Wheat Gluten Nanocomposite Films as Food-Contact Materials: Migration Tests and Impact of a Novel Food Stabilization Technology (High Pressure)

M. Mauricio-Iglesias, S. Peyron, V. Guillard, N. Gontard

Unité Mixte de Recherche 1208, Ingénierie des Agropolymères et Technologies Emergentes, Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Institut National de la Recherche Agronomique, UMR 1208 IATE (Agropolymer Engineering and Emerging Technology), University of Montpellier 2, CIRAD, INRA, Montpellier Supagro CC023, Place Eugène Bataillon F-34095, Montpellier, France

Received 16 July 2009; accepted 11 October 2009
DOI 10.1002/app.31647
Published online 20 January 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The aptitude of a novel biodegradable material [wheat gluten/montmorillonite (MMT)] to be used as a food-contact material was assessed with a focus on mass transfer from the film into foodstuff (migration). Special attention was paid to the impact of high-pressure treatments and subsequent storage. Several aspects were treated: the migration of a model molecule (Uvitex OB), MMT migration, protein migration, and overall migration. The results showed that overall migration and protein migration were high; on the contrary, MMT and Uvitex OB migration was low or not detectable. No difference was found between the high-pressure-treated samples and the control, except for the migration of MMT. Two solid simulants (agar gel and Tenax) were also tested to emphasize the need of new migration tests adapted to water-sensitive materials. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2526–2535, 2010

Key words: biopolymers; films; nanocomposites; proteins

INTRODUCTION

The introduction of new materials and technologies has deeply changed the panorama of food packaging. For instance, the use of nanocomposites and bioplastics is one of the latest trends in food packaging technologies and is intended to improve the performance of currently used materials[1] and/or produce environmentally friendly packaging. Another tendency aimed at reducing costs and energy is making packed foodstuff endure a food stabilization treatment[2] [thermal treatment, ionizing treatment, or high-pressure (HP) processing] with the additional advantage of preventing potential ensuing contamination. For instance, batch HP processing, which is an interesting technology allowing the preservation of the nutritional and organoleptic features of fresh foodstuff,[3] requires food to be packed before the treatment. However, the use of these technologies and/or new materials leads to a real challenge in the assessment of the compliance of food-contact materials (FCMs).

European regulation 1935/2004 sets the general guidelines for the compliance of FCMs: they "shall not transfer their components into the food in quantities that could endanger human health" or change the composition of food. For some FCMs (plastics, ceramics, and regenerated cellulose), the guidelines for determining transfer from the packaging into food are well established. Yet, the recommended migration tests are applicable only to water-resistant materials even though the use of water-sensitive materials, such as paper and board, is particularly widespread. Some of the new bioplastics in development have low water resistance as well. In the case of water-sensitive materials intended only to contain dry foodstuffs, migration values tend to be particularly high in common migration tests designed for plastic materials (European regulation 2002/72). So far, the only alternative test recommended for such materials considers the use of the solid fatty simulant Tenax [modified poly(phenylene oxide); Varian, Houten, the Netherlands] according to the conditions set in the European Committee for Standardization norm CEN/TC 172.[4] However, such a limited approach fails to reproduce the variety of foodstuff packed in these materials and imitates only dry fatty products. To extend the possibilities of solid food testing to nonfatty and intermediate water activity products, polysaccharide-based gels have also been used as food simulants.[5] In this study, agar gel was used to simulate high water activity food, as successfully done previously by Guillard et al.[6]

Correspondence to: V. Guillard (guillard@univ-montp2.fr).
Contract grant sponsor: Commission of the European Communities (through Framework 6, Priority 5, "Food Quality and Safety"); contract grant number: Q FP6-CT-2006-015710.

© 2010 Wiley Periodicals, Inc.

For a couple of years, reinforcement by nanofillers has appeared to be an interesting strategy for improving the functional properties of synthetic and biosourced materials.[1] For example, the addition of montmorillonite (MMT) to wheat gluten (WG) films was proven to improve the mechanical and barrier properties for water and aroma compounds.[7,8] WG is a byproduct of the wheat starch industry, is commercially available at low cost (1 €/kg), and displays unique viscoelastic properties and low water solubility. Gluten is a mixture of two main proteins, gliadins (monomeric proteins) and glutenins (larger polypeptide chains linked with disulfide bonds forming a macropolymer). With respect to its filmogen properties, WG is an interesting raw material that can be used as a food packaging material. Gluten proteins are naturally linked by disulfide bonds, although heating increases the disulfide interchange, and this leads to the formation of a three-dimensional macromolecular network and thus severely modifies some of its mechanical properties.[9] To date, very little has been studied about the effect of high-pressure/temperature (HP/T) treatments on WG. Apichartsrangkoon et al.[10] studied the effect on hydrated WG of HP/T for several pressures, times, and temperatures up to 800 MPa, 60°C, and 50 min. The results showed that all the treatments could alter the WG structure if they were held for 50 min. The increase in hardness, caused by crosslinking due to disulfide bonding, was significant only under hard temperature conditions (from 400 MPa and 60°C). Kieffer et al.[11] came to the same conclusion and pointed out that the influence of HP lies mainly on glutenin because it has a higher number of thiol groups. However, the effect of HP/T on processed WG films has never been studied.

WG-based films exhibit interesting gas-barrier properties, such as a selectivity to oxygen and carbon dioxide[12] that is especially suitable for the conservation of fruits and vegetables, even at a high relative humidity (RH).[13] Although WG-based films have great potential as bioplastics, their low mechanical resistance and high water sensitivity restrict their utilization to a narrow range of applications (especially dry and intermediate-aw products). With the aim of broadening the applications of WG films, Angellier-Coussy et al.[14] added MMT to modify its mechanical properties and water sensitivity.

The objective of this work was to study the suitability of WG–MMT nanocomposite materials as FCMs. To this end, several aspects of mass transfer from the film into food were treated: overall migration, protein migration, specific surrogate migration, and nanofiller migration. The effect of an HP/T treatment on the food/packaging interactions was also examined, and special attention was paid to the behavior of the nanoparticles (MMT) after treatment and during storage. Indeed, even if the use of nanoparticles is very promising in the food packaging field, very little is known about the potential release of these nanoparticles from FCMs and their subsequent effect on foodstuff. Actually, to our knowledge, only one publication[15] has dealt with the migration of nanoparticles: a net increase of the silicon content of vegetables packed with a starch biocomposite was detected.

The classical migration tests with food-simulating liquids (FSLs), as recommended by regulations, were used, and we discuss their convenience for such water-sensitive FCMs and outline proposals for alternative tests.

EXPERIMENTAL

Chemicals

All chemicals were reagent-grade or were of the highest purity available. 2,5-Bis-(5-*tert*-butyl benzoxazol-2-yl) thiophen (Uvitex OB; 430.6 g/mol), sodium azide (99%), *n*-heptane (99%), and Coomassie (Buchs, Switzerland) Brilliant Blue G-250 were purchased from Fluka. Octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate (Irganox 1076; 530 g/mol), a bovine serum albumin standard (1 mg/mL in 0.15*M* NaCl, 0.05% NaN_3), a bicinchoninic acid (BCA) solution, a copper sulfate solution [4% (w/v)], sodium dodecyl sulfate (SDS; 99%), dithiothreitol (99%), glyceryl triheptadecanoate (ca. 99%), sodium sulfate (99%), and a potassium hydroxide solution (1.0*M*) were purchased from Sigma-Aldrich. Methanol (99.9%) was purchased from Fisher. Ethanol (99.8 vol %), acetic acid (99–100 vol %), and a boron trifluoride/methanol complex [13–15% (w/v)] were purchased from Riedel-de Haën. Pentane (99%) was purchased from Merck. Sodium hydroxide (98%) was purchased from BDH (England). Vital WG was provided by Amylum (Aalst, Belgium). Its protein content was 77% (dry matter) according to the manufacturer. WG contained a 3.59% concentration of proteins insoluble in SDS, an approximately 42% concentration of glutenins, and a 46% concentration of gliadins. Anhydrous glycerol (Fluka and Sigma-Aldrich Chemie, Steinheim, Germany; purity = 98%) was used as a plasticizer. Sodium MMT 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/mL, and a pH of 9.3 at 100 g/L (208C).

Film preparation

The preparation of the WG films is explained in detail elsewhere[14] and is just described here briefly.

It is a suitable method for the production of films at a laboratory or pilot scale but is not suitable for large industrial manufacturing. WG and MMT powders were mixed with glycerol [37.5% (w/w)] 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 a controller unit (PL2000, Brabender). Glycerol and water were first introduced into the mixer, and then the WG/MMT/Uvitex OB mixture was added. The mixing chamber (50 cm^3) 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 of 15°C with a Julabo (Seelbach, Germany) F34 cryostat and water circulation in the double chamber of the mixer. The torque and temperature of the dough were continuously recorded during the mixing process.

The glycerol ratio was relative to the amount of WG, whereas the MMT content [5% (w/w)] and the Uvitex OB content [1% (w/w)] were relative to the total weight of the material (50 g). After mixing, the materials were pressed at 150 bar for 5 min at 120°C between two Teflon 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 average thickness of the resulting films was 230 μm.

HP/T treatment

Two types of HP treatments were performed at different temperatures to imitate sterilization and pasteurization:

- High-pressure/high-temperature (HP/HT) treatment: 5 min at 800 MPa and 115°C with the pressure building up at 800 MPa/min and a starting temperature of 90°C that rose to 115°C because of adiabatic heating. It was performed in a Resato (Wageningen, the Netherlands) hydrostatic pressure apparatus in A&F. The pressurization fluid was water.
- High-pressure/low-temperature (HP/LT) treatment: 5 min at 800 MPa and 40°C with the pressure building up at 300 MPa/min and a starting temperature of 20°C that rose to 40°C because of adiabatic heating. It was performed at the Institut de Recerca i Tecnologia Agroalimentàries (Monells, Spain) with a hydrostatic pressure apparatus: Thiot Ingenierie-NC (Bretenoux, France/Burgos, Spain) hyperbaric HP equipment with a chamber volume of 2 L. The pressurization fluid was Priplast 3019 (Uniqema, Snaith, England).

In each case, the gluten samples and the corresponding volume of the FSL were packed in a bioriented polypropylene bag [Mobil Plastics Europe, Luxembourg (supplied by Danone Vitapole, Paris, France)], and as a precaution, this one was overpacked in a metalized polyester bag (150 × 200 mm^2, 120-μm thickness; Sacoliva, Barcelona, Spain).

Migration tests

In accordance with the conditions set in directives 85/572/EEC[16] and 2002/72/EC,[17] strips of gluten films were immersed in a volume of the FSL and stored at 40°C. The tests were done in the same FSL used in the treatment for treated samples. A ratio of 6 dm^2 to 1000 mL of FSL was respected. Two types of test were done: (1) MMT and overall migration tests (film surface = 60 cm^2, FSL volume = 100 mL) and (2) protein and Uvitex OB migration tests (film surface = 3.5 cm^2, FSL volume = 6 mL). Four FSLs were used: distilled water, 3% acetic acid (w/v), 15% ethanol (v/v), and olive oil. To prevent microbial development in the gluten samples, all the FSLs except olive oil contained sodium azide [0.02% (w/v)]. The time of exposure varied with the test. The overall and MMT component migration was measured just after the HP/T treatment and after the HP/T treatment plus 10 days of storage at 40°C. For the determination of proteins and Uvitex OB, the tests were also done after 3 and 5 days of storage.

Determination of the overall migration

In aqueous simulants

The overall migration was determined in aqueous FSLs (water, 3% acetic acid, and 15% ethanol) in contact with the sample after the HP/T treatment and 10 days of storage at 40°C. The volume was 50 mL of the FSL plus 3 volumes of 10 mL of Milli-Q grade water to rinse the flask used for the test. This volume was poured into a Petri dish and put into an oven at 103°C until a constant weight was reached.

In olive oil

For olive oil tests, overall migration was determined as the difference in the weight of the film and further quantification of absorbed or stuck olive oil; the method was adapted from the Community Reference Laboratory for Food Contact Materials.[18]

Extraction. The WG samples were weighed after the migration test, and olive oil was extracted with a Soxhlet kit for 4 h with 200 mL of pentane as a solvent. Before the extraction, 10 mL of an internal standard solution (glyceryl triheptadecanoate in *n*-heptane; 2.0 mg/mL) had been added to the film.

Methylation of triglycerides. After extraction, the recovered solvent was evaporated in a rotary evaporator to a tenth of the initial volume, and 10 mL of

1.0*M* potassium hydroxide was added. The mixture was then boiled with refluxing for 10 min, and a 5-mL solution of a boron trifluoride/methanol complex [13–15% (w/v)] was slowly poured by the cooling tube to turn the triglycerides into more volatile methyl esters. Five minutes after the ebullition resumed, the mixture was removed from heat. A saturated solution of sodium sulfate was added until the upper organic layer containing the esters reached the top of the flask and was easily removed.

Determination of methyl ester in solution. The quantity of esters in solution was determined with a Varian 3800 GC-FID equipped with a DB-23 column (60 m × 0.32 mm i.d., 0.25 μm; Varian) and a flame ionization detector (hydrogen, 30 mL/min; air, 300 mL/min). Hydrogen was used as a carrier gas with a flow rate of 1.7 mL/min. The oven temperature was held for 1 min at 140°C, ramped at 5°C/min to 200°C, and maintained for 8 min. Finally, the temperature was raised again at 5°C/min to 220°C and then maintained for 30 min. The injector temperature was 220°C, and the detector was at 240°C. Injection was done in a split mode with a ratio of 1 : 40. The quantification of the total methyl esters was made by a comparison with the internal standard. Three replicates were made for each experiment.

In solid simulants of food

Tenax [modified poly(phenylene oxide)] and agar gel [1% (w/w) and 0.02% (w/v) sodium azide] were used to simulate contact with solid food. The method to determine overall migration in Tenax was adapted from standard CEN/TC 172[4] and is briefly described here. A Petri dish that was 68 mm in diameter was filled with 1.54 g of Tenax powder and covered with a disc of a film sample (70-mm diameter, 1.33 ± 0.04 g). The resulting surface/mass ratio was approximately 200 dm^2/kg, larger than that for an FSL to take into account the lower apparent density of a powder such as Tenax. A second larger Petri dish (76-mm diameter) was put on the sample to close the system, and the whole was overturned to ensure close contact between the powder and the film. The migration cells were then stocked at a controlled RH (0 and 80%) and 40°C. All the components of the migration cells, including the film samples and the Tenax, had been previously equilibrated to that RH. After 10 days, the Tenax powder was desorbed in 2 × 20 mL of *n*-pentane, which was subsequently evaporated until dryness. The dry residue was compared to a control (same experiment without a film), and the difference between the two was evaluated as the overall migration.

The experimental setup was equivalent for the agar gels [1% (w/w) agar–agar and 0.02% (w/v) sodium azide], which were intended to imitate high-aw-content food. The inner Petri dish was completely filled with the agar gel (ca. 45 g; surface/mass ratio = 8 dm^2/kg) and kept at 80% RH and 40°C. The overall migration was calculated in both the film and the agar gel as the difference in the dry materials of the studied sample and a control.

Quantification of aluminum and silicon

For all the FSLs except olive oil, the quantity of aluminum and silicon was directly determined with 50 mL of the FSL. In the case of olive oil, 50 mL of the sample was previously calcined in a muffle furnace at 550°C for 20 min, and the ashes were dissolved in an acid solution. For both the acid solution and the aqueous FSL, the analyses were then carried out at IPL Meditérranée Laboratories (Montpellier, France) by inductively coupled plasma according to ISO method NF-ISO 11885+15587-1.

The lower limits of quantification of the method were 0.03 mg/L for aluminum and 0.20 mg/L for silicon.

Quantification of proteins

In aqueous simulants

The determination of the quantity of total proteins was slightly adapted from Smith et al.[19] as follows. The standard for the calibration was prepared with bovine serum albumin and the corresponding FSL for water and 15% ethanol. In the case of 3% acetic acid, the samples were neutralized to pH 6.5 with a 5% NaOH solution and then compared to a standard in deionized water. The sample (25 μL) was diluted 1 : 6, added to 175 μL of a BCA–copper sulfate (50 : 1) solution, and put for 40 min in a stove at 40°C. The resulting complex was measured by colorimetry at 562 nm in a Multiskan spectrophotometer (Thermo Scientific, Waltham, MA).

In olive oil

Given the high amount of compounds in olive oil capable of interaction in the colorimetric reaction, the determination of proteins was performed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE).

The separation of proteins from olive oil was done according to Hidalgo et al.;[20] it was evaporated to dryness with an N_2 flux and dissolved in a 10% SDS solution. Electrophoresis was then performed according to Laemmli[21] with 4.5% stacking polyacrylamide gels and in a polyacrylamide gradient from 5 to 20% under reduction (5% dithiothreitol, 4 min, and 100°C). Gels were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in a 25% (v/v)

methanol/7.5% (v/v) acetic acid solution. The solution containing the proteins (20 μL) was injected.

Quantification of Uvitex OB

The extraction of the remaining Uvitex OB in the gluten samples was carried out with 20 mL of 70% ethanol (v/v) for 20 h at 70°C. Calibration of the extraction procedure showed that these conditions enabled stable and reproducible recovery (88.5 ± 0.6%). An internal standard solution of Irganox 1076 was prepared in absolute ethanol. Each solution (200 μL) was put together with an automatic injector. This solution (20 μL) was analyzed by reverse-phase high-performance liquid chromatography with an Alltima (Alltech, Deerfield, IL) C_{18} column (5 μm, 250 × 4.6 mm), isocratic elution of 98% ethanol/2% water, and ultraviolet detection (280 nm for Irganox 1076 and 374 nm for Uvitex OB). The limit of quantification of the high-performance liquid chromatography method was evaluated to 0.02% (w/w).

RESULTS AND DISCUSSION

WG-based materials containing 5 wt % MMT were prepared by thermoforming. This amount of MMT was chosen on the basis of the results of previous studies[14] to modify its mechanical properties. Moreover, in this study, Uvitex OB [1% (w/w)] was added to the film formulation as a surrogate of 430 g/mol to follow the specific migration of an approved nonpolar FCM additive. Uvitex OB is an optical brightener and ultraviolet stabilizer commonly used for polyolefins and is approved for FCMs with a specific migration limit of 0.6 mg/kg (2002/72/EEC). The tests of the specific migration of Uvitex OB were intended to determine the behavior and transfer of a medium-weight molecule added to the WG film as a surrogate of other specific additives.

WG–MMT nanocomposite materials were subjected to two HP/T treatments intended to perform pasteurization (800 MPa, 5 min, and 20–40°C) and sterilization (800 MPa, 5 min, and 90–115°C).[3] The films could not stand the hard conditions of the sterilization, and the films melted and got stuck to the polypropylene overpackaging. Therefore, this study deals only with the effects of HP/T pasteurization conditions as HP/T sterilization was not possible. Thus, a case study is presented in which the specific migration of an additive, nanocomposite migration, and protein and overall migration are assessed, and the effects of the MMT addition and the HP/T pasteurization on the packaging are discussed.

Uvitex OB specific migration tests

The initial content of Uvitex OB in the WG samples was determined to be 0.92 ± 0.05% (w/w) on a dry basis, whereas the lowest value found after the migration tests was 0.87%. Such results showed that the release was very low, and indeed, no trend of desorption could be made out. This behavior was observed regardless of the FSL, HP/T, or addition of MMT.

Olive oil does not enter into the WG film and, at least apparently, does not modify it either. In contrast, up to 60% Uvitex OB is lost in linear low-density polyethylene films subjected to the same test,[22] and this suggests that, at least for medium to large molecules, WG is a better barrier than linear low-density polyethylene. It is interesting to compare this behavior of WG to that of classic thermoplastic films. Most of these materials and their most common additives are highly nonpolar. Therefore, oil or other fatty products in contact may diffuse and plastify the film and thus trigger the diffusion of an additive. In most cases, this additive will be, in turn, soluble in oil and fatty materials and then will be quickly released. In contrast, WG appears to be a material resistant to nonpolar products.

An additional point that should be emphasized is that even if the apparent behavior is the same for all the FSLs, the underlying reasons are different. When WG films are exposed to an aqueous FSL, water diffuses into the film and acts as a plasticizer of WG: it modifies the inner structure and diminishes the barrier properties.[23] However, the release of Uvitex OB was not detected because its solubility in water is very low (even in 15% ethanol).

MMT migration tests

MMT is a clay mineral consisting of layers in which two silica tetrahedra enclose an alumina octahedron. The layers are about a nanometer large and are bonded by van der Waals forces and hydrated cations located between them; Al^{3+}, Na^{+}, Mg^{2+}, Ca^{2+}, and H^{+} are some of the most common. The general formula for these sheets is $[Al_{1.67}(Mg_{0.33}Na_{0.33})]Si_4O_{10}(OH)_2xH_2O$ with variable quantities of H_2O and interlayer cations. Hence, given that they are present in the highest quantities, aluminum and silicon were chosen as markers to follow the MMT migration.

Aluminum (Fig. 1) was found in significant quantities only in the acidic simulant as expected because aluminum is more soluble at a low pH. In this simulant, the quantities found after HP/LT and in the control were the same.

Silicon (Fig. 2) was found in a higher quantity than aluminum in all cases, and the highest concentrations were detected in 3% acetic acid too. Moreover, the amount of silicon detected was higher for all the FSLs after the HP/T treatment.

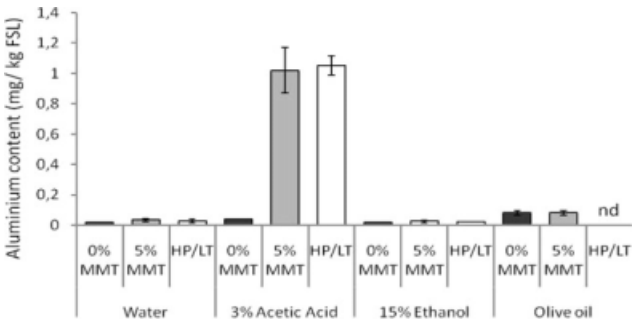

Figure 1 Concentration of aluminum in the FSL after the HP/LT treatment (800 MPa, 40°C) or the control (0.1 MPa, 40°C) versus the film without MMT. All were stored for 10 days at 40°C. The aluminum content in HP/T-treated olive oil was not determined.

However, the results for aluminum and silicon are not in agreement, and this makes it difficult to figure out which one is the more accurate marker for MMT. For example, if silicon is chosen as the marker, MMT particles are supposed to migrate 12 times more in distilled water than if aluminum is chosen as the marker. In both cases, the differences observed between the samples that did not contain MMT and those that did showed that virtually all the aluminum found came from MMT as well as most of the silicon. In Figure 1, all the bars apart from the samples containing MMT and immersed in 3% acetic acid represent values around the quantification limit of aluminum for this method (0.03 mg/L).

The fact that uneven quantities of aluminum and silicon were found suggests that MMT would be released not intact but after chemical modifications that might be caused by an acidic medium. Indeed, one of the most interesting properties of MMT is the possibility of exchanging the cations located in the interlayer space. The high concentration of H^+ in the 3% acetic acid solution (pH $\sim$ 2.9) could have substituted some of the exchangeable Al^{3+} (situated in the interlayer space), and this could explain their apparition in acidic media. However, that does not explain why aluminum is not present in the other media as silicon is, provided that both constitute the MMT sheets. During the processing of the film and mainly during the HP treatment, MMT undergoes mechanical tensions that may affect the structure of MMT.[24] Although the nature of these modifications is not well known, they might explain the higher concentrations of silicon in comparison with aluminum and, furthermore, the increase in silicon found after HP/LT. This possibility has to be further investigated in future work.

The effect of an HP/LT treatment on the release of MMT is not clear then, and it is evidenced that respective releases of aluminum and silicon are based on specific ways. The only conclusion that can be drawn for both is that migration was the highest in 3% acetic acid. Given that aluminum occurs in a ratio of 0.123 g of aluminum/g of MMT and silicon occurs in a ratio of 0.306 g of silicon/g of MMT, the highest value of migration obtained can be delimited between 8 (aluminum as the marker) and 15 mg (silicon as the marker) of MMT/kg of FSL.

Concerning food regulation, to date there is no specific migration limit on aluminum or silicon. However, the European Food Safety Authority has issued an opinion on the safety of aluminum from dietary intake and has established a tolerable weekly intake of 1 mg/kg of body weight/week (i.e., 60 mg/week/adult).[25] This is, in the worst case approach, equivalent to 8.6 mg of aluminum/kg of food, which is much higher than the values obtained here. Even for the highest values obtained in this work, the MMT migration of WG films is far from that limit. However, not only the amount of elements (aluminum and silicon in this case) should be taken into account when nanoparticles are used; the specific migration of the particles themselves must also be considered. Indeed, because of its highly developed surface, specific toxicology issues could appear, and the migration of nanoparticles should be quantified instead of a mere determination of its constituent elements, but it depends on the structure and size of the nanoparticle.[26] Tiede et al.[27] pointed out the importance (and difficulty) of developing analytical methods that provide information about the size and shape of nanoparticles in food matrices and the environment to carry out a reliable risk assessment. In conclusion, the potential modifications of the structure with HP treatments confirm the necessity of a thorough assessment of migration together with toxicological analysis whenever nanocomposites may be used in HP treatments.

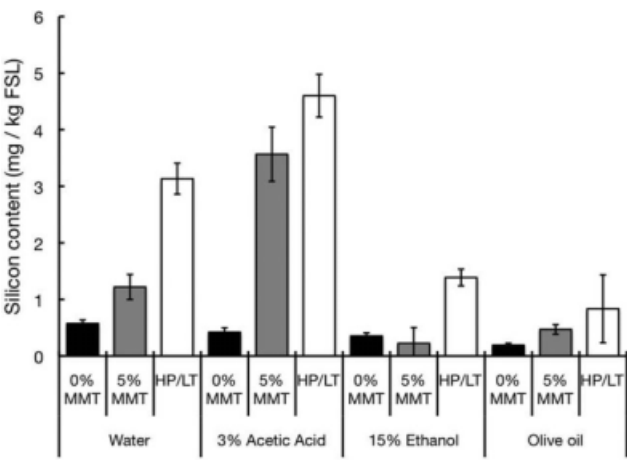

Figure 2 Concentration of silicon in the FSL after the HP/LT treatment (800 MPa, 40°C) or the control (0.1 MPa, 40°C) versus the film without MMT. All were stored for 10 days at 40°C.

TABLE I
Composition of the WG Used in the Preparation of the Films (Data from the Supplier) and Calculated Composition of the Films

		%	
		In WG	In the film
WG (68.3%)	Proteins	76.5	52.2
	Carbohydrates	11.8	8.1
	Lipids	5.0	3.4
	Ash	0.8	0.5
	Other	5.9	4.0
Glycerol		37.5	37.5
Uvitex OB		0.8	0.8
MMT		5.0	5.0

Protein and overall migration

WG–MMT films are composed of a variety of substances of different natures (Table I). Among these components, carbohydrates and glycerol are soluble in aqueous solvents, and part of the protein fraction may also solubilize, particularly in acidic and alcoholic media. Consequently, the results of overall and protein migration (Fig. 3) are high in comparison with conventional plastic materials because the standard tests (40°C, 10 days, and liquid media) are especially aggressive with water-sensitive materials. It is important to point out the need for the development and standardization of tests conceived for such materials under different conditions.

The samples had an average mass of 0.95 g. According to the migration results (and with consideration of the mass ratio of the sample to the FSL), the loss of mass from the film to the FSL roughly accounted for 10% in water and 20% in 3% acetic acid and 15% ethanol. Of these, the protein fraction

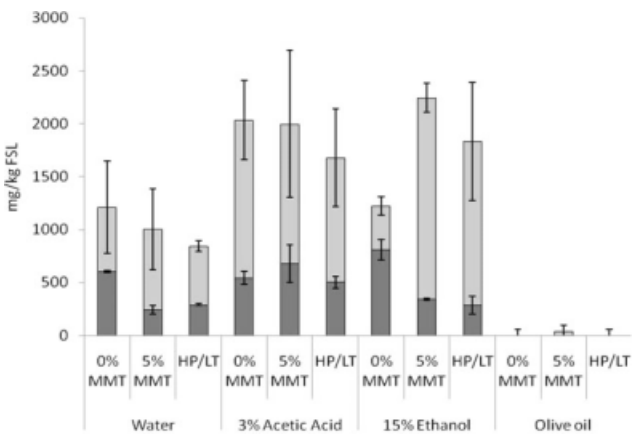

Figure 3 Overall migration and protein migration for samples containing 0 or 5% (w/w) MMT after 10 days of storage at 40°C. HP/T-treated samples contained 5% (w/w) MMT. The contribution of protein migration is represented as a darker region in each of the bars.

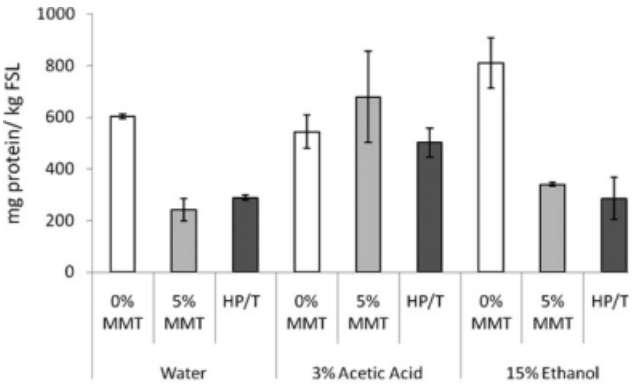

Figure 4 Migration of proteins in the aqueous FSL for samples containing 0 or 5% (w/w) MMT after 10 days of storage at 40°C.

accounted for only 4, 6, and 5% of the total losses in water, 3% acetic acid, and 15% ethanol, respectively. The losses in olive oil were negligible. This result shows that even if proteins are evidently the main components of WG–MMT, migration in aqueous simulants is not at all dependent only on protein migration but is also dependent on the other fractions. To obtain better insight, it could have been very interesting to determine the contents of the other fractions, in particular glycerol, which is present in the films at the second mass fraction.

According to the results in Figure 4, MMT addition seemed to decrease the amount of protein migration in water and in the 15% ethanol FSL, whereas no effect was detected in 3% acetic acid. It is well established that gliadins are more easily transferred to water and especially to 15% ethanol than glutenins. By definition, gliadins are characterized as the soluble fraction in 60% ethanol.[28] Gliadins would thus be responsible for a major part of protein migration to water and 15% ethanol. In the case of WG–MMT nanocomposite films, the addition of MMT would have a positive effect on gliadin migration and thus on overall protein migration. In this respect, Tunc et al.[7] showed that an increasing amount of MMT decreases the water uptake of WG films. On the contrary, both gliadins and glutenins are sensitive to a decrease in pH, and this effect fades in 3% acetic acid. It is important to point out that the results for protein migration do not follow the same tendency as those for overall migration. In effect, the trends of a reduction of protein migration in water and 15% ethanol when MMT is added are no longer observed for overall migration (Fig. 3). Other components (Table I) may have an important contribution to the overall migration values.

To evaluate the evolution of the protein content in the FSL throughout storage after treatment, the quantification of proteins was done just after the treatment (HP/LT and control) and after 3, 5, and

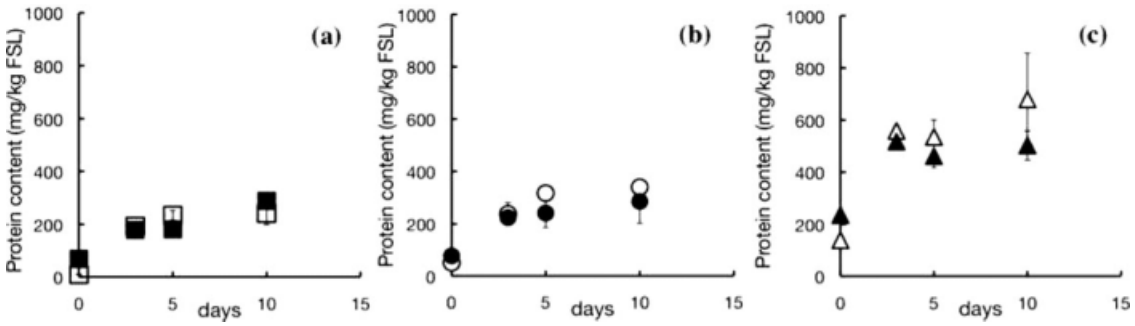

Figure 5 Release of proteins from samples containing 5% (w/w) MMT in (a) water, (b) 15% ethanol, and (c) 3% acetic acid. White symbols represent the HP/LT treatment (800 MPa, 40°C); black symbols represent the control (0.1 MPa, 40°C). After the treatment, both types were stored at 40°C.

10 days of storage at 40°C. A migration kinetic profile could then be outlined (Fig. 5). The release of proteins was fast; that is, roughly 70% of the final value was detected after 3 days of storage, and 90% was detected after 5 days. For the aqueous FSL (Fig. 4), the highest values were obtained for 3% acetic acid (500–700 mg/kg), and the lowest were obtained for water (200–300 mg/kg). In all cases, no significant difference was observed between the control and the HP/LT samples. Interestingly, if gliadins are the main proteins transferred in the FSL during storage, the HP treatment is expected to have little influence. Indeed, according to Kiefer et al.,[11] HP treatments mainly affect glutenins. It is not surprising that the treatment did not modify the transfer of proteins then. For olive oil, the protein content could not be determined by the BCA method because two difficulties arose: (1) the interference of many of the olive oil compounds in a colorimetric method and (2) the difficulty of separating with efficacy the proteins from the rest of the olive oil. To determine a lower threshold of WG in olive oil samples, SDS-PAGE was selected as a highly selective tool. According to the experimental conditions, the quantities found in all cases had to be far below 0.02 g/L (the most diluted standard tested) because no trace of color was left by the samples in the SDS-PAGE gel (Fig. 6). In conclusion, the tests of protein migration show the high sensitivity of WG toward water and its resistance to olive oil (fat simulant). This behavior is rather different from that of common plastic materials, which are generally more sensitive to apolar substances.

With respect to overall migration, no significant difference between the samples was observed for water or 3% acetic acid, regardless of the MMT content or the HP/T treatment. However, both factors were suspected to increase the resistance of the film to aqueous media. The addition of MMT is known to decrease the swelling in cast WG films,[7] and HP/T treatments increase the disulfide interchange in WG proteins.[11] There is a clear effect of MMT on overall migration in 15% ethanol, but we do not have any clue about this behavior. Among the aqueous FSLs, the highest migration values were detected in 3% acetic acid and 15% ethanol between 1500 and 2000 mg/kg, and they roughly doubled the results obtained in water. Several reasons account for this: WG proteins are more soluble in acidic solutions than in water; gliadins, some of the components of WG, are soluble in alcoholic solutions;[28] and the lipid fraction (Table I) is not soluble in water. Therefore, any factor contributing to a decrease in the pH of an aqueous solution will increase the solubilization of WG, and the addition

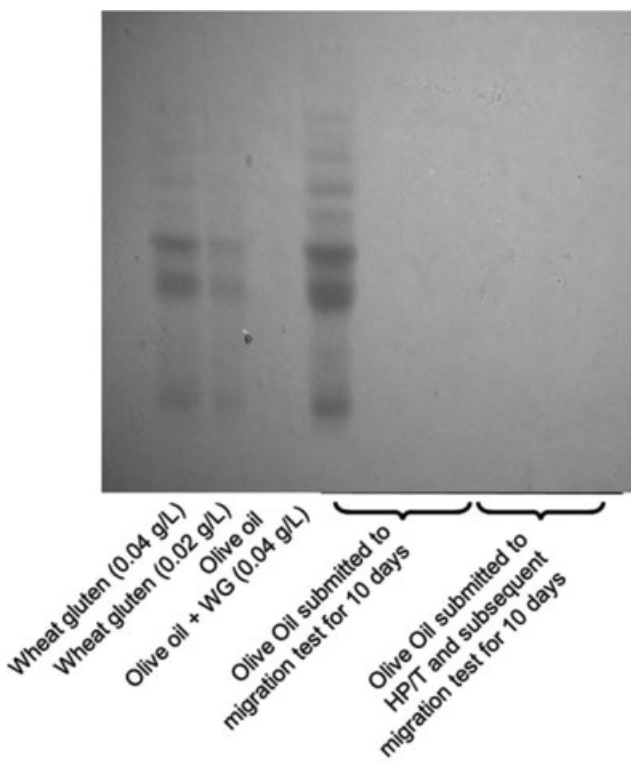

Figure 6 SDS-PAGE pattern of a WG solution, olive oil, olive oil including WG, and olive oil subjected to contact with WG for 10 days after the HP/T treatment.

TABLE II
Overall Migration of Samples Containing 5% (w/w) MMT in Food-Simulating Solids After 10 Days of Storage at 40°C at Different RH Values (0 and 80%)*

	Overall migration (mg/6 dm^2 of film)[a]
Tenax (0% RH)	28 ± 11
Tenax (80% RH)	48 ± 13
Agar gel (80% HR)	3849 ± 433

[a] Note that 6 dm^2 corresponds to the surface of film equivalent to 1 kg of FSL according to the ratio followed throughout this article.

* The samples were not submitted to HP treatments.

of ethanol will solubilize some of the gliadins and the lipid fraction. For olive oil, overall migration was evaluated as close to the limit of quantification (evaluated as 53 mg/kg of olive oil), and this showed the excellent resistance of the film to fatty material.

An emerging issue of food safety is how to deal with the compliance of all the novel materials recently developed for advanced packaging applications. Most of these have complex structures and are especially tailored for a specific purpose, and this makes it even more difficult to develop standard and universal migration tests. To imitate the actual conditions of use for WG-based films, food-simulating solids were also tested. Tenax [modified poly (phenylene oxide)] and agar gel were selected to simulate nonpolar and polar foodstuffs, respectively. These two materials also represented two types of food product structures: a powder (for Tenax) and a gel that could be of great importance with respect to migration evaluation because close contact between the film and the product will increase the transfer. An FCM and solid food simulant were put in contact in a migration cell as described previously by the European Committee for Standardization.[4] A low RH (0%) and a high RH (80%) were chosen as storage conditions for Tenax to simulate the packing conditions of dry and intermediate water activity products; only the high value of RH was chosen for the agar gel model food. As shown in Table II, overall migration from the FCM to the solid food simulants was successfully evaluated on the control system (without the HP/T treatment). Unfortunately, the integrity of the system was not preserved anymore during the HP/T treatment; that is, some of the agar gel was poured out of the cell, and the Tenax powder was compacted and was strongly stuck to the film sample. This prevented its separation unless the film was torn.

The results of overall migration with Tenax and agar gel (Table II) underpin the previous statements about the sensitivity of WG films toward humidity and moisture. Indeed, migration in Tenax was much lower than in the agar gel and in the aqueous FSL in both cases (0 and 80% RH). Likewise, migration at 0% RH was significantly lower than at 80% RH, and this confirmed the influence of humidity on the WG film structure and showed that WG films are potentially suitable for the packaging of dry products (or products that do not release water). Migration in the agar gel was much higher than in Tenax and, unexpectedly, even higher than in the aqueous FSL. A likely hypothesis is that the agar gel in contact with the gluten film got stuck to the film and pulled out a part of the film when they were split. This unexpected result points out another potential failing of the standard migration tests: the assumption that the transfer from the packaging to foodstuff takes place exclusively by diffusion (or rather by mass transfer). This is indeed the case in liquids, but it would be interesting to also pay attention to the effects of solids on FCMs. Some solid food products may abrade, scratch, or simply get stuck to the surface and mechanically pull out a part of the material; that is, the layer of nonstick pans is eroded often because of cleaning but also because of cooking.

In conclusion, even though an outstanding effort has been made in the last decades to ensure the compliance of FCMs in the most representative cases, there is still a lot of work to do to take into account more particular or new situations.

CONCLUSIONS

A full case study of a potential novel packaging material has been presented that deals with migration in service life and a stabilization process (HP/LT). No effect of HP/T treatments was observed on overall migration and protein migration from the WG nanocomposite films into four standard FSLs. It was nonetheless pointed out that the classic migration tests were not suitable for water-sensitive materials, and to replace them, migration tests were carried out with solid food simulants (agar gel and Tenax). Both gave a satisfactory performance for the migration tests at atmospheric pressure, but they failed when subjected to HP/LT treatments. This fact points out the difficulty of designing universal migration tests and the necessity of adapting them to specific situations, especially because the complexity of packaging materials increases more and more.

On the other hand, the release of nanoparticles was particularly studied, but the influence of HP/T treatments on the release of MMT could not be clarified because of the different results obtained with the markers used (aluminum and silicon). Because silicon was found in higher quantities after HP/LT treatments, a modification of the structure of MMT

is hypothesized, and it will be addressed in future work to assess the compliance of nanocomposite materials undergoing HP treatments.

The authors acknowledge Sana Raouche for her help with the electrophoresis experiments.

References

1. Ray, S. S.; Bousmina, M. Prog Mater Sci 2005, 50, 962.
2. Guillard, V.; Mauricio-Iglesias, M.; Gontard, N. Crit Rev Food Sci Nutr, to appear.
3. Rastogi, N. K.; Raghavarao, K.; Balasubramaniam, V. M.; Niranjan, K.; Knorr, D. Crit Rev Food Sci Nutr 2007, 47, 69.
4. CEN/TC 172: Paper and Board Intended To Come into Contact with Foodstuffs—Conditions for Determination of Migration from Paper and Board Using Modified Polyphenylene Oxide (MPPO) as a Simulant; European Committee for Standardization: Brussels, Belgium, 2003.
5. Lagaron, J. M.; Fernandez-Saiz, P.; Ocio, M. J. J Agric Food Chem 2007, 55, 2554.
6. Guillard, V.; Broyart, B.; Bonazzi, C.; Guilbert, S.; Gontard, N. J Food Sci 2003, 68, 958.
7. Tunc, S.; Angellier, H.; Cahyana, Y.; Chalier, P.; Gontard, N.; Gastaldi, E. J Membrane Sci 2007, 289, 159.
8. Olabarrieta, I.; Gallstedt, M.; Ispizua, I.; Sarasua, J. R.; Hedenqvist, M. S. J Agric Food Chem 2006, 54, 1283.
9. Shewry, P. R.; Tatham, A. S. J Cereal Sci 1997, 25, 207.
10. Apichartsrangkoon, A.; Ledward, D. A.; Bell, A. E.; Brennan, J. G. Food Chem 1998, 63, 215.
11. Kieffer, R.; Schurer, F.; Köhler, P.; Wieser, H. J Cereal Sci 2007, 45, 285.
12. Mujica Paz, H.; Gontard, N. J Agric Food Chem 1997, 45, 4101.
13. Barron, C.; Varoquaux, P.; Guilbert, S.; Gontard, N.; Gouble, B. J Food Sci 2002, 67, 251.
14. Angellier-Coussy, H.; Torres-Giner, S.; Morel, M. H.; Gontard, N.; Gastaldi, E. J Appl Polym Sci 2008, 107, 487.
15. Avella, M.; De Vlieger, J. J.; Errico, M. E.; Fischer, S.; Vacca, P.; Volpe, M. G. Food Chem 2005, 93, 467.
16. 85/572/EEC Council Directive of 19 December 1985 Laying Down the List of Simulants To Be Used for Testing Migration of Constituents of Plastic Materials and Articles Intended To Come into Contact with Foodstuffs; Official Journal of the European Union, 1985.
17. 2002/72/EC Commission Directive of 6 August 2002 Relating to Plastic Materials and Articles Intended To Come into Contact with Foodstuffs; Official Journal of the European Union, 2002.
18. Community Reference Laboratory for Food Contact Materials. Method for the Determination of Overall Migration into Olive Oil by Total Immersion. http://crl-fcm.jrc.it/files/om2_oil_immersion.pdf (accessed June 2009).
19. Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C. Anal Biochem 1985, 150, 76.
20. Hidalgo, F. J.; Alaiz, M.; Zamora, R. Anal Chem 2001, 73, 698.
21. Laemmli, U. K. Nature 1970, 227, 680.
22. Mauricio-Iglesias, M.; Jansana, S.; Guillard, V.; Peyron, S.; Gontard, N. Food Addit Contam A 2010, 27, 118.
23. Gontard, N.; Thibault, R.; Cuq, B.; Guilbert, S. J Agric Food Chem 1996, 44, 1064.
24. Hrachova, J.; Komadel, P.; Fajnor, V. S. Mater Lett 2007, 61, 3361.
25. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials. EFSA J 2008, 754, 1.
26. Limbach, L. K.; Li, Y. C.; Grass, R. N.; Brunner, T. J.; Hintermann, M. A.; Muller, M.; Gunther, D.; Stark, W. J. Environ Sci Technol 2005, 39, 9370.
27. Tiede, K.; Hassellöv, M.; Breitbarth, E.; Chaudhry, Q.; Boxall, A. B. A. J Chromatogr A 2009, 1216, 503.
28. Guilbert, S.; Gontard, N.; Morel, M. H.; Chalier, P.; Micard, V.; Redl, A. In Protein-Based Films and Coatings; Gennadios, A., Ed.; CRC: Boca Raton, Florida, 2002.