

# Functional Properties of Thermoformed Wheat Gluten/Montmorillonite Materials with Respect to Formulation and Processing Conditions

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**ABSTRACT:** Wheat gluten (WG)/montmorillonite (MMT) films were prepared by a thermomechanical process consisting of first mixing the components in a two-blade, counter-rotating device and second thermoforming the obtained dough. A significant loss in protein solubility due to the formation and rearrangement of disulfide bonds was observed upon mixing and thermoforming. In the range of studied glycerol contents (25–42.8 wt %), it was shown that glycerol had no significant effect on the mechanical properties or water sensitivity of WG-based films. Increasing the thermoforming temperature from 60 to

120°C led to considerable improvements of the mechanical properties (increases in both the stress and strain at break) and a significant reduction of the water sensitivity. The introduction of MMT (up to 5 wt %) allowed the achievement of mechanical properties that were not possible by just the variation of the glycerol content and the processing temperature. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 107: 487–496, 2008

**Key words:** clay; films; mechanical properties; nanocomposites; proteins

## INTRODUCTION

The increasing demand for biodegradable products based on renewable resources has led to the consideration of proteins as raw materials.<sup>1</sup> Among proteins, wheat gluten (WG) is a byproduct of the wheat starch industry that is commercially available at low cost (1 €/kg) and displays unique viscoelastic properties and low water solubility.<sup>2</sup> Gluten is a mixture of two main proteins, that is, gliadins and glutenins. Gliadins are monomeric proteins (molecular weight = 15,000–85,000). Glutenins consist of polypeptide chains linked together with disulfide bonds, thus forming a macropolymer (the molecular weight ranges from  $15 \times 10^4$  to  $>1,000,000$ ). Gluten proteins can undergo disulfide interchange upon heating, which leads to the formation of a three-dimensional macromolecular network.<sup>3</sup> Protein aggregation involves two steps: first, a reversible change in protein conformation, and second, an aggregation reaction through disulfide bonding.<sup>4</sup> For glutenins, crosslinking reactions occur above 60–70°C, whereas for gliadins, the reactive zone is clearly evidenced around 90°C. It is now well established that, con-

trary to other thermoplastic materials, the viscosity of gluten increases upon heating because of crosslinking reactions.<sup>5,6</sup>

WG-based films exhibit interesting gas-barrier properties, such as selectivity toward oxygen and carbon dioxide at a high relative humidity (RH).<sup>7</sup> Although WG-based films have great potential for bioplastics, their low mechanical resistance and high water sensitivity restrict their utilization to a narrow range of applications. Reinforcement by nanofillers appears to be an interesting strategy to improve the functional properties of WG-based films.<sup>8</sup>

As largely described in the literature, the incorporation of low contents of layered silicates on a nanometer scale into a polymeric matrix results in remarkable enhancements of mechanical, barrier, and thermal properties.<sup>9</sup> Such singular properties originate from the nature of the filler and its nanodispersion in the matrix. An optimal structure for nanocomposite formation is characterized by the coexistence of intercalated tactoids and exfoliated/disordered stacks of layers. Montmorillonite (MMT), displaying a smectite-type structure, is one of the most commonly used layered silicates because of its high aspect ratio and quite low cost. In its pristine form, MMT is miscible with hydrophilic polymers such as agropolymers. Most of the studies involving layered silicates as reinforcing agents concern synthetic polymers.<sup>9,10</sup> Just a few works deal with

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nanocomposites made from layered silicates (natural or synthetic, organically modified or not) and agropolymers such as starch,<sup>11–17</sup> chitosan,<sup>18</sup> gelatin,<sup>19</sup> whey,<sup>20</sup> soy protein,<sup>21</sup> and WG.<sup>22</sup> Most of these authors have confirmed material property improvements (chiefly in Young's modulus and water resistance) in the presence of nanodispersed clays.

Several methods have been considered to prepare polymer/layered silicate nanocomposites with an optimal dispersion of nanofillers.<sup>9</sup> Two of them are compatible with the production of WG-based materials: (1) the melt-intercalation technique,<sup>23</sup> which is compatible with the preparation of WG-based films via a dry, that is, thermomechanical, process based on the thermoplastic properties of gluten proteins at low water concentrations, and (2) the intercalation of a polymer from solution, which is compatible with the preparation of WG-based films via a wet process, commonly called casting, based on the dispersion of proteins in a solvent. This process is commonly preferred for making protein-based materials.<sup>8,18,22</sup> So far, no work has been devoted to the study of the properties of WG/unmodified MMT nanocomposites prepared by the dry process.

The purpose of this work was to elaborate WG/unmodified MMT films by a thermomechanical process and to study the influence of MMT nanoparticles on the mechanical properties and water sensitivity of the obtained materials. To see if the effect of MMT was original, the effects of both the plasticizer content and thermoforming temperature were also investigated. Because processing conditions are a key parameter of WG-based material properties, the gluten network evolution was characterized upon the mixing and thermoforming steps. Changes in the thermoformed film properties were examined by consideration being given to biochemical changes observed in gluten proteins. Finally, color, another characteristic that could limit the use of WG-based materials, was evaluated.

## EXPERIMENTAL

### Materials

Vital WG was provided by Amylum (Aalst, Belgium). Its protein content was 77% (dry matter) according to the manufacturer. WG contained 3.59% proteins insoluble in sodium dodecyl sulfate (SDS), about 42% glutenins, and 46% gliadins. Anhydrous glycerol (Fluka, Sigma-Aldrich Chemie, Steinheim, Germany; purity  $\geq 98\%$ ) was used as a plasticizer. Sodium montmorillonite without organic modification (i.e., MMT) was supplied by Süd-Chemie (Moosburg, Germany) under reference Nanofil EXU 757. MMT particles were characterized by a cationic exchange capacity of 80 mequiv/100 g, an interlayer

distance of 1 nm, a specific weight of 2.6 g/cm<sup>3</sup>, and a pH of 9.3 at 100 g/L (20°C).

### Film preparation

WG and MMT powders were mixed with glycerol in a two-blade, counter-rotating batch mixer turning at a 3 : 2 differential speed (Plasticorder W50, Brabender, Duisburg, Germany) connected to a computer interface and controller unit (PL2000, Brabender). Glycerol was first introduced into the mixer, and then the WG/MMT mixture added. The mixing chamber (volume = 50 cm<sup>3</sup>) was filled with a constant total mass of 50 g. Mixing was carried at a speed of 100 rpm for 25 min. The mixing chamber was regulated at the regulation temperature ( $T_{\text{reg}}$ ) of 15°C with a Julabo F34 (Seelbach, Germany) cryostat and water circulation in the double chamber of the mixer.  $T_{\text{reg}}$  was as low as possible to increase shear stress during mixing<sup>24</sup> and thus favor the dispersion of MMT in WG. The torque and material temperature were continuously recorded during the mixing process. The specific mechanical energy (SME; kJ/g) input to the material was computed from the torque curve as follows:

$$\text{SME} = \frac{\omega}{m} \int_0^{t_{\text{max}}} C(t) dt \quad (1)$$

where  $\omega$  is the rotor speed (rad/s),  $m$  is the sample mass (g),  $C(t)$  is the torque at time  $t$  (N m), and  $t_{\text{max}}$  is the mixing time (s).

The glycerol ratio was relative to the amount of WG, whereas the MMT content was relative to the total weight of the material (50 g).

After mixing, the materials were pressed at 150 bar for 5 min at different temperatures (60, 80, 100, and 120°C) between two Teflon-coated plates with a heated hydraulic press (PLM 10 T, Techmo, Nazelles, France). A 0.2-mm Teflon frame was placed between the two plates to control the thickness of the films.

The following codification is used in this article. WG-37.2-2-60 corresponds to WG plasticized with 37.2 wt % glycerol, filled with 2 wt % MMT, and thermoformed at 60°C.

### Gluten protein solubility

Materials (before and after heat pressing) were analyzed according to Domenek et al.<sup>4</sup> Briefly, ground samples (160 mg) were stirred for 80 min at 60°C into 20 mL of 0.1M sodium phosphate buffer (pH = 6.9) containing 1% SDS and subsequently centrifuged at 18,000 rpm for 30 min. The supernatant liquid contained the SDS-soluble protein fraction ( $F_s$ ).

The SDS-insoluble protein fraction ( $F_i$ ) was extracted with 5 mL of SDS phosphate buffer containing 20 mmol/L dithioerythriol under stirring for 60 min at 60°C and tip-sonicated for 3 min. The supernatant liquid (500  $\mu$ L, centrifugation for 30 min at 18,000 rpm) was mixed with 500  $\mu$ L of SDS phosphate buffer containing 40 mmol/L iodoacetamide. Both extracts (20  $\mu$ L),  $F_s$  and  $F_i$ , were submitted to size exclusion high-performance liquid chromatography.<sup>4</sup>

### Water uptake (WU)

WU was gravimetrically determined at room temperature by immersion in distilled water. Circular films (diameter = 20 mm) were dried over  $P_2O_5$  before testing. After being weighed with a four-digit balance, dry samples were immersed in distilled water. Samples were removed after 24 h of immersion (at equilibrium) and weighed after the careful removal of the excess water with filter paper. Swollen samples were subsequently dried over  $P_2O_5$  at room temperature for 1 week and reweighed. WU was calculated as follows:

$$\text{WU (\%)} = \frac{m_\infty}{m_d} \times 100 \quad (2)$$

where  $m_\infty$  is the weight of the wet swollen sample and  $m_d$  is the weight of the dried swollen sample.

### Water vapor sorption (WVS)

First, circular films (diameter = 20 mm) were dried over  $P_2O_5$  at room temperature for 1 week. After being weighed with a four-digit balance, samples were stored over  $K_2SO_4$  saturated salt (98% RH) for about 1 week until a constant weight was obtained. WVS was expressed as follows:

$$\text{WVS (\%)} = \frac{m_\infty}{m_0} \times 100 \quad (3)$$

where  $m_0$  is the weight of the dry sample and  $m_\infty$  is the weight of the water vapor sorbed at the equilibrium.

### Tensile tests

Tensile tests were conducted with a Rheo (Champlan, France) TAXT2 rheometer. WG-based films were first cut into rectangular strips 10 mm wide and stored at 20°C and 75% RH for 1 week. The initial gap between the jaws was adjusted to 40 mm. Force–elongation curves were obtained for each sample at 20°C and with a crosshead speed of 0.2 mm/s. The nominal strain and stress were calculated as follows: strain = elongation/initial gap between the jaws, and stress = force/initial cross section. The

Nominal Young's modulus and ultimate tensile properties, that is, the strain at break and stress at break, were also characterized. Data were averaged over 10 specimens.

### Color analysis

Commission Internationale de l'Eclairage color attributes ( $L^*$ ,  $a^*$ , and  $b^*$ ) were measured with a Minolta chroma meter (model CR-310; Minolta Camera Co., Osaka, Japan). Triplicate measurements were taken along the film with three superposed white paper sheets as background ( $L_0^* = 93.33$ ,  $a_0^* = 1.24$ ,  $b_0^* = -3.45$ ). The color parameters were expressed as a single value ( $\Delta E$ ), which defined the total color difference:

$$\Delta E = \left[ (L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2 \right]^{0.5} \quad (4)$$

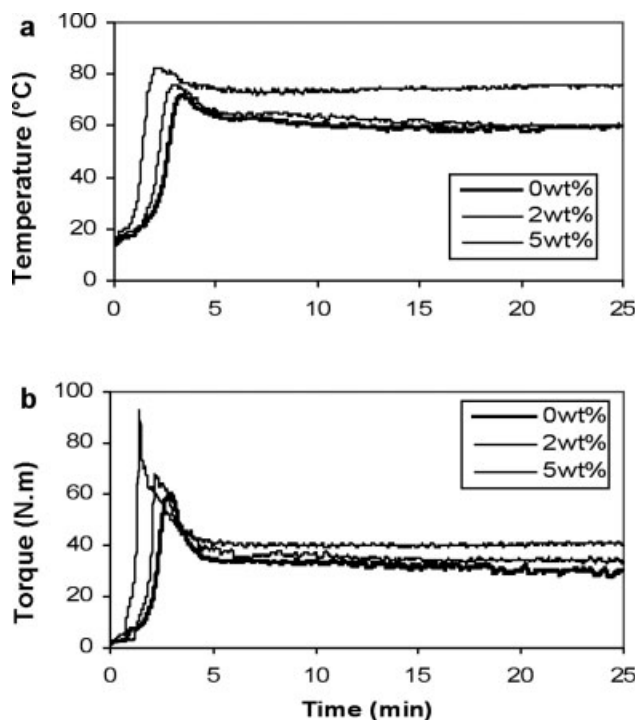
## RESULTS AND DISCUSSION

### Mixing curves

Typical changes in the temperature and torque versus the mixing time for WG/glycerol blends with an increasing amount of MMT are shown in Figure 1. Despite the thermostatic control of the mixing chamber, the temperature sharply rose from 15 ( $T_{\text{reg}}$ ) to 70–85°C [the peak temperature ( $T_{\text{peak}}$ )], depending on the blend composition. After this maximum, the temperature decreased rapidly until reaching a plateau [plateau temperature ( $T_{\text{plateau}}$ )] at values between 60 and 85°C. Heat dissipation led to stabilized temperatures 45–70°C higher than  $T_{\text{reg}}$ . The stabilized temperatures were the real temperatures at which the materials were processed. Torque curves presented an evolution similar to that of temperature curves. They were also characterized by a maximum [peak torque ( $C_{\text{peak}}$ )] followed by a drop until reaching a plateau after about 5 min of mixing [plateau torque ( $C_{\text{plateau}}$ )].

Increasing MMT contents led to a continuous increase in both torque and temperature values (Fig. 1 and Table I). Changes in torque curves resulted in an increase in the total specific mechanical energy ( $\text{SME}_{\text{total}}$ ) provided to the material (Table I). The observed effects of MMT are connected to changes in the rheological behavior of the gluten material. If the MMT content increases, the shear viscosity of the material is known to increase,<sup>25</sup> thus leading to an increase in the viscous dissipation of mechanical energy input and therefore in temperature.<sup>5,6</sup>

Decreasing glycerol contents had the same effect as increasing MMT contents (Table II). The increase in both torque and temperature values with decreasing glycerol contents was due to the plasticized



**Figure 1** Typical changes in (a) temperature and (b) torque versus the mixing time of WG/glycerol blends with increasing amounts of MMT.

effect of glycerol. If the glycerol content decreases, the glass-transition temperature of the material increases. Then, a higher temperature is needed to enable the mixing of the material.

A torque increase characterized the formation of a cohesive blend.<sup>6</sup> The feature was accelerated with increasing MMT content (Fig. 1). For instance, the peak occurred after 3 min of mixing for the unfilled blend (sample WG-37.2-0), although it occurred after only 1.5 min of mixing for the blend containing 5 wt % MMT (sample WG-37.2-5). This phenomenon was characterized by a significant decrease in the specific mechanical energy provided to the material at the peak ( $SME_{peak}$ ) with an increasing MMT content. For the blend containing 5 wt % MMT, the torque increase was very sharp, and this is typical of a loading peak generally observed when blends are not plasticized enough.<sup>26</sup> This suggests an antiplasticizing effect of MMT.

### Gluten network evolution upon mixing

Changes in the gluten structure upon mixing were evaluated from a biochemical point of view through the determination of  $F_i$  of mixed doughs (Tables I and II).  $F_i$  is related to the aggregation level of proteins in the material.<sup>3</sup>

As already shown by several authors,<sup>24,27,28</sup> mixing considerably increased  $F_i$  compared with that of

the original WG sample (Tables I and II). The loss in protein solubility is directly related to the increasing density of network entanglements resulting from the formation and rearrangement of disulfide bridges due to sulhydryl/disulfide interchange reactions.<sup>3</sup> During mixing, gluten can disaggregate because of shearing<sup>6,29,30</sup> but can also aggregate by means of disulfide bonds if the temperature is higher than 60°C,<sup>5</sup> which was always the case in this study. Consequently, the resulting molecular size distribution profile of gluten just after mixing will depend on the equilibrium between both shearing and temperature effects. In this work, the two effects, shearing and temperature, that occurred during mixing were not distinguishable.

Increasing MMT content led to a rise in  $F_i$  (Table I). For gluten blends filled with 5 wt % MMT, a higher amount of SDS-insoluble proteins was obtained (49.91%) in comparison with that of the unfilled blend (31.88%). The temperature reached during mixing was higher in the presence of MMT ( $T_{peak} = 71.4^\circ\text{C}$  and  $T_{plateau} = 59.6^\circ\text{C}$  for the WG/glycerol blend vs  $T_{peak} = 82.5^\circ\text{C}$  and  $T_{plateau} = 77.8^\circ\text{C}$  for the WG/glycerol/MMT blend). Decreasing glycerol content also led to a significant increase in  $F_i$  linked to an increase in  $T_{peak}$  and  $C_{peak}$ . In that case,  $T_{peak}$  and  $C_{peak}$  continuously increased, whereas  $T_{plateau}$  and  $C_{plateau}$  seemed to reach limit values (Table II). For example, blends plasticized with 37.2 or 42.8 wt % glycerol had about the same temperature and torque at the plateau (Table II) but significantly different  $T_{peak}$  (71.4 and 67.5°C, respectively),  $C_{peak}$  (59.4 and 53.3 N m, respectively), and  $F_i$  values (31.88 and 24.51%, respectively). This means that  $F_i$  was directly linked to what occurred at the transient peak during mixing.

Consequently,  $T_{peak}$  was considered to calculate the activation energy ( $E_a$ ) of the thermomechanical treatment. As already shown by Pomet et al.,<sup>24</sup>  $F_i$  can be fitted with an Arrhenius law as expressed by the following equation:

$$F_i(\%) = A \exp(-E_a/RT_{peak}) \quad (5)$$

**TABLE I**  
Mixing Characteristics (Torques, SMEs, and Temperatures) and  $F_i$  Values of Mixed WG/Glycerol/MMT Blends

Glycerol/gluten	37.2 wt %		
	0 wt %	2 wt %	5 wt %
$C_{peak}$ (N m)	59.5 ± 1.7	72.9 ± 7.2	96.5 ± 4.7
$C_{plateau}$ (N m)	29.8 ± 1.9	35.7 ± 2.7	39.9 ± 0.7
$SME_{peak}$ (kJ/kg)	0.64 ± 0.07	0.50 ± 0.05	0.320 ± 0.02
$SME_{total}$ (kJ/kg)	9.50 ± 0.35	11.72 ± 0.94	12.52 ± 0.29
$T_{peak}$ (°C)	71.4 ± 0.6	75.8 ± 0.1	82.5 ± 0.1
$T_{plateau}$ (°C)	59.6 ± 0.5	61.9 ± 2.6	77.8 ± 3.1
$F_i$ (%)	31.88	41.44	49.91

**TABLE II**  
**Mixing Characteristics (Torques, SMEs, and Temperatures) and  $F_i$  Values of Mixed WG/Glycerol Blends**

MMT/total Glycerol/gluten	0 wt %				
	25 wt %	30 wt %	34.7 wt %	37.2 wt %	42.8 wt %
$C_{\text{peak}}$ (N m)	81.2 ± 5.3	73.7 ± 2.7	63.6 ± 0.8	59.4 ± 1.7	53.3 ± 0.2
$C_{\text{plateau}}$ (N m)	39.3 ± 4.4	35.7 ± 1.6	28.9 ± 3.3	29.8 ± 1.9	28.1 ± 0.8
$SME_{\text{peak}}$ (kJ/g)	0.59 ± 0.07	0.65 ± 0.03	0.62 ± 0.18	0.64 ± 0.07	0.59 ± 0.06
$SME_{\text{total}}$ (kJ/g)	12.40 ± 2.38	11.95 ± 0.56	10.22 ± 0.45	9.50 ± 0.35	8.46 ± 0.03
$T_{\text{peak}}$ (°C)	85.5 ± 2.6	80.1 ± 0.1	74.3 ± 0.4	71.4 ± 0.6	67.5 ± 0.3
$T_{\text{plateau}}$ (°C)	84.2 ± 3.7	82.1 ± 1.6	72.3 ± 0.1	59.6 ± 0.5	58.6 ± 0.8
$F_i$ (%)	50.89	41.52	34.40	31.88	24.51

where  $A$  is the frequency factor for the reaction leading to the insolubilization of gluten proteins and  $R$  is the universal gas constant ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ).  $E_a$  is presented in joules per mole, and  $T_{\text{peak}}$  is presented in kelvins. Through the plotting of  $\ln F_i$  as a function of  $1/T_{\text{peak}}$ , a linear curve was obtained with a correlation coefficient ( $R^2$ ) of 0.94 [ $R^2 = 0.96$  or  $0.98$  if only the data collected for various MMT contents (Table I) or various glycerol contents (Table II) are considered]. This means that the mixing profiles well reflected the biochemical changes. The obtained  $E_a$  value was  $39.8 \text{ kJ/mol}$ , which was comparable to the  $E_a$  value calculated by Redl et al.<sup>27</sup> ( $33 \text{ kJ/mol}$ ). When only the data collected for various MMT contents or only the data collected for various glycerol contents were considered, similar  $E_a$  values were calculated ( $38.9$  and  $40.2 \text{ kJ/mol}$ , respectively). This confirmed that the effects of increasing MMT or decreasing glycerol were similar.

### Gluten network evolution upon thermoforming

WG/glycerol/MMT blends were thermoformed at four different temperatures: 60, 80, 100, and  $120^\circ\text{C}$ . A high-temperature limit of  $120^\circ\text{C}$  was chosen because above this temperature, WG begins to degrade.<sup>31</sup> It is noteworthy that homogeneous films were obtained, even at 60 and  $80^\circ\text{C}$ . As previously shown by Gällstedt et al.,<sup>32</sup> when the components are mixed in a mortar with a pestle, a minimum temperature of  $90^\circ\text{C}$  is required to obtain homogeneous films because of a low degree of denaturation and incomplete plasticization of proteins below this temperature. In the absence of mechanical mixing, a higher thermoforming temperature is needed to give sufficient mobility to proteins to attain molecular unfolding and intermolecular disulfide crosslinks. This shows that dough mixing in a two-blade, counter-rotating batch allows decreasing the low-temperature limit of the processing window of thermoforming.

$F_i$  in the films and the difference from the  $F_i$  values measured for the corresponding mixed dough ( $\Delta F_i$ ) are given in Table III. An increase in the ther-

moforming temperature from 60 to  $120^\circ\text{C}$  led to an increase in  $F_i$ . For any given temperature, no significant effect of MMT was noted. Biochemical changes induced by temperature suggested the occurrence of heat-activated reactions during thermoforming.

Changes in  $\Delta F_i$  with the thermoforming temperature from 60 to  $120^\circ\text{C}$  were only continuous for unfilled films (Table III). In the case of filled materials, significant increases in  $\Delta F_i$  were noted only from 100 and  $120^\circ\text{C}$  for MMT contents of 2 and 5 wt %, respectively. Changes in  $\Delta F_i$  clearly depended on the  $T_{\text{peak}}$  and  $T_{\text{plateau}}$  values reached during mixing. An increase in  $F_i$  upon thermoforming was observed only if the heat treatment applied during thermoforming was more severe than that provided during mixing.

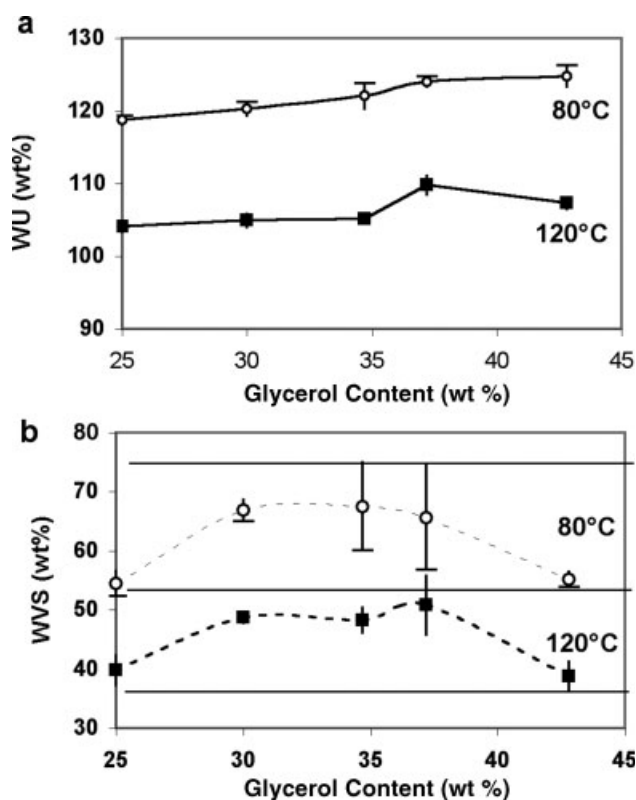
At  $120^\circ\text{C}$ , it can be underlined that changes in  $\Delta F_i$  were more pronounced whatever the MMT content was. This can be related to the formation of additional covalent bonds such as intermolecular isopeptide bonds or methylene bridges that are known to occur upon severe thermomechanical treatments.<sup>30,32–34</sup>

### Water sensitivity of thermoformed films

The water sensitivity of thermoformed films was evaluated through the measurement of WU and WVS. Thermoformed WG-based films retained their shape when dipped into water. In the case of cast

**TABLE III**  
 **$F_i$  and  $\Delta F_i$  for Thermoformed WG-Based Films**

Sample	$F_i$ (%)	$\Delta F_i$ (%)
WG-37.2-0-60	40.03	8.15
WG-37.2-0-80	47.21	15.33
WG-37.2-0-100	48.38	16.50
WG-37.2-0-120	74.87	42.99
WG-37.2-2-60	40.41	-1.03
WG-37.2-2-80	40.66	-0.78
WG-37.2-2-100	49.65	8.21
WG-37.2-2-120	77.03	35.59
WG-37.2-5-60	48.90	-1.01
WG-37.2-5-80	49.41	-0.5
WG-37.2-5-100	49.82	-0.09
WG-37.2-5-120	71.64	21.73



**Figure 2** Evolution of (a) WU and (b) WVS versus the glycerol content for WG films thermoformed at (○) 80 and (■) 120°C.

films, the introduction of MMT was needed to prevent the disruption of the gluten matrix in water.<sup>8</sup>

The effect of the glycerol content on the water sensitivity of WG-based films is illustrated in Figure 2. A slight increase in WU with increasing glycerol content was noted for both studied temperatures, 80 and 120°C [Fig. 2(a)]. This behavior was due to the hygroscopic effect of glycerol. Because of the high standard deviation values of WVS, no significant effect was identified [Fig. 2(b)].

The effect of the thermoforming temperature on the water sensitivity of WG films was the same whatever the composition of the films was, that is, the filler and glycerol contents. An increase in temperature led to a continuous diminution of both WU and WVS (Fig. 3). The decreased water sensitivity was strongly related to increased protein crosslinking.<sup>26,31</sup> The formation of covalent bonds within the films led to a decrease in the hydrophilicity of the films.<sup>35</sup>

The introduction of MMT had no significant effect on the water sensitivity of WG-based films. Just a slight decrease in WU was noted with increasing filler content. In contrast to cast films,<sup>8</sup> unfilled thermoformed films already have quite good water resistance because of the formation of a polymer covalent network.

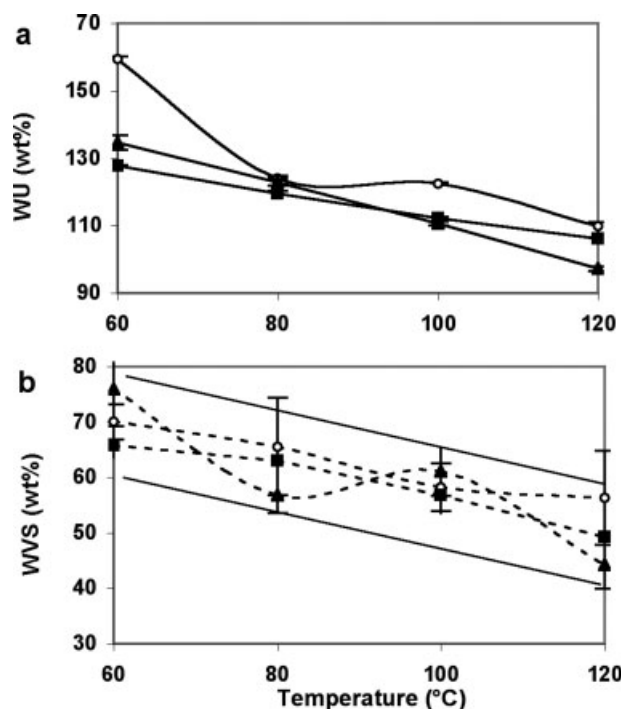
### Mechanical properties of WG-based thermoformed films

The mechanical properties of thermoformed WG-based films were assessed with tensile tests performed at 20°C and 75% RH. Tensile properties, that is, the Young's modulus, nominal stress at break, and nominal strain at break, are given with their standard deviations in Tables IV and V.

#### Effect of the glycerol content

The mechanical properties of WG-based films were almost unaffected by the content variation within the studied range of glycerol concentrations (Table IV), in contrast to the results of Mangavel et al.<sup>36</sup> showing a significant decrease in both the modulus and stress at break for increasing glycerol contents. However, it is noteworthy that their range of studied glycerol concentrations was larger (from 0.3 to 1.35 mol/mol of amino acid) than ours (from 0.39 to 0.56 mol/mol of amino acid, assuming a mean molecular weight of 121 g/mol per amino acid).

In our study, films were stored at 75% RH before being tested. The films being quite thin (ca. 300 μm thick), such an RH was needed to perform correct tensile tests. At 75% RH, WG-based films contained  $0.24 \pm 0.02$  g of water/g of material depending on the composition and the thermoforming temperature, that is,  $1.6 \pm 0.1$  mol of water/mol of amino



**Figure 3** Evolution of (a) WU and (b) WVS versus the thermoforming temperature for WG films filled with (○) 0, (■) 2, and (▲) 5 wt % MMT.

**TABLE IV**  
**Tensile Properties of WG Films Plasticized with Different Ratios of Glycerol and Thermoformed at 80 and 120°C**

Sample	Young's modulus (MPa)	Stress at break (MPa)	Strain at break (%)
WG-25-0-80	1.9 ± 0.7	0.51 ± 0.07	29.8 ± 3.6
WG-30-0-80	2.4 ± 0.3	0.59 ± 0.08	44.8 ± 5.2
WG-34.7-0-80	2.3 ± 0.3	0.53 ± 0.06	38.5 ± 7.9
WG-37.2-0-80	1.1 ± 0.5	0.31 ± 0.03	32.7 ± 7.3
WG-42.8-0-80	0.9 ± 0.2	0.25 ± 0.5	41.5 ± 7.8
WG-25-0-120	5.1 ± 2.1	1.70 ± 0.27	82.0 ± 9.2
WG-30-0-120	7.0 ± 1.6	2.02 ± 0.23	84.9 ± 7.1
WG-34.7-0-120	6.0 ± 1.4	1.76 ± 0.37	88.2 ± 13.1
WG-37.2-0-120	6.5 ± 1.1	1.61 ± 0.25	85.7 ± 10.1
WG-42.8-0-120	5.4 ± 1.8	1.74 ± 0.40	102.2 ± 13.7

acid. It is known that stress and strain values reach a plateau beyond a threshold value of the plasticizer concentration due to a saturation of the protein network by the plasticizer molecules.<sup>37</sup> In our case, this might be located on the plateau, thus preventing the observation of any change in the mechanical properties within the glycerol content variation.

#### Effect of the thermoforming temperature

The effects of the heat treatment on the mechanical properties of WG-based films were evidenced by changes in stress–strain curves as a function of the thermoforming temperature (Fig. 4). An increase in the temperature led to a significant increase in both the stress and strain at break (Fig. 4) and in Young's modulus (Table V). The effect of the thermoforming temperature was the same whatever the filler content (Table V) and the glycerol content were (Table IV). However, the rise in the stress at break and Young's modulus was more pronounced for the lowest filler contents and the highest glycerol contents, that is, for the lowest  $F_i$  values of mixed doughs.

The temperature effect on the stress at break and tensile modulus has been largely reported in the literature<sup>35,36,38</sup> and is related to a higher density of both intramolecular and intermolecular crosslinkages. The fact that the strain at break increased at

the same time as the stress at break and the modulus appeared is an original and interesting result. Most of the time, the increase in the stress and modulus is coupled with a strain decrease.<sup>32,35,38–40</sup> The increase in the strain at break may be related to a longer mean length of the gluten macromolecules. It seems that the mechanical treatment applied in the two-blade, counter-rotating mixer was responsive for such singular properties. First, mechanical energy provided by mixing might increase the molecular mobility of proteins, thus enhancing the exposure of reactive sites and the formation of long polypeptide chains. Second, polymeric chains might be oriented under shearing, this favoring the increase in the chain mean length upon thermoforming. Indeed, the heat treatment involved reorganization of intramolecular disulfide bonds to intermolecular disulfide bonds via thiol–disulfide exchange reactions.<sup>34</sup>

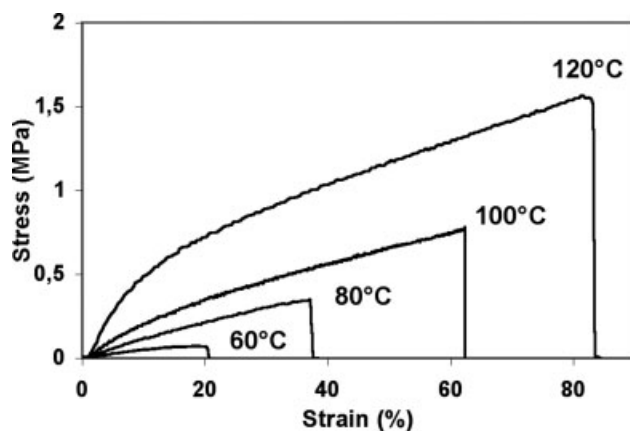
Changes in the mechanical properties were particularly important at 120°C. This behavior may be related to the formation of isopeptide bonds that is supposed to take place at this temperature.<sup>33</sup>

#### Effect of the filler content

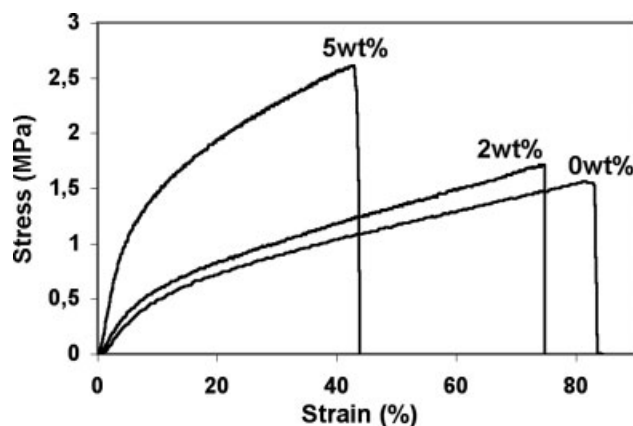
The effect of the introduction of MMT into WG on the stress–strain curves of thermoformed films is

**TABLE V**  
**Tensile Properties of Thermoformed WG-Based Films**

Sample	Young's modulus (MPa)	Stress at break (MPa)	Strain at break (%)
WG-37.2-0-60	0.5 ± 0.2	0.10 ± 0.02	24.1 ± 3.6
WG-37.2-0-80	1.1 ± 0.5	0.31 ± 0.03	32.7 ± 7.3
WG-37.2-0-100	1.9 ± 0.4	0.76 ± 0.04	59.9 ± 8.7
WG-37.2-0-120	6.5 ± 1.1	1.61 ± 0.25	85.7 ± 10.1
WG-37.2-2-60	1.2 ± 0.1	0.20 ± 0.04	28.6 ± 8.8
WG-37.2-2-80	1.7 ± 0.3	0.32 ± 0.04	29.8 ± 5.0
WG-37.2-2-100	4.5 ± 0.5	0.64 ± 0.08	32.3 ± 6.0
WG-37.2-2-120	9.5 ± 1.6	1.75 ± 0.24	70.0 ± 5.6
WG-37.2-5-60	3.2 ± 1.1	0.26 ± 0.04	11.9 ± 2.2
WG-37.2-5-80	6.4 ± 1.2	0.66 ± 0.07	23.1 ± 2.1
WG-37.2-5-100	10.0 ± 1.2	0.88 ± 0.08	22.7 ± 1.6
WG-37.2-5-120	23.6 ± 5.2	2.53 ± 0.16	41.7 ± 4.3



**Figure 4** Typical curves of the stress versus the strain for unfilled WG films thermoformed at 60, 80, 100, and 120°C.



**Figure 5** Typical curves of the stress versus the strain for WG films filled with 0, 2, or 5 wt % MMT and thermoformed at 120°C.

illustrated in Figure 5. As expected, the introduction of MMT into WG resulted in more resistant but more brittle materials, which were characterized by a higher Young's modulus and stress at break and a lower strain at break (Table V). The relative increase in Young's modulus was more important than that in the stress at break. The reinforcing effect of MMT was really significant for a filler content of 5 wt %, as already shown for nanocomposite films obtained by casting,<sup>8</sup> and for decreasing thermoforming temperatures. For example, the increase in Young's modulus was two times higher for WG/glycerol/5 wt % MMT thermoformed at 60°C than at 120°C. For increasing temperatures, the reinforcing effect of MMT due to the establishment of interactions between WG and MMT was in competition with that of the protein covalent network.

The relative effect of temperature was higher than that of MMT. Increasing the temperature from 60 to 120°C allowed enhancing Young's modulus by 7–13 times and the stress at break by 9.7–16 times, depending on the MMT content, whereas filling WG with 5 wt % MMT nanoparticles allowed only increasing Young's modulus by 3.6–6.4 times and

the stress at break by 1.6–2.6 times according to the thermoforming temperature. Furthermore, the original effect of temperature was the increase in the strain at the same time as the stress and modulus. However, it is noteworthy that the reinforcement of WG by MMT allowed achieving strain at break, stress at break, and Young's modulus values that were not achievable by just the variation of the glycerol content and the processing temperature.

#### Film color of thermoformed films

The influence of both the thermoforming temperature and MMT content on Commission Internationale de l'Éclairage color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , and  $\Delta E$ ) of WG-based films are presented in Table VI.  $L^*$ , representing the luminance, ranges from 0 (black) to 100 (white). The two chromatic components,  $a^*$  (from green to red) and  $b^*$  (from blue to yellow), range from  $-120$  to  $+120$ .<sup>41</sup>

The appearance of the WG-based films was yellow-brown and translucent. This yellow-brown color was attributed to the Maillard reaction that occurred

**TABLE VI**  
Color Attributes ( $L^*$ ,  $a^*$ ,  $b^*$ , and  $\Delta E$ ) of Thermoformed WG-Based Films

Sample	$L^*$	$a^*$	$b^*$	$\Delta E$
WG-37.2-0-60	63.78 ± 1.44	8.74 ± 0.64	35.01 ± 2.61	49.12 ± 2.95
WG-37.2-0-80	73.54 ± 1.60	4.59 ± 0.62	34.52 ± 0.34	42.94 ± 0.48
WG-37.2-0-100	77.30 ± 1.40	3.14 ± 0.66	33.15 ± 1.83	39.98 ± 2.26
WG-37.2-0-120	76.11 ± 1.71	4.68 ± 1.28	36.44 ± 2.81	43.56 ± 3.34
WG-37.2-2-60	66.62 ± 1.91	7.13 ± 0.80	37.86 ± 0.60	49.52 ± 1.60
WG-37.2-2-80	72.34 ± 1.38	4.62 ± 0.55	34.76 ± 1.08	43.70 ± 1.64
WG-37.2-2-100	75.91 ± 1.97	3.37 ± 0.95	32.66 ± 1.74	40.12 ± 2.47
WG-37.2-2-120	73.74 ± 2.23	5.11 ± 1.19	36.22 ± 1.48	44.39 ± 2.41
WG-37.2-5-60	65.31 ± 2.02	7.11 ± 1.35	37.35 ± 0.21	49.83 ± 1.46
WG-37.2-5-80	70.92 ± 2.13	5.09 ± 1.14	35.03 ± 0.74	44.68 ± 1.80
WG-37.2-5-100	71.95 ± 5.98	4.76 ± 2.72	34.28 ± 3.96	43.57 ± 6.59
WG-37.2-5-120	64.10 ± 1.84	9.92 ± 1.02	38.11 ± 0.13	51.53 ± 1.14



during the preparation of the thermoformed WG-based films.<sup>42</sup> Films obtained at 60°C exhibited some little imperfections possibly caused by inadequate network formation. A significant change in the color coordinates was observed only for films thermoformed at 120°C (Table VI), as already reported in the literature.<sup>32,37,39,40</sup> The darkness of WG films with increasing temperature was related to a higher network density and Maillard reactions.<sup>43–45</sup>

The introduction of MMT resulted in a slight decrease in  $L^*$ , which meant that films became darker, and a slight increase in  $a^*$  and  $b^*$ , which meant that films became more yellow-brown. This effect was more intense when the curing temperature increased. This cannot be attributed to an improved polymerization of gluten proteins because it was shown that  $F_i$  was not affected by the presence of MMT. Changes in the color coordinates of WG-based films may be due to the yellow-brown color of MMT nanoparticles.

## CONCLUSIONS

WG/MMT composite materials have been successfully prepared by a dry process consisting of a thermomechanical treatment in a Brabender followed by a thermoforming step. Films were thermoformed at temperatures as low as 60°C.

At 75% RH and in the range of studied glycerol concentrations (25–42.8 wt %), MMT contents (0–5 wt %), and thermoforming temperatures (60–120°C), it was demonstrated that glycerol had no influence on the mechanical properties of WG-based films, whereas the thermoforming temperature and MMT led to significant and interesting changes. The fact that glycerol had no influence on the final properties is not uninteresting. This means that we are able to decrease the blend viscosity, thus allowing the introduction of high MMT contents. An increase in the thermoforming temperature led to a rise in both the resistance and deformability of WG-based materials. Biochemical analysis has shown that the improved network resistance upon thermoforming was related to the establishment of a covalent network resulting from the formation and rearrangement of disulfide bridges. The increase in deformability was attributed to the thermomechanical treatment applied in the two-blade, counter-rotating mixer. It was supposed that shearing might favor the parallel orientation of proteins chains, thus leading to an increase in the mean length of the polymer. Finally, even if the relative effect of the thermoforming temperature was higher than that of MMT, it is noteworthy that the introduction of MMT allows achieving original mechanical properties.

Considering the water sensitivity, we found that only the thermoforming temperature had a notable

positive effect on the water sensitivity of WG-based films.

The elaboration of WG-based nanocomposites allows increasing the window of functional properties of WG-based films, thus opening new field of applications. The limiting aspect of the use of such materials in packaging, for example, might be the color changes induced by the use of high thermoforming temperatures and/or high filler contents.

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