Chitosan Films Barrier improvement

Chitosan is produced commercialy by deacetylation of chitin (a molecule derivative from glucose, and the second most plentiful natural polysaccharide found on our planet after cellulose) and chitosan films have been studied for food preservation, since they are biocompatible, biodegradable, and bioactive. However, their performance, in terms of water and gases barrier properties, needs to be improved.

1. Introduction

The food industry employs distinct types of materials in which glass and metal containers are highlighted for their excellent physical, gas, and water barrier properties, thus becoming important options to be considered by the food and beverage packaging. However, this type of materials have some negative aspects associated; they have increased transportation costs, demand excessive energy to recycle, and if not properly disposed of, may pose a risk to the environment because they do not decompose or decompose slowly in landfills or in the environment, releasing polluting substances [1]. Over the past century, plastic packaging has been increasingly replacing these traditional materials, due to their flexibility, variability in size and shape, thermal stability, and barrier properties. Despite all the appealing aspects undoubtedly connected to conventional plastic packaging, their non-renewable character, unsustainable use, and brief lifetime associated with their resistance to degradation caused a severe and realistic environmental problem, bringing out the responsibility to uncover creative and better ways to improve the disposal systems [2]. To fulfill the task of attenuating the ecological impact successfully and reduce the waste associated with the end of life of single-use plastics, novel bio-based food packaging systems should be intensely studied and optimized before being addressed for commercial purposes, ensuring that they are fully biodegradable and that no harmful toxicological and ecotoxicological effects arise from their use and disposal [3]. Bio-based plastics/polymers, recurrently expressed in literature as bioplastics or biopolymers, have recently been appointed as the logical candidates to replace conventional plastics due to their renewability, high abundance, accessibility, low cost, reduced toxicity, and biodegradable character [1]. The biopolymers normally used are polysaccharides (e.g., chitosan or pectin), lipids (e.g., natural waxes), proteins (e.g., whey protein), polyesters produced by microorganisms (e.g., polyhydroxybutyrate), synthesized from monomers (e.g., polylactic acid) or composites [4][5][6][7][8][9]. Chitin, a molecule derivative from glucose, is the second most plentiful natural polysaccharide found on our planet after cellulose and is mainly located in marine arthropods (i.e., more specifically in the exoskeleton of crustaceans) and in many other invertebrates, such as the cell walls of some fungi and algae. When submitting chitin to a fully or partially N-deacetylation, the acetyl group ($-COCH_3$) from the amino group ($-NH_2$) at the C-2 position is removed and a new bioactive polymer is designated as chitosan (Ch) arises. Structurally, Ch is a linear cationic polysaccharide with variable molecular weight (MW), assembled by D-glucosamine and N-acetyl-d-glucosamine units bonded by β-1,4 glycosidic linkages, with one amino (NH_2) group and two hydroxyl (OH) groups in each repeating glycosidic units [10][11][12]. The ratio of D-glucosamine units to the sum of D-glucosamine and N-acetyl-d-glucosamine units that constitute the Ch chain is defined as the degree of deacetylation (DD). Pure Ch is insoluble in water, alkaline solutions, and even in organic solvents; however, when the DD reaches about 50%, it becomes soluble in slightly acidic environments (below its pKa ~6.3). As a result, Ch forms a highly versatile viscous solution after being dissolved due to the amino groups in the chain that protonate ($-NH_3^+$), increasing intermolecular electric repulsions and resulting in a polycationic soluble polysaccharide, allowing it to activate reactive locations for a variety of new group attachments using mild reaction conditions [11][12]. Films and coatings, specifically made of chitosan and derivatives, have been extensively studied in the past decades for fresh and processed food conservation [13][14][15] because they possess the beneficial aspects inherently associated with polysaccharides. The combination of these properties with the advantageous film-forming feature and antimicrobial/antioxidant activity unlocks the door to the introduction of suitable chitosan active films and coatings on valuable scientific and technological markets such as food applications, but also pharmaceutical and biomedical applications [16].

A correct evaluation of the polymer barrier and mechanical properties is crucial to estimate the product-package shelf life and its maneuverability. The permeability through plastic materials depends on several factors, namely, (i) the permeant properties (molecule size and nature); (ii) material/polymer characteristics (molecular orientation, crystallinity, free volume, and chain stiffness); and (iii) external conditions (such as temperature and moisture) [17]. The temperature and humidity circumstances to which the food product will be exposed in the supply chain are vital for calculating the required barrier so that it applies to the conditions expected. Furthermore, the specific barrier requirement of a package system depends upon the food characteristics and the intended end-use applications [17][18][19][20]. The two main permeants studied in food packaging applications are water vapor and oxygen, because those gases can move from the internal or external environment through the polymer package wall, they result in possible negative changes in product quality and shelf-life and in the polymer itself. Moreover, the study of the permeability of oxygen (OP) is important because the oxidation is related to the odor, color, taste, and deterioration of food; therefore, packages must provide a barrier to this gas [18][21].

Petroleum-derived plastics often present a good water vapor barrier but poor oxygen block properties. On the contrary, the biopolymeric matrix (i.e., chitosan) is generally attractive due to its good obstacle characteristics against oxygen. In this subject, due to the well-ordered hydrogen-bonded network configuration, polysaccharides unveiled to be an effective barrier to gas transference, such as O_2 and CO_2, preventing oxidative rancidity, and surface-browning. In contrast, they typically own a hydrophilic nature resulting in poor water vapor and moisture barrier properties, which can turn into a dilemma in the preservation of perishable fresh-cut products [11][19][20][21]. Together with the barrier properties, mechanical properties (characterized by tensile strength, elastic modulus, elongation at break) also play an important role in the development of novel biodegradable plastic materials. When compared to plastics, bioplastics still present critical structural flaws coupled with processing challenges, which diminish their industrial applicability [22]. Blends of chitosan and anionic polymers could be a solution, showing improved mechanical and barrier properties when compared to chitosan films. This can be attributed to the formation of polyelectrolyte complexes through electrostatic interactions between the protonated amino groups of chitosan and the negatively charged side-chain assemblies of the other biopolymer. Better performance in terms of water vapor permeability and lower water solubility have been reported for combinations of chitosan with other polysaccharides, such as starch, pectin, or alginate compared to chitosan films [20].

To make it possible for biopolymer-based food packaging to go from concept to reality, it is fundamental to strengthen their composition, modifying and improving their properties. Therefore, another undergoing solution is the use of organic or inorganic nanoparticles to enhance the properties of the films, as was demonstrated in the study by Jamróz et al. [23]. The insertion of homogeneously dispersed nanoparticles, with a high aspect ratio and high surface area, into the biopolymer matrix is seen as a promising innovation, creating a novel functional class of materials, named bionanocomposites [2][11][24][25]. Polymer nanocomposites are mixtures of a certain polymer (or biopolymer) reinforced with small quantities of nanosized inorganic or organic (or bio-based organic) nanofillers with a particular size, geometry, and surface chemistry [26]. Nanotechnology represents the manipulation of matter with at least one dimension sized from 1 to 100 nanometers, and nanomaterials are characterized for having different physical, chemical, and biological properties than bulk material [18]. Thus, nanotechnology plays an important role as an alternative strategy to reinforce bio-based films through the incorporation of nanomaterials into the polymeric chain to enhance their mechanical and barrier properties [27]. After being reinforced with nanofillers, the chitosan-based nanocomposites are capable to be applied in various scientific areas and industries such as paper and food packaging, flexible electronics, sensing and biosensing, energy harvesting, liquid crystals, biomedicine and cosmetics, catalysis, adsorption, separation, decontamination, or filtration systems [28][29][30][31][32][33].

2. Nanotechnology as Reinforcement

2.1. Barrier Properties

The incorporation of nano-sized particles is under intense investigation for enhancement of barrier properties of packaging. The barrier properties of polymers can be significantly altered by the inclusion of inorganic platelets with a high aspect ratio to alter the diffusion path of gas molecules. Various models have been proposed to predict the permeability of platelet-filled composites as listed in Table 1. These models are generally based on random, parallel platelets perpendicular to the permeation direction (random in only two directions). At a high aspect ratio, significant decreases in permeability are predicted and observed in practice [34].

Table 1. Prediction models for barrier properties of nanocomposites (adapted from [34]).

Model	Filler Type	Aspect Ratio (α)	Formula
Nielsen	Ribbon	α = w/t (length is infinite, w is the width, t is the thickness)	$(P0/P)(1 − \phi) = 1 + \alpha\phi/2$
Cussler (regular array)	Ribbon	α = w/t (length is infinite, w is the width, t is the thickness)	$(P0/P)(1 − \phi) = 1 + (\alpha\phi)2/4$
Cussler (random array)	Ribbon	α = w/t (length is infinite, w is the width, t is the thickness)	$(P0/P)(1 − \phi) = (1 + \alpha\phi/3)2$
Gusev and Lusti	Disk	α = d/t (circular shape, d is the diameter, t is the thickness)	$(P0/P)(1 − \phi) = \exp[(\alpha\phi/3.47)0.71]$
Fredrickson and Bicerano	Disk	α = d/t (circular shape, d is the diameter, t is the thickness)	$(P0/P)(1 − \phi) = 4(1 + x + 0.1245x2)/(2 + x)2$, where x = $\alpha\phi/2\ln(\alpha/2)$
Bharadwaj	Disk	α = d/t (circular shape, d is the diameter, t is the thickness)	$(P0/P)(1 − \phi) = 1 + 0.667\alpha\phi(S + 0.5)$, where S is the orientation factor from -0.5 to 1.

P—permeability of composites; P0—permeability of pure polymer; ϕ—volume fraction. Common fillers used to prepare nanocomposites for food packaging applications are nanoclays, cellulose nanofibers and nanocrystals, carbon nanotubes, and metal oxides. Table 2, Table 3 and Table 4 show the effect of different nanofillers and compositions on water vapor permeability (WVP) and oxygen permeability (OP) through chitosan nanocomposite films.

Table 2. Barrier properties of chitosan films reinforced with nanoclays.

Formulation	Incorporation Method	Water Vapor Permeability (Percentage Relatively to Control)	Oxygen Permeability (Percentage Relatively to Control)	Ref
2% (w/v) Ch (deacetylation degree of 75–85%); Glycerol 25% (w/w Ch)	Mechanical stirring and ultrasonic homogenizer	Ch + 1% MMTNa: Decreased 31% Ch + 3% MMTNa: Decreased 56% Ch + 5% MMTNa: Decreased 38%	Not Performed	[39]
1.5% (w/v) Ch (deacetylation degree of 75%); Glycerol 30% (w/w Ch)	Rotor–stator homogenizer (Ultra-Turrax) and ultrasonic homogenizer	Ch + 2.5% MMTNa: Increased 25%	Ch + 2.5% MMTNa: Decreased 47%	[25]
1% (w/v) Ch (deacetylation degree of about 75%); Glycerol 40% (w/w Ch)	Mechanical stirring and ultrasonic homogenizer	Ch + 5% HNT: Decreased 2% Ch + 10% HNT: Decreased 5% Ch + 15% HNT: Decreased 10% Ch + 20% HNT: Decreased 13% Ch + 25% HNT: Decreased 14% Ch + 30% HNT: Decreased 16%	Not Performed	[40]

Formulation	Incorporation Method	Water Vapor Permeability (Percentage Relatively to Control)	Oxygen Permeability (Percentage Relatively to Control)	Ref
2% (w/v) Ch (deacetylation degree greater than 75%); Glycerol 25% (w/w Ch)	Mechanical stirring and ultrasonic probes	Ch + 1% MMT: Decreased 28%	Ch + 1%MMT: Decreased 12%	[41]
		Ch + 3% MMT: Decreased 37%	Ch + 3%MMT: Decreased 55%	
		Ch + 5% MMT: Decreased 17%	Ch + 5%MMT: Decreased 37%	
		Ch + 1% MMTCuO-20: Decreased 26%	Ch + 1%MMTCuO-20: Decreased 31%	
		Ch + 3% MMTCuO-20: Decreased 39%	Ch + 3%MMTCuO-20: Decreased 60%	
		Ch + 5% MMTCuO-20: Decreased 35%	Ch + 5%MMTCuO-20: Decreased 49%	
		Ch + 1% MMTCuO-90: Decreased 72%	Ch + 1%MMTCuO-90: Decreased 47%	
		Ch + 3% MMTCuO-90: Decreased 77%	Ch + 3%MMTCuO-90: Decreased 79%	
		Ch + 5% MMTCuO-90: Decreased 76%	Ch + 5%MMTCuO-90: Decreased 55%	
1% (w/w) Ch (deacetylation degree greater than 85%); Glycerol 30% (w/w Ch) of chitosan	Nanoparticles were dispersed stirred at 400 rotational frequency and immersed in an ultrasound bath	Ch + 0.5% MMT: Increased 16%	Ch + 0.5% MMT: Increased 89%	[42]
		Ch + 1% MMT: Decreased 19%	Ch + 1% MMT: Increased 225%	
3% (v/v) Ch (deacetylation degree of about 95%); Polycaprolactone 12% (w/w Ch)	Mechanical stirring and ultrasonic homogenizer	Ch + 2% HNT: Decreased 15% Ch + 4% HNT: Decreased 23% Ch + 6% HNT: Decreased 25%	Not Performed	[43]
1.5% (w/w) Ch (deacetylation degree of about 90%)	Mechanical stirring and ultrasonic homogenizer	Ch + 0.5% MMTNa: Decreased 2.7%	Ch + 0.5% MMTNa: Decreased 32%	[37]
		Ch + 1% MMTNa: Decreased 7.4%	Ch + 1% MMTNa: Decreased 61%	
		Ch + 2% MMTNa: Decreased 4.0%	Ch + 2% MMTNa: Decreased 51%	
0.2% (v/v) Ch (deacetylation degree of about 75%); carboxymethyl cellulose 1% (w/w Ch)	Mechanical stirring	Ch + 5% HNT: Decreased 60% Ch + 7% HNT: Decreased 71% Ch + 10% HNT: Decreased 75%	Not Performed	[44]
2% (w/w) Ch (deacetylation degree of about 90%)	Mechanical stirring	Ch + 25% HNT: Decreased 6,1% Ch + 42% HNT: Decreased 15% Ch + 66% HNT: Decreased 29% Ch + 100% HNT: Decreased 20%	Not Performed	[45]

Ch—chitosan; MMTNa—sodium montmorillonite; HNT—halloysite nanotubes; MMTCuO—montmorillonite with copper (II) oxide.

Table 3. Barrier properties of chitosan films reinforced with cellulose nanoparticles.

Formulation	Incorporation Method	Water Vapor Permeability (Percentage Relatively to Control)	Oxygen Permeability (Percentage Relatively to Control)	Ref
2% (w/v) Ch (deacetylation degree not specified)	Mechanical stirring and ultrasonic homogenizer	Ch + 1% CNC: Decreased 34% Ch + 3% CNC: Decreased 16% Ch + 5% CNC: Decreased 38% Ch + 10% CNC: Decreased 25%	Not Performed	[53]
1% (w/v) Ch (deacetylation degree of 90%)	Mechanical stirring and ultrasonic homogenizer	Ch + 2% CNC: Decreased 24% Ch + 4% CNC: Decreased 29% Ch + 6% CNC: Decreased 34% Ch + 8% CNC: Decreased 37%	Not Performed	[54]
3% (w/v) Ch (degree of deacetylation 94%);	Mechanical stirring	Ch + 10% CNC: Decreased 32% Ch + 20% CNC: Decreased 28%	Not Performed	[55]
2% (w/v) Ch (deacetylation degree not specified)	Mechanical stirring	Ch + 10% CGTOCNF: Decreased 6% Ch + 17% CGTOCNF: Increased 2% Ch + 25% CGTOCNF: Decreased 4% Ch + 33% CGTOCNF: Decreased 7%	Ch + 10% CGTOCNF: Increased 13% Ch + 17% CGTOCNF: Increased 16% Ch + 25% CGTOCNF: Increased 3% Ch + 33% CGTOCNF: Increased 62%	[56]
1% (w/v) Ch (deacetylation degree 75–85%);	Nanoparticles were dispersed with the aid of mechanical stirring	Ch + 2% BCNC: Decreased 9% Ch + 4% BCNC: Decreased 20% Ch + 6% BCNC: Decreased 27%	Not Performed	[57]

Ch—chitosan; CNC—cellulose nanocrystals; CGTOCNF—curcumin-grafted; BCNC—bacterial nanocellulose.

Table 4. Barrier properties of chitosan films reinforced with metal oxide nanoparticles and carbon nanotubes.

Formulation	Incorporation Method	Water Vapor Permeability (Percentage Relatively to Control)	Oxygen Permeability (Percentage Relatively to Control)	Ref
1% (w/v) Ch; 2% (w/v) purified CNT in dimethylformamide; 6 different PLA/CNT /Ch concentrations (0, 1, 3, 5, 7, 9% Ch)	Nanotubes were dispersed with stirring and electrospinning	PLA/CNTs/Ch-1%: Decreased 30% PLA/CNTs/Ch-3%: Decreased 64% PLA/CNTs/Ch-5%: Decreased 71% PLA/CNTs/Ch-7%: Decreased 75% PLA/CNTs/Ch-9%: Decreased 54%	Not performed	[58]
1 g of Ch in the 1% acetic acid solution;	Metal oxides were sonicated to be dispersed	Ch + 0.1% ZnONP: Decreased 21% Ch + 0.3% ZnONP: Decreased 31% Ch + 0.5% ZnONP: Decreased 56%	Not performed	[59]

Formulation	Incorporation Method	Water Vapor Permeability (Percentage Relatively to Control)	Oxygen Permeability (Percentage Relatively to Control)	Ref
2% (w/v) Ch	Metal oxides were dispersed with stirring	Ch + 30ZnO: Decreased 66% Ch + 50ZnO: Decreased 77% Ch + 70ZnO: Decreased 87%	Ch + 30ZnO: Decreased 7.5% Ch + 50ZnO: Decreased 10% Ch + 70ZnO: Decreased 41%	[60]
0.5% (w/v) Ch; 1% cellulose acetate (CelAc) solution; 0.25% glycerol	Metal oxides were dispersed in formic acid and after were mixed with the polymer solution	Ch-CelAc-CeO_2-0.1%: Increased 36% Ch-CelAc-CeO_2-1%: Increased 157%	Not performed	[61]
Ch (0.2 g) was dispersed in 50.0 mL acidic water (0.5% v/v acetic acid	Metal oxides were dispersed in water and sonicated in an ultrasonic bath	CMC-Ch-OL-ZnONPs 0.5%: Increased 5.1% CMC-Ch-OL-ZnONPs 1%: Increased 20% CMC-Ch-OL-ZnONPs 2%: Increased 28%	Not performed	[14]
1.5% (w/v) Ch in a 1% (v/v) l-(β)-lactic acid; 0.5% glycerol (w/v); 3 different acetylene flux (4 sccm, 12 sccm, 20 sccm)	Carbon-based coatings were dispersed with radio-frequency reactive magnetron sputtering	Ch-Acetylene 4 sccm: Increased 34% Ch-Acetylene 4 sccm: Increased 12% Ch-Acetylene 4 sccm: Increased 11%	Ch-Acetylene 4 sccm: Increased 14% Ch-Acetylene 12 sccm: Decreased 42% Ch-Acetylene 20 sccm: Decreased 81%	[62]
1% (w/v) Ch; 20% glycerol (w/v Ch)	Metal oxides weredispersed with refluxed	ChG7ZnO: Decreased 81% (The other results are not showed)	Not performed	[63]
0.6% (w/v) Ch; 4 different ZnONP concentrations (0.165, 0.33, 0.66, 0.99g in 15 mL Water)	Metal oxides were dispersed with vigorous stirring	Ch/ZnO 0.165 g: Decreased 13% Ch/ZnO 0.33 g: Decreased 17% Ch/ZnO 0.66 g: Decreased 8.0% Ch/ZnO 0.99 g: Decreased 6.8%	Not performed	[64]
1% (w/v) Ch and 1% (w/w of Ch) of MgO NP	Metal oxides weredispersed with vigorous stirring	Decreased 53%	Not performed	[65]

Ch—chitosan; PLA—polylactic acid; CNTs—carbon nanotubes; ZnO—zinc Oxide; CelAc—cellulose acetate; CeO_2—cerium (IV) oxide; CMC—carboxymethylcellulose; OL—oleic acid.

The amount of nanofillers ranges between 0.5% (*w/w*) and 10% *w/w* of chitosan, except in a few cases in which concentrations are up to 30% (*w/w*). It can also be seen that most studies present only WVP results. Although OP is an important parameter because it will influence the oxidation of the food products and consequently, the product shelf life is seldom reported [35].

In most studies, a decrease of permeability with the increase of filler amount may be observed. This is due to the increase in the diffusion path that slows the permeation of gases through the polymer barrier. In some, this trend happens until a critical nanoparticle content, after which a constant or a small increase of permeability is detected. This behavior has been explained with deficient nanoparticles' exfoliation or a decrease in aspect ratio. Moreover, when inorganic particles are included in a polymer matrix, their effect on the composite permeability is related to the type of interaction of the particles with the permeant species, and with the polymer and the resulting internal morphology. For instance, regarding the barrier to water vapor, the water adsorbs to the polymer and hydrophilic nanoparticles. The swelling of both solids may create unfilled zones around the particles where water vapor diffusion will be made easier and higher. From a given particle content, the increase in the diffusion path due to the impermeable barrier is nullified by the contribution of these unfilled diffusion zones, leading to stability or even an increase in permeability [17].

The barrier properties of chitosan films with different nanofillers are presented and discussed in the following subchapters.

2.1.1. Nanoclays

Montmorillonite (MMT) is a hydrated, alumina–silicate layered clay, and its negative charge is balanced with exchangeable cations, as Na^+ and Ca^{2+}. It is one of the most used clays because of its low cost, high active surface, and its capacity to improve the mechanical and barrier properties of chitosan nanocomposites [11]. The use of nanoclays in films has several advantages due to its low cost, availability, and good surface area. The addition of montmorillonite gives the films a lighter composition and less risk of microbial contamination, for the packaging and for the food packaged by this film, improving the shelf life of the food packaged [36]. Regarding toxicity, the MMT does not appear to be toxic and can be used widely in the food industry [37].

Nanoclays can provide the enhancement of the water barrier properties of bio-based films by the increase on the tortuous path of the water vapor molecules through the polymeric chain [38]. Exfoliated structures, in comparison with the intercalated and micro-phase separated structures, are more efficient to prevent the film from being water-permeable [11] [38]. Because the permeability to water vapor is related to the deterioration of food, films intended to be in direct contact with food must have a good barrier to this water vapor [21]. Some examples of reinforced chitosan films with MMT or halloysite nanotubes (HNT) are shown in Table 2 and described below.

Films prepared with 1% (*w/v* Ch), 30% glycerol, and two different MMT at 0.5 (*w/w)* and 1% (*w/w* Ch), demonstrated a decrease in WVP proportionally to the amount of MMT added in the matrix (Table 2) [19]. According to Llanos et al. (2018) [39], this phenomenon occurred due to the tortuous path provided by the MMT nanoparticles that are dispersed in the chitosan matrix, which makes the water vapor more difficult to diffuse through the film. The WPV was determined using the gravimetric method. This study also evaluated the material's oxygen permeability and the presence of nanoclay. Contrary to what was expected, the OP increased, because the nanoparticles (NPs) increased the porosity of the film, changing its microstructure [39]. The addition of MMT can improve the barrier properties of films; however, this nanoclay generally does not enhance the antimicrobial properties of chitosan films. Thus, it can be interesting to add antimicrobial agents, such as metal oxide nanoparticles or essential oils, to confer this antimicrobial activity to the packaging [11]. Copper oxide nanoparticles are known for their antimicrobial activity; however, the release of copper ions toward the food can cause leaching, enhance the food deterioration process, and pose some toxicity. Thus, the combination with MMT with exchangeable positively charged ions, such as Na^+ or Ca^{2+}, can prevent copper ions from being released [40].

Bionanocomposites based on chitosan (2% *w/v*) were formulated with the addition of 25% (*w/w* Ch) of glycerol and three different MMT concentrations (1%, 3%, and 5% *w/w* Ch) with or without CuO NPs. Two complexes were studied MMTCuO-20 and MMTCuO-90 (produced differently regarding the synthesizing time inside the muffle furnace, 20 min or 90 min, respectively) [40]. In this case, the WPV study was also conducted by the gravimetric method and the OP study by the ASTM D3985-05 [41] method. Overall, the addition of pure nanoclay decreased the WVP in all treatments; however, the lowest result was achieved with the addition of 3% of MMT (a decrease of 37% in comparison to the control sample). At the level of 5% MMT, the WPV was slightly superior to the composites with 3%, which was related to the possible agglomeration of the NPs. When the complex with MMT and CuO NPs were used, the same pattern was observed; however, CuO NPs were able to reduce even more the material WPV (reaching 77% reduction), and MMTCuO-90 at 3% was the best formulation. These results were attributed to the presence of hydrogen interactions between chitosan, glycerol, and an MMT–CuO, which decreased the number of hydroxyl groups in the film to react with the water, and the building of a tortuous path, that makes the diffusion of water vapor difficult [40]. Concerning OP, the addition of MMT to the matrix formed by chitosan and glycerol also reduced the oxygen permeability. The best result was also with the addition of MMT–CuO because the groups present in chitosan have a strong attraction with MMT–CuO, which closes the net of the film, preventing the entry of oxygen [40].

Contradictory behavior is also found in literature, as in Souza et al. (2019) [25] and Xu et al. (2018) [37], which did not observe a statistical difference in the WVP of chitosan films incorporated with different amounts of MMT (varying from 0.5–2.5% *w/w* Ch). However, the OP in both studies was reduced around 32–61% in comparison to pristine chitosan film. As described before, it seems that when the amount of MMT incorporated is increased the enhancement ability diminishes, probably due to the agglomeration effect. In addition, within the formulations assessed, the incorporation of 1% of MMT was demonstrated to be the best reinforcement formula [25][37].

Halloysite nanotubes (HNTs), $Al_2Si_2O_5(OH)_4 \cdot nH_2O$, belongs to a subgroup of kaolin clay, which is generally recognized as a safe (GRAS) food packaging material due to its non-toxicity and biocompatibility [42]. HNTs show a nanotubular morphology, with an outer tube diameter of nanometric dimension, whereas the length can range typically from hundreds of nanometers to about 1.5 µm [42]. Some of the advantages of HNTs are their hydrophilic characteristic, high dispersibility (easily exfoliated because of its structure with high aspect ratio), thermal stability, and non-toxicity [42][43].

In vitro and in vivo studies with HNTs demonstrated their biocompatibility with tissues and cells, thus showing that they are also interesting options for film production. In the formulation was added 0.2% of chitosan, carboxymethyl cellulose (CMX) in the proportion of 1:2 to chitosan and HNT in three different concentrations, 5%, 7.5%, and 10% (w/w Ch) [44]. The authors concluded that with the addition of HNT, the chitosan and CMX films have a greater barrier to water vapor due to the dispersion of the nanoclay in the mixture. This phenomenon was attributed to the more tortuous paths due to the hydrophilic interaction between chitosan and HNT, which also caused the reduction of the hydrophilic parts, thus making the interaction with water vapor more difficult and, consequently, the diffusion process more difficult to occur [44].

2.1.2. Cellulosic Nanofibers and Nanocrystals

Nanocellulose (NC), either as nanofibers or nanocrystals, are also nanomaterials commonly used to strengthen bio-based films by the improvement on their mechanical properties and the reduction on the WVP and OP [45], which are the main drawbacks of biopolymers' application in the packaging industry [27]. They are an environmentally friendly nanofiller that can be highlighted due to their biodegradability, low density, nontoxicity, excellent mechanical properties, and high aspect ratio that guarantee a huge interface with the polymeric matrix [46][47][48].

As previously described for the nanoclays, the decrease in the WVP or OP is generally attributed to the tortuosity created by, among other factors, the incorporation of nanocellulose, and this reinforcement is a topic that merits further research attention [49]. The types of nanocellulose used for this purpose vary from the source, namely, bacterial or extracted from biomass, and configuration (in crystals or fibers). The content of NC used in the film is generally low, between 0–35% (w/w of polymer), as described in Table 3.

The trend observed is that despite the type or source of NC used, WVP reduces with the incorporation of nanocellulose particles until a maximum (in general below 10% w/w), from which with the increase of the amount incorporated the barrier properties reduces. In the study of Corsello et al. (2017) [50], bionanocomposites based on chitosan incorporated with cellulose nanocrystals (CNC) at four increasing levels (1%, 3%, 5%, and 10% w/w Ch) showed a reduction in the WVP up to 38% in comparison to pristine chitosan films. The minimum permeability to water vapor was observed with the amount of 5% CNC, and when 10% was incorporated, the WVP decreased to 25%. The author attributed the WVP reduction to the presence of NC, which increased the tortuosity in the polymer matrix films, leading to slower water vapor diffusion processes [50][51]. In contrast, excessive NC destroyed the polymeric network structure of chitosan, allowing for the formation of gaps for water vapor molecules to pass through [52].

Other factors might account for this reduction in the WVP, such as (i) the increase in the polymer's crystallinity (acting as crystalline fillers that increases the distance for the molecules of water to pass through, i.e., a more tortuous path); (ii) the improvement on the film uniformity, and iii) making the film tough enough to decrease the frequency of cracks or other defects that could lead to a high gas permeability [49][52].

The study of OP is scarce, and few studies investigate it simultaneously with WVP, as in the study of Zhang et al. (2021) [52]. The author proposed a novel bionanocomposite of chitosan reinforced with curcumin grafted TEMPO-oxidized cellulose nanofibers (CGTOCNF) at 10%, 17%, 25%, and 33% (w/w Ch). Regarding WVP, similar behavior was reported, with a reduction in WVP with CGTOCNF incorporation. On the other hand, OP of the bionanocomposites increased with the incorporation of the nanoparticle complex; thus, the addition of CGTOCNF had a more powerful destructive effect than improved crystallinity for the polymer network, and the hydrophobicity of CGTOCNF was not sufficient to counterbalance this, contributing less to the oxygen barrier [52].

2.1.3. Metal Oxides Nanoparticles and Carbon Nanotubes

Metal oxide nanoparticles and carbon nanotubes are other classes of NPs that can be used to improve the quality of the films in relation to their oxygen and water vapor barrier properties. Some examples of the latest studies on this matter are shown in Table 4.

According to the study by Sanuja et al. (2015) [53], who evaluated the WVP of chitosan films incorporated with three different concentrations of zinc oxide nanoparticles (ZnONP) (0.1%; 0.3%, and 0.5%), there is a decrease in the material's WVP with the increment of the NP concentration in the formulation (up to 56% lower than pristine chitosan films). This behavior was attributed to the fact that chitosan has high hydrophilic properties, so it is easier to interact with the hydrogen in the water molecule, and with the addition of ZnO NPs, its dispersion in the matrix forms a more efficient barrier to contain the water vapor permeability. In this experiment, neem essential oil was also added to the chitosan formulation with 0.5% ZnO NPs. Because it presents even more hydrophobic characteristics and interacts with chitosan, it reduced WVP even further [53].

The use of ZnO NPs is very efficient to increase the antimicrobial properties of the film and its barrier properties, including WVP and OP, and that is the reason many studies with the reinforcement of chitosan with this NP are found in the literature. Moreover, ZnO NPs have antioxidant properties and are non-toxic when added to the film, being recognized as GRAS by the US Department of Food and Drug Administration (FDA). Chitosan films with ZnO NPs and gallic acid allowed the reduction of WVP in relation to the pure chitosan film, according to the study published by Yadav et al. (2021) [54]. This occurred due to the construction of complex paths for the passage of water and the occupation of spaces (porous) in the macromolecular structure. Concerning oxygen permeability, with the addition of ZnO NPs, the OP values reduced up to 41% when compared to pristine chitosan film [54].

In another study also using ZnO NPs to improve the barrier properties of the chitosan film, a decrease in WVP with the addition of ZnO NPs was reported [55]. According to the authors, when ZnO NPs are incorporated, structures with greater firmness are formed due to the interaction of the metal oxide NP and chitosan, generating a more complex path for the water molecules to diffuse through. In this experiment, silver nanoparticles (AgNPs) were also added to the formulation, i.e. composites of Chitosan-AgNPs and Chitosan-AgNPs-ZnNPs were evaluated. The addition of both nanoparticles to chitosan also showed satisfactory and better values than just the use of one of the compounds. Moreover, they also included citronella essential oil, and this formulation containing the four components presented the lowest value of WVP [55].

The use of carbon nanotubes (CNT) in the formulation of bio-based films was reported by Liu et al. (2019) [56]. The authors combined CNT with a polymeric blend of chitosan (in different percentages 1–9% *w/v* film-forming dispersion) and polylactic acid (PLA). When CNT was added to the formulation with chitosan, the resistance of the formed film was 90% greater than the pristine chitosan film, which corroborates with the WPV results. The lowest value of WVP was acquired when the amount of chitosan was 7% (*w/v* film-forming dispersion). This decrease in WVP was possible due to the reduction in the pore diameter of the fiber, making it difficult for water molecules to pass. In addition, because of the hydrophilicity, there was a certain absorption of the water vapor by the chitosan. However, if the amount of chitosan increases a lot, this hydrophilic characteristic can damage the water vapor barrier, and this may be one of the reasons related to the increase in WVP by adding 9% (*w/v* film-forming dispersion) chitosan in the formulation. In addition, the viscosity of the film can increase much more with the greater addition of chitosan, making it very porous and impairing its quality [56].

In another study that addresses chitosan films together with carbon-based coatings, no significant difference was observed in relation to the WVP. However, concerning OP, there was a decrease in this permeability when the addition of carbon-based coatings occurred, according to Fernandes et al. (2018) [57]. For this research, three different acetylene fluxes in standard cubic centimeters per minute (4 sccm, 12 sccm, and 20 sccm) were used to apply the CNT coating. The lowest OP value was acquired with the largest acetylene flux, 20 sccm, due to the presence of less porosity in the film. This is because the carbon coating reduces the affinity between the oxygen and chitosan molecules. The difference between the permeability of the film for water vapor and oxygen can be explained due to the high solubility of chitosan in water, which promotes diffusion with the micro-cracks that may exist in the film, and because the O_2 molecules do not degrade chitosan, this allows it to have greater coverage of the surface by the carbon coatings, having a greater barrier to oxygen [57].

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