mg	Yes
PLA-PF3*	1.8%	8.3±0.8 mg/dm^2	Yes
PLA-PF1*	1.8%	9.6±1.9 mg/dm^2	Yes
PLA-PF2*	5.5%	31.9±7.4 mg/dm^2	Yes
LDH spike	5.0 mg	5.2±0.2 mg	Yes

Table 1: Total migration and chemical detection of clay using ICP-MS. Other SCP clays (Cloisite™ 15A and 20A) were included for comparison. Films PLA-PF1*, 2* and 3* contained laurate-modified LDH.

3 Toxicology

As a result of findings from the PLA/clay processing trials, Cloisite™ 30B and the unmodified Cloisite™ Na^+ were chosen as the focus for the toxicological studies at DTU Food. The first step was to run in-vitro tests on genotoxicity based upon a Salmonella/microsome assay and also a comet test for DNA damage. The results of the latter showed that, unlike Cloisite™ Na^+, Cloisite™ 30B induced DNA damage and that this could be due to the quaternary ammonium modifier on the clay. Consequently, a series of in-vivo tests were conducted and, in this case, the comet assay showed that Cloisite™ 30B did not induce DNA damage. Furthermore, neither type of clay induced inflammatory or immuno responses in blood samples from test rats and elemental analysis showed that there was no clay uptake in the liver or kidney of these animals. These results were viewed as positive in terms of the overall project activities; however, they do not in themselves give the complete green light to the clays in question. The reason for this is that the tests conducted were not fully to OECD guidelines, further work is needed to determine whether Cloisite™ 30B causes DNA damage in-vitro and in-vivo, and more robust mutation frequency assays meeting OECD guidelines should ideally be undertaken.

4 Meat packaging shelf life trial

Following the polymer processing, nanoparticle characterization, migration and genotoxicity studies and as planned, a five-week modified atmosphere meat packaging shelf life trial was set up in the final year of the project in a cooperative exercise run by DMRI and Færch Plast A/S.

PLA films produced in a trial at Kunststof Kemi in mid-2010 were selected and thermoformed into trays at Færch Plast. Four film types were used: PET control, PLA reference, PLA/5% Cloisite™ 30B and PLA/5% chitosan-Cloisite™. The last of these was produced using a chitosan-intercalated Cloisite™ clay prepared in the laboratory at Risø DTU and was included as an alternative because final conclusions about the toxicity of Cloisite™ 30B were still to be determined at that time. The trial was set up to include both fresh meat and processed meat and the conclusions were as follows: i) PLA/chitosan-clay films gave an unacceptable aroma when packaged meat trays were opened, ii) oxygen concentrations in the test packages based on PLA and the Cloisite™ clays were significantly reduced but this had little effect on meat colour maintenance, iii) the oxygen barrier effects had no influence on the microbiological shelf life of either the fresh or processed meat, iv) rancidity due to oxidation of proteins and fat was not found in any of the packaged meat products. The outcome of the trial was that none of the PLA trays performed as well overall as the PET control, indicating that, in the case of the PLA films, oxygen permeability and other factors still need to be optimised.

5 *Layer-by-layer coating technique*

During the course of the project, the barrier properties of commercially coated PLA films had been evaluated (e.g., Oxaqua PLA09); however, it seemed unlikely that these materials would perform sufficiently well at high relative humidity or retain barrier properties after thermoforming. Nevertheless, given that PLA/Cloisite™ 30B films did not exhibit the oxygen barrier effect targeted at the project outset, the concept of achieving a high-barrier material through layer-by-layer coating was examined in the last year of the project. The approach involved sequential coating of PLA films with a chitosan solution and a suspension of Cloisite™ Na^+ in water. In cooperation with other partners in Copenhagen, the fundamentals of this approach were studied using a quartz crystal microbalance technique. The oxygen barrier improvement as a function of relative humidity is shown in Figure 2, confirming that this approach can in principle be very effective.

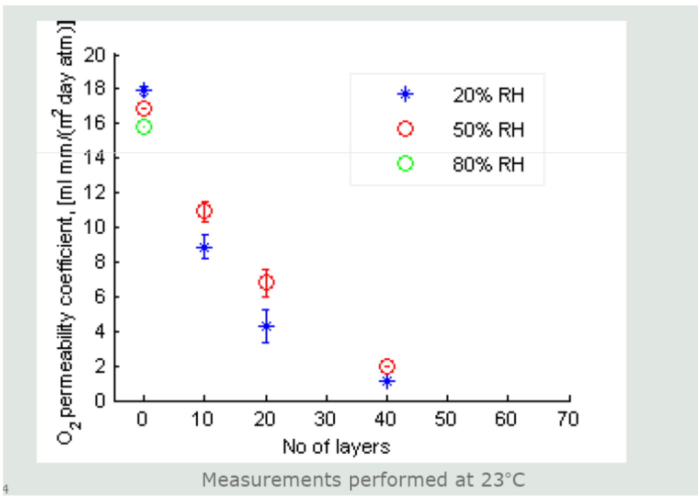

Figure 2: Oxygen permeability of layer-by-layer coated PLA films as a function of the number of chitosan/clay layers

The layer-by-layer approach requires further investigation and improvement, especially in respect to higher water vapour barrier properties and in determining whether or not such a system could be designed to withstand thermoforming. These topics will potentially be the subject of further investigations in a subsequent research project.

Industrial and societal results

The NanoPack project delivered new knowledge in respect to the potential for use of PLA nanocomposites in food packaging, particularly in the key topics of particle migration and toxicity which are becoming of increasing interest to all stakeholders (i.e., industry, consumers, regulatory bodies, other government agencies). As has been discussed at a number of recent conferences, there is still much work to be done to clarify the risks associated with nanotechnology in the food packaging industry. Although a new polylactide nanocomposite packaging product was not an outcome of the project, advances were made in regards to coating technology which, with further development, could be a promising alternative and which should be the subject of further research. Similarly, a significant contribution has been made in terms of new knowledge regarding the use of LDH clays in combination with PLA.

Research education

The NanoPack project results were built upon very significant contributions from two PhD students (DTU Food and KU LIFE) as well as two post docs (Risø DTU and KU LIFE/Risø DTU). The activity of these individuals spanned the range from analytical technique development, clay synthesis and polymer processing through to layer-by-layer coating technology and film characterization techniques. Two three-week student projects were also undertaken at DTU in 2009-2010 and involved bench-scale PLA/clay melt processing trials.

Collaboration

National and international collaboration featuring in the NanoPack project can be summarised as follows:

- Andy Baker, Thermofisher, UK: advice and access to melt extrusion facilities
- Stine Lausten, Kunststof Kemi A/S, Nykøbing Mors: access to and assistance with PLA nanocomposite extrusion processing.
- Andy Burrows, Center for Electron Nanoscopy (CEN), DTU, Lyngby: access to state-of-the-art scanning and transmission electron microscopy facilities.
- Peter Weidler and Frank Friedrich, Karlsruhe Institute of Technology (KIT) Germany: Discussions and assistance with electron microscopy.
- Anna Åkesson[1], Marite Cardenas[1] and Sanja Bulut[2], KU Institite of Chemistry and Nanoscience Center[1] and DTU Nanotech[2]: cooperation on layer-by-layer coating.

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