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Helene Angellier-Coussy, Emmanuelle Gastaldi

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Corresponding Author	Family Name	Angellier-Coussy
	Particle	
	Given Name	Hélène
	Suffix	
	Division	
	Organization	UMR IATE, Université Montpellier II, CC023
	Address	pl. E. Bataillon, 34095, Montpellier Cedex, France
	Email	helene.coussy@univ-montp2.fr
Author	Family Name	Gastaldi
	Particle	
	Given Name	Emmanuelle
	Suffix	
	Division	
	Organization	UMR IATE, Université Montpellier II, CC023
	Address	pl. E. Bataillon, 34095, Montpellier Cedex, France
	Email	
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Chapter 12

Protein/Clay Nano-Biocomposites

Hélène Angellier-Coussy and Emmanuelle Gastaldi

Abstract In the current context, protein-based materials might be considered as an alternative to the petroleum-based plastics since fully biodegradable and characterized by remarkable functional properties that can be exploited in a wide range of non-food applications. To improve their performances that are often restricted by high water sensitivity and low mechanical properties, a relevant strategy consisted in the development of protein/clay nanocomposite. For this purpose, several examples of protein-based nano-biocomposites were presented with a special attention for the methods used for the incorporation of layered silicates (organically modified or not) into the matrices and the ultimate functional properties exhibited by the resulting materials. In terms of mechanical properties, the addition of nanoclays leads to a significant improvement of material performance with an increase of Young's modulus and tensile strength ranging between 1.5 and 2 times. As regards as barrier properties, the improvement appeared quite moderate in spite of a rather good dispersion of layered silicates that would be expected to result in a tortuous pathway limiting diffusion of gases molecules. Thus, a two-fold reduction in water vapour permeability was obtained, and the same or no effect in the case of permeability toward O_2 and CO_2 .

List of Abbreviations

DSC	Differential scanning calorimetry
FTIR	Fourier transformed infrared
MMT	Montmorillonite
OMMT	Organically modified montmorillonite
RH	Relative humidity

H. A.-Coussy (✉) · E. Gastaldi
UMR IATE, Université Montpellier II, CC023, pl. E. Bataillon,
34095 Montpellier Cedex, France
e-mail: helene.coussy@univ-montp2.fr



27	SEM	Scanning electron microscopy
28	SPI	Soy protein isolate
29	TEM	Transmission electron microscopy
30	TGA	Thermogravimetric analysis
31	WG	Wheat gluten
32	WPI	Whey protein isolate
33	WVP	Water vapour permeability
34	XRD	X-ray diffraction
36		

37 12.1 Introduction

38 Among agricultural products, proteins have long and empirically been used as raw
39 materials in a wide range of non-food applications such as adhesives, glues, paints,
40 leather finishes, textile fibers, paper coatings, photographic supports and various
41 moulded plastic items (buttons, door handles, jewellery...). However, in the 1960s
42 the competition with synthetic plastics led the interest for protein-based materials
43 to markedly decrease until a recent renewal of interest for renewable and biode-
44 gradable materials, over the last two decades, in response to raising awareness on
45 environmental issues. Owing to their good film-forming properties, several
46 proteins stemming from animal (casein, whey proteins, gelatin, collagen, egg
47 white, keratin and fish myofibrillar proteins) and vegetable (soy proteins, wheat
48 gluten, corn zein and pea protein isolates) sources have received attention for
49 production of films or coatings. Proteins can be defined as natural polymers able to
50 form amorphous three-dimensional structures mainly stabilized by non-covalent
51 interactions. In contrary to most other biodegradable polymers, they are hetero-
52 polymers constituted of different amino acids and characterized by specific levels
53 of structure ensuring various interactions and bindings differing in position, type,
54 and energy. Protein-based materials can be prepared using either a solvent process
55 also called casting or a common thermoplastic process such as extrusion or
56 thermomolding. Their formulation often requires the addition of plasticizers to
57 allow their thermo-processability and impart film flexibility. Resulting protein-
58 based materials are characterized by remarkable functional properties that are
59 highly dependant on structural heterogeneity, especially on interactions between
60 polypeptide chains, thermal sensitivity, and hydrophilic behaviour of proteins.
61 Protein-based materials are fully biodegradable without toxic products release.
62 However serious concerns remain regarding their performance, mainly including
63 their high water sensitivity and low mechanical properties.

64 As largely described in the literature, the incorporation of layered silicates at
65 the nanoscale level into a polymeric matrix often results in significant enhance-
66 ment of mechanical, barrier and thermal properties. The high affinity of clays to
67 proteins and amino acids bearing neutral, positive, and even negative charges were

68 initially noted in soils and already largely used in winemaking for protein
69 precipitation, which hints the possibility to prepare protein-based nanocomposite
70 materials. This strategy has begun to be explored in the field of protein-based
71 materials for gelatin less than ten years ago (first publications in 2002). The
72 development of protein/clay nano-biocomposites has been yet devoted to almost
73 all proteins previously mentioned with the intention of mainly improving
74 mechanical and barrier properties. Protein-based nano-biocomposites are also
75 developed for the controlled release of active agents, for example for antimicrobial
76 applications.

77 After a short presentation of the protein structure, which is at the root of
78 the properties of protein-based materials, the second part of the chapter will
79 be devoted to the possible processing routes to prepare protein-based nano-
80 biocomposites. The third part will focus on the structure/properties relationships of
81 proteins (gelatin, milk proteins, soy proteins, corn zein and wheat gluten)/clay
82 nano-biocomposites.

83 12.2 Structure of Proteins

84 Proteins are heteropolymers constituted of 20 different amino acids and charac-
85 terized by different levels of structure that permit various interactions and bindings
86 differing in position, type, and energy. The functionalities of proteins-based
87 materials mainly depend on the composition of the amino acids constituting their
88 primary sequence and more precisely on the chemical reactivity of the amino acids
89 lateral groups i.e. polar or hydrophobic, ionised or not, acid or basic, aromatic or
90 aliphatic (Table 12.1). According to the chemical specificity of the amino acids
91 constituting proteins, different types of interactions could be established leading to
92 intra and inter-polypeptidic chain linkages. Among them, the most representative
93 are non-covalent bonds since considered as critical in stabilizing protein three-
94 dimensional structure that can be described at four levels i.e., from primary
95 structure to secondary, tertiary and quaternary structure [1]. The secondary
96 structure of proteins corresponds to the spatial arrangement of the polypeptide
97 chains, which results from the establishment of periodic hydrogen bindings
98 involving carbonyl and amine groups (CO-NH) of peptide bonds. We distinguish
99 the α -helix and the β -sheets structures. Proteins containing a relatively large
100 proportion of proline and hydroxyproline may be less prone to adopt an ordered
101 structure due to the disruptive effect of these amino acid residues on typical
102 secondary structure. The tertiary structure of proteins results from diverse inter-
103 actions (hydrogen, hydrophobic, electrostatic, covalent...) between amino acids
104 belonging to the same polypeptide chain but being not neighbours. Finally, the
105 quaternary structure generally results from non-covalent interactions between
106 polypeptide chains. This latter structure is closely related to the functional
107 properties of the final protein-based materials.

Table 12.1 Characteristics of the main proteins used to prepare protein/clay nano-biocomposites

Proteins	Amino acid ratios			Sub-units		
	Ionized polar ^a	Non- ionized polar ^a	Apolar ^a	Name	Rate (%)	Molecular weight (kDa)
Gelatine [46, 47]	29.1	27.6	43.3			3–200
Whey proteins [48, 49]	28.5	25	42.5	α -lactalbumin β -lactoglobulin	60 20	14 18
Soy proteins [50]	47	16.7	35	Glycinin β -conglycinine	40 35	350 185
Wheat gluten [51]	18	41	38.3	Gliadin Glutenin	45 45	30–80 200–2000
Corn zein [51]	15	38	45	α -zein	80	23–27

^a Ionized polar: Glu, Asp, Lys, Arg, His; Non-ionized polar: Gly, Asn, Gln, Ser, Thr, Tyr; Apolar: Ala, Val, Leu, Ileu, Pro, Phe, Met, Trp





108 Amino acids are generally classified in groups depending on their ability to
109 establish interactions via either hydrogen bonds (involving non-ionized polar amino
110 acids, 8–40 kJ.mol⁻¹ [1]), ionic interactions (implying ionized polar amino
111 acids, 42–84 kJ.mol⁻¹ [1]), hydrophobic interactions (between apolar amino acids,
112 4–12 kJ.mol⁻¹ [1]) or covalent linkages (amino acids involved in disulphide bonds,
113 330–380 kJ.mol⁻¹ [1]) (Table 12.1). Although the non-covalent bonds are weak
114 chemical forces ranging from 4 to 30 kJ.mol⁻¹ [1], they impart stability to the
115 structures generated by their collective action. Van der Waals forces (involving both
116 polar and apolar non-ionised amino acids, 0.4-4 kJ.mol⁻¹ [1]) are also implicated but
117 their effect on protein structure is minor. Their highly heterogeneous structure
118 characterized by the absence of repeating units along the polypeptide chains render
119 ineffective Van der Waals interactions that mainly act at short distance with a very
120 low energy input. In aqueous medium proteins generally adopt a conformation in
121 which polar amino acids are located at the interface with solvent whereas the
122 hydrophobic parts of the polypeptide chains tend to avoid solvent by burying within
123 the structure. Depending on the polarity, pH and ionic force of the solvent used, the
124 respective contributions of these interactions can be modified. In pH conditions far
125 from its isoelectric point, proteins are stabilized in the medium since the interactions
126 established between protein and solvent are greater than those formed between
127 amino acids. In addition to non-covalent interactions, covalent disulphide bonds
128 formed by oxidation of two thiol groups also play an important role in the stability of
129 the proteins containing cysteine residues.

130 The functional properties of the final materials will depend on the respective
131 contribution of these different kinds of interactions that are closely related to the
132 characteristics of the protein raw material but that can also be modulated by the
133 conditions used to process and store the protein-based materials. Furthermore, this
134 heterogeneous structure offers many opportunities for cross-linking or chemical
135 grafting.

136 12.3 Preparation of Protein-Based Nano-Biocomposites

137 The formation of a macromolecular network from proteins requires three steps:
138 first the rupture of low-energy intermolecular bonds that stabilize polymers in the
139 native state, secondly the arrangement and the orientation of polymer chains
140 (shaping), and finally the formation of a three-dimensional network stabilized by
141 new interactions and bonds after the agent that breaks intermolecular bonds is
142 removed [2]. Due to the high glass transition temperature of proteins (above their
143 temperature of degradation), their formulation often requires the addition of
144 plasticizers to allow their thermo-processability and impart material flexibility.
145 Besides water molecules that could be considered as the natural plasticizer of most
146 proteins, the most usual plasticizers are polyols (glycerol, sorbitol), mono-, di-,
147 and oligo-saccharides, and fatty acids.

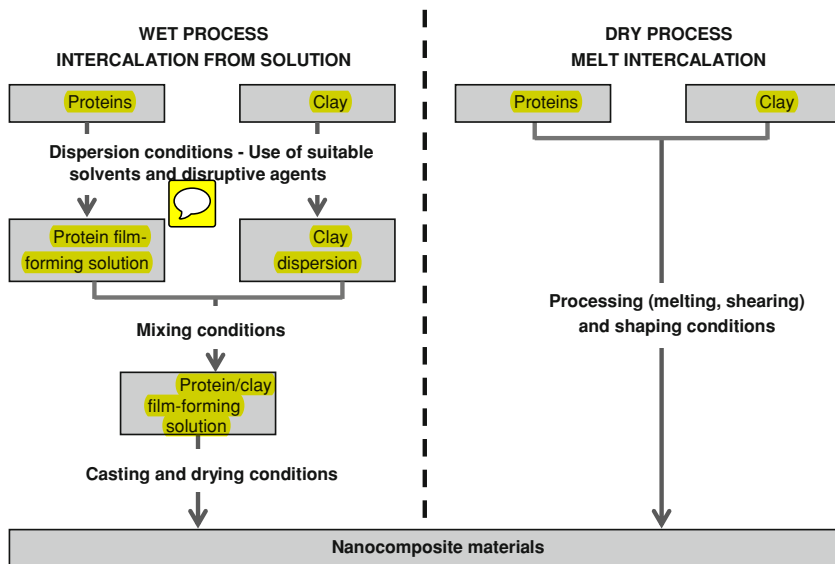


Fig. 12.1 Schematic representation of the two main technological processes used to prepare protein/clay nano-biocomposite materials

148 Several methods have been considered to prepare polymer/layered silicate
 149 nanocomposites with an optimal dispersion state of nanofillers [3]. Two of them
 150 are compatible with the production of protein-based materials: (1) the intercalation
 151 of proteins from solution, which is compatible with the preparation of protein-based
 152 materials via a wet process, commonly called casting, based on the disper-
 153 sion or solubilisation of proteins in a solvent, and (2) the melt-intercalation
 154 technique, which is compatible with the preparation of protein-based materials via
 155 a dry, that is, thermo-mechanical process based on the thermoplastic properties of
 156 some proteins. These two processes are schematically represented on Fig. 12.1.

157 **12.3.1 Wet Process/Intercalation from Solution**

158 The wet process is suitable for all proteins. The solvents used to prepare protein
 159 film-forming solutions are generally based on water and ethanol, and occasionally
 160 acetone. Proteins are required to unfold and realign before a new three-dimensional
 161 network can be formed, and then stabilized by new inter- and intra-molecular
 162 interaction. Depending on the amino acid sequence, dispersing proteins in solution
 163 may involve the addition of solvents, disruptive agents, pH adjustment by the
 164 addition of acids or bases, or ionic strength control by electrolyte addition [2]. Then,
 165 the functional properties of protein-based films prepared by casting depend on
 166 protein concentration in solution, pH, additives, solvent polarity, drying rate and

Table 12.2 Main proteins used as polymeric materials to develop agromaterials: tested methods to obtain nano-biocomposites

Proteins	Solution intercalation via casting	Solution intercalation followed by solvent evaporation, then shaping of the material using a dry process (thermoforming or extrusion)	Melt intercalation using a thermo-mechanical process (thermoforming or extrusion)	Melt intercalation using a thermo-mechanical process, then shaping via casting
Gelatine	[10–16]	–	–	–
Whey proteins	[19–21]	–	–	–
Soy proteins	[26]	[27, 30]	–	[28, 29]
Corn zein	[31]	[31]	–	–
Wheat gluten	[39, 40, 41, 44]	[52]	[33, 42]	–

167 temperature [2]. Ideally, layered silicates are exfoliated into single layers using a
 168 solvent in which the proteins are easy to disperse. Proteins then adsorb onto the
 169 delaminated surfaces of clay and when the solvent is evaporated, the sheets
 170 reassemble, sandwiching proteins (Fig. 12.1). To facilitate introduction of proteins
 171 into clay galleries, it is possible to play on the pH value of the film-forming solution to
 172 positively increase the protein net charge in favour of electrostatic interactions
 173 established with the negatively charged clay surfaces. To prepare optimized film-
 174 forming solutions, the conditions are retained in such a way to favour proteins
 175 unfolding together with a minimal viscosity. So far, the intercalation of layered
 176 silicates from solution was largely preferred for making protein-based nano-
 177 biocomposites (Table 12.2) for several reasons, including the non-necessity to use
 178 plasticizers, the easiness of processing and the possibility to achieve well exfoliated
 179 nanocomposite structures due to the capacity of layered silicates to swell in an
 180 appropriate solvent (such as water). In some cases, solution intercalation followed by
 181 solvent evaporation (often by freeze-drying) is only considered as a preliminary step
 182 allowing obtaining good nanoclay dispersion, the resulting dried powders being then
 183 shaped using a dry process (Fig. 12.1, Table 12.2).

184 12.3.2 Dry Process/Melt-Intercalation

185 Thermoplastic properties of proteins mainly depend on the type and the density of
 186 intermolecular interactions. Extrusion requires the formation of a homogeneous
 187 protein melt implying processing temperature above the protein softening point. The
 188 fact that proteins contain a wide range of inter-molecular interactions reduces chain
 189 mobility, increases viscosity and results in a high glass transition temperature often
 190 above the decomposition temperature. Furthermore, the heat sensitivity of some
 191 proteins that aggregate and cross-link upon heating restricts the window of feasible

192 processing conditions. Because chain mobility is a key parameter to achieve
193 successful extrusion, protein/protein interactions can become unfavourable and
194 should thus be controlled during processing. For this purpose, chemical additives,
195 such as sodium sulphite, sodium dodecyl sulphate and urea, known to disrupt
196 covalent and non-covalent bonds are often used as processing aids in combination
197 with plasticizers [4]. Although most of proteins are suitable for the extrusion process,
198 to date, only wheat gluten and soy proteins have led to nano-biocomposites by using a
199 dry process, such as extrusion, roller milling or thermo-molding (Table 12.2).
200 To prepare nanocomposite materials, layered silicates are mixed with the protein
201 matrix in the molten state under heating and shearing (Fig. 12.1). In the case of
202 proteins containing cysteine residues (such as soy and wheat gluten) the thermal
203 treatment not only allows polymer melting but also induces cross-linking reactions
204 between thiol groups (from rather low temperatures; 70°C in the case of wheat gluten
205 [5], for instance). Thus, extrusion conditions (temperature and shearing) should be
206 optimised in order to limit the temperature-induced cross-linking and favour the
207 shear-induced alignment of polymer chains preventing the viscosity increase and
208 allowing processability. When clay layers surface is sufficiently compatible with the
209 protein, polypeptide chains can crawl into the galleries and lead to either an inter-
210 calated or exfoliated nanocomposite structure depending on the degree of penetra-
211 tion of the polymer into the clay interlayer. According to Vaia and Giannelis [6],
212 interplay of entropic and energetic factors determines the outcome of polymer
213 intercalation. Exfoliation or complete layer separation depends on the establishment
214 of very favourable interactions between polymer and clay surfaces to overcome the
215 entropy loss associated with polymer confinement and penetration between the clay
216 layers. In the case of clays modified with alkylammonium, the increased conforma-
217 tional freedom of the surfactant chain induced by the clay layers separation might
218 compensate additionally the entropy decrease associated with the polymer chain
219 penetration between the clay. The kinetic of intercalation of the polymer chains
220 between the clay layers also depends on the polymer mobility that is related with the
221 temperature difference between the processing temperature and the polymer glass
222 temperature [7]. Although still too little used to prepare protein-based nanocom-
223 posites, the polymer melt intercalation is considered as a more efficient and
224 environmentally-benign alternative than the polymer intercalation from solution
225 since requiring no solvent. Moreover, dry processes are all available at the industrial
226 scale and commonly used in plastics processing.

227 **12.4 Structure/Properties Relationships of Protein-Based** 228 **Nano-Biocomposites**

229 ***12.4.1 Gelatine-Based Nano-Biocomposites***

230 Gelatine is a protein resulting from partial thermal or chemical hydrolysis of
231 collagen polypeptide chains stemming from animal skin, bones and cartilages.
232 For commercial production of gelatine, two processes are mainly used differing by

the pH conditions; type A gelatine involves an acid extraction whereas type B gelatine is obtained by an alkaline lime followed by a solubilisation at near neutral pH at 60–90°C [8]. Gelatine was one of the first food protein to be used as raw material not only for food applications but also in the pharmaceutical and photographic film industries due to large availability, low production cost (between 1 and 5 €/kg depending on the animal origin and the targeted application, food or non-food uses) and excellent film forming ability. Gelatine has been successfully used to form, by casting, films that are transparent, flexible, water-resistant and impermeable to oxygen [2]. Although gelatine can be used as a valuable biopolymer in tissue engineering, its poor mechanical properties, especially in the wet state, and its high water sensitivity, revealed by a high water swelling, restrict its application as a structural biomaterial such as osteosynthetic devices. The development of gelatine/clay nano-biocomposites provided some new opportunities to overcome these limitations and widen end-uses. They are considered as the first group of nano-biocomposites deliberately created from the assembling of a natural polymer and a layered solid. Gelatine/clay nanocomposites can only be prepared using a solvent intercalation based on an ion exchange process. In this manner, the control of pH is crucial because it is required to protonate the amino groups, in order to intercalate positively charged gelatine by replacing the interlayer cation when the pH of the solution is kept below the isoelectric point of the protein [9].

Zheng et al. first prepared gelatine/sodium MMT nano-biocomposites using a solution intercalation method [10] and MMT contents up to 20 wt%. The work mainly focused on thermal and mechanical properties. XRD results revealed that an intercalated or partially exfoliated structure was achieved. DSC and TGA results indicated that thermal properties of gelatine were significantly improved (the onset thermal decomposition temperature is 40°C higher than that of the pure gelatine for a filler content of 30 wt%), as later confirmed by Martucci et al. [11]. The tensile strength and Young's modulus were also improved (Table 12.3), reaching maximum values for MMT content of 17 wt%. The wet mechanical strength was also significantly improved, which was mainly attributed to the nano-dispersion of MMT within the gelatine matrix and the barrier effect of MMT sheets to solvent. A significant enhancement of both the Young's modulus and the tensile strength by about a factor 2 without sacrificing toughness was further confirmed by many authors for other sources of gelatine at a low MMT loading (5 wt%) [12–14] (Table 12.3).

Concerning barrier properties, several studies showed that the introduction of MMT led to a significant reduction in the water sensibility and oxygen permeability of gelatine due to the creation of an exfoliated nanocomposite structure (Table 12.3). [15] et al. first showed that the incorporation of natural MMT led to a significant decrease of both the swelling rate and the maximum solvent uptake [15]. Then, Bae et al. [13] and Martucci and Ruseckaite [16] showed that water vapour permeability could be reduced by about a factor 2 for a 5 wt% MMT filler content. The effect of clay on the oxygen barrier properties is not so pronounced, probably due to an already very low permeability of the neat matrix (Table 12.3).



Table 12.3 Mechanical and barrier properties of gelatine/clay nano-biocomposites: Young's modulus (E), tensile strength (TS), deformation at break (ϵ), water vapour permeability (WVP), oxygen permeability (PO₂) and maximum water uptake (W)

Sample	E (GPa)	TS (MPa)	ϵ (%)	WVP (mol/ (m.s.Pa)	P _{O₂} (mol/ (m.s.Pa)	W (%)	Ref
Bovine skin gelatine (type B) (25 wt% glycerol)	0.9	49.3	–	–	–	778	[10, 15]
–5 wt% Na ⁺ MMT	1.6	78.9	–	–	–	599	
–17 wt% Na ⁺ MMT	2.0	89.1	–	–	–	376	
Gelatine (type 4) (no plasticizer)	3.3	88.9	9.6	–	–	–	[12] ^a
–5 wt% Cloisite Na ⁺	5.9	97.4	3	–	–	–	
–10 wt% Cloisite Na ⁺	8.3	110.8	0.9	–	–	–	
Bloom fish gelatine (20 wt% glycerol)	–	30.3 ± 2.4	48 ± 4	17.3 × 10 ⁻¹²	2.08 × 10 ⁻¹⁸	–	[13] ^b
–5 wt% Cloisite Na ⁺	–	40.9 ± 5.3	38 ± 3	7.6 × 10 ⁻¹²	9.8 × 10 ⁻¹⁹	–	
Porcine skin gelatine (type A)	2.1 ± 0.2	–	3.3	–	–	–	[14]
–5 wt% sepiolite	3.3 ± 0.1	–	2.5	–	–	–	
Bovine hide gelatine (type B)	–	–	–	13.7 × 10 ⁻¹²	6.87 × 10 ⁻¹⁷	–	[16] ^c
–5 wt% Na ⁺ MMT	–	–	–	7.7 × 10 ⁻¹²	5.58 × 10 ⁻¹⁷	–	

^a Mechanical properties evaluated at 50 %RH

^b Mechanical properties and P_{O₂} measured at 50 % RH

^c WVP measured between 0 and 65 % RH and P_{O₂} at 65 % RH. Such details were not specified for other data

278 12.4.2 Milk Proteins-Based Nano-Biocomposites

279 Milk has a protein content of about 33 g.L⁻¹ and contains two main types of proteins:
 280 caseins which represent about 80 % of total milk protein and whey proteins (20 % of
 281 total) that principally consist of β -lactoglobulin, α -lactalbumin and serum albumin.
 282 Caseins are proteins of major importance in the food industry due to their key role in
 283 the cheese making process. Whey proteins are by-products of the cheese or casein



284 manufacture that remain in milk serum after pH/rennet-induced casein coagulation.
285 Increased production of whey because of expanding cheese manufacture has led to
286 identify new uses for whey proteins. This also involves that whey proteins are available
287 at lower price than caseins. The film-forming properties of milk proteins have been
288 indirectly demonstrated by films formed on processing equipment surfaces and at air-
289 water interfaces during heating of non-fat milk [2]. Caseins and whey proteins have
290 been used to produce transparent, flexible, colourless and odourless edible films and
291 coatings displaying excellent oxygen, aroma and oil barrier properties at low RH, but
292 poor moisture barrier properties as related to their high hydrophilicity and water
293 solubility. To remedy this drawback, several attempts have been made through the
294 incorporation of hydrophobic materials such as lipids in whey and casein film for-
295 mulation [17, 18]. However, phase separation often occurs and limits the efficiency of
296 lipids addition demonstrating the need to develop other strategies to improve water
297 resistance. The creation of a nanocomposite structure via the incorporation of layered
298 silicate fillers in film formulation can be considered as a convenient way to reach this
299 goal. Though, up to date, very few studies have been reported on whey protein/clay
300 nanocomposite materials and not any on casein/clay nanocomposite materials. This is
301 probably due to the high nutritional values and sensory acceptance of casein and whey
302 protein that render them more suitable for edible purposes. In fact, edible applications
303 are not compatible with the nanocomposite route.

304 Among studies already published on whey protein based nanocomposites, the
305 works of Sothornvit et al. [19, 20] and Hedenqvist et al. [21] constitute the only
306 available data reporting the effect of clay on the transport properties of whey-based
307 materials. In all studies, nanocomposite films were obtained by the casting process
308 from solutions in which whey protein isolates (WPI) were dispersed in water and
309 heat denatured at temperature above 73°C [22] to allow unfolding of their globular
310 native structure and unmasking of most of the hydrophobic and sulfhydryl groups
311 hidden in the core of the structure. WPI film forming solutions were then mixed
312 with a water suspension of montmorillonites.

313 In Sothornvit et al. studies, different types of MMT were tested; an unmodified
314 clay (Cloisite Na⁺) and two organically modified MMT (Cloisite 30B and Cloisite
315 20A) with a clay loading of 5 wt% [19] or up to 20 wt% [20]. In spite of the lack
316 of morphological and structural analysis provided by the authors, the fact that
317 mechanical properties (tensile strength and elongation at break) of nanocomposites
318 were either unchanged (Cloisite Na⁺ and Cloisite 30B) or decreased (Cloisite 20A)
319 as compared to the neat matrix drops hints that a composite rather than a nano-
320 composite structure was achieved [19]. In the case of nanocomposite films pre-
321 pared with Cloisite 30B, other results showed a dramatic decrease in tensile
322 properties for clay loadings higher than 5 wt% [20]. The MMT effect on the water
323 vapour permeability (WVP) indicated a small but significant decrease of a 1.4 and
324 1.6 factor with Cloisite Na⁺ at 5 wt% and Cloisite 30B at 20 wt% respectively,
325 whereas no effect was observed with Cloisite 30B and 20A at 5 wt%. According to
326 the authors, the improvement of the WVP could be directly related to the
327 hydrophilicity of the nanoclays that largely influences their compatibility with the
328 WPI matrix and the level of dispersion.

329 In the work reported by Hedenqvist et al. [21] the montmorillonites used were
330 previously intercalated with poly(vinylidene pyrrolidone) which is water soluble
331 and acts as surfactant by easily adsorbing onto the MMT Na⁺ surface. Clay filler
332 loadings tested were up to 4.8 vol% MMT as expressed in inorganic content. This
333 study mainly focused on the effect of clay content on the methanol diffusion
334 through the films in relation to the morphology of the composite and the thermal
335 properties of the polymer matrix. Thermal analysis confirmed that the polymer
336 matrix was influenced by the presence of the nanoparticles as evidenced by a
337 reduction of the glass transition temperature indicating a reduction of the
338 segmental mobility in the nanocomposite. They also noted that the absence of a
339 significant increase in relaxed rubber modulus in the nanocomposites suggests that
340 the reinforcing effect of the filler was limited, due to an incomplete exfoliation of
341 clay nanoparticles, as confirmed by TEM analysis. Finally, a small but significant
342 reduction (by almost two orders of magnitude) in the average methanol diffusivity
343 was obtained for the highest MMT content (4.8vol%). According to these authors,
344 the effect of nanoclays on the penetrant diffusivity was due to a reduction of the
345 segmental mobility that reflects interactions between the filler particles and the
346 polymer matrix and the tortuosity induced by MMT that extends the diffusion
347 pathway of the penetrant molecules.

348 Beside evaluating the effect of nanoclays on mechanical and transport prop-
349 erties, Sothornvit et al. also studied the antimicrobial properties of WPI films
350 reinforced with Cloisite Na⁺, Cloisite 30B and Cloisite 20A [19, 20]. Results
351 obtained showed that WPI/Cloisite 30B nanocomposite films exhibited a signifi-
352 cant bacteriostatic effect against Gram-positive bacteria (*L. monocytogenes*) and
353 no effect on Gram-negative bacteria (*E. coli* O157:H7) whereas, no antimicrobial
354 activity was observed with WPI films reinforced with Cloisite Na⁺ and Cloisite
355 20A. According to another study focusing on the antimicrobial activity of
356 nanoclays alone [23], this antimicrobial effect was mainly ascribed to the
357 quaternary ammonium group carried by the organically modified clays that might
358 be able to disrupt bacterial cell membranes to cause cell lysis. This hypothesis is
359 supported by the fact that among the different unmodified and organically modified
360 clays studied, the unmodified one was the only clay to display no antimicrobial
361 activity [23].

362 **12.4.3 Soy-Based Nano-Biocomposites**

363 Most of the proteins in soybean can be classified as globulin. Protein in the form of
364 meal is one of the typical end products from soybean industrial processing. Protein
365 meal can further be concentrated for the production of commercially available soy
366 protein concentrates and isolates. Because soy protein isolates (SPI) are a highly
367 refined class of soybean proteins that contains more than 90% protein and 18
368 different amino acids, they became the favourite choice of researchers. The film-
369 forming properties of soy proteins have been traditionally used in Asia to obtain

Table 12.4 Mechanical properties (Young's modulus (E), tensile strength (TS), deformation at break (ϵ)) and water vapour permeability (WVP) properties of soy protein isolate (SPI)/clay nano-biocomposites

Sample	E (MPa)	TS (MPa)	ϵ (%)	WVP (mol/(m.s.Pa))	Reference
SPI (glycerol)	–	7.4	68	17×10^{-11}	[26] ^a
–10 wt% Na ⁺ MMT	–	5.7	16	20×10^{-11}	
–10 wt% OMMT	–	9.4	54	16×10^{-11}	
–10 wt% bentonite	–	9.7	58	8.4×10^{-11}	
SPI (30 wt% glycerol)	180	8.7	88	–	[27] ^b
–4 wt% Na ⁺ MMT	260	10.3	63	–	
–8 wt% Na ⁺ MMT	340	12.2	32	–	
–12 wt% Na ⁺ MMT	420	14	15	–	
–16 wt% Na ⁺ MMT	540	15.4	8	–	
–20 wt% Na ⁺ MMT	587	14.5	6	–	
SPI (30 wt% glycerol)	180	6.8	108	–	[30] ^b
–4 wt% rectorite	360	9	57	–	
–8 wt% rectorite	450	11.8	21	–	
–12 wt% rectorite	560	12.8	12	–	
–16 wt% rectorite	620	10	8	–	
–20 wt% rectorite	580	9.4	7	–	
SPI (15 wt% glycerol)	–	2.3 ± 0.5	11.8 ± 0.4	$5.85 \pm 0.17 \times 10^{-11}$	[28] ^c
–5 wt% Na + MMT	–	6.3 ± 0.9	65 ± 5	$4.56 \pm 0.15 \times 10^{-11}$	
–10 wt% Na + MMT	–	12.6 ± 0.6	24 ± 5	$3.85 \pm 0.12 \times 10^{-11}$	
–15 wt% Na + MMT	–	15.6 ± 1.7	18 ± 3	$3.34 \pm 0.09 \times 10^{-11}$	
SPI (15 wt% glycerol)	–	2.3 ± 0.5	11.8 ± 0.4	$5.85 \pm 0.17 \times 10^{-11}$	[29] ^c
–5 wt% Cloisite 30B	–	15.1 ± 0.9	82 ± 3	$4.75 \pm 0.08 \times 10^{-11}$	
–10 wt% Cloisite 30B	–	16.2 ± 0.8	104 ± 5	$4.11 \pm 0.12 \times 10^{-11}$	
–15 wt% Cloisite 30B	–	18.6 ± 0.3	55 ± 3	$3.59 \pm 0.14 \times 10^{-11}$	

^a Mechanical properties measured on films stored at 50%RH and WVP according to the ASTM Method E 96–95

^b Mechanical properties measured on films stored at 0%RH

^c mechanical properties measured at 65%RH and WVP between 65 and 100 %RH

370 edible films by collecting the lipo-proteic skin formed after boiling soya milk [2].
 371 Soy-based materials can be prepared using either a wet or a dry process
 372 (Table 12.1). In the casting process, heating up to 80°C is essential for unfolding
 373 proteins and unmasking sulfhydryl groups and hydrophobic side chains that will be
 374 involved in the resulting protein network [24]. The concentration of total solids
 375 should not exceed 9 %, to avoid gelation and thickening upon heating, and film
 376 formation is favoured in slightly basic conditions. SPI-based films alone possess
 377 poor flexibility. Therefore, plasticizers are usually used to overcome the brittleness,
 378 leading unavoidably to a significant decrease in their tensile strength
 379 [25]. Furthermore, they display high water vapour permeability due to the
 380 hydrophilic nature of proteins. Several authors succeeded in improving these latter
 381 properties by introducing unmodified montmorillonites [26–28], organically
 382 modified montmorillonites (OMMT) [26, 29] or rectorite [30] (Table 12.4).

383 Whatever the type of MMT used and the MMT loading (from 5 to 15 wt%), highly
384 exfoliated and intercalated structures have been successfully prepared either via a
385 solution intercalation process in a neutral aqueous media without any special aid
386 [26, 27, 30] or via melt extrusion [28, 29]. In fact, in most studies, both the dry and the
387 wet processes were used; the first one with the objective to exfoliate clays in the SPI
388 matrix, and the second to obtain ultimate nanocomposite materials [27–30]. According
389 to Chen and Zhang [27], the high affinity between soy proteins and the MMT layers was
390 due to two types of interactions: hydrogen bonding between the –NH and Si–O groups
391 and electrostatic interactions between the positive-charge-rich domains of soy protein
392 and the negatively charged MMT layers as evidenced by zeta-potential measurements
393 and FTIR analysis. In spite of the negative charge of soy proteins at neutral pH, the
394 heterogeneous distribution of the surface positive charges provided the possibility for
395 soy proteins to intercalate and exfoliate negatively charged MMT layers [27].
396 However, structures and properties were shown to strongly depend on the filler content
397 used. An increase in the filler content up to 12 wt% led to a significant increase in both
398 the Young's modulus and the tensile strength (Table 12.4).

399 It is accompanied by a drop in the deformation at break due to the restricted
400 segmental motion of soy proteins and the establishment of favourable interactions
401 between the clay sheets and the protein chains. For filler content as high as
402 12–16 wt%, all the mechanical properties were degraded, due to phenomenon of
403 MMT aggregation leading to a phase separation that occurred in the soy protein
404 matrix [27, 30].

405 The introduction of clay led to a small but significant reduction of the water
406 vapour permeability (Table 12.4). However, filler contents higher than 10 wt% are
407 required to get a decrease of about a factor 2. Authors classically attribute this
408 effect to the creation of a tortuous pathway for water vapour to diffuse [28, 29].

409 12.4.4 Corn Zein-Based Nano-Biocomposites

410 Zein is the major corn protein fraction (prolamin fraction) commercially produced
411 from corn gluten meal, a by-product of corn wet-milling processing. Corn gluten
412 meal contains about 55–60 % proteins and is available at relatively low price;
413 600–700 dollar/metric ton depending on the grade (food or not). Zein-based
414 materials can be formed by different processes including casting and thermoplastic
415 processing. Due to its low content in polar amino acids and high amount of apolar
416 amino acids, zein-based films are not water-soluble and exhibit remarkable grease-
417 resistance properties [2]. However, zein-based materials although less water
418 sensitive than other biopolymers still show high water vapour permeability and low
419 tensile strength when compared with commodity polymers. As it was the case for
420 other proteins, its inherent brittleness may be improved by the use of plasticizers,
421 which, on the other hand, further decrease water vapour and gas barrier properties.

422 To our knowledge, only one article has been recently published on the devel-
423 opment of nano-biocomposite materials to improve the properties of zein-based

424 films [31]. In this study, zein/organically-modified MMT nano-biocomposite films
425 were successfully produced from two different processes, (i) solvent casting and
426 (ii) blown extrusion involving precipitating zein protein from solution into zein
427 resin and blowing into zein balloon, which resembled the melt intercalation
428 method. Both techniques resulted in partially exfoliated structures, as demon-
429 strated by XRD analysis and TEM observations. The introduction of nanoclays did
430 not affect the translucency and the yellow colour of the films. The thermal stability
431 of zein-based films was significantly improved for films prepared using the blown
432 technique since a loading of only 5 wt% led to a delay of about 10°C of the
433 temperature corresponding to a 20% weight loss. However, the improvement of
434 mechanical and barrier properties of the zein-based films did not evolve linearly
435 with the MMT content. Luecha et al. showed that for the solvent casting technique,
436 critical MMT contents of 5 wt% and 3 wt% resulted in optimal improvement of
437 tensile strength and water vapour barrier properties, respectively [31]. The limi-
438 tation of the blown technique was the restriction to use very low filler contents
439 (below 3 wt%).

440 **12.4.5 Wheat Gluten-Based Nano-Biocomposites**

441 Wheat gluten (WG) has been widely investigated as a protein source because it is
442 annually renewable and readily available at a reasonable cost (from 1 to 1.3 €/kg).
443 Wheat gluten proteins consist of monomeric gliadins with a molecular weight
444 ranging from 15000 to 85000 and a mixture of glutenin polymers with a molecular
445 weight between about 80000 to several million (Table 12.1). WG proteins can
446 undergo disulphide interchange upon heating, which leads to the formation of a
447 covalent three-dimensional macromolecular network. The reactivity of the WG
448 proteins toward chemical and temperature allows preparing WG-based materials
449 by using two technological approaches: either a solvent-based process or a com-
450 mon thermo-mechanical process based on the thermoplastic properties of WG.
451 With respect to its unique viscoelastic and film-forming properties, WG is an
452 interesting raw material that can be used as a food packaging material. WG based-
453 films present an attractive combination of strength and flexibility [32, 33], a high
454 gas (oxygen and carbon dioxide) permeability in dry condition and a significant
455 gas perm-selectivity at high relative humidity [34–36], and good grease [36] and
456 aroma barrier properties [37] which are key functional properties for food quality
457 preservation. They also are translucent [33] and can be heat-sealed [38].
458 A downside to the use of WG-based materials is their *per se* reactivity and thus
459 lower inertia than most conventional petrochemical-based plastics. As mentioned
460 above for soy proteins-based films, WG-based films are sensitive towards water
461 due to the hydrophilic nature of many amino acids constituting the protein chains
462 and to the need for adding hydrophilic plasticizer. As a consequence, their
463 mechanical properties and water vapour barrier properties in high moisture

Table 12.5 Mechanical properties (Young's modulus (E), tensile strength (TS), deformation at break (ϵ)) and water vapour permeability (WVP) properties of wheat gluten/clay nanobiocomposites

Sample	E (MPa)	TS (MPa)	ϵ (%)	WVP (mol/(m.s.Pa))	Reference
WG (10 wt% glycerol)	3.7	1.9	58	1.4×10^{-11}	[40] ^{a, c}
-2.5 wt% Na ⁺ MMT	5.6	2.4	55	1.4×10^{-11}	
-5 wt% Na ⁺ MMT	10.6	4.7	16	6.6×10^{-12}	
-7.5 wt% Na ⁺ MMT	11.4	3.6	15	7.2×10^{-12}	
WG (12 wt% water/15 wt% glycerol)	65.4	5.8	106	-	[41] ^b
-3 wt% C30B	91.8	9	78	-	
WG (T = 120°C; 37.2 wt% glycerol)	6.5	1.6	86	-	[33] ^c
-2 wt% Na + MMT	9.5	1.8	70	-	
-5 wt% Na + MMT	23.6	2.5	42	-	
WG (T = 80°C; 37.2 wt% glycerol)	1.1	0.31	33	-	[33] ^c
-2 wt% Na + MMT	1.7	0.32	30	-	
-5 wt% Na + MMT	6.4	0.66	23	-	
WG (no plasticizer)	737	48	-	-	[52] ^d
-3 wt% attapulgite	691	50	-	-	
-7 wt% attapulgite	773	59	-	-	
-10 wt% attapulgite	783	57	-	-	
-20 wt% attapulgite	770	49	-	-	

Mechanical properties evaluated at ^a 70 %RH

^b 50 %RH

^c 75 %RH

^d 53 %RH

^e WVP measured between 0 and 100 % RH

464 conditions are relatively poor as compared to synthetic films such as low-density
 465 polyethylene.

466 The development of nanocomposite structures has been recently applied to
 467 WG-based materials to improve moisture resistance and mechanical properties
 468 [39–42] as well as to create antimicrobial delivery systems [43, 44]. All the studies
 469 devoted to WG-based nanocomposites involve the use of both unmodified and
 470 organically modified montmorillonites. The properties of these nanocomposite
 471 materials are directly related to their structure and, in this respect, the state of
 472 dispersion (intercalation/exfoliation) of the inorganic phase seems to be a key
 473 parameter [45]. The level of exfoliation highly depends on the nature of the
 474 montmorillonites. Almost fully exfoliated structures were obtained in the case of
 475 unmodified sodium MMT (Nanofil EXM 757 from Süd-Chemie (from [40]) or
 476 Cloisite Na⁺ from Southern Clay Products, Inc (from [39]). Contrarily, the pres-
 477 ence of both stacks on TEM pictures and a d_{001} peak characteristic of an inter-
 478 calated structure on XRD patterns evidenced that organically modified
 479 montmorillonites (Cloisite 10A [39] and Cloisite 30B [41]) were not well

480 exfoliated. This highlighted that the affinity between wheat proteins and nanoclays
481 is a key parameter governing the state of dispersion of the nanoclays. The use of
482 unmodified MMT, which are naturally hydrophilic and thus, compatible with
483 proteins seems to be more appropriate.

484 All studies demonstrated that the introduction of MMT nanoparticles in
485 WG-based materials resulted in significant improvement in tensile strength and
486 Young's modulus with a simultaneous decrease in deformability due to increased
487 interfacial interactions (Table 12.5). An optimal filler content of around 5 wt%
488 was required to achieve the greatest improvement of mechanical properties, due to
489 the possibility of layered silicates to form a tridimensional network (Table 12.5).
490 In the case of organically modified MMT, the presence of quaternary alkylam-
491 monium might act as a plasticizer in the WG matrix, which would play a negative
492 role in achieving higher strength enhancement in the nanocomposites [41].

493 According to other studies, the moisture sensitivity of WG materials was effi-
494 ciently reduced when wheat gluten proteins were combined with unmodified MMT
495 [39, 40, 42]. For example, the liquid water uptake in cast WG-based nanocom-
496 posite films and liquid water diffusivity in thermoformed films were both divided
497 by a factor 2 for respectively 10 wt% and 21.9 wt% of unmodified MMT [40, 42].
498 A decrease in WVP from 1.6×10^{-11} to 0.6×10^{-11} mol.m⁻¹.s⁻¹.Pa⁻¹ between
499 the neat matrix and the nanocomposite (from 5 to 10 wt% of MMT) might be
500 related to the establishment of hydrophilic interactions between gluten proteins
501 and nanoclays, which resulted in a lower availability of the hydrophilic sites for
502 water vapour. As regards to O₂ and CO₂ permeability (evaluated at 25°C and
503 80–90 % RH), no significant effect was observed for WG cast films over the range
504 of studied MMT contents (up to 10 wt%) [40]. However, increased barrier prop-
505 erties towards 2-heptanone were also obtained for MMT contents ≥ 5 wt%. This
506 decrease in permeability was ascribed to a tortuosity effect liable for a significant
507 diffusivity decrease while increasing MMT contents [40].

508 Besides the improvement of protein based materials performance, the addition
509 of clays could also be used to control the release of active compound. In this
510 context, an antimicrobial delivery system based on WG as matrix, unmodified
511 MMT as structuring agent and carvacrol as antimicrobial compound have been
512 investigated [44, 45]. In the presence of 5 wt% MMT, particle size measurements
513 and microscopic observations pointed out large aggregated structures that were
514 supposed to entrap carvacrol molecules. Such structures could be responsible for
515 the increased carvacrol retention as exhibited by both WG film-forming solutions
516 and cast films. Further indications of this assumption were provided by wide angle
517 X-ray analysis that confirmed the establishment of specific interactions between
518 MMT, carvacrol and WG. The effect of MMT on the carvacrol release from wheat
519 gluten coated papers was studied under controlled humidity from 40 to 100 % RH
520 in a second part of this study [44]. Results indicated an increase of the carvacrol
521 diffusivities while increasing RH and this RH-induced effect was accentuated in
522 the presence of 5 wt% of MMT. As evidenced by scanning electron microscopy
523 (SEM) and TEM, specific aggregated structures were formed in the presence of
524 carvacrol and 5 wt% MMT creating a preferential pathway for carvacrol diffusion.

525 The antimicrobial efficiency of the MMT-WG-coated papers toward *Escherichia*
526 *coli* was assessed and showed that the antimicrobial effect was related to the
527 carvacrol diffusivity. The diffusivity coefficients were utilized to optimize the
528 packaging characteristics required to develop an efficient anti-microbial system
529 and were finally validated against *Botrytis cinerea*.

530 12.5 Conclusion

531 In the current context, societal concerns and growing environment awareness have
532 triggered the search for new products and processing that could be considered as
533 an alternative to the existing petroleum-based plastics. In this chapter, several
534 examples of protein-based nano-biocomposites have been presented with a special
535 attention for the methods used for the incorporation of layered silicates (organi-
536 cally modified or not) into the biodegradable matrices and the ultimate functional
537 properties exhibited by these new kind of materials. The majority of the studies are
538 devoted to the influence of filler content on mechanical properties, with very few
539 data concerning other properties such as barrier properties.

540 One of the advantages of layered silicates as nanofiller is to ensure polymeric
541 material reinforcement in two dimensions without requiring a special process to
542 prepare the materials. Whatever the protein used, unmodified clays which are
543 naturally hydrophilic and thus, compatible with proteins seem to be fully appro-
544 priate for the preparation of protein-based nano-biocomposites displaying both a
545 good level of clay dispersion and a low environmental impact. The rather good
546 dispersion of layered silicates leads to the formation of an expected tortuous path
547 limiting the diffusion of small gases molecules, and thus to a significant decrease
548 of permeability towards gases (oxygen, water vapour) and aroma compounds. The
549 addition of nanoclays also leads to a significant improvement of material perfor-
550 mance in terms of mechanical properties. The improved properties reported above
551 are generally attained at low silicates contents (≈ 5 wt%) as compared to that of
552 conventional composites. However, although nanoclays are well dispersed within
553 the protein matrix, the resulting improvement of barrier properties is quite moder-
554 ate and does not exceed a two-fold reduction in the case of water vapour, and the
555 same effect or even no effect in the case of O_2 and CO_2 . Concerning the effect on
556 mechanical properties, the relative increase in Young's modulus and in tensile
557 strength range between 1.5 and 2 times, with often a dramatic decrease in elon-
558 gation for filler contents of 5 wt% and 50 %RH.

559 It seems that it would be more relevant to develop the "nano-biocomposite"
560 strategy for other purposes than the improvement of mechanical and barrier
561 properties of protein-based materials. The introduction of nanoclays in protein-
562 based matrices could provide new additional functionalities such as UV protection
563 or controlled delivery of active agents (antimicrobial compounds, agrochemicals,
564 fertilizers or drugs). These properties are highly demanded in polymers in general,
565 and all the more in the case of bio-sourced polymers with interests in a wide range
566 of applications such as packaging, medicine and agriculture.

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Chapter No.: 12

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