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Influence of Microwave Heating Time on the Structure and Properties of Chitosan Films

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ABSTRACT: The aim of this study was to investigate chitosan film behavior during microwave heating for 10 different heating times from 0 to 40 min. Chitosan films were produced by casting. Their structure and properties were investigated with several techniques, including Fourier transform infrared spectroscopy and differential scanning calorimetry, but also by the measurement of the film color and the mechanical properties or by the study of the rheological properties of the rehydrated films. An original technique of gas chromatography (electronic nose) was used to analyze the film odor and highlight the presence of volatile compounds related to the Maillard reaction occurring during film heating. The results show that structural modifications occurred in two steps; this affected the polymer structure, such as the crystallization and chain scission. The appearance of the neoformed compounds was also observed and must be controlled to guarantee the safety of this food-contact packaging material. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40779.

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INTRODUCTION

Nowadays, with increased environmental concerns and growing questioning about an oil-based materials future, there has been a huge development in the bioplastics industry. Bioplastics are supposed to be more ecofriendly, with a lower impact on the environment; dozens of green plastics have emerged in the past 15 years.[1,2] Although those materials represent a great opportunity and meet the demands of greener chemistry, biopolymers are not necessarily safe or inert.[3]

Because of the opportunity for these materials to be used as food packaging materials,[4] their behavior during aging or processing, such as microwave heating, need to be investigated to guarantee consumer safety. Because of the bisphenol A crisis,[5–9] concerns about the behavior of polymer materials used in food packaging have increased and if traditional oil-based polymers have already been tested under microwave irradiation,[10–14] biopolymer behavior is still barely known. Only a few publications have reported chitosan molecular weight degradation with microwave irradiation.[15,16]

Furthermore, microwave irradiation with commercial domestic microwave ovens has received increasing interest because of the noticeable enhancements in chemical reactions and significant effects over conventional reactions.[17,18] Microwave heating is qualified as internal heating and is supposed to increase the reaction rate compared to classical methods.[19] The study of biopolymer behavior under microwave irradiation is not only a way to test the material in actual use conditions but also a way to simulate accelerated aging.[20]

Chitosan is a biodegradable and biocompatible biopolymer;[21] this allows for its use in a wide range of applications, including pharmaceuticals, biotechnology, food, agriculture, and tissue engineering.[22] Chitosan is a polysaccharide (a copolymer of 1–4 linked β-D-glucosamine and β-D-*N*-acetylglucosamine) obtained from chitin deacetylation extracted from crustacean shells, such as crabs or shrimps.[23] This material has been widely studied because of its ability to act as an antimicrobial film[24] and its opportunities for use in food contact.

The aim of this study was to identify microwave treatment consequences on chitosan films. The material structure was investigated before and after treatment, and the consequences on structure and properties were evaluated.

EXPERIMENTAL

Chitosan and Casting Supplies

The chitosan (Sigma-Aldrich, Saint-Quentin, France) used during the experiments was a biochemical product extracted from

shrimp cells and had a viscosity of less than 200mPa s in 1% acetic acid (99% pure, Chem-Lab, Belgium) at 20°C according to the supplier. Petri dishes (Optilux), provided by Nunclon (Fisher, Roskilde, Denmark), were 10-mm high with a diameter of 90 mm.

Film Production

Chitosan is a highly basic polysaccharide and is insoluble in water; it becomes soluble in diluted acids, such as acetic or formic acid.[25] A film-forming solution was prepared by the dissolution of 1 g of chitosan in a 1% v/v acetic acid 99% pure (Chem-Lab, Belgium) aqueous solution with a heating magnetic stirrer (Fisher Bio-Block Scientific) for 24 h at 20°C and 350 rpm. After mixing, the solution was filtered *in vacuo* on a 5-μm nitrocellulose filter (Millipore, France) and was finally degassed before it was poured into Petri dishes and dried at 20°C and 50% relative humidity for at least 48 h.

Film Conditioning

Microwave treatment involves water molecule agitation and causes heating. As humidity plays an important part during microwave processing, this parameter appeared to be very important to control. After a 48-h drying period, each film was conditioned in a controlled activity water atmosphere at 20°C and in darkness. $Mg(NO_3)_2$ salt (Sigma-Aldrich, Saint-Quentin, France) was used to adjust the water activity at 0.53 in a climatic chamber at 20°C.

Microwave Processing

A microwave device (Matfer, France) was used with different heating times (0, 4, 8, 12, 15, 20, 25, 30, 35, and 40 min) at a controlled power (800 W). A volume of 600 mL of distilled water at 20°C was put into a beaker, and nothing was done to prevent vapor production and diffusion into the microwave oven during heating. Chitosan films were put into the microwave device individually, and heating treatment was applied. Finally, the surface temperature of the film before and after microwave treatment was measured with an IR thermometer (ThermoFlash). This process simulated microwave heating of a highly humid food product.

Film Thickness Measurement

The films thickness was determined according to the standard NF Q 03–016 with a manual micrometer (Mitutoyo, Kawasaki, Japan), equipped with a head of 5 mm in diameter with a 1-μm sensitivity. The thickness was measured in 10 randomly selected points on each film, and the average value was calculated.

Moisture Content (MC) Measurement

Three samples of each microwave heating time tested were weighed (m_0) and were then dried in oven at 102°C for 3 h (according to ASTM D 644-99). The films were then weighed again (m_f) to determine their MC. MC was calculated as the percentage of weight loss relative to the original weight:

$$MC(\%) = \frac{(m_0 - m_f)}{m_f} \times 100 \quad (1)$$

Film Color Measurement

Measurements were carried out with a Minolta CM CR-210 colorimeter (Minolta, Colombes, France) with the Commission Internationale de l'Eclairage (CIE) Lab color scale. The results were the average of at least 10 points on each film, and three films for each heating time were tested. The color differences (ΔE) were calculated as follows:

$$\Delta E = \sqrt{(L_t - L_{t0})^2 + (a_t - a_{t0})^2 + (b_t - b_{t0})^2} \quad (2)$$

where L_t is L value after t minutes of heating; L_{t0} is L value at $t0$; a_t is a value after t minutes of heating; a_{t0} is a value at $t0$; b_t is b value after t minutes of heating; b_{t0} is b value at $t0$, where L^* is the lightness (from 0 for black to 100 for white). The a^* value is assigned to redness (positive a^* values) or greenness (negative a^* values) in the sample. Finally, the b^* value describes the yellowness (positive b^* values) or blueness (negative b^* values) in the sample.

The yellowing index (YE)[17] was measured in accordance with ASTM D 1925 as follows:

$$YE = \frac{100 * (C_x X - C_z Z)}{Y} \quad (3)$$

where X, Y, and Z are the CIE tristimuli values and the coefficients C_x and C_z depend on the illuminant and observation angle. The illuminant used was D65, and the observation angle was 10°; the involving C_x and C_z were 1.3013 and 1.1498, respectively, in this case.

Electronic Nose/Headspace Chromatography Associated with the Volatile Compound Library

An electronic nose Heracles II (Alpha MOS, Toulouse, France) was used to detect the off odors produced during heating. It consisted of double-column gas chromatography with hydrogen (flame ionization detector grade) used as the gas carrier.

An amount of 0.30 g of the chitosan film was sealed hermetically in a vial and heated at 40°C for 1 h to allow volatile compound release. A volume of 1 mL of a headspace atmosphere was injected into the device via a syringe to an injector, where a flash evaporation process happened. Volatile compounds from the sample headspace then passed through an adsorbent trap to be concentrated. The system was finally reheated to release trapped compounds, which were subsequently injected in two short capillary columns (2-m long each), one apolar DB-5 and the second one with a medium polarity DB-1701. Two flame ionization detectors in parallel provided two chromatograms, which were later analyzed with Heracles software. The coupling of two columns allowed a sharper identification of the volatile compounds.

Film Fourier Transform Infrared (FTIR) Analysis

The biopolymer film structure was investigated by FTIR spectroscopy in attenuated total reflection mode before and after microwave treatment. Measurements were performed at 20°C with a Tensor 27 mid-FTIR Bruker spectrometer (Bruker, Karlsruhe, Germany) equipped with a platinum attenuated total reflection optical cell and an RT-Dla Triglycine Sulfate (TGS) detector (Bruker, Karlsruhe, Germany). The diaphragm was set to 6 mm. The scanning rate was 10 kHz, and 64 scans were performed both for reference (t_0), where t_0 is equal to 0-minute-heating time and for the samples (microwave-heated) from 3800 to 900 cm^{-1} with a 4-cm^{-1} resolution. All of the data

Table I. Characteristics of Chitosan Films During Microwave Treatment (800 W and 100% RH)

	Microwave treatment time (min)								
	0	4	8	12	15	20	25	30	35	40
Film thickness (μm)	48 ± 5	47 ± 3	46 ± 4	46 ± 4	46 ± 3	47 ± 7	46 ± 4	46 ± 5	47 ± 4	46 ± 4
Film water content (%)	12.8 ± 0.3	11.1 ± 1.1	11.9 ± 2.1	9.9 ± 0.7	9.6 ± 0.7	10.0 ± 1.0	10.4 ± 0.9	12.4 ± 2.6	11.1 ± 2.8	9.9 ± 1.4
Film surface temperature (°C)	20	50 ± 5	83 ± 1	98 ± 1	100 ± 2	108 ± 2	111 ± 3	117 ± 0	111 ± 3	115 ± 1
DSC										
Moisture peak temperature (°C)	79.7	NM	NM	92.1	NM	94.0	NM	93.3	NM	96.4
Degradation peak temperature (°C)	297.4	NM	NM	295.8	NM	295.9	NM	291.9	NM	286.7
Glass-transition temperature (°C)	Glass transition not observable on DSC thermogram							142	NM	NM

NM, not measured.

treatments were carried out with OPUS software (Bruker, Karlsruhe, Germany). The raw absorbance spectra were smoothed with a 9-point Savitsky–Golay smoothing function. Elastic baseline correction was applied to the spectra.

Differential Scanning Calorimetry (DSC)

We performed thermal analysis by sealing 5.5–8 mg of the chitosan film sample in an aluminum pan. The pan was put in the DSC device (Netzsch, Germany) and heated under protective nitrogen in three steps. First, the sample was heated from 25 to 180°C at a rate of 10°C/min to eliminate its thermal history. Then, it was cooled from 180 to 25°C at a rate of 30°C/min. Finally, the sample was heated from 20 to 350°C at the same rate of 10°C/min.

Rheological Behavior

Rheological measurements were performed with a Kinexus rotational rheometer (Malvern Instruments, KNX 2100, United Kingdom) on rehydrated films solutions. A Kinexus rheometer (Malvern Instruments, Orsay, France) was equipped with a temperature control unit (Peltier effect) for a very accurate temperature ramp. The rheological properties (viscous and elastic components and the angle phase) were measured at 20°C with cone (CP2/50 SC0029SS) and plate (PL65 SO381SS) geometry. The solutions were prepared in 1% v/v acetic acid solution at a 2% w/v chitosan film concentration.

Various shear rates ($\dot{\gamma}$) were applied to the samples from 10^{-1} to 10^{2} s^{-1}. The power law model[26] was applied to the raw data:

$$\sigma = K(\dot{\gamma})^{n} \tag{4}$$

where σ is the shear stress (Pa), K is the consistency coefficient, and n is the flow-behavior or power law index. The n parameter is representative of the viscosity and rheological behavior of the solution, that is, $n = 1$ when the solution is a Newtonian fluid, $n < 1$ when it is a pseudo-plastic fluid (shear thinning), and $n > 1$ when it is a dilatant fluid (shear thickening).

Molecular Weight Determination of Chitosan

Size exclusion chromatography (SEC) with multiangle laser light-scattering detectors (MALLS) was used to study the molecular weight distributions of chitosan according to the method described by Nguyen et al.[27] The refractive index (RI) was measured with a microviscosimeter, and the molecular weights were determined relative to chitosan standards (American Polymer Standard). For every sample, chitosan solutions (1 mg/mL) were prepared by direct dissolution in acetate buffer (0.3*M* acetic acid and 0.3*M* sodium acetate, pH 4) at room temperature. The acetate buffer used for dissolution was from the same batch as the mobile phase used for SEC. The mobile phase was filtered before use through a hydrophilic Millipore membrane with a 0.45-μm pore size (47-mm in diameter, Millipore). Analyses were performed at 30°C at a flow rate of 0.7 mL/min with a sample injection volume of 100 μL with a Viscotek A4000M column f (7.8 mm *i.d.* and 30 cm length). Chitosans with different molecular weights were used as standards to determine the sample molecular weights.

Mechanical Property Evaluation

Mechanical characterization tests, specifically traction, were performed in collaboration with ProViSys Engineering (http://

www.provisys-eng.com). The samples were 40-mm wide, 30-mm high, and 48-μm thick. On each sample, four position dot points were drawn and used as markers for the extensometer. Traction was performed at constant strain rate (10^{-4} s^{-1}) on a traction device (Instron 5800 series, ITW, Elancourt, France) equipped with an original extensometer (VidéoTraction system). This software allows real-time monitoring of localized deformation (throughout markers placed on the specimen) and regulates the machine speed at a constant strain rate.

Statistical Analysis

Experimental values were given as means plus or minus the standard deviations. Analysis of variance was used to compare the mean differences of the samples. Differences at $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

The microwave heating process was designed to simulate humid food cooking and to limit the influence of water and temperature on the study. Indeed, the film surface temperature increased with the microwave treatment time. It reached 100°C at 15 min of heating and finally stabilized around 110°C from 20 min of treatment. Thus, the inside of the microwave oven was full of steam; this allowed us to assume that the relative humidity remained constant during the whole heating process (100%). This hypothesis was confirmed by film humidity measurements at each heating time; these remained constant around 11% (Table I). Moreover, the chitosan film thickness was not affected by the microwave heating time and remained stable at 47 ± 0.7 μm; this indicated that the chitosan films did not swell (Table I).

Thermal Properties of the Chitosan Films

The thermograms obtained by DSC measurement on the chitosan films showed three events. During the first heating, the unique endothermic peak was attributed to absorbed moisture evaporation in the chitosan film. The cooling curve showed an exothermal peak corresponding to partial polymer crystallization. The glass transition was hard to detect in chitosan. During the second heating, a slight change in the baseline inclination at 140°C indicated the film glass transition. This result was consistent with the glass-transition temperature found in the literature; even several different values[28,29] were reported.[30] Finally, when the temperature increased, chitosan film degradation occurred, starting at 290°C.

Chitosan is mainly amorphous, so a clearly defined glass transition could be expected on a DSC thermogram. However, the glass transition remained nonmeasurable until long time heating. The main hypothesis for this phenomenon was a polymeric chain size homogenization. Indeed, the DSC device had a limited sensitivity and was not able to detect a discreet heating flow. We assumed that tge chitosan film was initially made of different sized chains. During microwave treatment, small chains easily tended to join each other, whereas the long polymeric chains seemed to be broken. This led to polymeric chain size homogenization, with a more accurate glass-transition temperature, which became detectable by the DSC device. The hypothesis of depolymerization occurring during microwave heating was consistent with previous work showing that chitosan is susceptible to depolymerization and that the phenomenon was enhanced with increased temperature.[31] Thus, several studies showed that low-mass chitosan is more soluble at neutral pH; this makes it potentially more available *in vivo*[32] and explains why it has been reported as causing more cell damage.[33,34]

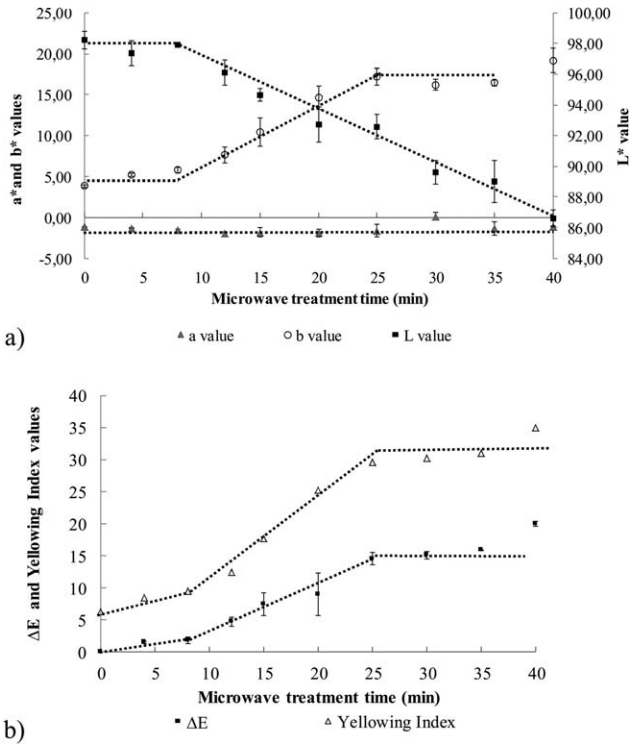

Figure 1. Chitosan film color measurements during the microwave treatment: (a) a^*, b^*, and L^* and (b) ΔE and YE.

Film Color

The L^*, a^*, and b^* values during microwave heating are presented in Figure 1(a). It appeared that the a^* values, which were slightly negative and close to zero, remained stable during heating, whereas the positive b^* values increased. On the contrary, the L^* value tended to decrease because of film darkening, which became stronger during heating. To be more accurate, the b value did not increase linearly. The curve showed three slopes corresponding to three steps of yellowing. The first step, from 0 to 8 min, showed the beginning of film yellowing. The phenomenon accelerated from 8 to 25 min, as described by the second slope in Figure 1(a). Finally, for long-time heating (from 25 to 40 min), the yellowing was intense and became stable.

To confirm this hypothesis, yellowing index (YE) was calculated in accordance with ASTM D 1925 and is presented in Figure 1(b) and compared to the color difference ΔE to show the global difference in color during heating. Both ΔE and YE increased during heating, and their respective slope showed the same behavior.

ΔE increased with the microwave heating time up to 20. It is important to point out that a ΔE value below 2 is considered imperceptible to the human eye. In this case, the film yellowing was perceptible after 4 min of microwave heating.

This phenomenon led us to conclude that the film color significantly changed during microwave heating. The yellowing was

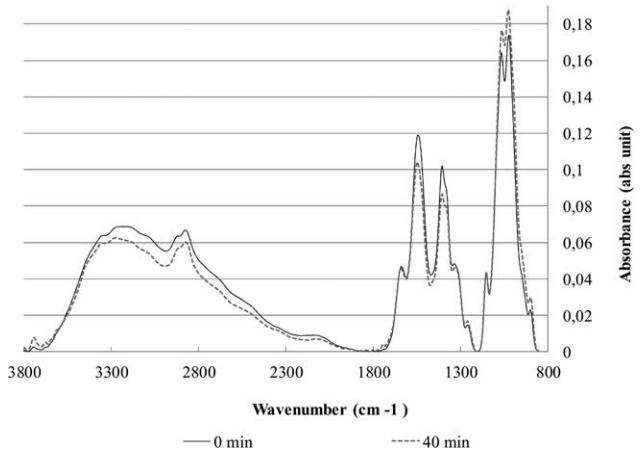

Figure 2. FTIR spectra between 800 and 3800 cm^{-1} for the chitosan films that were microwave-heated for 0 and 40 min as examples of the 10 heating times studied. The spectrum shown for each heating time is the average spectrum obtained from at least three samples.

the main component of color change and was initiated from the very beginning of heating, first slowly, then more quickly, and finally remained stable for long-duration heating. This result has implications in both consumer confidence and safety. A film color change, even for a short heating time, means that the film endures chemical changes that are possibly detrimental for its quality and maybe its use safety. We assumed that neoformed compounds could appear, for instance, Maillard reaction products responsible for film coloration, as previously described in the literature.[35–37]

Film Structure Changes

FTIR spectroscopy has been shown to be a powerful, rapid, easy to apply, and nondestructive tool for studying the polysaccharide structure. Figure 2 displays the chitosan film spectra obtained for 0 and 40 min of microwave heating. Each spectrum is the average spectra of at least three spectra, and the standard deviation is not pictured because of the perfect superposition of the different repetitions for each heating time.

Principal component analysis (PCA) analysis of the FTIR spectra (0, 4, 8, 12, 15, 20, 25, 30, 35, and 40 min of heating) was performed with The Unscrambler software. The data set for PCA calculation contained 10 rows corresponding to the number of samples studied and 814 columns referring to the wave numbers (cm^{-1}). The score plot obtained is shown in Figure 3. On this chart, each point corresponds to each different sample from different heating times. It revealed that each spectrum was significantly different from the reference (nonheated chitosan film, t_0); this means that the FTIR spectra was modified, even for short microwave heating times.

The longer the chitosan film was heated, the statistically more different the sample became from reference. This phenomenon was amplified with increased microwave heating time; that is, the longer a chitosan film was heated, the more the FTIR spectra was modified. From PCA analysis, three groups of samples emerged, as presented in Figure 3. The first group only contained the reference sample, t_0. The second group contained samples heated from 4 to 25 min; these were statistically close to each other from the FTIR spectral analysis point of view. Finally, the third and last group included samples heated for 30, 35, and 40 min.

This categorization into three groups was based on the statistical analysis performed on the film FTIR spectra. Interestingly, these three groups of samples were observable on the color analysis, especially with the b^* values (i.e., the yellowing of the chitosan film); this led us to the conclusion that some structural changes highlighted by FTIR spectroscopy were responsible for the film color change.

From a qualitative point of view, the spectral global shape remained the same between 0 and 40 min of heating (Figure 2). Some peaks appeared to be modified by microwave heating and changed according to the heating treatment time. Particularly, a large wave around 3232 cm^{-1} corresponding to the hydroxyl bonds remained quite stable with heating time. These hydroxyl bonds are usually related to water in the sample. The microwave process did not significantly affect the sample humidity; this was in agreement with the film water contents (Table I).

The peak at 1026 cm^{-1} was identified as -OH elongation vibrations and increased during microwave heating. The breaking of the C-O-C glycosidic bond led to chain scission on the chitosan polymeric chain. We confirmed this phenomenon by following the 1060-cm^{-1} peak corresponding to -OH, which increased with microwave heating time. This was consistent with the former hypothesis of polymeric chain scission and the creation of hydroxyl groups, and it was also consistent with previous work on chitosan solutions, which showed molecular weight degradation with microwave irradiation according to Wasikiewicz and Yeates.[15]

The peak at 1542 cm^{-1} corresponded to the amide-II band and decreased during microwave treatment; this was consistent with chitosan deacetylation.[38] As previously shown, this decrease occurred in two steps corresponding to a slow decrease between 4 and 25 min; then, there was a steeper slope for 30, 35, and 40

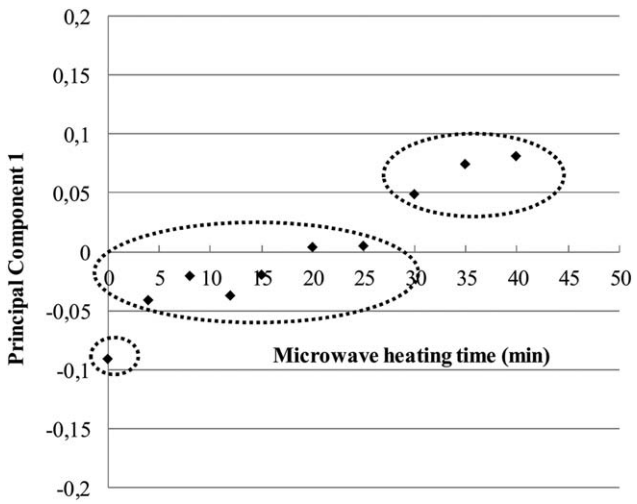

Figure 3. Score plot extracted from the principal component analysis of 10 average FTIR spectra corresponding to different heating times ranging from 0 to 40 min. Each circle regroups the FTIR spectra that were considered statistically closed by PCA.

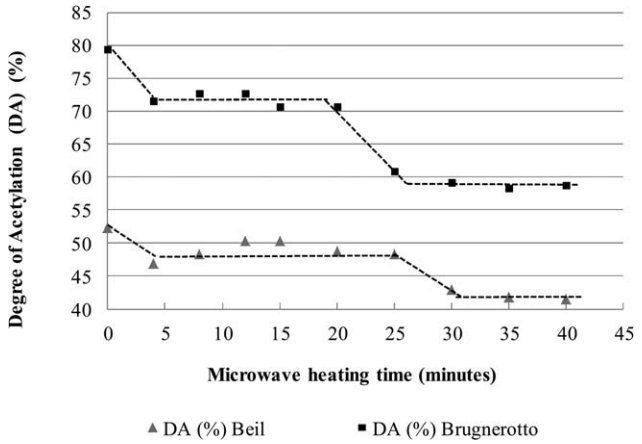

Figure 4. Calculation of the DA of the chitosan films during microwave heating according to Beil[39] and Brugnerotto.[40]

min of microwave heating. The peak at 898 cm^{-1} increased by almost 60% between 0 and 40 min. This peak was related to ring stretching. In the meantime, peak at 1405 cm^{-1} related to $-CH_2$ and $-CH_3$ decreased by 15%. All of this information led to the hypothesis that chitosan was deacetylated during heating.

To confirm this hypothesis, the degree of acetylation (DA) was calculated. Previous works investigated the possibility of determining the deacetylation degree (DD) with IR spectra and showed good correlation,[38] for example, with a ratio between the absorbance at 1539 cm^{-1} corresponding to the -NH bending in amide groups and the absorbance at 2869 cm^{-1} corresponding to the -CH stretching band.[39]

Two different methods were used to calculate DD according to Beil[39] and Brugnerotto.[40] These methods are based on different peaks of interest in the FTIR spectra. As shown in Figure 4, DA tended to decrease with microwave heating time. DD was not the same, depending on the method of calculation, but in both case, it decreased. Again, we discerned three steps. The first step was the beginning of deacetylation, from 0 to 4 min of microwave heating. DA remained stable during heating continued until 20 or 25 min (depending on the method of calculation), where the deacetylation decreased again and reached a second equilibrium for a long heating time.

Rheological Behavior

To confirm the chain-scission hypothesis, a rheological study was performed on a rehydrated film, as solution obtained by film dissolution should show viscosity modification if the polymer structure is degraded.

Table II shows the rheological results obtained for a solution of rehydrated chitosan films after different microwave heating times (0, 15, and 30 min). The power law model was applied on the raw data.

The *n* parameter was always below 1; this indicated a non-Newtonian pseudo-plastic fluid in every case. The *n* parameter decreased with increasing microwave heating time. Two parameters affected the chitosan solution viscosity: the molecular weight and DD. According to Mucha,[41] this indicated that DD decreased. As the chain-scission phenomenon had a higher impact on the viscosity than DD, the apparent viscosity decreased although the chitosan deacetylation increased (i.e., DA decreased).

Molecular Weight Determination of Chitosan

SEC–MALLS was used to determine the weight-average molecular weights before and after microwave heating to confirm the chain-scission hypothesis. It decreased from 271 kDa (before microwave heating) to a value of 175 kDa after 40 min of microwave heating. Moreover, the polydispersity index also decreased from 41 (before heating) to 16 (after 40 min of heating). This led us to confirm the previous hypothesis; that is, the polymer chain scission and homogenization in the size of the polymeric chains were strongly linked to chain scission.

Electronic Nose/Headspace Chromatography Associated with Volatile Compound Library

The off flavor in the chitosan films could have been responsible for a loss of consumer confidence. Moreover, because of the fact that the polymer structure was modified by microwave heating, neoformed compounds could be produced and could be potentially hazardous. To check this point, electronic nose analysis was performed not only on odorous volatiles but also on nonodorous compounds; this allowed the analysis of potential hazardous nonodorous compounds.

On the chromatograms obtained after electric nose analysis, several peaks were observed as displays in Table III.

The first identified peak corresponded to acetaldehyde, and α-aminoacetaldehyde has been found in the literature to be an intermediate product of glucosamine degradation in water; this leads to the final production of pyrazine.[42] This explains the high amount of acetaldehyde found for nonheated chitosan. The acetaldehyde peaks decreased between 0 and 30 min of microwave heating; this was consistent with the pyrazine formation observed and described hereafter. Trimethylamine,[43] a volatile compound found in sea products, was strongly present in the chitosan film before microwave heating but vanished after treatment.

A third peak was not identified by the electric nose. Indeed, the software used the volatile compounds library, crossed information obtained on the two columns of the system, and proposed numerous possibilities to identify this unknown peak. None of these propositions could have been crossed with literature data to formally identify this peak. This is one of the weaknesses of an electric nose based on literature data.

After microwave heating, several pyrazine or pyridine derivatives were found and resulted from the thermal processing of glucosamine.[42,44–47] These molecules consisted of a heterocyclic

Table II. Rheological Behavior of the Chitosan Solutions as a Function of the Microwave Heating Time

Time (min)	*K*	*n*	χ^2	R^2
0	0.170 ± 0.001	0.946 ± 0.022	418 ± 91	0.999
15	0.550 ± 0.033	0.896 ± 0.018	259 ± 11	0.999
20	0.313 ± 0.004	0.790 ± 0.021	307 ± 53	0.996

χ^2 = Chi-squared.

Table III. Volatile Compounds Identified During the Microwave Heating of the Chitosan Film with an Electronic Nose Device

Compound	RI	Formula	Olfactive notes (when identified)	Peak area	
				t_0 (reference sample)	t_{30} (sample heated during 30 minutes)
Acetaldehyde	431	C_2H_4O	—	6633 ± 850	3722 ± 945
Trimethylamine	490	C_3H_9N	Ammonia, fish	2708 ± 422	810 ± 195
Unidentified compound	566	—	—	1465 ± 482	ND
Pyridine	750	C_5H_5N	—	ND	308 ± 74
2-Methylpyridine	816	C_6H_7N	Hazelnut popcorn	ND	1142 ± 291
4-Pyridine carbonitrile	978	$C_6H_4N_2$	—	670 ± 159	980 ± 3
Pyrazine trimethylisopentyl	1386	$C_{12}H_{20}N_2$	—	ND	5273 ± 3659

ND, not detected.

aromatic organic compound with one or several nitrogen atoms in different positions on the aromatic ring. These compounds were strongly correlated with the occurrence of the Maillard reaction and were responsible for aroma development in processed food.[48] To allow the Maillard reaction, three elements were needed: amine, reductor sugar, and heat. A Fehling liquor test was performed to confirm the presence of the reductor sugars; amines were present because of the chitosan structure itself, and the films reached 100–110°C during the microwave heating time. Moreover, as some residual acetic acid in the films could enhance the reaction kinetics, the Maillard reaction products could be produced. Those compounds were already identified in literature for the high-temperature heating of glucosamine until the pyrolysis of chitosan or chitin.[44,49,50]

The presence of these compounds was consistent with previous FTIR results, which indicated that the ring-stretching vibration strongly increased with heating time. Indeed, the pyrazine and derivatives were the aromatic compounds observed at 898 cm^{-1} in the FTIR spectra.

Young's Modulus Determination

The Young's modulus was chosen as a relevant parameter to track the chitosan films' mechanical properties decrease during microwave heating. Indeed, the other usual parameters, such as the tensile strength or elongation at break, could not be determined because of the brittleness of the chitosan films after microwave treatment. The Young's modulus appeared to be the more repeatable measurement to perform on the chitosan films. It was determined as the slope of the regression line obtained with the stress–strain curve of the samples heated for different times (Figure 5).

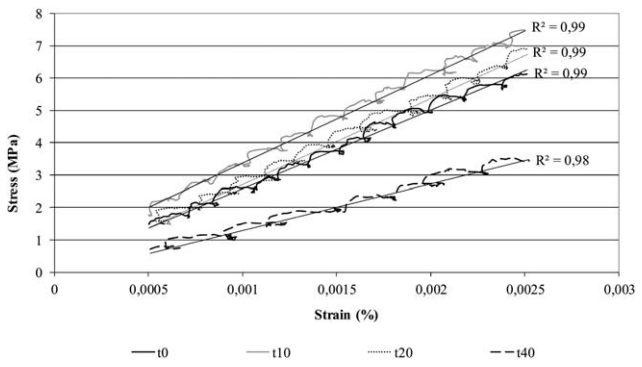

Figure 5. Linear part (elastic phase) of the stress–strain curves obtained from the tensile tests of the chitosan films at different microwave heating times. For each sample, the equation of the regression line was determined, with the slope representing the Young's modulus.

Before microwave heating, the chitosan films had a Young modulus of 2471 ± 344 MPa. After 10 min of heating, it was 2617 ± 386 MPa and 2517 ± 335 MPa after 20 min. Interestingly, the Young's modulus decreased after 40 min of heating to 1482 ± 256 MPa.

The Young's modulus appeared to remain stable for 20 min and fell after 40 min of microwave heating. This was consistent with the sample categorization into three groups revealed by the PCA analysis. We assumed that before 20 min of heating, the moisture and heat levels were low enough for the chitosan film to stay under its glass-transition state. Beyond 20 min, the glass transition was crossed, and the chitosan structure was affected. This led to film brittleness with the Young's modulus decrease. This led us to conclude that the structure modifications highlighted by FTIR spectroscopy affected the mechanical properties of the chitosan films for long heating times.

CONCLUSIONS

Several modifications occurred in chitosan films during microwave treatment. The first important modification was polymeric chain scission, as observed in the FTIR results and confirmed by rheological study and the SEC–MALLS results. Depolymerization led to a deep change in the polymer structure, which was confirmed with DSC. The second important modification occurring in the chitosan film during microwave heating was chitosan deacetylation, which occurred in three steps: a first decrease, stabilization, and a final decrease. Deacetylation occurred in the chitosan films before depolymerization and led to the Maillard reaction and volatile compound release. The polymer remained stable until long-chain chitosan depolymerization happened; this allowed a higher mobility in the polymer structure and a resumption of deacetylation.

Meanwhile, volatile compounds were produced, as highlighted by film yellowing; this was responsible for the off flavor and was potentially hazardous for food contact.

In this study, several modifications were shown during the microwave heating of the chitosan film to change its structure

and properties. Although chitosan is a biopolymer that is widely used with a wide range of applications, some chemical reactions could occur, and these may affect consumer satisfaction or safety. It is crucial to go further in the qualification and quantification of these phenomena, especially as these phenomena start within the first minute of microwave heating.

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