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Characterization of Cellulose Nanocrystals and PLA Based Thin Films with either Silver or Antimicrobial Peptide

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Abstract: *Sustainable polylactic acid (PLA) antimicrobial-based plastics were engineered with a cellulose nanocrystals (CNC) filler. The small weight fractions (< 10 % w/w in total of CNC) and antibacterial agents were added in the different systems. Silver (Ag) nanomaterial was used as a positive control. A peptide sequence known to exhibit antimicrobial activity was integrated into the PLA based systems. The plastics were previously characterized in an earlier publication from our group. That publication focused on estimating the thermal and mechanical stability of the plastics, the oxygen and water vapour permeability, and the morphological arrangement of the components within the plastics. Hence, in this study, our team completed antimicrobial testing against four strains of organisms commonly encountered in the food industry. All the systems with either Ag (higher concentration systems only) or peptide were characterized with significant inhibition of microbial growth over different time periods. Further, another important parameter was to estimate whether exposure to a medium concentration within the package will reduce the integrity of the plastic systems. Thus, plastics exposed to different solvents were assessed for migration rates. The thermal and mechanical properties after soaking were determined. All systems were characterized with migration rates below values reported by international bodies. Finally, in most cases, when the plastics were exposed to the solvents, they were maintained their mechanical and thermal properties overtime.*

Keywords*: Cellulose nanocrystals, poly (lactic) acid, antibacterial properties, thermal and mechanical properties*

I. Introduction

Currently, one of the main areas of research focuses on the development of renewable and biodegradable based plastics. Dorgan et al. (2001) comprehensively reviewed the prospects of using polylactides as an environmental benign plastic. They stated that polylactides are an appealing option because they are manufactured from renewable sources, sequester large quantities of carbon dioxide during the production of these renewable materials, are easily degraded, and have comparable mechanical properties to polyolefin based materials that are currently in demand. One such polymer is polylactic acid (PLA), a biodegradable thermoplastic polyester produced by fermentation of corn starch (Fortunati et al. 2012).

PLA is commercialized and has different applications in the manufacturing industry such as containers, drinking cups, or packaging materials for fresh fruits and vegetables (Rapa et al. 2016, Girdthep et al. 2016). Nevertheless, there are a few key limitations affecting the potential of PLA based plastics. These include poor thermal stability and poor barrier properties (Fortunati et al. 2012, Fortunati et al. 2013). As a result, several research teams have focused on adding nanomaterials to aid with the mitigation of these shortcomings. For instance, Trifol et al. (2015) engineered PLA based plastics with nanocellulose/nanoclay fillers. They used Cloisite C30B and nanocellulose (either acetylated nanocellulose or nanocrystalline cellulose) at varying percentages and investigated the barrier and thermomechanical properties. Their research team reported a synergistic effectbetween the oxygen transmission and the water vapour transmission rates, with reductions greater than 90 %. Further, the plastics were characterized with improved thermomechanical resistance and improved crystallization. In a similar case, Fortunati et al. (2013) studied the effect of reacting the cellulose nanomaterials with a surfactant prior to integration with PLA and silver (Ag-potential antimicrobial agent). They found that the resulting plastics were characterized with increased barrier effect while none of the plastic systems exceeded the migration levels stipulated by governing international bodies.

However, improving the limitations of PLA based structures is only one focus area. Another key aspect is inducing antibacterial properties into the plastics for food and medical applications. Different strategies have been implemented to impart these properties into the plastics. For example, teams have triedcoating the plastics with an active agent (Salmieri et al. 2014, Tawakkal et al. 2014), inserting sachets into the packaged goods (Tian et al.

2013), and inserting bioactive component into the packaging material itself (Fortunati et al. 2012, 2013). Given that the phenomenon of bacterial contamination is a surface based mechanism, it is in our best opinion that incorporation of the bioactive agent into the film will impart many advantages when compared to the other strategies.

In the past, there has been the incorporation of different types of bioactive agents have been incorporated into PLA based plastics. There has also been an investigation into the efficacy of enzymes (Arrnentano et al. 2013), bacteriocin (Ming et al. 1997), and organic acids (Weng Chen, 1997). With the rapid acceptance and potential of silver compounds, much of the research with those materials was less focused on over time. Silver based agents are well researched because they are easily inserted into the plastics and have various applications.Since the acceptance from the Food and Drug Administration /Centre for Food Safety and Applied Nutrition (FDA/CFSAN-USA) of silver zeolites in all food contact applications (and as an active ingredient in bottled water), the research in this space has evolved. But, silver based materials are characterized with very low migration rates from the polymeric matrix and there is an active debate whether the small quantities of silver released can significantly address the safety concerns when used for food applications.

In this research study, the main aim is to build on a previous communication (George et al. 2017) where we studied the thermal, mechanical, morphological, and barrier properties of PLA based plastics. The plastics were fortified with cellulose nanocrystals and one of two antimicrobial agents (either silver nanoparticles or a peptide) and the properties studied. In this communication, we seek to study the migration properties (overall and specific) and the antimicrobial effect of the blends. Also, we determined whether soaking the plastics in ethanol or isooctane (used for migratory studies) significantly influences the thermal and mechanical properties. This study is of utmost importance because it is assumed that Ag nanoparticle or peptide will not leach into the food material above specified quantities established for food consumption. The study will provide important information on the sustainability of these two antimicrobial agents, one an established researched item (Ag) and the other a novel material (peptide).

II. Experimental

2.1 Materials

Cellulose nanocrystals (CNC) and PLA (NatureWorks LLC, Minnetonka, USA) were obtained in-kind from a pilot scale facility at Alberta Innovates Technology Futures, Alberta, Canada. The PLA factsheet specified a glass temperature of 50-60 °C, a melt temperature of 145-170 °C, a melt density of (200°C) of 1.2 g/cc, and a pellet bulk density of 0.79-0.85 kg/L. Silver nanopowder (< 100 nm, Mol. wt. 107.87 g/mol), nitric acid (70 %, Mol. wt. 63.01 g/mol), ethanol (absolute > 99.8 %, Mol. wt. 46.07 g/mol), and chloroform (anhydrous, > 99 %, Mol. wt. 119.38) were sourced from Sigma-Aldrich (Minnesota, USA).

For the peptide synthesis, Fmoc-leu-OH (Mol. wt. 353.4 g/mol), Fmoc-lys(BOC)-OH (Mol. wt. 468.5 g/mol), HCTU (Mol. wt. 413.69 g/mol) were sourced from Sigma-Aldrich (Minnesota, USA). Also, Fmoc-Leu-wan resin (0.57 mmol/g, 100-200 mesh) and 4-methylmropholine (> 98 %, Mol. wt. 101.15 g/mol) were obtained from Sigma-Aldrich (Minnesota, USA). Hardy Diagnostics (Santa Maria, CA). supplied the Mueller Hinton Broth, Criterion™ Dehydrated Culture Media (2kg). *Staphylococcus aureus* (ATCC6538), *Klebsiella poeumoniae* (ATCC4352), *Salmonella typhimurium*,and *Escherichia. Coli* (O157: H7 ATCC-35150) were obtained from ATCC.

2.2 Methods

2.2.1 CNC production

Samples of cellulose nanocrystals (CNC) were obtained from a pilot scale facility located at Alberta Innovates Technology Futures, Edmonton, Canada. The samples were stored at 4 °C until utilization. The CNC samples were obtained via hydrolysis of dissolving pulp. Reaction parameters of 120 minutes, 45 °C, stirred at 200 rpm, and a sulfuric acid concentration of 64 % (wt.) were used to produce the samples. An acid: pulp consistency of 12:1 was used for all experiments. Samples were finally spray dried for transport and storage.

2.2.2 Peptide synthesis

E14LKK is a 14 residue, magainin-class peptide with broad-spectrum demonstrated antimicrobial activity (Steven and Hotchkiss, 2008). It was synthesized by following the standard Fmoc (9-fluroenylmethoxycarbonyl)-based solid-phase peptide synthesis (SPPS) protocol (Fields and Noble, 1994). The freeze-dried peptide was re-suspended before addition to the plastics.

2.2.3 Experimental design for thin film production

Thin films were made from PLA and CNC. Solvent casting was used to produce all films. Plastics were fitted with silver nanoparticles or a peptide. The experimental design is presented in Table 1 (George, Shen, and Montemagno, 2017).

Table 1. Experimental design

System	Wt. PLA (mg)	Wt. CNC (%)	Wt. Ag (%) or Peptide (%)[1]
PLA[2]	1000 mg	-	-
PLA_CNC_1.0%CNC	980 mg	1.0	-
PLA_CNC_2.5%CNC	975 mg	2.5	-
PLA_CNC_5.0%CNC	950 mg	5.0	-
PLA_CNC_1.0%CNC_xAg	980 mg	1.0	0.25, 0.75, 1.25
PLA_CNC_2.5%CNC_xAg	975 mg	2.5	0.25, 0.75, 1.25
PLA_CNC_5.0%CNC_xAg	950 mg	5.0	0.25, 0.75, 1.25

[1] Given these components were used in such low weight relative to CNC and PLA, they were not calculated into the final weight.
[2] Control for CNC based PLA plastics

As can be appreciated, many films were formulated because of the number of parameters covered. For any given system, the required amount of PLA was dissolved in 20 mL chloroform and stirred for 2 hours. Depending on the system, a specific amount of CNC (and in some cases either Ag or peptide) were weighed separately and slowly added to the PLA on the stir plate. The sample was vigorously stirred for another 2 hours (4 hours when Ag or peptides were added). Immediately after stirring, the PLA mix was poured onto a Pyrex 9 cm petri dish (all sample types were done in triplicate). A smooth stirring rod was used to evenly distribute the PLA mix onto the bottom of the petri dish. All petri dishes were covered with aluminium foil to facilitate slow evaporation of chloroform in a fume hood. After 24 hours (36 hours in some instances, when peptides were used), the plastics were peeled from the bottom of the dishes and conditioned in a vacuum over at 40 °C for 8 hours. Samples were sealed in bags and stored for subsequent analysis.

2.2.2 Overall migration per specimen

The overall migration rates of the different plastics were performed in 5 % (v/v) ethanol (stimulant A), 10 % (v/v) ethanol (stimulant B), and isooctane (stimulant C). For each sample, triplicate rectangular strips (10 cm^2 total area) were immersed in glass tubes containing approximately 15 mL of each stimulant. All samples exposed to ethanol were kept in a controlled atmosphere at 40 °C for 10 days (Fortunati et al. 2013). These reaction conditions were adopted from Regulation EU No. 10/2011 (Commission Regulation EU 10/2011). On the other hand, samples exposed to isooctane were kept at 20 °C for two days, based on the European Standard EN 1186-1:2002. After the incubation time, each sample was removed from the stimulant. The remaining stimulant was either air-dried or in a vacuum oven at 40 °C (three consecutive measurements within ± 2 % of overall weight signified constant weight). The residues were weighed using an analytical balance with ± 0.1 mg precision. The overall migration rate was reported in $\mu g\ kg^{-1}$.

2.2.3 Specific migration per specimen

A) Ag based films

The specific migration of the different samples was estimated analysing the Ag^+ released in the stimulants by InductiveCoupled Plasma spectroscopy with Mass Spectrometry detection (ICP-MS). For samples exposed to ethanol stimulant, no further treatment was necessary after removing the stimulant (as done above). On the other hand, for samples exposed to isooctane, 5 mL of 1 % (w/v) nitric acid was added to the residue of each tube. A kinetic study was performed on selected systems (randomly selected), where the specific migration was determined at different time periods (Ethanol systems-2, 4, 6, 8, and 10 days; isooctane system-8, 16, 24, 36, 48 hours). An ICP-MS was used for all analysis. Each experimental run was conducted in the presence of argon (0.40 L/min at 2 °C) at 1600 W of radiofrequency. A calibration curve was obtained using Ag^+ in a concentration range between 10 $\mu g\ kg^{-1}$ and 1200 $\mu g\ kg^{-1}$. Each standard was done in triplicate. In this specific case, the LOD and LOQ were calculated as 2 and 8 times, respectively, the standard deviation for the blank measurements.

The diffusion coefficients were calculated from the beginning of the migration process by using a migration model based on the Fick's second law and previously described by the equation 1 (Chung et al. 2002).

$$\frac{M_f}{M_i} = \frac{2}{L_p}\left(\frac{Dt}{\pi}\right)^{0,5}$$

Where M_f is the amount of Ag^+ in stimulant after exposure and M_i is the amount Ag^+ of in the plastics before exposure to the stimulant. $L_p$is the thickness of a given film and D is the corresponding diffusion coefficient.

B) Peptide based plastics (HPLC determination of [peptide]

The method reported by Di Lorenzo et al. (2014) was closely followed to determine the concentration of peptide leached from the plastic samples after exposure. Specifically, amino acid and peptide quantification recovered from the different systems were analysed using a HPLC equipped with a fluorichrom detector (excitation 340 nm; emission 450 nm). Samples were derivatized using the O-phthadialdehyde method as reported by Mekonnen et al. (2013). Sample separations were done via a Supelcosil 3 micron LC-18 reverse phase column (4.6 mm × 150 mm; Supelco) equipped with a Supelco guard column (4.6 mm × 50 mm, Supelco, Oakville, ON, Canada) packed with Supelco LC-18 reverse phase packing (20–40 μm). Finally, the derivatized samples were eluted with a gradient composed of 0.10 M sodium acetatetrihydrate buffer (eluent A) and methanol (eluent B) for a total analysis time of 45 min.

2.2.4 Mechanical properties after exposure to stimulants

he mechanical properties of the plastics exposed to the 10 % (vol.) ethanol and isooctane stimulants were determined. ASTM D 882 Standard Test method for the tensile properties of thin plastic sheeting was followed for all samples. An Instron dual column table-top universal testing (System 3365) equipped with a 5 kN capacity, 1000 mm/min maximum crosshead speed, and vertical test space of 1192 mm was used for all tests. Prior to testing, all samples were conditioned at 40 °C for 8 hours. Depending on the precision of the measurements, up to 10 tests were performed per sample. Cast films were cut into 5 by 50 mm pieces. The thickness, ranging from 65 to 90 μm, was determined using a micrometer. For samples, where the thickness was < 65 μm, an assumption was made that the thickness was 65 μm. A head speed of 10 mm/min was used for all measurements.

2.2.5 Thermal properties after exposure to stimulants

The thermal properties of the plastics exposed to the 10 % (v/v) ethanol and isooctane stimulants were determined. This was done to determine whether agents added to packaging materials would influence the final properties of the plastics. Thermal gravimetric analysis (TGA) was used to determine the effect of the different plastic types on the thermal stability. All samples were tested using a Thermal Analysis Instruments TGA Q50 (TA Instruments) apparatus under a flow of nitrogen to study the effects of heating on stability of the different thin films. Platinum pans were used given their high temperature resistance and the ease of cleaning. The temperature range selected was from room temperature to 600 °C at a rate of 10 °C increase per minute. Triplicate runs were done for each level per treatment. All results were reproduced to 5 % error or better (George et al. 2016). Differential scanning calorimetry (DSC) was used to investigate the thermal transitions for each sample. These measurements were performed using a Thermal Analysis Instruments DSC Q2000 (TA Instruments) in the temperature range of 25-300 °C at a heating rate of 10 °C per minute. Approximately 5-10 mg of sample was used for each analysis. For each sample, triplicate runs were done.

2.2.6 Antimicrobial activity

The antimicrobial activity of the different plastics was studied against some of the most common food borne pathogens. The standard test method for determining the antimicrobial activity of incorporated antimicrobial agent(s) in polymeric or hydrophobic materials (ASTM E2180) was closely followed. The pathogens studied were *Klebsiella Pneumonia (ATCC4352), E. Coli O159:H7 (ATCC35150), Listeria Monocytogenes (ATCC13932), and Salmonella typhimurium (ATCC14028*) were obtained from ATCC. All these organisms are considered Biosafety Level 2; hence all work was done under strict work conditions housed at the National Research Council of Canada Building (NINT), University of Alberta. Mueller Hinton Broth, Criterion™ Dehydrated Culture Media (2 kg) supplied by Hardy Diagnostics was used for all cultures. The Clinical & Laboratory Standards Institute (CLSL) protocol was carefully followed. That is, broth macro dilution method was used to evaluate the antimicrobial activity of the different plastic systems. The starting inoculum required a bacterial suspension of 1×10^8 CFU/ml. Before

determining the antimicrobial activity for the different plastics, the relationship between OD600 and CFU was determined for each strain. This was done to determine what dilution factor needed to be used to determine the CFU. In the bullet points to follow, a simple outline of the different steps is presented.

Calibration and work up of different strains.

1) A bacterial strain was streaked on an MHB agar plate to isolate single colonies. Plates were Incubated overnight at 37 °C.
2) One single colony was picked and transferred into a 50 ml test tube containing 10 ml MHB.
3) Step 2 was repeated a few times to get enough inoculums.
4) The broth was incubated at 37 °C in a shaker at 225 rpm. The turbidity of the samples was checked often. Depending on the strain examined, after 3-6 hours a visual change in turbidity was observed.
5) The OD600 of the cell cultures were then measured. At each time, 1 mL culture was removed for measurement of OD600 and 100 µL was removed to culture for plating. The 100 µL was plated using a dilution of 10-5 to 10-7 (using MHB medium) on each MHB agar plate. The idea was to correlate the OD600 values with the CFU value. A curve was drawn to fit the data points of different OD600 against the corresponding CFU. Care was taken to ensure the OD600 does not exceed 1.
6) The OD600 value that corresponds to the cell concentration of 1×10^8 CFU/mL was estimated.

Determining antimicrobial activity of the different plastic system.

A bacterial strain was streaked on an MHB agar plate. This was done to isolate single colonies. The samples were incubated overnight at 37 °C.

1) A single colony was picked and transferred into a test tube containing 10 mL MHB.
2) The broth was incubated at 37 °C in a shaker at 225 rpm. The cultures were grownto the estimated OD600 that corresponds to 1×10^8CFU/mL.
3) The cultures were diluted in 300 mL MHB medium to give a cell concentration of 5×10^5 CFU/mL based on the OD600-CFU curve. For instance, if the culture was 1×10^8 CFU/mL, 1.5 mL of the culture was added to 300 mL MHB medium. The diluted culture was then mixed thoroughly.
4) 10 µL culture from Step 4 was removed and a dilution of a 1:100 was made using MHB medium. 100 µL of the dilution was plated (using MHB medium) on an MHB agar plate. This step was repeated three times to make replicates. The plates were incubated at 37 °C overnight.
5) Finally, 10 mL of the diluted culture was added to each of the 50 mL test tube containing the plastic sample.
6) The test tubes were Incubatedat 37 °C in a shaker at 225 rpm. 10 µL sample was removed from each test tube at specific time points (such as 4hr, 8hr, 12hr). Based on the turbidity of the cultures, 100 µL of the 10-5 to 10-7 dilutions (using MHB medium) was plated on each MHB agar plate. The plates were incubated at 37 °C overnight.
7) The colonies on every plate were counted and the CFUs were calculated.

2.3 Statistical analysis

In all cases, experiments were replicated at least three times and the results are expressed as mean value ± standard deviation. The major aspects of the statistical analysis were done using SAS Version 9.4. All data sets that were normally distributed were analysed using t-test embedded in Microsoft Excel. For those systems that were not normally distributed, to identify significant differences the Kruskal Wallis Test was applied to the data populations involved, with a 95 % confidence level ($P < 0.05$).

III. Results And Discussion

3.1 Overall migration rate of different plastic systems

Migration is the quantity of material that transfers from a material into a surrounding solvent system. In the case of packaging materials, the addition of additives, such as CNC, Ag, and or peptide can be leached into the food material. Hence, it is of paramount importance to understand the quantities that can potentially leach into the packaged material and the whether these values are within internationally accepted ranges. Migration has attracted widespread focus for the past decade with respect to packaging films used for food applications. This is because producers and consumers are worried whether the leached additives can affect the flavour and shelf life of the packaged foodstuff (Muratore and Zarba, 2011, Palma et al. 2015). In this study, the overall and specific migration of Ag or peptide based PLA + CNC based plastics wasdetermined. Results of the overall migration for the silver and peptide based blends in a) 5 % (vol.) ethanol, b) 10 % ethanol (vol.), and c) isooctane are presented in Figure 1 and 2, respectively.

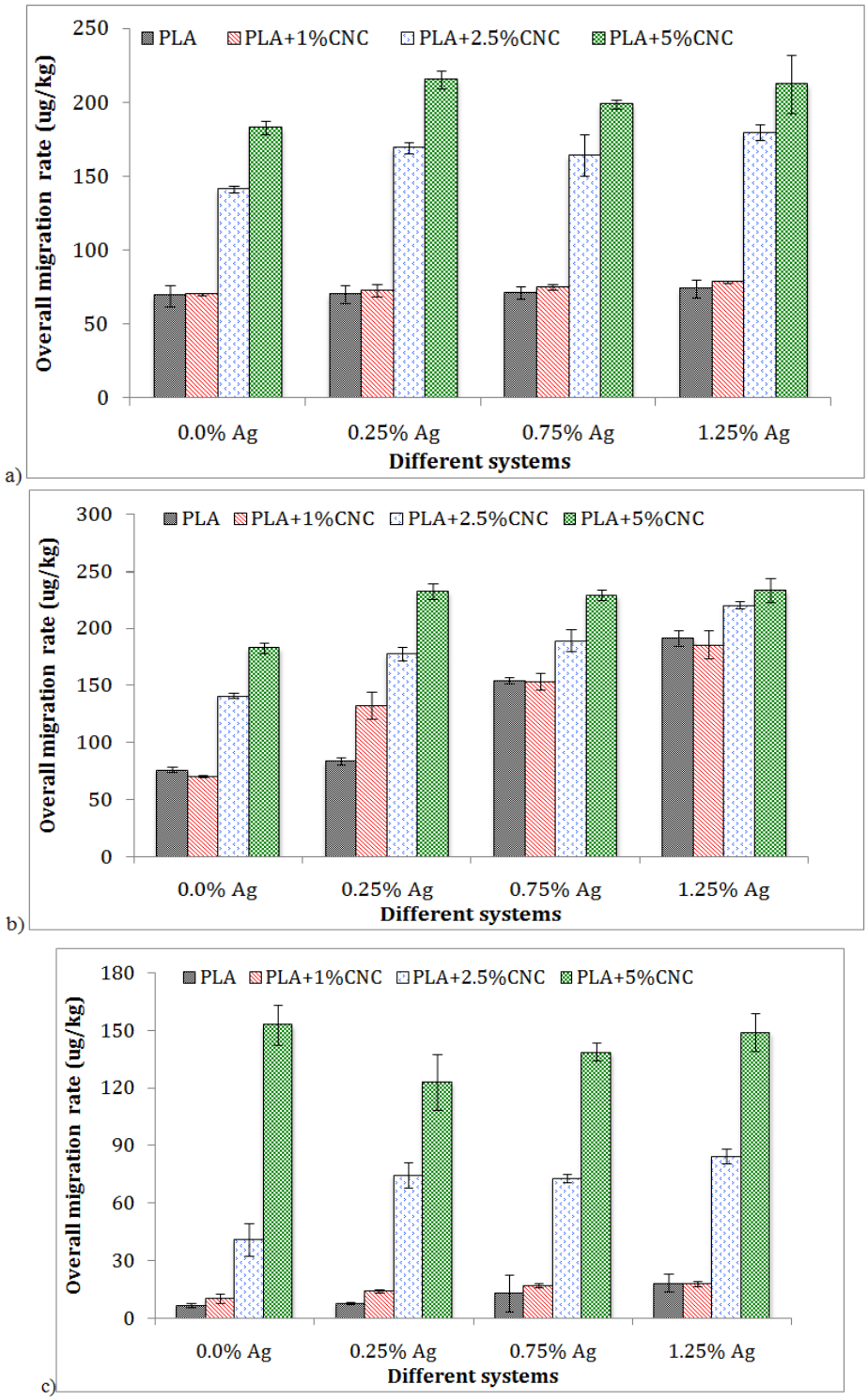


Figure 1. Overall migration (ug/kg) for PLA + CNC + Ag based plastics exposed to a) 5 % (vol.) ethanol, b) 10 % ethanol (vol.), and c) isooctane

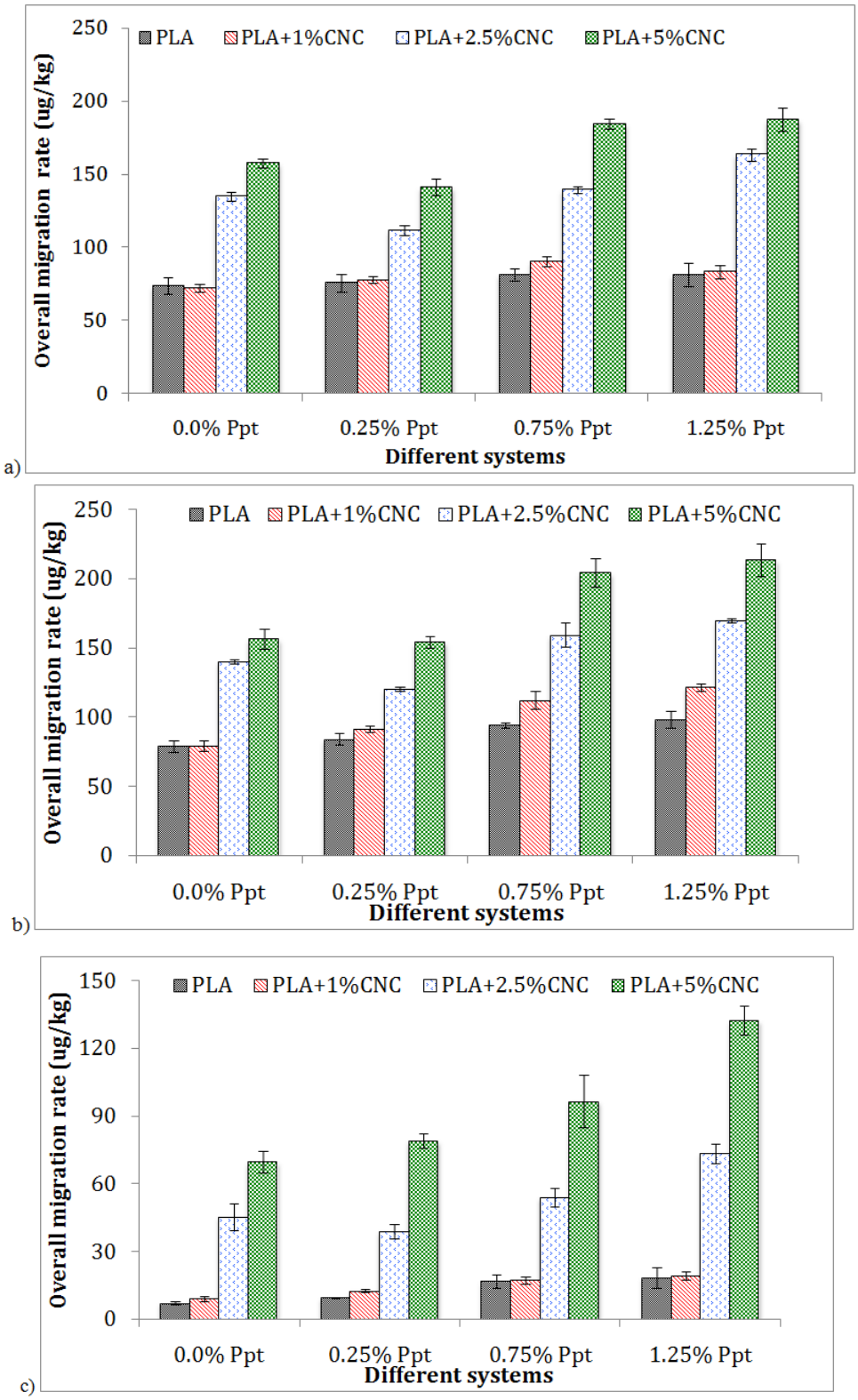


Figure 2. Overall migration (ug/kg) for PLA + CNC + Ppt based plastics exposed to a) 5 % (vol.) ethanol, b) 10 % ethanol (vol.), and c) isooctane

The control samples (PLA and PLA + Ag/Ppt) were characterized with similar migratory concentrations, below 100 ug/kg when exposed to 5 % (v/v) ethanol. On the other hand, the concentration of the additives in the solvents significantly increased when the concentration of Ag increased in 10 % (v/v) ethanol. With respect to isooctane, the controls were fairly within range of each other, even as the concentration of Ag increased. As expected, as the concentrations of both CNC and Ag increased, so did the overall migration rate for all systems. Interestingly, in isooctane, samples were characterized with an increase in overall migration rate, but the systems reached a threshold of about 120 ug/kg, when PLA + 5 % CNC + any [Ag] were studied.

When studying the trends in Figure 2, it can be observed that the controls for PLA + CNC + Ppt systems behaved similarly to the previously reported systems. But, it was observed, unlike with Ag, there was no significant increase in the overall migration rate for these plastics when exposed to 10 % (v/v) ethanol (controls only, there were significant increases in the other systems- 5 % ethanol and isooctane). In summary, higher migration rates were observed for samples fortified with CNC and or Ag/Ppt when compared to the pristine PLA or PLA + CNC systems. However, importantly, non of the systems studied exceeded the overall migration limit quoted by the United States Department of Agriculture (20 mg/kg) and the European Food Safety Authority (60 mg/kg).

The values reported in this communication are very similar to those reported by Fortunati et al. (2013) when they investigated the migration rates for PLA and CNC based ternary plastics. Specifically, when the plastics were placed in 5 or 10 % (v/v) ethanol, the values were comparable to those they reported. But, when the samples were placed in isooctane, the values reported in this communication are significantly lower. In fact, in most cases, there was an observed reduction of approximately 25-35 % for most systems. In summary, the overall migration rate for the different systems studied are within accepted ranges for food material packaging within North America and Europe. Also, the values in most instances are comparable to those previously published for the ethanol stimulant systems, but the values reported when the plastics are in contact with isooctane are significantly lower.

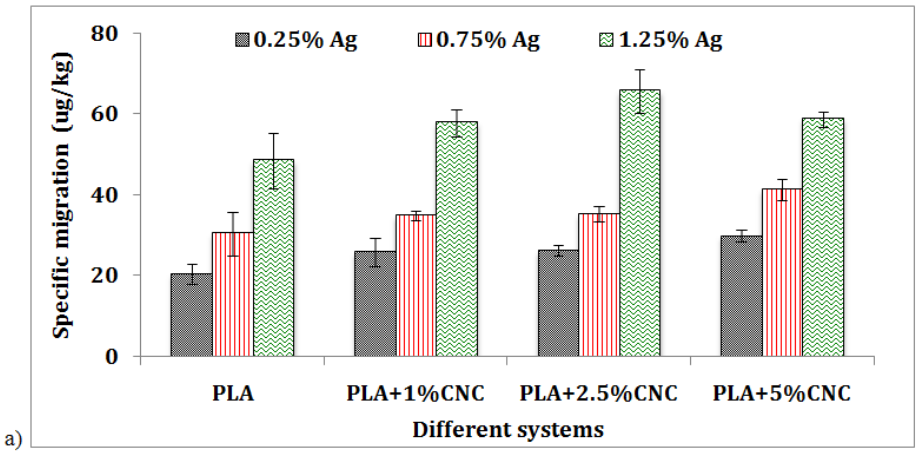
3.2 Specific migration properties

Materials intended for food packaging need to be evaluated for their specific migratory rates. The European Food Safety Authority demands a general specific migration limit of 0.05 mg of silver per kg of food (Fortunati et al. 2013). The specific migration of the silver and peptide based blends in a) 5 % (vol.) ethanol, b) 10 % ethanol (vol.), and c) isooctane are presented in Figure 3 and 4, respectively.

PLA based plastics with 0.25 % (w/w) Ag with any [CNC] were characterized within range of each other, for each stimulant system. As the [Ag] increased, the specific migration of those samples increased as the [CNC] increased for all systems. For most systems, specific migration values reached approximately 60 ug/kg in 5 or 10 % (v/v) ethanol and 16 ug/kg for isooctane. The values reported for isooctane systems were significantly lowered than those reported by Fortunati et al. (2013), but the values when the systems were exposed to ethanol are higher for this study. Nevertheless, the pristine CNC based system reported in this study outperformed their modified CNC based systems, in all cases (when 5 % CNC was used).

Peptide based plastics exhibited similar properties to those for the Ag based plastics. In fact, as the [Ppt] increased, so did the specific migration of the plastics. Careful observation of both peptide and Ag based systems would reveal that as the [Ag] or [Ppt] increases, so does the specific migration rate, indicating that the [CNC] had little or no effect (this is expected because for the specific migration rates, the concentration of [Ag] or [Ppt] were specifically monitored). Although, not being monitored directly, it is plausible that increasing [CNC] can affect the rate of release of either antimicrobial agent from the plastics. That is, addition of CNC can result in morphological changes induced by higher cellulosic content, triggering easier release of bonded or trapped Ag/Ppt particles.

The specific migration of the additives is governed by diffusion and the migration models are based on Fick's second law. In fact, Fick's Law has been used in the past to determine the diffusion coefficients based on carefully planned experiments (Fernandez et al. 2010). When calculated, it was determined that the diffusion coefficients for the different samples were within the range of $(3.50 \pm 0.04) \times 10^{-16}$ cm^2/s to $(1.49 \pm 0.39) \times 10^{-19}$ cm^2/s. These values are in agreement with those reported by Fortunati et al. (2013), for their unmodified CNC based systems. In summary, it was found that incorporation of increasing [CNC] into the PLA matrix accelerated the release of the different additives. Additionally, the plastics behaved differently when exposed to ethanol versus isooctane, with the former having a greater leaching capacity. Nevertheless, the values reported in this communication are within ranges recommended by international bodies.



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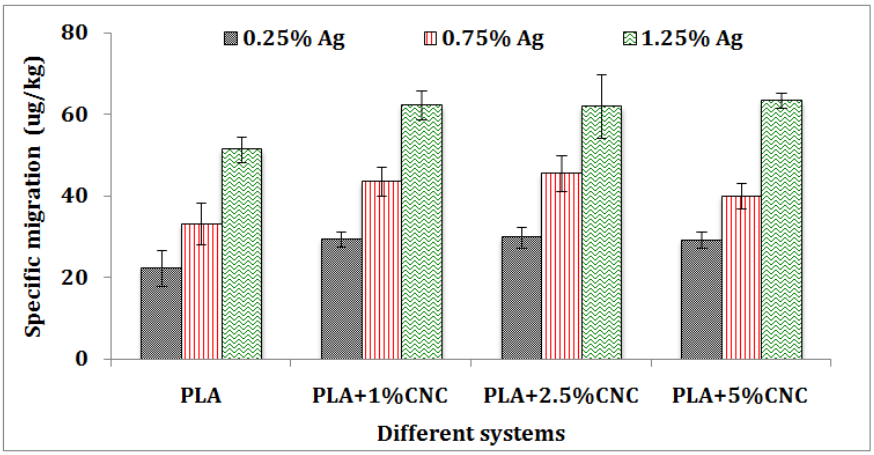

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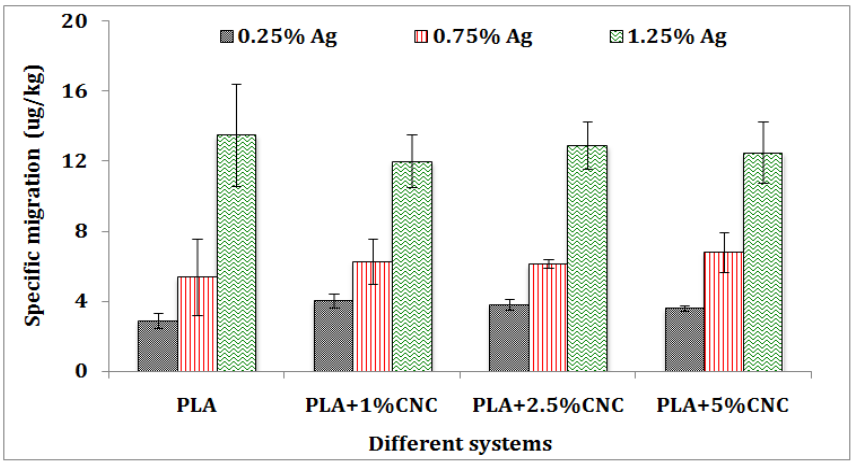
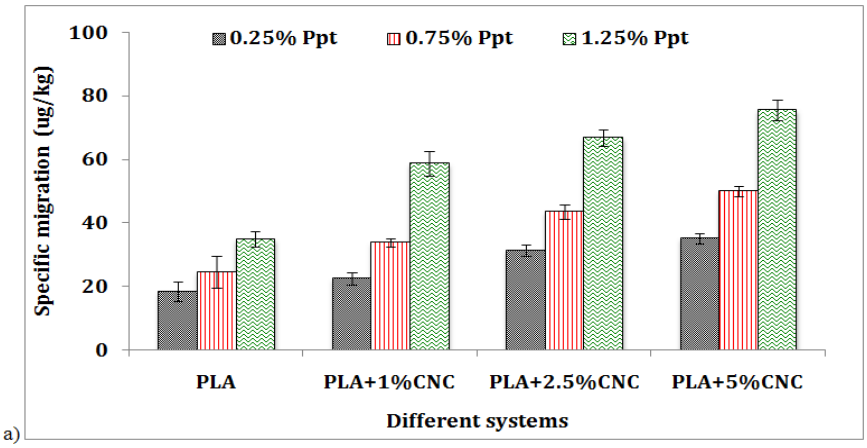
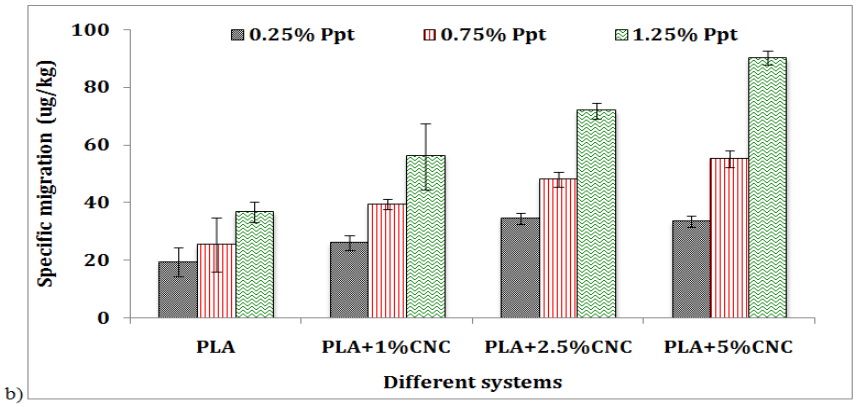


c)

Figure 3 Specific migration (ug/kg) for PLA + CNC + Ag based plastics exposed to a) 5 % (vol.) ethanol, b) 10 % ethanol (vol.), and c) isooctane





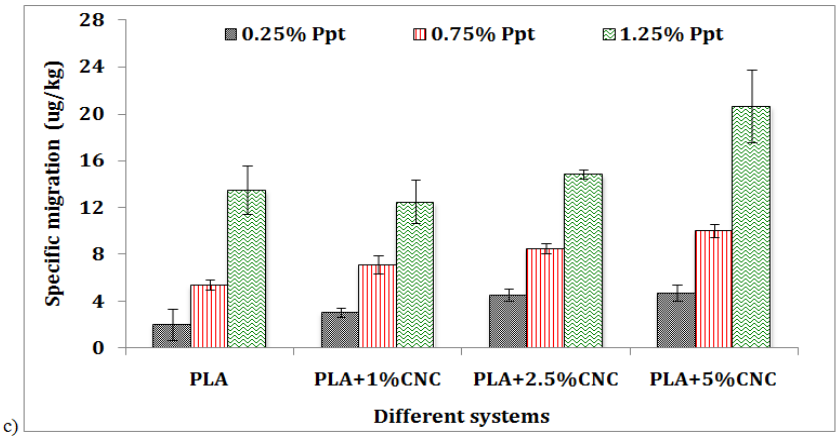


Figure 3 Specific migration (ug/kg) for PLA + CNC + Ppt based plastics exposed to a) 5 % (vol.) ethanol, b) 10 % ethanol (vol.), and c) isooctane

3.3 Mechanical properties after exposure to solvents

In most instances, there was no significant difference between the results obtained for plastics soaked in 5 and 10 % (vol.) ethanol. As a result, samples exposed to 10 % (vol.) ethanol and isooctane is presented in the communication here on. For Tables 2-5, the results are presented as mean / standard deviation. All values highlighted are significantly different from the controls for that specific system at $P < 0.05$.

Table 2. Mechanical property of the CNC+PLA+Ag based plastics systems: original, exposure to 10 % (vol.) ethanol, and exposure to isooctane

Sample	Sub-sample	Original			10 % ethanol			Isooctane		
		Tensile strength (MPa)	Modulus (MPa)	% Elongation	Tensile strength (MPa)	Modulus (MPa)	% Elongation	Tensile strength (MPa)	Modulus (MPa)	% Elongation
PLA	0 % Ag	19.4 / 0.51	2514 / 212	6.14 / 0.20	19.2 / 0.81	2356.8 / 32.4	7.54 / 0.70	19.1 / 0.21	2593.8 / 42.1	6.21 / 0.45
	0.25% Ag	17.5 / 1.47	2468 / 108	6.02 / 0.58	17.1 / 1.07	2405.2 / 74.5	6.52 / 1.28	18.1 / 1.29	2365.2 / 52.5	6.23 / 0.32
	0.75% Ag	20.1 / 0.89	2399 / 130	6.59 / 1.05	19.1 / 0.49	2320.8 / 53.9	7.19 / 0.09	19.9 / 0.19	2321.8 / 49.1	6.29 / 1.85
	1.25% Ag	21.3 / 1.91	2601 / 101	6.16 / 1.53	19.3 / 1.01	**2334.4 / 34.9**	7.41 / 2.73	18.2 / 2.11	**2304.4 / 31.1**	6.10 / 0.53
PLA+1 %CNC	0 % Ag	17.5 / 1.47	2645/ 39	4.48 / 0.33	17.1 / 1.97	2505.2 / 19.8	6.22 / 1.33	17.2 / 1.13	2625.2 / 79.8	6.29 / 1.03
	0.25% Ag	18.7 / 0.39	2659 / 166	4.99 / 0.21	17.9 / 1.19	2525.0 / 26.6	5.41 / 0.71	18.0 / 0.12	2619.0 / 32.6	5.54 / 1.01
	0.75% Ag	17.4 / 0.64	2681 / 200	4.98 / 0.32	17.0 / 0.54	2619.6 / 90.5	5.10 / 0.52	16.9 / 0.24	2569.6 / 50.1	5.40 / 0.12
	1.25% Ag	17.6 / 0.70	2687 / 87	5.12 / 0.41	16.1 / 0.21	**2324.6 / 37.2**	5.28 / 0.46	16.2 / 0.45	**2139.9 / 27.2**	5.82 / 0.21
PLA+2 .5% CNC	0 % Ag	22.1 / 0.80	2700 / 33	4.55 / 1.05	21.1 / 1.80	2650.8 / 83.9	6.39 / 1.45	21.9 / 0.50	2644.8 / 23.9	6.17 / 1.75
	0.25% Ag	21.5 / 1.74	2711 / 22	4.79 / 0.42	20.5 / 0.76	2701.2 / 42.7	6.18 / 0.39	20.1 / 0.44	2701.2 / 29.7	6.14 / 0.12
	0.75% Ag	22.9 / 0.70	2696 / 131	5.38 / 0.33	21.0 / 0.10	2613.0 / 26.6	6.19 / 0.63	19.3 / 1.40	2503.0 / 66.5	5.89 / 0.13
	1.25% Ag	22.3 / 0.69	2721 / 37	4.20 / 0.20	20.1 / 0.99	**2592.0 / 17.9**	5.90 / 0.50	19.0 / 0.19	**2412.8 / 37.5**	5.85 / 0.17
PLA+5 %CNC	0 % Ag	23.1 / 1.11	2864 / 24	5.10 / 0.53	22.3 / 0.11	2834.4 / 94.2	7.19 / 0.23	22.9 / 0.54	2754.4 / 94.2	7.96 / 1.33
	0.25% Ag	23.3 / 0.75	2899 / 45	4.86 / 0.30	23.4 / 1.45	2789.2 / 95.8	7.46 / 1.36	18.4 / 1.45	**2707.2 / 15.8**	**8.06 / 1.30**
	0.75% Ag	22.7 / 0.64	2985 / 26	5.04 / 0.34	20.7 / 0.14	**2705.6 / 59.8**	7.04 / 1.35	17.4/ 0.25	**2577.6 / 76.8**	**9.14 / 0.54**
	1.25% Ag	24.1 / 1.03	2945 / 20	5.14 / 0.26	23.4 / 0.48	**2696.0 / 32.5**	6.44 / 0.19	17.1 / 0.99	**2610.0 / 49.3**	**9.04 / 1.26**

Table 3. Mechanical property of the CNC+PLA+Ppt based plastics systems: original, exposure to 10 % (vol.) ethanol, and exposure to isooctane

Sample	Sub-sample	Original			10 % ethanol			Isooctane		
		Tensile strength (MPa)	Modulus (MPa)	% Elongation	Tensile strength (MPa)	Modulus (MPa)	% Elongation	Tensile strength (MPa)	Modulus (MPa)	% Elongation
PLA	0 % Ppt	19.2 / 0.41	2396 / 29	5.99 / 0.41	19.9 / 0.49	2169 / 101	8.01 / 0.77	18.4 / 0.49	2109 / 44	7.99 / 0.11
	0.25% Ppt	16.5 / 3.47	2315 / 41	6.09 / 0.28	16.8 / 0.33	2149 /45	8.11 / 1.06	15.1 / 0.51	2145 / 39	8.01 / 009
	0.75% Ppt	17.1 / 1.85	2360 / 98	6.79 / 1.41	16.3 / 1.12	2249 / 80	7.24 / 1.44	15.9 / 0.04	2216 / 14	7.29 / 0.15
	1.25% Ppt	18.3 / 4.91	2348 / 74	6.56 / 2.53	17.1 / 0.05	2199 / 93	7.33 / 0.54	16.4 / 0.66	2011 / 61	7.84 / 0.08
PLA+1% CNC	0 % Ppt	19.5 / 1.07	2395 / 21	6.02 / 0.63	19.0 / 0.39	2289 / 49	6.99 / 0.16	18.4 / 0.48	**2014 / 49**	7.59 / 0.28
	0.25% Ppt	18.7 / 0.39	2559 / 46	5.94 / 0.21	18.0 / 0.43	2381 / 94	7.10 / 0.34	17.1 / 0.25	**2011 / 105**	7.66 / 0.54
	0.75% Ppt	19.4 / 2.34	2349 / 20	6.90 / 2.32	18.1 / 0.04	2159 /118	7.31 / 0.65	17.8 / 0.04	**1994 / 14**	7.99 / 0.51
	1.25% Ppt	17.1 / 3.70	2499 / 37	6.92 / 0.09	17.3 / 0.41	2351 / 83	6.18 / 0.31	16.8 / 0.09	**1999 / 91**	**7.91 / 0.25**
PLA+2.5 % CNC	0 % Ppt	22.4 / 0.60	2511 / 31	6.59 / 0.59	20.1 / 1.04	2491 / 49	6.71 / 0.01	20.3 / 0.07	**2359 / 18**	6.29 / 0.41
	0.25% Ppt	20.1 / 1.34	2601 / 84	6.71 / 0.22	18.2 / 0.46	2484 / 99	7.98 / 0.49	18.0 / 0.15	**2310 / 47**	6.38 / 0.19
	0.75% Ppt	19.3 / 0.24	2649 / 36	6.45 / 0.23	18.9 / 1.28	**2345 / 48**	9.24 / 1.05	17.3 / 0.19	**2301 / 99**	6.40 / 0.99
	1.25% Ppt	15.3 / 0.39	2224 / 17	9.20 / 0.41	**13.4 / 0.03**	**1910 / 16**	9.44 / 0.99	**11.4 / 0.41**	**1841 / 51**	8.54 / 0.45
PLA+5% CNC	0 % Ppt	22.1 / 0.21	2844 / 29	5.46 / 0.13	20.1 / 0.08	2611 / 44	7.81 / 0.09	19.4 / 0.14	**2644 / 55**	5.99 / 0.15
	0.25% Ppt	22.3 / 0.49	2817 / 15	5.09 / 1.09	18.3 / 0.16	2719 / 67	7.15 / 0.19	18.0 / 0.44	**2610 / 59**	6.16 / 0.41
	0.75% Ppt	18.0 / 0.29	2605 / 46	5.99 / 0.54	**14.3 / 0.38**	**2105 / 82**	**8.69 / 0.05**	**12.4 / 0.41**	**2045 / 23**	7.11 / 0.18
	1.25% Ppt	16.1 / 0.73	2536 / 29	6.31 / 0.27	**12.7 / 0.11**	**1955 / 81**	**11.1 / 0.16**	**12.1 / 0.05**	**1855 / 61**	**12.0 / 1.40**

An interesting question arises when one considers whether the structural integrity of the engineered plastics is compromised when in contact with packaging solvents and the food product. As a result, in Tables 2-3, the mechanical properties of the different systems after exposure to the stimulants are carefully presented and compared to the controls (tested as obtained, no exposure to solvents). As can be observed, for the Ag based plastics, as the [Ag] increases at higher [CNC], there was a corresponding decrease in the modulus after being exposed to 10 % (v/v) ethanol. Specifically, in all cases where plastics were made with 1.25 % Ag, there were significant reduction in

the modulus when compared to the virgin plastics (not exposed to stimulants). Additionally, those samples characterized with a reduced modulus exhibited significant increases in elongation at break. The peptide-based plastics were characterized with lower tensile strength and modulus when compared to the Ag based counterparts, prior to being exposed to the stimulants. Nevertheless, these plastics were characterized with similar decreases as the Ag based plastics. The key difference being the peptide-based plastics when exposed to either 10 % (v/v) ethanol or isooctane were characterized with both a decrease in tensile strength and modulus. This was obvious for the plastics made with > 0.75 % peptide and > 2.5 % CNC. In summary, most systems when exposed to the different stimulants were characterized with no major changes in mechanical integrity. The main exceptions being when high concentrations of CNC (> 2.5 %) and high concentration of Ag (>0.75 %) were incorporated into the plastics, the modulus and to some extent the tensile strength were reduced. Nevertheless, in cases, where more pliable or ductile plastics are needed, these systems, with significant increases in elongation can be used.

3.4 Thermal properties after exposure to different solvents

The thermal stability and transitions of the different plastic formulations after exposure to the solvent systems were characterized using TGA and DSC, respectively. The data gathered for both the Ag and Ppt based systems are represented in Table 4 and 5. DTG curves of neat PLA and the different plastic formulations were previously published in Part 1 of this work (George, Shen, and Montemagno, 2016). The main question being addressed in this section was whether exposure to these stimulants will compromise the thermal integrity of the plastics. Systems with PLA + CNC + Ag were in most cases unaffected after exposure to the different solvents.Plastics with 1.25 % (w/w) of Ag wasused and exposed to 10 % (v/v) ethanol were characterized with reduced temperature for 10 % degradation and melting endotherm. Also, when exposed to isooctane, plastics with 1.25 % (w/w) Ag or 5 % CNC (w/w) with any percentage of Ag were characterized with reduced temperature for 10 % degradation. Hence, systems with high [Ag] resulted in plausible lost of thermal integrity. The reason for this may be the introduction of higher concentrations induces flaws into the macrostructure of the plastics, enable faster degradation. Similarly, plastics made from PLA + CNC + Ppt were characterized with comparable properties to those above. Specifically, plastics made with 5 % CNC (w/w) with 0.75 or 1.25 % (w/w) Ppt were characterized with reduced temperature for 10 % degradation when exposed to either solvent system. In fact, when exposed to isooctane, there were significant reductions in the percentage degradation with 1.25 % Ppt at any given % of CNC addition. In summary, it has been demonstrated, apart from adding high concentrations of either Ag or Ppt, most systems were thermally stable after exposure to the different solvents. A plausible reason for the observed decreases at those levels may have been the lost of the Ag or Ppt over time that can weaken the macrostructure of the plastics.

Table 4. Thermal property of the CNC+PLA+Ag based plastics systems: original, exposure to 10 % (vol.) ethanol and exposure to isooctane

		Original			10 % ethanol			Isooctane		
	Sub-sample	Tg (°C)	Melting endotherm (Tm)	Temp. 10 % degradation	Tg (°C)	Melting endotherm (Tm)	Temp. 10 % degradation	Tg (°C)	Melting endotherm (Tm)	Temp. 10 % degradation
PLA	0% Ag	52.9 / 2.35	150.3 / 1.99	327.4 / 3.68	51.4 / 1.34	148.3 / 1.09	320.4 / 5.48	51.4 / 0.45	143.4 / 2.58	321.4 / 4.31
	0.25% Ag	52.8 / 3.91	148.3 / 4.88	330.2 / 5.33	53.3 / 2.45	147.4 / 2.48	322.5 / 3.19	50.3 / 2.58	141.5 / 4.16	320.4 / 1.48
	0.75% Ag	51.4 / 6.34	140.5 / 9.33	319.4 / 5.99	51.9 / 0.94	145.3 / 1.99	318.4 / 1.99	49.3 / 4.31	142.5 / 3.56	319.4 / 3.59
	1.25% Ag	52.3 / 4.23	142.5 / 2.53	324.7 / 6.90	52.6 / 0.49	143.8 / 1.84	315.4 / 3.95	50.3 / 4.10	140.3 / 2.58	316.9 / 6.94
PLA+1 %CNC	0% Ag	55.1 / 0.45	154.8 / 0.56	332.3 / 5.13	53.2 / 0.99	150.1 / 0.44	324.4 / 2.89	48.3 / 3.24	148.5 / 2.58	323.5 / 4.58
	0.25% Ag	54.8 / 2.60	156.8 / 2.43	332.3 / 1.53	53.9 / 2.84	151.4 / 2.94	325.3 / 8.85	49.3 / 1.58	149.6 / 0.58	321.4 / 4.58
	0.75% Ag	52.2 / 2.21	155.2 / 1.75	344.7 / 3.51	51.4 / 2.46	153.3 / 1.44	328.4 / 9.64	50.3 / 1.03	150.6 / 3.27	**324.5 / 1.59**
	1.25% Ag	53.2 / 1.17	158.6 / 0.71	343.3 / 2.52	50.2 / 1.39	**153.5 / 0.44**	**331.8 / 3.39**	50.9 / 0.44	**151.4 / 3.58**	**324.5 / 1.94**
PLA+2.5 % CNC	0% Ag	53.7 / 0.57	154.5 / 0.72	343.0 / 2.64	50.4 / 2.35	151.4 / 2.48	333.2 / 4.58	50.5 / 1.49	148.4 / 4.85	331.4 / 4.49
	0.25% Ag	55.0 / 1.31	157.7 / 2.12	343.3 / 4.99	54.5 / 1.11	154.3 / 0.99	336.3 / 1.64	51.4 / 0.45	149.5 / 3.94	332.4 / 6.89
	0.75% Ag	55.0 / 2.51	153.8 / 2.51	341.7 / 2.08	53.1 / 1.63	151.3 / 5.43	336.1 / 4.39	51.9 / 4.48	151.9 / 3.25	**331.4 / 3.48**
	1.25% Ag	54.4 / 1.70	153.2 / 2.18	343.7 / 1.53	50.4 / 1.83	**147.3 / 0.93**	**334.4 / 2.54**	50.3 / 3.57	148.4 / 5.24	**330.4 / 1.58**
PLA+5 %CNC	0% Ag	52.3 / 0.72	147.3 / 1.51	346.0 / 2.65	51.4 / 0.58	145.6 / 0.84	335.3 / 3.94	49.5 / 0.38	143.5 / 4.31	**334.4 / 1.91**
	0.25% Ag	51.4 / 0.49	150.3 / 3.21	343.7 / 2.52	50.2 / 0.49	148.6 / 6.41	332.5 / 8.32	50.3 / 1.57	144.3 / 0.45	**330.4 / 1.54**
	0.75% Ag	52.6 / 1.30	148.4 / 0.93	340.7 / 1.53	47.6 / 2.39	143.5 / 9.43	331.4 / 5.93	48.5 / 1.91	140.5 / 2.59	**328.3 / 4.15**
	1.25% Ag	48.3 / 0.85	149.9 / 1.15	344.7 / 2.08	47.5 / 0.99	**140.2 / 3.21**	**333.6 / 3.53**	47.3 / 0.34	**140.1 / 1.58**	**324.5 / 8.31**

Table 5. Thermal property of the CNC+PLA+Ppt based plastics systems: original, exposure to 10 % (vol.) ethanol and exposure to isooctane

Sample	Sub-sample	Original			10 % ethanol			Isooctane		
		Tg (°C)	Melting endotherm (Tm)	Temp. 10 % degradation	Tg (°C)	Melting endotherm (Tm)	Temp. 10 % degradation	Tg (°C)	Melting endotherm (Tm)	Temp. 10 % degradation
PLA	0% Ppt	52.9 / 2.35	153.3 / 1.49	323.1 / 3.91	50.3 / 2.45	150.2 / 3.94	325.4 / 1.58	49.3 / 4.14	149.8 / 3.58	320.4 / 4.49
	0.25% Ppt	49.8 / 4.85	145.3 / 9.88	329.4 / 6.39	50.9 / 3.19	151.4 / 2.80	328.4 / 4.14	50.6 / 2.59	147.5 / 5.29	324.5 / 5.14
	0.75% Ppt	50.4 / 2.14	144.5 / 4.49	321.1 / 2.09	49.3 / 1.09	148.5 / 1.48	325.6 / 4.99	51.6 / 3.50	149.5 / 3.16	324.8 / 3.11
	1.25% Ppt	50.0 / 1.23	146.1 / 3.53	323.6 / 1.40	51.4 / 2.18	145.9 / 4.18	328.3 / 8.14	50.4 / 4.58	145.6 / 8.43	321.5 / 4.59
PLA+1 %CNC	0% Ppt	54.2 / 0.17	153.8 / 1.06	334.3 / 2.93	51.3 / 3.91	151.2 / 2.68	331.9 / 2.58	49.8 / 1.49	149.3 / 3.28	330.8 / 3.48
	0.25% Ppt	54.9 / 0.65	159.9 / 2.71	335.1 / 0.51	51.9 / 2.11	153.1 / 1.48	334.5 / 1.99	51.5 / 1.44	150.3 / 1.99	332.5 / 9.38
	0.75% Ppt	51.1 / 0.29	158.1 / 2.35	338.5 / 1.99	50.3 / 1.99	154.3 / 4.69	332.8 / 6.31	52.6 / 3.14	154.1 / 2.48	329.4 / 5.39
	1.25% Ppt	54.9 / 1.47	159.7 / 1.70	341.1 / 1.02	53.7 / 2.81	153.5 / 6.19	337.4 / 3.58	50.4 / 3.18	153.4 / 0.99	**334.5 / 2.19**
PLA+2.5 % CNC	0% Ppt	54.1 / 1.52	155.1 / 1.32	335.0 / 1.69	51.4 / 3.81	150.3 / 3.33	336.4 / 3.84	52.4 / 4.11	151.3 / 0.39	329.1 / 0.49
	0.25% Ppt	51.4 / 0.91	152.8 / 2.91	338.1 / 3.09	52.9 / 1.03	150.9 / 1.94	332.9 / 2.49	53.6 / 1.09	149.3 / 2.48	328.3 / 0.47
	0.75% Ppt	53.0 / 1.56	154.8 / 3.79	341.9 / 6.08	51.0 / 2.48	153.3 / 4.38	333.7 / 0.90	54.3 / 3.14	148.3 / 2.40	331.3 / 4.95
	1.25% Ppt	53.9 / 2.50	154.4 / 3.10	339.7 / 5.51	49.1 / 0.99	150.7 / 9.45	331.4 / 1.03	50.4 / 2.00	145.4 / 0.58	**330.1 / 1.48**
PLA+5 %CNC	0% Ppt	54.1 / 1.72	146.1 / 2.51	338.0 / 1.62	52.4 / 2.01	144.3 / 4.16	334.3 / 2.59	51.9 / 2.48	145.2 / 5.39	328.3 / 3.14
	0.25% Ppt	52.9 / 1.41	148.3 / 2.01	341.7 / 0.52	50.1 / 0.93	143.4 / 5.49	338.9 / 1.83	52.5 / 0.48	140.3 / 0.48	**331.7 / 0.55**
	0.75% Ppt	53.9 / 1.90	146.7 / 0.13	340.5 / 1.10	49.1 / 2.39	143.4 / 2.41	**331.4 / 1.04**	49.8 / 3.10	**135.6 / 0.68**	**330.4 / 2.48**
	1.25% Ppt	49.3 / 1.89	149.1 / 3.16	344.3 / 1.18	48.9 / 2.99	142.5 / 3.84	**333.7 / 2.38**	47.4 / 0.91	**137.4 / 3.18**	**333.5 / 1.47**

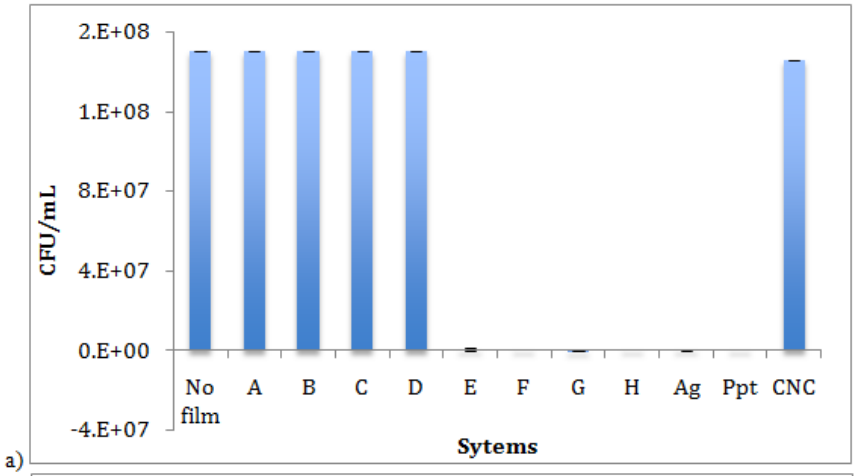
3.5 Antimicrobial activity

The antimicrobial activity of the different plastic systems were evaluated against four bacteria systems known to be problems when it comes to food and consumer goods production. Letters in Figure 4 represents the different systems. Summaries of the different representation are given in Table 6.

Table 6. Systems designation

Symbol	Systems[a]
A	PLA
B	PLA + CNC
C	PLA + CNC + 0.25 % Ag
D	PLA + CNC + 0.75 % Ag
E	PLA + CNC + 1.25 % Ag
F	PLA + CNC + 0.25 % Ppt
G	PLA + CNC + 0.75 % Ppt
H	PLA + CNC + 1.25 % Ppt

[a] In the systems outlined here, % are based on (w/w) and in all cases systems with 5 % (w/w) CNC were tested (preliminary tests indicated changing the [CNC] had little effect on the activity)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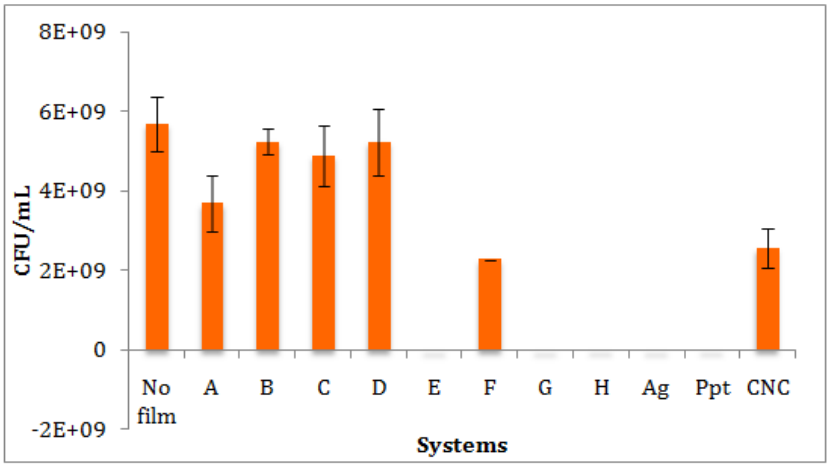
A
B
C
D
E
F
G
H
Ag
Ppt
CNC
Sytems

a)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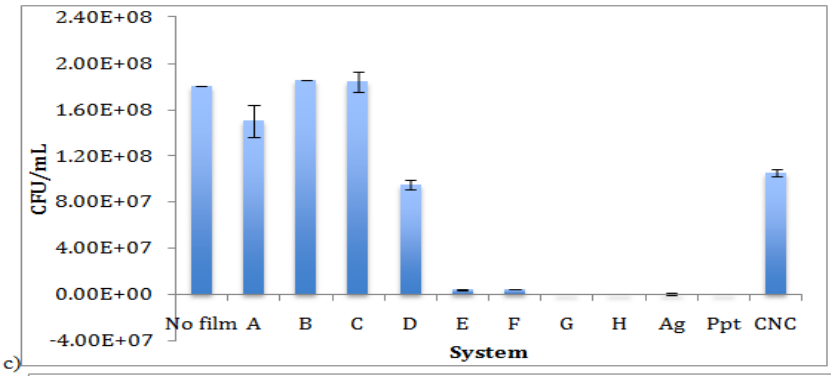
G
H
Ag
Ppt
CNC
Systems

b)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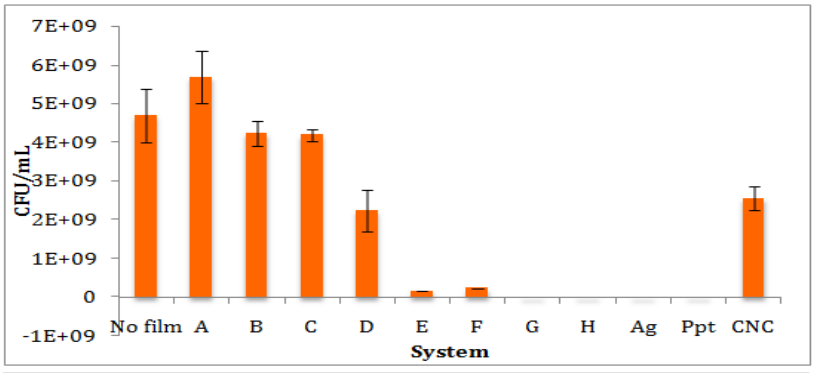
CFU/mL
No film
A
B
C
D
E
F
G
H
Ag
Ppt
CNC
System

c)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E
F
G
H
Ag
Ppt
CNC
System

d)

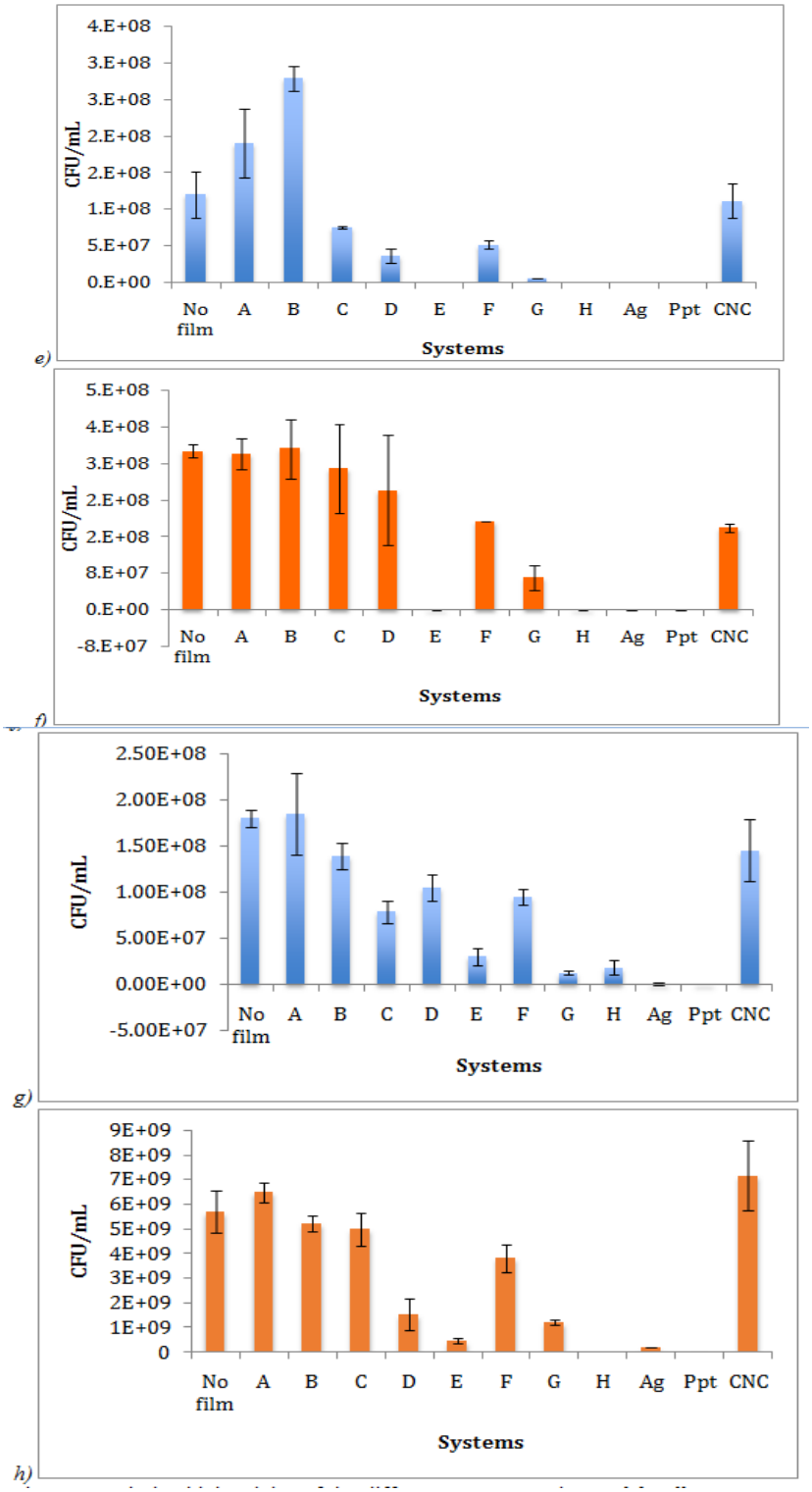

Figure 4 Antimicrobial activity of the different systems against: *Klebsiella pneumoniae* at a) 3 hours, b) 24 hours incubation, *E.coli* at c) 3 hours, d) 24 hours incubation, *Listeria monocytogenes* e) 24 hours, f) 48 hours, and *Salmonella spp.* g) 3 hours and h) 24 hours

Contrary to what has been previously published, where clay and PLA based plastics were evaluated for activity against similar strains of bacteria (Rhim, Hong, and Ha, 2009), this communication clearly outlined that systems with high [Ag] or [peptide] resulted in significant inhibition of bacterial growth. They alluded to the hydrophobicity of the polymer, which prevented microbial access and inhibition. Busolo et al. (2010) studied the antimicrobial effect of PLA manufactured with a silver-based silicate layered arrangement, they found that composites with at least 10 mg of silver based nanoclay was needed for inhibition to acceptable levels. Hence, it is feasible to assume that the integration of Ag or any other antimicrobial agent is more dependent on the mode of introduction into the composite rather than the nature of the polymer being used.

Silver was used in this study as a positive control because it has been successfully demonstrated that it has antibacterial effects when combined with PLA and other fillers (Busolo et al. 2010, Fortunati et al. 2012). But, unlike previous studies, this communication presents plastics with high transparency because of the low concentrations of fillers and active ingredient used. Nevertheless, all systems with peptide were characterized with prolong inhibitory effects against all four systems of bacteria. Systems with 0.75 or 1.25 % (w/w) peptide were characterized with complete bacterial inhibition. In conclusion, depending on the targeted application, peptide based PLA plastics can be developed with antimicrobial characteristics against a wide range of bacteria.

IV. Conclusions

This work builds on an earlier publication and clearly highlights that the migration properties of the plastics are within limits specified by international bodies. Further, given the low concentrations of additives used (CNC and antimicrobial agent- Ag or peptide), the results presented here are significantly lower than what other teams previously publish. Also, the mechanical and thermal integrity of the plastics are in many cases preserved with the addition of the antimicrobial agents, except in cases where the systems were fortified with higher concentration of CNC and active agent. But, as has been presented, systems with low fractions of CNC and high active agent content does not significantly comprise the integrity of the plastics. Finally, all systems with at least 0.75 % peptide were characterised with inhibition of all bacterial strains tested against. This study presents a simple and proven methodology to render plain PLA based plastics very valuable for applications where food safety is a concern.

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