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Physicochemical properties of edible films from chitosan composites obtained by microwave heating

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Summary

Chitosan films were prepared by casting, using microwave and dried by air convection. No scientific literature covers the use of microwave heating in the preparation of chitosan films by casting technique. Effects of heating time, molecular weight and plasticizer on structure, thermal behavior, surface, barrier properties and light transmission were investigated. Heating time showed that the microwave heating did not affect the structural composition and thermal decomposition of chitosan films. UV-vis light barrier properties, equilibrium moisture content and water vapor permeability varied significantly with the heating treatment. Surface film analysis revealed no captured differences between different heating treatments.

Introduction

Chitosan, copolymer of β-(1-4)-2-acetamido-2-deoxy-β-D-glucose and β-(1-4)-2-amine-2-deoxy-β-D-glucose, has attention for its film-forming capacity [1-2]. Film formation from chitosan is a process involving the amine group protonation, followed by salt formation from the weak acid solvent. Currently, the most commonly method used to prepare films from chitosan and derivatives is the casting, with conventional heating [3-6]. Srinivasa et al. [7] reported the use of IR dried for film formation, like faster and superior in preserving desirable functional characteristics of films. Microwave heating is a thermal treatment with several advantages, as fast heating, power transference efficacy, selective heating, low cost and easy manipulation [8-9]. Mechanism of microwave heating is based in the heat and mass transference or interactions between microwave energy and individual polar molecules. In the case of polymers, the mechanism involves the dipolo reorientation in the electric field imposed. It is well known, that the movement of isolated individual molecules is not responsible for heat generation upon microwave absorption in liquids, but the collective movement inside the liquid [10]. The main advantage of microwave heating is the quick propagation rate through the material, so that heating occurs in the bulk of material, and not just on the surface as it happens by conventional heating [11-12]. Electromagnetic heating is mainly a result of the internal heat generation, exhibiting unique properties such as the great penetration depth and the selective heating of different components having different dielectric properties [9]. Modifications of chitosan and film formation have been reported by the use of UV and IR irradiation, X-ray diffraction pattern of oven-dried films showed a different crystalline nature, respect to the IR dried. Barrier properties of films can be modified by the type of drying, for example, water vapor and oxygen transmission rate values were slightly reduced for example, in IR-dried films [7]. Therefore, the goal of this study was to investigate the structural, microsurface and barrier properties as affected by combination of molecular weight and plasticizers incorporation with the heating in the film formation.

Experimental

Materials

Chitosan (95% deacetylation degree, with 400,000 Da and 170,000 Da molecular weight) were gently provided by Quitoquímica Co. (Coronel, VIII Región, Chile).
D(-)Sorbitol and D(-) Fructose were purchased from Sigma Chemicals (St. Louis, USA). Acetic acid was purchased from Caledon (Georgetown, Canada). All chemicals were analytical grade.

Preparation of chitosan solutions and film formation

Six chitosan solutions (2%) were prepared. Each solution was prepared by dissolving chitosan (HMW and MMW) in 1% acetic acid with 13 h. stirring. Solutions were filtered through a Schott filter. D(-)Sorbitol and D(-) Fructose were added as plasticizer at 0.5% v/v and homogenized by stirring for 15 h. Films were cast and dried by two types of heating: microwave and air convection. Film-forming solutions (30 mL) were poured in the casting plate and dried for 12 h at 35°C in an air convection oven [6]. Films obtained by microwave heating were dried for 10 min at full power, using a Thomas microwave/convection oven (model TH-MO17D, Robert Thomas-Germany, 2450 MHz) and left to final dry at atmospheric conditions for 2 h. The heating was carried out one by one and stabilized until room temperature. Films were placed in polystyrene bags and stored at room temperature.

Analysis techniques

Film thickness was determined with a hand electronic digital micrometer. Shown values are an average of 10 measurements. The infrared spectra were recorded from 4000 to 100 cm^{-1} on a Nicolet Nexus spectrometer at room temperature (Nicolet Instrument Co., USA). These films were pressed into pellets with KBr crystal. Thermal analysis of films was performed using a Thermogravimeter Analyzer Perkin Elmer TGA 7. All experiments were carried out under a nitrogen atmosphere. All specimens were weighted in the range of 3-4 mg and heated to 580°C at a rate of 25°C/min.

Water solubility

Samples of pre-weighed films were immersed in distilled water (pH 7) at 25°C. At 60 min, chitosan films were removed from the bath and the water excess was eliminated with filter paper. Finally, chitosan films were dried until constant weight in order to determine the weight of dried film.

Equilibrium moisture content

Equilibrium moisture content was determined according to Pinotti et al. 2007 [13]. The weight loss of films was measured upon drying until constant weight at 110°C. Equilibrium moisture content (%) was calculated as follows:

$$\mathrm{EMC}(\%) = \left(\frac{\text{Initial sample weight}}{\text{dry sample}} - 1 \right) \mathrm{x}\, 100 \tag{1}$$

Water vapor transmission rate (WVTR)

WVTR was determined according to ISO 2528 standard method, 1995(E) with WVP Correction Method [14]. Films with an area of 0.001 m^2 were exposed for 24 h at 20°C and 90% RH before determination. Films were mounted on cups filled with water. The cups were placed, at 20°C and 90% RH in desiccators. Weight cups were measured at 24h intervals. Weight loss vs. time plots and simple linear regression were used to found the slope and to calculate water vapor transmission rate (WVTR).

Surface microstructure

Scanning electron microscopy (SEM) was used to examine representative film surfaces, samples were-sputter coated with gold and scanned using a JEOL-JEM 6380 LV, using an accelerating voltage of 20 kV.

Opacity, light transmission and transparency

Opacity was measured according to the method described by Cho and Rhee [15]. Films were cut into rectangles (1.0 cm×3.0 cm) and placed in the film holder device. A spectrum of each film was recorded using a spectrophotometer (2450 UV-vis spectrophotometer Shimadzu Corp, Kyoto Japan). The area under the absorbance curve from 400 to 800 nm was taken as the opacity of film. It was expressed as absorbance unit (AU) × nm/unit thickness (μm). Measures of opacity were performed at 0, 7, 14, 21 and 28 days of storage at 23°C in polystyrene bags. Absorbance values were normalized, so that no absorbance scale is given in the figure 5. Transparency of films was calculated as follows:

$$\text{Transparency} = \left(\frac{\text{Absorbance (nm OD)}}{\text{Thickness } (\mu\text{m})} \right) \tag{2}$$

Results and Discussion

Preliminary tests showed that microwave heating time did not affect the structural properties of chitosan films: high molecular weight chitosan-acetic acid (HMW CS-AA), high molecular weight chitosan-acetic acid-sorbitol (HMW CS-AA-Sor), high molecular weight chitosan-acetic acid-fructose (HMW CS-AA-Fruc), medium molecular weight chitosan-acetic acid (MMW CS-AA), medium molecular weight chitosan-acetic acid-sorbitol (MMW CS-AA-Sor) and medium molecular weight chitosan-acetic acid-fructose (MMW CS-AA-Fruc). Reasonable time of microwave heating for water loss is 10 min at full power, for 30 mL of film-forming solution. Information can be obtained from the intensity peaks of FT-IR spectra, because this parameter is directly related with water loss and carboxyl group loss, belonging to acetate. Evidence of water loss can be observed by the decreasing of bands near to

3200 – 3400 cm^{-1}. Moreover, -OH stretch band position taken-drove to lower frequency with increased H-bonding in matrix. For 10 min, this band is shifted to higher frequency than 4 min, similar behavior is showed to coupling of C–N axial stretching. Important evidence of residual acetic acid loss is the presence of intense bands near 1620 and 1530 cm^{-1}, not present in the microwave heating of 10 min. Table 1 shows the IR spectral evolution of films for different microwave drying times in order to obtain chitosan films (high molecular weight, HMW CS-AA) without plasticizer. The main aim was to find such characteristics that would show differences in both heating treatments. Film thickness is an important factor because affects directly two barrier properties: water and light.

As shown (Tab. 2), heating treatment had no significant effects on film thickness, which remained unchanged for plasticizer addition. Solutions without plasticizer with higher pH produced films with higher thickness and solutions with lower pH produced films with lower thickness. However, for solutions with plasticizer, thickness-pH relationship is reverse. Molecular interactions can affect thickness considering the polarity of plasticizers in combination with the heating treatment.

Table 1. Characteristic absorption bands (cm-1) in infrared spectra, position and intensity peaks are summarized.

Assignment	4 min		6 min		8 min		10 min	
	Pos.	Intens.	Pos.	Intens.	Pos.	Intens.	Pos.	Intens.
-OH and –NH$_2$ stretches overlapping	3275	3.7	3258	3.3	-------	------	3291	3.2
-C–H axial stretching	2966	3.5	2935	3.4	2997	1.7	2933	6.2
C=O stretch (acetate, amide I)	1627	2.1	1628	1.5	1629	1.1	1625	1.1
C=O stretch (amide II)	1531	5.8	1533	2.4	1540	2.6	1519	1.7
Coupling of C–N axial stretching	1413	2.2	1412	1.5	1412	1.1	1416	1.2
polysaccharide skeleton, (glycosidic bonds, C–O and C–O–C stretching)	1152	2.0	1.152	1.6	1152	1.4	1152	4.6

In microwave heating, alcohol molecules can be aligned with the field, permitting a slowly evaporation, which could control the dried and therefore the thickness. The mean thickness of films was 0.055 mm for HMW CS and 0.054 mm for MMW CS. For air convection, film mean thickness of films was 0.056 mm and 0.054 mm for microwave heating. Thickness variations were observed during storage. The first week of storage, films from MMW CS from air convection showed a loss mass during the first and third week of storage, whereas, MMW CS films from microwave heating lost mass only during the first week. HMW CS films from air convection lost mass during the second and last week and films from microwave heating lost mass during the first and second week of storage. Figure 1 shows the variation of film thickness through 28 days of storage in polystyrene bags.

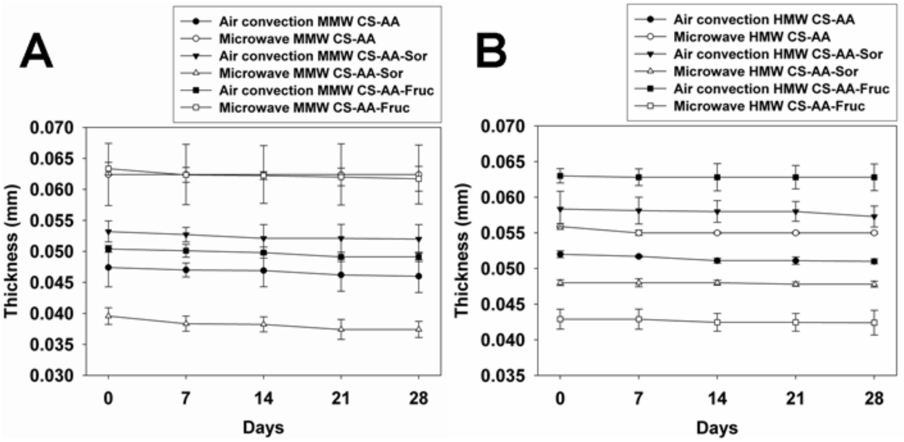

Figure 1. Thickness variation (A) Films from MMW CS and (B) Films from HMW CS.

Air convection dry does not remove humidity in the same way as microwave treatment. It is possible that some part of humidity content is coming from the absorption of moisture in air convection heating. Air convection heating is not suitable for elimination of water and residual volatile compounds. The hygroscopic nature of chitosan is a factor that may affect several properties of films, such as permeability, thickness and stability. In general, films from carbohydrates are ineffective water vapor barriers due to their high hydrophilicity. Effects of heating treatment in WVP of films can be compared in Table 2.

Films from microwave heating showed higher WVP, compared to air convection films, this effect can be modified by the time of microwave heating and by addition of additives. Water vapor permeability has also shown to be directly related to the molecular weight; films with sorbitol and fructose had a higher hydrophilic nature in comparison with films without plasticizer, for both heating treatments. Films with sorbitol had lower WVP compared to films containing fructose, probably due to linear chain of sorbitol binding chitosan by hydrogen bonding, decreasing the selectivity of film toward water in active sites of chitosan, no effect is showed for films with fructose, due to steric impediment caused by the ring. These results are according to Srinivasa et al., 2007 [7].

Absorptions peaks of chitosan films with plasticizer from microwave and conventional heating are shown in Fig. 2. The structure modification of films by heating treatment was investigated by comparing the FTIR spectra of films as shown in Fig. 2. No structural modification can be observed between heating, for both molecular weights.

Thermal analysis showed film stability up to 60°C. HMW Chitosan powder showed two thermal events, the first, attributed to humidity and the second event (about

300°C) attributed to decomposition of glucose chains. HMW CS-AA films from air convection without plasticizer showed 3 thermal events, identified at (a) 120°C with mass loss of 19% (b) 208°C with mass loss of 7.1%, and (c) 303°C with mass loss of 37%. The first thermal event was related to water evaporation present in the films. The second thermal event started over 160°C, related to acetate loss of chitosan salt. The third event started over 280°C, related to decomposition of glucosamine chains. A decrease of decomposition temperature of glucosamine units can be observed, respect to chitosan powder.

Table 2. Physicochemical properties of films from air convection and microwave heating.

Films	pH film-forming solution	WVP gm/m^{2}dayKPa	Water solubility	Thickness mm	Equilibrium moisture content (%)
Air convection					
HMW CS-AA	4.60	0.00591±0.000	Dissolved	0.052±0.000	15.719±0.355
HMW CS-AA-Sor	4.45	0.00102±0.000	Dissolved	0.057±0.001	22.096± 0.631
HMW CS-AA-Fruc	4.56	0.00186±0.001	Dissolved	0.068±0.002	16.065±0.065
MMW CS-AA	4.63	0.00109±0.001	Dissolved	0.047±0.003	20.000±0.000
MMW CS-AA-Sor	4.49	0.00918±0.002	Dissolved	0.053±0.002	30.706±1.057
MMW CS-AA-Fruc	4.61	0.00677±0.001	Dissolved	0.058±0.003	30.952±1.944
Microwave					
HMW CS-AA	4.60	0.00724±0.002	Dissolved	0.057±0.000	15.684±0.435
HMW CS-AA-Sor	4.45	0.00094±0.000	Dissolved	0.048±0.000	16.0781±1.058
HMW CS-AA-Fruc	4.56	0.00289±0.001	Dissolved	0.050±0.001	5.444±1.277
MMW CS-AA	4.63	0.00630±0.002	Dissolved	0.065±0.004	18.824±0.961
MMW CS-AA-Sor	4.49	0.00659±0.001	Dissolved	0.040±0.001	27.922±0.530
MMW CS-AA-Fruc	4.61	0.01774±0.001	Dissolved	0.063±0.001	28.636±1.113

The first weight loss was observed 30°C higher with respect to temperature assigned for chitosan powder and this shift could belong to water and acetate loss. Microwave treatment showed a shift over 54°C, respect to chitosan powder, without significant mass loss, for HMW CS-AA film. This can be attributed to least amount of evaporating moisture for films with microwave treatment, in comparison with films dried by air convection; microwave heating makes temperature increasing to evaporate the bound water, because higher amount of unbound water was eliminated by microwave heating, whereas air convection dried eliminates water in accessible sites of the polymer. All films showed a decrease of decomposition temperature of glucose chains, respect to chitosan powder. In the films with plasticizers, the mass loss below 100°C was mainly ascribed to water humidity loss and over 100°C was related to crystallization water, acetate and plasticizers loss. Thermal behavior of first event for films with sorbitol is similar to films without plasticizer, for both heating treatments and molecular weights.

Moreover, the first thermal event for films with fructose as plasticizer was at lower temperature than sorbitol, ascribed to plasticizer and acetate loss.

The presence of a strong thermal event for films with fructose, in all cases, might be due to weak interactions between fructose and chitosan, and be considered as a proof of their poor complexation and immiscibility. In case of films from HMW CS-AA-Sor, the second thermal event is similar to decomposition of films from HMW CS without plasticizer. This pattern confirms the interactions between chitosan and sorbitol, and might be considered as a proof of good miscibility and complexation. Third thermal event for films from HMW CS was observed about 300°C with maximal rate at 303 and 319°C either for air convection or microwave, respectively. Miscibility and complexation are inherent phenomena to film formation, and were not modified by heating treatment.

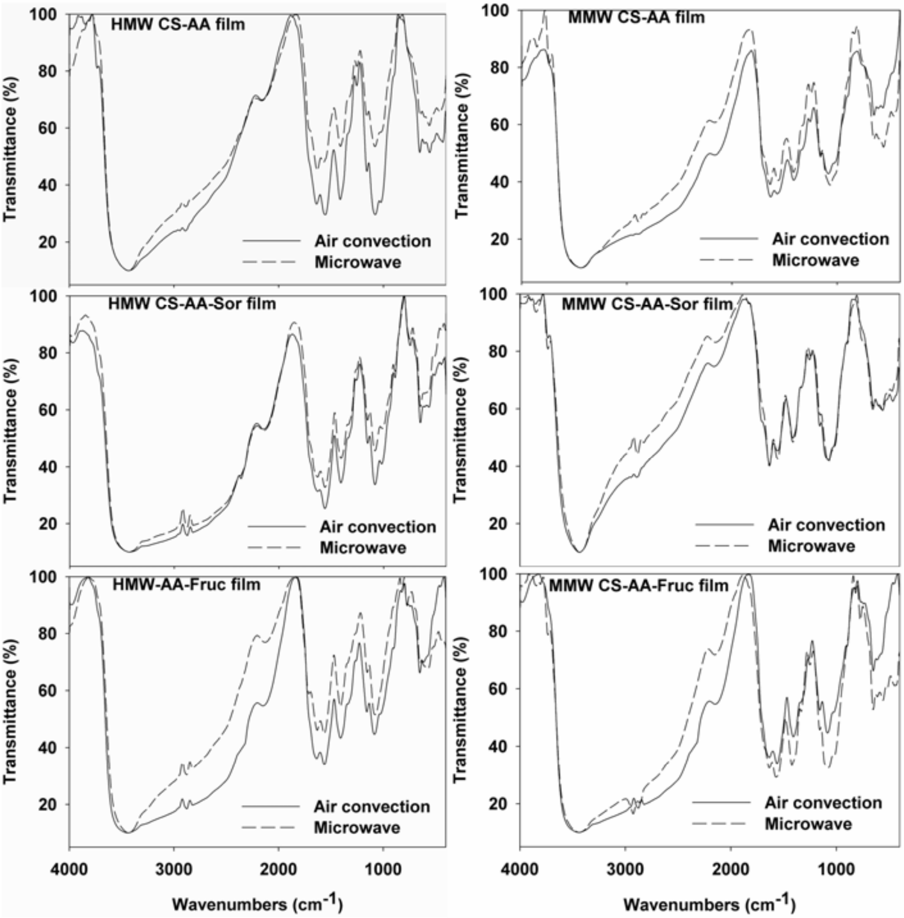

Figure 2. Characteristic absorption bands in infrared spectra (cm^{-1}).

For air convection cases, in the TGA curve, the weight loss is higher, in comparison with the weight loss occurred in films from microwave heating, probably due to efficient evaporation of solvent and acetic acid residual in the cast by microwave.

Table 3 summarizes the thermal behavior of chitosan powder and chitosan films from air convection and microwave heating.

Table 3. Thermal behavior of chitosan films from air convection and microwave heating

Sample	**Thermal behavior**					
	°C	**% Weight loss**	**°C**	**% Weight loss**	**°C**	**% Weight loss**
HMW Chitosan (powder)	87.5	6.9	--	--	352.4	50.3
MMW Chitosan (powder)	90.6	5.9	--	--	355.0	50.9
Air convection						
HMW CS-AA Film	117	19.1	208.1	7.1	303.3	37.0
HMW CS-AA-Sor Film	--	--	206.5	21.9	302.0	41.2
HMW CS-AA-Fruc Film	160.9	37	--	--	285.9	25.5
MMW CS-AA Film	125	20.3	199.5	9.1	320.3	35.0
MMW CS-AA-Sor Film	134.4	16.7	211.7	5.88	324.1	40.1
MMWCS-AA-Fruc Film	153.9	54.2	--	--	310	10.3
Microwave						
HMW CS-AA Film	141	17	209.4	8.7	319.7	38.1
HMW CS-AA-Sor Film	--	--	212.3	17.3	298.3	44.5
HMW CS-AA-Fruc Film	159.6	16.8	--	--	291.9	41.3
MMW CS-AA Film	121.9	16.0	208.7	6.33	324.9	40.1
MMW CS-AA-Sor Film			198.9	26.7	325.8	40.3
MMWCS-AA-Fruc Film	146.87	7.0	--	--	300.0	43.6

Light barrier properties of films in the UV range (200-400 nm) are important characteristics in order to avoid phenomena such as lipid oxidation induced by UV light in the food systems. However, the mechanisms of blocking of ultraviolet light can compromise the visual transparency, interesting sensorial factor for consumers, and the major goal within the package industry. Fig. 3 shows the variation of opacity values of films during 28 days. The opacity values of films from air convection had the lowest among all samples, however, films from microwave not shown much higher values than films from air convection. Variation of opacity through 28 days of storage was not significant for all cases. Films from microwave heating shown an increasing absorption of UV-light (200–350 nm), while keeping very high optical quality. The HMW CS-AA-Fruc and MMW CS-AA-Sor films from microwave heating presented a much better UV-absorption as shown in Figure 4 A and B. All films, from microwave and air convection, showed very high transparency in the visible region of the spectra (400–700 nm).

The HMW CS AA Fruc film microwave combines the advantages of UV-protective material, showing very strong absorption in the whole UV region and high transparency in the visible region of the spectra, at the same time, the absorption of HMW CS AA Fruc film from microwave heating covers much more UV region than that of HMW CS-AA-Fruc film from air convection, blocking the major contribution of sun to damaging UV light (290–350 nm).

Below 250 nm, HMW CS AA Fruc film air convection shows much stronger absorption than HMW CS-AA film air convection, but above 370 nm, it shows much weaker absorption.

The UV-absorption performance of the HMW CS films can be listed as following: HMW CS AA Fruc (MH) > HMW CS-AA Fruc (AC) > HMW CS-AA (AC) > HMW CS- AA-Sor (MH) > HMW CS-AA (MH) > MW CS-AA-Sor (AC). For MMW CS films, follow the trend: MMW CS-AA-Sor (MH) > MMW CS- AA-Fruc (AC) > MMW CS-AA-Fruc (MH) > MMW CS-AA (MH)> MMW CS-AA- SOR (AC)> MMW CS-AA (AC). AC and MH are abbreviations of air convection and microwave heating, respectively.

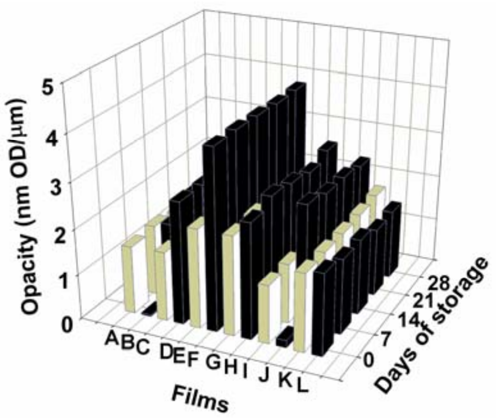

Figure 3. Variation of opacity along storage. White bars: air convection, black bars: microwave heating. HMW CS-AA (A and B), HMW CS-AA-Sor (C and D), HMW CS-AA-Fruc (E and F), MMW CS-AA, (G and H), MMW CS-AA-Sor (I and J), MMW CS-AA-Fruc (K and L).

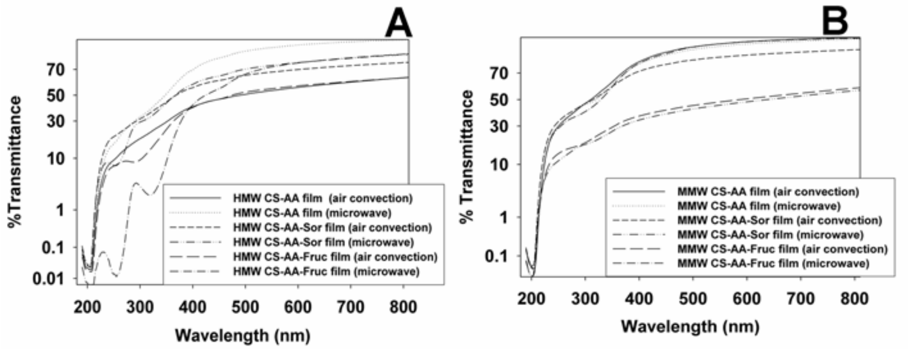

Figure 4. UV-vis % transmittance spectra of films. (A) Films from high molecular weight CS and (B) films from medium molecular weight CS.

Figure 5 shows the transparency values along of UV-vis spectra for all films. In general, chitosan films are no good light barriers in the UV range. Chitosan films from microwave heating showed the better performance in terms of transparency, blocking effectively the UV light. The strong absorbance in UV range and weak absorbance in visible range are good indicators of barrier light and transparency, respectively.

Chitosan films from air convection showed weak absorbance in UV range and high transparency in the visible range. Molecular weight of chitosan did not affect this behavior, films from high and medium molecular weight showed better performance with microwave heating. Addition of sorbitol produces the same behavior, better performance for HMW and MMW CS films from microwave heating in UV range, however, for visible range the best performance is showed by air convection for MMW CS film, similar to fructose, only for MMW CS. On the other hand, HMW CS-AA-Fruc from microwave heating showed the best performance in UV range.

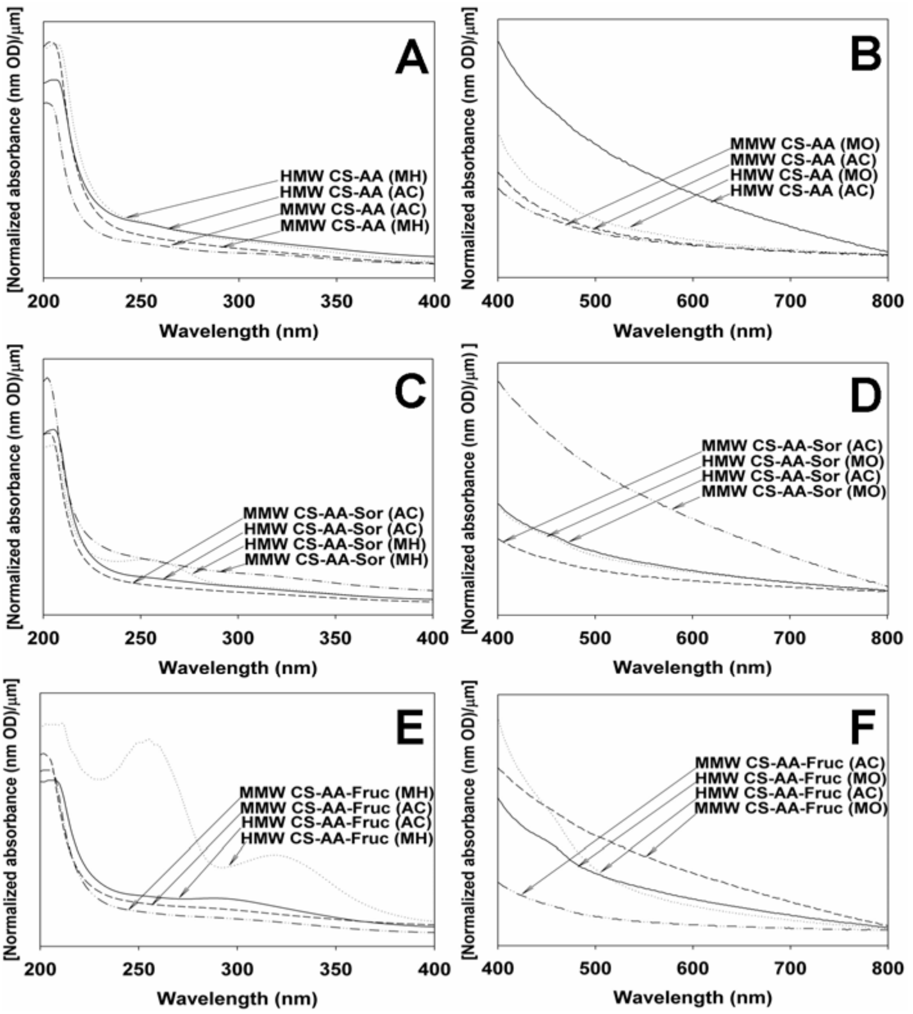

Figure 5. Transparency of films. (A), (C) and (E) UV range; (B), (D) and (F) vis range.

Figure 6 shows the scanning electronic micrographics of film surface. Pure chitosan film from high and medium molecular weight showed a homogeneous surface and no characteristic change in morphology was observed for both heating. However, small protuberances were seen in major quantity in air convection, for all films. For this case, were scanned 3 different surfaces to evaluate the behavior in all films, with a similar pattern, in order to avoid subjective error criteria. On introducing sorbitol and fructose as plasticizer, into the film forming solution, smooth surface was observed for films from microwave, it is possible that, this heating favors the plasticizers miscibility with the chitosan solutions, avoiding the formation of micro-dispersed globules.

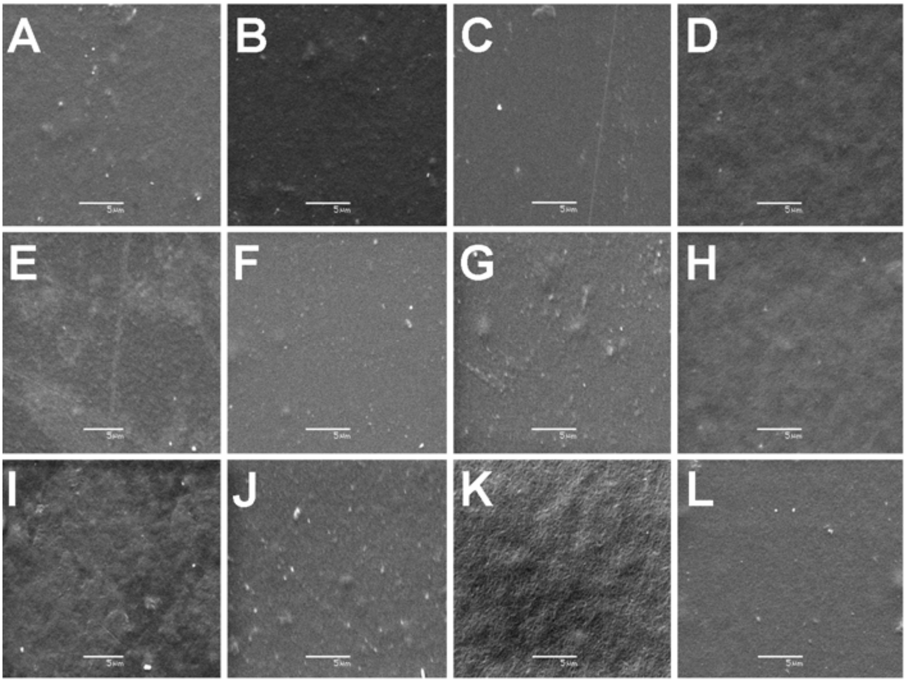

Figure 6. Micrographs of film surface. Air convection (A) HMW CS-AA, (E) HMW CS-AA-Sor, (I) HMW CS-AA-Fruc, (C) MMW CS-AA, (G) MMW CS-AA-Sor (K) MMW CS-AA-Fruc. Microwave heating (B) HMW CS-AA, (F) HMW CS-AA-Sor, (J) HMW CS-AA-Fruc, (D) MMW CS-AA, (H) MMW CS-AA-Sor and (L) MMW CS-AA-Fruc.

Conclusions

Chitosan films were successfully obtained by solution-casting technique assisted with microwave heating, offering a great advantage in terms of time and effectiveness. Film formation is carried out with microwave heating faster than with air convection heating, increasing 6 times the casting speed. Results showed that there are no significant structural and thermal behavior differences in their morphological and physicochemical properties. However, improvements on smooth surface and UV-vis light barrier properties of films were observed for microwave heating. The presence of plasticizers (sorbitol and fructose) improves the surface quality of the films.

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