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Application of nanoclays in food packaging

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ABSTRACT

Nanoclays have received special attention due to their wide range of industrial applications due to some inherent properties. Nowadays one of the most active areas of nanoclays research is their employ in food packaging. In this paper, at first, the reasons of nanoclay applications are introduced. Then critical issue in developing food packaging materials are reviewed to minimize gasses and other small molecules transfer between out-side packaging environment and food. The reason for improvement of permeability of films is discussed. The permeability of nanocomposite films may be determined using two factors: diffusion and solubility coefficients. Then antimicrobial activity of clay-based nanocomposites and its mechanism and factors influencing on it are mentioned. The way of evaluation of mechanical properties of the nanocomposite films and strategies for improvement are introduced. Finally, other applications of nanoclays e.g. controlled release of various bioactive molecules and development of reinforced food contact materials have been introduced.
Keywords: *Nanoclay; food packaging.*

1. INTRODUCTION

Nanotechnology include the characterization, fabrication and/ or manipulation of structures, devices or materials that have at least one dimension lower than 1–100 nm in length. When the particle size is reduced below this threshold, the resulting material exhibits physical and chemical properties that are significantly different from the properties of macroscale materials composed of the same substance [1]. Among all nanoparticles, nanoclays have received special attention in a wide range of industrial applications because of their low cost, abundance in nature, commercial availability, high strength and stability, effectiveness at low filler loadings, simple processing, high aspect ratio of individual platelets and let them be modified easily [2-4]. These material originated from clay fraction of soil, consisting of SiO_4 tetrahedral and Al3+ octahedral sheets of one to a few نnanometers thick and hundreds to thousands of nanometers in measure [5]. Nanoclays result in good dispersion with water-soluble polymers due to their hydrophilic nature while they are adverse with the wide range of hydrophobic polymers and need to be modified with organic surfactants in order to improve their interaction with organic material [6]. Nanoclays, organically modified nanoclays and polymer layered nanocomposites are used in different areas.

The first report on the application of clay minerals was about the absorption of DNA by montmorillonite. Organoclays have potential utility in manufacture of inks, paints, greases, bone implant, filler for dental adhesives and cosmetics and also these hydrophobic material are commonly used in remediation of environmental pollutants because of their unique sorption capabilities. For instance, as a sorbent in the removal of phenolic compounds and pesticides from water [7, 8]. Park et al [9] reviewed the application of organoclays for the adsorption of organic contaminants contains aromatic organic compounds, phenolic compounds, pesticides, herbicides, hormones and antibiotics from aqueous media. In addition other adsorption applications of these compounds include removing oil, grease, heavy metal, polychlorinated biphenyl and mycotoxins. Nano clays such as montmorillonite, bentonite, and hydrated sodium calcium alumino silicate (HSCAS) used as anticaking agents for animal feed, have been reported as an additive to inhibit or at least diminish disease associated with multi-mycotoxins in high risk population [10]. Other potential applications of nanoclays are the controlled release of various bioactive molecules and development of reinforced food contact materials which are completely investigated in the present review.

Nowadays the most active area of nanoclays research is their employ in food packaging. The incorporation of nanoclays into polymeric systems results in an enhancement in their barrier, mechanical and thermal properties, as well as causes an improvement in the antimicrobial properties and modulates the release of antimicrobial agents from packaging [11, 12]. The earliest successful example of a polymer–clay nanocomposite was a nylon-clay hybrid found and developed at Toyota Central Research and Development Laboratories in 1986 [13]. However, studies on the development of PCNs based food packaging materials have been published only since the late 1990s [14].

2. STRUCTURE OF CLAY AND SILICATE NANOCOMPOSITES

The naturally abundant clay minerals ordinarily have a stacked aggregated of silicate layers as tetrahedral and octahedral sheets with nanometric thickness. This stacking result in regular van der Waals gaps between the layers called interlayer or gallery[15]. The sheet-structured hydrous silicates are generally referred to as phyllosilicates.

Upon the number and arrangement of octahedral sheets and/or tetrahedral sheets, Clay minerals are divided into three

major categories: 1:1, 2:1 and 2:1:1 arrangement. 1:1 clay would consist of one tetrahedral sheet and one octahedral sheet and 2:1 , combined by two tetrahedral layers organized by Si and O atoms, fused with an octahedral layer with aluminium and magnesium atoms bonded to oxygen and hydroxyl groups while 2:1:1 clay would consist of octahedral sheet adjacent to a 2:1 layer [16,17]. The structural family of the 1:1 layered silicate is called kaolinite group with the general formula of $Al_2Si_2O_5(OH)_4$. This group includes the dioctahedral minerals (e.g. kaolinite, dickite, nacrite, and halloysite), and the trioctahedral minerals (e.g. antigorite, chamosite, chrysotile, and cronstedite). This group includes the dioctahedral and the trioctahedral minerals. Various smectite types including dioctahedral minerals (e.g. montmorillonite, beidellite, nontronite, and bentonite), and trioctahedral minerals (e.g. hectorite, saponite, and sauconite) belong to the structural family of the 2:1 layered silicate. They contain highly anisotropic platelets separated by thin layers of water with a general formula $(Ca,Na,H) (Al,Mg,Fe,Zn)_2 (Si,Al)_4O_{10}(OH)_2$-$xH_2O$, where x shows different levels of water affiliated to the mineral. These clay platelets contain two tetrahedral sheets of silica and an octahedral sheet of alumina or magnesia. Each platelet has an average thickness around 1 nm and average lateral dimensions ranging between a few tens of nm to several microns, depending on clay type [5, 9]. The forms of each platelet have a net negative charge due to an isomorphic substitute of Al^{+3} in the octahedral layer by Mg^{+2} or Fe^{+2}, and Mg^{+2} by Li^+ which is quantified as cation-exchange capacity (CEC) and varies from layer to layer. The imbalance of the surface negative charges is counter balanced by exchangeable cations (typically Na^+ and Ca^{2+}) located in the interlayer space between platelets [20].

The attendance of mineral cations on the planar surface of layers increases hydrophilic character and therefore makes them incompatible with the wide range of hydrophobic polymers [5, 21]. One of the most common methods to promote miscibility and adaptability between clays and polymers is to convert the hydrophilic silicate surface to organophilic. It can be typically achieved by replacing these cations with organic cationic surfactants such as organic ammonium ions bearing longaliphatic chains, leading to organo-modified layered silicates (OMLS) with increased interlayer spacing, hydrophobic surface and improved interactions with organic polymers [22]. The surfactants lower the surface energy of the platelets, resulting in an increase in the layer spacing (d-spacing). The more the modifier is compatible with organophilic matrix, the more it facilitates clay dispersion, it means that appropriate selection of organoclay depends on the type of polymer matrix used. For instance, to improve the dispersion, the polar surface of pristine montmorillonite is usually functionalized with organic cations such as stearyl dihydroxyethyl ammonium chloride [23], distearyl dimethyl ammonium chloride [23] and quaternary ammonium [22], although sulfonium and phosphonium can also be used [23].

3. FABRICATION OF POLYMER/ CLAY NANOCOMPOSITES

The structure and properties of the resulting material depend on the state of the nanoclay in the nanocomposite, i.e., if it is exfoliate or intercalate [24]. With the incorporation of nanomaterials, three structural arrangements can be defined in the nanocomposites, namely tactoid, intercalated, and exfoliated [25].

The structure and morphology of the nanocomposites can be determined by X-ray diffraction (XRD), both wide angle (WAXS) and small angle (SAXS), scanning electron microscopy (SEM), transmission electr-=on microscopy (TEM), infrared spectroscopy (IR), and atomic force microscopy (AFM) [24, 26, 27]. Tactoid structures (microcomposites) is obtained when the presence of particles (agglomerates>100 nm) in the material is observed. Clay nanoplate-lets tend to be agglomerated, especially when dispersed in nonpolar polymer environments due to large surface energies. Agglomeration of clay platelets leads to agglomerated morphology with reduced aspect ratios. In this case the interlayer space of the clay gallery does not extend and no true nanocomposites are formed because of poor dependency between polymer and clay [28].

Intercalated nanocomposites are the result of balance dependency among of and clay observed when the sheets of the clay are intercalated by the polymer, but they are stacked in a group of layers whose thickness is less than 100 nm. In this case the polymer chains penetrate between the silicate layers keeping the clay stacks (tactoids) virtually unchanged except for a moderate expansion of interlayer spaces. Moreover a laminated structure is obtained when the clay platelets are totally delaminated and separated from one another by a distance greater than a few nanometers, with a typical size below 20 nm. In exfoliated nanocomposites, platelets have very favorable interactions with the polymer matrix and are distributed homogeneously entirely the polymer phase with no memory of the original clay tactoids to take full benefit of nanoclays high surface area [20, 29]. The delaminated structure is the most suitable to achieve better properties.

It is difficult to form either an intercalated or exfoliated structure of nanoclay by using incorporation of existing/traditional techniques because of the large lateral sidelong of the layers, high intrinsic viscosity of the polymer and a strong attitude of clay platelets to agglomerate [30]. One alternative to deal with these problems is using conventional shear devices such as extruders, mixers, ultrasonicators, ball milling, high pressure fluidizators, etc. to create alternating pressure cycles, which overcome the bonding forces and break the agglomerates and so improve the degree of exfoliation [3]. Other solutions include preparing a clay suspension containing a lower percentage of clay and a low concentration polymer solution and processing with plasticizers. Preparing the nanocomposites in diluted aqueous solutions followed by plasticization results in well-dispersed nanocomposites due to lower viscosity of solution and higher miscibility of polymer with clay [31].

Best effects are generally observed at low loads, clay was dispersed more uniformly via polymer matrix at low concentrations but presumably at higher nanoclay concentrations, the adsorbent force between the clay and the polymer matrix is disordered by the tactoid form of the clay. It was presumed that plasticizers might also partake in the interactions between polymer and clay and thus could most affect the formation of nanostructure and further influence the obstacle, mechanical, and thermal properties of the nanocomposite films. Several researchers

working on plasticized starch–clay nanocomposite films reported that plasticizers comfort stronger interactions between the starch matrix and clay surface due to the strong polar–polar interactions between starch, plasticizer, and clay surface, and further affected the performance of nanocomposite films [32-34]. They deduce that combination of sufficient glycerol (5% w/w) into the starch–nanoclay samples have led to an exfoliated structure, whereas with combine an extra value of glycerol, caused a reduction in exfoliation of clays in the starch matrix. This phenomenon has been explained by two reasons. The primary reason is that the excess amount of glycerol resulted in large structures by developing hydrogen bridges, hence negatively effective the starch chain mobility; and second, formation of hydrogen bonds between starch and glycerol might compete with interactions between glycerol, starch, and the clay surface and reduced of absorbing forces between starch and clay[33-35].

On the other hand, efficient delamination of clay layers into the polymer to achieve fully exfoliated morphologies is hindered by the hydrophobic- hydrophilic character of polymers and clays. These mismatches can be overbearing by organically modification of the polar clay surface with various cationic surfactants including quaternary ammonium, phosphonium and immidazoliumions etc. to render it more hydrophobic. The complexing agent here is achieved uniform distribution of clays into the polymer matrix. While advances have been made toward understanding the factors which influence the degree of clay platelet exfoliation in PNC films, these relationships are complex and difficult to control in practice.

How the clay platelets are distributed throughout the matrix and, therefore, the properties of the resulting materials are influenced by the type of processing used to provide polymer–clay nanocomposite. Polymer clay nanocomposites are fabricated by different methods, containing polymerization of a monomer in the attendance of clay (in situ polymerization), polymer intercalation in solution, emulsion polymerization in the presence of layered silicates and by dispersing the nanoclay into the molten polymer (melt Intercalation). These techniques may be more or less adaptable for different filler/polymers systems [36].

It has been reported that among the fabrication techniques, melt intercalation is the most environmentally benign method for polymer nanocomposites formation [37]. This method has already been applied in the manufacturing of conventional films and the main benefits are the versatility, efficiency, and scale-up simplification [38]. The polymer and silicate layers are heated upper the glass transition or melting temperature and mixed together in the molten state by using single screw extrusion, double screw extrusion, or manual mixing [39]. Top-down approaches to PNC fabrication are limited in their ability to provide consistently impressive improvements in oxygen and moisture vapor permeabilities [40]. For example solvent casting technique is one of the so-called top-down methods which has been extensively used to prepare starch-based films [32] and also PLA-based and gelatin –based [41] composite films with different types of nanoclays for research purposes. This technique comprising the steps of gelatinization, pasting, bursting of granules, and starch retrogradation, has some troubles involved in: (i) combination materials of different natures; (ii) film removal from the support; and (iii) scale up from laboratory to industrial scale [38]. Layer-by-layer (LBL) assembly is a bottom-up strategy to fabricate multilayer film structures with pre-defined component organization at the nanoscale. Multilayer films are constructed as follows. A prepared substrate (quartz or polymer such as PET, e.g.) is submerged in a solution of a positively charged polymer, rinsed, dried, and then submerged in a solution of negatively charged clay platelets. Each cycle of alternating submerging leads to the formation of a single clay-polymer bilayer, and cycles are repeated until the desired number of bilayers is achieved. Bilayers are held together by electrostatic bind between the polymer and clay layers, which have opposite polarity [40].

4. OBSTACLE ACTION OF CLAY AND SILICATE NANOCOMPOSITES

The permeability of polymeric materials is a critical issue when developing food packaging materials where efficient barrier properties are desired to minimize gasses and other small molecules transfer between out-side packaging environment and packaged food. The permeability of the polymer films is usually affiliate on a large number of pertinent agents, containing: free volume hole sizes, degree of polymer crystallinity and motion, and specific polymer–polymer and polymer–gas interactions, polarity and structural features of polymeric side chains, hydrogen bonding characteristics, molecular weight and polydispersity, degree of branching or cross-linking, processing methodology, method of synthesis, and although permeability to one migrant can also be complicated by the presence of other migrants. The overall diffusion rate is also straightly related to the film thickness [42, 43].

Actually, no known pure polymer exhibits complete impermeability to atmospheric gasses, water vapor, or natural substances contained within the food being packaged or even the packaging material itself. Thus to deal with their inherent permeability complex multilayer films or polymer blends are often utilized. For example, in an application where ultrahigh oxygen barriers are required over a large humidity range, a high oxygen barrier, water sensitive material like EVOH can be sandwiched between two layers composed of a relatively hydrophobic polymer such as polyethylene [44].

Direct polymer blending is also a useful approach to achieve desired gas barrier. While they provide remarkable improvement in barrier properties, but they possess higher production and material costs and require the use of additional additives and adhesion. Thus polymer nanocomposites (PNCs) are introduced as the latest alternative to overcome the aforementioned problems. Nanoclays have been widely studied as regards the barrier properties due to their unique platelet-like structure and There have been many researches involving the use of nanoclay as rigid impermeable fillers in the development of nanocomposites to reduce the permeability of gases, like oxygen and carbon dioxide; organic vapour and moisture that are hazardous to packaged material [45]. Significant improvement in barrier properties through the integration of nanoclays into the polymer matrix can be achieved by two specific way. The primary way by which nanofillers influence the barrier properties is mainly attributed to the creation of tortuous pathway for permeate

diffusion due to the impermeable clay layers distributed in the polymer matrix consequently increasing the effective diffusion path length. Several theoretical models have been proposed to explain the effect of dispersed nanomaterials on the mean path length for diffusing molecules. Nielsen proposed the first simplest model assumes that fillers are evenly dispersed throughout the matrix and take the shape of rectangular platelets of uniform size. Furthermore, supposes that the penetrant diffusion rate is only influenced by the tortuosity of the path. This model shows that the barrier effectiveness is expected to increase as the particles become more anisotropic or plate-like in shape and the filler aspect ratio (length divided by width) increases, a prediction which has been experimentally verified [43].

It is worth noting that in practice, the Nielsen model is valid only for small filler loading percentages but higher loading levels result in deviation between the experimental data and predicted results due to the particle agglomeration, which in turn effectively reduces mean particle aspect ratio [43]. As all of these models are based on the assumption that the impermeable clay platelets are completely exfoliated into the polymer matrix and it is not always the case, many deviations can be observed owing to less exfoliation or poor clay orientation. To deal with aforementioned problems and improve Nielsen's model a new model provides a correction factor applicable to Nielsen's model. In this model, the proportion of ''interphase'' regions in which the nanoclay surface and any organic modifiers interact directly with the polymeric host material, acts as a governing factor in addition to the tortuous path [46].

The second way by which nanoparticulates influence the barrier properties is by causing changes to the polymer matrix itself at the polymer-nanofiller interface. When the polymer–nanoparticle interactions are favorable, polymer chains located close to each nanoparticle can be partially immobilized. This immobilization results in the fact that permeant molecules traveling through these interfacial zones have attenuated hopping rates as observed by positron annihilation lifetime spectroscopy (PALS)[47, 48]. Some recent efforts at modeling the effect of the interfacial regions [43] on the diffusivity properties of migrant gasses through polymer films have been made but considering the relevant factors are not always easily the case [49]. It is also worth mentioning that polymer crystallinity plays a major role in barrier properties and crystalline regions of semicrystalline polymers act as nanoscale fillers [50]. Moreover it is hypothesized that nanoclayactesas nucleating agents increasing the rate of the formation of the crystallites and results improvement in barrier property [51].

The permeability of nanocomposite films may be determined using two factors: diffusion and solubility coefficients. Hence, a reduction in the nanocomposite was caused by the combined effect of the reductions in both the solubility coefficient and the effective diffusion coefficient [52-54]. A reduction in the solubility coefficient of the composite-films seems to be caused by the reduction of available hydroxyl groups, as a consequence of their interactions with the nanoclay [55]. Furthermore the compatibilizers or plasticizers used to efficiently and uniformly incorporate the filler into the matrix, can also affect the diffusivity or solubility of permeates [40]. Carmen et al [56] working with starch/clay nanocomposite films, reported that the addition of

nanoclay lead to a remarkable decrease in the water vapor permeability and this effect was more intense in the nanocomposite films plasticized with glycerol. This behavior may indicates that the nanoclay dispersion procedure increases the nanoclay–glycerol interactions and improves the intercalation process thus reduces the accessibility of hydroxyl groups to interaction with water and results in a less water vapor permeability. However, It was observed that the lowest water vapor permeability would be attained for nanocomposite films with sufficient glycerol (5%) but in higher glycerol content, WVP increased because of the decreased extent of clay exfoliation and also increased the hydrophilicity of the starch films. They provided more active sites by exposing hydrophilic hydroxyl groups in which the water molecules could be absorbed [33]. They also found that the use of two different plasticizers (urea and formamide) with amide groups at 15% level, has led to lower WVP as compared to glycerol at the same level. That meant the new plasticizers (urea and formamide) improved the balance of interactions between starch, plasticizer and clay, and allowed more plasticizer and starch to diffuse together inside the layers of silicates so increased the degree of clay exfoliation.

Kanmani and Rhim [41], who worked on developing gelatin based active nanocomposite films containing AgNPs and nanoclay, concluded that the water vapor permeability of the gelatin film reduced by incorporation of clay and AgNPs. This behavior is mainly attributed to the water vapor impermeable silicate layered nanoclay and AgNPs which create a tortuous pathway for water vapor diffusion [57]. Furthermore, dispersion of the nanoparticles in the polymer matrix as discontinuous particles prevents the mobility of polymer strains and so positively affect the WVP of the polymeric films [58]. Research conducted by [59] on assessment of water vapor permeability (WVP) of PLA films incorporated with different types of clay (Cloisite Na+, 30B and 20A) showed a decrease in the water vapor permeability of nanocomposite films with the incorporation of organically modified nanoclays, i.e., Cloisite 20A and 30B, reduced, while that of films incorporated with unmodified natural nanoclay, CloisiteNa, increased slightly. This result can be explained by the hydrophobicity of organically modified nanoclays and hydrophilicity of unmodified nanoclay. On the other hand, among the organically modified nanoclays, Cloisite 20A is more hydrophobic and has more compatibility with PLA polymer matrix. Thus Cloisite 20A was the most effective in improving the water vapor barrier property [60]. Furthermore they examined the effect of clay concentration using Cloisite 20A and resulted that with an increase in clay content, WVP of nanocomposite films decreased significantly. Casariego et al, showed that this behavior can be attributed to the quality of clay dispersionin polymer [61].

It seems that when the concentration of nanoclay reaches a certain level, its orientation changes and nanoclay probably aggregates. A similar study was carried out by Shin et al [62] at developing nanocomposite films based on the combination of different nanoclays (Cloisite Na+, 30B and 20A) into polymeric blends of apple peel powder and carboxymethylcell-ulose (CMC). The incorporation of nanoclays provided an effective barrier against water and oxygen in the CMC films, regardless of the type of nanoclay used. This implies that the layered silicates could be regarded as an impermeable obstacle to the motion of water or gas

molecules and thus improve the barrier properties [63]. The CMC films composited with Cloisite Na+ had the WVP values remarkably lower than those with Cloisite 20A or Cloisite 30B. This result is most likely attributed to the surface hydrophilicity or hydrophobicity of the clay used due to hydrophilic natural of (Cloisite Na^+) and hydrophobic nature of organically modified nanoclays such as Cloisite 30B and Cloisite 20A [64].

Abdollahi et al [12] evaluated the combined effect of clay (MMT) and rosemary essential oil (REO) on the barrier properties of the chitosan film. For this mean, water vapor permeability (WVP) of chitosan films incorporated with three levels of MMT (1, 3 and 5% w/w) and REO (0.5, 1, and 1.5% v/v), was investigated. The results showed that WVP of films decreased by incorporating 1 or 3 % w/w MMT and this phenomenon became more intense with addition of 1% or 1.5% REO. As mentioned previously, the earlier decrease in WVP is due to creation of tortuous path for the molecule diffusion by clay. Further decreasing of WVP in the presence of REO is related to the hydrophobic nature of REO which may increase film hydrophobicity. Moreover this reduction is also due to the formation of the cross-link network induced by the hydrogen bonds between the chitosan and REO which limits the availability of hydrogen groups to form hydrophilic bonding with water, subsequently leads to less WVP [65].

5. ANTIMICROBIAL ACTIVITY OF NANOCLAYS

Antimicrobial activity of clay-based nanocomposites has been widely studied. It has been stated that they may have direct or indirect antimicrobial function by themselves, moreover, they also influence on increasing retention of antimicrobial agents by polymer matrix or exhibiting controlled release capacity of them with interest.

5.1. Antimicrobial activity of nanoclay in single form.

According to research conducted by the group [66, 67] on the clay included nanocomposite film, even though the same basic MMT structure, organically modified MMT such as Cloisite 30B and Cloisite 20A have shown distinctive antibacterial activity, while no antibacterial activity has been observed with unmodified MMT. Organically modified nanoclay inactivated all bacteria. The main place of action is cell wall [68]. At physiological condition bacteria have negatively charged cell wall and pristine montmorillonite typically has negatively charged surface so under these conditions due to electrostatic repulsion forces interaction between them is not favorable. Modification of clays with quaternary ammonium groups converts them to the material with both positively charged and hydrophobic surface thus promotes their affinity to bacterial cell wall. Among the quaternary ammonium groups, the alkylammonium cations alkyl chains between 8-18 carbon in length are the most active ones. It has been reported that Cloisite 30B showed superior antimicrobial activity than Cloisite 20A, which was ascribed to their unlike hydrophobicity. Due to the more hydrophobic nature of Cloisite 20A, bacteria cannot easily be adsorbed in to the nanoclay surface therefore the antimicrobial function reduces. The consistent results have been found by Rhim and Hong [59] in a later research, which showed only poly (lactic acid) (PLA)/ Cloisite 30B composite films exhibited a bacteriostatic effect against L. monocytogenes. In this study, they also found that small amounts of organically modified nanoclay powders alone could inactive both Gram-positive and Gram-negative bacteria. However, when they were incorporated into the PLA matrix, their efficacy has been reduced. This behavior was related to hydrophobic nature of PLA, which may prevented bacteria to access the nanocomposite film surface, whereupon the interaction between the surfactants of the organoclay and bacteria occurs. Sothornvit et al [69] have also reported that whey protein isolate/organoclay composite films had a noticeable bacteriostatic effect against L. monocytogenes.

5.2. Antimicrobial activity of nanoclays in combined form.

As mentioned previously there are a certain number of reports about antimicrobial activity of organically modified silicate layers, while unmodified silicate layers are not antimicrobial by themselves. Thus it will be of great importance to combine silicate layers with natural antibacterial agents such as copper and silver ion or macromolecule polymers with antimicrobial property. In this regards, a series of studies have been carried out on antimicrobial activity of chitosan intercalated with rectorite (REC) by Wang et al. [48, 49, 70] and MMT by Rhim et al. [66] and Han [68] providing new organic–inorganic hybrid materials with dual antibacterial benefit of organic and inorganic antimicrobial factors. Chitosan (CS) is the second most abundant cationic polysaccharide in nature after cellulose. It can be applied widely in different applications especially as an antimicrobial agent in packaging. It has been found that CS has some advantages over other type of polymers used as packaging material due to its antibacterial behavior and chelating bivalent minerals [71]. Higher antibacterial activity, a broader spectrum of activity, a higher killing rate and lower toxicity towards mammalian cells make it preferable than other types of disinfactants [49]. CS showed antimicrobial function against Wide range of microorganisms including bacteria, fungi and algae. Despite all these advantages, its application has been limited due to insolubility in water. Therefore to deal with this limitation, it is necessary to modify CS surface with a cationic group like quaternary ammonium group which produces water soluble derivative of CS, quaternized chitosan (HTCC), with better bacteria deterrence. It has been suggested that the mechanism of the antimicrobial activity of HTCC is contributed to hydrophobicity and cationic charge of the introduced quaternary ammonium group. The positively charged glucosamine monomer on the C-2 in CS molecule at below pH 6.3 [49] makes this polycationic biocideable to interact with polymers at the negatively charged cell surface of the bacteria which changes the permeability of the cell membrane of the microorganisms and results in the leakage of intercellular components and so the death of the cell [72]. Moreover, HTCC can apply strong restraint efficacy on growth of microorganisms by its long chain alkyls with hydrophobicity that can easily infiltrate into the cell membrane [73]. The enhancement in antimicrobial activity of chitosan and its derivative by adding REC and/or organically modified REC (OREC) has been reported [48, 49, 70, 73, 74]. Addition of OMMT to HTCC produced nanocomposite with more positive potential than OMMT, so promoted its affinity to the bacteria with negative charge by electrostatic forces. It has been clearly demonstrated that the positive charge of HTCC/ OMMT

brought bacteria on the clay surface thereupon HTCC exerts its antimicrobial function [74].

The better inhibitory effect of these complexes on bacterial growth was Caused higher surface area to volume ratio resulted from layered silicates dispersion which caused effective contact and interplay with bacteria. Absorbency between silicate and bacteria was considered as main factor for the antimicrobial activity of these nanocomposites. Chitosan around the layered silicate might have more opportunity to exert its inhibitory effect on bacterial growth. Moreover, aggregation of chitosan chains in the interlayer of the silicate increased positive charge (amino group) density in each unit volume which resulted in a better inhibitory effect on the growth of bacteria. Based on the above reports, the antibacterial process in these systems may inclusive two stage: the first step was the adsorption and immobilization of the bacteria from solution to the surface of layered silicate by Using of the hydrophobic and electrostatic forces; the second stage is that CS and/or HTCC could exert antimicrobial activity.

In the study of Wang et al [49], it was also reported that the nanocomposites with the highest amount of OREC and those with the largest interlayer distance showed the strongest inhibitory activity bacterial growth. It was ascribed to magnification of specific surface area which resulted in more bacteria to be adsorbed and immobilized on the surface of layered silicates; as well as, more CS chains were aggregated and ordered in the interlayer of the silicate, hence positive charge density in each unit volume increased.

It has been reported that modification of nanoclays with silver [75, 76] and copper [77] could potentially lead to the development of novel antimicrobial food packaging and films offering strong synergies between two naturally sourced materials. Silver has unique antimicrobial properties to a wide range of microorganisms but recognized as safe for human being. Antimicrobial activity of silver has been ascribed to different mechanisms, namely: (a) interaction of AgNPs with phosphorous and sulfur containing compounds of proteins and DNA, preventing DNA replication, and leading to cell death. In addition, the positively charged AgNPs are believed to bind with negatively charged bacterial cell membranes, which disrupt cell walls and surface proteins leading to cell death [78].

Raffi et al. [79] reported that the penetration of AgNPs into the bacteria could inactivate the enzymes and produce H2O2 leading to cell death. It was suggested that supporting silver by nanoclay helps to prevent particle agglomeration and high efficiency for longer period of time due to Continuous and constant release of silver biocide in a concentration level that can apply an antimicrobial effect. According the study of Praus et al.

[80] which investigated the antimicrobial activities of some chemical compounds, ions and elemental silver stabilized on nanoclay, antibacterial compounds are effective just when released from the inorganic carrier. Regarding this context restrictions have been currently referenced by the European Food Safety Agency (EFSA) for silver migration. This agency defined a general specific migration limit of 0.05 mg of silver per kg of food. Busolo et al [81] carried out a research on development of polylactic acid (PLA) biocomposites containing a novel silver-based antimicrobial layered silicate additive for use in active food packaging applications. These nanocomposites coatings presented strong antimicrobial activity against Gram-negative Salmonella spp. Moreover in this paper silver migration from the films was measured by stripping voltammetry. It was shown that migration levels of silver were within the permitted specific migration levels and it was also approved that those levels of acceptable migration can be sufficient to exhibit strong antimicrobial activity.

Silver loaded montmorillonite were used to extend the shelf life of cheese without affecting its quality [82], to increase the stability of a kiwi-pineapple salad [75] and in the further study of same group [83] to prolong the refrigerated shelf life of fresh-cut carrots from 4 days of control samples to 70 days. The antimicrobial properties of low density polyethylene (LDPE) nanocomposite films containing Ag modified Cloisite 30B and Ag modified pristine montmorillonite compared to nanocomposite films containing Cloisite 30B. In this paper antimicrobial effect of nanocomposite films were studied against E. coli ATCC 25923 as a gram negative bacteria and S. aureus ATCC 29523 as a gram positive bacteria. The nanocomposite films containing Cloisite 30B-Ag exhibited the most antimicrobial effect. This phenomenon is ascribed to the addition of the antimicrobial effect of Ag particles to Cloisite 30B clay. Similarly, the nanocomposite films containing MMT-Ag also showed more antimicrobial effect than that of nanocomposite films containing Cloisite 30B. The authors postulated that the good antimicrobial action of nanocomposite films was also attributable to the better distribution for nanoclay particles. Thus uniform and exfoliated structure for nanocomposite films containing Cloisite 30B-Ag proven by the XRD patterns, could be another reason for the good antimicrobial effect of these films.

Bruna et al [77] tested the antibacterial efficiency of nanocomposite films prepared by incorporating copper-modified MMT (MMT-Cu2+) into LDPE by melt mixing in an extruder at 200° C. They indicated that enhancement of MMT-Cu2+ proportion resulted in higher antibacterial activity and bacterial colonies reduced by 94% when 4% of MMT-Cu2+ was added to the polymer.

6. MECHANICAL PROPERTIES

Mechanical properties of the films were usually improved by incorporation of nanoclays into matrices, especially when nanoclay exfoliation occurred [55, 84-86]. The mechanical properties of the nanocomposite films are generally evaluated by designation the tensile strength (TS), Young's modulus (YM) and percent elongation at break (EB). Generally, improvement in the mechanical properties of the nanocomposite films are closely related to the interfacial interaction between the polymer matrix and nanosized fillers and good dispersion of clay layers throughout the polymer matrix [87, 88]. Furthermore, the large aspect ratio of the nanoclay can cause an enhancement in the mechanical properties of the resulting nanocomposite films [33].

At the uniform dispersion of nanoclay, the nanocomposite films showed an enhancing trend for TS and YM.

This behavior was related to higher aspect ratio and surface area which increased surface interaction between polymer matrix and nano-sized clays, moreover formation of hydrogen bonds between them. Increase in TS is advantageous for food packaging applications as it strengthens packaging material against the normal stress encountered during food handling, shipping, and transportation [85]. In the case of EB, a decreasing trend was found by addition of nanoclay. This can be attributed to the formation of new nucleation sites and crystallites growth resulting in higher brittleness of nanocomposites [32]. EB indicates the flexibility of films and is directly related to the biodegradability of the films, thus the reduction in this property results in higher rate of biodegradability and can be beneficial for food packaging [89].

Increasing trends for TS and YM and decreasing trend for EB were observed by integration of nanoclay into bio-based polymers such as gelatin [85, 90], wheat gluten [91], starch [55, 89, 92] and chitosan [12, 66]. Voon et al. [85] investigated the effect of halloysite nanoclay on the mechanical properties of bovine gelatin films. It was reported that addition of 5% w/w halloysite nanoclay increased tensile strength and elastic modulus from 9.19 MPa and 1.32 MPa% to 13.39 MPa and 2.99 MPa%, respectively, and decreased elongation at break from 80.80 to 55.72 % as compared with the control film. The effects of halloysite nanoclay on physicochemical properties of potato starch based films were studied [89]. They reported that addition of 5% w/w halloysite nanoclay increased the TS by about 34%, decreased EB by about 35.3 % and increased YM about 2-fold compared with the control film. In contrast, the opposite trend was observed in some studies and the TS of nanocomposite films decreased with the clay content while EB increased [59]. The incorporation of Cloisite Na+, Cloisite 20A and Cloisite 30B into PLA films resulted in a reduction in the TS by about 19, 10 and 19 %, respectively, compared to control.

Sothornvit et al [93] showed that addition of Cloisite 30B into whey protein isolate film dramatically decreased the TS at contents higher than 5% w/w while EB slightly increased. Such reduction trend has been also found in a recent study performed by Echeverr´ıa et al [94] who investigated the effect of MMT addition to soy protein based films on the physicochemical properties. The authors reported that addition of 5% w/w MMT decreased the TS compared with control film. This fact might be attributed to incomplete intercalation or exfoliation of the clay silicate layers in the polymer matrix and the brittle nature of the clay [93]. Indeed, the improvement in mechanical properties is only obtained at lower loading in which nanoclay exfoliation occurs. While addition of more amount of clay into the matrix led to a deterioration effect on mechanical properties due to appearance of clay stacks and even aggregates [32]. In addition the clay content, type of nanoclay, plasticizer and also processing method affect the mechanical properties.

Abdollahi et al [12] reported that incorporating REO (rosemary essential oil), even at a low amount, increased the TS of nanocomposite and the highest TS was obtained by incorporating 5% of MMT and 0.5% of REO into chitosan matrix. The authors suggested that the low amount of REO improved interactions between nanofiller and matrix because of altering the hydrogen-bonding network within the material. As well as, the EB showed an enhancement, and it has been noted that REO acted as a plasticizer. The proper choice of clay type with considering its compatibility with polymer matrix can beneficially improve mechanical properties. In this regard, cloisite 20A was the most effective in maintaining the TS property of PLA among the tested nanoclays [94] and Cloisite Na+ showed the better mechanical in combination with Barley protein (BP) films compared to Cloisite 30B [95]. These differences were ascribed to different chemical structures resulting different degree of interaction between the polymer and nanoclay. So, hydrophilic BP was more compatible with inorganic and hydrophilic Cloisite Na$^+$ and hydrophobic PLA was more compatible with hydrophobic cloisite 20A.

Similarly Nayak et al [96] also found that with addition of cloisite 30B to polycaprolactone (PCL)/soy protein isolate (SPI) blend increased TS significantly. Shin et al [62] carried out research at developing nanocomposite films based on combination of different nanoclays (Cloisite Na+, 30B and 20A) into polymeric blends of apple peel powder (APP) and carboxymethyl cellulose (CMC). They reported that among all the tested films, NaD (Cloisite Na+ in distilled water) composite films exhibited the highest TS value while the other CMC nanocomposite films showed lower TS value than that of a neat CMC. This is indirect evidence that the exfoliated NaD nanoclays are able to form chemical bonds with CMC [20]. Even though the Cloisite Na+ nanoclay was well exfoliated in the ethanol, the NaE (Cloisite Na+ in ethanol) film showed the TS value lower than that of the NaD film. This was related to incomplete dissolving of CMC in ethanol. It was also reported that addition of nanoclays increased the EB% by more than 400% regardless of the clay type. The nanoclay particles themselves acting as a plasticizer and enhancing the flexibility of the CMC films; whether they are exfoliated or not [62].

7. ASSESSMENT OF NANOCLAY MIGRATION

The migration of nanoclays from nanocomposite of food package is a main risk for consumer. Avella et al [84] investigated the immigration of MMT Ingredients like magnesium, iron and silicon by putting the biodegradable starch/clay nanocomposite films into contact with vegetables and stimulants. The authors reported low overall migration levels for these elements. Similarly, Mauricio-Iglesias et al. [97] working on MMT/wheat gluten nanocomposite films found a very low migration of aluminum and silicon into the food stimulants that were within the limits set by the food packaging legislation. Low migration of nanoclays can be attributed to their structure. In a migration study conducted by Schmidt et al [98], the migration level of Cloisite 30B from PLA films into the 95% ethanol was 6.7 ± 0.5 mg/dm2 when stored at 40 °C for 10 days. This research group also performed the first investigation on migration properties of PLA and laurate-modified Mg-Al layered double hydroxide (LDH-C12) films. They reported relatively low total migration values for LDH from PLA-LDH-C12 nanocomposite films [99].

Maisanaba et al [100] also tested the migration of two modified clays, Clay1 and Clay2, into distilled water from PLA bottles. The authors detected the overall migration value of 0.1 ± 0.2 mg/dm2 in all samples, after 10 days of storage at 40 °C. Busolo and Lagaron [101] tested the immigration from neat processed HDPE and HDPE-iron-based kaolinite films into water and isooctane as food simulants for aqueous and fatty foods, respectively, using inductively coupled plasma mass spectroscopy (ICP-MS). The results indicated very small migration values for iron (from active ingredient) and aluminum (from clay migration).

Farhoodi et al [102] also evaluated the migration of aluminum and silicon from poly(ethylene terephthalate) (PET)/ Cloisite 20A nanocomposite bottles into acidic food stimulant by ICP-MS. The authors found that after 90 days storage at 25° C and 45° C, the migration values of silicon, as the main element of clay nanoparticles, were 6.0 and 9.5 ppm, correspondingly. It was mentioned that higher interactivity of nanoclay surface and polymer chains led to the less migration of nanoparticles into a contact solution. Thus the release process of nanoclays was correlated to interactions amongst the polymer, the nanoclay and the foods/food stimulants as reported by Xia et al [4]. This study was performed on migration assessment of OMMT and surfactants from two types of nanocomposite films: polypropylene (PP) and polyamide 6 (PA6) with 3% and 5% (w/w) OMMT, respectively, into ethanol as a fatty-food stimulant at 70 °C. In spite of the less initial nanoclay loading, more nanoparticles were released from PP-clay films (0.15 mg L-1) than from PA6-clay films (0.10 mg L-1). The authors attributed that fact to the poor interaction between the nanoclay and PP. A substantial release for surfactants compared to nanoparticles was also reported that was due to the diffusion behavior of these small molecules and changes in nanoclay structure as exposed to ethanol. The migration values obtained in mentioned studies were in accordance with the EU legislation [103], which establishes an overall migration limit (OML) of 10 mg of substance per 1 dm2 of the plastic surface. As mentioned previously, platelet shaped clays are nanoscale in one dimension and as much as several microns in other two directions. It was suggested that the large and irregularly shape of nanoclays caused to lower their motions through the rigid polymer environment. However, it has been demonstrated that high mechanical shear forces used to achieve uniform dispersion of clays during manufacture made clay platelets separate into residual fragments such as silicon and aluminum which resist many chemical digestion procedures and have high natural abundance. However it should be needed to assess the potential risk of these substances on a case-by-case basis.

8. TOXICOLOGICAL CONSIDERATIONS OF NANO-CLAYS

Nanoclay may show different toxic kinetic profile in comparison to microparticle [100, 104] because they may penetrate into cells, come into contact with biomolecules and may affect cellular functions [4]. Human exposure to nanoclays takes place via inhalation through the respiratory route and ingestion through the oral pathway. These particles can be distributed to several organs such as liver, lung, kidney and evoke adverse effects (In vitro toxic). Thus, it would be necessary to evaluate the risk assessment and the toxicological profile of nanoclays, claimed by the European Food Safety Authority. In this sense, appropriate in vivo and in vitro studies on different cell models such as human epithelial cell line [99], human hepatic cell line HepG2 and human intestinal Caco-2 Cells [11, 106], human embryonic kidney (HEK) cell line, and cervical cancer cell line [107] have been performed to address uncertainties about the health effects. Adverse health effects of nanoclays depend on size, shape, aspect ratio, electrostatic charge, concentration, time and cell line [105, 107]. For instance, the platelet structured cytotoxic effects of nanoclays were more than tubular ones [105].

Rawat et al [107], assessed the cytotoxicity of MMT and laponite in human embryonic kidney (HEK) and cervical cancer SiHa cell lines. In this research the cytotoxicity of nanoclays followed the order of MMT−HEK > Laponite−HEK > MMT−SiHa > Laponite−SiHa, which presented aspect ratio and cell line affiliate toxicity. It was demonstrated that HEK cell lines were more susceptible than SiHa cell lines to nanclays and also more cytotoxic effects were dedicated to MMT as compared to laponite due to its higher aspect ratio. In general, the cytotoxicity on HEK and SiHa cell lines was observed at 0.05% concentration of both MMT and laponite within 24h of exposure time.

Verma et al [105], the cytotoxicity of platelet and tubular structured nanoclays on cultured human lung epithelial cells A549 were investigated. The authors reported the significant cytotoxic effects on cultured human lung epithelial cells A549 at 25 μg/mL and 250 μg/mL of platelet and tubular type nanoclays, respectively. Lordan et al [11] evaluated the cytotoxicity of Cloisite Na+ and Cloisite 93A in the human hepatic HepG2 cell line within the 24 h exposure time. They found a reduction of 23 and 37% in cell viability at the highest concentration of Cloisite Na+ and Cloisite 93A (1 μg/mL), respectively. Maisanaba et al. [100] moreover studied the cytotoxicity of Cloisite Na+ and Cloisite 30B in HepG2 cells. The authors reported that only Cloisite 30B induced toxic effects and a significant reduction of viability was observed at the concentration of 62.5 μg/mL. In addition, Sharma et al., [88] and Gutiérrez-Praena et al. [108], investigated the cytotoxicity of the same clays in Caco-2 cells. The results of these studies showed no cytotoxic effects for Cloisite Na+ whereas, in the case of Cloisite 30B, substantial differences with respect to the control were observed. These differences could be attributed to the content of modifiers which has a major role in the toxicity of clays [100]. Furthermore, the cytotoxicity of two modified clays, Clay1 and Clay2, on both Caco-2 and HepG2 cell lines were evaluated by Jorda-Beneyto et al. [109]. The concentration ranges of 0 to 8 μg/mL and 0 to 125 μg/mL were used for Clay1 and Clay2, respectively, due to their hydrophobicity. In the case of Clay1, a significant decrease in cell viability was only observed in HepG2 cells at the highest concentration assayed (8 μg/mL) within the 24 and 48 h exposure times. Although Clay2 induced cytotoxicity in Caco-2 cells at concentration of 40 and 20 μg/mL and in HepG2 cells at concentration of 62.5 and 62.5 μg/mL after 24 and 48 h exposure times, respectively. A higher cytotoxicity of CLAY2 in comparison with CLAY1 was derived from the different modifiers used to modify the montmorillonite. Regarding CLAY2, Caco-2 cells were more sensitive than the HepG2 cell line. Similarly, in the research carried out by Houtman et al., [106] significant

cytotoxicity effects were observed in both Caco-2 and HepG2 cell lines exposed to CLAY2 in a range of 0 to 125 µg/mL during 24 and 48 h. Also no toxic effects were recorded for Cloisite®20A and CLAY1 at the ranges of 0–62.5 µg/mL and 0 to 8 µg/mL, respectively. In their work, Maisanaba et al. [100] also evaluated the cytotoxicity and mutagenicity of migration extracts obtained from a nanocomposite material with poly (lactic) acid (PLA) and two modified clays, Clay1 and Clay2, to assess the real exposure. In summary, results showed no cytotoxic and genotoxic effects with the migrations extracts. Apart from the in vitro studies, in addition important that evaluate the toxicity in vivo experimental models to fully mimic the complicated in vivo system. Unmodified Montmorillonite clay was tested for toxicological effects in rats and did not show any effect at doses up to 143 mg/kg bw after oral exposure for 72 h [110]. Moreover *in vitro & in vivo* safety evaluations on cytotoxicity and genotoxicity of exfoliated silica plates were performed by Li et al. [111]. Results showed a reduction of 30 and 40% in cell viability in Chinese hamster ovary (CHO) cells at the highest concentration of 1000

µg/mL after 12 and 24 h incubation times, respectively. Potential acute oral toxicity in rats was evaluated by three oral doses of 1500, 3000, and 5700 mg/kg. All the treatment groups didn't show any mortalities, clinical signs or macroscopic changes. In the research conducted by Baek et al. [112], MMT was given to mice at a single dose of up to 1000 mg/kg for 14 days. There wasn't evidence of any record of any fatality or unusual or symptoms during 14 days post administration. Thus, the LD50 values of MMT were estimated to be more than 1,000 mg/kg. Maisanaba et al. [113] exposed Wistar rats to Clay1 in a diet at 40 mg/kg/d for 90 days without histophatological damage. This research group also performed a histopathological study of the migration extract of an organomodified clay nanocomposite (PLA-Clay1) in several organs of Wistar rats after 90 d of oral exposure and didn't find any significant adverse effects [114]. Sharma et al investigated the in-vivo genotoxic and inflammatory potential of Cloisite® 30B in liver, kidney and colon of Wistar rats at doses ranging from 250 to 1000 mg/kg body weight twice 24 h apart by oral gavage. Results showed no abnormalities in clinical appearance [115].

9. CONCLUSIONS

Organically modified nanoclay inactivated both Gram-positive and Gram-negative bacteria by interacting with their cell wall and this effect was more pronounced against Gram-positive bacteria. It has been demonstrated that the antimicrobial function of organically modified nanoclay is ascribed to the quaternary ammonium groups. The efficient antimicrobial activity of nanoclays depends on their affinity and miscibility with microorganisms and they interact to bacterial surface by both hydrophobic and electrostatic interactions. Silver-modified clays may be prepared by a cation exchange method and their applications as antimicrobial agents in food packaging have been published. Considering the increased use of nanoclays, the

evaluation of consumer exposure to these compounds would be of great importance. The migration of nanoclays from nanocomposite intended for food packaging application is considered as a main risk of consumer exposure to these nanoclays. Although the nanoscale formulation of clays confers advantages to use of these particles, it alters their toxic kinetic profile. Moreover owing to their smaller size, higher surface area and greater reactivity, nanoclays can penetrate into cells, come into contact with biomolecules and may affect cellular functions. Adverse health effects of nanoclays were dependent on size, shape, aspect ratio, electrostatic charge, concentration, time and cell line.

5. REFERENCES

1. Roco, M.C.; Mirkin, C.A.; Hersam, M.C. Nanotechnology research directions for societal needs in 2020. *World Technology Evaluation Center, INC.* **2010**, *13*, 897-919, https://doi.org/10.1007/s11051-011-0275-5.

2. Blumstein, A. Polymerization of adsorbed monolayers. II. Thermal degradation of the inserted polymer. *J. Polym. Sci., Part A: Gen. Pap* **1965**, *3*, 2665-2672, https://doi.org/10.1002/pol.1965.100030721.

3. Vartiainen, J.; Tuominen, M.; Nättinen, K. Bio-hybrid nanocomposite coatings from sonicated chitosan and nanoclay. *J. Appl. Poly. Sci* **2010**, *116*, 3638-3647, http://dx.doi.org/10.1002/app.31922.

4. Xia, Y.; Rubino, M.; Auras, R. Release of nanoclay and surfactant from polymer–clay nanocomposites into a food simulant. *Environ. Sci. Technol* **2014**, *48*, 13617-24, https://doi.org/10.1021/es502622c.

5. Pavlidou, S.; Papaspyrides, C. A review on polymer–layered silicate nanocomposites. *Prog. Polym. Sci* **2008**, *33,* 1119-98, https://doi.org/10.1016/j.progpolymsci.2008.07.008.

6. LeBaron, P.C.; Wang, Z.; Pinnavaia, T.J. Polymer-layered silicate nanocomposites: an overview. *Appl. Clay Sci* **1999**, *15*, 11-29, https://doi.org/10.1016/S0169-1317(99)00017-4.

7. Patel, H.A.; Somani, R.S.; Bajaj, H.C.; Jasra, R.V. Nanoclays for polymer nanocomposites, paints.; inks.; greases and cosmetics formulations.; drug delivery vehicle and waste water

treatment. *Bull. Mater. Sci* **2006**, *29*, 133-45, https://doi.org/10.1007/BF02704606.

8. Wiles, M.C.; Huebner, H.J.; McDonald, T.J.; Donnelly, K.C.; Phillips, T.D. Matrix-immobilized organoclay for the sorption of polycyclic aromatic hydrocarbons and pentachlorophenol from groundwater. *Chemosphere* **2005**, *59*, 1455-64, https://doi.org/10.1016/j.chemosphere.2004.12.022.

9. Park, Y.; Ayoko, GA.; Frost, RL. Application of organoclays for the adsorption of recalcitrant organic molecules from aqueous media. *J. Colloid Interface Sci* **2011**, *354*, 292-305, https://doi.org/10.1016/j.jcis.2010.09.068.

10. Abdel-Wahhab, M.A.; El-Denshary, E.S.; El-Nekeety, A.A.; Abdel-Wahhab, K.G.; Hamzawy, M.A.; Elyamany, M.F.; Hassan, N.S.; Manaa, F.A.; Shaiea, M.N.Q.; Gado, R.A.; Zawrah, M.F. Efficacy of Organo-Modified Nano Montmorillonite to Protect against the Cumulative Health Risk of Aflatoxin B1 and Ochratoxin A in Rats. *Soft Nanosci. Lett* **2015**, *5*, http://dx.doi.org/10.4236/snl.2015.52004.

11. Lordan, S.; Kennedy, J.E.; Higginbotham, C.L. Cytotoxic effects induced by unmodified and organically modified nanoclays in the human hepatic HepG2 cell line. *J. Appl. Toxicol* **2011**, *31*, 27-35, https://doi.org/10.1002/jat.1564.

12. Abdollahi, M.; Rezaei, M.; Farzi, G. A novel active bionanocomposite film incorporating rosemary essential oil and nanoclay into chitosan. *J. Food Eng* **2012**, *111*, 343-50, https://doi.org/10.1016/j.jfoodeng.2012.02.012.

13. Kawasumi, M. The discovery of polymer-clay hybrids. *J. Polym. Sci., Part A: Polym. Chem* **2004**, *42*, 819-24, https://doi.org/10.1002/pola.10961.

14. Ray, S.; Quek, S.Y.; Easteal, A.; Chen X.D. The potential use of polymer-clay nanocomposites in food packaging. *Int. J. Food Eng* **2006**, 2, https://doi.org/10.2202/1556-3758.1149.

15. Ray, S.S; Ojijo, V. A brief overview of layered silicates and polymer/layered silicate nanocomposite formation. *J InProcess. Polym. Nanocompos* 2018, 57-86, https://doi.org/10.1007/978-3-319-97779-9.

16. Yin, X.; Gupta, V.; Du, H.;Wang, H.;Miller, J. Surface charge and wetting characteristics of layered silicate minerals. *J. Adv. Colloid Interface Sci* 2012, 179-182, 43-50, https://doi.org/10.1016/j.cis.2012.06.004.

17. Chen, X.; Peng, Y. Managing clay minerals in froth flotation-A critical review. *J. Miner. Process. Extr. Metall. Rev* **2018**, *39*, 289-307, https://doi.org/10.1080/08827508.2018.1433175.

18. De Azeredo, H.M. Nanocomposites for food packaging applications. *Food Res. Int* **2009**, *42*, 1240-53, https://doi.org/10.1016/j.foodres.2009.03.019.

19. Paul, S.A.; Boudenne, A.; Ibos, L.; Candau, Y.; Joseph, K.; Thomas, S. Effect of fiber loading and chemical treatments on thermophysical properties of banana fiber/polypropylene commingled composite materials. *Composites, Part A* **2008**, *39*, 1582-8, https://doi.org/10.1016/j.compositesa.2008.06.004.

20. Ray, S.S.; Okamoto, M. Polymer/layered silicate nanocomposites: a review from preparation to processing. P*rog. Polym. Sci* **2003**, *28*, 1539-641, https://doi.org/10.1016/j.progpolymsci.2003.08.002.

21. Bagdi, K.; Müller, P.; Pukánszky, B. Thermoplastic starch/layered silicate composites: structure, interaction, properties. *Compos. Interfaces* **2006**, *13*, 1-17, https://doi.org/10.1163/156855406774964364.

22. Chivrac, F.; Pollet, E.; Schmutz, M.; Avérous, L. New approach to elaborate exfoliated starch-based nanobiocomposites. *Biomacromolecules* **2008**, *9*, 896-900, http://dx.doi.org/10.1021/bm7012668.

23. Barick, A.; Tripathy, D. Effect of organically modified layered silicate nanoclay on the dynamic viscoelastic properties of thermoplastic polyurethane nanocomposites. *Appl. Clay Sci* **2011**, *52*, 312-21, https://doi.org/10.1016/j.clay.2011.03.010.

24. Bhiwankar, N.N.; Weiss, R. Melt intercalation/exfoliation of polystyrene–sodium-montmorillonite nanocomposites using sulfonated polystyrene ionomer compatibilizers. *Polymer* **2006**, *47*, 6684-91, https://doi.org/10.1016/j.polymer.2006.07.017.

25. Lee, SM.; Tiwari, D. Organo and inorgano-organo-modified clays in the remediation of aqueous solutions: an overview. *J Appl. Clay Sci* 2012, 59-60, 84-102, https://doi.org/10.1016/j.clay.2012.02.006.

26. Ray, SS.; Ojijo, V. A brief overview of layered silicates and polymer/layered silicate nanocomposite formation. J In Process. Polym. Nanocompos 2018, 277, 57-86, https://doi.org/10.1007/978-3-319-97779-9_3.

27. Enobeidallah, B.; Benhamida, A.; Dorigato, A.; Sola, A.; Messori, M.; Pegoretti, A. Structure and Properties of Polyamide 11 Nanocomposites Filled with Fibrous Palygorskite Clay. J. Renewable Mater 2019, 7, 89-102, https://doi.org/10.32604/jrm.2019.00136.

28. Alexandre, M.; Dubois, P. Polymer-layered silicate nanocomposites: preparation, properties and uses of a new class of materials. *Materials Science and Engineering: R: Reports* **2000**, *28*, 1-63, https://doi.org/10.1016/S0927-796X(00)00012-7.

29. Ray, S.S.; Bousmina, M. Biodegradable polymers and their layered silicate nanocomposites: in greening the 21st century

materials world. *Prog. Mater. Sci* **2005**, *50*, 962-1079, https://doi.org/10.1016/j.pmatsci.2005.05.002.

30. Hussain, F.; Hojjati, M.; Okamoto, M.; Gorga, R.E. Polymer-matrix nanocomposites, processing.; manufacturing.; and application: an overview. *J. Compos. Mater* **2006**, *40*, 1511-75, https://doi.org/10.1177/0021998306067321.

31. Chung, Y.L.; Ansari, S.; Estevez, L.; Hayrapetyan, S.; Giannelis, E.P.; Lai, H.M. Preparation and properties of biodegradable starch–clay nanocomposites. *Carbohydr. Polym* **2010**, *79*, 391-6, https://doi.org/10.1016/j.carbpol.2009.08.021.

32. Majdzadeh-Ardakani, K.; Navarchian, A.H.; Sadeghi, F. Optimization of mechanical properties of thermoplastic starch/clay nanocomposites. *Carbohydr. Polym* **2010**, *79*, 547-54, https://doi.org/10.1016/j.carbpol.2009.09.001.

33. Tang, X.; Alavi, S.; Herald, T.J. Barrier and mechanical properties of starch-clay nanocomposite films. *Cereal Chem* **2008**, *85*, 433-9, https://doi.org/10.1094/CCHEM-85-3-0433.

34. Chiou, B.S.; Wood, D.; Yee, E.; Imam, S.H.; Glenn, G.M.; Orts, W.J. Extruded starch–nanoclay nanocomposites: Effects of glycerol and nanoclay concentration. *Polym. Eng. Sci* **2007**, *47*, 1898-904, https://doi.org/10.1002/pen.20903.

35. Pandey, J.K.; Singh, R.P. Green nanocomposites from renewable resources: effect of plasticizer on the structure and material properties of clay-filled starch. *Starch-Stärke* **2005**, *57*, 8-15, https://doi.org/10.1002/star.200400313.

36. Khan, WS.; Hamadneh, NN.; Khan, WA. Polymer nanocomposites–synthesis techniques, classification and properties. J. Sci. Appl. Tailored. Nanostruct. OCP 2016, 50-67,http://www.onecentralpress.com/wp-content/uploads/2016/12/Chapter-4-TN-.pdf

37. Madhumitha, G.; Fowsiya, J.; Mohana Roopan, S.; Thakur, VK. Recent advances in starch–clay nanocomposites. *Int. J. Polym. Anal. Charact* 2018, 23, 331-345, https://doi.org/10.1080/1023666X.2018.1447260

38. Chen, B.; Evans, J.R. Thermoplastic starch–clay nanocomposites and their characteristics. *Carbohydr. Polym* **2005**, *61*, 455-63, https://doi.org/10.1016/j.carbpol.2005.06.020.

39. Arora, A.; Choudhary, V.; Sharma, D. Effect of clay content and clay/surfactant on the mechanical, thermal and barrier properties of polystyrene/organoclay nanocomposites. *J. Polym. Res* **2011**, *18*, 843-57, https://doi.org/10.1007/s10965-010-9481-6.

40. Duncan, T.V. Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors. *J. Colloid Interface Sci* **2011**, *363*, 1-24, https://doi.org/10.1016/j.jcis.2011.07.017.

41. Kanmani, P.; Rhim, J.W. Physicochemical properties of gelatin/silver nanoparticle antimicrobial composite films. *Food Chem* **2014**, *148*, 162-9, https://doi.org/10.1016/j.foodchem.2013.10.047.

42. Thellen, C.; Schirmer, S.; Ratto, J.A.; Finnigan, B.; Schmidt, D. Co-extrusion of multilayer poly (m-xylylene adipimide) nanocomposite films for high oxygen barrier packaging applications. *J. Membr. Sci* **2009**, *340*, 45-51, https://doi.org/10.1016/j.memsci.2009.05.011.

43. Choudalakis, G.; Gotsis, A. Permeability of polymer/clay nanocomposites: a review. *Eur. Polym. J* **2009**, *45*, 967-84, https://doi.org/10.1016/j.eurpolymj.2009.01.027.

44. Kollen, W.; Gray, D. Controlled humidity for oxygen barrier determinations. *J. Plast. Film Sheeting* **1991**, *7*, 103-17, https://doi.org/10.1177/875608799100700203.

45. Silvestre, C.; Duraccio, D.; Cimmino, S. Food packaging based on polymer nanomaterials. *Prog.Polym.Sci* **2011**, *36*, 1766-82, https://doi.org/10.1016/j.progpolymsci.2011.02.003.

46. Adame, D.; Beall, G.W. Direct measurement of the constrained polymer region in polyamide/clay nanocomposites

and the implications for gas diffusion. *Appl. Clay Sci* **2009**, *42*, 545-52, https://doi.org/10.1016/j.clay.2008.03.005.

47. Garcia, M.; Barsema, J.; Galindo, R.E.; Cangialosi, D.; Garcia-Turiel, J.; van Zyl, W.E.; Verweij, H.H.; Blank, D. Hybrid organic inorganic nylon-6/SiO2 nanocomposites: Transport properties. *Polym. Eng. Sci* **2004**, *44*, 1240-6, http://dx.doi.org/10.1002/pen.20119.

48. Wang, X.; Du, Y.; Luo, J.; Lin, B.; Kennedy, J.F. Chitosan/organic rectorite nanocomposite films: Structure.; characteristic and drug delivery behaviour. *Carbohydr. Polym* **2007**, *69*, 41-9, https://doi.org/10.1016/j.carbpol.2006.08.025.

49. Wang, X.; Du, Y.; Luo, J.; Yang, J.; Wang, W.; Kennedy, J.F. A novel biopolymer/rectorite nanocomposite with antimicrobial activity. *Carbohydr. Polym* **2009**, *77*, 449-56, https://doi.org/10.1016/j.carbpol.2009.01.015.

50. Christenson, E.M.; Anderson, J.M.; Hiltner, A.; Baer, E. Relationship between nanoscale deformation processes and elastic behavior of polyurethane elastomers. *Polymer* **2005**, *46*, 11744-54, https://doi.org/10.1016/j.polymer.2005.08.083.

51. Yahiaoui, F.; Benhacine, F.; Ferfera-Harrar, H.; Habi, A.; Hadj-Hamou, A.S.; Grohens, Y. Development of antimicrobial PCL/nanoclay nanocomposite films with enhanced mechanical and water vapor barrier properties for packaging applications. *Polym. Bull* **2015**, *72*, 235-54, https://doi.org/10.1007/s00289-014-1269-0.

52. Larotonda, F.; Matsui, K.; Sobral, P.; Laurindo, J. Hygroscopicity and water vapor permeability of Kraft paper impregnated with starch acetate. *J. Food Eng* **2005**, *71*, 394-402, https://doi.org/10.1016/j.jfoodeng.2004.11.002.

53. Müller, C.M.; Yamashita, F.; Laurindo, J.B. Evaluation of the effects of glycerol and sorbitol concentration and water activity on the water barrier properties of cassava starch films through a solubility approach. *Carbohydr. Polym* **2008**, *72*, 82-7, https://doi.org/10.1016/j.carbpol.2007.07.026.

54. Müller, CM.; Laurindo, JB.; Yamashita, F. Effect of cellulose fibers addition on the mechanical properties and water vapor barrier of starch-based films. *Food Hydrocolloids* 2009, *23*, 1328-33, https://doi.org/10.1016/j.foodhyd.2008.09.002.

55. Dean, K.; Yu, L.; Wu, D.Y. Preparation and characterization of melt-extruded thermoplastic starch/clay nanocomposites. *Compos. Sci. Technol* **2007**, *67*, 413-21, https://doi.org/10.1016/j.compscitech.2006.09.003.

56. Müller, C.M.; Laurindo, J.B.; Yamashita, F. Effect of nanoclay incorporation method on mechanical and water vapor barrier properties of starch-based films. *Ind. Crops Prod.* **2011**, *33,* 605-10, https://doi.org/10.1016/j.indcrop.2010.12.021.

57. Sorrentino, A.; Gorrasi, G.; Tortora, M.; Vittoria, V. Barrier properties of polymer/clay nanocomposites. *Polym. Nanocompos* **2006**, *11*, 273-92.

58. Su, J.F.; Huang, Z.; Zhao, Y.H.; Yuan, X.Y.; Wang, X.Y.; Li, M. Moisture sorption and water vapor permeability of soy protein isolate/poly (vinyl alcohol)/glycerol blend films. *Ind. Crops Prod* **2010**, *31*, 266-76, https://doi.org/10.1016/j.indcrop.2009.11.010.

59. Rhim, J.W.; Hong, S.I.; Ha, C.S. Tensile, water vapor barrier and antimicrobial properties of PLA/nanoclay composite films. *LWT-Food Science and Technology* **2009**, *42*, 612-7, https://doi.org/10.1016/j.lwt.2008.02.015.

60. Aldana, D.; Villa, E.; De Dios, Hernández, M.; Sánchez, G.; Cruz, Q.; Gallardo, S.; Castillo, H.; Casarrubias, L. Barrier properties of polylactic acid in cellulose based packages using montmorillonite as filler. *J. polym* 2014, 6, 2386-2403, https://doi.org/10.3390/polym6092386

61. Casariego, A.; Souza, B.; Cerqueira, M.; Teixeira, J.; Cruz, L.; Díaz, R.; Vicente, A.A. Chitosan/clay films' properties as affected by biopolymer and clay micro/nanoparticles'

concentrations. *Food Hydrocolloids* **2009**, *23*, 1895-902, https://doi.org/10.1016/j.foodhyd.2009.02.007.

62. Shin, S.H.; Kim, S.J.; Lee, S.H.; Park, K.M.; Han, J. Apple peel and carboxymethylcellulose-based nanocomposite films containing different nanoclays. *Journal of food science* **2014**, *79*, 342-53, https://doi.org/10.1111/1750-3841.12356.

63. Frounchi, M.; Dourbash, A. Oxygen barrier properties of poly (ethylene terephthalate) nanocomposite films. *Macromol. Mater. Eng* **2009**, *294*, 68-74, https://doi.org/10.1002/mame.200800238.

64. Rhim, J.W.; Lee, S.B.; Hong, S.I. Preparation and characterization of agar/clay nanocomposite films: the effect of clay type. *Journal of food science* **2011**, *76*, 40-8, https://doi.org/10.1111/j.1750-3841.2011.02049.x.

65. Siripatrawan, U.; Harte, B.R. Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids* **2010**, *24*, 770-5, https://doi.org/10.1016/j.foodhyd.2010.04.003.

66. Rhim, J.W.; Hong, S.I.; Park, H.M. Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *J. Agric. Food Chem* **2006**, *54*, 5814-22, https://doi.org/10.1021/jf060658h.

67. Hong, SI.; Rhim, J.W. Antimicrobial activity of organically modified nano-clays. *J.Nanosci.Nanotechnol* **2008**, *8*, 5818-24, https://doi.org/10.1166/jnn.2008.248.

68. Han, Y.S.; Lee, S.H.; Choi, K.H.; Park, I. Preparation and characterization of chitosan–clay nanocomposites with antimicrobial activity. *J. Phys. Chem. Solid* **2010**, *71*, 464-7, https://doi.org/10.1016/j.jpcs.2009.12.012.

69. Sothornvit, R.; Hong, S.I.; An, D.J.; Rhim, J.W. Effect of clay content on the physical and antimicrobial properties of whey protein isolate/organo-clay composite films. *LWT-Food Science and Technology* **2010**, *43*, 279-84, https://doi.org/10.1016/j.lwt.2009.08.010.

70. Wang, X.; Du, Y.; Yang, J.; Wang, X.; Shi, X.; Hu, Y. Preparation.; characterization and antimicrobial activity of chitosan/layered silicate nanocomposites. *Polymer* **2006**, *47*, 6738-44, https://doi.org/10.1016/j.polymer.2006.07.026.

71. Aider, M. Chitosan application for active bio-based films production and potential in the food industry. *LWT-Food Science and Technology* **2010**, *43*, 837-42, https://doi.org/10.1016/j.lwt.2010.01.021

72. Rabea, E.; Badawy, M.; Rogge, T.; Stevens, C.; Smagghe, G.; Höfte, M.; et al. Synthesis and biological activity of new chitosan derivatives against pest insects and fungi. *Commun. Agr. Appl. Biol. Sci* **2003**, *68*, 135-8, https://doi.org/10.1021/bm0344295.

73. Deng, H.; Lin, P.; Xin, S.; Huang, R.; Li, W.; Du, Y.; Zhou, X.; Yang, J. Quaternized chitosan-layered silicate intercalated composites based nanofibrous mats and their antibacterial activity. *Carbohydr. Polym* **2012**, *89*, 307-13, https://doi.org/10.1016/j.carbpol.2012.02.009.

74. Deng, H.; Wang, X.; Liu, P.; Ding, B.; Du, Y.; Li, G.; Hu, X.; Yang, J. Enhanced bacterial inhibition activity of layer-by-layer structured polysaccharide film-coated cellulose nanofibrous mats via addition of layered silicate. *Carbohydr.Polym* **2011**, *83*, 239-45, https://doi.org/10.1016/j.carbpol.2010.07.042.

75. Costa, C.; Conte, A.; Buonocore, G.; Del Nobile, M. Antimicrobial silver-montmorillonite nanoparticles to prolong the shelf life of fresh fruit salad. *Int. J. Food Microbiol* **2011**, *148,* 164-7, https://doi.org/10.1016/j.ijfoodmicro.2011.05.018.

76. Armstrong, G.; Thornton, R.; Ryan, M.P.; Laffir, F.; Russell, R.J.; Bala, T.; Keely, K.; Babu Formulation of epoxy–polyester powder coatings containing silver-modified nanoclays and evaluation of their antimicrobial properties. *Polym. Bull* **2012**, *68*, 1951-63, https://doi.org/10.1007/s00289-011-0695-5.

77. Bruna, J.; Peñaloza, A.; Guarda, A.; Rodríguez, F.; Galotto, M. Development of MtCu2+/LDPE nanocomposites with antimicrobial activity for potential use in food packaging. *Applied clay science* **2012**, *58*, 79-87, https://doi.org/10.1016/j.clay.2012.01.016.

78. Sui, Z.; Chen, X.; Wang, L.; Xu, L.; Zhuang, W.; Chai, Y.; Yang, C.J. Capping effect of CTAB on positively charged Ag nanoparticles. *Physica E Low Dimens. Syst Nanostruct* **2006**, *33*, 308, https://doi.org/10.1016/j.physe.2006.03.151.

79. Raffi, M.; Hussain, F.; Bhatti, T.; Akhter, J.; Hameed, A.; Hasan, M. Antibacterial characterization of silver nanoparticles against *E. coli* ATCC-15224. *J. Mater. Sci. Technol* **2008**, *24*, 192-6,

80. Malachová, K.; Praus, P.; Pavlíčková, Z.; Turicová, M. Activity of antibacterial compounds immobilised on montmorillonite. *Appl. Clay Sci* **2009**, *43*, 364-8, https://doi.org/10.1016/j.clay.2008.11.003.

81. Busolo, M.A.; Fernandez, P.; Ocio, M.J.; Lagaron, J.M. Novel silver-based nanoclay as an antimicrobial in polylactic acid food packaging coatings. *Food Addit. Contam* **2010**, *27*, 1617-26, https://doi.org/10.1080/19440049.2010.506601.

82. Incoronato, A.; Buonocore, G.; Conte, A.; Lavorgna, M.; Del Nobile M. Active systems based on silver-montmorillonite nanoparticles embedded into bio-based polymer matrices for packaging applications. *J. Food Prot* **2010**, *73*, 2256-62, https://doi.org/10.4315/0362-028X-73.12.2256.

83. Costa, C.; Conte, A.; Buonocore, G.; Lavorgna, M.; Del Nobile, M.A. Calcium-alginate coating loaded with silver-montmorillonite nanoparticles to prolong the shelf-life of fresh-cut carrots. *Food Res. Int* **2012**, *48*, 164-9, https://doi.org/10.1016/j.foodres.2012.03.001.

84. Avella, M.; De Vlieger, J.J.; Errico, ME.; Fischer, S.; Vacca, P.; Volpe, M.G. Biodegradable starch/clay nanocomposite films for food packaging applications. *Food chemistry* **2005**, *93*, 467-74, https://doi.org/10.1016/j.foodchem.2004.10.024.

85. Voon, H.C.; Bhat, R.; Easa, A.M.; Liong, M.; Karim, A. Effect of addition of halloysite nanoclay and SiO 2 nanoparticles on barrier and mechanical properties of bovine gelatin films. *Food Bioprocess Technol* **2012**, *5*, 1766-74, http://dx.doi.org/10.1007/s11947-010-0461-y.

86. Heydari, A.; Alemzadeh, I.; Vossoughi, M. Functional properties of biodegradable corn starch nanocomposites for food packaging applications. *Materials & Design* **2013**, *50*, 954-61, https://doi.org/10.1016/j.matdes.2013.03.084.

87. Zhang, Y.; Choi, JR.; Park, SJ. Interlayer polymerization in amine-terminated macromolecular chain-grafted expanded graphite for fabricating highly thermal conductive and physically strong thermoset composites for thermal management applications. Composites, Part A**2018**,109,498-506, https://doi.org/10.1016/j.compositesa.2018.04.001

88. Gutiérrez, M.Q.; Echeverría, I.; Ihl, M.; Bifani, V.; Mauri, A.N. Carboxymethylcellulose–montmorillonite nanocomposite films activated with murta (Ugni molinae Turcz) leaves extract. *Carbohydr. Polym* **2012**, *87*, 1495-502, https://doi.org/10.1016/j.carbpol.2011.09.040.

89. Sadegh-Hassani, F.; Nafchi, A.M. Preparation and characterization of bionanocomposite films based on potato starch/halloysite nanoclay. *Int. J. Biol. Macromol* **2014**, *67*, 458-62, https://doi.org/10.1016/j.ijbiomac.2014.04.009.

90. Rao, Y. Gelatin–clay nanocomposites of improved properties. *Polymer* **2007**, *48*, 5369-75, https://doi.org/10.1016/j.polymer.2007.06.068.

91. Tunc, S.; Angellier, H.; Cahyana, Y.; Chalier, P.; Gontard, N.; Gastaldi E. Functional properties of wheat gluten/montmorillonite nanocomposite films processed by casting. *J. Membr. Sci* **2007**, *289*, 159-68, https://doi.org/10.1016/j.memsci.2006.11.050.

92. Cyras, V.P.; Manfredi, L.B.; Ton-That, M.T.; Vázquez, A. Physical and mechanical properties of thermoplastic starch/montmorillonite nanocomposite films. *Carbohydrate Polymers* **2008**, *73*, 55-63, https://doi.org/10.1016/j.carbpol.2007.11.014.

93. Sothornvit, R.; Rhim, J.W.; Hong, S.I. Effect of nano-clay type on the physical and antimicrobial properties of whey protein isolate/clay composite films. *J. Food Eng* **2009**, *91*, 468-73, https://doi.org/10.1016/j.jfoodeng.2008.09.026.

94. Echeverría, I.; Eisenberg, P.; Mauri, A.N. Nanocomposites films based on soy proteins and montmorillonite processed by casting. *J. Membr. Sci* **2014**, *449*, 15-26, https://doi.org/10.1016/j.memsci.2013.08.006.

95. Shin, Y.J.; Song, H.Y.; Jo, W.S.; Lee, M.J.; Song, K.B. Physical properties of a barley protein/nano-clay composite film containing grapefruit seed extract and antimicrobial benefits for packaging of *Agaricus bisporus*. *Int. J. Food Sci. Technol* **2013**, *48*, 1736-43, https://doi.org/10.1111/ijfs.12145.

96. Nayak, P.; Sasmal, A.; Nayak, P.; Sahoo, S.; Mishra, J.; Kang, S.; Lee, J.W.; Chang, Y.W. Nanocomposites from polycaprolactone (PCL)/soy protein isolate (SPI) blend with organoclay. *Polym. Plast. Technol. Eng* **2008**, *47*, 600-5, https://doi.org/10.1080/03602550802059402.

97. Mauricio-Iglesias, M.; Peyron, S.; Guillard, V.; Gontard, N. Wheat gluten nanocomposite films as food-contact materials: Migration tests and impact of a novel food stabilization technology (high pressure). *J. Appl. Polym. Sci* **2010**, *116*, 2526-35, https://doi.org/10.1002/app.31647.

98. Schmidt, B.; Petersen, J.H.; Bender Koch, C.; Plackett, D.; Johansen, N.R.; Katiyar, V.; Larsen, E.H. Combining asymmetrical flow field-flow fractionation with light-scattering and inductively coupled plasma mass spectrometric detection for characterization of nanoclay used in biopolymer nanocomposites. *Food Addit. Contam* **2009**, *26*, 1619-27, https://doi.org/10.1080/02652030903225740.

99. Schmidt, B.; Katiyar, V.; Plackett, D.; Larsen, E.H.; Gerds, N.; Koch, C.B.; Petersen, J.H. Migration of nanosized layered double hydroxide platelets from polylactide nanocomposite films. *Food Additives & Contaminants: Part A* **2011**, 28, 956-66, https://doi.org/10.1080/19440049.2011.572927.

100. Maisanaba, S.; Pichardo, S.; Jordá-Beneyto, M.; Aucejo, S.; Cameán, A.M.; Jos Á. Cytotoxicity and mutagenicity studies on migration extracts from nanocomposites with potential use in food packaging. *Food Chem. Toxicol* **2014**, *66*, 366-72, https://doi.org/10.1016/j.fct.2014.02.011,

101. Busolo, M.A.; Lagaron, J.M. Oxygen scavenging polyolefin nanocomposite films containing an iron modified kaolinite of interest in active food packaging applications. *Innovative Food Sci. Emerging Technol* **2012**, *16*, 211-7, https://doi.org/10.1016/j.ifset.2012.06.008.

102. Farhoodi, M.; Mousavi, S.M.; Sotudeh-Gharebagh, R.; Emam-Djomeh, Z.; Oromiehie, A. Migration of aluminum and silicon from PET/clay nanocomposite bottles into acidic food simulant. *Packag. Technol. Sci* **2014**, *27*, 161-8, https://doi.org/10.1002/pts.2017.

103. Commission E. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Official Journal of the European Union* **2011**, *12*, 1-89.

104. EFSA. The potential risks arising from nanoscience and nanotechnologies on food and feed safety (Scientific Opinion). *EFSA J* **2009**, *7*, 958, https://doi.org/10.2903/j.efsa.2009.958.

105. Verma,, N.K.; Conroy, J.; Lyons, PE.; Coleman, J.; O'sullivan, M.P.; Kornfeld, H.; Kelleher, D.; Volkov, Y. Autophagy induction by silver nanowires: A new aspect in the biocompatibility assessment of nanocomposite thin films.

Toxicol. Appl. Pharmacol **2012**, *264*, 451-61, https://doi.org/10.1016/j.taap.2012.08.023.

106. Houtman, J.; Maisanaba, S.; Puerto, M.; Gutiérrez-Praena, D.; Jordá, M.; Aucejo, S.; Jos, A. Toxicity assessment of organomodified clays used in food contact materials on human target cell lines. *Appl. Clay Sci* **2014**, 90, 150-8, https://doi.org/10.1016/j.clay.2014.01.009.

107. Rawat, K.; Agarwal, S.; Tyagi, A.; Verma, A.K.; Bohidar, H. Aspect ratio dependent cytotoxicity and antimicrobial properties of nanoclay. *Appl. Biochem. Biotechnol* **2014**, *174*, 936-44, https://doi.org/10.1007/s12010-014-0983-2.

108. Sharma, A.K.; Schmidt, B.; Frandsen, H.; Jacobsen, N.R.; Larsen, E.H.; Binderup, M.L. Genotoxicity of unmodified and organo-modified montmorillonite. *Mutat. Res., Genet. Toxicol. Environ. Mutagen* **2010**, *700*, 18-25, https://doi.org/10.1016/j.mrgentox.2010.04.021.

109. Jorda-Beneyto M.; Ortuño N.; Devis A.; Aucejo S.; Puerto M.; Gutiérrez-Praena, D.; Houtman, J.; Pichardo, S.; Maisanaba, S.; Jos, A. Use of nanoclay platelets in food packaging materials: technical and cytotoxicity approach. *Food Additives & Contaminants: Part A* **2014**, *31*, 354-63, https://doi.org/10.1080/19440049.2013.874045.

110. Lee, Y.H.; Kuo, T.F.; Chen, B.Y.; Feng, Y.K.; Wen, Y.R.; Lin, W.C.; Lin, F.H. Toxicity assessment of montmorillonite as a drug carrier for pharmaceutical applications: yeast and rats model. *Biomedical Engineering: Applications, Basis and Communications* **2005**, *17*, 72-8, https://doi.org/10.4015/S1016237205000111.

111. Li, P.R.; Wei, J.C.; Chiu, Y.F.; Su, H.L.; Peng, F.C.; Lin, J.J. Evaluation on cytotoxicity and genotoxicity of the exfoliated silicate nanoclay. *ACS Appl. Mater. Interfaces* **2010**, *2*, 1608-13, https://doi.org/10.1021/am1001162.

112. Baek, M.; Lee, J.A.; Choi, S.J. Toxicological effects of a cationic clay.; montmorillonite in vitro and in vivo. *Mol. Cell. Toxicol* **2012**, 8, 95-101, https://doi.org/10.1007/s13273-012-0012-x.

113. Maisanaba, S.; Gutiérrez-Praena, D.; Puerto, M.; Moyano, R.; Blanco, A.; Jordá, M.; Camean, A.M.; Aucejo, S.; Jos, A. Effects of the subchronic exposure to an organomodified clay mineral for food packaging applications on Wistar rats. *Appl. Clay Sci* **2014**, *95*, 37-40, https://doi.org/10.1016/j.clay.2014.04.006.

114. Maisanaba, S.; Gutiérrez-Praena, D.; Puerto, M.; Llana-Ruiz-Cabello, M.; Pichardo, S.; Moyano, R.; Blanco, A.; Jorda-Beneyto, M.; Jos, A. In vivo toxicity evaluation of the migration extract of an organomodified clay–poly (lactic) acid nanocomposite. *J. Toxicol. Environ. Health, Part A* **2014**, *77*, 731-46, https://doi.org/10.1080/15287394.2014.890987

115. Sharma, A.K.; Mortensen, A.; Schmidt, B.; Frandsen, H.; Hadrup, N.; Larsen, EH.; et al. In-vivo study of genotoxic and inflammatory effects of the organo-modified Montmorillonite Cloisite® 30B. *Mutat. Res., Genet. Toxicol. Environ. Mutagen* **2014**, *770*, 66-71, https://doi.org/10.1016/j.mrgentox.2014.04.023.

6. ACKNOWLEDGEMENTS